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New Perspectives

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Contributors
Ajaz Ahmad, Ali Irfan, Ali Ramazani, Amol T. Mahajan, Emilio Mateev, Farheen Farooqui, Huma Hameed, Laila Rubab, Lubna Khan, Magdi E.A. Zaki, Mohammad Raish, Nawal Kishore Sahu, Pinki Pal, Raid Abdel-Jalil, Rashid Ali, Sadia Rani, Sami A. Al-Hussain, Samina Aslam, Sandeep Chaudhary, Seyedmohammad Hosseininezhad, Shazia Akram Ghumman, Shazia Perveen, Thuraya Al-Harthy, Wajdi Zoghaib, Yousef A. Bin Jardan, Zeemal Seemab Amin

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Meet the editor

Dr. Rashid Ali is a pioneering researcher engaged inorganic and supramolecular chemistry. He obtained his Ph.D. from the Indian Institute of Technology Bombay (IITB), Mumbai, Maharashtra, India. He has more than 13 years of research experience, including 11 months as a postdoctoral fellow at Sookmyung Women's University, Seoul, South Korea, and 4 months as a SERB-SIRE Visiting Scientist in Prof. L. Greb's research group at Heidelberg University, Germany. He has published more than seventy-five original research papers, three books, and a dozen book chapters.He isan assistant-professor in the Department of Chemistry, Jamia Millia Islamia, New Delhi, India.

Contents

Preface

Heterocyclic compounds play a leading role in preparative chemistry, with great significance across industrial, biological, pharmacological, and everyday applications. Notably, most drugs and bioactive molecules are heterocyclic in nature, with about 70% of all agrochemicals and pharmaceuticals featuring at least one heterocyclic moiety within their structures. This makes the design, fabrication, transformation, and exploration of heterocyclic compounds foundational to organic synthesis. Creating valuable heterocyclic scaffolds that are cost-effective as well as efficient is a primary goal for industry, while the synthesis of complex heterocyclic structures that display unique molecular architectures is a challenge for academic research.

In view of the importance of heterocycles in diverse areas, and to highlight new advances in heterocyclic chemistry, we have assembled this book to showcase innovative perspectives in the field. This book includes seven chapters. Chapter 1 provides an overview of the Structure-Activity Relationship (SAR) of macrocyclic anticancer agents. Chapter 2 discusses the application of heterocycle-based ionic liquids (ILs) in transdermal drug delivery. Chapter 3 focuses on targeting tyrosinase. Chapter 4 examines the biological activities of benzazoles. Chapter 5 describes the anticancer activities of pyridine derivatives. Chapters 6 and 7 cover recent advances in furan derivatives and pyrrolidines, respectively.

I am very grateful to all the contributors to this book. Without their efforts and expertise, this book would not have been possible.

I express my heartfelt thanks to my mother Rabeda Khatoon, my wife Saba Khan, and my lovely daughters Naira Khan and Samaira Khan. Finally, I wish to thank my family members, relatives, colleagues, and friends for their continuous motivation and encouragement.

Rashid Ali
Faculty of Sciences,
Department of Chemistry,
Jamia Millia Islamia,
New Delhi, India

Chapter 1

From Rings to Remedies: Investigating the Structure-Activity Relationship of Macrocyclic Anticancer Agents

Sadia Rani, Samina Aslam, Ali Irfan, Emilio Mateev, Sami A. Al-Hussain and Magdi E.A. Zaki

Abstract

The profound pharmacological attributes of macrocyclic compounds have spurred their transformation into pharmaceutical drugs. Within conformationally pre-organized ring structures, the macrocycle's intricate functions and stereochemical complexity contribute to a heightened affinity and selectivity for protein targets. Simultaneously, they maintain sufficient bioavailability to penetrate intracellular locations. As a result, the construction of macrocycles emerges as an optimal strategy for addressing the challenge of "undruggable" targets like cancer. Cancer stands as the second most prevalent and formidable threat to human life, prompting researchers to channel their efforts toward the extraction and synthesis of effective therapeutic drugs designed on macrocyles to combat various types of cancer cells. Many macrocyclic drugs have been licensed by the Food and Drug Administration (FDA) for the treatment of cancer patients. Nonetheless, the significance of these compounds in the production of cancer therapeutics is still undervalued. According to recent research, macrocyclic compounds can be a useful tactic in the fight against drug resistance in the treatment of cancer. This chapter aims to present bits of evidence about the uses of macrocyclic compounds as potential cancer treatments. By providing more innovative approaches to aid cancer patients and society as a whole, this chapter will hopefully stimulate greater interest in the development of macrocyclic medicines for cancer therapy.

Keywords: synthetic macrocyles, natural macrocycles, anticancer agents, kinase inhibitor, SAR

1. Introduction

Macrocycles have been referred as a ring structure containing 12 or more atoms [1]. Although there are differences of opinion regarding the ring size criteria for defining macrocycles, this alternative highlights the qualitative differences in

behavior between large macrocyclic rings (≥12 atoms) and medium rings (8–11 atoms). Because of their constrained rotation, macrocycles have a structure that allows for some conformational pre-organization. Numerous naturally occurring compounds possess a macrocyclic core, indicating that the synthesis of secondary metabolites derived from these scaffolds could confer an evolutionary benefit [1, 2]. Macrocycles can belong to a variety of classifications, such as peptidic and nonpeptidic natural products, synthetic peptides and non-natural macrocycles [3]. Repeating patterns in the distribution of charge and polarity within the molecule are a significant feature of many macrocycles. A polar, hydrophilic side and an apolar, lipophilic side are present in many structures. A general structure **A** with an endocyclic small heterocycle is often accompanied by another small heterocyclic (such as an oxazole, imidazole, thiazole, sugar, etc.) or substituted aromatic moiety, which may be endo- or exo-cyclic (**Figure 1**) [2].

Macrocycles have physicochemical and pharmacokinetic characteristics similar to those of drugs, including high lipophilicity, solubility, bioavailability and metabolic stability. Macrocyclic substances have demonstrated therapeutic potential, however their potential for discovering new medicinal molecules has been under-explored and inadequately studied. There are numerous causes for this. The pharmaceutical industry has been reluctant to explore natural products more and more because of the challenges they pose in the analogue synthesis process due to their structural complexity. Additionally, it is now common practice to screen compounds that comply with the rule of 5-compliant compounds preferentially. Nonetheless, a number of research organizations are looking at the possibility of using synthetic macrocycles for drug discovery, and they have demonstrated that these substances can offer good target selectivity and affinity in structures with acceptable drug-like characteristics. There are currently several synthetic macrocycles undergoing active preclinical and clinical research that have no relation to natural compounds [1]. There are known to be more than 100,000 secondary metabolites of natural products, and about 3% of these are macrocycles [2]. Although the proportion of macrocycles in this class of natural products is small, it contains a potent subset of medicines that are used to treat cancer, combat emerging infectious pathogens, and modify immune system responses. A number of cancer medications that are either approved for clinical use or have advanced to the late stages of clinical development are the result of the exploitation of natural product macrocycles.

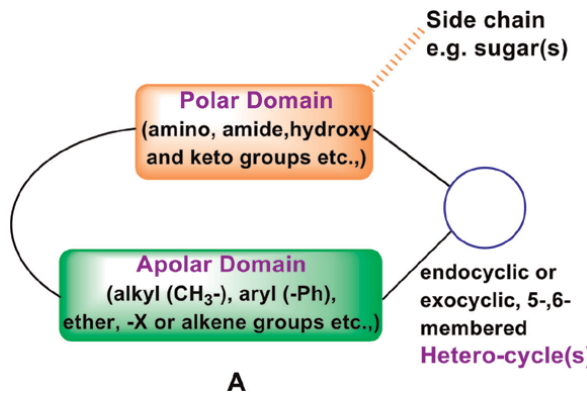


Figure 1.
A distribution of polarity domains present in numerous natural macrocycles [2].

2. Advantages of using macrocyclic molecules as drugs

The use of macrocyclic compounds as pharmaceuticals has several benefits: First, in order to manage their structural flexibilities, macrocyclic molecules often have more limited conformations. Strong binding affinities and excellent selectivity to target proteins are made possible by their tight conformations. Second, compared to conventional small-molecule medicines or large biologics, macrocyclic compounds have unusual pharmacological properties due to their unique structural features [4]. Through macrocyclization, molecules can lose some of their degree of freedom, which can improve their oral bioavailabilities, cellular penetration, polarity, metabolic stabilities, and pharmacokinetic and pharmacodynamic properties [1, 3, 5, 6]. Macroscopic molecules have molecular weights ranging from 300 to 2000 Dalton. They often have molecular weights that are lower than acyclic peptides, which typically have molecular weights of more than 2000 Dalton. Their lifetime in vivo is increased by their decreased molecular weights, which also provide more effective pharmacological properties, such as permeabilities and reduced susceptibility to proteolytic breakdown [7]. Moreover, macrocycles are the tiniest examples of biomolecules with functional sub-domains; they are not merely larger forms of tiny molecules [1]. In summary, throughout the past 20 years, medicinal chemistry has focused a lot of attention on macrocyclic molecule medicines due to their many benefits.

The second most prevalent disease that poses a threat to human life is cancer. The prevalence of cancer is rapidly rising in the whole world [8]. In 2022, there were 0.6 million cancer-related fatalities and 1.9 million new cancer diagnoses in the US, according to cancer statistics [9]. The annual cost of cancer is likewise very high. Thus, there is a large global market for cancer medications, and their sales are high. Thus, there is a great deal of interest in drug research to develop different cancer therapies. Researchers have recently concentrated their efforts on creating effective medicines that can treat various cancer cells. The majority of research has gone toward creating medications that treat cancer in its early stages, as these medications have attracted more attention than those that treat the disease in its later stages [10]. Numerous macrocyclic compounds, including pacritinib, have demonstrated efficacy as medications for the treatment of cancer patients. Several efforts have been undertaken to create innovative macrocyclic drugs to treat cancer patients, as the unmet demands for cancer therapy in clinics continue to rise. For the purpose of developing possible anticancer derivatives, macrocyclic molecules are typically preferred, particularly in the chemical, biological, and medicinal domains [6, 11].

3. Why macrocylic compounds are used as anti-cancer agents?

Targeting proteins with expanded binding sites, like class B G-protein-coupled receptors (GPCRs), protein-protein interactions, and certain enzymes, is a very challenging task for small molecule drug developers [12]. Designing anticancer drugs presents especially challenging conditions. The most widely used “biological agents” to modulate these targets have a number of drawbacks, such as high cost, low oral bioavailability, lack of cell permeability, and decreased patient compliance. Due to their degree of structural pre-organization, macrocyclic compounds minimize the significant entropy loss during binding by enabling important functional groups to interact across extended binding regions in the protein [3]. A molecule must take on a bioactive shape in order to bind to a target protein. Because the ligand-protein binding

reduces the number of conformations the unbound molecule can adopt, there is a lower entropic cost. Macrocycles are conformationally confined, yet not totally stiff, with limited internal bond rotations. Macrocycles are theoretically adaptable compounds that possess sufficient flexibility to effectively engage with flexible binding sites in proteins while also reducing the internal entropy loss linked to the ligand's transition from the unbound to the bound state. A decrease in a receptor's entire mobility can have a positive enthalpic contribution even though it is an unfavorable entropic alteration because it can strengthen hydrogen bonding and other intermolecular interactions with a ligand. Because of these properties of macrocyclic molecules, "molecular macrocyclization" is an important strategy for resolving the aforementioned issues [13]. Consequently, further research should be done on the relationships that macrocycles have with the proteins that they target [13].

3.1 Role of macrocycles in diseases especially in cancer and cancer multi drug resistance (MDR)

The primary therapeutic indication for which macrocyclic drugs are used (representing 44.4% of all macrocyclic pharmaceuticals) is an infectious disease. While antibacterial agents make up the majority of this class, antifungals (8.3%) and antivirals (6.9%) are also significant. The remaining three primary therapeutic indications are immunosuppressants (5.6%), autoimmune diseases (5.6%), and oncology (20.8%). 13 "Other" minor indications, or 23.6% of the total number of indications, also employ macrocyclic drugs. Antidiuretics, persistent pain, hereditary obesity, heart failure, etc. are some of these indications. **Table 1** provides a comprehensive list of therapeutic indications and targets for the macrocyclic medicinal products [14].

Drug	Target	Therapeutic indications	Drug	Target	Therapeutic indications
Capreomycin	16S/23S rRNA (cytidine-2'-O)-methyltransferase TlyA	Infection: Antibacterial	Nystatin	Ergosterol	Infection: Antifungal
			Natamycin		
Oritavancin	NAM/NAG peptide (D-Ala-D-Ala)		Amphotericin B		
Vancomycin			Micafungin	1,3-beta-glucan synthase component (FKS1)	
Dalbavancin			Anidulafungin		
Telavancin			Caspofungin		
Bacitracin	C55-isoprenyl pyrophosphate		Lanreotide	Somatostatin receptor (SSTR)	Acromegaly
Azithromycin	23S ribosomal RNA (50S)		Cyclosporin	Calcium signal-modulating cyclophilin ligand (CAMLG), Calcineurin subunit B (CNB)	Autoimmune diseases
Erythromycin			Voclosporin		
Telithromycin			Pimecrolimus	FKBP12, Calcineurin subunit B (CNB)	
Dirithromycin			Tacrolimus		
Clarithromycin			Plecanatide	Guanylate cyclase soluble subunit alpha-2 (GUCY1A2)	Chronic Idiopathic Constipation (CIC)

Drug	Target	Therapeutic indications	Drug	Target	Therapeutic indications
Polymyxin B	Bacterial membrane		Nesiritide	Atrial natriuretic peptide receptor	Heart failure
Daptomycin			Oxytocin	Oxytocin receptor	Induction of labor
Colistimethate			Vosoritide	Atrial natriuretic peptide receptor	Achondroplasia
Fidaxomicin	RNA polymerase		Hydroxocobalamin	Methionine synthase Methylmalonyl-CoA mutase Methionine synthase reductase (mitochondrial)	Vitamin B12 deficiency
Rifamycin			Cyanocobalamin		
Rifampicin					
Rifapentine Rifabutin			Verteporfin	NA (Reactive oxygen species)	Macular degeneration
			Bremelanotide	Melanocortin receptor	Premenopausal women (with hypoactive sexual desire disorder)
Rifaximin			Ziconotide	Voltage-dependent N-type calcium channel subunit alpha-1B	Chronic pain
Dalfopristin	Streptogramin A acetyltransferase		Setmelanotide	Melanocortin receptor	Genetic obesity
Ixabepilone	Tubulin	Oncology	Pasireotide	Somatostatin receptor	Cushing's disease
Eribulin					
Lutetium Lu-177 Vipivotide Tetraxetan	Prostate-specific antigen		Everolimus	FKBP12, Serine/threonine-protein kinase mTOR	Immunosuppressant
Lorlatinib	ALK receptor		Sirolimus		
Lutetium Lu 177 Dotatate	Somatostatin receptor		Tacrolimus	FKBP12, Calcineurin subunit B	
Lanreotide			Cyclosporin		
Octreotide			Voxilaprevir	HCV NS3/4A protease	Infection: Antiviral (Hepatitis C)
Plerixafor	CXCR4 chemokine receptor		Grazoprevir		
Histone	deacetylase 1,2 (HDAC) Romidepsin		Glecaprevir		
Porfimer sodium	NA (Reactive oxygen species) (ROS)		Simeprevir		
Dactinomycin	DNA		Paritaprevir		
Temsirolimus	FKBP12, Serine/threonine-protein kinase mTOR	Ivermectin	Glutamate-gated chloride channel (GluCl), GABA-A gated chloride channel	Infection: Antiparasitic	

Drug	Target	Therapeutic indications	Drug	Target	Therapeutic indications
Sirolimus			Moxidectin	GABA-A gated chloride channel	
Everolimus			Eptifibatide	Integrin beta-3 (CD61)	Acute coronary syndrome
Pacritinib	Tyrosine-protein kinase JAK2 Receptor-type tyrosine-protein kinase FLT3		Desmopressin Vasopressin	Vasopressin receptors	Antidiuretic

[a]*NA: Target not available.*

Table 1.
FDA-approved macrocyclic drugs and their related targets therapeutic indications[a].

Natural products or rational innovations are potential sources of macrocyclic compounds. The majority of clinical prospects and macrocycles that have been approved by the Food and Drug Administration (FDA) are obtained as natural products due to their complicated synthetic design [14, 15]. One well-known example of a macrocyclic molecular drug is erythromycin. It provides people with penicillin allergies with additional options by efficiently treating Gram-positive bacterial infections [16]. Many macrocyclic compounds have since been created to treat a wide range of maladies. Biologically active macrocycles have become more common in medicinal chemistry literature over the last few years [1, 6]. Scheme 1 illustrates a few examples of biologically active macrocyclic compounds which possess medical importance (**Figure 2**) [2].

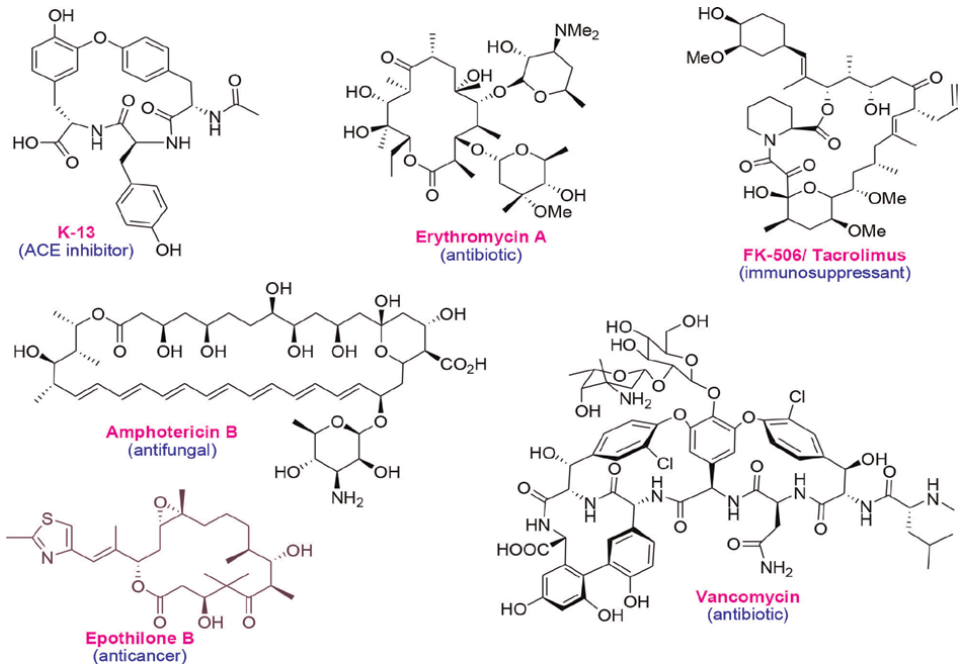


Figure 2.
Some naturally occurring macrocycles displaying a variety of medicinally important biological activities.

4. Macrocycles can modulate challenging targets

Drug resistance, pharmacological ineffectiveness and systemic toxicity of given medications are common challenges related to cancer therapy [17]. It is also very important to find new anticancer agents as pharmacological leads because confusing characteristics such the numerous signaling nature of pathways and the propensity of most cancer cells to change have impeded the search for an effective therapeutic agent to treat cancers [18]. Multi-target macrocyclic inhibitors are one way to get beyond these obstacles and succeed in the battle against different types of cancer.

Targets that have proven extremely difficult for conventional small-molecule drug development have been repeatedly successfully targeted by macrocycles, particularly when it comes to modifying macromolecular processes like protein–protein interactions. Macrocycles are useful for controlling a variety of macromolecular interactions; frequently, they do this by giving their targets additional interaction surfaces that might lead to a complex's gain-of-function that is dependent on the macrocycle. The potential of several types of macrocyclic natural products to alter the dynamics of microtubules in mammalian cells and impede the growth of tumors is presently being studied [19]. Once more, the α and β subunits of the tubulin heterodimer modify the protein-protein interactions responsible for these effects. Epothilone B, a macrocycle originating from myxobacteria, connects itself at the tubulin subunit interface (a region that is shared by the taxol binding site). Widespread reorganization of the α–β junction disrupts general microtubule dynamics while stabilizing the dimer [20]. Ixabepilone has been licensed for the treatment of metastatic breast cancer [21]. Although there are numerous examples of non-macrocyclic microtubule disruptors, the state of our knowledge suggests that macrocycles are widely found in nature and are used to modify protein–protein interactions between microtubule subunits.

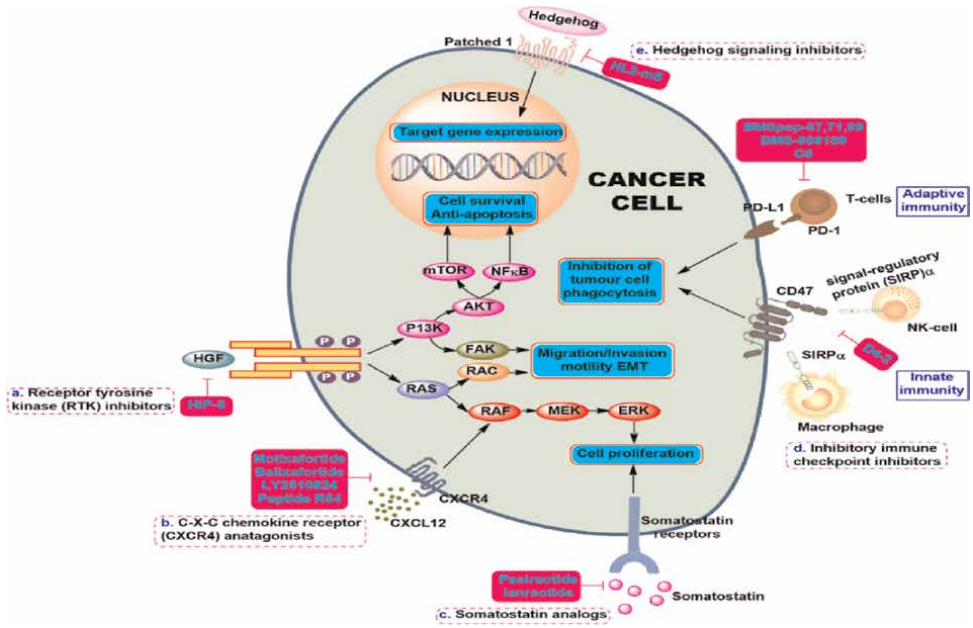
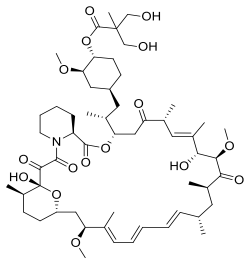


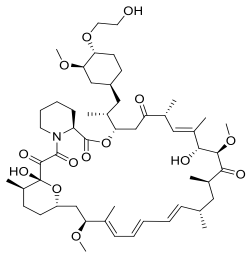
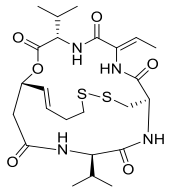
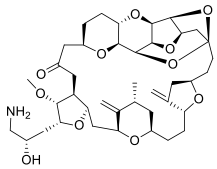
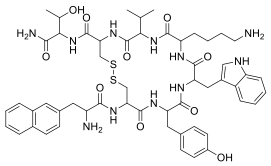
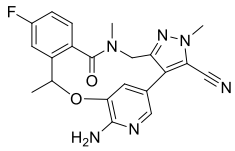
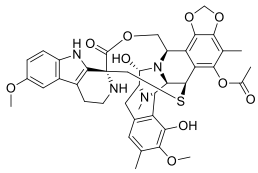
Figure 3.
Extracellular protein–protein interactions targeted by macrocyclic compounds [22].

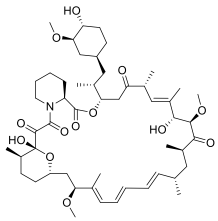
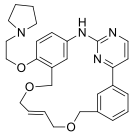
Figure 3 displays the extracellular proteins that are targeted by macrocyclic peptides, the associated signaling pathways, and their physiological roles. The hepatocyte growth factor (HGF)-mesenchymal-epithelial transition tyrosine kinase receptor (MET) interaction is essential for cancer cell proliferation, migration, and invasion. HiP-8 (Receptor tyrosine kinase (RTK) inhibitors) can disrupt this connection (**Figure 3a**). C-X-C chemokine receptor (CXCR4) antagonists such as motixafortide, balixfortide, LY2510924, and Pep R54 can block the connection between CXCR4 and CXCL12, which is necessary for the growth of cancer cells (**Figure 3b**). Somatostatin analogs include pasireotide and lanreotide. To stop cell division, they can prevent endogenous somatostatin from activating somatostatin receptors (**Figure 3c**). The interaction between programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1), which negatively modulates the adaptive immune systems, can be inhibited by BMSpep-57,77,99, BMS-986189, and C8 (inhibitory immune checkpoint inhibitors). D4–2 has the ability to block the connection between CD47 and SIRPα, which releases a "do not eat me" signal that prevents immune cells from identifying and eliminating cancer cells. For cancer immunotherapy, the PD-1/PD-L1 and CD47/SIRPα domains are essential elements (**Figure 3d**). Hedgehog (HH) signaling protein inhibitors, such as HL2-m5, can prevent the HH pathway from activating, which controls the expression of target genes (**Figure 3e**) [22].

4.1 Macrocyclic compounds targeting cancer development and to overcome drug resistance

The development of cancer is characterized by 14 key functional features, as stated in the new hallmarks of cancer [23]. Numerous proteins that are essential to various cancer pathways have been effectively targeted by macrocyclic compounds. Among these targets are histone deacetylase (HDAC), cyclin-dependent kinases (CDKs), Janus kinase (JAK2), and mammalian target of rapamycin (mTOR). However, patients with cancer now have a higher quality of life thanks to targeted therapy, particularly those with nonsmall cell lung cancer (NSCLC). It has been demonstrated that macrocyclic compounds are effective cancer treatment agents. From 2007 to 2022, the US FDA authorized nine macrocyclic medications for use in cancer patients (**Table 2**). Several drugs target essential proteins implicated in the genesis of cancer. For instance, Ribulin (Halaven) was authorized in 2010 for the treatment of patients

Drug name and structure	Target	Indications	MW	First approval	References
Temsirolimus 	mTORC1	RCC	1030	2007	[24]

Drug name and structure	Target	Indications	MW	First approval	References
Eeverolimus 	mTORC1	RCC, breast cancer, neuroendocrine tumors	958	2009	[24–27]
Romidepsin 	HDAC1/2	Cutaneous/peripheral T-cell lymphoma	541	2009	[28]
Eribulin 	Microtubule	Breast cancer; liposarcoma	826	2010	[29, 30]
Lanreotide 	Growth hormone	Neuroendocrine tumors, acromegaly	1096	2014	[22]
Lorlatinib 	ALK/ROS1	Nonsmall-cell lung cancer	406	2018	[31]
Lurbinectedin 	Transcription	Small-cell lung cancer	785	2020	[32, 33]

Drug name and structure	Target	Indications	MW	First approval	References
Sirolimus 	mTORC1	Perivascular epithelioid cell tumor	914	2021	[34]
Pacritinib 	JAK2/FLT3	Myelofibrosis	473	2022	[35]

FDA, Food and Drug Administration; RCC, renal cell carcinoma; MW, molecular weight.

Table 2.
Macrocyclic drugs approved by FDA (2007–2022) for cancer therapy.

with liposarcoma and in 2010 for patients with fatal or metastatic breast cancer. In 2014, Lorenotide received approval for the treatment of gastroenteropancreatic neuroendocrine tumors, or GEP-NETs. In 2020, the FDA authorized Libirectedin (a macrolide) for the treatment of SCLC. The FDA authorized paritinib in 2022 to treat MF, a rare form of leukemia.

Targeted therapy's long-term success is limited by drug resistance that eventually developed in cancer patients after these medications were first used. When it comes to combating drug resistance, particularly pocket-alteration-mediated drug resistance, macrocyclic compounds exhibit more potency than acyclic molecules due to their smaller and more compact structures. Patients with NSCLC who have activating EGFR mutations and fusion proteins containing tropomyosin receptor kinase (TRK) or anaplastic lymphoma kinase (ALK) are successful cases. To avoid confusion, only macrocyclic compounds that can inhibit kinases with acquired resistance mutations were included in this category. This is because several drug targets that are crucial for the development of cancer are also involved in the drug resistance for other target proteins. For instance, patients who have drug resistance from EGFR-targeted therapy may benefit from a combination of mTOR inhibitors due to enhanced mTOR signaling [36].

The FDA approved a single macrocyclic compound to overcome drug resistance in cancer-targeted therapy. Lorlatinib is a small, compact drug with good brain penetration that potently suppresses the growth of resistant malignancies that have relapsed after earlier-generation therapy. In order to overcome resistance to the previous generation of acyclic ALK-specific inhibitor medicines, lorlatinib was licensed (2018) for the treatment of ALK-positive NSCLC [36]. SB1578 and zotiraciclib, two novel JAK2 inhibitors that target the development of cancer, as well as novel inhibitors of ALK, TRK, and EGFR that target the development of drug resistance to targeted therapy, are now in the exploratory stage. Several newly created substances are still in the preclinical phase. The EGFR inhibitor BI-4020, for instance, has not yet been the subject of any published clinical trials. Phase 1 or phase 2 clinical trials are now in

progress on a number of these compounds. Repotrectinib, for instance, inhibits a number of ALK, ROS1, and TRK kinase resistant mutant proteins. To investigate the effectiveness of repotrectinib in patients with solid tumors, six clinical trials are presently accepting new participants; the majority of these trials are in phase 1 or phase 1/2. Patients with advanced solid tumors containing ALK, ROS1, and NTRK1–3 rearrangements (TRIDENT–1) are enrolled in an ongoing phase 1/2 clinical trial (NCT03093116). Patients who had relapsed on repotrectinib but were TKI-naïve or NTRK+ had durable responses [37]. The discovery of macrocyclic compounds, such as zotizalkib, repotrectinib, lorlatinib, and BI-4020 illustrates the substantial advantages of macrocyclic drugs in enhancing affinity and selectivity in overcoming drug resistance, owing to their powerful actions against resistant proteins. Historically, drug development has relied more on logical design than on random screening. The rational design of macrocyclic structures accelerates the process of discovering novel drugs. **Table 2** lists the macrocyclic compounds that are used to target the development of cancer and to combat drug resistance in cancer cells.

5. Types of macrocyclic anti-cancer agents

Many synthetic and naturally occurring macrocyclic anticancer drugs are now in use, and others are continually being sought after [38].

5.1 Natural macrocyclic anti-cancer agents

Natural products have long been used for medical purposes [39]. Natural products have proven enormously beneficial in the treatment of cancer. The two most well-known of these are taxol from Taxus baccata, which is used to treat cervical cancer, and the vinca alkaloids from Catharanthus roseus (Vinca rosea), which are used to treat leukemia [40, 41]. Thus, natural chemicals from plant, soil, marine, fungal, and animal sources cannot be ignored in the quest for multitarget anticancer agents. For example, one naturally occurring macrocyclic drug that has shown strong inhibition against various cancer cell types is rapamycin.

5.1.1 Natural macrocycles as anti-colon and anti-cervical cancer agents

Sea squirt depsipeptides are known as didemnins. They are active against B16 melanoma and P388 lymphocytic leukemia, among other malignancies [42]. It has been reported that didemnins B (**Figure 4**) target DNA functioning [43]. Through the combined suppression of eukaryotic translation elongation factor-1α (EEF1A1) and palmitoylprotein thioesterase 1 (PPT1), it disrupts the cell cycle and prevents DNA synthesis at the elongation phase [44]. Moreover, it inhibits protein synthesis by blocking the translocation required for eukaryotic elongation factor (Eef-2) and activates caspases, which triggers apoptosis [45].

5.1.2 Natural macrocycles as anti-renal cell cancer agents

Temsirolimus (**Figure 5**) is an analog of rapamycin, a natural substance. For the treatment of adult patients with advanced RCC, temsirolimus is prescribed. The enzyme that controls cell growth and proliferation, known as mTOR, is inhibited by temsirolimus (Torisel®; Wyeth Pharmaceuticals, Inc., Madison, NJ). Through mTOR

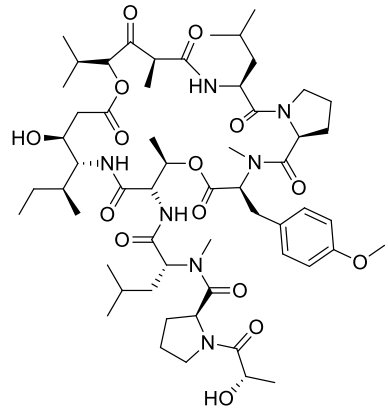


Figure 4.
Structure of Didemnin B acting as an active anti-colon and anti-cervical cancer agent.

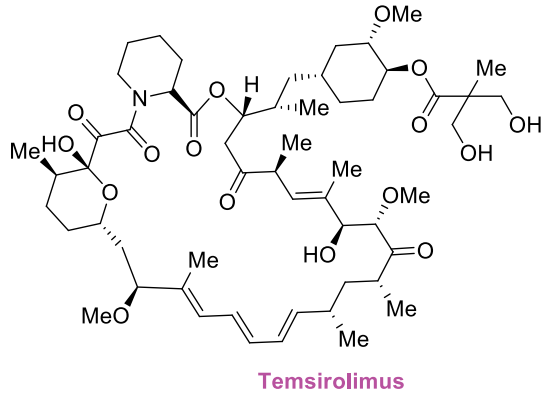


Figure 5.
Structure of Temsirolimus acting as an anti-renal cell cancer agent.

inhibition, temsirolimus stops cells from progressing from the G1 to the S phase of the cell cycle. It also inhibits mTOR-dependent protein translation, which is triggered by growth factor stimulation of cells, which has an impact on cell proliferation [24].

5.1.3 Natural macrocycles as anti-pancreatic and anti-pulmonary cell cancer agents

Cyclic hexapeptides from a deep-water sponge belonging to the species Microscleroderma are known as microsclerodermins [46]. Microsclerodermin A and B (**Figure 6**) inhibit the transcriptional activity of NF-κB, which results in a decrease in the amount of phosphorylated (active) NF-κB (nuclear factor kappa B) cells in the AsPC-1 cell line. Additionally, they significantly induce apoptosis in the AsPC-1, MIA PaCa-2, BxPC-3, and PANC-1 pancreatic cancer cell lines. The expression of proteins in the glycogen synthase kinase 3 pathway was likewise controlled by these anti-cancer agents according to further research into their mode of action [47].

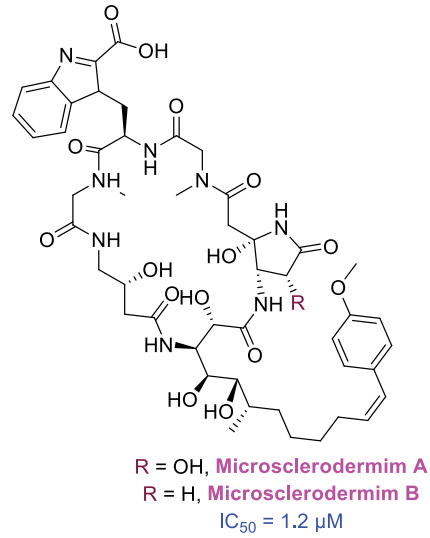


Figure 6.
Structure of Microsclerodermim acting as an anti-pancreatic and anti-pulmonary cell cancer agent.

5.1.4 Natural macrocycles as anti-prostate cancer cell agents

A trisoxazole macrolide called halichondramide (**Figure 7**), is derived from the marine sponge Chondrosia corticate. A range of cancer cells are resistant to its antiproliferative properties. For example, it modulates the epithelial-tomesenchymal transition, which results in an antimetastatic impact on human prostate cancer cells. HCA had a strong inhibitory effect on PC3 cell growth, with an IC_{50} of 0.81 μM. This compound has cytotoxic effects via suppressing the Akt/mTOR pathway, and it blocks the G_2/M phase by upregulating the expression of the proteins GADD45 and p53 [48].

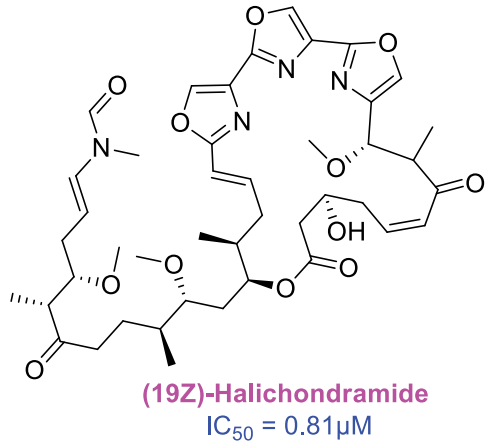


Figure 7.
Structure of Halichondramide acting as an anti-prostate cancer cell agent.

5.1.5 Natural macrocycles as anti-brain cell cancer agents

A polyketide called Candidaspongiolide (**Figure 8**) was isolated from Candidaspongia sp. In both U251 and HCT116 cells, it suppresses protein synthesis and triggers apoptosis, with the latter occurring partly through a caspase 12—dependent mechanism [49]. Furthermore, this compound selectively suppresses the proliferation of human melanoma cells in contrast to cell lines of lung and breast cancer [50].

5.2 Synthetic macrocycles

The following examples show that synthetic macrocycles can offer disease-relevant targets appealing ligands, with these compounds offering drug-like stability and bioavailability together with high levels of target affinity and selectivity. Recent research has focused a lot of emphasis on macrocyclic peptides as significant cancer therapy agents, mostly due to their decreased toxicity to normal cells and synthetic accessibility. For example, a macrocycle-quinoxalinone class pan-Cdk inhibitor acts as antitumor agent [51]. Below are a few examples of synthetic macrocycles used as anticancer agents.

5.2.1 Synthetic macrocycle as anti-breast and anti-CNS cancer agents

A new acylated cyclopentapeptide namely, Cyclo-(*N* $^{\alpha}$-dipicolinoyl)-*bis*-[L-Leu--DL-Nval]-L-Lys OMe (**Figure 9**) showed early signs of promising cytotoxic action. The molecule exhibited possible anti-proliferative effects, primarily attributed to DNA intercalation, and metal sensor properties, specifically for lead cations (a pollutant) [52].

5.2.2 Synthetic macrocycle as anti-hepatic and anti-breast cancer agents

Novel macrocyclic compounds of pyridoheptapeptides (**Figure 10**) have been generated. The anticancer potential of these heptapeptidopyridine compounds was assessed in comparison to commonly used cancer-fighting drugs. The antitumor potential of each produced molecule was assessed using two human cancer cell lines, MCF-7 and HepG-2. Both anti-hepatic and anti-breast cancer activities were demonstrated by **1a–c**. Only anti-hepatic cancer action has been shown by **2a–c** [53].

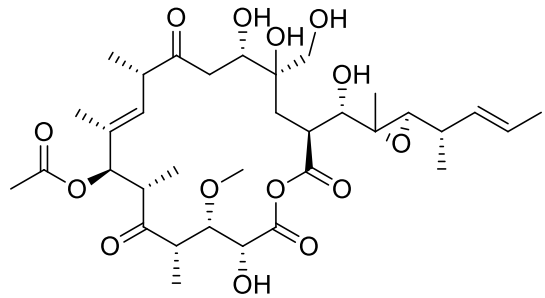


Figure 8.
Structure of Candidaspongiolide acting as an anti-brain cell cancer agent.

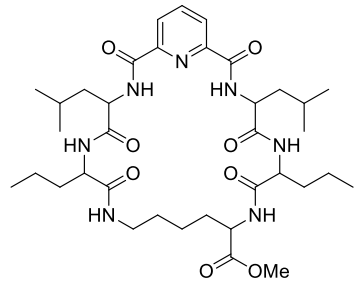


Figure 9.
Structure of Cyclo-(N$^{\alpha}$-dipicolinoyl)-bis-[L-Leu-DL-Nval]-L-Lys OMe acting as an anti-breast and anti-CNS cancer agent.

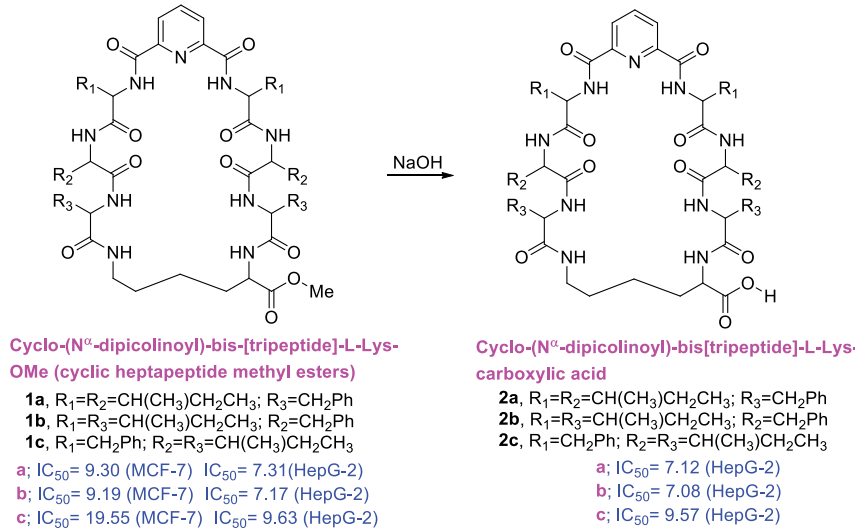


Figure 10.
Structure of pyridoheptapeptides acting as anti-hepatic and anti-breast cancer agents.

5.2.3 Synthetic macrocylces as anti-leukemia agents

N-(2-aminophenyl) benzamide acridine (**Figure 11**) demonstrated multi-targeting ability against HDAC (IC_{50} = 87 nM), transmembrane ligand-activated receptor tyrosine kinase (FLT3) (IC_{50} = 87 nM), and Janus kinase 2 (IC_{50} = 686 nM), exhibiting a high lethal effect on human erythroleukemia (HEL) cells and the human acute myeloid leukemia cell line MV4–11. This compound functions by inhibiting Topoisomerase 1 and HDAC, which stops cell proliferation (IC_{50} 0.12–0.35 μM) that is caused by G_0/G_1 stoppage of the cell cycle [54]. Innovative pyrimidine-based macrocycle SB1518 exhibits a distinct kinase profile with selective inhibition of fms-like tyrosine kinase-3 (FLT3; IC_{50} = 22 nM) and Janus Kinase-2 (JAK2; IC_{50} = 23 and 19 nM for $JAK2^{WT}$ and $JAK2^{V617F}$, respectively) within the JAK family (IC_{50} = 1280, 520 and 50 nM for JAK1, JK3, and TYK2, respectively). Clinical trials including myelofibrosis and lymphoma patients are also validating this drug [55].

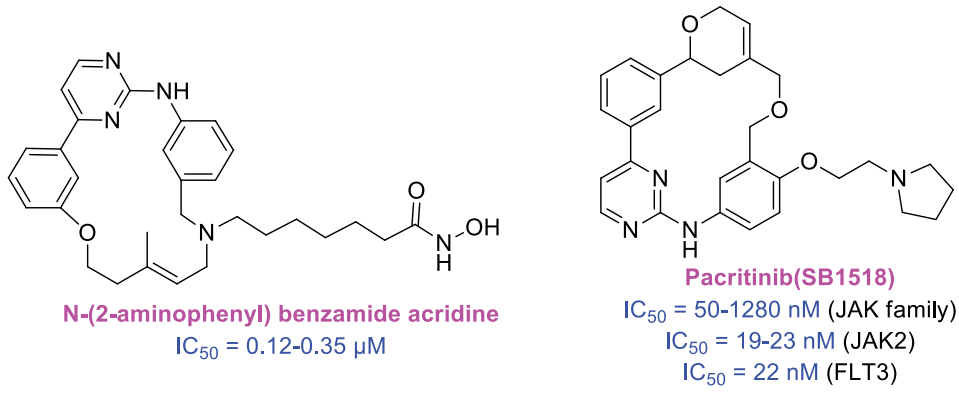


Figure 11.
Structures of N-(2-aminophenyl) benzamide acridine and Pacritinib acting as as anti-leukemia agents.

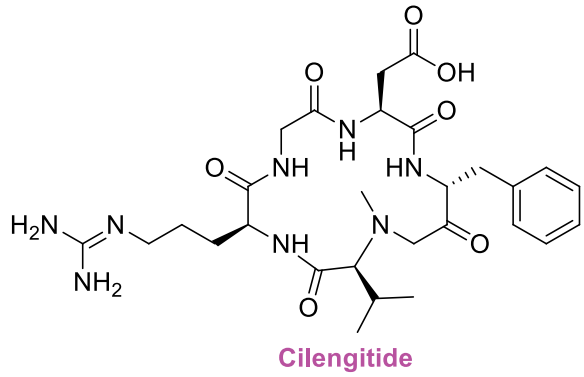


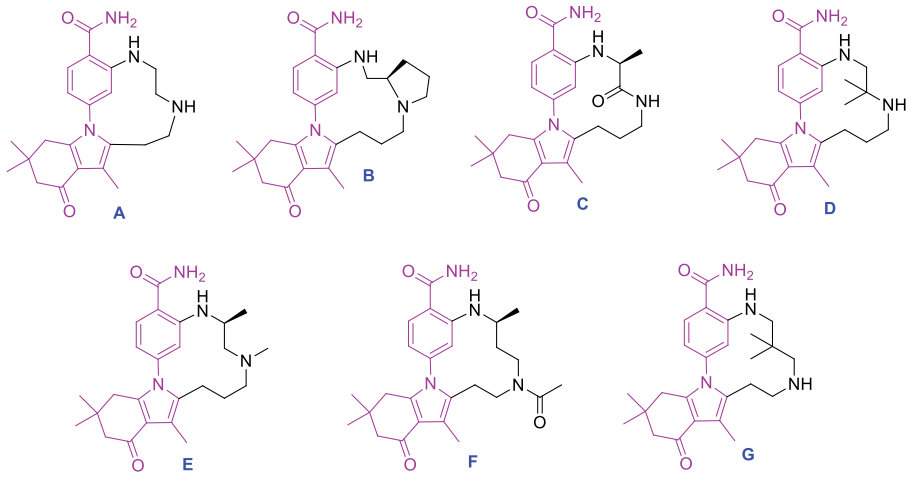
Figure 12.
Structure of Cilengitide as anti-angiogenic agents.

5.2.4 Synthetic macrocylces as anti-angiogenic agents

A cyclic pentapeptide called cilengitide (**Figure 12**) is presently being studied in phase II clinical trials for glioblastomas and in phase III trials for a number of different malignancies. This drug targets the integrins αvβ3, αvβ5, and α5β1, making it the first small molecule anti-angiogenic agent [56, 57].

6. Effect of varying ring size and linker functionalization of macrocyclic drug on structure–activity relationships (SARs)

Studies of the structure–activity relationship show that a macrocyclic structure's inhibitory action is influenced by its ring size and ring functionalities. **Table 3** provides a detailed description of the impact of altering a macrocyclic linker, encompassing information on Hsp90 inhibition, cell-growth inhibition, water solubility, and microsomal stability. The first steps in determining the ideal linker length included developing macrocycles with 11–13 atoms that had an amine inside the linker. Various potencies, aqueous solubilities (starting at 100 μg/ml), and metabolic



Compound	Ring size	Target activity Hsp90 IC50 (μM)	Rat liver microsomal stability t1/2 (min)	Aqueous solubility (μg/ml)	cLog P	Cell growth inhibition HCT116 EC_{50} (μM)
A	11	12.44	15	>100	3.1	
B	12	0.096	6	2324	4.3	0.032
C	12	0.082	>30	>100	3.5	0.056
D	12	0.14	15	52	4.4	0.08
E	12	0.15	11	31	4.4	0.08
F	12	0.11	9	19	2.8	0.15
G	13	0.29	3	6	4.7	0.21

Table 3.
SARs for macrocyclic (A-G) Hsp90 inhibitors and differ in terms of linker functionalization and ring size.

stabilities were noted in relation to the macrocyclic tether's length, composition, and substitution. Despite the complicated appearance of SARs, potency was found to be sensitive to the degree and chirality of alkyl substitution in the linker, indicating that conformational preferences may change and that affinity within the series may be driven by steric problems. It has been found that the best platform for multiparameter optimization is a 12-membered macrocycle. Interestingly, compounds **A**, **D** and **G** show that as the macrocycle size expanded, the water solubility and rat liver microsome stability of this series dropped. This may possibly be a result of the smaller macrocycle pushing the biaryl system to twist and break planarity, which has been demonstrated to promote solubility, even though it is partially reflecting rising lipophilicity with increasing ring size and substitution [58]. A more planar biaryl system ought to be possible due to the larger macrocycles.

7. Conclusions

To sum up, macrocyclic compounds show promise as cancer treatment agents. Macrocyclic compounds are being developed for cancer therapy at a rapid pace due to

the increasing need for medications to treat cancer. One of the many cancer treatment approaches that is accessible is chemotherapy. Nevertheless, a number of existing chemotherapeutic drugs have significant side effects, such as drug resistance, inefficiency, and intricately linked pathways in the etiology of cancer disorders. It has been suggested that multi-target macrocyclic compounds are an efficient way to treat cancer. Several kinds of synthetic and naturally occurring macrocyclic compounds possessing multi-targeting capabilities have been assessed in this chapter as possible chemotherapeutic treatments for diverse types of cancer. These substances work by inhibiting cell development, causing apoptosis, and reducing cell metastasis, among other effects.

8. Future perspective

This chapter is intended to serve as a guide for the development of macrocyclic compounds. The structural class of macrocycles offers substantial promise for the development of new drugs. Despite their current lack of exploration, it is expected that in the upcoming years, interest in and success with this class of compounds will increase significantly. More medications based on macrocyclic compounds with particular curative powers will be created in the future.

Author details

Sadia Rani[1], Samina Aslam[1], Ali Irfan[2*], Emilio Mateev[3], Sami A. Al-Hussain[4] and Magdi E.A. Zaki[4*]

1 Department of Chemistry, The Women University, Multan, Pakistan

2 Department of Chemistry, Government College University Faisalabad, Faisalabad, Pakistan

3 Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Medical University, Sofia, Bulgaria

4 Department of Chemistry, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia

*Address all correspondence to: raialiirfan@gmail.com and mezaki@imamu.edu.sa

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Chapter 2

Heterocycles-Based Ionic Liquids (ILs) in Transdermal Drug Delivery

Lubna Khan, Rashid Ali and Farheen Farooqui

Abstract

Transdermal drug delivery systems (TDDSs) have become immensely popular over the past few years owing to their safe and noninvasive administration of the drugs across the skin. The TDDSs have provided a better surrogate pathway over conventional routes such as skin patches and injections, thereby resulting in superior and easier acceptance by the patients, minimized side effects, and controlled delivery rates. While TDDSs present these advantages, they also come with their limitations, specifically in delivering both small and macro drug molecules that exhibit moderate solubility in water and/or commonly used volatile organic solvents. To subdue this obstacle, ionic liquids (ILs) are being considered as the potential media not only for the syntheses of drugs but also as suitable carriers for the efficient delivery of both small as well as macromolecules. In this particular book chapter, we have discussed the transdermal drug delivery (TDD) of various partially soluble drugs such as acyclovir, anti-inflammatory drugs like diclofenac and ibuprofen, various anticancer drugs, etc., through heterocyclic-based ILs. Moreover, some green routes for ILs syntheses, including fatty acid-based "*amino acid ionic liquids*" (FAAAE-ILs) and "*magnetic surface-active ionic liquid surfactants*" (MSAIL), have also been discussed highlighting their function as the potential transdermal drug delivery agent.

Keywords: drug delivery, heterocycles, ionic liquids (ILs), drugs, green media, synthesis

1. Introduction

Notably, heterocycles represent a vital and quite wide-ranging class of organic compounds playing a critical role in our daily lives. They have found prevalent applications in agrochemicals, medicinal chemistry, materials sciences, organic synthesis, supramolecular chemistry, etc. [1]. Among the heterocyclic systems, structurally diverse ionic liquids (ILs) have found significant roles not only in organic syntheses but also in drug delivery besides many more promising applications [2, 3].

Over the years, ILs have engrossed a remarkable interest of the research community because of their unique biochemical properties they possess [4]. Noticeably, ILs were initially discovered by Paul Walden in 1914 [5], while he was researching

the "molten salts" (MSs), and realized $[EtNH_3][NO_3]$, afterward a plethora of room temperature ILs have successfully been revealed by researchers worldwide [6]. It is to be pointed out that at present, ILs have immensely grown both in academia as well as at the industry level due to their fascinating signatures [7]. Remarkably, ILs are considered as new greener solvents *in lieu* of common volatile organic solvents (they often are toxic, flammable, and highly volatile) in the domain of chemistry and biochemistry in general and organic syntheses in particular. Due to extensive uses of the ILs in a wide range of fields, in 2003, they were labeled as "*solvents of the future*" [8]. Generally, ILs exist in the liquid state at ambient temperature consisting of "*cations and anion*," but they are quite different from MSs—as detailed and described by Seddon [9]. They are commonly defined as liquids below an arbitrary temperature (373.15 K), though this particular temperature constraint is not necessary for a substance to be considered an IL [10].

Importantly, the distinctive assets between the MSs and ILs are; the MSs are highly viscous with comparatively high melting temperature besides being corrosive liquid medium, whereas ILs devise high thermal stability, low melting temperature, are noninflammable, and have negligible vapor pressure [11]. The most distinctive features of ILs are—they comprised "*bulky organic cations*" (imidazolium, pyrrolidinium, thiazolium, triazolium, tetraalkylammonium, etc.), **Figure 1** and bulky inorganic and/or organic anionic counterparts (trifluoroacetate, alkyl sulfate, phosphates, aluminates, etc.), **Figure 2** [12–14]. Interestingly, ILs can be improved/modified depending upon their applications, just by changing the structure of ILs, for instance by selecting the appropriate cation or anion or through the proper substituent in the molecules of the cations and/or the anions.

Noticeably, unique physicochemical characteristics of the ILs lead to a wide range of potential applications spanning from the electrochemistry, analytical chemistry, supramolecular chemistry, physical chemistry, medical chemistry, engineering

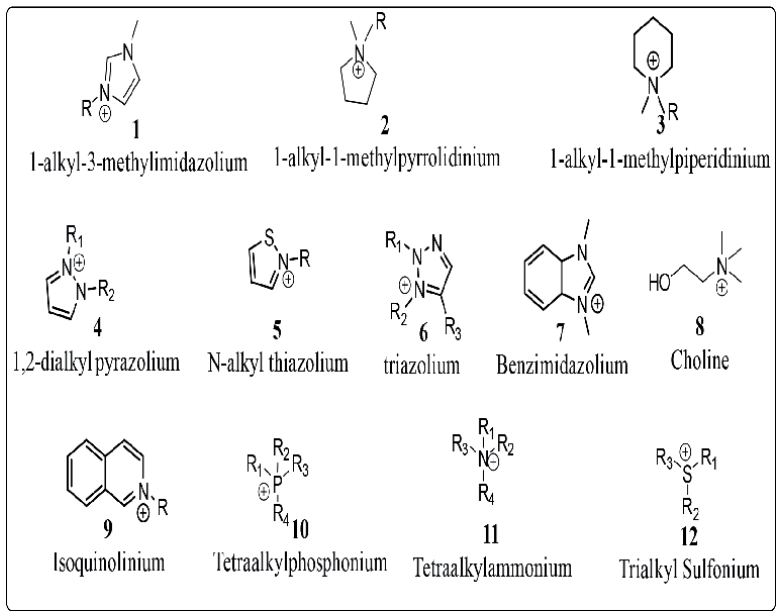

Figure 1.
Structures of some commonly found bulky organic cations in ILs.

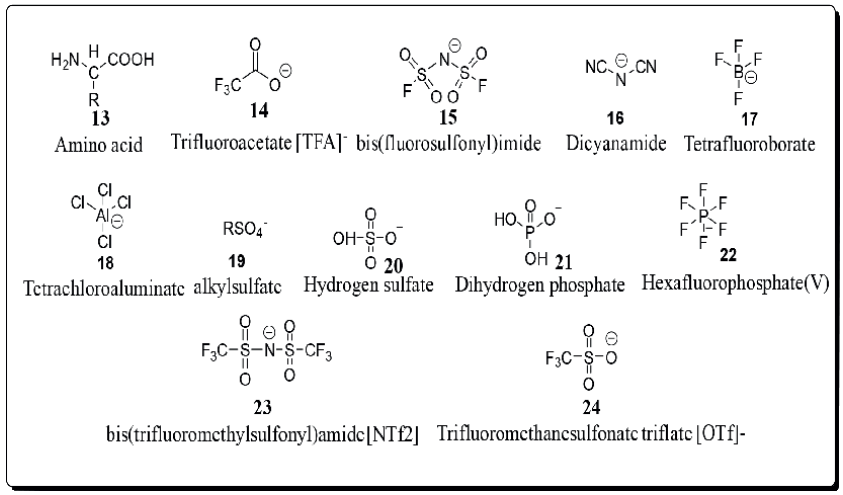

Figure 2.
Structures of some frequently used bulky counteranions in ILs.

chemistry, and pharmaceutical chemistry to the solvent systems and/or catalysts [15]. As far as synthetic organic chemistry perspective is concerned, ILs have successfully been employed in a plethora of vital organic transformations (**Figure 3**), such as Beckmann rearrangement, Diels-Alder reaction, Henry reaction, Friedel-Crafts sulfonylation and sulfamoylation, Fischer indole synthesis, Knoevenagel condensation, Markovnikov addition, Mannich-type reaction, etc. [16–18]. Moreover, in recent years, ILs have drawn a great interest in the arena of biomedicine—owing to their

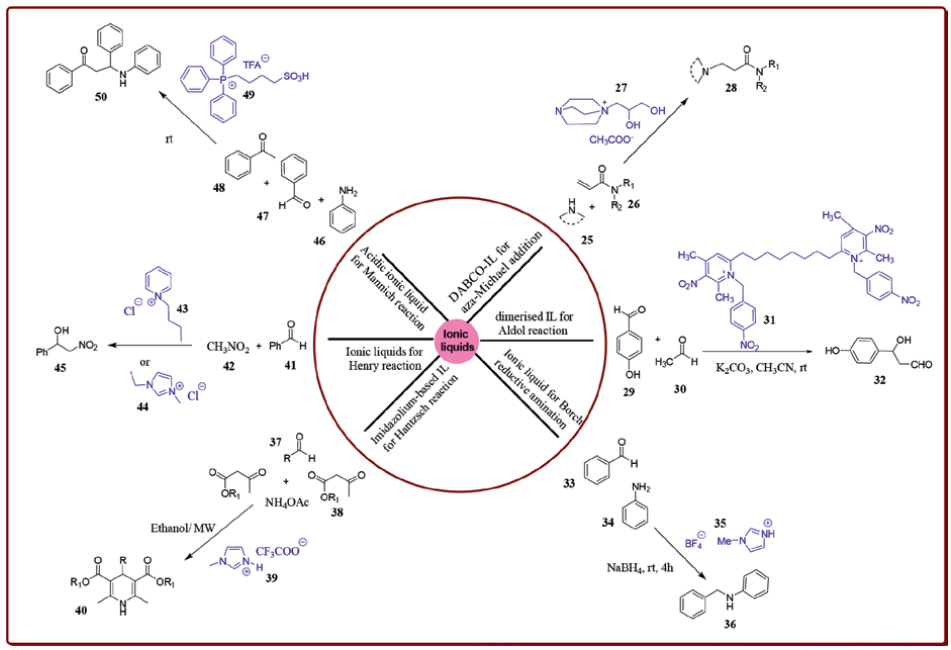

Figure 3.
Role of ILs in catalyzing various crucial organic named reactions.

potential uses in the drug delivery. Importantly, ILs are being considered as potential media for drug synthesis as well as suitable carriers for the effective and selective delivery of both small as well as macromolecules. In addition to drug delivery systems, ILs drugs have also been risen in the biomedical analytics, sensors, excipients, and stabilizers of the important biomolecules.

Based upon various properties of ILs, they can be classified into numerous categories. Some of them are task-specific, chiral, metallic, neutral, or basic ILs, and some may be protic, acidic, or supported ILs [19].

- *Task-specific ILs*: Task-specific ILs are being used in the organic synthesis, for example, in esterification reaction, dehydration reaction, etc. One such example of the task-specific ILs is 3-sulfopropyl tri-phenyl phosphonium *p*-toluene sulfonate.

- *Chiral ILs*: Chiral ILs are mostly used in stereoselective polymerization, liquid chiral chromatography, nuclear magnetic resonance (NMR) chiral discrimination, etc.

- *Neutral ILs*: Anions and cations in the neutral ILs are bonded with weak electrostatic forces, which result in lowering the melting points and viscosity. Therefore, they are employed in the applications demanding robust electrochemical and thermal stability.

- *Protic ILs*: The presence of Brønsted acidic proton(s) in these ILs has opened up the room for a series of reactions, such as hydrolysis, dehydration, and many more [20].

- *Basic ILs*: Basic ILs replaced inorganic bases in reactions, such as aldol condensation, Michael addition, *aza*-Michael, Markovnikov addition, etc., because of its noncorrosive and nonvolatile properties.

On the other front, green chemistry focuses upon the principles to reduce or eliminate hazardous chemical and/or processes for the sake of environment as well as humanity [21–24]. ILs have negligible vapor pressure at ambient temperature, leading to believe them as the "green solvents" due to their low atmospheric pollution; however, the manufacture, uses, and disposal of any solvent must also be taken into account before considering them as eco-friendly. Due to their diverse rising applications, various studies have reported that ILs toxicity is becoming an alarming problem for the environment, especially for aquatic organisms. In many cases, it has been noticed that the replacement of common organic solvents with ILs was even more unsafe than the former one. One such example is carcinogenic chromium (IV) salts, used in chromium electroplating processes [25]. Therefore, it is strictly advised to check thoroughly the toxicity test of the given IL before branding them as greener solvents.

2. Preparations of some important heterocyclic-based ionic liquids

Undoubtedly, heterocyclic compounds are omnipresent, for example, most of the natural products, drug molecules, and even our body also contains heterocyclic scaffold(s) [19, 26, 27]. Moreover, a plethora of vital synthetic molecules of particular

interest also consist of the heterocyclic ring system(s) in their structure [28–34]. Noticeably, most of the ILs also comprise the heterocyclic ring in their cationic portion, as detailed in **Figure 1**. In this particular section, we have shed light onto the preparation of some vital and commonly used ILs. The general synthetic pathway for the preparation of ILs can be represented as shown in **Figure 4**.

The task-specific ILs **59** were prepared by Huang et al. in the year 2012 (**Figure 5**) [35]. Toward this mission, the authors have commenced from the commercially available starting material, namely 4-formylphenol derivatives (**55**), by reacting it with the 1,4-dibromobutane (**56**) in the presence of NaOH/tetrabutylammonium bromide (TBAB) under microwave condition to give the intermediate compound **57**. Next, reaction of **57** with 1-methylimidazole (**58**) under microwave (MW) afforded the anticipated IL derivatives **59**.

On the other hand, imidazolium-based chiral ILs **63** and SiO_2-supported anchored ILs **67** have successfully been revealed by the research groups of Cárdenas and Mehnert (**Figure 6**) [36–38]. As can be inspected from **Figure 6**, these crucial ILs were assembled in three steps both involving alkylation and salt metathesis reactions as key steps.

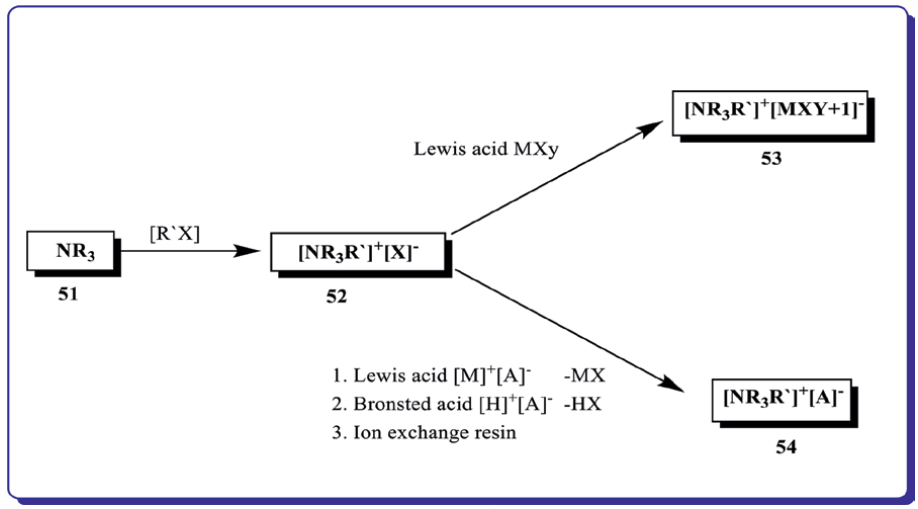

Figure 4.
Common method for the preparation of nitrogen containing ILs.

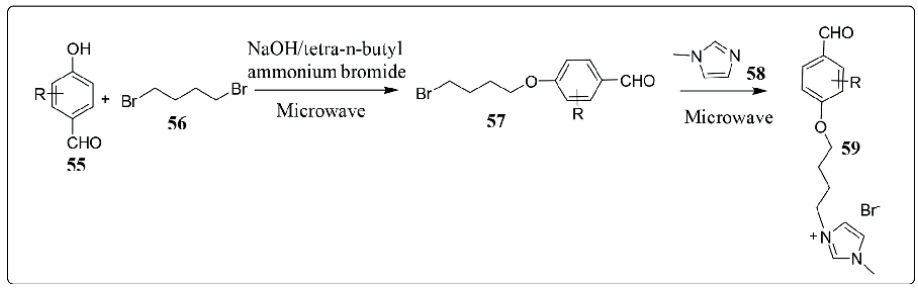

Figure 5.
Synthesis of benzaldehyde-based task-specific ILs **59**.

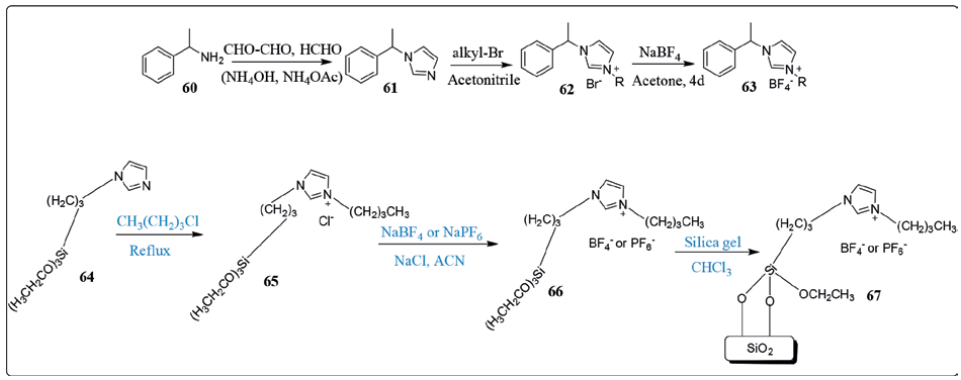

Figure 6.
Synthesis of imidazole-based chiral ILs **63** *and* SiO_2*-supported IL* **67**.

3. Transdermal drug delivery of sparingly soluble drugs in ILs

In the field of pharmacy and pharmaceutical technology, intelligent delivery of drugs has become a quite challenging task. Target-specific drug delivery has gathered attention in pharmacotherapy and has become a promising research area. Skin being the largest organ of the body, with an average total area of 20 square feet, comprises of two important layers: (1) epidermis and (2) dermis, so the skin becomes the apt and logical target for drug delivery. Although, the outermost layer of skin, stratum corneum (SC), that varies in thickness depending on the region of body causes obstacle. The structurally well-organized corneocytes with lipid layer in the SC provide the most formidable barrier for the systemic distribution of drug molecules. To surmount this barrier function, diverse strategies, encompassing both physical methods (electroporation, iontophoresis, and ultrasound) and chemical approaches (nanoparticles, prodrugs, and penetration enhancers), have been utilized, individually and in combination, to enhance drug permeation [39, 40]. Hence, formulating biocompatible drug delivery systems for both the small and macro drug molecules, especially those with limited solubility in water and most organic solvents, is a complex undertaking. The challenge lies in achieving this without compromising the efficacy and safety of the drugs.

Transdermal drug delivery systems (TDDSs) have gained recognition, owing to their innocuous and nondisruptive administration of the drugs across the system. They provide an alternative route over oral and injection, have improved patient compliance, and minimized undesirable side effects [41, 42]. Although, the oral route offers some advantages like portability, predetermined doses, and patient self-administration; most therapeutic drugs (peptides and proteins) cannot be delivered orally, since they can go through rapid degradation in the stomach and upper section of the intestines (**Figure 7**) [43]. In a recent study, researchers have demonstrated the efficacy of ILs and their role in transdermal drug deliveries (TDDs), thus highlighting their advantages over the conventional permeation enhancing methods [44]. Even the administration of drugs via injections has its own limitations, such as needle phobia, resulting in lower patient adherence and the requirement of a trained professional for administration. Rationally, these limitations due to conventional routes can be potentially overcome by transdermal drug delivery systems.

In recent years, transdermal patches have become a popular choice for drug delivery, as they provide reduced gastrointestinal side effects, reduced peak plasma

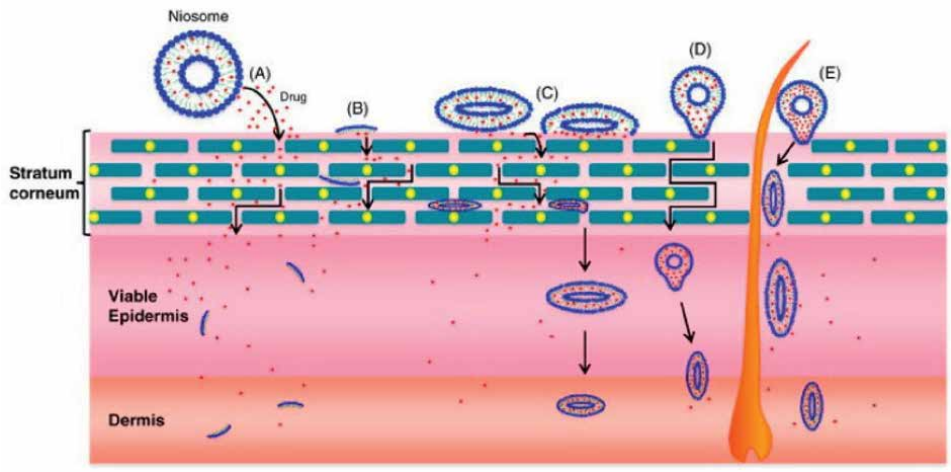

Figure 7.
Representation of mechanisms of action of (A) release of drug molecules are through niosomes; (B) niosome constituents acting as penetration enhancer; (C) niosome interaction with the stratum corneum; (D) niosome penetration across intact skin; (E) niosome permeation through hair follicles. Source: Reprinted with the permission from Ref. [43], copyright 2014 Elsevier.

concentrations, minimal drug therapy, as well as avoiding first hepatic elimination. In addition to their simplified application, cost-effectiveness, and tolerance, TDDSs come with their own limitations. Many small and macro drug moieties are nearly insoluble in water and most organic solvents, so they require the aid of chemical permeation enhancers (CPEs) for their transportation. The most common CPEs are ethanol, terpenoids, sulfoxide, and menthol ester derivatives to augment penetration through the stratum corneum (SC) [45]. The presence of corneocytes with lipid layers in the SC causes hindrance for most of the drugs. In spite of a large number of penetration enhancers, very few of them are launched in the market due to their skin toxicity or irritation in most of the cases.

To overcome these obstacles, ILs-mediated drug delivery is introduced, since they offer numerous advantages over CPEs as they have shown an enhancing capacity of drug permeation. ILs are considered novel and green solvents *in lieu* of organic solvents, which are often toxic and flammable. Although the applications of ILs in the field of pharmaceutical have risen over the years, however, many of these options are not deemed environmentally and geologically friendly. The imidazolium, quinolinium, pyridinium, and fluorinated derivatives of ILs are not as biodegradable and toxic as they are considered [46, 47]. But, ILs comprising of organic anions (such as carboxylate, phosphate, acetate, and amino acids) and cations (amino acid ester, choline, piperidinium, and pyrrolidinium) are superior as they are nontoxic and biodegradable, as well [48, 49].

4. Transdermal drug delivery of sparingly soluble drugs

Delivering a scarcely soluble drug has always been a challenging task, but this has been overcome by the usage of the ILs. These ILs have shown remarkable delivering property without affecting the other cells of the system.

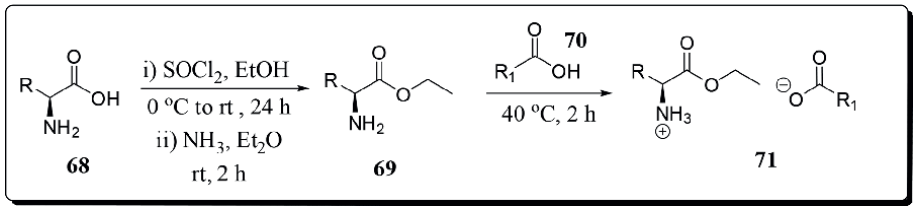

Figure 8.
Synthetic scheme for the hydrophobic ionic liquids.

4.1 Anti-inflammatory drug delivery systems

Moshikur et al. have synthesized and characterized eight green hydrophobic fatty acid-based amino acid ionic liquids (FAAAE-ILs) that exist in a liquid state at 25°C and showed desirable thermal as well as physicochemical properties in the transdermal drug delivery (**Figure 8**). The formed ILs **71**, permitted high ibuprofen solubility owing to the hydrogen bonding interactions, and were found to be more effective in enhancing the diffusion of drug molecules than the conventional CPE transcutol by inducing fluidization within the intracellular lipid matrix of the stratum corneum. The *l*-proline ethyl ester linoleate ([L-ProEt][Lin])-based formulation outperformed the *d*-proline ethyl ester linoleate, *l*-leucine ethyl ester linoleate, and alanine ethyl ester linoleate formulations. Notably, compared to CPE-containing formulations, the identical FAAAE-IL ([L-ProEt][Lin]) significantly improved the peptide permeation through pig skin. Moreover, the findings shed fresh light onto the conventional ILs and suggested that the newly developed FAAAE-ILs (**Figures 9** and **10**)—emerge as a promising transdermal alternative to traditional chemical enhancers by exhibiting the potency to surmount the barrier of transdermal macromolecule distribution [50].

Mahkam and his teammates have reported two ILs monomers, 1-(4-vinylbenzyl)-4-(dimethylamino)-pyridinium hexafluorophosphate **81** (VDPH) and 1-(4-vinylbenzyl)-3-methyl imidazolium hexafluorophosphate **85** (VMIH), in addition to their polymers **88** and **91**, respectively (**Figures 11, 12,** and **13**). The free radical polymerization reactions at 70°C produced homopolymers of VDPH, VMIH, and their copolymers using methyl styrene. Furthermore, naproxen (anionic drug) was efficiently packed into the positive charge polymers (PCPs) and stayed within them under acidic conditions (pH 2–6.5) (**Figure 14**). This loading of drug molecules can be enhanced by increasing the positive charge density by raising the amount of IL groups. The dispersion of hydrolyzing agents is increased on the polymer, and the

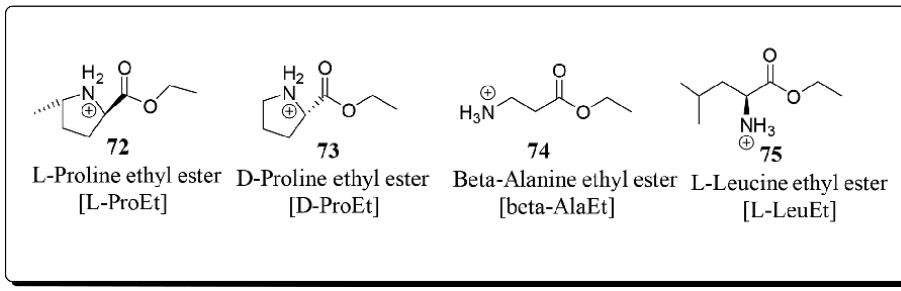

Figure 9.
Amino acid ester cations of developed FAAAE-ILs.

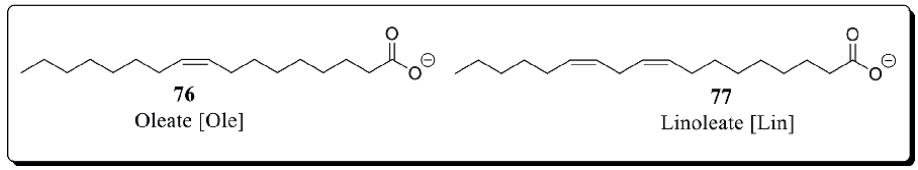

Figure 10.
Amino acid ester anions of the synthesized FAAAE-ILs.

hydrolysis rate is raised by raising the ionic strength at the pH > 6. These PCPs are well suited for anionic drug delivery targeting colon by capitalizing on the variation in hydrolysis rates between weakly acidic and neutral pH values [51].

Shishu and his coworkers have detailed the IL-in-water (IL/w) microemulsion (ME), proficient in solubilizing Etodolac (ETO), a drug with limited water solubility for topical delivery. This formulation employed BMIMPF6 (1-butyl-3-methylimidazolium hexafluorophosphate) as IL, Tween 80 (surfactant), and a co-surfactant ethanol [52]. Particle size, transmission electron microscopy (TEM), pH, zeta potential, and conductometric investigations were performed for the prepared formulation. *Ex vivo* drug infusion tests via rat skin were carried out using a Franz diffusion cell. The microemulsion formulated with the IL-in-water (IL/w) system exhibited the highest average cumulative percent permeation at 99.030 ± 0.921%, surpassing the oily solution (48.830 ± 2.488%) and

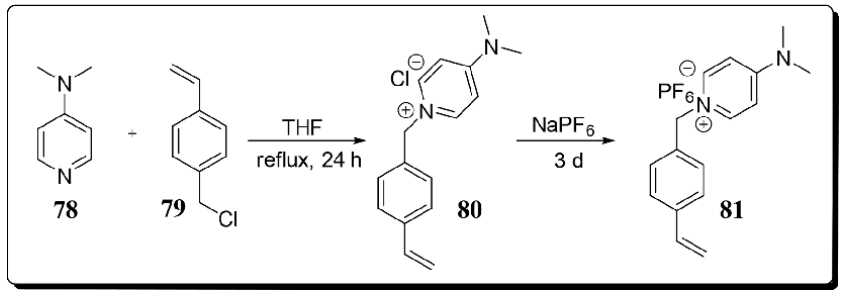

Figure 11.
Synthetic scheme for the preparation of VDPH monomer **81.**

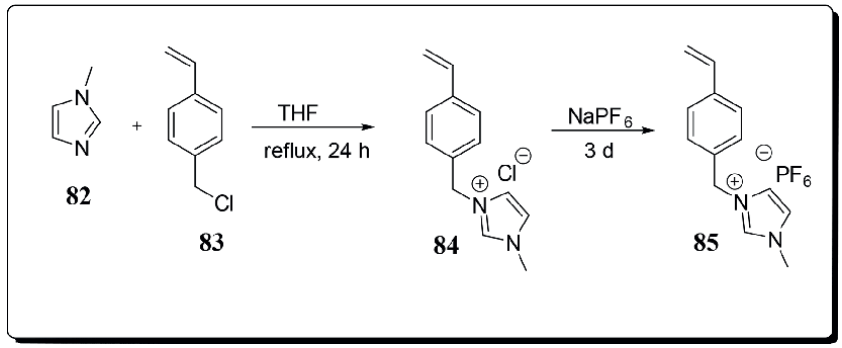

Figure 12.
Synthesis of VMIH monomer **85.**

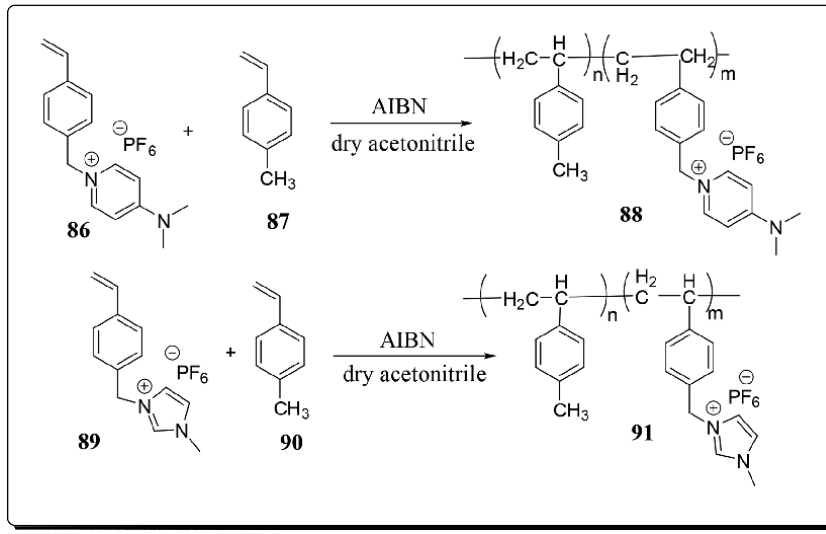

Figure 13.
Synthesis of the positive charge polymer (PCP) **91**.

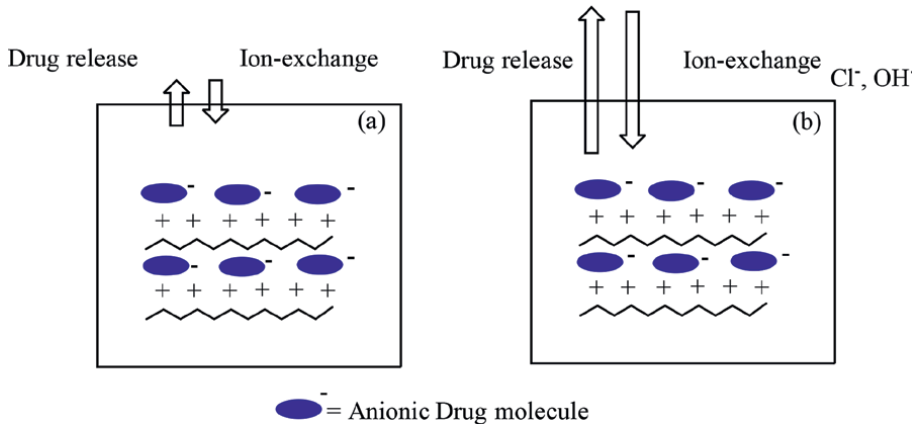

Figure 14.
Representation for the release of naproxen adsorbed in the PCP sample.

the oil-in-water (o/w) ME (61.548 ± 1.875%) of ETO. It was further manifested that ETO-loaded IL/w microemulsion showed efficacy in reducing inflammation without causing any undesirable variation in the skin.

Later, Suksaeree and co-workers have prepared and investigated the transdermal patches used for lidocaine-diclofenac drug delivery comprising of polymer matrix (pectin and Eudragit® NE 30 D) and plasticizer (glycerin) [53]. This lidocaine-diclofenac IL drug was prepared through an ion-pair reaction between the hydrochloride and sodium salts of lidocaine-diclofenac, respectively. The attributes defining the transdermal patch characteristics were dependent on the quantity of Eudragit® NE 30 D, and the loading of lidocaine-diclofenac IL drug. Although raising the amount of Eudragit® NE 30 D in the transdermal fragments reveals drug's crystal characteristics but it also tends to decrease the drug release from the

patches. The drug concentration in these patches was found to be 1.88–2.11 mg/cm^2 for lidocaine and 2.33–2.64 mg/cm^2 for diclofenac. Nevertheless, the use of these polymeric matrices incorporating IL drugs for transdermal delivery of lidocaine and diclofenac led to regulate drug release, presenting advantages for forthcoming research.

4.2 Drug delivery of antibiotics

On the other hand, Gao et al. have proposed a microwave (MW)-induced hydrogel antibacterial approach by synthesizing VACPHs (hydrogels) that combine the benefits of microwave thermal conversion with that of drug delivery [54]. These hydrogels were synthesized via the copolymerization of a MW-active IL, vinylbenzyl trimethylammonium chloride **92** (VBTMACl), and [2-(methacryloyloxy) ethyl]trimethylammonium chloride (ChMACl) **94** with acrylic acid **93** (**Figure 12**). Polyvinylpyrrolidone **95** (PVP) was added to improve the mechanical stability of VACPHs. The moiety **92** enhances the thermal conversion capability of hydrogen, whereas **94** is responsible for the transdermal drug delivery. Moreover, the VACPHs with MW and LEVO (levofloxacin drug) reduce *Staphylococcus aureus* colonization, and hence, disclosed VACPHs MW therapeutic platform represents a viable technique for tissue infection (**Figure 15**) [54].

Isa and his group have analyzed the properties of mesoporous silica nanoparticles (MSNs) using three different parameters: template amount, triethanolamine (TEA) amount, and reaction temperature through the Box-Behnken Design (BBD). The properties, such as surface area and the particle size, are best represented by linear and quadratic models, respectively, which are highly influenced by the temperature variable. In accordance with the drug loading and drug release method, 37% of the drug (quercetin) were efficiently contained in MSNs, with 32% being released within 48 hours. This demonstrates the potency of MSN as a drug delivery agent [55].

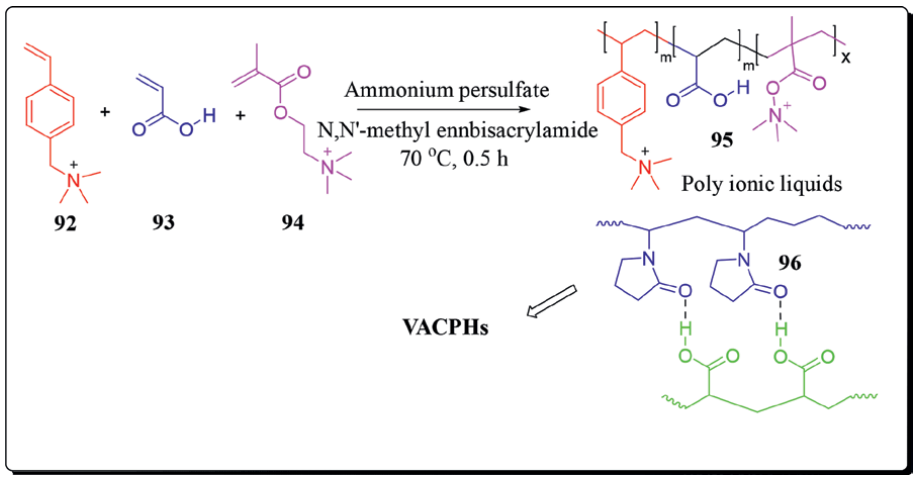

Figure 15.
Synthesis of VACPHs hydrogels.

4.3 Anticancerous drug delivery

In a separate report, Shu et al. have documented 11 imidazolium-based ILs, serving not only as the precursors for carbon dots (IL-CDs) preparation but also tend to regulate their properties [56]. In a hydrothermal environment, sulfuric acid carbonization of the IL precursors results in hydrophilic (IL-HCDs) **97** and hydrophobic (IL-OCDs) **98** carbon dots (**Figure 16**). The IL-OCD quantum yields depend on both anionic and cationic moieties. Notably, longer side chains of cations in imidazolium ILs and lesser nucleophilicity of the anions created intensely fluorescent IL-OCDs. Moreover, the ILs **97** and **98** showed low cytotoxicity, albeit the former has the lowest. Nevertheless, IL-OCDs have been found to increase the intracellular transport of the anticancer medication that disrupts the affected cells.

Remarkably, Goto and his teammates have described the transformation of Methotrexate (MTX) into an array of five ILs consisting of a cationic moiety, such as tetramethylammonium (TMA), cholinium (Cho), tetrabutylphosphonium (TBP) or an amino acid ester, and an anionic component (MTX). Each MTX-based IL's tissue distribution, pharmacokinetics, biocompatibility, and anticancer effectiveness was studied to assess its utility as a medication. As per the pharmacokinetics study, IL[ProEt][MTX] allowed persistent MTX discharge and showed 4.6-fold better oral bioavailability than MTX sodium. Moreover, the IL-based MTX solution was found to have higher antitumor efficacy than MTX sodium, implying that the MTX-ILs had a synergistic antitumor impact in C57BL/6 mice (**Figure 17**), which in turn ensures a promising platform for boosting the hydrophobic drug's oral absorption and targeted delivery to the tumor regions. These findings imply that MTX-based ILs offer an extensible method for improving the oral bioavailability of inadequately soluble MTX [53, 57].

Kulshreshta et. al. created a valine-based surfactant $[ValCl_6][Cl]$ along with its magnetic surface-active IL surfactant (MSAIL) $[ValCl_6][FeCl_4]$ and evaluated their drug delivery potential [58]. Specific conductivity, surface tension, and pyrene fluorescence were used to study the self-assembly behavior of $[ValCl_6][Cl]$ and $[ValC1_6]$

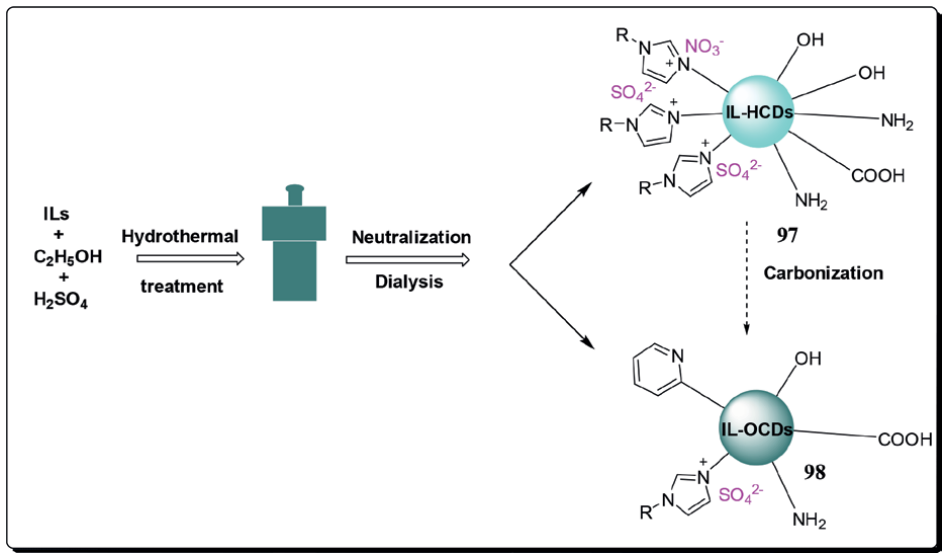

Figure 16.
Synthesis of IL-HCDs and IL-OCDs.

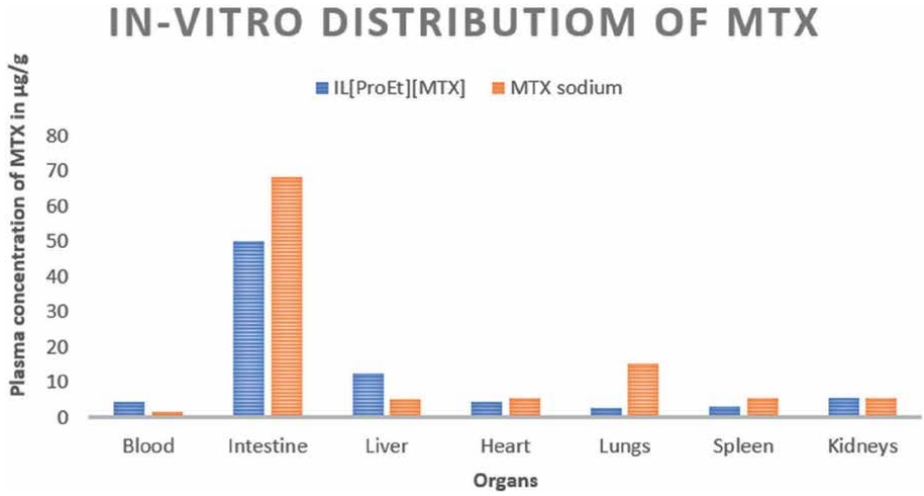

Figure 17.
At 2.0 hours postoral administration of 50 mg/kg MTX to mice, MTX distribution in major organs was analyzed (ns = nonsignificant; n = 4; mean ± SD).

$[FeCl_4]$, and relevant parameters were obtained. The biocompatibility of $[ValC1_6]$ $[FeCl_4]$ was demonstrated through its physicochemical interaction with animal DNA using zeta potential, circular dichroism (CD), ethidium bromide exclusion assay, and agarose gel electrophoresis. Rheology and vibrating sample magnetometry were used to assess magnetic behavior and the gel strength of the generated magnetoresponsive biocomposite hydrogels. The produced magnetic biocomposite gel was used as a drug carrier for ornidazole (69.06%) and for an anticancer drug, 5-fluorouracil (78.03%), with extremely high loading efficiency. Both the drugs' release kinetics follow the Korsmeyer-Peppas paradigm. These magnetic biocomposite gels, devoid of nanoparticles, can be employed for *in vivo* investigations and applications including drug transport and tissue engineering.

Jahanshahi et al. have developed a process for synthesizing fluorinated graphene (FG) utilizing synthesized acidic IL **100** and a solid fluorine source (NH_4F) at 80°C (**Figure 18**). This procedure was initiated by the oxidation of graphite resulting in graphene oxide **99** (GO) followed by a mild-temperature fluorination with an acidic IL, as it allows increased protonation of epoxide and hydroxyl groups that in turn boosts the fluorinating proficiency of the reaction. X–ray photoelectron spectroscopy (XPS) further revealed that the FG **105** showed the maximum degree of fluorination (66.4 wt.% of F) with F/C ratio of 2.2. As a result, the generated FG nanosheets demonstrated a greater Curcumin (a natural anticancer drug) loading efficacy (78.43%), and also these FG nanocarriers loaded with Curcumin precisely transported the drug to the nuclei of cancer cells, causing death of PC-3 cells. Concludingly, the posited approach for preparing FG is proved to be promising in the broad range of organic compounds for further implications in varied disciplines of research [59].

4.4 Antiviral drug delivery

In another report, the research group of Goto has described the IL-based ternary (IL-EtOH-IPM) systems that are thermodynamically stable and optically transparent with an array of IL pertinence. It comprises biocompatible ILs, a co-solvent isopropyl

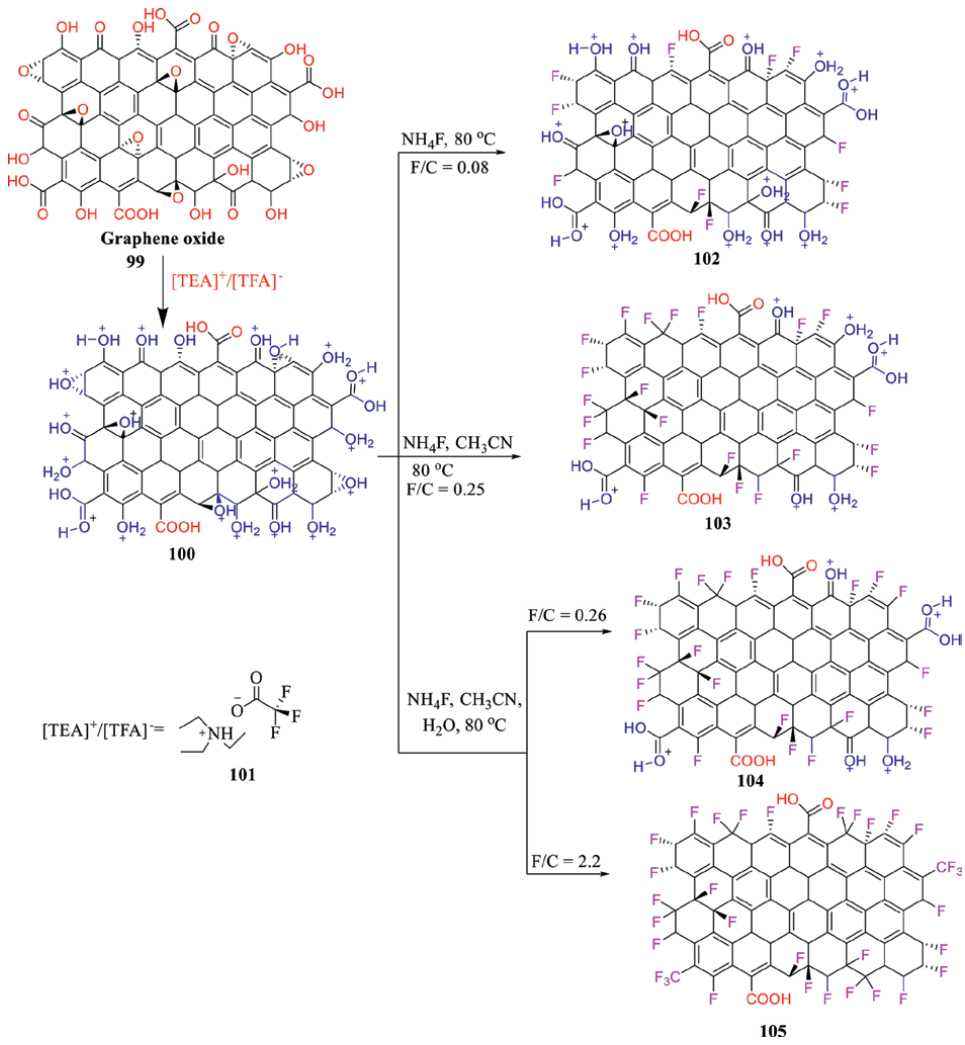

Figure 18.
Synthesis of fluorinated graphene products.

myristate (IPM), and ethanol that can significantly dissolve the moderately soluble drug acyclovir (ACV). *In vitro* drug testing revealed that these ILs have shown a remarkable increase in ACV permeation through the skin. The ILs' biocompatibility was proven against fibroblast cells (L-929) when compared to commercially available ILs [C1mim][DMP] and [Bmim][Cl]. Furthermore, the skin irritation studies performed on the human epidermis model (LabCyte EPI-MODEL) revealed that the suggested IL-EtOH-IPM ternary system's safety and toxicity is equivalent to that of TDDSs [60].

5. Conclusions and outlook

In summary, we have highlighted the role of ILs in the transportation of an array of drugs that demonstrated inefficient delivery through conventional methods. The studies revealed that the ionic liquids displayed the characteristics of a potent drug

carrier and are highly recommended for poorly soluble drugs. The scope of these carriers is impeccable in the medical and biochemistry research areas. We hope that this particular chapter will be useful to the readers to further expand the horizon of the ionic liquids in drug delivery systems.

Acknowledgements

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Conflicts of interest

The authors declare no conflicts of interest.

Author details

Lubna Khan, Rashid Ali* and Farheen Farooqui
Organic and Supramolecular Functional Materials Research Laboratory, Department of Chemistry, Jamia Millia Islamia, New Delhi, India

*Address all correspondence to: rali1@jmi.ac.in

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Chapter 3

Targeting Tyrosinase: Heterocyclic Compounds in the Spotlight

Laila Rubab, Ali Irfan, Mohammad Raish, Huma Hameed, Shazia Akram Ghumman, Samina Aslam, Shazia Perveen, Ajaz Ahmad, Zeemal Seemab Amin and Yousef A. Bin Jardan

Abstract

Tyrosinase (TYR) is a multifunctional, glycosylated, copper-containing oxidase and metalloenzyme that falls within the type-3 copper protein family. The primary function of tyrosinase is the catalytic oxidation of two consecutive steps involved in the biosynthesis of melanin. TYR is responsible for the enzymatic browning of fruits and vegetables and hyperpigmentation in human skin, which results in economic loss as well as skin cancer in humans. Consequently, tyrosinase inhibitors (TYRIs) emerge as potential chemotherapeutic skin whitening and browning inhibitors in fruits, as well as anti-melanogenic substances for treating melanoma. The development of novel inhibitors with lesser side effects or without side effects remains a current topic in medicinal chemistry because already reported tyrosinase inhibitors showed side effects. Heterocycles emerged as novel tyrosinase inhibitors that possess different bioactive functionalities and substitution patterns that play a fundamental role in their anti-tyrosinase activity. Therefore, focusing this chapter on TYRI-bearing heterocycles proves to be valuable and inspirational for the scientific community, as it offers insights for designing new generations of molecules capable of inhibiting or even degrading tyrosinase. The researchers are encouraged to develop new efficient and potent heterocyclic tyrosinase inhibitors for use in foods, cosmetics, and to treat skin cancer.

Keywords: human tyrosinase tyrosinase inhibitors, mushroom tyrosinase inhibitors, bacterial tyrosinase inhibitors, nitrogen heterocycles, oxygen heterocycles, sulfur heterocycles, SAR

1. Introduction

The tyrosinases (TYRs) are oxidoreductase proteins that are binuclear copper-containing metalloenzymes that contain two copper ions in their active sites and are part of all life domains, such as humans, plants, arthropods, mammals, fungi, bacteria, and prokaryotes [1]. In mammals, tyrosinases catalyze the synthesis of melanin in skin and hair color while in bacteria, tyrosinase protects DNA from UV damage. In plants, fruits, and vegetables, tyrsoinases are responsible for browning and cell damage, while in arthropods, sponges, and many other invertebrates, tyrsoinases play a vital role in sclerotization, wound healing, and the primary immune response, respectively [2–4].

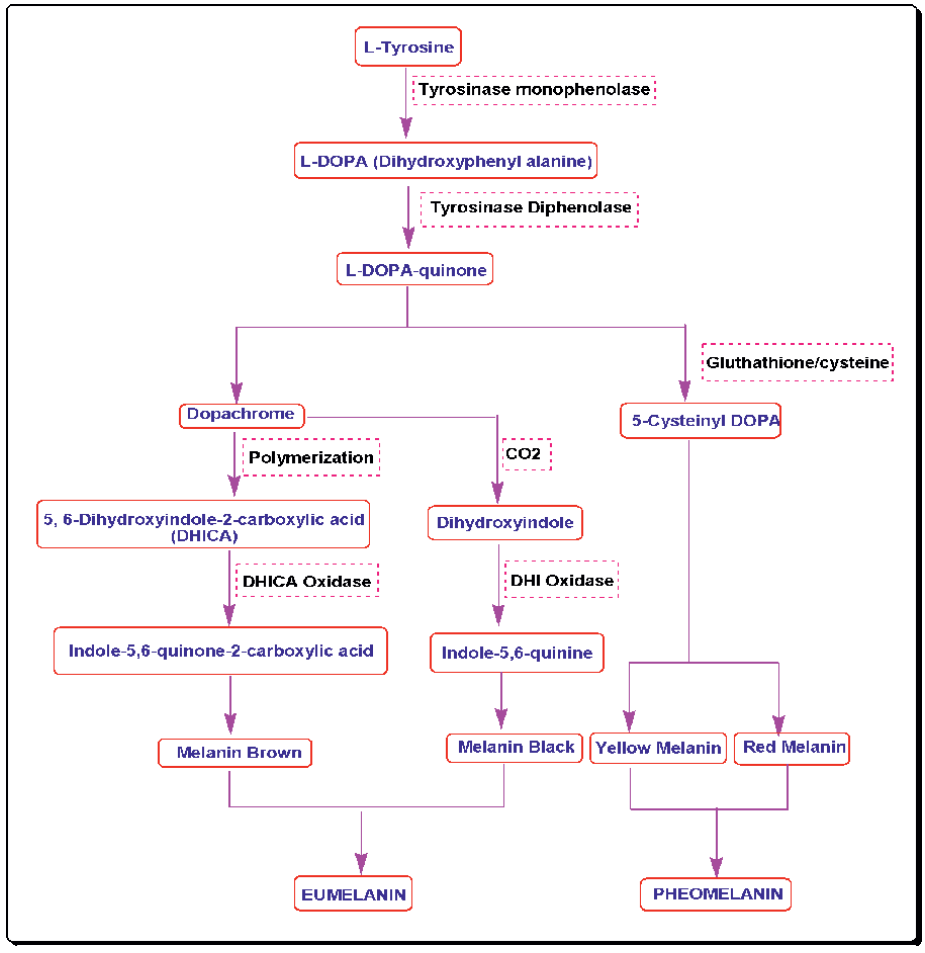

Figure 1.
Biosynthesis of different types of melanin.

The structure of the tyrosinase enzyme can be divided into three distinct parts, namely:

- *N*-terminal domain
- Central domain
- *C*-terminal domain

The active site of the tyrosinase enzyme is the central domain, which is characterized by six conserved histidine residues and coordinates with two copper-oxidizing enzymes present in the active site [5].

1.1 Role of tyrosinase in the biosynthesis of melanin

"Tyrosinase enzyme as catalyst is involved in the biosynthesis of melanin which catalyzes process through the two oxidation steps."

- Tyrosine hydroxylase (monophenolase activity)
- *o*-Diphenol oxidase, catechol oxidase or DOPA oxidase (diphenolase activity) [1, 6]

The melanin biosynthesis process is outlined in **Figure 1**, in which tyrosinase catalyzes the oxidation step of L-tyrosine to L-DOPA, termed monophenolase activity. In the next step, L-DOPA is oxidized to quinone in the presence of tyrosinase as a catalyst. This formation of quinone serves as a substrate for subsequent steps that are involved in the synthesis of different types of melanin, such as eumelanin (which changes from brown to black) and pheomelanin (which changes from yellow to red), as depicted in **Figure 1**. Eumelanin is stronger in color than pheomelanin [6–8].

1.2 Dermal and epidermal melanin reduction

Melanins, the multifaceted compounds integral to these protective processes, exhibit distinct structural characteristics, notably represented by eumelanin, pheomelanin, and neuromelanin. These compounds collectively contribute to the intricate defense mechanisms against UV-induced damage and oxidative stress [9]. The intricate interplay of melanogenesis in maintaining skin homeostasis underscores the delicate balance between protective adaptation and the potential for harmful hyperpigmentation disorders as shown in **Figure 2**. L-tyrosine is converted into L-DOPA and these are the first step for the formation of dopaquinone and quinone is used as a substrate for additional melanin production. Hydroquinone, Deoxy Arbutin and Kojic acid like substances are responsible for disruption in conversion of L-TYR to L-DOPA. This disruption reduced activity of TRP1/2 in production melanin. This catalysis process is entirely dependent on copper ions, which is also disrupted due to Deoxy Arbutin. The melanosome membrane isoform-I of tyrosine hydroxylase converts the L-tyrosine substrate to the L-DOPA intermediate product, which stimulates tyrosinase enzyme activity. Hydroquinone and Kojic

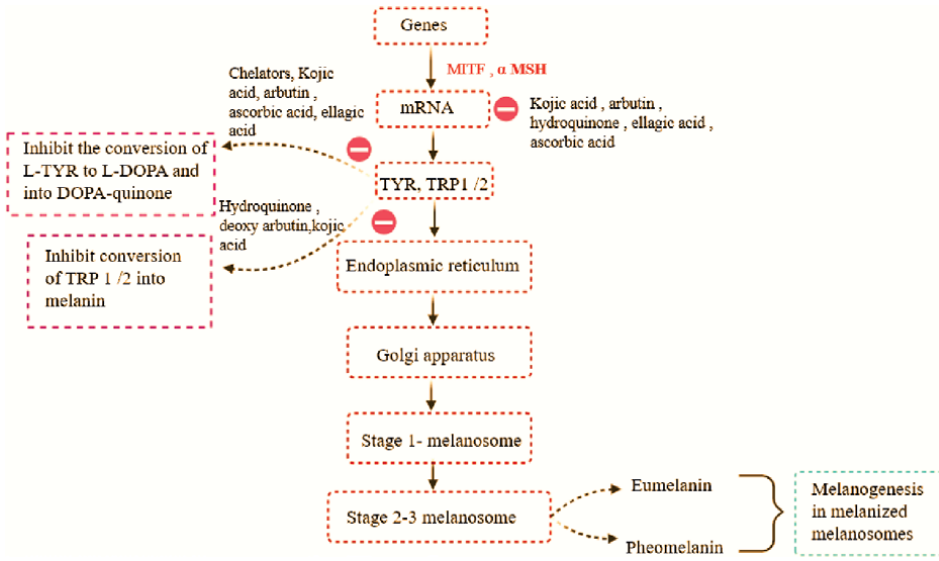

Figure 2.
Strategies for dermal and epidermal melanin reduction.

acid mainly interact with tyrosinase enzyme activity and block it, ultimately leads to reduction in dermal and epidermal melanin [10]. TRP-1 activates and stabilizes the tyrosinase enzyme and the formation of melanosomes endosomes, as well as increasing the eumelanin to pheomelanin ratio and substrate peroxidation levels. But, Substances like Hydroquinone, Deoxy Arbutin and Kojic acid reduces the formation of melanocytes/melanosomes and ultimately, decrease the level of Eumelanin and Pheomelanin [11].

1.3 Signaling pathway (melanogenesis at the transcriptional level)

For cellular signaling in melanogenesis, the initial receptor MC1-R has predominantly been activated by its relevant and endogenous agonists [12]. MC1-R is a G protein that is coupled to adenylyl cyclase and predominantly expressed in dermal melanocytes. MC1-R regulates melanocytic differentiation and consequently determines the direction of skin phototype via interactions with adrenocorticotropic hormone (ACTH) and Alpha-Melanocytes stimulating hormone (α-MSH) [13], ACTH and α-MSH are melanocortins produced by enzymatic proopiomelanocortin (POMC) cleavage [14]. To compensate for their pituitary production in melanogenesis, melanocytes and keratinocytes produce them on the skin [15]. ACTH sustained administration have all been shown to cause skin hyperpigmentation [16]. MC1-R activators induce adenylate cyclase activity and consequently increase Cyclic adenosine monophosphate (cAMP) level. cAMP is a second messenger present in cell to regulate cell signaling. Cyclic adenosine 3′, 5′-monophosphate activates protein kinase A (PKA) activity [17]. PKA catalyzes phosphor-CREB (cAMP-response element binding protein) formation [18], which is crucial for a cAMP response element to upregulate specific transcriptional factors such as microphthalmia-associated transcription factor (MTTF) via the downstream signaling of mitogen-activated protein kinases/extracellular-signal-regulated kinases (MAPKs/ERK). That further controls the level TRP 1/2, the main enzymes involved in melanogenesis process as indicated in **Figure 3**.

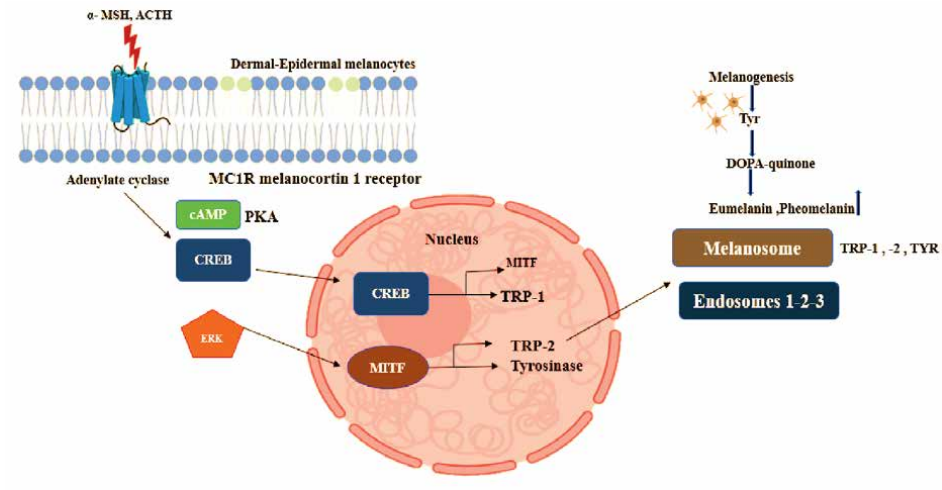

Figure 3.
Signaling pathway (Melanogenesis at the transcriptional level).

1.4 Sources of tyrosinase

As already discussed, tyrosinases are extensively distributed in all life domains, from microorganisms to highly advanced living organisms such as humans and mammals. Following are the major sources of tyrosinase enzymes:

1.4.1 Bacterial strains as sources of tyrosinase

Bacterial tyrosinases are involved in melanin production, and for the first time, tyrosinases were isolated, purified, and well characterized from Streptomyce species [19]. This enzyme is also reported in many other bacterial strains, such as *Sinorhizobium meliloti, Symbiobacterium thermophilum, Thermomicrobium roseum, Verrucomicrobium spinosum, Rhizobium, Pseudomonas maltophilia, Bacillus thuringiensis, Ralstonia solanacearum, Pseudomonas putida, etc.* [20, 21].

1.4.2 Fungal strains as sources of tyrosinase

Fungal strains such as *Agaricus bisporus, Lentinula edodes, Portabella mushrooms, Lentinula boryana, Neurospora crassa, Pycnoporus sanguineus, Amanita muscaria,* and *Aspergillus oryzae etc*. are valuable and significant sources isolation, purification, characterization and applications of tyrosinase enzymes [21, 22].

1.4.3 Plants as a source of tyrosinase

In the class of plants, fruits and vegetables are the major and most significant source of the tyrosinase enzyme. Tyrosinase is localized in the chloroplast, while its substrates are localized in the vacuoles of the plants. The most potent source of tyrosinase is the Portulaca grandiflora (Portulacaceae) species [21, 23]. Tyrsosinases generally cause the undesirable browning of farm products, which destroys the nutritional and market value of vegetables and fruits and results in economic loss [24]. Janovitz-Klapp et al. (1989) extracted tyrosinase from the Monastrell grape [25]. Tyrosinase enzymes are also extracted from sunflower, *solanum melongena*, and apple [26, 27].

2. Tyrosinase inhibitors (TYRIs)

Tyrosinase inhibitors are widely applied in the cosmetic industry and agriculture [18, 28], which can be synthesized or extracted from natural sources. The inhibitory activity of the tyrosinase enzyme is due to one of the below-mentioned reasons:

1. Reduction of dopaquinone by reducing agents such as ascorbic acid, etc.

2. Scavenging activity of compounds by reacting with *o*-dopaquinone to produce colorless products such as thio moiety-containing molecules.

3. Good-affinity alternative substrates for the tyrosinase enzyme form different products instead of dopachrome, such as phenol compounds.

4. Denaturing of the tyrosinase enzyme by using non-specific enzyme activators such as acids or bases has the capability to act as tyrosinase inhibitors.

5. Mechanism-based tyrosinase enzyme inhibitors or specific tyrosinase enzyme activating substrates, which, upon reaction with the tyrosinase enzyme, resulted in a suicide reaction to inhibit tyrosinase catalytic activity.

6. Specific and true tyrosinase inhibitors bind directly with the enzyme and inhibit its catalytic activity [28–30].

3. Heterocycles as tyrosinase inhibitors

Heterocycles are cyclic organic compounds that possess one or more heteroatoms in their rings, such as sulfur (S), nitrogen (N), and oxygen (O), but rings containing other heteroatoms like selenium (Se), magnesium (Mg), and phosphorus (P) can also be found [31]. Heterocycles are core parts of many natural scaffolds that are significant for life on earth, like chlorophyll, DNA, heme groups, proteins, RNA, and vitamins. It is noteworthy that over 90% of new drugs are estimated to incorporate a heterocyclic moiety [32]. For this reason, we report in this chapter recent advances in heterocycles as tyrosinase inhibitors (TYRIs). Heterocycles play a promising chemotherapeutic role in the treatment of melanoma skin cancer and as skin whitening agents. Different heterocyclic compounds which are already reported as standard skin whitening agents and tyrosinase inhibitors are given in **Figure 4** [8, 18, 30, 32–34].

3.1 Ascorbic acid (vitamin C) as representative heterocyclic inhibitor

Ascorbic acid, also known as vitamin C, is a water-soluble vitamin with a high antioxidant potential (18) [35]. It has been shown to inhibit melanin synthesis by transforming dopaquinone into L-DOPA. The in vitro inhibitory activity of ascorbic acid towards tyrosinase was determined using a microtiter plate assay with an ELISA microplate reader, while its in silico inhibitory effect was investigated through molecular docking simulations using Molegro Virtual Docker (MVD) [36]. In vitro assays have revealed that ascorbic acid, or vitamin C, interacts with the active site of tyrosinase through hydrophobic interactions with specific amino acid residues, namely Phe264, His263, Ser282, and Val283 [37]. Additionally, the O5 atoms of ascorbic acid form two distant hydrogen bonds with the copper (Cu) ions at the active site, with distances of 3.57 and 3.41 Å. This interaction with the copper ions inhibits the action of the tyrosinase enzyme, consequently reducing melanin formation [38]. The instability of ascorbic acid in aqueous environments is attributed to its oxidation to dehydroascorbic acid, which is a reversible reaction, and subsequently to 2,3-diketo-L-gulonic acid, an irreversible reaction leading to the loss of its physiological properties. Despite its instability, ascorbic acid has demonstrated good photoprotective ability against ultraviolet A-mediated phototoxicity [39]. Ascorbic acid has been shown to indirectly inhibit the activity of tyrosinase due to its antioxidant capacity, thereby reducing melanogenesis. However, conflicting findings exist regarding its stability and its potential to act as a pro-oxidant, which may lead to increased proliferation and melanin content in melanoma cells [40].

Ascorbic acid has been shown to stimulate the activity and expression of tyrosinase in B16F10 cells through the activation of p38 mitogen-activated protein kinase

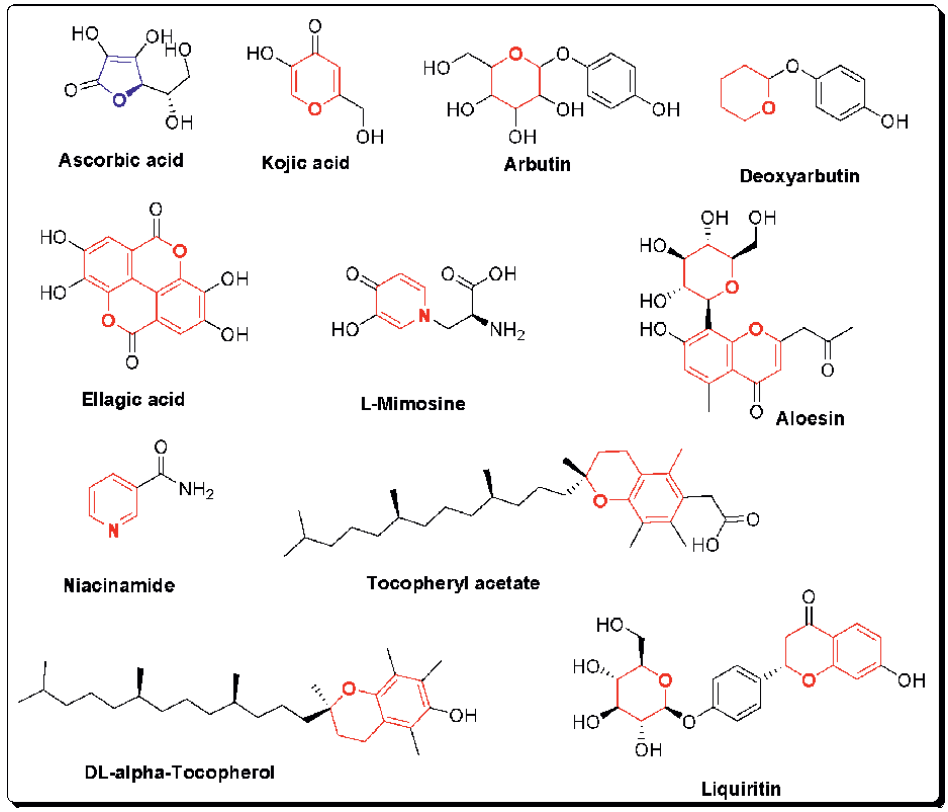

Figure 4.
Structures of standard heterocyclic tyrosinase inhibitors.

(MAPK) signaling [41]. This leads to the up-regulation of tyrosinase and the expression of melanogenic regulatory factors such as tyrosinase-related protein-1 (TRP-1), dihydroxyphenylalaminechrome tautomerase (TRP-2), and microphthalmia-associated transcription factor (MITF) [42]. Additionally, ascorbic acid induces phosphorylation of p38 MAPK, which is involved in the regulation of tyrosinase activity. However, the inhibition of the p38 MAPK pathway by SB203580 leads to the suppression of tyrosinase, TRP-1, and TRP-2 expression in cells treated with ascorbic acid [43]. Furthermore, combined treatment with *N*-acetyl-L-cysteine and/or desferrioxamine mesylate attenuates the stimulating effect of ascorbic acid on tyrosinase activation in the cell [44] as depicted in **Figure 5**.

3.2 Kojic acid as representative heterocyclic inhibitor

Kojic acid is a natural compound that has been extensively studied as a competitive inhibitor of tyrosinase, an enzyme responsible for melanin production in human melanocytes. It prevents melanin formation by reversibly inhibiting tyrosinase [45]. Kojic acid exhibits a competitive inhibitory effect on monophenolase activity and a mixed inhibitory effect on the diphenolase activity of mushroom tyrosinase. Its ability to chelate copper at the active site of the enzyme may explain its competitive inhibitory effect [46]. Additionally, kojic acid is reported to be a slow-binding inhibitor of the diphenolase activity of tyrosinase, requiring the active form of tyrosinase to be present

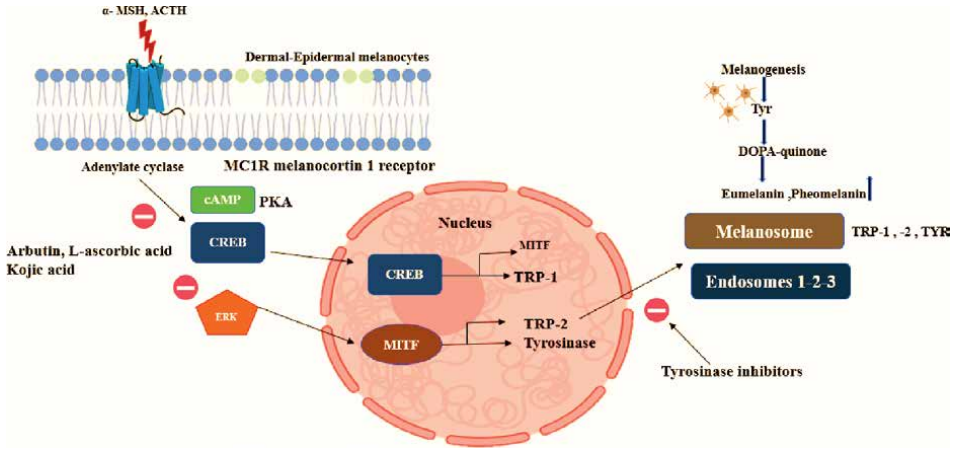

Figure 5.
Inhibiting melanogenesis at the transcriptional level.

before binding to the enzyme can occur [30]. Kojic acid has been shown to downregulate the expression of melanogenesis-related genes such as tyrosinase and tyrosinase related protein –1 (TRP-1) at the transcriptional level by disrupting the CREB and MAPK/ERK signaling pathways, leading to reduced melanin production in skin cells [47] as displayed in **Figure 5**.

3.3 Heterocycles as bacterial tyrosinase (bTYR) inhibitors

Bacterial strains have the ability to produce melanin due to the presence of the tyrosinase enzyme, which has been known for a long time. The latest example is the *proteobacterium Brevundimonas* sp. SG [48]. Bacterial tyrosinase (bTYR) is classified into five types based on the arrangement of domains and the possible requirement of secondary helper proteins known as caddie proteins, which are essential for enzyme activity [49]. Researchers usually utilize bTYRs to determine the inhibitory chemotherapeutic efficacy of heterocyclic or other tyrosinase-inhibiting agents.

Zahoor et al. reported the synthesis of a series of 1-tosyl piperazine-dithiocarbamate acetamide hybrids and screened for their in vitro bacterial tyrosinase inhibitory activity. Among all these derivatives, 4-methoxy-containing piperazine-dithiocarbamate acetamide scaffold **1** showed the most powerful tyrosinase inhibitory potential with an IC_{50} value of 6.88 ± 0.11 μM when compared with standard reference drugs kojic acid having an IC_{50} value of 30.34 ± 0.75 μM and ascorbic acid having an IC_{50} value of 11.5 ± 1.00 μM, respectively. The SAR studies revealed that compound **1**, due to substitution of the methoxy (MeO) group at the *p*-position of the aromatic ring and electron donation through resonance/conjugation effects, demonstrated outstanding inhibitory activity compared to all other derivatives as well as higher than standard drugs kojic acid and ascorbic acid (Vitamin C) [50] as depicted in **Figure 6**. In another study, Irfan et al. reported a new series of 10 heterocyclic hybrids incorporating furan-oxadiazole *S*-alkylated amide linkages. These hybrids were strategically designed and evaluated for their therapeutic potential in targeting pharmacologically significant bacterial tyrosinase enzyme inhibition. Among the compounds, compound **2** emerged as the most potent bacterial tyrosinase inhibtior, demonstrating remarkable tyrosinase inhibitory efficacy with an IC_{50}

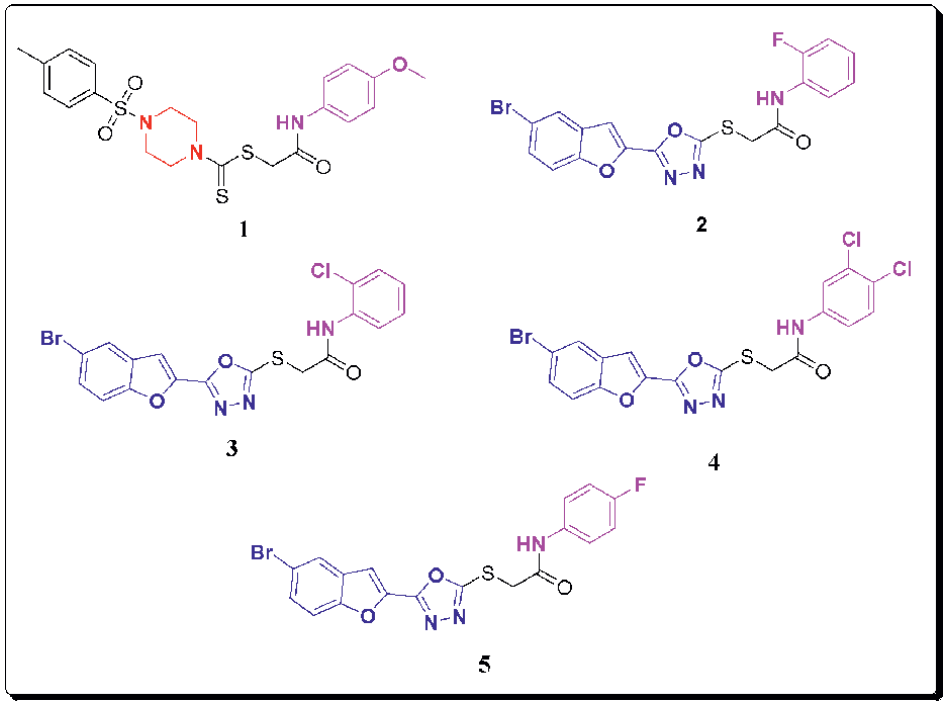

Figure 6.
Structures of heterocyclic bacterial tyrosinase inhibitors.

value of 11 ± 0.25 μM. This efficacy surpassed that of the reference drug, ascorbic acid, which had an IC_{50} value of 11.5 ± 0.1 μM as displayed in **Figure 6**. Additionally, benzofuran-1,3,4-oxadiazole compounds **3–5** displayed significant inhibitory activity against the tyrosinase enzyme, with IC_{50} values ranging from 12.4 ± 0.0 to 15.5 ± 0.0 μM as shown in **Figure 3**. The findings suggest that these newly designed compounds have promising potential for further development as inhibitors of the bacterial tyrosinase enzyme. The results from SAR studies revealed that compounds, which incorporate electron-withdrawing halogen groups (EWD) present on phenyl groups, demonstrated noteworthy inhibitory effectiveness ranging from good to excellent [51].

3.4 Heterocycles as mushroom tyrosinase (mTYR) inhibitors

Mushroom tyrosinase is usually used to assess the inhibitory chemotherapeutic activity of TYRIs instead of human tyrosinase. Despite variation between human and mushroom tyrosinases, the use of commercially available mTYR facilitates the direct screening of potential TYRIs. Compounds identified through mushroom tyrosinse screening have demonstrated chemotherapeutic capability to inhibit the hTYR enzyme, leading to skin whitening and anti-melanogenic effects within human cell lines [52]. Already reported heterocyclic compounds such as ascorbic acid (vitamin C), kojic acid, arbutin, deoxyarbutin, ellagic acid, L-mimosine, lloesine, niacinamide, tocopheryl acetate, vitamin E (DL-alpha-tocopheryl), liquiritin, etc., as displayed in **Figure 1**, are well-established whitening agents and served as positive controls in in vitro assays for evaluating tyrosinase inhibitory activity [53].

3.4.1 Triazole and pyrazole derivatives as mushroom tyrosinase inhibitors

A series of some *S*-alkylated 2-aminothiazole-ethyltriazole structural motifs was prepared by Butt et al. The newly reported derivatives were studied for their mushroom tyrosinase inhibitory activity. Among the synthesized scaffolds, the 4-({5-[(2,4-dichlorobenzyl)sulfanyl]-4-ethyl-4*H*-1,2,4-triazol-3-yl}methyl)-1,3-thiazol-2-amine analogue (6) displaying outstanding tyrosinase inhibitory potency with an IC_{50} value of 0.0018 ± 0.0005 μM against tyrosinase enzyme in comparison with standard drug kojic acid (IC_{50} = 16.8320 ± 1.1600 μM). The SAR analysis resulted in the finding that **6** showed the highest tyrosinase inhibition activity due to the presence of dichloro moieties on the *ortho* and *para*-positions of the benzylic ring [54].

Ashooriha et al. synthesized a series of novel natural product conjugates containing phenolic and also various derivatives related to phenolic and enolic analogues such as umbelliferone, sesamol, thymol, carvacrol, eugenol, isoeugenol, vanillin, isovanillin, apocynin, syringaldehyde, 4-hydroxybenzaldehyde, 4-hydroxyacetophenone, methylparaben, and 4-hydroxycoumarin (4-coumarinol) and evaluated for their mushroom tyrosinase activity *in vitro* towards tyrosinase inhibitor. These all-natural products were linked with the side chain of the tyrosinase inhibitor kojic acid. Among all these, the triazole moiety containing apocynin motif **7**, displaying an IC_{50} value of 0.03 ± 0.11 μM, and the 4-coumarinol motif **8**, having an IC_{50} range of 0.02 ± 0.001 μM, demonstrated superb tyrosinase activity when compared with standard kojic acid, with an IC_{50} range of 9.28 ± 2.04 μM, respectively. The analogue **8** showed >460 times greater activity as compared to the standard compound kojic acid. The SAR study showed that the introduction of the methoxy group at the *ortho*-position of analogue **7** and the 4-oxycoumarin moiety at compound **8** increased the inhibitory potency against [55]. In the next study, Saeed and coworkers prepared seventeen novel coumarinyl-pyrazolinyls containing thiazole structural motifs and evaluated their mushroom tyrosinase inhibitory potency. All synthesized derivatives exhibited good activity as tyrosinase enzyme inhibitors. The 3-(5-(4-(benzyloxy)-3-methoxyphenyl)-1-(4-(4-bromophenyl) thiazol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one **9** having an IC_{50} range of 0.00458 ± 0.00022 μM demonstrated powerful tyrosinase inhibitory activity in comparison with standard compound kojic acid with an IC_{50} range of 16.84 ± 0.052 μM. The structure–activity relationship (SAR) showed that the inhibition activity was significantly influenced by the substituent's nature around the aromatic rings. Electron-withdrawing groups had a more pronounced impact on the electronic density of the aromatic ring as compared to electron-donating moieties. The scaffold **9**, which exhibited the highest potency, contained both benzyloxy and methoxy groups. The synergistic effect observed in this compound could be attributed to the electron-donating abilities of these two groups through resonance [56]. Boatenget et al. reported a series of 25 azole derivatives, which were evaluated for their *in vitro* and *in silico* anti-tyrosinase activity against mushroom tyrosinase inhibitors. Among all these, 3-methyl-1,5-diphenyl-1*H*-pyrazole **10** showed excellent tyrosinase inhibitory activity, having an IC_{50} value of 15.9 ± 1.2 μM and a 50% maximal inhibition concentration of dead cells as compared to the standard drugs arbutin and kojic acid, with IC_{50} values of 91 μM and 31 μM, respectively. The SAR showed scaffold **10** showed potent activity due to the introduction of an electron-donating CH_3 group on the para-position of the *N1*-phenyl ring [57]. Vanjare et al. synthesized a series of some novel 1,2,4-triazole hybrid structures, which were evaluated for their mushroom tyrosinase inhibition potential. The

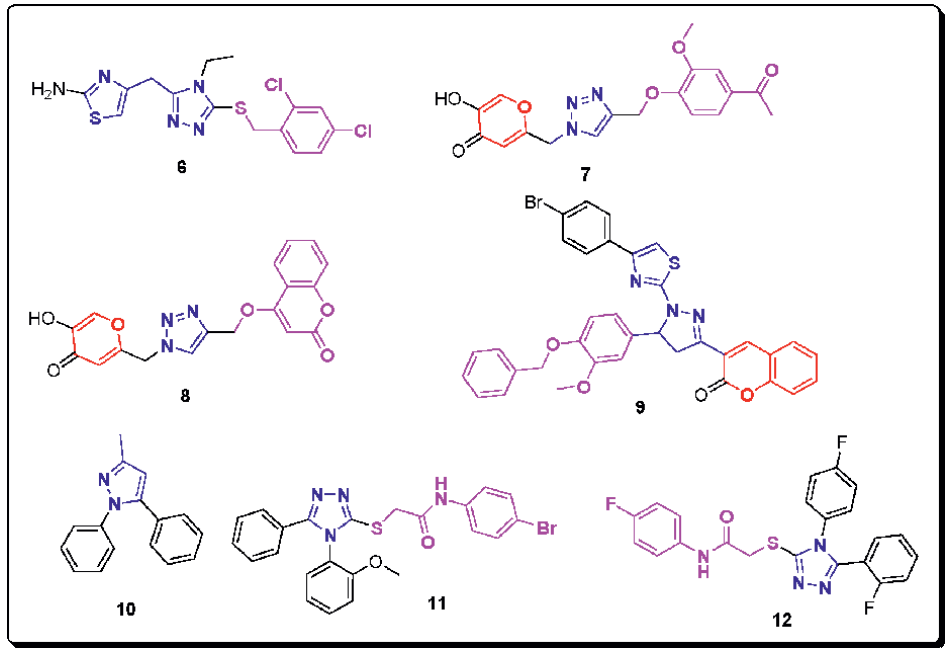

Figure 7.
Structures of triazole and pyrazole moieties containing heterocyclic mTYRIs.

N-(4-bromo-phenyl)-2-(4-(2-methoxyphenyl)-5-phenyl-4*H*-1,2,4-triazol-3-ylthio) acetamide scaffold **11** from the series of newly reported derivatives demonstrated 3500-fold stronger tyrosinase inhibition efficacy with IC_{50} value 0.0048 ± 0.0016 μM towards mushroom tyrosinase enzyme as compared to standard reference compound kojic acid having IC_{50} value 16.8320 ± 1.1600 μM. The SAR studies revealed that the analogue **11** tyrosinase activity increases due to the introduction of the bromo moiety at the *para*-position of the phenyl ring [58]. A novel series of nine 1,2,4-triazole-containing hybrids was reported by Hassan et al. and studied for their mushroom tyrosinase inhibitory activity. Among all synthesized derivatives, *N*-(4-fluorophenyl)-2-(5-(2-fluorophenyl)-4-(4-fluorophenyl)-4*H*-1,2,4-triazol-3-ylthio) acetamide **12** demonstrated the strongest tyrosinase inhibition potential with an IC_{50} range of 0.098 ± 0.009 μM in comparison with the reference drug kojic acid with an IC_{50} value of 16.832 ± 1.161 μM. The SAR investigations demonstrated that the presence of a fluoro moiety on compound **12** enhanced the inhibition of tyrosinase [59]. The structures of all the azole moiety containing mushroom tyrosinase inhibitors (6–12) are displayed in **Figure 7**.

3.4.2 Thiazole, imidazole, indole, and oxazole derivatives as mushroom tyrosinase inhibitors

Shehzadi et al. afforded the synthesis of imidazole-2-thiones, thiazolidinones, and thiazolidin-2-imines-based *N*-and *S*-containing 5-membered heterocyclic compounds, which show significant biological activities. The two newly reported thiazolidine-2-imine derivatives **13** and **14** were screened for their *in vitro* and *in silico* tyrosinase inhibitory activity. The synthesized thiazolidine-2-imines demonstrated strong inhibition activity against mushroom tyrosinase *in vitro*, with IC_{50}

values of 1.151 ± 1.25 μM and 2.079 ± 0.87 μM compared to the commonly used anti-pigment agent, kojic acid, with IC_{50} values of 16.031 ± 1.27 μM, respectively. The SAR showed that different functional groups like methyl and bromo on the benzene ring increase biological activity. Due to substitution of the CH_3 group at the *para* position of the benzene ring, scaffold **13** showed greater activity than scaffold **14** (**Figure 8**) [60]. In another study, Choi et al. furnished a novel series of sixteen (*Z*)-5-(substituted benzylidene)-3-phenyl-2-thioxooxazolidin-4-one derivatives based on β-phenyl-α, β-unsaturated carbonyl compounds and evaluated their mushroom tyrosinase inhibitory activities. Among all these compounds, (*Z*)-5-(2,4-dihydroxybenzylidene)-3-phenyl-2-thioxooxazolidin-4-one **15** and (*Z*)-5-(2-hydroxy-benzylidene)-3-phenyl-2-thioxooxazolidin-4-one **16**, (**Figure 8**) with IC_{50} values of 4.70 ± 0.40 μM and 11.18 ± 0.54 μM, demonstrated 4.9-fold and 2.1-fold stronger inhibition of tyrosinase activity as compared to standard drug kojic acid with IC_{50} value 23.18 ± 0.11 μM, respectively. The SAR studies revealed that compound 1c showed enhanced tyrosinase inhibitory activity due to the presence of a 2,4-dihydroxyphenyl group [61]. In the next study, Choi and his colleagues afforded several benzylidene groups containing 5,6-dihydroimindazo[2,1-b] thiazol-3(2*H*)-one (DHIT) analogues, which were demonstrated for their potential tyrosinase inhibition. The three newly reported DHIT compounds, (*Z*)-2-(4-hydroxybenzylidene)-5,6-dihydroimidazo[2,1-b]thiazol-3(2*H*)-one **17**, (*Z*)-2-(2,4-dihydroxybenzylidene)-5,6-dihydro-imidazo[2,1-b]thiazol-3(2H)-one **18**, and (*Z*)-2-(3-hydroxy-4-methoxybenzylidene)-5,6-dihydroimidazo[2,1-b]thiazol-3(2*H*)-one **19** showed powerful mushroom tyrosinase inhibitory activity with IC_{50}

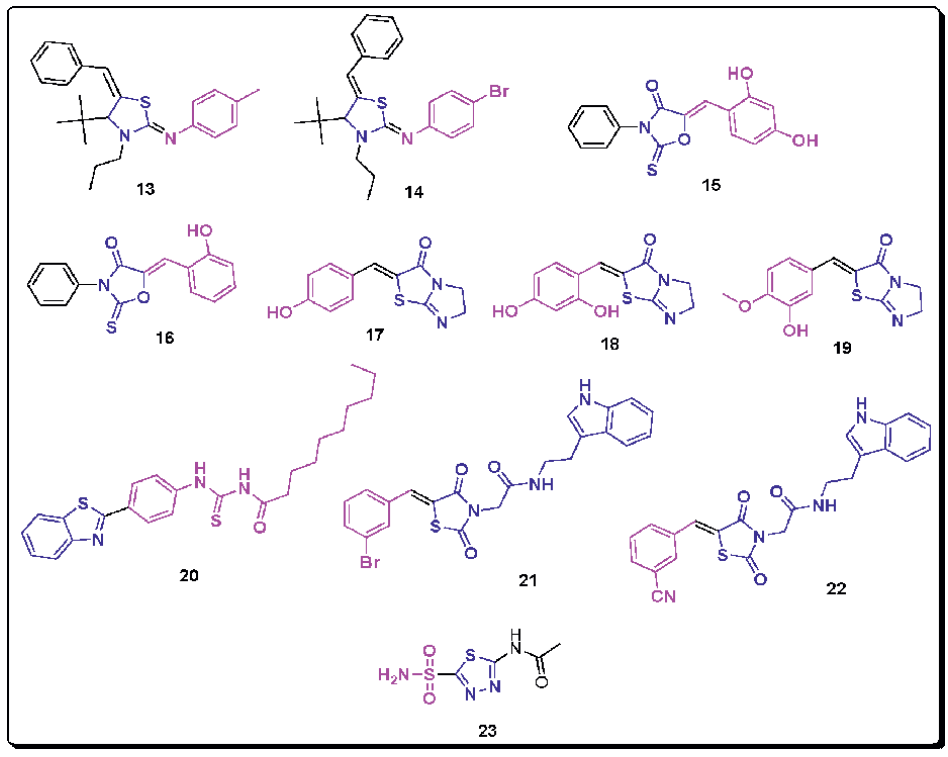

Figure 8.
Structures of Thiazole, imidazole, indole, and oxazole containing heterocyclic mTYRIs.

ranges of 36.14 ± 3.90 μM (40% inhibition), 0.88 ± 0.91 μM (96% inhibition), and 17.10 ± 1.01 μM (73% inhibition) when compared with standard reference compound kojic acid having IC_{50} value 84.41 ± 2.87 μM (30% inhibition), respectively. The SAR showed that the presence of a 4-hydroxyl group on the phenyl ring of analogue **17**, a 2,4-dihydroxyl moiety on the phenyl ring of scaffold **18**, (**Figure 8**) and a 3-hydroxy-4-methoxyl group on the phenyl ring of compound **19** (**Figure 8**) were responsible for their potent mTYR inhibitory activity [62]. Ujan synthesized the nine different derivatives of benzothiazole-thiourea bearing aromatic and aliphatic side chains and demonstrated their mushroom tyrosinase activity *in vitro.* The 1-(4-(benzo[d]thiazol-2-yl)phenyl)-3-decanoylthiourea compound **20** (**Figure 8**) was the most active in the series of compounds, displaying an IC_{50} value of 1.3431 ± 0.0254 μM against a tyrosinase inhibitor when compared with the standard drug kojic acid, which has an IC_{50} range of 16.8320 ± 1.1600 μM. The SAR showed scaffold **20** showed potent tyrosinase activity due to the presence of lengthy alkyl chains, thiourea, and benzothiazole moieties [63]. A structurally unique series of novel indole-thiazolidine-2,4-dione hybrids was afforded by Lu and coworkers. The synthesized structural motifs were evaluated for their mushroom tyrosinase inhibitory activity. The scaffolds (*Z*)-*N*-(2-(1*H*-indol-3-yl)ethyl)-2-(5-(3-bromobenzylidene)-2,4-dioxothiazolidin-3-yl)acetamide **21** and (*Z*)-*N*-(2-(1*H*-indol-3-yl)ethyl)-2-(5-(3-cyanobenzylidene)-2,4-dioxothiazolidin-3-yl)-acetamide **22**, possessed excellent tyrosinase inhibitory potency among all the 26 screened compounds. The thiazolidine compounds **21** and **22** (**Figure 8**) displaying IC_{50} ranges of 13.3 μM and 11.2 μM in comparison to the IC_{50} value of standard drug kojic acid (15.6 μM), respectively. The SAR showed that the introduction of the bromine group on the *meta*-position of the benzene ring of analogue **21** and the cyanide moiety on the *meta*-position of the benzene ring of compound **22** (**Figure 8**) were responsible for their potent tyrosinase inhibitory activity [64]. Abbas et al. reported the inhibitory potential of acetazolamide (ACZ) **23** (**Figure 8**) via enzyme kinetic, in vitro, in vivo and in silico studies by screening against mushroom tyrosinase inhibitory activity. The ACZ exhibited most potent tyrosinase inhibitory activity, displaying IC50 range of 7.89 ± 0.24 μM in comparison to standard drug kojic acid which had an IC_{50} value 16.84 ± 0.64 μM [65].

3.4.3 Benzothiazepine, oxazine, and quinoline derivatives as mushroom tyrosinase inhibitors

Al-Rooqi et al. described the synthesis of fourteen novel 2,3-dihydro-1,5-benzothiazepine analogues and demonstrated their mushroom tyrosinase activity via in vitro and in silico studies. Among all the synthesized structural hybrids, the compound (*E*)-2-(3,4-dimethoxyphenyl)-4-*p*-tolyl-2,3-dihydrobenzo[b][1,4]thiazepine **24** (**Figure 9**) demonstrated outstanding TYR inhibitory efficacy having lowest IC_{50} value 1.21 μM in comparison to the reference standard kojic acid which had an IC_{50} value of 16.69 μM. The SAR analysis showed that in structural motif **24** introduction of a methoxy (−OCH3) substituent on the meta and para positions of phenyl ring B, along with p-CH3 moiety attachment on ring C, resulted in a 14-fold increases in inhibitory activity compared to the standard drug kojic acid [66]. A new novel series of substituted 1,3-oxazine-tetrazole structural motifs (3a-k) were prepared by Qamar and his colleagues and evaluated these synthesized scaffolds for their mushroom tyrosinase inhibitory activity. Among all the target compounds, 5-(2*H*-tetrazol-5-yl)-4-thioxo-2-(2-bromophenyl)-4,5-dihydro-1,3-oxazin-6-one **25** (**Figure 9**) showed remarkably excellent tyrosinase inhibitory activity having IC_{50} value = 0.0371 ± 0.0018 μM when it

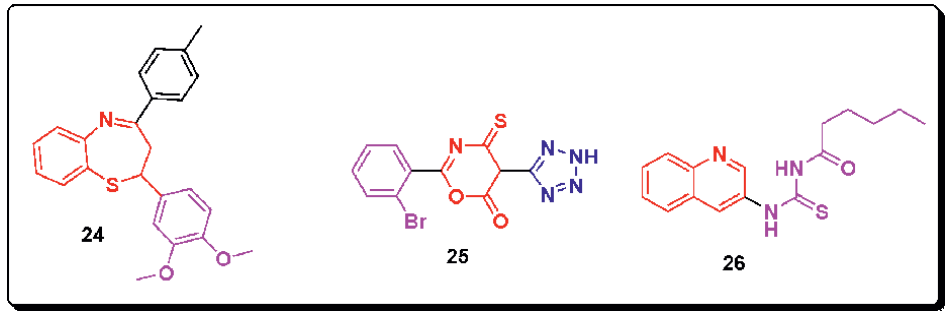

Figure 9.
Structures of benzothiazepine, oxazine, and quinoline based heterocyclic mTYRIs.

compared to standard reference drug kojic acid with an IC_{50} value = 16.832 ± 0.73 μM. The SAR showed that compound **25** containing 2-bromophenyl moiety exhibited stronger inhibitory activity [67]. Mustafa et al. reported variety of alkyl and aryl groups containing novel quinolinyl bearing acyl thiourea hybrids and determined their mushroom tyrosinase inhibition potential. Among all tested derivatives, *N*-(quinolin-3-ylcarbamothioyl)hexanamide **26** (**Figure 9**) possessed most potent tyrosinase inhibitory activity with an IC50 value 0.0070 ± 0.0098 μM when compared with standard kojic acid with IC50 range 16.8320 ± 0.0621 μM. The SAR studies resulted that the nature of functionalities around the acyl thiourea moiety greatly affected the tyrosinase inhibition activity. The sterically hindered substituents like long alkyl chains in the compounds resulted in diminishing the inhibitory effect. The compound **26** containing moderate alkyl chain, thiourea and quinoline moiety, interact strongly and occupy the whole pocket of receptor [68].

3.4.4 Piperzine, fluoroquinolone, pyrimidine, and pyridine derivatives as mTYRIs inhibitors

Raza et al. synthesized the structurally unique series of *N*-(substituted-phenyl)-4-(4-phenyl-1-piper-azinyl)butanamide derivatives, which were studied for their in vitro mushroom tyrosinase inhibitory activity. From all reported derivatives, the structural motif *N*-(2,5-Dimethylphenyl)-4-(4-phenyl-1-piperazinyl)butanamide **27** (**Figure 10**) demonstrated outstanding tyrosinase inhibitory activity, having an IC_{50} of 0.258 ± 0.024 μM that was more active than the reference drug kojic acid, with an IC_{50} value of 16.841 ± 1.146 μM. The SAR analysis revealed that the scaffold **27** activity increases due to the substitution of two methyl moieties on the 2 and 5 positions of the benzene ring and also shows strong interactions and occupies the whole pocket of the receptor [69]. Abbasi et al. developed a novel series of sulfonamide structural motifs fused with piperazine and heterocyclic secondary amines and studied their outstanding mushroom tyrosinase inhibition potential. Among the all afforded derivatives, the 1-phenyl-4-[4-(1-piperidinylsulfonyl)benzyl]-piperazine scaffold **28** (**Figure 10**) exhibited the most potent activity with an IC_{50} value of 0.0586 ± 0.0033 μM against tyrosinase inhibitor. The standard reference compound kojic acid possessed an IC_{50} value of 16.8320 ± 1.1600 μM towards tyrosinase inhibitors. The SAR study confirmed that analogue **28** demonstrated good tyrosinase activity was due to the substitution of a piperidinyl ring in its structure [70]. Alyami et al. reported seven novel fluoroquinolone drugs such as ciprofloxacin, enoxacin

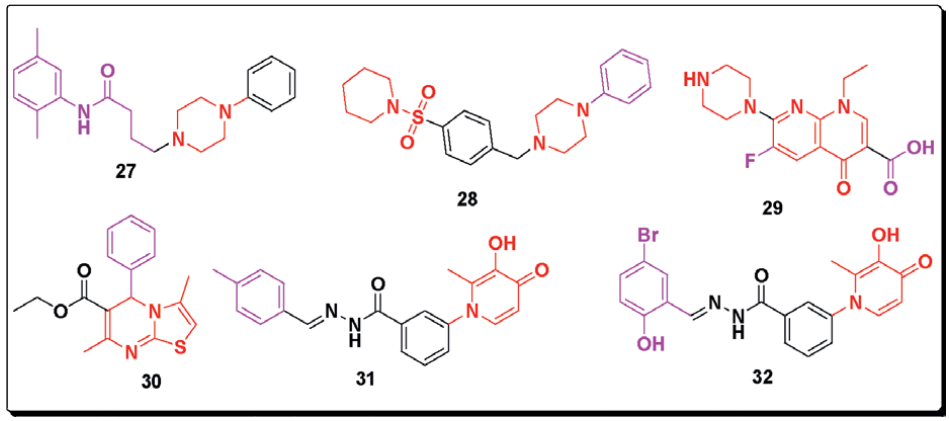

Figure 10.
Structures of piperzine, fluoroquinolone, pyrimidine, and pyridine based heterocyclic mTYRIs.

sesquihydrate, gemifloxacin, levofloxacin, moxifloxacin, ofloxacin, and sparfloxacin, which were evaluated for their mushroom tyrosinase inhibition potential via *in vitro* study. Among all the tested drugs, enoxacin sesquihydrate **29** (**Figure 10**) screened for outstanding tyrosinase inhibitory potency, displaying an IC_{50} ± SEM value of 28 ± 4 μM as compared to reference standard hydroquinone along with an IC_{50} value of 170 μM. The SAR result revealed that enoxacin sesquihydrate showed increased activity due to the presence of more bulky floro and carbonyl groups in the structure [71]. A series of some thiazolopyrimidine hybrids were reported by Ghasemi et al. and screened for their mushroom tyrosinase potency against tyrosinase inhibitors. The ethyl 3,7-dimethyl-5-phenyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate **30** (**Figure 10**), among the series of synthesized molecules, exhibited excellent activity, having an IC_{50} range of 28.50 μM, and was more active than the standard reference kojic acid, having an IC_{50} range of 43.50 μM. The structure–activity relationship (SAR) revealed that the unsubstituted analogue **30** possessed the most potent activity as compared to those compounds having electron withdrawing halogen substituents attached to the phenyl ring [72]. Hassani et al. synthesized a novel class of 3-hydroxypyridine-4-one hybrids bearing benzyl hydrazide moieties and examined their mushroom tyrosinase inhibitory activity. Among all the derivatives, 3-(3-hydroxy-2-methyl-4-oxopyridin-1(4*H*)-yl)-*N*′-(4-methylbenzylidene)benzo-hydrazide **31** (**Figure 10**) and 3-(3-hydroxy-2-methyl-4-oxopyridin-1(4*H*)-yl)-*N*′-(5-bromo-2-hydroxybenzyl-idene)benzohydrazi-de structural motif **32** demonstrated the excellent tyrosinase activity, displaying IC_{50} ranges of 26.36 and 25.29 μM in comparison to the positive control reference drug kojic acid (IC50 = 16.68 μM), respectively. The SAR study found that in mono-substituted series 1, hybrid **31** showed better tyrosinase activity due to the introduction of an electron-donating methyl moiety at the para-position of the benzene ring, while in di-substituted series 2, scaffold **32** exhibited potent activity due to the substitution of the brome and hydroxyl motives at the 2, 5 position of the phenyl ring [73].

3.5 Heterocycles as human tyrosinase (hTYR) inhibitors

Throughout the years, mTYRs have been extensively utilized to assess the skin-whitening capacity (depigmenting efficacy) of skin-whitening agents. Numerous

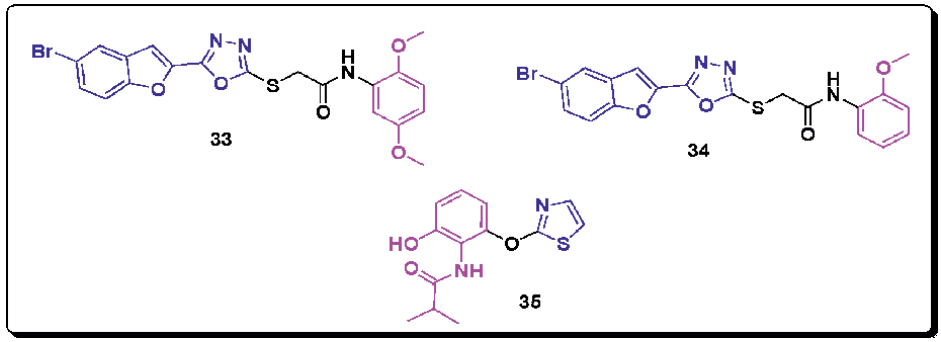

Figure 11.
Structures of amide moiety containing heterocycles as hTYRIs.

studies have highlighted the significant structural differences between mushroom tyrosinase and human tyrosinase isoforms, therefore necessitating diverse structural hybrids for the inhibition of hTYR [74]. In the literature, a very limited number of heterocyclic compounds are reported as human tyrosinase inhibitors as compared to mushroom tyrosinase [75]. Irfan et al. reported furan-1,3,4-oxadiazole-tethered *N*-phenylacetamide hybrid structures, which involved evaluating the inhibitory potential against human tyrosinase (hTYR) and human tyrosinase-related protein-1 (hTYRP1). Among the potential molecules, 2,5-dimethoxy containing furan-1,3,4-oxadiazole **33** (**Figure 11**) demonstrated a higher binding affinity of 11.50 kcal/mol towards hTYRP1, and 2-methoxy containing furan-1,3,4-oxadiazole **34** (**Figure 11**) screened the most favorable binding affinity −13.30 kcal/mol against hTYR when compared with standard inhibitor drug kojic acid having binding affinities of −8.90 kcal/mol and −6.62 Kcal/mol, respectively [76]. Mann and colleagues screened a library of 50, 000 compounds using recombinant hTYR. Among the screened compounds, the thiamidol scaffold **35** (**Figure 11**) emerged as the most potent chemotherapeutic anti-hTYR inhibiting agent and demonstrated an excellent IC_{50} value of 1.1 μM, while in the case of mTYR enzyme, compound **35** displayed very low inhibition activity with an IC_{50} value of 108 μM. Additionally, thiamidol was screened against melanocyte cultures to inhibit melanogenesis (IC_{50} = 0.9 μM) which showed the most promising chemotherapeutic potency as compared to the reference drug resorcinol (IC_{50} = 16.3 μM) [77, 78].

4. Conclusions

The tyrosinase enzyme has diversified applications in the chemical industries, agriculture, and pharmaceutical fields. Tyrosinase is a copper-containing enzyme that is part of all life domains, such as humans, plants, arthropods, mammals, fungi, bacteria, and prokaryotes. This book chapter discusses the origin of tyrosinase, its structure, its role in melanin biosynthesis, strategies for dermal and epidermal melanin reduction, the signaling pathway (melanogenesis at the transcriptional level), sources of tyrosinases, the inhibitory mechanism of tyrosinase, inhibiting melanogenesis at the transcriptional level, the mechanism of action of ascorbic acid and kojic acid as tyrosinase inhibitors, heterocycles as bacterial tyrosinase inhibitors, mashroom tyrosinase inhibitors, and human tyrsoinase inhibitors. In this chapter, the structure–activity relationship of the most bioactive heterocycles is reported against bTYR, mTYR, and

hTYR, showing how different moieties, functionalities, and positions of substituents will affect the therapeutic efficacy of heterocyclic compounds. This will help the scientific community working in the field of medicinal and pharmaceutical chemistry to design novel heterocyclic derivatives as tyrosinase inhibitors with an excellent and promising medicinal and pharmacological profile, as well as the agriculture field.

Conflict of interest

The authors declare no conflict of interest.

Author details

Laila Rubab[1], Ali Irfan[2*], Mohammad Raish[3], Huma Hameed[4], Shazia Akram Ghumman[5], Samina Aslam[6*], Shazia Perveen[7], Ajaz Ahmad[8], Zeemal Seemab Amin[9] and Yousef A. Bin Jardan[2*]

1 Department of Chemistry, The University of Lahore-Sargodha Campus, Sargodha, Pakistan

2 Department of Chemistry, Government College University Faisalabad, Faisalabad, Pakistan

3 Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

4 Faculty of Pharmaceutical Sciences, University of Central Punjab, Lahore, Pakistan

5 College of Pharmacy, University of Sargodha, Sargodha, Pakistan

6 Department of Chemistry, The Women University Multan, Pakistan

7 Department of Zoology, The Women University Multan, Pakistan

8 Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

9 School of Biochemistry Minhaj University Lahore, Pakistan

*Address all correspondence to: raialiirfan@gmail.com, drsamina.chem@wum.edu.pk and ybinjardan@ksu.edu.sa

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Chapter 4

The Impact of Incorporating Piperazine on Biological Activities of Benzazoles

Thuraya Al-Harthy, Wajdi Zoghaib and Raid Abdel-Jalil

Abstract

Heterocycles are widely distributed compounds in natural products and are involved in many biological processes. Its uses have been extended to different fields, including industry, medicine, and agriculture sectors. Benzazole is one of the popular heterocycle scaffolds known as a privilege structure which is commonly found in many pharmaceutical agents. Another outstanding scaffold is piperazine that is known as a distinguishable motif in drug design with a wide range of biological activities. One of the fruitful approaches in the drug design is a hybridization of privilege structures in one skeleton which are believed to grant a characteristic feature with improved or more selective biological activities than the two scaffolds. The effect that piperazine imparted while introduced into a benzazole has drawn attention since first used in the nineteenth century. Numerous research has been performed discussing the synthesis and biological activities of benzazoles containing piperazine. In this chapter, we will highlight a general introduction about chemistry and structure of piperazine, and its importance in medicinal chemistry and benzazole as well. Next, several studies will be discussed that highlight the importance of incorporating piperazine in benzazole skeletons, benzimidazole, benzothiazole, and benzoxazole, and biological activity inherited from this combination.

Keywords: benzazole, benzoxazole, benzothiazole, benzimidazole, piperazine, biological activity

1. Introduction

Heterocycles are paramount scaffolds that are extremely involved in various biochemical reactions. The ability of many heterocycles to produce stable complexes with metal ions has great biochemical significance. The heterocycle containing nitrogen could behave as acid and base depending on the type of nitrogen. Due to the presence of lone pairs, heterocycles can coordinate with practically all metal ions. Hydrophobic properties of heterocycles affect various biological and chemical processes, including the behavior of heterocyclic compounds. It can enhance the binding to specific targets. The hydrophobic interaction of the heterocycle can be exploited which

favors its interaction with the hydrophobic active site and affects its solubility in water. Hydrophobic interaction can be engaged in hydrogen bonding or π-π stacking, depending on their specific structures. These interactions contribute to the overall stability and properties of the compounds [1].

In this chapter, we will be focusing on two magnificent heterocycles, piperazine and benzazole scaffold, and their importance in medicinal chemistry.

1.1 Chemistry of piperazine

Piperazine is a non-aromatic six-membered nitrogen heterocycle called hexahydropyrazine containing two opposed primary nitrogen atoms at positions 1 and 4 with a chemical formula $C_4H_{10}N_2$ as shown in **Figure 1**. Piperazine has cyclohexane-like structure that adopts spatial conformation, chair, and boat conformation [2].

The piperazine moiety is found in a wide range of natural products [3] which make it a remarkable scaffold in different aspects of our life. That is because it has fascinating properties and one of these properties is the presence of two basic nitrogen atoms and two pKa values. Generally, piperazine is a weak base in which the first acid dissociation constant (pKa_1) of piperazine is 5.333 larger than the second acid dissociation (pKa_2) 9.731 at 25°C [4].

The presence of two nitrogen atoms provides a larger polar surface where the first nitrogen atom acts as hydrogen bond donors/acceptors, and this increases the ability to form hydrogen bonding. As a result of that, these hydrogen bonds enhance the water solubility and binding with biomolecules structure, compared to the other six-membered rings e.g., piperidine and morphine, the additional nitrogen in piperazine allows for adjusting 3D geometry at the distal position of the six-membered rings [5].

About 30% of drug candidates failed because of pharmacokinetic properties reasons [6]. Thus, understanding the pKa of piperazine and its derivatives is critical in the medicinal field, especially in drug design, as the protonation influences the ligand-receptor interaction, and this sequence affects its bioavailability and pharmacokinetics properties. Thus, we can conclude that pKa of a drug influences lipophilicity, solubility, protein binding and permeability. As a sequence, this will in turn directly affect pharmacokinetic (PK) characteristics such as absorption, distribution, metabolism, and excretion (ADME) [7].

Piperazine has such characteristic features which make a popular skeleton in the industrial field as well. It can be used as an inexpensive, environmentally organocatalyst [8–10] in synthesis of some heterocycles and synthesis of polymer [11, 12]. Besides that, it required short reaction time, simple procedure, available, highly yielded, and easy in separation without chromatographic separation.

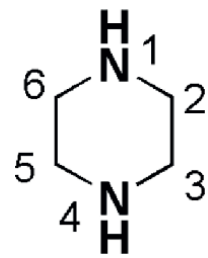

Figure 1.
Piperazine structure.

Piperazine moiety provides a versatile platform for further functionalization of the two nitrogen atoms. The amine group can undergo several reactions, amine coupling reactions, acylation, alkylation, and other transformations. Piperazine can be easily replaced by another amine heterocyclic, as bioisostere, to maintain or improve biological activity.

Despite the wide usage of piperazine-containing compounds in marketed drugs, its structure is limited to substituent in nitrogen site. The limitation can be summarized in the hindrance of nitrogen, limited availability of starting material, and long procedure synthesis. As such, the piperazine's structure has a limitation in functionality and thus diversity. Thus, further functionalization of the C–H bond adjacent to nitrogen has become a topic of interest that was discussed lately [5].

1.2 The importance of piperazine in medicinal chemistry

In general, nitrogen heterocycles have become indispensable constituents that are widely sound in many biomolecules and other sectors in our life. In drug design, nitrogen heterocycles are considered as a privileged structure that is commonly available in many commercial drugs. Among the commercially approved drugs by US FDA, the vast majority are nitrogen heterocycles that account for about 75% of marketed drugs. Piperazine is considered the third most common nitrogen heterocycles that found in 59 marketed drugs [13].

In the 1950s piperazine was first introduced as an anthelmintic agent in medicinal field. Over time, the piperazine derivatives have been expanded with different moieties for various medical purposes. The N-arylpiperazine-containing compounds are available in more than 50 commercial medicinal drugs which are exhibiting diverse medicinal uses [14].

Aryl piperazine skeletons are found in many therapeutic agents that target CNS receptors such as various subtypes of serotoninergic, adrenergic, and dopaminergic receptors such as natural agonists, e.g. 5HT6-serotonin, dopamine, and adrenaline, respectively. In the nervous system, it can mimic the interactions and the conformational features of several bio-targets.

Regardless of the progress done in the pharmacological properties of arylpiperazine containing compounds, the ionization constant of arylpiperazine is not getting much attention. In pursuance of their research, Lacivita et al. have reported a novel series of 1-(substitutedphenyl)-4 propylpiperazines (**1**) to study the effect of manipulation of the substituent on the phenyl ring on the basicity of the N-propyl-substituted nitrogen [15]. Based on the compiled data, the electronic properties of the substituent on the phenyl ring are not the only feature that modulates the basicity of the *N*-4 nitrogen of the piperazine. The substituent at the ortho position has increased the basicity of the compound. This result can be related to steric and conformational effects not to its electronic properties. On the other hand, the meta and para positions show a slight decrease in the pKa and that was qualitatively related to electronic properties of the substituent.

As mentioned above, the availability of two ionizable sites on piperazine contributes to affect the acid dissociation constant (pKa) which is very crucial in absorption and distribution processes as it influences the physicochemical properties, lipophilicity, and solubility in water.

Hydrophobic interactions and π-π stacking of piperazine are important, hydrogen bonding, electrostatic interactions, and steric effects also play roles in determining the overall binding affinity and specificity of ligands for their target enzymes [16].

Many studies proved the binding affinity of N-phenylpiperazine derivatives to certain enzymes such as monoamine oxidase (MAO) A and B and receptors of dopamine and serotonin receptors. Thus, N-phenylpiperazine is utilized in drug design in order to improve the pharmacokinetic (selectivity) properties [17].

An example of such modification of arylpiperazine was done by Chen et al. where a new series of arylpiperazine derivatives is combined with saccharin motif and tested for anti-prostate cancer. This combination with piperazine shows high potential cytotoxic activities against DU145 cells (half maximal inhibitory concentration (IC_{50}) < 2 μM). Interestingly, the position on arylpiperazine plays a role in activity and selectivity in which position-4 with fluoro group (**2**) exhibited potent cytotoxic activity and excellent selectivity compared to the other derivatives [18].

Furthermore, Clark et al. have reported a novel series of α7 neuronal nicotinic acetylcholine receptor (nAChR) modulators based on the 2-((pyridin-3-yloxy) methyl) piperazine derivatives. Some previous studies propose that these receptors are ligand-gated ion channels that are involved in disorders of the central nervous system. The structure of these modulators is designed by a combination of different potential scaffold that are potent in Alzheimer's disease and schizophrenia treatment and piperazine moiety that serves as a hydrogen bond acceptor. Among these modulators, compounds (**3** and **4**) have the potential for α7 nAChR with good selectivity and good oral bioavailability (**Figure 2**) [19].

Numerous studies revealed that the incorporation of piperazine with heterocycles improves biological activities where the potency was attributed to the presence of piperazine moiety. Scanning throughout literature, piperazine has a wide range of activities such as anticancer [20–23], anti-inflammatory [24–26], anticonvulsant [27–29], anti-HIV [30, 31], antidepressant [32–34], antitubercular [35–37], antifungal [38–40], anti-obesity [41, 42], and antimalarial [43–45].

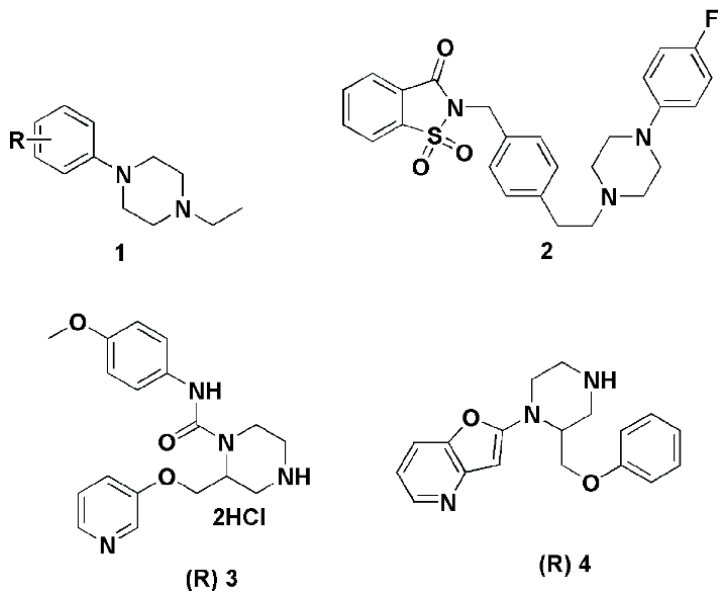

Figure 2.
Arylpiperazine derivatives.

2. Benzazole scaffold

Benzazole skeleton is a class of aromatic heterocyclic compounds that are composed of benzene fused with azole ring structure. There are several types of benzazole, benzimidazole, benzothiazole, benzoxazole, and benzotriazole in which azole structure varied to be imidazole, thiazole, and oxazole, respectively (**Figure 3**).

There is no doubt that benzazole unit has granted significant attention in various sectors, medicine, industrial, and agricultural fields. It is a popular scaffold in material science and that is because of its high quantum yield, ease synthesis, and thermal stability. Thus, it can be incorporated in polymers as additives to enhance its thermal stability [46–48]. Due to its aromaticity, tends to be stable so it can be utilized in the synthesis of liquid crystal and conducting polymer with specific features. Moreover, it is used as chromophores in dyes and pigments in fluorescent agents, luminescent material, and photovoltaic Devices [49–51].

During the past years, benzazole scaffold has been utilized with different strategies in drug design. To start with, benzazole scaffold known as privileged structure that is commonly utilize as core structure in drug design. That's because the former is a structural bioisosteres of biomolecules, nucleotide macromolecules, e.g., adenine and guanine and this structural similarity allows benzazole-containing compounds to interact with biomolecules in biological system. The employment of the privilege structure concept can be alone or combined with another molecular strategy like bioisosterism which is another fruitful strategy of molecular modification in drug discovery [52, 53]. The structure-activity relationship (SAR) stands as an exceptional strategy that has been exploited in drug design in which the strategic modification of different type substituents can lead to significant alteration of molecules' biological activity. These approaches are used alone or combined in drug design based on the hypothesis that similar molecules tend to behave similarly and hence obtain similar biological activities.

As the azole structure is modified to be imidazole, thiazole, and oxazole, that can provide variety in structure and hence in their physicochemical properties which subsequently affect its biological action. For example, benzimidazole scaffold is considered acidic due to the NH group and weakly basic in nature with the ionization

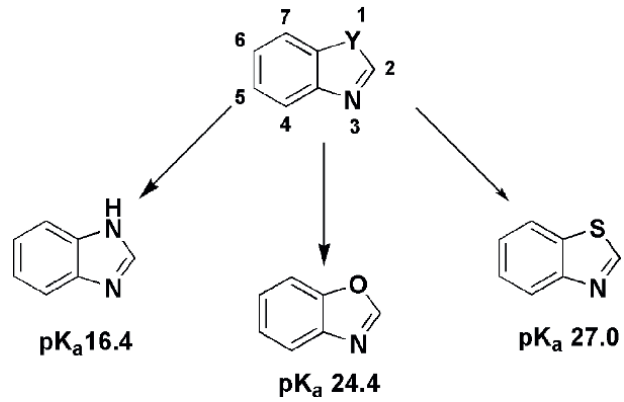

Figure 3.
Benzazole structures.

constant (pK_a) 12.8, and its conjugate acid is 5.6. In the other scaffolds, benzoxazole has pK_a = 24.4 and the benzothiazole is the more basic with pK_a = 27.0.

Herein, compiled literature regarding the importance of incorporation piperazine with benzazole derivatives will be discussed individually in the following sections.

2.1 Benzothiazole containing piperazine

To start with, a new set of 36 new benzothiazole derivatives were synthesized by Xie's et al. and subjected for antitumor activity. Antitumor SAR analysis was obtained using various substituents at 2-,5-, and 6- positions and the results revealed that the presence of two phenyl rings at positions 2 and 5 along with N-methylpiperazine moiety at position-6 in benzothiazole derivative (**5**) (GI_{50} = 0.87 μM) is crucial for antitumor activity [54]. The results of this study provide a starting point for future lead compound optimization and finding new antitumor agents.

Another study to be mentioned was reported by Gurdal et al. in which a new series of benzothiazole-piperazine analogs were screened against three different cancer cell lines. Among the whole of synthesized compounds, compound (**6**) showed the highest cytotoxicity against the tested cancer cell lines and causes apoptosis by cell cycle arrest at subG1 phase [14]. The data analysis to understand the mechanism of cytotoxicity, fluorescence-activated cell sorting analysis (FACS), shows an increase in subG1 phased cells indicating the subG1 cell cycle arrest compared to control cells. This result is an indication that the presence of cells arrested at the subG1 phase supports the induction of apoptotic cell death in those cells treated with compound (**6**).

Analysis of SAR by Nagarapu et al. identified that both nucleus benzothiazole and substituent methyl-piperazine moieties are essential for enhancing the cytotoxic activity [55]. Compound (**7**) exhibited a promising cytotoxicity specifically against human breast adenocarcinoma cell lines MDA-MB-231 and MCF-7. Compared with to the secondary amines, the methyl piperazine moiety shows more potential inhibitory activity against human-tested cancer cell lines.

As mentioned above, arylpiperazine is a good choice template for various biological targets. One attempt in piperazine modification to find cytotoxic agents for better treatment of cancer, a combination of more than one pharmacophore, with different mode of action could lead to potent drugs. For instance, Murty et al. have combined arylpiperazines, benzothiazoles/benzoxazoles, and substituted 1,3,4-oxadiazol-2-thiol scaffold. Some compounds show interesting cytotoxicity while the benzothiazole (**8**) with *N*-phenylacetamide displayed the highest cytotoxicity against five cancer cell lines [56].

The importance of bearing N-methylpiperaizne is discussed in the study done by Abdelgawad et al. who prepared a new derivative of benzazoles that targets epidermal growth factor receptor (EGFR) represents for cancer treatment. Their finding showed that the derivatives containing *N*-methylpiprazine-1-yl-acetamide derivative for both benzoxazole (**9**) and benzothiazole (**10**) were the most potent cytotoxic against breast cancer cell lines with IC50 values 12 nM and 10 nM, respectively [57].

Karaca et al. have designed a new series of enzyme inhibitors against Alzheimer's disease. They have utilized the dual acetylcholinesterase (AChE)–monoamine oxidase B (MAO-B) inhibitors which are known as a new approach in the treatment of Alzheimer disease (AD) [58]. The amine motif e.g. piperazine or piperidine in the structure of donepezil is essential for the interaction with the AChE enzyme binding site called catalytic active site (CAS) region of the enzyme. This chemical structure provides flexibility which enables the proper binding to gorge of the enzyme active site. Among the

synthesized inhibitors, compound (**11**) displayed inhibitory activity against AChE and MAO-B enzymes with IC_{50} values of 23.4 + 1.1 nM and 40.3 + 1.7 nM, respectively.

It is worth mentioning the study done by Ktadna et al. in which they combined pyrimidine/benzothiazole piperazinyl moieties with flavones to enhance the antiradical and antioxidant activity of flavones as both piperazine and benzothiazole are known for their antioxidant activity [59]. Several sets of 2(2-hydroxyphenyl) pyrimidine/benzothiazole piperazinyl-substituted flavones were prepared and evaluated the antioxidant activity. Among the synthesized molecules, (**12–14**) containing benzothiazole and piperazine moieties show a higher antioxidant activity compared to pyrimidine analogous and 3BTA in particular the most potent one. These results can serve as an opening for preparing new compounds containing flavonoid—piperazine moieties with more effective and less toxic potential candidates for anti-inflammatory and anticancer drugs.

In another study of piperazine containing benzothiazole, Al-Harthy et al. introduced a new compound of 2-aminobenzothiazole (**15**) as intermediate and a library of benzothiazole Schiff bases with piperazine at position-6 and underwent cytotoxicity assays [60]. Al least one compound (**16**) fluorine at position 4 of phenyl exhibited a selective antitumor activity against DMS-53 human lung cancer cell line in comparison to primary HLMVECs. In addition, a new series from the same intermediate (**15**) was proposed with the same research team of 5-fluoro-6-(4 methylpiperazin-1-yl)-substitutedphenylbenzo[d]thiazoles and screened against different bacteria and fungi strains. Among, two compounds, (**17** and **18**) show potent bacterial growth inhibition against Gram-positive bacteria *S. aureus* with MIC 32 μg/cm^3 compared to 10 μg/cm^3 for tamoxifen used as a positive cytotoxicity standard (**Figure 4**) [61].

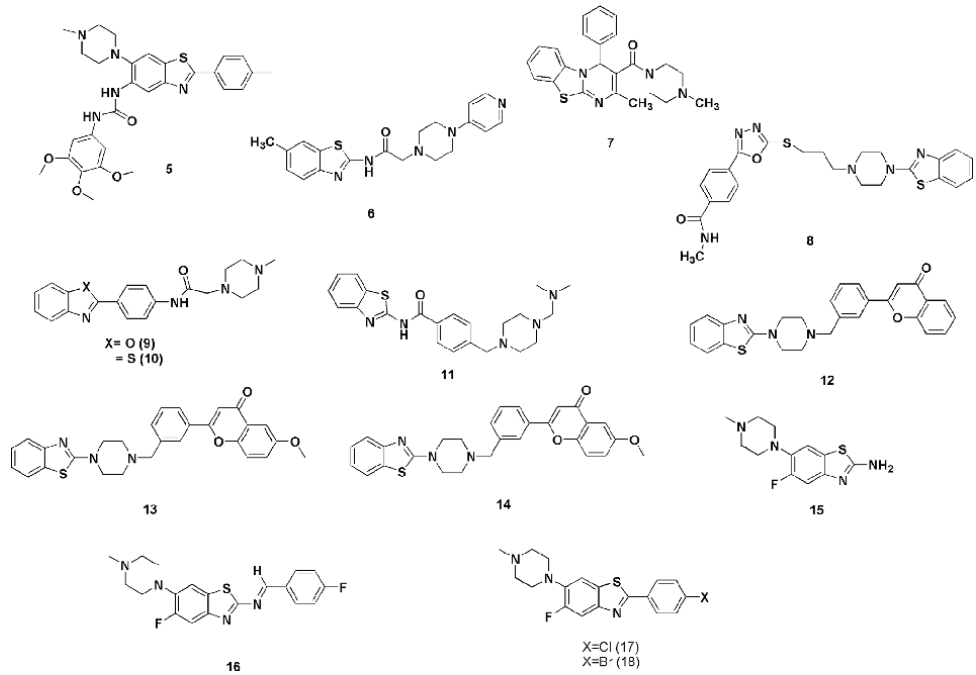

Figure 4.
Benzothiazole derivatives incorporating piperazine.

2.2 Benzimidazoles containing piperazine

To begin with, Hu et al. disclosed a series of 1-cyano-2-amino-benzimidazole derivatives which was investigated against cancer cell lines. Several compounds showed certain cytotoxicity, and compound (**19**) was the most promising analogues. Based on extensive SAR studies, the presence of methyl piperazine was crucial in enhancing the potency against A549, K562, and PC-3 cell cancer cell lines with IC_{50} values of 6.48, 2.69, and 18.51 μmol L-1, respectively [62]. In addition, they have examined the influence of compound (**19**) on the growth and division of K562 cells by measuring the DNA content of eukaryotic cells. The results show an arrest of the cells in the G2/M phase of the cell cycle by the examined compound.

It is well-known that Hoechst 33342 and 33,258 are adenine-thymine-specific dyes that stain DNA by binding to its minor groove. In Hoechst 33342, the ethoxy group has a role in inducing apoptosis and causing cell death in HL-60 cells unlike in Hoechst 33258. To mimic their biological activity, Alp et al. reported a synthesis and biological evaluation of 5-(4-methylpiperazin-1-yl)-2-phenyl-1H-benzimidazoles. At least four compounds (**20–23**) exhibited significant antiproliferative activities against the cancer cell lines with IC50 values 1.86 ± 0.09, 1.86 ± 0.20, 1.70 ± 0.11, and 1.56 ± 0.09 μM, respectively [63].

As part of their efforts in synthesizing benzimidazole and alkoxyamine derivatives for antifungal activities, Jin et al. and his team have investigated the effect of appendage piperazine moiety on a series of α-alkoxyimino-(1H-benzoimidazol-2-yl) acetonitriles. The derivatives (**24** and **25**) showed high antifungal activities against *B. cinerea* with EC50 7.14 and 13.99 μg/mL respectively, which is more potent than the standard used in their investigation [64].

Nimesh et al. prepared a potential topoisomerase IA inhibitors of bisbenzimidazoles. The *in vivo* antimicrobial testing data shows that 5-(4-propylpiperazin-1-yl)-2-[2′-(4-ethoxyphenyl)-5′-benzimidazolyl]benzimidazole (**26**) is an efficient candidate as antibacterial agent [65].

Recently, Zhang et al. have designed a new series of benzimidazole quinolones as potential antibacterial agents to improve the quinolone's resistance against bacteria by utilizing the benzimidazole ring at the 7-position of quinolone core. The quinolone derivative (**27**) shows significant antibacterial potency which was more effective than norfloxacin, ciprofloxacin, and clinafloxacin [66].

Basavaraja et al. have proposed unique piperazine-linked benzimidazole analogs and evaluated for their antibacterial, anthelmintic, and anticancer properties [67]. Regarding the antibacterial activity, compound (**28**) exhibits the most potent activity that similar to procaine penicillin and Streptomycin which is referred to as the piperazine attached to the methoxy group. While compounds (**29** and **30**) show a potential antifungal activity which can be attributed to the resonance phenyl and nitro groups connected to benzimidazole through the piperazine motif. For the anthelmintic activity, compound (**31**) showed excellent activity compared to the standard drug Albendazole. Compound (**32**) with methyl piperazine, exhibited excellent activity against only the MCF7 cell lines, with an IC_{50} value of 9.32 μg/mL. Interestingly, compound (**30**) with a phenyl ring attached with piperazine, showed excellent activity against human liver (HUH7) and breast cancer (MCF7) cell lines, with IC_{50} values of 6.41 and 9.70 μg/mL, respectively.

Bemonyl, Mecarbinzid, Carbendazim, and Debacarb are one of the most effective fungicide agents in plants that contain benzimidazole derivatives. This benzimidazole with carbamate at position 2 was utilized to prepare a novel series of 2-carbamate

benzimidazoles that have been posted by Al-Harthy et al. and evaluated for antifungal activity. Compounds (**33** and **34**) were the most efficacious, which resulted in a 96% growth inhibition in Pythium at 100 mg L^{-1} [68]. In pursuit their effort in preparing bioactive, these 2-carbamate benzimidazoles have been investigated for potential α-glucosidase inhibitory behavior. It was found that the most effective inhibitors in this series were (**35** and **36**) with IC_{50} values of 118 and 155 μM, respectively. The data collected found out that compound (**35**) competes with the substrate in binding to the enzyme active site as a competitive inhibitor and the docking results revealed an interesting interaction between these two benzimidazoles and the enzyme active site (**Figure 5**) [69].

2.3 Benzoxazoles containing piperazine

For benzoxazole's derivatives, Erol et al. have synthesized new derivatives of benzoxazoles, 2-(p-substitutedphenyl)-5-(2-substitutedacetamido) benzoxazole and evaluated for their antimicrobial activities on 10 different microorganisms. In general, the benzoxazole derivatives revealed a weak activity against *S. aureus* and MRSA with MIC: 256 μg/m while the antifungal activities of the compounds against *C. albicans* and *C. albicans* ranged between 64 and 128 μg/mL. The cytotoxic activities were investigated on (breast cancer cell line) MCF-7 and (lung cancer cell line) A549 cell lines by the MTT method. From the series, compound (**37**) exhibits the best cytotoxicity on MCF-7 and A549 cell lines, with a reduction of 70% and 71.29% viability.

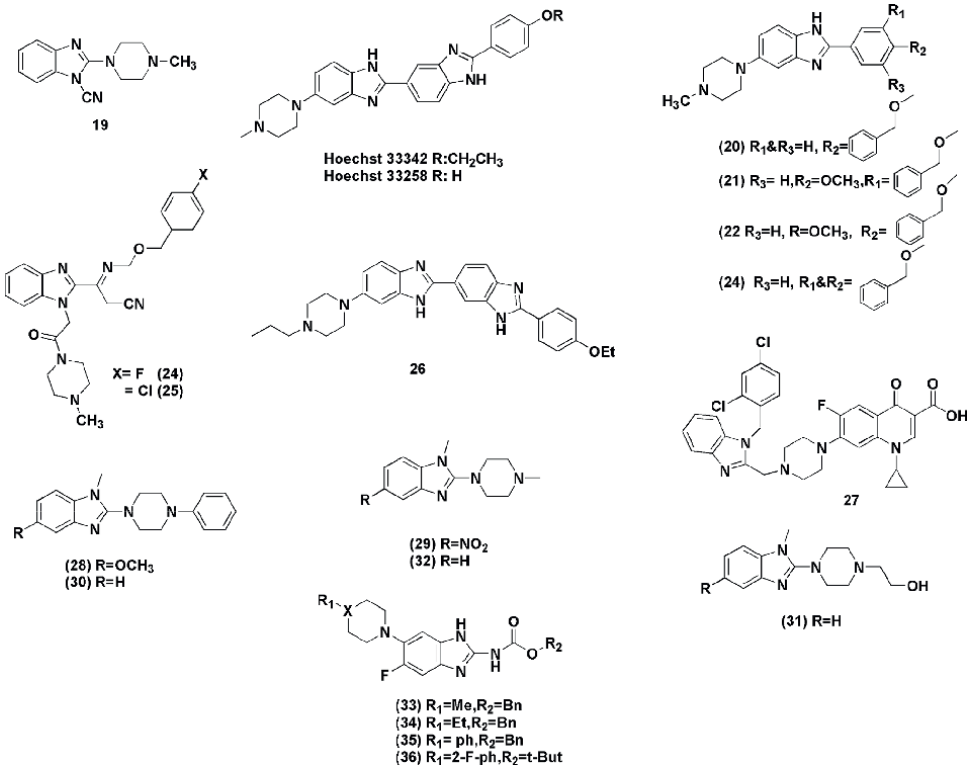

Figure 5.
Benzimidazole derivatives incorporating piperazine.

An excellent predictive ADME profile was shown for all compounds within the series which is a promising candidate for further and extensive study [70].

Moheson et al. have proposed a new series of 2-substituted benzoxazole derivatives and their anticancer activities were evaluated *in vitro* against two human breast cancer cell lines based on their previous work, that substituent at 4-position of the 2-phenyl ring with alter functionality has a role in enhancing the anticancer activity. Thus, the 2-phenylbenzoazole derivatives were designed to be attached with dithiocarbamate and flexible amide $NHCOCH_2$ spacer. Their findings revealed that when amide was introduced, the 4-(2-ethoxyphenyl)piperazinyl moiety at position-4 grant molecule (**38**) a moderate to weak activity against both cell lines (IC_{50} = 132.619 and 62.081 μM against MCF-7 and MDB-MB-231 cell lines, respectively. For dithiocarbamate, compound (**39**) substituted with 2-phenyl piperazinyl moiety exhibits high selectivity compared to other analogous, where it is highly potent against MDA-MB-231 cells and it is inactive against MCF-7 cells [71].

Extensive effort was performed by Liu et al. to find potential candidates for the treatment of prion diseases. They found that the presence of hydrogen bond acceptor (HBA) is critical for anti-prion potency so benzoxazole has been introduced where oxygen and nitrogen can act as hydrogen bond acceptors and benzene fused structure adds additional binding affinity. They reported SAR study on different arylpiperazines and their findings showed that compounds (**40** and **41**) have enhanced anti-prion activity, PK profile, and good central nervous system (CNS) penetration [72]. They have concluded that the HBA effect mainly from nitrogen atom as benzazole was replaced with a more hydrophobic moiety, benzothiazole, it has little impact on anti-prion activity. The nitrogen on pyridine attached to piperazine has a role in forming hydrogen bonds with putative target compared with other analogous.

The solubility of some anticancer agents causes difficulty for *in vivo* studies. For this reason, some functional groups can be introduced to enhance the solubility e.g., piperazine or its derivatives. Based on their previous study, Xiang and his team are trying to overcome the poor solubility in which, N-methyl-piperazine moiety has been introduced at the 2-position and 6-position to a promising lead compound to enhance its solubility. Impressive results have been obtained from this modification shown in compounds (**42–44**), the placement of *N*-methyl-piperazine at position 6 has decreased the potency. On the other hand, where it was placed at position 2 enhanced solubility (and hence drug delivery) and antitumor activity. As the benzothiazole scaffold was replaced by benzoxazole and benzimidazole, it retained its anticancer activity. A new phenomenon was observed for the first time, that is, cytosolic vacuolization after treatment compared to their previous work which is worth further study [73].

In pursue with their ongoing work on synthesis of benzazole scaffolds, Al-Harty et al. have synthesized novel benzoxazoles appendaged with different aryl-piperazine moieties at position 6. Generally, cytotoxicity assay was performed at 50 μM since almost all tested compounds above 50 μM precipitated in cell-culture media. In other words, 43 and 42% of hepatocytes and cancer cells, respectively. The CT_{50} value of ~50 μM could only be obtained for (**45**), and it shows no selectivity in killing health and cancer cells. At low concentration, 10 μM, compounds (**46–49**) were highly toxic to lung cancer cells, killing 30–40% of all cancer cells at this low concentration. Concentrations below 10 μM were not toxic for both [74].

Siracusa and his research team have proposed a new series of different benzazole analogues that are linked with arylpiperazine by different thioalkyl chains and evaluated for their radioligand binding affinities [75]. Many compounds show an

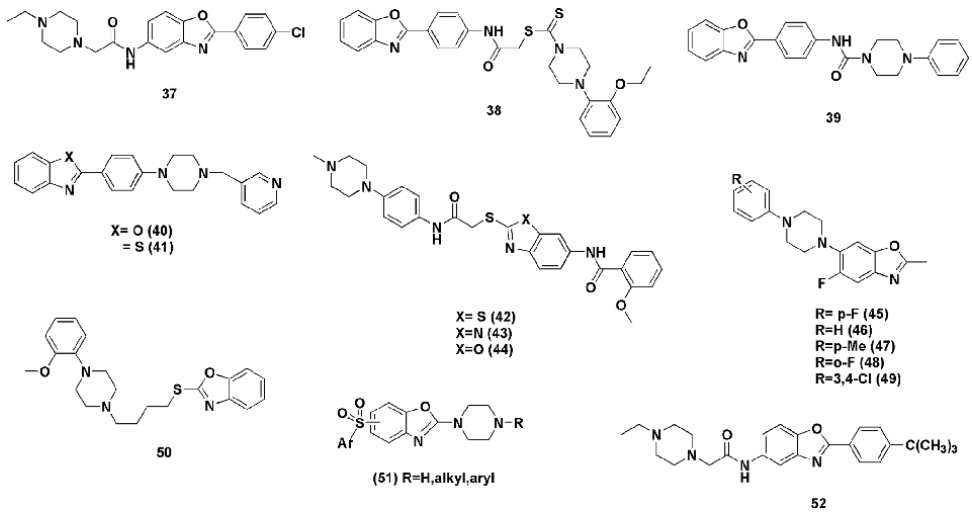

Figure 6.
Benzoxazole derivatives incorporating piperazine.

interesting binding profile for the 5-HT_{1A}R and good selectivity over 5-HT_{2A}, α1, D1, and D_2 receptors. Out of the synthesized benzazole and benzothiazole scaffold being potent ligands than benzimidazole and the compound (**50**) displayed higher affinity at D_1, D_2 dopaminergic, and 5-HT_{2A} serotonergic receptors and selectivity at 5-HT_{1A} receptor over all the tested receptors. This high-affinity binding can be referred to as the remarkable π-π stacking in compared to other teste analogs.

In addition, another work was done by Liu et al. to identify potent and selective ligands as effective treatments for central nervous system diseases (CNS) such as Alzheimer's disease (AD) and schizophrenia. Their focus was on the synthesis of a novel series of benzoxazole derivatives as 5-HT_6 ligands. The former derivatives (**51**) have demonstrated full antagonism as determined by blockage of 5-HT-induced cyclic AMP (cAMP) formation [76].

Moreover, another study targeting Acetylcholinesterase (Ach), Butyrylcholinesterase (BCh) and Tyrosinase, which play an important role in the development of Alzheimer disease (AD), was reported by Celik et al. The *in vitro* enzyme inhibition activity revealed that the inhibitor effect of against ACh and tyrosinase enzyme was very low and compound (**52**) with ethyl piperazine moiety inhibited the BCh enzyme at a concentration of 50 mM by 54 ± 0.75%. The calculated data collected found that the compound (**52**) highest BChE inhibitory activity, has the ability to form hydrogen bonding and has more electronic structure stability compared to standard drug, galantamine (**Figure 6**) [77].

3. Conclusions

In summary in this work, we emphasis the significance added to biological activity of benzazole when piperazine is within the structures. The strategic choice of heterocycle, and substituent around plays a vital role in modifying by increasing, decreasing, or maintaining the biological activity. In terms of future regard, structural modification at strategic position is becoming a promising direction in drug discovery. Bioisosterism is utilized as well in drug design to maintain or enhance the

biological activity of a molecule while improving other properties such as toxicity, metabolic stability, or pharmacokinetics. From the literature discussed, we can value the importance of piperazine and benzazole when they are engaged in one scaffold. Hence, this will be a useful tool in predicting the biological activity of the compound which in sequence minimizes the cost and time for the designing step. In the above discussed studies, till date, piperazine still owing the top priority in the drug design when incorporated with other heterocycles.

Conflict of interest

The authors declare that there is no conflict of interest.

Author details

Thuraya Al-Harthy[1*], Wajdi Zoghaib[2] and Raid Abdel-Jalil[2]

1 Basic and Applied Sciences Department, College of Applied and Health Sciences, A'Sharqiyah University, Ibra, Oman

2 Chemistry Department, College of Science, Sultan Qaboos University, Al-Khod, Muscat, Oman

*Address all correspondence to: thuraya.alharthy@asu.edu.om

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Chapter 5

Pharmaceutically Privileged Bioactive Pyridine Derivatives as Anticancer Agents: Synthetic Developments and Biological Applications

Nawal Kishore Sahu, Amol T. Mahajan and Sandeep Chaudhary

Abstract

Pyridine is an *N*-containing heterocyclic compound that exists naturally and accounts for a wide range of biological activities. The medicinal and pharmacological features of the substituted pyridine derivatives make them as an important scaffold for consideration in synthetic organic chemistry. Numerous pyridine derivatives have been established to inhibit kinases, androgen receptors, tubulin polymerization, topoisomerase enzyme, human carbonic anhydrase, and several additional targets against cancer. The privileged scaffold pyridine has consistently functioned in a wide range of FDA-approved pharmaceutical candidates. Researchers are currently focusing on exploring the new synthetic method for the development of novel pyridine molecules with additional moieties for cancer treatment. This section discusses the synthesis of bioactive pyridine derivatives, and their biological expansions as anticancer agents.

Keywords: *N*-heterocyclic, pyridine, *in vitro*, *in vivo*, anticancer, molecular docking, enzyme, receptors, cancer cell lines

1. Introduction

Pyridine is an *N*-containing heterocyclic organic compound with the chemical formula C_5H_5N, i.e., structurally related to benzene, with one sp^2-hybridized carbon replaced by a sp^2-hybridized nitrogen atom. It was initially isolated from picoline by Thomas Anderson [1]. Wilhelm Korner (1869) and James Dewar (1871) identified the structure of pyridine [2]. William Ramsay synthesized pyridine in a red-hot iron-tube furnace by combining acetylene and hydrogen cyanide [3]. This was the first synthetic heteroaromatic compound. Till today, Pyridine serves as a pioneer in the field of chemistry, contributing as a reagent, solvent, and catalyst. Pyridine serves as an important heterocyclic moiety that is present, as a part or as a whole, in many natural products/therapeutics/drugs. It performs a substantial part in the

catalysis of chemical and biological processes. The pyridine ring system is widely dispersed in nature, i.e., existence in the vitamins B_3 and B_6 (niacin and pyridoxine), alkaloids nicotine, and others. Therefore, research on pyridine scaffold based on these developments is an evergreen subject with increased expectations for addressing various disease-related challenges. Pyridine derivatives exhibit outstanding biological applications, including anticancer, antiviral, antioxidant, antiarthritic, antibacterial, antifungal, analgesic, anticonvulsant, antithrombotic, ulcerogenic, antiparkinsonian, antiglycation, anti-inflammatory, and several others activities [4, 5]. Therefore, the pyridine ring system and its derivatives play an essential role in synthetic organic chemistry due to their medicinal and pharmacological properties.

Cancer is a life-threatening disease that affects people all around the world. Covering studies on 185 nations globally and 36 different cancer types, the International Association of Research on Cancer's GLOBOCAN 2020 report includes the high-alarming statistics; as a result, 19.3 million new cancer cases have been diagnosed, with 10 million deaths [6]. Significant progress has been made in the field of cancer cell biology research since the 1950s, but the majority of cancer therapies still involve radiation therapy, chemotherapy, and major surgery, with chemotherapy remaining the most widely used pharmacological approach. These strategies have a number of drawbacks when used in actual practice. The people belonging to all age groups are susceptible to cancer, even fetuses; however, the risk increases with an increase in age for the majority of forms. Over the years, research has revealed an apparent link between inflammation and cancer, leading to the conclusion that cancer is an inflammatory disease. The presence of heteroatoms, especially heterocyclic blocks, offers a promising chance to discover novel compounds for cancer therapy [7]. Subsequently, the privileged scaffold pyridine has continuously functioned in a broad spectrum of medication candidates approved by the FDA. In medicinal chemistry research, pyridine, in its several equivalent forms, holds a significant place as a valuable source of clinically effective drugs. In the pharmaceutical sector, it comprises the essential backbones of over 7000 existent drugs. The marketed anticancer drugs which are having pyridine scaffolds are: Sorafenib (Nexavar®) **1**, Crizotnib (Xalkori®) **2,** Regorafenib (Stivarga®) **3**, Vismodegib (Erivedge®) **4**, (**Figure 1**) [8–10]. The FDA has authorized numerous drugs for the treatment of cancer in recent years, including Abemaciclib (2015), Lorlatinib (2018), Apalutamide (2018), and Ivosidenib (2019). While a great deal of progress has been made in the discovery of

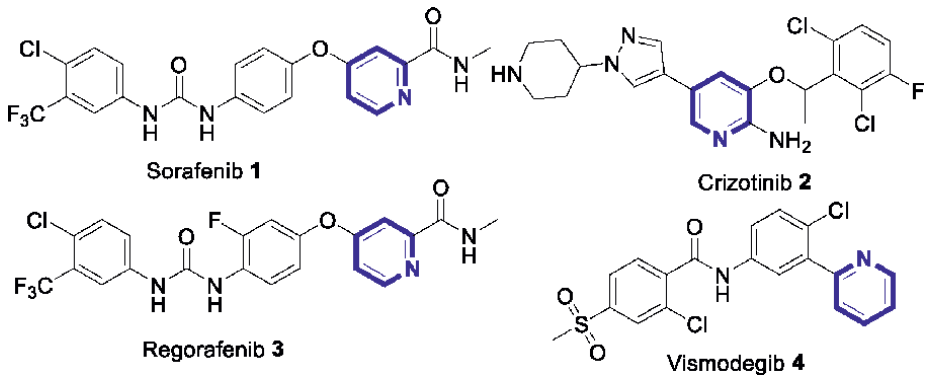

Figure 1.
Marketed anticancer drugs with pyridine.

several chemotherapeutical medicines, optimal therapeutics—that is, highly effective drugs with few adverse effects—still remain unattainable. It is nevertheless urged that research on novel bioactive substances be advanced.

2. Synthetic developments of anticancer pyridine derivatives

Pyridine derivatives are the most significant heterocyclic compounds that serve as an important component for anticancer pharmaceuticals. Various methods have been reported in the literature for the synthesis of pyridine derivatives followed by evaluation of their anticancer activities.

Rani et al. [11] reported the synthesis of novel imidazo [1,2-a] pyridine derivatives containing amide functional group 15, and used the MTT assay with etoposide as the standard reference drug to assess their anticancer activities against breast (MCF-7 and MDA-MB-231), lung (A549), and prostate (DU-145) cancer cell lines (**Figure 2**). Among all the synthesized compounds, the most potent one in the series demonstrated the potent anticancer effects against MCF-7 with an IC50 values of 0.021 ± 0.0012 μM, while on other cell lines like MDA-MB-231, A549, DU-145, it shows 0.95 ± 0.039 μM, 0.091 ± 0.0053 μM, and 0.24 ± 0.032 μM, respectively. Moreover, anticancer activity of these compound against four human cancer cell lines: MCF-7, MDA-MB-231 (breast cancer), A549 (lung cancer), and DU-145 (prostate cancer) was evaluated by using MTT assay.

The one-pot synthesis pathway for amino cyanopyridine derivatives **19** was described by Ashmawy *et al.* [12]. It begins with *α,β*-unsaturated ketone intermediate **16**, which is formed by the Claisen–Schmidt condensation reaction between the acetylthiazole derivative and substituted benzaldehyde derivatives. The corresponding pyridine derivatives were then obtained by performing Michael addition reaction by reacting intermediate with malononitrile in the presence of ammonium acetate, followed by cyclization, auto-oxidation, and ultimately tautomerization (**Figure 3**). The tested compounds recorded better anticancer activity as compared to the standard drug. Consequently, the present study demonstrated that the thiazolyl pyridines are found to be potent EGFR inhibitors and open the door for the synthesis of other

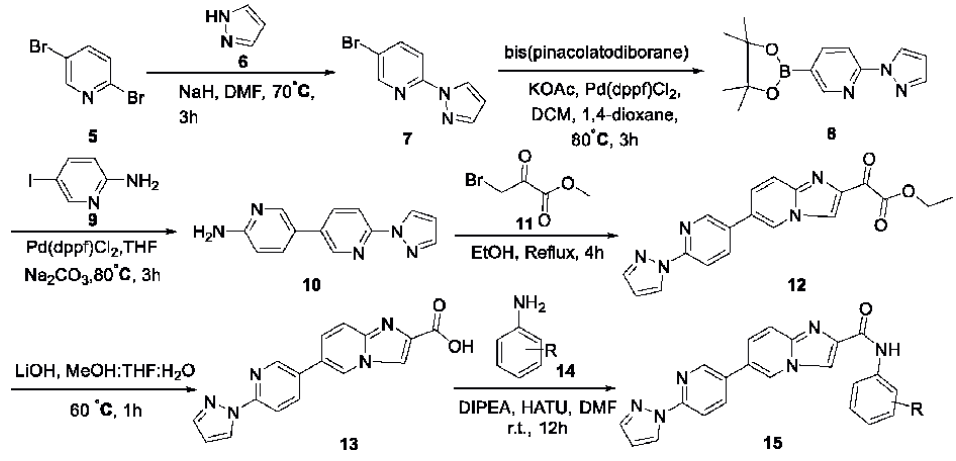

Figure 2.
Synthesis of imidazo pyridines-based amide derivatives.

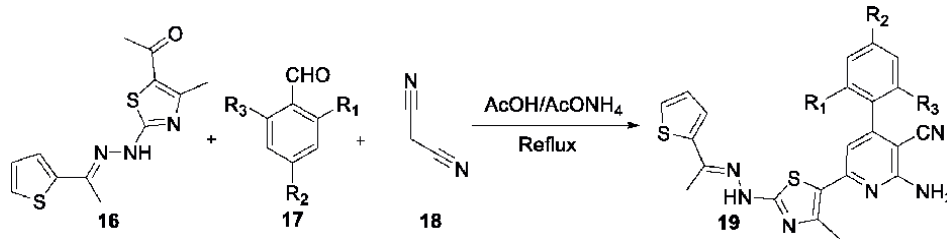

Figure 3.
Synthesis of amino-cyanopyridine derivatives.

similar kind of compounds based on the same scaffold, which could ultimately lead to the development of a successful treatment of the lung cancer.

Adarsh *et al.* [13] explored the medicinal chemistry perspective of pyrido [2,3-*d*] pyrimidines as anticancer agents. Kisliuk *et al.* [14] developed a novel method for synthesizing pyrido [2,3-*d*]pyrimidine-2,4-diamines **24** by reacting 2,4,6-triamino-pyrimidine **20** with the sodium salt of nitromalonaldehyde **21** to form 2,4-diamino 6-nitropyrido [2,3-*d*] pyrimidine **22.** Then, the molecule **22** was reduced to its 6-amino derivative by using RANEY® Ni. The desired product **23** was obtained by reductive amination of various aldehydes (ArCHO = 3,4,5-trimethoxybenzaldehyde). In the last step, formaldehyde was employed to *N*-methylate **23** in the presence of sodium cyanoborohydride (**Figure 4**). They have discussed a variety of anticancer targets of pyrido [2,3-d] pyrimidine in this study, such as a wide variety of kinases like cyclin-dependent kinase, phosphatidylinositol-3 kinase, extracellular regulated protein kinases, ABL kinase, p38 mitogen-activated protein kinases. Along with this, some other targets include phosphodiesterase, mammalian target of rapamycin KRAS, and fibroblast growth factor receptors, DHFR, etc. Also, the author has demonstrated the structure-activity relationship with respect to the mechanism of action for above-mentioned targets. The *N*-heterocyclic compounds, particularly pyrido [2,3-*d*] pyrimidines, are a broad class of substances with a variety of biological characteristics. These derivatives have demonstrated significant anticancer activities [15, 16].

Naggar and his coworkers [17] performed the synthesis of a novel series of pyridine-ureas **32** toward the development of effective anticancer agents (**Figure 5**). All newly developed compounds were tested for their anticancer activity against the breast cancer MCF-7 cell line. Some compounds were found potent against MCF-7

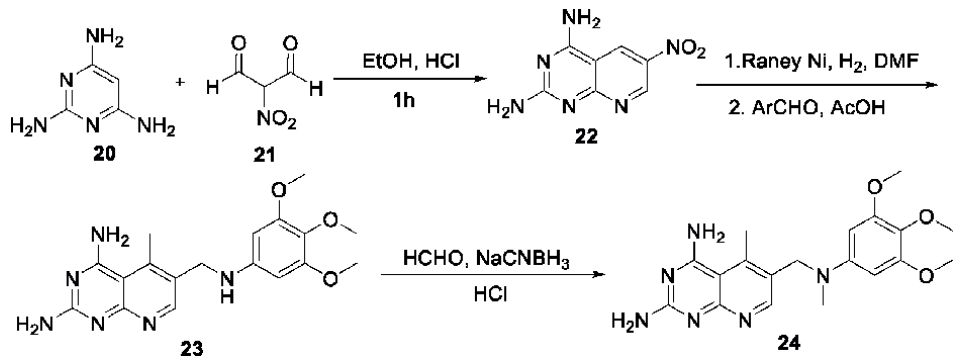

Figure 4.
Synthesis of pyrido [2,3-d] pyrimidine diamine derivative.

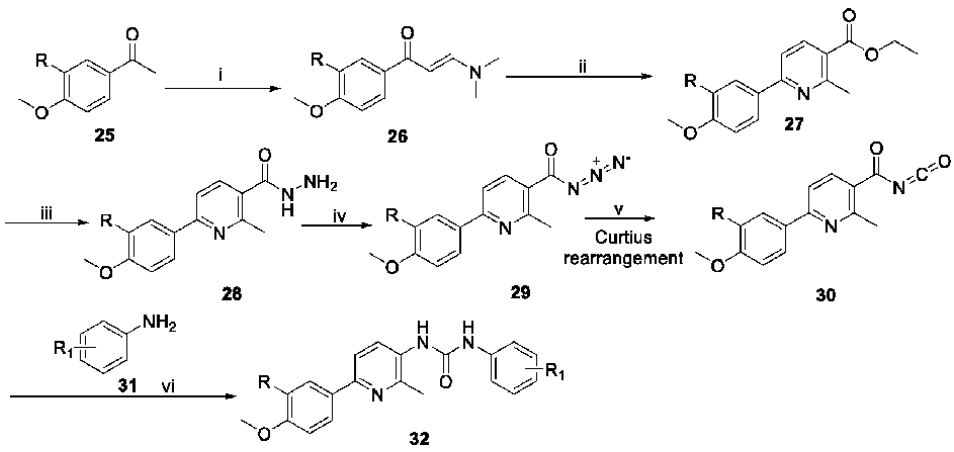

Figure 5.
Synthesis of target pyridine-ureas; Reagents and conditions: (i) DMF-DMA, xylene, reflux 7 h; (ii) NH_4OAc, AcOH, reflux 4 h; (iii) Methanol, $NH_2NH_2{\cdot}H_2O$, reflux 2 h; (iv) $NaNO_2$, AcOH, stirring 2 h; (v) Xylene, reflux 1 h; (vi) Xylene, reflux 3 h.

cells (IC50 = 0.22 and 1.88 μM after 48 h treatment; 0.11 and 0.80 μM after 72 h therapy, respectively), it shows excellent activity compared to the standard reference drug doxorubicin (IC50 = 1.93 μM). According to the US-NCI approach, some of the compounds were the most effective anticancer drugs in the NCI assay, with mean inhibitions of 43–49%, respectively. The most active pyridines were found in accordance with Lipinski and Veber rules. Finally, numerous ADME characteristics were predicted for the active pyridines using a theoretical kinetic analysis (**Figure 6**).

Khalaf and coworkers [18] had synthesized several dipyridyl **39** or pyridinyl sugar hydrazones **40** coupled to thienyl or methylfuryl rings, along with that pyridine thioglycosides fused with naphthyl and furyl systems in a multistep approach. The synthesized compounds demonstrated higher cytotoxicity against a variety of cancer cell types. The anticancer activity of the products was assessed using the MTT assay on cancel cell lines PC3, A549, and HCT116, which stand for human prostatic adenocarcinoma, adenocarcinomic human alveolar basal epithelial, and human colorectal carcinoma, respectively. Also, to check the selectivity index, their effect on normal cell line RPE1 human normal retinal pigmented epithelial cell line has been demonstrated.

Hagras and coworkers [19] described the design, synthesis, molecular docking, and anticancer evaluation of new trimethoxyphenyl pyridine derivatives **44**, **45** as tubulin targeting agents and apoptosis inducers. The compounds were tested *in vitro* for their anti-proliferative activities against hepatocellular carcinoma (HepG-2), colorectal carcinoma (HCT-116), and breast cancer (MCF-7) cancer cells. Almost all of the new trimethoxyphenyl pyridine derivatives demonstrated considerable cytotoxicity and tubulin inhibitory activities (**Figure 7**).

Icsel et al. [20] reported the synthesis of novel 5-fluorouracil complexes of Zn(II) with pyridine-based ligands and evaluated their potential as anticancer agents. This sequence contains numerous unique Zn(II) complexes of 5-fluorouracilate (5-FU), namely $[Zn(5\text{-}FU)_2(bpy)]$ **48**, $[Zn(5\text{-}FU)_2(phen)]$ **49**, $[Zn(5\text{-}FU)_2(dpya)].H_2O$ **50**, $[Zn(5\text{-}FU)_2(bpyma)].2H_2O$ **51,** and $[Zn(5\text{-}FU)_2(terpy)].H_2O$ **52**, which were produced and structurally studied using spectroscopic techniques and X-ray crystallography. The anticancer potentials of the soluble complexes were examined in cancer cells

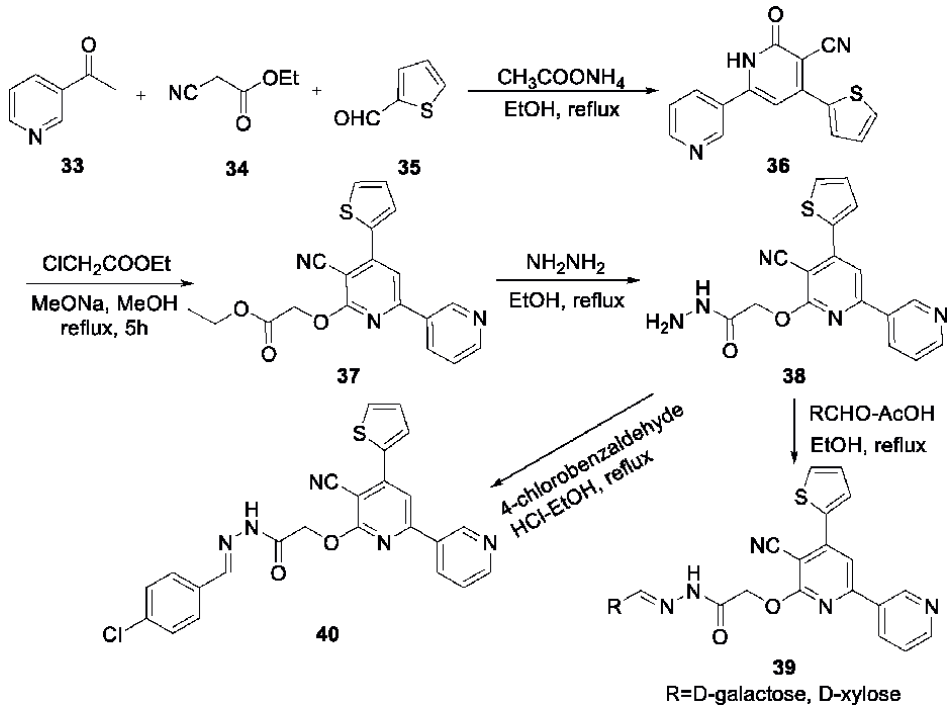

Figure 6.
Synthesis of most active anticancer agent dipyridinyl-thiazole sugar and acrylidine derivatives.

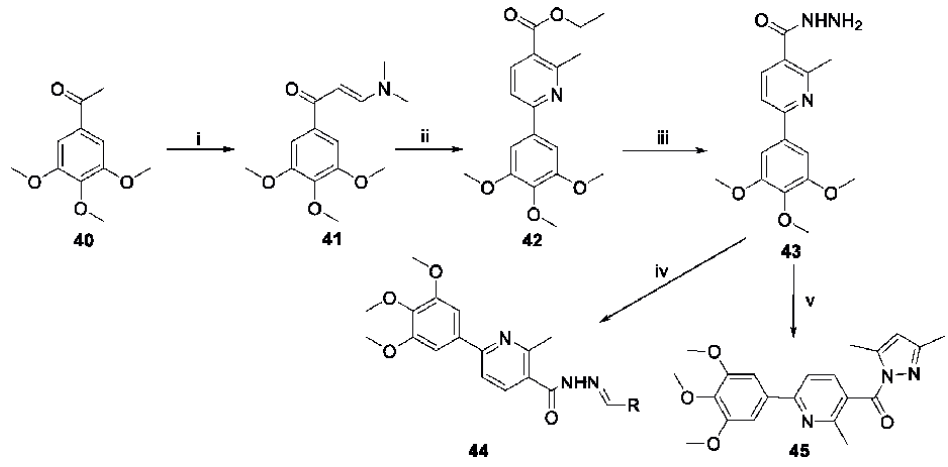

Figure 7.
Reagents and conditions; (i) DMF-DMA, 80°C, 8 h. (ii) Glacial acetic acid, Ammonium acetate, ethyl acetoacetate, reflux, overnight. (iii) Ethanol, NH2NH2, reflux, 8 h. (iv) Aldehyde, Ethanol, glacial acetic acid, reflux, 8–12 h. (v) Acetyl acetone, glacial acetic acid, 100°C, 7 h.

against lung (A549), breast (MDA-MB-231), colon (HCT116), and prostate (DU145) cancer cell lines in which most of the compounds exhibited the good to moderate anticancer potential values (**Figure 8**).

Wu and coworkers [21] reported the development of a multi-target anticancer Sn(II) pyridine-2-carboxaldehyde thiosemicarbazone complex **56**, investigated their anticancer

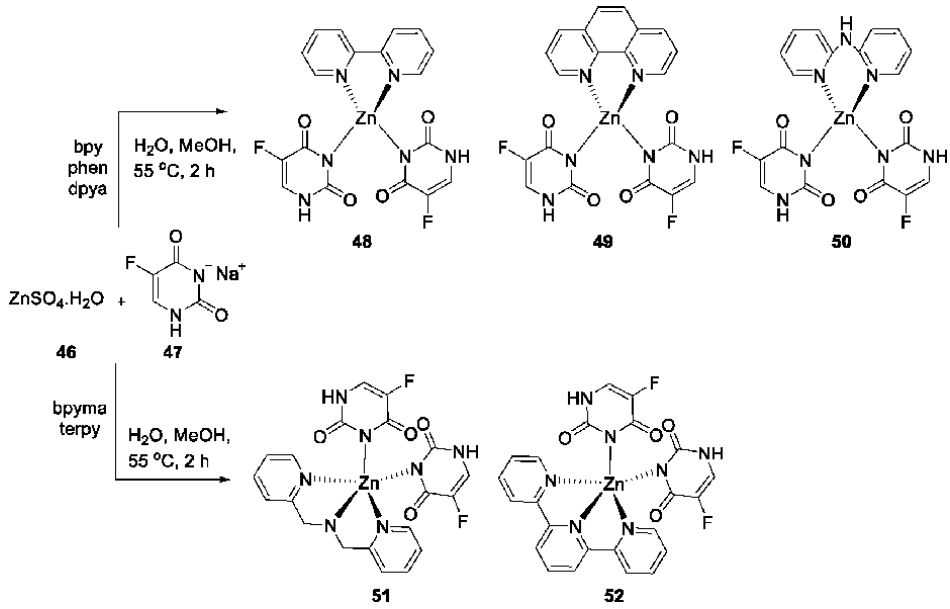

Figure 8.
Synthesis of Zn (II) 5-FU complexes bearing pyridine-based ligands.

activities [especially against human cervical cancer (HeLa) cell lines] and explored their potential molecular mechanisms. The idea was to use ligands with anti-cancer activity to chelate innocuous metals in order to create potent multi-target anticancer pharmaceuticals. In total, five Sn(II) pyridine-2-carboxaldehyde thiosemicarbazone complexes were produced with this reaction and were verified by single crystal X-ray crystallographic analysis. Among these complexes, **56e** was found to be excellent at suppressing the angiogenesis and restricting the metastasis of cancer cells *in vitro*. Along with that, it also shows the inhibitory effect against tumor growth *in vivo* very effectively. To evaluate the antitumor activity of **56e** *in vivo*, the HeLa tumor xenograft model was used [22]. The anticancer action of **56e** is attributed to several mechanisms, including DNA damage, apoptosis induction, and inhibition of anti-apoptotic Bcl-xL protein, metalloproteinase MMP2, and topoisomerase II activities (**Figure 9**).

Boraei et al. [23] described the synthesis of new substituted pyridine derivatives as *in vitro*, *in vivo*, and *in silico* potent anti-liver cancer agents through apoptosis induction. They synthesized a series of 4,6-diaryl-3-cyano-2-pyridones derivatives **67**, **68** using various aromatic aldehydes **57**, ketones **58**, ethyl cyanoacetate **59**, and ammonium acetate in butanol using one-pot four component reaction (MCR). The activity has been enhanced by using structural modifications on 4,6-diphenyl-3-cyano-2-pyridone **61**. It was found that pyridone alkylation proceeds on oxygen. Therefore, alkylation in the presence of K_2CO_3 afforded the *O*-alkylated products **63**. After being synthesized and cyclized, acetoxy hydrazide **64** yielded 1,3,4-oxadiazolethione **65**, which alkylated on sulfur to yield **67**. The synthesized derivatives were subjected to cytotoxic screening against HepG2 and THLE-2 cells, some of compounds had a remarkable cytotoxic activity with an IC50 value range of 10.7–13.9 μM. Compound **64** excellent IC50 value of 7.26 compared to 5-FU (IC50 = 6.98 μM) against the HepG2 cell line. Though it was nontoxic against THLE2 cells, it readily inhibited cell growth by 76.76%. Furthermore, it markedly increased the rate of apoptotic liver cancer

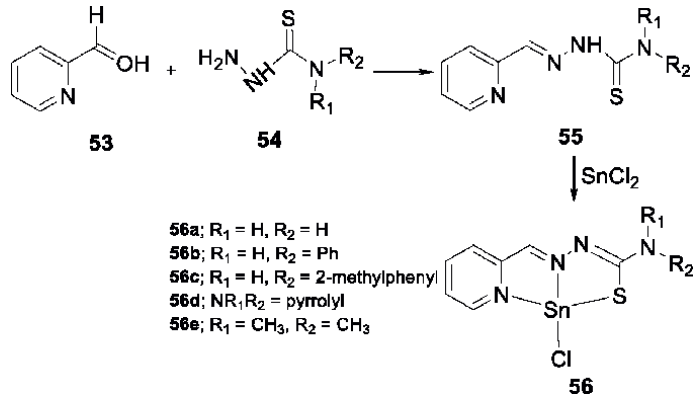

Figure 9.
Synthesis of Sn(II) pyridine-2-carboxaldehyde thiosemicarbazone complex.

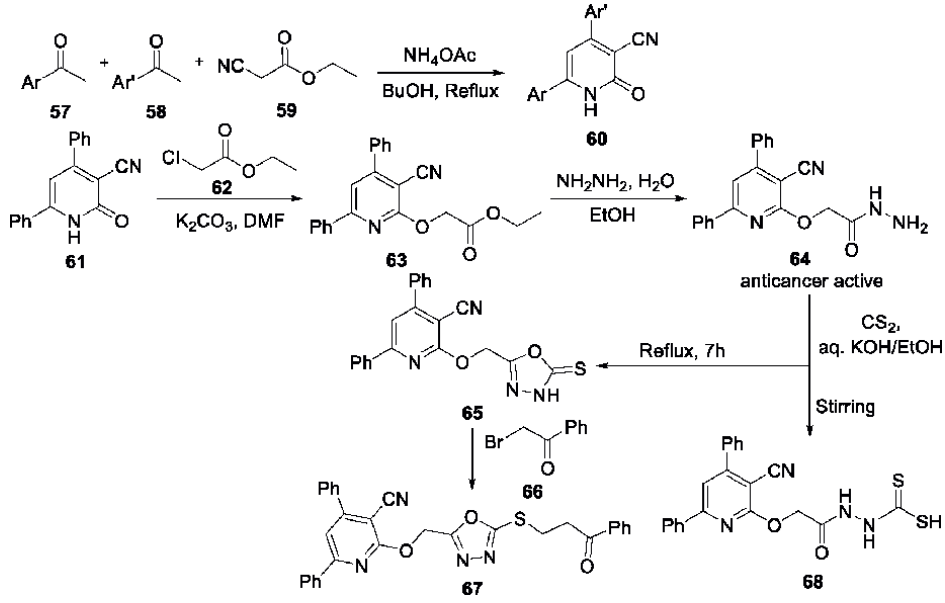

Figure 10.
Alkylation of 4,6-diphenyl-3-cyano-2-pyridones with ethyl chloroacetate, and further, hydrazinolysis of the ester.

cell death by 49.78-fold and arrested the cell cycle at pre-G1 with 35.16% of the cell population, as opposed to 1.57% for the control. Additionally, by upregulating P53 and other relevant genes and inhibiting anti-apoptotic genes through PIM-1 inhibition, it verified intrinsic apoptosis (**Figure 10**).

According to Climova et al. [24], newly synthetic ligands based on Zn(II) pyrazine and pyridine derivatives **74** were designed and produced, and their anticancer activity was studied. Three novel pyrazine and pyridine derivatives, i.e., carbohydrazonamide derivative **L1**, picolinohydrazonamide derivative **L2**, and 4-chloropicolinohydrazonamide **L3**, were previously described and based on these derivatives, Cu coordination compounds were also reported with their anticancer activity [25]. In addition, they explored three other chemical ligands of a similar nature in their work: **L4** pyrrolidine-based picolinohydrazonamide, **L5** pyrazine-based carbohydrazonamide,

and **L6** pyrrolidine pyrazine-based carbohydrazonamide. Furthermore, they synthesized six new Zn coordination compounds based on three newly reported chemical ligands and the three previously mentioned. The following process was used to create novel Zn (II) coordination compounds: A homogeneous solution was prepared by dissolving 60 mg of each ligand (**L1**: 0.231 mmol, **L2:** 0.196 mmol, **L3**: 0.237 mmol, **L4**: 0.186 mmol, **L5**: 0.204 mmol, and **L6**: 0.204 mmol) in 25 mL of methanol at 50°C while stirring continuously. Each of the next ligand solutions established anhydrous $ZnCl_2$ solution (10 mL) in methanol (equimolar quantities with the ligand). The resulting mixtures were stirred for 3 h at a steady temperature of 50°C. Following this, they were refrigerated for 10 days at 4°C to precipitate. After the samples containing the ligands **L2**, **L3**, **L4**, and **L6** developed precipitates in solution, they were filtered, cleaned with methanol, and allowed to air dry. A precipitate occurred in the case of **L1** and **L5**, where the solutions were allowed to slowly evaporate for a week. The crystal structures for $Zn(L_2)Cl_2$, $Zn(L_3)Cl_2$, and $Zn(L_5)_2Cl_2$ complexes were determined. Compounds have more promising anticancer properties when compared to their corresponding ligands. The complex demonstrated cytotoxicity against two glioblastoma cell lines, with the U87 showing comparable populations of early and late-apoptotic cells and LN229 showing primarily late-apoptotic cell population (**Figure 11**).

Helal et al. [26] explored the synthesis of a novel series of pyridine derivatives **80**, **81,** which were screened for their anticancer, anti-inflammatory, and analgesic activity. The reaction of methyl 2-aminobenzoate **75** with diethylmalonate or ethyl cyanacetate **76** in refluxing m-xylene at 150°C provided the essential starting material in good yield (80%) (**Figure 12**). Substituted-2-methoxycarbonyl acetanilide **77** was reacted with a-substituted cinnamonitriles **78** in ethanolic piperidine solution to yield the necessary pyridone of type **80**, **81** under reflux conditions [27]. The elemental and spectral data of the synthesized pyridones were used to confirm their structures. Pyridones were expected to occur by the Michael addition of active methylene groups from cinnamonitriles derivatives, followed by intramolecular cyclization of **79** and the removal of the HX molecule. The synthesized compounds were studied for their various *in vivo* anti-inflammatory and analgesic properties as well as their anticancer properties *in vitro*. The anti-inflammatory activity of the test substances was assessed using carrageenan-induced paw edema in rats. The SAR studies showed that all compounds have moderate to good activity. The molecular modeling was performed to validate their wet results.

Kutlu et al. [28] reported the development of pyridine derivative-based platinum complexes **84**, **86** and conducted DFT and initial anticancer activity assessments. Both the bis(2-fluoropyridine) dichloroplatinum (II) **84** and bis(2- amino-3-methyl

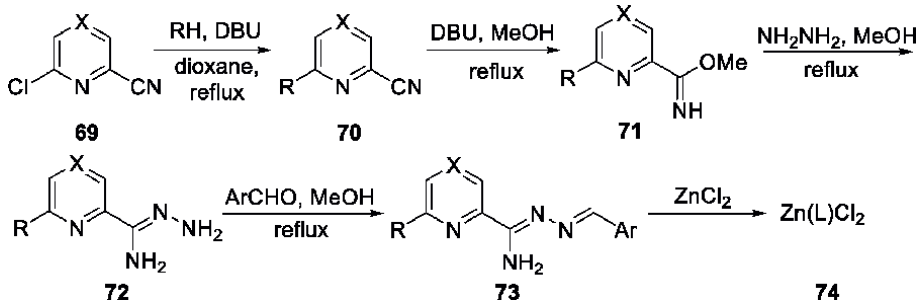

Figure 11.
Synthesis of novel Zn (ii) pyridine-based complexes for anticancer agents.

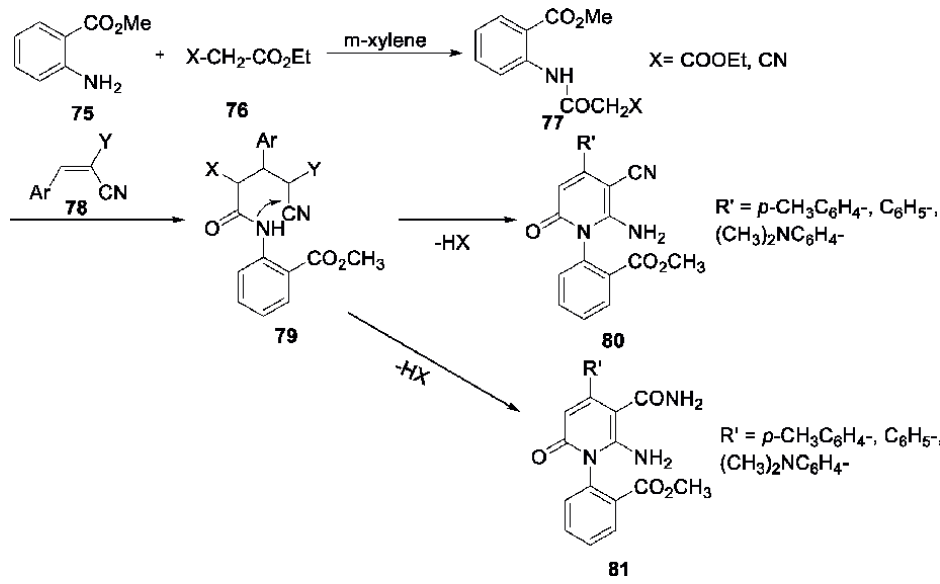

Figure 12.
Synthesis of novel pyridine derivatives.

pyridine)dichloroplatinum(II) **86**, $[PtCl_2L^1{}_2]$ and $[PtCl_2L^2{}_2]$, have been prepared by dissolving $K_2[PtCl_4]$ **82** and stirring continuously for 2 h at room temperature. Subsequently, dropwise additions of ethanolic solution of 2-fluoropyridine **83** for $[PtCl_2L^1{}_2]$ and 2-amino-3-methylpyridine **85** for $[PtCl_2L^2{}_2]$ were made to this solution while refluxing for 2 h. The precipitates were then separated and dried for 2 h at 100°C. The black and brownish yellow solids for $[PtCl_2L^1{}_2]$ and $[PtCl_2L^2{}_2]$ were obtained respectively. The complexes were studied utilizing FT-IR, UV-Vis, ^{1}H NMR, ^{13}C NMR, and elemental analytical techniques, as well as theoretical computations. The probable molecular structure of the most stable compounds was computed using the DFT/MPW1PW91 method with the LanL2DZ basis set. When the complexes were evaluated for cytotoxicity against the colon cancer cell line DLD-1, it was discovered that the $[PtCl_2L^1{}_2]$ complex outperformed $[PtCl_2L^2{}_2]$ over a 24-hour incubation

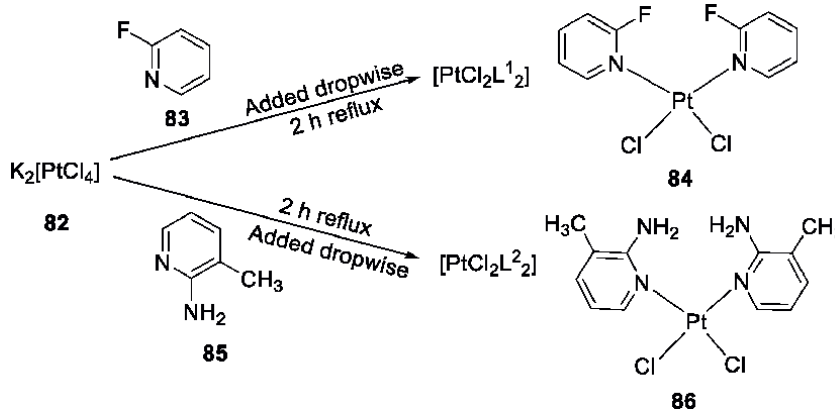

Figure 13.
Synthesis of Pyridine derivative platinum complexes.

period. The outcomes indicated that the synthesized platinum complexes formed from pyridine could be useful as chemotherapeutic agents in the treatment of colon cancer (**Figure 13**).

3. Anticancer activities of other heterocyclic integrating pyridine moiety

Liu et al. [29] described the synthesis of pyridine-2-carboxaldehyde thiosemicarbazone derivatives. In mice with L1210 leukemia, the anti-neoplastic potential of the synthesized compounds was estimated. The antitumor activity of 3-amino derivative compounds **87** and **88** was comparable against L1210 leukemia. The 5-amino derivatives **89**, **90**, and 5-hydroxy amino derivatives **91** behaved similarly to the 5-HP anti-neoplastic agents. Nicolaou and coworkers [30] reported pyridine epothilones and examined their potential cytotoxicity toward multiple human cancer cell lines. Owing to the pyridyl methyl group at position 6th and the nitrogen ring at position 5th, compounds **93** and **94** showed the maximum activity, respectively. Jong-Keun Son et al. [31] synthesized a series of a 2,6-diaryl-substituted pyridines derivatives **92** and recognized their cytotoxic efficacy against numerous human cancer cell lines. Promising cytotoxic topoisomerase I inhibitory action was shown by compounds containing their substituents.

Romagnoli et al. [32] described the evaluation of a series of 2-amino-3-(3′,4′,5′-trimethoxybenzoyl)-6-substituted-4,5,6,7-tetrahydrothieno[2,3-*c*] pyridine derivatives **95** for ant tubulin activities against numerous cancer cell lines. Liou et al. [33] developed a novel derivative of indoline-sulfonamide **96** and studied its anticancer properties on various human cancer cell lines *via* disruption of microtubules. Several pyridine phenyl urea derivatives were recognized by Eldehna and coworkers. The *in vivo* activity of the compounds produced was assessed on A549 and HCT-116 cancer cell lines, with doxorubicin as a positive control. In both cell lines, compound **97** is recognized as the lead molecule among the phenyl area derivatives. The lead substance causes apoptosis in HCT-116 cells, as demonstrated by increased levels of proapoptotic proteins and decreased expression of the anti-apoptotic Bcl-2 protein. Furthermore, active molecules prevented the G2/M phase of the cell cycle [34]. Durgapal et al. [35] designed and produced the 3-aminomethyl pyridine derivatives **98** and compounds were evaluated for *in vitro* anti-proliferative and DNA binding activities. *In vitro* activity was assessed using two cancer cell lines, A549 and MCF-7, with 5-fluorouracil serving as a positive control. The compound **98a** was determined to be a lead molecule and is more active than 5-fluorouracil. The subsequent examination of this molecule in a DNA binding experiment revealed that compound **98a** is two-fold more active than compound **98b**. Compound **98b** was further evaluated using various tests, and it showed to be efficient.

Nagababu et al. [36] synthesized the cobalt (III) pyridine complexes **99** and **100** and showed their anticancer activities. Chalcone pyridine analogues were reported by Xu and coworkers [37] as anti-tubulin agents. A series of imidazo [1,2-*a*] pyridine scaffolds **101** were synthesized by Hayakawa and coworkers [38], and their inhibitory properties against p110a (an emerging target for cancer therapy) were examined. It was only the thiazolyl imidazo[1,2-*a*]pyridine molecule that suppressed the proliferation of tumor cells. The most active compound was compound **102**, which binds to the tubulin colchicine site to efficiently inhibit tubulin polymerization activities. Furthermore, research on cellular mechanisms has shown that cell cycle arrest takes place at the G2/M phase. Interestingly, compound **102** had a more potent *in vivo* effectiveness than CA-4.

Gomha et al. synthesized a variety of thiadiazolyl-pyridine derivatives. Two cancer cell lines, A549 and HepG2, were used to examine the anticancer activity of the synthesized compounds, with cisplatin serving as a standard reference. Among all compounds, compound **103** was the leading molecule in the HepG2 cell line and the most active in the A549 cell line [39].

Jian et al. [40] synthesized a series of pyrazolo[3,4-*b*]pyridine-bridged derivatives **104** of combretastatin A-4, obtaining 3,4,5-trimethoxylphenyl groups, and evaluated for their anti-proliferative and tubulin polymerization-inhibiting properties. Based on biological evaluation, a few potential compounds showed significant anti-proliferative activity against four cell lines, namely MDA-MB-231, MCF-7, Kyse150, and HeLa *via* G_2/M cell cycle stage arrest. The newly developed pyrido[2,1-*b*]quinazoline fused compounds **105** with promising therapeutic characteristics were disclosed by Bathula *et al.* [41]. The cytotoxic properties of these developed compounds were evaluated using *in vitro* experiments, against a variety of malignant cell lines, such as NCI-H460, A549, HCT-15, HT-29, HFL, and DU-145. Compound 11-(1-benzyl-1*H*-indol-3-yl)-2,3,4,11-tetrahydro-1*H*-pyrido[2,1-*b*]quinazoline **105** was shown to have a potent anticlonogenic activity against lung cancer cells and to have good cytotoxic impact against the lung cancer cell lines A549 and NCI-H460.

Murugavel and coworkers [42] described the biological evaluation and computational quantum compound analysis of a newly synthesized sulfur thiophene heterocyclic analogue that consists of 1,2,3-triazole and pyridine components, i.e., BTPT [2-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-6-methoxy-4-(thiophen-2-yl) pyridine] **106**. The drug resemblance abilities of BTPT were examined by the use of *in silico* medicinal ADMET attribute evaluation. The molecular docking was done by using human topoisomerase IIa targeting ATP binding site. The three human malignant cell lines PC-3, A549, and MDAMB-231 were subjected to the MTT assay as part of the BTPT/doxorubicin in vitro cytotoxicity test. By contrast with the well-known cancer drug doxorubicin, the lead substance BTPT revealed significant cytotoxicity against MDAMB-231 (a breast cancer cell), moderate action with A-549 (a human lung cancer cell), and minimal inhibition with PC-3 (a human prostate cancer cell). It was proposed that BTPT would be a promising anticancer pharmaceutical option.

Zhang and coworkers [43] designed and synthesized two novel series of triazolopyridazine/−pyrimidine derivatives **107** and evaluated their inhibitory action over c-Met kinase *in vitro*, along with three cancer cell lines (A549, HeLa, and MCF-7; these are malignant cell lines with overexpressed c-Met) and one normal cell line (LO2; normal human hepatocytes). Based on pharmacological data, it was found that most of the compounds tested showed modest cytotoxicity, with **107** being the most potent, showing significant cytotoxicity against MCF-7, A549, and HeLa cell lines. According to the results of apoptosis and the cell cycle distribution, **107** may cause late-apoptotic action in A549 cells and expedite cells arrest in the G0/G1 phase. According to the reported results, **107** has the potential to develop into a strong class II c-Met inhibitor (**Figure 14**).

Amin *et al.* [44] synthesized a novel series of tetralin-6-yl pyridines derivatives and assessed their anti-proliferative efficacy *in vitro* using HepG2 and MCF-7cell lines. It was established by the data that compound **109** was selective for breast cancer and compound **108** was showed potent results for liver cancer. The isatin-pyridine derivatives were reported as anti-proliferative agents by Eldehna and coworkers [45] and evaluated their *in vitro* anticancer activity of the compounds utilizing HepG2, A549, and MCF-7 cancer cell lines. Compound **110** was the most effective isatin derivative against HepG2 cancer cell lines when compared to the standard drug

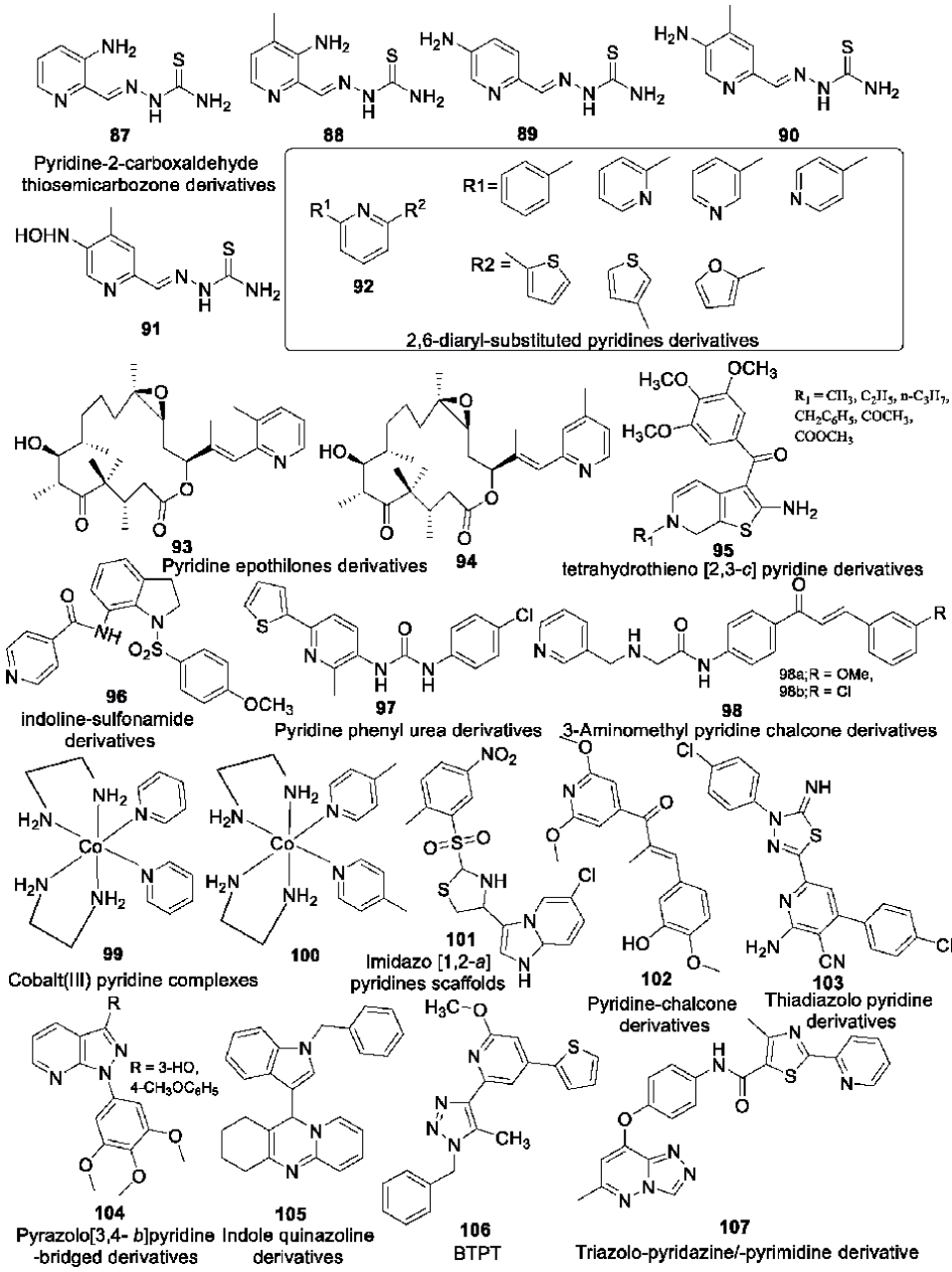

Figure 14.
Pyridine and its metal complexes as anticancer agents.

doxorubicin. Compound **111**, on the other hand, showed good activity against the A549 and MCF-7 cell lines. Fu and groups [46] showed a few examples of pyridine-based tetraindole derivatives and were tested on triple-negative breast cancer cell lines and adenocarcinoma cell lines. Compound **112**, one of the synthetic derivatives, showed preferential cytotoxicity against breast cancer cell lines relative to normal cell lines. Furthermore, it has been demonstrated that its mode of action involves the G2/M phase of cell cycle arrest and successfully prevents cancer cell metastasis.

A series of pyridine derivatives of curcumin were designed and synthesized by Zhou and coworkers [47] as inhibitors of human prostate cancer. Curcumin analogues have an impact on the CWR-22Rvl human prostate cancer cell line. Compounds **113**–**115** were identified as the most potent compounds of the series. The inhibitory effects of these drugs were assessed utilizing an androgen receptor-linked luciferase assay. The results showed that compounds **113**–**115** had a good inhibitory impact (**Figure 15**).

Fayed *et al.* [48] developed a new series of coumarin-pyridine-fused pyridine hybrids **116**–**118** and evaluated them against the human cancer cell lines HCT-116, MCF-7, A549, and HepG-2 to establish anticancer properties. As VEGFR-2 kinase inhibitors, Zeidan et al. [49] designed and synthesized two series of picolinamide derivatives **119**–**121** that developed dithiocarbamate and thiourea derivatives. In addition to their ability to inhibit VEGFR-2, all the newly synthesized compounds were evaluated for cytotoxicity against the A549 malignant cell line. Compounds **119**, **120**, and **121** showed possible inhibitory activity against VEGFR-2 kinase as compared to the control drug sorafenib. Furthermore, these compounds were tested for antitumor

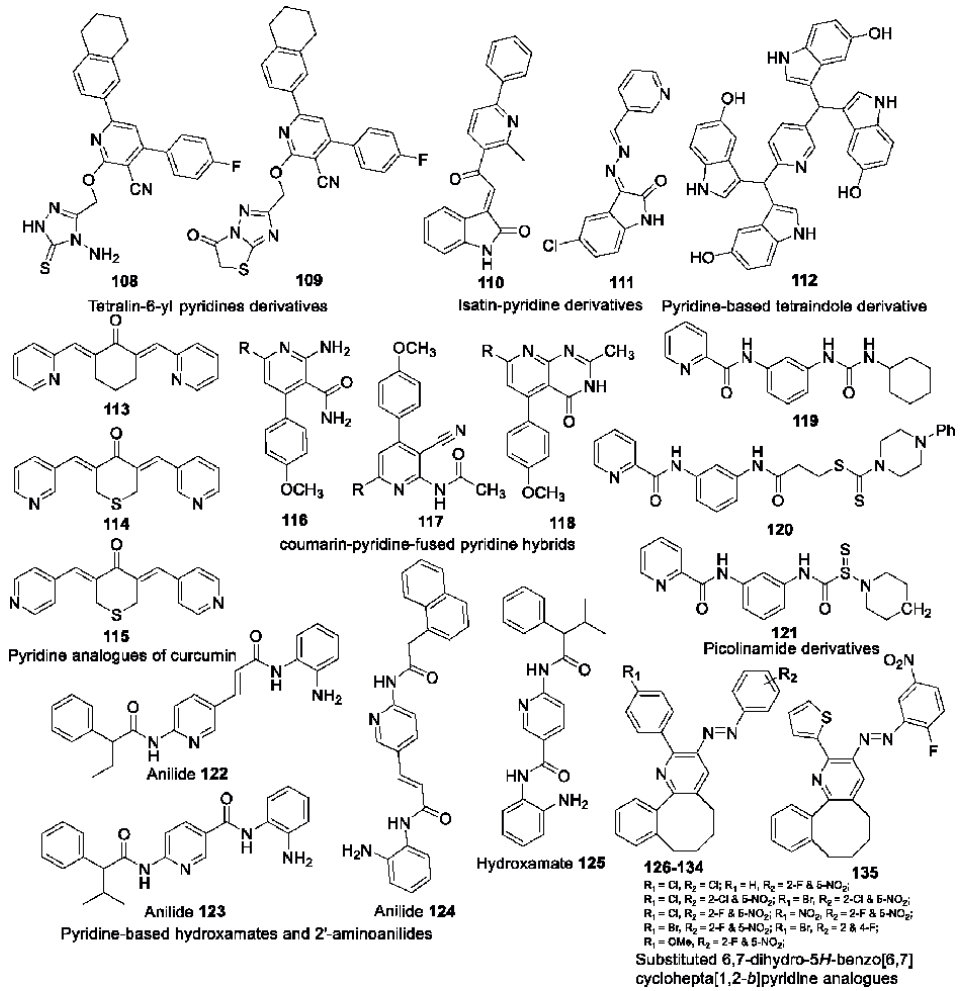

Figure 15.
Pyridine derivatives as anticancer compounds.

activity against malignant cell lines derived from various sources, including OVCAR-3, Panc-1, HT29, and 786-O, with compound **119** causing a considerable cell death in the majority of cases. Zwergel and group [50] produced four aza-analogues similar to the regioisomers from the *N*-hydroxy-3-(4-(2-phenylbutanoyl) amino) phenyl) acrylamide containing pyridine nucleus as previously described by the same group as an HDAC inhibitor. Additionally, tests against K562, HCT116, and A549 cancer cells, hydroxamate **125**, and anilide **122**, **123**, and **124** demonstrated promising antiproliferative activities.

Behbehani *et al.* [51] explored a unique synthetic protocol that uses a novel, accessible, and effective approach for synthesizing substituted 6,7-dihydro-5H-benzo[6,7] cyclohepta[1,2-b]pyridines derivatives **126**–**135**. All the synthesized compounds were assessed for cytotoxicity studies against the A549 (lung cancer) and MCF-7 (breast cancer) cell lines by MTT colorimetric assay. When tested on MCF-7 and A549 malignant cells, the results showed that pyridine derivatives had good anticancer efficacy.

4. Conclusions

The study enlists promising consolidated results of various syntheses of pyridine derivatives and their anticancer properties, demonstrating the role of the pyridine nucleus in the development of anticancer pharmaceuticals. In the field of organic and medicinal chemistry, pyridine is a fascinating moiety that has already demonstrated its importance in the pharmaceutical sector with over 7000 medicines, including cancer treatments. This book chapter highlights promising pyridine derivatives as anticancer agents that played significant advancements in the chemotherapy of cancer.

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Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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Author details

Nawal Kishore Sahu[1], Amol T. Mahajan[2] and Sandeep Chaudhary[2]*

1 Department of Chemistry, Engineering College, Bharatpur (A Constituent College of Rajasthan Technical University Kota), Bharatpur, Rajasthan, India

2 Laboratory of Bioactive Heterocycles and Catalysis (BHC lab), Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Raebareli (NIPER-R), Transit Campus, Lucknow, UP, India

*Address all correspondence to: schaudhary.chy@niperraebareli.edu.in and sandeep.chaudhary2311@gmail.com

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Chapter 6

Recent Advances in Syntheses and Antibacterial Activity of Novel Furan Derivatives

Pinki Pal

Abstract

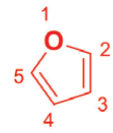
Microbial resistance has turned into a global issue due to the ineffectiveness of currently available antimicrobial medicines. In the realm of medicinal chemistry, furan derivatives have taken on a special position. An essential synthetic technique in the search for new drugs is the inclusion of the furan nucleus. Due to the remarkable therapeutic efficacy of furan-related medicines, medicinal chemists have been inspired to create numerous innovative antibacterial agents. Due to the numerous methods by which furans derivatives can be made as well as their numerous structural reactions, the field of organic chemistry and medicinal chemistry offers a wide range of prospects. To combat the enduring issue of microbial resistance, the crucial facts presented in this chapter may aid in the creation of more effective and secure antimicrobial agents.

Keywords: furan, furan derivatives, antibacterial activity, gram-positive bacteria, gram-negative bacteria

1. Introduction

Antimicrobial drugs are one of the most powerful tools in the fight against bacterial strain-caused infection. There is an urgent need to find new antimicrobial compounds to treat multi-resistant illnesses with distinct mechanisms of action, as evidenced by the rise in drug resistance to clinically utilized anti-infectives. Furan-containing compounds exhibit a wide range of advantageous biological and pharmacological characteristics, and as a result, they have been employed as medicines in a number of distinct disease areas [1].

The Latin word *furfur*, which implies bran, is where the name furan originates. The earliest furan derivative was 2-furoic acid, which was described by Carl Wilhelm Scheele in 1780 [2]. Furan is a class of organic compounds of the heterocyclic aromatic series characterized by a ring structure composed of one oxygen and four carbon atoms (**Figure 1**). The most fundamental member of the furans family is "furan," with a boiling point of 31.36°C and is a colorless, volatile and mildly toxic liquid. Out of all the 5-membered heterocyclic compounds, furan is the most reactive.



Furan (1)

Figure 1.
Structure of furan 1.

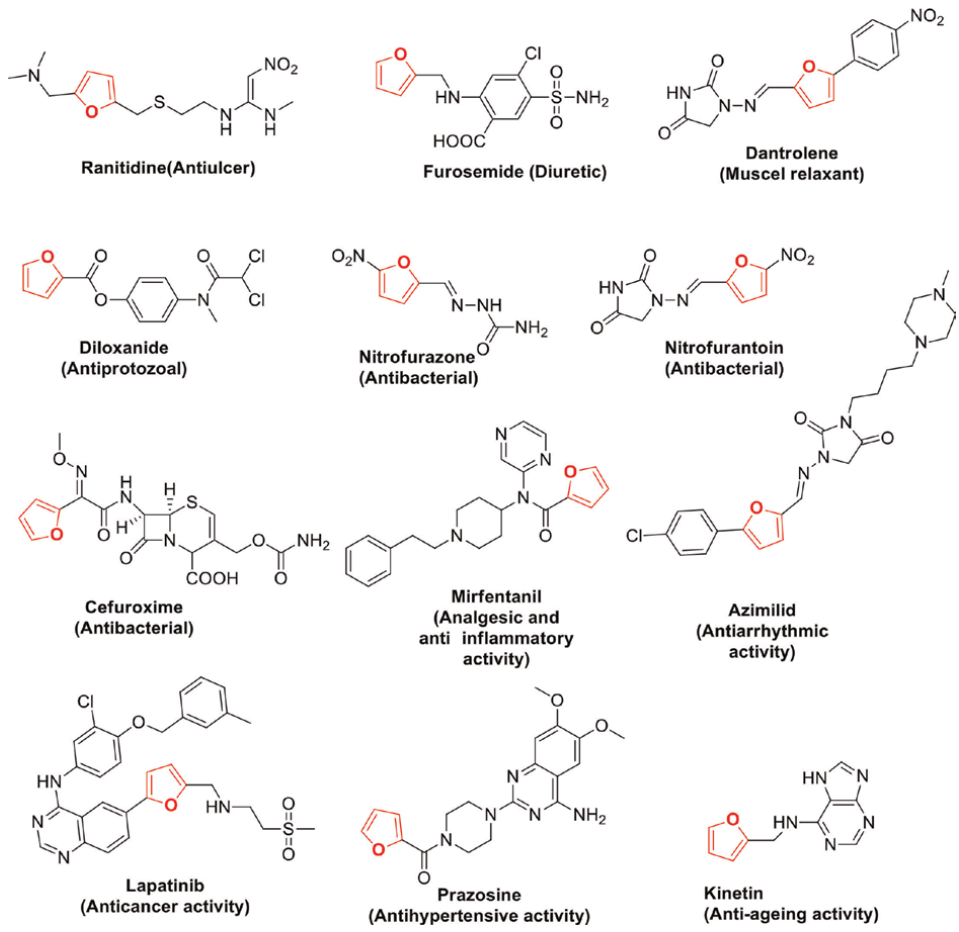


Figure 2.
Clinically approved drugs containing furan ring.

Furan has a variety of therapeutic advantages, such as anti-ulcer [3], diuretic [4], muscle relaxant [5], anti-protozoal [6], antibacterial or antifungal or antiviral [7, 8], anti-inflammatory, analgesic, antidepressant, anti-anxiolytic, anti-parkinsonian, anti-glaucoma, antihypertensive, anti-aging and anticancer (**Figure 2**) [9].

Several market drugs, such as morphine, citalopram, ramelteon, amiodarone and darifenacin, contain benzofuran or a dihydrobenzofuran moiety (**Figure 3**).

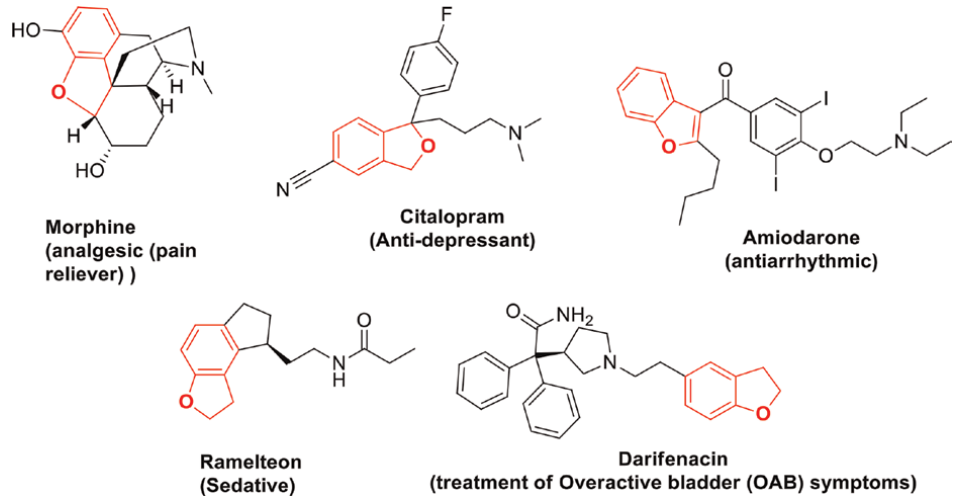


Figure 3.
Market drugs containing benzofuran moiety.

2. Literature reports on recent developments in furan derivatives syntheses and their antibacterial efficacy

In 2020, Altintop et al. designed and synthesized a new series of ten 4-[2-((5-Arylfuran-2-yl)methylene)hydrazinyl]benzonitrile derivatives **4a–j** in one step *via* the reaction of 4-cyanophenylhydrazine hydrochloride **3** with 5-arylfurfurals **2a–j** (**Figure 4**) and screened for *in-vitro* activity against *Staphylococcus aureus* (NRRL B-767), *Listeria monocytogenes* (ATCC 7644), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Micrococcus luteus* (NRRL B-4375), *Bacillus subtilis* (NRS-744) and *Candida albicans* (ATCC 90028), using streptomycin and ketoconazole as standard [10].

According to their bioassay results, the antifungal effects of the compounds were more significant than their antibacterial effects. Compound **4e** was the most potent antifungal agent against *Candida albicans*, *Trichoderma harzianum* and *Fusarium species*, whereas compound **4j** was the most effective antifungal agent on *Aspergillus ochraceus* [10].

In the year 2020, Hassan and team designed and synthesized a series of thirteen nitrofurantoin analogues containing furan and pyrazole scaffolds as N-aryl-3-(arylamino)-5-(((5-substituted furan-2-yl)methylene)amino)-1H-pyrazole-4-carboxamide (**11a–g** and **13a–f**) by the condensation of 5-Amino-1*H*-pyrazole-4-carboxamides (**9a–g**) with 5-nitrofuran-2-carbaldehyde (**10**) or 5-methylfuran-2-carbaldehyde (**12**) (**Figures 5-7**) [11].

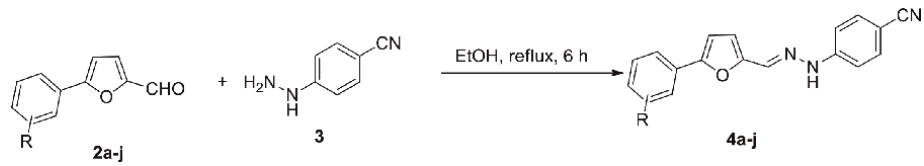


Figure 4.
*The synthetic route for the preparation of compounds **4a–j**.*

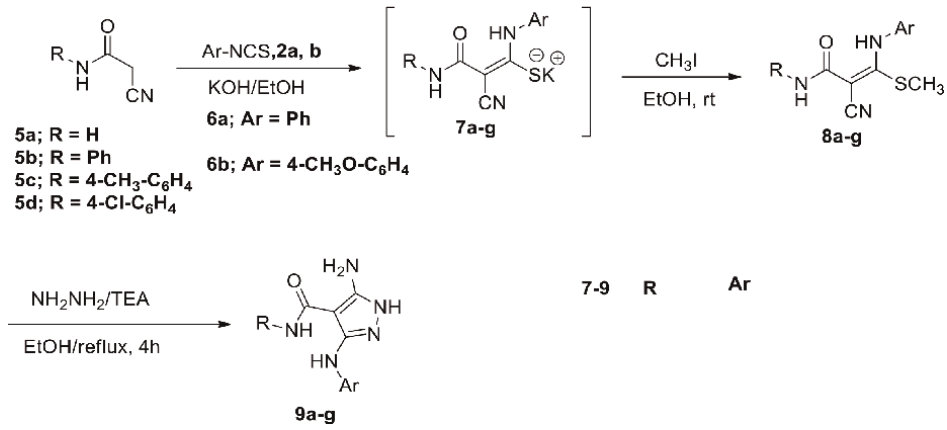


Figure 5.
*Synthesis of compounds **9a–g**.*

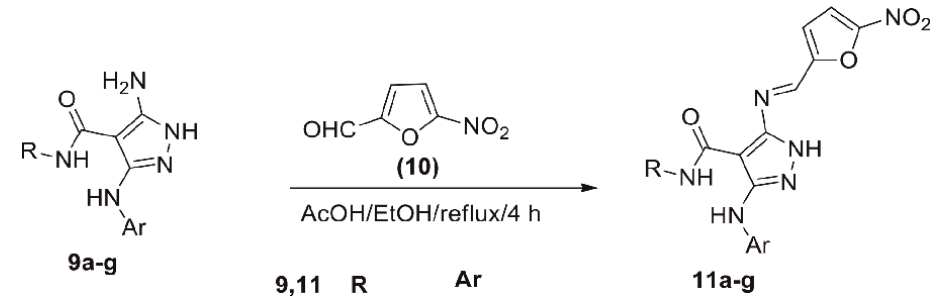


Figure 6.
*Synthesis of nitrofurantoin analogues **11a–g**.*

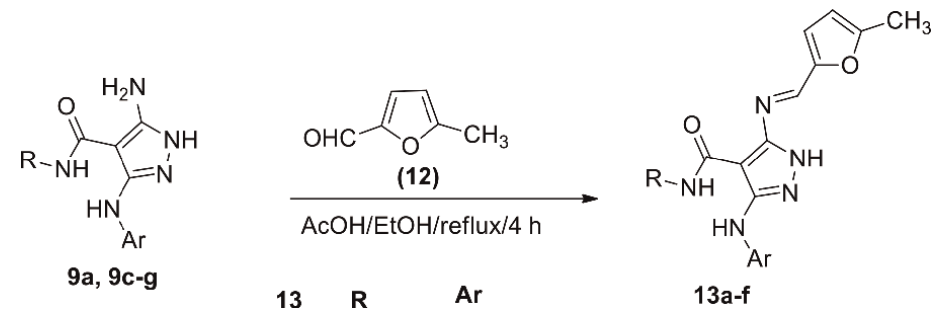


Figure 7.
*Synthesis of compounds **9a–f**.*

All the synthesized compounds were evaluated for their antibacterial properties against gram-negative bacteria *Escherichia coli*, *Salmonella typhimurium* and gram-negative bacteria *Staphylococcus aureus* and *Streptococcus faecium* using nitrofurantoin antibiotic as standard [11].

The results showed that four compounds (**11a, 11b, 11f** and **11g**) exhibit good antibacterial activities against *Escherichia coli*, while compounds **11b, 11c** and **11e** exhibited moderate activity compared to nitrofurantoin. They reported compounds **11a–g** showed good activity against *Salmonella typhimurium*. Two compounds **13b** and

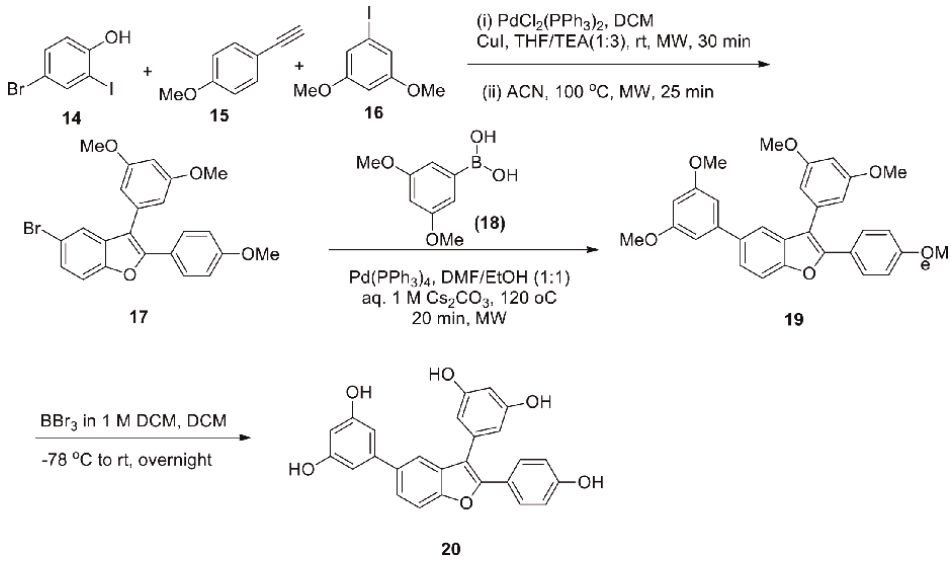


Figure 8.
Synthesis of compound **20**.

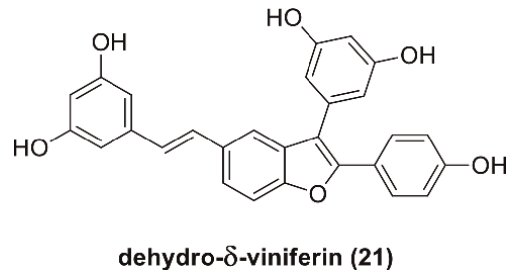


Figure 9.
Structure of dehydro-δ-viniferin **21**.

13d were moderately active against *Salmonella typhimurium*. All synthesized nitrofurantoin analogues were biologically inert against *Staphylococcus aureus* and *Streptococcus faecium* and the nitrofurantoin antibiotic as well was inactive against these gram-positive bacteria [11].

In the year 2021, Dallavalle et al. designed and synthesized stilbenoid dehydro-δ-viniferin analogues and isosteres, which were evaluated for antibacterial activity against *S. aureus* ATCC29213.

By reacting 4-bromo-2-iodophenol (**14**), 4-ethynylanisole (**15**), and 3,5-dimethoxy-1-iodobenzene (**16**), they synthesized the bromo functionalized intermediate **17** (**Figure 8**). Suzuki-coupling of compound **17** with (3,5-dimethoxyphenyl)boronic acid (**18**) in the presence of $Pd(PPh_3)_4$ and aqueous 1 M Cs_2CO_3 in a mixture DMF/EtOH (1:1), under microwave irradiation, for 20 min at 120°C afforded compound **19**. Final demethylation with BBr_3 provided **20** (**Figure 8**), as a simplified analogue of dehydro-δ-viniferin (**21**, **Figure 9**) [12].

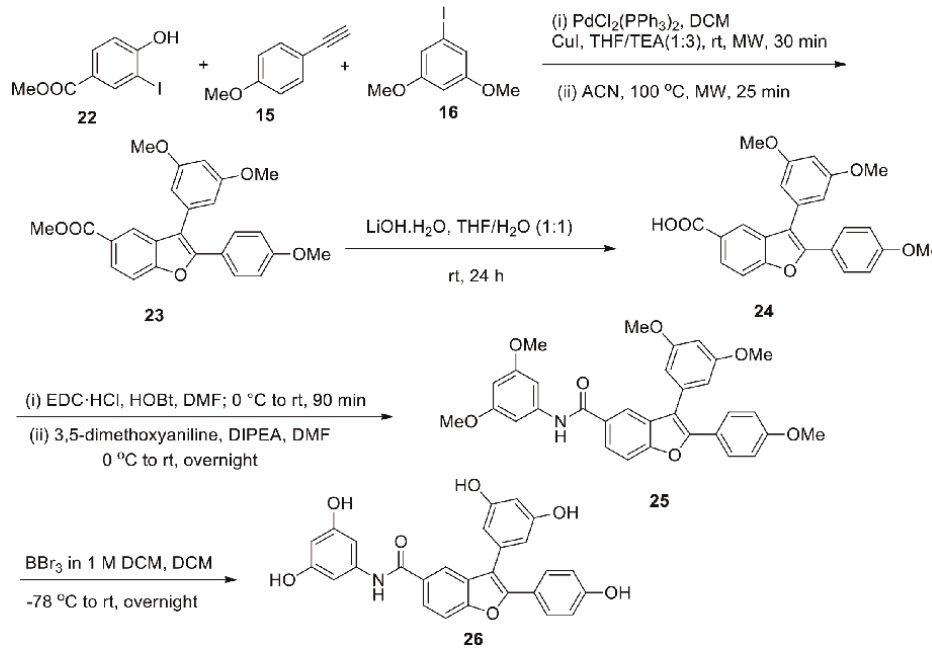


Figure 10.
Synthesis of compound **26**.

The desired benzofuran **23** was produced using the Sonogashira/Cacchi type cyclization of the commercially available methyl 4-hydroxy-3-iodobenzoate (**22**), 4-ethynylanisole (**15**) and 3,5-dimethoxy-1-iodobenze (**16**). Lithium hydroxide mediated hydrolysis of the ester **23** produced corresponding carboxylic acid **24**, which on reaction with 3,5-dimethoxyaniline, in presence of EDC•HCl and HOBt gave **25**, which was demethylated to afford **26** an amide isosteres (**Figure 10**).

They further continued their work with intermediate **23**, which on $LiAlH_4$ reduction formed compound **27**. The reaction of compound **27** with PBr_3 followed by triethyl phosphite at 130°C for overnight furnished phosphonate **28**. The Horner-Wadsworth-Emmons (HWE) reaction of **28** with 4-methoxybenzaldehyde formed the desired stilbene **29**, only the "*E*" isomer. Demethylation with BBr_3 gave only degradation products.

To get the desired compound **30**, they selected bromo derivative **17** as the starting material. The initial deprotection of bromo derivative **17**, followed by Heck reaction with 4-hydroxystyrene gave a mixture of **30** and its isomer **31** (**Figure 11**).

Another synthetic route started with 2-iodo-4-methylphenol (**33**), which was produced in excellent yields by combining *para*-cresol (**32**) with *N*-iodosuccinimide and *para*-toluenesulfonic acid in acetonitrile (**Figure 12**). The desired benzofuran derivative **34** was obtained by one-pot Sonogashira-Cacchi reaction conditions using compound **33** with 4-ethynylanisole (**15**) and 3,5-dimethoxy-1-iodobenze (**16**). Compound **35** was produced by a smooth demethylation of intermediate **34**. The protection of free hydroxy groups of **35** with *tert*-butyldimethylsilylchloride (TBDMSCl), imidazole in 1,2-dichloroethane at 60°C gave compound **36** in a good yield. The radical bromination of the methyl group of **36** with NBS/AIBN in CCl_4

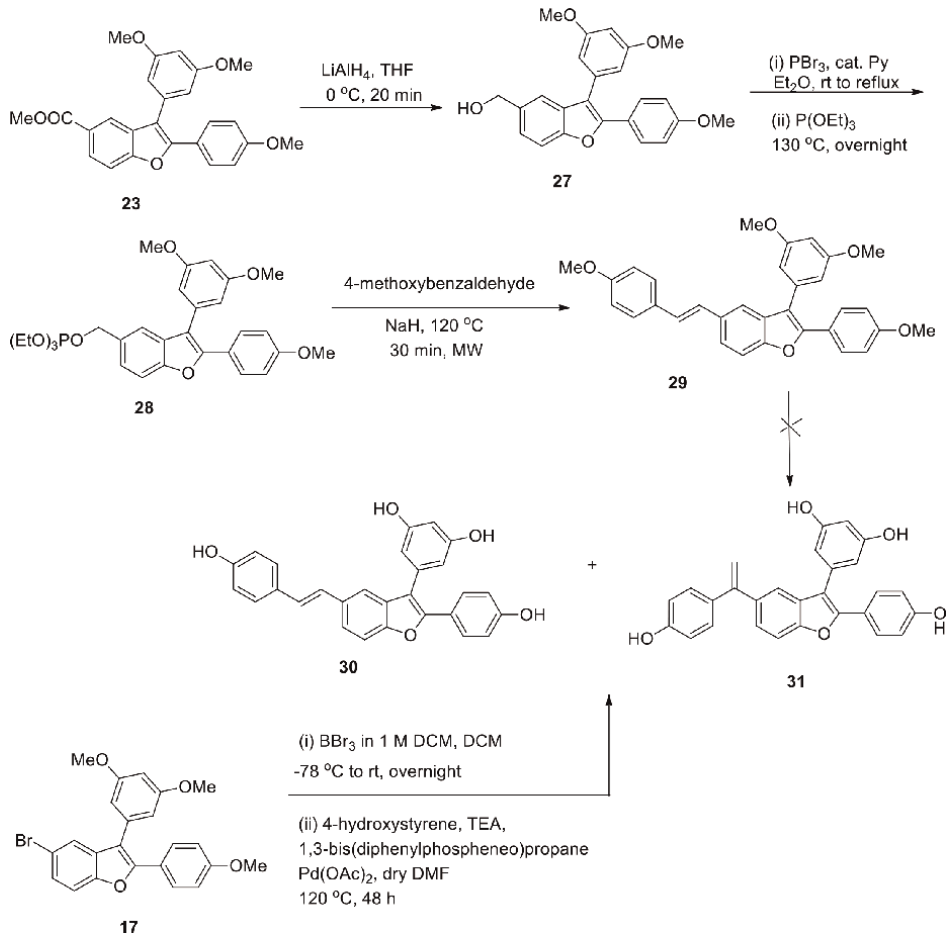


Figure 11.
*Synthesis of compound **30**.*

under reflux gave a brominated intermediate, which was converted into the corresponding phosphonate **37** with triethyl phosphite at 130°C, which on reaction with 3,4-bis((tert-butyldimethylsilyl)oxy)benzaldehyde in presence of NaH in THF followed by desilylation with tetrabutylammonium fluoride (TBAF) furnished compound **38**.

Demethylation of compound **17** followed by protection of free hydroxyl group by TBDMSCl formed silyl ether **39**.

The alkyne **42** was obtained from starting material 3,5-dihydroxy benzaldehyde **40**, which was silylated and then subjected to Corey-Fuchs reaction condition to yield compound **41**. Treatment of **41** with LDA-formed alkyne **42**. Finally, Sonogashira coupling of bromo derivative **39** with alkyne **42**, in the presence of $Pd(PPh_3)_4$/CuI in triethylamine under reflux condition, followed by desilylation with KF formed desired compound **43** (**Figure 13**).

Finally, Pd/C catalyzed hydrogenation of dehydro-δ-viniferin **21** yielded compound **44** having a saturated chain in place of the stilbene double bond. By following the same procedure, δ-viniferin **45** was hydrogenated to produce compound **46**, which had a cleaved dihydrobenzofuran ring (**Figure 14**).

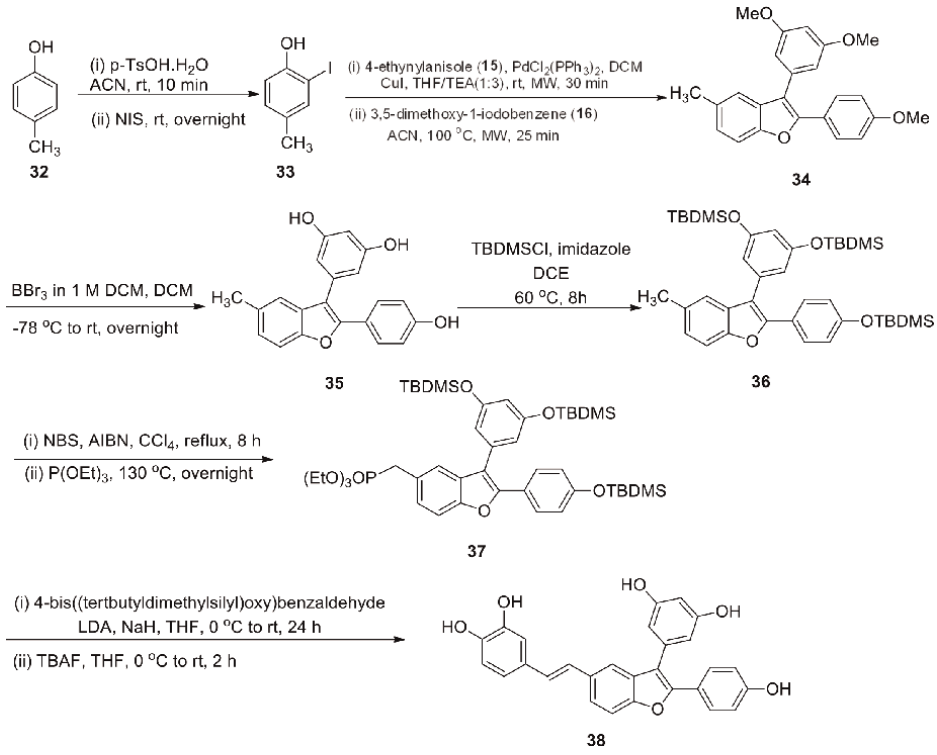


Figure 12.
Synthesis of compound ***38****.*

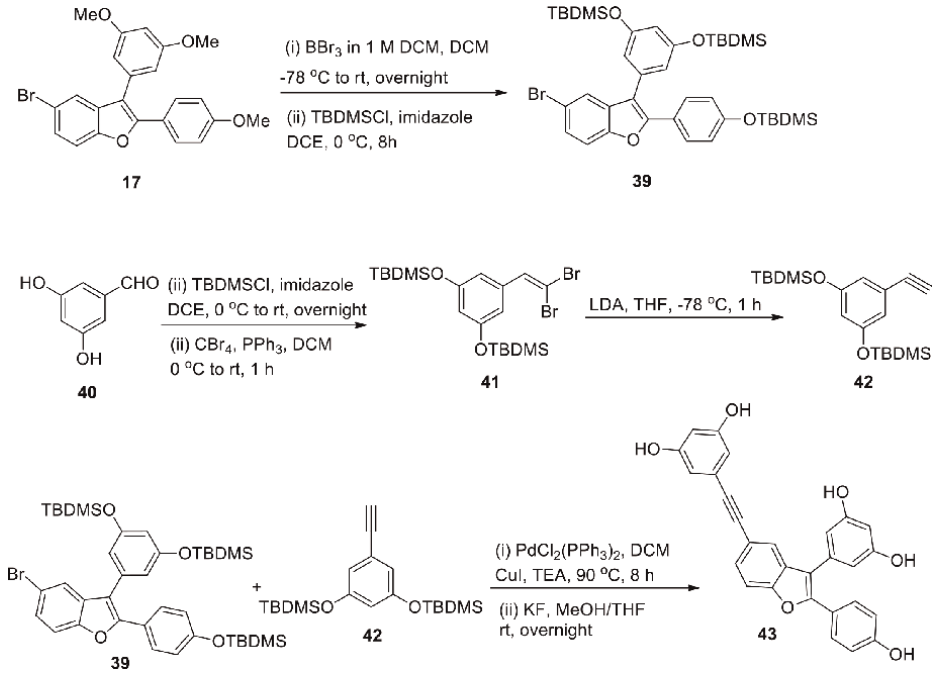


Figure 13.
Synthesis of compound ***43****.*

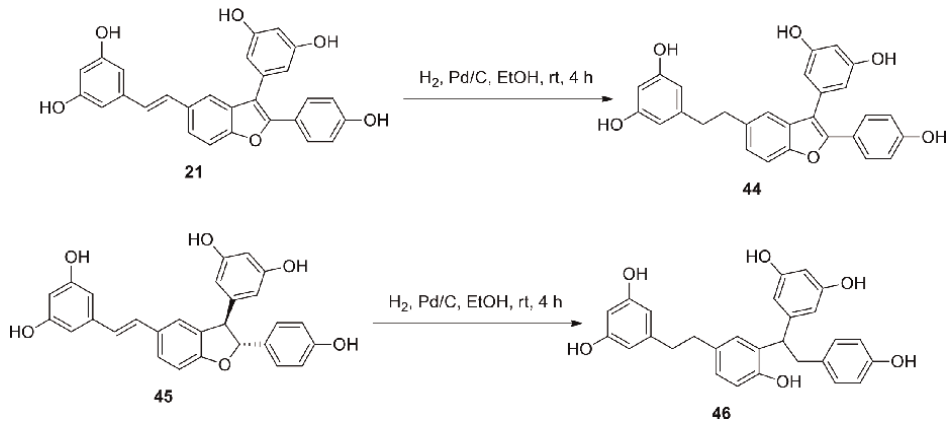


Figure 14.
*Synthesis of compound **44** and **46**.*

The model compound **21** and six newly synthesized analogues (**20**, **26**, **38**, **43**, **44**, **46**) were screened for their anti-bacterial efficacy against *S. aureus* ATCC29213, using tobramycin as standard. Compounds **20**, **43** and **44** showed significant activity whereas compounds **26** and **38** were not successful in terms of activity. Compound **46** displayed very low anti-bacterial activity compared to other synthesized compounds [12].

In the year 2021, Oliveira and colleagues synthesized eighteen arylfuran derivatives and tested their anti-bacterial efficacy against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Meerwein arylation of furfural **47** with the haloarene-diazonium salt using $CuCl_2$ as a catalyst yielded 5-arylfurfural derivatives **48** and **49**, which on $AgNO_3$ oxidation resulted in the formation of the corresponding carboxylic acid **50** and **51**, respectively. Finally, compounds **50** and **51** were converted to amides **52–55** via carbodiimide/N-hydroxysuccinimide coupling (**Figure 15**) [13].

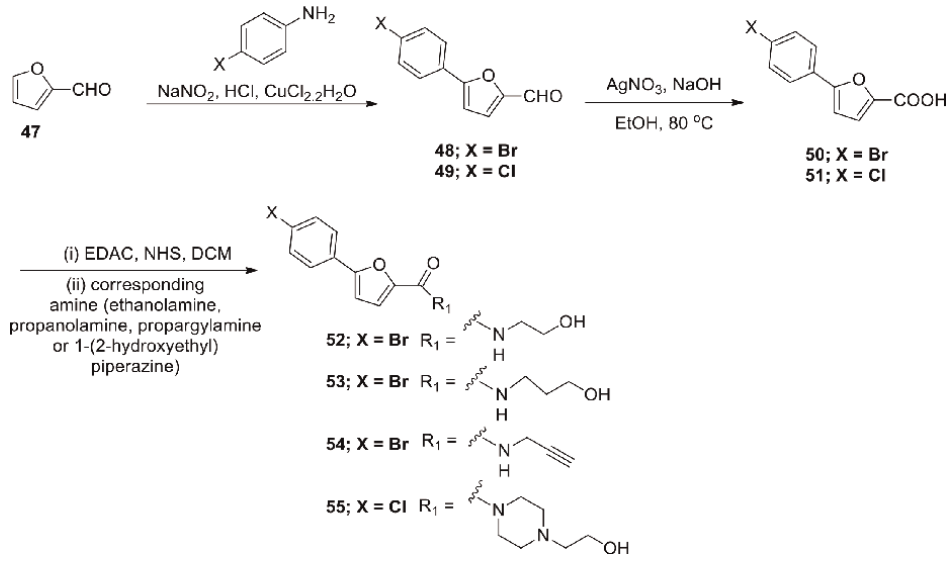


Figure 15.
*Synthesis of compounds **52–55**.*

Morpholine (**56**) and chloroacetic anhydride were used as starting materials in the two-step synthesis of the azide derivative **58**. The peracetylated glycosyl bromide **60** was converted to azide **61** by reaction with NaN_3 in acetone/H_2O at room temperature. The click reaction between the propargyl amide **54** and the azide derivatives **58** and **61**, was used to create the furan-triazole derivatives **62** and **63** (**Figure 16**).

The reaction of arylfuran **49** with 1-aminohydantoin formed hydantoin derivative **64.** Treatment of arylfuran **49** with corresponding amines to Schiff bases which on *insitu* $NaBH_4$ reduction formed amines **65–73,** respectively (**Figure 17**).

The *in vitro* antibacterial activity of each synthesized aryl furan derivative was evaluated against *S. aureus* (ATCC 29213TM), *E. coli* (ATCC 25922TM), and *P. aeruginosa* (ATCC 27853TM). The aryl furan derivative **73** was found to possess considerable activity against both gram-negative and gram-positive bacteria, indicating a broad spectrum of action of this novel compound [13].

In the year 2022, Almasirad et al. designed and synthesized twenty-five new 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylimino)thiazolidin-4-one derivatives bearing an aryl or heteroaryl methylene group on position 5 of thiazolidinone and evaluated their anti-microbial activity against *S. aureus* ATCC 6538, *MRSA* ATCC 33591, *S. epidermidis* ATCC 12228, *M. luteus* ATCC 9341, *B. subitilis* ATCC 6633, *B. cereus* PTCC 1247, *E. faecalis* ATCC 11700, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *K. pneumonia* ATCC 10031, *S. typhimurium* ATCC 14028, using ampicillin as

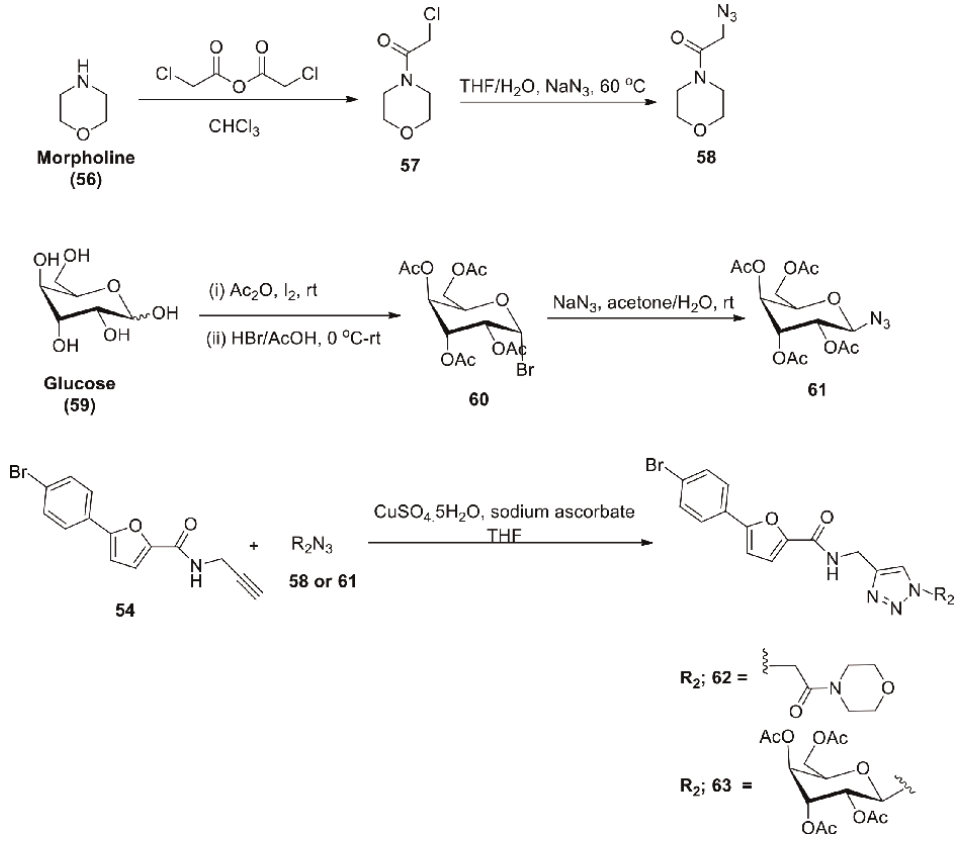


Figure 16.
Synthesis of compounds 58, 61–63.

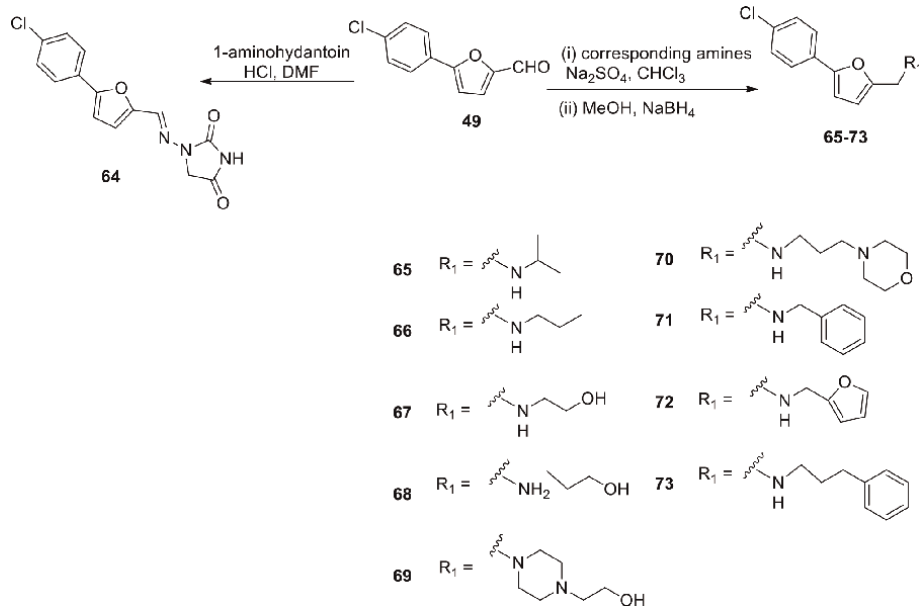


Figure 17.
*Synthesis of compounds **64–73**.*

standard. These compounds were also tested against three clinically isolated metronidazole-resistant strains of *Helicobacter pylori*.

They started a reaction with commercially available 5-nitrofuran-2-carbaldehyde **74** and thiosemicarbazide **75** in refluxing ethanol under acidic conditions to yield compound **76**, which on oxidative cyclization in the presence of ferric ammonium sulfate (FAS) formed compound **77**. Compound **77** on the reaction with chloroacetyl chloride in dry toluene at 80–90°C gave intermediate **78**, which was subsequently treated with ammonium thiocyanate in refluxing ethanol to afford 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-ylimino)thiazolidin-4-one **79**. Finally, compound **79** was reacted with respective aromatic or heteroaromatic aldehydes in the acidic conditions to obtain the final compounds **80–104** (**Figure 18**) [14].

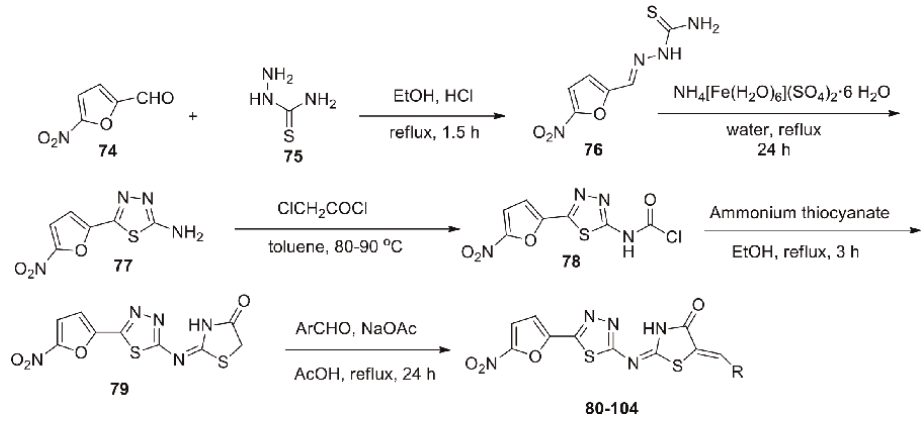


Figure 18.
*Synthesis of compounds **80–104**.*

The findings of the MIC testing revealed that most compounds had more potent antimicrobial effects against *MRSA, S. epidermidis* and *B. cereus* than the reference antibiotic, ampicillin and compounds **90** and **101** were the most active. The anti-*H. pylori* assay showed that compounds **81**, **82**, **93** and **102** had strong growth inhibitory activity against three metronidazole-resistant strains. According to their findings, it appeared that 2-(5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol2-ylimino)thiazolidin-4-one derivatives with small aryl or heteroaryl groups and non-bulky non-polar substituents are more effective at inhibiting the growth of gram-positive bacteria. On the other hand, the small polar substituents on the *para* position of aryl or heteroaryl methylene groups showed increased anti-*H. pylori* activity.

In the same year, Latha et al. focused on the synthesis of naphthofuran derivatives **110a–d** and **111a–d**, started from ethyl 1-aminonaphtho[2,1-*b*]furan-2-carboxylate **105**, which on reaction with acetyl chloride formed 1-acetamidonaphtho[2,1-*b*]furan-2-carboxylate **106**. Nitration of **106** yielded 5-nitro derivative **107**, which on treatment with hydrazine hydrate formed compound **108**. Schiff bases, bearing napthofuran derivatives **110a–d,** were prepared by the reaction of compound **108** with differently substituted benzaldehydes **109a–d** in ethanol as a solvent. Finally, the compounds **110a–d** were transformed to 1-acetamido-5-nitro-N-(5-oxo-2-phenylthiazolidin-3-yl) naphtha [2,1-*b*]furan-2-carboxamide and its derivatives **111a–d** on treatment with anhydrous $ZnCl_2$ and mercaptoacetic acid in dioxane (**Figure 19**).

The synthesized naphthofuran derivatives **110a–d** and **111a–d** were used for the study of antibacterial activity against both gram-positive bacteria *S. aureus*, *Streptococci* and gram-negative bacteria *E. coli* and *Pseudomonas.* All the compounds exhibited good activity against both gram-positive and gram-negative organisms [15].

Benfodda and team, synthesized three furan derivatives **114a–c** using Suzuki–Miyaura cross-coupling reaction starting from 2-bromo-5-nitro furan (**112**) with 2-hydroxy phenyl boronic acid (**113a**), 3-hydroxy-phenyl boronic acid (**113b**) and 4-hydroxy phenyl boronic acid (**113c**) under microwave irradiation in presence of

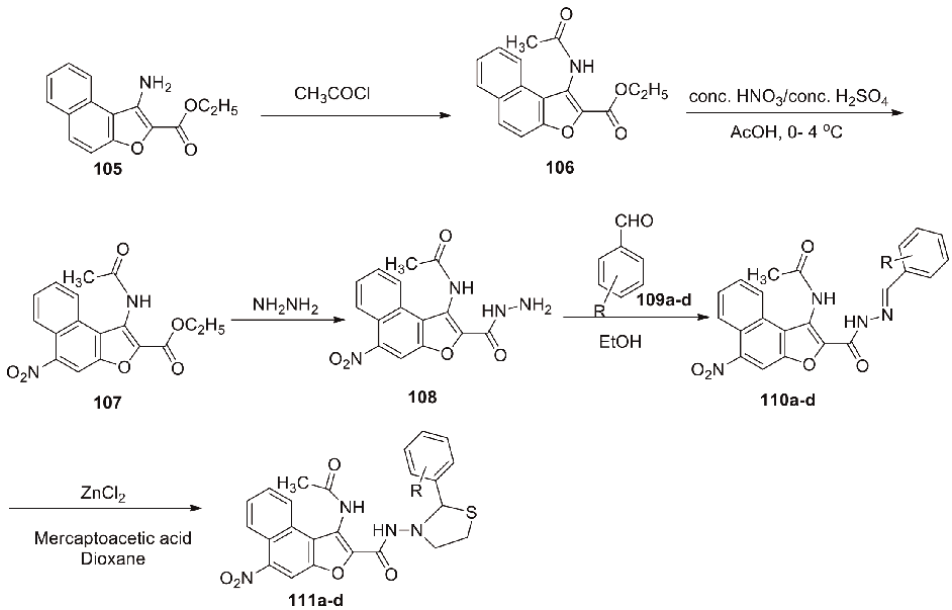


Figure 19.
Synthesis of compounds ***111a–d.***

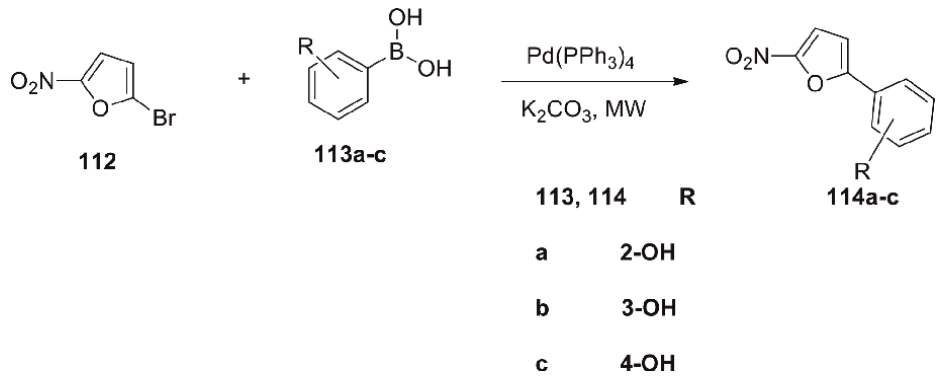


Figure 20.
Synthesis of compounds ***114a–c.***

$Pd(PPh_3)_4/K_2CO_3$ (**Figure 20**) [16]. All these synthesized compounds were tested against gram-positive bacteria *B. subtilis, S. aureus, B. anthracis, S. pyogenes, S. agalactiae, E. faecalis* and gram-negative bacteria *S. enterica, E. coli*. Compound **114b** significantly inhibited gram-positive bacteria *B. anthracis, S. pyogenes* with a minimal inhibitory value of 0.097 g/mL. and gram-negative bacteria *S. enterica* with a minimal inhibitory concentration of 0.78 g/mL.

3. Conclusion

In conclusion, the goal of this chapter was to highlight a few attractive synthetic techniques for furan derivatives that have recently been shown to have potent antibacterial properties. The many synthetic methods discussed in this chapter will motivate researchers to devise, design, and synthesize a large variety of novel compounds using the furan moiety as a useful framework to create efficient and less harmful next-generation antimicrobial drug systems. The purpose of this chapter is to pique the interest of the synthetic and medicinal chemistry communities in the quest for much-needed drugs that use the potentially bioactive furan as a building block.

Author details

Pinki Pal
Department of Chemistry, Presidency University, Bengaluru, India

*Address all correspondence to: pinkipal@gmail.com

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Chapter 7

Recent Advances in the Synthesis of Pyrrolidines

Seyedmohammad Hosseininezhad and Ali Ramazani

Abstract

The pyrrolidine ring is one of the important and valuable heterocyclic compounds. This five-membered ring containing nitrogen is also known as tetrahydropyrrole. This heterocyclic scaffold is present in medicinal and biological molecules as well as bioactive compounds and alkaloids because of the properties and characteristics of this ring. Considering the sensitivity of the subject and the importance of this structure, the recent advances in the synthesis of these types of structures are very important. In this chapter, we reviewed the recent advances in the synthesis of pyrrolidines along with the mechanisms and limitations, with a detailed discussion from 2019 to 2023.

Keywords: organic synthesis, diastereoselective, enantioselective, cyclization, 1,3-dipolar cycloaddition, [3 + 2] cycloaddition, drug delivery, spiropyrrolidines, heterocyclics, chiral

1. Introduction

The use of nitrogen-containing heterocycles is increasingly growing, which is also important in the development of clinically active drugs. These cases are evident according to the available reports [1–6]. The combination and integration of heteroatomic parts are not arbitrary but act as a tool to change the physicochemical parameters to reach the optimal ADME/Tox conditions for drug candidates [7, 8]. Tajabadi and colleagues [9] reported that out of 15,822 scaffolds, 70% of them are related to non-flat natural products. That this source can be used to design novel synthetic molecules [10]. According to the reports provided by researchers, the pyrrolidine ring has been seen abundantly in NPs, especially in alkaloids that are isolated from microorganisms or even plants [11–13]. Tetrahydropyrrole is another name for the pyrrolidine ring. This compound is one of the most important five-membered heterocycles that contain a nitrogen hetero atom. This structure acts as an active nucleus with medicinal and biological properties [14, 15]. This ring is one of the important heterocycles that are used and paid attention to in the science of drug design and pharmacy, and this is not accidental [14]. This significance is said to be no accident as this ring is present in 37 FDA-approved drugs and also ranks first among the five most common nitrogen heterocycles that are non-aromatic [16]. Today, this ring is evaluated in pharmaceutical research and development in the synthesis and development of novel drugs [17, 18]. Some pyrrolidine derivatives are used as pharmacophore groups, with some having antibacterial [19], antifungal [20], antimalarial [21], antiviral [22],

anti-inflammatory [23], antioxidant [24], and antitumoral [25] activities, and also others derivatives showed diverse enzyme inhibitory effects [26–29]. Furthermore, drugs such as pacritinib, futibatinib, and daridorexant, which have a pyrrolidine ring in their structure, were approved by the FDA in 2022 [30]. Molecules based on the pyrrolidine ring have a complex and diverse nature that allows chemists to design drugs that are more potent and less toxic by considering quantitative structure-activity relationship and structure-activity relationship [16, 18, 31]. **Figure 1** shows drugs that have a pyrrolidine ring in their structure. In addition, the application of pyrrolidine and its derivatives is wide; for instance, it is used as a chiral controller in asymmetric synthesis, as a catalyst, and also as a ligand [32–34].

Pyrrolidine rings have attracted the attention of many chemists, and this has led researchers to put new methods for the synthesis of these compounds on the agenda, so that the synthesis of pyrrolidines has been investigated by microwave, and this indicates. It is possible to synthesize these structures by various methods [35]. The synthesis of pyrrolidines is usually asymmetric, and of course, there are methods that have problems. One of the popular methods is asymmetric lithiation of N-Boc pyrrolidine. Meanwhile, the disadvantages of cryogenic temperatures and pyrophoric reagents are known [36–39]. Moreover, organocatalytic approaches, asymmetric azomethine cycloaddition to olefins, as well as aza-Michael reactions such as asymmetric addition to nitroalkenes are among the reactions that tend to produce products that require further reactions. Meanwhile, the intramolecular aza-Michael cyclization is an alternative method. In addition, pyrrolidines can be synthesized with good yield through racemic reactions [40–47]. Synthesis of spiro pyrrolidines and substituted

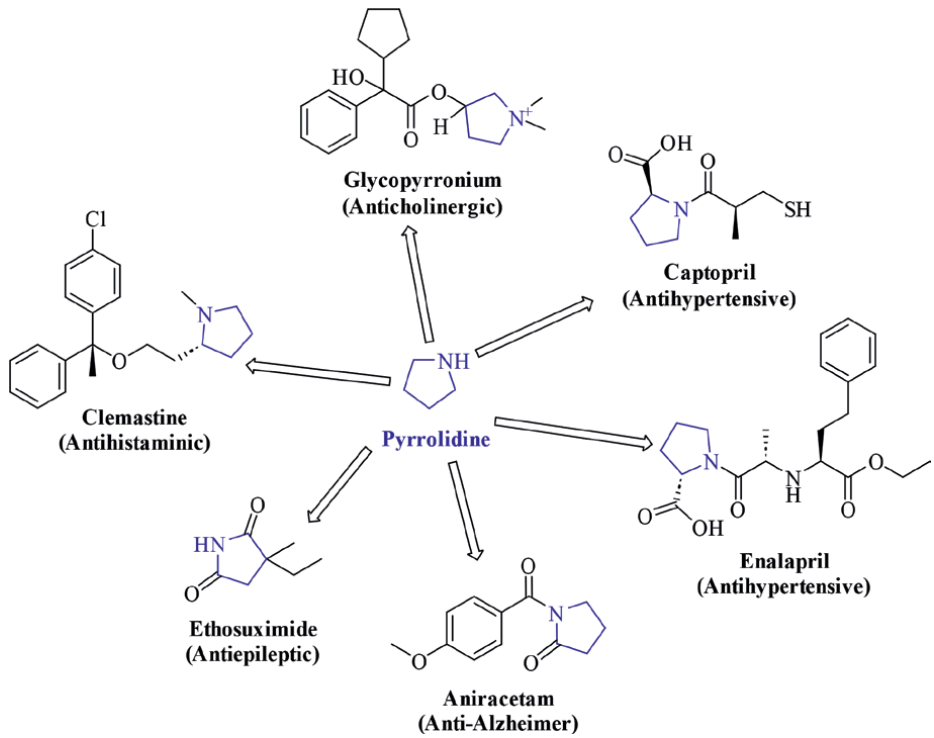

Figure 1.
The well-known drugs contain a pyrrolidine ring.

pyrrolidines has been limited to the use of chiral auxiliaries on nitrogen. Also, another case is the formation of specific substitution patterns of pyrrolidine. It should be noted that these structures have attracted the attention of many researchers due to their value in drug discovery projects. Although novel synthesis methods of pyrrolidines have been reported, few of them are asymmetric [48–63].

It is interesting to note that the synthesis of new derivatives of pyrrolidines in the form of enantiopure is of interest to chemists. Also, in medicinal chemistry, there is a great interest in custom-made amino acids that have a pyrrolidine ring [64]. Among the important and interesting procedures for the synthesis of pyrrolidines, the 1,3-dipolar cycloaddition (1,3-DC) reaction of azomethine ylides with dipolarophiles (electron-deficient) can be mentioned [65, 66]. Via this reaction, a maximum of four chirality centers is created in one step. Also, two C–C bonds are created, so it can be said that this method is economical [67]. Many studies have been reported on the use of this method, in which transition-metal-chiral ligand catalyst was used for the enantioselective synthesis of pyrrolidines. It should be noted that many studies have been reported on the use of 1,3-DC reactions for the synthesis of aryl-substituted pyrrolidines. On the other hand, there are not many studies on the synthesis of pyrrolidines substituted with heteroaryls. These deficiencies in the case of heteroaryl-substituted pyrrolidines are related to reasons such as the preparation of the desired imines, which are less stable than aryl-substituted imines. In addition, the enantioselectivity is low, which is related to the additional coordination of hetero atoms with the metal catalyst [68].

In the last several years, the reactions that have revolutionized the field of retrosynthesis are catalytic C–H functionalization reactions. Nowadays, C–H bonds have the ability to create synthetic strategies with better atom economy because these bonds are considered as functional groups. By using this newly created paradigm, it can be used to novel bond disconnections in heterocycle chemistry, and in this way, nitrogen-containing heterocycles can be synthesized [69–73]. There are three methods for the synthesis of pyrrolidines. One of them was reported by Daugulis and Chen, who were able to synthesize pyrrolidines through C–H activation reactions [74–76]. Inspired by the Hofmann-Löffler-Freytag reaction, stoichiometric methods were developed that rely on intramolecular H atom transfer. In addition, the 1,5 route is preferred [77–90]. The use of metal-catalyzed intramolecular C(sp^3)–H insertion of nitrenes is the latest approach [91–100]. Recently, enantioselective versions of this nitrene transfer have been reported. These reported reactions are limited in terms of enantioselectivity and yield. Asymmetric pyrrolidine syntheses via C–H activation process are rare occurrences [101–104].

In this chapter, we have reviewed the latest developments for the synthesis of di-, tri-, tetra-, and pentasubstituted pyrrolidines and the synthesis of 2-aryl-substituted as well as spiro pyrrolidines.

2. Spiro pyrrolidines

Zhu and co-workers synthesized spiro pyrrolidines **4** (up to 98% yield) via a reaction between aldehydes **2**, exocyclic alkenes **1**, and amino esters **3** that demonstrated highly enantio- (up to >99% ee) and diastereoselective (all >20:1 dr) (**Figure 2**). This reaction was made possible by using bifunctional phosphonium salt catalysts. This is the first instance of a catalytic asymmetric three-component reaction using a phase-transfer catalysis system, which is worth noting. This process was done via the 1,3-DC reactions [105].

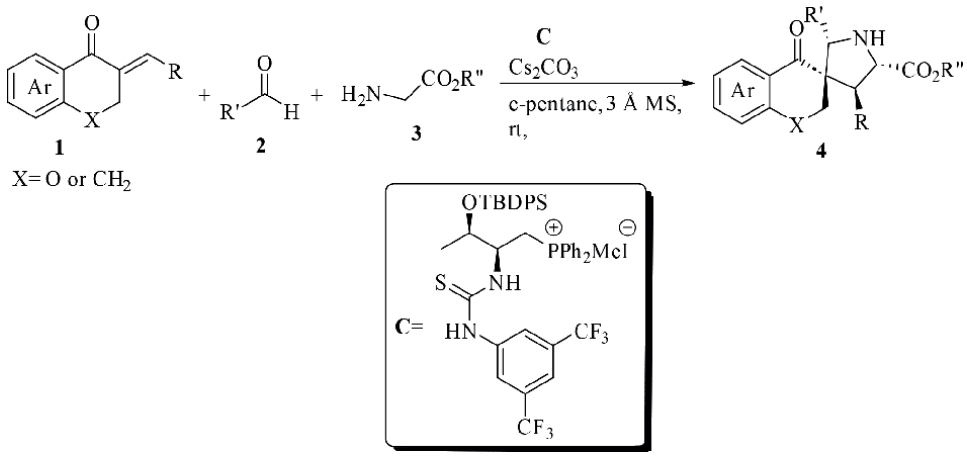

Figure 2.
Synthesis of spiro pyrrolidines by a phase-transfer catalysis system [105].

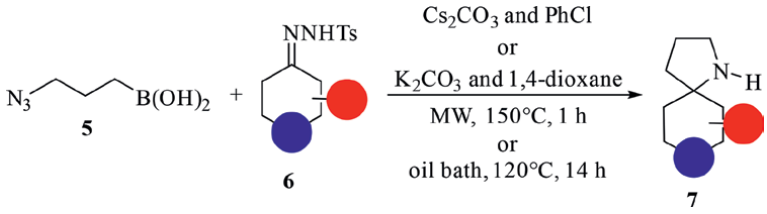

Figure 3.
Synthesis of spiropyrrolidine derivatives under two optional conditions [106].

Llpez and co-workers synthesized spiropyrrolidine derivatives **7** from arylboronic acid **5** and N-sulfonylhydrazone derivatives **6** (**Figure 3**). In this report, depending on the type of specific substrate that was chosen, the conditions were variable and optional. In this way, the choice of bases, solvents, and even the thermal conditions of the reaction can vary depending on the type of selected substrate. The reaction can be performed in the presence of two optional base namely Cs_2CO_3 or K_2CO_3, and two optional solvents, namely 1,4-dioxane or chlorobenzene. Also, heating procedure can be performed under MW conditions at 150°C for 1 h or conventional heating at 120°C for 14 h. No transition-metal catalyst was needed to proceed with the reaction. The desired spiropyrrolidine derivatives were obtained in 45–95% yields. It should be noted that this method can be utilized to generate alkaloid steroids from steroid N-tosylhydrazone derivatives [106].

3. Substituted pyrrolidines

3.1 2-Aryl-substituted pyrrolidines

In 2020, Zhang et al. by two imine reductases (IREDs), synthesized chiral 2-aryl-substituted pyrrolidine derivatives (**9** and **9'**) from imines **8** (**Figure 4**). To synthesize (R)-2-aryl-substituted pyrrolidine derivatives **9'**, imines **8** underwent ScIR in the presence of $NADP^+$, GDH, and glucose. Whereas, to synthesize (S)-2-Aryl-substituted pyrrolidines **9**, imines **8** underwent SvIR in the presence of $NADP^+$, GDH,

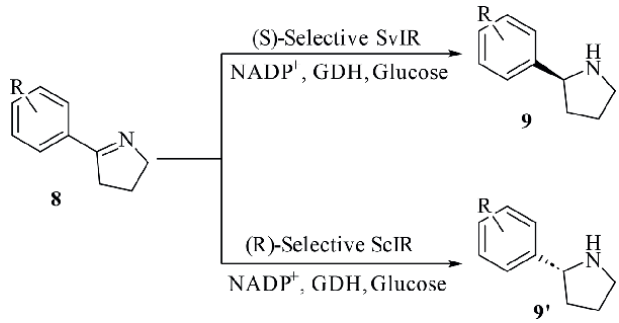

Figure 4.
Synthesis of chiral 2-aryl-substituted pyrrolidines under imine reductases (IREDs) [107].

and glucose. According to biotransformation results, to generate 2-aryl-substituted pyrroline, ScIR enzyme and SvIR enzyme were strictly stereoselective and gave the desired products in 60–80% yields with >99% ee. This study showed that IREDs can be impressive in the stereoselective synthesis of pharmaceutically relevant chiral amines [107].

Pharmaceutically relevant enantioenriched 2-aryl-substituted pyrrolidines **11** were synthesized via the enantioselective hydrosilylation of cyclic imines **10** by a chiral zinc complex (**Figure 5**), which this method was first reported by Węglarz et al. 2-aryl-substituted pyrrolidine derivatives were obtained in 72–97% yields with 62–99% ee. To generate 2-aryl-substituted pyrrolidine derivatives, (S,S)-ProPhenol L, and $ZnEt_2$ were reacted in THF at 4°C for 48 h, which containing specified imine derivatives **10** and $(EtO)_2MeSiH$. Chiral pyrrolidines produced by this method are versatile scaffolds for further functionality and have the property of being converted into pharmaceutical drugs or biologically active compounds. It should be noted that the imine containing the furan ring did not produce the desired pyrrolidine, which was probably related to the additional coordination of the Lewis acid by the hetero atom. Also, the six-membered cyclic imine derivatives were tested using this method and produced the racemic amine derivatives [108].

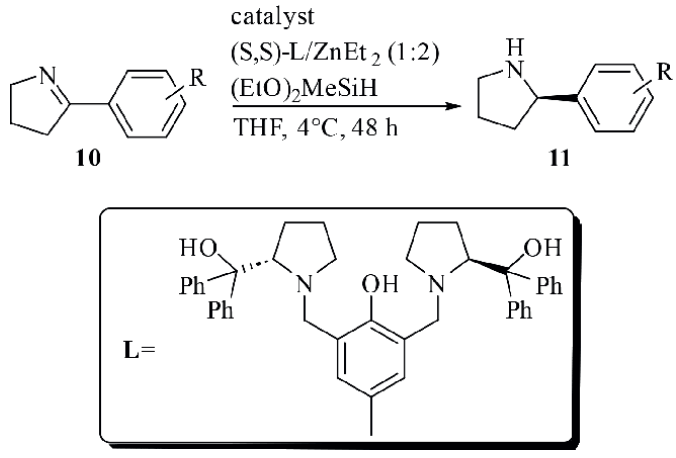

Figure 5.
Synthesis of enantioenriched 2-aryl-substituted pyrrolidines under zinc catalyst [108].

In 2022, α-chiral pyrrolidine derivatives via a highly diastereoselective, two-step continuous flow protocol were reported by Shan and co-workers (**Figure 6**). In this way, for synthesis of the desired pyrrolidine derivatives, was used N-(tert-butylsulfinyl)-chloroimine. The reaction condition was also mild. The desired pyrrolidine derivatives were obtained in 40–87% yields. In addition, the diastereoselectivities were acceptable. In the following, free pyrrolidines were generated via a removal t-Bu-sulfinyl group under acidic conditions. According to the reported results, B and (S)-B were generated from A and (S_S,S)-A. In the first step, A and (S_S,S)-A were treated with HCl (2 M in MeOH). Next, alkalization was occurred by using NaOH [109].

Florentino and co-workers synthesized 2,2-disubstituted pyrrolidine derivatives **14** via a reaction of compound **12** and compound **13** in the presence of K_2CO_3 in 1,4-dioxane at 150°C (**Figure 7**). This process is a domino reaction that involves three steps: (1) formation of diazoalkane, (2) the diazocompound intermolecular carboborylation, and (3) the azide intramolecular carborylation. The reaction resulted in the formation of a Csp^3–Csp^3 and a Csp^3–N bond on the same C atom. The reaction was performed using microwave activation and has a broad range of applicability for both reaction partners. It should be noted that the reaction proceeded without the need for any transition-metal catalyst. Moreover, the outcome of the process was found to be significantly influenced by the electronic properties of the compound **13**. The reactions with arylboronic acids that were rich in electrons resulted in the formation of pyrrolidines **14** as the only reaction product. However, a combination of pyrrolidine **14** and open-chain product **15** was produced when electron-donating groups were not present or electron-withdrawing substituents were present. It is worth noting that an increasing ratio of product **15** was obtained with the more electron-withdrawing character of the substituent. The proposed reaction mechanism has been shown in **Figure 7B**.

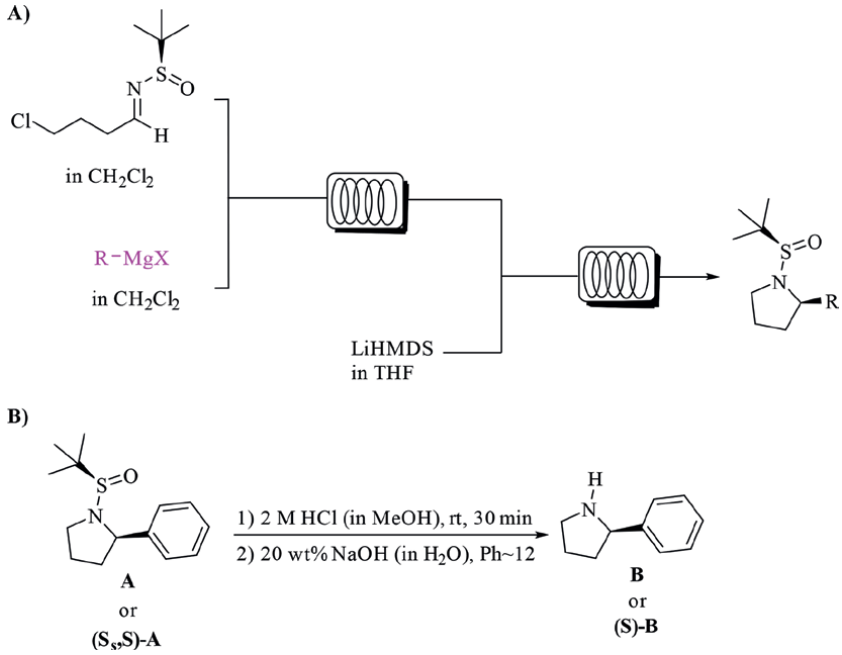

Figure 6.
(A) Synthesis of α-chiral pyrrolidine derivatives via a two-step continuous flow protocol, (B) synthesis of free pyrrolidine derivatives via a removal t-Bu-sulfinyl group [109].

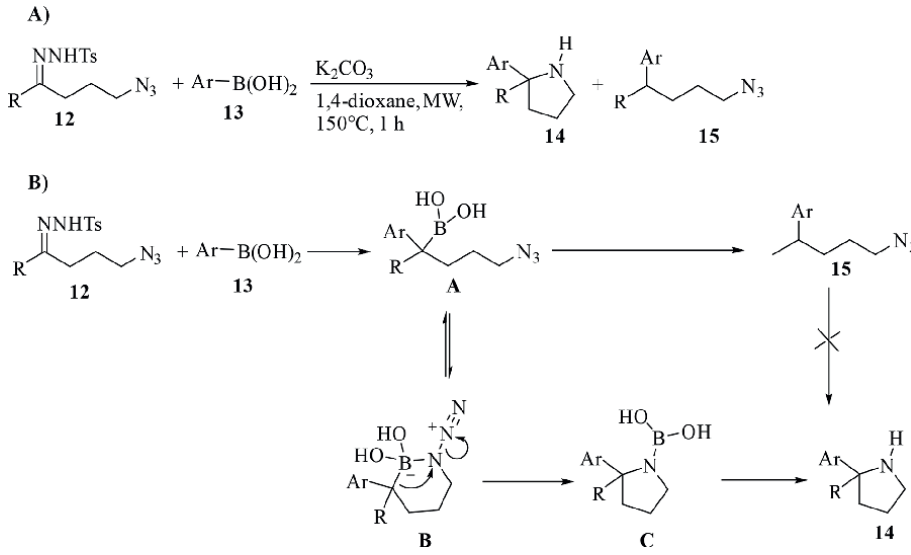

Figure 7.
(A) Synthesis of 2,2-disubstituted pyrrolidine derivatives from γ-azido-N-tosylhydrazones and (B) proposed reaction mechanism for the formation of compounds 3 and 4 [110].

The formation of compound **A** was resulted via the process of combining compound **12** with compound **13** through reductive arylation. According to this process, the desired azide **15** was obtained. When **A** generated, boronate **B** formed via the coordination of the azide to the boron center. Next, N-boropyrrolidine **C** was produced from complex **B** via the 1,2-migration of the tertiary carbon substituent, along with the removal of N_2. Lastly, upon hydrolysis of intermediate **C**, pyrrolidine **14** was generated. It is worth mentioning that the 1,2-migration reaction and the rates of the protodeboronation may determine the ratio of the products **14** and **15** [110].

3.2 Disubstituted

Lazib et al. synthesized chiral disubstituted pyrrolidine derivatives **18** from hydrocarbon derivatives **16** via two steps (**Figure 8**). Initially, a stereo- and regioselective catalytic nitrene C–H insertion was done. After that, a subsequent diastereoselective cyclization was carried out. To generate the desired **18**, amine derivatives **17** were mixed in the presence of Ag and 3-$ClC_6H_4I(mCBA)_2$ in $C_6H_5CH_3$ at 90°C. When the hypervalent iodine reagent was used by itself, the trans isomer was obtained with complete selectivity. In contrast, in the absence of the hypervalent iodine reagent, a much lower dr was given. To remove the sulfonimidoyl group, magnesium in dry MeOH was used and underwent sonication, which produced the free NH-pyrrolidines **A** and **B** in 76% and 66% yields, respectively [111].

Müller et al. synthesized pyrrolidine derivatives **21** (up to 97% yield) from olefins **19** (**Figure 9**). This reaction was done via a Pd-catalyzed cyclization. To generate pyrrolidine derivatives **21**, iodoalkynes **20** and olefin derivatives **19** were reacted together in the presence of K_2CO_3 and $Pd(PPh_3)Cl_2$ or $Pd(OAc)_2$ in DCE at 80°C for 24 h. It should be noted that the combination of **19** with **20** plays an essential role in the reaction. In addition, the reaction's remarkable feature was the versatility of the picolinamide directing group. This function is essential for the synthesis of complex alkenes based on C–H activation, as well as for the subsequent aminoalkynylation reaction [112].

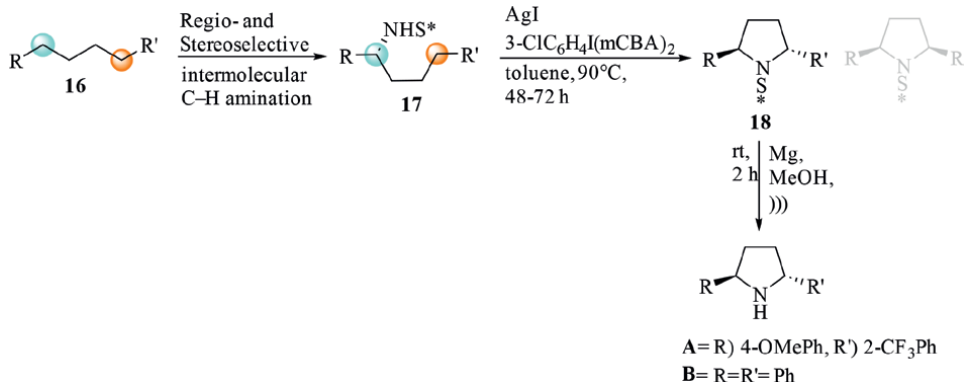

Figure 8.
Synthesis of chiral disubstituted pyrrolidine derivatives from hydrocarbons [111].

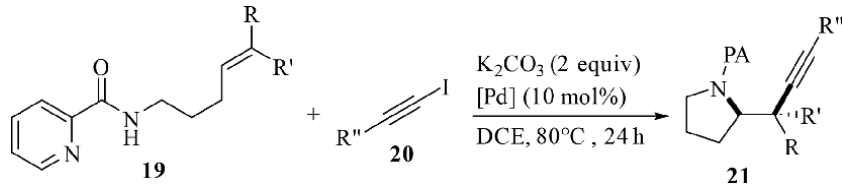

Figure 9.
Synthesis of pyrrolidine derivatives from iodoalkynes under Pd catalyst [112].

Liashuk and co-workers, via a reaction between alkenyl boronates **23** and compound **22** were synthesized the 3-borylated pyrrolidine derivatives (**24 or 24'**) (**Figure 10A**). In this method, LiF was utilized in dimethyl sulfoxide (DMSO) which the process was applicable for most β-(het)aryl-substituted alkenyl boropinacolates **A**. This process was done via 1,3-dipolar reaction and generated the desired pyrrolidine derivatives in a diastereoselective manner. Also, *N*-debenzylated products **25** (40–92%) were synthesized successfully by using $Pd(OH)_2$ in producing the desired bifunctional building blocks on a scale of up to 130 grams, which was a significant addition to the synthetic chemist's toolkit (**Figure 10B**). In the case of **A**, electron-rich p-$Me_2NC_6H_4$-, p-$MeOC_6H_4$-, and pyrazol-4-yl-substituted alkene derivatives showed improper cycloaddition partners [113].

Quintavalla et al. reported a novel procedure for the synthesis of chiral α-disubstituted β-homoprolines **30** with high enantioselectivity (**Figure 11**). In this reaction, chiral sulfinimine **26** and allyl bromide **27** were mixed in solvents (THF, DMF, and H_2O) in the presence of with or without additives at different temperatures for different times. Also, several metals were used (Mg, In, and Zn). According to the results, two products were generated. When indium was added to the reactants in THF at 60°C for 6 h, product **29** was produced as a single diastereoisomer (dr > 99:1). In contrast, when zinc/LiCl in DMF was used, product **28** was generated as a single diastereoisomer (dr > 99:1). When desired precursors **29** were produced, the desired β-homoproline derivatives **30** via a three-step tandem reaction of standard synthetic transformations (**Figure 11B**). In this reaction, by considering the presence of a quite sterically hindered quaternary in the reaction center and also ozonolysis and Pinnick oxidation, the desired products **30** were obtained in 50–55% yields [114].

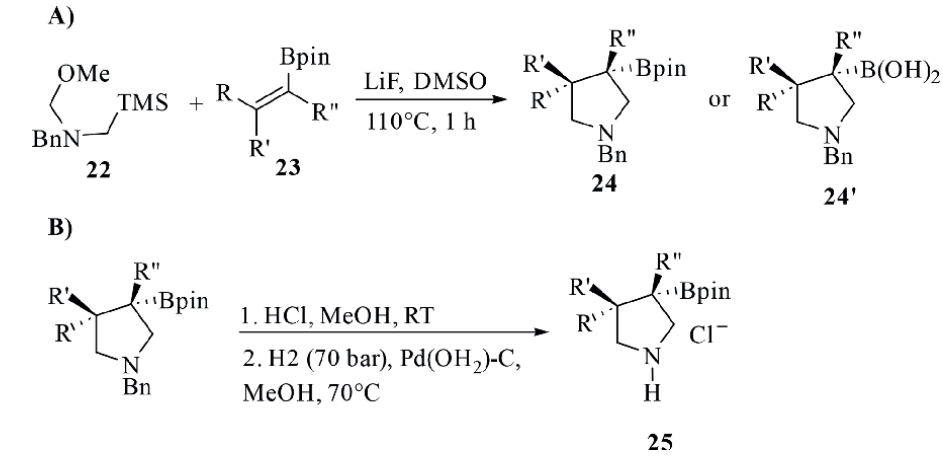

Figure 10.
(A) Synthesis of 3-borylated pyrrolidine derivatives, (B) synthesis of N-debenzylated products [113].

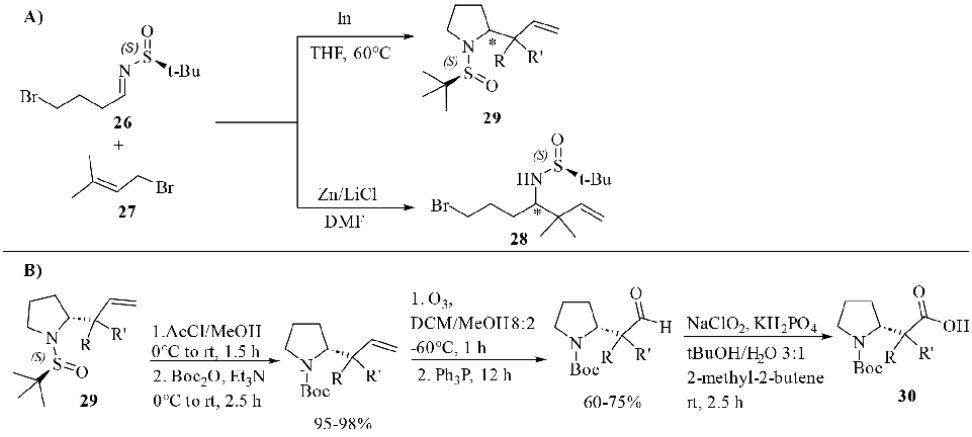

Figure 11.
(A) The prenylation reaction of chiral sulfinimine 3, (B) synthesis of α-disubstituted β-homoprolines 7 from precursors 6 [114].

3.3 Tri-substituted

Shi and co-workers developed a new method to simplify the synthesis of chiral pyrrolidine derivatives (**32** and **34**) (**Figure 12**). Chiral sulfonamide derivatives as N nucleophiles were used to induce high levels of diastereoselectivity. It should be noted that the annulating reagent **Z** was a novel and versatile crystalline compound that remained stable at room temperature for over a year. The reaction of annulation was a wide scope (**Figure 12A**), as it has produced pyrrolidine derivatives up to 84% yields with up to >95:5 dr using a variety of singly and doubly activated Michael acceptors (**Figure 12B**). The use of sulfinamide group resulted in the formation of crystalline products. This approach to annulation has enabled the synthesis of numerous pyrrolidine-containing molecules on a gram scale, paving the way for a medicinal chemistry campaign. This has significantly decreased the time and costs required to access biologically active RORγt inverse agonists [115].

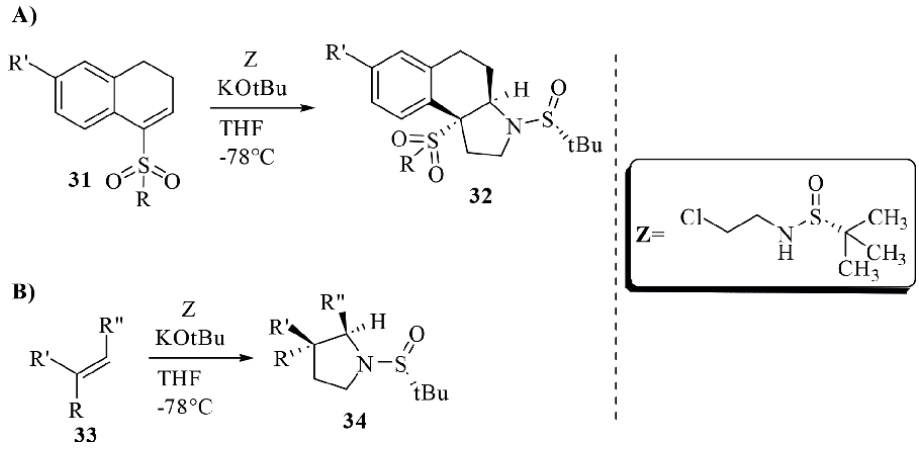

Figure 12.
Synthesis of chiral pyrrolidines from chiral sulfonamides, (A) scope of the annulation reaction, (B) scope of the annulation reaction with different Michael acceptors [115].

Kim et al. synthesized pyrrolidine derivatives **36** with excellent dia- and enantioselectivity along with vicinal quaternary-quaternary or quaternary-tertiary stereocenters via an intramolecular memory of chirality (MOC) S_N2' reaction of acyclic α-amino ester **35** (**Figure 13**). Various functional groups were tested in the reaction and produced the desired pyrrolidine derivatives in 80–99% yields with 94–>99% ee. It should be noted that pyrrolidines with vicinal stereocenters were synthesized in a single operation, and this was achieved by the effect of a single chiral center in the substrate. To generate the desired pyrrolidine derivatives, the α-substituent of α-amino ester derivatives were reacted with KHMDS in a THF/DMF mixture at −60°C for 2 h. According to the mechanism of MOC cyclization provided by authors (**Figure 14**), by deprotonation of conformer **A**, enolate **C** is formed, which is more favorable than enolate **ent-C**, which is the result of conformer **B**. It should be noted that the steric interaction between the KHMDS and the N-protecting group makes the deprotonation of **B** unfavorable. The major diastereomer **36** was obtained from enolate conformer C-I due to have the low steric repulsions. In contrast, conformer C-II has steric compression between allyl chloride and ester enolate moieties [116].

In 2022, Yoshimura et al. synthesized stereoselective pyrrolidines **38** containing a tetrasubstituted carbon stereocenter via an Au-catalyzed sequential alkyne hydroamination/iminium ion formation/allylation reaction (**Figure 15**). To generate pyrrolidines, alkyne derivatives were mixed with Ph_3PAuCl, $AgSbF_6$, $allylSiMe_3$, and benzoic acid in DCE at 60°C for 12–14 h. In this reaction, trifluoroacetyl-protected alkyne did not generate the desired pyrrolidine. It should be noted that

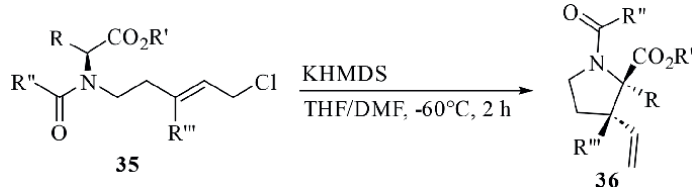

Figure 13.
Synthesis of pyrrolidine via the MOC SN2′ reaction [116].

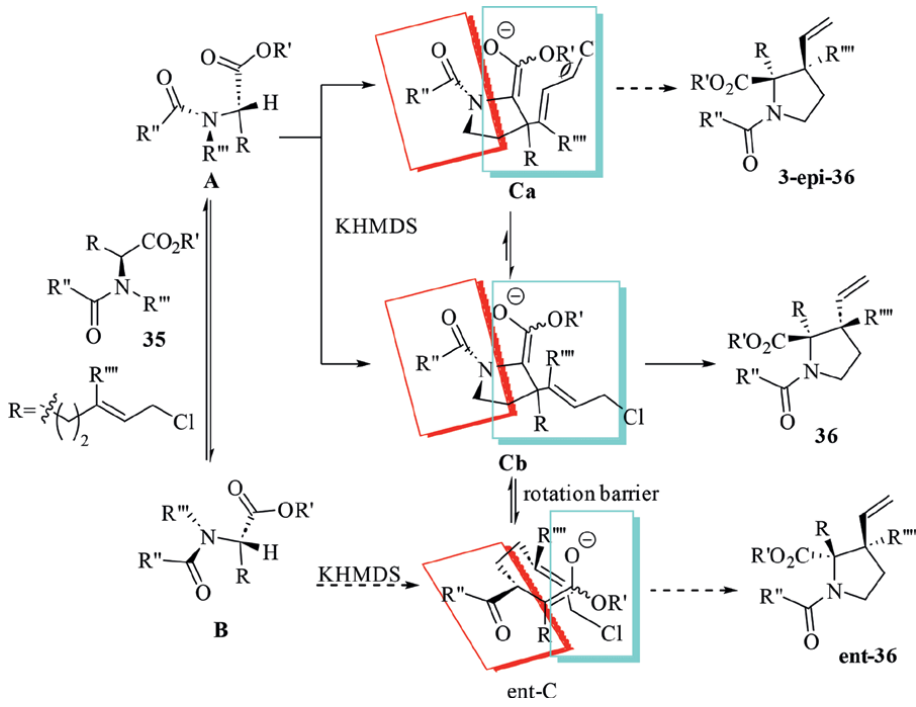

Figure 14.
Proposed mechanism for the synthesis of pyrrolidines [116].

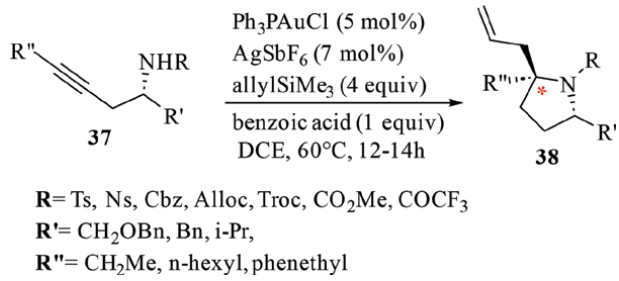

Figure 15.
Synthesis of pyrrolidine derivatives under Au catalyst [117].

electron-withdrawing protecting substituents (trifluoroacetyl and Ns) in this sequential reaction were not acceptable due to their detrimental effect on the iminium ion formation process. The results showed that the formation of iminium ions may be dependent on the double-bond electron density [117].

In 2023, pyrrolidine derivatives (up to 94% yield) containing an α-quaternary stereocenter were synthesized via an intramolecular radical amination of tertiary C(sp^3)–H bonds in N-chlorosulfonamide derivatives **39**, catalyzed by Cu and phosphoric acid (**Figure 16A**). While functionalizing tertiary C–H bonds was difficult, this method offered a new approach to this challenge by using a tandem radical intramolecular 1,5-HAT and C–N bond formation. To synthesize desired pyrrolidines, N-chlorosulfonamide derivatives in the presence of CuTc, AgOTf, and L were mixed in the desired solvent at room temperature. Various aryl-substituted sulfonyl, which

contained a variety of groups, were tolerated well in the reaction and produced the desired pyrrolidines. Also, the tertiary C(sp^3)–H moieties were tested and all substituents produced the desired pyrrolidines. To obtain enantioenriched α-quaternary pyrrolidines **40'**, N-chlorosulfonamide **39'** underwent intramolecular amination asymmetrically, with different aryl-substituted sulfonyl groups present and produced the desired α-quaternary pyrrolidine derivatives with 61–81% ee (**Figure 16B**). According to the proposed mechanism (**Figure 17**), Cu(I) and L, via a single-electron transfer process with **39** generated the compound **A** and Cu(II) phosphate. In this step, the formed chloride anion, along with AgCl, is removed by Ag(I) salt. Then, radical **A** generated tertiary C-centered radical intermediate **B** via the 1,5-HAT process. Finally, the desired **40** was obtained via the reaction between intermediate **B** and Cu(II) phosphate, which after produced the intermediate **C**. It should be noted that the enantioselectivity of the amination reaction was controlled by a catalytic system consisting of Cu(I) and L [118].

3.4 Tetrasubstituted

Rigotti and co-workers described a simple and flexible method for producing pyrrolidine-BODIPY derivatives **43** with high yields (up to 89%) and excellent stereoselectivities (up to 98% ee) (**Figure 18**). In this type of reaction, the BODIPY

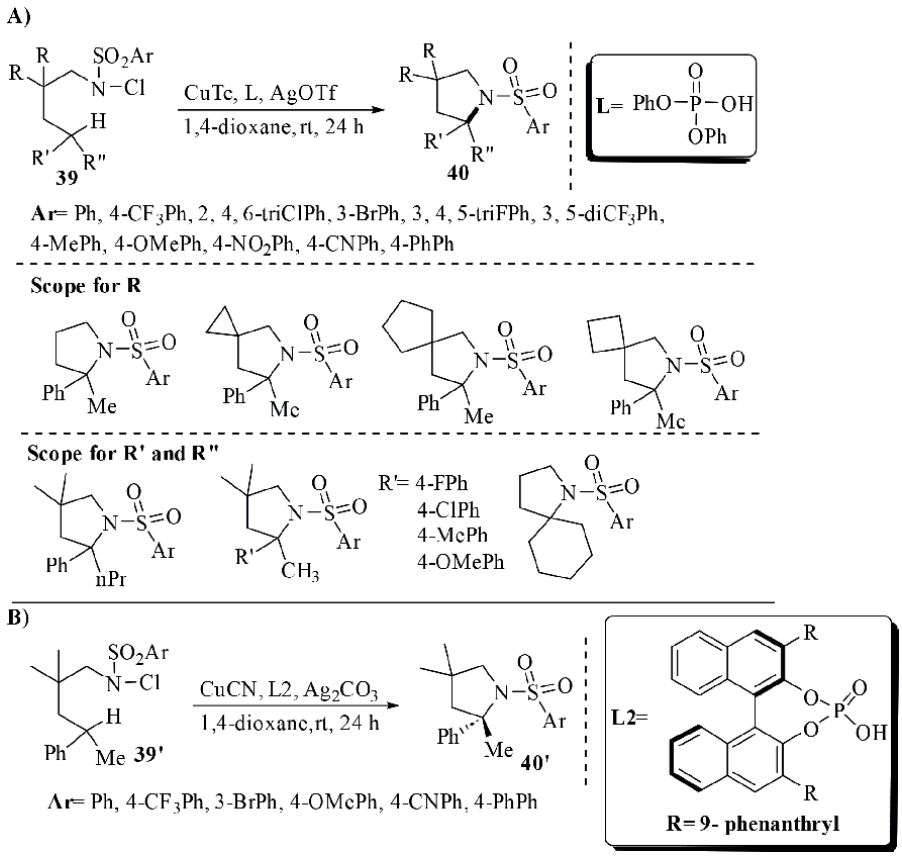

Figure 16.
(A) The investigation of intramolecular amination of tertiary C(sp^3)–H bonds, (B) the investigation of enantioselective tertiary C(sp^3)–H amination [118].

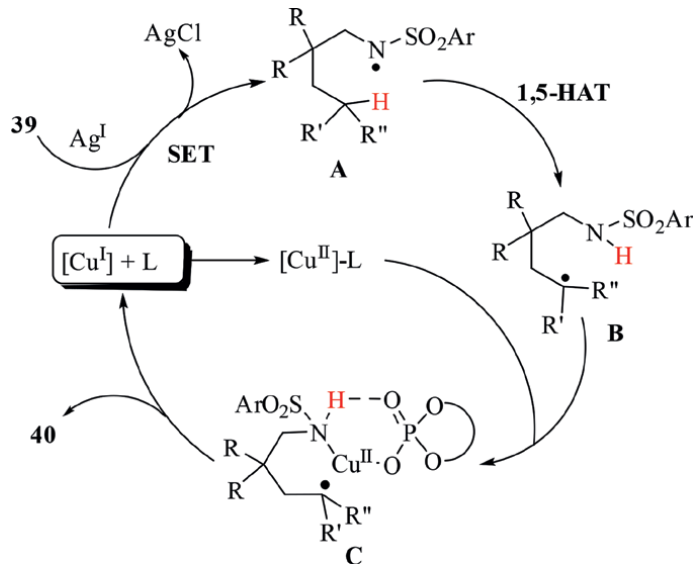

Figure 17.
Proposed mechanism for the synthesis of pyrrolidine derivatives containing an α-quaternary stereocenter [118].

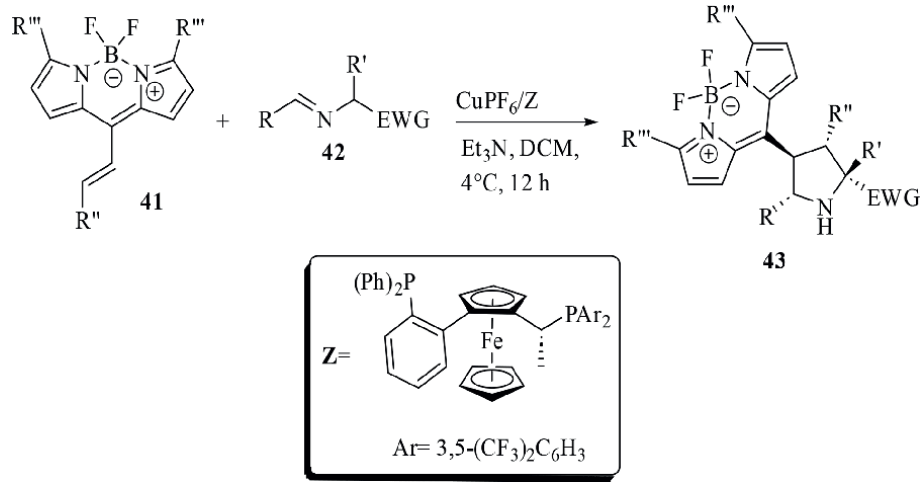

Figure 18.
Synthesis of pyrrolidine-BODIPY derivatives from imines and double bonds [119].

group was discovered to activate double bonds by serving as an electron-withdrawing group. However, DFT results showed that the BODIPY moiety was less reactive than a NO_2 group in this specific [3 + 2] cycloaddition. The reason for this was that the intermediate produced in the first step of the cycloaddition was highly stable in the case of BODIPY-alkene due to the significant charge separation between the catalyst/Z/dipole complex and the dipolarophile, resulting in a strong electrostatic stabilization. Three distinct cell lines were used to test the derivatives and were found to selectively accumulate in the lysosomes. The synthesized BODIPY derivatives had excellent fluorescent properties and could be utilized for imaging these organelles in live cells [119].

Amador et al. reported a new procedure for the synthesis of pyrrolidines **46** via the [3 + 2] cycloaddition of cyclopropyl ketone derivatives **44** and hydrazine derivatives **45** (**Figure 19**). Photoredox catalysis was used in the reaction, which produced a distonic radical anion intermediate, and this intermediate could engage relatively unreactive C−N bonds. Finally, this process can obviate the need for an amine co-reductant. To generate pyrrolidines, cyclopropane (1 equiv) and hydrazone (2 equiv) were mixed together in the presence of $Yb(OTf)_3$ and $Ir(4\text{-}CF_3\text{-}ppy)_3$ in THF underwent blue LED irradiation. The desired pyrrolidines were generated in 31–85% yields [120].

In 2021, Beksultanova et al. synthesized enantioselective heteroaryl-substituted pyrrolidines **49** under ferrocenyl aziridinyl methanol-Ag catalyst systems (**Figure 20**). To obtain desired pyrrolidines, imine derivatives **48**, and dipolarophile derivatives **47** were mixed together in the presence of the catalyst in toluene under nitrogen atmosphere. This reaction was carried out by 1,3-DC reaction of azomethine ylide derivatives, which contain electron-deficient dipolarophiles. According to the results, enantioselectivities of the reaction were low (ee:4-76%) but yields were acceptable to good (22–89%).It should be noted that the ee of solid cycloadducts was increased by crystallization up to 95% [68].

Iddum and co-workers synthesized tetrasubstituted pyrrolidine derivatives via the reaction of aryl carbaldehyde, chalcone, and primary amine (**Figure 21**). In this three-component reaction, different bases were tested such as Cs_2CO_3, K_2CO_3, and $NaHCO_3$. Also, I_2 in toluene as an additive was tested. When Cs_2CO_3 was used as a base, the desired pyrrolidine derivatives **50** were obtained in 47–74% yields with a high diastereomeric ratio (>9:1). It is probable that the reaction consisted of a [3 + 2] cycloaddition reaction between chalcone and 1,3-dipolar N-benzylidene benzylamine, which was generated in situ from aldehyde and amine. Importantly, when 1,4-diazabicyclo[2.2.2]octane (DABCO) as a base was used in the reaction, pyrrolidine **51** was produced as a major product in 55–69% yields, along with product **52**, but the amount of it was traced. Also, no pyrrolidine **50** was detected in this reaction. It should be noted that when using trimethylamine instead of DABCO, the yields were decreased. When 4-dimethylaminopyridine (DMAP) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was used as a base in the reaction with two equivalents of aryl carbaldehyde, the fully substituted dihydropyrrole 8 in 52–76% yields was observed [121].

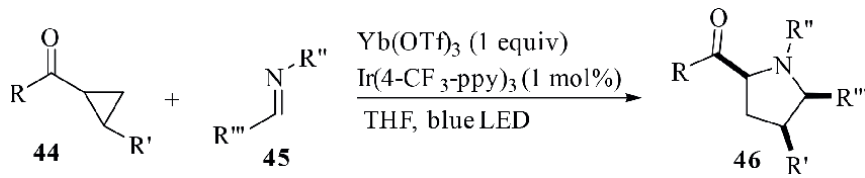

Figure 19.
Synthesis of pyrrolidines under photoredox catalysis [120].

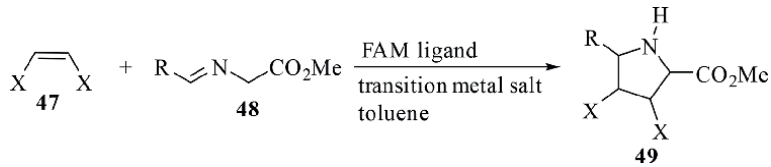

Figure 20.
Synthesis of pyrrolidines under ferrocenyl aziridinyl methanol-Ag catalyst systems [68].

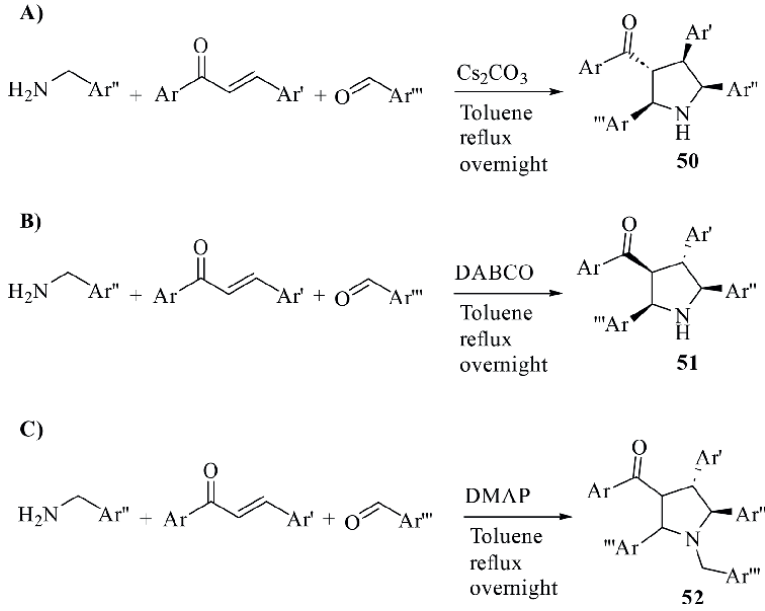

Figure 21.
(A) Synthesis of tetrasubstituted pyrrolidine derivatives under Cs_2CO_3, (B) synthesis of tetrasubstituted pyrrolidine derivatives under DABCO, (C) synthesis of pyrrolidine derivatives under DMAP [121].

Zhang et al. synthesized highly functionalized chiral pyrrolidine derivatives **55** via a phosphine-catalyzed highly diastereoselective and enantioselective (3 + 2) annulation between N-tosylaldimine derivatives **53** and vinylcyclopropane derivatives **54** up to >20:1 dr and 98% ee, respectively (**Figure 22**). To produce the pyrrolidines, N-tosylaldimine derivatives and vinylcyclopropane derivatives reacted together in the presence of phosphine **A**, and $MgSO_4$ in PhCl at room temperature for 4 h. This method pioneered the use of cyclopropane substrates in phosphine-mediated asymmetric transformation. Results demonstrated that cyclopropanes had excellent potential as powerful synthons in phosphine catalysis [122].

Imino ester derivatives **56** and chiral N-tert-butanesulfinyl imine derivatives **57** were reacted with Ag_2CO_3 in $C_6H_5CH_3$ at room temperature and synthesized the densely substituted pyrrolidines **58** (30–83% yields and up to >99% dr) via a [3 + 2] cycloaddition reaction (**Figure 23**). This reaction occurred by 1,3-dipolar cycloadditions with azomethylene ylides. It should be noted that the use of Ag_2CO_3 led to proline derivatives with up to four stereogenic centers in the pyrrolidine

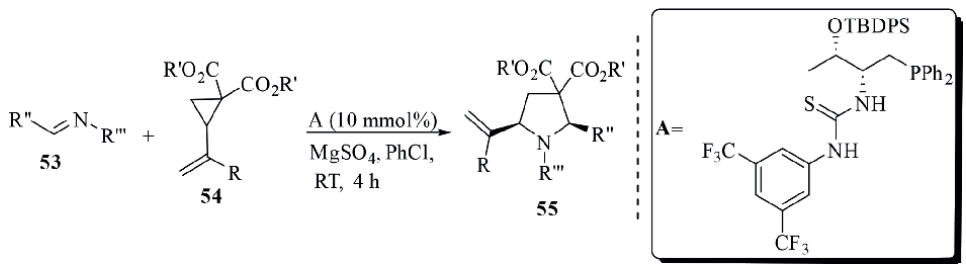

Figure 22.
Synthesis of chiral pyrrolidine under phosphine catalyst [122].

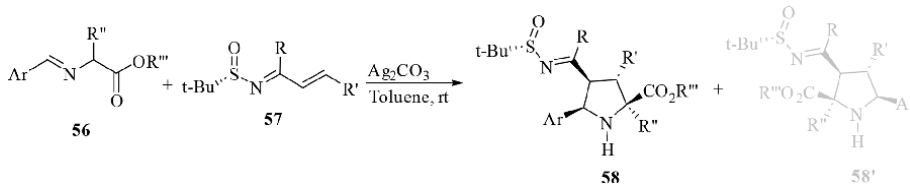

Figure 23.
Diastereoselective synthesis of densely substituted pyrrolidines in the presence of Ag_2CO_3 by 1,3-dipolar cycloadditions [123].

ring with high regio and diastereoselectivities. In addition, the sulfinyl group with (S)-configuration could induce a (2S,3R,4S,5R) absolute configuration in the final pyrrolidine derivatives [123].

Beksultanova and Dogan synthesized chiral pyrrolidine derivatives **61** via the reaction between N-methylmaleimide **59** as the dipolarophile and aryl-substituted azomethine ylides **60** in the presence of AgOAc and ligand in toluene from range of 0°C to room temperature for 20 h (**Figure 24A**). It should be noted that this process was done via the 1,3-dipolar cycloaddition reactions. Enantioselectivities of the corresponding products were acceptable and led to endo products. In addition, the yields of the desired products were obtained up to 98%. In the case of the ee, upon crystallization, it can be raised up to >99%. According to the reported transition situation, cycloaddition took place from the si-side of the imine nitrogen. This process was done via a coordination between Ag ion and imine, dipolarophile, and the corresponding ligand (**Figure 24B**) [124].

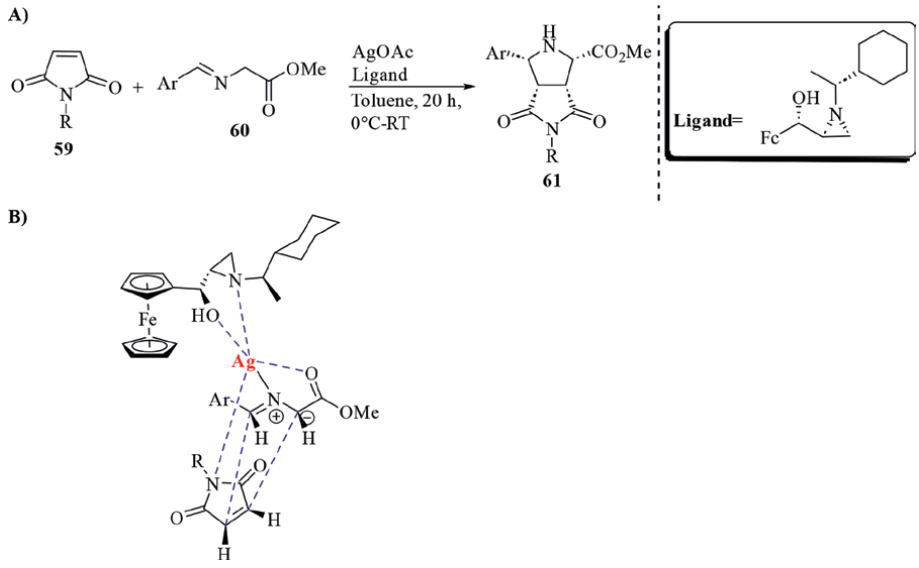

Figure 24.
(A) Synthesis of chiral pyrrolidine derivatives via the 1,3-dipolar cycloaddition reactions, (B) proposed transition state model [124].

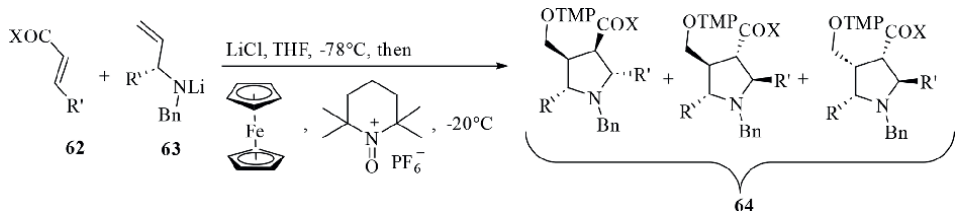

Figure 25.
Synthesis of pentasubstituted pyrrolidines under an N-oxopiperidinium salt [125].

3.5 Pentasubstituted

Hidasová and co-workers developed a method for synthesizing N,2,3,4,5-pentasubstituted pyrrolidine derivatives **64** in a single step (**Figure 25**). The synthesis involved a series of chemical reactions including oxidative single-electron transfer-catalyzed tandemaza-Michael addition, radical 5-exo cyclization, and oxygenation. The use of chiral allylamines asymmetric conjugate additions to β-substituted-α,β-unsaturated esters determined the stereochemistry of the product. The substituent R′ determined the configuration of the two stereocenters next to the amine function, while the radical 5-exo cyclization step generated two more stereocenters in a diastereoselective manner. This method allowed for the synthesis of pyrrolidine derivatives with four contiguous stereogenic centers in a single step. In this reaction, lithium amide derivatives **62** and compounds **63** were reacted together in the presence of ferrocene and N-oxopiperidinium salt, which acted concomitantly as oxygenation reagent and stoichiometric oxidant. The desired pyrrolidine derivatives were obtained in 33–66% yields and good to excellent diastereoselectivity [125].

4. Conclusion

The latest and most innovative methods reported by researchers from 2019 to 2023 are discussed in this chapter. Various procedures have been reported for the synthesis of these structures, each of which has unique characteristics. The types of substrates were effective in choosing the type of solvent, the type of base, and even the reaction conditions. IREDs were effective in the stereoselective synthesis of pyrrolidines. Some of the reported reactions were also used in gram scale and this showed the applicability of these methods. The use of green methods such as microwaves was also effective in the synthesis of these structures, and good results have been reported by researchers, so it is suggested that studies move toward the use of green methods. Various reactions in which catalysts were used were also reported, but the methods that reached the pyrrolidine field without the use of catalysts were superior because they are better in terms of economic efficiency and also do not have the complexity of interpreting the reaction mechanism and ease of work. Therefore, it is suggested to design reactions that minimize the need for catalysts or at least use green catalysts to improve the reactions.

Conflict of interest

The authors declare no conflict of interest.

Author details

Seyedmohammad Hosseininezhad[1] and Ali Ramazani[1,2*]

1 The Organic Chemistry Research Laboratory (OCRL), Department of Chemistry, Faculty of Science, University of Zanjan, Zanjan, Iran

2 The Convergent Sciences and Technologies Laboratory (CSTL), Research Institute of Modern Biological Techniques (RIMBT), University of Zanjan, Zanjan, Iran

*Address all correspondence to: aliramazani@gmail.com

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