Section C

Chapter 6. Introduction of (1$H$-benzo[d]imidazol-2-yl)amino-pyrimidine derivatives
6.0 Introduction

Benzimidazole is a fused aromatic imidazole ring system where a benzene ring is fused to the 4 and 5 positions of an imidazole ring. Benzimidazoles are also known as benziminazoles and 1,3-benzodiazoles.\textsuperscript{1, 2} (Figure 1) They possess both acidic and basic characteristics. The NH group present in benzimidazoles is relatively strongly acidic and also weakly basic. Another characteristic of benzimidazoles is that they have the capacity to form salts. Benzimidazoles with unsubstituted NH groups exhibit fast prototropic tautomerism, which leads to equilibrium mixtures of asymmetrically substituted compounds.\textsuperscript{1}

![Figure 1](image)

The benzimidazole scaffold is a useful structural motif for the development of molecules of pharmaceutical or biological interest.\textsuperscript{3–5} Appropriately substituted benzimidazole derivatives have found diverse therapeutic applications such as in antiulcers,\textsuperscript{3} antihypertensives,\textsuperscript{6,7} anti-HIV,\textsuperscript{8} antiinflammatory,\textsuperscript{9} anticancers,\textsuperscript{10} antioxidant,\textsuperscript{11} antitrichinellosis,\textsuperscript{12} and anxiolytics.\textsuperscript{13} The optimization of benzimidazole-based structures has resulted in various drugs which are currently in the market, such as omeprazole 1 (proton pump inhibitor), pimobendan 2 (ionodilator), and mebendazole 3 (anthelmintic) (Figure 2).

![Figure 2](image)
6.1 Benzimidazoles

Benzimidazole-derived alkaloids are rare in nature, and only few examples of these natural products can be found in the literature. On other hand, the occurrence of the imidazole skeleton in various natural sources is quite common.\textsuperscript{14–16} The benzimidazole alkaloid kealiiquinone (Figure 3) was isolated from a yellow button-like Micronesian sponge species of \textit{Leucetta}.\textsuperscript{16}

Recently, Nakamura \textit{et al.} has successfully synthesized a regioisomer of kealiiquinone.\textsuperscript{17} The kealiiquinone 4 and its synthetic regioisomer 5 both have relatively weak activities against a panel of 39 human cancer cell lines but are considered to have a unique mechanism of action.\textsuperscript{17}

![Figure 3 Benzimidazole alkaloid and its regioisomer](image)

Makaluvamines (pyrroloiminoquinones) 6 (Figure 4) was isolated from a Fijian sponge in the early 1990s display in vitro cytotoxicity against human colon tumor cell lines and also inhibited human topoisomerase II in vitro. The benzimidazole analog of this indole-based marine natural product, imidazoquinoxalinone 7, has been synthesized by LaBarbera D.V. and co-workers.\textsuperscript{18}

![Figure 4](image)
In comparison with the natural inositol 1,4,5-triphosphate, the adenophostins 8 (Figure 5) exhibit higher receptor binding activity and Ca\(^{2+}\) mobilizing potencies and thus have significant biological importance. A total synthesis of a benzimidazole analog of adenophostin A 9 was described by Shuto et al.\(^{19}\)

![Figure 5 Adenophostin and its benzimidazole analog](image)

### 6.2 Synthetic Methodologies for Benzimidazoles

Most commonly benzimidazoles have been prepared from the reaction of 1,2-diaminobenzenes with carboxylic acids under harsh dehydrating reaction conditions, utilized strong acids such as polyphosphoric acid, hydrochloric acid, boric acid, or p-toluenesulfonic acid.\(^{20}\) However, the use of milder reagents, particularly Lewis acids,\(^{21}\) inorganic clays,\(^{22}\) or mineral acids,\(^{23}\) has improved both the yield and purity of this reaction.\(^{24}\) On the other hand, the synthesis of benzimidazoles via the condensation of 1,2-diaminobenzenes with aldehydes requires an oxidative reagent to generate the benzimidazole nucleus. Various oxidative reagents, such as nitrobenzene, benzoquinone, sodium metabisulfite, mercuric oxide, lead tetraacetate, iodine, copper(II) acetate, indium perfluorooctane sulfonates, ytterbium perfluorooctane sulfonates, and even air, have been employed for this purpose.\(^{25}\) Moreover, a variety of benzimidazoles could also be produced via coupling of 1,2-diaminobenzenes with carboxylic acid derivatives such as nitriles, imidates, orthoesters, anhydrides or lactones.\(^{26}\)

In recent years, some innovative and improved pathways for the synthesis of benzimidazoles have been developed and these are discussed in the following.
A palladium-catalyzed N-arylation reaction provided a novel synthesis of benzimidazoles from (o-bromophenyl)amidine precursors under microwave irradiation. The route was found to be flexible with respect to various substituents and allowed for the preparation of highly substituted benzimidazoles, including N-substituted examples (Scheme 1). The method was later improved and optimized to achieve the rapid formation of benzimidazoles in high yield. It has been found that 50% aqueous dimethyl ether (DME) is an optimal solvent for the reaction and that catalyst loading of palladium can be reduced to 1 mol%.

Recently, 2-alkyl- and 2-aryl-substituted benzimidazole derivatives have been synthesized from 1,2-diaminobenzene and its corresponding acids in the presence of polyphosphoric acid using microwave assisted methods (Scheme 2). The reaction time required for the synthesis of benzimidazole derivatives was reduced to minutes by this method compared to conventional synthesis, which required up to four hours of heating to complete the reaction. Furthermore, it was found that the application of microwave irradiation increased yields by 10–50%.

Conventional condensation of 1,2-diaminobenzene with 6-fluoro-3,4-dihydro-2H-chroman-2-carboxylic acid under Phillip’s conditions or using Eaton’s reagent (1 : 10 mixture of phosphorus pentoxide/methanesulfonic acid) yielded 2-(6-fluorochroman-2-yl)-1H-benzimidazole (Scheme 3).
Recently, microwave-assisted synthesis of 2-(alkyloxyaryl)-1H-benzimidazole derivatives related to the natural stilbenoid family has been reported (Scheme 4).\(^{31}\)

\[\text{Scheme 4}\]

Recently, a facile, rapid one-pot procedure for the generation of 2-substituted benzimidazoles directly from 2-nitroanilines using a microwave procedure has been demonstrated (Scheme 5). An advantage of this approach is that the intermediate N-acyl derivatives need not be isolated prior to cyclization.\(^{32}\)

\[\text{Scheme 5}\]

Classical condensation-cyclization reactions using 1,2-diaminobenzenes, 2-mercaptoacetic acid and appropriately substituted aromatic aldehydes in dry benzene under reflux required a long reaction time to afford the thiazobenzimidazoles, which are potent anti-HIV agents. (Scheme 6)\(^{33}\)
Functionalization of C–H bonds of heterocycles to C-arylation is an important synthetic reaction and is used to build important bioactive structures. Palladium catalyst and copper-mediated C-2 arylations of benzimidazole with aryl iodides under ligandless and base-free conditions have been described by Bellina et al. (Scheme 7).34

6.3 Pharmacological profile of Benzimidazoles

6.3.1 Antibacterial and Antifungal Agents

2-substituted benzimidazole derivatives are known to possess varied biological activities.35 Recently, an efficient and rapid synthesis of novel benzimidazole azetidin-2-ones 10 has been established,36 and antibacterial screening revealed that all newly synthesized azetidin-2-ones 10 exhibited potent antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. Among all of the compounds investigated, 10i and 10j exhibited the greatest antibacterial activity against Gram-negative *E. coli* as compared to the antibiotic streptomycin.36 In addition, 5-fluoro benzimidazole carboxamide derivatives 1137 and benzimidazole isoxazolines 12 38 were reported to show antibacterial and antifungal activities.
6.3.2 Anthelmintic Agents

Bearing in mind previous benzimidazole anthelmintics (e.g., albendazole, mebendazole), the search for new anthelmintic drugs is being actively pursued. Synthetic benzimidazole piperazine derivatives exhibited 50% anthelmintic activity in mice infected with *Syphacia obvelata*. Furthermore, piperazine derivatives of 5(6)-substituted-(1H-benzimidazol-2-ylthio) acetic acids \(13-15\) \(^{40}\) and benzimidazolyl crotonic acid anilide \(16\) have shown good anthelmintic activity \(^{41}\) (Figure 7).
6.3.3 Anti-inflammatory and Antiulcer Agents

Structure–activity relationship studies of the 5,6-dialkoxy-2-thiobenzimidazole derivatives 17 have revealed that compounds 17a–k possess pronounced anti-inflammatory properties (Figure 10). Using the carrageenan model, the most significant anti-inflammatory effects were observed for compounds 17a, 17d, 17h, 17i, and 17j. While using the bentonite model, the maximum activities were observed for compounds 17e and 17h. These results indicated that benzimidazoles are promising leads for the development of new anti-inflammatory agents.
In addition, N-benzoyl and N-tosyl benzimidazole compounds 18 showed significant anti-inflammatory activity, as indicated by ear swelling induced by xylene in mice, and their ulcer indices were all lower than those of aspirin. Furthermore, N-morpholinomethylbenzimidazole 19 and its derivatives have been recently reported to show significant anti-inflammatory activity.

6.3.4 Cytotoxic and Antitumor Agents

Novel bisbenzimidazoles with general formula 20–23 incorporating benzimidazole, pyridoimidazole, and imidazoquinone moieties as one of the units of bisbenzimidazole with a piperazinyl functional group have been synthesized (Figure 9). The series of bisbenzimidazoles contains different leaving groups along with \( p \)-methoxy substituents. The latter may be expected to have some influence on the nitrogen lone pair and consequently on the binding characteristics of the ligand. These novel bisbenzimidazoles are found to be actively cytotoxic against many human cancer cell lines, with GI\(_{50}\) values of between 0.01 and 100 \( \mu \)M, especially in the cases of renal cancer, CNS cancer, colon cancer, melanoma, and breast cancer cell lines.
In addition, the alkyl-linked bisbenzimidazole 24\textsuperscript{46} and thiazolylbenzimidazole-4,7-diones 25\textsuperscript{47} exhibited cytotoxic activity against tumor cell lines (Figure 10).

Moreover, novel head-to-head bisbenzimidazole compound 26 showed potent growth inhibition in human ovarian carcinoma cell lines (IC\textsubscript{50} = 200–300 nM), with no significant cross-resistance in two acquired cisplatin-resistant cell lines and a low level of cross-resistance in the \textit{p}-glycoprotein over expressing doxorubicin-resistant cell line. In addition, compound 26 was found to have significant in vivo activity in the allowed fiber assay and tumor xenografts.\textsuperscript{48-50}
6.4 Introduction of Pyrimidine

Pyrimidine is a heterocyclic aromatic organic compound similar to benzene and pyridine, containing two nitrogen atoms at positions 1 and 3 of the six-member ring.\textsuperscript{51} It is isomeric with two other forms of diazine. A pyrimidine has many properties in common with pyridine, as the number of nitrogen atoms in the ring increases the ring pi (\(\pi\)) electrons become less energetic and electrophilic aromatic substitution gets more difficult while nucleophilic aromatic substitution gets easier.

Azaheterocycles constitute a very important class of compounds. In particular, pyrimidine derivatives include a large number of natural products, pharmaceuticals, and functional materials (Figure 13).\textsuperscript{52} Several examples of pharmaceutically important compounds include trimethoprim 27,\textsuperscript{53} sulfadiazine 28,\textsuperscript{54} Gleevec (29, imatinib mesilate),\textsuperscript{55} and Xeloda (30, capecitabine).\textsuperscript{56}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig12}
\caption{Figure 12}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig13}
\caption{Figure 13 Representative compounds containing a pyrimidine substructure.}
\end{figure}
6.5 Pyrimidine Natural Products

In nature, the pyrimidine ring is synthesized from glutamine, bicarbonate, and aspartate.\textsuperscript{57} These starting materials are converted to orotate (31, Figure 14). Several (mainly uracil, thymine and cytosine) pyrimidines have been isolated from the nucleic acid hydrolysates. The nucleic acid are essential constituent of all cell and thus of all living matter cytosine is found to be present in both types of nucleic acid i.e. ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) while uracil present only in RNA and thymine only in DNA.\textsuperscript{58}

![Figure 14](image)

In addition, Pyrimidine ring is also found in vitamin like thiamine 32, riboflavin 33 and folic acid 34.\textsuperscript{59} Barbitone1 35, the first barbiturate hypnotic sedative and anticonvulsant is a pyrimidine derivative.\textsuperscript{60}

![Figure 15](image)
6.6 Synthetic Methodologies for Pyrimidines

In 1818, Brugnatelli synthesized the first pyrimidine derivative, alloxan by nitric acid oxidative degradation of uric acid (Scheme 8).\textsuperscript{61} Another early report, by Frankland and Kolbe in 1848, described the first synthesis of a pyrimidine cyanalkine by heating propionitrile with potassium metal (Scheme 8).\textsuperscript{62}

![Scheme 8 Early reports on the synthesis of pyrimidine](image)

Nitriles are a common N–C source and have been used to form pyrimidines in many syntheses. Cyanamide is a particularly useful nitrile derivative in the synthesis of pyrimidines as illustrated in Scheme 9.\textsuperscript{63}

![Scheme 9](image)

The 4,6-di-substituted pyrimidines and 2-amino-4,6-di-substituted pyrimidines formed by the reaction of chalcone with thiourea and guanidine hydrochloride in presence of sodium hydroxide.(Scheme 10)\textsuperscript{64}
Anilido compound are produced by treatment of different aryl amine with ethylacetoacetate which cyclized with various aromatic aldehyde and thiourea furnishing corresponding pyrimidinethione derivatives (Scheme 11).\textsuperscript{65}

Bag Seema et al. has reported single step reaction for 2,4-diaminopyrimidine from guanidine and orthoester in presence of sodium ethanoate in ethanol as solvent (Scheme 12).\textsuperscript{66}

4-Aryl-2-anilinopyrimidines and 2,4-dianilinopyrimidines (i.e., DAPYs) represent privileged structures\textsuperscript{67} found in an ever increasing number of drug-like molecules including VEGF\textsuperscript{68} and CDK\textsuperscript{69} inhibitors, reverse transcriptase inhibitors (e.g., dapivirine), and tyrosine kinase inhibitors (e.g., Gleevec). Brian I. Bliss et al. describe the convenient preparation of novel 4-aryl-2-(heteroarylamino)-pyrimidines and 4-anilino-2-(heteroarylamino)-pyrimidines.\textsuperscript{70} (Scheme 13)
Terry V. Hughes and co-workers have synthesized 4-Aryl-5-cyano-2-aminopyrimidines as VEGF-R2 inhibitors.\textsuperscript{71} The key step involved reaction of a vinylogous amide with a guanidinium salt to form the pyrimidine ring. Specifically, conversion of an aryl methyl ester 3 (R\textsubscript{1} = aryl) to the corresponding $\alpha$-cyanoketone was achieved via formation of the lithium salt of acetonitrile by treatment with n-BuLi at 78 °C followed by reaction with the ester at 45 °C. Subsequent treatment of the $\alpha$-cyanoketone with N, N-dimethylformamide diethyl acetal (DMF-DEA) formed a vinylogous amide in situ that was reacted with guanidine nitrate in DMF at 100 °C to form the 2-amino-4-aryl-5-cyanopyrimidine. The Sandmeyer reaction of the aminopyrimidine was accomplished smoothly afford the 2-chloropyrimidine. The displacement of the Cl of pyrimidine with aliphatic amines proceeded at room temperature and with aromatic amines in refluxing THF to afford the pharmacophore. (Scheme 14)
Giblin et al.\textsuperscript{72} developed an efficient synthesis of 2-anilino pyrimidine derivatives has been achieved via reaction of 2-chloro-4-trifluoromethyl pyrimidine ester and aromatic amine in dioxane. (Scheme 15) These compounds were found very potent analgesic in the FCA model of inflammatory pain and have a high therapeutic index and a promising pharmacokinetic profile in the rat.

The prepared 2-anilino pyrimidine ester and amide derivatives showed micromolar potency at the CB2 receptor and good selectivity against CB1.\textsuperscript{73}
Youssef et al.\textsuperscript{74} recently reported the synthesis and anticancer, antimicrobial activity of 4-amino-2-(benzo[d]thiazol-2-ylamino)pyrimidine-5-carbonitrile derivative. This compound was prepared from 2-guanidinobenzothiazole with ethoxymethylene malononitrile in presence of anhydrous potassium carbonate reflux in absolute ethanol. (Scheme 16)

Sherif et al.\textsuperscript{75} has designed, synthesized and investigated the anti-HIV activity of some new 4-amino-2-(benzoxazol-2-ylamino)-pyrimidines-5-carbonitrile derivatives, which was found to inhibit the spread of the HIV infection by 95 \% in MT\textsuperscript{4} cell culture. The 2-(benzoxazol-2-ylamino)-pyrimidines derivative was prepared by conventional method as described below. (Scheme 17)
6.7 Pharmacological Profile of Pyrimidines

6.7.1 Antineoplastics and anticancer agents

There are a large number of pyrimidine-based antimetabolites. They are usually structurally related to the endogenous substrates that they antagonize. One of the early metabolites prepared was 5-fluorouracil\textsuperscript{76} (5-FU, 36a), a pyrimidine derivative. 5-Thiouracil 36b also exhibits some useful antineoplastic activities.\textsuperscript{77}

![Scheme 17](image)

There are many more in recent times, like nimustine 37\textsuperscript{78}, uramustine 38\textsuperscript{79} and trimetrixate 39\textsuperscript{80}. It is mainly used as an anticancer agent and also exhibits significant therapeutic effects in patients with herpes virus infections and herpes encephalitis.

![Figure 17](image)
6.7.2 Antibacterials and antiprotozoals

In 1948, Hitchings made an important observation that a large number of 2,4-diaminopyrimidines and some 2-amino-4-hydroxypyrimidines are antagonists of folic acid.\textsuperscript{81} Since then, a large number of 2,4-diaminopyrimidines have been synthesized as antifolates. It was eventually proved that these pyrimidines are inhibitors of the enzyme dihydrofolate reductase (DHFR).\textsuperscript{82} Notable amongst the 2,4-diaminopyrimidine drugs are pyrimethamine \textsuperscript{40}, a selective inhibitor of the DHFR of malarial plasmodia; trimethoprim \textsuperscript{41}, an antibacterial drug which selectively inhibits bacterial DHFR.

![Figure 18](image)

6.7.3 Sulfur drugs

Pyrimidine derivatives of sulfur drugs, namely sulfadiazine, sulfamerazine and sulfadimidine are superior to many other sulfonamides and are used in some acute UT infections, cerebrospinal meningitis and for patients allergic to pencillins.\textsuperscript{83} Sulfonamide–trimethoprim combinations are used extensively for opportunistic infections in patients with AIDS.\textsuperscript{84} Sulfadoxine\textsuperscript{85} \textsuperscript{42a}, a short and intermediate acting sulfonamide with a half-life of 7–9 days is used for malarial prophylaxis. Sulfisomidine \textsuperscript{42b} with a halflife of 7 h is used as a combination sulfur therapy in veterinary medicine.\textsuperscript{86} Sulfadiazine \textsuperscript{43a}, sulfamerzine \textsuperscript{43b} and sulfadimidine \textsuperscript{43c} possess good water solubility and therefore carry minimum risk of kidney damage, which makes them safe even for patients with impaired renal functions.
6.7.4 Antivirals and anti-AIDS

Pyrimidine derivatives have generated widespread interest due to their antiviral properties. 5-Iododeoxyuridine\(^\text{87}\) \(44\) is an antiviral agent of high selectivity. 5-Trifluromethyl-2’-deoxyuridine (F3 TDR, \(45\)) has been found useful against infections resistant to IDU therapy.\(^\text{87}\)

Several members of a series of acyclic nucleosides, which contain a pyrimidine ring, are found to be effective antiviral. (Figure 21)
6.7.5 Antifungals

Pyrimidines also exhibit antifungal properties. Flucytosine 50\textsuperscript{88} is a fluorinated pyrimidine used as nucleosidal antifungal agent for the treatment of serious systemic infections caused by susceptible strains of candida and cryptococcus.\textsuperscript{89}
6.8 Research Aim

Three component condensations with the participation of C–H acids, aldehydes or orthoesters, and N-containing mono- or binucleophiles lead to a variety of derivatives, which possess a wide spectrum of biological activity.\textsuperscript{90} For example, interaction of aromatic aldehydes, (thio)urea, and β-diketones in Biginelli conditions gave dihydro(thia)pyrimidones, which are calcium channel activators, antagonists of adrenoreceptors, etc.\textsuperscript{91-93}

While development of important methodologies for the synthesis of pyrimidines enjoys a rich history, the discovery of new strategies for the convergent synthesis of pyrimidines remains a vibrant area of chemical research.

At present there are no reports for the use of such reactions on guanidines, in this work, the three component condensation of benzoimidazole-2-guanidines with orthoesters and active methylene compounds containing a carbonyl function were studied. (Figure 23)

The chemical synthesis and characterization of Substituted-(1\textit{H}-benzo[\textit{d}]imidazol-2-yl)amino-pyrimidine derivatives are described in Chapter 7.