CHAPTER 1

Introduction
1. Introduction

1.1 Thiazole

Thiazole is a heterocyclic compound featuring both nitrogen and sulfur atom as part of the aromatic five-membered ring. Thiazole and related compounds are called 1,3-azoles (nitrogen and one other heteroatom in a five-membered ring). They are isomeric with the 1,2-azoles, the nitrogen and sulfur compound being called isothiazole. The numbering system is shown below for naming derivatives of thiazole.

![Thiazole Resonance Structures](image)

Thiazole is aromatic on the basis of delocalization of a lone pair of electrons from the sulfur atom completing the needed 6π electrons to satisfy Hückel’s rule. The resonance forms

All electrophilic and nucleophilic reactions of thiazole and its derivatives can be explained on the basis of these resonance structures.

Thiazole is a clear to pale yellow liquid with a boiling point of 116-118 °C. Its specific gravity is 1.2 and it is sparingly soluble in water. It is soluble in alcohol and ether. The odour of thiazole is similar to pyridine. It is used as an intermediate to manufacture synthetic drugs, fungicides, and dyes.

1.1.1 Natural sources

A thiazole ring is found naturally in the essential vitamin B₁₂ (Thiamin).

![Thiamin](image)

Thiamin is a water soluble vitamin that helps the body release energy from carbohydrates during metabolism. It also helps in the normal functioning of the nervous
system by its role in the synthesis of acetylcholine, a neurotransmitter. Thiamin is found mostly in pasta and breads made from refined flours. It is also found in ready-to-eat cereals and in navy and kidney beans.

Thiazoles are found in a variety of specialized products, often fused with benzene derivatives, the so-called benzothiazoles. In addition to vitamin B\textsubscript{1}, the thiazole ring is found in epothilone. Other important thiazole derivatives are benzothiazoles, for example, the firefly chemical luciferin. Whereas thiazoles are well represented in biomolecules, oxazoles are not. The anticonvulsant riluzole, the antiparkinsonian talipexole, the antischistosomal miridazole, the anthelmintic tiabendazole, the anti-ulcer alizatidine, the vitamin B\textsubscript{1}, the antibacterial sulfathiazole, and the antiviral ritonavir can be cited (Fig. 1) [1].

![Fig. 1: Natural and synthetic products containing thiazole](image)

1.1.2 Applications

In the case of natural products, thiazole is present as a subunit in a large number of terrestrial and marine compounds, with different biological activities that represent a very important field in drug discovery. Thiazole ring also finds applications in other fields,
such as polymers, liquid crystals, photonucleases, fluorescent dyes, insecticides and antioxidant [2] (Fig. 2).

1.1.3 Biological activities

1.1.3.1 Anticonvulsant activity

Satoh et al. [3] identified 4-fluoro-N-[4-[6-(isopropylamino)-pyrimidin-4-yl]-1,3-thiazol-2-yl]-N-methyl benzamide (1) as a potent mGluR₁ antagonist as PET tracer, it would have great potential for elucidation of mGluR₁ functions in human. Agarwal et al. [4] synthesized a series of 5-[(N-substituted benzylidenylimino) amino]-2-oxo/thiobarbituric acids and screened, *in vivo* for anticonvulsant and acute toxicity studies. The compounds 2a and 2b found to be more potent.

1.1.3.2 Antimicrobial activity

Abdel-Wahab et al. [5] synthesized various pyrazoline incorporated thiazole derivatives 3(a-d) and screened for antibacterial and antifungal activity against *Escherichia coli* and *Aspergillus niger*. A series of arylidene-2-(4-(4-
methoxy/bromophenyl) thiazol-2-yl)hydrazines and 1-(4-(4-methoxy/bromo phenyl)-thiazol-2-yl)-2-cyclohexylidene/cyclopentylidene hydrazines were synthesized, and screened for antimicrobial and antifungal activities by Bharti et al. [6]. Among the tested compounds 4(a-c), 5(a-b) and 6(a-b) were more potent.

1.1.3.3 Anti-inflammatory activity

Sondhi et al. [7] reported variety of \( N\)-(4-phenyl-3-(2',3',4'-\text{un})substituted phenyl)thiazol-2(3H)-ylidene)-2,4-(un)substituted acridin-9-amine and 1-[(2,4-(un)substituted acridin-9-yl)-3-(4-phenyl-3-(2',3',4'-\text{un})substituted phenyl)thiazol-2(3H)-ylidene]isothiourea derivatives and screened for anti-inflammatory, analgesic and kinase (CDK1, CDK5 and GSK3) inhibition activities. Out of these, compounds 7 and 8 showed potent activity.

1.1.3.4 Anticancer activity

A series of 3,4-diarylthiazol-2(3H)-ones and three 3,4-diarylthiazol-2(3H)-imines were synthesized and evaluated by Liu et al. [8] for their cytotoxicity in a panel of human cancer cell lines. Two compounds 9 and 10 showed potential anticancer activity against human CEM cells with IC\(_{50}\) values of 0.12 and 0.24 \(\mu\)M, respectively.
Havrylyuk et al. [9] synthesized a series of 5-arylidene derivatives and evaluated them for antitumor activity. Among the tested compounds, 2-\{2-[3-(benzothiazol-2-ylamino)-4-oxo-2-thioxothiazolidin-5-ylidene methyl]-4-chlorophenoxy\}-N-(4-methoxyphenyl) acetamide (11) were found to be the most active with log GI$_{50}$ and log TGI values 5.38 and 4.45 respectively. Shao et al. [10] synthesized novel ferrocenyl containing thiazole derivatives from 2-amino-4-ferrocenyl-5-(1H-1,2,4-triazole-1-yl)-1,3-thiazole and substituted benzoyl chloride and evaluated of anticancer activities. Thiazole 12a and 12b showed good inhibition percentages against human cancer cell lines.

Marini et al. [11] studied the incorporation of planar heterocyclic thiazole nucleus in place of one of the amine in the clinically ineffective trans-[PtCl$_2$(NH$_3$)$_2$] (transplatin) to obtain compound 13. On the basis of results obtained, they concluded that such compounds significantly enhanced anticancer activity.

### 1.1.3.5 Antidiabetic activity

The optimization of the led GK (Glucokinase) activator to 3-[(1S)-2-hydroxy-1-methylethoxy]-5-[4-(methylsulfonyl) phenoxy]-N-1,3-thiazol-2-ylbenzamide (14), a potent GK activator was described by Iino et al. [12]. Following oral administration, this compound exhibited robust glucose lowering in diabetic model rodents. Identification and synthesis of novel 3-alkoxy-5-phenoxy-N-thiazolyl benzamides as glucokinase activators were described by Iino et al. [13]. Removal of an aniline structure of the prototype led and incorporation of an alkoxy or phenoxy substituent led to the identification of 3-
isopropoxy-5-[4-(methylsulfonyl)phenoxy]-N-(4-methyl-1,3-thiazol-2-yl)benzamide (15) as a novel, potent, and orally bioavailable GK activator.

![Chemical structures](image)

1.1.3.6 Anti-HIV activity

Rawal et al. [14] synthesized a series of 2-(2,6-dibromophenyl)-3-heteroaryl-1,3-thiazolidin-4-one and evaluated as selective human immunodeficiency virus type-1 reverse transcriptase (HIV-1, RT) enzyme inhibitors. *In vitro* cell assay showed that eight compounds 16(a-h) effectively inhibited HIV-1 replication at 20-320 nM concentrations with minimal cytotoxicity in MT-4 as well as in CEM cells.

Rawal et al. [15] synthesized a series of 2-arylsulfonyl-1,3-thiazolidin-4-ones. Compounds having isothiourea or thiourea functional group showed high anti-HIV-1 activity. *In vitro* tests showed that the compound 17 exhibited $EC_{50}$ at 0.26 l M with minimal toxicity in MT-4 cells as compared to 0.35 μM for thiazobenzimidazole (TBZ).

![Chemical structures](image)

16a, R=furan-2ylmethyl, 16b, R=pyridin-2-yl 16c, R=6-methyl-pyridin-2-yl 16d, R=pyrimidin-2yl 16e, R=4-methyl-pyrimidin-2yl, 16f, R=4,6-dimethyl-pyrimidin-2-yl 16g, R=4-methyl-6-trifluoromethylpyrimidin-2yl, 16h, R=4,5,6-trimethylpyrimidin-2-yl

Masuda et al. [16] synthesized various N-3-alkylated thiazolidene sulfonamide. The effects of different bases and solvents were investigated, and the NaH–THF combination was found to be the most effective at conferring high yields and endo-selectivity. Among the tested compounds an endo-alkylated compound 18 found to be showed more potent antiretroviral activity.
1.1.3.7 Anti-Alzheimer activity

A novel clubbed triazolylthiazole series of cdk5/p25 inhibitors, potentially useful for the treatment of Alzheimer’s disease, was disclosed by Shiradkar et al. [17]. Evaluation of the SAR of substitution within these series had allowed the identification of compounds 19a and 19b which significantly reduce brain cdk5/p25 and thus have potential as possible treatments for Alzheimer’s disease.

Helal et al. [18] used high-throughput screening with cyclin-dependent kinase 5 (cdk5)/p25 that led to the discovery of N-(5-isopropyl-thiazol-2-yl)isobutyramide (20). This compound was an equipotent inhibitor of cdk5 and cyclin-dependent kinase 2 (cdk2)/cyclin E (IC$_{50}$ = ca. 320 nM).

\[
\begin{align*}
19(a-b) \\
19a, R=NHCOCH_2Cl \\
19b, R=NHCOCH_3
\end{align*}
\]

1.1.3.8 Antihypertensive activity

Some 1-(4-arylthiazol-2-yl)-3,5-diaryl-2-pyrazoline derivatives were synthesized by Zitouni et al. [19] by reacting 1-thiocarbamoyl-3,5-diaryl-2-pyrazoline derivatives with phenacetylbromide. The hypotensive activities were evaluated by using the tail-cuff method. An increase in the hypotensive activity of the compounds 21(a-d) has been observed.

Abdel-Wahab et al. [20] synthesized potent derivative of thiazolylmalonamide, tetrachloroisindolylimide, and triazole and evaluated for antihypertensive α-blocking activity and low toxicity. Among these compounds, compound 22 found to be more potent.

\[
\begin{align*}
21(a-d) \\
21a, R_1=H, R_2=H, R_3=H, R_4=H \\
21b, R_1=CH_3, R_2=CH_3, R_3=H, R_4=OCH_3 \\
21c, R_1=H, R_2=H, R_3=OCH_3, R_4=OCH_3 \\
21d, R_1 & & R_2=-CH_2^-, R_3=H, R_4=OCH_3
\end{align*}
\]
1.1.3.9 Antioxidant activity

Shih et al. [21] synthesized 3-aryl-4-heterocyclic sydnones derivatives. The antioxidant activity of synthesized compounds was evaluated. Among these compounds, 4-methyl-2-[(3-arylhydrazono-4-ylmethylene)hydrazono]-2,3-dihydro-thiazole-5-carboxylic acid ethyl ester 23(a-d) and 4-phenyl-2-[(3-arylhydrazono-4-ylmethylene)hydrazono]-2,3-dihydro-thiazoles 24(a-d) exhibited the potent DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, comparable to that of vitamin E.
1.2 Dihydropyrimidines

Nature is diversified with different variety of natural products with number of scaffolds and unique structures with specific activity. Among all the structures known, available and identified, heterocyclic scaffolds are ubiquitous in pharmaceuticals, natural products and biologically active compounds. A variety of methods are used to prepare these heterocyclic compounds with different core structure, the synthetic access to polysubstituted-polyfunctionalized heterocyclic derivatives remains a serious challenge to scientific community. Although multistep sequences are widespread in the literature.

The synthetic variables that have to be optimized are time, costs, overall yield, and simplicity of performance, safety, and environmental acceptability. Thus, multicomponent reactions (MCRs) are one-pot reaction procedures, which are easier to perform, isolate and diversify than multistep syntheses. Thus MCR strategy is a highly desirable approach in drug discovery development in the context of rapid identification, structural diversification and optimization of biologically active lead compounds of potential therapeutic importance within a short span of time which can generate large number of libraries of heterocyclic compounds with the aid of high-throughput biological screening [22].

Hantzsch, Knoevenagel and Biginelli reactions have some similarity; as each one of these employs aldehyde, acetoacetic ester (active methylene compound). The earliest of these seems to be the discovery of the Hantzsch reaction which was reported in 1881 [23] wherein Hantzsch heated acetoacetic ester, an ammonia source, and an aldehyde, to obtain the now well-known dihydropyridines or Hantzsch pyridines [23]. A decade later the Italian chemist P. Biginelli [24] reacted same two components in equimolar ratio *viz.* acetoacetic ester, aldehyde and third component as urea in acidic alcoholic solution to obtain a new compound, the now well-known 3,4-dihydropyrimidin-2(1H)-ones or Biginelli compounds 26 which are obvious aza-analogues of the Hantzsch dihydropyridinines. Biginelli did not detect any Hantzsch dihydropyridines 25 as byproducts.
He apparently did this reaction in a multicomponent way, and currently the development of MCRs is an integral part of numerous research efforts around the world involved in the drug development programs to achieve synthetic targets in expeditious way.

In recent years, interest has also been focused on aza-analogs of 1,4-dihydropyridines such as dihydropyrimidines (DHPMs), which exhibit a pharmacological profile similar to classical dihydropyridine calcium channel modulators [25-29]. These inherently asymmetric dihydropyrimidine derivatives are not only better calcium channel blockers, but have also been extensively studied to expand the existing SARs and to get further insight into molecular interactions at the receptor level [25-29].

1.2.1 Discovery

In 1893, an Italian chemist Pietro Biginelli from University of Florence reported one of the most important MCRs that allow the synthesis of dihydropyrimidinones and their sulfur analogues for the first time, which has given birth to this Biginelli reaction. In honor to his novel and excellent discovery this reaction is named as Biginelli reaction [24] after him.

1.2.2 Synthesis

The DHPMs can be easily synthesized by the Biginelli dihydropyrimidine synthesis [24, 30], which is a very simple one-pot, acid-catalyzed condensation reaction. Such a synthesis is an example of a multicomponent reaction (MCR) where three or more reactants come together in a single reaction vessel to form new products that contain portions of all the components. The original Biginelli DHPM synthesis [24] involved simple heating of a mixture of three components which were ethylacetooacetate (27), benzaldehyde (28) and urea/thiourea (29) which may be regarded as the building blocks.
The mixture was dissolved in ethanol along with a catalytic amount of HCl and refluxed to get the product (30).

![Fig. 2: Synthesis of 3,4 dihydropyrimidine-2-thiones](image)

The heterocyclic scaffold in 30 is given the acronym DHPM in literature. It is evident from Fig. 2 that the variation of all the three building blocks allows access to a large number of multifunctionalized pyrimidine derivatives [31]. Consequently, the number of publications and patents describing the synthesis of novel DHPM analogs is constantly growing. Given the diversity in building block selection that is tolerated in the Biginelli reaction, a large number of DHPM derivatives of the general formula 31 can be synthesized [32]:

\[ \text{R}_1 = \text{H, alkyl} \]
\[ \text{R}_4 = \text{H, alkyl, (het) aryl, carbohydrate} \]
\[ \text{R}_6 = \text{H, alkyl, aryl} \]
\[ \text{E} = \text{ester, acyl, amide, nitro, nitrile, phosphono} \]
\[ \text{X} = \text{O, S, NR} \]

Hence from compound 31 it is clear that a much larger number of very interesting heterocycles having the DHPM scaffold can be obtained by chemical functionalization of the six diversity points around the DHPM core.

### 1.2.3 Mechanism

The reaction begins with protonation of the aldehyde by the acid and is followed by attack of the amine from urea. Proton transfer steps then result in a protonated alcohol which leaves as water to form an N-acyliminium ion intermediate. The intermediate is then attacked by the enol form of the β-keto ester. Reaction of the other amine group to the carbonyl produces a cyclic intermediate. Proton transfer steps, the release of water, and deprotonation result in the final pyrimidone product [24].
1.2.4 Pharmacology

Biginelli reaction is a three component one pot reaction, variation of all three building blocks, viz. active methylene, urea, aldehyde component lead to extension of the scope original multi-component resulting in large molecular diversity of dihydropyrimidines. The biological investigation of these various molecules showed activities like antifilarial [33], antihypertensive [34,35], anti-HIV [36,37], antitumor [38], anti-malarial [39], anti-epileptics [40], anti-inflammatory [41], antimicrobial [42] and antitubercular [43] activity.

1.2.4.1 Antifilarial agents

Singh et al. [33] synthesized a series of 2-sulfanyl-6-methyl-1, 4-dihydropyrimidines by alkylation of 5-methyl-6-phenyl-2-thioxo-1, 2, 3, 6- tetrahydropyrimidine-4-carboxylic
acid ethyl esters. Compound 32 was evaluated for their antifilarial activity against adult parasites of human lymphatic filarial parasite Brugia malayi (sub-periodic strain) *in vitro* and *in vivo* at various concentrations. From the synthesized compounds showing potent *in vitro* antifilarial activities were screened *in vivo* against *B. malayi* in Mastomys coucha model to see the effect of the compounds on parasitological parameters. It is evident from the observation that a significant effect on adult worms (50%; *P* < 0.001) was shown by compound 32 at 100 mg/kg.

\[
\text{32}
\]

### 1.2.4.2 Antihypertensive agents

Alam *et al.* [34] synthesized a number of 5-(4-substituted phenyl)-2-(substituted benzylsulfanyl)-4-(substituted-phenyl)-6-methyl-1,4-dihydro-5-pyrimidine carboxamides. Keeping in view the structural requirements as suggested in the pharmacophore model for antihypertensive activity. All the synthesized compounds were tested for antihypertensive activity by non-invasive blood pressure (NIBP) measurements (tail-cuff method) in rats. Almost all the tested compounds displayed considerable decrease in the blood pressure as compared to control. Thirteen compounds showed significant antihypertensive activity comparable to the standard drug nifedipine. Out of all the compounds synthesized only compounds 33-37 show promising anti-hypertensive drug activity.

\[
\text{33-37}
\]

Chikhale *et al.* [35] synthesized fifteen new ethyl 6-methyl-2-methoxy-3-(substituted 1-phenylethanone)-4-(substituted phenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates compounds following a two step sequence. All these compounds were tested for
antihypertensive activity by non-invasive tail-cuff method and evaluated by carotid artery cannulation method for determining the diastolic blood pressure. Hypertension was induced by DOCA-salt. It is observed that compounds 38 and 39 possessed novel antihypertensive activity.

1.2.4.3 Anti-HIV agents

No et al. [36] synthesized 3, 4-dihydropyrimidin-2(1H)-ones (DHPMs) were selected and derivatized through a HIV-1 replication assay based on GFP reporter cells, where compounds (R/S)-40/41 exhibited significant inhibition of HIV-1 replication in vitro, with a good safety profile. Chiral separation of each enantiomer by fractional crystallization showed that only the (S)-40, enantiomer on C-4 in dihydropyrimidinone ring shows potent anti-HIV activity.

He et al. [37] synthesized novel dihydro-aryl/alkylsulfanyl-cyclohexylmethyl-oxopyrimidines (S-DACOs) was synthesized and evaluated with C8166 cells infected by the HIV-1 IIIB in vitro, using Nevirapine (NVP) and Zidovudine (AZT) as positive control. The anti-HIV screening results revealed that C-6-cyclohexyl methyl substituted pyrimidinones possessed higher selective index than its 6-aryl methyl counter-parts. Compounds 42, 43, 44 and 45 showed potent anti-HIV activities.
1.2.4.4 Antitumor activity

It is documented that, Human kinesin Eg5, plays an essential role in mitosis by establishing the bipolar spindle, which has proven to be an interesting drug target for the development of cancer chemotherapeutics. In this development, Monastrol 46 is the first Biginelli compound, which has excellent anticancer activity. Further a series of compounds for their ability to inhibit Eg5 activity has been investigated using two in vitro steady-state ATPase assays (basal and microtubule-stimulated) as well as a cell-based assay. In an attempt, another dihydropyrimidine i.e. furyl derivative 47 appeared more potent than monastrol by a fivefold factor [38].

![Structures of monastrol and furyl derivative](image)

1.2.4.5 Anti-malarial activity

Chiang et al. [39] described pyrimidinone-amide derivatives of DHPMs 48, 49 and 50, a new class of Hsp70 modulators, could inhibit the replication of the pathogenic *P. falciparum* stages in human red blood cells. These three molecules acts as good anti-malarial agents.

![Structures of pyrimidinone-amide derivatives](image)

1.2.4.6 Anti-epileptics

Phenobarbital 51 is well known drug for epilepsy, when one sees Biginelli compounds it has similar structural framework and as a natural tendency, when compounds of type 52 were examined for epilepsy they have shown promising anti-epilepsy activity [40].
1.2.4.7 Anti-inflammatory activity

A series of compounds 3-(4,6-disubstituted-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl) propanoic acid derivatives 53-55 were screened for their anti-inflammatory activity using rat paw edema method. Most of the compounds from the series showed significant anti-inflammatory activity [41].

1.2.4.8 Anti-microbial activity

Biginelli compounds have been multi-functionalized with isoxazole amines i.e. 56 has shown anti-microbial apart from anti-bacterial, anti-fungal, and anti-malarial activities [42].

1.2.4.9 Anti-tubercular activity

Dihydropyrimidines (30 examples) also were evaluated for their antitubercular activity against Mycobacterium tuberculosis H37Rv. This study was in vitro only. Only two
compounds, \(57a\) and \(57b\) were shown to be the most active compounds and found to be more potent than isoniazid. Compounds \(58\) and \(59\) with 2,3-dimethylphenyl and 3,4-dimethyl carbamoyl side chain, respectively, showed 65% and 63% inhibition against \(Mycobacterium\) \(tuberculosis\) \(H37Rv\) [43].

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1.2.4.10 Potassium channel antagonists

Vaccaro et al. [44] synthesized a series of dihydropyrazolo pyrimidine inhibitors of \(Kv1.5\) (IKur). Compounds \(60\) and \(61\) were evaluated for selectivity versus a panel of ion channels. Compounds \(60\) and \(61\) are greater than 50 fold selective for \(Kv1.5\) versus hERG, \(I_{N_{a}}\), \(I_{C_{a}}\) (L-type), \(I_{K_{s}}\), and \(I_{K_{1}}\) ion channels. The ion channel selectivity of these compounds suggests that they may be useful for the treatment of atrial fibrillation without the risk of ventricular proarrhythmia. Lloyd et al. [45] designed and synthesized series of pyrazolo dihydropyrimidines as \(Kv1.5\) blockers which led towards the discovery of \(62\) as a potent and selective antagonist. This compound showed atrial selective prolongation of effective refractory period in rabbits and was selected for clinical development.

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1.3 Thiazolo[3,2-a]pyrimidines

The literature on thiazolo[3,2-a]pyrimidines has been reviewed mainly due to the interesting biological and pharmaceutical activities associated with this ring system.

![Chemical structures of thiazolo[3,2-a]pyrimidines](image)

5H-Thiazolo[3,2-a]pyrimidine  
5H-Thiazolo[3,2-c]pyrimidine

6H-Thiazolo[3,4-a]pyrimidine  
3H-Thiazolo[3,4-c]pyrimidine

1.3.1 Methods of preparation

The synthesis of the thiazolopyrimidines has already been reported in the literature [46-50]. The following routes have been employed.

i. **Azole approach**: Starting from the thiazole ring and subsequent construction of the pyrimidine ring in the terminal step.

ii. **Azine approach**: Starting from pyrimidine ring and subsequent construction of the thiazole in the terminal step.

iii. **Retro Diels-Alder reaction**: both azole and azine approaches were considered as major methods for synthesis of the thiazolopyrimidines. Whereas, the retro Diels-Alder reaction was considered a minor method.

1.3.1.1 Azole approaches

Thiazolo[3,2-a]pyrimidine 64 was prepared by fusing 2-aminothiazole 63 with diethyl malonate in an oil bath at 240 °C for 3 h and the compound 65 was obtained by refluxing 63 with ethyl benzylidene cyanoacetate in presence of piperidine as a catalyst in ethanol about 10 h [51].
1.3.1.2 Azine approach

Pyrimidinethione derivatives 66-68 were alkylated with monochloroacetic acid or chloroacetyl chloride and then cyclized to give thiazolopyrimidine derivatives [52-65]. Thus, pyrimidinethione 66 reacted with monochloroacetic acid or chloro acetyl chloride in DMF [52] or in an acetic anhydride/pyridine mixture [54,56] to give thiazolopyrimidines 69 and 70. Alkylation with monochloroacetic acid in the presence of an aromatic aldehyde [52-54,57,59-63,65], gave the ylidene derivatives 71. Similarly, pyrimidinethione derivatives 67 and 68 reacted with monochloroacetic acid in acetic acid/acetic anhydride/sodium acetate mixture [57,58] or with chloroacetyl chloride in dry dioxane and with aromatic aldehyde to give the corresponding thiazolopyrimidines.

1.3.1.3 By Retro Diels-Alder reaction

The reaction of ethyl 3-exo-aminobicyclo[2,2,1]hept-5-ene-2-exo-carboxylate 72 and the diendo counterpart 73 with chloroethyl isothiocyanate yielded the teracycles 74 and 75, respectively. When compounds 74 and 75 were melted at 140 °C for 20 min, bicycles 76 were formed in good yield, by eliminating cyclopentadiene [66].
1.3.2 Chemical reactivity

1.3.2.1 Reaction with amines

Thiazolopyrimidine derivative 77 reacted with 78 and 79 to give the tricyclic alkyl substituent 80 and 81, respectively [67,68].

Thiazolopyrimidine carboxylate 82 reacted with 4-chloroaniline in refluxing bromobenzene to give compound 83 [69].

The active methylene in the thiazolopyrimidine derivative 84 readily condensed with an aromatic aldehyde in acidic medium to give an ylidene derivative 85. In addition, α, β-unsaturated ketone obtained was used to synthesize fused heterocyclic systems in order to give conclusive proof for ylidene structure. Thus, the ylidene derivative 85 reacted with hydrazine hydrate to give 86 [70].
The isocyanate 87 reacted with the morpholine, aminothiazole, methyl-aminoisoxazole and 2-aminopyridine to give the ureas 88-91, respectively [71].

1.3.2.2 Michael Addition Reactions

Thiazolopyrimidines 92 reacted with cinnamoniitrile derivatives in refluxing pyridine to give 93. Alternatively the reaction of malononitrile with 94 in refluxing ethanolic piperidine gave the same reaction product 93 [72].
1.3.2.3 Ring Transformations

Although it was reported [70] that oxothiazolopyrimidine reacted with hydrazine hydrate to give the cyclized amino derivative. Shridhar et al. [71] reported that the hydrazinolysis of ethyl thiazolopyrimidine carboxylate 95 instead of giving 96, led to the formation of pyrimidinotriazine hydrazide 97. This was believed to take place through the initial opening of thiazole ring in 95 with hydrazine hydrate followed by recyclization with the elimination of hydrogen sulfide [71].

![Chemical structures showing ring transformations](image)

1.3.3 Basicity of thiazole heterocyclic compounds

The potentiometric titration method of Albert and Serjeant has been used in this determination, so that the pKa’s have been corrected for ionic-strength effects. Because of their poor solubilities in water, the pKa of the phenyl derivative has been measured in 50% aqueous ethanol. The pKa values of thiazolo[3,2-a]pyrimidine with methyl and phenyl substituents are shown below [73].

![Chemical structures showing basicity of thiazole compounds](image)

1.3.4 Applications

Thiazolopyrimidines have been used as a coating dye for optical recording materials [74]. On the material comprising a support coated with silver halide, the layer containing the thiazolopyrimidinium salt shows a clear reversal image on high luminescence [75]. Cyanine dyes based on mesoionic thiazolo[3,2-a]pyrimidine derivatives [76-78] or thiazolo[3,4-a]pyrimidine have been reported [79].

Thiazolopyrimidine 98 is used in the manufacture of a medicament for the therapy of hyperalgesic pain conditions and their symptoms [80].

![Chemical structure of thiazolopyrimidine 98](image)
Setoperone (99) is a compound that is a ligand to the 5-HT$_{2A}$ receptor. It can be radiolabeled with the radioisotope fluorine-18 and used as a radioligand with positron emission tomography (PET). Several research studies have used the radiolabeled setoperone in neuroimaging for the studying neuropsychiatric disorders, such as depression [81] or schizophrenia [82].

![Chemical structures](99.png)

Ritanserin (100) is a serotonin antagonist with possibilities for the treatment of many neurological disorders. When used together with typical antipsychotics in the treatment of schizophrenia, it is able to decrease negative symptoms and adds some "atypicality" as parkinsonism is slightly decreased.

Ritanserin (100) may also be effective in the prophylaxis of chronic migraine headaches. Its efficacy may be explained by its ability to antagonize 5-HT$_2$ receptors [83]. Ritanserin acts as a 5-HT$_{2A}$ ($K_i=0.45$ nM) and 5-HT$_{2C}$ receptor ($K_i=0.71$ nM) antagonist, with higher affinity for the former site [84].

1.3.4.1 Synthesis of condensed thiazolo-[3,2-a]pyrimidine systems

Synthesis of thiazolo[3,2-a]pyrimidines systems represented by the general formula

![Chemical structures](100.png)

The C, D, E and F rings represent arene, heteroarene, cycloalkene, etc.

1.3.4.1.1 Synthesis of tricyclic thiazolo[3,2-a]pyrimidines

Antaki and Petrow [85] reported the first synthesis of a tricyclic thiazolopyrimidinone, i.e. 2-methyl-4-oxo-benzothiazolo[3,2-a]pyrimidine (102) by condensing 2-aminobenzothiazole (101) with ethyl p-aminocrotonate. Ogura et al. [86] reported the synthesis of 4-ethoxycarbonyl-2H-benzothiazolo[3,2-a]pyrimidin-2-one (103) by condensation of 2-
aminobenzothiazole with diethyl acetylenedicarboxylate (DEAD) and performed a comparative study of the product structure with that obtained by condensation of benzothiazole with diethyl (ethoxymethylene)malonate. In the former reaction, the 2-oxo compound 102 was obtained, while the 4-oxo derivative (103) was the product in the latter condensation.

\[
\begin{align*}
\text{SN} & \\
\text{N} & \\
\text{S} & \\
\text{N} & \\
\text{O} & \\
\text{O} & \\
\text{N} & \\
\text{H} & \\
\text{N} & \\
\text{S} & \\
\text{N} & \\
\text{O} & \\
\text{O} & \\
\text{N} & \\
\text{N} & \\
\text{H} & \\
\text{S} & \\
\text{C} & \\
\text{H} & \\
\end{align*}
\]

1.3.4.1.2 Synthesis of tetra cyclic thiazolo[3,2-a]pyrimidines

Abdel-Razik [87] reported the fused tetra cyclic compounds 105-108 containing two thiazole moieties via cyclization reaction of compound 104 with \(\alpha\)-haloactive-methylene compounds like monochloro acetic acid, chloro acetone, phenacyl bromide and chloro acetonitrile respectively.

\[
\begin{align*}
\text{SN} & \\
\text{N} & \\
\text{S} & \\
\text{N} & \\
\text{O} & \\
\text{O} & \\
\text{N} & \\
\text{H} & \\
\text{N} & \\
\text{S} & \\
\text{N} & \\
\text{O} & \\
\text{O} & \\
\text{N} & \\
\text{N} & \\
\text{H} & \\
\text{S} & \\
\end{align*}
\]

1.3.4.1.3 Synthesis of pentacyclic thiazolo[3,2-a]pyrimidines

Ramadas et al. [88] obtained the pentacyclic thiazolopyrimidone 9-methyl-6H, 11H-naphtho[2′,1′,5′,6′]thiopyrano[4′,3′,4,5]thiazolo[3,2-a]pyrimidin1-1-one (110) by condensation of the corresponding tetracyclic 2-aminothiazole derivative (109) with ethyl acetoacetate in the presence of excess p-toluenesulfonic acid.
1.3.4.1.4 Synthesis of hexacyclic thiazolo[3,2-a]pyrimidines

Abu-Hashem et al. [89] reported the synthesis of some hexacyclic thiazolo[3,2-a]pyrimidines (112–115). Compounds 112-115 were obtained by refluxing 111 with 2-chlorobenzaldehyde, 2-chlorocyclohex-1-ene carbaldehyde, 2-chlorobenzoic acid, and 2, 4-dichlorobenzoic acid.

1.3.5 Biological activities

1.3.5.1 Antimicrobial activity

El-Emary and Abdel-Mohsen [90] reported the synthesis and antimicrobial activities of some thiazolo[3,2-a]pyrimidine derivatives. The results revealed that all tested compounds exhibit moderate to strong activity against E. coli and were inactive against B. cereus. However, only compounds 116-118, showed considerable potency against two fungal species used (Botrytis and Geotrichum candidum).

Youssef et al. [51] have developed reactions of aminothiazole and synthesis of thiazolo[3,2-a]pyrimidine. Some of the prepared compounds were tested for their
antimicrobial activity against six fungal (Aspergillus flavus, Aspergillus niger, Candida albicans, Geotrichum candidum, Scopulariopsis brevicaulis and Trichophyton rubrum) and five bacterial (Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Serratia marcescens and Escherichia coli) species. Compound 119 showed a wide spectrum of antifungal action but a narrow spectrum of antibacterial effects with minimum inhibitory concentrations (MIC) ranging from 5 to 50 mg/cm$^3$.

Flefel et al. [91] prepared some new series of thiazolopyrimidines. These compounds were evaluated in vitro against one strain of Gram-negative bacteria (Escherichia coli.) and two strains of fungi, Aspergillus fumigates and Candida albicans, Colimex, and Floconazole were used as references. Compound 121 exerted significant antibacterial activity against Escherichia coli in range of 90 µg/mL. On the other hand, compound 120 showed antifungal activities against Aspergillus fumigates in range of 50 µg/mL. Also compounds 120 and 122 exerted significant antifungal activity against Candida albicans in range of 20 µg/mL.

Coumarinyl thiazolo-[3,2-a]-pyrimidines have been synthesized from 3-bromoacetyl coumarin usingazole and azine approaches by Yaragatti et al. [92] screened them for their antibacterial activities. compounds 123c, 123d, 124a and 124b showed a good activity against P. vulgaris, whereas all the compounds 123(a–d) and 124(a–d) showed a very good activity against Staphylococcus aureus the best results were obtained for compounds 123b and 124b, which were active against both C. albicans and P.
chrysogenum at 25 μg/mL concentration. In the thiazolo-[3,2-a]-pyrimidinyl-5-ones, chloro, bromo and nitro substituted was found to be effective.

Ashok et al. [57] have reported a new series of new 2-(arylidine)-5-(4-methylthiophenyl)-6-carboethoxy-7-methyl-5H-thiazolo[3,2-a]pyrimidine-3(1H)-ones. The newly synthesized compounds 125(a-d) were screened for their antibacterial and antifungal activities and have exhibited moderate to excellent growth inhibition of bacteria and fungi.

### 1.3.5.2 Antimalarial activity

Fathima et al. [93] reported one pot synthesis of a series of pluripotent (E)-1-(3-methyl-5-aryl-7-styryl-5H-thiazolo[3,2-a]pyrimidin-6-yl)-3-arylprop-2-en-1-ones. The newly synthesized compounds were evaluated for antimalarial activity against Plasmodium falciparum and as HIV-RT inhibitors. Most of the compound displayed potent antimalarial activity with IC50< 2 mg/mL. Compounds 126-128 showed better activity against P. falciparum K1 strains in comparison to standard drug chloroquine.

### 1.3.5.3 Anti-inflammatory and Analgesic activity

Abu-Hashem et al. [89] synthesized a variety of tera and penta cyclic thiazolopyrimidine derivative and these were subjected to anti-inflammatory and analgesic activities. The anti-inflammatory activity data indicated that all the tested
compounds protected rats from carrageenan-induced inflammation. Compounds 129-131 showed similar and higher anti-inflammatory activity than diclofenac sodium. The result of analgesic activity revealed that all tested compounds exhibited significant activity. Compound 131 exhibited activities higher than the reference drug in peripheral analgesic activity testing.

Amr et al. [94] reported analgesic activity of some selected compounds. The analgesic activities of compounds 132-134 approached those of Valdecoxib+, and showed 62-84% activity as compared to Valdecoxib+ (¼100%).

Antiparkinsonian activity measured by the ability of compounds to protect animals against the parkinsonian like signs induced by agonists. Compounds 132 and 133 showed nearly no antiparkinsonian activities. Compound 134 is the most potent antiparkinsonian agent’s relative potencies to Benzatropine [94].

Alam et al. [95] reported a new series of thiazolo[3,2-a]pyrimidine derivatives. Anti-inflammatory activity was assessed by the rat paw edema method. All compounds were tested at a dose of 25 mg/kg p.o and have shown considerable anti-inflammatory activity in the range of 5-60%. The compounds 5-(4-chlorophenyl)-2-(4-florobenzylidene)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluoro phenyl)amide (135) and 2-(4-chlorobenzylidene)-5-(4-fluorophenyl)-7-methyl-3-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid (4-fluorophenyl)amide (136)
were found to possess significant anti-inflammatory comparable to those for the standard drug phenylbutazone. In addition to anti-inflammatory activity, compounds 135 and 136 were found to possess antinociceptive, ulcerogenic activity and acute toxicity studies.

Antinociceptive activity of compounds 135 and 136 were found to be the most potent, with 172 and 189% comparable to the standard drug diclofenac [95].

The ulcerogenic activity of the most active compounds 135 and 136 was assessed in albino rats 8 h after compound administration intraperitoneally and the results are reported. Interestingly, both the compounds displayed a better safety profile than the standard drug phenylbutazone. The UD\(_{50}\) values of compounds 135 and 136 were found to be 240.36 and 174.63 mg/kg, much higher than that of the standard drug (UD\(_{50}\) = 70.25 mg/kg). This shows that these compounds are less prone to cause ulcers and are relatively safer agents [95].

Acute toxicity studies were performed in albino mice and the approximate lethal dose (ALD\(_{50}\)) was determined 24 h after drug administration. Results are reported. Both compounds showed ALD\(_{50}\) values ([1000 mg/kg) comparable to that of the standard drug phenylbutazone [95].

Hu et al. [96] synthesized a series of thiazolopyrimidine derivatives and screened for anti-inflammatory activities. A majority of these compounds showed excellent inhibition on the expression of TNF-\(\alpha\) and IL-6 in LPS-stimulated macrophages. Discussions are given regarding the structure activity relationships. Compounds 137 and 138 inhibited LPS-induced TNF-\(\alpha\) and IL-6 release in a dose-dependent manner. Furthermore, 137 exhibited a significant protection against LPS-induced septic death in mouse model.
1.3.5.4 Anticancer activity

Mohamed et al. [97] synthesized some new derivatives containing tetra hydro pyridine fused thiazolopyrimidines and screened for their anticancer activity utilizing 59 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate as well as kidney. From the in vitro observed data it has been noticed that, the selected compounds 139(b-d), 140a, 140b, 140d, 141(b-d), 142(b-d), 143b and 143d seem to be the most active prepared derivatives against all the tested cell lines.

Al-Omary et al. [98] developed a novel series of thiazolo[2,3-b]quinazoline and pyrido[4,3-d]thiazolo[3,2-a]pyrimidine analogues. The obtained compounds were evaluated for their in-vitro antitumor activity at the National Cancer Institute (NCI) 60
cell lines panel assay. Among them, four compounds 144-147 showed remarkable broad-spectrum antitumor activity. Two compounds 144 and 145 were almost nine fold more active than 5-FU, with GI₅₀, TGI, and LC₅₀ values of 2.5, >100, >100; and 2.4, 9.1, 36.2 mM, respectively. While 146 and 147 are almost seven fold more active than 5-FU, with GI₅₀, TGI, and LC₅₀ values of 2.9, 12.4, 46.6 and 3.0, 16.3, 54.0 mM, respectively.

1.3.5.5 Antioxidant and cytotoxic activity

Abu-Hashem et al. [89] developed new synthetic strategy to polyfunctionalized heterocycles such as the synthesis of thiazolopyrimidines. The series of compounds 148, 149, 151b, 151c, 152a and 152b exhibited a high antioxidant activity and protect the DNA. The series of compounds 150 and 152(a-c) proved to have the best cytotoxic activity.

1.3.5.6 Tuberculosis activity

Jean kumar et al. [99] synthesized compounds evaluated in vitro for their ability to inhibit CysK₁, activity against M. tuberculosis and cytotoxicity as steps towards the derivation of structure–activity relationships (SAR) and lead optimization. Compound 8-nitro-4-(2-(trifluoromethyl)phenyl)-4,4a-dihydro-2H-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine-2,5(3H)-dione (153) emerged as the most promising lead with an IC₅₀ of
17.7, 1 M for purified CysK₁ and MIC of 7.6, 1 M for *M. tuberculosis*, with little or no cytotoxicity (>50 μM).

1.3.5.7 mGlu2/3 receptor negative allosteric modulators (NAMS)

Wichmann and co-workers disclosed the first series of mGlu2/3 receptor NAMs to be identified, based around a 5Hthiazolo[3,2-a]pyrimidine scaffold 154 and 155 [100]. These compounds were identified and characterized utilizing a [35S]-GTPγS binding assay on rat mGlu2 receptor transfected CHO cell membranes by determining the inhibition of the mGlu2/3 receptor agonist (1S,3R)-ACPD-induced GTPγS binding response. From this report, compounds 154 and 155 demonstrated the most potent inhibition of GTPγS binding, with IC₅₀ values of 1.5 and 1.0 μM, respectively [101]. Compound 154 was further characterized and found to have an IC₅₀ value of 10 μM for reversal of (1S,3R)-ACPD-induced inhibition of forskolin-stimulated cAMP production in CHO cells transfected with rat mGlu2 receptors. Compound 155 was also demonstrated to be inactive toward mGlu1a and mGlu4a receptors. These compounds represent the first example of non-amino acid antagonists of mGlu2/3 receptors. The mGlu2/3 receptor NAMs could be useful for the treatment of memory deficits seen in Alzheimer’s, aging, schizophrenia, and other neuropsychiatric conditions.

Adam *et al.* [102] filed US patent for phenyl substituted thiazolo pyrimidine derivatives (156) synthesized from DHPM. These compounds and their slats are novel and are distinguished by valuable therapeutic properties. Specifically it has been found that the compound of Markus structure given below was metabotropic glutamate receptor
antagonists. These compounds are capable of high affinity binding to group II mGluR receptors.

1.3.5.8 CDC25 phosphatases inhibitor

Montes et al. [103] reported thiazolopyrimidine structure based compound 157 as CDC25 phosphatases inhibitor. CDC25 phosphatases play critical roles in cell cycle regulation and are attractive targets for anticancer therapies. Several small non-peptide molecules are known to inhibit CDC25, but many of them appear to form a covalent bond with the enzyme or act through oxidation of the thiolate group of the catalytic cysteine.

Duval et al. [104] recently identified benzylidene-thiazolopyrimidines (BTP) (158 and 159) as novel heterocyclic inhibitors of CDC25 enzymes. Analysis of the SARs in this series suggests that an extended conjugation through a substituted 2-thiocinnamamide system is a critical feature for CDC25 inhibition.
1.4 X-ray Crystallography

Crystallography is the science that examines the arrangement of atoms in solids. The word "crystallography" derives from the Greek words *crystallon* = cold drop / frozen drop, with its meaning extending to all solids with some degree of transparency, and *grapho* = write. A more comprehensive definition is: "Crystallography is the science of condensed matter with emphasis on the atomic or molecular structure and its relation to physical and chemical properties.

The knowledge of accurate molecular structures is a prerequisite for rational drug design and for structure based functional studies to aid the development of effective therapeutic agents and drugs. Crystallography can reliably provide the answer to many structure related questions, from global folds to atomic details of bonding. In contrast to NMR (which is a spectroscopic method), no size limitation exists for the molecule or complex to be studied. The price for the high accuracy of crystallographic structures is that a good crystal must be found, and that limited information about the molecule's dynamic behavior in solution is available from one single diffraction experiment. In the core regions of the molecules, X-ray and NMR structures agree very well, and enzymes maintain their activity even in crystals, which often requires the design of non-reactive substrates to study enzyme mechanisms.

A brief outline of the concepts behind the experimental set-up, data-collection strategies, structure solution methods, refinement and molecular self-assembly based on intermolecular interactions, with emphasis on hydrogen bonded interactions are discussed in this chapter.

1.4.1 Information from a single crystal structure determination

- Crystal system, Bravais-type, space group, metrics, lattice parameters
- Crystallographic density and “chemical composition“
- Symmetry of molecules
- Constitution and absolute configuration of a chemical compound
- Three-dimensional structure and packing of building blocks
- Precise and accurate bond lengths
- Conformation of molecules (torsion angles)
- Intermolecular bonding parameters
- van der Waals radii
- Volume of molecules
• Electron distribution
• Dynamic in crystalline solids
• Static and dynamic disordering in crystalline solids

1.4.2 Samples, instrumentation and data collection

The samples are unfracturated and optically clear single crystals. Their size should be between 0.1 and 0.2 millimeters in the three directions of space. They are normally selected using an optical microscope (x40) equipped with a polarizing attachment and observing if light extinguishes regularly every 90º when turning the stage of the microscope.

A selected crystal is fixed on the tip of a thin glass fiber using epoxy or cement, or in a loop including specific oil, which fits into the goniometer head in the diffractometer. The crystal is then aligned along the beam direction.

It is necessary to know the stability properties of the crystals. Crystals can be sensitive to light, air or moisture, or susceptible to loss of crystallization solvent. If so, a special treatment is required.

For example, they can be mounted inside sealed glass capillaries or the data collection can be performed at low temperature. Fig. 3 depicts the X-ray single crystal diffractometer Bruker AXS SMART APEX system. It is a 3-axis goniometer module with SMART APEX detector, it works with the usual monochromatic MoKa radiation (\(\lambda=0.7108\ \text{Å}\)).

![Fig. 3: Single crystal X-ray diffractometer](image)

Once the crystal is mounted on the diffractometer, the appropriate parameters for each measurement such as the distance to the detector and the space of the Ewald sphere are selected, and the intensity data is collected. Data are typically obtained between 3º and 30º 2θ when using molybdenum radiation. Generally, a complete data collection may
require between 3 to 12 h, depending on the specimen and the diffractometer. Some of the measured intensities enable the calculation of the unit cell parameters. Then all the intensities are indexed and a list of observed hkl reflections is obtained.

1.4.3 Structure determination methodology

The intensity of x-rays in a diffraction pattern depending only upon the crystal structure is referred to as called the structure factor:

\[ F_{hkl} = \sum_{j=1}^{N} f_j \exp \left( 2\pi i (hx_j + ky_j + lz_j) \right) \]  

(1)

Where \( h, k \) and \( l \) are the indices of the diffraction planes (Bragg reflections), \( N \) is the number of atoms in the cell and \((x_j, y_j, z_j)\) are the fractional coordinates of the \( j \)th atom with scattering factor \( f_j \). Each structure factor represents a diffracted beam which has an amplitude \( |F_{hkl}|\) and a relative phase \( \phi_{hkl} \).

The crystal structure can be obtained from the diffraction pattern if the electron density function is calculated at every point in a single unit cell:

\[ \rho(x, y, z) = \frac{1}{V} \sum_{h} \sum_{k} \sum_{l} |F_{hkl}| \cos \left[ 2\pi (hx_j + ky_j + lz_j) \right] - \phi_{hkl} \]  

(2)

Where the summation is over all values of \( h, k \) and \( l \) and \( V \) is the volume of the unit cell. Since X-rays are diffracted from the whole crystal, the calculation yields the contents of the unit cell averaged over the whole crystal. In practice, the calculation of the electron density produces maps. The maxima on these maps represent the position of the atoms in the cell.

The structure factors are reciprocal space vectors whereas the electron density is from the real space. The diffraction pattern is the Fourier transform of the electron density and the electron density is the inverse Fourier transform of the diffraction pattern. The measured intensities of a diffraction pattern enable the determination of only the structure factor amplitudes but not their phases. The calculation of the electron density is not then obtained directly from experimental measurements and the phases must be obtained by other methods. This is the so called phase problem. The most usual methods to overcome the phase problem are direct methods and methods based on the Patterson function. The former are the most important in chemical crystallography and the latter are currently applied when some heavy atoms are present. The phases are obtained approximately and have to be improved. With the calculated phases and structure factors amplitudes, a first
electron density map is calculated, also approximate, from which the atomic positions will be obtained.

The next step is the completion of the structure by Fourier synthesis and refinement of the structural parameters to optimize the fitting between the observed and calculated intensities in the diffraction pattern. The refinement cycles include positional atomic parameters and anisotropic vibration parameters. Finally, the hydrogen atom positions, if present, are determined or calculated. The structural refinement is evaluated from the agreement between the calculated and the measured structure factors. The refinement is considered finished when the following essential conditions are fulfilled:

- The agreement factors are small enough.
- The structural model is chemically appropriate.
- The estimated standard deviations of all geometrical parameters are as small as possible.
- The peaks remaining in the electron density map are as small as possible.

Once the structure is determined and refined several geometrical parameters such as bond lengths, bond angles, torsion angles, π-stacking and hydrogen-bonding are evaluated and appropriate tables and graphics representing the structure are prepared. A standard file (CIF: crystal information file) containing all the information of the structure is created and can be used to evaluate their quality and possible problems.

1.4.4 Program used for structural analysis:

Data collection: SMART [105]
Data Reduction: SAINT-Plus [105]
Absorption correction: SADABS [105]
Structure solution: SHELXS-97, SIR-92
Structure refinement: SHELXL-97 [106]
Graphics: ORTEP3 [107], CAMERON [108], Available from PLATON [109] \{WINGX [107]
Mean plane calculation: PARST [110]
CIF generation: ENCIPHER

Available from program suite
1.4.5 Limitations of X-ray structure analysis.

(1) Liquids and gases lack 3-dimensional order, and therefore cannot be used in diffraction experiments in the same way as crystals.

(2) It is not easy to locate light atoms in the presence of heavy atoms.
   a. Difference-Fourier maps alleviate the situation to some extent, but the atomic positions are not precise.
   b. The least-squares refinement of light-atom parameters is not always successful.

(3) Hydrogen atoms are particularly difficult to precisely locate because of their poor scattering power and the fact that the center of the hydrogen atom does not generally coincide with the maximum of its electron density.

(4) In general, bond distances determined by X-ray diffraction indicate distances between the centers of gravity of the electron clouds, which may not be the same as the inter nuclear distances.

1.4.6 Intermolecular interactions

Nature utilizes various weak interactions such as hydrogen bonds, relatively weak coordinate covalent bonds, van der Waals and columbic interactions, hydrophobic interactions, etc. for various fundamental biological processes [111].

1.4.6.1 Hydrogen bonding: A–H···B

A hydrogen bond can be defined as A–H···B where A and B are atoms that have electronegativities higher than hydrogen, such as carbon, nitrogen, phosphorus, oxygen, sulfur, selenium, fluorine, chlorine, bromine, and iodine [112]. The hydrogen bond is mainly electrostatic in nature, where the hydrogen atom has a partial positive charge $\delta^+$, while the more electronegative atoms each have a partial negative charge $\delta^-$.

$$\delta^- \overset{\delta^+}{\smile} \delta^- \quad \overset{\delta^+}{A \smile H \cdots B}$$
The failure to recognize these distinctions has led to some popular misconceptions in the past.

i. Hydrogen bonds are almost linear.

ii. The distance between the donor $A$ and acceptor $B$ atoms should be less than the sum of the van der Waals radii of $A$ and $B$.

1.4.6.2 Categories

Hydrogen-bond energies extend from about 15-40 kcal/mol for strong bonds, to 4-15 kcal/mol for moderate bonds and 1-4 kcal/mol for weak bonds.

1. Strong hydrogen bonds are formed by groups in which there is a deficiency of electron density in the donor group ($-\text{O-H}^+$ or $-\text{N-H}^+$) or an excess of electron density in the acceptor group ($\text{F}^-, \text{OH}^-, \text{O-C}^-, \text{O-P}^-$ or $\text{N}\text{R}_2$).
   
   a. Ionic hydrogen bonds.
   
   b. Forced strong hydrogen bonds.

2. Moderate hydrogen bonds are formed generally by neutral donor ($-\text{O-H}$ or $-\text{N-H}$) and acceptor (OR$_2$, O=C, or NR$_2$) groups.
a. These are the most common hydrogen bonds. ⇒ normal hydrogen bonds.

b. They are important and essential components of the structures and functions of biological molecules.

3. **Weak hydrogen bonds** are formed when the hydrogen atom is covalently bonded to a slightly more electro neutral atom relative to hydrogen (C–H or Si–H) or when the acceptor group has no lone-pair electrons but has π electrons (C≡C or aromatic rings).

### 1.4.6.3. General hydrogen bond rules

It is convenient to have a set of guidelines at hand when predicting hydrogen-bond patterns in the solid state. A Cambridge Structural Database (CSD) analysis of hydrogen bond patterns indicates preferred hydrogen-bond motifs and hydrogen-bond selectivity for certain functional groups or for sets of functional groups. From this analysis came about the formulation of three general hydrogen bond rules [113]. The first rule was developed by Donohue upon observation of only a handful of organic crystal structures: “all acidic hydrogens available in a molecule will be used in hydrogen bonding in the crystal structure of that compound” [114]. This rule is the most useful of all the hydrogen bond rules. The second and third rules were formulated by Etter based on her work on organic co-crystals: “all good acceptors will be used in hydrogen bonding when there are available hydrogen-bond donors”, and “the best hydrogen-bond donor and the best hydrogen-bond acceptor will preferentially form hydrogen bonds to one another” [113, 115,116]. These rules are based on energetically favourable types of hydrogen bonds and also reflect crystal packing patterns of organic crystal structures.

### 1.4.6.4. Graph set notation

Etter formulated a useful method for describing hydrogen-bond patterns in crystal structures based on graph set notation [113,117]. These patterns can be classified according to one of four descriptors: chains (C), rings (R), dimers (D), or intramolecular (S). Following these descriptors, the number of hydrogen-bond acceptors (superscript) and donors (subscript) are designated. The total number of atoms involved in the motif is then provided in parentheses (Fig. 4)

$$G^a_d (n)$$
Fig. 4: Some examples of graph set notation in hydrogen-bond patterns

1.4.6.5 van der Waals forces

Dispersion forces, the most important of the nondirectional forces, are net attractive forces generated by temporary charge imbalances among molecules. These forces arise due to the rapidly pulsating field associated with the internal motion of the electrons relative to the nucleus in the molecule. The oscillating dipole polarizes the other atom. The coupling of the dipoles of the two atoms results in an attractive force between those atoms.

When two interacting atoms come very close together, their electron clouds overlap, resulting in very strong repulsion. These repulsive forces operate at short intermolecular separations and vary approximately with reciprocal twelfth power of distance between the interacting atoms. These forces are responsible for non bonded strain and steric hindrance.

The repulsive and dispersive forces are collectively referred to as the van der Waals forces. These are long-range forces. van der Waals forces include dipole-dipole interactions, dipole-induced dipole interactions, induced dipole-induced dipole interactions, and \( \pi \cdots \pi \) interactions. Of these, \( \pi \cdots \pi \) interactions have been common in some of the structures discussed here.
1.4.6.6. π - π interactions

These are interactions specific to aromatic π-systems. When the two π-atoms are oriented in a face-to-face fashion (angle = 0, offset = 0), repulsion is observed; however, when one πatom is rotated by up to 90° with respect to the other, an attractive geometry is formed. Another attractive geometry is formed when one π-atom is offset laterally with respect to the other. These results for non-polarized π-systems can be summarized with three rules: (1) π...π repulsion dominates in a face-to-face π-stacked geometry; (2) π...σ attraction dominates in an edge-on or T-shaped geometry; and (3) π...σ attraction dominates in an offset π-stacked geometry [118].

In the presence of polarizing groups (i.e. electron donors and acceptors), π...π interactions can adopt six possible orientations, which are as shown in Fig. 5.

![Fig. 5: Possible orientations for the π...π interactions between polarized π-systems, where R₁ and R₂ are polarizing groups.](image)

Based on the above three rules, offset stacking (orientations 3-6) are attractive, while face-to-face stacking geometries (orientations 1 and 2) are repulsive.
References: