2.1 INTRODUCTION

Azole derivatives have gained significance importance in the field of synthetic and medicinal research owing to their immense biological properties [1-5]. Among them, thiazoles are the five-membered heterocyclic compounds which contain sulphur and nitrogen at the position-1 and position-3, respectively. The general structure of thiazole ring is shown in Figure 1.

![Thiazole structure](image)

**Fig. 1**

Thiazole ring is found in a large number of natural products [6-7]. Thiazole and its derivatives were known to possess a wide range of biological properties. Some potent drugs bearing thiazole nucleus include Fanetizole (anti-inflammatory agent), Tiazofurin (antineoplastic agent), Penicillin (potent antibiotic), Sulfatiazol (antimicrobial) and Abafungin (antifungal) [8-10]. It has been reported that substitution at position-2 and -4 of thiazole ring leads to the development of many bioactive agents. 2,4-Disubstituted thiazoles displayed antimicrobial [11], anti-inflammatory [12], anticancer [13], antimalarial [14], antimycobacterial [15], chemopreventive [16] and antioxidant [17] properties. Moreover, they act as inhibitor of metastatic cancer cell migration and invasion [18], ovarian cancer cell growth inhibitor [19], P-glycoprotein inhibitor [20] besides corticotropin-releasing factor 1 receptor antagonists [21]. The most common approach used to construct thiazole system is Hantzsch thiazole synthesis which involves the reaction of thioureas or thioamides with $\alpha$-haloketones [22]. The synthetic and biological developments of some 2,4-disubstituted thiazole derivatives are described below.

**Synthetic and biological developments of 2,4-disubstituted thiazoles**

Maillard *et al.* have synthesized some novel 2-hydrazino-1,3-thiazole derivatives 3 in 37-92% yield by the reaction of various thiosemicarbazones 1 with 2-chloro-1-(2-hydroxy-5-methoxyphenyl)ethanone 2 using ethanol as a solvent [23] (Scheme-1). The products 3 were screened for their antifungal activity against eight *Candida* spp.
strains such as \textit{C. albicans}, \textit{C. glabrata}, \textit{C. krusei} and \textit{C. parapsilosis} and compared with five reference drugs such as Amphotericin B, Itraconazole, Fluconazole, Voriconazole and Caspofungin. The antifungal results revealed that compound 3 bearing \(R = \text{H} \) and \(R' = \text{indol-3-yl}\) displayed potent activity (MIC = 0.25 to > 16 \(\mu\text{g/ml}\)) against the \textit{C. albicans} spp. strains and may act as new lead for the development of potent antifungal agents in future. Cytotoxic effect of 3 against mouse fibroblast (NIH/3T3) cell line showed that it displayed weak cytotoxicity = 0.5 \(\mu\text{g/mL}\) with more than 83% of cell viability.

\[ \text{R} = \text{Me, H, cyclohexyl} \]
\[ \text{R'} = \text{indanyl, 1,3 - benzodioxol-5-yl, cyclohexyl, napht-2-yl, indol-3-yl, imidazo[1,2-a]pyridin-3-yl, benzofuran-2-yl, pyrrol-3-yl, fur-2-yl, imidazol-5-yl, 3-methyl-6-phenylimidazo[2,1-b]thiazol-5-yl} \]

\textbf{Scheme-1}

Condensation of various 5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-thiocarboxamides 4 with 3-(2-bromoacetyl)coumarins afforded 2-(5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazoles 5 [24] (\textbf{Scheme-2}).

\[ \text{R} = \text{CF}_3, 4-\text{ClPh}, 4-\text{BrPh}, 4-\text{FPh} \]
\[ \text{R'} = \text{H, Cl, Br} \]

\textbf{Scheme-2}

The antibacterial activity of 5 was evaluated against three Gram-positive bacterial strains namely, \textit{S. aureus}, \textit{B. subtilis}, \textit{S. epidermidis} and three Gram-negative strains namely, \textit{K. aerogenes}, \textit{E. coli}, \textit{P. mirabilis} and \textit{P. aeruginosa} using agar well diffusion method. In addition, \textit{in vivo} anti-inflammatory activity was also determined using carrageenan-induced paw oedema method. In antibacterial screening, it was
found that all compounds displayed potent antibacterial activity. However, compounds having \((R = \text{CF}_3, R' = \text{H})\) and \((R = \text{CF}_3, R' = \text{Cl})\) showed 83% and 86% anti-inflammatory activity, respectively when compared to indomethacin (94%).

The treatment of thionicotinamide 6 with \(p\)-chloroacetylacetanilide 7 or 3-bromoacetylcoumarin or 3-chloroacetylcetone 8 using triethylamine in ethanol afforded the corresponding 2-(3-pyridyl)thiazoles 9-11, respectively [25] (Scheme-3).

The synthesized compounds (9-11) were evaluated for their \textit{in vitro} antimicrobial screening by using the disc diffusion assay method against ten bacterial strains i.e \textit{S. aureus}, \textit{S. epidermidis}, \textit{S. pyogenes}, \textit{B. subtilis} and \textit{E. faecalis} (Gram-positive bacteria) and \textit{N. gonorrhoeae}, \textit{P. vulgaris}, \textit{K. pneumonia}, \textit{S. flexneri} and \textit{P. aeruginosa} (Gram-negative bacteria) and five fungal human pathogenic strains i.e \textit{A. fumigates}, \textit{A. clavatus}, \textit{C. albicans}, \textit{G. candidum} and \textit{P. marneffei}. The standards used for antibacterial activity Ampicillin and Gentamycin, however, Amphotericin B was used in the antifungal screening. Among all, the compound 11 was found to be the most potent antimicrobial agent which inhibited \textit{S. aureus} and \textit{S. epidermidis} with MIC = 0.97 \(\mu\text{g/ml}\), \textit{K. pneumonia} with MIC = 1.95 \(\mu\text{g/ml}\) and \textit{G. candidum} with MIC = 1.95 \(\mu\text{g/ml}\).

![Scheme-3](image-url)

4-(4-Arylthiazol-2-yl)amino-4-oxobutanoic acid 14 and 2-(4-chloro-6-[4-aryl-1,3-thiazol-2-yl]aminopyrimidin-2-yl-sulfanyl)octanoic acid derivatives 15 were synthesized using 4-(2-chlorophenyl)thiazol-2-amines 13 as intermediates which were
obtained by the treatment of various $\alpha$-bromoketones with thiourea 12 [26] (Scheme-4). The compounds 14 were screened for their inhibitory effect on the production of LPS-stimulated TNF-\(\alpha\) in RAW 264.7 cells in order to check their potential in the treatment of type II diabetes. Among all synthesized derivatives, the compound 14 having 3,4-difluorophenyl substitution displayed 92.35\% inhibitory effect at a dose level of 10 $\mu$mol/ml. However, the compound 15 was evaluated to assess microsomal prostaglandin E2 synthase-1 (mPGES-1) and 5-lipoxygenase (5-LO) inhibitor potential [27]. It was found that compound 15 bearing naphthalen-2-yl displayed potent dual mPGES-1/5-LO inhibitory activity with IC\(_{50}\) = 0.2-0.4 \(\mu\)M.

Gaikwad et al. have prepared 1-(2-(1H-benzo[d][1,2,3]triazol-1-yl)-1-(4-substitutedphenyl) ethylidene)-2-(4-(substitutedphenyl) thiazol-2-y1)hydrazines 17 by the condensation of 1-(2-(1H-benzo[d][1,2,3]triazol-1-yl)-1-(4-substituted phenyl)ethylidene)thiosemicarbazides 16 with appropriate $\alpha$-bromoketones in refluxing ethanol [28] (Scheme-5). The synthesized compounds 17 were screened for their in vitro antibacterial activity against four bacterial strains viz. \textit{B. subtilis}, \textit{S. aureus}, \textit{E. coli} and \textit{P. aeruginosa} and two fungal strains namely, \textit{C. albicans} and \textit{A. niger}. The results of antibacterial activity indicated that most of the synthesized compounds displayed moderate to good activity against the tested bacterial strains as well as the fungal strains. The presence of fluoro, chloro and bromo group enhanced the antibacterial and antifungal activity irrespective of their position in the molecule.
Some substituted phenoxythiazole derivatives **20** have been synthesized by the treatment of 2-(substituted phenoxy) ethanethioamides **18** with α-bromoketones [29] (Scheme-6). The compounds **20** were evaluated for their Glutathione S-transferase pi (GSTpi) inhibitory potential because GSTpi enzyme protects cells from death and detoxifies chemotherapeutic agents in cancer cells [29]. From this investigation, it was found that three compounds having (R = Ph and R' = R'' = R''' = CH₃), (R = Ph, R' = H, R'' = Cl and R''' = CH₃) and (R = Ph, R' = H and R''/ R''' = CH₃) exhibited highest inhibitory potential in the range of 44.0-54.1% in comparison to ethacrynic acid (24.2% inhibition) at 1μM concentration.

Selective human monoamine oxidase B inhibitors, (4-aryl-2-cycloalkylidenhydrazinylthiazoles) **22** were synthesized by the reaction of thiosemicarbazones **21** with α-haloketones in methanol under microwave irradiation [30]. It was found that compounds **22** containing 2-methylcyclopentyldiene
substituent were found as more potent inhibitors than the compounds bearing 3-
methylycyclopentylidene substituent (Scheme-7).

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{N} \quad \text{S} \quad \text{H}_2\text{N} \\
\text{C} & \quad \text{S} \quad \text{NH}_2 \\
\end{align*}
\]

\[
\begin{align*}
\text{R} & \quad \text{O} \\
\text{X} & \quad \text{X} = \text{Cl, Br} \\
\text{R} & \quad \text{R} = 4-\text{F, 4-CN, 4-NO}_2, \text{2,4-F} \\
\end{align*}
\]

Scheme-7

The condensation of \(\alpha\)-bromoketones with \(N\)-arylthioureas 23 in ethanol under microwave irradiation afforded \(N,4\)-diaryl-1,3-thiazole-2-amines 24 [31]. (Scheme-8)

The compounds 24 were tested to investigate their activity and selectivity towards COX-1, COX-2, LO-5, LO-12 and LO-15 enzymes in order to assess their potential in treating eicosanoid-mediated diseases. The results revealed that compound 24 having (\(R = \text{phenyl and } R' = \text{OH}\)) was found active against all the enzymes while 24 having (\(R = 4\)-chlorophenyl and \(R' = \text{OH}\)) was found active and selective against 5-LO and COX-2 enzymes with an IC\(_{50}\) value of 0.9 ± 0.2 \(\mu\)M (5-LO) and a residual activity of 9.1 ± 1.1% at 10 \(\mu\)M.

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{N} \quad \text{S} \quad \text{N} \\
\text{H} & \quad \text{S} \quad \text{N} \\
\text{R} & \quad \text{R} \\
\end{align*}
\]

Scheme-8

Therefore, these two compounds may act as new lead in the treatment of eicosanoid-mediated diseases. On the other hand, Siddiqui et al. used the intermediate 24 in order to construct 3-[4-(substituted phenyl)-1,3-thiazol-2-ylamino]-4-(substituted phenyl)-4,5-dihydro-1\(H\)-1,2,4-triazole-5-thiones 25 which exhibited anticonvulsant activity in
two seizure models *i.e.* maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ) [32]. Among the compounds 25, the compounds having \((R = \text{Cl, } R' = 4\text{-OCH}_3)\) and \((R = \text{Br, } R' = 2\text{-CH}_3)\) exhibited potent anticonvulsant activity with \(ED_{50} = 23.9\) and 13.4 mg/kg, respectively in MES model and 178.6 and 81.6 mg/kg, respectively in scPTZ model.

Chimenti *et al.* synthesized \([4-(4'\text{-substituted-phenyl})\text{thiazol-2-yl}]\text{hydrazine derivatives 27}\) by the reaction of thiosemicarbazones 26 with \(\alpha\text{-bromo-4-methyl/4-methoxyacetophenone [33]}\) (Scheme-9). The synthesized compounds 27 were evaluated for their *in vitro* antifungal activity against 20 clinical isolates of pathogenic *Candida spp.* and compared their potential with reference drugs, Clotrimazole and Fluconazole. The antifungal screening results revealed that most of the synthesized compounds displayed selective inhibitory activity especially against *Candida albicans* and *Candida glabrata*.

\[\text{Het} = \text{fur-2-yl, thien-2-yl, pyridin-2-yl, naphth-1-yl, naphth-2-yl, benzodioxol-5-yl, indol-3-yl, coumarin-3-yl}\]

\[R = \text{H, CH}_3; R' = 4\text{-OCH}_3, 4\text{-CH}_3\]

**Scheme-9**

A series of novel \(p\text{-toluenesulfonyl hydrazino thiazoles 29 and 30}\) were synthesized to evaluate their anticancer potential against prostate DU-145 and hepatocarcinoma HepG2 cancer cell lines using \(2,3\text{-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt (XTT) assay [34]}\) (Scheme-10). The treatment of \(p\text{-toluenesulfonylthiosemicarbazide 28}\) with various \(\alpha\text{-bromoketones using a mixture of anhydrous DMF and anhydrous acetone afforded 4-substituted-2-[2-(p-toluenesulfonyl)-hydrazino]-thiazoles 29 which on acetylation furnished 4-substituted-2-[2-(p-toluenesulfonyl)-N,N-diacetyl-hydrazino]-thiazoles 30 [33]. Among all, the compounds 29a, 29c and 30a showed significant activity against both the cell lines with \(IC_{50} < 10 \mu\text{M}\).
The reaction of 3-aryl-1H-pyrazole-4-carbaldehyde thiosemicarbazones 31 with 2,4-dichlorophenacyl chloride 32 and 6-H/Br-3-(bromoacetyl)-2H-chromen-2-one afforded 2,4-disubstituted thiazoles 33 and 34, respectively [35] (Scheme-11). Two compounds 33 and 34 were screened for antibacterial potential against four bacterial strains viz. *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. The results revealed that compounds having 2,5-dichlorothiophene and 2,4-dichlorophenyl substituents showed significant antibacterial activity against all tested microorganisms.

Sarojini *et al.* synthesized 2-substituted 4-(2,5-dichlorothienyl)-1,3-thiazoles 36 by the reaction of 2-bromo-1-(2,5-dichlorothien-3-yl)ethanone with thiourea or substituted thioamides 35 in the presence of potassium hydroxide in refluxing ethanol.
The compounds 36 were screened for antimicrobial potential against bacterial strains namely, *E. coli*, *S. aureus*, *P. aeruginosa*, and *K. pneumoniae* as well as fungal strains *i.e.* *A. flavus*, *A. fumigates*, *P. marneffei* and *T. mentagrophytes*. Among all, the compound having 8-quinolinyl moiety was found to be the most potent antimicrobial agent. Moreover, molecular docking studies results revealed that this compound showed minimum binding and docking energy and may be considered as a good inhibitor of D-fructose-6-phosphateamido-transferase (GlcN-6-P).

Some novel 2,4-disubstituted thiazoles 38 as potential FabH inhibitors were synthesized by the treatment of various thiosemicarbazones 37 with different α-haloketones [37] (Scheme-13). The synthesized compounds were screened for their antibacterial potential against three Gram positive bacterial strains namely, *B. subtilis*, *S. aureus* and *S. faecalis* and three Gram negative bacterial strains namely, *E. coli*, *P. aeruginosa*, and *E. cloacae*.

Among all the synthesized compounds 38, two compounds *i.e.* (E)-2,4-dibromo-6-((2-(4-(3,4-dichlorophenyl)thiazol-2-yl)hydrazono)methyl)phenol and (E)-4-bromo-2-((2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)methyl)phenol exhibited potent
inhibitory effect against *E. coli*. Furthermore, from the results of *E. coli* FabH inhibitory activity and molecular docking study, it has been found that these compounds act as potent FabH inhibitors.

Mjambili *et al.* synthesized 2-substituted-amino-4-(2-pyridyl) thiazole scaffolds 41 by the reaction of 2-bromo-1-(pyridin-2-yl)ethanone with thiourea 12 followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) mediated coupling in the presence of mono substituted carboxylic acids 40 to achieve the targeted compounds [38] (Scheme-14). The synthesized compounds were screened for their *in vitro* antimycobacterial activity against the *Mycobacterium tuberculosis* H37Rv strain and antiplasmodial activity against the chloroquine sensitive NF54 *Plasmodium falciparum* strain. It was found that most of the synthesized compounds displayed potent inhibitory potential against the two strains used.

![Scheme-14](image)

The treatment of 3-aryl-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamides 42 with α-bromoketones using DMF yielded 4-aryl-2-(3-aryl-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazoles 43 [39] (Scheme-15).

![Scheme-15](image)

All the compounds 43 were evaluated for BRAFV600E inhibitory activity and antiproliferative activity against WM266.4 human melanoma and breast cancer MCF-7 cell lines. Among all, the compound having R = Cl and R' = CF₃ was found to be the most potent BRAFV600E inhibitor with IC₅₀ = 0.051 µM. It also displayed
excellent antiproliferative activity with IC<sub>50</sub> = 0.12 µM for WM266.4 cell line and with IC<sub>50</sub> = 0.16 µM for MCF-7 cell line.

1-(4-(2-Substituted thiazol-4-yl)-phenethyl)-4-(3-(4-substituted piperazin-1-yl)alkyl)-piperazine analogues 46 were synthesized via four step procedure using 4-(4-(2-chloroethyl)phenyl)-2-substitutedthiazoles 45 as an intermediate which was obtained by the reaction of 2-chloro-1-(4-(2-chloroethyl)phenyl)ethanone 44 with thioureas / thioamides 35 [40] (Scheme-16). The targeted compounds 46 were evaluated for antitubercular activity and cytotoxicity against *Mycobacterium tuberculosis* H37Rv strain and mouse macrophage (RAW264.7) cell lines, respectively. The results revealed that among all, compound having R = NHCH<sub>3</sub>, R' = (Ph)<sub>2</sub>CH and n = 3 was found to be the most potent which inhibited 99% growth of *M. tuberculosis* H37Rv strain. In addition, in cytotoxic study it showed selectivity index greater than 30 indicating the suitability of compound in an endeavour to attain lead molecule for further drug development.

![Scheme-16](image)

2-(Pyridin-2-yl)-1,3-thiazoles 48 were synthesized by the reaction of 2-(pyridin-2-yl)thiosemicarbazones 47 with various α-bromoketones in the presence of calcium carbonate using propan-2-ol as a solvent under ultrasound irradiation [41] (Scheme-17). It was found that two compounds namely 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(2,4-dichlorophenyl)-1,3-thiazole and 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(3,4-dichlorophenyl)-1,3-thiazole displayed potent antitrypanosoma cruzi activity with IC<sub>50</sub> = 1.2 µM even more than Benznidazole (IC<sub>50</sub> = 6.2 µM), a standard drug.
Inoue et al. synthesized N-{4-[2-(4-([amino(imino)methyl]-amino)phenyl)ethyl]-1,3-thiazol-2-yl}acetamide 51 via multistep procedure using (2-amino-1,3-thiazol-4-yl)methyl acetate hydrochloride 50 an intermediate which was obtained by cyclization of 3-chloro-2-oxopropyl acetate 49 with 12 in ethanol [42] (Scheme-18). The compounds 51 were evaluated for vascular adhesion protein-1 (VAP-1) inhibitors for the treatment of diabetic macular edema and it was found that it displayed excellent VAP-1 inhibitory effect with IC$_{50}$ = 0.23 µM besides significant inhibitory effect on ocular permeability in streptozotocin (STZ)-induced diabetic rats.

Scheme-18

1-(4-(4'-Chlorophenyl)-2-thiazolyl)-3-aryl-5-(2-butyl-4-chloro-1H-imidazol-5-yl)-2-pyrazolines 55 were synthesized by the treatment of p-chlorophenacyl bromide with thiosemicarbazide 52 using polyethylene glycol to obtain 4-(4'-chlorophenyl)-2-hydrazino-thiazole 53 which was further reacted with 1-(substituted phenyl)-3-(2-butyl-4-chloro-1H-imidazol-5-yl)-2-propen-1-ones 54 in the presence of sodium hydroxide using polyethylene glycol to afford the targeted compounds [43] (Scheme-19). The synthesized compounds 55 were screened for their antimicrobial activities against E. coli, S. typhi, S. aureus, B. subtilis, A. niger, T. viridae, P. chrysogenum, F. moniliforme and C. albicans. The results revealed that compounds having 2-hydroxy-3-iodo-5-chloro-phenyl, 2-hydroxy-3-bromo-5-chloro-phenyl, 2-hydroxy-3,5-di-iodo-
phenyl, 2-hydroxy-3,5-dibromo-phenyl and 2-hydroxy-3,5-dichloro-phenyl displayed good antifungal and antibacterial activity.

Scheme-19

2-Aryl-N-(4-(4-((piperidine-1-carbonyl)thiazol-2-yl)benzyl)acetamides 59 were synthesized via using ethyl 2-(4-((1,3-dioxoisindolin-2-yl)methyl)phenyl)thiazole-4-carboxylate 58 which was prepared by the reaction of ethyl bromopyruvate 56 with thioamide derivative 57 [44] (Scheme-20). The anticancer activity of 59 was evaluated against three cell lines viz. T47D breast, Caco-2 colorectal and HT-29 colon cancer cell lines. From this investigation, the compound having 3-fluoro substituent was found to be more potent against all the cell lines having IC50 value 10 µg/ml while the compounds having 4-methoxy substituent was found active against Caco-2 cell line. The compound bearing 2-methoxy substituent was found potent against HT-29 and T47D cell lines.
3-(Benzofuran-2-yl)-4,5-dihydro-5-arylpyrazole-1-carbothioamides 60 were treated with α-bromoketones in ethanol to afford 3-(benzofuran-2-yl)-1-(4-(4-aryl)thiazol-2-yl)-5-(4-aryl)-4,5-dihydro-1H-pyrazoles 61 [45] (Scheme-21). The synthesized compounds 61 were evaluated for their in vitro antimicrobial activity against Gram-positive bacterial strains viz. *S. aureus*, *B. subtilis*, Gram-negative bacterial strains viz. *E. coli*, and fungal strains viz. *C. albicans* and *A. niger*. The results revealed that compound having Ar = Ar' = Ph displayed excellent activity against *S. aureus* and *E. coli*. However, two compounds having (Ar = 4-Cl-Ph, Ar' = Ph) and (Ar = 4-Cl-Ph, Ar' = 4-Br-Ph) exhibited potent antifungal activity against *C. albicans* even more than Fluconazole, a standard antifungal drug.

\[ \text{Ar} = \text{Ph, 4-ClPh}; \text{Ar'} = \text{Ph, 4-Br-Ph} \]

**Scheme-21**

4-(2-Morpholinoethoxy)benzothioamide 62 was treated with ethyl-4-chloroacetate 63 to afford ethyl 2-(2-(4-(2-morpholinoethoxy)phenyl)thiazol-4-yl)acetate 64 which was used to synthesize *N*-benzyl substituted [(2-morpholinoethoxy)phenyl]thiazol-4-yl]acetamide derivatives 65 [46] (Scheme-22).

\[ \text{R} = \text{H, 4-F, 2-Cl, 3,4-di-Cl, 4-CH}_3 \]

**Scheme-22**

The compounds 65 were evaluated for c-Src kinase inhibitory activity in NIH3T3/c-Src527F and SYF/c-Src527F cells. The results shown that compound having unsubstituted phenyl ring was found to be the most potent with GI$_{50}$ values of 1.34 µM and 2.30 µM in NIH3T3/c-Src527F and SYF/c-Src527F cells, respectively. In addition, the anticancer activity of compounds was evaluated against human colon
(HT-29), breast cancer (BT-20) and leukemia (CCRF-CEM) cancer cell lines. From this investigation, it has been found that 4-fluorobenzylthiazolyl displayed 64-71% cell proliferation inhibition of BT-20 and CCRF cells at concentration of 50 µM.

A new series of \(N\)-(1-arylethylidene)-\(N’\)-(4-arylthiazol-2-yl)hydrazine derivatives \(67\) was synthesized by the treatment of 1-(1-arylethylidene)thiosemicarbazides \(66\) with \(\alpha\)-bromoketones [47] (Scheme-23). The synthesized compounds \(67\) were screened for their antitubercular activity against \textit{Mycobacterium tuberculosis} H37Rv strain. It was found that compounds having 2-pyridyl and 2-hydroxy-5-methoxyphenyl group were found most potent with IC\(_{50}\) = 6.22 and 6.78 µg/ml, respectively besides low cytotoxicity (CC\(_{50}\): > 40 µg/mL).

\[
\begin{align*}
\text{Ar}_N\text{H} & \quad \text{N} \quad \text{S} \quad \text{NH}_2 \\
+ \quad \text{Ar’}_\text{Br} & \quad \text{O} \quad \text{propan-2-ol} \quad \text{reflux} \\
\rightarrow & \quad \text{Ar’}_N\text{H} & \quad \text{N} \quad \text{S} \quad \text{Ar} \\
\end{align*}
\]

\(\text{Ar} = 2\text{-pyridinyl}, 3\text{-pyridinyl}, 4\text{-pyridinyl} ; \text{Ar’} = 2\text{-thiophenyl}, 2\text{-hydroxy-5-methoxyphenyl}\)

Scheme-23

Juneja \textit{et al}. synthesized diaminocinnamoylthiazoles \(70\) by the treatment of bromomethyl styryl ketones \(68\) with 1-(4-chlorophenyl)-3-(\(N\)-nitroamidino)thiourea \(69\) in the presence of triethylene amine using \(N,N\)-dimethylformamide [48] (Scheme-24). The cytotoxic and apoptotic effects of compounds \(70\) were studied on HeLa cells. Both the compounds (\(R = \text{OH}, R’ = \text{OMe}\)) and (\(R = \text{OMe}, R’ = \text{OH}\)) inhibited the cell growth with IC\(_{50}\) = 60 and 30 µM, respectively besides inducing apoptosis of HeLa cells.

\[
\begin{align*}
\text{R’}_N\text{O}_\text{Br} & \quad \text{O}_2\text{NHN} & \quad \text{O}_2\text{NHN} \\
+ \quad \text{NH}_2\text{NH} & \quad \text{NH}_2\text{NH} & \quad \text{N,N-DMF} \\
\rightarrow & \quad \text{R’}_N\text{O} & \quad \text{R’}_N\text{O} \\
\end{align*}
\]

\(\text{R} = \text{R’} = \text{OH}, \text{OMe}\)

Scheme-24

Some novel thiazolyl-pyrazoline derivatives \(72\) containing benzodioxole were synthesized by the treatment of pyrazoline thiocarboxamides \(71\) bearing benzodioxole moiety with \(\alpha\)-bromoketones in ethanol under reflux conditions [49] (Scheme-25). The synthesized compounds \(72\) were screened for anticancer activities against MCF-7 human breast cancer and B16-F10 mouse melanoma cell lines as well as HER-2
inhibitory activity. Among all, the compound 2-(5-(benzo[d][1,3]dioxol-5-yl)-3-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-(4-bromophenyl)thiazole displayed potent \textit{in vitro} anticancer activity against MCF-7 and B16-F10 cell line with IC$_{50}$ value of 0.09 µM and 0.12 µM, respectively in reference to Erlotinib (IC$_{50}$ = 0.02 µM and 0.05 µM against MCF-7 and B16-F10, respectively), as the standard drug used. Moreover, this compound was found potent to inhibit the autophosphorylation of HER-2 kinases with IC$_{50}$ = 0.18 µM.

![Scheme-25](image)

Thiosemicarbazones 73 were treated with 2-bromacetophenone in propanol under ultrasound irradiation to give 2-imino-1,3-thiazoles 74 [50] (Scheme-26). The synthesized compounds 74 were evaluated for anti-trypanosoma cruzi activity against epimastigotes (axenic culture) and bloodstream trypomastigotes of the Y strain. It was observed that compounds bearing 4-pyridinyl and 3-nitrophenyl substituents exhibited high trypanocidal activity with IC$_{50}$ values of 3.9 and 3.8 µM for bloodstream Y strain trypomastigotes, respectively.

![Scheme-26](image)

Basarab \textit{et al.} synthesized 2,4-disubstituted thiazoles 78, as potent ATP competitive dual targeting type II topoisomerase inhibiting antibacterial agents, by using 1-(5-bromo-4-(4-tert-butylthiazol-2-yl)pyridin-2-yl)-3-ethylurea 77 [51] (Scheme-27). The treatment of 5-bromo-2-(3-ethylureido)pyridine-4-carbothioamide 75 with 1-bromo-3,3-dimethylbutan-2-one 76 in the presence of triethylamine using trifluoroacetic acid (TFAA) under reflux afforded the targeted compound 78.
Scheme-27

75 + 76 → 77

TFAA, Et₃N, CH₃CN, 80 °C
2. RESULTS AND DISCUSSION

2.2A Synthesis, Characterization and Biological Evaluation of Some Novel Oxazolidinone-Thiazole Hybrids

2.2A.1 Chemistry

Owing to diverse biological properties, oxazolidinone and thiazole containing motifs have gained a great attention of scientific community in the field of medicinal chemistry. Oxazolidinone derivatives exhibited a wide range of applications in the field of antimicrobial, anticancer and anti-tubercular research [52-56]. Designing efficient antimicrobial agents with different mechanism of action could be the potential approach to resolve the issues related to the resistance of pathogens. Compounds bearing oxazolidinone moiety as antimicrobial agents contributed a lot in this field [52, 53, 55-57]. They exhibited very high potential against Gram-positive multidrug resistant strains. Linezolid I (marketed under the trade name zyvox), (Figure 2) a potent antimicrobial was the first member of oxazolidinone family approved by the US Food and Drug Administration (FDA) for the treatment of serious infection caused by Gram-positive bacteria [54, 55]. It inhibits protein synthesis prior to chain initiation step by binding to the 50S ribosomal subunit to prevent formation of 70S initiation complex [55]. Some other biologically active oxazolidinone pharmacores include Eperezolid II and Posizolid III, which were used as promising antibiotics to treat bacterial infections while Torezolid IV was used in the treatment of complicated skin infections [54, 56] (Figure 2).

![Fig.2 Structure of oxazolidinone containing potent pharmacores](image)

In addition, thiazole containing motifs have also attracted much interest over the years due to their role in the development of pharmacologically active compounds [11-17]. It has been reported that several potent drugs like Penicillin (a potent antibiotic),
Sulfathiazole (antimicrobial) and Abafungin (antifungal) possess thiazole nucleus [8-10].

As discussed in previous chapter, the development of some novel DNA damage protecting agents constitutes an another challenging area in the field of medicinal chemistry. An exposure to UV radiations leads to the formation of intra and intermolecular cross-links in DNA besides its degradation and thus creates the problems for genetic engineers in performing the gel electrophoresis experiments [58, 59]. UV irradiation is also responsible for generation of reactive oxygen species and reactive nitrogen species which may result in serious disorders for example erythema, edema, hyperpigmentation, immunosuppression, photoaging, skin cancer, cataract formation and retinal degeneration, etc [60]. In literature, it has been reported that some heterocyclic or non heterocyclic compounds resolved such problems to some extent but still more research work is required in this field to develop newer and efficient protecting agents [60].

Though nature provided each cell a protective mechanism (known as antioxidant mechanism) against harmful effects of free radicals, administration of antioxidants plays an important role in protecting the biological targets when the normal antioxidant defense mechanism fails [61, 62]. Therefore, considerable studies have been focused on the development of novel and safer antioxidants with lesser toxicity in view of treating various serious disorders like coronary heart diseases, ulcers, cancers and neurodegenerative diseases produced by excessive formation of these radicals [61, 62].

Prompted from the above facts and in continuation to our ongoing interest in the synthesis of new biologically active compounds, it was planned to synthesize some novel structural hybrids by incorporating both thiazole and oxazolidinone moiety. The compound 4-(4'-aminobenzyl)oxazolidin-2-one **84** was used as a key intermediate to synthesize the targeted oxazolidinone-thiazole hybrid compounds. The compound **84** was obtained via four-step synthetic route starting from 4-nitrophenylalanine monohydrate **79** according to the literature procedure [63] (Scheme-28). Initially, the compound **79** was treated with thionyl chloride in methanol under reflux conditions to obtain methyl-4-nitrophenylalaninate hydrochloride **80** which on further reduction with sodium borohydride gave 2-amino-3-(4-nitrophenyl)propanol **81**. The reaction of **81** with triphosgene **82** in the presence of potassium carbonate using toluene afforded 4-(4'-nitrobenzyl)oxazolidin-2-one **83**.
which was converted into 84 by hydrogenation in presence of raney nickel. However, α-bromoketones were prepared by the bromination of arylmethylketones as reported in the previous Chapter.

![Scheme-28 Synthesis of 4-(4'-aminobenzyl)oxazolidin-2-one 84](image)

The synthesis of novel oxazolidinone-thiazole hybrid compounds 87a-m has been accomplished according to Scheme-29. The reaction of 4-(4'-aminobenzyl)oxazolidin-2-one 84 with ammonium thiocyanate was performed in a solution of hydrochloric acid and water under reflux conditions to obtain the thiourea derivative 85. Further, treatment of 85 with α-bromoketones 86 gave exclusively 4-(4'-(4''-(aryl/heteroaryl) thiazol-2-ylamino)benzyl)oxazolidin-2-ones 87.

The structures of the compounds 85 and 87 were established on the basis of the combined use of IR, NMR (1H & 13C) spectroscopy and mass spectrometry. An appearance of C = C, C = N and C = O stretching frequency bands at 1543, 1589 and 1728 cm⁻¹, respectively indicated the formation of 85. In the 1H NMR spectrum, a singlet at δ 4.80 due to NH₂ protons was disappeared, however, two singlets at δ 7.13 and 7.26 for two NH₂ protons were appeared and thus supported the formation of 85 (Figure 3). Appearance of two signals may be due to the existence of resonance between the unshared electron pair on nitrogen and the thiocarbonyl group which restricted the C-N bond rotation and thus makes the hydrogens of NH₂ group nonequivalent. The formation of 85 was further supported by its 13C NMR and mass spectral data, which showed a signal at δ 180.91 due to thiocarbonyl carbon (Figure 4) and a molecular ion peak at m/z = 251 (M⁺) in accordance to molecular formula C₁₁H₁₃N₃O₂S, respectively.
The disappearance of two signals for NH$_2$ protons and an appearance of a characteristic singlet at $\delta$ 6.96 due to thiazole proton (H-5") in the $^1$H NMR spectrum confirmed the formation of 87a (Figure 5). In the IR spectrum, appearance of C=C, C=N and C=O stretching vibrational bands at 1551, 1605 and 1728 cm$^{-1}$, respectively further supported the structure of 87a. In $^{13}$C NMR spectrum, observation of three characteristic signals at $\delta$ 162.92, 150.27 and 100.99 due to C-2', C-4', and C-5', respectively provided the firm evidence in support of the formation of thiazole nucleus (Figure 6). Further confirmation of 87a was supported by its mass spectral data which showed a molecular ion peak at m/z = 365 (M$^+$) in accordance with molecular formula C$_{20}$H$_{19}$N$_3$O$_2$S.

Scheme-29 Synthesis of oxazolidinone-thiazole hybrid compounds 87
Fig. 3 The $^1$H NMR spectrum of the compound 85

Fig. 4 The $^{13}$C NMR spectrum of the compound 85
Fig. 5 The $^1$H NMR spectrum of compound 87a

Fig. 6 The $^{13}$C NMR spectrum of the compound 87a
2.2A.2 Biological Evaluation

2.2A.2.1 Antimicrobial activity

Thirteen newly synthesized compounds were screened to explore their in vitro antibacterial and antifungal activity against four bacterial and two yeast strains, respectively through agar-diffusion method. In this investigation, Ciprofloxacin and Amphotericin-B were used as positive control for bacteria and yeasts, respectively. Preliminary results were recorded by measuring the inhibition zones (IZ) of bacterial or fungal growth around the wells for each tested compound at 400 µg/100 µl. From these results, it has been found that most of the compounds were found active against the bacterial and yeasts strains (Table-1 and Figure 7, 8). Solutions for the measurement of minimum inhibitory concentration (MIC) in µg/100 µl were obtained using two-fold serial dilution method for those compounds which have displayed significant growth inhibition zones (>16 mm) (Table-2).

Table 1 Inhibition zone diameter of 87a-m using agar well diffusion method at 400 µg / 100 µl

<table>
<thead>
<tr>
<th>Compound</th>
<th>Diameter of growth of inhibition zone (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td></td>
<td>B. subtilis</td>
</tr>
<tr>
<td>87a</td>
<td>-</td>
</tr>
<tr>
<td>87b</td>
<td>15</td>
</tr>
<tr>
<td>87c</td>
<td>17</td>
</tr>
<tr>
<td>87d</td>
<td>18</td>
</tr>
<tr>
<td>87e</td>
<td>18</td>
</tr>
<tr>
<td>87f</td>
<td>20</td>
</tr>
<tr>
<td>87g</td>
<td>25</td>
</tr>
<tr>
<td>87h</td>
<td>25</td>
</tr>
<tr>
<td>87i</td>
<td>29</td>
</tr>
<tr>
<td>87j</td>
<td>20</td>
</tr>
<tr>
<td>87k</td>
<td>15</td>
</tr>
<tr>
<td>87l</td>
<td>19</td>
</tr>
<tr>
<td>87m</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>24.0</td>
</tr>
<tr>
<td>Amphotericin-B</td>
<td>-</td>
</tr>
</tbody>
</table>

*Values, including diameter of the well (8mm), are means of three replicates, -: no activity.

On the basis of MIC values, it has been observed that compound 87i exhibited promising inhibitory potential against B. subtilis with MIC = 6.25, equivalent to the standard drug, Ciprofloxacin while compounds 87g and 87h displayed 50% inhibition against the same strain with MIC = 12.5 in comparison to Ciprofloxacin. The moderate inhibitory profile was exhibited by 87f against B. subtilis MIC = 25, which was about 25% of the standard drug. Furthermore, compounds 87j and 87l inhibited
the growth of *B. subtilis* with MIC = 25. Weak inhibitory effects were shown by 87c-e against *B. subtilis* with MIC = 50. On the other hand, 87i exhibited two-fold lesser (MIC = 12.5) and 87f, 87g four-fold lesser inhibitory potential against *S. aureus* in comparison to the standard drug. The compounds 87d and 87h exhibited weak inhibitory effect against *S. aureus* with MIC = 50, which was about 12.5% in comparison to the standard drug, however, 87c, 87e and 87k were found inactive against the same strain. Against *E. coli*, compounds 87c, 87i and 87g exhibited two-fold, four-fold and eight-fold lesser inhibitory potential, respectively. Compounds, 87g as well as 87i (MIC = 25), have shown 50% while 87f and 87h (MIC = 50) have displayed 25% inhibitory action against *P. aeruginosa*.

The antifungal evaluation revealed that compound 87i exhibited equipotent inhibitory action with MIC equal to 12.5 against *C. albicans* as well as *S. cerevisiae*. The compounds 87g and 87h exhibited half of inhibitory potential (MIC = 25) against *C. albicans* in comparison to Amphotericin-B, the standard drug. The compounds 87f-h displayed two-fold lesser (MIC = 25) and 87b-e displayed four-fold lesser inhibitory potential (MIC = 50) against *S. cerevisiae*. The compounds 87a and 87j-m did not show any inhibitory action against both the fungal strains. Among all synthesized compounds, 87c, 87j and 87l were found to be the most potent class of antibacterial agents while 87f-i were emerged as the most potent antimicrobial agents.

The results drawn from the antimicrobial screening (on the basis of MIC values) demonstrated the following assumptions about the structural-activity relationship (SAR).

- Antimicrobial potential was found remarkable, when biphenyl substituent is attached to thiazole moiety in comparison to phenyl, 2/3/4-(halo/ methyl/ nitro)substituted phenyl, naphthyl, chromenyl and pyranyl substituents.
- In case of compounds bearing halo-substituents, the observed order of antimicrobial potential was: *o*-fluorophenyl > *m*-chlorophenyl > *p*-fluorophenyl > *p*-chlorophenyl > *p*-bromophenyl.
- High antibacterial action was observed in case of the compound bearing Ar = 2-naphthyl or pyranyl moiety in comparison to 1-naphthyl and chromenyl group, respectively.
- On the basis of zone of inhibition, it has been found that electron-withdrawing substituents (NO₂) attached to phenyl ring of thiazole moiety enhance the
activity against bacterial strains in comparison to phenyl and $p$-methyl phenyl substituent.

- In general, it was found that phenyl substitution on position-4 of the thiazole moiety increases antimicrobial potential in comparison to naphthyl, pyranyl and chromenyl substitution.

Fig. 7 Comparison of zones of inhibition (in mm) of $87a-m$ in reference to Ciprofloxacin

Fig. 8 Comparison of zones of inhibition (in mm) of $87a-m$ in reference to Amphotericin-B
2.2A.2.2 Scavenging effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

Antioxidant potential of all the synthesized were studied in the concentration range of 50-400 µg/ml using DPPH assay (Table-3). Among all the synthesized compounds, 87d and 87j exhibited excellent antioxidant profile at 50 µg/ml even more than ascorbic acid (reference compound). However, the compounds 87a-c, 87e-g, 87k and 87l displayed good antioxidant profile close to ascorbic acid at 50 µg/ml. In addition, compound 87m exhibited very low antioxidant activity whereas 87h and 87i were found inactive.

The overall conclusion drawn from the results of antioxidant activity:

- In case of halophenyl substituents attached to thiazole moiety, the order of antioxidant potential was observed as: p-chlorophenyl > p-fluorophenyl > p-bromophenyl > o-fluorophenyl. However, antioxidant potential was diminished in case of m-chlorophenyl substituent.
- The antioxidant potential was found less, when Ar is phenyl, p-methylphenyl and p-nitrophenyl in comparison to Ar = 2/4-halophenyl substituents.
- The compounds having 2-naphthyl substituent attached to thiazole moiety was found to possess higher antioxidant potential in comparison to 1-naphthyl but in case of biphenyl substituent, antioxidant potential was completely diminished.
Antioxidant potential was found higher in pyranyl substituent in comparison to chromenyl substituent.

Table 3 The *in vitro* free radical-scavenging activity of compounds, measured by DPPH assay

<table>
<thead>
<tr>
<th>Compds</th>
<th>(%) Scavenging Concentrations (µg/ml)*</th>
<th>IC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>87a</td>
<td>49.71 ± 0.54</td>
<td>56.38 ± 0.53</td>
</tr>
<tr>
<td>87b</td>
<td>50.69 ± 0.32</td>
<td>65.5 ± 0.35</td>
</tr>
<tr>
<td>87c</td>
<td>50.56 ± 0.77</td>
<td>66.01 ± 0.79</td>
</tr>
<tr>
<td>87d</td>
<td>70.47 ± 0.38</td>
<td>73.37 ± 0.52</td>
</tr>
<tr>
<td>87e</td>
<td>54.13 ± 0.33</td>
<td>66.9 ± 0.08</td>
</tr>
<tr>
<td>87f</td>
<td>55.48 ± 0.44</td>
<td>78.05 ± 0.26</td>
</tr>
<tr>
<td>87g</td>
<td>53.37 ± 0.66</td>
<td>61.55 ± 0.77</td>
</tr>
<tr>
<td>87h</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>87i</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>87j</td>
<td>69.39 ± 0.22</td>
<td>71.45 ± 0.14</td>
</tr>
<tr>
<td>87k</td>
<td>50.88 ± 0.31</td>
<td>57.41 ± 0.21</td>
</tr>
<tr>
<td>87l</td>
<td>46.47 ± 0.66</td>
<td>48.63 ± 0.51</td>
</tr>
<tr>
<td>87m</td>
<td>18.5 ± 0.28</td>
<td>35.40 ± 0.55</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>58 ± 0.6</td>
<td>70 ± 4.5</td>
</tr>
</tbody>
</table>

*Data expressed as means of three replicates ± SD, -: not active.*

2.2A.2.3 Effect of compounds 87a-m on plasmid DNA under UV-irradiation

To observe the effects of 87a-m as DNA photocleaving and DNA damage protecting agents, agarose gel electrophoresis was used (Figure 9). It has been reported in literature that on single strand breaking the supercoiled (SC) form changes into the more relaxed open circular (OC) form, however, double strand breaking leads to the transformation of the SC form into the linear (LC) form. In the present investigation, it has been observed that in absence of UV-irradiation, plasmid DNA existed in SC form (lane 1) but on exposure to UV light, most of it was transformed into OC form along with a very less intense LC form as shown in lane-2.

The protecting potential of the test compounds was assessed by comparing the appearance of bands and their intensities appeared in control (C) and test compounds in presence of UV-irradiation.

In this study, it has been found that compounds 87a-m displayed very high DNA damage protecting effect from the effect of UV radiation at 60 µg concentration as they prevented the DNA degradation and preserved the initial supercoiled form when compared with control. In addition to DNA damage protecting effect, compound 87m also displayed very high fluorescence effect whereas 87l showed less fluorescence.
effect. The reason of exhibiting fluorescence may be due to the presence of conjugation in chromenyl (87m) and pyranyl (87l) systems.

Fig. 9 Effects of compounds on plasmid DNA under UV-irradiation (87a-m): Lane-1: DNA + DMSO without UV, Lane-2: DNA + DMSO + UV, Lane-3: DNA + 87b + UV, Lane-4: DNA + 87e + UV, Lane-5: DNA + 87d + UV, Lane-6: DNA + 87f + UV, Lane-7: DNA + 87a + UV, Lane-8: DNA + 87c + UV, Lane-9: DNA + 87j + UV, Lane-10: DNA + 87k + UV, Lane-11: DNA + 87h + UV, Lane-12: DNA + 87l + UV, Lane-13: DNA + 87i + UV, Lane-14: DNA + 87m + UV, Lane-15: DNA + 87g + UV

2.2A.3 Conclusion
Total thirteen novel oxazolidinone-thiazole based compounds 87a-m have been synthesized and their structures were established on the basis of rigorous analysis of IR, NMR (1H and 13C), mass spectral data as well as their elemental percentages. A structural-activity relationship revealed that compound 87i containing biphenyl substituent attached to position-4 of thiazole moiety exhibited excellent inhibitory action against the tested bacterial and fungal strains. In addition, compounds having para-fluorophenyl (87f), ortho-fluorophenyl (87g) and meta-chlorophenyl (87h) substituents on the thiazole moiety also displayed promising antimicrobial activities. The compound 87c exhibited high inhibitory action against E. coli and moderate inhibitory potential was exhibited by 87j and 87l selectively against B. subtilis. Therefore, these compounds could serve as new and potent antimicrobial leads in future. Apart from this, all compounds 87a-m displayed a very high level of DNA damage protecting ability by preventing the SC form in comparison with control. In addition to DNA damage protecting effect, 87m and 87l also displayed fluorescence effect. Therefore, the compounds 87a-m can act as template for the synthesis of potent DNA damage protecting agents which may serve as potent anti-UV materials in the future. On the other hand, compounds 87l and 87m may be used in the development of new fluorescent materials. The compounds having p-chlorophenyl
(87d) and 2-naphthyl (87j) substituent on the thiazole moiety exhibited very high antioxidant profile even more than ascorbic acid and thus may serve as anticancer or antioxidant agents in future.

2.2B Synthesis, Characterization and Biological Evaluation of Some Novel (E)-2-(3,5-Dimethyl-4-(aryldiazenyl)-1H-pyrazol-1-yl)-4-arylthiazoles

2.2B.1 Chemistry

Thiazoles, pyrazoles and thiazole-pyrazole hybrids have attracted great attention over the years due to their remarkable biological activities. Motifs containing thiazole nucleus exhibited antitrypanosomal [66], antimicrobial [67], anticancer [68], anti-inflammatory [69] and antiviral [70] properties. Some potent drugs like Fanetizole (anti-inflammatory agent), Tiazofurin (antineoplastic agent), Penicillin (potent antibiotic), Sulfatiazol (antimicrobial) and Abafungin (antifungal) also possessed thiazole ring [8-10]. After the discovery of pyrazofurin (a potent antimicrobial), usefulness of pyrazole derivatives in the field of medicine has been much explored [71]. An extensive part of research in this field revealed that 4-arylazo substituted pyrazole derivatives were found to act as analgesic [72], cytotoxic [73], anti-staphylococcal [74], antioxidant [71], and CDK2-cyclin E inhibiting agents [75]. In literature, 3,5-dimethyl substituted 4-arylazopyrazole derivatives were also reported to exhibit high antimicrobial and antibacterial potential [71, 76, 77] (Figure 10).

![Fig.10 4-Arylazo-3,5-dimethylpyrazoles (V-VIII) associated with antimicrobial, antibacterial and antioxidant property](image-url)
Moreover, pyrazole linked thiazoles exhibited antibacterial [78], antimicrobial [79, 80] and ΔF508-CFTR corrector activities [81] besides treating cardiovascular diseases [82]. Some important examples of pyrazolylthiazole derivatives associated with significant antibacterial [78] and antimicrobial profile [79, 80] are shown in (Figure 11).

As already discussed, a great attention has been paid worldwide towards the developments of DNA damage protecting and DNA photocleaving agents [59, 83]. It has been reported that exposure to UV radiations resulted in serious disorders like erythema, edema, hyperpigmentation, immunosuppression, photoaging, skin cancer, cataract formation and retinal degeneration, etc [60, 84]. Though in recent years some heterocyclic or non heterocyclic compounds have been used to protect the DNA from UV-induced damage, developments of newer and efficient protecting agents are still needed [59]. Furthermore, photocleaving agents generate some structural modifications in DNA under UV irradiation and thus contributed in development of antitumor drugs [83]. The literature survey revealed that compounds bearing pyrazole [85] and thiazole [86, 87] nuclei act as efficient DNA photocleaving agents.

It has already been reported that some 3,5-dimethyl-4-arylazopyrazole derivatives [69] (V) (Figure 10) and thiazole derivatives were proved as an efficient class of antioxidants [88]. Though nature already provided each cell a protective mechanism (known as antioxidant mechanism), administration of antioxidants played an important role in protecting the biological targets when the normal antioxidant defense mechanism fails [61].

Promoted from the above facts, it was planned to synthesize some novel 4-aryl-2-(3,5-dimethyl-1H-pyrazol-1-yl)thiazole derivatives bearing arylazo group at position-4 of the pyrazole moiety under solvent free conditions. The two main objectives of the
study include, to observe the influence of 4-arylazo group on the Hantzsch thiazole approach in both the solvent free as well as solvent mediated conditions, and to explore the biological potential of the target compound with an expectation to find a new class of bioactive compounds.

The starting precursors, 86 and 91 used to synthesize 92 were obtained by the reported methods [64, 65, 89, 90]. Various primary aromatic amines 88 on diazotization followed by coupling with acetyl acetone 89 yielded 3-arylazopentane-2,4-diones 90 which on treatment with thiosemicarbazide 52 afforded 91 (Scheme-30).

The synthesis of novel (E)-2-(3,5-dimethyl-4-(aryldiazenyl)-1H-pyrazol-1-yl)-4-arylthiazoles 92a-p has been accomplished via Hantzsch thiazole approach under solvent free conditions (Scheme-31).

![Scheme-30](image)

Scheme-30 (E)-2''-(3,5-dimethyl-4-(aryldiazenyl)-1H-pyrazol-1-yl)-4''-arylthiazole 92a

To obtain the product (E)-2''-(3,5-dimethyl-4-(p-tolyl diazenyl)-1H-pyrazol-1-yl)-4''-phenylthiazole 92a, the reactant (E)-3,5-dimethyl-4-(p-tolyl diazenyl)-1H-pyrazole-1-carbothioamide 91a was ground with phenacyl bromide 86b in the presence of sodium carbonate at 100 °C temperature. The product 92a of high purity was formed within 10-15 minutes under solvent free conditions (Scheme-31). The structure of 92a was established on the basis of a combined use of IR, NMR (1H & 13C), COSY, ROESY, HSQC and HMBC spectroscopy. The disappearance of N-H stretching bands at 3140 and 3387 cm⁻¹ due to NH₂ group of carbothioamide indicated the formation of compound 92a. In the 1H NMR spectrum, disappearance of two signals at δ 6.96 and 8.69 for NH₂ protons and an appearance of singlet at δ 7.21 due to thiazole proton (H-5'') confirmed the formation of 92a (Figure 12). The structure of 92a was further supported by its 13C NMR spectrum in which three characteristic
signals of thiazole system at $\delta$ 161.67, 152.79 and 109.10 were appeared due to C-2', C-4' and C-5', respectively (Figure 13).

![Chemical structure](image)

For compds (91)  
- a: R = CH$_3$,  
- b: H,  
- c: F

For compds (92)  
- a: R = CH$_3$,  
- b: H,  
- c: F

**Scheme-31** Solvent free synthesis of (E)-2-(3,5-dimethyl-4-(aryldiazenyl)-1H-pyrazol-1-yl)-4-arylthiazoles 92a-p

The chemical shifts for four methyl protons of 92e were assigned by analyzing its $^1$H-$^1$H COSY, HMQC and HMBC spectral data. In the COSY spectrum, 3'''', 5'''-H protons have shown a correlation with 2''', 6'''-H protons and vice-versa while 3', 5'-H protons showed the correlation with 2', 6'-H protons. The HMBC spectrum revealed that the protons 3'''', 5'''-H have shown a correlation with 4'''-CH$_3$ protons while protons 3', 5'-H showed the correlation with 4'-CH$_3$ protons (Figure 14). The 4'''-CH$_3$ protons exhibited the correlation with C-3'''', C-5'''', C-4''' and C-1''' carbons while the correlation of 4''-CH$_3$ protons was observed with C-3'', C-5'', C-4'' and C-1'' carbons (Figure 15). A correlation of 3'-CH$_3$ protons with C-3' and C-4' carbons has also been observed. The 5'-CH$_3$ protons were found to be correlated with C-4' and C-5' carbons. The correlation of 5'-carbon with CH$_2$ protons was also observed. In the ROSEY spectrum, the protons 3' and 5'-H have shown a strong spacial relationship with 4'-CH$_3$ protons while protons 3''' and 5'''-H showed the spacial relationship with 4'''-CH$_3$ protons. In order to generalize the protocol, various $\alpha$-bromoketones 86 were treated with different pyrazole-1-carbothioamides 91 under the similar reaction...
conditions to achieve pyrazol-1-ylthiazoles \(92\). The \(^{13}\)C NMR spectral results of all the synthesized compounds \(92a-p\) are presented in Table-4 and Table-5.

In the present investigation, initially an attempt to perform the reaction of \(91a\) with \(86b\) in ethanol under reflux conditions was made which resulted in an exclusive formation of a mixture of thiocyanatoketone \(93\) and cleaved pyrazole \(94\). The results were supported by the appearance of a characteristic signal at \(\delta\ 4.72\) (s, 2H, CH\(_2\)) of \(93\) as well as a signal at \(\delta\ 2.52\) (s, 6H, 3,5-CH\(_3\)) and 2.34 (s, 3H, 4'-CH\(_3\)) of \(94\) in the \(^1\)H NMR spectrum of the crude reaction mixture (Scheme-32). This observation was consistent to the previously reported results based on the reaction of 4-unsubstituted pyrazole-1-carbothioamide \([91]\). In this study, it has been found that arylazo group present at position-4 of the pyrazole moiety did not play any role in preventing the cleavage of C-N bond in the reaction of differently substituted pyrazole-1-carbothioamides with \(\alpha\)-bromoketones.

Scheme-32 The reaction of \((E)-3,5\text{-dimethyl-4-}(p\text{-tolyldiazenyl})-1H\text{-pyrazole-1-carbothioamide}\ 91a\) with phenacyl bromide \(86b\) under solvent mediated conditions
**Fig. 13** $^1$H NMR spectrum of the compound 92a

**Fig. 14** $^{13}$C NMR spectrum of the compound 92a
**Fig.14** The HMBC spectrum of the compound 92e

**Fig.15** The HMBC spectrum of the compound 92e
### Table 4 $^{13}$C NMR data of the synthesized compounds 92a-h

<table>
<thead>
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<th>Compds</th>
<th>92a</th>
<th>92b</th>
<th>92c</th>
<th>92d</th>
<th>92e</th>
<th>92f</th>
<th>92g</th>
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<td>122.00</td>
<td>122.07</td>
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<td>122.11</td>
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<td>21.46</td>
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<tr>
<td>C-2''</td>
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<td>134.16</td>
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<td>127.61</td>
<td>127.25</td>
<td>127.73 (d, $^J_{CF} = 9.05$ Hz)</td>
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<td>131.50*</td>
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<td>128.96</td>
<td>115.68 (d, $^J_{CF} = 22.13$ Hz)</td>
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<td>123.88*</td>
<td>128.79</td>
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<tr>
<td>C-4'''</td>
<td>128.31</td>
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<td>162.75 (d, $^J_{CF} = 247.50$ Hz)</td>
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</tr>
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<td>C-6'''</td>
<td>126.03</td>
<td>127.61</td>
<td>127.25</td>
<td>127.73 (d, $^J_{CF} = 9.05$ Hz)</td>
<td>125.90</td>
<td>128.44*</td>
<td>126.03</td>
<td>127.57</td>
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<tr>
<td>C-7''''</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>128.44*</td>
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</tr>
<tr>
<td>C-8''''</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>126.22*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C-9''''</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>133.22*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C-10''''</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>133.56*</td>
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</tr>
<tr>
<td>CH$_3$-4''''</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>21.30</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

For compounds 92a, 92c-f and 92g-h CDCl$_3$ and in case of 92b mixture of CDCl$_3$ and trifluoroacetic acid (TFA) was used as a solvent.*Exchangeable.
### Table 5: $^{13}$C NMR data of the synthesized compounds 92i-p

<table>
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<tr>
<th>Compds</th>
<th>92i</th>
<th>92j</th>
<th>92l</th>
<th>92m</th>
<th>92n</th>
<th>92o</th>
<th>92p</th>
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<td>137.19</td>
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<td>12.03</td>
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<td>153.35</td>
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<td>149.88</td>
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<td>122.12</td>
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<td>123.91</td>
<td>123.93</td>
<td>123.93</td>
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<td>122.12</td>
<td>123.91</td>
<td>123.93</td>
<td>123.93</td>
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<td>CH$_3$-4'</td>
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<td>125.93</td>
<td>131.49*</td>
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<td>128.80</td>
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<td>129.45</td>
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<td>-</td>
<td>133.57*</td>
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<td>CH$_3$-4''</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>21.34</td>
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<td>-</td>
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</tbody>
</table>

For compounds 92i-l and 92m-p, CDC$_3$ was used as a solvent. *Exchangeable.
2.2B.2 Biological Evaluation

2.2B.2.1 Antimicrobial activity

To explore the antimicrobial potential, sixteen newly synthesized compounds were screened for their in vitro antibacterial and antifungal activity through agar-diffusion method using Ciprofloxacin and Amphotericin-B as positive controls for bacteria and yeasts, respectively. The preliminary results were recorded by measuring the inhibition zones (IZ) of bacterial or fungal growth around the wells for each tested compound at 400 µg/100 µl. From the preliminary study, it has been found that all the compounds (except 92c and 92e) were found active against the yeast strain viz. C. albicans (IZ in the diameter range = 12-50 mm) (Table-6 and Figure 16). Among them, compound 92l showed a very big size inhibitory zone of diameter 50 mm while 92g-k displayed zones of 21-25 mm in reference to Amphotericin-B, the standard drug (IZ = 16.6 mm). Three compounds 92a, 92b and 92d were found active against E. coli (IZ = 12 mm) and none of the compounds possess activity against B. subtilis, S. aureus, P. aeruginosa and S. cerevisiae. The minimum inhibitory concentration (MIC) in µg/100 µl was measured using two-fold serial dilution method for those compounds which have displayed appreciable inhibitory zones (> 16 mm) (Table-7).

Table 6 Inhibition zone diameter of 92a, 92b, 92d and 92g-p (in mm) using agar well diffusion method at 400 µg/100 µl

<table>
<thead>
<tr>
<th>Compds (92)</th>
<th>a</th>
<th>b</th>
<th>d</th>
<th>f</th>
<th>g</th>
<th>h</th>
<th>i</th>
<th>j</th>
<th>k</th>
<th>l</th>
<th>m</th>
<th>n</th>
<th>o</th>
<th>p</th>
<th>Standard</th>
</tr>
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<tr>
<td>IZ(\wedge)</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25.0 (Ciprofloxacin)</td>
</tr>
<tr>
<td>E. coli</td>
<td>12</td>
<td>14</td>
<td>13</td>
<td>12</td>
<td>24</td>
<td>21</td>
<td>22</td>
<td>24</td>
<td>25</td>
<td>50</td>
<td>12</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td>16.6 (Amphotericin-B)</td>
</tr>
<tr>
<td>IZ(\wedge)</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>C. albicans</td>
<td>12</td>
<td>12</td>
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</tr>
</tbody>
</table>

- : No activity; \(^a\)Values, including diameter of the well (8mm), are means of three replicate; None of the compounds produce inhibition zone against Gram positive bacterial strains, Gram negative strain viz. P. aeruginosa and yeast strain viz. S. cerevisiae.

From the MIC results, it has been found that 92l exhibited two-times high inhibitory potential (MIC = 6.25) against C. albicans in comparison to the standard drug (MIC = 12.5) while compound 92k (MIC = 12.5) was found equipotent. On the other hand,
the compounds 92g-j exhibited two-fold lesser inhibitory action with MIC = 25 against *C. albicans* as compared to the reference drug.

The results drawn from the antimicrobial screening (on the basis of MIC value) demonstrated the following assumptions about the structural-activity relationship (SAR).

- Most of the synthesized compounds were found active selectively against the fungal strain, *C. albicans*.
- It has been observed that unsubstituted phenyl ring (R = H) of the arylazo moiety may be responsible for the higher antifungal potential.
- Substitution on position-4 of the phenyl ring in arylazo group (R = CH₃ and F) leads to diminish the antifungal potential.
- Among active compounds, antifungal potential was found to be higher than the standard drug, Amphotericin-B, when Ar is naphthyl in comparison to substituted phenyl.
- High inhibitory potential was observed in case of the presence of 4-methylphenyl substituent on thiazole moiety in comparison to phenyl and 4-halophenyl groups.
- In conclusion, compounds 92g-l may serve as an excellent class of antifungal agents in future especially *C. albicans* related problems.

![Fig.16](image)

**Fig.16** Comparison of zones of inhibition (in mm) of 92a, 92b, 92d and 92g-p in reference to Amphotericin-B (antifungal drug)
Table 7 Antimicrobial activity (expressed as MIC) of compounds 92g-l against *C. albicans*

<table>
<thead>
<tr>
<th>Compds</th>
<th>92g</th>
<th>92h</th>
<th>92i</th>
<th>92j</th>
<th>92k</th>
<th>92l</th>
<th>Amphotericin-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (in µg / 100 µl)</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

2.2B.2.2 Effects of compounds 92 on plasmid DNA under UV-irradiation

It has been reported in literature that breaking of single strand changes the supercoiled (SC) form into more relaxed open circular (OC) form, however, breaking of double strand leads to conversion of SC form into the linear (LC) form. In the present investigation, it has been observed that in absence of UV-irradiation, plasmid DNA existed in SC form (lane 1) but on exposure to UV light most of it was transformed into OC form along with a very less intense LC form as shown in lane-2. The potential of the test compounds were assessed by comparing the bands appeared in control (C) and test compounds in presence of UV-irradiation. The effects of compounds 92a-p at 40 µg concentration on plasmid DNA under UV-irradiation were studied using agarose gel electrophoresis method (Figure 17).

![Fig. 17 Effects of compounds 92a-p on plasmid DNA under UV-irradiation](image)

Among 92a-f, the compounds 92d and 92e protected the DNA from UV damaging effects as observed from the prevention of the DNA degradation by preserving initial supercoiled conformation in comparison to control while 92a, 92c and 92f compounds...
were found to behave like control (C). However, in 92b the intensity of SC was completely diminished and OC form was reduced in comparison to control which indicated that it exhibited partial photocleaving effect. In case of 92g-l, the compound 92j exhibited very high protective effect as indicated by an appearance of high intensity SC form along with a less intense OC form, in comparison to 92g, 92i and 92k that displayed moderate protective effects. The compound 92l showed less protective and more photocleaving effect as indicated by less intense SC as well as OC bands, may be due to degradation of these forms into smaller pieces. On the other hand, in case of 92m-p, the compounds 92m and 92o protected the DNA from UV radiation. On the other hand, 92n and 92p displayed opposite effects. In compound 92n, the intensity of OC form was decreased in comparison to control (C) may be due to degradation of OC form while in 92p along with decrease in intensity of OC form, increase in intensity of LC form was also observed. In this investigation, among all compounds, 92j and 92n was found to be the most potent DNA damage protecting and DNA photocleavage agent, respectively.

Some important points drawn from the study are given below.

- In case of compounds having R = CH₃, more DNA damage protecting effects were observed, when p-fluorophenyl and p-methylphenyl groups are attached to the thiazole moiety. However, partial photocleavage activity was observed in case of the compounds bearing p-bromophenyl group. Protective as well as photocleaving effects were not observed when phenyl, p-chlorophenyl and 2-naphthyl groups are linked with the thiazole ring.

- In case of compounds having R = H, increase in DNA damage protecting effect was observed in presence of p-fluorophenyl moiety attached to thiazole nucleus while phenyl, p-chlorophenyl and p-methylphenyl groups were found to be responsible to show moderate protective effects. Lesser protective and more photocleaving effect was observed for the compound bearing 2-naphthyl moiety.

- In compounds bearing R = F, DNA damage protecting effect was observed when Ar is phenyl and p-chlorophenyl. However, p-bromophenyl and p-fluorophenyl moieties were found to be associated with significant DNA photocleaving effects
2.2B.2.3 Scavenging effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical
Antioxidant potential of all the synthesized were studied in the concentration range of 50-400 µg/ml using DPPH assay and none was found active in scavenging DPPH radical.

The overall effects of substitution pattern on the biological activities are summarized in Figure 18.

![Figure 18 Substitution pattern / bioactivity](image)

2.2B.3 Conclusion
In conclusion, a series of sixteen novel (E)-2-(3,5-dimethyl-4-(aryldiazenyl)-1H-pyrazol-1-yl)-4-arylthiazoles 92a-p has been synthesized under mild and greener reaction conditions. The structures of the compounds were established on the basis of rigorous analysis of their IR, NMR (¹H and ¹³C), COSY, ROSEY, HSQC and HMBC spectral data as well as elemental percentages. A structural-activity relationship revealed that the compounds bearing aryl groups at position-4 of the thiazole nucleus
in presence of unsubstituted arylazo moiety (R = H) attached to position-4 of the pyrazole ring were emerged as potent antifungal agents which possessed very high inhibitory action selectively against *C. albicans*, a yeast strain. The compound 92l was found to be the most active agent even higher than the reference antifungal drug. The compound 92k bearing R = H and Ar = p-methylphenyl exhibited inhibitory potency similar to the standard drug against *C. albicans* while compounds 92g-j having R = H and Ar = phenyl and 4-halophenyl exhibited moderate inhibitory potency against *C. albicans*, which was 50% of the standard drug. Therefore, the compounds 92l and 92k could serve as new lead to act as antifungal agents in future. In DNA based study, the compounds 92j and 92n were found as the most potent DNA damage protecting and DNA photocleavage agents, respectively. Therefore, the compound 92j may act as a template for the synthesis of newer potent DNA protecting agents and can be used as potent anti-UV agents while 92k may provide the skeleton for synthesis of newer and potent DNA-photocleaving agents which may serve as potent anticancer/antitumor agents in the future.
2.3 EXPERIMENTAL

Melting points were determined by open capillary method and are uncorrected. The FT-IR spectra of the compounds were recorded on FT-Infra-Red Spectrometer Model RZX (Perkin Elmer) using KBr pellets. The $^1$H and $^{13}$C NMR spectra were recorded on Bruker Advance II 400 NMR Spectrometer at 400 MHz and 100 MHz, respectively; chemical shifts are expressed on $\delta$-scale downfield from TMS as an internal standard. Mass spectra were recorded on Waters Micromass Q-Tof Micro Mass spectrometer equipped with electrospray ionization (ESI) and atmospheric pressure chemical ionization sources having mass range of 4000 amu in quadruple and 20000 amu in ToF. Thermo Scientific (FLASH 2000) CHN Elemental Analyser was used to determine percentages of C, H and N with an accuracy of 0.3%. The absorbance for the antioxidant activity was recorded on spectrod 250 analytikjena UV spectrophotometer.

2.3A Chemistry

2.3A.1 Synthesis of oxazolidinone thiazole hybrids (87)

Synthesis of methyl-4-nitrophenylalaninate (80)

To suspension of 4-nitrophenylalanine (30 g, 0.1315 mol) in chilled methanol (250 ml), thionyl chloride (23.48 g, 0.1974 mol) was added dropwise while stirring. The reaction mixture was refluxed for 6-7 h. After the completion of reaction, solvent was concentrated at reduced pressure and slurry thus obtained was cooled, stirred for 10 min and filtered to obtain methyl-4-nitrophenylalaninate.

Yield: 76%, m.p. 134 °C, Lit m.p. 134 °C [63]

IR ($\nu_{\text{max}}, \text{cm}^{-1}$): 1744 (CO str.), 3200 (NH$_2$ symm. str.), 3472 (NH$_2$ asymm. str.)

$^1$H NMR (400 MHz; CDCl$_3$ + DMSO-$d_6$, $\delta_H$): 3.28-3.43 (m, 4H, 2,4-$H$), 3.69 (s, 3H, C$_3$H$_3$), 4.35-4.39 (m, 1H, 3-$H$), 7.59 (d, 2H, 2', 6'-$H$, $^3J_{H-H} = 8.72$ Hz), 8.12 (d, 2H, 3', 5'-$H$, $^3J_{H-H} = 8.80$ Hz), 8.89 (bs, 2H, NH, D$_2$O exchangeable)

$^{13}$C NMR (100 MHz; CDCl$_3$ + DMSO-$d_6$, $\delta_C$): 35.27, 35.27, 50.62, 52.74, 123.44, 130.84, 143.02, 146.74, 168.92

Synthesis of 2-amino-3-(4-nitrophenyl)propanol (81)

To the solution of methyl-4-nitrophenylalaninate hydrochloride (10 g, 0.038 mol) in a mixture of methanol (96 ml) and water (84 ml) cooled below 0 °C, sodium
borohydride (5.8 g, 0.153 mol) was added. The temperature was maintained for 1 h after that temperature was raised to room temperature and maintained for 2 h followed by heating to 50 °C. After that the reaction mixture was cooled and filtered to remove the inorganic solid. The filtrate so obtained was concentrated under reduced pressure to obtain a slurry followed by cooling at room temperature to get precipitate. The precipitated solid was filtered and washed with water to get the desired 2-amino-3-(4-nitrophenyl)propanol.

Yield: 79%, m.p. 132 °C, Lit m.p. 136 °C [63]

IR (ν max, cm⁻¹): 3105 (OH str.), 3296 (NH₂ symm. str.), 3351 (NH₂ asymm. str.)

¹H NMR (400 MHz; CDCl₃ + DMSO-d₆, δH): 2.52-3.31 (m, 5H, 1,2,3-H), 3.32 (s, 1H, OH, D₂O exchangeable), 7.48 (d, 2H, 2',6'-H, 3J H-H = 8.70 Hz), 8.13 (d, 2H, 3', 5'-H, 3J H-H = 8.80 Hz), 8.26 (bs, 2H, NH, D₂O exchangeable)

¹³C NMR (100 MHz; CDCl₃ + DMSO-d₆, δC): 40.11, 54.12, 65.81, 123.00, 130.34, 145.77, 148.59

Synthesis of 4-(4'-nitrobenzyl)oxazolidin-2-one (83)

To suspension of 2-amino-3-(4-nitrophenyl)propanol (6.5 g, 0.0331 mol) in toluene (35.7 ml) potassium carbonate was added in lots at 10-15 °C,. Solution of triphosgene (3.83 g, 0.0129 mol) in toluene (19.5 ml) was added to the reaction mixture by maintaining temperature at 10-15 °C. After the completion of reaction, water (55.2 ml) was added and temperature was raised to 20-25 °C. The solid obtained was filtered, washed with water and dried. The obtained solid was stirred in water (65.0 ml) at 50-55 °C for 1 h, after that it was filtered and washed with water to obtain the desired product.

Yield: 73%, m.p. 119 °C, Lit m.p. 120 °C [63]

IR (ν max, cm⁻¹): 1747 (CO str.), 3397 (NH str.), 1349 (NO₂ symm. str.), 1511 (NO₂ asymm. str.)

¹H NMR (400 MHz; CDCl₃ + DMSO-d₆, δH): 2.96 (m, 2H, 6-Ha, Hb), 4.02-4.35 (m, 3H, 4, 5-H), 7.53 (d, 2H, 2', 6'-H, 3J H-H = 8.28 Hz), 7.83 (s, 1H, NH), 8.17 (d, 2H, 2', 6'-H, 3J H-H = 8.20 Hz)

¹³C NMR (100 MHz; CDCl₃ + DMSO-d₆, δC): 40.12 (C-6), 52.18 (C-4), 67.94 (C-5),
123.24, 130.61, 144.77, 146.35, 158.51 (C-2)

**Anal. Caled** for C$_{10}$H$_{12}$N$_2$O$_2$ (%): C, 62.50; H, 6.25; N, 14.58. Found (%): C, 62.42;

**Synthesis of 4-(4'-aminobenzyl)oxazolidin-2-one (84)**

To the solution of 4-(4'-nitrobenzyl)oxazolidin-2-one (6.5 g, 0.0293 mol) in methanol (6.5 ml), Raney nickel (1.3 cm$^3$) was added. Hydrogenation was done in the presence of hydrogen of (6 Kg) at 30 ºC for 6-7 h. After the completion of reaction, the reaction mass was filtered through hyflobed and washed with methanol (19.5 ml). The filtrate obtained was evaporated under reduced pressure to distill off methanol. To the residue obtained ethylacetate (13.0 ml) was added and heated at 50-55 ºC for 1 h to obtain a slurry. The reaction mixture was cooled and solid obtained was filtered and washed with chilled ethylacetate to get the pure product.

![Structure](image)

**Yield:** 75%; **m.p.** 117 ºC

**IR** ($\nu_{\text{max}}, \text{cm}^{-1}$): 1746 (CO str.), 1612 (C = N str.), 1512 (C = C str.), 3298 (NH$_2$ symm. str.), 3483 (NH$_2$ asymm. str.)

**$^1$H NMR** (400 MHz; CDCl$_3$ + DMSO-$d_6$, $\delta_H$): 2.51-2.56 (m, 1H, 6-H$_a^*$), 2.66 (dd, 1H, 6-H$_b^*$, $^2$J$_{Hb-Ha} = 13.64$ Hz, $^3$J$_{Hb-H} = 4.52$ Hz), 3.88-3.96 (m, 2H, 5-H), 4.19-4.23 (m, 1H, 4-H), 4.80 (s, 2H, 4'-NH), 6.51 (d, 2H, 3', 5'-H, $^3$J$_{H-H} = 8.28$ Hz), 6.86 (d, 2H, 2', 6'-H, $^3$J$_{H-H} = 8.20$ Hz), 7.68 (s, 1H, 3-NH)

**$^{13}$C NMR** (100 MHz; CDCl$_3$ + DMSO-$d_6$, $\delta_C$): 40.14* (C-6), 52.90 (C-4), 68.01 (C-5), 114.06, 123.12, 129.66, 146.98, 158.68 (C-2)

**Synthesis of 4'-(2''-oxooxazolidin-4''-yl)methylphenylthiourea (85)**

A mixture of 4-(4'-aminobenzyl)oxazolidin-2-one (0.1 mol), HCl (9 ml) and water (25 ml) was heated on a water bath for 10 min and ammonium thiocyanate (0.3 mol) was added while stirring. The reaction mixture was refluxed for 2 h and then cooled to room temperature. The separated product was filtered, washed with water to obtain the crude product.
Yield: 76%; m.p. 116 °C

**IR** ($v_{\text{max}}, \text{ cm}^{-1}$): 1749 (CO str.), 1589 (C = N str.), 1543 (C = C str.), 3297 (NH$_2$ symm. str.), 3483 (NH$_2$ asymm. str.)

**$^1H$ NMR** (400 MHz; CDCl$_3$ + DMSO-$d_6$, $\delta_H$): 2.71-2.82 (m, 2H, 6''-H), 3.97-4.07 (m, 2H, 5''-H), 4.26-4.30 (m, 1H, 4''-H), 7.13 (s, 1H, NH), 7.18 (d, 2H, 3', 5'-H, $^3J_{\text{H-H}} = 8.32$ Hz), 7.26 (s, 1H, NH), 7.35 (d, 2H, 2', 6'-H, $^3J_{\text{H-H}} = 8.16$ Hz), 7.71 (s, 1H, 1'-NH), 9.68 (s, 1H, 3''-NH)

**$^{13}C$ NMR** (100 MHz; CDCl$_3$ + DMSO-$d_6$, $\delta_C$): 40.13 (C-6''), 52.65 (C-4''), 68.09 (C-5''), 123.14, 129.53, 132.65, 137.46, 158.60 (C-2''), 180.91 (C-1)

**MS**: m/z 251 (M$^+$)

**Anal. Calcd** for C$_{11}$H$_{13}$N$_3$O$_2$S (%): C, 52.59; H, 5.18; N, 16.73. Found (%): C, 52.57; H, 5.15; N, 16.70

**Synthesis of α-bromoketones (86)** [55, 56]

General procedure: Added dropwise a solution of bromine (12.5 ml) in glacial acetic acid (20 ml) into the precooled (10-15 °C) solution of an appropriate arylmethylketone (0.25 mol) dissolved in 80 ml of acetic acid while stirring. The resultant solution was stirred for 3 h at the same temperature. After completion of the reaction, reaction mixture was poured in ice cold water and stirred for 5 min, solid thus obtained was filtered, washed with 50% ethanol and dried. Recrystallized the crude product from 95% ethanol.

**Table-8** Melting point data of α-bromoketones 86

<table>
<thead>
<tr>
<th>Compounds</th>
<th>m.p. (°C)</th>
<th>Lit m.p. (°C) [Ref]</th>
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<tbody>
<tr>
<td>86a</td>
<td>47</td>
<td>46-48 [92]</td>
</tr>
<tr>
<td>86b</td>
<td>48</td>
<td>48-50 [92]</td>
</tr>
<tr>
<td>86c</td>
<td>94</td>
<td>-</td>
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<td>86d</td>
<td>95-96</td>
<td>93-96 [92]</td>
</tr>
<tr>
<td>86e</td>
<td>47</td>
<td>46-48 [92]</td>
</tr>
<tr>
<td>86f</td>
<td>47</td>
<td>47-49 [92]</td>
</tr>
<tr>
<td>86g</td>
<td>Low melting</td>
<td>-</td>
</tr>
<tr>
<td>86h</td>
<td>Low melting</td>
<td>-</td>
</tr>
<tr>
<td>86i</td>
<td>128</td>
<td>-</td>
</tr>
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</table>
Synthesis of 4-(4''-(ary/heteroaryl)thiazol-2-ylamino)benzyl)oxazolidin-2-ones 87

General procedure: An ethanolic solution of the compound 85 (0.1 mol) and an appropriate α-bromoketone 86 (0.15 mol) in the presence of sodium bicarbonate (0.15 mol) was refluxed for 3 h. The reaction was monitored by TLC using a mixture of chloroform and methanol (9:1) as an eluent. After completion of the reaction, the solvent was concentrated under reduced pressure. The solid thus obtained was filtered, washed with chilled ethanol and dried. Finally, treatment of the crude solid with petroleum ether gave the pure product.

4-(4''-(4''-p-Tolylthiazol-2''-ylamino)benzyl)oxazolidin-2-one (87a)

Yield: 84%; m.p. 222-224 °C

\[
\begin{align*}
\text{IR} (\nu_{\text{max}}, \text{cm}^{-1}): & \quad 1750 (\text{CO str.}), 1605 (\text{C = N str.}), \\
& \quad 1551 (\text{C = C str.}), 3298 (\text{NH str.}) \\
\text{1H NMR} (400 MHz; CDCl}_3 + \text{DMSO-d}_6, \delta_H): & \quad 2.33 (s, 3H, 4'''-\text{C}_H_3), 2.68 (dd, 1H, 6-\text{Ha\*}, 2J_{\text{Ha-Hb}} = 13.92 Hz, 3J_{\text{HB-Ha}} = 4.84 Hz), 3.98-4.04 (m, 2H, 2H, 5'-\text{H}), 4.21-4.28 (m, 1H, 4'-\text{H}), 6.96 (s, 1H, 5''-\text{H}), 7.14 (d, 2H, 2', 6'-\text{H}, 3J_{\text{H-H}} = 8.44 Hz), 7.17 (d, 2H, 3''', 5'''-\text{H}, 3J_{\text{H-H}} = 8.12 Hz), 7.65 (d, 2H, 3', 5'-\text{H}, 3J_{\text{H-H}} = 8.40 Hz), 7.68 (s, 1H, \text{N-H}), 7.75 (d, 2H, 2''', 6'''-\text{H}, 3J_{\text{H-H}} = 8.04 Hz), 10.03 (s, 1H, 3-NH)
\end{align*}
\]

\[
\begin{align*}
\text{13C NMR} (100 MHz; CDCl}_3 + \text{DMSO-d}_6, \delta_C): & \quad 20.84, 40.22 (C-6), 52.82 (C-4), 68.08 (C-5), 100.99 (C-5'''), 116.85, 125.47, 128.59, 128.89, 129.57, 131.83, 136.63, 139.87, 150.27 (C-4''), 158.69 (C-2), 162.92 (C-2''') \\
\text{MS: m/z 365 (M\textsuperscript+)} \\
\text{Anal. Calcd for C}_{20}H_{19}N_3O_2S (%):} & \quad \text{C, 65.75; H, 5.21; N, 11.51. Found (%): C, 65.71; H, 5.15; N, 11.48}
\end{align*}
\]
4-(4"-(4"-Phenylthiazol-2"-ylamino)benzyl)oxazolidin-2-one (87b)

Yield: 82%; m.p. 104°C

IR (ν_max, cm⁻¹): 1747 (CO str.), 1608 (C = N str.), 1551 (C = C str.), 3297 (NH str.)

¹H NMR (400 MHz; CDCl₃ + DMSO-d₆, δ_H): 2.71 (dd, 1H, 6-Hₐ, ²J_Hₐ-Hₐ = 13.52 Hz, ³J_Hₐ-Hₐ = 6.68 Hz), 2.82 (dd, 1H, 6-Hₐ, ²J_Hₐ-Hₐ = 13.72 Hz, ³J_Hₐ-Hₐ = 4.88 Hz), 4.00-4.06 (m, 2H, 5-H), 4.26-4.30 (m, 1H, 4-H), 7.17 (s, 1H, 5"-H), 7.20 (d, 2H, 2', 6'-H, ³J_H-H = 8.44 Hz), 7.28-7.32 (m, 1H, 4"-H), 7.39-7.43 (m, 2H, 3", 5"-H), 7.67 (d, 2H, 3', 5'-H, ³J_H-H = 8.40 Hz), 7.74 (s, 1H, NH), 7.90 (d, 2H, 2", 6"-H, ³J_H-H = 7.28 Hz), 10.19 (s, 1H, 3-NH)

¹³C NMR (100 MHz; CDCl₃, δ_C): 40.66 (C-6), 53.71 (C-4), 69.55 (C-5), 101.40, 118.68, 126.07, 128.39, 128.80, 130.08, 130.57, 133.47, 139.05, 149.59, 159.72 (C-2), 164.88 (C-2")

MS: m/z 351 (M⁺)

Anal. Calcd for C₁₉H₁₇N₃O₂S (%): C, 64.96; H, 4.84; N, 11.97. Found (%): C, 64.92; H, 4.81; N, 11.92

4-(4"-(4"-Nitrophenyl)thiazol-2"-ylamino)benzyl)oxazolidin-2-one (87c)

Yield: 83%; m.p. 218 °C

IR (ν_max, cm⁻¹): 1746 (CO str.), 1597 (C = N str.), 1543 (C = C str.), 3299 (NH str.), 1346 (NO₂ symm. str.), 1511 (NO₂ asymm. str.)

¹H NMR (400 MHz; CDCl₃ + DMSO-d₆, δ_H):

2.70 (dd, 1H, 6-Hₐ, ²J_Hₐ-Hₐ = 13.76 Hz, ³J_Hₐ-Hₐ = 6.84 Hz), 2.80 (dd, 1H, 6-Hₐ, ²J_Hₐ-Hₐ = 13.68 Hz, ³J_Hₐ-Hₐ = 4.76 Hz), 3.98-4.04 (m, 2H, 5-H), 4.23-4.27 (m, 1H, 4-H), 7.18 (d, 2H, 2', 6'-H, ³J_H-H = 8.48 Hz), 7.51 (s, 1H, 5"-H), 7.65 (d, 2H, 3', 5'-H, ³J_H-H = 8.44 Hz), 7.72 (s, 1H, NH), 8.12 (d, 2H, 2", 6"-H, ³J_H-H = 8.92 Hz), 8.23 (d, 2H, 3", 5"-H, ³J_H-H = 8.92 Hz), 10.23 (s, 1H, 3-NH)

¹³C NMR (100 MHz; CDCl₃ + DMSO-d₆, δ_C): 40.20 (C-6), 52.73 (C-4), 68.02 (C-5), 107.10 (C-5"), 117.03, 123.75, 126.27, 129.08, 129.71, 139.55, 140.45, 146.11, 148.08, 158.65 (C-2), 163.41 (C-2")
**CHAPTER-2**

**EXPERIMENTAL**

**MS**: m/z 396 (M⁺)

**Anal. Calcd** for C₁₉H₁₆N₄O₄S (%): C, 57.58; H, 4.04; N, 16.16. Found (%): C, 57.52; H, 4.01; N, 16.14

4-(4′-(4″-(4'''-Chlorophenyl)thiazol-2″-ylamino)benzyl)oxazolidin-2-one (87d)

**Yield**: 86%; **m.p.** 209-211 °C

**IR** (υmax, cm⁻¹): 1745 (CO str.), 1600 (C = N str.), 1548 (C = C str.), 3293 (NH str.), 3485 (NH str.)

**¹H NMR** (400 MHz; CDCl₃ + DMSO-d₆, δH): 2.71 (dd, 1H, 6-Hₐ*, 2J₇ₙₐ-Hₐ = 13.76 Hz, 3J₇ₙₐ-Hₐ = 6.88 Hz), 2.82 (dd, 1H, 6-Hₐ*, 2J₇ₙₐ-Hₐ = 13.72 Hz, 3J₇ₙₐ-Hₐ = 4.80 Hz), 4.00-4.06 (m, 2H, 5-H), 4.25-4.31 (m, 1H, 4-H), 7.19 (d, 2H, 2', 6'-H, 3J₇ₙₐ-Hₐ = 8.48 Hz), 7.20 (s, 1H, 5″-H), 7.41 (d, 2H, 3″, 5″-H, 3J₇ₙₐ-Hₐ = 8.52 Hz), 7.67 (d, 2H, 3', 5'-H, 3J₇ₙₐ-Hₐ = 8.48 Hz), 7.75 (s, 1H, NH), 7.91 (d, 2H, 2″, 6″-H, 3J₇ₙₐ-Hₐ = 8.52 Hz), 10.16 (s, 1H, 3-NH)

**¹³C NMR** (100 MHz; CDCl₃ + DMSO-d₆, δC): 40.19 (C-6), 52.74 (C-4), 68.02 (C-5), 102.89 (C-5″), 116.89, 127.11, 128.34, 128.81, 129.67, 132.06, 133.25, 139.72, 148.91, 158.65 (C-2), 163.14 (C-2″)

**MS**: m/z 385 (M⁺)

**Anal. Calcd** for C₁₉H₁₆ClN₃O₂S (%): C, 59.22; H, 4.16; N, 10.91. Found (%): C, 59.13; H, 4.08; N, 10.82

4-(4′-(4″-(4'''-Bromophenyl)thiazol-2″-ylamino)benzyl)oxazolidin-2-one (87e)

**Yield**: 85%; **m.p.** 204-207 °C

**IR** (υmax, cm⁻¹): 1749 (CO str.), 1615 (C = N str.), 1556 (C = C str.), 3293 (NH str.)

**¹H NMR** (400 MHz; CDCl₃ + DMSO-d₆, δH): 2.71 (dd, 1H, 6-Hₐ*, 2J₇ₙₐ-Hₐ = 13.64 Hz, 3J₇ₙₐ-Hₐ = 4.88 Hz), 2.80 (dd, 1H, 6-Hₐ*, 2J₇ₙₐ-Hₐ = 13.72 Hz, 3J₇ₙₐ-Hₐ = 4.88 Hz), 3.99-4.06 (m, 2H, 5-H),
4.25-4.29 (m, 1H, 4-H), 7.20 (d, 2H, 2', 6'-H, 3 J_{H-H} = 8.48 Hz), 7.29 (s, 1H, 5''-H), 7.58 (d, 2H, 3'', 5''-H, 3 J_{H-H} = 8.56 Hz), 7.66 (d, 2H, 3', 5'-H, 3 J_{H-H} = 8.52 Hz), 7.76 (s, 1H, NH), 7.86 (d, 2H. 2''', 6'''-H, 3 J_{H-H} = 8.52 Hz), 10.21 (s, 1H, 3-NH)

^{13}C NMR (100 MHz; CDCl$_3$ + DMSO-d$_6$, $\delta$C): 40.14 (C-6), 52.67 (C-4), 68.02 (C-5), 103.30 (C-5''), 116.89, 120.49, 127.52, 128.97, 131.35, 133.67, 139.70, 148.90, 158.64 (C-2), 163.18 (C-2''

MS: m/z 429 (M^+)

Anal. Calcd for C$_{19}$H$_{16}$BrN$_3$O$_2$S (%): C, 53.15; H, 3.72; N, 9.79. Found (%): C, 53.13; H, 3.66; N, 9.72

4-(4''-(4'''-Fluorophenyl)thiazol-2''-ylamino)benzyl)oxazolidin-2-one (87f)

Yield: 82%; m.p. 97 °C

IR (v$_{max}$, cm$^{-1}$): 1750 (CO str.), 1604 (C = N str.), 1553 (C = C str.), 3299 (NH str.)

$^{1}$H NMR (400 MHz; CDCl$_3$ + DMSO-d$_6$, $\delta$H): 2.73 (dd, 1H, 6-H$_a^*$, 2 J$_{H_a-H_b}$ = 13.92 Hz, 3 J$_{H_b-H_a}$ = 6.88 Hz), 2.85 (dd, 1H, 6-H$_b^*$, 2 J$_{H_b-H_a}$ = 13.76 Hz, 3 J$_{H_a-H_b}$ = 5.12 Hz), 4.02-4.08 (m, 2H, 5-H), 4.27-4.32 (m, 1H, 4-H), 6.96 (s, 1H, 5''-H), 7.09-7.17 (m, 4H, 2', 6', 3''', 5'''-H), 7.63 (s, 1H, NH), 7.67 (d, 2H, 3', 5'-H, 3 J$_{H-H}$ = 8.48 Hz), 7.88-7.93 (m, 2H, 2''', 6'''-H), 10.01 (s, 1H, 3-NH)

$^{13}$C NMR (100 MHz; CDCl$_3$ + DMSO-d$_6$, $\delta$C): 40.24 (C-6), 52.88 (C-4), 68.14 (C-5), 101.32 (C-5''), 114.98 (d, 2 J$_{C-F}$ = 21.13 Hz), 116.96, 127.31 (d, 3 J$_{C-F}$ = 8.05 Hz), 128.61, 129.47, 130.93 (4 J$_{C-F}$ = 3.07 Hz), 139.78, 149.21, 158.74 (C-2), 161.61 (1 J$_{C-F}$ = 246.49 Hz), 163.17 (C-2'')

MS: m/z 369 (M^+)

Anal. Calcd for C$_{19}$H$_{16}$FN$_3$O$_2$S (%): C, 61.79; H, 4.34; N, 11.38. Found (%): C, 61.72; H, 4.33; N, 11.31

4-(4''-(2'''-Fluorophenyl)thiazol-2''-ylamino)benzyl)oxazolidin-2-one (87g)
Yield: 85%; m.p. 72-74 °C
IR (ν_max, cm⁻¹): 1747 (CO str.), 1604 (C = N str.), 1555 (C = C str.), 3294 (NH str.)
₁H NMR (400 MHz; CDCl₃, δ_H): 2.66-2.77 (m, 2H, 6-H), 3.94-4.10 (m, 2H, 5-H), 4.37-4.41 (m, 1H, 4-
H), 5.93 (s, 1H, NH), 7.02-7.08 (m, 4H, 2', 6', 5'', 3'''-H), 7.11-7.15 (m, 1H, 5''-H), 7.18-7.23 (m, 1H, 4'''-H), 7.30 (d, 2H, 3', 5'-H, ³J_H-H = 8.40 Hz), 7.88 (s, 1H, 3-NH), 7.98-8.01 (m, 1H, 6''-H)
¹³C NMR (100 MHz; CDCl₃, δ_C): 40.78 (C-6), 53.83 (C-4), 69.65 (C-5), 106.96 (⁴J_C-F = 15.09 Hz) (C-5''), 115.97 (d, ²J_C-F = 23.14 Hz), 118.33, 122.28 (d, ²J_C-F = 11.06 Hz), 124.34 (d, ³J_C-F = 4.02 Hz), 128.92, 128.97 (d, ³J_C-F = 9.05 Hz), 129.76 (d, ³J_C-F = 3.02 Hz), 129.97, 139.58, 144.77, 159.58 (C-2), 160.28 (¹J_C-F = 250.52 Hz), 163.29 (C-2')
MS: m/z 369 (M⁺)

Anal. Calcd for C₁₉H₁₆FN₃O₂S (%): C, 61.79; H, 4.34; N, 11.38. Found (%): C, 61.72; H, 4.33; N, 11.31

4-(4'-4''-(3'''-Chlorophenyl)thiazol-2''-ylamino)benzyl)oxazolidin-2-one (87h)
Yield: 82%; m.p. 143 °C
IR (ν_max, cm⁻¹): 1756 (CO str.), 1600 (C = N str.), 1553 (C = C str.), 3293 (NH str.)
₁H NMR (400 MHz; CDCl₃ + DMSO-d₆, δ_H): 2.67-2.70 (m, 1H, 6-Hₘ), 2.78-2.81 (m, 1H, 6-Hₗ), 3.98 (appeared as bs, 5-H), 4.21-4.23 (m, 1H, 4-H), 7.05 (s, 1H, 5''-H), 7.11 (d, 2H, 2', 6'-
H, ³J_H-H = 7.96 Hz), 7.19-7.21 (m, 1H, 5''-H), 7.29-7.33 (m, 1H, 4''-H), 7.59 (s, 1H, NH), 7.60 (d, 2H, 3', 5'-H, ³J_H-H = 7.88 Hz), 7.74-7.76 (m, 1H, 6''-H), 7.82 (s, 1H, 2''-H), 9.99 (s, 1H, 3-NH)
¹³C NMR (400 MHz; CDCl₃ + DMSO-d₆, δ_C): 40.25 (C-6), 52.88 (C-4), 68.13 (C-5), 103.12 (C-5''), 116.96, 123.78, 125.34, 126.92, 128.70, 129.51, 129.73, 133.64, 136.35, 139.69, 148.72, 158.75 (C-2), 163.22 (C-2')
MS: m/z 385 (M⁺)

Anal. Calcd for C₁₉H₁₆ClN₃O₂S (%): C, 59.22; H, 4.16; N, 10.91. Found (%): C,
4-(4"-(4''-(Biphenyl-4'''-yl)thiazol-2''-ylamino)benzyl)oxazolidin-2-one (87i)

**Yield:** 82%; **m.p.** 104 °C  
**IR** ($\nu_{\text{max}}, \text{cm}^{-1}$): 1752 (CO str.), 1603 (C = N str.), 1551 (C = C str.), 3299 (NH str.)

$^1$H NMR (400 MHz; CDCl$_3$ + DMSO-$d_6$, $\delta_H$):
- 2.70 (dd, 1H, 6-$H_a$*, $^2J_{Ha-Hb} = 13.72$ Hz, $^3J_{Ha-H} = 6.60$ Hz),
- 2.80 (dd, 1H, 6-$H_b$*, $^2J_{Hb-Ha} = 13.80$ Hz),
- 3.98 - 4.04 (m, 2H, 5-\(H\)),
- 4.25 - 4.28 (m, 1H, 4-\(H\)),
- 7.16 (d, 2H, 2', 6'-\(H\), $^3J_{Hb-H} = 4.80$ Hz),
- 3.98-4.04 (m, 2H, 5-\(H\)),
- 4.25-4.28 (m, 1H, 4-\(H\)),
- 7.16 (d, 2H, 2', 6'-\(H\))

$^1$C NMR (100 MHz; CDCl$_3$ + DMSO-$d_6$, $\delta_C$): 40.16 (C-6), 52.69 (C-4), 68.04 (C-5), 111.29, 117.43, 126.08, 126.40, 127.34, 128.70, 129.18, 129.57, 129.71, 132.74, 133.05, 139.24, 139.79, 152.02, 158.66 (C-2), 163.34 (C-2''')

**MS:** m/z 427 (M$^+$)

**Anal. Calcd** for C$_{25}$H$_{21}$N$_3$O$_2$S (%): C, 70.26; H, 4.92; N, 9.84. Found (%): C, 70.23; H, 4.88; N, 9.82

4-(4"-(Naphthalen-2''-yl)thiazol-2''-ylamino)benzyl)oxazolidin-2-one (87j)

**Yield:** 83%; **m.p.** 180-183 °C  
**IR** ($\nu_{\text{max}}, \text{cm}^{-1}$): 1745 (CO str.), 1607 (C = N str.), 1552 (C = C str.), 3291 (NH str.)

$^1$H NMR (400 MHz; CDCl$_3$ + DMSO-$d_6$, $\delta_H$):
- 2.70 (dd, 1H, 6-$H_a$*, $^2J_{Ha-Hb} = 13.84$ Hz, $^3J_{Ha-H} = 6.92$ Hz),
- 2.81 (dd, 1H, 6-$H_b$*, $^2J_{Hb-Ha} = 13.68$ Hz, $^3J_{Hb-H} = 4.72$
- 4.00-4.06 (m, 1H, 5-\(H\)),
- 4.25-4.30 (m, 1H, 4-\(H\)),
- 7.22 (d, 2H, 2', 6'-\(H\), $^3J_{Hb-H} = 8.44$ Hz),
- 7.33 (s, 1H, 5''-\(H\)),
- 7.45-8.05 (m, 6H, 3''', 4''', 5'''', 6''', 7''', 8'''-\(H\)),
- 7.77 (s, 1H, NH),
- 8.43 (s, 1H, 1'''-\(H\)),
- 10.22 (s, 1H, 3-NH)

$^1$C NMR (100 MHz; CDCl$_3$ + DMSO-$d_6$, $\delta_C$): 40.18, 52.75 (C-4), 68.07 (C-5), 103.12 (C-5''), 116.95, 123.99, 124.18, 125.75, 126.19, 127.43, 127.91, 128.03,
CHAPTER 2

EXPERIMENTAL

128.92, 129.79, 131.98, 132.41, 133.14, 150.10, 158.66 (C-2), 163.14 (C-2"

**MS:** m/z 427 (M⁺)

**Anal. Calcd** for C₂₅H₂₁N₃O₂S (%): C, 70.26; H, 4.92; N, 9.84. Found (%): C, 70.23; H, 4.88; N, 9.82

4-(4'-(4''-(Naphthalen-1'''-yl)thiazol-2''-ylamino)benzyl)oxazolidin-2-one (87k)

**Yield:** 79%; **m.p.:** 101 °C

**IR** (νmax, cm⁻¹): 1749 (CO str.), 1605 (C = N str.), 1550 (C = C str.), 3297 (NH str.)

**1H NMR** (400 MHz; DMSO-d₆, δH): 2.57-2.70 (m, 2H, 6-H), 3.88-3.96 (m, 2H, 5-H), 4.16-4.19 (m, 1H, 4-H), 7.03 (s, 1H, 5''-H), 7.09 (d, 2H, 2', 6'-H, 3JHH = 8.48 Hz), 7.37-7.93 (m, 6H, 3'', 4'', 5'', 6'', 7'', 8''-H), 7.56 (d, 2H, 3', 5'-H, 3JHH = 8.52 Hz), 7.69 (s, 1H, NH), 8.37-8.42 (m, 1H, 2''-H), 10.26 (s, 1H, 3-NH)

**13C NMR** (100 MHz; DMSO-d₆, δC): 40.12 (C-6), 52.56 (C-4), 68.01 (C-5), 94.32, 100.94, 102.75, 118.28, 130.38, 131.22, 138.40, 142.09, 158.62 (C-2), 162.88 (C-2"

**Yield:** 81%; **m.p.:** 261 °C

**IR** (νmax, cm⁻¹): 1748 (CO str.), 1609 (C = N str.), 1552 (C = C str.), 3295 (NH str.)

**1H NMR** (400 MHz; DMSO-d₆, δH) 2.24 (s, 3H, CH₃), 2.71-2.82 (m, 2H, 6-H), 3.98-4.07 (m, 2H, 5-H), 4.27-4.30 (m, 1H, 4-H), 6.21 (s, 1H, 5''-H), 7.28 (appeared as s, 4H, 2', 3', 5', 6'-H), 7.42 (s, 1H, 5''-H), 7.81 (s, 1H, NH), 10.61 (s, 1H, 3-NH), 14.74 (s, 1H, 2''-OH)

**13C NMR** (100 MHz; DMSO-d₆, δC) 40.08* (C-6), 52.46 (C-4), 68.01 (C-5), 94.32, 100.94, 102.75, 118.28, 130.38, 131.22, 138.40, 142.09, 158.62 (C-2), 161.35 (C-2")
162.00, 163.54, 168.45

**MS**: m/z 399 (M⁺)

**Anal. Calcd** for C₁₉H₁₇N₃O₅S (%): C, 57.12; H, 4.26; N, 10.53. Found (%): C, 57.10; H, 4.21; N, 10.46

4-(4’-(4’’-(2’’-Oxo-2’’H-chromen-3’’-yl)thiazol-2’’-ylamino)benzyl)oxazolidin-2-one (87m)

**Yield**: 85%; **m.p.** 229 °C decomposed

**IR** (νmax, cm⁻¹): 1743 (CO str.), 1598 (C = N str.), 1552 (C = C str.), 3297 (NH str.)

**¹H NMR** (400 MHz; CDCl₃ + DMSO-d₆, δH): 2.72 (dd, 1H, 6-Hₐ*, 2Jₕₐ-Hₜ = 13.68 Hz, 6.76 Hz), 2.81 (dd, 1H, 6-Hₜ*, 2Jₕₜ-Hₕ = 13.72 Hz, 7.93 (m, 1H, 5’’-H), 8.68 (s, 1H, 4’’-H), 10.30 (s, 1H, 3-NH)

**¹³C NMR** (100 MHz; CDCl₃ + DMSO-d₆, δC): 40.14 (C-6), 52.68 (C-4), 68.03 (C-5), 109.63 (C-5’’), 115.80, 117.13, 119.24, 120.33, 124.59, 128.80, 129.26, 129.95, 131.52, 138.48, 139.56, 143.60, 152.28, 158.61 (C-2), 158.75, 162.45 (C-2’’)

**MS**: m/z 419 (M⁺)

**Anal. Calcd** for C₂₂H₁₇N₃O₄S (%): C, 63.00; H, 4.06; N, 10.02. Found (%): C, 62.98; H, 4.00; N, 10.00

2.3A.2 Synthesis of pyrazolyl thiazoles (92a-p)

**Synthesis of 3-arylazopentane-2,4-diones (90)**

Aniline / substituted aniline (0.02 mol) was dissolved in a mixture of conc. HCl and water (20 ml, 1:1). It was cooled to 0 °C and a cold aq. solution of sodium nitrite (1.38 g, 0.02 mol in 10 ml water) was added to it slowly by maintaining the temperature upto 5 °C. The cold diazotized solution was added dropwise to a precooled mixture of acetylacetone (0.02 mol) and sodium acetate (10 g) in 20 ml of 50% ethanol. The stirring was continued for 1 h and the crystals separated were filtered, washed with water, dried and recrystallized from ethanol to yield 90.

**Table-9 Physical data of 3-arylazopentane-2,4-diones 90**

<table>
<thead>
<tr>
<th>Compds</th>
<th>m. p. (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90a</td>
<td>80-82</td>
<td>89</td>
</tr>
</tbody>
</table>
Synthesis of \((E)-3,5\text{-dimethyl-4-}(\text{aryldiazenyl})-1H\text{-pyrazole-1-carbothioamide} \) (91)

To the stirred solution of compound 90 in ethanol, thiosemicarbazide dissolved in a mixture of conc. HCl and water was added. The reaction mixture was stirred at 45 °C. After completion of reaction, solid obtained was filtered and washed with chilled ethanol to obtain the crude product which was recrystallized from ethanol.

| Table-10 Physical data of \((E)-3,5\text{-dimethyl-4-}(\text{aryldiazenyl})-1H\text{-pyrazole-1-carbothioamides} \) (91) |
|----------------|----------------|----------------|
| Compds         | m.p. (°C)      | Yield (%)      |
| 91a            | 227            | 87             |
| 91b            | 215 (decomp)   | 82             |
| 91c            | 229            | 86             |

Synthesis of \((E)-2''-(3,5\text{-dimethyl-4-}(\text{aryldiazenyl})-1H\text{-pyrazol-1-yl})-4''-arylthiazoles \) (92a-p)

In a dried mortar, a mixture of \((E)-3,5\text{-dimethyl-4-}(\text{p-tolyldiazenyl})-1H\text{-pyrazole-1-carbothioamide} \) 91a (0.1 mol), phenacyl bromide 86b (0.1 mol) and sodium carbonate (0.6 mol) was ground for 10-15 minutes at 100 ºC. After that the reaction mixture was poured in water to remove the sodium carbonate, dried, and recrystallized the crude product from ethanol.

\((E)-2''-(3,5\text{-Dimethyl-4-}(\text{p-tolyldiazenyl})-1H\text{-pyrazol-1-yl})-4''-\text{phenylthiazole} \) (92a)

Yield: 82%; m.p. 206-207 °C
IR \((\nu_{\text{max}}, \text{cm}^{-1})\): 1697 (C = N str.), 1566 (C = C str.)

\(^1\text{H NMR} \) (400 MHz; CDCl\textsubscript{3}, \(\delta_{\text{H}}\)): 2.36 (s, 3H, 4'-CH\textsubscript{3}), 2.51 (s, 3H, 3-CH\textsubscript{3}), 3.09 (s, 3H, 5-CH\textsubscript{3}), 2.51 (s, 3H, 3-CH\textsubscript{3}), 3.09 (s, 3H, 5-CH\textsubscript{3}), 7.21 (s, 1H, 5''-H), 7.22 (d, 2H, 3', 5'-H, \(3J_{\text{H-H}} = 8.12\text{ Hz}\)), 7.29-7.31 (m, 1H, 4'''-H), 7.36-7.40 (m, 2H, 3'''', 5''''-H), 7.68 (d, 2H, 2', 6'-H, \(3J_{\text{H-H}} = 8.24\text{ Hz}\)), 7.84-7.86 (m, 2H, 2'', 6'''-H)

MS: m/z 373 (M\textsuperscript{+})
**Anal. Calcd** for C$_{21}$H$_{19}$N$_5$S (%): C, 67.56; H, 5.09; N, 18.77. Found (%): C, 67.52; H, 5.08; N, 18.72

(E)-4''-(4'''-Bromophenyl)-2''-(3,5-dimethyl-4-(p-tolyl diazenyl)-1H-pyrazol-1-yl) thiazole (92b)

Yield: 83%; m.p. 214-216 °C

IR ($v_{max}$, cm$^{-1}$): 1692 (C = N str.), 1563 (C = C str.)

$^1$H NMR (400 MHz; CDCl$_3$ + TFA, $\delta_H$): 2.53 (s, 3H, 4'-CH$_3$), 2.87 (s, 3H, 3-CH$_3$), 3.29 (s, 3H, 5-CH$_3$), 7.47 (d, 2H, 3', 5'-H, $^3J_{H-H} = 8.12$ Hz), 7.64 (d, 2H, 3'', 5''-H, $^3J_{H-H} = 8.12$ Hz), 7.65 (s, 1H, 5''-H), 7.78-7.84 (m, 4H, 2', 6', 2'', 6''-H)

MS: m/z 453 (M$^+$)

**Anal. Calcd** for C$_{21}$H$_{18}$BrN$_5$S (%): C, 55.63; H, 3.97; N, 15.45. Found (%): C, 55.61; H, 3.95; N, 15.41

(E)-4''-(4'''-Chlorophenyl)-2''-(3,5-dimethyl-4-(p-tolyl diazenyl)-1H-pyrazol-1-yl) thiazole (92c)

Yield: 84%; m.p. 210-211 °C

IR ($v_{max}$, cm$^{-1}$): 1693 (C = N str.), 1563 (C = C str.)

$^1$H NMR (400 MHz; CDCl$_3$ + DMSO-$d_6$, $\delta_H$): 2.53 (s, 3H, 4'-CH$_3$), 2.56 (s, 3H, 3-CH$_3$), 3.14 (s, 3H, 5-CH$_3$). 7.30-7.44 (m, 2H, 3', 5'-H), 7.66 (s, 1H, 5''-H), 7.72-7.74 (m, 2H, 3'', 5''-H), 7.95 (m, 4H, 2', 6', 2'', 6''-H)

MS: m/z 407.5 (M$^+$)

**Anal. Calcd** for C$_{21}$H$_{18}$ClN$_5$S (%): C, 61.84; H, 4.42; N, 17.18. Found (%): C, 61.82; H, 4.41; N, 17.13

(E)-2''-(3,5-Dimethyl-4-(p-tolyl diazenyl)-1H-pyrazol-1-yl)-4''-(4'''-fluorophenyl) thiazole (92d)
Yield: 82%; m.p. 184-185 °C  
**IR** ($v_{\text{max}}, \text{cm}^{-1}$): 1695 (C = N str.), 1565 (C = C str.)

1H NMR (400 MHz; CDCl$_3$, $\delta_H$): 2.31 (s, 3H, 4'-CH$_3$), 2.46 (s, 3H, 3-CH$_3$), 3.01 (s, 3H, 5-CH$_3$), 7.00-7.04 (m, 3H, 3''', 5''' - H), 7.05 (s, 1H, 5'' - H), 7.17 (d, 2H, 3', 5'-H, $^3J_{H-H} = 8.24$ Hz), 7.63 (d, 2H, 2', 6'-H, $^3J_{H-H} = 7.96$ Hz), 7.74-7.77 (m, 2H, 2'', 6'' - H)

**MS**: m/z 391 (M$^+$)

**Anal. Calcd** for C$_{21}$H$_{18}$FN$_5$S (%): C, 64.45; H, 4.60; N, 17.90. Found (%): C, 64.44; H, 4.56; N, 17.88

(E)-2''-(3,5-Dimethyl-4-(p-tolyldiazenyl)-1H-pyrazol-1-yl)-4''-p-tolylthiazole (92e)

Yield: 84%; m.p. 187-189 °C  
**IR** ($v_{\text{max}}, \text{cm}^{-1}$): 1698 (C = N str.), 1565 (C = C str.)

1H NMR (400 MHz; CDCl$_3$, $\delta_H$): 2.40 (s, 3H, 4''-CH$_3$), 2.43 (s, 3H, 4'-CH$_3$), 2.58 (s, 3H, 3-CH$_3$), 3.16 (s, 3H, 5-CH$_3$), 7.21 (s, 1H, 5'' - H), 7.25 (d, 2H, 3'', 5'' - H, $^3J_{H-H} = 8.12$ Hz), 7.29 (d, 2H, 3', 5'-H, $^3J_{H-H} = 8.20$ Hz), 7.75 (d, 2H, 2', 6'-H, $^3J_{H-H} = 8.20$ Hz), 7.81 (d, 2H, 2'', 6'' - H, $^3J_{H-H} = 8.12$ Hz)

**MS**: m/z 387 (M$^+$)

**Anal. Calcd** for C$_{22}$H$_{21}$N$_5$S (%): C, 68.22; H, 5.43; N, 18.09. Found (%): C, 68.20; H, 5.41; N, 18.07

2''-(3,5-Dimethyl-4-(p-tolyldiazenyl)-1H-pyrazol-1-yl)-4''-(naphthalen-2''-yl)thiazole (92f)

Yield: 78%; m.p. 176-179 °C  
**IR** ($v_{\text{max}}, \text{cm}^{-1}$): 1696 (C = N str.), 1566 (C = C str.)

1H NMR (400 MHz; CDCl$_3$, $\delta_H$): 2.40 (s, 3H, 4''-CH$_3$), 2.55 (s, 3H, 3-CH$_3$), 3.15 (s, 3H, 5-CH$_3$), 7.26 (d, 2H, 3', 5'-H, $^3J_{H-H} = 7.80$ Hz), 7.31 (s, 1H, 5'' - H), 7.45-7.97 (m, 6H, 3'', 4'', 5'', 6'', 7'', 8'' - H), 7.73 (d, 2H, 2', 6'-H, $^3J_{H-H} = 7.80$ Hz), 8.34 (s, 1H, 1'' - H)

**MS**: m/z 423 (M$^+$)
Anal. Calcd for C_{25}H_{21}N_{5}S (%): C, 70.92; H, 4.96; N, 16.55. Found (%): C, 70.90; H, 4.92; N, 16.52

(E)-2''-(3,5-Dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-4''-phenylthiazole (92g)

Yield: 80%; m.p. 168-170 °C
IR (ν_{max}, cm\(^{-1}\)): 1694 (C = N str.), 1564 (C = C str.)

\(^1\)H NMR (400 MHz; CDCl\(_3\), δ_H): 2.57 (s, 3H, 3''-CH\(_3\)), 3.15 (s, 3H, 5'-CH\(_3\)), 7.23 (s, 1H, 5''-H), 7.32-7.36 (m, 1H, 4''-H), 7.38-7.49 (m, 5H, 3', 5', 4', 3'', 5''-H), 7.83 (d, 2H, 2', 6'-H, \(^3\)J\(_{H-H} = 7.52\) Hz), 7.89 (d, 2H, 2'', 6''-H, \(^3\)J\(_{H-H} = 7.40\) Hz)
MS: m/z 359 (M\(^+\))

Anal. Calcd for C\(_{20}\)H\(_{17}\)N\(_5\)S (%): C, 66.85; H, 4.74; N, 19.50. Found (%): C, 66.80; H, 4.70; N, 19.48

(E)-4''-(4'''-Bromophenyl)-2''-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)thiazole (92h)

Yield: 84%; m.p. 200-201 °C
IR (ν_{max}, cm\(^{-1}\)): 1694 (C = N str.), 1564 (C = C str.)

\(^1\)H NMR (400 MHz; CDCl\(_3\), δ_H): 2.58 (s, 3H, 3''-CH\(_3\)), 3.15 (s, 3H, 5'-CH\(_3\)), 7.27 (s, 1H, 5''-H), 7.40-7.44 (m, 1H, 4'-H), 7.48-7.51 (m, 2H, 3', 5'-H), 7.55-7.58 (m, 2H, 3'', 5''-H), 7.76-7.79 (m, 2H, 2', 6'-H), 7.83-7.85 (m, 2'', 6''-H)
MS: m/z 439 (M\(^+\))

Anal. Calcd for C\(_{20}\)H\(_{16}\)BrN\(_5\)S (%): C, 54.67; H, 3.64; N, 15.95. Found (%): C, 54.65; H, 3.61; N, 15.92

(E)-4''-(4'''-Chlorophenyl)-2''-(3,5-dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)thiazole (92i)

Yield: 85%; m.p. 188-189 °C
IR (ν_{max}, cm\(^{-1}\)): 1694 (C = N str.), 1566 (C = C str.)

\(^1\)H NMR (400 MHz; CDCl\(_3\), δ_H): 2.56 (s, 3H, 3''-CH\(_3\)), 3.12 (s, 3H, 5'-CH\(_3\)), 7.21 (s, 1H, 5''-H), 7.37-7.42 (m, 3H, 4', 3'', 5''-H), 7.46-7.50 (m, 2H, 3', 5'-H), 7.79-7.84 (m,
4H, 2', 6', 2'', 6''-H)

**MS**: m/z 393.5 (M⁺)

**Anal. Calcd** for C₂₀H₁₆ClN₅S (%): C, 61.07; H, 4.07; N, 17.79. Found (%): C, 61.04; H, 4.08; N, 17.75

(E)-2''-(3,5-Dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-4''-(4'''-fluorophenyl)thiazole (92j)

![Chemical structure](attachment:image.png)

**Yield**: 83%; **m.p.**: 164-165 °C

**IR** (ν<sub>max</sub>, cm⁻¹): 1697 (C = N str.), 1566 (C = C str.)

**¹H NMR** (400 MHz; CDCl₃, δ<sub>H</sub>): 2.54 (s, 3H, 3-CH₃), 3.11 (s, 3H, 5-CH₃), 7.07-7.12 (m, 2H, 3'', 5''-H), 7.13 (s, 1H, 5''-H), 7.37-7.41 (m, 1H, 4'-H), 7.45-7.48 (m, 2H, 3', 5'-H), 7.80-7.85 (m, 2', 6', 4'', 2'', 6''-H)

**MS**: m/z 377 (M⁺)

**Anal. Calcd** for C₂₀H₁₆FN₅S (%): C, 63.66; H, 4.24; N, 18.57. Found (%): C, 63.61; H, 4.22; N, 18.55

(E)-2''-(3,5-Dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-4''-p-tolylthiazole (92k)

![Chemical structure](attachment:image.png)

**Yield**: 87%; **m.p.**: 167 °C

**IR** (ν<sub>max</sub>, cm⁻¹): 1698 (C = N str.), 1568 (C = C str.)

**¹H NMR** (400 MHz; CDCl₃, δ<sub>H</sub>): 2.27 (s, 3H, 4''-CH₃), 2.46 (s, 3H, 3-CH₃), 3.03 (s, 3H, 5-CH₃), 7.05 (s, 1H, 5''-H), 7.12 (d, 2H, 3'', 5''-H, 3<sup>J</sup>ₗ-H-H = 8.00 Hz), 7.28-7.31 (m, 1H, 4'-H), 7.35-7.39 (m, 2H, 3', 5'-H), 7.67 (d, 2H, 2'', 6''-H, 3<sup>J</sup>ₗ-H-H = 8.00 Hz), 7.72 (d, 2H, 2', 6'-H, 3<sup>J</sup>ₗ-H-H = 7.56 Hz)

**MS**: m/z 373 (M⁺)

**Anal. Calcd** for C₂₁H₁₉N₅S (%): C, 67.56; H, 5.09; N, 18.77. Found (%): C, 67.55; H, 5.03; N, 18.72

2''-(3,5-Dimethyl-4-(phenyldiazenyl)-1H-pyrazol-1-yl)-4''-(naphthalen-2''-yl)thiazole (92l)

![Chemical structure](attachment:image.png)

**Yield**: 80%; **m.p.**: 180-181 °C

**IR** (ν<sub>max</sub>, cm⁻¹): 1698 (C = N str.), 1567 (C = C str.)
1H NMR (400 MHz; CDCl₃, δ_H): 2.59 (s, 3H, 3-CH₃), 3.21 (s, 3H, 5-CH₃), 7.37 (s, 1H, 5''-H), 7.40-7.44 (m, 1H, 4'-H), 7.48-7.98 (m, 10H, 2', 3', 5', 6', 3'', 4'', 5'', 6'', 7'', 8''-H), 8.39 (s, 1H, 1'''-H)

MS: m/z 409 (M⁺)

Anal. Calcd for C₂₄H₁₉N₅S (%): C, 70.42; H, 4.65; N, 17.11. Found (%): C, 70.39; H, 4.62; N, 17.07

(E)-2''-(4-((4'-Fluorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)-4''-phenyl thiazole (92m)

Yield: 82%; m.p. 194 °C

IR (ν_max, cm⁻¹): 1696 (C = N str.), 1562 (C = C str.)

1H NMR (400 MHz; CDCl₃, δ_H): 2.56 (s, 3H, 3-CH₃), 3.15 (s, 3H, 5-CH₃), 7.14-7.19 (m, 2H, 3', 5'-H), 7.27 (s, 1H, 5''-H), 7.34-7.37 (m, 1H, 4''-H), 7.43-7.47 (m, 2H, 3'', 5''-H), 7.83-7.86 (m, 2H, 2', 6'-H), 7.90-7.92 (m, 2H, 2'', 6''-H)

MS: m/z 377 (M⁺)

Anal. Calcd for C₂₀H₁₆FN₅S (%): C, 63.66; H, 4.24; N, 18.57. Found (%): C, 63.61; H, 4.22; N, 18.52

(E)-4''-(4''-Bromophenyl)-2''-(4-((4'-fluorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl) thiazole (92n)

Yield: 86%; m.p. 201-202 °C

IR (ν_max, cm⁻¹): 1698 (C = N str.), 1562 (C = C str.)

1H NMR (400 MHz; CDCl₃, δ_H): 2.56 (s, 3H, 3-CH₃), 3.13 (s, 3H, 5-CH₃), 7.14-7.19 (m, 2H, 3', 5'-H), 7.26 (s, 1H, 5''-H), 7.54-7.58 (m, 2H, 3'', 5''-H), 7.75-7.78 (m, 2H, 2'', 6''-H), 7.83-7.86 (m, 2H, 2', 6'-H)

MS: m/z 457 (M⁺)

Anal. Calcd for C₂₀H₁₅BrFN₅S (%): C, 52.52; H, 3.28; N, 15.32. Found (%): C, 52.48; H, 3.26; N, 15.29
(E)-4''-(4'''-Chlorophenyl)-2''-(4-((4'-fluorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl) thiazole (92o)

Yield: 86%; m.p. 198 °C
IR (ν_{max}, cm^{-1}): 1698 (C = N str.), 1562 (C = C str.)

\(^1\)H NMR (400 MHz; CDCl_{3}, δ_H): 2.55 (s, 3H, 3'-C\text{H}_3), 3.12 (s, 3H, 5-C\text{H}_3), 7.14-7.18 (m, 2H, 3', 5'-H), 7.24 (s, 1H, 5''-H), 7.38-7.42 (m, 2H, 3'', 5''-H), 7.80-7.85 (m, 4H, 2', 6', 2'', 6''-H)

MS: m/z 411.5 (M^+)

Anal. Calcd for C\textsubscript{20}H\textsubscript{15}ClF\textsubscript{5}N\textsubscript{5}S (%): C, 58.32; H, 3.65; N, 17.01. Found (%): C, 58.29; H, 3.62; N, 17.00

(E)-4''-(4'''-Fluorophenyl)-2''-(4-((4'-fluorophenyl)diazenyl)-3,5-dimethyl-1H-pyrazol-1-yl)thiazole (92p)

Yield: 81%; m.p. 197-198 °C
IR (ν_{max}, cm^{-1}): 1694 (C = N str.), 1562 (C = C str.)

\(^1\)H NMR (400 MHz; CDCl_{3}, δ_H): 2.54 (s, 3H, 3'-C\text{H}_3), 3.11 (s, 3H, 5-C\text{H}_3), 7.09-7.17 (m, 4H, 3', 5'; 3'', 5''-H), 7.16 (s, 1H, 5''-H), 7.80-7.87 (m, 4H, 2'', 6'', 2', 6'-H)

MS: m/z 395 (M^+)

Anal. Calcd for C\textsubscript{20}H\textsubscript{15}F\textsubscript{2}N\textsubscript{5}S (%): C, 60.76; H, 3.80; N, 17.72. Found (%): C, 60.73; H, 3.76; N, 17.70

2.3B Biological Evaluation

2.3B.1 Antimicrobial activity

Test microorganisms: On the basis of clinical importance in causing diseases in humans, total six microbial strains were selected. These strains include two Gram-positive bacteria viz. *Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121, two Gram-negative bacteria viz. *Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741 and two yeasts namely *Candida albicans* MTCC 227 and *Saccharomyces cerevisiae* MTCC 170. All the microbial cultures were purchased from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh.
Subculturing of bacterial strains were done on Nutrient Agar (NA) while yeasts were carried on Malt Extract Agar (MEA) plates.

**Determination of zones of inhibition**

The zones of inhibition of all the synthesized compounds was measured by the agar well diffusion method [95]. The inoculum suspensions of the test microorganisms were prepared by using 16 h old cultures adjusted to $10^8$ cfu/ml by referring the 0.5 McFarland standards. Total 20 ml of agar medium (NA in case of antibacterial and MEA in case of antifungal) was poured into each petri plate and then plates were swabbed with 100 µl inocula of the test microorganisms and kept for 15 min for adsorption. Wells were bored into the seeded agar plates using a sterile cork borer of diameter (8 mm) and these were loaded with a 100 µl volume with concentration of 4.0 mg/ml of each compound reconstituted in the dimethylsulphoxide (DMSO). The incubation of all the plates was carried at 37 °C for 24 h. Antimicrobial activity of each compound against the selected organisms was evaluated by measuring the zone of inhibition with zone reader (Hi Antibiotic zone scale) Ciprofloxacin and Amphotericin-B were used as positive control for bacterial and yeast strains, respectively. This procedure was performed in three replicate plates for each organism.

**Determination of minimum inhibitory concentration (MIC)**

MIC is the lowest concentration of an antimicrobial compound that inhibits the visible growth of the microorganisms after incubation. MIC of the various compounds against bacterial and yeast strains was tested through a modified agar well diffusion method [95]. In this protocol, a two-fold serial dilution of each compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 4 to 0.0625 mg/ml. A 100 µl volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100µl of standardized inoculum ($10^8$ cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 37 °C for 24 h and observed for the inhibition zones. MIC, taken as the lowest concentration of the chemical compound that inhibited the growth of the microbes which was shown by a clear zone of inhibition and was recorded for each test organism. Ciprofloxacin and Amphotericin-B were used as positive controls while DMSO was used as a negative control in this investigation.
2.3B.2 Free radical-scavenging of the compounds using DPPH analysis

The DPPH free radical scavenging activity is based on the fact that methanolic solution of DPPH which imparts vivid purple color and gives strong absorption band at 515 nm, gets reduced in the presence of an antioxidant compound [96]. Different concentrations of the compounds under evaluation (50-400 µg/ml) were added to 4 ml of a DPPH solution (120 µM) in methanol and incubated at 37 °C temperature for 30 min dark. The absorbance was determined at 515 nm and the percentage free radical scavenging (%) was calculated according the following equation:

\[
\text{Scavenging \%} = \left[ \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100
\]

where, \(A_{\text{control}}\) is the absorbance of the control reaction (containing all reagents except the test compounds), and \(A_{\text{sample}}\) is the absorbance of the test compound. Ascorbic acid was used as a positive control and tests were conducted in triplicate.

2.3B.3 Effects of compounds on plasmid DNA under UV irradiation

Treatment of plasmid DNA with the samples

The stock solutions for all tested compounds were prepared by dissolving 0.005 g of compound in 0.5 ml of DMSO. All synthesized compounds (40-60 µg) in DMSO were added separately to volume of 2µl containing plasmid DNA in TE (Tris 10 mM, EDTA 0.01 mM, pH 8.0) buffer. The same volume of DMSO as used to make the solution of the test compounds was added into A and control C. The reaction volumes except A were held in caps of polyethylene microcentrifuge tubes, which were irradiated directly on the surface of a trans-illuminator (8000 mW/cm) at 360 nm for 30 min at room temperature. After that A, control (C) and test samples were incubated at 37 °C for 0.5 h.

Agarose gel electrophoresis

After the treatment, electrophoresis was performed according to the given procedure mentioned [97].

To a 2 ml 50X tris-acetate EDTA buffer (TAE) (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH: 8.0), added 98 ml of autoclaved water to make it 1X TAE buffer. Agarose (0.8 g) was dissolved by boiling to the resultant mixture. When the gel attained 55 °C temperature, 10 mg/ml of ethidium bromide (ETBR) was added. The treated DNA sample mixed with 6X loading dye (0.25%) bromophenol blue added
and then it was poured into gel cassette fitted with a comb. The gel was then allowed to solidify. The comb was carefully removed and the gel was placed over electrophoresis chamber flooded with tris-acetate EDTA buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH: and 30% glycerol) was carefully loaded into the wells along with control (C) and A, and electrophoresis was carried out at 5V/cm for 2.0 h and the bands were observed under UV transilluminator.
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