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Thiazoles: A Valuable Insight into the Recent Advances and Biological Activities

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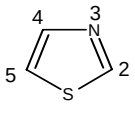
ABSTRACT
Thiazoles displayed broad range of biological activities and found in many potent biologically active molecules such as Sulfathiazol (antimicrobial drug), Ritonavir (antiretroviral drug), Abafungin (antifungal drug) and Tiazofurin (antineoplastic drug). So far, modifications of the thiazole ring have proven highly effective with improved potency and lesser toxicity. The present review highlights the recently synthesized thiazoles possessing important biological activities.

Keywords: Thiazoles derivatives; Biological activities.

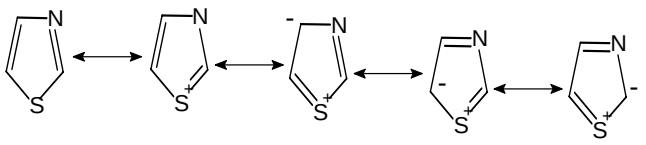
INTRODUCTION

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



1
Numbering system of thiazole ring

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



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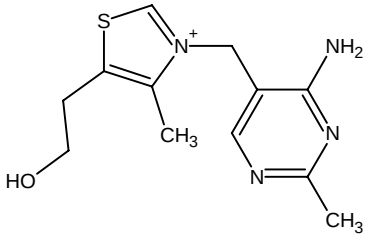
Thiazole is a clear to pale yellow liquid with a boiling point of 116-118°C. Its specific gravity is 1.2 and it is sparingly soluble in water. It is soluble in alcohol and ether. The odor of thiazole is similar to pyridine. It is used as an intermediate to manufacture synthetic drugs, fungicides, and dyes. A thiazole ring is found naturally in the essential vitamin B_1 (thiamin).



Thiamin

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

1.2. BIOLOGICAL ACTIVITIES

Thiazoles are important class of heterocyclic compounds, found in many potent biologically active molecules such as Sulfathiazol (antimicrobial drug), Ritonavir (antiretroviral drug), Abafungin (antifungal drug) with trade name Abasol cream and Bleomycine and Tiazofurin (antineoplastic drug).
It has been noticed continuously over the years that interesting biological activities [1-2] were associated with

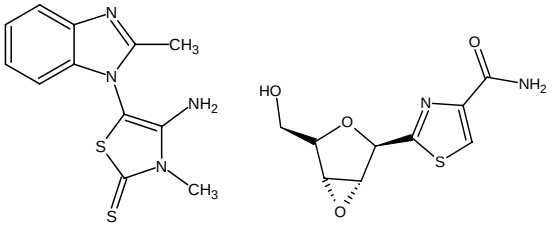
thiazole derivatives. Recently the applications of thiazoles were found in drug development for the treatment of allergies [3], hypertension [4], inflammation [5], schizophrenia [6], bacterial [7], HIV infections [8], hypnotics [9] and more recently for the treatment of pain [10], as fibrinogen receptor antagonists with antithrombotic activity [11] and as new inhibitors of bacterial DNA gyrase B. [12] A brief review of thiazoles associated with large number of biological activities is presented below.

1.2.1. Antitumor activity

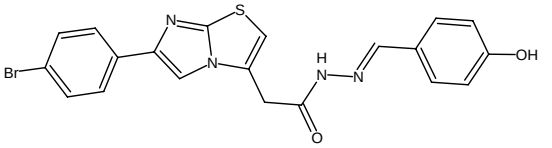
Ramla et al [13] synthesized a variety of 1-substituted-2-methyl-5-nitrobenzimidazoles and evaluated them for anti-tumor activity. The anti-tumor effect of compound [**1**] was found to be significant.

Popsavin et al [14] reported a set of 2-(2, 3-anhydrofuranosyl) thiazole-4-carboxamide (2', 3'-anhydro tiazofurin) derivatives and screened them for their anti-tumor activity. The most active compound was found to be [**2**] against K_{562} malignant cells, with IC_{50} vlues ranging from 0.09-0.49 μM.

Gulsory et al [15] presented a series of arylidene hydrazides from [6-(4-bromophenyl) imidazol-3yl] acetic acid hydrazide. The synthesized compounds were evaluated one dose primary cytotoxicity assay. Compound [**3**] demonstrated the most effective agents on a prostate cancer cell lines.



[**1**] [**2**]



[**3**]

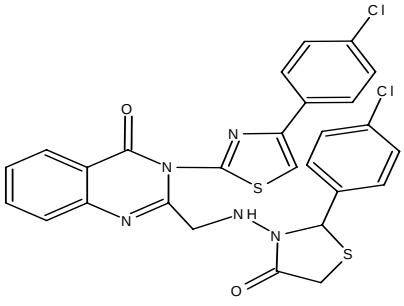
1.2.2. Anti-inflammatory activity

Kumar et al [16] synthesized a group of 3-[4'(*p*-chlorophenyl) thiazol-2'-yl]-2-[(substituted azetidinone/thiazolidinone)-aminomethy]-6-bromoquinazolin-4-ones and screened them for anti-inflammatory and analgesic activities. Compound [**4**] was found to be highly active in both the activities. They found that the presence of thiazolidinone ring have shown much better anti-inflammatory as well as analgesic activity at 50 mg/kg po as compared to their parent compounds. Compound substituted with chloro group at 2nd position of phenyl ring has shown almost equal anti-inflammatory activity to that of the standard drug phenylbutazone at 50 mg/kg.

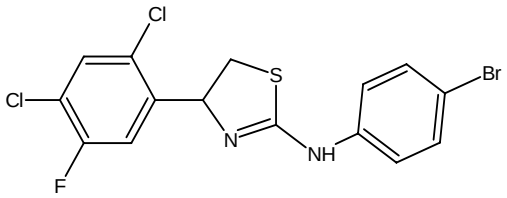
Holla et al [17] reported different series of arylaminothiazoles, arylidene/5-aryl-2-furfurylidene hydrazinothiazoles and screened them for their antibacterial and anti-inflammatory activities. Two of the newly synthesized compounds [**5**] and [**6**] showed anti-inflammatory activity comparable with that of ibuprofen.

Kalkhambkar et al [18] reported triheterocyclic thiazoles containing coumarin and carbostyril (1-aza coumarin). The newly synthesized compounds were tested for their *in vitro* analgesic and anti-inflammatory activities. Among the tested compounds, [**7**] and [**8**] significantly inhibited the acetic acid induced writhing.

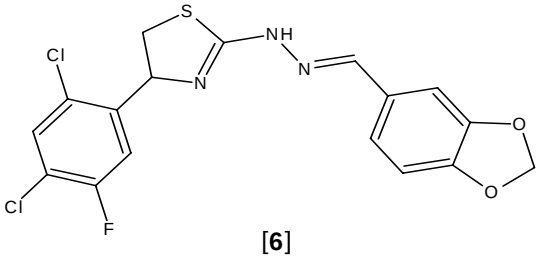
Rostom et al [19] reported two groups of structure hybrids comprising basically the antipyrine moiety attached to polysubstituted thiazole or 2, 5-disubstituted-1, 3, 4-thiadiazole counterparts through various linkages. Twelve compounds were evaluated for their anti-inflammatory activity, ulcerogenic effects and acute toxicity. The analgesic activity of the same compounds was also evaluated. Additionally, their *in vitro* antimicrobial activity was evaluated. Some compounds [**9**] and [**10**] displayed remarkable anti-inflammatory and analgesic profiles with a fast onset of action together with a super GI safety profile and safety margin. Additionally, some compounds exhibited broad-spectrum antimicrobial activity.



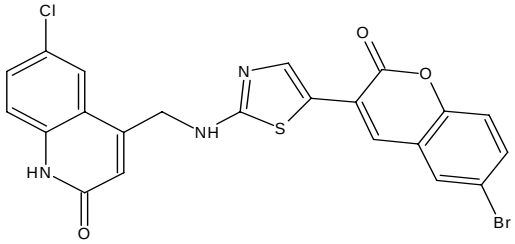
[**4**]



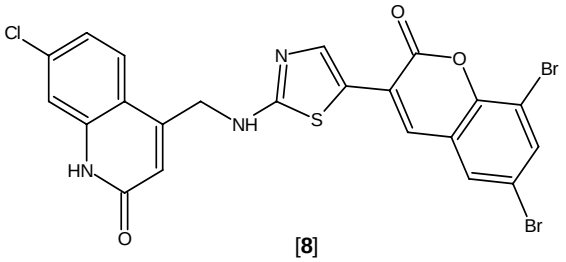
[**5**]



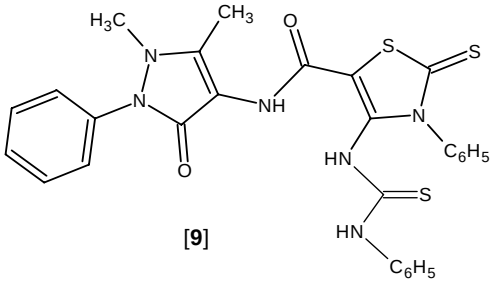
[**6**]



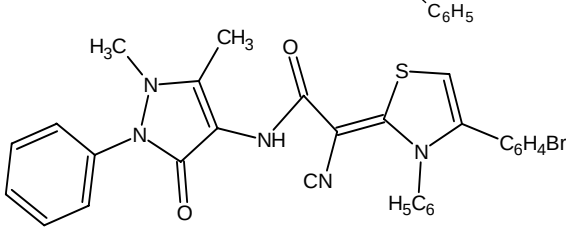
[**7**]



[**8**]



[**9**]



[**10**]

1.2.3. Antimicrobial activity

Pandeya et al [20] prepared a series of Schiff and Mannich bases derived from isatin derivatives and *N*-[4-(4'chlorophenyl) thiazol-2-yl] thiosemicarbazide. Investigation of antimicrobial activity of compounds was done by agar dilution method against 28 pathogenic bacteria, 8 pathogenic fungi and anti-HIV-1 (IIIB) in MT-4 cells. Among the compounds tested [**11**] showed the most favorable antimicrobial activity.

Shiradkar et al [21] reported a series of *N*-{4-[(4-amino-5-sulphanyl-4*H*-1, 2, 4-triazol-3-yl) methyl]-1, 3-thiazol-2-yl}-2-substituted amide derivatives. The compounds were tested for their preliminary *in vitro* antibacterial activity against *S. aureus*, *E. coli*, *P. aeroginosa* and *S. typhosa* and then were screened for antitubercular activity against *M. tuberculae* $H_{37}Rv$ strain by both micro dilution assay method. Compound [**12**] and [**13**] showed best activity. They revealed that the compounds that have shown more than 90% inhibition were obtained by S-alkylation with acetonitrile. It was noted that the cyano group may not have any role in increase in the activity. When the sulfhydryl group were optimized and investigated, it resulted into the loss of activity.

Xin et al [22] reported sixteen novel oxazolidinone analogue containing substituted thiazole/ fused bicyclic [imidazo[1,2-b] pyradazine/imidazo [2,1-b] thiazole groups were designed and synthesized. All the compounds were evaluated for their *in vitro* antibacterial activity against *S. aureus*. Among them compound [**14**] displayed promising antibacterial activity comparable to that of linezolid.

Vicini et al [23] produced a new set of 2-thiazolylimino-5-arylidene-4-thiazolidinones and assayed *in vitro* for their antimicrobial activity against Gram positive and Gram negative bacteria, yeast and mould. All the compounds especially compound [**15**] exhibited potent against Gram positive bacteria. They have studied the structure-activity relationship and found that the 5-arylidene derivatives showed a significant antibacterial efficacy greater than that of the parent compound suggesting that the unsubstituted and substituted 5-arylidene moiety plays an important role in enhancing the antimicrobial properties of this class of compounds.

Dundar et al [24] presented a set of thiazolyl thiazolidine-2,4-dione derivatives and screened them for their antibacterial and antifungal activities against methicillin resistant *S. aureus*, *E. coli* and *C. albicans*. All the compounds particularly [**16**] were found to be moderately potent against screened microorganisms. The structure-activity relationships showed that the anti-fungal activity of the substituents at the phenyl ring is H, Cl, Br, *o*,*p*-diCl > F, NO_2 for benzylic 2,4-TZD compounds. As for phenacyl 2,4-TZD compounds, it is Cl, Br > H, F, *o*,*p*-di-Cl, NO_2.

Cukurovali et al [25] reported a series of Schiff bases containing 2, 4-disubstituted thiazole and cyclobutane rings and hydrazones moieties in the same molecule and evaluated them for antibacterial and antifungal activities. Among the tested compounds, the most effective compound providing a MIC value of 16 μg ml^{-1} was found to be [**17**] against *C. tropicalis* and *B. subtilis*. They studied the lowest effective substance against all the microorganisms and found that despite all the substances have very similar structures, their antibacterial and antifungal activities are very different. Most of them demonstrate weak activity against gram-positive and gram-negative bacteria and fungi in comparison to the reference drugs.

Zitouni et al [26] reported new thiazole derivatives of triazoles and evaluated for antifungal and antibacterial activity. Their antimicrobial activities against *Candida albicans* (two strains), *C. glabrata*, *E. coli*, *S. aureus*, *P. aeruginosa* were investigated. The results showed that some of the compounds [**18**] have very strong antifungal activity.

Abdel-Wahab et al [27] synthesized a series of 1-(benzofuran-2-yl)-4-nitro-3-arylbutan-1-ones and 3-(benzofuran-2-yl)-4,5-dihydro-5-aryl-1-[4-(aryl)-1,3-thiazol-2-yl]-1*H*-pyrazoles. All the synthesized compounds were screened for their antibacterial and antifungal activities. Compound [**19**] showed a significant activity against *E. coli* higher than that of the control drug, whereas antifungal activity against *Aspergillus niger* was also exhibited by some of the compounds equal to that of the reference drug.

Karegoudar et al [28] synthesized a series of novel 4-aryl-2-(2, 3, 5-trichlorophenylidenehydrazino)-1, 3-thiazoles in good yield. The newly synthesized compounds were screened for their antibacterial and antifungal activities. Preliminary results reveal that derivatives of synthesized compound [**20**] are showing promising antimicrobial activity.

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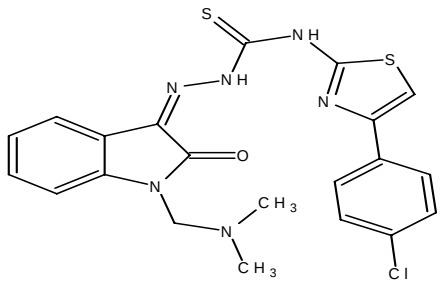
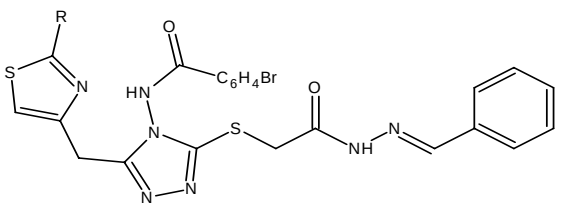
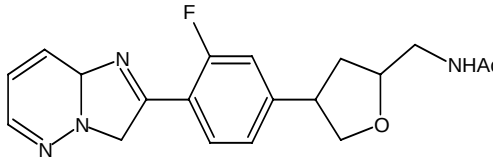


[**11**]

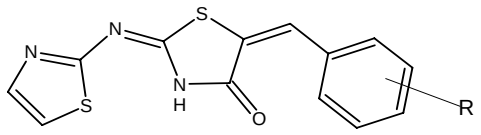


[**12**] R=NHCOCH$_3$, Ar=3-NO$_2$.C$_6$H$_4$

[**13**] R=NHCOC$_6$H$_5$, Ar=3-NO$_2$.C$_6$H$_4$

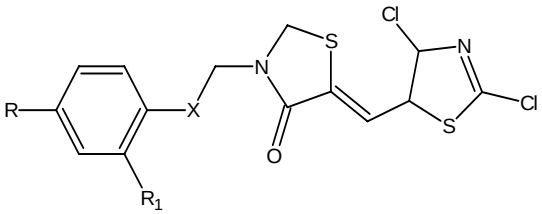


[**14**]



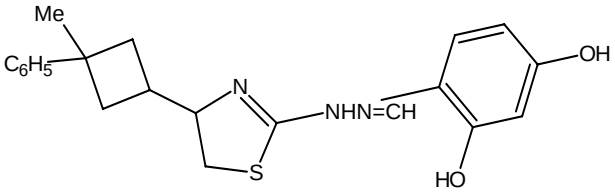
R = H, OH, OCH_3, NO_2, Cl

[**15**]

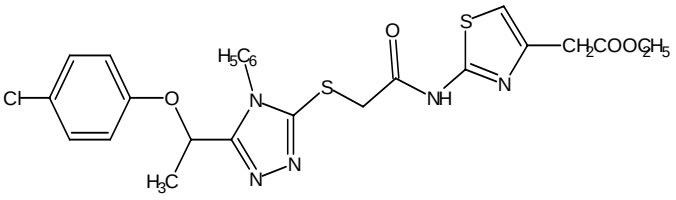


R = H, F, Cl, Br, NO_2: R_1 = H, Cl: X = CH_2, CO

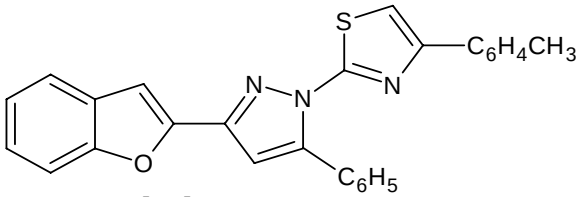
[**16**]



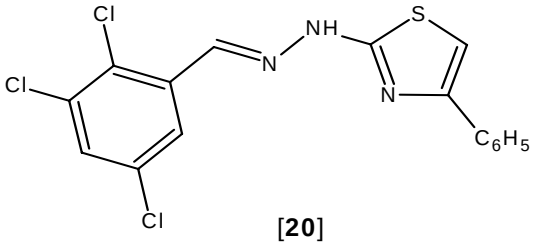
[**17**]



[**18**]



[**19**]



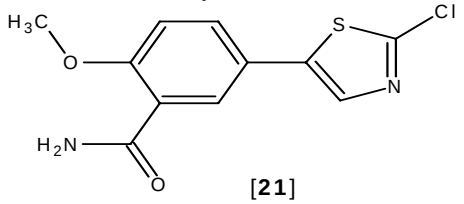
[**20**]

1.2.4. Antifungal activity

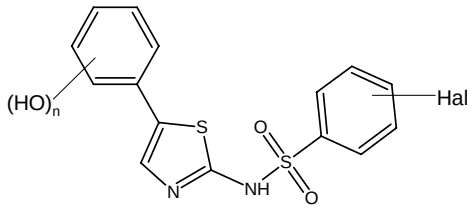
Narayana et al [29] prepared a series of 5-{2-[(*N*-substituted aryl) amino]-1, 3-thiazol-5-yl} 2-hydroxy benzamides by reacting 5-(bromoacetyl) salicylamide with thiourea, thioformamide, thioalkylamide and substituted thioureas in absolute ethanol. These compounds were converted to 5-(2-substituted–1, 3-thiazol-5-yl)-2-alkoxybenzamides and 5-(2-*N*-(substituted aryl)-1, 3-thiazol-5-yl)-2-alkoxy benzamides by reacting with *n*-alkylbromides in presence of a base. The newly synthesized compounds were screened for their antifungal activity. The derivatives of compound [**21**] exhibited significant activity.

Beuchet et al [30] synthesized polymethoxylated and polyhydroxylated derivatives of 2-amino-4-arylthiazoles bearing a halogenobenzenesulfonamide moiety at position 2 as azole antifungal analogues. *In vitro* assays against various pathogenic fungal strains (*Candida* and *Trichophyton* species) showed no activity in comparison to econazole as reference.

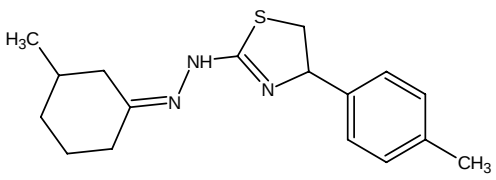
Chimenti et al [31] reported the synthesis of a novel series of 2-thiazolylhydrazone derivatives and the influence of the substituents on the thiazole ring on antifungal activity. All synthesized compounds were screened for their *in vitro* activities against 22 clinical isolates of *Candida* sp., representing six different species, compared to clotrimazole as a reference compound. Some of the tested compounds were found to possess significant antifungal activity when compared to clotrimazole, in particular compound [**23**] which exhibited higher potency against most of the *Candida* sp. considered. The compounds that were most active as anti-Candida agents were also submitted to cytotoxic screening by the Trypan Blue dye exclusion assay and in general they were shown to induce low cytotoxic effects.



[**21**]



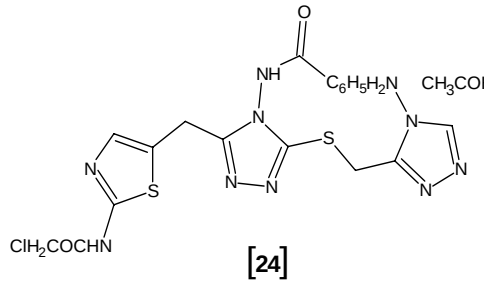
[**22**]



[**23**]

1.2.5. Antitubercular activity

Shiradkar et al [32] synthesized a series of *N*-{4-[(4-amino-5-sulfanyl-4H-1, 2, 4-triazol-3-yl) methyl]-1, 3-thiazol-2-yl}-2-substitutedamide [**24**], [**25**] and [**26**] derivatives in good yields. The compounds were evaluated for their preliminary



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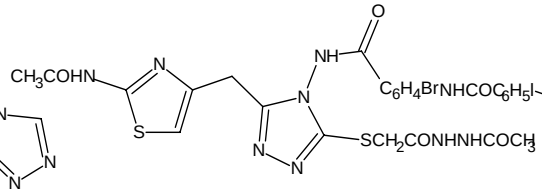

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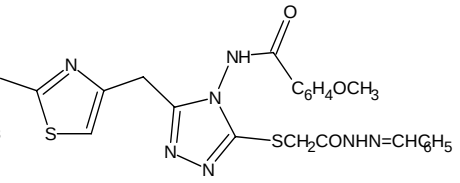

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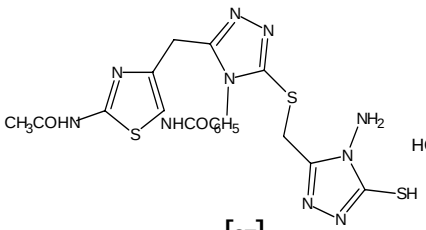

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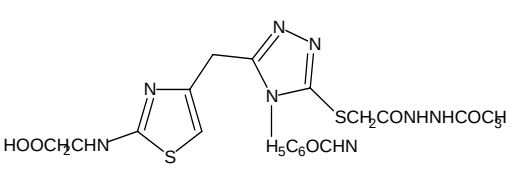

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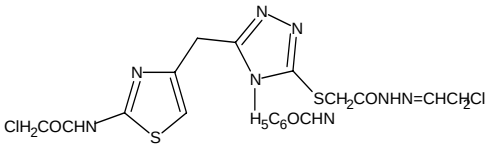

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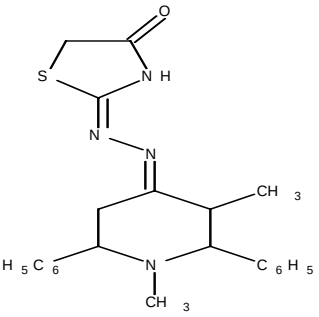
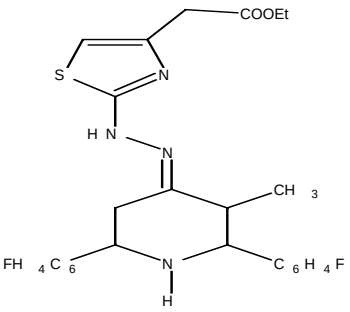
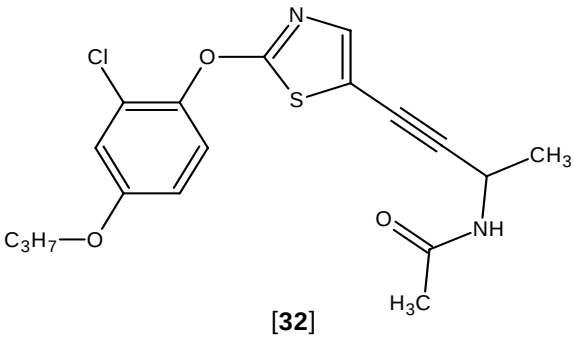


[30]



[31]



[32]

in vitro antibacterial activity against *S. aureus, E. coli, P. aeruginosa* and *S. typhosa* and then were screened for antitubercular activity against *Mycobacterium tuberculosis* H37 Rv strain by broth microdilution assay method. The antibacterial data of the tested compounds indicated that most of the synthesized compounds showed better activity against bacteria compared to reference drugs. The *in vitro* antitubercular activity reports of tested compounds against *M. tuberculosis* strain H37 Rv showed moderate to better activity. It was noted that the cyano group may not have any role in increase in the activity. When the sulfhydryl group were optimized and investigated, it resulted into the loss of activity.

Shiradkar et al [33] reported the synthesis of thiazolyl triazole derivatives, starting from ethyl acetoacetate, by microwave organic reaction enhancement method (MORE). Results of investigations of their antimycobacterial and antimicrobial activities were also produced. Many compounds [**27**], [**28**], [**29**] have shown promising activity while others were inactive. They found that two compounds that have shown 97% and 100% inhibition were obtained by the S-alkylation with acetonitrile. When the acetate derivatives were converted into the hydrazide derivatives, the antimycobacterial activity was quite interesting as all of these compounds have shown inhibition above 90 %.

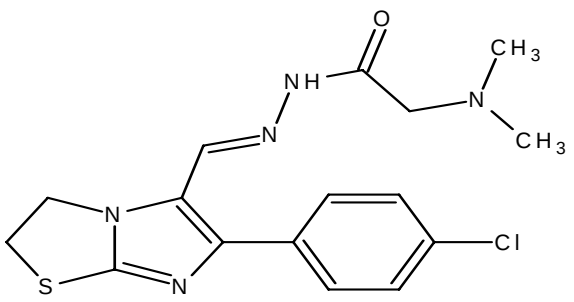
Aridoss et al [34] synthesized some new thiazolidinones and thiazoles based on t-3-alkyl-r-2,c-6-diarylpiperidin-4-ones and evaluated them for antimycobacterial and antimicrobial activity and it was revealed after screening that substitution of electron withdrawing or donating substituents at the para position of the phenyl groups besides methyl group at N-1 and C-3 exerted better biological profiles [**30**], [**31**].

1.2.6. Acetyl-Co-A carboxylase inhibitors

Clark et al [35] presented a new series of phenoxy thiazolyl derivatives and screened them for their acetyl-Co-A carboxylase inhibitory profile. Compound [**32**] was found to be highly active in the inhibition of acetyl-Co-A carboxylase isozyme.

1.2.7. Diuretic activity

Andreani et al [36] synthesized a series of imidazo[2,1-b] thiazole acetohydrazones and screened them for their diuretic activity. A potent diuretic activity was confirmed for the 2-methyl derivative bearing a phenyl ring at position C-6 [**33**]. Evaluation of the diuretic activity of both the saturated compounds and their unsaturated analogues shows that among the 6-position substituents, which were synthesized, a phenyl or substituted phenyl group was superior. This was confirmed by the results obtained with the analogues, 2- and 3-methyl derivatives which, considering the dose employed and the acute toxicity were the most promising derivatives.

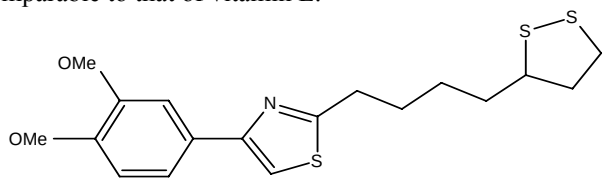


[33]

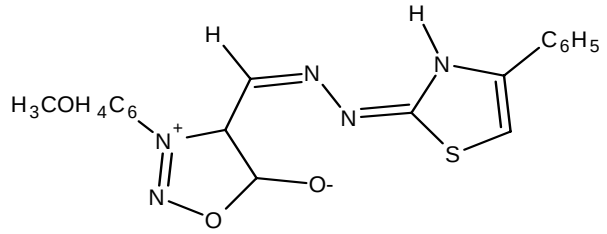
1.2.8. Neuroprotective and antioxidant activity

Koufaki et al [37] designed synthesized new analogues containing 1, 2-dithiolane derivatives and screened for neuroprotective activity. Compound [**34**] was found to be highly neuroprotective. The structure-activity relationship revealed that when the amide functionality was replaced by the tetrazole ring, they were found to be the strngest neuroprotectant, while the 1, 3, 4-oxadiazole derivative was somewhat less potent. Thus, it appeared that the replacement of the amide functionality by the aromatic heterocycles conveyed greater neuroprotective activity to the resulting compounds.

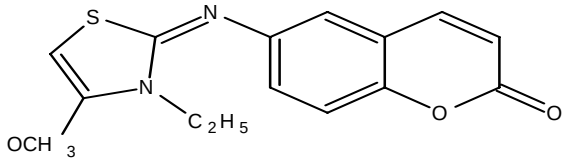
Shih et al [38] synthesized a series of sydnonyl substituted thiazolidinone and thiazoline derivatives and evaluated them for antioxidant activity. The antioxidant activity of derivatives of compound [**35**] exhibited the significant DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity, comparable to that of vitamin E.



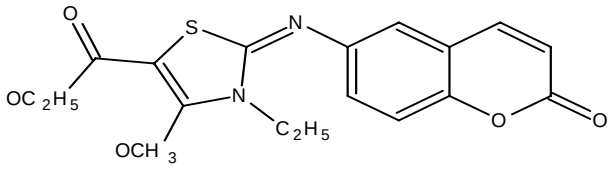
[**34**]



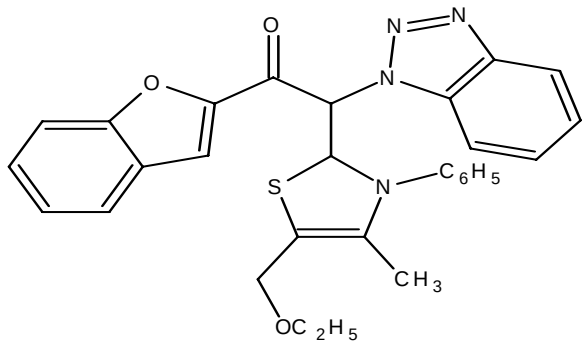
[**35**]



[**36**]



[**37**]



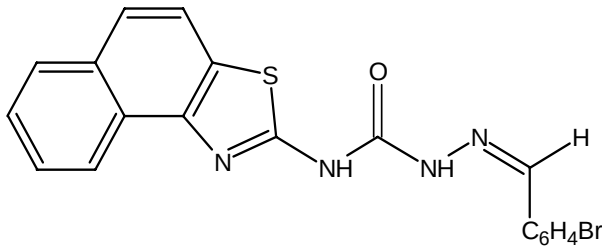
[**38**]

1.2.9. Anticonvulsant activity

Amin et al [39] reported some new substituted coumarinyl thiazolines, coumarinyl thiazolidin-4-ones and substituted chromenothiazoles and evaluated for anticonvulsant activity. Compounds [**36**] and [**37**] were the most active of the series against PTZ induced seizures

Dawood et al [40] reported a series of newly synthesized compounds and evaluated them for anti-inflammatory and anticonvulsant activity. The newly synthesized compounds [**38**] were found to possess anticonvulsant and anti-inflammatory activities with the same mechanism of action of selective COX-2 inhibitors. From the structure-activity relationship viewpoint, the anti-inflammatory activity of 5-acetyl-1, 3, 4-thiadiazole derivatives were found to be high in the case of unsubstituted phenyl derivatives and decreases with substitution in the order H > 4-CH_3 > 4-Cl. Also, the anti-inflammatory effect of the thiazolidine ester derivative is higher than that of its acetyl derivatives. In addition, the chlorinated ester derivatives of 1, 3, 4-thiadiazole system was found to be more effective than its non-chlorinated derivatives.

Azam et al [41] designed and synthesized a series of N^4-(naphtha[1,2-*d*]thiazol-2-yl)semicarbazides [**39**] and evaluated for their anticonvulsant and neurotoxicity studies. The biochemical estimations of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) from brain homogenate for selected compounds were performed to study their antioxidant property.



[**39**]

1.3. CURRENT ASPECTS OF THIAZOLE

Zhu et al [42] have performed the structure-based 3D-QSAR studies on 20 thiazoles against their binding affinities to the 5-HT_3 receptor with comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA). The thiazoles were initially docked into the binding pocket of a human 5-HT_{3A} receptor homology model, constructed on the basis of the crystal structure of the snail acetylcholine binding protein (AChBP), using the GOLD program. The docked conformations were then extracted and used to build the 3D-QSAR models, with cross-validated τ^2_{cv} values 0.785 and 0.744 for CoMFA and CoMSIA, respectively. An additional five molecules were used to validate the models further, giving satisfactory predictive r^2 values of 0.582 and 0.804 for CoMFA and CoMSIA, respectively. The results would be helpful for the discovery of new potent and selective 5-HT_3 receptor antagonists.

Deeb et al [43] have performed the QSAR analysis of a set of 96 heterocyclics with antifungal activity. The results revealed that pyridine ring was more favorable than benzene as the 6-membered ring, for high activity, but thiazole was

unfavorable as the 5-membered ring relative to imidazole or oxazole. Methylene was the spacer leading to the highest activity. The descriptors used were indicator variables, which account for identity of substituent, lipophilicity and volume of substituent, and total polarizability.

This has been noticed so far, that modifications on thiazole moiety displayed valuable biological activities. It will be interesting to observe that these modifications can be utilized as potent therapeutic agents in future. Thus the quest to explore many more modifications on thiazole moiety needs to be continued.

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Original article

Synthesis, anti-bacterial and anti-fungal activities of some novel Schiff bases containing 2,4-disubstituted thiazole ring

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ABSTRACT

A series of arylidene-2-(4-(4-methoxy/bromophenyl) thiazol-2-yl) hydrazines (**4a**–**z**) and 1-(4-(4-methoxy/bromophenyl) thiazol-2-yl)-2-cyclohexylidene/cyclopentylidene hydrazines (**5a**–**b**/**6a**–**b**) were synthesized, characterized and screened for their antimicrobial activities. The structures of synthesized compounds were established by spectroscopic (FT-IR, ^{1}H NMR, ^{13}C NMR, Mass) and elemental analyses. Both the anti-bacterial and anti-fungal activities with MIC values of compounds were evaluated. The results of anti-bacterial screening reveal that among all the compounds screened eight compounds showed moderate to good anti-bacterial activity while ten of the newly synthesized compounds displayed good to excellent anti-fungal activity. Among the tested compounds, the most effective compounds with MIC value in the range of 6.25–25 μg/ml are **4a**, **4n**, **4z**, **5a**, **5b**, **6a** and **6b** against three fungal strains viz. *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus flavus*.

1. Introduction

Schiff bases have gained importance because of physiological and pharmacological activities associated with them. Compounds containing azomethine group (–C═N–) in the structure are known as Schiff bases, which are usually synthesized by the condensation of primary amines and active carbonyl groups. Schiff bases are well known for their pharmacological properties as anti-bacterial, anti-fungal, anti-cancer and anti-viral agents [1,2]. Similarly, the occurrence of thiazole ring system in numerous biologically active molecules has been recognized which plays an important role in animal and plant kingdom. Different thiazole bearing compounds possess activities such as anti-bacterial [3], anti-fungal [4], anti-inflammatory [5], antihypertensive [6], anti-HIV [7], antitumor [8–11], antifilarial [10,11], anticonvulsant [12], herbicidal, insecticidal, schistosomicidal and anthelmintic [13]. The presence of thiazole ring in vitamin B_1 and its coenzyme play an important role as electron sink and for the decarboxylation of α-keto acids, respectively [14]. Many biologically active products, such as Bleomycin and Tiazofurin (antineoplastic agents) [15], Ritonavir (anti-HIV drug) [16], Fanetizole and Meloxicam (anti-inflammatory agents) [17,18], Nizatidine (antiulcer agent) [19], imidacloprid (insecticide) and penicillin (antibiotic) are some examples of thiazole bearing products. Thiazole derivatives are also widely used for the synthesis of antibiotic sulphathiazole [20], and with polyoxygenated phenyl component they showed promising anti-fungal activity [21]. Thiazole nucleus as ligand of estrogen receptors [22] and also as novel class of antagonists for adenosine receptors [23] is known. Screening of 2,4-disubstituted thiazoles as latent pharmacophores for diacylhydrazine of SC-51089, a potential PGE_2 antagonist have been reported [24]. The exciting results of 2,4-disubstituted thiazoles as a novel class of Src homology 2 (SH2) inhibitors for the treatment of osteoporosis and breast cancer have also been reported [25]. Synthesis of thiazole derivatives by various methods and their biological evaluation have been described by many researchers [26–39]. Thus the thiazole nucleus has attracted much interest in the development of pharmacologically active compounds. Since the thiazole moiety seems to be a possible pharmacophore in various pharmacologically active agents, we decided to synthesize compounds with this functionality coupled with Schiff base as possible antimicrobial agents which could furnish better therapeutic results.

2. Chemistry

In the present work, arylidene-2-(4-(4-methoxy/bromophenyl) thiazol-2-yl) hydrazines (**4a**–**z**) and 1-(4-(4-methoxy/bromophenyl) thiazol-2-yl)-2-cyclohexylidene/cyclopentylidene

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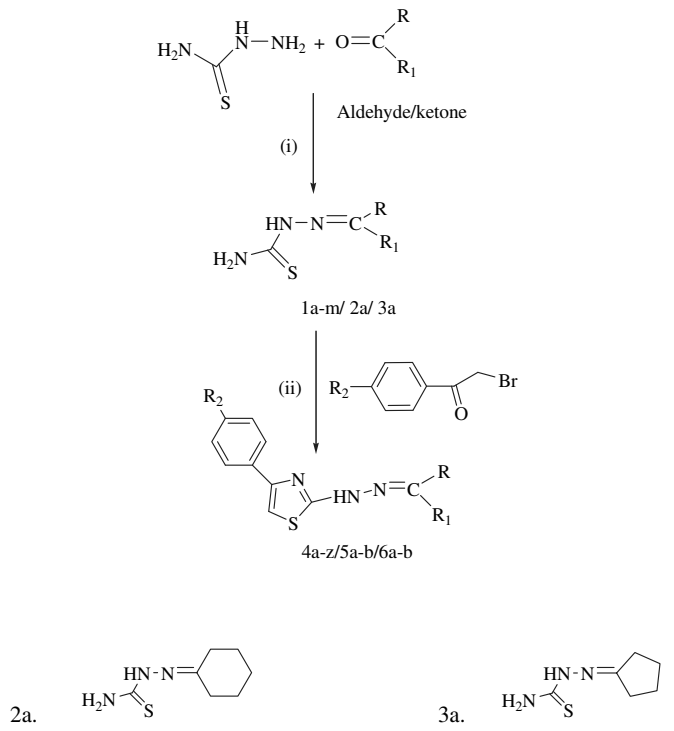
hydrazines (**5a–b**/**6a–b**) were prepared by cyclization of thiosemicarbazone with substituted phenacyl bromide in accordance with the method described in the literature [34,40–45]. The synthetic route of compounds is outlined in Scheme 1. Compounds **5a–b**/**6a–b** were prepared by above method by taking cyclohexanone/cyclopentanone as starting material. The final product was obtained in two steps. In the first step, we have synthesized Schiff base of thiosemicarbazone (**1a–m**/**2a**/**3a**) by condensing substituted aldehyde/ketone with thiosemicarbazide in suitable solvent (methanol/ethanol) in the presence of few drops of glacial acetic acid as catalyst. In the second step, equimolar quantities of thiosemicarbazone thus obtained in first step (**1a–m**/**2a**/**3a**) and substituted phenacyl bromide (4-methoxy/bromo-phenacyl bromide) were refluxed and neutralized with $NaHCO_3/K_2CO_3$ to obtain desired compound (**4a–z**/**5a–b**/**6a–b**) in 58–86% yields. The chemical structures of the synthesized compounds were established by spectroscopic (FT-IR, ^{1}H NMR, ^{13}C NMR, Mass) and elemental analyses.

3. Pharmacology

Anti-bacterial activity of newly synthesized compounds **4a–z**/**5a–b**/**6a–b** was evaluated against various pathogenic bacterial strains (Gram-negative and Gram-positive) viz., *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Salmonella typhi* (*S. typhi*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Vibrio cholerae* (*V. cholerae*). Anti-fungal activity of the above compounds **4a–z**/**5a–b**/**6a–b** was evaluated against fungal strains viz. *Candida albicans* (*C. albicans*), *Cryptococcus neoformans* (*C. neoformans*), *Aspergillus flavus* (*A. flavus*) and *Chrysosporium tropicum* (*C. tropicum*). The anti-bacterial and anti-fungal activities were evaluated by agar disc diffusion method as per the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1997) [46]. The solvent, DMSO used for the preparation of compounds did not show inhibition against the tested organisms.



Scheme 1. Reagents and conditions: (i) CH_3OH/C_2H_5OH, AcOH, reflux, 4–6 h; (ii) CH_3OH/C_2H_5OH, reflux, 4–10 h, $NaHCO_3/K_2CO_3$.

4. Results and discussion

4.1. Chemistry

The FT-IR spectra of Schiff bases of thiosemicarbazone (**1a–m**) showed absorption bands at 3120–3330 cm^{-1} for –NH– and –NH_2, at 2950–3070 cm^{-1} for aromatic C–H and at 1590–1670 cm^{-1} for azomethine group (–CH═N–). The absence of absorption band at 1700–1750 cm^{-1} also confirms the conversion of –CHO/C═O group to –CH═N– group. The nuclear magnetic resonance (^{1}H NMR) spectra of the compounds were recorded in DMSO-d_6/$CDCl_3$ solvents and the structural assignments are given in Section 6. The ^{1}H NMR spectra of hydrazones showed peaks of aromatic, hydrazide (NH), amine (NH_2) and imine (–N═CH–) proton. These were all singlets and each one indicating intensity of one proton in 300 MHz ^{1}H NMR. This also indicates the formation of hydrogen bonding (H···S) in the thioamide part of the molecule which gives broad singlet. All of these protons, except azomethine proton are D_2O exchangeable, –OH group signal(s) in the compounds **4c**, **4e**, **4p** and **4r** are broad singlets due to the presence of intramolecular hydrogen bonding [47]. The ^{1}H NMR spectrum of thiazoles (**4a–z**/**5a–b**/**6a–b**) showed sharp singlet at δ 7.6–7.8 indicating the presence of azomethine (–CH═N–) proton and singlet at δ 7.8–7.9 for hydrazide (NH) proton. The sharp singlet at δ 3.8–3.9 indicated the presence of –OCH_3 group attached to the benzene ring. The four aromatic protons of *p*-anisyl moiety resonated as two doublets at δ 6.8–6.9 and 7.9–8.2, respectively. The appearance of multiplets at δ 6.9–7.3 was due to aromatic protons. Moreover, ^{13}C NMR spectra showed the signals in the range of δ 114.4–114.9 ppm and at δ 132.8–133.1 ppm due to aryl carbon and azomethine carbon, respectively. The peak appearing in the range of δ 160–165 ppm corresponds to C_2 of the thiazole ring. In the mass spectrum, compound **4c** showed peak at *m*/*z* 326 (M + 1, 100%), which matches with its molecular formula $C_{17}H_{15}N_3O_2S$. A peak at *m*/*z* 340 (M + 1, 100%) was observed for compounds **4d** which is in conformity with the molecular formula $C_{18}H_{17}N_3O_2S$. FT-IR, ^{1}H NMR, ^{13}C NMR, Mass spectral data and elemental analysis results are in agreement with the proposed structures. Physicochemical data and results of elemental analysis of the compounds are listed in Table 1. The spectral data of all the compounds are given in Section 6.

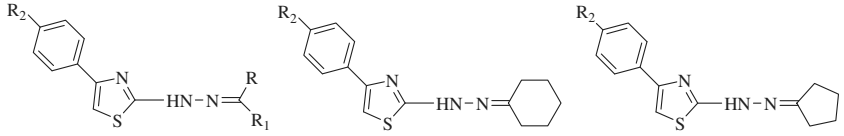
4.2. Anti-bacterial activity

The results of anti-bacterial screening of all the newly synthesized compounds are presented in Table 2. Some of them showed moderate to good activity with MIC value in the range of 12.5–50 μg/ml in DMSO. Particularly, compound 2-(2-(4-(4-bromophenyl) thiazol-2-yl) hydrazono)-1,2-diphenylethanol (**4z**) showed good activity (zone of inhibition up to 17–20 mm at concentration of 12.5 μg/ml) against *S. aureus* and *V. cholerae*. Compound 2-(2-(4-(4-methoxyphenyl) thiazol-2-yl) hydrazono)-1,2-diphenylethanol (**4m**) showed good activity against *V. cholerae* (zone of inhibition up to 16–19 mm at concentration of 25 μg/ml) while compounds **4d**, **4e**, **4f**, **4j**, **4o** and **4p** showed moderate activity against few bacterial strains.

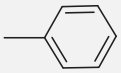
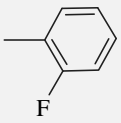
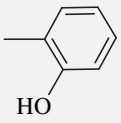
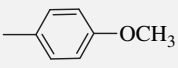
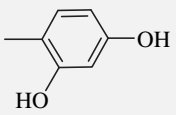
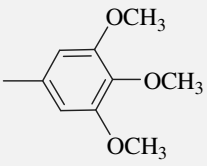
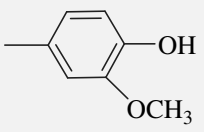
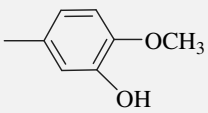
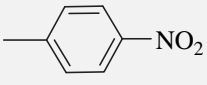
4.3. Anti-fungal activity

The results of anti-fungal screening of all the newly synthesized compounds are presented in Table 2. Some of them showed good to excellent activity with MIC value in the range of 6.25–25 μg/ml in

Table 1
Analytical and physicochemical data of the synthesized compounds.



4a-z **5a-b** **6a-b**

Compd.	R	R_1	R_2	Molecular formula	M.W.[c]	M.p.(°C)[b]/Crystallization solvent	Yield (%)	% Analysis of C, H, N found (Calc.)[a]		
								C	H	N
4a	H		$-OCH_3$	$C_{17}H_{15}N_3OS$	309.39	189–190/$CHCl_3$	72	66.00(66.01)	4.86(4.89)	13.57(13.58)
4b	H		$-OCH_3$	$C_{17}H_{14}FN_3OS$	327.38	127–129/C_2H_5OH	84	62.37(62.39)	4.31(4.32)	12.84(12.86)
4c	H		$-OCH_3$	$C_{17}H_{15}N_3O_2S$	325.38	183–185/$CHCl_3$	75	62.75(62.77)	4.65(4.68)	12.91(12.93)
4d	H		$-OCH_3$	$C_{18}H_{17}N_3O_2S$	339.41	173–175/$CHCl_3$	75	63.70(63.71)	5.05(5.07)	12.37(12.38)
4e	H		$-OCH_3$	$C_{17}H_{15}N_3O_3S$	341.38	80–82/$CHCl_3$	62	59.81(59.83)	4.43(4.44)	12.30(12.31)
4f	H		$-OCH_3$	$C_{20}H_{21}N_3O_4S$	399.46	234–236/$CHCl_3$	67	60.11(60.13)	5.30(5.32)	10.52(10.54)
4g	H		$-OCH_3$	$C_{18}H_{17}N_3O_3S$	355.41	182–183/$CHCl_3$	58	60.83(60.85)	4.82(4.84)	11.80(11.82)
4h	H		$-OCH_3$	$C_{18}H_{17}N_3O_3S$	355.41	191–193/$CHCl_3$	72	60.85(60.83)	4.82(4.84)	11.80(11.82)
4i	H		$-OCH_3$	$C_{17}H_{14}N_4O_3S$	354.38	100–101/$CHCl_3$	82	57.60(57.62)	3.98(4.00)	15.80(15.81)

(*continued on next page*)

Table 1 (*continued*)

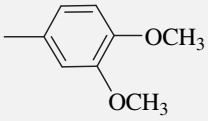
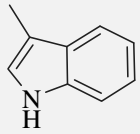
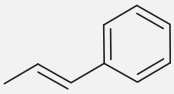
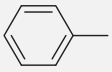
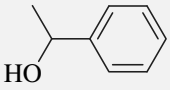
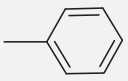
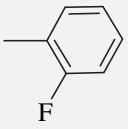
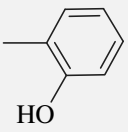
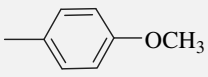
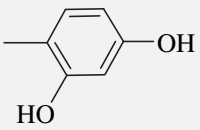
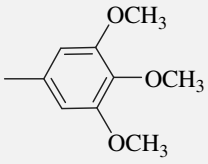
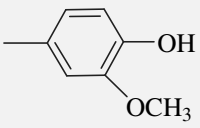
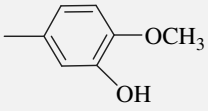
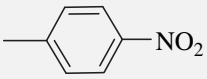
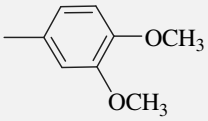
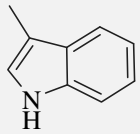
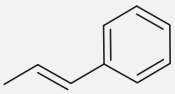
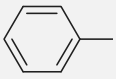
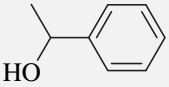
Compd.	R	R_1	R_2	Molecular formula	M.W.[c]	M.p.(°C)[b]/Crystallization solvent	Yield (%)	% Analysis of C, H, N found (Calc.)[a]		
								C	H	N
4j	H		$-OCH_3$	$C_{19}H_{19}N_3O_3S$	369.44	205–208/$CHCl_3$	62	61.75(61.77)	5.15(5.18)	11.35(11.37)
4k	H		$-OCH_3$	$C_{19}H_{16}N_4OS$	348.42	207–208/C_2H_5OH	72	68.46(68.45)	4.52(4.53)	12.58(12.60)
4l	H		$-OCH_3$	$C_{19}H_{17}N_3OS$	335.42	192–194/$CHCl_3$	86	68.00(68.03)	5.10(5.11)	12.51(12.53)
4m			$-OCH_3$	$C_{24}H_{21}N_3O_2S$	415.51	207–209/$CHCl_3$	71	69.40(69.39)	5.06(5.07)	10.11(10.13)
4n	H		–Br	$C_{16}H_{12}BrN_3S$	358.26	239–240/$CHCl_3$	67	53.60(53.64)	3.36(3.38)	11.73(11.73)
4o	H		–Br	$C_{16}H_{11}BrFN_3S$	376.25	192–195/C_2H_5OH	75	51.10(51.08)	2.95(2.97)	11.16(11.17)
4p	H		–Br	$C_{16}H_{12}BrN_3OS$	374.25	223–225/$CHCl_3$	71	51.35(51.37)	3.22(3.25)	11.23(11.26)
4q	H		–Br	$C_{17}H_{14}BrN_3OS$	388.28	212–215/$CHCl_3$	72	52.58(52.59)	3.63(3.65)	10.82(10.84)
4r	H		–Br	$C_{16}H_{12}BrN_3O_2S$	390.25	230–231/$CHCl_3$	67	49.34(49.37)	3.09(3.10)	10.77(10.77)
4s	H		–Br	$C_{19}H_{18}BrN_3O_3S$	448.33	151–153/$CHCl_3$	71	50.92(50.90)	4.04(4.05)	9.39(9.37)
4t	H		–Br	$C_{17}H_{14}BrN_3O_2S$	404.28	185–187/$CHCl_3$	64	50.49(50.50)	3.45(3.49)	10.35(10.39)

Table 1 (*continued*)

Compd.	R	R_1	R_2	Molecular formula	M.W.[c]	M.p.(°C)[b]/Crystallization solvent	Yield (%)	% Analysis of C, H, N found (Calc.)[a]		
								C	H	N
4u	H		–Br	$C_{17}H_{14}BrN_3O_2S$	404.28	198–200/$CHCl_3$	68	50.48(50.50)	3.46(3.49)	10.38(10.39)
4v	H		–Br	$C_{16}H_{11}BrN_4O_2S$	403.25	159–161/$CHCl_3$	80	47.65(47.66)	2.74(2.75)	13.87(13.89)
4w	H		–Br	$C_{18}H_{16}BrN_3O_2S$	418.31	205–208/$CHCl_3$	63	51.65(51.68)	3.85(3.86)	10.03(10.05)
4x	H		–Br	$C_{18}H_{13}BrN_4S$	397.29	190–192/C_2H_5OH	69	56.54(56.55)	3.15(3.16)	11.00(10.99)
4y	H		–Br	$C_{18}H_{14}BrN_3S$	384.29	217–219/$CHCl_3$	78	56.25(56.26)	3.66(3.67)	10.94(10.93)
4z			–Br	$C_{23}H_{18}BrN_3OS$	464.38	206–208/$CHCl_3$	78	59.47(59.49)	3.90(3.91)	9.07(9.05)
5a	–	–	$-OCH_3$	$C_{16}H_{19}N_3OS$	301.41	152–153/$CHCl_3$	72	63.75(63.76)	6.33(6.35)	13.92(13.94)
5b	–	–	–Br	$C_{15}H_{16}BrN_3S$	350.28	187–188/$CHCl_3$	69	51.41(51.43)	4.61(4.60)	11.98(12.00)
6a	–	–	$-OCH_3$	$C_{15}H_{17}N_3OS$	287.38	208–209/$CHCl_3$	72	62.67(62.69)	5.97(5.96)	14.61(14.62)
6b	–	–	–Br	$C_{14}H_{14}BrN_3S$	336.25	213–215/$CHCl_3$	72	50.00(50.01)	4.18(4.20)	12.49(12.50)

[a] Elemental analyses for C, H and N were within ±0.03% of the theoretical value.
[b] Melting point of the compound at their decomposition.
[c] Molecular weight of the compound.

DMSO. Compounds 1-benzylidene-2-(4-(4-bromophenyl)thiazol-2-yl)hydrazine (**4n**), 2-(2-(4-(4-bromophenyl)thiazol-2-yl)hydrazono)-1,2-diphenylethanol (**4z**), 1-cyclohexylidene-2-(4-(4-methoxyphenyl) thiazol-2-yl) hydrazine (**5a**) and 1-(4-(4-bromophenyl) thiazol-2-yl)-2-cyclohexylidenehydrazine (**5b**) exhibited good activity against all the four fungal strains viz. *C. albicans*, *C. neoformans*, *A. flavus* and *C. tropicum* taken in these studies. Compounds 1-cyclopentylidene-2-(4-(4-methoxyphenyl) thiazol-2-yl) hydrazine (**6a**) and 1-(4-(4-bromophenyl) thiazol-2-yl)-2-cyclopentylidenehydrazine (**6b**) showed excellent activity against *C. albicans*, *C. neoformans* and *A. flavus* but no activity against *C. tropicum*. Compound 1-benzylidene-2-(4-(4-methoxyphenyl) thiazol-2-yl) hydrazine (**4a**) showed good activity against *C. albicans*, *C. neoformans*, and *A. flavus* but no activity against *C. tropicum*. Compound 2-({2-[4-(4-methoxyphenyl)-1,3-thiazol-2-yl] hydrazinylidene} methyl) phenol (**4c**) showed moderate activity against *C. albicans*, *C. neoformans*, and *C. tropicum* but no activity against *A. flavus*. Compound 2-(2-(4-(4-methoxyphenyl) thiazol-2-yl) hydrazono)-1,2-diphenylethanol (**4m**) showed moderate activity only against *A. flavus* while N-((1H-indol-3-yl) methylene)-4-(4-bromophenyl) thiazol-2-amine (**4x**) showed moderate activity against *C. neoformans*.

5. Conclusion

The novel Schiff bases containing 2,4-disubstituted thiazole ring were synthesized by cyclization of Schiff bases of thiosemicarbazone by treating with substituted phenacyl bromide and were studied for their antimicrobial activity. The results of anti-bacterial screening reveal that among all the compounds screened, compounds **4d**, **4e**, **4f**, **4j**, **4o** and **4p** showed moderate anti-bacterial activity while compound **4m** and **4z** displayed good anti-bacterial activity when compared with ciprofloxacin used as standard. Particularly, compound **4z** which is carrying imino-1,2-diphenylethanol substituent appears to exhibit the highest anti-bacterial activity (zone of inhibition up to 17–20 mm at concentration of 12.5 μg/ml) against *S. aureus* and *V. cholerae*. Other compounds containing either electron withdrawing substituent on phenyl ring (flouro, bromo, nitro) or electron donating group (methoxy) do not significantly increase the anti-bacterial activity.

The results of anti-fungal screening showed that compounds **4a**, **4c**, **4m**, **4n**, **4z**, **5a**, **5b**, **6a** and **6b** have moderate to excellent anti-fungal activity. Compound **4m** showed moderate activity against *A. flavus* while **4x** showed moderate activity against *C. neoformans*.

Table 2
Anti-bacterial and anti-fungal activities of compounds **4a–z**, **5a–b** and **6a–b**.

Compd.	Microbial species									
	Bacteria						Fungi			
	E. coli	*S. aureus*	*S. typhi*	*P. aeruginosa*	*K. pneumoniae*	*V. cholerae*	*C. albicans*	*C. neoformans*	*A. flavus*	*C. tropicum*
4a	–	–	–	–	–	–	13–16(12.5)	14–17(12.5)	13–16(12.5)	–
4b	–	–	–	–	–	–	–	–	–	–
4c	–	–	–	–	–	–	11–14(25)	12–15(25)	–	13–16(25)
4d	–	<10(50)	–	–	–	–	–	–	–	–
4e	–	–	–	<10(50)	–	–	–	–	–	–
4f	–	<10(50)	–	–	–	–	–	–	–	–
4g	–	–	–	–	–	–	–	–	–	–
4h	–	–	–	–	–	–	–	–	–	–
4i	–	–	–	–	–	–	–	–	–	–
4j	–	<10(50)	–	–	–	–	–	–	–	–
4k	–	–	–	–	–	–	–	–	–	–
4l	–	–	–	–	–	–	–	–	–	–
4m	–	–	–	–	–	16–19(25)	–	–	13–16(25)	–
4n	–	–	–	–	–	–	15–18(12.5)	15–18(12.5)	16–19(12.5)	14–17(25)
4o	–	–	–	–	<10(50)	–	–	–	–	–
4p	–	–	–	–	<10(50)	–	–	–	–	–
4q	–	–	–	–	–	–	–	–	–	–
4r	–	–	–	–	–	–	–	–	–	–
4s	–	–	–	–	–	–	–	–	–	–
4t	–	–	–	–	–	–	–	–	–	–
4u	–	–	–	–	–	–	–	–	–	–
4v	–	–	–	–	–	–				
4w	–	–	–	–	–	–	–	–	–	–
4x	–	–	–	–	–	–	–	13–16(25)	–	–
4y	–	–	–	–	–	–	–	–	–	–
4z	–	17–20(12.5)	–	–	–	17–20(12.5)	19–22(6.25)	21–24(6.25)	17–20(6.25)	16–19(6.25)
5a	–	–	–	–	–	–	16–18(12.5)	17–19(12.5)	18–20(12.5)	16–18(12.5)
5b	–	–	–	–	–	–	17–19(12.5)	16–18(12.5)	16–18(12.5)	14–17(12.5)
6a		–	–		–	–	17–20(12.5)	18–20(12.5)	18–20(12.5)	–
6b	–	–	–	–	–	–	16–18(12.5)	14–17(12.5)	15–18(12.5)	–
Ciprofloxacin	24–29(6.25)	25–28(6.25)	18–22(6.25)	24–27(3.12)	23–26(6.25)	19–22(6.25)	–	–	–	–
Fluconazole	–	–	–	–	–	–	22–25(6.25)	22–25(6.25)	19–23(6.25)	20–24(6.25)
DMSO	–	–	–	–	–	–	–	–	–	–

MIC values are given in brackets. MIC (μg/ml) = Minimum inhibitory concentration, i.e. the lowest concentration of drug which completely inhibit bacterial/fungal growth. Ciprofloxacin and fluconazole were used as standard for anti-bacterial and anti-fungal activity, respectively. Diameter of inhibition zone was measured in mm.

Compound **4c** showed moderate activity against three fungal strains viz. *C. albicans*, *C. neoformans* and *C. tropicum*. Compounds **4n**, **4z**, **5a** and **5b** showed excellent anti-fungal activity against all the four fungal strains viz. *C. albicans*, *C. neoformans*, *A. flavus* and *C. tropicum* and were comparable to that of standard drug fluconazole. Compounds **4a**, **6a** and **6b** showed good activity against *C. albicans*, *C. neoformans* and *A. flavus* but no activity against *C. tropicum*.

Structure activity relationship (SAR) studies from the results of the antimicrobial activity revealed that unsubstituted phenyl group or cyclohexanimine/cyclopentaniminyl substituent at C-8 position in the target compounds showed excellent anti-fungal activity. From these results, it may be concluded that introduction of cyclic ring in the Schiff bases containing 2,4-disubstituted thiazole at C-8 position may contribute for enhanced anti-fungal effect. Efforts are on to prepare compounds with this type of functionality and also to screen the compounds against some plant pathogenic fungal strains for their broad spectrum of activities.

6. Experimental protocols

All the chemicals and solvents used for this work were obtained from Merck (Germany), S.D. Fine (Mumbai), Hi-media (Mumbai) and Aldrich chemical company (U.S.A.). The chemicals purchased were of analytical reagent grade or were purified by standard methods prior to use [48]. Melting points of the synthesized compounds were determined in open-glass capillaries on Stuart-SMP10 melting point apparatus and are uncorrected. IR absorption spectra were recorded on Shimadzu FTIR-8400s using KBr pellets in the range of 4000–400 cm^{-1}, ^1H NMR and ^{13}C NMR spectra were recorded on the JEOL AL300 FTNMR spectrometer operating at 300 MHz and TMS (tetramethylsilane) as an internal standard. The ^1H NMR and ^{13}C NMR chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si). The splitting patterns are designated as follows; s, singlet; d, doublet; m, multiplet. Mass spectra were recorded on VG-AUTOSPEC spectrometer. IR, ^1H NMR, ^{13}C NMR and Mass spectra were consistent with the assigned structures. Elemental analyses (C, H, N) were done on a CHN rapid analyzer. All the new compounds gave C, H and N analysis within ±0.03% of the theoretical values. Purity of the compounds was checked by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} precoated sheets in chloroform/methanol mixture and spots were developed using iodine vapours/ultraviolet light as visualizing agent.

6.1. General procedure for the synthesis of Schiff bases of thiosemicarbazone (*1a–m/2a/3a*)

A mixture of equimolar quantities of substituted aldehyde/ketone (0.01 mol) in ethanol/methanol (20 ml) and thiosemicarbazide (0.01 mol) in ethanol (20 ml) was refluxed on a water bath for 4–6 h in the presence of few drops of glacial acetic acid as catalyst. The progress of reaction was monitored by TLC at appropriate time interval. After completion of reaction, the solution was cooled, solid thus separated was washed with ice-cold water (3 × 50 ml) and dried. Finally, the product thus obtained was recrystallized from ethanol.

*6.2. General procedure for the synthesis of arylidene-2-(4-(4-methoxy/bromophenyl) thiazol-2-yl) hydrazines (**4a–z**)*

A mixture of equimolar quantities of thiosemicarbazone (**1a–m**) (0.01 mol) in ethanol/methanol (20 ml) and substituted phenacyl bromide (0.01 mol) in methanol was refluxed on a water bath for 4–10 h. The progress of reaction was monitored by TLC at appropriate time interval. The excess of solvent was distilled off and the solid that separated was collected by filtration, suspended in water and neutralized with $NaHCO_3/K_2CO_3$ to get the desired product (**4a–z**). The product was recrystallized from ethanol/chloroform.

*6.3. General procedure for the synthesis of 1-(4-(4-methoxy/bromophenyl) thiazol-2-yl)-2-cyclohexylidene/cyclopentylidene hydrazines (**5a–b**/**6a–b**)*

A mixture of equimolar quantities of thiosemicarbazone (**2a**/**3a**) (0.01 mol) in ethanol/methanol (20 ml) and substituted phenacyl bromide (0.01 mol) in methanol was refluxed on a water bath for 4–10 h. The progress of reaction was monitored by TLC at appropriate time interval. The solution was poured on to the crushed ice and kept aside. The precipitate thus separated was collected by filtration, suspended in water and neutralized with $NaHCO_3/K_2CO_3$ to get the desired product (**5a–b**/**6a–b**). The product was recrystallized from chloroform.

6.3.1. Characterization data of compounds are given below

*6.3.1.1. 1-Benzylidene-2-(4-(4-methoxyphenyl) thiazol-2-yl) hydrazine (**4a**).* IR (KBr, ν_{max} cm^{-1}): 3348, 3271 (–NH), 1621 (C═N azomethine), 837 and 763; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.8 (s, 3H, OCH_3), 6.9 (d, 2H, *p*-anisyl), 7.1–7.6 (m, 5H, phenyl H), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.0 (d, 2H, *p*-anisyl), 8.2 (s, 1H, –N═CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 58.5 (OCH_3), 102.5 (thiazole-*C*-5), 115.7, 126.2, 129.0 (Ar–*C*H), 141.1 (H*C*═N), 148.0 (thiazole-*C*-4), 167.1 (Ar–*C*–OCH_3), 170 (thiazole-*C*-2); MS (*m*/*z*, %): 310 (M + 1, 100).

*6.3.1.2. 1-(2-Fluorobenzylidene)-2-(4-(4-methoxyphenyl) thiazol-2-yl) hydrazine (**4b**).* IR (KBr, ν_{max} cm^{-1}): 3267 (–NH), 1604 (C═N azomethine), 835 and 761; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.8 (s, 3H, OCH_3), 7.0 (d, 2H, *p*-anisyl), 7.6 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.4 (d, 2H, *p*-anisyl), 10.0 (s, 1H, –N═CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 55.5 (OCH_3), 76.5, 99.3 (thiazole–*C*-5), 114.9 (Aryl–*C*H), 116.3, 124.5, 127.3 (Aryl–*C*H), 133.1 (H*C*═N), 161.1(thiazole–*C*-2); MS (*m*/*z*, %): 328 (M + 1, 100).

*6.3.1.3. 2-({2-[4-(4-Methoxyphenyl)-1, 3-thiazol-2-yl]hydrazinylidene}-methyl)phenol (**4c**).* IR (KBr, ν_{max} cm^{-1}): 3541 (–OH, br.), 1618 (C═N azomethine), 1504 (aromatic C═C), 833 and 754; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.6 (s, 3H, OCH_3), 7.3 (d, 2H, *p*-anisyl), 7.6 (s, 1H, –N═CH), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.0 (d, 2H, *p*-anisyl), 9.7 (s, 1H, phenolic –OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 55.1 (OCH_3), 76.5, 114.4 (Aryl–*C*H), 131.4 (Aryl–*C*), 132.8 (H*C*═N), 149.8 (thiazole–*C*-4), 153.3, 153.6, 159.7, 160.7 (Ar–*C*–OCH_3), 171.4 (thiazole–*C*-2); MS (*m*/*z*, %): 326 (M + 1, 100), 327 (M + 2, 15).

*6.3.1.4. 1-(4-Methoxybenzylidene)-2-(4-(4-methoxyphenyl) thiazol-2-yl) hydrazine (**4d**).* IR (KBr, ν_{max} cm^{-1}): 3298 (–NH), 1600 (C═N azomethine), 1562 (aromatic C═C), 916 and 829; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.8 (s, 3H, OCH_3), 6.8 (d, 2H, *p*-anisyl), 7.1–7.5 (m, 4H, Ar–H), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 7.9 (d, 2H, *p*-anisyl), 8.2 (s, 1H, –N═CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 55.5 (OCH_3), 100.2 (thiazole–*C*-5), 114.5 (Aryl–*C*H), 128.4 (Aryl–*C*H), 142.8 (H*C*═N), 148.8 (thiazole–*C*-4), 160.8 (Ar–*C*–OCH_3), 172.0 (thiazole–*C*-2); MS (*m*/*z*, %): 340 (M + 1, 100), 341 (M + 2, 30), 342 (M + 3, 10).

*6.3.1.5. 4-({2-[4-(4-Methoxyphenyl)-1,3-thiazol-2-yl]hydrazinylidene}-methyl)benzene-1,3-diol (**4e**).* IR (KBr, ν_{max} cm^{-1}): 3414 (–OH, br.), 3121 (–NH), 1599 (C═N azomethine), 1506 (aromatic C═C) and 833; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.9 (s, 3H, OCH_3), 7.2 (d, 2H, *p*-anisyl), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 7.9 (d, 2H, *p*-anisyl), 8.7 (s, 1H, –N═CH), 11.52 (s, 1H, *o*-OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 56.0 (OCH_3), 105.4 (thiazole–*C*-5), 111.0–132.2 (Ar–*C*H), 143.0 (H*C*═N), 147.8 (thiazole–*C*-4), 159.9 (Ar–*C*–OH), 161.8 (Ar–*C*–OCH_3), 172.5 (thiazole–*C*-2); MS (*m*/*z*, %): 342 (M + 1, 100).

*6.3.1.6. 1-(3,4,5-Trimethoxybenzylidene)-2-(4-(4-methoxyphenyl)thiazol-2-yl) hydrazine (**4f**).* IR (KBr, ν_{max} cm^{-1}): 3324 (–NH), 1620 (C═N azomethine), 1508 (aromatic C═C), 831 and 748; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.8 (s, 3H, OCH_3), 6.5–7.3 (m, 4H, ArH), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 7.9 (d, 2H, *p*-anisyl), 8.4 (s, 1H, –N═CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 56.5 (OCH_3), 101.3 (thiazole–*C*-5), 115.5, 129.0 (Ar–*C*H), 143.1 (H*C*═N), 148.7 (thiazole–*C*-4), 151, 155.4, 160.0 (Ar–*C*–OCH_3), 172.2 (thiazole–*C*-2); MS (*m*/*z*, %): 400 (M + 1, 100).

*6.3.1.7. 2-Methoxy-4-({2-[4-(4-methoxyphenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl)-phenol (**4g**).* IR (KBr, ν_{max} cm^{-1}): 3298 (–NH–), 1600 (C═N azomethine), 1562 (aromatic C═C), 829 and 704; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.8 (s, 3H, OCH_3), 6.8–7.3 (d, 2H, *p*-anisyl), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 7.9 (d, 2H, *p*-anisyl), 8.6 (s, 1H, –N═CH), 9.8 (s, 1H, *p*-OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 55.9 (OCH_3), 100.3 (thiazole–*C*-5), 113.5, 128.6 (Ar–*C*H), 143.3 (H*C*═N), 148.6 (thiazole–*C*-4), 151.7, 159.8 (Ar–*C*–OCH_3), 171.1 (thiazole–*C*-2); MS (*m*/*z*, %): 356 (M + 1, 100).

*6.3.1.8. 2-Methoxy-5-({2-[4-(4-methoxyphenyl)-1,3-thiazol-2-yl]hydrazinylidene}methyl) phenol (**4h**).* IR (KBr, ν_{max} cm^{-1}): 3419 (–OH), 1624 (C═N azomethine), 1510 (aromatic C═C) and 619; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.7 (s, 3H, OCH_3), 7.1 (d, 2H, *p*-anisyl), 7.3 (d, 2H, *p*-anisyl), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.5 (s, 1H, –N═CH), 11.0 (s, 1H, *m*-OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 56.0 (OCH_3), 106.0 (thiazole–*C*-5), 114.6, 123.2, 128.5 (Ar–*C*H), 141.3 (H*C*═N), 147.8 (thiazole–*C*-4), 156.5, 159.7 (Ar–C–OCH_3), 170.1 (thiazole–*C*-2); MS (*m*/*z*, %): 356 (M + 1, 100), 357 (M + 2, 25), 358 (M + 3, 15).

*6.3.1.9. 1-(4-Nitrobenzylidene)-2-(4-(4-methoxyphenyl)thiazol-2-yl)-hydrazine (**4i**).* IR (KBr, ν_{max} cm^{-1}): 3124 (–NH), 1622 (C═N, azomethine), 1516 (aromatic C═C), 927, 849 and 787; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.7 (s, 3H, OCH_3), 7.1 (d, 2H, *p*-anisyl), 7.3 (d, 2H, *p*-anisyl), 7.4–7.6 (m, 4H, Ar–H), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.4 (s, 1H, –N═CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 58.5 (OCH_3), 105.3 (thiazole–*C*-5), 115.7, 126.2, 129.0 (Ar–*C*H), 141.1 (H*C*═N), 148.0 (thiazole–*C*-4), 167.1 (thiazole–*C*-2); MS (*m*/*z*, %): 355 (M + 1, 100).

*6.3.1.10. 1-(3,4-Dimethoxybenzylidene)-2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazine (**4j**).* IR (KBr, ν_{max} cm^{-1}): 3319 (–NH), 1620 (C═N azomethine), 1508 (aromatic C═C), 831 and 748; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.7 (s, 3H, OCH_3), 7.1 (d, 2H, *p*-anisyl), 7.3 (d, 2H, *p*-anisyl), 7.5–7.6 (m, 3H, Ar–H), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 7.9 (d, 2H, *p*-anisyl), 8.2 (s, 1H, –N═CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 56.5 (OCH_3), 101.3 (thiazole–*C*-5), 115.5, 129.0 (Ar–*C*H), 143.1 (H*C*═N), 148.7 (thiazole–*C*-4), 155.4, 160.0 (Ar–*C*–OCH_3), 172.2 (thiazole–*C*-2); MS (*m*/*z*, %): 370 (M + 1, 100), 371 (M + 2, 30).

*6.3.1.11. 1-((1H-indol-3-yl)methylene)-2-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazine (**4k**).* IR (KBr, ν_{max} cm^{-1}): 3157 (–NH), 1607

(C=N azomethine), 1549 (aromatic C=C), 939 and 743; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.1 (d, 2H, *p*-anisyl), 7.3 (d, 2H, *p*-anisyl), 7.9 (s, 1H, –N=CH), 8.4 (1H, s, indole–H), 9.6 (s, 1H, thiazole H), 10.5 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 43.3, 51.2, 56.8 (OCH_3), 109.4 (thiazole–*C*-5), 116.4, 128.5 (Ar–*C*H), 145.1 (H*C*=N), 148.7 (thiazole–*C*-4), 155.4, 161.0 (Ar–*C*–OCH_3), 172.1 (thiazole–*C*-2); MS (*m*/*z*, %): 349 (M + 1, 100).

*6.3.1.12. 1-(4-(4-Methoxyphenyl) thiazol-2-yl)-2-(3-phenylallylidene) hydrazine (**4l**).* IR (KBr, ν_{max} cm^{-1}): 3202 (–NH), 1620 (C=N azomethine), 1562 (aromatic C=C), 973, 834 and 754; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.8 (s, 3H, OCH_3), 7.1–7.6 (m, 7H, aromatic), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 7.9 (d, 2H, *p*-anisyl), 8.7 (s, 1H, –N=CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 55.2 (OCH_3), 114.1 (thiazole–*C*-5), 124.8, 126.7, 127.3, 127.8, 128.6, 136.1 (Ar–*C*H), 137.1 (H*C*=N), 145.0 (thiazole–*C*-4), 159.6 (Ar–*C*–OCH_3), 168.0 (thiazole–*C*-2); MS (*m*/*z*, %): 335 (M^+, 20).

*6.3.1.13. 2-(2-(4-(4-Methoxyphenyl) thiazol-2-yl) hydrazono)-1,2-diphenylethanol (**4m**).* IR (KBr, ν_{max} cm^{-1}): 3122 (–NH), 1608 (C=N azomethine), 1583 (aromatic C=C) and 750; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.8 (s, 3H, OCH_3), 6.9 (d, 2H, *p*-anisyl), 7.3 (d, 2H, *p*-anisyl), 7.4–7.6 (m, 10H, phenyl), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 11.7 (s, 1H, –N=CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 56.2 (OCH_3), 114.2 (thiazole–*C*-5), 125.0–129.3 (Ar–*C*H), 135.5 (Ar–*C*), 139.8 (H*C*=N), 150.8 (thiazole–*C*-4), 159.3 (Ar–*C*–OCH_3), 169.1 (thiazole–*C*-2); MS (*m*/*z*, %): 416 (M + 1, 100), 417 (M + 2, 35).

*6.3.1.14. 1-Benzylidene-2-(4-(4-bromophenyl)thiazol-2-yl)hydrazine-(**4n**).* IR (KBr, ν_{max} cm^{-1}): 3332 (–NH), 1627 (C=N azomethine), 834 and 761; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.1–7.3 (m, 5H, phenyl), 7.4 (d, 2H, *p*-bromophenyl), 7.5 (s, 1H, –N=CH), 7.7 (d, 2H, *p*-bromophenyl), 7.8 (s, 1H, thiazole H), 8.0 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 38.6, 39.0, 40.3, 126.5, 128.8, 129.7 (Ar–*C*H), 130.7, 131.8, 132.4 (Ar–*C*), 134.7 (H*C*=N), 165.3 (thiazole–*C*-4), 187.4 (thiazole–*C*-2); MS (*m*/*z*, %): 359 (M + 1, 100).

*6.3.1.15. 1-(2-Fluorobenzylidene)-2-(4-(4-bromophenyl)thiazol-2-yl)-hydrazine (**4o**).* IR (KBr, ν_{max} cm^{-1}): 3069 (–NH), 1620 (C=N azomethine), 1581 (aromatic C=C), 918, 821 and 752; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.0–7.2 (m, 4H, *o*-fluorophenyl), 7.3 (d, 2H, *p*-bromophenyl), 7.6 (d, 2H, *p*-bromophenyl), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.5 (s, 1H, –N=CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 100.3 (thiazole–*C*-5), 116.3, 118, 124.6, 129.3 (Ar–*C*H), 133.1 (H*C*=N), 143 (thiazole–*C*-4), 148, 161.1 (thiazole–*C*-2); MS (*m*/*z*, %): 378 (M + 1, 100), 380 (M + 3).

*6.3.1.16. 2-({2-[4-(4-Bromophenyl)-1,3-thiazol-2-yl]hydrazinylidene}-methyl)phenol (**4p**).* IR (KBr, ν_{max} cm^{-1}): 3402 (–OH, br.), 3149 (–NH), 1602 (C=N azomethine), 1562 (aromatic C=C) and 821; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.8 (d, 2H, *p*-bromophenyl), 7.2 (s, 1H, –N=CH), 7.4 (d, 2H, *p*-bromophenyl), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 9.5 (s, 1H, phenolic –OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 100.0 (thiazole–*C*-5), 128.4 (Ar–*C*H), 131.4 (Ar–*C*), 143.8 (H*C*=N), 149.8 (thiazole–*C*-4), 153.6, 159.7 (Ar–*C*–OH), 160.2 (thiazole–*C*-2); MS (*m*/*z*, %): 375 (M + 1, 100).

*6.3.1.17. 1-(4-Methoxybenzylidene)-2-(4-(4-bromophenyl) thiazol-2-yl) hydrazine (**4q**).* IR (KBr, ν_{max} cm^{-1}): 3301 (–NH), 1620 (C=N azomethine), 1554 (aromatic C=C), 916, 830; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.8 (s, 3H, OCH_3), 6.7–7.4 (m, 4H, Ar–H), 7.6 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.7 (s, 1H, –N=CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 55.3 (OCH_3), 76.5, 114.2 (Aryl–*C*H), 127.5, 131.9 (Aryl–*C*), 143 (H*C*=N); MS (*m*/*z*, %): 389 (M + 1, 100), 390 (M + 2, 20).

*6.3.1.18. 4-({2-[4-(4-Bromophenyl)-1,3-thiazol-2-yl]hydrazinylidene}-methyl)benzene-1,3-diol (**4r**).* IR (KBr, ν_{max} cm^{-1}): 3414 (–OH, br.), 3298 (–NH), 1600 (C=N azomethine), 1562 (aromatic C=C), 829 and 704; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.2–8.0 (m, 7H, Ar–H), 8.1 (s, 1H, thiazole H), 8.2 (s, 1H, NH), 9.9 (s, 1H, –N=CH), 12.1 (s, 1H, *o*-OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 104.0 (thiazole–*C*-5), 125.5, 128.5 (Ar–*C*H), 132.2 (Ar–*C*), 143.0 (H*C*=N), 147.8 (thiazole–*C*-4), 155.3, 160.9 (thiazole–*C*-2); MS (*m*/*z*, %): 391 (M + 1, 100).

*6.3.1.19. 1-(3,4,5-Trimethoxybenzylidene)-2-(4-(4-bromophenyl)thiazol-2-yl)hydrazine (**4s**).* IR (KBr, ν_{max} cm^{-1}): 3326 (–NH), 1622 (C=N azomethine), 1517 (aromatic C=C), 835 and 747; ^{1}H NMR ($CDCl_3$) δ (ppm): 3.8–3.9 (m, 9H, OCH_3), 6.9–7.5 (m, 6H, Ar–H), 7.7 (s, 1H, thiazole H), 8.0 (s, 1H, NH), 8.4 (s, 1H, –N=CH); ^{13}C NMR ($CDCl_3$) δ (ppm): 56.0 (OCH_3), 104.3 (thiazole–*C*-5), 118.5, 123.0, 129.0 (Ar–*C*H), 131.0 (Ar–*C*), 143.4 (HC=N), 148.7 (thiazole–*C*-4), 160.0 (Ar–*C*–OCH_3), 170.0 (thiazole–*C*-2); MS (*m*/*z*, %): 448 (M^+, 90), 449 (M + 1, 25), 450 (M + 2).

*6.3.1.20. 4-({2-[4-(4-Bromophenyl)-1,3-thiazol-2-yl]hydrazinylidene}-methyl)-2-methoxyphenol (**4t**).* IR (KBr, ν_{max} cm^{-1}): 3298 (–NH–), 1600 (C=N azomethine), 1562 (aromatic C=C), 829 and 704; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.8 (s, 3H, OCH_3), 6.8–7.3 (m, 7H, Ar–H), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.9 (s, 1H, –N=CH), 11.2 (s, 1H, *p*-OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 56.4 (OCH_3), 102.3 (thiazole–*C*-5), 116.5, 127.6 (Ar–*C*H), 144.3 (HC=N), 148.6 (thiazole–*C*-4), 151.7, 158.7 (Ar–*C*–OCH_3), 169.7 (thiazole–*C*-2); MS (*m*/*z*, %): 404 (M^+, 100).

*6.3.1.21. 5-({2-[4-(4-Bromophenyl)-1,3-thiazol-2-yl]hydrazinylidene}-methyl)-2-methoxyphenol (**4u**).* IR (KBr, ν_{max} cm^{-1}) 3298 (–NH), 1600 (C=N azomethine), 1562 (aromatic C=C), 829 and 704; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.8 (s, 3H, OCH_3), 6.8 (d, 2H, *p*-bromophenyl), 7.4 (d, 2H, *p*-bromophenyl), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.0 (s, 1H, –N=CH), 10.3 (s, 1H, *m*-OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 56.2 (OCH_3), 100.3 (thiazole–*C*-5), 113.5, 128.6 (Ar–*C*H), 143.4 (HC=N), 148.4 (thiazole–*C*-4), 151.3, 159.8 (Ar–*C*–OCH_3), 170.1 (thiazole–*C*-2); MS (*m*/*z*, %): 404 (M^+, 20).

*6.3.1.22. 1-(4-Nitrobenzylidene)-2-(4-(4-bromophenyl)thiazol-2-yl)-hydrazine (**4v**).* IR (KBr, ν_{max} cm^{-1}): 3292 (–NH), 1564 (C=N azomethine), 1506 (aromatic C=C), 1120, 839 and 734; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.9 (d, 2H, *p*-bromophenyl), 7.3 (d, 2H, *p*-bromophenyl), 7.4–7.6 (m, 4H, aromatic), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.6 (s, 1H, –N=CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 108.2 (thiazole–*C*-5), 115.7, 126.2, 129.0 (Ar–*C*H), 141.1 (HC=N), 148.0 (thiazole–*C*-4), 151.0 (Ar–*C*–NO_2), 167.1 (thiazole–*C*-2); MS (*m*/*z*, %): 404 (M + 1, 100).

*6.3.1.23. 1-(3, 4-Dimethoxybenzylidene)-2-(4-(4-bromophenyl) thiazol-2-yl) hydrazine (**4w**).* IR (KBr, ν_{max} cm^{-1}): 3217 (–NH), 1621 (C=N azomethine), 1506 (aromatic C=C), 833 and 750; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.7 (s, 3H, OCH_3), 7.1 (d, 2H, *p*-bromophenyl), 7.4 (d, 2H, *p*-bromophenyl), 7.5–7.6 (m, 3H, Ar–H), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.5 (s, 1H, –N=CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 56.5 (OCH_3), 101.3 (thiazole–*C*-5), 115.5, 129.0 (Ar–*C*H), 143.1 (HC=N), 148.7 (thiazole–*C*-4), 160.0 (Ar–*C*–OCH_3), 172.0 (thiazole–*C*-2); MS (*m*/*z*, %): 419 (M + 1, 100).

*6.3.1.24. 1-((1H-indol-3-yl)methylene)-2-(4-(4-bromophenyl)thiazol-2-yl)hydrazine (**4x**).* IR (KBr, ν_{max} cm^{-1}): 3135 (–NH), 1566 (C=N azomethine), 975, 830, 762 and 698; ^{1}H NMR (DMSO-d_6) δ (ppm): 6.9 (d, 2H, *p*-bromophenyl), 7.2–7.6 (m, 7H, Ar–H), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 8.2 (1H, d, indole–H), 8.8 (s, 1H, –N=CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 107.6 (thiazole–*C*-5),

117.0, 123.0, 131.0 (Ar–*C*H), 144.1 (H*C*=N), 148.9 (thiazole–*C*-4), 154.4, 161.0 (thiazole–*C*-2); MS (*m*/*z*, %): 391 (100%), 397 (M^+, 44).

*6.3.1.25. 1-(4-(4-Bromophenyl) thiazol-2-yl)-2-(3-phenylallylidene) hydrazine (**4y**).* IR (KBr, ν_{max} cm^{-1}): 3290 (–NH), 1566 (C=N azomethine), 970, 829, 721 and 687; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.8 (d, 2H, *p*-bromophenyl), 7.0–7.3 (m, 5H, phenyl), 7.4 (d, 2H, *p*-bromophenyl), 7.7 (s, 1H, thiazole H), 7.8 (s, 1H, NH), 9.2 (s, 1H, –N=CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 101.3 (thiazole–*C*-5), 115.5, 123.0, 126.1, 129.0 (Ar–*C*H), 139 (Ar–*C*), 139.1 (H*C*=N), 148.7 (thiazole–*C*-4), 155.4, 161.0 (thiazole–*C*-2); MS (*m*/*z*, %): 385 (M + 1, 100).

*6.3.1.26. 2-(2-(4-(4-Bromophenyl)thiazol-2-yl)hydrazono)-1,2-diphenylethanol (**4z**).* IR (KBr, ν_{max} cm^{-1}): 3130 (–NH), 1600 (C=N azomethine), 887, 744 and 696; ^{1}H NMR (DMSO-d_6) δ (ppm): 6.8 (d, 2H, *p*-bromophenyl), 7.4 (d, 2H, *p*-bromophenyl), 7.5–7.8 (m, 10H, aromatic), 7.9 (s, 1H, thiazole), 8.0 (s, 1H, NH), 11.87 (s, 1H, –N=CH); ^{13}C NMR (DMSO-d_6) δ (ppm): 100.7 (thiazole–*C*-5), 116.9, 126.9, 129.3 (Ar–*C*H), 141.8 (H*C*=N), 147.8 (thiazole–*C*-4), 169.1 (thiazole–*C*-2); MS (*m*/*z*, %): 464 (M^+, 80), 466 (M + 2, 100).

*6.3.1.27. 1-Cyclohexylidene-2-(4-(4-methoxyphenyl) thiazol-2-yl) hydrazine (**5a**).* IR (KBr, ν_{max} cm^{-1}) 3068 (–NH), 1612 (C=N azomethine), 1510 (aromatic C=C), 829 and 754; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.6–2.5 (m, 10H, cyclohexylidene–H), 3.8 (s, 3H, OCH_3), 7.8 (s, 1H, thiazole H), 8.4 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 24.8, 25.7, 27.8, 34.5, 38.9, 39.7, 40.3, 55.2 (OCH_3), 101.0 (thiazole–*C*-5), 114.2 (Aryl–*C*H), 127.2 (Aryl–*C*H), 159.5 (thiazole–*C*-4), 169.6 (thiazole–*C*-2); MS (*m*/*z*, %): 302 (M + 1, 100), 303 (M + 2).

*6.3.1.28. 1-(4-(4-Bromophenyl) thiazol-2-yl)-2-cyclohexylidenehydrazine (**5b**).* IR (KBr, ν_{max} cm^{-1}): 3265 (–NH), 1625 (C=N azomethine), 837 and 763; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.7–2.5 (m, 10H, cyclohexylidene–H), 7.8 (s, 1H, thiazole H), 8.0 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 25.0–38.8, 40.3, 111.0 (thiazole–*C*-5), 120.2, 127.9 (Ar–*C*H), 167.5 (thiazole–*C*-4), 173.0 (thiazole–*C*-2); MS (*m*/*z*, %): 351 (M + 1, 100).

*6.3.1.29. 1-Cyclopentylidene-2-(4-(4-methoxyphenyl) thiazol-2-yl) hydrazine (**6a**).* IR (KBr, ν_{max} cm^{-1}): 3213 (–NH), 1616 (C=N azomethine), 1008, 820 and 765; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.7–2.6 (m, 8H, cyclopentylidene–H), 3.5 (s, 3H, OCH_3), 7.2–7.5 (m, 4H, *p*-bromophenyl), 7.8 (s, 1H, thiazole H), 8.2 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 25.8, 28.1, 34.6, 39.1, 39.8, 40.8, 56.2 (OCH_3), 108.0 (thiazole–*C*-5), 115.3, 127.8 (Ar–*C*H), 161.0 (thiazole–*C*-4), 169.6 (thiazole–*C*-2); MS (*m*/*z*, %): 288 (M + 1, 100), 289 (M + 2, 20).

*6.3.1.30. 1-(4-(4-Bromophenyl) thiazol-2-yl)-2-cyclopentylidenehydrazine (**6b**).* IR (KBr, ν_{max} cm^{-1}): 3213 (–NH), 1610 (C=N azomethine), 1003, 819 and 763; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.6–2.5 (m, 8H, cyclopentylidene–H), 7.5 (d, 2H, *p*-bromophenyl), 7.7 (d, 2H, *p*-bromophenyl), 8.5 (s, 1H, NH), 10.6 (s, 1H, thiazole H); ^{13}C NMR (DMSO-d_6) δ (ppm): 24.0, 25.7, 27.8, 34.5, 38.9, 39.7, 40.3, 107.0 (thiazole–*C*-5), 114.2, 127.2 (Aryl–*C*H), 159.5 (thiazole–*C*-4), 170.5 (thiazole–*C*-2); MS (*m*/*z*, %): 336 (M^+, 70), 338 (M + 2, 100).

6.4. Experimental procedure for antimicrobial activity

6.4.1. Disc diffusion method

The antimicrobial activity of newly synthesized compounds was evaluated according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1997) using the agar disc diffusion method [46]. Briefly, a 24/48 h-old culture of selected bacteria/fungi was mixed with sterile physiological saline (0.85%) and the turbidity was adjusted to the standard inoculum of MacFarland scale 0.5 [$\sim 10^6$ colony forming units (CFU) per millilitre]. Petri plates containing 20 mL of Mueller Hinton Agar (MHA, Hi-Media) were used for all the bacteria tested. Fungi were cultured in Sabouraud's dextrose agar (SDA)/potato dextrose agar (PDA) (Hi-Media) and were purified by single spore isolation technique [49]. The inoculum was spread on the surface of the solidified media and Whatman no. 1 filter paper discs (6 mm in diameter) impregnated with the test compound (20 μl/disc) were placed on the plates. Ciprofloxacin (5 μg/disc, Hi-Media) was used as positive control for bacteria. Fluconazole (10 μg/disc, Hi-Media), was used as positive control for fungi. A paper disc impregnated with dimethylsulfoxide (DMSO) was used as negative control. Plates inoculated with the bacteria were incubated for 24 h at 37 °C and the fungal culture was incubated for 72 h at 25 °C. The inhibition zone diameters were measured in millimeters. All the tests were performed in triplicate and the average was taken as final reading.

6.4.2. Determination of MIC

Minimum inhibitory concentration (MIC) of any compound is defined as the lowest concentration which completely inhibits visible growth (turbidity on liquid media). MIC values were determined by testing performed according to the guidelines of NCCLS document M27-A [46]. Solutions of the test compounds, ciprofloxacin and fluconazole were prepared in DMSO at a concentration of 100 μg/ml. From this stock solution, serial dilutions of the compounds (50, 25… 3.12 μg/ml) were prepared to determine the MIC. All determinations were done in triplicates and the average was taken as final reading. The standard antibiotic, ciprofloxacin (100 μg/ml) for bacteria and fluconazole (100 μg/ml) for fungi were used as positive controls and 100 μl of DMSO used as a negative control. At the end of the incubation period, the MIC values were determined.

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Synthesis and in vitro activity of 2-thiazolylhydrazone derivatives compared with the activity of clotrimazole against clinical isolates of *Candida* spp.

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Abstract—In this paper, we report on the synthesis of a novel series of 2-thiazolylhydrazone derivatives and the influence of the substituents on the thiazole ring on antifungal activity. All synthesized compounds were screened for their in vitro activities against 22 clinical isolates of *Candida* spp., representing six different species, compared to clotrimazole as a reference compound. Some of the tested compounds were found to possess significant antifungal activity when compared to clotrimazole, in particular compound **14** which exhibited higher potency against most of the *Candida* spp. considered. The compounds that were most active as anti-*Candida* agents were also submitted to cytotoxic screening by the Trypan Blue dye exclusion assay and in general they were shown to induce low cytotoxic effects.

Although *Candida* spp. are present as commensal flora in 30–60% of healthy individuals,[1] they can become infective depending on predisposing conditions related to the host, such as systemic disease leading to immunosupression, and local conditions.[2] As a matter of fact, the pathogenicity of *Candida* spp. is affected by several virulence factors, such as the ability to adhere to epithelial and endothelial cells,[3] germination, extracellular proteinases and phospholipases, and phenotypic switching.[4]

Over the past few decades, the frequency of systematic fungal infections has progressively increased, due to the larger population of immunologically compromised hosts (AIDS, cancer, and transplant), thanks to advances in supportive therapy and an increasing elderly population.

In addition, the widespread use of antimicrobial agents for prophylaxis and treatment has led to less effective antibiotics, not only because many induced side effects but also due to the emergence of drug resistant microorganisms.[5–7]

Large-scale surveillance for fungal bloodstream infections has been performed worldwide by a number of organizations, including the European Confederation of Medical Mycology (ECMM),[8] the Centres for Disease Control and Prevention (CDC),[9] and the National Epidemiology of Mycoses Survey (NEMIS).[10] These studies have demonstrated an increasing incidence of drug-resistant fungal pathogens. As a matter of fact, a significant number of yeast species (*Candida glabrata*, *Candida krusei*, *Candida guilliermondii*, *Candida lusitaniae*) have been shown to exhibit primary resistance to amphotericin B, while *C. glabrata* and *C. krusei* have

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been found to be intrinsically less susceptible to triazoles than the same *Candida albicans*. It has also been found that *Candida dubliniensis* can rapidly develop in vitro stable resistance to fluconazole.[11,12] Furthermore, *C. albicans* intrinsically resistant strains have been observed as part of a commensal microflora or due to acquisition from the environment or from other individuals.[13]

In addition, despite advances in therapy thanks to the introduction of fluconazole and itraconazole, the development of new, more potent azoles, such as voriconazole and posaconazole, and the discovery of a new class of echinocandins,[14] mortality rates for invasive fungal infections today can reach 90% in immunocompromised patients especially.[15]

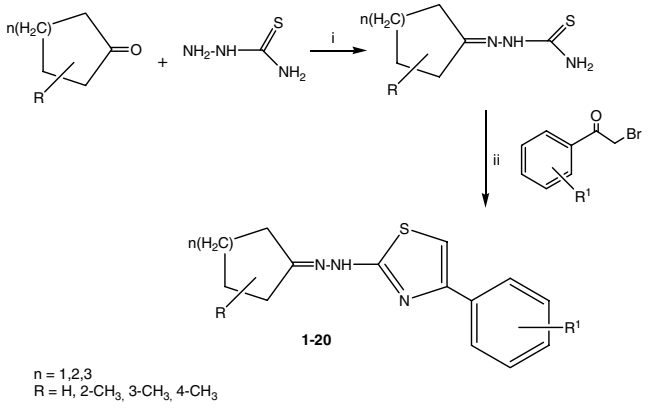
Apart from this, antifungal chemotherapy could be associated with a low toxicological profile, determining termination of the treatment.[16]

Another important issue is the clinical outcome due to several factors such as host defenses, the virulence of the fungal pathogen, drug efficacy/toxicity, and drug interactions.[17,18] Besides, pharmacokinetic studies have shown a variability of at least 50% between individuals;[19,20] in particular, plasma levels for voriconazole can vary from 74% to 100%.[21] These variations could account for the lack of efficacy and the toxicity.

As a consequence of the toxicity of the currently used polyene antifungal drugs and the emergence of candidal species resistant to azole-based agents, there is an urgent need for investigating alternative drug therapies.

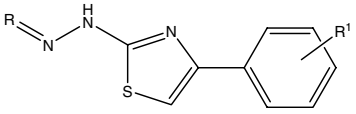
It has recently been reported in the literature that phenyl-thiazole analogues can be used as efficient antifungal agents.[22] Moreover, a large number of substituted benzothiazole,[23] thiazole derivatives of triazoles,[24] thiazolylhydrazones[25] have been shown to exhibit significant antimicrobial activity against a variety of fungal strains.

Moving from these literature indications and pursuing our research in the field,[26,27] in this report we describe the synthesis and antimicrobial evaluation of a new series of 2-thiazolylhydrazone derivatives.



Scheme 1. Synthesis of 2-thiazolylhydrazone derivatives **1**–**20**. Reagents: (i) isopropyl alcohol, AcOH; (ii) isopropyl alcohol.

Table 1. Chemical and physical data of derivatives **1**–**20**



Compound	R	R^1	M.W.	Mp (°C)	% Yield
1	Cyclopentyl	4-CH_3	352.27[a]	218–220	71
2	Cyclopentyl	4-OCH_3	368.27[a]	214–217	88
3	Cyclopentyl	3-NO_2	383.27[a]	187–190	69
4	Cyclopentyl	4-NO_2	383.27[a]	219–220	80
5	Cyclopentyl	4-CN	363.28[a]	207–208	67
6	Cyclopentyl	4-Cl	372.70[a]	223–225	58
7	Cyclopentyl	4-Br	417.16[a]	213–215	66
8	Cyclopentyl	4-C_6H_5	414.35[a]	235–237	58
9	Cyclohexyl-2-CH_3	4-CH_3	380.34[a]	160–162	64
10	Cyclohexyl-2-CH_3	4-OCH_3	396.34[a]	135–137	94
11	Cyclohexyl-2-CH_3	3-NO_2	411.31[a]	165–166	60
12	Cyclohexyl-2-CH_3	4-Br	445.21[a]	130–133	71
13	Cyclohexyl-2-CH_3	4-C_6H_5	442.41[a]	150–152	87
14	Cyclohexyl-3-CH_3	4-CH_3	380.34[a]	171–174	95
15	Cyclohexyl-3-CH_3	4-OCH_3	396.34[a]	162–165	84
16	Cyclohexyl-3-CH_3	4-CN	391.32[a]	189–190	98
17	Cyclohexyl-3-CH_3	4-Cl	400.76[a]	195–199	84
18	Cyclohexyl-3-CH_3	4-F	339.85[b]	192–195	77
19	Cyclohexyl-4-CH_3	4-Cl	400.76[a]	177–179	92
20[28]	Cycloheptyl	H	285.41	184–185	97

[a] Chloridrate.
[b] Bromidrate.

The cytotoxic activity of selected compounds that showed good activity against *Candida* species was evaluated: we incubated them in the presence of an immortalized hybrid cell line displaying an endothelial phenotype, EAhy 926, derived from the fusion of human umbilical vein endothelial cells (HUVEC) with a lung carcinoma cells and determined their cell viability by the Trypan Blue dye exclusion assay.

Table 2. ^{1}H NMR data of derivatives **1**–**20**

Compound	^{1}H NMR δ (ppm)
1[a]	1.80 (q, 2H, cyclopentyl), 1.92 (q, 2H, cyclopentyl), 2.35 (s, 3H, 4′-CH_3-phenyl), 2.50 (t, 2H, cyclopentyl), 2.62 (t, 2H, cyclopentyl), 6.67 (s, 1H, C_5H-thiaz.), 7.22 (d, J = 7.9 Hz, 2H, Ar), 7.55 (d, J = 7.9 Hz, 2H, Ar), 12.13 (br s, 1H, NH, D_2O exch.)
2[a]	1.85 (q, 2H, cyclopentyl), 1.90 (q, 2H, cyclopentyl), 2.50 (t, 2H, cyclopentyl), 2.63 (t, 2H, cyclopentyl), 3.83 (s, 3H, 4′-OCH_3-phenyl), 6.51 (s, 1H, C_5H-thiaz.), 6.96 (d, J = 7.9 Hz, 2H, Ar), 7.63 (d, J = 7.9 Hz, 2H, Ar), 12.13 (br s, 1H, NH, D_2O exch.)
3[a]	1.90 (q, 2H, cyclopentyl), 1.97 (q, 2H, cyclopentyl), 2.58 (t, 2H, cyclopentyl), 2.68 (t, 2H, cyclopentyl), 6.96 (s, 1H, C_5H-thiaz.), 7.77 (t, 1H, Ar), 8.20 (t, 1H, Ar), 8.31 (d, J = 7.9 Hz, 1H, Ar), 8.53 (d, J = 1.68 Hz, 1H, Ar), 12.13 (br s, 1H, NH, D_2O exch.)
4[a]	1.91 (q, 2H, cyclopentyl), 2.01 (q, 2H, cyclopentyl), 2.57 (t, 2H, cyclopentyl), 2.66 (t, 2H, cyclopentyl), 6.97 (s, 1H, C_5H-thiaz.), 7.94 (d, J = 8.8 Hz, 2H, Ar), 8.35 (d, J = 8.8 Hz, 2H), 12.40 (br s, 1H, NH, D_2O exch.)
5[b]	1.83 (q, 2H, cyclopentyl), 1.87 (q, 2H, cyclopentyl), 2.37 (t, 4H, cyclopentyl), 7.66 (s, 1H, C_5H-thiaz.), 7.96 (d, J = 8.4 Hz, 2H, Ar), 8.12 (d, J = 8.4 Hz, 2H, Ar), 10.83 (br s, 1H, NH, D_2O exch.)
6[b]	1.72 (q, 2H, cyclopentyl), 1.78 (q, 2H, cyclopentyl), 2.37 (t, 4H, cyclopentyl), 7.30 (s, 1H, C_5H-thiaz.), 7.44 (d, J = 8.4 Hz, 2H, Ar), 7.84 (d, J = 8.4 Hz, 2H, Ar), 10.70 (br s, 1H, NH, D_2O exch.)
7[b]	1.72 (q, 2H, cyclopentyl), 1.80 (q, 2H, cyclopentyl), 2.37 (t, 4H, cyclopentyl), 7.31 (s, 1H, C_5H-thiaz.), 7.57 (d, J = 8.4 Hz, 2H, Ar), 7.77 (d, J = 8.4 Hz, 2H, Ar), 10.65 (br s, 1H, NH, D_2O exch.)
8[a]	1.87 (q, 2H, cyclopentyl), 1.94 (q, 2H, cyclopentyl), 2.52 (t, 2H, cyclopentyl), 2.66 (t, 2H, cyclopentyl), 6.72 (s, 1H, C_5H-thiaz.), 7.38 (d, J = 7.1 Hz, 1H, Ar), 7.47 (t, 2H, Ar), 7.59 (d, J = 7.1 Hz, 2H, Ar), 7.69 (d, J = 7.9 Hz, 2H, Ar), 7.77 (d, J = 7.9 Hz, 2H, Ar), 12.24 (br s, 1H, NH, D_2O exch.)
9[a]	1.16 (d, J = 6.3 Hz, 3H, CH_3), 1.24–1.42 (m, 1H, cyclohexyl), 1.57–1.66 (m, 2H, cyclohexyl), 1.79–1.86 (m, 1H, cyclohexyl), 1.98–2.03 (m, 2H, cyclohexyl), 2.17–2.27 (m, 1H, cyclohexyl), 2.41 (s, 3H, 4′-CH_3-phenyl), 2.43–2.49 (m, 1H, cyclohexyl), 3.04–3.10 (m, 1H, cyclohexyl), 6.66 (s, 1H, C_5H-thiaz.), 7.28 (d, J = 8.4 Hz, 2H, Ar), 7.60 (d, J = .4 Hz, 2H, Ar), 12.50 (s, 1H, NH, D_2O exch.)
10[a]	1.11 (d, J = 6.3 Hz, 3H, CH_3), 1.17–1.37 (m, 1H, cyclohexyl), 1.48–1.61 (m, 2H, cyclohexyl), 1.75–1.81 (m, 1H, cyclohexyl), 1.95–1.99 (m, 2H, cyclohexyl), 2.11–2.21 (m, 1H, cyclohexyl), 2.35–2.44 (m, 1H, cyclohexyl), 2.97–3.03 (m, 1H, cyclohexyl), 3.81 (s, 3H, 4′-OCH_3-phenyl), 7.53 (s, 1H, C_5H-thiaz.), 6.94 (d, J = 8.8 Hz, 2H, Ar), 7.60 (d, J = 8.8 Hz, 2H, Ar), 12.37 (s, 1H, NH, D_2O exch.)
11[b]	1.08 (d, J = 6.5 Hz, 3H, CH_3), 1.16–1.26 (m, 1H, cyclohexyl), 1.37–1.59 (m, 2H, cyclohexyl), 1.69–2.02 (m, 4H, cyclohexyl), 2.31–2.42 (m, 1H, cyclohexyl), 2.91–2.96 (m, 1H, cyclohexyl), 7.56 (s, 1H, C_5H-thiaz.), 7.70 (t, 1H, Ar), 8.12 (d, J = 8.1 Hz, 1H, Ar), 8.27 (d, J = 8.1 Hz, 1H, Ar), 8.68 (s, 1H, Ar), 10.95 (s, 1H, NH, D_2O exch.)
12[a]	1.15 (d, J = 6.3 Hz, 3H, CH_3), 1.26–1.38 (m, 1H, cyclohexyl), 1.54–1.65 (m, 2H, cyclohexyl), 1.75–1.81 (m, 1H, cyclohexyl), 1.92–1.98 (m, 2H, cyclohexyl), 2.13–2.23 (m, 1H, cyclohexyl), 2.39–2.47 (m, 1H, cyclohexyl), 2.97–3.02 (m, 1H, cyclohexyl), 6.76 (s, 1H, C_5H-thiaz.), 7.59 (s, 4H, Ar), 12.41 (s, 1H, NH, D_2O exch.)
13[a]	1.13 (d, J = 6.3 Hz, 3H, CH_3), 1.26–1.39 (m, 1H, cyclohexyl), 1.54–1.61 (m, 2H, cyclohexyl), 1.79–1.83 (m, 1H, cyclohexyl), 1.89–1.97 (m, 2H, cyclohexyl), 2.17–2.22 (m, 1H, cyclohexyl), 2.38–2.41 (m, 1H, cyclohexyl), 3.01–3.05 (m, 1H, cyclohexyl), 6.75 (s, 1H, C_5H-thiaz.), 7.36 (d, J = 7.1 Hz, 1H, Ar), 7.46 (t, 2H, Ar), 7.59 (d, J = 7.1 Hz, 2H, Ar), 7.69 (d, J = 8.4 Hz, 2H, Ar), 7.78 (d, J = 8.4 Hz, 2H, Ar), 12.48 (s, 1H, NH, D_2O exch.)
14[a]	1.16 (d, J = 6.4 Hz, 3H, CH_3), 1.35 (s, 1H, cyclohexyl), 1.60–1.62 (m, 2H, cyclohexyl), 1.81 (s, 2H, cyclohexyl), 1.98–1.99 (m, 2H, cyclohexyl), 2.20–2.22 (m, 1H, cyclohexyl), 2.42 (d, J = 6.1 Hz, 3H, 4′-CH_3-phenyl), 3.03–3.04 (m, 1H, cyclohexyl), 6.63 (s, 1H, C_5H-thiaz.), 7.28 (s, 2H, Ar), 7.60 (s, 2H, Ar), 12.52 (s, 1H, NH, D_2O exch.), 13.56 (s, 1H, NH, D_2O exch.)
15[a]	1.06 (d, J = 6.2 Hz, 3H, CH_3), 1.20–1.21 (m, 1H, cyclohexyl), 1.81–1.95 (m, 6H, cyclohexyl), 2.52 (t, 1H, cyclohexyl), 3.05–3.06 (m, 1H, cyclohexyl), 3.85 (s, 3H, 4′-OCH_3-phenyl), 6.52 (s, 1H, C_5H-thiaz.), 6.99 (d, J = 8.0 Hz, 2H, Ar), 7.65 (d, J = 8.6 Hz, 2H, Ar), 12.44 (s, 1H, NH, D_2O exch.), 13.58 (s, 1H, NH, D_2O exch.)
16[a]	1.19 (d, J = 6.4 Hz, 3H, CH_3), 1.57–1.61 (m, 3H, cyclohexyl), 1.83–1.85 (m, 1H, cyclohexyl), 2.00–2.03 (m, 2H, cyclohexyl), 2.19–2.21 (m, 1H, cyclohexyl), 2.50–2.52 (m, 1H, cyclohexyl), 3.00–3.01 (m, 1H, cyclohexyl), 6.87 (s, 1H, C_5H-thiaz.), 7.79 (d, J = 7.8 Hz, 2H, Ar), 7.86 (s, 2H, Ar), 12.48 (s, 1H, NH, D_2O exch.), 14.01 (br s, 1H, NH, D_2O exch.)
17[a]	1.64 (d, J = 6.5 Hz, 3H, CH_3), 1.25–1.26 (m, 1H, cyclohexyl), 1.69–1.71 (m, 1H, cyclohexyl), 1.98–1.99 (m, 2H, cyclohexyl), 2.20–2.21 (m, 2H, cyclohexyl), 2.50–2.52 (m, 2H, cyclohexyl), 2.95–2.96 (m, 1H, cyclohexyl), 6.68 (s, 1H, C_5H-thiaz.), 7.47 (d, J = 8.6 Hz, 2H, Ar), 7.67 (d, J = 8.5 Hz, 2H, Ar), 12.50 (s, 1H, NH, D_2O exch.), 13.80 (s, 1H, NH, D_2O exch.)
18[a]	1.16 (d, J = 6.5 Hz, 3H, CH_3), 1.58–1.59 (m, 3H, cyclohexyl), 1.98–1.99 (m, 1H, cyclohexyl), 2.05–20.7 (m, 2H, cyclohexyl), 2.25–2.27 (m, 1H, cyclohexyl), 2.40–2.41 (m, 1H, cyclohexyl), 3.00–3.01 (m, 1H, cyclohexyl), 6.62 (s, 1H, C_5H-thiaz.), 7.18 (d, J = 6.1 Hz, 2H, Ar), 7.70 (d, J = 5.3 Hz, 2H, Ar), 12.71 (s, 1H, NH, D_2O exch.), 14.30 (s, 1H, NH, D_2O exch.)
19[a]	0.99 (d, J = 6.1 Hz, 3H, CH_3), 1.20–1.21 (m, 2H, cyclohexyl), 1.60–1.61 (m, 1H, cyclohexyl), 2.00–2.01 (t, 2H, cyclohexyl), 2.20–2.30 (m, 2H, cyclohexyl), 2.60–2.61 (m, 1H, cyclohexyl), 3.09–3.10 (m, 1H, cyclohexyl), 6.68 (s, 1H, C_5H-thiaz.), 7.47–7.51 (m, 2H, Ar), 7.71 (d, J = 6.6 Hz, 2H, Ar), 12.55 (s, 1H, NH, D_2O exch.), 13.75 (s, 1H, NH, D_2O exch.)
20[a]	1.58–1.63 (m, 6H, cycloheptyl), 1.70–1.71 (m, 2H, cycloheptyl), 2.55–2.56 (m, 2H, cycloheptyl), 2.70–2.72 (m, 2H, cycloheptyl), 6.80 (s, 1H, C_5H-thiaz.), 7.48–7.50 (m, 3H, Ar), 7.73 (d, J = 7.9 Hz, 2H, Ar), 12.40 (s, 1H, NH, D_2O exch.), 13.80 (br s, 1H, NH, D_2O exch.)

[a] $CDCl_3$.
[b] DMSO-d_6.

2-Thiazolylhydrazone derivatives **1**–**20** were synthesized as reported in our previous communications.[28,29]

Cyclic ketones or aryl aldehydes reacted directly with thiosemicarbazide and the obtained thiosemicarbazones subsequently reacted with α-halogenoketones to yield the 4-substituted thiazole ring derivatives as shown in Scheme 1. In the synthesis of all compounds isopropyl alcohol proved to be the best solvent for our purpose. As a matter of fact, the reaction products precipitate on cooling down and can be filtered and purified by crystallization from ethanol or ethanol/isopropanol. All synthesized compounds were fully characterized by analytical and spectral data as listed in Tables 1 and 2.

All synthesized compounds were evaluated for antifungal activity and compared with the reference compound clotrimazole (Table 3).[30,31]

The newly prepared compounds were dissolved in dimethylsulfoxide (DMSO) and their in vitro activity was evaluated against a total of 22 strains of *Candida* species. The included isolates were *Candida albicans* (8 strains), *Candida glabrata* (4), *Candida krusei* (3), *Candida tropicalis* (3), *Candida sakè* (2), and *Candida parapsilosis* (2). The isolates were collected from specimens of patients at the 'Azienda Policlinico Umberto I°' of Rome 'La Sapienza' University and were obtained from haematology/oncology and surgery departments, which also included an intensive care unit. In particular, the samples were isolated from the upper and lower respiratory tract, blood, and indwelling venous catheters; the intensive care unit accounted for 65% of the cases (15/22 isolates). The isolates were identified by conventional methodologies. Prior to testing, each isolate was subcultured on a qualified medium to ensure purity and optimal growth.

The data reported in Table 3 show that cyclopentyl derivatives **1**–**6** had good anti-*C. albicans* and anti-*C. glabrata* activity. In particular compounds **1**, **2**, and **6** were very active against both strains, while compounds **3**, **4**, and **5** showed good activity only against *C. glabrata*. Furthermore, cyclohexyl substituted derivatives **9**–**19** showed good anti-*C. albicans* activity, in particular with compounds **9**–**11** and **14**–**19**. Some derivatives, especially **9** and **10**, also showed good anti-*C. glabrata* activity. Cycloheptyl derivative **20** showed good anti-*C. albicans* activity. Among all synthesized compounds, we found the antifungal activity showed by compound **14**, 2-(3-methylcyclohexyl)-1-[4-(4-methylphenyl)-2-thiazolyl]hydrazone, particularly interesting. Compound **14** was more active than the clotrimazole reference compound against *C. albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. sakè*.

The cytotoxic profile of the compounds showed that the derivatives were less toxic at concentrations below 0.5 μg/mL with a percentage of viable cells of 73.0 and 94.7 (Table 4).[32–34]

In conclusion, a series of novel 2-thiazolylhydrazone derivatives was assessed for antifungal activity on 22 *Candida* strains. Compound **14** was found to have good antifungal activity and may be used as a good reference for identifying features of the structure that could be important for antifungal activity.

Table 3. Minimal inhibitory concentration (MIC)[a] of compounds **1**–**20** and clotrimazole against 22 strains of *Candida* species

Compound	Tested fungi (MIC[a] μg/mL)					
	C. *albicans* (8 strains)	C. *glabrata* (4 strains)	*C. tropicalis* (3 strains)	*C. krusei* (3 strains)	*C. parapsilosis* (2 strains)	*C. sakè* (2 strains)
1	0.25–2	0.50–2	4–16	64–128	128	16–32
2	0.50–2	2–4	64–128	32–64	8–16	32
3	1–8	0.50–2	64–128	>128	>128	16–32
4	8–32	0.50–2	64–128	32–64	>128	16
5	2–8	0.50–2	32–64	32–64	128	32
6	0.25–2	0.50–2	32–64	64–128	8–16	16
7	2–8	2–8	128–>128	>128	128	32
8	16–64	>128	64–128	>128	>128	32
9	0.25–2	0.50–2	2–4	64	8	32
10	0.50–2	0.25–2	8	16	8	32
11	0.50–2	16–64	64	>128	8–16	16–32
12	4–8	2–4	32–64	64–128	8–16	16
13	32–64	64–128	64	16	16	16–32
14	0.50–2	32–64	0.50–2	0.50–2	0.50–1	2–4
15	0.50–2	>128	2–4	2–4	1–2	8–16
16	0.50–2	>128	2–4	4–8	0.50–1	4–8
17	0.50–2	>128	2–4	4–8	1	2
18	0.50–4	>128	4–16	4–16	1	8
19	0.50–2	32–64	2–4	05–1	8	2
20	0.50–2	>128	8–16	8–16	8–16	16–32
Clo[b]	0.50–4	4–8	4–8	4–8	4–8	8–16

[a] Range value.
[b] Clotrimazole.

Table 4. Cytotoxic effect of selected compounds tested on EAhy 926 cells after 24 h of incubation at 37 °C, using Trypan Blue exclusion test, expressed as cell survival fraction[a] (%)

Compound	Concentration[b] (μg/mL)			
	50	5	0.5	0.05
1	75.0 ± 3.2	80.0 ± 4.1	82.0 ± 4.6	85.4 ± 5.0
2	66.0 ± 3.2	76.6 ± 4.0	82.4 ± 2.8	85.0 ± 2.2
5	76.0 ± 4.6	81.0 ± 3.2	84.7 ± 2.8	88.9 ± 6.0
6	64.9 ± 4.2	81.0 ± 3.0	89.2 ± 2.8	86.0 ± 2.6
9	64.0 ± 3.2	63.6 ± 3.6	78.5 ± 4.2	85.7 ± 3.6
10	75.0 ± 4.0	83.3 ± 3.6	84.0 ± 3.2	86.0 ± 4.8
11	83.3 ± 3.6	85.7 ± 4.2	86.0 ± 3.8	88.8 ± 2.2
14	38.5 ± 4.3	72.7 ± 3.1	83.3 ± 3.6	84.0 ± 2.0
15	54.5 ± 3.2	72.7 ± 3.8	80.0 ± 1.9	83.3 ± 2.2
16	25.0 ± 1.9	50.0 ± 2.5	68.7 ± 2.9	68.8 ± 1.4
17	28.6 ± 2.8	50.0 ± 1.9	70.0 ± 4.2	73.0 ± 3.1
18	52.9 ± 2.7	60.0 ± 1.5	76.9 ± 1.7	80.0 ± 2.0
20	54.5 ± 5.0	80.0 ± 3.9	87.5 ± 1.8	94.7 ± 2.5

[a] Cells incubated with culture medium alone represented the controls and the cell viability was always greater than 97%.

[b] Data represent the arithmetic means ± SD of at least three independent experiments.

Moreover other tested compounds may be good candidates for further investigation.

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30. *Antifungal activity:* All synthesized derivatives **1**–**20** were evaluated for their antifungal activity dissolved in dimethylsulfoxide (DMSO). The in vitro antifungal activities of the compounds were determined with the broth microdilution method with Sabouraud dextrose broth (BBL Microbiology Systems, Cockeysville, MD) as recommended by the NCCLS.[31] Microtiter plates containing serial dilutions of each compound were inoculated with each organism to yield the appropriate density (10^3/mL) in a 100 μL final volume; each plate included positive controls (fungi without a compound) and a negative control (medium only). The plates were incubated for 24 h at 37 °C. The MIC for all isolates was defined as the lowest concentration of antifungal agent that completely inhibited growth of the organism, as detected by the unaided eye.
31. National Committee for Clinical Laboratory Standards 2003. Performance standards for antimicrobial susceptibility testing. Supplement M100-S13. National Committee for Clinical Laboratory Standards, Wayne, PA.

32. *In vitro cytotoxicity:* The cytotoxicity of the newly synthesized compounds under investigation was tested against EAhy, human cell line obtained from a hybridoma between HUVEC and epithelial cells from a lung carcinoma. The viability of cells exposed to test compounds was estimated by the Trypan Blue dye exclusion assay. Cell lines were maintained as adherent type cultures under humidified atmosphere in 5% CO_2 at 37 °C, Dulbecco's modified Eagle's culture medium (high glucose) supplemented with 2 mM L-Glutamine, HAT supplement and containing antibiotic mixture. Experiments were performed in cells grown to 60–70% confluency.[33] The stock solutions of the investigated compounds were prepared in sterile dimethylsulfoxide (DMSO) and the successive dilutions were made in culture medium; the DMSO percent present in culture medium never exceeded 0.5%. EAhy cells in the exponential phase of growth (1×10^5/mL) were seeded into 24-well microplate. and incubated for 24 h with four different concentrations of the compounds (50–0.05 μg/mL). Some plates containing cells alone or cells and DMSO represented the controls. After the incubation period, cells were mechanically scraped off from the plates and an aliquot was diluted (1:1) with a solution 0.4% Trypan Blue Stain. After few minutes at rt cells were counted under an optical microscope in a Thoma hemocytometer chamber by two different operators. On the basis that Trypan Blue is a vital dye[34] and can enter and interact with the cells unless the plasmatic membrane is damaged, blue stained cells were considered as dead. Values are expressed as % of viable cells. Cell viability in control samples was always 97–98%.
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