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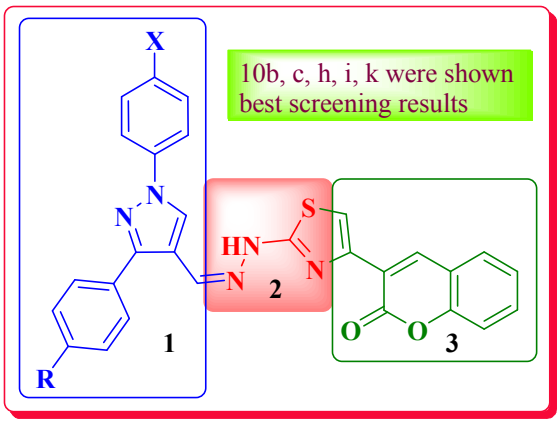

A new series 3-{2-[N′-(1,3-disubstituted-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (**10a-l**) were synthesized by multi-step reaction. All the synthesized compounds were characterized and were screened for their *in-vitro* antibacterial and antifungal studies against various microorganisms. Most of the compounds found to be biologically active.

1,3,4-Trisubstituted pyrazole bearing 4-(chromen-2-one) thiazole: Synthesis, characterization and its biological studies

N. Harikrishna[a, b], Arun M. Isloor[a, *], K. Ananda[c], Abdulrahman Obaid[d] and Hoong-Kun Fun[d,e]

[a]*Medicinal Chemistry Laboratory, Department of Chemistry, National Institute of Technology Karnataka, Surathkal, Mangalore 575025, India.*

[b]*SeQuent Scientific Limited, Baikampady, Mangalore 575011, India.*

[c]*Biological Sciences, Poornaprajna Institute of Scientific Research, Bangalore 562110, India.*

[d]*Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box. 2457 - Riyadh 11451, Kingdom of Saudi Arabia.*

[e]*X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, Penang 11800, Malaysia.*

Abstract

A new series 3-{2-[N′-(1,3-disubstituted-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (**10a-l**) were synthesized by multi-step reaction. All the synthesized compounds were characterized by IR, NMR, Mass spectral studies, followed by elemental analysis. The newly synthesized thiazole compounds were screened for their *in-vitro* antibacterial and antifungal studies against various microorganisms. Antimicrobial studies carried out by the well diffusion method, showed very good zone of inhibition for both bacteria (at a range of 20-50 mm diameter) and fungi (at a range of 10-30 mm diameter). Minimum Inhibitory Concentration (MIC) required for the 100% inhibition of bacteria and fungi was found to be as low as 15.6 μg/ml for a few of the synthesized compounds.

Key words: Thiazole, pyrazole, coumarin, antimicrobial study.

* Corresponding author. Tel.: +91 824 2474000; Fax: +91 824 2474033. E-mail address: isloor@yahoo.com(A.M. Isloor)

1. Introduction

The treatment of infectious diseases still remains as an important and challenging problem to the medicinal chemists due to the emerging infectious diseases and increasing number of multi-drug resistant microbial pathogens. In spite of the discovery of a large number of antibiotics and chemotherapeutics, the emergence of old and new antibiotic resistant bacterial strains constitutes a substantial need for the new class of potent antimicrobial agents.[1] Heterocyclic compounds play an important role in designing of a new class of structural entities of medicinal importance with new mechanisms of action. These heterocyclic compounds are well known to possess diverse pharmacological properties.

Thiazoles are members of the azole heterocycles that includes imidazoles and oxazoles. Thiazole can also be considered a functional group. Commercial significant thiazoles include mainly dyes and fungicides. Thifluzamide, Tricyclazole, and Thiabendazole are marketed for control of various agricultural pests. Another widely used thiazole derivative is the non-steroidal anti-inflammatory drug Meloxicam. In recent years, thiazoles and their derivatives have attracted medicinal chemists because of their varied biological activities such as for the treatment of allergies,[2] hypertension,[3] inflammation,[4] schizophrenia,[5] antibacterial,[6] HIV infections,[7] hypnotics,[8] and more recently for the treatment of pain,[9] as fibrinogen receptor antagonists with antithrombotic activity,[10] and as new inhibitors of bacterial DNA gyrase B.[11]

Further, derivatives of pyrazoles have shown significant pharmacological activities, such as anti-microbial,[12] analgesic,[13] anti-inflammatory,[14] and anticancer.[15] This gave a great impetus to the search for potential pharmacologically active drugs carrying pyrazole substituents. The search for novel antimicrobial and analgesic agents devoid of side effects continues to be an active area of research in medicinal chemistry.[1] Although new and many more expensive drugs have been developed, their cost is beyond the reach of common man. As a consequence, these trends have emphasized the pressing need for new, more effective, cheaper and safe antimicrobial agents.

Although we have newer, less toxic antimicrobial agents that are available for clinical use, their clinical efficacy in some invasive fungal infections, is not optimal.[16] In recent years, the widespread use of antimicrobial agents has resulted in the development of resistance to these drugs by pathogenic microorganisms, causing an increase in morbidity and mortality.[17] Thus, intense efforts in antimicrobial drug discovery are still needed to develop more promising and

effective antimicrobial agents for use in the clinical arena. Therefore, novel, effective and cheaper antimicrobial drugs are the immediate need of present days. Keeping in view of this and in continuation of our research on pharmacologically potent molecules,[18, 19, 20] we planned to combine the pyrazole and thiazole nucleus with the hope of enhancing the biological activities of the new molecule. We hereby report the synthesis and antimicrobial property of some new 1,3,4-trisubstituted pyrazole bearing 4-(chromen-2-one) thiazoles. These compounds were evaluated for their antimicrobial properties against *Mycobacterium smegmatis* (antituberculosis bacteria), *Staphylococcus aureus* (Gram positive bacteria) and *Candida albicans* (fungi). Figure-1 reprsents a few of the commercially important heterocyclic based drugs.

Figure-1

2. Results and discussion

2.1. Chemistry

The targeted compounds 3-{2-[N′-(1,3-disubstituted-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (**10a-l**) were synthesized by using the chemistry outlined in the Scheme-1. The basic pyrazole skeleton, *i.e.* 1,3-disubstituted pyrazole-4-carbaldehydes (**4a-l**) were synthesized by condensation of substituted acetophenone **1** with 4-substituted phenylhydrazine hydrochloride **2** in the presence of catalytic amount of sodium hydroxide solution in acetic acid media. Which on stirring for 1 h at ambient temperature, yielded the hydrazone derivative **3** in high yield. Further, on treatment with DMF/$POCl_3$ by the Vilsmeier-Haack reaction at 70 °C, gave the 1,3-disubstituted pyrazole-4-carbaldehydes (**4a-l**) in moderate to good yields from the corresponding hydrazone derivative **3**.[21, 22]

The other key starting material 3-(bromoacetyl)-2*H*-chromen-2-one **8,** was prepared by the bromination of 3-acetyl-2*H*-chromen-2-one **7** in chloroform media, which was prepared by Knoevenagel condensation of salicyladehyde **5** with ethylacetoacetate **6** with piperidine as a base in ethanol media.[23, 24]

The targeted pyrazole bearing thiazole derivatives (**10a-l**) were prepared as shown in Scheme-1. The 1,3-disubstituted pyrazole-4-carbaldehydes (**4a-l**) reacted with thiosemicarbazide to give thiosemicarbazones (**9a-l**) respectively. Which on reacting with 3-(bromoacetyl)-2*H*-chromen-2-one **8** in ethanol media, under reflux temperature to give 3-{2-[N′-(1,3-disubstituted-

1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (**10a-l**) in reasonably good yields (85-96%). The newly synthesized compounds were characterized by IR, NMR, mass spectra and C, H, N elemental analyses.

Scheme-1

Formation of 1,3-disubstituted pyrazole-4-carbaldehydes (**4a-l**) were confirmed by recording their IR, ^{1}H NMR, ^{13}C NMR and MS spectrum. The IR data for compound **4c** was confirmed by the peak observed at 1672 cm^{-1} which is due to -CHO stretching of aldehyde group. Band at 1522 cm^{-1} is showing the presence of -C=N group. The ^{1}H NMR spectrum of **4c** showed a singlet at δ 2.43 corresponding to the -CH_3 proton. A singlet at δ 10.05 is due to aldehyde proton of pyrazole moiety. Another singlet at δ 8.52 is due to the pyrazole-5H proton. In ^{13}C NMR spectrum, peak at 185.29 showed the presence of aldehyde carbon, other peaks for **4c** showed at 154.86, 139.31, 139.05, 130.82, 129.63, 129.45, 128.82, 128.45, 127.87, 122.45, 119.72, 21.34. The mass spectrum of **4c** showed a molecular ion peak at *m/z* = 263.2 (M^+), which is confirmed with the molecular formula $C_{17}H_{14}N_2O$. The characterization data of the synthesized compounds (**4a-l**) were presented in Table-1.

Table-1

Formation of 3-(bromoacetyl)-2*H*-chromen-2-one **8** was confirmed by recording their IR, ^{1}H NMR and mass spectra. The formation of 3-(bromoacetyl)-2*H*-chromen-2-one was confirmed by the peak observed at 1735 cm^{-1} in the IR spectrum, which is due to the -C=O stretching of keto group. The ^{1}H NMR spectrum of **8** showed a singlet at δ 4.76 corresponding to the-CH_2 group. A singlet at δ 8.65 is due to chromen-2-one-4H. The mass spectrum of **8** showed a molecular ion peak at *m/z* = 266.9 (M^-), which has confirmed the molecular formula $C_{11}H_7BrO_3$.

Formation of 3-{2-[N′-(1,3-disubstituted-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (**10a-l**) were confirmed by recording their IR, ^{1}H NMR, ^{13}C NMR and mass spectra. IR analysis of compound **10a** showed the peak at 3412 cm^{-1}, which was due to the -NH group. Another absorption band at 3140 cm^{-1} was due to the -C-H stretching of the aromatic ring. The absorption band at 1720 cm^{-1} was due to the -C=O group, band at 1627 cm^{-1} due to the -C=N group, -C=C stretching was observed at 1497 cm^{-1} and an absorption band at 1094 cm^{-1} was due to the -C-O stretching, which confirmed the formation of compound **10a**.

The ^{1}H NMR spectrum of **10a** in DMSO-d_6 solvent showed a singlet at δ 7.76 which was attributed to the thiazole -5H proton, another singlet attributed at δ 8.53 which was due to the chromen-2-one-4H proton. The characteristic peak of pyrazole-5H proton was observed as a singlet at δ 8.88 and the -NH proton was attributed at δ 12.03. The detailed ^{1}H NMR resonances are summarized in the experimental section.

The ^{13}C NMR spectrum of compound **10a** shows the peaks at 167.89, 159.22, 152.75, 151.36, 144.31, 139.50, 138.60, 135.64, 132.69, 132.14, 130.06, 129.29, 129.06, 128.95, 128.13, 127.35, 125.17, 120.99, 119.66, 119.19, 117.33, 116.34, 110.89.

The mass spectrum of **10a** showed a molecular ion peak at *m/z* = 490.1 (M^+). This in turn confirmed the formation of a compound having the molecular formula $C_{28}H_{19}N_5O_2S$. The characterization data of the newly synthesized compounds **10a-l** were presented in Table-2.

Table-2

2.2. Biological results

Antibacterial activity of all the compounds against *Mycobacterium smegmatis* was plotted in a bar diagram and represented in Figure-2. The Figure-3 represents the zone of inhibition for *Staphylococcus aureus* at three different concentrations (1, 0.5 and 0.25 mg/ml) with a standard deviation of triplicate values. Antifungal zone of inhibition showed by the synthetic compounds represented in Figure-4. Figure-5 represents the MIC of the synthesized thiazole derivatives against the microorganisms. Antibacterial standard (Ciprofloxacin, ABS) and antifungal standard (Fluconazole, AFS) were tested at a concentration of 10 mg/ml. Most of the compounds in this series showed good microbial inhibition for all the tested microorganisms such as *Mycobacterium smegmatis* (antituberculosis bacteria), *Staphylococcus aureus* (Gram positive bacteria) and *Candida albicans* (fungi) in disc diffusion method. Compounds **10b**, **10c**, **10h**, **10i** and **10k** showed good zone of inhibition among all other compounds with a zone of inhibition up to 50 mm. The structural activity relationship of the synthesized compounds was explained based on the tested microorganisms. Among all the compounds, **10b** has shown the best inhibition against all the tested microorganisms. This is due to the presence of 4-chlorophenyl at first position of the pyrazole ring. The presence of *p*-tolyl group (weak electron donating group) at the third position of pyrazole has enhanced the activity of **10c** against all the tested microorganisms. The compound **10h** has shown significant inhibition against Gram-positive bacteria

Staphylococcus aureus, which is due to the presence of 4-chlorophenyl group at first and third position of the pyrazole ring respectively. The compound **10i** has shown good inhibition on *Mycobacterium smegmatis,* which is due to the presence of 4-bromophenyl group at third position and phenyl group at the first position of pyrazole ring. The compound **10j** showed higher inhibition of bacteria and less for fungi indicating that, the compound can be a better antibacterial than antifungal due to the presence of 4-chlorophenyl and 4-bromophenyl at the first and third position of pyrazole respectively. **10k** has shown best inhibition on fungi *Candida albicans,* which is due to the presence of 4-fluorophenyl group at the third position of the pyrazole ring. Among all, the majority of the compounds were active against bacteria as well as fungi. This indicates that, the synthesized compounds are biologically active as antimicrobials. Tuberculosis variant bacteria *M. smegmatis* was inhibited to the maximum extent by most of the compounds. The MIC of **10b** and **10c** showed that, they are most active against bacteria *P. aeruginosa*, *M. smegmatis* and fungi *C. albicans* with value of 15.6 µg/ml. The same compounds were shown moderate MIC value of 31.25 µg/ml against *S. aureus* (Gram positive bacteria). Minimum inhibitory concentration assay conducted for three bacteria and one fungi is represented in Figure-5. Results of antimicrobial studies have been tabulated in Table-3 and 4.

Figure-2

Figure-3

Figure-4

Figure-5

Table-3

Table-4

3. Conclusions

A new series of 3-{2-[N′-(1,3-disubstituted-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (**10a-l**) derivatives were synthesized in good yields. They were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS spectrometry and elemental analysis. Targeted compounds were investigated for their *in-vitro* antibacterial and anti-fungal activities by standard well plate method and proved to be very good antimicrobial compounds. The compounds **10b, 10c, 10h** and **10i** exhibited the best inhibition against all the tested microorganisms. The compound **10k** has showed the excellent inhibition on fungi *Candida albicans* due to the presence of the high

electronegativity of fluorine presence in the phenyl group at the third position of pyrazole. The compounds **10b**, **10c** showed the best MIC against tested bacteria and fungi. The similarity between **10b** and **10c** is the pyrazole ring having phenyl substitution either at first or third position. Few of the synthesized compounds such as **10b**, **10c**, **10h**, **10i** and **10k** have shown the least MIC value (as low as 15.6 µg mL^{-1}) against most of the microorganisms. Inhibition of *M. smegmatis* a counterpart of tuberculosis bacteria indicates that, synthesized pyrazole containing thiazole compounds are promising drug molecules for treating tuberculosis. Most of the compounds shown a wide range of antimicrobial activity, which leads to the conclusion that, these compounds might be an excellent antimicrobial compound to combat with multidrug resistant microorganisms.

4. Experimental

4.1. Analysis and instruments

All the chemicals were purchased from Sigma Aldrich, Merck and AVRA-India. Commercial grade solvents used for the reactions were distilled before use. Melting points were determined by open capillary method and were uncorrected. The IR spectrums (in KBr pellet) were recorded on a Perkin-Elmer FTIR-4000-400 cm^{-1} spectrophotometer. NMR spectra were recorded on a Bruker Avance-400 spectrometer (400 MHz) for ^{1}H NMR and ^{13}C NMR using tetramethylsilane (TMS) as internal standard. Chemical shift and coupling constants are recorded in units of δ (ppm) and Hz, respectively. The mass spectrum was recorded on a LC-MS Applied biosystems MDS SCIEX-API 4000 spectrometer. Elemental analysis was performed on a Flash EA 1112 series CHNS-O analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated, ready made aluminium sheets (Merck F_{254}). The names of the structures were mentioned as per chemdraw.

4.2 General procedure for the synthesis of 1,3-disubstituted-4-pyrazole carbaldehydes (4a-l)

A mixture of substituted acetophenone (**1a-l**) (0.01 mol) and 4-substituted phenylhydrazine hydrochloride **2** (0.1 mol) in acetic acid (10 ml) was stirred with 10% sodium hydroxide solution (0.5 ml) at an ambient temperature for 1 h. The obtained solid hydrazone intermediate **3a-l** was filtered, washed with pre-chilled acetic acid (2 ml) to give off-white free flow solid. Yield: 90-96%.

DMF (1.16 ml, 0.015 mol) was taken in a round bottom flask, cooled to -5 °C using ice-salt mixture and added $POCl_3$ (2.8 ml, 0.03mol) in about 1.5 h maintaining the temperature. The hydrazone intermediate (**3a-l**) (0.005 mol) was added at 0 °C and heated the reaction mass to 70 °C for 3 h. After the completion of the reaction, the reaction mixture was cooled and poured into ice-chilled water. Neutralized with 10% sodium bicarbonate solution and the solid product was stirred for one hour at ambient temperature and filtered. Further, it was washed with distilled water and re-crystallized the crude material from methanol. Yield: 66-91%. The characterization data of the synthesized aldehydes are presented below.

4.2.1. 1,3-Diphenyl-1*H*-pyrazole-4-carbaldehyde (4a). IR (KBrν_{max} cm^{-1}): 3125 (C-H-str), 1672 (C=O str), 1524 (C=N str), 1227 (C-O str).

4.2.2. 1-Phenyl-3-(*p*-tolyl)-1*H*-pyrazole-4-carbaldehyde (4c). IR (KBrν_{max} cm^{-1}): 3123 (C-H-str), 1672 (C=O str), 1522 (C=N str), 1227 (C-O str); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 2.43 (s, 3H, -CH_3), 7.30-7.32 (d, 2H, Ar-H, J = 7.9 Hz), 7.37-7.40 (t, 1H, Ar-H, J = 7.4 Hz), 7.49-7.52 (t, 2H, Ar-H, J = 7.9 Hz), 7.71-7.72 (d, 2H, Ar-H, J = 8.1 Hz), 7.78-7.80 (d, 2H, Ar-H, J = 7.5 Hz), 8.52 (s, 1H, pyrazole-5H), 10.05 (s, 1H, -CHO); ^{13}CNMR (100 MHz, $CDCl_3$, ppm): δ 185.29, 154.86, 139.31, 139.05, 130.82, 129.63, 129.45, 128.82, 128.45, 127.87, 122.45, 119.72, 21.34; MS: *m/z* = 263.2 (M^+), ANAL. Calcd.for $C_{17}H_{14}N_2O$; calcd: C, 77.84; H, 5.38; N, 10.68; found: C, 77.85; H, 5.38; N, 10.70.

4.2.3. 1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-1*H*-pyrazole-4-carbaldehyde (4f). IR (KBrν_{max} cm^{-1}): 3125 (C-H-str), 1674 (C=O str), 1524 (C=C str), 1225 (C-O str).

4.2.4. 1,3-Bis-(4-chlorophenyl)-1*H*-pyrazole-4-carbaldehyde (4h). IR (KBrν_{max} cm^{-1}): 3125 (C-H-str), 1684 (C=O str), 1528 (C=C str), 1223 (C-O str).

4.2.5. 3-(4-Bromophenyl)-1-(4-chlorophenyl)-1*H*-pyrazole-4-carbaldehyde (4j). IR (KBrν_{max} cm^{-1}): 3124 (C-H-str), 1682 (C=O str), 1525 (C=C str), 1220 (C-O str);^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 7.48-7.50 (dd, 2H, Ar-H), 7.62-7.64 (dd, 2H, Ar-H), 7.72-7.76 (m, 4H, Ar-H), 8.50 (s, 1H, pyrazole-5H), 10.03 (s, 1H, -CHO); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 184.25, 153.37, 137.38, 133.80, 131.92, 131.84, 130.38, 130.05, 129.85, 123.86, 122.72, 120.81; MS: *m/z* = 361.0 (M^+), ANAL. Calcd.for $C_{16}H_{10}BrClN_2O$; calcd: C, 53.14; H, 2.79; N, 7.75.; found: C, 53.20; H, 2.80; N, 7.72.

4.3. Synthesis of 3-(2-Bromo acetyl)-chromen-2-one (8)

A mixture of salicyladehyde **5** (5.3 ml, 0.05 mol), ethylacetoacetate **6** (6.37 ml, 0.05 mol) in the presence of catalytic amount of piperidine (5% mol) in ethanol (20 ml) was stirred for 1 h at ambient temperature. The yellow colored precipitation was filtered and washed with ethanol to give 3-(2-acetyl)-chromen-2-one **7** as yellow colored solid having melting point of 121-122 °C (lit. range: 120-122 °C).[28] The compound **7** was dissolved in 20 ml of chloroform and added an equivalent amount of bromine in 2 ml chloroform at ambient temperature. The reaction mixture was heated to 60 °C for 1h and cooled to 20 °C to get the solid material. Filter the compound and washed with pre-chilled chloroform to get white to almost white color solid melting at 162-164 °C.[29]

4.3.1 3-(2-Bromo acetyl)-chromen-2-one (8). IR (KBrν_{max} cm^{-1}): 3022 (C-H-str), 1735 (C=O str), 1551 (C=C str), 1176 (C-O str); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 4.76 (s, 2H, -CH_2), 7.37-7.42 (t, 2H, Ar-H, J = 10.6), 7.69-7.71 (d, 2H, Ar-H, J = 7.2 Hz), 8.65 (s, 1H, Ar-H); MS: m/z = 266.9 (M^-).

4.4. 3-{2-[N′-(1,3-Disubstituted-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (10a-l):

A mixture of 1,3-disubstituted pyrazole-4-carbaldehyde (**4a-l**) (0.01 mol), thiosemicarbazide (0.91 g, 0.01 mol) in ethanol (10 ml) were stirred in the presence of sodium acetate (0.82 g, 0.01 mol) at reflux temperature for 5 h. The reaction mass was cooled and quenched into water to get the semicarbazone intermediate (**9a-l**) as a white solid. The compound (**9a-l**) (0.005 mol) was taken in ethanol (10 ml) and added equimolar amount of **8** (1.3 g, 0.005 mol)**.** The reaction mixture was heated at reflux temperature for 3 h and monitored by TLC [Hexane:Ethylacetate (4:1)]. The solid mass was cooled to room temperature and stirred for 0.5 h. The solid product was filtered and washed with pre-chilled ethanol (~10 °C).

4.4.1. 3-{2-[N′-(1,3-Diphenyl-11*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (10a). IR (KBrν_{max} cm^{-1}): 3377 (N-H str), 3143 (Ar-H-str), 1726 (C=O str), 1633 (C=N str), 1501 (C=C str), 1215 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 7.36-7.40 (m, 2H, Ar-H), 7.44-7.50 (m, 2H, Ar-H), 7.52-7.56 (m, 4H, Ar-H), 7.60-7.63 (m, 1H, Ar-H), 7.76 (s, 1H, thiazole-5H), 7.77-7.79 (d, 2H, Ar-H, J = 8.0 Hz), 7.85-7.87 (d, 1H, chromen-2-

one-5H, *J* = 7.8 Hz), 7.98-8.00 (d, 2H, Ar-H, *J* = 8.4 Hz), 8.19 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.92 (s, 1H, pyrazol-5H), 12.03 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 167.89, 159.22, 152.75, 151.36, 144.31, 139.50, 138.60, 135.64, 132.69, 132.14, 130.06, 129.29, 126.06, 128.95, 128.13, 127.35, 125.17, 120.99, 119.66, 119.19, 117.33, 116.34, 110.89; MS: *m/z* = 490.1 (M^+), ANAL. Calcd.for $C_{28}H_{19}N_5O_2S$;calcd: C, 68.70; H, 3.91; N, 14.31; found: C, 68.65; H, 3.90; N, 14.28.

4.4.2. 3-(2-{N′-[1-(4-Chlorophenyl)-3-phenyl-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (10b). IR (KBrν_{max} cm^{-1}): 3412 (N-H str), 3140 (Ar-H-str), 1720 (C=O str), 1627 (C=N str), 1497 (C=C str), 1094 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 7.37-7.41 (t, 1H, chromen-2-one-6H, *J* = 8.0 Hz), 7.44-7.48 (m, 2H, Ar-H), 7.49-7.56 (m, 2H, Ar-H), 7.60-7.65 (m, 3H, Ar-H), 7.76-7.79 (m, 3H, Ar-H), 7.85-7.87 (d, 1H, chromen-2-one-5H, *J* = 9.1 Hz), 8.03-8.05 (d, 2H, Ar-H, *J* = 9.0 Hz), 8.18 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.94 (s, 1H, pyrazol-5H), 12.03 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 167.88, 159.24, 152.75, 151.65, 144.31, 138.63, 138.31, 135.43, 132.49. 132.16, 131.46, 126.97, 129.27, 129.10, 128.95, 128.24, 125.20, 120.96, 120.83, 119.64, 117.66, 116.34, 110.92; MS: *m/z* = 524.0 (M^+), ANAL. Calcd.for $C_{28}H_{18}ClN_5O_2S$; calcd: C, 64.18; H, 3.46; N, 13.37; found: C, 64.21; H, 3.45; N, 13.38.

4.4.3. 3-{2-[N′-(1-phenyl-3-*p*-tolyl-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (10c). IR (KBrν_{max} cm^{-1}): 3443 (N-H str), 3239 (Ar-H-str), 1708 (C=O str), 1601 (C=N str), 1503 (C=C str), 1106 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 2.40 (s, 3H, -CH_3), 7.33-7.41 (m, 4H, Ar-H), 7.44-7.46 (d, 1H, chromen-2-one-8H, *J* = 8.3 Hz), 7.52-7.56 (t, 2H, Ar-H, *J* = 8.0 Hz), 7.60-7.65 (t, 1H, chromen-2-one-7H, *J* = 8.6 Hz), 7.67-7.69 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.76 (s, 1H, thiazole-5H), 7.85-7.87 (d, 1H, chromen-2-one-5H, *J* = 9.1 Hz), 7.98-8.00 (d, 2H, Ar-H, *J* = 7.7 Hz), 8.18 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.88 (s, 1H, pyrazol-5H), 12.01 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ167.91, 159.23, 152.74, 151.38, 144.39, 139.51, 138.58, 138.41, 135.75, 132.13, 130.04, 129.82, 129.28, 128.79, 127.92, 127.28, 125.17, 121.00, 119.65, 119.15, 117.25, 116.34, 110.89, 21.37; MS: *m/z* = 502.2 (M^-), ANAL. Calcd.for $C_{29}H_{21}N_5O_2S$; calcd: C, 69.17; H, 4.20; N, 13.91; found: C, 69.20; H, 4.21; N, 13.92.

4.4.4. 3-(2-{N′-[1-(4-Chlorophenyl)-3-*p*-tolyl-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (10d). IR (KBrν_{max} cm^{-1}): 3413 (N-H str), 3145 (Ar-H-str), 1719 (C=O str), 1631 (C=N str), 1500 (C=C str), 1092 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 2.41 (s, 3H, -CH_3), 7.34-7.36 (d, 2H, Ar-H, J = 7.6 Hz), 7.39-7.41 (t, 1H, chromen-2-one-6H, J = 7.6 Hz), 7.45-7.47 (d, 1H, chromen-2-one-8H, J = 8.4 Hz), 7.59-7.61 (d, 2H, Ar-H, J = 8.0 Hz), 7.63 (t, 1H, chromen-2-one-7H, merged), 7.67-7.69 (d, 2H, Ar-H, J = 7.2 Hz), 7.77 (s, 1H, thiazole-5H), 7.85-7.87 (d, 1H, chromen-2-one-5H, J = 7.6 Hz), 8.03-8.05 (d, 2H, Ar-H, J = 7.6 Hz), 8.18 (s, 1H, -CH=N), 8.54 (s, 1H, chromen-2-one-4H), 8.91 (s, 1H, pyrazol-5H), 12.03 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 167.88, 159.23, 152.77, 151.66, 144.32, 138.60, 138.55, 138.35, 135.57, 132.13, 131.36, 129.94, 129.67, 129.29, 128.80, 128.08, 125.18, 120.99, 120.79, 119.66, 117.57, 116.35, 110.93, 21.39; MS: *m/z* = 538.2 (M^+), ANAL. Calcd.for $C_{29}H_{20}ClN_5O_2S$; calcd: C, 64.74; H, 3.75; N, 13.02; found: C, 64.76; H, 3.75; N, 13.05.

4.4.5. 3-(2-{N′-[3-(4-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (10e). IR (KBrν_{max} cm^{-1}): 3426 (N-H str), 3142 (Ar-H-str), 1719 (C=O str), 1607 (C=N str), 1500 (C=C str), 1106 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 3.84 (s, 3H, -OCH_3), 7.08-7.10 (d, 2H, Ar-H, J = 8.8 Hz), 7.34-7.41 (m, 2H, Ar-H), 7.44-7.46 (d, 1H, chromen-2-one-8H, J = 8.3 Hz), 7.51-7.55 (t, 2H, Ar-H, J = 8.0 Hz), 7.60-7.65 (t, 1H, chromen-2-one-7H, J = 8.6 Hz), 7.72-7.76 (m, 3H, Ar-H), 7.84-7.87 (d, 1H, chromen-2-one-5H, J = 9.2 Hz), 7.97-7.99 (d, 2H, Ar-H, J = 8.7 Hz), 8.17 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.91 (s, 1H, pyrazol-5H), 12.03 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 167.91, 159.98, 159.22, 152.75, 151.19, 144.31, 139.52, 138.84, 132.13, 130.26, 130.04, 129.29, 128.08, 127.21, 125.17, 125.07, 121.01, 119.66, 119.08, 117.05, 116.34, 114.48, 110.92, 55.73; MS: *m/z* = 520.3 (M^+), ANAL. Calcd.for $C_{29}H_{21}N_5O_3S$; calcd: C, 67.04; H, 4.07; N, 13.48; found: C, 67.11; H, 4.08; N, 13.46.

4.4.6. 3-(2-{N′-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (10f). IR (KBrν_{max} cm^{-1}): 3425 (N-H str), 3144 (Ar-H-str), 1716 (C=O str), 1608 (C=N str), 1496 (C=C str), 1097 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 3.85 (s, 3H, -OCH_3), 7.08-7.10 (d, 2H, Ar-H, J = 7.6 Hz), 7.39-7.41 (t, 1H, chromen-2-one-6H, J = 6.8 Hz), 7.45-7.47 (d, 1H, chromen-2-one-8H, J = 8.0 Hz), 7.59-

7.63 (m, 3H, Ar-H), 7.73-7.75 (d, 2H, Ar-H, J = 8.4 Hz), 7.77 (s, 1H, thiazole-5H), 7.85-7.87 (d, 1H, chromen-2-one-5H, J = 7.6 Hz), 8.17 (s, 1H, -CH=N), 8.20-8.40 (d, 2H, Ar-H, J = 8.0 Hz), 8.54 (s, 1H, chromen-2-one-4H), 8.91 (s, 1H, pyrazol-5H), 12.03 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 167.90, 160.08, 159.23, 152.77, 151.47, 144.32, 138.60, 138.37, 135.66, 132.14, 131.28, 130.29, 129.94, 129.29, 128.23, 125.18, 124.90, 121.01, 120.73, 119.67, 117.40, 116.35, 114.52, 110.95, 55.76; MS: m/z =555.3 (M^+), ANAL. Calcd.for $C_{29}H_{20}ClN_5O_3S$; calcd: C, 62.87; H, 3.64; N, 12.64; found: C, 62.88; H, 3.65; N, 12.63.

4.4.7. 3-(2-{N′-[3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (10g). IR (KBrν_{max} cm^{-1}): 3410 (N-H str), 3141 (Ar-H-str), 1719 (C=O str), 1623 (C=N str), 1496 (C=C str), 1095 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 7.39 (m, 2H, Ar-H), 7.45-7.47 (d, 1H, chromen-2-one-8H, J = 8.0 Hz), 7.54-7.57 (t, 2H, Ar-H, J = 7.4 Hz), 7.60-7.61 (m, 3H, Ar-H), 7.77 (s, 1H, thiazole-5H), 7.85-7.89 (t, 3H, Ar-H, J = 8.0 Hz), 7.98-8.00 (d, 2H, Ar-H, J = 7.6 Hz), 8.19 (s, 1H, -CH=N), 8.54 (s, 1H, chromen-2-one-4H), 8.93 (s, 1H, pyrazol-5H), 12.05 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 167.89, 159.22, 152.78, 149.91, 139.42, 138.62, 135.46, 133.71, 132.16, 131.68, 130.73, 130.10, 129.30, 129.04, 127.48, 125.19, 121.01, 119.67, 119.22, 117.43, 116.36, 110.99; MS: m/z = 524.3 (M^+), ANAL. Calcd.for $C_{28}H_{18}ClN_5O_2S$; calcd: C, 64.18; H, 3.46; N, 13.37; found: C, 64.21; H, 3.46; N, 13.38.

4.4.8. 3-(2-{N′-[1,3-Bis-(4-Chlorophenyl)-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (10h). IR (KBrν_{max} cm^{-1}): 3419 (N-H str), 3145 (Ar-H-str), 1717 (C=O str), 1623 (C=N str), 1496 (C=C str), 1095 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 7.39-7.41 (t, 1H, chromen-2-one-6H, J = 6.0 Hz), 7.44-7.46 (d, 1H, chromen-2-one-8H, J = 8.4 Hz), 7.60-7.62 (m, 5H, Ar-H), 7.77 (s, 1H, thiazole-5H), 7.86 (m, 3H, Ar-H), 8.01-8.04 (d, 2H, Ar-H, J = 8.4 Hz), 8.17 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.96 (s, 1H, pyrazol-5H), 12.05 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 167.84, 159.21, 152.77, 150.16, 144.32, 138.60, 138.22, 135.22, 133.81, 132.14, 131.55, 131.48, 130.73, 129.99, 129.28, 129.14, 129.05, 125.17, 120.99, 120.81, 119.65, 117.72, 116.35, 110.99; MS: m/z = 558.2 (M^+), ANAL. Calcd.for $C_{28}H_{17}Cl_2N_5O_2S$; calcd: C, 60.22; H, 3.07; N, 12.54; found: C, 60.19; H, 3.06; N, 12.55.

4.4.9. 3-(2-{N′-[3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (10i). IR (KBrν_{max} cm^{-1}): 3427 (N-H str), 3153 (Ar-H-str), 1720 (C=O str), 1608 (C=N str), 1499 (C=C str), 1099 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 7.39 (m, 2H, Ar-H), 7.45-7.47 (d, 1H, chromen-2-one-8H, J = 7.6 Hz), 7.54-7.55 (t, 2H, Ar-H, J = 6.8 Hz), 7.61-7.65 (t, 1H, chromen-2-one-7H, J = 7.2 Hz), 7.73-7.75 (d, 2H, Ar-H, J = 8.0 Hz), 7.77 (s, 1H, thiazole-5H), 7.81-7.83 (d, 2H, Ar-H, J = 8.0 Hz), 7.85-7.87 (d, 1H, chromen-2-one-5H, J = 6.8 Hz), 7.98-8.00 (d, 2H, Ar-H, J = 7.2 Hz), 8.18 (s, 1H, -CH=N), 8.54 (s, 1H, chromen-2-one-4H), 8.93 (s, 1H, pyrazol-5H), 12.06 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 166.80, 158.14, 151.70, 148.87, 143.24, 138.34, 137.54, 134.38, 131.07, 130.95, 130.88, 129.92, 129.02, 128.22, 127.98, 126.40, 124.10, 121.29, 119.92, 118.59, 118.14, 116.35, 115.28, 109.90; MS: *m/z* = 568.2 (M$^+$), ANAL. Calcd.for $C_{28}H_{18}BrN_5O_2S$; calcd: C, 59.16; H, 3.19; N, 12.32; found: C, 59.17; H, 3.20; N, 12.31.

4.4.10. 3-(2-{N′-[3-(4-Bromophenyl)-1-(4-chlorophenyl)-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (10j). IR (KBrν_{max} cm^{-1}): 3418 (N-H str), 3145 (Ar-H-str), 1717 (C=O str), 1624 (C=N str), 1496 (C=C str), 1097 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 7.37-7.41 (t, 1H, chromen-2-one-6H, J = 8.0 Hz), 7.44-7.46 (d, 1H, chromen-2-one-8H, J = 8.2 Hz), 7.60-7.65 (m, 3H, Ar-H), 7.72-7.74 (d, 2H, Ar-H, J = 8.6 Hz), 7.76 (s, 1H, thiazol-5H), 7.79-7.81 (d, 2H, Ar-H, J = 8.6 Hz), 7.84-7.87 (d, 1H, chromen-2-one-5H, J = 9.1 Hz), 8.01-8.03 (d, 2H, Ar-H, J = 8.9 Hz), 8.16 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.96 (s, 1H, pyrazol-5H), 12.06 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 167.83, 159.20, 152.76, 150.21, 144.32, 138.59, 138.22, 135.19, 132.13, 131.97, 131.82, 131.56, 130.98, 129.99, 129.28, 129.14, 125.17, 122.48, 120.98, 120.82, 119.65, 117.70, 116.34, 111.00; MS: *m/z* = 602.2 (M$^+$), ANAL. Calcd.for $C_{28}H_{17}BrClN_5O_2S$; calcd: C, 55.78; H, 2.84; N, 11.62; found: C, 55.80; H, 2.85; N, 11.61.

4.4.11. 3-(2-{N′-[3-(4-Fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl-methylene]-hydrazino}-thiazol-4-yl}-chromen-2-one (10k). IR (KBrν_{max} cm^{-1}): 3414 (N-H str), 3138 (Ar-H-str), 1723 (C=O str), 1605 (C=N str), 1501 (C=C str), 1099 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 7.38-7.40 (m, 4H, Ar-H), 7.45-7.47 (d, 1H, chromen-2-one-8H, J = 8.4 Hz), 7.54-7.56 (t, 2H, Ar-H, J = 7.6 Hz), 7.62-7.66 (t, 1H, chromen-2-one-7H, J = 7.4 Hz), 7.76 (s, 1H, thiazole-5H), 7.87-7.88 (m, 3H, Ar-H), 7.98-8.00 (d, 2H, Ar-H, J = 7.2 Hz), 8.18 (s, 1H, -

CH=N), 8.54 (s, 1H, chromen-2-one-4H), 8.92 (s, 1H, pyrazol-5H), 12.04 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ167.92, 159.23, 152.78, 150.26, 144.34, 139.46, 138.61, 135.53, 131.13, 130.09, 129.30, 128.81, 127.39, 125.19, 121.02, 119.68, 119.17, 117.28, 116.36, 116.03, 115.81, 110.96; MS: *m/z* = 508.2 (M^+), ANAL. Calcd.for $C_{28}H_{18}FN_5O_2S$; calcd: C, 66.26; H, 3.57; N, 13.80; found: C, 66.31; H, 3.59; N, 13.82.

4.4.12. 3-(2-{N′-[1-(4-Chlorophenyl)-3-(4-fluorophenyl)-1*H*-pyrazol-4-yl-methylene)-hydrazino]-thiazol-4-yl}-chromen-2-one (10l). IR (KBrν_{max} cm^{-1}): 3417 (N-H str), 3173 (Ar-H-str), 1719 (C=O str), 1623 (C=N str), 1497 (C=C str), 1094 (C-O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 7.35-7.41 (m, 3H, Ar-H), 7.44-7.46 (d, 1H, chromen-2-one-8H, *J* = 8.3 Hz), 7.59-7.61 (d, 2H, Ar-H, *J* = 6.6 Hz), 7.62-7.65 (t, 1H, chromen-2-one-6H, *J* = 6.3 Hz), 7.76 (s, 1H, thiazol-5H), 7.84-7.88 (m, 3H, Ar-H,), 8.01-8.03 (d, 2H, Ar-H, *J* = 9.0 Hz), 8.15 (s, 1H, -CH=N), 8.53 (s, 1H, chromen-2-one-4H), 8.95 (s, 1H, pyrazol-5H), 12.04 (s, 1H, -NH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 166.81, 158.30, 154.19, 151.70, 149.45, 143.29, 140.73, 137.77, 137.26, 134.31, 131.08, 130.41, 130.15, 130.06, 128.92, 124.11, 119.92, 119.73, 118.79, 116.51, 115.28, 114.98, 114.77, 109.91; MS: *m/z* = 542.1 (M^+), ANAL. Calcd.for $C_{28}H_{17}ClFN_5O_2S$; calcd: C, 62.05; H, 3.16; N, 12.92; found: C, 62.06; H, 3.16; N, 12.89.

5. Antibacterial and antifungal studies

Antimicrobial screening for the newly synthesized compounds **10a-10l** were determined by the nutrient plate well diffusion method using a protocol explained elsewhere.[30] Antibacterial activity against 12 h old cultures of Gram-positive *Staphylococcus aureus* (MTCC 3160) and Tuberculosis variant bacteria *Mycobacterium smegmatis* (MTCC 994) was determined by inhibition method. Antifungal activity of these compounds was also carried out against pathogenic fungi *Candida albicans* (MTCC 7253). All the bacterial and fungal cultures were purchased from the microbial type culture collection, IMTECH, Chandigarh, India and maintained the cultures as per the standard protocol. Nutrient agar plates were prepared and 100 µl of 0.5 McFarland standard of microbial culture was spread over agar medium. Using a sterile cork borer 5 mm wells were made in the agar media. Working solution of the compounds was prepared in DMSO and 10-20 mg/ml solution was used for the test depending on the solubility of the compound. Test compound volume of 50, 25 and 12.5µl was transferred to separate wells in triplicates. Then agar plates were incubated in an incubator at 37 °C for 12 hrs and observed in

the zone of inhibition. DMSO was used as negative control. Ciprofloxacin and Flucanazole were used as antibacterial and antifungal standards respectively. Inhibition zone was measured in millimeter using a scale.

6. Minimum Inhibitory Concentration (MIC)

All the synthetic thiazole compounds showed substantial inhibition of both bacteria and fungi, were tested further for the Minimum Inhibitory Concentration (MIC). Standard pathogenic bacterial strains *Mycobacterium smegmatis, Pseudomonas aeruginosa* (MTCC 2640) and Gram positive *Staphylococcus aureus* were used in the MIC of bacteria. The MIC for fungal strain *Candida albicans* was also determined. The Resazurin reduction method was used for determining the MIC in 96 well microplates.[31, 32] All the compounds were dissolved in DMSO having a final stock concentration of 10 mg/ml. Fifty microlitres of this stock solution was serially diluted to eight times and 50 µL of each serially diluted compound was added to microplate wells. All the microbial cultures were grown in nutrient agar to reach 0.5 McFarland concentrations and 50 µL of this culture was added to each well. Microplate contents were mixed well and incubated at room temperature for 12 hours. After 12 hrs of incubation, 10 µL of mixture from each well was spread on the agar plate and checked for the Colony Forming Units (CFU). Further 30 µL of 0.1 % Resazurin solution was added to each well and incubated up to another 24 hours. Microplate well contents were observed in the change in colour from blue to pink. Those wells that have microbes growing will change the blue Resazurin into pink color. The well, which remains blue after 24 hours of incubation indicates there are no microorganisms survived in the well, the minimum concentration where no microbial growth found are considered as MIC value.

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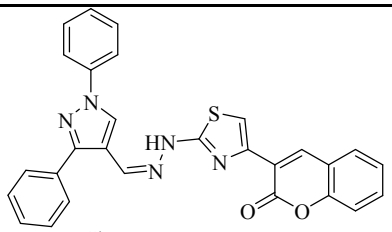
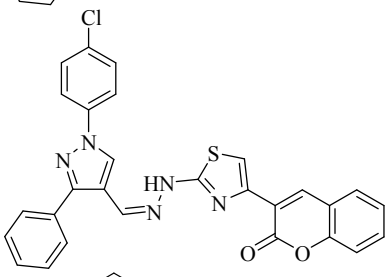
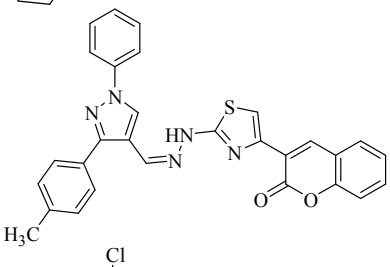
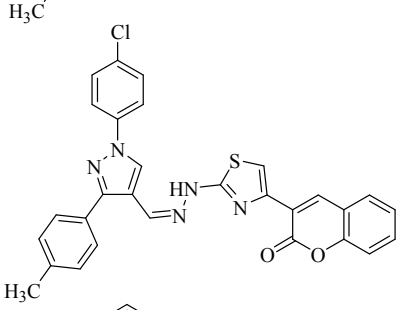
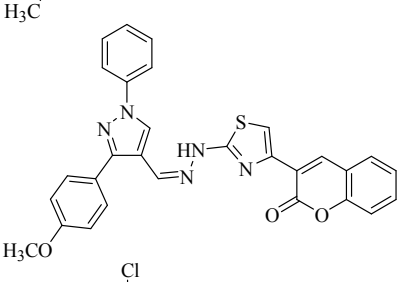
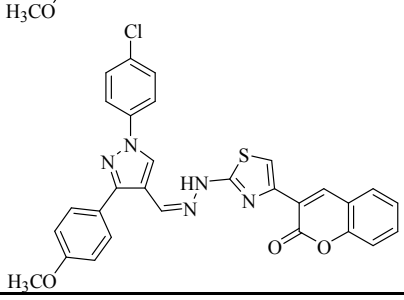
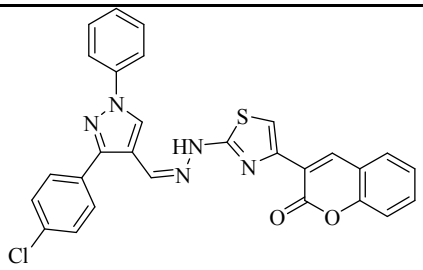
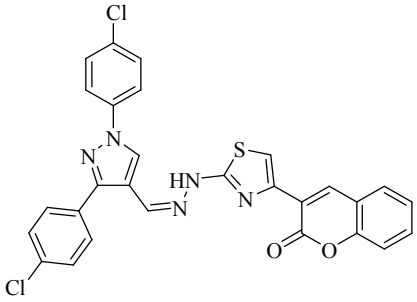
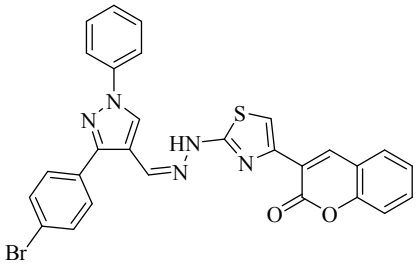
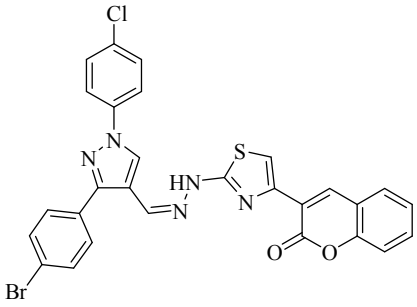
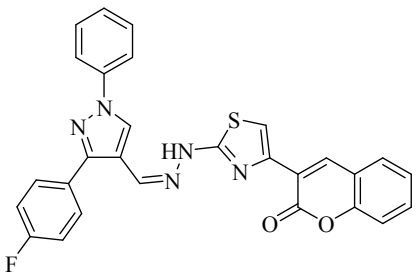
Table 1 Characterization data of the compounds **4a-l**.

Comp. no	R	X	M.F	M.Wt	M.p (°C)	Yield (%)
4a	H	H	$C_{16}H_{12}N_2O$	248.28	144-146 [22]	87
4b	H	Cl	$C_{16}H_{11}ClN_2O$	282.72	145-146	91
4c	CH_3	H	$C_{17}H_{14}N_2O$	262.31	121-123	88
4d	CH_3	Cl	$C_{17}H_{13}ClN_2O$	296.75	133-134[23]	90
4e	OCH_3	H	$C_{17}H_{14}N_2O_2$	278.31	137-137	76
4f	OCH_3	Cl	$C_{17}H_{13}ClN_2O_2$	312.75	142-143[23]	89
4g	Cl	H	$C_{16}H_{11}ClN_2O$	282.72	139-140 [24]	89
4h	Cl	Cl	$C_{16}H_{10}Cl_2N_2O$	317.17	177-178	85
4i	Br	H	$C_{16}H_{11}BrN_2O$	327.18	139-140	69
4j	Br	Cl	$C_{16}H_{10}BrClN_2O$	361.62	156-157	79
4k	F	H	$C_{16}H_{11}FN_2O$	266.27	157-159	77
4l	F	Cl	$C_{16}H_{10}ClFN_2O$	300.71	204-205	66

Table 2 Characterization data of the compounds **10a-l**

Compounds	R/X	Structure of derivative	M.F/ M.Wt	M.P (°C)	Color & nature
10a	H/H		$C_{28}H_{19}N_5O_2S$/ 489.55	188-190 (decomp.)	Yellow solid
10b	H/Cl		$C_{28}H_{18}ClN_5O_2S$/ 523.99	242-244 (decomp.)	Yellow solid
10c	CH_3/H		$C_{29}H_{21}N_5O_2S$/ 503.57	224-226 (decomp.)	Yellow solid
10d	CH_3/Cl		$C_{29}H_{20}ClN_5O_2S$/ 538.02	238-240 (decomp.)	Yellow solid
10e	OCH_3/H		$C_{29}H_{21}N_5O_3S$/ 519.57	225-227 (decomp.)	Yellow solid
10f	OCH_3/Cl		$C_{29}H_{20}ClN_5O_3S$/ 554.02	242-245 (decomp.)	Yellow solid

10g	Cl/H		$C_{28}H_{18}ClN_5O_2S$/ 523.99	243-245 (decomp.)	Reddish yellow solid
10h	Cl/Cl		$C_{28}H_{17}Cl_2N_5O_2S$/ 558.44	249-251 (decomp.)	Yellow solid
10i	Br/H		$C_{28}H_{18}BrN_5O_2S$/ 568.44	234-236 (decomp.)	Reddish yellow solid
10j	Br/Cl		$C_{28}H_{17}BrClN_5O_2S$/ 602.89	249-251 (decomp.)	Yellow solid
10k	F/H		$C_{28}H_{18}FN_5O_2S$/ 507.54	232-235 (decomp.)	Reddish yellow solid

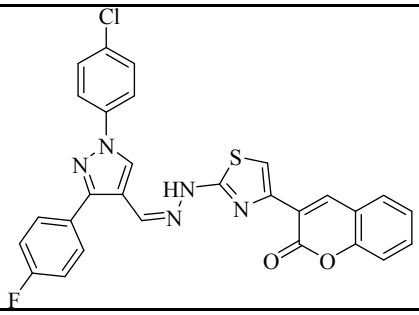
10l	F/Cl		$C_{28}H_{17}ClFN_5O_2S$/ 541.98	252-255 (decomp.)	Yellow solid

Table 3: Antimicrobial activity of the compounds **10a-l** using well diffusion assay

Synthesized Compounds	Inhibition Zone in mm; Mean±SD								
	Mycobacterium smegmatis			*Staphylococcus aureus*			*Candida albicans*		
	50 µL	**25 µL**	**12.5µL**	**50 µL**	**25 µL**	**12.5µL**	**50 µL**	**25 µL**	**12.5µL**
10a	22.67±1.15	20.00±1.00	16.00±0.00	18.33±0.58	15.67±0.58	14.00±0.00	9.67±0.58	--	--
10b	47.00±1.00	38.67±1.15	29.33±1.15	32.67±0.58	28.33±0.58	26.00±0.00	28.67±0.58	27.67±0.58	27.67±0.58
10c	49.00±1.00	44.67±1.15	42.33±2.08	31.67±0.58	24.33±0.58	22.33±0.58	29.33±0.58	27.33±0.58	27.33±0.58
10d	29.00±1.00	27.00±1.00	25.00±1.00	30.33±0.58	27.33±1.15	22.33±0.58	12.33±0.58	10.67±0.58	10.67±0.58
10e	33.67±1.53	30.00±2.00	25.00±1.00	24.33±0.58	22.00±1.00	20.37±0.58	17.67±0.58	12.50±0.50	12.50±0.50
10f	34.33±0.58	31.00±1.00	27.00±1.00	27.33±0.58	21.67±0.58	19.67±0.58	13.67±0.58	12.67±0.58	12.67±0.58
10g	25.00±1.00	23.67±0.58	20.00±1.00	23.00±1.00	19.83±0.76	19.00±0.00	19.67±0.58	16.67±0.58	16.67±0.58
10h	46.67±0.58	42.33±0.58	38.67±0.58	34.00±1.00	30.67±0.58	29.33±0.58	28.33±0.58	26.67±0.58	26.67±0.58
10i	49.00±1.00	47.33±0.58	40.67±1.53	32.00±1.00	29.33±0.58	27.00±1.00	29.00±1.00	26.67±0.58	26.67±0.58
10j	46.67±0.58	40.00±1.00	31.67±0.58	30.67±0.58	27.67±1.15	25.67±0.58	13.00±0.00	11.67±0.58	11.67±0.58
10k	40.33±1.15	34.67±0.58	31.33±0.58	33.00±1.00	30.67±0.58	28.33±1.15	29.37±1.15	26.67±0.58	26.67±0.58
10l	34.33±0.58	32.33±0.58	27.67±0.58	26.67±0.58	24.67±0.58	22.33±0.58	15.67±0.58	13.67±0.58	13.67±0.58
Control	00	00	00	00	00	00	00	00	00
ABS/AFS		46.67±0.58			33.33±0.58			30.27±1.55	

ABS; antibacterial standard Ciprofloxacin; **AFS;** anti-fungal standard Fluconazole; **--:** not detected inhibition; **control;** dimethylsulfoxide

Table 4: The Minimum Inhibitory Concentration (MIC) for the compounds **10 a-l**

	MIC in µg/mL			
Compound	*S. aureus*	*P. aeruginosa*	*M. smegmatis*	*C. albicans*
10a	31.25	62.5	62.5	31.25
10b	31.25	15.6	15.6	15.6
10c	31.25	15.6	15.6	15.6
10d	62.5	500	31.25	15.6
10e	15.6	62.5	15.6	15.6
10f	62.5	>500	62.5	31.25
10g	62.5	62.5	62.5	31.25
10h	15.6	15.6	31.25	31.25
10i	15.6	15.6	31.25	31.25
10j	31.25	62.5	62.5	62.5
10k	31.25	15.6	31.25	15.6
10l	62.5	125	62.5	62.5
ABS	<5	<5	<5	--
AFS	--	--	--	<10

ABS; antibacterial standard Ciprofloxacin; AFS; anti-fungal standard Fluconazole; --: not detected inhibition; control; dimethylsulfoxide

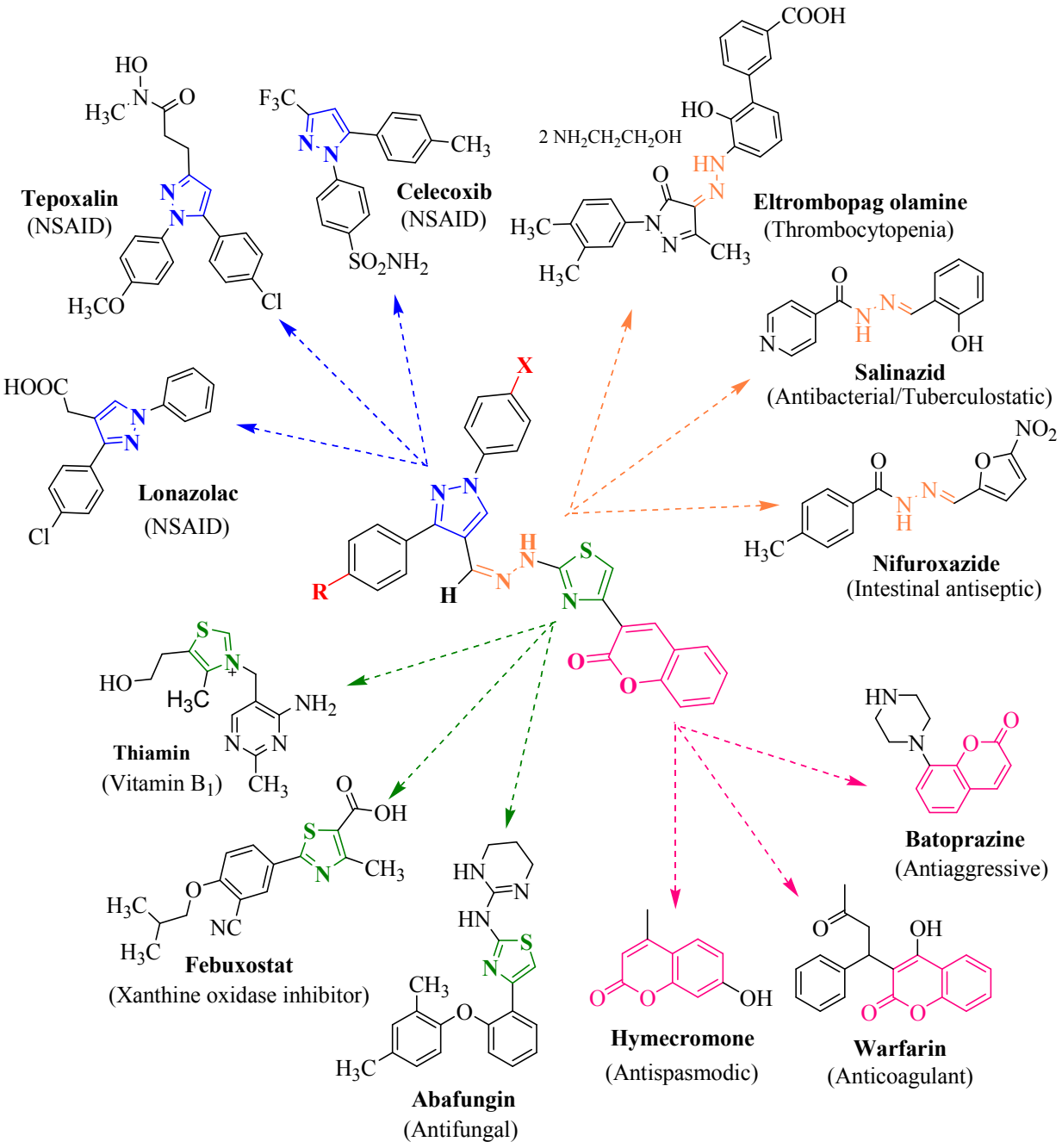


Figure 1: Structural importance of few of the commercial drug molecules

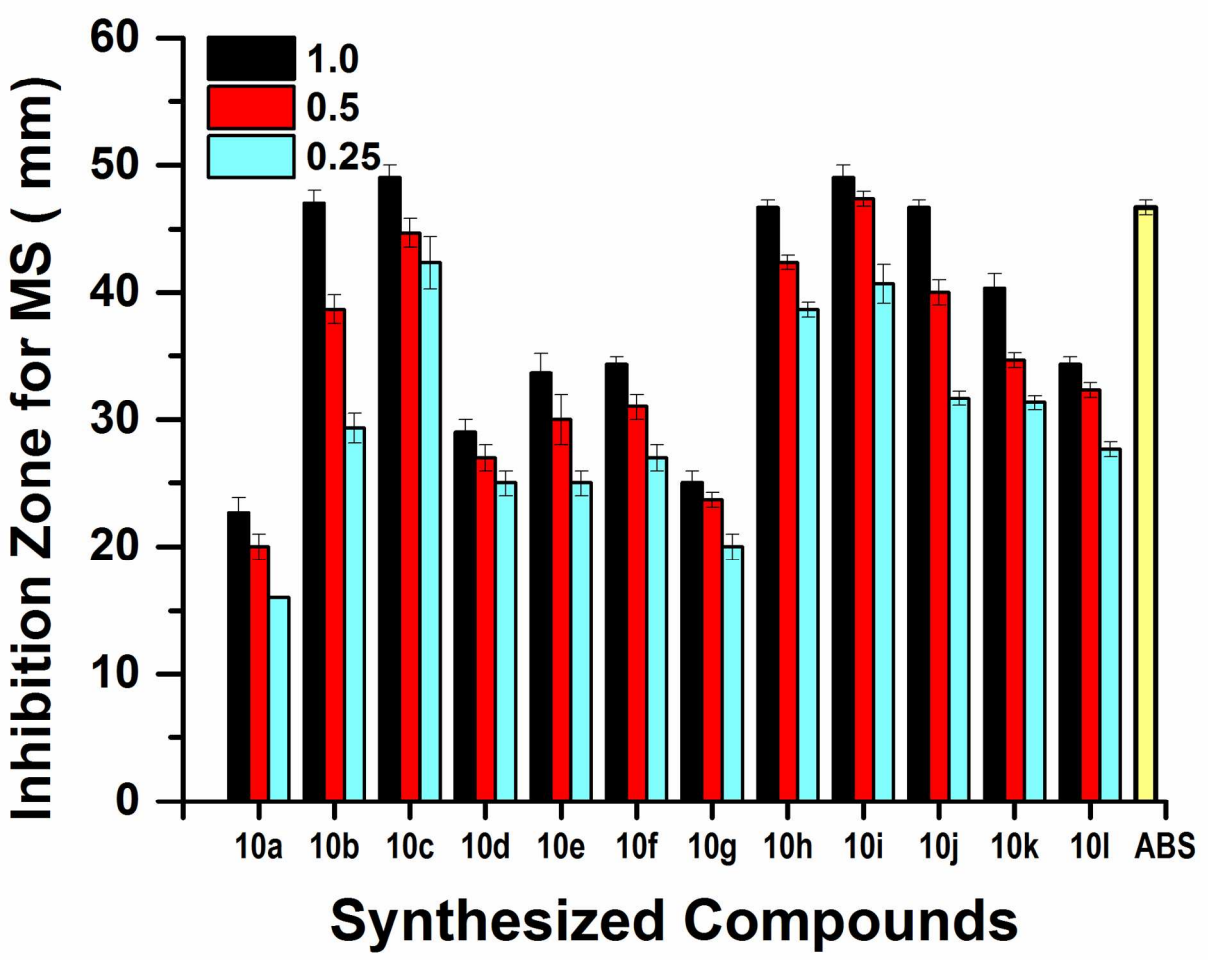


Figure 2: Anti-bacterial activity of pyrazole containing thiazole derivatives against *Mycobacterium smegmatis* (MS) by the well diffusion method. Three different concentrations (1, 0.5 and 0.25 mg/ml) of compound was tested against the *M.smegmatis* in the well diffusion method along with the standard antibacterial compound Ciprofloxacin (ABS) at 10 mg/mL concentration.

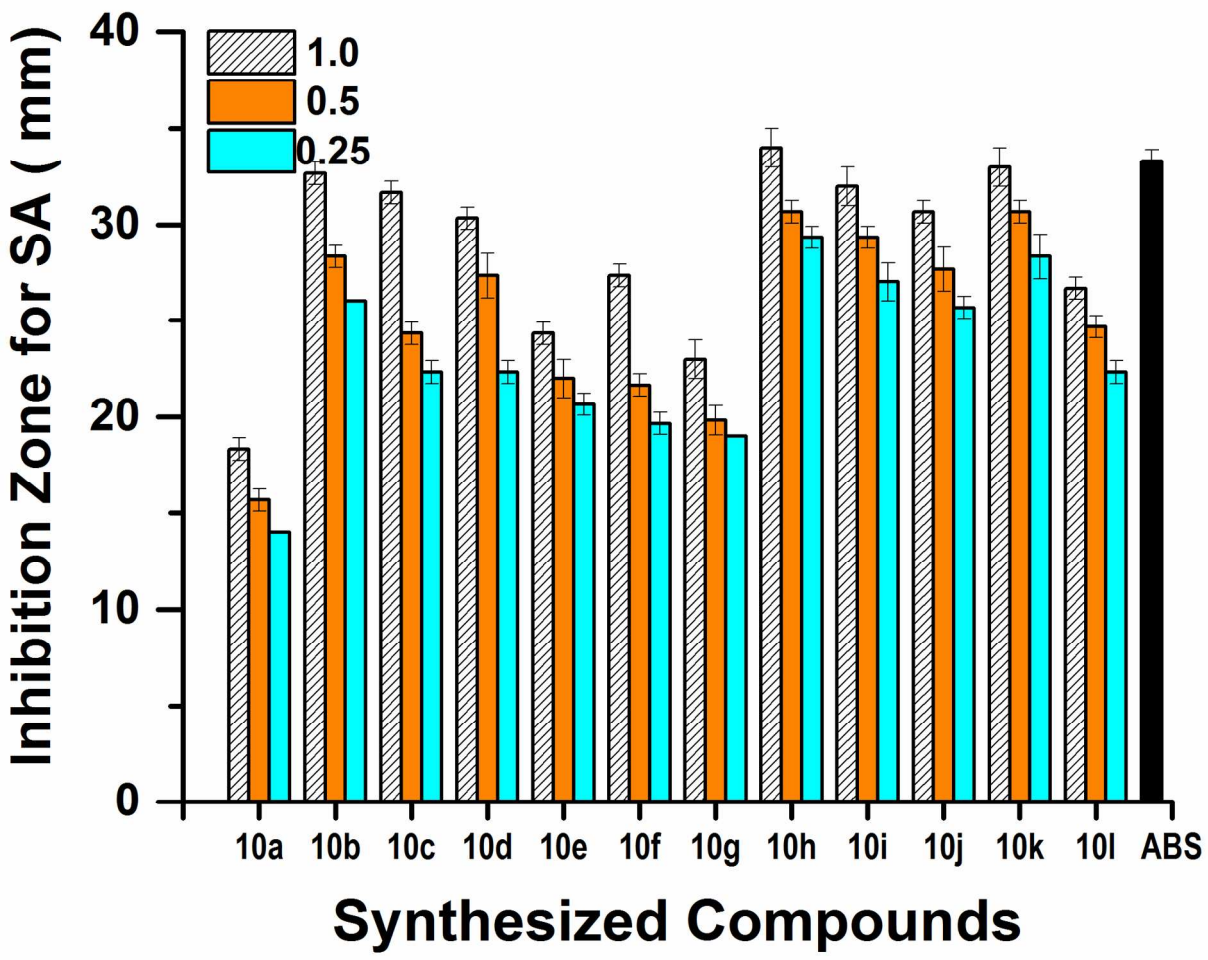


Figure 3: Anti-bacterial activity of pyrazole containing thiazole derivatives against *Staphylococcus aureus* (SA) by the well diffusion method. Three different concentrations (1, 0.5 and 0.25 mg/ml) of compound were tested against the *S. aureus* by well diffusion method along with the standard antibacterial compound Ciprofloxacin (ABS) at 10 mg/mL concentration.

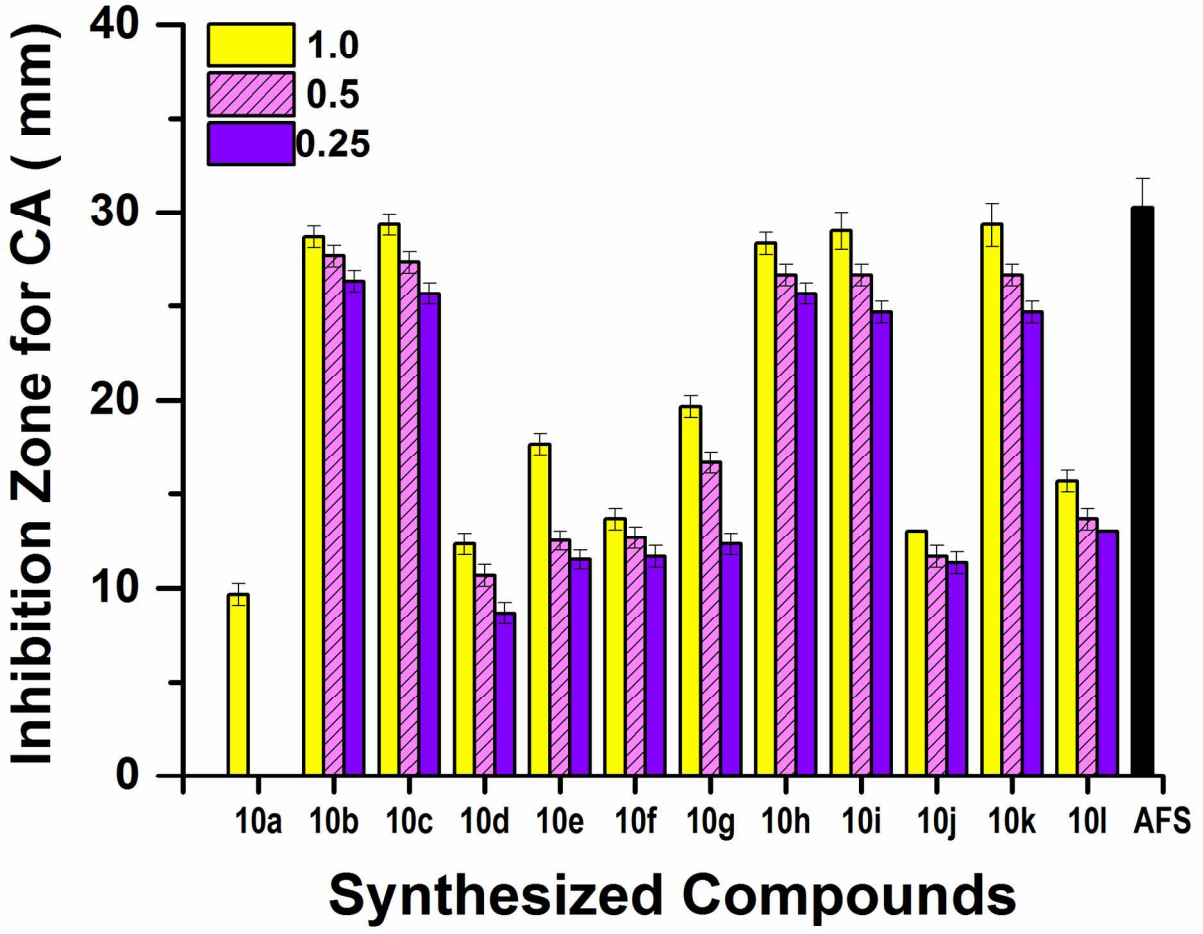


Figure 4: Anti-fungal activity of pyrazole containing thiazole derivatives against *Candida albicans* (CA) by the well diffusion method. Three different concentrations (1, 0.5 and 0.25 mg/ml) of compound were tested against the *Candida albicans* by the well diffusion method along with the standard antifungal compound Fluconazole (AFS) at 10 mg/mL concentration.

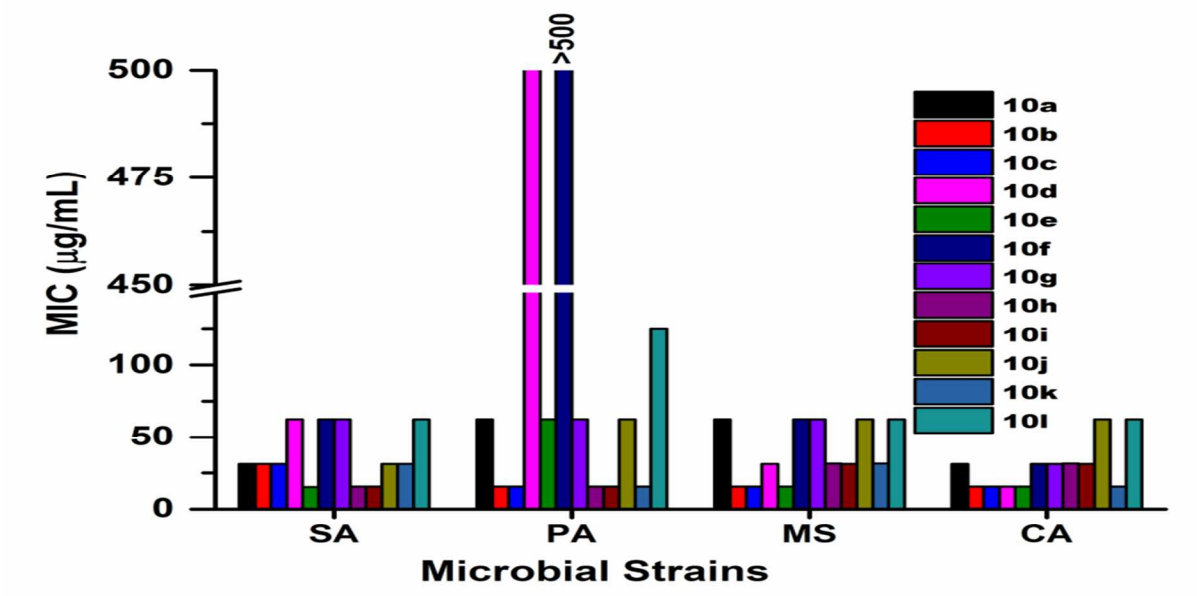
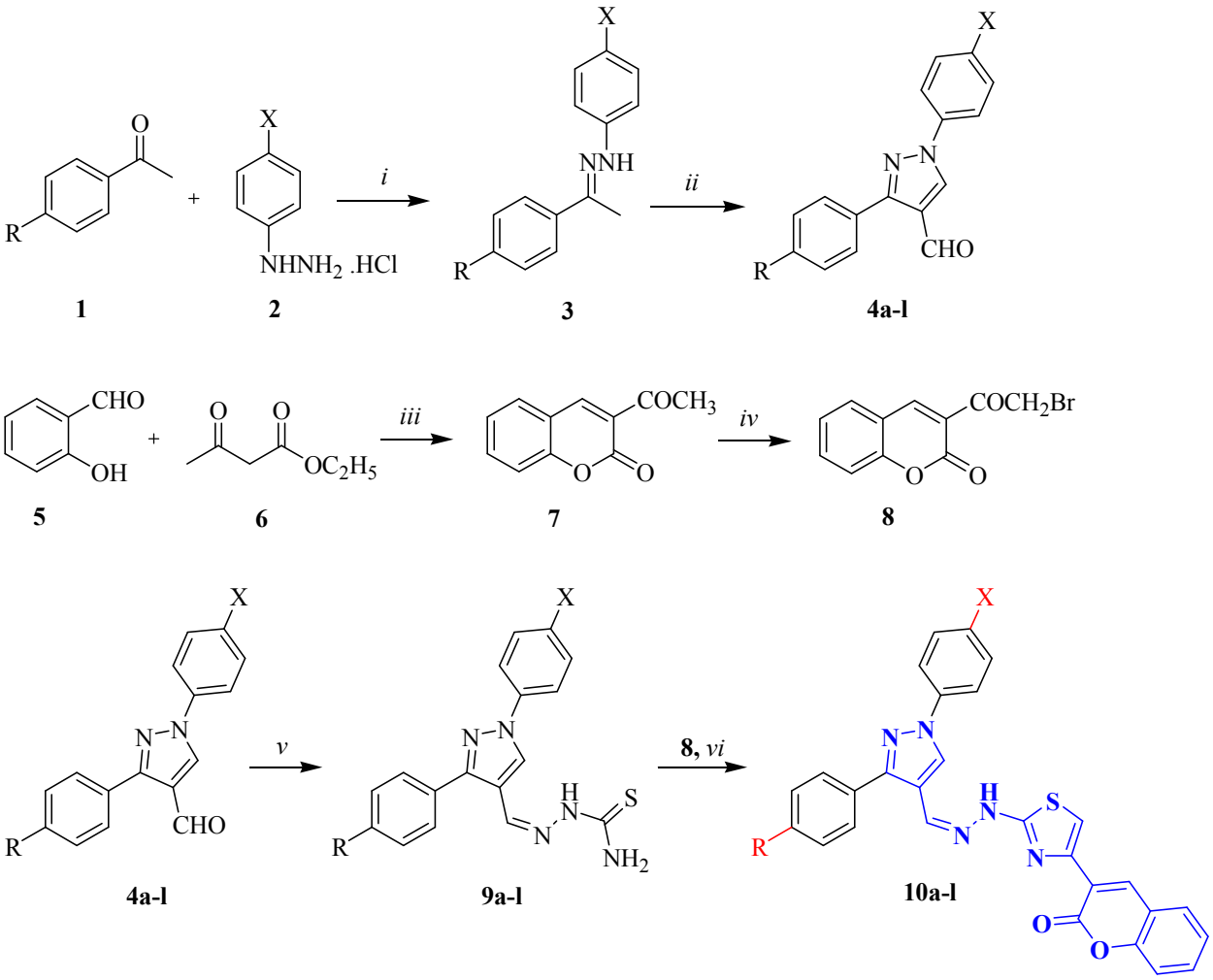


Figure 5: Minimum Inhibitory Concentration (MIC) of pyrazole containing thiazole derivatives against microorganisms. MIC for serially diluted compounds were tested for three bacteria SA, *Staphylococcus aureus*; PA, *Pseudomonas aeruginosa* and MS, *Mycobacterium smegmatis* and fungi CA, *Candida albicans.*



Where, R= H, CH_3, OCH_3, Cl, Br, F and X= H, Cl

i= AcOH, sodium hydroxide sol., RT, 1 h; *ii*= DMF, $POCl_3$, 70 ^oC, 3 h; *iii*= EtOH, piperidine, RT, 1 h; *iv*= $CHCl_3$, Br_2, 60 ^oC, 1 h; *v*= Thiosemicarbazide, EtOH, NaOAc, reflux, 5 h; *vi*= EtOH, reflux, 3 h.

Scheme 1: Schematic route for the synthesis of pyrazole bearing thiazole derivatives