Synthesis, Nematicidal and Antimicrobial Properties of Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-methylphenyl)-2(aryl)-tetrahydro-2*H*-pyrazolo[3,4-*d*]thiazol-5-yl]phenyl]methanes

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A new series of bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-methylphenyl)-2-(aryl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methanes 6a—r was synthesized by the reaction of arylidine derivative of methylene-bis-thiazolidinones 5a—c with aryl/alkyl hydrazines. Chemical structures of all the new compounds were established by IR, ^{1}H-NMR, ^{13}C-NMR, MS and elemental data. The compounds 6a—r were evaluated for their nematicidal activity against *Ditylenchus myceliophagus* and *Caenorhabdites elegans* by aqueous *in vitro* screening technique. Amongst them compounds containing *N*-benzylpyrazole moiety (6d, 6j, 6p), and *N*-methylpyrazole moiety (6f, 6l, 6r) showed significant nematicidal activity against both the test nematodes with LD_{50} 160—210 ppm, almost equal to the oxamyl standard. Further, these compounds 6a—r were screened for their antibacterial (MZI, MIC and MBC) activity against three representative Gram-positive bacteria *viz. Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 11), *Staphylococcus aureus* (MTCC 96) and three Gram-negative bacteria *viz. Pseudomonas aeruginosa* (MTCC 741), *Klebsiella aerogenes* (MTCC 39), *Chromobacterium violaceum* (MTCC 2656) and also screened for their antifungal (MZI, MIC and MFC) activity against four fungal organisms *viz. Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185) and *Trichophyton mentagrophytes* (IFO 40996). Most of these new compounds showed appreciable activity against test bacteria and fungi, and emerged as potential molecules for further development.

Key words pyrazolo[3,4-*d*]thiazole; nematicidal activity; antimicrobial activity

Thiazoles are familiar group of heterocyclic compounds possessing a wide variety of biological activities and their utility as medicine is very much established.[1,2] Thiazole nucleus is also an integral part of all the available penicillins which have revolutionized the therapy of bacterial diseases.[3—5] Further, there has been considerable interest in the chemistry of thiazolidin-4-one ring system, which is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activities.[6—8] Thiazolidin-4-one ring also occurs in nature; thus actithiazic acid [(−)2-(5-carboxypentyl)thiazolidin-4-one] isolated from *Streptomyces* strains exhibits highly specific *in vitro* activity against *Mycobacterium tuberculosis*.[9,10] Thiazolidin-4-one derivatives are known to exhibit diverse bioactivities such as anticonvulsant,[11] antidiarrheal,[12] anti-platelet activating factor,[13—15] antihistaminic,[16,17] antimicrobial,[18,19] antidiabetic,[20] cyclooxygenase (COX) inhibitory,[21] Ca^{2+} channel blocker,[22] platelet activating factor (PAF) antagonist,[23] cardioprotective,[24] anti-ischemic,[25] anticancer,[26] tumor necrosis factor-α antagonist[27] and nematicidal activities.[28]

Pyrazole and their derivatives could be considered as possible antimicrobial agents.[29—31] The other activities include antidepressant,[32] inhibitors of protein kinases,[33] antiaggregating,[34] antiarthritic[35] and cerebroprotectors.[36] Some aryl pyrazoles[37] were reported to have non-nucleoside human immunodeficiency virus-1 (HIV-1) reverse transcriptase inhibitory,[38] COX-2 inhibitory[39—41] activator of the nitric oxide receptor and soluble guanylate cyclase activity.[42]

Nematodes are tiny worms, some of them are plant parasites, and can play an important role in the predisposition of the host plant to the invasion by secondary pathogens.[43] Plants attacked by nematodes show retarded growth and development, as well as loss in the quality and quantity of the harvest. The nematicide use is slated for reduction due to environmental problems, and human and animal health concerns. For example, effective nematicides such as dibromochloropropane (DBCP) and ethylenedibromide (EDB) have been withdrawn from the market due to their deleterious effects on humans and the environment. Methyl bromide, the most effective and widely used fumigant for soil borne pests, including nematodes, has already been banned. The use of nonfumigant nematicides, based on organophosphates and carbamates, is expected to increase the withdrawal of methyl bromide, which will bring about new environmental concerns. In fact, the highly toxic Aldicarb used to control insects and nematodes has been detected in ground water.[44] Therefore, alternative nematode control methods or less toxic nematicides need to be developed.[45] One way of searching for such nematicidal compounds is to screen naturally occurring compounds in plants. Several such compounds, *e.g.* alkaloids, phenols, sesquiterpenes, diterpenes, polyacetylenes and thienyl derivatives have nematicidal activity.[46] For example, α-terthienyl is a highly effective nematicidal compound.[47] Other compounds with nematicidal activity have been isolated from plants, mainly from the family Asteraceae.[46] However, compounds of plant origin and their analogs have not been developed into commercial nematicides, hence there is a need to develop commercial synthesis.

Following the successful introduction of antimicrobial and nematicidal agents, inspired by the biological profile of thiazoles/thiazolidinones, pyrazoles, and in the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores in one frame, may lead to compounds with interesting biological profiles, it was thought of interest to accommodate thiazolidinone and pyrazole moieties in a single molecular frame work to synthesize the bis-compounds for enhancing biological activity. A few examples of pyrazolothiazoles were reported in the

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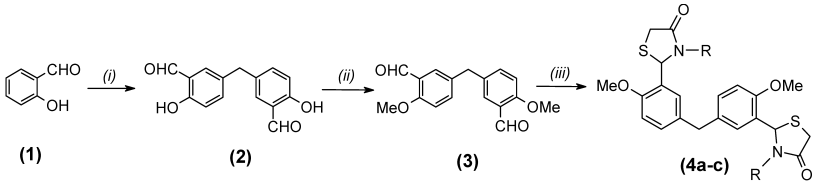
literature,[48—50] however, no detailed design and study of biological properties specially on nematicidal activity was made. This fact aroused our interest to design and synthesize some of the interesting and new bis-pyrazolothiazoles from bis-salicylaldehyde and to evaluate their nematicidal activity along with antimicrobial activities.

We have reported some of our work on the synthesis, transformations and biological properties of various heterocycles derived form bis-salicylaldehyde, some of these compounds were screened for their antimicrobial activities, and has been found that some of them showed positive fungicidal and bactericidal activity.[51—54] The biological significance of such compounds impelled us to continue working on the synthesis of new and novel heterocycles. For this purpose we use bis-salicylaldehyde as a suitable starting material. The present investigation deals with the use of bis-salicylaldehyde in the synthesis of some of the interesting bis-pyrazolothiazoles of expected pharmacological action and to study their effect on nematodes, bacteria and fungi.

Results and Discussion

Synthesis Condensation of salicylaldehyde **1** with trioxane in the presence of a mixture of concentrated sulphuric acid and acetic acid gave methylene-bis-salicylaldehyde **2** in good yield.[55] Compound **2** was on reaction with methyl iodide in the presence of K_2CO_3 in *N*,*N*-dimethylformamide (DMF) at room temperature furnished 5-(3-formyl-4-methoxybenzyl)-2-methoxybenzaldehyde **3**. The one-pot synthesis of methylene-bis-thiazolidinone derivatives **4a**—**c** was carried out by the condensation-cyclization reaction between compound **3**, primary aromatic amine and mercaptoacetic acid in the presence of $ZnCl_2$ under reflux or microwave irradiation conditions (Chart 1). In the "classical" method, the reactions were performed in dry toluene at reflux for a long time (2—4 h), often leading to degradation processes and consequent low yields of isolated products, whereas with the application of microwave assisted technology, the reactions were completed within 4—7 min and the compounds, isolated by conventional work-up, obtained in satisfactory yields, often higher than those achieved by the traditional methods.

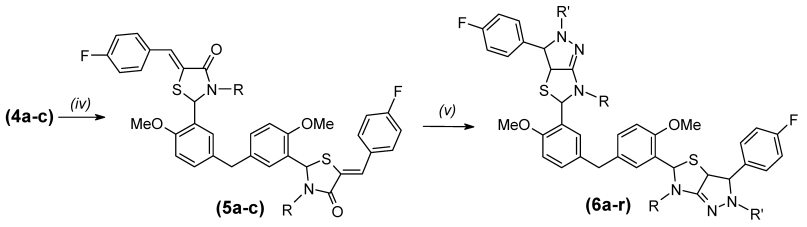
Compounds **4a**—**c** when reacted with *p*-fluorobenzaldehyde in the presence of anhydrous NaOAc in glacial acetic acid at reflux tempereature afforded arylidine derivatives of methylene-bis-thiazolidinones **5a**—**c**. Further, these compounds on cyclocondensation with aryl/alkyl hydrazines in the presence of anhydrous NaOAc in glacial acetic acid at reflux temperature gave bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-methylphenyl)-2-(aryl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methanes **6a**—**r** in good to excellent yields (Chart 2). The versatility of the reaction is well demonstrated by the fact that both aromatic hydrazines such as phenylhydrazine, *p*-methoxyphenylhydrazine, *p*-chlorophenylhydrazine and aliphatic hydrazines such as benzylhydrazine, isopropylhydrazine, methylhydrazine afforded their corresponding compounds **6a**—**r** in good yields. The



4: R = **a**) 4-MeC_6H_4; **b**) 4-Cl-C_6H_4; **c**) 4-NO_2-C_6H_4

Reagents and conditions: (*i*) trioxane, H_2SO_4/AcOH, reflux, 81%; (*ii*) MeI, K_2CO_3, DMF, rt, 83%; (*iii*) R-NH_2, HS-CH_2-COOH, $ZnCl_2$ / PhMe, reflux/MWI, 81-90%.

Chart 1



5: R = **a**) 4-Me-C_6H_4; **b**) 4-Cl-C_6H_4; **c**) 4-NO_2-C_6H_4

6	R	R'	6	R	R'	6	R	R'
a	4-Me-C_6H_4-	C_6H_5-	**g**	4-Cl-C_6H_4-	C_6H_5-	**m**	4-NO_2-C_6H_4-	C_6H_5-
b	4-Me-C_6H_4-	4-MeO-C_6H_4-	**h**	4-Cl-C_6H_4-	4-MeO-C_6H_4-	**n**	4-NO_2-C_6H_4-	4-MeO-C_6H_4-
c	4-Me-C_6H_4-	4-Cl-C_6H_4-	**i**	4-Cl-C_6H_4-	4-Cl-C_6H_4-	**o**	4-NO_2-C_6H_4-	4-Cl-C_6H_4-
d	4-Me-C_6H_4-	C_6H_5-CH_2-	**j**	4-Cl-C_6H_4-	C_6H_5-CH_2-	**p**	4-NO_2-C_6H_4-	C_6H_5-CH_2-
e	4-Me-C_6H_4-	$(CH_3)_2$-CH-	**K**	4-Cl-C_6H_4-	$(CH_3)_2$-CH-	**q**	4-NO_2-C_6H_4-	$(CH_3)_2$-CH-
f	4-Me-C_6H_4-	CH_3-	**l**	4-Cl-C_6H_4-	CH_3-	**r**	4-NO_2-C_6H_4-	CH_3-

Reagents and conditions: (*iv*) 4-F-C_6H_4-CHO, AcOH / NaOAc, reflux, 82-88%; (*v*) R'-$NHNH_2$.HCl, AcOH / NaOAc, reflux, 67-76%.

Chart 2

structures of newly described compounds were confirmed by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR and MS spectral data.

In the IR spectra of compounds **6a**—**r** disappearance of amide carbonyl (C=O) absorption band at about 1700 cm^{-1}, which was present in compounds **5a**—**c**, confirmed the cyclization of involvement of α,β-unsaturated carbonyl system, and the bands around 1360—1337 cm^{-1} characteristic for N–C–S bending vibration provided confirmatory evidence for ring closure. In addition, the absorption bands corresponding to C=N of the pyrazole moiety were observed at about 1600 cm^{-1}. Further, support was obtained from the ^{1}H-NMR spectra, the N–CH–S protons of thiazole ring appeared at 7.40—7.38 ppm as a singlet, 5-CH fused protons at 4.20 ppm as a doublet and CH–N–R_2 protons of pyrazole ring at 5.25 ppm as a doublet. These signals demonstrate that the cyclization step has occurred. In the ^{13}C-NMR spectra, the prominent signals corresponding to the carbons of thiazolopyrazole ring in all the compounds observed nearly at 147.0, 71.1, 62.6, and 56.5 ppm, are proof of further evidence of their structures. In summary, all the synthesized compounds exhibited satisfactory spectral data consistent with their structures.

Biological Properties. Nematicidal Activity All the newly synthesized compounds **6a**—**r** were assayed for their nematicidal activity against *Ditylenchus myceliophagus* and *Caenorhabdites elegans* by aqueous *in vitro* screening technique[56] at various concentrations. The results have been expressed in terms of LD_{50} (ppm) *i.e.* median lethal dose at which 50% nematodes became immobile (dead). The LD_{50} values given in Table 1 show that the series of compounds **6d**, **6j** and **6p** containing *N*-benzylpyrazole moiety, and compounds **6f**, **6l** and **6r** containing *N*-methylpyrazole moiety are the most effective against *D. myceliophagus* and *C. elegans* with LD_{50} between 160 and 190 ppm, 190 and 210 ppm respectively, which are nearly equal to the standard. Further, the activity of compounds **6a**—**r** with N–R is almost the same however, is different with N–R′. The carbamoyl oxime nematicide oxamyl (Vydate L Du Pont®; 24% oxamyl in methanol) was used for comparative treatment.

Antibacterial Activity All the compounds **6a**—**r** were assayed for their antibacterial activity against three representative Gram-positive bacteria *viz. Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 11) and *Staphylococcus aureus* (MTCC 96), and three Gram-negative bacteria *viz. Pseudomonas aeruginosa* (MTCC 741), *Klebsiella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656) by disc diffusion method,[57,58] and the mean inhibition zone data are reported in Table 2. In addition, the minimum inhibitory concentration (MIC, μg/ml, *i.e.* minimum concentration required to inhibiting the growth of bacteria) and the minimum bacterial concentration (MBC, μg/ml, *i.e.* the lowest concentration of the drug/compound at which 99.9% inoculums were killed) of all the compounds were determined by the broth dilution method.[59] The MIC and MBC data are presented in Table 3. All assays included the solvent and reference controls. Streptomycin and penicillin were used as standard drugs.

Table 1. Median Lethal Dose LD_{50} (ppm) of Compounds (**6a**—**r**) against Tested Nematodes

Compound	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**	**6h**	**6i**	**6j**	**6k**	**6l**	**6m**	**6n**	**6o**	**6p**	**6q**	**6r**	Oxamyl
D. myceliophagus	850	950	240	190	550	160	810	900	290	180	520	190	800	920	230	190	510	180	150
C. elegans	670	870	360	210	600	200	650	820	380	200	670	210	620	810	350	190	600	200	180

LD_{50}, median lethal dose (the concentration at which 50% nematodes became immobile).

Table 2. Inhibitory Zone Diameters (mm) of Compounds (**6a**—**r**) against Tested Bacterial Strains by Disc Diffusion Method

Compound	Mean zone inhibition (MZI) in mm[a]					
	B. subtilis	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
6a	14	16	27	18	17	19
6b	15	15	26	18	20	17
6c	17	17	29	11	18	21
6d	20	14	24	14	22	17
6e	13	13	23	23	19	16
6f	13	13	25	14	20	18
6g	18	21	20	19	22	20
6h	17	16	28	20	21	23
6i	11	19	22	18	19	18
6j	23	23	30	18	23	18
6k	12	11	13	13	20	16
6l	21	20	20	21	21	19
6m	21	22	20	11	24	11
6n	14	20	21	9	21	16
6o	21	16	16	16	20	16
6p	15	21	19	10	19	13
6q	18	13	23	10	17	15
6r	16	20	21	12	19	18
Streptomycin	30	20	41	15	25	20
Penicillin	25	28	40	25	30	25

Streptomycin (100 μg/disc) and penicillin (100 μg/disc) were used as positive reference; compounds **6a**—**r** (300 μg/disc). *a*) Values are mean (n=3).

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) in μg/ml of Compounds (**6a**—**r**)

Compound	*B. subtilis*		*B. sphaericus*		*S. aureus*		*P. aeruginosa*		*K. aerogenes*		*C. violaceum*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
6a	12.5	25.0	25.0	50.0	12.5	12.5	12.5	50.0	12.5	25.0	25.0	25.0
6b	25.0	25.0	50.0	50.0	25.0	25.0	12.5	25.0	25.0	25.0	6.25	12.5
6c	12.5	25.0	12.5	25.0	12.5	25.0	6.25	25.0	12.5	50.0	12.5	25.0
6d	6.25	12.5	6.25	12.5	6.25	6.25	12.5	25.0	12.5	50.0	12.5	12.5
6e	25.0	50.0	50.0	50.0	25.0	50.0	6.25	12.5	12.5	12.5	25.0	25.0
6f	6.25	50.0	12.5	25.5	12.5	25.0	12.5	12.5	6.25	12.5	6.25	12.5
6g	6.25	25.0	12.5	50.0	25.0	50.0	6.25	12.5	12.5	25.0	12.5	25.0
6h	12.5	12.5	12.5	50.0	12.5	25.0	12.5	50.0	12.5	25.0	6.25	50.0
6i	12.5	25.0	6.25	25.0	12.5	50.0	50.0	50.0	12.5	12.5	25.0	50.0
6j	6.25	12.5	6.25	50.0	6.25	25.0	12.5	50.0	6.25	12.5	6.25	6.25
6k	12.5	25.0	25.0	50.0	12.5	25.0	25.0	25.0	25.0	50.0	12.5	25.0
6l	12.5	50.0	12.5	25.0	12.5	12.5	12.5	25.0	12.5	25.0	12.5	50.0
6m	12.5	25.0	6.25	12.5	6.25	25.0	6.25	25.0	12.5	25.0	25.0	50.0
6n	12.5	25.0	12.5	50.0	12.5	25.0	12.5	25.0	6.25	12.5	12.5	25.0
6o	6.25	50.0	12.5	50.0	6.25	25.0	50.0	50.0	12.5	25.0	12.5	50.0
6p	6.25	12.5	12.5	50.0	12.5	50.0	12.5	50.0	12.5	25.0	6.25	12.5
6q	25.0	25.0	50.0	50.0	25.0	50.0	25.0	25.0	12.5	25.0	25.0	50.0
6r	6.25	6.25	12.5	25.0	12.5	25.0	12.5	12.5	12.5	25.0	12.5	12.5
Streptomycin	6.25	12.5	6.25	25.0	6.25	12.5	1.56	6.25	1.56	6.25	3.12	6.25
Penicillin	1.56	6.25	3.12	25.0	1.56	6.25	6.25	12.5	6.25	12.5	12.5	12.5

MIC, minimum inhibitory concentration (the lowest concentration that inhibited the bacterial growth). MBC, minimum bacterial concentration (the lowest concentration at which no bacterial growth was observed).

The investigation of antibacterial screening data reveal that almost all the compounds **6a**—**r** are active and showing moderate to good antibacterial activity. Compounds **6d**, **6j**, **6l**, **6m** and **6o** showed good zone of inhibition against *B. subtilis*, compounds **6c** and **6j** were active against *S. aureus*, compounds **6c**, **6g** and **6h** were active against *C. violaceum*, and compounds **6g**, **6j** and **6m** were the most active against *K. aerogenes*, *P. aeruginosa* and *B. sphaericus* (Table 2). The MIC and MBC values of compounds **6a**—**r** (Table 3) indicate that the compounds **6d** and **6j** showed good inhibition against Gram-positive bacteria at 6.25 μg/ml concentration. Most of the compounds exhibited good antibacterial activity almost equivalent to that of standard. The MBC of few compounds is the same as MIC but for many compounds it is two to four-folds higher than the corresponding MIC value.

Antifungal Activity The compounds **6a**—**r** were also screened for their antifungal activity against four fungal organisms *viz. Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185) and *Trichophyton mentagrophytes* (IFO 40996) in dimethyl sulfoxide (DMSO) by agar diffusion method.[60] Amphotericin B was used as a standard drug and the zones of fungal inhibition values are reported in Table 4. In addition, the MIC and MFC (minimum fungicidal concentration, *i.e.* the lowest concentration of the compound at which 99.9% of inoculums were killed) values determined by the broth dilution method[59] and are recorded in Table 5.

The antifungal screening data reported in Table 4 and Table 5 reveal that most of the new compounds are active and having moderate to good antifungal activity. Among the screened compounds, the compound **6f** in which thiazolopyrazole moiety bearing *p*-methylphenyl and *p*-fluorophenyl nucleus on nitrogen showed highest activity against all the microorganisms employed. The activity of this compound is almost equal to the standard. Compounds **6d** and **6j** containing *N*-benzylpyrazole moiety showed good inhibition towards *C. albicans* at the concentration of 3.12 μg/ml, which is less than amphotericin B standard. The MFC of some compounds is the same as MIC but for many compounds it is two to four-folds higher than the corresponding MIC value.

Table 4. Inhibitory Zone Diameters (mm) of Compounds (**6a**—**r**) against Tested Fungal Strains by Disc Diffusion Method

Compound	Mean zone inhibition (MZI) in mm[a]			
	C. albicans	*A. fumigatus*	*T. rubrum*	*T. mentagrophytes*
6a	11	10	—	9
6b	9	15	13	12
6c	13	11	11	10
6d	22	16	15	16
6e	—	13	—	—
6f	20	20	20	18
6g	—	—	17	—
6h	14	—	—	—
6i	—	—	15	—
6j	21	17	14	16
6k	10	—	11	12
6l	18	15	13	16
6m	8	11	10	9
6n	13	—	—	—
6o	15	—	—	—
6p	19	16	11	13
6q	—	—	9	—
6r	21	14	15	14
Amphotericin B	25	20	20	18

Amphotericin B (100 μg/disc) was used as positive reference; compounds **6a**—**r** (300 μg/disc). —, indicates no sensitivity or MZI lower that 7 mm. *a*) Values are mean (n=3).

Conclusion

In conclusion, a new series of bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-methylphenyl)-2-(aryl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methanes **6a**—**r** have been synthesized and evaluated for their nematicidal ac-

Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) in μg/ml of Compounds (**6a**—**r**)

Compound	*C. albicans*		*A. fumigatus*		*T. rubrum*		*T. mentagrophytes*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
6a	—	—	25.0	25.0	25.0	50.0	25.0	25.0
6b	25.0	25.0	25.0	50.0	12.5	12.5	12.5	25.0
6c	12.5	25.0	6.25	12.5	6.25	12.5	6.25	12.5
6d	3.12	6.25	25.0	25.0	12.5	25.0	25.0	25.0
6e	12.5	25.0	—	—	—	—	—	—
6f	3.12	3.12	3.12	6.25	3.12	6.25	6.25	12.5
6g	6.25	6.25	12.5	25.0	12.5	12.5	6.25	12.5
6h	25.0	50.0	—	—	25.0	50.0	25.0	50.0
6i	12.5	12.5	25.0	25.0	12.5	12.5	25.0	50.0
6j	3.12	6.25	6.25	12.5	6.25	12.5	12.5	25.0
6k	12.5	25.0	25.0	25.0	—	—	25.0	50.0
6l	12.5	25.0	6.25	50.0	6.25	25.0	—	—
6m	12.5	25.0	12.5	50.0	6.25	25.0	12.5	12.5
6n	6.25	12.5	12.5	25.0	12.5	25.0	—	—
6o	25.0	25.0	25.0	50.0	25.0	50.0	6.25	12.5
6p	12.5	25.0	—	—	—	—	—	—
6q	25.0	25.0	25.0	50.0	12.5	25.0	12.5	25.0
6r	12.5	25.0	12.5	25.0	12.5	25.0	12.5	25.0
Amphotericin B	6.25	12.5	3.12	6.25	3.12	12.5	3.12	12.5

MIC, minimum inhibitory concentration (the lowest concentration that inhibited the fungal growth). MFC, minimum fungicidal concentration (the lowest concentration at which no fungal growth was observed). —, indicates fungal are resistant to the compound >100 μg/ml concentration.

tivity, most of the compounds showed appreciable nematicidal activity. The antibacterial (MZI, MIC and MBC) activity and antifungal (MZI, MIC and MFC) activity of these compounds were also evaluated against various bacteria and fungi. Many of the synthesized compounds showed good activity against the test bacteria and fungi and emerged as potential molecules for further development.

Experimental

General Reagents were commercial grade and were used as supplied or were prepared according to procedures described in literature. Solvents except analytical reagent grade were dried and purified according to literature when necessary. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel F_{254} plates from Merck and compounds visualized either by exposure to UV light or dipping in 1% aqueous potassium permanganate solution. Chromatographic columns 70—230 mesh silica gel for separations were used. Melting points were determined through a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded using KBr disk on a Perkin-Elmer FTIR spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are reported in δ ppm units with respect to TMS as internal standard and coupling constants (J) are reported in Hz units. Mass spectra were recorded on a VG micro mass 7070H spectrometer. Elemental analyses (C, H, N) determined by means of a Perkin-Elmer 240 CHN elemental analyzer, were within $\pm$0.4% of theory.

Typical Procedure. Bis-[4-methoxy-3-[3-(aryl)-1,3-thiazolan-4-one]phenyl]methanes (4a—c) To a stirred mixture of compound **3** (5 mmol), aromatic amine (10 mmol) and thioglycolic acid (15 mmol) in dry toluene (10 ml), $ZnCl_2$ (5 mmol) was added after 2 min and irradiated in a microwave oven at 280 W for 4—7 min at 110 °C. After cooling, the filtrate was concentrated to dryness under reduced pressure and the residue was taken-up in ethyl acetate. The ethyl acetate layer was washed with brine, 5% sodium bicarbonate solution and finally with brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness at reduced pressure. The crude product thus obtained was purified by column chromatography on silica gel with hexane–ethyl acetate as eluent to afford pure compounds.

Bis-[4-methoxy-3-[3-(4-methylphenyl)-1,3-thiazolan-4-one]phenyl]-methane (4a) This was obtained by reacting compound **3** (1.42 g), *p*-methylaniline (1.07 g) and thioglycolic acid (1.41 g) as described in the typical procedure and isolated as brown solid. Yield 82%, mp 180—182 °C. IR (KBr) cm^{-1}: 3012, 2988, 1698, 1612, 1475, 1410, 1220, 689. ^{1}H-NMR (DMSO-d_6) δ: 7.39 (d, J=8.3 Hz, 4H, Ar-H), 7.15 (d, J=8.3 Hz, 4H, Ar-H), 7.10—6.70 (m, 6H, Ar-H), 6.07 (s, 2H, CH–S), 4.13 (s, 6H, OCH_3), 3.80 (s, 4H, CH_2), 3.36 (s, 2H, CH_2), 2.02 (s, 6H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 171.1, 152.3, 139.8, 136.9, 135.1, 133.7, 128.7, 124.3, 122.1, 113.4, 109.2, 64.2, 54.5, 41.1, 35.6, 19.7. MS *m*/*z*: 612 (M^+). *Anal.* Calcd for $C_{35}H_{34}N_2O_4S_2$: C, 68.83; H, 5.61; N, 4.59. Found: C, 68.73; H, 5.50; N, 4.52.

Bis-[4-methoxy-3-[3-(4-chlorophenyl)-1,3-thiazolan-4-one]phenyl]-methane (4b) This was obtained by reacting compound **3** (1.42 g), *p*-chloroaniline (1.27 g) and thioglycolic acid (1.41 g) as described in the typical procedure and isolated as yellow solid. Yield 81%, mp 214—216 °C. IR (KBr) cm^{-1}: 3015, 2987, 1714, 1605, 1481, 1410, 1221, 746, 690. ^{1}H-NMR (DMSO-d_6) δ: 7.52 (d, J=8.7 Hz, 4H, Ar-H), 7.21 (d, J=8.7 Hz, 4H, Ar-H), 7.10—6.70 (m, 6H, Ar-H), 6.06 (s, 2H, CH–S), 4.11 (s, 6H, OCH_3), 3.77 (s, 4H, CH_2), 3.36 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 171.1, 152.9, 138.7, 136.3, 135.3, 132.1, 128.7, 127.6, 123.5, 121.8, 109.3, 63.7, 54.3, 41.1, 35.9. MS *m*/*z*: 652 (M^+). *Anal.* Calcd for $C_{33}H_{28}Cl_2N_2O_4S_2$: C, 60.83; H, 4.33; N, 4.30. Found: C, 60.71; H, 4.25; N, 4.22.

Bis-[4-methoxy-3-[3-(4-nitrophenyl)-1,3-thiazolan-4-one]phenyl]-methane (4c) This was obtained by reacting compound **3** (1.42 g), *p*-nitroaniline (1.38 g) and thioglycolic acid (1.41 g) as described in the typical procedure and isolated as brown solid. Yield 79%, mp 197—199 °C. IR (KBr) cm^{-1}: 3016, 2992, 1695, 1610, 1510, 1479, 1410, 1318, 1224, 682. ^{1}H-NMR (DMSO-d_6) δ: 8.11 (d, J=9.1 Hz, 4H, Ar-H), 7.77 (d, J=9.1 Hz, 4H, Ar-H), 7.10—6.70 (m, 6H, Ar-H), 6.08 (s, 2H, CH–S), 4.12 (s, 6H, OCH_3), 3.77 (s, 4H, CH_2), 3.36 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 171.2, 152.5, 142.1, 138.9, 136.1, 135.7, 133.1, 128.3, 126.1, 123.9, 109.2, 63.3, 54.1, 41.1, 35.3. MS *m*/*z*: 674 (M^+). *Anal.* Calcd for $C_{33}H_{28}N_4O_8S_2$: C, 58.92; H, 4.20; N, 8.33. Found: C, 58.10; H, 4.11; N, 8.20.

Typical Procedure. Bis-[4-methoxy-3-[2-(*Z*)-5-(4-fluorophenyl)-methylidene-3-(aryl)-1,3-thiazo-lan-4-one]phenyl]methane (5a—c) A mixture of compound **4** (5 mmol), *p*-fluorobenzaldehyde (10 mmol) and sodium acetate (5 mmol) in anhydrous glacial acetic acid (10 ml), was refluxed for 3 h. The reaction mixture was concentrated and then poured into ice cold water, the solid thus separated, was filtered, washed with water, the crude product thus obtained was purified by column chromatography on silica gel with hexane–ethyl acetate as eluent to afford pure compounds.

Bis-[4-methoxy-3-[2-(*Z*)-5-(4-fluorophenyl)methylidene-3-(4-methylphenyl)-1,3-thiazolan-4-one]phenyl]methane (5a) This was obtained by reacting compound **4a** (3.6 g) and *p*-fluorobenzaldehyde (1.24 g) as described in the typical procedure and isolated as brown solid. Yield 88%, mp 167—169 °C. IR (KBr) cm^{-1}: 3062, 2990, 1720, 1530, 1270, 685. ^{1}H-NMR (DMSO-d_6) δ: 7.81, (s, 2H, CH=C), 7.27 (d, J=8.3 Hz, 4H, Ar-H), 7.55—7.50 (m, 8H, Ar-H), 7.15—6.90 (m, 8H, Ar-H+CH–S), 6.70 (s, 2H, Ar-H), 6.61 (d, J=9.1 Hz, 2H, Ar-H), 4.10 (s, 6H, OCH_3), 3.81 (s, 2H, CH_2), 2.10 (s, 6H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 165.4, 161.3, 154.0, 142.7,

137.8, 136.7, 135.7, 134.5, 131.9, 130.5, 128.6, 126.9, 125.0, 123.2, 117.1, 112.5, 64.0, 56.1, 42.0, 20.3. MS *m/z*: 822 (M^+). *Anal.* Calcd for $C_{49}H_{40}F_2N_2O_4S_2$: C, 71.51; H, 4.90; N, 3.40. Found: C, 71.46; H, 4.92; N, 3.34.

Bis-[4-methoxy-3-[2-(*Z*)-5-(4-fluorophenyl)methylidene-3-(4-chlorophenyl)-1,3-thiazolan-4-one]phenyl]methane (5b) This was obtained by reacting compound **4b** (3.26 g) and *p*-fluorobenzaldehyde (1.24 g) as described in the typical procedure and isolated as brown solid. Yield 84%, mp 237—239 °C. IR (KBr) cm^{-1}: 3035, 2989, 1719, 1535, 1182, 747, 686. ^{1}H-NMR (DMSO-d_6) δ: 7.71, (s, 2H, CH=C), 7.63 (d, J=8.6 Hz, 4H, Ar-H), 7.51 (d, J=8.3 Hz, 4H, Ar-H), 7.29—7.23 (m, 8H, Ar-H), 7.12 (d, J=9.1 Hz, 2H, Ar-H), 6.92 (s, 2H, CH–S), 6.69 (s, 2H, Ar-H), 6.61 (d, J=9.1 Hz, 2H, Ar-H), 4.12 (s, 6H, OCH_3), 3.81 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 165.7, 161.8, 154.0, 139.1, 137.9, 135.3, 134.5, 133.4, 131.7, 130.6, 128.9, 127.1, 126.6, 124.9, 122.7, 117.0, 112.9, 64.0, 56.1, 42.0. MS *m/z*: 864 (M^+). *Anal.* Calcd for $C_{47}H_{34}Cl_2F_2N_2O_4S_2$: C, 65.36; H, 3.97; N, 3.24. Found: C, 65.37; H, 3.91; N, 3.19.

Bis-[4-methoxy-3-[2-(*Z*)-5-(4-fluorophenyl)methylidene-3-(4-nitrophenyl)-1,3-thiazolan-4-one]phenyl]methane (5c) This was obtained by reacting compound **4c** (3.37 g) and *p*-fluorobenzaldehyde (1.24 g) as described in the typical procedure and isolated as brown solid. Yield 82%, mp 289—291 °C. IR (KBr) cm^{-1}: 3036, 2995, 1720, 1542, 1535, 1340, 1187, 684. ^{1}H-NMR (DMSO-d_6) δ: 8.18 (d, J=8.7 Hz, 4H, Ar-H), 7.80—7.70 (m, 6H, Ar-H+CH=C), 7.47 (d, J=8.4 Hz, 4H, Ar-H), 7.32 (d, J=8.4 Hz, 4H, Ar-H), 7.14 (d, J=9.1 Hz, 2H, Ar-H), 6.92 (s, 2H, CH–S), 6.60—6.57 (m, 4H, Ar-H), 4.19 (s, 6H, OCH_3), 3.80 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 165.6, 161.7, 154.0, 146.3, 145.6, 137.8, 135.2, 134.5, 130.7, 129.3, 128.7, 128.0, 126.7, 125.1, 123.1, 117.1, 112.5, 64.0, 56.1, 42.0. MS *m/z*: 884 (M^+). *Anal.* Calcd for $C_{47}H_{34}F_2N_4O_8S_2$: C, 63.79; H, 3.87; N, 6.33. Found: C, 63.73; H, 3.89; N, 6.27.

Typical Procedure. Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-methylphenyl)-2-(aryl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methanes (6a—r) A mixture of compound **5** (5 mmol), aryl/alkyl hydrazine (10 mmol) and anhydrous sodium acetate (5 mmol) in glacial acetic acid (20 ml), was refluxed for 7 h. The reaction mixture was concentrated and cooled to room temperature, the solid thus separated, was filtered, washed thoroughly with water, the crude product thus obtained was purified by column chromatography on silica gel with hexane–ethyl acetate as eluent to afford pure compounds.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-methylphenyl)-2-phenyl-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6a) This was obtained by reacting compound **5a** (2.0 g) and phenylhydrazine (0.54 g) as described in the typical procedure and isolated as brown solid. Yield 69%, mp 256—258 °C. IR (KBr) cm^{-1}: 3078, 1598, 1328, 1256. ^{1}H-NMR (DMSO-d_6) δ: 7.40 (s, 2H, CH–S), 7.29—7.24 (m, 6H, Ar-H), 7.18 (m, 8H, Ar-H), 7.14—7.05 (m, 14H, Ar-H), 6.70 (d, J=9.1 Hz, 2H, Ar-H), 6.65 (s, 2H, Ar-H), 5.25 (d, J=1.8 Hz, 2H, CH–N), 4.20 (d, J=1.8 Hz, 2H, CH–S), 4.09 (s, 6H, OCH_3), 3.42 (s, 2H, CH_2), 2.21 (s, 6H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 164.4, 154.1, 147.0, 146.3, 142.7, 136.4, 135.7, 134.6, 132.0, 129.7, 129.3, 128.0, 127.4, 124.9, 122.6, 119.0, 117.6, 114.8, 110.6, 71.1, 62.6, 56.5, 54.6, 42.0, 22.0. MS *m/z*: 1004 (M^+). *Anal.* Calcd for $C_{61}H_{52}F_2N_6O_2S_2$: C, 73.03; H, 5.22; N, 8.38. Found: C, 72.96; H, 5.27; N, 8.32.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-methylphenyl)-2-(4-methoxyphenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6b) This was obtained by reacting compound **5a** (2.0 g) and *p*-methoxyphenylhydrazine (0.69 g) as described in the typical procedure and isolated as dark yellow solid. Yield 70%, mp 281—283 °C. IR (KBr) cm^{-1}: 3065, 1596, 1327, 1256. ^{1}H-NMR (DMSO-d_6) δ: 7.38 (s, 2H, CH–S), 7.21 (d, J=9.1 Hz, 2H, Ar-H), 7.20—7.10 (m, 16H, Ar-H), 6.90 (d, J=8.9 Hz, 4H, Ar-H), 6.86 (d, J=8.9 Hz, 4H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 5.18 (d, J=1.7 Hz, 2H, CH–N), 4.21 (d, J=1.7 Hz, 2H, CH–S), 4.09 (s, 6H, OCH_3), 3.70 (s, 6H, OCH_3), 3.41 (s, 2H, CH_2), 2.21 (s, 6H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 159.3, 156.0, 154.0, 146.3, 142.7, 141.2, 136.4, 135.7, 134.7, 132.0, 129.3, 128.1, 127.4, 124.9, 122.6, 121.9, 117.6, 114.7, 110.5, 71.1, 62.6, 56.5, 55.9, 54.0, 42.1, 22.0. MS *m/z*: 1064 (M^+). *Anal.* Calcd for $C_{63}H_{56}F_2N_6O_3S_2$: C, 71.17; H, 5.31; N, 7.90. Found: C, 71.12; H, 5.27; N, 7.86.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-methylphenyl)-2-(4-chlorophenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6c) This was obtained by reacting compound **5a** (2.0 g) and *p*-chlorophenylhydrazine (0.71 g) as described in the typical procedure and isolated as brown solid. Yield 67%, mp 267—269 °C. IR (KBr) cm^{-1}: 3064, 1580, 1327, 1260, 748. ^{1}H-NMR (DMSO-d_6) δ: 7.50 (d, J=8.6 Hz, 4H, Ar-H), 7.36 (s, 2H, CH–S), 7.30—7.25 (m, 6H, Ar-H), 7.20—7.10 (m, 16H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 5.18 (d, J=1.7 Hz, 2H, CH–N), 4.21 (d, J=1.7 Hz, 2H, CH–S), 4.09 (s, 6H, OCH_3), 3.41 (s, 2H, CH_2), 2.21 (s, 6H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 159.3, 154.0, 146.2, 142.7, 140.6, 136.4, 135.7, 134.5, 132.0, 130.1, 129.2, 128.9, 128.1, 127.4, 125.0, 123.5, 122.6, 117.6, 110.6, 71.1, 62.5, 56.5, 55.0, 42.0, 22.0. MS *m/z*: 1072 (M^+). *Anal.* Calcd for $C_{61}H_{50}Cl_2F_2N_6O_2S_2$: C, 68.34; H, 4.70; N, 7.84. Found: C, 68.29; H, 4.77; N, 7.86.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-methylphenyl)-2-benzyl-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6d) This was obtained by reacting compound **5a** (2.0 g) and *p*-benzylhydrazine (0.62 g) as described in the typical procedure and isolated as brown solid. Yield 72%, mp 286—288 °C. IR (KBr) cm^{-1}: 3062, 2972, 1578, 1327, 1270. ^{1}H-NMR (DMSO-d_6) δ: 7.38 (s, 2H, CH–S), 7.30—7.20 (m, 12H, Ar-H), 7.15—7.10 (m, 16H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 4.95 (d, J=1.8 Hz, 2H, CH–N), 4.20 (s, 4H, CH_2), 4.09 (s, 6H, OCH_3), 4.00 (d, J=1.8 Hz, 2H, CH–S), 3.41 (s, 2H, CH_2), 2.21 (s, 6H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 166.2, 154.0, 146.3, 142.7, 136.7, 136.4, 135.6, 134.7, 133.1, 129.3, 128.7, 128.4,128.0, 127.5, 125.0, 122.6, 116.4, 110.5, 71.2, 63.1, 56.1, 55.5, 54.7, 41.9, 22.0. MS *m/z*: 1032 (M^+). *Anal.* Calcd for $C_{63}H_{56}F_2N_6O_2S_2$: C, 73.37; H, 5.47; N, 8.15. Found: C, 73.29; H, 5.41; N, 8.20.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-2-isopropyl-6-(4-methylphenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6e) This was obtained by reacting compound **5a** (2.0 g) and isopropylhydrazine (0.37 g) as described in the typical procedure and isolated as yellow solid. Yield 76%, mp 249—251 °C. IR (KBr) cm^{-1}: 3065, 2980, 1572, 1327, 1270. ^{1}H-NMR (DMSO-d_6) δ: 7.36 (s, 2H, CH-S), 7.21 (d, J=9.1 Hz, 2H, Ar-H), 7.15—7.10 (m, 12H, Ar-H), 7.00 (d, J=8.6 Hz, 4H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 4.89 (d, J=1.8 Hz, 2H, CH–N), 4.14 (s, 6H, OCH_3), 4.09 (d, J=1.8 Hz, 2H, CH–S), 3.41 (s, 2H, CH_2), 3.11 (m, 2H, CH–N), 2.21 (s, 6H, CH_3), 0.98 (d, J=6.3 Hz, 12H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 164.7, 154.0, 147.8, 142.7, 136.4, 135.7, 134.7, 133.1, 129.1, 128.5, 128.2, 125.0, 122.6, 116.4, 110.5, 71.3, 59.2, 58.6, 54.7, 52.4, 42.0, 22.1, 20.2. MS *m/z*: 936 (M^+). *Anal.* Calcd for $C_{51}H_{48}F_2N_6O_2S_2$: C, 69.68; H, 5.50; N, 9.56. Found: C, 69.70; H, 5.45; N, 9.51.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-2-methyl-6-(4-methylphenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6f) This was obtained by reacting compound **5a** (2.0 g) and methylhydrazine (0.23 g) as described in the typical procedure and isolated as yellow solid. Yield 66%, mp 229—231 °C. IR (KBr) cm^{-1}: 3062, 2969, 1571, 1327, 1266. ^{1}H-NMR (DMSO-d_6) δ: 7.36 (s, 2H, CH–S), 7.21 (d, J=9.1 Hz, 2H, Ar-H), 7.15—7.05 (m, 16H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 4.71 (d, J=1.8 Hz, 2H, CH–N), 4.11 (s, 6H, OCH_3), 4.00 (d, J=1.8 Hz, 2H, CH–S), 3.41 (s, 2H, CH_2), 2.70 (s, 6H, N–CH_3), 2.21 (s, 6H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 164.7, 154.0, 142.7, 142.2, 136.4, 135.6, 134.7, 133.1, 129.1, 128.1, 128.4, 125.0, 122.6, 116.4, 110.6, 71.3, 62.5, 54.7, 52.5, 41.7, 35.7, 22.0. MS *m/z*: 880 (M^+). *Anal.* Calcd for $C_{51}H_{48}F_2N_6O_2S_2$: C, 69.68; H, 5.50; N, 9.56. Found: C, 69.70; H, 5.45; N, 9.51.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-chlorophenyl)-2-phenyl-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6g) This was obtained by reacting compound **5b** (2.1 g) and phenylhydrazine (0.54 g) as described in the typical procedure and isolated as brown solid. Yield 68%, mp 249—251 °C. IR (KBr) cm^{-1}: 3078, 1598, 1572, 1327, 746. ^{1}H-NMR (DMSO-d_6) δ: 7.42 (d, J=8.6 Hz, 4H, Ar-H), 7.36 (s, 2H, CH–S), 7.30—7.20 (m, 18H, Ar-H), 7.10—7.05 (m, 6H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 5.18 (d, J=1.8 Hz, 2H, CH–N), 4.21 (d, J=1.8 Hz, 2H, CH–S), 4.20 (s, 6H, OCH_3), 3.41 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 166.0, 154.0, 147.8, 145.3, 142.6, 135.6, 134.7, 133.1, 132.4, 131.6, 128.9, 127.1, 126.6, 124.9, 122.6, 118.9, 117.4, 114.9, 110.6, 71.3, 62.5, 56.8, 54.7, 42.0. MS *m/z*: 1044 (M^+). *Anal.* Calcd for $C_{59}H_{46}Cl_2F_2N_6O_2S_2$: C, 67.87; H, 4.44; N, 8.05. Found: C, 67.85; H, 4.48; N, 8.00.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-chlorophenyl)-2-(4-methoxyphenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6h) This was obtained by reacting compound **5b** (2.1 g) and *p*-methoxyphenylhydrazine (0.69 g) as described in the typical procedure and isolated as brown solid. Yield 70%, mp 274—276 °C. IR (KBr) cm^{-1}: 3065, 1579, 1562, 1327, 747. ^{1}H-NMR (DMSO-d_6) δ: 7.42 (d, J=8.6 Hz, 4H, Ar-H), 7.36 (s, 2H, CH–S), 7.29 (d, J=8.6 Hz, 4H, Ar-H), 7.20—7.15 (m, 10H, Ar-H), 6.92 (d, J=8.8 Hz, 4H, Ar-H), 6.80—6.70 (m, 8H, Ar-H), 5.18 (d, J=1.8 Hz, 2H, CH–N), 4.21 (d, J=1.8 Hz, 2H, Ar-H), 4.11 (s, 6H, OCH_3), 3.70 (s, 6H, OCH_3), 3.41 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 166.0, 155.9, 154.0, 146.2, 142.4, 140.5, 135.4, 134.7, 133.2, 131.6, 130.4, 127.1, 126.3, 124.9, 123.0, 122.6, 117.4, 113.7, 110.5, 71.2,

62.4, 56.8, 55.0, 54.3, 42.0. MS *m/z*: 1104 (M^+). *Anal.* Calcd for $C_{61}H_{50}Cl_2F_2N_6O_4S_2$: C, 66.36; H, 4.56; N, 7.61. Found: C, 66.30; H, 4.51; N, 7.56.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-chlorophenyl)-2-(4-chlorophenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6i) This was obtained by reacting compound **5b** (2.1 g) and *p*-chlorophenylhydrazine (0.71 g) as described in the typical procedure and isolated as brown solid. Yield 76%, mp 289—291 °C. IR (KBr) cm^{-1}: 3068, 1594, 1562, 1327, 741. ^{1}H-NMR (DMSO-d_6) δ: 7.51 (d, J=8.8 Hz, 4H, Ar-H), 7.42 (d, J=8.8 Hz, 4H, Ar-H), 7.36 (s, 2H, CH–S), 7.25 (d, J=8.8 Hz, 4H, Ar-H), 7.20—7.15 (m, 14H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 5.18 (d, J=1.8 Hz, 2H, CH–N), 4.22 (d, J=1.8 Hz, 2H, CH–S), 4.12 (s, 6H, OCH_3), 3.41 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 166.0, 154.0, 146.9, 146.5, 142.5, 135.6, 134.5, 133.1, 131.6, 130.9, 130.0, 128.1, 127.1, 126.6, 124.9, 124.0, 122.6, 117.4, 110.5, 71.3, 61.0, 56.8, 55.0, 42.0. MS *m/z*: 1112 (M^+). *Anal.* Calcd for $C_{59}H_{44}Cl_4F_2N_6O_2S_2$: C, 63.67; H, 3.98; N, 7.55. Found: C, 63.62; H, 3.99; N, 7.50.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-chlorophenyl)-2-benzyl-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6j) This was obtained by reacting compound **5b** (2.1 g) and benzylhydrazine (0.62 g) as described in the typical procedure and isolated as yellow solid. Yield 69%, mp 257—259 °C. IR (KBr) cm^{-1}: 3067, 1596, 1571, 746. ^{1}H-NMR (DMSO-d_6) δ: 7.42 (d, J=8.8 Hz, 4H, Ar-H), 7.36 (s, 2H, CH–S), 7.30—7.20 (m, 16H, Ar-H), 7.10—7.05 (m, 8H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 4.87 (d, J=1.8 Hz, 2H, CH–N), 4.20 (s, 6H, OCH_3), 4.12 (s, 4H, CH_2), 3.98 (d, J=1.8 Hz, 2H, CH–S), 3.41 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 166.0, 154.0, 146.2, 142.5, 135.8, 134.9, 134.1, 133.1, 131.9, 131.0, 128.6, 128.0, 127.9, 127.0, 126.6, 124.9, 122.6, 117.4, 110.6, 71.2, 62.7, 56.7, 55.0, 54.2, 42.0. MS *m/z*: 1072 (M^+). *Anal.* Calcd for $C_{61}H_{50}Cl_2F_2N_6O_2S_2$: C, 68.34; H, 4.70; N, 7.84. Found: C, 68.40; H, 4.66; N, 7.85.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-2-isopropyl-6-(4-chlorophenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6k) This was obtained by reacting compound **5b** (2.1 g) and isoproylhydrazine (0.37 g) as described in the typical procedure and isolated as dark yellow solid. Yield 67%, mp 238—239 °C. IR (KBr) cm^{-1}: 3067, 1596, 1571, 1327, 746. ^{1}H-NMR (DMSO-d_6) δ: 7.43 (d, J=8.8 Hz, 4H, Ar-H), 7.36 (s, 2H, CH–S), 7.25 (d, J=8.8 Hz, 4H, Ar-H), 7.21 (d, J=9.1 Hz, 2H, Ar-H), 7.10—7.05 (m, 8H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 4.78 (d, J=1.8 Hz, 2H, CH–N), 4.17 (s, 6H, OCH_3), 4.09 (d, J=1.8 Hz, 2H, CH–S), 3.41 (s, 2H, CH_2), 3.11 (m, 2H, CH–N), 0.98 (d, J=6.4 Hz, 12H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 166.0, 154.0, 142.7, 142.1, 135.6, 134.7, 133.1, 132.0, 131.5, 127.1, 126.6, 125.0, 122.6, 117.4, 110.5, 71.2, 60.2, 58.9, 56.1, 54.6, 42.1, 20.1. MS *m/z*: 976 (M^+). *Anal.* Calcd for $C_{53}H_{50}Cl_2F_2N_6O_2S_2$: C, 65.22; H, 5.16; N, 8.61. Found: C, 65.20; H, 5.10; N, 8.65.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-2-methyl-6-(4-chlorophenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6l) This was obtained by reacting compound **5b** (2.1 g) and methylhydrazine (0.23 g) as described in the typical procedure and isolated as yellow solid. Yield 71%, mp 290—291 °C. IR (KBr) cm^{-1}: 3078, 1597, 1572, 746. ^{1}H-NMR (DMSO-d_6) δ: 7.42 (d, J=8.8 Hz, 4H, Ar-H), 7.36 (s, 2H, CH–S), 7.24 (d, J=8.8 Hz, 4H, Ar-H), 7.19 (d, J=9.1 Hz, 2H, Ar-H), 7.10—7.05 (m, 8H, Ar-H), 6.70—6.65 (m, 2H, Ar-H), 4.71 (d, J=1.8 Hz, 2H, CH–N), 4.11 (s, 6H, OCH_3), 4.00 (d, J=1.8 Hz, 2H, CH–S), 3.41 (s, 2H, CH_2), 2.59 (s, 6H, N–CH_3). ^{13}C-NMR (DMSO-d_6) δ: 166.0, 154.0, 148.7, 142.5, 135.6, 134.7, 133.1, 132.0, 131.5, 127.1, 126.6, 125.0, 122.5, 117.4, 110.5, 71.3, 62.3, 54.6, 52.3, 42.0, 35.8. MS *m/z*: 920 (M^+). *Anal.* Calcd for $C_{49}H_{42}Cl_2F_2N_6O_2S_2$: C, 63.98; H, 4.60; N, 9.14. Found: C, 63.92; H, 4.69; N, 9.10.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-nitrophenyl)-2-phenyl-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6m) This was obtained by reacting compound **5c** (2.16 g) and phenylhydrazine (0.54 g) as described in the typical procedure and isolated as brown solid. Yield 74%, mp 241—243 °C. IR (KBr) cm^{-1}: 3072, 1598, 1579. ^{1}H-NMR (DMSO-d_6) δ: 8.22 (d, J=9.1 Hz, 4H, Ar-H), 7.40—7.35 (m, 6H, Ar-H+CH–S), 7.30 (d, J=8.4 Hz, 4H, Ar-H), 7.20—7.15 (m, 10H, Ar-H), 7.10—7.00 (m, 6H, Ar-H), 6.70—6.65 (m, 2H, Ar-H), 5.18 (d, J=1.8 Hz, 2H, CH–N), 4.27 (d, J=1.8 Hz, 2H, CH–S), 4.12 (s, 6H, OCH_3), 3.41 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 165.1, 154.0, 148.2, 147.4, 142.3, 141.1, 135.6, 134.3, 132.0, 127.5, 128.0, 127.0, 126.1, 125.2, 122.6, 118.6, 117.5, 114.9, 110.6, 71.2, 62.3, 54.6, 52.3, 42.1. MS *m/z*: 1066 (M^+). *Anal.* Calcd for $C_{59}H_{46}F_2N_8O_6S_2$: C, 66.53; H, 4.35; N, 10.52. Found: C, 66.50; H, 4.30; N, 10.56.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-nitrophenyl)-2-(4-methoxyphenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6n) This was obtained by reacting compound **5c** (2.16 g) and *p*-methoxyphenylhydrazine (0.69 g) as described in the typical procedure and isolated as yellow solid. Yield 76%, mp 265—267 °C. IR (KBr) cm^{-1}: 3071, 1597, 1579, 1532. ^{1}H-NMR (DMSO-d_6) δ: 8.22 (d, J=9.1 Hz, 4H, Ar-H), 7.40—7.35 (m, 6H, Ar-H+CH–S), 7.24 (d, J=9.1 Hz, 2H, Ar-H), 7.20—7.15 (m, 8H, Ar-H), 6.92 (d, J=9.0 Hz, 4H, Ar-H), 6.80 (d, J=9.0 Hz, 4H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 5.24 (d, J=1.8 Hz, 2H, CH-N), 4.21 (d, J=1.8 Hz, 2H, CH–S), 4.14 (s, 6H, OCH_3), 3.60 (s, 6H, OCH_3), 3.41 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 165.1, 156.7, 154.0, 147.4, 142.1, 141.1, 135.7, 134.3, 132.0, 128.0, 127.0, 126.1, 125.0, 122.9, 122.1, 117.5, 113.7, 110.5, 71.2, 62.4, 56.7, 54.9, 54.1, 42.0. MS *m/z*: 1126 (M^+). *Anal.* Calcd for $C_{61}H_{50}F_2N_8O_8S_2$: C, 65.11; H, 4.48; N, 9.96. Found: C, 65.05; H, 4.42; N, 9.90.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-nitrophenyl)-2-(4-chlorophenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6o) This was obtained by reacting compound **5c** (2.16 g) and *p*-chlorophenylhydrazine (0.71 g) as described in the typical procedure and isolated as black solid. Yield 72%, mp 285—287 °C. IR (KBr) cm^{-1}: 3069, 1598, 1577, 1532. ^{1}H-NMR (DMSO-d_6) δ: 8.24 (d, J=9.1 Hz, 4H, Ar-H), 7.43 (d, J=8.8 Hz, 4H, Ar-H), 7.40—7.35 (m, 6H, Ar-H+CH–S), 7.25 (d, J=8.8 Hz, 4H, Ar-H), 7.20—7.15 (m, 10H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 5.24 (d, J=1.8 Hz, 2H, CH–N), 4.29 (d, J=1.8 Hz, 2H, CH–S), 4.12 (s, 6H, OCH_3), 3.41 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 165.1, 154.0, 147.3, 146.7, 145.6, 141.2, 135.7, 134.4, 132.0, 129.9, 129.6, 127.9, 127.0, 126.1, 124.8, 124.0, 122.5, 117.4, 110.6, 70.3, 62.4, 55.0, 54.7, 42.0. MS *m/z*: 1134 (M^+). *Anal.* Calcd for $C_{59}H_{44}Cl_2F_2N_8O_6S_2$: C, 62.49; H, 3.91; N, 9.88. Found: C, 62.42; H, 3.94; N, 9.83.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-nitrophenyl)-2-benzyl-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6p) This was obtained by reacting compound **5c** (2.16 g) and benzylhydrazine (0.62 g) as described in the typical procedure and isolated as yellow solid. Yield 74%, mp 275—277 °C. IR (KBr) cm^{-1}: 3067, 1596, 1579, 1327. ^{1}H-NMR (DMSO-d_6) δ: 8.24 (d, J=9.1 Hz, 4H, Ar-H), 7.40—7.35 (m, 6H, Ar-H+CH–S), 7.30—7.20 (m, 12H, Ar-H), 7.10—7.05 (m, 8H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 4.87 (d, J=1.8 Hz, 2H, CH–N), 4.26 (s, 4H, CH_2), 4.14 (s, 6H, OCH_3), 4.00 (d, J=1.8 Hz, 2H, CH–S), 3.41 (s, 2H, CH_2). ^{13}C-NMR (DMSO-d_6) δ: 165.1, 154.0, 147.4, 145.6, 141.2, 137.0, 135.7, 134.5, 132.0, 128.1, 128.0, 128.5, 127.6, 127.0, 126.2, 124.8, 122.6, 117.4, 110.6, 70.5, 62.7, 56.1, 54.7, 53.2, 42.0. MS *m/z*: 1094 (M^+). *Anal.* Calcd for $C_{61}H_{50}F_2N_8O_6S_2$: C, 67.02; H, 4.61; N, 10.25. Found: C, 67.00; H, 4.55; N, 10.21.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-2-isopropyl-6-(4-nitrophenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6q) This was obtained by reacting compound **5c** (2.16 g) and isopropylhydrazine (0.37 g) as described in the typical procedure and isolated as brown solid. Yield 71%, mp 221—223 °C. IR (KBr) cm^{-1}: 3070, 1598, 1576, 1532. ^{1}H-NMR (DMSO-d_6) δ: 8.24 (d, J=9.1 Hz, 4H, Ar-H), 7.40—7.35 (m, 6H, Ar-H+CH–S), 7.18 (d, J=8.6 Hz, 2H, Ar-H), 7.10—7.05 (m, 8H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 4.87 (d, J=1.8 Hz, 2H, CH–N), 4.16 (s, 6H, OCH_3), 4.07 (d, J=1.8 Hz, 2H, CH–S), 3.41 (s, 2H, CH_2), 3.11 (m, 2H, CH-N), 0.99 (s, 12H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 165.1, 154.0, 147.4, 142.1, 141.1, 135.7, 134.2, 132.0, 127.9, 127.0, 124.8, 126.2, 122.6, 117.4, 110.6, 71.1, 60.1, 58.4, 56.3, 54.7, 42.0, 20.6. MS *m/z*: 998 (M^+). *Anal.* Calcd for $C_{53}H_{50}F_2N_8O_6S_2$: C, 63.84; H, 5.05; N, 11.24. Found: C, 63.80; H, 5.00; N, 11.20.

Bis-[4-methoxy-3-[3-(4-fluorophenyl)-2-methyl-6-(4-nitrophenyl)-3,3a,5,6-tetrahydro-2*H*-pyrazolo[3,4-*d*][1,3]thiazol-5-yl]phenyl]methane (6r) This was obtained by reacting compound **5c** (2.16 g) and methylhydrazine (0.23 g) as described in the typical procedure and isolated as brown solid. Yield 67%, mp 241—243 °C. IR (KBr) cm^{-1}: 3070, 1597, 1532, 1327. ^{1}H-NMR (DMSO-d_6) δ: 8.24 (d, J=9.1 Hz, 4H, Ar-H), 7.40—7.35 (m, 6H, Ar-H+CH–S), 7.18 (d, J=8.6 Hz, 2H, Ar-H), 7.10—7.05 (m, 8H, Ar-H), 6.70—6.65 (m, 4H, Ar-H), 4.81 (d, J=1.8 Hz, 2H, CH–N), 4.14 (s, 6H, OCH_3), 4.00 (d, J=1.8 Hz, 2H, CH–S), 3.41 (s, 2H, CH_2), 2.61 (s, 6H, N–CH_3). ^{13}C-NMR (DMSO-d_6) δ: 165.1, 154.0, 148.0, 147.4, 141.2, 135.7, 134.2, 132.0, 127.9, 127.0, 126.1, 124.8, 122.6, 117.4, 110.6, 70.7, 62.6, 55.0, 52.7, 42.0, 36.7. MS *m/z*: 942 (M^+). *Anal.* Calcd for $C_{49}H_{42}F_2N_8O_6S_2$: C, 62.54; H, 4.50; N, 11.91. Found: C, 62.50; H, 4.48; N, 11.95.

Antibacterial Assay For the antibacterial assay standard inoculums (1—2×10^7 colony forming unit (c.f.u.)/ml 0.5 McFarland standards) were introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The discs measuring

6.26 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. The plates were inverted and incubated for 24 h at 37 °C. The inhibition zones were measured and compared with the standards. For the determination of MIC Bacteria were grown over night in Luria Bertani (LB) broth at 37 °C, harvested by centrifugation, and then washed twice with sterile distilled water. Stock solutions of the series of compounds were prepared in DMSO. Each stock solution was diluted with standard method broth (Difco) to prepare serial two-fold dilutions in the range of 50—0.8 μg/ml. Ten microtiters of the broth containing about 10^5 c.f.u./ml of test bacteria wasere added to each well of 96-well microtiter plate. Culture plates were incubated for 24 h at 37 °C, and the growth was monitored visually and spectrometrically. The lowest concentration required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC, μg/ml), were determined and compared with the standards. To obtain the minimum bacterial concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18—24 h of incubation at 35 °C.

Antifungal Assay For the antifungal assay Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Twenty millilitres of agar media was poured into each petri-dish, excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 °C for 3—4 d. MIC of compounds **6a**—**r** was determined by the broth dilution method.[38] The *C. albicans* was grown for 48 h at 28 °C in YPD broth (1% yeast extract, 2% peptone, and 2% dextrose), harvested by centrifugation and then washed twice with sterile distilled water. *A. fumigatus*, *T. rubrum* and *T. mentagrophytes* were plated in potato dextrose agar (PDA) (Difco) and incubated at 28 °C for two weeks. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculums size of 10^5 spores/ml. Each test compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to prepare serial two-fold dilutions in the range 100 to 0.8 μg/ml. Ten microtiters of the broth containing about 10^3 (for yeast) and 10^4 (for filamentous fungi) cells/ml of test fungi was added to each well of a 96-well microtiter plate. Culture plates were incubated for about 48—72 h at 28 °C. To obtain the minimum fungicidal concentration (MFC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 48 h of incubation at 35 °C.

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