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Synthesis and biological evaluation of some novel thiazole substituted benzotriazole derivatives

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ABSTRACT

A series of novel hybrid molecules **4a–y** containing thiazole and benzotriazole templates were designed and synthesized. The structures of the synthesized compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the synthesized compounds were tested for their antimicrobial activity (zone of inhibition) against Gram-positive, Gram-negative strains of bacteria as well as fungal strains. After that minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and minimum fungicidal concentrations (MFCs) of all the synthesized compounds were determined. The investigation of antimicrobial screening data revealed that most of the tested compounds showed moderate to good microbial inhibitions.

The chemistry of carbon–nitrogen double bond of hydrazone is becoming the backbone of condensation reaction in benzo-fused N-heterocycles.[1] Hydrazone containing azomethine (–NH–N=C) constitutes an important class of compounds for new drug development.[2] Many researchers have synthesized these compounds as target structures and evaluated their biological activities. Hydrazones have been reported to possess, antimicrobial,[3] antitubercular,[4] anticonvulsant,[5] analgesic,[6] antiinflammatory,[7] antiplatelet,[8] anticancer,[9] antiviral,[10] antitumoral,[11]and antimalarial[12] activities.

One of the most common classes of antifungal agents is azoles. For over a decade, azoles have been a mainstay of the antifungal armamentarium. The triazole derivatives,[13–17] are known to exhibit various pharmacological properties. The most important use, however, is as antimycotics in drugs such as fluconazole, itraconazole and voriconazole[18] and so on, (Fig. 1) currently play a leading role in the treatment of invasive fungal infections.[19] These antifungal drugs act by competitive inhibition of cytochrome P450 14α-demethylase (CYP51), a necessary enzyme in the biosynthesis of ergosterol which is the primary membrane sterol in fungi.[20] CYP51 is a member of the cytochrome P450 super-family, which is widely distributed in different biological kingdoms, being found in animals, plants, fungi, yeast, lower eukaryotes and bacteria,[21] and considered to be the most ancient member of the super-family.[22] In all cases CYP51 catalyzes a three-step reaction of sterol 14α-demethylation. The 14α-methyl group is converted to an alcohol, then to an aldehyde, and is removed as formic acid in the final step.[23] Recent studies showed that typical azole inhibitors were able to fit the putative active site of CYP51 by a combination of heme coordination, hydrogen bonding, π–π stacking and hydrophobic interactions.[24–26]

Benzotriazole represent an overwhelming and rapid developing field in modern heterocyclic chemistry. From literature it is predictable that, benzotriazoles represent important pharmacophores, and play a vital role as medicinal agents. A degree of respectability has been bestowed for benzotriazole derivatives due to their wide range of biological activities such as antiprotozoal agents[27] (inhibitors of *Acanthamoeba castellani*), antimicrobial,[28] antiinflammatory,[29] antitumor[30] and etc. Several derivatives of benzotriazoles are reported as agonists of peroxisome proliferator activated receptors.[31] Synthesis and biological activity of benzotriazole analogous as inhibitors of the NT pase/helicase and of some related flavivade has been extensively investigated.[32]

Triazole and thiazole[33–37] derivatives represent a major chemical group as biologically active agent. Triazoles, in particular, substituted 1,2,4-triazoles and the open-chain thiosemicarbazide counterparts of 1,2,4-triazole, are among the various heterocycles that have received the much attention during the last two decades as potential antimicrobial agents.[38–41] Thiazole moiety has already been reported for its antimicrobial activity.[42,43] Thiazole ring is an

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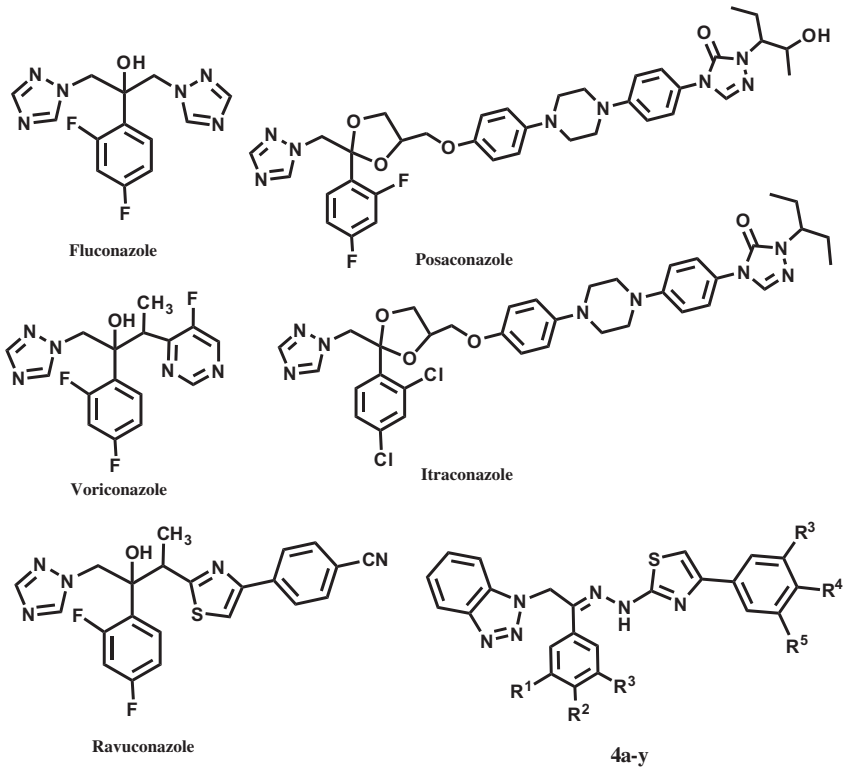
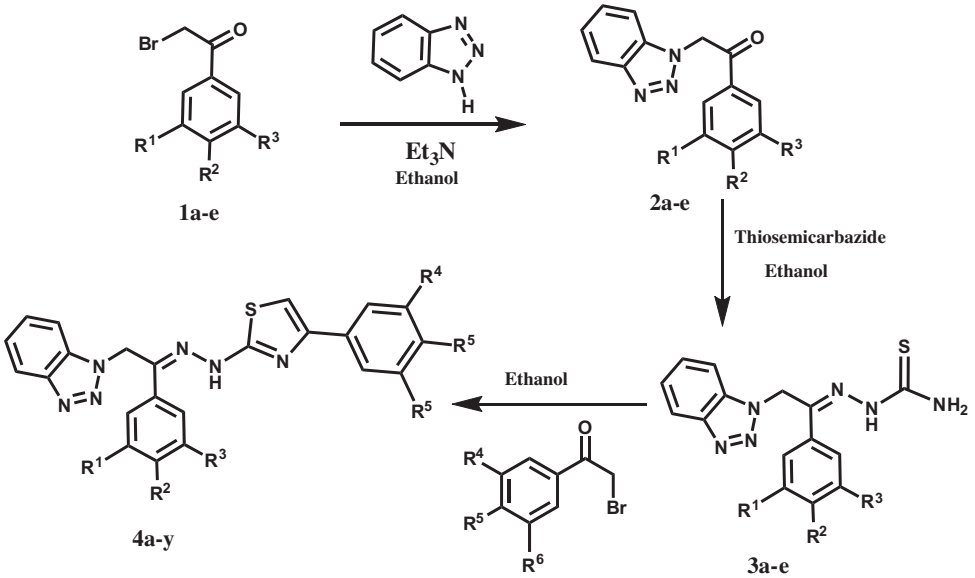


Figure 1. Structures of the antifungal agent fluconazole, posaconazole, voriconazole, itraconazole, ravuconazole and synthesized compounds **4a**–**y**.



Scheme 1. Synthetic route of **4a**–**y**.

important pharmacophore[44] and its coupling with other rings could furnish new biologically active compounds. Thiazole containing compounds exhibit a wide range of biological properties, such as antitumor,[45] anticonvulsant,[46] cardiotonic,[47] IMP dehydrogenase inhibiton,[48] analgesic,[49] anticancer.[50]

It was observed that, benzotriazole and thiazole rings present in the same molecule could be convenient models for investigation of their biological activity. Literature revealed that syntheses of such thiazolyl-benzotriazole showed anti-convulsant and anti-inflammatory activity,[51] anti-tumoral activity.[52] After extensive literature search, it was observed that, till date enough efforts have not been made to combine benzotriazole and thiazole moieties as a single molecular scaffold and to study its biological activity. In continuation with our earlier work,[53–55] we herein report the synthesis of new benzotriazole derivatives clubbed with thiazole moiety (Scheme 1) with the aim of investigating their antimicrobial activity.

Compound	R^1	R^2	R^3	R^4	R^5	R^6
4a	H	F	H	H	F	H
4b	H	F	H	H	Cl	H
4c	H	F	H	H	Br	H
4d	H	F	H	H	NO_2	H
4e	H	F	H	CF_3	H	CF_3
4f	H	Cl	H	H	F	H
4g	H	Cl	H	H	Cl	H
4h	H	Cl	H	H	Br	H
4i	H	Cl	H	H	NO_2	H
4j	H	Cl	H	CF_3	H	CF_3
4k	H	Br	H	H	F	H
4l	H	Br	H	H	Cl	H
4m	H	Br	H	H	Br	H
4n	H	Br	H	H	NO_2	H
4o	H	Br	H	CF_3	H	CF_3
4p	H	NO_2	H	H	F	H
4q	H	NO_2	H	H	Cl	H
4r	H	NO_2	H	H	Br	H
4s	H	NO_2	H	H	NO_2	H
4t	H	NO_2	H	CF_3	H	CF_3
4u	CF_3	H	CF_3	H	F	H
4v	CF_3	H	CF_3	H	Cl	H
4w	CF_3	H	CF_3	H	Br	H
4x	CF_3	H	CF_3	H	NO_2	H
4y	CF_3	H	CF_3	CF_3	H	CF_3

The synthetic route of compounds is outlined in Scheme 1. In the present work substituted phenacyl bromides **1a–e** and substituted 1-aryl-2-(1*H*-benzotriazole-1-yl) ethanones **2a–e** were obtained by literature method.[56] The 1-(2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(4-substitutedphenyl)ethylidene)thiosemicarbazide **3a–e** were prepared by reacting 1-aryl-2-(1*H*-benzotriazole-1-yl) ethanones **2a–e** with thiosemicarbazide in the presence of acetic acid. The condensation of **3a–e** with appropriate phenacyl bromide resulted in the formation of 1-(2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1-(4-substitutedphenyl)ethylidene)-2-(4-(substitutedphenyl) thiazol-2-yl)hydrazine **4a–y** as shown in Scheme 1. Analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR and EIMS) confirmed the structures of the new compounds.

The behavior of thiocarbamoyl functional group in thiosemicarbazone towards phenacyl bromide was investigated. Literature[57] reported three sets of experimental conditions used to the reaction of thiosemicarbazone with phenacyl bromide using pyridine as catalyst. In the literature[58] it was reported that thiosemicarbazone reacted with phenacyl bromide in absolute ethanol in the presence of fused sodium acetate at room temperature. We treated the thiosemicarbazones **3a–e** with phenacyl bromide in anhydrous ethanol under 70 °C for 15 min to give corresponding compounds in good yields without using catalyst (Scheme 1).

The structure of intermediates **3** were substantiated by IR, ^{1}H NMR. IR spectra of compounds **3** revealed in each case, absorption band in the region 3395–3215, 1613–1604 and 1276–1275 cm^{-1} corresponding to N–H, C=N and C=S, respectively. The structure of the title compounds have been confirmed by elemental analysis IR, ^{1}H NMR, ^{13}C NMR and MS. The IR spectra of these compounds show C=C/C=N absorption bands between 1610–1482 cm^{-1} and showed a broad band at 3118–3104 cm^{-1} due to N–H group absorption. In the NMR, title compounds exhibited broad singlet between δ 12.06–11.90 ppm, due to N–H protons, the presence of multiplet signal at δ 6.96–7.87 ppm was assigned to the aromatic protons and thiazole-H, the methylene absorption bands appeared as a singlet at δ 5.71–5.79. In MS spectra, molecular ion peaks of all title compounds were obtained from EI-MS, the presence of M+2 peaks are characteristics for the compound having chlorine and bromine atoms.

All the synthesized compounds were screened for their in vitro antibacterial activity against the standard strains *Bacillus subtilis* (2250), *Staphylococcus aureus* (2079), *Escherichia coli* (2109) and *Pseudomonas aeruginosa* (2036) and for their antifungal activity

Table 1
Antimicrobial screening of synthesized compounds **4a–y** (zone diameter of growth inhibition in mm)

Compounds	Microorganisms					
	S. aureus	*E. coli*	*B. subtilis*	*P. aeruginosa*	*A. niger*	*C. albicans*
4a	20.12	15.04	18.85	11.80	19.11	21.08
4b	25.88	–	–	19.14	21.44	20.18
4c	20.85	–	22.81	–	–	14.74
4d	–	–	14.86	–	16.25	18.71
4e	19.23	–	24.14	–	17.52	17.44
4f	24.68	17.58	16.92	14.31	20.08	21.33
4g	27.88	–	25.14	11.32	18.20	20.98
4h	–	–	13.76	–	15.25	18.01
4i	–	–	–	16.44	13.63	–
4j	–	21.12	–	–	–	13.71
4k	–	18.66	–	19.83	17.56	16.76
4l	–	21.84	–	21.22	15.25	–
4m	23.44	–	17.23	–	–	18.09
4n	–	–	–	13.18	–	–
4o	22.15	–	20.27	–	–	–
4p	26.48	–	–	20.72	17.76	12.25
4q	–	18.68	–	16.73	–	–
4r	22.58	–	15.28	–	–	17.32
4s	–	17.54	–	–	–	14.28
4t	–	14.96	–	–	17.88	19.02
4u	24.29	–	–	–	16.81	18.44
4v	–	18.68	–	–	19.66	–
4w	–	–	–	15.63	–	–
4x	–	13.14	–	–	–	–
4y	–	16.41	–	–	–	11.74
Nystatin	NA	NA	NA	NA	20.32	22.03
Chloramphenicol	31.4	27.55	29.24	23.87	NA	NA

[a] Chloramphenicol (128 μg/disc); Nystatin (128 μg/disc) were used as reference; synthesized compounds (128 μg/disc); NA = not applicable; (–) = inactive.

against *Candida albicans* (3471) and *Aspergillus niger* (545). All the strains were obtained from microbial type culture collection (MTCC) at the NCIM, Pune, India. For antibacterial activity solid medium used for the study were Muller-Hinton agar (Hi media) MHA and soybean casein digest agar (SCDA) and for antifungal activity solid medium used for the study were Potato dextrose agar (Hi media).

The antibacterial activity of all the newly synthesized compounds was done by the agar-well diffusion assay technique.[59,60] Twenty four-hour-old bacterial cultures of all test microorganisms were used as inoculums, which was adjusted to 0.5 McFarland standard, that is, 1.5×10^8 CFU/mL. The stock solutions of all test compounds (128 μg/mL) were prepared by dissolving 128 μg of the test compound in DMSO (1 mL). Chloramphenicol and DMSO were used as positive and negative controls, respectively. Twenty milliliter of molten and cooled MHA and 320 μL of each test bacterial culture were mixed (separate flasks were used for each bacterial culture) and poured in sterilized and labeled petri plates. The wells of 6 mm were punched in the solidified petri plates, aseptically. Fifty microlitres from stock solutions of all compounds as well as controls was added to each well of labeled petri plates and incubated at 35 °C for 24 h. The diameter of the zone of growth inhibition around each well was measured after incubation using vernier caliper (Table 1).

The minimum inhibitory concentration (MIC) of compounds against Gram-positive and Gram-negative test bacteria was determined by the method of NCCLS.[61] All the test cultures were streaked on SCDA and incubated overnight at 37 °C. Turbidity of all the bacterial cultures was adjusted to 0.5 McFarland standard by preparing bacterial suspension of 3–5 well isolated colonies of the same morphological type selected from an agar plate culture. The cultures were further diluted 10-fold to get an inoculums size of 1.2×10^7 CFU/mL. Stock solutions of 4 mg/mL of each compound was prepared in DMSO and was appropriately diluted to get a final concentration of 256, 128, 64, 32, 16, 8, 4, 2, 1 μg/mL. Standard antibiotic chloramphenicol were also diluted to get a final concentration in the same manner. Three hundred and twenty micro liters of each dilution was added to 20 mL molten and cooled MHA (separate flasks was taken for each dilution). After thorough mixing, the medium was poured in sterilized petri plates. The test bacterial cultures were spotted in a predefined pattern by ascetically transferring 5 mL of each bacterial culture on the surface of solidified agar plates and incubated at 35 °C for 24 h. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as MIC. To obtain the minimum bacterial concentration (MBC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of CFU was counted after 18–24 h of incubation at 35 °C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum bactericidal concentration are given in Table 2.

For the antifungal activity, Potato dextrose agar (Hi media) medium was used. This sterilized hot medium (15 mL) was pipette out into flat petri plates. When it solidified 15 mL of warm seeded agar was applied over it. The seeded agar was made by cooling the medium to 40 °C and then adding spore suspension to seeded medium. The spores were obtained from 10 days culture of *C. albicans* and *A. niger* species. The final inoculums size was adjusted to 1×10^6 spore mL^{-1}. Nystatin and DMSO were used as positive and negative controls, respectively.

Before the solidification of agar, the plate was tilted to ensure that coverage should be even. These petri plates were then put into the refrigerator upside down to prevent condensation of moisture. Concentration 128 μg/mL of the synthesized compounds were prepared by dissolving the required quantity of compounds in DMSO, sterilized Whatman filter paper number 541 discs were prepared by cutting 6 mm diameter were spread individually with needle and planted upon the chilled seeded medium. The culture plates were then incubated for 24–72 h at 37 °C and inhibition zone around each disc was measured from the centre of the discs. The diameter of growth inhibition zone was calculated by vernier caliper (Table 1).

Table 2
Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) results of **4a–y**

Compounds	Microorganisms							
	S. aureus		*E. coli*		*B. subtilis*		*P. aeruginosa*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
4a	64	128	128	128	64	128	128	256
4b	32	32	—	—	—	—	64	64
4c	64	128	—	—	64	128	—	—
4d	—	—	—	—	128	256	—	—
4e	128	128	—	—	32	64	—	—
4f	64	32	64	128	128	128	128	256
4g	64	32	—	—	32	64	128	128
4h	—	—	—	—	128	256	—	—
4i	—	—	—	—	—	—	64	128
4j	—	—	32	64	—	—	—	—
4k	—	—	128	128	—	—	32	64
4l	—	—	64	128	—	—	16	32
4m	32	64	—	—	128	256	—	—
4n	—	—	—	—	—	—	128	256
4o	64	64	—	—	64	128	—	—
4p	32	64	—	—	—	—	32	32
4q	—	—	64	128	—	—	64	128
4r	64	128	—	—	128	256	—	—
4s	—	—	128	256	—	—	—	—
4t	—	—	128	256	—	—	—	—
4u	32	64	—	—	—	—	—	—
4v	—	—	128	256	—	—	—	—
4w	—	—	—	—	—	—	64	128
4x	—	—	128	256	—	—	—	—
4y	—	—	128	256	—	—	—	—
Chloramphenicol	16	32	16	32	16	32	16	32

Chloramphenicol (μg/ml) were used as positive control; (—) = inactive; MIC (μg/ml) = minimum inhibitory concentration, that is, the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (μg/ml) = minimum bactericidal concentration, that is, the lowest concentration of the compound for killing the bacteria completely.

For minimum inhibitory concentrations (MIC) of synthesized compounds **4a–y** were determined in the range of concentrations from 256 to 1 μg/mL. The standardized micro broth dilution methods, were used according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standards NCCLS).[61] Table 3 summarizes the minimum concentration of each derivative necessary to completely inhibit (MIC_{90}) the growth of two standardized opportunistic pathogenic fungi including *C. albicans* and *A. Niger*. To obtain the minimum fungicidal concentration (MFC), 0.1 mL volume was taken from each tube and spread on agar plates. The number of CFU was counted after 48 h of incubation at 35 °C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 3. The ratio MFC/MIC was calculated in order to determine if the compound had a fungistatic (MFC/MIC $\geqslant 4$) or fungicidal (MFC/MIC $\leqslant 4$) activity and the results have been summarized in Table 3.

Careful analysis of the MICs in Table 2 and 3 provides some lead molecules with good antibacterial and antifungal activity. Of the compounds **4a–y** tested, compounds with electron-withdrawing F, Cl, Br, CF_3, and NO_2 at the phenyl ring expressed a moderate to good activity against most of the tested pathogens, they inhibited the Gram-positive and Gram-negative pathogens equally. Compounds **4a–y** required about 16–128 μg/mL against Gram-positive and Gram-negative bacteria as well as both the fungi species,

Table 3
Minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) and fungicidal/fungistatic activity (MFC/MIC) of **4a–y**

Compounds	Microorganisms					
	A. niger		*C. albicans*		*A. niger*	*C. albicans*
	MIC	MFC	MIC	MFC	MFC/MIC	MFC/MIC
4a	16	64	16	32	4	2
4b	16	16	16	64	1	4
4c	—	—	128	256	—	2
4d	32	128	32	64	4	2
4e	32	128	32	64	4	2
4f	16	32	16	64	2	4
4g	32	32	16	32	1	2
4h	64	64	32	128	1	4
4i	128	256	—	—	2	—
4j	—	—	64	128	—	2
4k	32	64	32	128	2	4
4l	64	128	—	—	2	—
4m	—	—	32	64	—	2
4n	—	—	—	—	—	—
4o	—	—	—	—	—	—
4p	32	64	128	256	2	2
4q	—	—	—	—	—	—
4r	—	—	32	64	—	2
4s	—	—	64	128	—	2
4t	32	64	128	256	2	2
4u	32	64	32	128	2	4
4v	16	64	—	—	4	—
4w	—	—	—	—	—	—
4x	—	—	—	—	—	—
4y	—	—	128	256	—	2
Nystatin	16	32	16	32	2	2

Nystatin (μg/ml) were used as positive control; (—) = inactive; MIC (μg/ml) = minimum inhibitory concentration, that is, the lowest concentration of the compound to inhibit the growth of fungus completely; MBC (μg/ml) = minimum bactericidal concentration, that is, the lowest concentration of the compound for killing the bacteria completely.

whereas**4b, 4f, 4g, 4m, 4p, 4u** required 32 μg/mL and**4a, 4e, 4o, 4r** required 64 μg/mL against *S. aureus*. Also compounds **4i** required 32 μg/mL, **4f, 4l, 4q** required 64 μg/mL and **4a, 4k, 4s, 4v, 4x, 4y** registered their MIC at 128 μg/mL against *E. coli*. However, the CF_3 substituent did not enhance the activity. Introduction of the F, Cl, Br, NO_2 substituent on the phenyl ring showed an improvement in its activity. Compounds **4e**, **4g** required 32 μg/mL, **4a, 4c, 4o** required 64 μg/mL and **4d, 4f, 4h, 4m, 4r** showed MIC at 128 μg/mL against *B. subtilis*. Compounds **4b, 4j, 4k, 4l, 4p, 4q, 4w** and **4a, 4f, 4g, 4n** registered MIC at 64 and 128 μg/mL against *P. aeruginusa* (they are four and eightfold less potent than chloramphenicol). The MBC of few compounds was found to be the same as MIC but in most of the compounds it was 2- or 4-folds higher than the corresponding MIC results.

Table 2 also describes the MIC of synthesized compounds for their antifungal activity. The introduction of F, Cl, Br, NO_2 substituents on phenyl ring exhibited moderate to good activity against *C. albicans* and *A. niger*, whereas, except the **4m, 4o, 4r, 4w, 4x** all other compounds showed moderate to good activity against *A. niger* and *C. albicans*. Of the fluoro and chloro substituted compounds **4a, 4b, 4f** registered a excellent activity against *A. niger* at 16 μg/mL, also compounds **4d, 4e, 4g, 4k, 4p, 4t, 4u** recorded good activity at 32 μg/mL and **4h, 4j, 4l** recorded moderate activity at 64 μg/mL which is fourfold lower than standard Nystatin. In addition compounds **4a, 4b, 4f, 4g** registered the MIC at 16 μg/mL, **4d, 4e, 4h, 4l, 4n, 4s, 4u, 4v** recorded at 32 μg/mL and**4c, 4i, 4q, 4t, 4x** registered the MIC at 128 μg/mL against *C. albicans*. Introduction of the halo substituent on the phenyl ring inhibited the growth of *A. niger* and *C. albicans*. The MFC of most of the compounds was two or three folds higher than the corresponding MIC results. Most of the synthesized compounds showed good fungicidal activity against the fungal strain.

In conclusion, a series of new **4a–y** were synthesized. The pharmacological studies were undertaken to evaluate the effect of substituents for their antimicrobial activities. Most of the synthesized compounds exhibited moderate to good activity towards Gram-positive and Gram-negative bacteria as well as both the fungi species. The enhancement in antibacterial and antifungal activity can be attributed to the presence of pharmacologically active F, Cl, Br groups irrespective of their position in the molecule.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.094. These data include MOL files and InChiKeys of the most important compounds described in this article.

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