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Synthesis and antimicrobial evaluation of benzothiazole linked isoxazole Schiff bases

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A new series of benzothiazole linked isoxazole Schiff base derivatives have been prepared and characterized by suitable spectroscopic methods *via* ^{1}H and ^{13}C NMR, ESI-MS and IR spectra. These compounds have been further screened for their antimicrobial activity against a panel of microorganisms. Among them, compounds **12d**, **12g** and **12l** demonstrate promising antimicrobial activity against all the tested strains with MIC values ranging between 3.9 – 62.5 µg/mL. Further, compounds **12d**, **12g** and **12l** exhibit promising antifungal activity with MIC values ranging between 7.8 – 32.5 µg/mL. Further studies are underway for determining the antifungal molecular mechanisms of these potential compounds.

Keywords: Benzothiazole, isoxazole, Schiff base, antimicrobial activity

Infectious diseases in mankind are becoming ever more challenging, primarily in the midst of multidrug resistance against the accessible antimicrobial drugs ensuing decreased effectiveness[1]. Further, Persistent fungal infections pretense an incessant as well as serious threat to individual's wellbeing and existence. Clinically, Candidiasis, Aspergillosis and Cryptococcosis are three most imperative fungal infections in immuno-compromised patients[2]. Recently, the rising morbidity and appearance of drug-resistance in life threatening fungal infections masquerade a momentous health predicament particularly in individuals with AIDS and cancer[3].

Isoxazole containing compounds exhibit diverse pharmacological properties such as anti-TB, antiviral, anti-proliferative, anti-inflammatory, antibacterial, antifungal activities, *etc*.[4-11]

Risperidone, used in the management schizophrenia and mania in adults possess isoxazole moiety[12]. Some of the marketed drugs possessing isoxazole scaffold such as Cycloserine, Sulphamethoxazole, Oxacillin, Cloxacillin and dicloxacillin (Figure 1) are used in the treatment of microbial infections. Muscimol[13] and mofezolac are used as sedatives. In addition, various drugs used against different diseases were found to contain this medicinally important scaffold.

Isoxazole is an integral part of a powerful antifungal drug, Micafungin[14] (Figure 2) available in the market. Therefore, this area of research signifies a challenging and demanding problem which should be tackled by discovering new antifungal drugs.

In this context, Santos and co-workers reported new isoxazole compounds with good antifungal activity than the standard Amphotericin B drug with MIC ranging from value of 0.2-47.9 µg/mL against *Candida parapsilosis* and *Candida glabrata*, respectively[15]. Later, Srinivas and co-workers prepared a new class of thiazolidinone clubbed isoxazole derivatives[16]. Out of which, some compounds demonstrated equipotent antifungal activity with the reference drug Amphotericin B against the tested fungi and surfaced as shows potential molecules for additional development.

On the other hand, Benzothiazole is the bicyclic fused ring, which contain benzene and heterocyclic five membered thiazole ring, while the core structure of thiazole and it's pharmacologically and biologically active properties are due to the presence of sulfur and nitrogen atoms present in the ring chemistry. Benzothiazoles are known to exhibit diverse biological activities *viz.* antiproliferative, antibacterial, anticonvulsant, antiretroviral, antimyco-

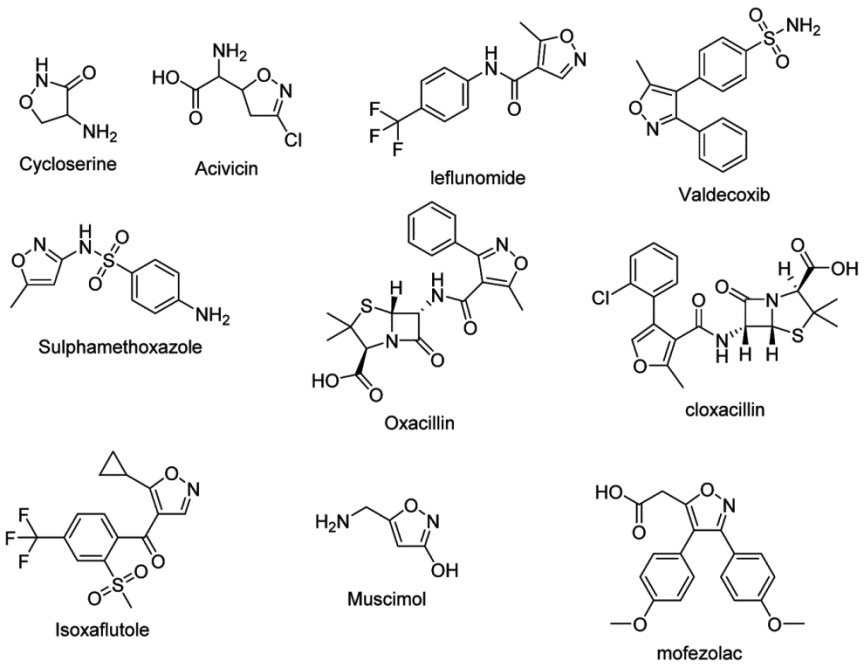


Figure 1 — Structures of Isoxazole containing drugs

bacterial, antiparasitic, analgesic, antiinflammtory, antidiabetic as well as fungicidal activities[17-21].

Although abundant efforts have been made to develop new arsenal of antifungal antibiotics, Benzothiazole (BTA) derivatives appear as one of the most therapeutic versatile antimicrobial compounds[22] and therefore, are constructive motif for exploring newer antimicrobials. Literature review has revealed that there is a significant potential for BTA derivatives in discovering newer agents to combat multidrug resistance.

Consequently, pyrazolinone/linked Benzothiazoles having antibacterial property against a range of tested organisms have been reported by Amir and co-workers. The prepared BTA-pyrazoline compounds bearing halogen groups at *para* position of the benzene ring displayed good to considerable anti-bacterial activities in the MIC range of 13.95-31.01 μM, respectively Figure 2 (I a-c). However, compounds with methyl group in the same position displayed decreased activity. Similar trend in anti-bacterial activity was observed in case of BTA-pyrazoles conjugates, compound (II,a-b)[23] (Figure 2). Overall, 6-chloroBenzothiazole groupwas found to be favorable for activity These compounds were also screened for antifungal activity against various tested fungi and the results revealed that these compounds exhibited promising antifungal activities, particularly, compounds (III, a,b) (Figure 2). SAR suggested that 2-mercapto derivatives were normally more active

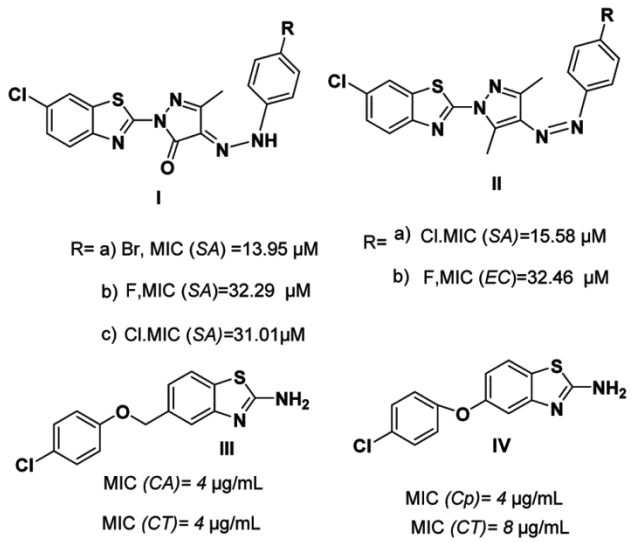


Figure 2 — Benzothiazole derivatives exhibiting antimicrobial activity

against bacteria, while the 2-amino ones were more potent against fungi. This highlights that enhanced antifungal activity can be achieved by incorporating bulky groups at the 6-position of the 2-aminobenzothiazole moiety.

Results and Discussion

Chemistry

The Synthesis of Benzothiazole linked Isoxazole Schiff bases **12a-p** was carried out by condensation

between an equivalent quantity of substituted 2-amino benzothiazole and 2-substituted 3-phenyl-1H-isoxazol-5-carbaldehyde **11a-d** in ethanol[24], as depicted in Scheme I. Isoxazole carbaldehydes **11a-d** were prepared initially by cyclizing substituted acetophenone **7a-d** with diethyl oxalate in the presence of newly prepared sodium ethanolate in ethanol to obtain diketo esters **8a-d** which were then cyclized with NH_2-NH_2.2HCl to yield ethyl 3-substituted phenyl-1*H*-isoxazol-5-carboxylates **9a-d** in good yields. The reduction of these carboxylates by $LiAlH_4$ furnished the corresponding alcohols **10a-d** which were selectively oxidized to substituted isoxazol-5-carbaldehydes **11a-d** by IBX in DMSO[25].

Biology

Antimicrobial activity

Minimum inhibitory concentration (MIC)

The prepared compounds were investigated for their *in vitro* antimicrobial activity using well diffusion method[26] against a panel of Gram-positive and Gram-negative bacterial strains along with a fungal strain, *Candida albicans*. Ciprofloxacin (CPZ) and Miconazole (MCZ) were used as drugs (Controls). Out of all the tested compounds, compounds **12d**, **12g**, and **12l** showed appreciable activities towards all the bacterial strains with MIC values ranging between 3.9-32.5 µg/mL, respectively as illustrated in Table I. Interestingly, **12d**, **12g**, and **12l** exhibited promising antifungal activity with MIC values ranging between 7.8 -31.2 µg/mL, respectively.

Minimum bactericidal concentration and Minimum Fungicidal concentration (MBC/MFC)

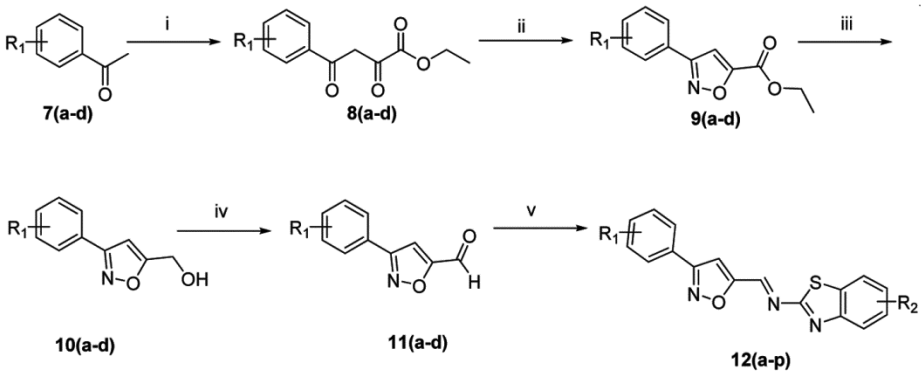
All the prepared compounds were screened for their Minimum bactericidal concentration Minimum Fungicidal concentration (MBC/MFC)[27]. Among them, compounds **12d**, **12g**, and **12l** demonstrated superior activities towards all the tested strains with MBC/MFC values ranging between 7.8 -31.2 µg/mL as mentioned in Table II.

Experimental Section

Chemistry

Preparation of ethyl 2,4-dioxo-4-(substituted phenyl) butanoates,8a-d

Initially sodium ethanolate was prepared *in situ* and diethyl oxalate (1.0 mol) was added slowly at 0°C. The stirring was continued for 15 minutes followed by the addition of different acetophenones **7a-d** (1.0 mol) slowly in small portions, maintaining the temperature at 0°C. After completion of addition, the stirring was continued for 4 h at RT. The progress of



7(a-d) to 11(a-d)

a = R_1(3,4(OCH_3)2) b = R_1(4-OCH_3)

c = R_1(3,4,5(OCH_3)3) d = R_1(3,4-OCH_2O-)

	R_1	R_2		R_1	R_2
12a	3,4(OCH_3)2	6-OCH_3	**12i**	3,4-(OCH_2O)	6-OCH_3
12b	3,4(OCH_3)2	6-F	**12j**	3,4-(OCH_2O)	6-F
12c	3,4(OCH_3)2	6-Cl	**12k**	3,4,5(OCH_3)3	6-OCH_3
12d	3,4(OCH_3)2	6-CH_3	**12l**	3,4,5(OCH_3)3	6-F
12e	4-OCH_3	6-F	**12m**	3,4,5(OCH_3)3	4-OCH_3
12f	3,4(OCH_3)2	6-CF_3	**12n**	3,4,5(OCH_3)3	6-Cl
12g	4-OCH_3	6-OCH_3	**12o**	3,4-(OCH_2O)	6-Cl
12h	4-OCH_3	5-Cl	**12p**	3,4-(OCH_2O)	6-CF_3

Reagents and conditions: (i) Diethyloxalate, NaOEt/EtOH, 4 h, 0°C-RT; (ii) NH_2OH•HCl, EtOH, reflux, 3 h; (iii) $LiAlH_4$/THF, 0°C-RT, 1 h; (iv) IBX/DMSO, 1 h, RT, 80-85%; (v) substituted aminobenzothiazoles, EtOH, 4 h, reflux.

Scheme I — Synthesis of Benzothizole linked Isoxazole Schiff bases **12a-p**

the reaction was monitored by TLC using ethyl acetate and hexane as the mobile phase. After completion of reaction, the reaction mixture was neutralized using dilute H_2SO_4 solution and further extracted with ethyl acetate followed by evaporation of the solvent under reduced pressure to obtain solid products **8a-d** in good yields (80–90%). These were taken as such for the next step without purification.

Preparation of ethyl 3-substituted isoxazole-5-carboxylates, 9a-d

To each of the ethyl 2,4-dioxo-4-(substituted phenyl)butanoates **8a-d** (1.0 mol) prepared in the previous step was added hydroxylamine hydrochloride (NH_2OH•HCl) (1.5 mol) in ethanol and refluxed for 3–4h. After completion of the reaction, the ethanol was removed under reduced pressure and then 150–200 mL of water was added to the residue followed by extraction with ethyl acetate (50 mL × 4). The organic layer was dried on anhydrous Na_2SO_4 and solvent was removed to obtain a crude compound that was further purified by column chromatography using an ethyl acetate and hexane solvent system (3:7). The pure compounds **9(a–d)** were eluted with 30–40% of ethyl acetate in hexane with good yields.

9a: Yellow colored solid. Yield 80.0%. Rf = 0.3 (30% ethyl acetate/ hexane); ^{1}H NMR (300 MHz, $CDCl_3$); δ 1.23-1.37 (t, 3H, *J*1= 6.7 Hz, *J*2 =7.5 Hz), 3.81 (s, 3H), 4.17-4.36 (q, 2H, *J*1 = 6.7 Hz, *J*2 = 7.5 Hz), 6.84 (s, 1H), 6.90 (d, 2H, *J* =2.2 Hz), 7.62 (d, 2H, *J*= 9.0 Hz); MS (ESI): *m/z* 248 [M +H].

9b: Pale yellow colored solid. Yield 80.0%. Rf = 0.3 (30% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$); δ 1.23-1.31 (t, 3H, *J* = 7.1 Hz), 3.85 (s, 3H), 3.90 (s, 3H), 4.19- 4.32 (q, 2H, *J*1 =.1 Hz), 6.88 (d, 1H, *J*=7.1 Hz), 6.94 (s, 1H), 7.21-7.28 (m, 1H), 7.29-7.34 (m, 1H); MS (ESI): *m/z* 278 [M + H].

9c: Pale yellow colored solid. Yield 75.0%. Rf = 0.3 (40% ethyl acetate/hexane); ^{1}H NMR (500 MHz, $CDCl_3$); δ 1.33-1.40 (t, 3H, *J*1 = 6.7 Hz, *J*2 = 7.5 Hz,), 4.33-4.45 (q, 2H, *J*1 = 6.7 Hz, *J*2 =7.5 Hz), 6.0 (s, 2H), 6.86 (d, 1H, *J* ¼ 8.3 Hz), 7.05 (s, 1H), 7.21-7.28 (m, 2H); MS (ESI): *m/z* 262 [M + H].

9d: Yellow colored solid. Yield 75.0%. Rf = 0.3 (40% ethyl acetate/ hexane); ^{1}H NMR (300 MHz, $CDCl_3$); δ 1.33-1.40 (t, 3H, *J*1= 6.7 Hz, *J*2 = 7.5 Hz), 3.88 (s, 3H), 3.95 (s, 6H), 4.33-4.45 (q, 2H, *J*1 =6.7 Hz, *J*2= 7.5 Hz), 7.01 (s, 2H), 7.05 (s, 1H); MS (ESI): *m/z* 308 [M + H].

Preparation of (3-substitutedphenyl-isoxazole-5-yl) methanols, 10a-d

To the ethyl 3-substituted isoxazole-5-carboxylates **9a-d**, obtained in the above step was added $LiAH_4$ (0.5 mol) in dry THF at 0°C and stirred for 1h at RT. Added saturated NH_4Cl solution drop wise to quench the unreacted $LiAlH_4$ and removed the THF under vacuum

Table I — Antimicrobial activity of the synthesized compounds

Compd	Minimum inhibitory concentration (μg/mL)								
	S. aureus	*Bacillus subtilis*	*S. aureus MLS16*	*Micrococcus luteus*	*Klebsiella pneumonia*	*Escherichia coli*	*Pseudom-onas aeruginosa*	*Salmon-ella Paratyphi*	*Candida albicans*
12a	>125	>125	>125	>125	7.8	>125	>125	>125	>125
12b	>125	>125	>125	>125	>125	>125	>125	>125	>125
12c	>125	>125	>125	>125	>125	>125	>125	>125	>125
12d	15.6	3.9	3.9	7.8	3.9	3.9	7.8	>125	7.8
12e	15.6	3.9	>125	15.6	3.9	3.9	7.8	>125	31.2
12f	>125	>125	>125	>125	>125	>125	>125	>125	>125
12g	>125	>125	>125	3.9	7.8	>125	3.9	>125	7.8
12h	>125	>125	>125	>125	3.9	3.9	>125	>125	>125
12i	7.8	>125	>125	15.6	3.9	3.9	15.6	>125	>125
12j	15.6	>125	>125	15.1	>125	>125	>125	>125	15.6
12k	15.6	3.9	3.9	7.8	3.9	7.8	7.8	7.8	15.6
12l	15.6	3.9	>125	7.8	>125	3.9	15.6	3.9	7.8
12m	>125	3.9	>125	7.8	3.9	7.8	15.6	15.6	31.2
12n	3.9	7.8	>125	>125	3.9	>125	7.8	15.6	31.2
12o	15.6	7.8	15.6	7.8	15.6	7.8	>125	>125	15.6
12p	7.8	7.8	15.6	7.8	15.6	15.6	>125	>125	31.2
Miconazole	–	–	–	–	–	–	–	–	3.9
Ciprofloxacin	0.9	0.9	0.9	0.9	0.9	0.9	0.9	–	–

Table II — Minimum bactericidal concentration Minimum Fungicidal concentration (MBC/MFC) (µg/mL) the synthesized compounds

Compd	Minimum bactericidal concentration Minimum Fungicidal concentration (MBC/MFC) (µg/mL)								
	Staphylo-coccus aureus	*Bacillus subtilis*	*S. aureus*	*Micrococcus luteus*	*Klebsiella pneumonia*	*Escherichia coli*	*Pseudom-onas aeruginosa*	*Salmon-ella Paratyphi*	*Candida albicans*
12a	>125	>125	>125	>125	15.6	>125	>125	>125	>125
12b	>125	>125	>125	>125	>125	>125	>125	>125	>125
12c	>125	>125	>125	>125	>125	>125	>125	>125	>125
12d	31.2	7.8	7.8	15.6	7.8	7.8	15.6	>125	15.6
12e	31.2	7.8	>125	31.2	7.8	7.8	15.6	>125	62.5
12f	>125	>125	>125	>125	>125	>125	>125	>125	>125
12g	>125	>125	>125	7.8	15.6	>125	7.8	>125	15.6
12h	>125	>125	>125	>125	7.8	7.8	>125	>125	>125
12i	15.6	>125	>125	31.2	7.8	7.8	31.2	>125	>125
12j	31.2	>125	>125	31.2	>125	>125	>125	>125	31.2
12k	31.2	7.8	7.8	15.6	7.8	15.6	15.6	15.6	31.2
12l	31.2	7.8	>125	15.6	>125	7.8	31.2	7.8	15.6
12m	>125	7.8	>125	15.6	7.8	15.6	15.6	31.2	62.5
12n	7.8	15.6	>125	>125	7.8	>125	15.6	62.5	62.5
12o	31.2	15.6	31.2	15.6	31.2	15.6	>125	>125	31.2
12p	15.6	15.6	31.2	15.6	31.2	31.2	>125	>125	62.5
Miconazole	–	–	–	–	–	–	–	–	7.8
Ciprofloxacin	0.9	0.9	0.9	0.9	0.9	0.9	0.9	–	–

then extracted with ethyl acetate (100 mL × 4). The organic layer was dried on anhydrous Na_2SO_4 and evaporated ethyl acetate to obtain colorless solid products of (3-substitutedphenyl-1*H*-pyrazol-5-yl) methanols**10a-d** in 70-80% yield. The alcohols produced in this step were pure, and no further purification was required. These compounds were taken as such for the next step.

Preparation of 3-subtitutedphenyl-isoxazole-5-carbaldehydes, 11a-d

To the (3-substitutedphenyl-1H-pyrazol-5-yl) methanols **10a-d** produced in the above step was added IBX (1.2 mol) in DMSO and stirred for 1h at RT. Added ice cold water to the reaction mixture and extracted with ethyl acetate (50 mL × 4). The organic layer was dried on anhydrous Na_2SO_4 and evaporated the ethyl acetate to obtain pure corresponding 3-subtitutedphenyl-1*H*-pyrazole- 5-carbaldehydes **11a-d** in good yields (80-85%). The obtained carbaldehydes were as such taken in the next step for the synthesis of pyrazoleoxindole conjugates **12a-p**.

11a: Yellow colored solid. 1.71 g, yield 85%. Rf = 0.3 (40% ethyl acetate/hexane); ^{1}H NMR (400 MHz, $CDCl_3$); δ 3.85 (s, 3H,), 7.78-7.82 (m, 1H), 7.86-7.93 (m, 2H), 7.95-8.03 (m, 2H), 9.95 (s, 1H); MS (ESI): *m/z* 204 [M + H].

11b: Yellow colored solid. 1.85 g, yield 82%. Rf = 0.4 (40% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$); δ 3.87 (s, 3H), 3.91 (s, 3H), 6.93-7.04 (m, 1H), 7.27-7.47 (m, 2H), 7.86-8.10 (m, 1H), 9.93 (s, 1H); MS (ESI): *m/z* 234 [M + H].

11c: Yellow colored solid. 2.09 g, yield 80%. Rf = 0.3 (40% ethyl acetate/hexane); ^{1}H NMR (400 MHz, $CDCl_3$); δ 3.82 (s, 3H), 3.92 (s, 6H), 6.85-7.25 (m, 3H,), 9.96 (s, 1H); MS (ESI): *m/z* 264 [M + H].

11d: Yellow colored solid. 1.83 g, yield 85%. Rf = 0.3 (40% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ d 6.03 (s, 2H), 7.35e7.45 (m, 1H), 7.87-7.94 (m, 2H), 8.10-8.17 (m, 1H), 10.2 (s, 1H); MS (ESI): *m/z* 219 [M +H].

General procedure for the synthesis of Benzothizole linked Isoxazole Schiff bases, 12a-p

Initially, equimolar quantities of substituted isoxazole carboxaldehydes (1.0 eq) and appropriate amino benzothiazoles (1.0 eq) were dissolved in absolute ethanol (6 mL) and the reaction was refluxed for 3-4 h. The progress of the reaction was monitored by TLC using ethyl acetate and hexane (1: 1) solvent system. Then the reaction was cooled to obtain the solid precipitate. The precipitate was filtered off, washed with ice cold water and recrystallized in small quantity of absolute ethanol to furnish the titled compounds **12a-p** in good to excellent yields.

Spectral Data

12a: Yellow solid. 81.5% yield; m.p. 184-185°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.81 (s, 6H), 3.84

(s, 3H), 6.76 (s, 1H), 6.85 (d, *J* = 8.3 Hz,1H), 6.88 (d, *J* = 8.8 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.30 -7.35 (m, 2H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$+DMSO-d_6): δ 55.5, 60.0, 100.9, 101.0, 102.3, 109.6, 109.7, 137.3, 152.9, 157.6, 159.5; MS (ESI): *m/z* 395.

12b: Yellow solid. 72.6% yield; m.p. 164-166°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.72 (s, 6H), 6.93 (d, *J* = 8.1 Hz,1H), 7.02 (s, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.71 (dd, *J* = 8.4 Hz and 8.4 Hz, 3H), 8.26 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$+DMSO-d_6): δ 54.7, 55.0, 99.8, 111.0, 113.7, 126.2, 127.4, 129.9, 131.7, 131.5, 155.5, 158.9; MS (ESI): *m/z* 383.

12c: Yellow solid. 71.5% yield; m.p. 142-143°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (s, 6H), 6.93 (d, *J* = 8.2 Hz,1H), 7.00 (s, 1H), 7.09 (d, *J*= 7.9 Hz, 1H), 7.35 (d, *J* = 8.5 Hz, 2H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 7.7 Hz, 1H), 7.81 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$+ DMSO-d_6): δ 55.3, 55.4, 100.7, 108.6, 111.7, 117.7, 124.6, 148.6; MS (ESI): *m/z* 418.

12d: Yellow solid. 71.0% yield; m.p. 169-170°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.35 (s, 3H), 3.73 (s, 6H), 6.93 (d, *J* = 9.0 Hz, 1H), 7.00 (s, 1H), 7.03 (d, *J* = 8.3 Hz, 1H), 7.13-7.23 (m, 1H), 7.34 (s, 2H), 7.44-7.53 (m, 1H), 7.56 (d, *J* = 8.3 Hz, 1H); ^{13}C NMR (125 MHz, , $CDCl_3$+ DMSO-d_6): δ 28.8, 54.7, 100.3, 113.9, 121.7, 126.3, 159.0; MS (ESI): *m/z* 379.

12e: Yellow solid. 61.5% yield; m.p. 154 – 186°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.77 (s, 3H), 6.95-6.98 (m, 1H), 7.02 (d, *J* = 8.3 Hz, 2H), 7.13-7.18 (m, 1H), 7.25-7.43 (m, 1H), 7.56-7.66 (m, 1H), 7.76 (t, *J* = 8.4 and 9.1 Hz, 2H), 8.25 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6): δ 54.6, 100.1, 113.7, 121.4, 126.2, 159.8; MS (ESI): *m/z* 352.

12f: Yellow solid. 69.8% yield; m.p. 174-175°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.75 (s, 6H), 6.94 (d, *J* = 8.1 Hz,1H), 7.01 (s, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 8.3 Hz, 2H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 7.9 Hz, 1H), 7.82 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6): δ 54.9, 55.5, 103.2, 108.7, 127.9, 128.0, 130.7, 131.5, 138.0, 139.1, 149.8, 159.8; MS (ESI): *m/z* 433.

12g: Yellow solid. 71.9% yield; m.p. 170 – 172°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.76 (s, 6H), 6.91 (s, 1H), 6.99 (d, *J* = 6.7 Hz, 1H), 7.09-7.20 (m, 1H), 7.33 (d, *J* = 5.8 Hz, 3H), 7.55-7.63 (m, 2H), 8.20 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$+ DMSO-d_6): δ 54.9, 59.9, 102.3, 113.3, 121.7, 130.1, 137.2, 152.8; MS (ESI) *m/z*: 365.

12h:Yellow solid. 82.5% yield; m.p. 189 – 192°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.77 (s, 3H), 6.90 (s, 2H), 7.16 (s, 1H), 7.40 (s, 1H), 7.54-7.68 (m, 2H), 7.72 (d, *J* = 8.3 Hz, 2H), 8.25 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$+DMSO-d_6): δ 54.8, 100.3, 109.4, 109.7, 113.9, 126.4, 130.2, 136.5, 136.6, 156.9, 159.1, 160.0; MS (ESI): *m/z* 369.

12i: Yellow solid. 82.1% yield; m.p. 164 – 165°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.74 (s, 3H), 6.03 (s, 2H), 6.74 (d, *J* = 8.1, 2H), 7.10 (s, 1H), 7.29 (d, *J* = 8.2 1 Hz, 2H), 7.94 (d, *J* = 8.1Hz, 2H), 8.28 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 56.7, 100.0, 105.8, 112.2, 128.4, 130.3, 130.7, 133.5, 136.2, 138.2, 139.1, 149.8, 158.8; MS (ESI): *m/z* 368.

12j: Yellow solid. 73.5% yield; m.p. 168 – 170°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.04 (s, 2H), 6.74-6.79 (m, 2H), 7.06 (s, 1H), 7.29 (t, *J* = 8.8 and 10.1 Hz, 2H), 7.90 (d, *J* = 8.4Hz, 2H), 8.26 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$+DMSO-d_6): δ 100.4, 100.7, 105.4, 108.1, 118.8, 147.0, 147.5; MS (ESI): *m/z* 367.

12k: Yellow solid. 71.8% yield; m.p. 187 – 189°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.72 (s, 6H), 3.73 (s, 3H), 3.75 (s, 3H), 6.93 (s, 1H), 6.97 (d, *J* = 7.7 Hz, 3H), 7.23 (d, *J* = 6.8 Hz, 2H), 8.21 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6): δ 56.3, 60.4, 102.2, 108.9, 113.7, 116.2, 124.3, 126.2, 130.3, 131.5, 132.1, 136.8, 149.8, 158.8; MS (ESI): *m/z* 425.

12l: Yellow solid. 83.8% yield; m.p. 174 – 177°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.80 (s, 3H), 3.86 (s, 6H), 6.79 (s, 1H), 6.86 (s, 1H), 7.09 (t, *J* = 8.1and 8.8 Hz, 1H), 7.30 (s, 1H), 7.63 (t, *J* = 7.5 and 8.1 Hz, 2H), 7.80 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 55.5, 60.0, 100.9, 102.2, 121.6, 137.2, 152.9; MS (ESI): *m/z* 413.

12m: Yellow solid. 77.3% yield; m.p. 184 – 185°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (s, 6H), 3.75 (s, 3H), 3.77 (s, 3H), 6.95 (s, 1H), 6.98 (d, *J* = 8.0 Hz, 3H), 7.25 (d, *J* = 7.8 Hz, 2H), 8.22 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 53.2, 55.8, 60.4, 103.2, 109.6, 116.2, 120.1, 124.5, 126.9, 128.3, 132.6, 135.2, 140.1, 149.8, 157.8; MS (ESI): *m/z* 425.

12n: Yellow solid. 81.5% yield; m.p. 184 – 185°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 3H), 3.84 (s, 6H), 6.81 (s, 1H), 6.84 (s, 2H), 7.09 (t, *J* = 8.0 and 8.2 Hz, 2H), 7.39 (s, 1H), 7.82 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 54.5, 55.6, 106.2, 111.7, 119.2, 126.3, 128.9, 131.7, 134.2, 135.0, 138.1, 149.8, 158.8; MS (ESI): *m/z* 431.

12o:White solid. 71.1% yield; m.p. 169 – 172°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.03 (s, 2H), 6.76-6.81 (m, 2H), 7.04 (s, 1H), 7.32 (d, *J* = 8.8 Hz, 2H), 7.93 (d, *J* = 8.1Hz, 2H), 8.24 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 100.1, 108.7, 113.3, 122.8, 127.3, 128.0, 131.7, 135.3, 138.1, 149.8, 160.1;MS (ESI):*m/z* 385.

12p:Yellow solid. 81.8% yield; m.p. 190 – 192°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.08 (s, 2H), 6.76 (d, *J* = 8.4 Hz, 2H), 7.08 (s, 1H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.90 (d, *J* = 8.4 Hz, 2H), 8.28 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 99.2, 106.7, 111.4, 120.3, 128.9, 132.7, 133.6, 135.8, 139.5, 144.8, 156.8; MS (ESI):*m/z* 418.

Biology

Antimicrobial activity

The antimicrobial activity of the synthesized hybrids was evaluated using well diffusion method against various pathogenic Gram positive and Gram negative bacterial strains and different Candida strains procured from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. Previously activated pathogenic strains including *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MLS-16 MTCC 2940, *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453, *Klebsiella planticola* MTCC 530, *Candida albicans* MTCC 3017 containing 1.5×10^8 cfu/mL (equal to 0.5 McFarland standard) were spread onto the surface of Mueller-Hinton agar plates using sterile cotton swabs. Afterwards in the agar plates, wells of 6 mm diameter were prepared using a cork borer and the compounds with different concentration (ranging from 125-0.97 µg/mL) prepared in DMSO (10%) were loaded in each well. Further, DMSO (negative control), ciprofloxacin and miconazole (positive controls) (ranging from 125-0.97 µg/mL) were run in parallel. Plates were thereafter incubated at 37°C for 24 h. All the experiments were conducted in triplicates and represented as mean values.

Minimum Bactericidal Concentration and Minimum Fungicidal concentration (MBC/MFC)

The effect of various synthesized derivatives on the viability of various strains was determined in terms of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The fungicidal assay was performed in sterile 2.0 mL microfuge tubes against a panel of different pathogenic strains which were cultured overnight in Mueller Hinton broth. MFC is the lowest concentration of compound required to kill a particular Candida strain under the above assay conditions. All the experiments were carried in triplicates and the mean values were determined.

Conclusion

To recapitulate, a new series of benzothiazole linked isoxazole Schiff base derivatives were synthesized and characterized by suitable spectra and were investigated for antimicrobial activity against a panel of pathogenic microorganisms. Among them, compounds **12d,12g** and **12l** were found to be prime candidates because of their promising antimicrobial activity against all the tested strains with MIC values ranging between 3.9 – 62.5 µg/mL. Further, compounds **12d**, **12g**, and **12l** exhibited promising antifungal activity with MIC values ranging between 7.8 - 32.5 µg/mL. These three leads could be considered as prime candidates for further modifications towards the exploration of newer antimicrobial agents.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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