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Short communication

Synthesis and antimicrobial activity of some new N-acyl substituted phenothiazines

Tanaji N. Bansode, Jayant V. Shelke, Vaijanath G. Dongre*

Department of Chemistry, University of Mumbai, Vidyanagari, Kalina, Santacruz (East), Mumbai 400 098, India

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ABSTRACT

A series of 2-substituted N-acylphenothiazines were synthesized by using imides, N-carboxymethyl imides and the structures of these newly synthesized compounds were confirmed by spectral and elemental analyses. All new compounds were tested for their antibacterial and antifungal activities. Some compounds showed promising antibacterial and antifungal activities.

1. Introduction

Phenothiazines have found widespread use in medicinal chemistry. Phenothiazine and related compounds have been reported to possess various diverse biological activities including tranquilizers [1], anti-inflammatory [2], antimalarial [3], antipsychotropic [4], antimicrobial [5], antitubercular [6–8], antitumor [9–11], antihistamine [12] and analgesic [13] properties. Phenothiazine derivatives having amino alkyl side chain connected to the nitrogen atom of heterocyclic unit playing crucial role in medicinal chemistry are reported in the literature [14,15].

Cyclic imides, such as succinimide, maleimide and phthalimide possess structural features, which confer potential biological activity [16] and pharmaceutical use. Their molecules contain an imide ring and the general structure –CO–N(R)–CO–, so that they are hydrophobic and neutral, and can therefore cross biological membranes in vivo. The various classes of cyclic imides have received attention due to their antibacterial, antifungal, analgesic [17] and antitumor activities [18,19].

As part of our project, we have synthesized some new N-acylphenothiazines with imides and subjected for antimicrobial activity. To our knowledge, compounds in which such aromatic systems connected to the N-acylphenothiazine unit are rarely studied even though N-acylated models equipped with 1,4-benzodioxan [20], furan [21] or benzofuran [22] aromatic units have shown interesting neurotropic properties. These examples prompted us to develop a synthetic strategy for N-acylphenothiazines using imides and N-carboxyimides and evaluate their antimicrobial activities.

2. Chemistry

The N-acyl derivatives of phenothiazines **3a–d, 4a–d** and **5a–d** (Scheme 1) were synthesized by two different methods. In method (A), the compounds (**2a–d**) were treated with different imides in presence of anhydrous K_2CO_3 at room temperature and in method (B), the compounds (**1a–d**) were treated with chlorides of acids **6**, **7**, and **8** in xylene at 120 °C. The yield of the products was found to be excellent by method (A). The reaction sequences are outlined in Scheme 1.

3. Result and discussion

Formation of N-acyl derivatives of phenothiazine **3**, **4** and **5** was confirmed on the basis of elemental analysis, IR and NMR. The IR spectra of compound (**3a**) did not show absorption in the range 3320–3340 cm^{-1} due to the absence N–H group, but instead

* Corresponding author. Tel.: +91 022 2652 60 91; fax: +91 022 2652 85 47.
E-mail address: dongrevg@yahoo.com (V.G. Dongre).

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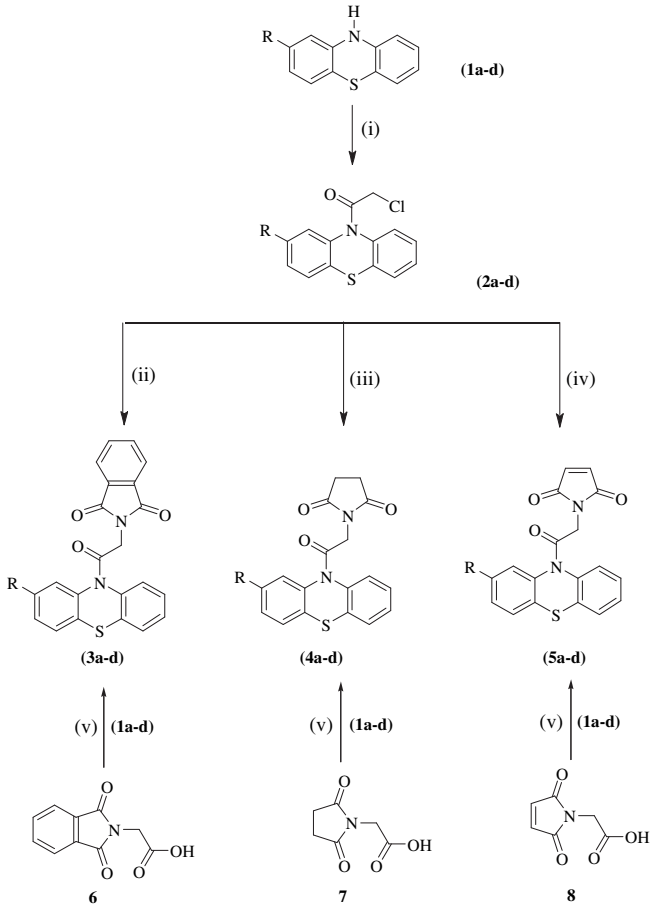

Scheme1. Synthetic route of compounds **3a–d**, **4a–d** and **5a–d**. R: a – H, b – Cl, c – CF_3, d – $COCH_3$. Reaction reagents and conditions: (i) $ClCH_2COCl$, C_6H_6, reflux 60 °C, 74%; (ii) phthalimide, K_2CO_3, DMSO, rt 3–4 h, 80%; (iii) succinimide, K_2CO_3, DMSO, rt, 3–4 h, 82%; (iv) maleimide, K_2CO_3, DMSO, rt, 3–4 h, 79%; (v) (a) PCl_5 in dry C_6H_6, reflux 80 °C, 1 h, (b) **1a–d** in xylene, reflux 120 °C, 2–3 h, 74%.

showed new peak at 1698 cm^{-1} due to amide and at 1769 cm^{-1} due to cyclic C═O stretch.

The ^1H NMR spectrum of **3a** showed a singlet at δ 4.50 due to the CH_2 protons. It showed two multiplets resonating at δ (7.88–7.71) and δ (7.60–7.32) due to phthalimide and phenothiazine ring protons respectively. In case of **3d**, **4d** and **5d** additional singlet at δ 2.44–2.48 showed due to acetyl protons.

Further evidence for the formation of compound (**3a**) was obtained by recording the mass spectra. The mass spectrum of compound **3a** showed a molecular ion peak at *m*/*z* 386 which is in conformity with the molecular formula $C_{22}H_{14}N_2O_3S$. The other fragmentation peaks are observed at 200 (26%), 188 (60%) and 160 (86%). The characterization data of N-acyl derivatives of phenothiazine **3a–d**, **4a–d** and **5a–d** are given in Table 1.

4. Conclusion

In conclusion, we have developed a simple and efficient method for the synthesis of N-acyl substituted phenothiazine derivatives. The antibacterial and antifungal activities were observed in all the tested compounds. Four compounds showed good bacterial inhibition almost equivalent to standard drug. As far as the antifungal screening results are concerned all compounds showed moderate to good activity against all fungal strains.

5. Experimental section

All chemicals were purchased from Aldrich and Merck Ltd, Mumbai (India), and were used without further purification. The melting points were determined in open capillaries using a Toshniwal melting point apparatus and are uncorrected. The IR spectra were recorded in the solid state as a KBr suspension on a Perkin–Elmer spectrum one FT-IR spectrophotometer and ^1H NMR spectra were obtained in $CDCl_3$ on a Brucker 400 MHz instrument using TMS as an internal standard (chemical shifts in δ, ppm), Mass spectra on a LCQ Adavantage Thermo Finnigen spectrometer. Elemental analysis was performed on Carlo Erba 1108 analyzer.

*5.1. General procedure for synthesis of N-(2-chloroacetyl) phenothiazines [23,24] (**2a–d**)*

To the solution of 10H-phenothiazines [25–27] **1a–d**, (0.01 mol) in dry benzene 50 ml, chloroacetyl chloride (0.01 mol) was added at 0–5 °C, the reaction mixture was refluxed under stirring for 3–4 h at 50–60 °C temperature. The resulting mixture was distilled off and poured on ice cold water. The solid thus obtained was recrystallized from ether.

*5.1.1. Compound **2a***

White powder, yield 85%, m.p. 100–102 °C, IR (KBr) cm^{-1}: 1693 (C═O), 2952 (CH_2), 3067 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.08–7.82 (m, 8H, Ar–H), 4.52 (s, 2H). Anal. calcd. for $C_{14}H_{10}ClNOS$: C, 60.98; H, 3.6; N, 5.08. Found: C, 61.03; H, 3.52; N, 5.19%.

*5.1.2. Compound **2b***

White powder, yield 63%, m.p. 115–118 °C, IR (KBr) cm^{-1}: 1670 (C═O), 2960 (CH_2), 3089 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.32–8.12 (m, 7H, Ar–H), 4.50 (s, 2H). Anal. calcd. for $C_{14}H_9Cl_2NOS$: C, 54.19; H, 2.90; N, 4.15. Found: C, 53.99; H, 3.12; N, 4.38%.

*5.1.3. Compound **2c***

Greenish white powder, yield 70%, m.p. 110–112 °C, IR (KBr) cm^{-1}: 1663 (C═O), 2961 (CH_2), 3040 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.14–7.89 (m, 7H, Ar-H), 4.58 (s, 2H). Anal. calcd. for $C_{15}H_9ClF_3NOS$: C, 52.40; H, 2.62; N, 4.07. Found: C, 52.59; H, 2.68; N, 4.13%.

*5.1.4. Compound **2d***

Faint Green powder, yield 74%, m.p. 136–140 °C, IR (KBr) cm^{-1}: 1666, 1681 (C═O), 2958 (CH_2), 2989 (CH_3), 3059 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.02–7.35 (m, 7H, Ar–H), 4.48 (s, 2H), 2.28 (s, 3H). Anal. calcd. for $C_{16}H_{12}ClNO_2S$: C, 60.47; H, 3.77; N, 4.40. Found: C, 60.31; H, 3.52; N, 4.48%.

*5.2. General procedure for synthesis of compounds (**3a–d**, **4a–d** and **5a–d**)*

Method (*A*). A mixture of N-(2-Chloroacetyl) phenothiazines **2a–d** (0.125 mmol), phthalimide (0.125 mmol) and anhydrous K_2CO_3 (0.125 mmol) in 20 ml DMSO was stirred at room temperature for 4–5 h. The reaction mixture was poured on water and crude precipitate was filtered to afford **3a–d**.

Similarly, compounds **4a–d** and **5a–d** were synthesized by using succinimide and maleimide respectively.

Method (*B*). PCl_5 (0.255 mmol) was added to 2-(1,3-dioxoisoindolin-2yl) acetic acid (**6**) (0.250 mmol) in dry benzene 20 ml and refluxed for 1 h at 70–80 °C, the solvent was removed under vacuum. Then phenothiazines **1a–d** (0.125 mmol) in dry xylene (10 ml) was added to reaction mixture. Then reaction mixture was refluxed further for 2–3 h at 120 °C. The reaction mixture was kept

Table 1
Physical data of compounds **3a–d**, **4a–d** and **5a–d**.

Compound no.	R	Mol. formula	M.p. (°C)	Yield (%)		Analysis (%)		
				Method		Calculated (found)		
				(a)	(b)	C	H	N
3a	H	$C_{22}H_{14}N_2O_3S$	232–234	82	77	68.38(68.48)	3.65(3.89)	7.25(6.99)
3b	Cl	$C_{22}H_{13}ClN_2O_3S$	228–230	76	72	62.78(62.69)	3.11(3.12)	6.66(6.74)
3c	CF_3	$C_{23}H_{13}F_3N_2O_3S$	238–240	82	69	60.79(60.85)	2.88(2.95)	6.16(6.26)
3d	Ac	$C_{24}H_{16}N_2O_4S$	224–226	80	77	67.28(67.25)	3.76(3.82)	6.54(6.24)
4a	H	$C_{18}H_{14}N_2O_3S$	178–182	87	80	63.89(63.78)	4.17(4.28)	8.28(8.19)
4b	Cl	$C_{18}H_{13}ClN_2O_3S$	170–172	85	84	57.99(60.11)	3.51(3.39)	7.51(7.59)
4c	CF_3	$C_{19}H_{13}F_3N_2O_3S$	102–106	74	70	56.16(56.10)	3.22(3.08)	6.89(6.77)
4d	Ac	$C_{20}H_{16}N_2O_4S$	80–82	82	72	63.14(63.18)	4.24(4.27)	7.36(7.37)
5a	H	$C_{18}H_{12}N_2O_3S$	189–192	81	79	64.27(64.08)	3.60(3.66)	8.33(8.23)
5b	Cl	$C_{18}H_{11}ClN_2O_3S$	158–162	69	66	58.30(58.21)	2.99(2.89)	7.55(7.50)
5c	CF_3	$C_{19}H_{11}F_3N_2O_3S$	142–146	78	73	56.44(56.32)	2.74(2.75)	6.93(6.89)
5d	Ac	$C_{20}H_{14}N_2O_4S$	108–112	86	77	63.48(63.32)	3.73(3.65)	7.40(7.43)

Ac – $COCH_3$.

overnight; the solid thus obtained was recrystallized from xylene with addition of activated carbon to afford **3a–d**.

Similarly, compounds **4a–d** and **5a–d** were synthesized by using 2-(2,5-dioxopyrrolidin-1-yl) acetic acid (**7**) and 2-(2,5-dioxo-2H-pyrrol-1(5H)-yl) acetic acid (**8**) respectively.

*5.2.1. 2-[Oxo-2-(10H-phenothiazin-10-yl)ethyl]-1H-isoindole-1,3(2H)-dione (**3a**)*

White powder, IR (KBr) cm^{-1}: 1698, 1715 (C=O), 1769 (cyclic C=O), 2937 (CH_2), 1586,1615 (C=C aromatic), 3059 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.88–7.71 (m, 4H, phthalimide), 7.60–7.32 (m, 8H, phenothiazine), 4.50 (s, 2H); MS: *m*/*z* (%): 387 [$M^+ + 1$] (100), 388 [$M^+ + 2$] (30), 188 (60), 160 (100).

*5.2.2. 2-[2-(2-Chloro-10H-phenothizin-10-yl)-2-oxoethyl]-1H-isoindole-1,3(2H)-dione (**3b**)*

White powder, IR (KBr) cm^{-1}: 1728 (C=O), 1771 (cyclic C=O), 2942 (CH_2), 1587, 1614 (C=C aromatic), 3068 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.87–7.72 (m, 4H, phthalimide), 7.57–7.31 (m, 7H, phenothiazine), 4.58 (s, 2H); MS: *m*/*z* (%): 422 [$M^+ + 1$] (96), 423 [$M^+ + 2$] (65), 188 (60), 160 (95).

*5.2.3. 2-{2-Oxo-2-[2-(trifluoromethyl)-10H-phenothiazin-10-yl] ethyl}-1H-isoindole-1,3(2H)-dione (**3c**)*

White powder, IR (KBr) cm^{-1}: 1723 (C=O), 1776 (cyclic C=O), 2944 (CH_2), 1582, 1608 (C=C aromatic), 3066 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.89–7.70 (m, 4H, phthalimide), 7.70–7.38 (m, 7H, phenothiazine), 4.59 (s, 2H); MS: *m*/*z* (%): 455 [$M^+ + 1$] (85), 266 (100), 247 (10), 235 (70), 198 (20), 160 (100).

*5.2.4. 2-[2-(2-Acetyl-10H-phenothiazin-10-yl)-2-oxoethyl]-1H-isoindole-1,3(2H)-dione (**3d**)*

White powder, IR (KBr) cm^{-1}: 1721, 1686 (C=O),1774 (Cyclic C=O), 2955 (CH_2), 2997 (CH_3), 1588, 1612 (C=C aromatic), 3059 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.91–7.69 (m, 4H, phthalimide), 7.82–7.48 (m, 7H, phenothiazine), 4.48 (s, 2H), 2.44 (s, 3H); MS: *m*/*z* (%): 429 [$M^+ + 1$] (100), 241 (35), 160 (50).

*5.2.5. 1-[2-Oxo-2-(10H-phenothiazin-10-yl)ethyl] pyrrolidine-2,5-dione (**4a**)*

White powder, IR (KBr) cm^{-1}: 1685, 1706 (C=O), 1777 (Cyclic C=O), 2941, 2977 (CH_2), 1589 (C=C aromatic), 3054 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.62–7.26 (m, 8H, phenothiazine), 4.40 (s, 2H), 2.79 (s, 4H, succinimide–CH_2); MS: *m*/*z* (%): 339 [$M^+ + 1$] (100), 340 [$M^+ + 2$] (20), 200 (30), 140 (100), 112 (35).

*5.2.6. 1-[2-(2-Chloro-10H-phenothiazin-10-yl)-2-oxoethyl] pyrrolidine-2,5-dione (**4b**)*

White powder, IR (KBr) cm^{-1}: 1709 (C=O), 1780 (cyclic C=O), 2938, 2967 (CH_2), 1577(C=C aromatic), 3062 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.81–7.35 (m, 7H, phenothiazine), 4.32 (s, 2H), 2.70 (s, 4H, succinimide–CH_2); MS: *m*/*z* (%): 374 [$M^+ + 1$] (85), 375 [$M^+ + 2$] (60), 140 (100), 112 (40).

*5.2.7. 1-{2-Oxo-2-[2-(trifluoromethyl)-10H-phenothiazin-10-yl]ethyl}pyrrolidine-2,5-dione (**4c**)*

White powder, IR (KBr) cm^{-1}: 1710 (C=O), 1781 (cyclic C=O), 2943, 2970 (CH_2), 1583 (C=C aromatic), 3068 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.82–7.44 (m, 7H, phenothiazine), 4.39 (s, 2H), 2.68 (s, 4H, succinimide–CH_2); MS: *m*/*z* (%): 407 [$M^+ + 1$] (100), 266 (70), 140 (100).

*5.2.8. 1-[2-(2-Acetyl-10H-phenothiazin-10-yl)-2-oxoethyl]pyrrolidine-2,5-dione (**4d**)*

White powder, IR (KBr) cm^{-1}: 1709, 1673 (C=O), 1778 (cyclic C=O), 2948, 2971 (CH_2), 1588 (C=C aromatic), 3058 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.80–7.46 (m, 7H, phenothiazine), 4.41 (s, 2H), 2.75 (s, 4H, succinimide–CH_2), 2.48 (s, 3H); MS: *m*/*z* (%): 381 [$M^+ + 1$] (100), 300 (20), 241 (90).

*5.2.9. 1-[2-Oxo-2-(10H-phenothiazin-10-yl)ethyl]-1H-pyrrole-2,5-dione (**5a**)*

White powder, IR (KBr) cm^{-1}: 1686, 1708 (C=O), 1779 (cyclic C=O), 2941, 2977 (CH_2), 1628 (C=C cyclic), 1589 (C=C aromatic), 3014 (C=C–H), 3054 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.62–7.26 (m, 8H, phenothiazine), 6.72 (s, 2H, olefinic–CH), 4.40 (s, 2H); MS: *m*/*z* (%): 337 [$M^+ + 1$] (100), 339 [$M^+ + 2$] (30), 200 (35), 138 (100), 110 (35).

*5.2.10. 1-[2-(2-Chloro-10H-phenothiazin-10-yl)-2-oxoethyl]-1H-pyrrole-2,5-dione (**5b**)*

White powder, IR (KBr) cm^{-1}: 1713 (C=O), 1778 (cyclic C=O), 2938, 2967 (CH_2), 1577 (C=C aromatic), 3062 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.81–7.35 (m, 7H, phenothiazine), 6.70 (s, 2H, olefinic–CH), 4.32 (s, 2H), MS: *m*/*z* (%): 372 [$M^+ + 1$] (85), 373 [$M^+ + 2$] (60), 140 (100), 112 (40).

*5.2.11. 1-{2-Oxo-2-[2-(trifluoromethyl)-10H-phenothiazin-10-yl] ethyl}-1H-pyrrole-2,5-dione (**5c**)*

White powder, IR (KBr) cm^{-1}: 1708 (C=O), 1781 (cyclic C=O), 2943, 2970 (CH_2), 1618 (C=C cyclic), 1583 (C=C aromatic), 3018 (C=C–H), 3068 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.82–7.44

(m, 7H, phenothiazine), 6.69 (s, 2H, olefinic–CH), 4.39 (s, 2H); MS: *m/z* (%): 405 [$M^+ + 1$] (100), 266 (70), 140 (100).

*5.2.12. 1-[2-(2-Acetyl-10H-phenothiazin-10-yl)-2-oxoethyl]-1H-pyrrole-2,5-dione (**5d**)*

White powder, IR (KBr) cm^{-1}: 1709, 1673 (C═O), 1778 (cyclic C═O), 2948, 2971 (CH_2), 1588 (C═C aromatic), 3058 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.80–7.46 (m, 7H, phenothiazine), 6.72(s, 2H, olefinic–CH), 4.41 (s, 2H), 2.44 (s, 3H); MS: *m/z* (%): 379 [$M^+ + 1$] (100), 300 (20), 241 (90).

5.3. General procedure of N-carboxy acids [28–30]

*5.3.1. 2-(1,3-Dioxoisoindolin-2yl) acetic acid (**6**)*

A mixture of equivalents amount of phthalic anhydride (0.05 mol) and glycine (0.05 mol) were refluxed in 40 ml toluene for 3 h. Toluene was removed under vacuum, followed by addition of 80 ml of water and 1.5 ml of concentrated HCl. The mixture was stirred well, filtered and recrystallized from ethanol.

White powder, yield 95%, m.p, 191–192 °C, IR (KBr) cm^{-1}: 1720 (C═O), 1766 (cyclic C═O), 3040 (Ar–H); ^1H NMR ($CDCl_3$): δ 7.60–7.95 (m, 4H, Ar–H), 4.57(s, 2H); MS: *m/z* (%): 161 [$M^+ - CO_2$] (90), Anal. calcd. for $C_{10}H_7NO_4$: C, 58.54; H, 3.44; N, 6.83. Found: C, 58.59; H, 3.32; N, 6.38%.

Similarly **7** and **8** were synthesized from succinic anhydride and maleic anhydride respectively.

*5.3.2. 2-(2,5-Dioxopyrrolidin-1-yl) acetic acid (**7**)*

White powder, yield 92%, m.p. 108–109 °C, IR (KBr) cm^{-1}: 1718 (C═O), 1768 (cyclic C═O), 2972 (CH_2); ^1H NMR ($CDCl_3$): δ 2.70 (s, 4H,), 4.07(s, 2H); MS: *m/z* (%): 113 [$M^+ - CO_2$] (96). Anal. calcd. for $C_6H_7NO_4$: C, 45.86; H, 4.49; N, 8.91. Found: C, 45.49; H, 4.25; N, 9.02%.

*5.3.3. 2-(2,5-Dioxo-2H-pyrrol-1(5H)-yl) acetic acid (**8**)*

White powder, yield 93% m.p 112–113 °C, IR (KBr) cm^{-1}: 1710 (C═O), 1776 (cyclic C═O), 3040 (C–H olefinic); ^1H NMR ($CDCl_3$): δ 6.70 (s, 2H, olefinic), 3.97(s, 2H); MS: *m/z* (%): 111 [$M^+ - CO_2$] (87), Anal. calcd. for $C_6H_5NO_4$: C, 46.5; H, 3.15; N, 9.10. Found: C, 46.44; H, 3.25; N, 9.10%.

6. Biological activity

6.1. Antibacterial studies

The newly synthesized compounds were screened for their antibacterial activity against *Bacillus subtilis* (ATCC-11774), *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923) and *Pseudomonas aeruginosa* (ATCC-27853) bacterial strains by disc diffusion method [31,32]. Discs measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heat at 140 °C for an hour. The test compounds were prepared with different concentrations using dimethylsulphoxide. One milliliter containing 100 times the amount of chemical in each disc was added to each bottle, which contains 100 discs. The discs of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 °C for 24 h. Ciprofloxacin was used as a standard drug. Solvent and growth controls were prepared and kept. Zones of inhibition and minimum inhibitory concentrations (MICs) were noted. The results of antibacterial studies are given in Table 2.

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. Compounds **3a**, **3b**, **3d**, **4a**, **4d**, **5a**, **5b**, **5c** are active at 10 μg/ml concentrations against *E. coli*. Compounds **3a**, **3b**, **3d** are active at 10 μg/ml against *S. aureus* and Compounds **3b** is active at 10 μg/ml against *P. aeruginosa* i.e. almost equivalent to standard. Compounds **3a**, **3d**, **4a**, **4c**, **5a**, **5c**, **5d** are active at 15 μg/ml against *B. subtilis*.

Table 2
Antibacterial activity of the compounds **3a–d**, **4a–d** and **5a–d**.

Compound no.	Antibacterial activity			
	B. subtilis	*E. coli*	*S. aureus*	*P. aeruginosa*
3a	17(15)	22(10)	20(10)	–
3b	13(30)	23(10)	19(10)	18(10)
3c	14(25)	–	–	–
3d	16(15)	21(10)	19(10)	–
4a	18(15)	24(10)	–	–
4b	14(30)	16(15)	12(30)	16(15)
4c	14(25)	21(20)	18(15)	19(15)
4d	–	24(10)	–	–
5a	16(15)	20(10)	15(15)	17(15)
5b	–	20(10)	18(15)	17(15)
5c	17(15)	19(10)	–	19(15)
5d	16(15)	18(20)	16(15)	17(15)
Standard	22(10)	25(10)	21(10)	23(10)

"–" Indicates bacteria are resistant to the compounds at concentration >50 μg/ml; MIC values are given in brackets.

6.2. Antifungal studies

Newly prepared compounds were screened for their antifungal activity against *Aspergillus niger* (NCIM no. 617), *Aspergillus flavus* (NCIM no. 524), *Aspergillus fumigatus* (NCIM no. 902), *Candida albicans* (NCIM no. 300) in DMSO by serial plate dilution method [33,34].

Sabourands agar media were prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting 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. Agar media (20 ml) were 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 2–3 days. Zone of inhibition and minimum inhibitory concentration (MIC) were noted. The activity of each compound was compared with fluconazole as the standard drug. The results of antifungal studies are given in Table 3. The antifungal screening data showed good activity. Compounds **3a**, **3b**, **3c**, **3d** are active at 10 μg/ml against

Table 3
Antifungal activity of the compounds **3a–d**, **4a–d** and **5a–d**.

Compound no.	Antifungal activity			
	A. niger	*A. fumigatus*	*A. flavus*	*C. albicans*
3a	23(10)	22(10)	20(15)	17(10)
3b	24(10)	21(10)	19(10)	18(10)
3c	21(10)	22(10)	18(10)	21(10)
3d	25(10)	22(10)	19(15)	14(10)
4a	19(20)	24(15)	19(20)	17(15)
4b	23(20)	12(25)	18(20)	13(25)
4c	21(20)	23(10)	19(15)	19(20)
4d	26(20)	20(25)	13(15)	–
5a	17(20)	18(20)	15(20)	17(20)
5b	18(20)	15(20)	16(20)	17(20)
5c	19(20)	19(20)	10(30)	19(20)
5d	–	18(25)	16(30)	–
Standard	23(10)	24(10)	20(10)	21(10)

"–" Indicates fungus are resistant to the compounds at concentration >50 μg/ml; MIC values are given in brackets.

A. niger, *A. flavus*, *A. fumigatus* and *C. albicans* i.e., almost equivalent to standard drug.

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