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Synthesis and biological activity of substituted 3-fluoro/3-trifluoromethyl 10*H*-phenothiazines, its ribofuranosides and sulfones

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ABSTRACT

The present article describes the synthesis of new fluorinated 10H-phenothiazines by Smiles rearrangement. The reaction of these synthesized phenothiazines with sugar (beta-D-ribofuranose 1-acetate-2,3,5-tribenzoate) afforded the new ribofuranosides and the oxidation of these synthesized phenothiazines with 30% hydrogen peroxide in glacial acetic acid resulted in the formation of 10*H*-phenothiazine sulfones. These compounds were evaluated for their antioxidant and antimicrobial activities (using broth microdilution method). The structural assignments of the synthesized compounds were made on the basis of elemental analysis and spectroscopic data.

1. Introduction

The vogue of constructing biologically active molecules such as phenothiazines, their ribofuranosides and sulfones by molecular modification has been of enormous interest in recent years because of their theoretical and structural implications, the diversity of synthetic methods used in their preparation, and their biological, pharmacological and industrial significance.

These moieties are extensively used as tranquilizers, sedatives, antibacterial, antifungal, antitumor, anticancer, CNS depressants, etc. A slight change in the substitution pattern in phenothiazine nucleus causes distinguishable difference in their biological activities [1–7]. Therefore, these observations prompted us to synthesize new fluorinated phenothiazines. Fluorinated phenothiazines serve as heterocyclic base for the formation of ribofuranosides on treatment with sugar. On refluxing with hydrogen peroxide in glacial acetic acid, 10*H*-phenothiazines yielded 10*H*-phenothiazine-5,5-dioxides. These prepared ribofuranosides and sulfones too possess similar chemotherapeutic activities. The synthesized compounds were screened for their antioxidant [8–12] and antimicrobial activities [13–16].

2. Results and discussion

Two series of fluorinated 10*H*-phenothiazines were prepared by treating 2-aminobenzenethiols (**1a–b**) with o-halonitrobenzenes (**2a–c**). In first series, substituted 10*H*-phenothiazine (**5a**) can be accomplished via Smiles rearrangement of substituted 2-formamido-2′-nitrodiphenylsulfide (**4a**). The formyl derivative was prepared by the formylation of 2-amino-2′-nitrodiphenylsulfide (**3a**), which in turn was prepared by condensation of 2-amino-5-methoxybenzenethiol (**1a**) with 1,4-difluoro-2-nitrobenzene (**2a**) in ethanolic sodium acetate solution. In second series, 10*H*-phenothiazines (**5b,c**) were prepared by condensation of 2-amino-5-methoxybenzenethiol (**1a**)/2-amino-5-bromo-3-ethylbenzenethiol (**1b**) with reactive halonitrobenzenes [2,4-dichloro-3,5-dinitrobenzotrifluoride (**2b**) and 4-chloro-3,5-dinitrobenzotrifluoride (**2c**)] respectively, (which have two nitro groups ortho to the reactive halogen atom) in ethanolic sodium hydroxide, where Smiles rearrangement occured in situ. Oxidation of 10H-phenothiazines (**5a–c**) with 30% hydrogen peroxide in glacial acetic acid yielded sulfones (**6a–c**). All synthesized phenothiazines (**5a–c**) were treated with β-D-ribofuranose-1-acetate-2,3,5-tribenzoate in toluene stirred in vacuum on an oil bath at 155–160 °C for 10 h to form ribofuranosides (**7a–c**) (Scheme 1).

The structures proposed for the synthesized compounds were well supported by elemental analysis and spectral data. The synthesized compounds were also screened for their antioxidant and antimicrobial activities (Scheme 2).

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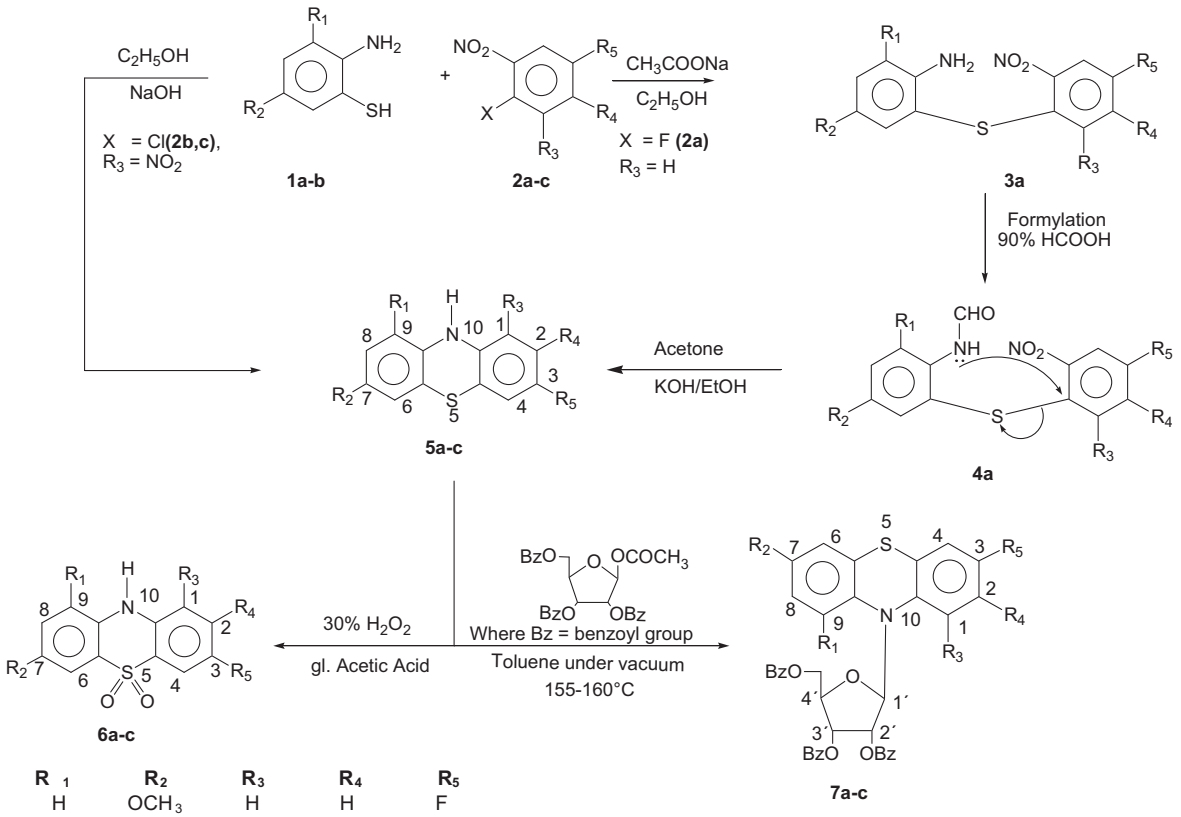
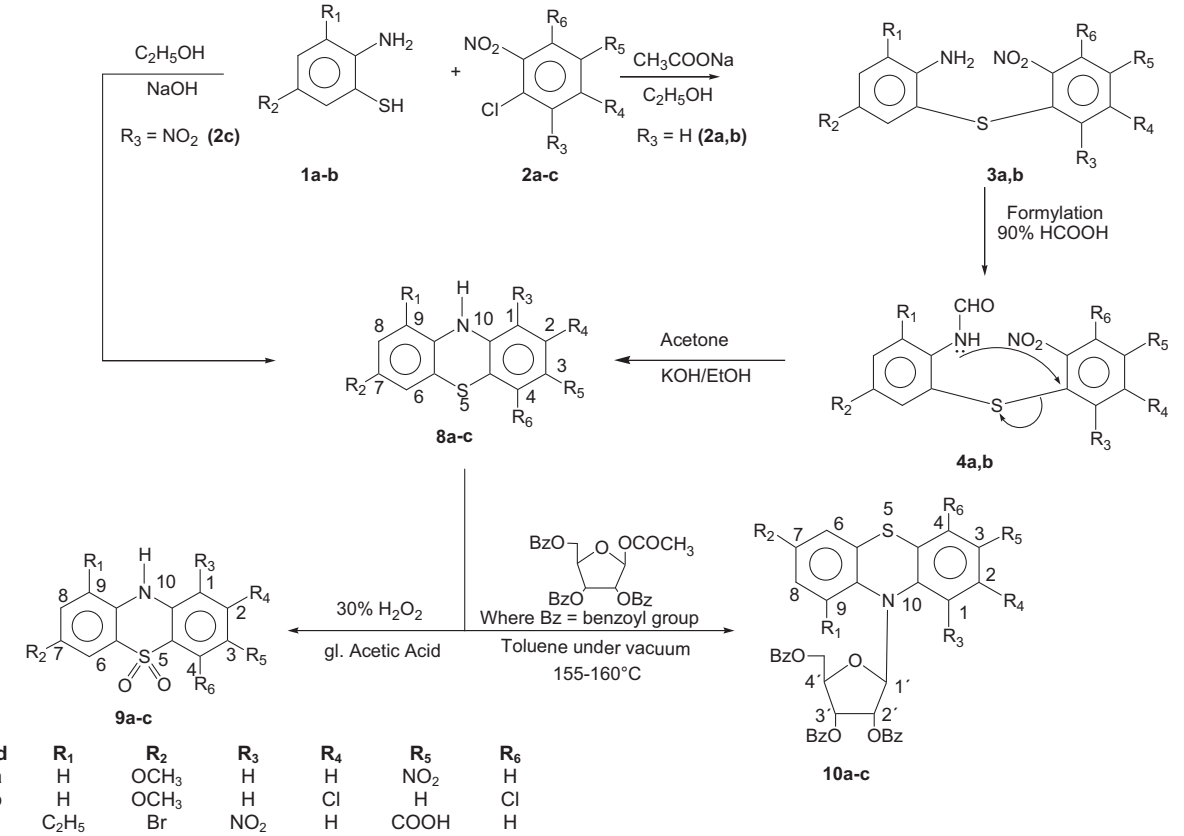


Compound	R 1	R2	R3	R4	R5
5a, 6a, 7a	H	OCH_3	H	H	F
5b, 6b, 7b	H	OCH_3	NO_2	Cl	CF_3
5c, 6c, 7c	C_2H_5	Br	NO_2	H	CF_3

Scheme 1.



Compound	R_1	R_2	R_3	R_4	R_5	R_6
8a, 9a, 10a	H	OCH_3	H	H	NO_2	H
8b, 9b, 10b	H	OCH_3	H	Cl	H	Cl
8c, 9c, 10c	C_2H_5	Br	NO_2	H	COOH	H

Scheme 2.

2.1. Antioxidant activity

The present study demonstrated that the synthesized compounds showed mixed radical scavenging activity in both DPPH and $ABTS^{\bullet+}$ assays. These compounds showed good chemopreventive and antigenotoxic effect.

(a) Compounds **5a** and **7a** showed strong radical scavenging activity in DPPH assay that have DPPH% inhibition ≥ 50.
(b) Compounds **5b**, **5c** and **7b** showed moderate radical scavenging activity in DPPH assay that have DPPH% inhibition ≥ 30.
(c) Compounds **6a**, **6b**, **6c** and **7c** showed mild radical scavenging activity in DPPH assay that have DPPH% inhibition < 30.
(d) Compounds **5a**, **5b**, **5c**, **7a** and **7b** were found to be more active in ABTSradicalradical$^{\bullet+}$ assays which showed much decline in graph.

2.2. Antimicrobial activity

The present paper is focused on the synthesis of novel fluorinated phenothiazine derivatives as possible antibacterial and antifungal agents. A series of novel compounds **5a–c**, **6a–c** and **7a–c** were prepared and tested for their in vitro antibacterial activity against the four strains of bacteria (two gram positive and two gram negative) and antifungal activity against the four strains of fungi. Three compounds of the obtained series showed high in vitro antimicrobial activity. Compounds **6a–c** showed excellent activity against all the four strains of bacteria (i.e. *Bacillus cereus*, *Staphylococcus aureus*, *E. coli* DH5 alpha and *Pseudomonas aeruginosa*). This indicated that all these three compounds showed in vitro antibacterial activity lower than that of ampicillin sodium salt. Compounds **5c** and **7c** showed good activity against all the four strains of bacteria indicating in vitro antibacterial activity slightly lower than that of ampicillin sodium salt. Compounds **5b**, **7b** and **5a**, **7a** showed moderate activity against all the four strains of bacteria. This indicated that these compounds showed in vitro antibacterial activity higher than ampicillin sodium salt.

Compounds **6a–c** showed excellent activity against all the four strains of fungi (*Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Rhizopus stolonifer*). This indicated that all these three compounds showed in vitro antifungal activity lower than that of Ketoconazole. Compounds **5c** and **7c** showed good activity against all the four strains of fungi indicating in vitro antifungal activity slightly lower than that of ketoconazole. Compounds **5b**, **7b** and **5a**, **7a** showed moderate activity against all the four strains of fungi. This indicated that these compounds showed in vitro antifungal activity higher than that of ketoconazole.

2.3. Structure activity relationships

Structure activity relationships between fluorinated derivatives (**6a–c**) and the corresponding non-fluorinated derivatives (**9a–c**) have been expressed in terms of pMIC by drawing comparison plots against different bacteria and fungi. Fluorinated compounds (**6a–c**) have superior antimicrobial properties (i.e. biologically more active) than their corresponding non-fluorinated compounds (**9a–c**) as it is evidenced by their antimicrobial activities expressed in terms of MIC (Table 3, and Tables 4–6 and Fig. 2).

3. Conclusion

The structures proposed for the synthesized compounds were well supported by elemental analysis and spectroscopic data. Regarding both DPPH and ABTS assays, compounds **5a** and **7a** showed excellent activity. Compound **6a–c** showed moderate activity as compared to their phenothiazines **5a–c** respectively due to electron withdrawing effect of oxygen in **6a–c**. Compounds **5a–c** showed good antioxidant activity as compared to their ribofuranosides **7a–c**, respectively due to slight electron withdrawing effect of ribofuranosyl moiety in the framework of **7a–c**. In general, the presence of electron donating groups on the aromatic ring increase the antioxidant activities of tested compounds as compared to compounds having electron withdrawing groups. Regarding antimicrobial activity, the MIC values of antibacterial and antifungal screening suggest that compounds **6a–c** exhibited significant antibacterial and antifungal activities as compared to their phenothiazines **5a–c**, respectively due to electron withdrawing effect of oxygen in **6a–c**. Compounds **5b**, **7b** and **5a**, **7a** bearing electron donating group like methoxy group exhibited moderate antibacterial and antifungal activities as compared to compounds **5c** and **7c** which showed good activity against all the four strains of bacteria and fungi, respectively. In general the presence of electron withdrawing group on the aromatic ring increase the antimicrobial activities of tested compounds as compared to compounds having electron donating groups.

4. Experimental

All the melting points were determined in open capillary tubes and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra (by broad band proton decoupling technique) were recorded on JEOL AL-300 spectrometer at frequencies of (300.40 MHz and 75.45 MHz) respectively, in Me_2SO-d_6/$CDCl_3$ using TMS (Tetramethyl silane) as an internal standard. IR spectra were recorded in KBr on SHIMADZU 8400 S FTIR spectrophotometer.^{19}F NMR spectra were recorded in $CDCl_3$ using CF_3COOH as standard compound. The FAB mass spectra were recorded on a JEOL SX 102/DA-600 mass spectrometer using Ar/Xe as FAB(Fast atom bombardment) gas. Purity of compounds were checked by TLC using silica gel ''G'' as adsorbent, visualizing these by UV light or in an iodine chamber.

4.1. General procedure for the synthesis of 2-amino-2′-nitrodiphenylsulfide (3a)

2-Amino-5-methoxybenzenethiol (**1a**) (0.01 mol) was dissolved in EtOH (20 mL) containing (0.01 mol) of anhydrous NaOAc in a 50 mL round bottom flask and 1,4-difluoro-2-nitrobenzene (**2a**) (0.01 mol) in 10 mL EtOH was added. The reaction mixture was refluxed for 4–5 h. The resultant solution was cooled and kept overnight in an ice chamber. The solid obtained was filtered, washed with 30% EtOH and recrystallised with MeOH.

4.2. General procedure for the synthesis of 2-formamido-2′-nitrodiphenylsulfide (4a)

The 2-amino-2′-nitrodiphenylsulfide (**3a**) (0.01 mol) obtained above was refluxed for 4 h in 90% HCOOH (20 mL). The contents were then poured into crushed ice and the solid was filtered, washed with H_2O and recrystallized from C_6H_6.

4.3. General procedure for the synthesis of fluorinated 10H-phenothiazine (5a)

Formyl derivative (**4a**) (0.01 mol) in acetone (15 mL) was refluxed and an alcoholic solution of KOH (0.2 g in 5 mL EtOH) was added. These were then heated for 30 min. After then a second lot of KOH (0.2 g in 5 mL EtOH was added and further refluxed for 4 h. The contents were poured into a beaker containing crushed ice and filtered. The residue was washed with cold H_2O, then with 30% EtOH and then crystallized from C_6H_6.

*4.3.1. 3-Fluoro-7-methoxy-10H-phenothiazine (**5a**)*

Black solid; m.p.: 90 °C; Yield: 67%, IR (KBr): ν 3360 (N–H), 1260 and 1040 (C–O–C), 2950 and 2835 (–OCH_3), 1072 (C–S), and 1270 cm^{-1} (C–F); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 8.92 (s, 1H, N–H), 7.01–6.35 (m, 6H, $J_{1,2}$ = 7.9 Hz, $J_{8,9}$ = 8 Hz, Ar–H), 3.72 (s, 3H, –OCH_3); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): δ 120.2 (C-1), 114.6 (C-2), 151.4 (C-3, d, $^1J_{CF}$ = 249.0 Hz), 118.2 (C-4), 117.6 (C-6), 152.4 (C-7), 113.2 (C-8), 118.9 (C-9), 123.1 (C-4a), 122.6 (C-5a), 137.8 (C-9a), 141.0 (C-10a), 55.7 (OCH_3 at C_7); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –109.367 (s, 1F, C–F); MS (FAB) 10 kV, *m/z* (rel. int.): 247 $[M]^+$ (100), 246 $[M–H]^+$ (60), 216 $[M–OCH_3]^+$ (45), 177 $[M–C_4H_3F]^+$ (75); "Anal. Calcd for $C_{13}H_{10}NOFS$: C, 63.15; H; 4.04; N, 5.66. Found: C, 63.38, H, 4.01; N, 5.61".

*4.4. General procedure for the synthesis of 1-nitro-10H-phenothiazines (**5b,c**)*

A mixture of 2-amino-5-methoxybenzenethiol (**1a**)/2-amino-5-bromo-3-ethylbenzenethiol (**1b**) (0.01 mol), NaOH (0.01 mol), 20 mL absolute alcohol and 0.01 mol of substituted reactive o-halonitrobenzene (**2b,c**) were taken in 50 mL round bottom flask fitted with reflux condensor. The colour of the solution darkened immediately. The reaction mixture was refluxed for 2 h, concentrated, cooled and filtered. The precipitate was washed well with hot H_2O, then with EtOH and crystallized from acetone.

*4.4.1. 2-Chloro-3-trifluoromethyl-7-methoxy-1-nitro-10H-phenothiazine (**5b**)*

Red solid; m.p.: 80 °C, yield: 82%, IR (KBr): ν 3280 (N–H), 1550 and 1320 (–NO_2), 1340 and 1140 (–CF_3), 2962 and 2872 (–OCH_3), 1252 and 1030 (C–O–C), 1084 (C–S), and 800 cm^{-1} (C–Cl); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 9.79 (s, 1H, N–H), 7.78–6.45 (m, 4H, $J_{8,9}$ = 8 Hz, Ar–H), 3.75 (s, 3H,–OCH_3); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): δ 138.9 (C-1), 125.3 (C-2), 122.5 (C-3), 136.4 (C-4), 117.5 (C-6), 152.3 (C-7), 113.2 (C-8), 119.4 (C-9), 120.8 (C-4a), 122.4 (C-5a), 137.8 (C-9a), 145.2 (C-10a), 108.5 (CF_3 at C_3, q, $^1J_{CF}$ = 270.8 Hz), 56.2 (OCH_3 at C_7); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –61.865 (s, 3F, CF_3); MS (FAB) 10 kV, *m/z* (rel. int.): 376 $[M]^+$ (100), 378 $[M+2]^+$ (33.33), 359 $[M–OH]^+$ (85), 346 $[M–NO]^+$ (62), 330 $[M–NO_2]^+$ (43), 329 $[M–HNO_2]^+$ (52); "Anal. Calcd for $C_{14}H_8N_2O_3ClF_3S$: C, 44.68; H, 2.12; N, 7.44, Found: C, 44.73; H, 2.15; N, 7.50".

*4.4.2. 7-Bromo-9-ethyl-3-trifluoromethyl-1-nitro-10H-phenothiazine (**5c**)*

Red solid; m.p.: 86 °C, yield: 78%, IR (KBr): ν 3320 (N–H), 1570 and 1340 (–NO_2), 1345 and 1120 (–CF_3), 2941 and 2880 (–CH_3), 1079 (C–S), and 590 cm^{-1} (C–Br); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 9.01 (s, 1H, N–H), 8.12–6.74 (m, 4H, Ar–H), 2.62 (q, 2H, $^3J_{HH}$ = 6.5 Hz, CH_2 of C_2H_5 at C_9), 1.29 (t, 3H, $^3J_{HH}$ = 6.5 Hz, CH_3 of C_2H_5 at C_9); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): δ 138.9 (C-1), 120.2 (C-2), 122.4 (C-3), 135.1 (C-4), 131.9 (C-6), 113.5 (C-7), 130.9 (C-8), 132.9 (C-9), 121.9 (C-4a), 123.6 (C-5a), 144.5 (C-9a), 144.8 (C-10a), 117.6 (CF_3 at C_3,q, $^1J_{CF}$ = 271.0 Hz) 19.1 (CH_2 of C_2H_5 at C_9), 15.8 (CH_3 of C_2H_5 at C_9); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –62.452 (s, 3F, CF_3); MS (FAB) 10 kV, *m/z* (rel. int.): 419 $[M]^+$ (100), 421 $[M+2]^+$ (100), 402 $[M–OH]^+$ (77), 389 $[M–NO]^+$ (40), 373 $[M–NO_2]^+$ (54), 372 $[M–HNO_2]^+$ (63); "Anal. Calcd for $C_{15}H_{10}N_2O_2F_3BrS$: C, 42.95; H, 2.38; N, 6.68, Found: C, 43.15; H, 2.33; N, 6.61".

*4.5. General procedure for the synthesis of fluorinated 10H-phenothiazine-5,5-dioxide (sulfones) (**6a–c**)*

A mixture of substituted fluorinated 10*H*-phenothiazines (**5a–c**) (0.01 mol), 20 mL glacial HOAc and 30% H_2O_2 (5 mL) in round bottom flask were refluxed for 15 min at 50–60 °C, then additional H_2O_2 (5 mL) was added. The reaction mixture was refluxed for 4 h. The contents were then poured into a beaker containing crushed ice. Residue obtained was filtered, washed with H_2O and crystallized from EtOH.

*4.5.1. 3-Fluoro-7-methoxy-10H-phenothiazine-5,5-dioxide (**6a**)*

Brown solid; m.p.: 250 °C; Yield: 45%, IR (KBr): ν 3360 (N–H), 2955 and 2841 (–OCH_3), 1278 (C–F), 1175 and 1140 (SO_2 sym), 580 and 560 (SO_2 bend), 1338, 1280 and 1260 (SO_2 asym), and 1080 cm^{-1} (C–S); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 9.15 (s, 1H, N–H), 8.01–6.40 (m, 6H, $J_{1,2}$ = 7.98 Hz, $J_{8,9}$ = 8.2 Hz, Ar–H), 3.72 (s, 3H,–OCH_3); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): δ 120.7 (C–1), 121.6 (C-2), 151.9 (C-3, d, $^1J_{CF}$ = 249.3 Hz), 113.7 (C-4), 113.4 (C-6), 152.8 (C-7), 119.7 (C-8), 119.3 (C-9), 129.8 (C-4a), 129.3 (C-5a), 133.3 (C-9a), 136.5 (C-10a), 55.7 (OCH_3 at C_7); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –108.967 (s, 1F, C–F); MS (FAB) 10 kV, *m/z* (rel. int.): 279 $[M]^+$ (100), 278 $[M–H]^+$ (34), 215 $[M–SO_2]^+$ (41), 188 $[M–SO_2–HCN]^+$ (52); "Anal. Calcd for $C_{13}H_{10}NO_3FS$: C, 55.91; H; 3.58; N, 5.01. Found: C, 55.84, H, 3.52; N, 5.07".

*4.5.2. 2-Chloro-3-trifluoromethyl-7-methoxy-1-nitro-10H-phenothiazine-5,5-dioxide (**6b**)*

Black solid; m.p.: 220 °C, yield: 53%, IR (KBr): ν 3281 (N–H), 1565 and 1340 (–NO_2), 1355 and 1150 (–CF_3), 2970 and 2881 (–OCH_3), 1180 and 1150 (SO_2 sym), 570 and 555 (SO_2 bend), 1340, 1292 and 1265 (SO_2 asym), and 1095 cm^{-1} (C–S); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 9.88 (s, 1H, N–H), 8.32–6.58 (m, 4H, $J_{8,9}$ = 8.16 Hz, Ar–H), 3.76 (s, 3H,–OCH_3); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): δ 139.8 (C-1), 131.8 (C-2), 122.9 (C-3), 131.9 (C-4), 113.1 (C-6), 152.7 (C-7), 120.2 (C-8), 119.8 (C-9), 127.5 (C-4a), 129.1 (C-5a), 133.3 (C-9a), 140.7 (C-10a), 109.0 (CF_3 at C_3, q, $^1J_{CF}$ = 270.84 Hz), 56.2 (OCH_3 at C_7); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –61.465 (s, 3F, CF_3); MS (FAB) 10 kV, *m/z* (rel. int.): 408.0 $[M]^+$ (100), 410.0 $[M+2]^+$ (33.33), 391 $[M–OH]^+$ (82), 378 $[M–NO]^+$ (61), 362 $[M–NO_2]^+$ (41), 361 $[M–HNO_2]^+$ (51), 344 $[M–SO_2]^+$ (38); "Anal. Calcd for $C_{14}H_8N_2O_5ClF_3S$: C, 41.17; H, 1.96; N, 6.86, Found: C, 41.29; H, 1.93; N, 6.79".

*4.5.3. 7-Bromo-9-ethyl-3-trifluoromethyl-1-nitro-10H-phenothiazine-5,5-dioxide (**6c**)*

Black solid; m.p.: 190 °C, yield: 59%, IR (KBr): ν 3320 (N–H), 1580 and 1346 (–NO_2), 1360 and 1130 (–CF_3), 2955 and 2887 (–CH_3), 1177 and 1165 (SO_2 sym), 590 and 570 (SO_2 bend), 1345, 1300 and 1262 (SO_2 asym), and 1090 cm^{-1} (C–S); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 9.80 (s, 1H, N–H), 8.33–6.95 (m, 4H, Ar–H), 2.64 (q, 2H, $^3J_{HH}$ = 6.51 Hz, CH_2 of C_2H_5 at C_9) 1.30 (t, 3H, $^3J_{HH}$ = 6.51 Hz, CH_3 of C_2H_5 at C_9); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): δ 139.6 (C-1), 126.7 (C-2), 118.1 (C-3), 130.6 (C-4), 127.4 (C-6), 113.9 (C-7), 137.4 (C-8), 133.3 (C-9), 128.6 (C-4a), 130.3 (C-5a), 140.1 (C-9a), 140.3 (C-10a), 122.8 (CF_3 at C_3, q, $^1J_{CF}$ = 271.6 Hz), 19.5 (CH_2 of C_2H_5 at C_9), 15.8 (CH_3 of C_2H_5 at C_9); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –62.052 (s, 3F, CF_3); MS (FAB) 10 kV, *m/z* (rel. int.): 451 $[M]^+$ (100), 453 $[M+2]^+$ (100), 434 $[M–OH]^+$ (74), 421 $[M–NO]^+$ (38), 405 $[M–NO_2]^+$ (52), 404 $[M–HNO_2]^+$ (59), 387 $[M–SO_2]^+$ (44); "Anal. Calcd for $C_{15}H_{10}N_2O_4F_3BrS$: C, 39.91; H, 2.21; N, 6.20, Found: C, 39.85; H, 2.16; N, 6.12.

*4.5.4. 7-Methoxy-3-nitro-10H-phenothiazine-5,5-dioxide (**9a**)*

IR (KBr): ν3335 (N–H), 2932 and 2830 (–OCH_3), 1556 and 1350 (–NO_2), 1160 and 1145 (SO_2 sym), 570 and 565 (SO_2 bend), 1352, 1282 and 1260 (SO_2 asym), and 1034 cm^{-1} (C–S); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 8.89 (s, 1H, N–H), 7.56–6.25 (m, 6H, $J_{1,2}$ = 8.12 Hz, $J_{8,9}$ = 8.12 Hz, Ar–H), 3.71 (s, 3H,–OCH_3); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): δ 119.3 (C-

1), 128.9 (C-2), 139.7 (C-3), 123.6 (C-4), 113.3 (C-6), 152.1 (C-7), 121.2 (C-8), 119.5 (C-9), 128.4 (C-4a), 129.5 (C-5a), 135.1 (C-9a), 146.7 (C-10a), 55.8 (OCH_3 at C_7); MS (FAB) 10 kV, *m/z* (rel. int.): 306 $[M]^+$ (100), 305 $[M\text{-}H]^+$ (26), 242 $[M\text{-}SO_2]^+$ (43), 215 $[M\text{-}SO_2\text{-}HCN]^+$ (60).

4.5.5. 2,4-Dichloro-7-methoxy-10H-phenothiazine-5,5-dioxide (**9b**)

IR (KBr): *ν*3352 (N–H), 2950 and 2836 (–OCH_3), 1185 and 1145 (SO_2 sym), 585 and 560 (SO_2 bend), 1348, 1285 and 1260 (SO_2 asym), and 1054 cm^{-1} (C–S); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, *δ* ppm from TMS): *δ* 9.07 (S, 1H, N–H), 7.69–6.35 (m, 5H, $J_{8,9}$ = 8.3 Hz, Ar–H), 3.75 (s, 3H, –OCH_3); ^{13}C NMR (75.45 MHz, $CDCl_3$, *δ* ppm from TMS): *δ* 118.1 (C-1), 140.8 (C-2), 120.6 (C-3), 135.2 (C-4), 113.0 (C-6), 152.2 (C-7), 121.4 (C-8), 120.2 (C-9), 125.9 (C-4a), 129.7 (C-5a), 134.8 (C-9a), 144.2 (C-10a), 56.0 (OCH_3 at C_7); MS (FAB) 10 kV, *m/z* (rel. int.): 330 $[M]^+$ (100), 332 $[M+2]^+$ (33.33), 329 $[M-H]^+$ (39), 266 $[M-SO_2]^+$ (61), 239 $[M-SO_2-HCN]^+$ (48).

4.5.6. 7-Bromo-3-carboxy-9-ethyl-1-nitro-10H-phenothiazine-5,5-dioxide (**9c**)

IR (KBr): *ν*3330 (N–H), 1565 and 1337 (–NO_2), 2960 and 2868 (–CH_3) 1155 and 1142 (SO_2 sym), 558 and 545 (SO_2 bend), 1348, 1280 and 1266 (SO_2 asym), and 1092 cm^{-1} (C–S); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, *δ* ppm from TMS): *δ* 9.69 (s, 1H, N–H), 8.48–6.90 (m, 4H, Ar–H), 11.20 (s, 1H, COOH), 2.68 (q, 2H, $^3J_{HH}$ = 6.76 Hz, CH_2 of C_2H_5 at C_9), 1.35 (t, 3H, $^3J_{HH}$ = 6.76 Hz, CH_3 of C_2H_5 at C_9); ^{13}C NMR (75.45 MHz, $CDCl_3$, *δ* ppm from TMS): *δ* 139.5 (C-1), 130.4 (C-2), 123.1 (C-3), 136.2 (C-4), 127.3 (C-6), 113.9 (C-7), 135.8 (C-8), 133.7 (C-9), 129.1 (C-4a), 130.0 (C-5a), 140.4 (C-9a), 141.6 (C-10a), 170.1 (COOH at C_3), 19.2 (CH_2 of C_2H_5 at C_9), 16.2 (CH_3 of C_2H_5 at C_9); MS (FAB) 10 kV, *m/z* (rel. int.): 427 $[M]^+$ (100), 429 $[M+2]^+$ (100), 410 $[M-OH]^+$ (76), 397 $[M-NO]^+$ (26), 381 $[M-NO_2]^+$ (55), 380 $[M-HNO_2]^+$ (44), 363 $[M-SO_2]^+$ (39).

4.6. General procedure for the synthesis of substituted fluorinated N-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl) phenothiazines (**7a–c**)

To the solution of (**5a–c**) (0.002 mol) in 5 mL $C_6H_5CH_3$, β-D-ribofuranose-1-acetate-2,3,5-tribenzoate (0.002 mol) was added and the contents were refluxed under vacuum with stirring on an oil bath at 155–160 °C for 15 min. The vacuum was broken and reaction mixture was protected from moisture by fitting a guard tube. Stirring was further continued for 10 h and a vacuum was applied for 10 min after every 1 h. The viscous mass obtained was dissolved in MeOH, boiled for 10 min and cooled to room temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in Et_2O, filtered, concentrated and kept in refrigerator overnight to afford crystalline ribofuranosides.

4.6.1. N-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-3-fluoro-7-methoxy-10H-phenothiazine (**7a**)

Red solid; m.p.: 96 °C; Yield: 68%, IR (KBr): *ν* 1145 (C–O–C), and 1273 cm^{-1} (C–F); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, *δ* ppm from TMS): *δ* 7.42–6.48 (m, 6H, $J_{1,2}$ = 7.92 Hz, $J_{8,9}$ = 8.04 Hz, Ar–H), 3.72 (s, 3H,–OCH_3); ^{13}C NMR (75.45 MHz, $CDCl_3$, *δ* ppm from TMS): *δ* 120.5 (C-1), 113.9 (C-2), 151.7 (C-3, d, $^1J_{CF}$ = 249.2 Hz), 118.4 (C-4), 117.5 (C-6), 152.5 (C-7), 113.9 (C-8), 118.9 (C-9), 123.5 (C-4a), 122.9 (C-5a), 137.6 (C-9a), 140.8 (C-10a), 70.1 (C-1′), 76.3 (C-2′), 72.5 (C-3′), 71.7 (C-4′), 55.7 (OCH_3 at C_7); ^{19}F NMR (282.65 MHz, $CDCl_3$): *δ* –109.359 (s, 1F, C–F); MS (FAB) 10 kV, *m/z* (rel. int.): 691 $[M]^+$ (100), 660 $[M-OCH_3]^+$ (33), 621 $[M-C_4H_3F]^+$ (72); "Anal. Calcd for $C_{39}H_{30}NO_8FS$: C, 67.72; H; 4.34; N, 2.02. Found: C, 67.84, H, 4.29; N, 2.07".

4.6.2. N-(2′,3′,5′-tri-O-benzoyl -β-D-ribofuranosyl)-2-chloro-3-trifluoromethyl-7-methoxy-1-nitro-10H-phenothiazine (**7b**)

Grey solid; m.p.: 120 °C, yield: 41%, IR (KBr): *ν* 1558 and 1315 (–NO_2), 1120 (C–O–C), 1344 and 1146 (–CF_3), and 810 cm^{-1} (C–Cl); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, *δ* ppm from TMS): *δ* 7.89–6.50 (m, 4H, $J_{8,9}$ = 8.05 Hz, Ar–H), 3.75 (s, 3H, –OCH_3); ^{13}C NMR (75.45 MHz, $CDCl_3$, *δ* ppm from TMS): *δ* 139.0 (C-1), 125.5 (C-2), 122.8 (C-3), 136.3 (C-4), 117.7 (C-6), 152.3 (C-7), 113.7 (C-8), 119.6 (C-9), 121.1 (C-4a), 122.6 (C-5a), 138.0 (C-9a), 145.2 (C-10a), 69.9 (C–1′), 76.3 (C–2′), 72.5 (C–3′), 71.6 (C–4′), 108.5 (CF_3 at C_3, q, $^1J_{CF}$ = 270.81 Hz), 56.2 (OCH_3 at C_7); ^{19}F NMR (282.65 MHz, $CDCl_3$): *δ* –61.854 (s, 3F, CF_3); MS (FAB) 10 kV, *m/z* (rel. int.): 820.5 $[M]^+$ (100), 822.5 $[M+2]^+$ (33.33), 803.5 $[M-OH]^+$ (76), 790.5 $[M-NO]^+$ (56), 774.5 $[M-NO_2]^+$ (45), 773.5 $[M-HNO_2]^+$ (50); "Anal. Calcd for $C_{40}H_{28}N_2O_{10}ClF_3S$: C, 58.50; H, 3.41; N, 3.41, Found: C, 58.59; H, 3.35; N, 3.44".

4.6.3. N-(2′,3′,5′-tri-O-benzoyl -β-D-ribofuranosyl)-7-bromo-9-ethyl-3-trifluoromethyl-1-nitro-10H-phenothiazine (**7c**)

Brown solid; m.p.: 110 °C, yield: 53%, IR (KBr): *ν* 1576 and 1330 (–NO_2), 1160 (C–O–C), 1350 and 1124 (–CF_3), 600 cm^{-1} (C–Br); ^1H NMR spectral data (300.40 MHz, Me_2SO-d_6, *δ* ppm from TMS): *δ* 8.20–6.83 (m, 4H, Ar–H), 2.62 (q, 2H, $^3J_{HH}$ = 6.5 Hz, CH_2 of C_2H_5 at C_9) 1.29 (t, 3H, $^3J_{HH}$ = 6.5 Hz, CH_3 of C_2H_5 at C_9); ^{13}C NMR (75.45 MHz, $CDCl_3$, *δ* ppm from TMS): *δ* 139.1 (C-1), 120.6 (C-2), 122.3 (C-3), 135.1 (C-4), 132.0 (C-6), 113.5 (C-7), 131.2 (C-8), 132.9 (C-9), 121.8 (C-4a), 123.6 (C-5a), 144.4 (C-9a), 144.8 (C-10a), 70.1 (C-1′), 76.3 (C-2′), 72.5 (C-3′), 71.7 (C-4′), 117.6 (CF_3 at C_3, q, $^1J_{CF}$ = 271.2 Hz), 19.4 (CH_2 of C_2H_5 at C_9), 15.8 (CH_3 of C_2H_5 at C_9); ^{19}F NMR (282.65 MHz, $CDCl_3$): *δ* –62.445 (s, 3F, CF_3); MS (FAB) 10 kV, *m/z* (rel. int.): 863 $[M]^+$ (100), 865 $[M+2]^+$ (100), 846 $[M-OH]^+$ (75), 833 $[M-NO]^+$ (47), 817 $[M-NO_2]^+$ (51), 816 $[M-HNO_2]^+$ (62); "Anal. Calcd for $C_{41}H_{30}N_2O_9F_3BrS$: C, 57.01; H, 3.47; N, 3.24, Found: C, 57.05; H, 3.41; N, 3.26".

4.7. Antioxidant activity

All the synthesized compounds were screened for their antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (Table 1) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) $ABTS^{\bullet+}$ radical cation decolorization assay (Table 2 and Fig. 1). In antioxidant activity assays (both DPPH $ABTS^{\bullet+}$ and assays), ascorbic acid was used as a positive control.

4.7.1. DPPH radical scavenging assay

Radical scavenging activity of synthesized compounds against stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined spectrophotometrically by the method described by Braca

Table 1
Antioxidant activity of synthesized compounds (DPPH assay).

Compd. no.	DPPH % inhibition[a] of 1 mg/mL of compound
5a	64.27 ± 0.08
5b	45.56 ± 1.50
5c	31.24 ± 0.08
6a	27.46 ± 0.26
6b	22.73 ± 1.02
6c	19.36 ± 1.20
7a	59.86 ± 0.07
7b	39.39 ± 0.18
7c	29.87 ± 0.05
Ascorbic acid	98.37 ± 0.02

[a] Inhibition (%) of DPPH radical scavenging activity of various compounds at particular concentration. Stock solution of crude compound was prepared as 1 mg/mL in CH_3OH. 10 μL of samples of particular concentration were added to 3 mL of 0.1 mM CH_3OH solution of DPPH·. After 30 min incubation in dark at room temperature, the absorbance was read against a blank at 517 nm.

Table 2
Antioxidant activity of synthesized compounds ($ABTS^{\bullet+}$ assay).

Compd. no.	$ABTS^{\bullet+}$ activity at different intervals (min)					
	Initial	0 min	1 min	2 min	4 min	6 min
5a	0.702	0.636	0.337	0.049	0.039	0.037
5b	0.710	0.698	0.415	0.225	0.175	0.105
5c	0.709	0.676	0.382	0.204	0.198	0.191
6a	0.710	0.682	0.469	0.350	0.286	0.225
6b	0.711	0.702	0.595	0.496	0.328	0.290
6c	0.715	0.712	0.629	0.575	0.466	0.325
7a	0.704	0.645	0.396	0.159	0.056	0.049
7b	0.705	0.658	0.280	0.235	0.205	0.182
7c	0.707	0.665	0.435	0.315	0.226	0.210
Ascorbic acid	0.717	0.34	0.049	0.039	0.03	0.028

et al. [10]. DPPH is stable free radical by virtue of delocalization of electron over the whole molecule. For hydrogen atom transfer, antioxidants quench the free radicals by donating hydrogen, while for single electron transfer, antioxidants transfer one electron to the radical.

A stock solution containing 1 mg/mL of the compound was prepared in MeOH. 10 μL of the solution were added to 3 mL of a 0.1 mM solution of DPPH in MeOH. After 30 min incubation in the dark at room temperature, the absorbance was read against a blank at 517 nm.

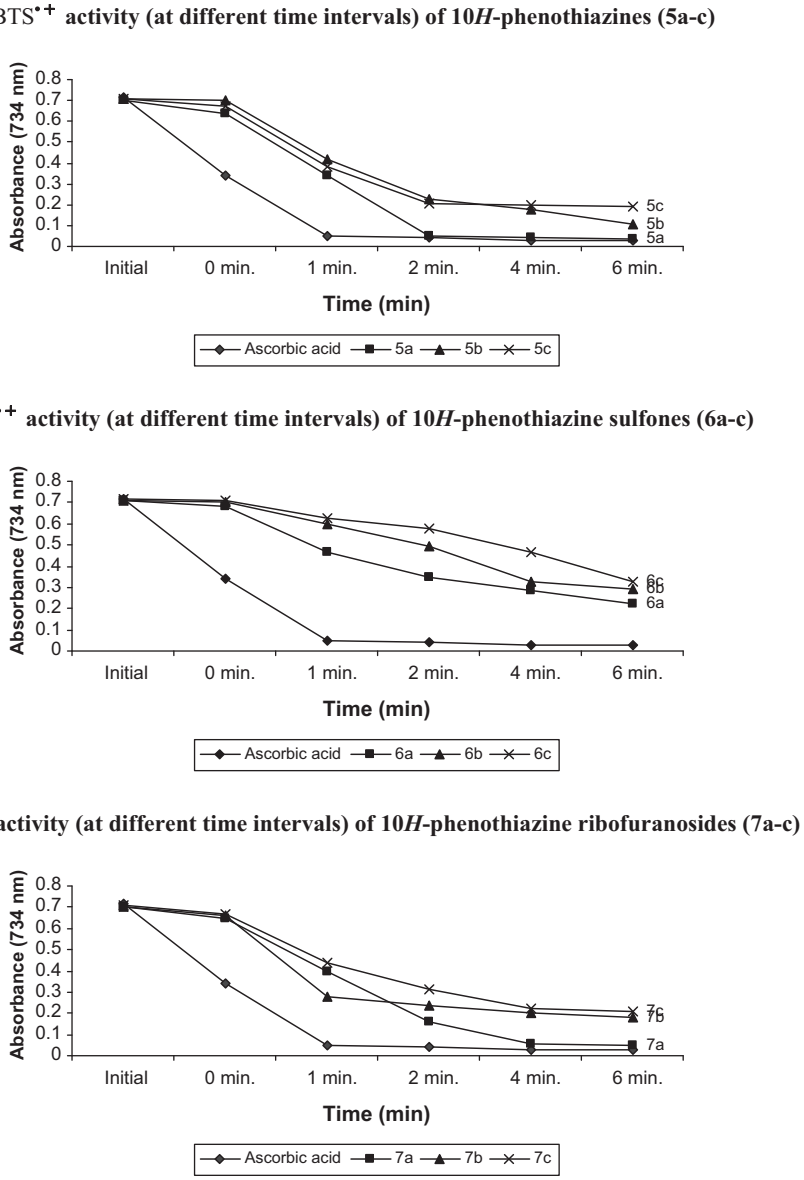


Fig. 1. The effect of time on the suppression of absorbance of ABTS by synthesized compounds. After addition of 1 mL of diluted ABTS solution (A 734 nm = 0.700 ± 0.020) to 10 μL of the compound the absorbance reading was taken at 30°C exactly 1 min., after initial mixing and every min. up to 6 min. All determinations were repeated three times.

Table 3
Antimicrobial activity of synthesized fluorinated compounds (in terms of MIC).

Compounds	Minimal inhibition concentrations of bacterial strains (MIC) in μg/mL				Minimal inhibition concentrations of fungal strains (MIC) in μg/mL			
	Bacillus cereus MTCC 4317	*Staphylococcus aureus* MTCC 3160	*E. coli* DH 5 alpha MTCC 1652	*Pseudomonas aeruginosa* MTCC 4676	*Aspergillus niger* MTCC 282	*Aspergillus flavus* MTCC 2456	*Fusarium oxysporum* MTCC 6659	*Rhizopus stolonifer* MTCC 2591
5a	50.1	78.9	79.7	74.8	53.1	112.8	84.3	63.5
5b	46.1	73.6	74.1	69.1	46.9	102.1	80.2	51.1
5c	35.1	58.6	62.1	57.4	36.9	96.1	74.2	46.9
6a	25.2	46.7	49.2	42.4	24.5	66.8	51.3	28.6
6b	21.4	42.6	43.1	36.5	20.2	61.1	48.1	24.2
6c	19.7	39.4	40.3	33.6	18.9	58.2	45.7	22.6
7a	49.4	76.8	78.1	73.5	51.2	111.6	82.4	61.2
7b	45.2	72.9	73.4	67.8	46.3	101.2	79.4	49.7
7c	34.6	57.9	61.3	56.6	36.2	95.4	73.8	46.2
Ampicillin sodium salt	38	64	64	60	–	–	–	–
Ketoconazole	–	–	–	–	38	95	74	46

The assay was carried out in triplicate and the percentage of inhibition was calculated using the following formula.

$$\text{Inhibition} = \frac{(AB - AA)}{AB} \times 100$$

where AB = Absorption of blank, AA = Absorption of test.

5. ABTS radical cation decolorization assay

The 2,2-azinobis(3-ethybenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\bullet+}$) decolorization test was also used to assess the antioxidant activity of the synthesized compounds. The $ABTS^{\bullet+}$ assay was carried out using the improved assay of Re et al. [9] $ABTS^{\bullet+}$ was generated by oxidation of ABTS with $K_2S_2O_8$. For this purpose, ABTS was dissolved in deionized H_2O at a concentration of 7 mM, and $K_2S_2O_8$ was added to a concentration of 2.45 mM. The reaction mixture was left at room temperature overnight (12–16 h) in the dark before use; the $ABTS^{\bullet+}$ solution then was diluted with EtOH to an absorbance of 0.700 ± 0.020 at 734 nm. After addition of 1 mL of the diluted ABTS solution to 10 μL of compound and mixing, absorbance readings were taken at 30 °C at intervals of exactly 1–6 min, later. All determinations were carried out in triplicate.

5.1. Antimicrobial activity

The minimal inhibition concentrations (MICs, $\mu g\ mL^{-1}$) of the chemical compounds assays were carried out by broth microdilution method as per NCCLS-1992 manual. Two Gram-positive (*Bacillus cereus* MTCC 4317 and *Staphylococcus aureus* MTCC 3160) and two Gram-negative (*Escherichia coli* DH5 alpha MTCC 1652 and *Pseudomonas aeruginosa* MTCC 4676) bacteria were used as quality control strains. For determining antifungal activities of the compounds, the following reference strains were tested: *A. niger* MTCC 282, *A. flavus* MTCC 2456, *F. oxysporum* MTCC 6659 and *R. stolonifer* MTCC 2591. Ampicillin Na salt and Ketoconazole were used as standard antibacterial and antifungal drugs, respectively. Solutions of test compounds and standard drugs were prepared in Me_2SO. Each synthesized drug was diluted obtaining 1000 μg/mL concentration as a stock solution. In primary screening, 500, 250 and 125 μg/mL concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100, 50, 25, 20, 15 μg/mL concentrations. The highest dilution showing at least 99% inhibition was taken as MIC. This means that the lowest concentration of each chemical compound in the tube with no growth (i.e. no turbidity) of inoculated bacteria/fungi was recorded as minimal inhibitory concentration of that compound. Antibacterial activities of the bacterial strains were carried out in Luria broth (Himedia) medium and all fungi were cultivated in Sabouraud Dextrose Agar (Himedia), at pH 6.9, with an inoculum of 10^8 cfu/mL by the spectrophotometric method and an aliquot of 10 μL was added to each tube of the serial dilution and incubated on a rotary shaker at 37 °C for 24 h at 150 rpm. At the end of incubation period, MIC values were recorded. The minimal inhibitory concentrations (MICs, $\mu g\ mL^{-1}$) of tested compounds against certain bacteria and fungi are shown in Table 3.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jfluchem.2011.04.012.

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