Synthesis and Biological Evaluation of New 1,4-Thiazine Containing Heterocyclic Compounds

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Abstract

4H-1,4-benzothiazines are prepared by condensation of 2-aminobenzenethiol with β-diketones / β-ketoesters in dimethyl sulfoxide. These prepared benzothiazines are used as base to prepare ribofuranosides by treating them with β-D-ribofuranosyl-1-acetate-2,3,5-tribenzoate. 4H-1,4-benzothiazines, on refluxing with hydrogen peroxide in glacial acetic acid gave 4H-1,4-benzothiazine-1,1-dioxides (sulfones). Their antioxidant and antimicrobial activity have also been evaluated. Structure of the synthesized compounds have been established by their spectral investigation.

Keywords: 4H-1,4-benzothiazines; Ribofuranosides; Antioxidant activity; Antimicrobial activity

Introduction

The synthesis of 4H-1,4-benzothiazines, their sulfones and ribofuranosides derivatives have attracted tremendous interest evidenced by a large number of publications and patents registered world wide. 4H-1,4-benzothiazines constitute an important class of heterocycles containing 1,4-thiazine ring fused to benzene ring. The oxidation of sulfide linkage in 4H-1,4-benzothiazines to dioxide leads to an important class of heterocyclic sulfones not only from medicinal and industrial point of view, but also from structural aspects. It has stimulated our interest to convert benzothiazines to sulfones to understand oxidation behaviour of 4H-1,4-benzothiazines and to investigate changes in infrared and nuclear magnetic resonance spectra caused by the conversion of sulfide linkage to sulfones. In ribofuranosides, ribosylation takes place mostly by replacing hydrogen atom attached to nitrogen in 4H-1,4-benzothiazines. All these compounds are of immense importance and are extensively employed as sedative, antispasmodic, antiulcer, blood cholesterol lowering, anticancer agents, antibacterial, antifungal and many other properties. A slight change in the substitution pattern in the benzothiazine nucleus cause distinguishable difference in their biological activities[1-13]. The newly synthesized compounds have been screened for antimicrobial and antioxidant activity[14-17].

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Material and methods

Experimental section

Melting points of synthesized compounds were determined in open capillaries and are uncorrected. The purity of the synthesized compounds was checked by TLC on silica gel 'G' coated glass plates, spotting these by UV light or in an iodine chamber. IR spectra were recorded in KBr on SHIMADZU FT-IR 8400 S spectrophotometer. ^{1}H NMR and ^{13}C NMR were recorded on JEOL AL-300 using TMS as internal standard in $CDCl_3$/DMSO-d_6. Mass spectra were recorded on JEOL SX 102/DA-600 using Argon/Xenon as FAB gas. All the compounds gave satisfactory elemental analysis.

Synthesis of substituted 4H-1,4-Benzothiazines **3a-d**

To 2-amino-4,6-dimethylbenzenethiol (0.01 mole) **1**, a stirred suspension of the appropriate **2a-d** in DMSO (5 ml) was added and the resulting mixture was refluxed for 20-40 minutes. The reaction mixture was concentrated, cooled down to room temperature and filtered. The product was washed with petroleum ether and crystallized from methanol.

Synthesis of 4H-1,4-benzothiazine sulfones **4a-d**

A mixture of substituted 4H-1,4-benzothiazines **3a-d** (0.01 mole), glacial acetic acid (20 ml) and 30% hydrogen peroxide (5 ml) was refluxed for 15 minutes. Heating was stopped and another lot of hydrogen peroxide (5 ml) was added. The reaction mixture was again refluxed for further 4 hrs. The content were poured into a beaker containing crushed ice. The yellow residue obtained was filtered and washed with water and recrystallized from ethanol.

Synthesis of N-(2´,3´,5´-tri-o-benzoyl-β-D-ribofuranosyl)-4H-1,4-benzothiazines **5a-b**

To a solution of **3a-b** (0.002 mole) in toluene (5ml), β-D-ribofuranose-1-acetate-2,3,5-tribenzoate (0.002 mole) was added and the contents were refluxed under vaccum with stirring in an oil bath at 155-160°C for 15 minutes. The vaccum was removed and the reaction mixture was protected from moisture by fitting a guard tube. Stirring was further continued for 10 hrs. and vaccum was applied for 10 min. at every hour. The viscous mass thus obtained was dissolved in methanol and 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 ether, filtered, concentrated and kept in refrigerator overnight to get crystalline ribofuranosides.

Antioxidant activity

All the synthesized compounds were screened for their antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and 2,2-azinobis (3-ethyl benzothiazoline-6-sulfonic acid) $ABTS^{\bullet+}$ radical cation decolorization assay.

DPPH Radical scavenging assay

Radical scavenging activity of synthesized compounds against stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined spectrophotometrically as described by Cuendet et al.[15] A stock solution containing 1 mg/ml of the compound was prepared in methanol. 50 μl of the solution were added to 5 ml of a 0.004% methanol solution of DPPH. After 30 min incubation in the dark at room temperature, the absorbance was read against a blank at 517 nm.

The assay was carried out in triplicate and the percentage of inhibition (table 1) was calculated using the following formula.

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

where

AB = Absorption of blank

AA = Absorption of test

Table 1: Antioxidant activity of synthesized compounds

Compd. No.	Compound		DPPH % inhibition of 1 mg/ml of the compound
	R_1	R_2	
3a	CH_3	CF_3	67.49 ± 0.04
3b	CH_3	$C_6H_3(CH_3)_2$ (m,p)	73.27 ± 0.09
3c	CH_3	$OCH(CH_3)_2$	72.61 ± 0.07
3d	C_3H_7	OCH_2CH_3	90.87 ± 0.02
4a	CH_3	CF_3	34.10 ± 0.08
4b	CH_3	$C_6H_3(CH_3)_2$ (m,p)	46.81 ± 1.2
4c	CH_3	$OCH(CH_3)_2$	15.40 ± 0.09
4d	C_3H_7	OCH_2CH_3	17.00 ± 1.1
5a	CH_3	CF_3	71.82 ± 0.05
5b	CH_3	$C_6H_3(CH_3)_2$ (m,p)	57.09 ± 0.06

Inhibition (%) of DPPH radical scavenging activity of various compounds at particular concentration. Stock solution of crude compound was prepared as 1mg/ml in methanol. Fifty microlitres of samples of particular concentration were added to 5 ml of 0.004% methanol solution of $DPPH^{\cdot}$. After 30 min. incubation in dark at room temperature, the absorbance was read against a blank at 517 nm.

ABTS Radical cation decolorization assay

The 2,2-azinobis(3-ethybenzothiazoline-6-sulphonic acid) radical cation (ABTS) decolorization test was also used to assess the antioxidant activity of synthesized compounds. The $ABTS^{\bullet+}$ assay was carried out using the improved assay of Re et al. [16] In short, $ABTS^{\bullet+}$ was generated by oxidation of 2,2-azinobis(3-ethybenzothiazoline-6-sulphonic acid) (ABTS) with potassium persulphate. For this purpose, 2,2-azinobis(3-ethybenzothiazoline-6-sulphonic acid) (ABTS) was dissolved in deionized water at a concentration of 7mM, and potassium persulphate 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 ethanol 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 (table 2 and figure 1).

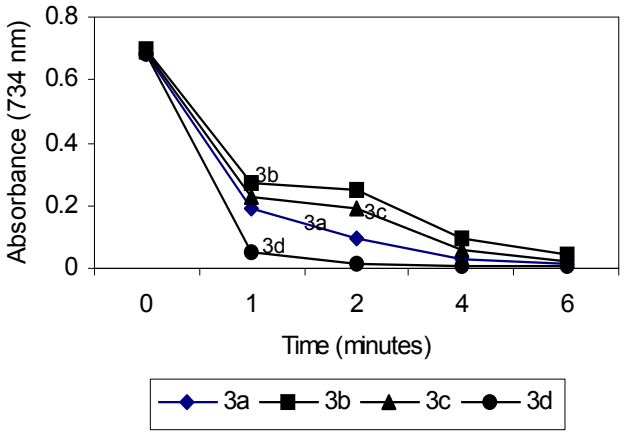
Table 2: Antioxidant activity of synthesized compounds ($ABTS^{\bullet+}$ assay).

Compd. No.	Compound		$ABTS^{\bullet+}$ Activity at different time intervals (minutes)				
	R_1	R_2	0 min.	1 min.	2 min.	4 min.	6 min.
3a	CH_3	CF_3	0.685	0.194	0.095	0.033	0.015
3b	CH_3	$C_6H_3(CH_3)_2$ (m,p)	0.696	0.273	0.253	0.097	0.041
3c	CH_3	$OCH(CH_3)_2$	0.688	0.228	0.194	0.062	0.024
3d	C_3H_7	OCH_2CH_3	0.686	0.055	0.014	0.005	0.005
4a	CH_3	CF_3	0.711	0.303	0.264	0.097	0.025
4b	CH_3	$C_6H_3(CH_3)_2$ (m,p)	0.713	0.225	0.182	0.062	0.021
4c	CH_3	$OCH(CH_3)_2$	0.695	0.231	0.175	0.041	0.029
4d	C_3H_7	OCH_2CH_3	0.697	0.335	0.321	0.213	0.16
5a	CH_3	CF_3	0.712	0.482	0.371	0.301	0.244
5b	CH_3	$C_6H_3(CH_3)_2$ (m,p)	0.695	0.109	0.09	0.002	0.002

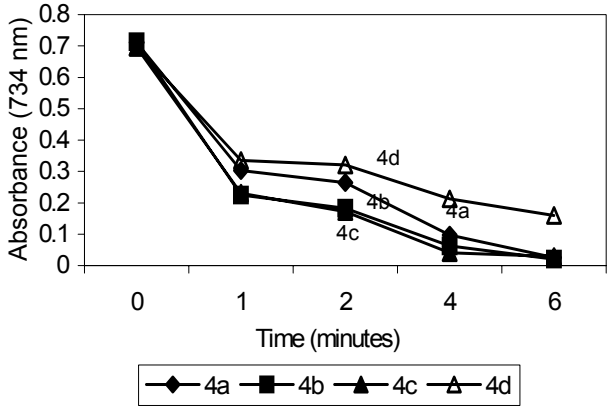
Antimicrobial activity

The synthesized compounds were tested for their antibacterial activity by using Paper Disc method[17] by measuring the zone of inhibition on agar plates with *Enterobacter*, *Coagulase positive Staphylococci*, *Coagulase negative staphylococci* as test organisms at concentration of 100 μg per disc using vancomycin and gatifloxacin as standard compounds and antifungal activity against *Candida albicans* at concentration of 100 μg/disc using flucanazole as standard compound (table 3).

$ABTS^{\bullet+}$ activity (at different time intervals) of 4H-1,4-benzothiazines (3a-d)



$ABTS^{\bullet+}$ activity (at different time intervals) of 4H-1,4-benzothiazine sulfones (4a-d)



$ABTS^{\bullet+}$ activity (at different time intervals) of 4H-1,4-benzothiazine nucleosides (5a-b)

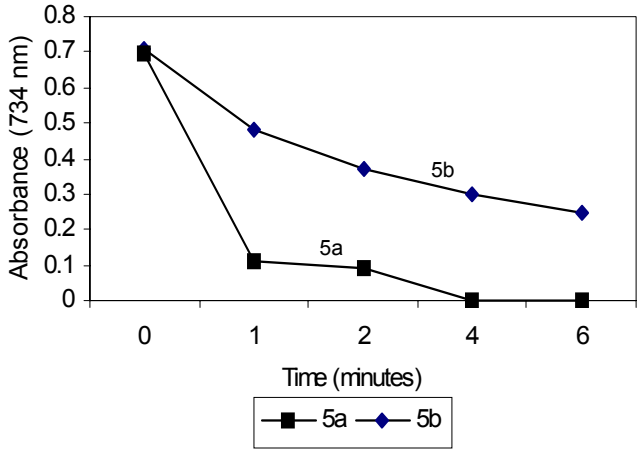


Figure1: The effect of time on the suppression of absorbance of ABTS by synthesized compounds. After addition of 1ml of diluted ABTS solution (A 734 nm = 0.700±0.020) to 10 μl of the compound the absorbance reading was taken at 30°C exactly 1 min., after initial mixing and up to 6 min. All determinations were carried out in triplicates.

Table 3: Antimicrobial activities of synthesized compounds.

Compd.	Compound		Antibacterial activity (zone of inhibition in mm)				Anti fungal activity (zone of inhibition in mm)
	R_1	R_2	*Enterobacter*	*Coagulase positive Staphylococci*	*Coagulase negative Staphylococci*		*Candida albicans*
3a	CH_3	CF_3	14	10	18		12
3b	CH_3	$C_6H_3(CH_3)_2$ (m,p)	12	15	14		10
3c	CH_3	$OCH(CH_3)_2$	10	11	16		10
3d	C_3H_7	OCH_2CH_3	15	12	18		11
4a	CH_3	CF_3	12	-	11		19
4b	CH_3	$C_6H_3(CH_3)_2$ (m,p)	11	10	-		-
4c	CH_3	$OCH(CH_3)_2$	-	10	13		-
4d	C_3H_7	OCH_2CH_3	-	11	12		10
5a	CH_3	CF_3	-	-	16		16
5b	CH_3	$C_6H_3(CH_3)_2$ (m,p)	-	11	19		12
Vancomycin			-	15	15		-
Gatafloxacin			17	-	-		-
Flucanazole			-	-	-		25

Note < 7 mm inactive; 7-9 mm weakly active; 10-12 mm, moderately active; > 12 mm, active

< 7mm, inactive; 7-11 mm, weakly active; 12-17 mm, moderately active; > 17 mm active

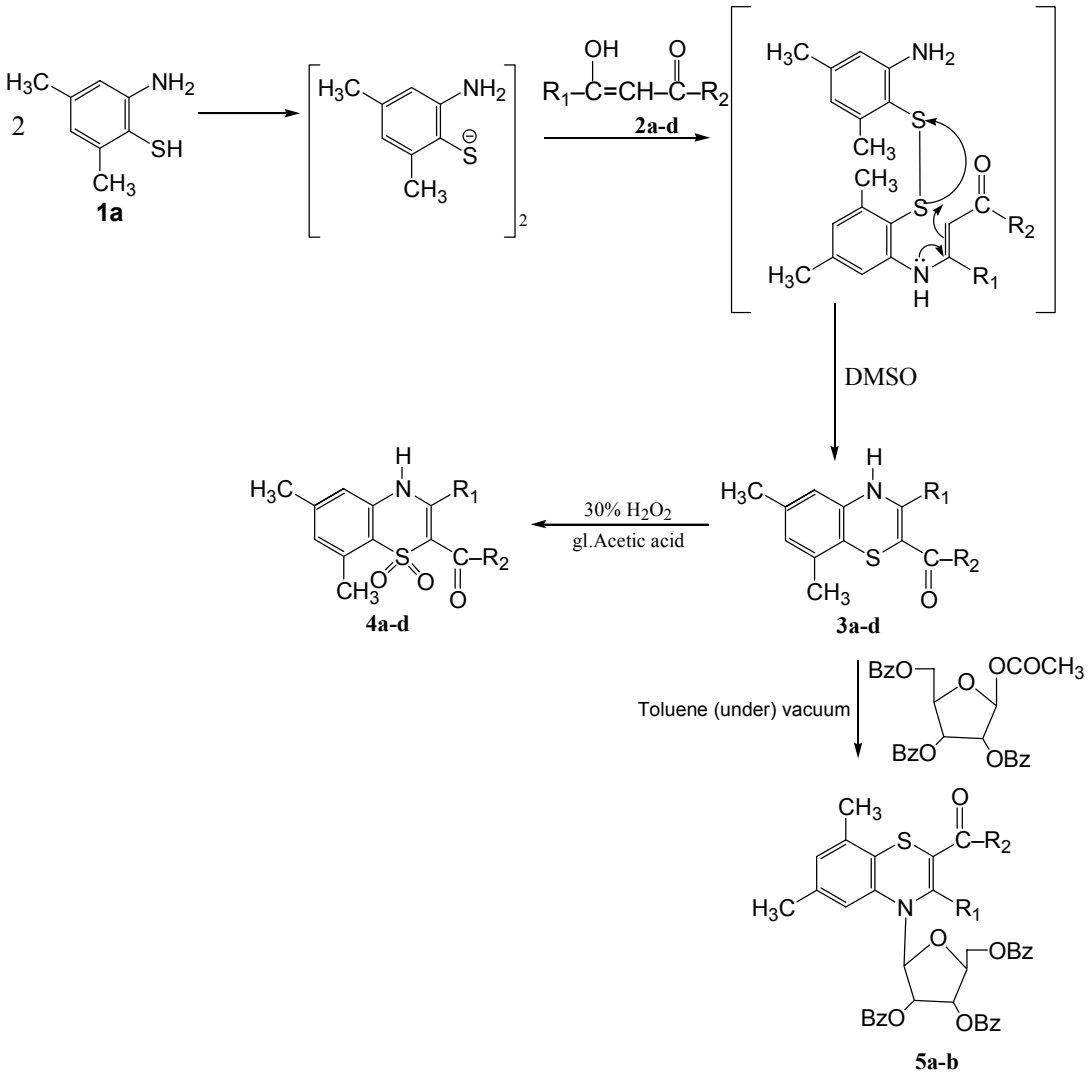
Results and Discussion

2-Amino-4,6-dimethylbenzenethiol **1** and the appropriate **2a-d** were refluxed in dimethyl sulfoxide which result in oxidative cyclization. The reaction is believed to proceed through the formation of intermediate enaminoketone. The bis-(2-aminophenyl) disulfides[18-19] were obtained which cyclizes to 4H-1,4-benzothiazine **3a-d** by scission of sulfur-sulfur bond due to high reactivity of α-position of enaminoketone system towards nucleophilic attack. Refluxing of compounds **3a-d** with 30% hydrogen peroxide in glacial acetic acid, the corresponding sulfones **4a-d** were obtained. Treatment of the pasty mixture of **3a-b** in toluene with β-D-ribofuranosyl-1-acetate-2,3,5-tribenzoate in vaccum gave the corresponding ribofuranosides **5a-b** (scheme-1).

The structure assignment of the synthesized compounds was established using spectroscopic data (table 5) and on basis of elemental analysis (table 4).

IR spectra

The IR spectral data of compound **3a-d** and **4a-d** exhibit a single sharp peak in the region 3430-3280 cm^{-1} corresponding to N–H stretching vibrations. The sharp bands observed in the region 1645-1565 cm^{-1} are due to >C=O stretching vibrations of carbonyl group. Compounds **4a-d** exhibit two intense peaks in the region 1365-1345cm^{-1} and 1190-1120 cm^{-1} for asymmetric and symmetric stretching vibration of sulfonyl group. In compounds **5a-b** the N–H band disappeared, suggesting its ribosylation. The bands due to C=O and C–O–C appeared at 1750-1745 cm^{-1} and 1190-1140 cm^{-1} respectively.



Compound	R_1	R_2
3a, 4a, 5a	CH_3	CF_3
3b, 4b, 5b	CH_3	$C_6H_3(CH_3)_2$ (m,p)
3c, 4c	CH_3	$OCH(CH_3)_2$
3d, 4d	C_3H_7	OCH_2CH_3

Scheme-1

^{1}H NMR spectra

The ^{1}H NMR spectra of compound **3a-d** and **4a-d** exhibit a singlet in the region δ 9.50-8.11 ppm due to N–H proton and a multiplet observed in the region δ 8.50-6.10 ppm corresponding to the aromatic proton.

In ribofuranosides **5a-b**, a multiplet appeared at δ 8.30-6.52 ppm due to aromatic protons. C_4'–H and CH_2 protons of the sugar moiety gave a multiplet in the region δ 4.29-4.90 ppm, while C_2'–H and C_3'–H signals appeared in the region δ 5.70-5.95 ppm as multiplet. The doublet in the region d 6.45-6.35 ppm is attributed to C_1'–H.

^{13}C NMR spectra

In the ^{13}C NMR spectra of compounds **3a-d, 4a-d** and **5a-b**, values for the aromatic carbons are seen between δ 90.2-163.1 ppm. For the carbonyl carbon at C-2, values are found between δ 165.0-196.5 ppm.

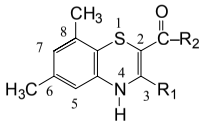
Mass spectra

In the mass spectra of 4H-1,4-benzothiazines molecular ion peaks are in accordance with their molecular weights. The base peak is obtained by fission of side chain at C_2 as acylium group (table 5).

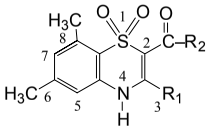
Table 4: Characterization data of synthesized compounds **3a-d**, **4a-d**, **5a-d.**

Compd. No.	Nomenclature	Compound		Mol. Formula	M.P. (°C)	Yield %	Elemental analysis Found (calcd.)		
		R_1	R_2				C	H	N
3a	2-(Trifluoroacetoxy)-3,6,8-trimethyl-4H-1,4-benzothiazine	CH_3	CF_3	$C_{13}H_{12}OF_3NS$	98	54	54.46 (54.35)	4.25 (4.18)	4.92 (4.87)
3b	2-(3´,4´-Dimethylbenzoyl)-3,6,8-trimethyl-4H-1,4-benzothiazine	CH_3	$C_6H_3(CH_3)_2$ (m,p)	$C_{20}H_{21}ONS$	104	75	74.38 (74.30)	6.56 (6.50)	4.37 (4.33)
3c	Methyl 6,8-dimethyl-3-isopropyl-4H-1,4-benzothiazine-2-carboxylate	CH_3	$OCH(CH_3)_2$	$C_{15}H_{19}O_2NS$	112	79	65.15 (64.98)	6.90 (6.85)	5.09 (5.05)
3d	Isopropyl 3,6,8-trimethyl-4H-1,4-benzothiazine-2-carboxylate	C_3H_7	OCH_2CH_3	$C_{16}H_{21}O_2NS$	95	84	66.10 (65.97)	7.25 (7.21)	4.84 (4.81)
4a	2-(Trifluoroacetoxy)-3,6,8-trimethyl-4H-1,4-benzothiazine-1,1-dioxide (sulfone)	CH_3	CF_3	$C_{13}H_{12}O_3F_3NS$	210	50	49.10 (48.90)	3.79 (3.76)	4.41 (4.38)
4b	2-(3´,4´-Dimethylbenzoyl)-3,6,8-trimethyl-4H-1,4-benzothiazine-1,1-dioxide (sulfone)	CH_3	$C_6H_3(CH_3)_2$ (m,p)	$C_{20}H_{21}O_3NS$	160	58	67.85 (67.60)	5.95 (5.91)	3.97 (3.94)
4c	Methyl 6,8-dimethyl-3-isopropyl-4H-1,4-benzothiazine-2-carboxylate-1,1-dioxide (sulfone)	CH_3	$OCH(CH_3)_2$	$C_{15}H_{19}O_4NS$	290	68	58.48 (58.25)	6.15 (6.14)	4.55 (4.53)
4d	Isopropyl 3,6,8-trimethyl-4H-1,4-benzothiazine-2-carboxylate-1,1-dioxide (sulfone)	C_3H_7	OCH_2CH_3	$C_{16}H_{21}O_4NS$	120	72	59.69 (59.44)	6.52 (6.50)	4.38 (4.33)
5a	N-(2´,3´,5´-tri-O-benzoyl)-β-D-ribofuranosyl-2-(Trifluoroacetoxy)-3,6,8-trimethyl-4H-1,4-benzothiazine	CH_3	CF_3	$C_{39}H_{32}O_8F_3NS$	118	42	63.93 (64.02)	4.45 (4.37)	1.94 (1.91)
5b	N-(2´,3´,5´-tri-O-benzoyl)-β-D-ribofuranosyl-2-(3´,4´-Dimethylbenzoyl)-3,6,8-trimethyl-4H-1,4-benzothiazine	CH_3	$C_6H_3(CH_3)_2$ (m,p)	$C_{46}H_{41}O_8NS$	104	48	72.04 (71.96)	5.39 (5.34)	1.83 (1.82)

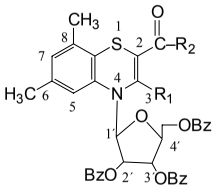
Table 5: Spectral data of synthesized compounds **3a-d**, **4a-d** and **5a-b**



3a-d



4a-d



5a-b

Comp.	^{1}H NMR (δ, ppm)	^{13}C NMR (δ, ppm)	Mass m/z (%)	IR ν(cm^{-1})
3a	8.95 (s, NH), 8.05-7.10 (m, Ar-H) 1.98 (s, CH_3 at C_3)	116.2 (C-2), 136.3 (C-3), 110.2 (C-5), 140.2 (C-6), 115.8 (C-7), 144.6 (C-8), 196.5 (C of $COCF_3$ at C_2), 128.8 (C of CF_3 at C_2), 16.2 (CH_3 at C–3), 18.2 (CH_3 at C-6), 16.1 (CH_3 at C-8)	287 (M$^+$), 245 (72), 202 (65), 160 (50), 97 (100) etc.	3310 (N–H), 1680 (C=O), 1350, 1150 (–CF_3), 2890 (–CH_3)
3b	8.52 (s, >N–H), 7.63-6.39 (m, Ar-H), 2.10 (s, CH_3 at C_3) 1.41 (s, –CH_3 at meta position of –$COC_6H_3(CH_3)_2$ (m,p) at C_2), 1.50 (s, –CH_3 protons at para position of –$COC_6H_3(CH_3)_2$ (m, p) at (C_2)	112.9 (C-2), 138.6 (C–3), 112.1 (C-5), 139.1 (C-6), 116.2 (C-7), 143.2 (C-8), 187.0 [C of CO–$C_6H_3(CH_3)_2$], 16.5 (CH_3 at C-3), 18.5 (CH_3 at C-6), 14.9 (CH_3 at C-8), C-1′ at C-2 (133.6), 130.3 (C-2′ at C-2), 138.9 (C–3′ at C-2), 144.2 (C-4′ at C-2), 129.6 (C-5′ at C-2), 126.6 (C-6′ at C-2)	323 (M$^+$), 281 (75), 190 (60), 148 (52), 133 (100), 105 (45), 79 (42) etc.	3355 (N–H), 1655 (C=O), 2900 (–CH_3)
3c	8.11 (s, >N–H), 7.61-6.37 (m, Ar-H), 2.30 (s, –CH_3 at C_3), 4.74 (septet, CH of $OCH(CH_3)_2$ at C_2), 1.94 (d, CH_3 of OCH $(CH_3)_2$ at C_2)	107.2 (C-2), 138.6 (C-3), 113.1 (C-5), 135.2 (C-6), 120.2 (C-7), 139.1 (C-8), 166.0 (C at C-2), 68.3 (CH of COOCH $(CH_3)_2$ at C-2), 22.0 (CH_3 of $COOCH(CH_3)_2$ at C-2), 21.4 (CH_3 at C-6), 15.2 (CH_3 at C-8)	277 (M$^+$), 235 (78), 190 (62), 149 (50), 87 (100) etc.	3315 (N–H), 1595 (C=O), 2910 (–CH_3)
3d	8.42 (s, >N–H), 7.5-6.4 (m, Ar-H), 2.02 (t, CH_2 (terminal) of C_3H_7 at C_3), 1.21 (sextet, CH_2 (center) of C_3H_7 at C_3), 4.72 (q, CH_2 of OCH_2CH_3 at C_2), 1.10 (t, CH_3 of C_3H_7 at C_3)	107.2 (C-2), 139.2 (C-3), 111.2 (C-5), 135.8 (C-6), 121.8 (C-7), 137.2 (C-8), 165.0 (C at C-2), 59.2 (CH_2 of $COOCH_2CH_3$), 17.6 (CH_3 of $COOCH_2CH_3$ at C-2), 20.1 (CH_3 at C-6), 16.2 (CH_3 at C-8), 21.0 (CH_2 of C_3H_7 at C-3), 18.4 (CH_2 of C_3H_7 at C-3), 15.2 (CH_3 of C_3H_7 at C-3)	291 (M+), 249 (70), 218 (68), 176 (55), 73 (100) etc.	3350 (N–H), 1650 (C=O), 2870 (–CH_3)
4a	9.10 (s, >N–H), 7.95-6.9 (m, Ar-H), 2.02 (s, CH_3 at C_3)	102.4 (C-2), 160.1 (C-3), 118.1 (C-5), 145.1 (C-6), 117.1 (C-7), 147.1 (C-8), 195.1 (C of $COCF_3$ at C_2), 130.5 (C of CF_3 at C_2), 16.0 (CH_3 at C–3), 18.1 (CH_3 at C-6), 16.5 (CH_3 at C-8)	319 (M$^+$), 277 (75), 234 (60), 192 (45), 97 (100) etc.	3380 (N–H), 1710 (C=O), 1060 (C-S)
4b	8.6 (s, >N–H), 7.5-6.45 (m, Ar-H), 2.15 (s, CH_3 at C_3), 1.45 (s, CH_3 at meta proton of –$COC_6H_3(CH_3)_2$ (m,p) at C_2), 1.52 (s, –CH_3 protons at p-position of –$COC_6H_3(CH_3)_2$ (m,p) at C_2	98.1 (C-2), 162.1 (C–3), 113.5 (C-5), 141.1 (C-6), 117.5 (C-7), 145.7 (C-8), 191.2 [C of CO–$C_6H_3(CH_3)_2$], 16.1 (CH_3 at C-3), 18.1 (CH_3 at C-6), 15.1 (CH_3 at C-8), 133.0 (C-1′ at C-2), 131.0 (C-2′ at C-2), 140.2 (C–3′ at C-2), 145.8 (C-4′ at C-2), 130.1 (C-5′ at C-2), 126.12 (C-6′ at C-2)	355 (M$^+$), 354 (71), 313 (64), 222 (50), 180 (45), 133 (100 etc.	3390 (N–H), 1690 (C=O), 1070 (C–S)
4c	8.32 (s, >N–H), 7.52-6.25 (m, Ar-H), 2.35 (s, CH_3 at C_3), 4.78 (septet, CH of $COOCH(CH_3)_2$ at C_2), 1.95 (d, CH_3 of $CH(CH_3)_2$ at C_2)	90.2 (C-2), 161.4 (C-3), 113.8 (C-5), 143.4 (C-6), 120.9 (C-7), 136.4 (C-8), 165.0 (C at C-2), 59.6 (CH at COOCH $(CH_3)_2$ at C-2), 25.1 (CH_3 of $COOCH(CH_3)_2$ at C-2), 21.2 (CH_3 at C-6), 15.2 (CH_3 at C-8)	309 (M$^+$), 267 (80), 222 (60), 181 (50), 87 (100) etc.	3420 (N–H), 1610 (C=O), 1065 (C–S)
4d	8.72 (s, >N–H), 7.6-6.35 (m, Ar-H), 2.05 (t, –CH_2 (terminal) of C_3H_7 at C_3), 1.22 (sextet, –CH_2 (center) of C_3H_7 at C_3), 1.11 (t, –CH_3 of C_3H_7 at C_3), 4.8 (q, CH_2 of OCH_2CH_3 at C_2	92.4 (C-2), 163.1 (C-3), 113.0 (C-5), 144.2 (C-6), 122.2 (C-7), 135.1 (C-8), 168.0 (C at C-2), 56.8 (CH_2 of $COOCH_2CH_3$), 18.1 (CH_3 of $COOCH_2CH_3$ at C-2), 20.5 (CH_3 at C-6), 15.5 (CH_3 at C-8), 22.5 (CH_2 of C_3H_7 at C-3), 17.1 (CH_2 of C_3H_7 at C-3), 15.0 (CH_3 of C_3H_7 at C-3)	323 (M$^+$), 281 (72), 250 (62), 208 (48), 73 (100) etc.	3400 (N–H), 1700 (C=O), 1090 (C–S)
5a	8.54-6.52 (m, Ar-H), 1.95 (s, CH3 at C_3)	116.2 (C-2), 136.3 (C-3), 110.3 (C-5), 135.2 (C-6), 118.6 (C-7), 139.1 (C-8), 14.6 (CH_3 at C-3), 182.2 (CO at C-2), 128.9 (CF_3 at C-2), 14.0 (CH_3 at C-8), 16.2 (CH_3 at C-6), 92.2 (C-1′), 75.17 (C-2′), 69.7 (C-3′), 64.4 (C-4′)	731 (M$^+$), 646 (60), 97 (100) etc.	1690 (C=O), 1360, 1170 (–CF_3 str.), 2895 (–CH_3 str.), 1160 (C–O–C str.)
5b	8.29-6.31 (m, Ar-H), 2.05 (s, CH_3 at C_3), 1.45 (s, –CH_3 at m-position of –$COC_6H_3(CH_3)_2$ at C_2, 1.53 (s, –CH_3 at p-position of –$COC_6H_3(CH_3)_2$ at C_2	115.1 (C-2), 138.2 (C-3), 111.0 (C-5), 136.1 (C-6), 120.2 (C-7), 138.2 (C-8), 15.1 (CH_3 at C-3), 185.1 (CO at C-2), 14.5 (CH_3 at C-8), 16.5 (CH_3 at C-6), 91.0 (C-1′), 76.1 (C-2′), 68.1 (C-3′), 65.1 (C-4′)	767 (M$^+$), 634 (55), 133 (100), 105 (20) etc.	1660 (C=O), 2910 (–CH_3 str.), 1150 (C–O–C str.)

Biological activity

All the synthesized compounds were screened for their antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radial scavenging assay and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation decolorization assay and also screened for their antimicrobial activity.

The present study showed that the synthesized compounds showed mixed radical scavenging activity in both DPPH and $ABTS^{\bullet+}$ assay, as follows:

(a) Compounds (3a, 3b, 3c, 3d, 5a, 5b) showed strong radical scavenging activity in DPPH assay that have DPPH% inhibition $\geq$ 50.

(b) Compounds (4a, 4b) showed moderate radical scavenging activity in DPPH assay that have DPPH% inhibition $\geq$ 30.

(c) Compounds (4c, 4d) showed mild radical scavenging activity in DPPH assay that have DPPH% inhibition < 30.

(d) Compounds (3a, 3b, 3c, 3d, 5a, 5b) were found to be more active in $ABTS^{\bullet+}$ assay which showed much decline in graph.

The results showed that the synthesized compounds (3a, 3b, 3c, 3d, 5a, 5b) have good radical scavenging activity in DPPH assay and were found to be more active in $ABTS^{\bullet+}$ assay.

The results of antibacterial screening indicated that good activity was shown by compounds 3a, 3d against *Enterobacter*, compound 3b showed good activity against *Coagulase positive staphylococci* and compounds 3a, 3b, 3c, 3d, 5a, 5b showed good activity against *Coagulase negative staphylococci*. Other compounds showed moderate to less activity against all bacterial strains. Regarding antifungal activity all compounds were found moderate to less active against fungus *Candida albicans*.

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