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Synthesis and pharmacological activities of 1-(1-naphtho[2,1-*b*]furan-2-yl)ethylidene)(arylsubstituted)thiosemicarbazide derivatives and 1-(1-naphtho[2,1-*b*]furan-2-yl)ethylideneamino)-2-thioxoarylsubstituted imidazolidin-4-one derivatives

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Some new naphtho[2,1-*b*]furan derivatives containing carbothiamide group and imidazolidine heterocycle have been synthesized. Antimicrobial and pharmacological activities of the synthesized compounds have been carried out. The newly synthesized compounds have been characterized by IR, ^{1}H NMR and mass spectral studies.

Keywords: Naphtho[2,1-b]furan, carbothiamide, imidazolidin, antimicrobial, pharmacological activities

Carbothioamides exhibit high range of biological activities including anti TB[1], antioxidant[2], antiulcer[3], and anticancer[4-6] activities. Naphtho[2,1-*b*]furan derivatives were known to show various biological[7] and pharmacological activities[8-11].

Imidazolidins are important heterocycles found in many biologically active compounds. They exhibit high range of biological activities including anticonvulsant[12], antihypertensive[13] and antiulcer[14,15] activities. Naphtho[2,1-*b*]furan derivatives were known to show various biological and pharmacological activities. Imidazolioline moiety plays a vital role in medicinal chemistry. Hence it was thought to synthesize aryl derivatives of Naphtho[2,1-*b*]furan carbothioamide and naphtho[2,1-*b*] furan derivatives with imidazolidine ring by simple method and screened them for pharmacological activities.

Results and Discussion

Carbothioamides and imidazolidins found to show a wide range of biological and pharmacological activities. In view of this we synthesized 1-(1-naphtho[2,1-*b*]furan-2-yl)ethylidene)(arylsubstituted) thio semicarbazide derivatives and 1-(1-naphtho[2,1-*b*]furan-2-yl)ethylideneamino)-2-thioxoarylsubstituted imidazolidin-4-one derivatives starting from 2-hydroxy-1-naphthaldehyde **2** with the use of chloroacetone, hydrazine hydrate and various aryl isothiocyanates by following simple reaction methods.

The compound 1-(naphtho[2,1-*b*]furan-2-yl) ethanone **3** was synthesized by condensing 2-hydroxy-1-naphthaldehyde **2** with chloroacetone in presence of potassium carbonate in acetone. The compound **3** on treatment with hydrazine hydrate in presence of catalytic amount of acid produced 1-(1-naphtho[2,1-*b*]furan-2-yl)ethylidene)hydrazine **4**. The compound **4** on treatment with 4-methylphenylisothiocyante in DMF produced **5f**. Similar method was employed to get compounds **5(a-e)** from **4** with phenyl isothiocyanate, 4-fluorophenyl isothiocyanate, 3-chlorophenylisothiocyanate, 4-nitrophenylisothiocyanate and benzylisothiocyanate. The selection of arylisothiocyanates was based on electron withdrawing and electron donating groups/which could enable to study structure activity relationship during the evaluation of pharmacological activities. The compound **5a** on treatment with chloroacetyl chloride in 1,4-dioxan medium produced **6a**. Similar method was employed to get compounds **6(b-f)** from **4** with chloroacetyl chloride. The newly synthesized compounds were evaluated for

antimicrobial, antioxidant, antitubercular and anthelmintic activities. The compounds **5e, 6d** and **6e** were found to exhibit significant antibacterial activity against *P. aerugenasa* whereas the remaining compounds found to be considerable active. The compounds **6c** and **6e** showed noticeable antifungal activity against *A. niger* and *C. lunata.* The rest of the compounds showed considerable antifungal activity. The compounds **5d** and **6d** showed potent antioxidant activity. The remaining compounds were found to show considerable antioxidant activity. All the newly synthesised compounds **5(a-f)** and **6(a-f)** showed powerful anti TB activity and considerable anthelmintic activity. It is observed that electron withdrawing and electron releasing groups resulted in enhancement of activity.

Materials and Methods

The entire chemicals used were of AR grade. Melting points were recorded in open capillaries and are uncorrected. IR spectra were recorded in Nicolet 5700 FT–IR instrument (Nicolet, Madison, WI, USA) as using KBr pellets. The ^{1}H NMR and ^{13}C NMR spectra are recorded on VNMRS-400 Agilent-NMR instrument using TMS as internal reference (ppm). Mass spectra were recorded using Water's SYNAPT G2 QTOF LCMS instrument. Purity of the compounds was checked by TLC.

Experimental Section

Synthesis of 1-(naphtho[2,1-*b*]furan-2-yl) ethanone 3

A mixture of 2-hydroxy-1-naphthaldehyde **2** (1.72g), chloroacetone (0.80mL) and anhydrous potassium carbonate (12.8 g) in dry acetone (50 mL) was refluxed for 24 hrs. The product **3** was extracted from acetone. Then acetone was evaporated to get impure product **3**. It was purified using hexane.

Synthesis of 1-(1-naphtho[2,1-*b*]furan-2-yl)ethylidene) hydrazine 4

1-(Naphtho[2,1-*b*]furan-2-yl)ethanone **3** (2.0g) was refluxed with hydrazine hydrate (2.0 g) in ethanol (30.0 mL) in presence of catalytic amount of conc. HCl for 2 hrs, and then cooled and poured into ice cold water to get a pale yellow colored 1-(1-naphtho[2,1-*b*]furan-2-yl)ethylidene)hydrazine **4**. The crude product so obtained was purified using ethanol.

Synthesis of 1-(1-naphtho[2,1-*b*]furan-2-yl) ethylidene) arylsubstitutedthiosemicarbazide derivatives 5(a-f)

1-(1-Naphtho[2,1-*b*]furan-2-yl)ethyl-idene) hydrazine **4** (0.3g) was dissolved in DMF (5 mL). To this 4-methyl phenylisothiocyanate (0.135 g) was added. The reaction mixture was stirred at room temperature until the completion of reaction, then poured into ice cold water to get 1-(1-naphtho[2,1-*b*]furan-2-yl)ethylidene)-2-(3-phenylprop-1-en-2-yl) hydrazine **5a**. It was purified using ethanol. The IR (KBr) spectrum of **5f** exhibited two absorption bands at 3291 cm^{-1} and 3392 cm^{-1} due to two –N–H bonds and an absorption band at 1963 cm^{-1} due to –C=S group. In ^{1}H NMR (DMSO) spectrum there are two singlets at δ 9.95 and δ 10.84 integrated for two –NH protons (D_2O exchangeable), and a multiplet at δ 7.1-8.2 integrated for eleven aromatic protons and two singlets at δ 2.3 and δ 2.47 due to two $–CH_3$ protons. It was further confirmed by the mass spectrum showing a (M+1) peak at 374. The remaining compounds **5(a-e)** were synthesised by following the same procedure followed for the preparation of **5f**.

Synthesis of 1-(1-naphtho[2,1-b]furan-2-yl) ethylideneamino)-2-thioxoarylsubstituted imidazolidin-4-one derivatives 6(a-f)

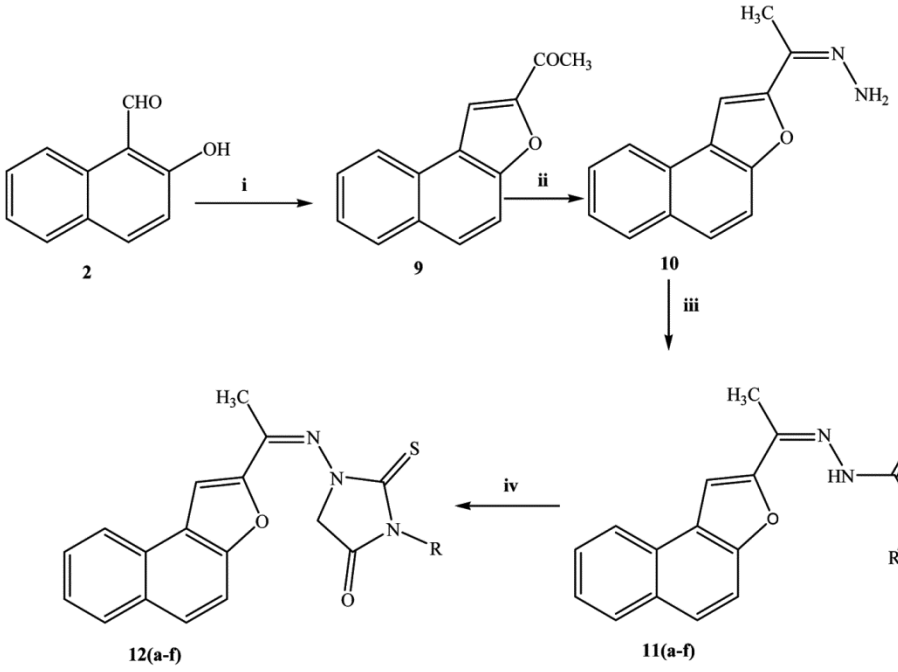
1-(1-Naphtho[2,1-*b*]furan-2-yl)ethyl-idene)-2-(3-phenylprop-1-en-2-yl)hydrazine **5a** (0.3 g) was dissolved in 1,4-dioxan (5 mL). To this chloroacetyl chloride (0.14 mL) was added. The reaction mixture was heated at 60°C for about 1 hrs. Then the reaction mixture was cooled and poured into ice cold water to get 1-(1-(naphtho[2,1-*b*]furan-2-yl)ethylideneamino)-3-phenyl-2-thioxoimidazolidin-4-one **6a**. The product obtained was filtered, washed with water and purified using hexane. The IR spectrum of **6a** exhibited one absorption band at 1712 cm^{-1} due to –C=O group and an absorption band at 2359 cm^{-1} due to –C=S group. In ^{1}H NMR spectrum there is a multiplet at δ 7.0-8.0 integrated for twelve aromatic protons, a singlet at δ 4.51 due to $–CH_2$ protons and a singlet at δ 3.24 due to $–CH_3$ protons. It was further confirmed by the mass spectrum showing a (M+1) peak at 400. Same procedure was employed to synthesize compounds **6(b-f)** from **5(b-f)**. Physical data of newly synthesized compounds were reported in Table I. The IR and ^{1}H NMR spectral data of compounds **5(a-e)** and **6(b-f)** is summarized in Table II. The synthetic route is shown in Scheme I.

Table I — Physical data of synthesized compounds **5a-f** and **6a–f**

Compd	R	m.p. (°C)	Yield (%)	Mol. Formula	Found (Calcd) %		
					C	H	N
5a	C_6H_5-	190	91.29	$C_{21}H_{17}N_3OS$	70.29 (70.17)	4.73 (4.77)	11.76 (11.69)
5b	4-F C_6H_5	236	96.01	$C_{21}H_{16}FN_3OS$	65.75 (66.83)	4.20 (4.27)	11.19 (11.13)
5c	3-Cl C_6H_5	237	90.22	$C_{21}H_{16}ClN_3OS$	64.10 (64.03)	4.01 (4.09)	10.70 (10.67)
5d	4-NO_2 C_6H_5	235	88.5	$C_{21}H_{16}N_4O_3S$	62.27 (62.36)	3.85 (3.99)	13.79 (13.85)
5e	C_6H_5 CH_2-	234	95.0	$C_{22}H_{19}N_3OS$	70.66 (70.75)	5.02 (5.13)	11.19 (11.25)
5f	4-$CH_3C_6H_5$	224	90.1	$C_{22}H_{19}N_3OS$	70.80 (70.75)	5. 02 (5.13)	11.19 (11.25)
6a	C_6H_5-	150	89.08	$C_{23}H_{17}N_3O_2S$	69.11 (69.15)	4.37 (4.29)	10.57 (10.52)
6b	4-F C_6H_5	140	85.05	$C_{23}H_{16}FN_3O_2S$	66.01 (66.17)	3.71 (3.86)	10.00 (10.07)
6c	3-Cl C_6H_5	110	85.71	$C_{23}H_{16}ClN_3O_2S$	63.58 (63.66)	3.63 (3.72)	9.57 (9.68)
6d	4-$NO_2C_6H_5$	120	87.39	$C_{23}H_{16}N_4O_4S$	62.07 (62.15)	3.55 (3.63)	12.53 (12.61)
6e	C_6H_5 CH_2-	130	80.09	$C_{24}H_{19}N_3O_2S$	69.60 (69.71)	4.56 (4.63)	10.07 (10.16)
6f	4-$CH_3C_6H_5$-	128	74.33	$C_{24}H_{19}N_3O_2S$	69.62 (69.71)	4.52 (4.63)	10.06 (10.16)

Table II — IR and ^{1}H NMR spectral data of compounds **5a-e** and **6b–f**

Compd	R	IR cm^{-1} 2–N-H	^{1}H NMR (δ, ppm)
5a	C_6H_5	3032(N-H) 3249(N-H)	δ 3.3 (s, 3H, CH_3), δ 7.13–8.26 (m, 12H ArH), δ 10.0 (s, 1H, NH) δ 10.9 (s, 1H, NH). m/z 360 (M+1)
5b	4-FC_6H_4	3059 3270	δ 3.29 (s, 3H, CH_3), δ 7.20–8.27 (m, 11H ArH), δ 10.0 (s, 1H, NH). 10.9 (s, 1H, NH). m/z 378 (M+1), 379 (M+2)
5c	3-ClC_6H_4	3047 3260	δ 2.48 (s, 3H, CH_3), δ 7.26–8.3 (m, 11H ArH), δ 10.13 (s, 1H, NH). and 11.03 (s, 1H, NH). m/z 393 (M+1), 394 (M+2)
5d	4-$NO_2C_6H_5$	3279 3850	δ 3.3 (s, 3H, CH_3), δ 7.5–8.3 (m, 11H ArH), δ 10.44 (s, 1H, NH). and 11.26 (s, 1H, NH). m/z 405 (M+1)
5e	$C_6H_5CH_2$-	3058 3228	δ 2.46 (s, 3H, CH_3), δ 4.9 (s, 2H, CH_2) δ 7.22–8.9 (m, 12H ArH), δ 8.95 (s, 1H, NH). and δ 10.63 (s, 1H, NH). m/z 394 (M+1)
6b	4-FC_6H_4	1751 (C=O)	δ 3.0 (s, 3H, CH_3), δ 4.45 (s, 2H, CH_2) δ 6.5–7.9 (m, 11H ArH) and m/z 378 (M+1) and 379 (M+2)
6c	3-ClC_6H4	1754 (C=O)	δ 2.52 (s, 3H, CH_3), δ 4.46 (s, 2H, CH_2) δ 6.52–7.9 (m, 11H ArH) and m/z 334 (M+1) and 335 (M+2)
6d	4-$NO_2C_6H_5$	1753 (C=O)	δ 2.53 (s, 3H, CH_3), δ 4.51 (s, 2H, CH_2) δ 6.38–8.07 (m, 12H ArH) and m/z 400 (M+1)
6e	$C_6H_5CH_2$-	1749 (C=O)	δ 2.45 (s, 3H, CH_3), δ 4.25 and 4.66 (s, 2H, CH_2) δ 7.30–8.42 (m, 12H ArH) and m/z 414 (M+1)
6f	4-$CH_3C_6H_4$	1752 (C=O)	δ 2.3 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), δ 4.48 (s, 2H, CH_2) δ 7.09–8.0 (m, 11H ArH) and m/z 414 (M+1)



Compd	R	Compd	R
5a	$-C_6H_5$	**5d**	$4\text{-}NO_2C_6H_4$
5b	$4\text{-}FC_6H_4$	**5e**	$C_6H_5CH_2$-
5c	$3\text{-}ClC_6H_4$	**5f**	$4\text{-}CH_3C_6H_4$

Reaction conditions: (i) $ClCH_2COCl$/anhyd. K_2CO_3, acetone, (ii) NH_2NH_2/ethanol, (iii) RNCS/glacial acetic acid, (iv) $ClCH_2COCl$/1,4-dioxane.

Scheme I — Synthetic route for the synthesis of target molecules **5a-f** and **6a-f**

Evaluation of pharmacological activities

The compounds encompassing naphthofuran have been known to exhibit wide spectrum of biological and pharmacological activities. Hence, it was intrigued to evaluate newly synthesized compounds for pharmacological activities by adopting literature procedure.

Antibacterial activity

The *in vitro* antibacterial activity was carried out against 24 hour old cultures of two bacteria by cup–plate method[16]. The newly synthesized compounds have been investigated for their antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Chloramphenicol was used as standard for measuring antibacterial activity. The compounds were tested at a concentration of 0.001 mol/mL in DMF against both the organisms. The zone of inhibition was compared with the standard drug after 24 hour of incubation at 25°C. The results were tabulated in Table III.

Table III — Antimicrobial activity data of the compounds **5a-f** and **6a-f**

Compd	Zone of Inhibition in mm			
	Antibacterial activity		Antifungal activity	
	P. aerugenosa	*S.aureus*	*A. niger*	*C. lunata*
Standard	24	26	23	24
DMF	Nil	Nil	Nil	Nil
5a	18	19	16	17
5b	19	18	17	16
5c	19	17	19	18
5d	19	19	18	17
5e	20	19	19	17
5f	18	19	17	18
6a	19	18	17	16
6b	18	18	16	17
6c	18	17	18	19
6d	20	19	18	18
6e	20	18	19	18
6f	19	18	18	17

Antifungal activities

The *in vitro* antifungal activity was carried out against 48 hour old cultures by cup–plate method. The newly synthesized compounds have been investigated for their antifungal activity against *Aspergillus niger* and *Curvularia lunata*. Fluconazole was used as standard for the evaluation of antifungal activity. The compounds were tested at a concentration of 0.001 mol/mL in DMF medium against both the organisms. The zone of inhibition was compared with the standard drug after 24 hrs of incubation at 25°C. The results are presented in Table III.

Antioxidant activity

The synthesized compounds were screened for antioxidant activity by DPPH method[17].

Reagents prepared

0.2 mM DPPH

0.1 M TrisHCl (Tris hydroxymethyl aminomethane hydrochloride); *p*H 7.4

Three tubes were taken and labeled as blank, control and test. In the blank tube 600µL of ethanol and 400µL of TrisHCl were taken. In the control tube 100µL of ethanol, 400 µL of Tris HCl and 500µL of DPPH solution of were taken and in the test tube 100 µL of sample (10 mg/mL), 400µL of Tris HCl and 500µL of DPPH solution was taken. All the tubes were mixed and kept in dark for 30mins. Then absorbance was measured at 490 nm. The inhibition ratio was calculated using the equation,

$$\text{Inhibition ratio} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c = Absorbance for control

A_s = Absorbance for sample$\frac{A_c - A_s}{A_c} \times 100$

Standard used is Ascorbic acid. Standard value for the antioxidant activity was 100% inhibition. The results obtained are tabulated in the Table IV.

Antitubercular activitiy

The anti mycobacterial activity of all the newly synthesised compounds were assessed against *M. tuberculosis* using microplate Alamar Blue assay (MABA). This methodology was non-toxic, uses a thermally stable reagent and showed remarkable correlation with proportional and BACTEC radiometric method[18,19].

Briefly, 200µL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 well plates received 100µL of the Middle brook 7H9 broth and serial dilution of compounds was made directly on plate. The final drug concentrations tested were 100 to 0.2µg/mL. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25µL of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 were added to the plate and incubated for 24 hrs.

A blue color in the well was interpreted as no bacterial growth and pink color was scored as growth. The MIC was defined as minimum inhibitory concentration, which prevented the color change from blue to pink. Standard Strain used is *Mycobacteria tuberculosis* (Vaccine strain, H37 RV strain).

Standard values for the Anti-TB test, which was performed were,

Pyrazinamide- 3.125µg/mL,

Ciprofloxacin-3.125µg/mL and

Streptomycin- 6.25µg/mL.

The Photograph and results of Standard Drugs and the synthesized compounds are shown in Table V and Table VI.

Table IV — Antioxidant activity data of the compounds **5a-f** and **6a-f**

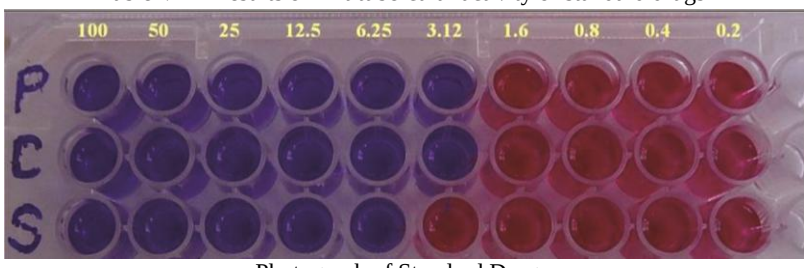
S. No.	Compd	% Inhibition
	Vitamin C (Standard)	100
1	**5a**	36.0
2	**5b**	15.2
3	**5c**	15.1
4	**5d**	40.2
5	**5e**	19.0
6	**5f**	39.5
7	**6a**	22.4
8	**6b**	35.4
9	**6c**	34.9
10	**6d**	53.2
11	**6e**	16.4
12	**6f**	29.8

Anthelmintic activity

Anthelmintic activity was evaluated by using *Pheritima posthuma* (class-Annelida and order-Oligichaeta).

The worms were washed with water to remove adhering materials and were sorted out for uniform size and length. The worms were kept in 6% dextrose solution for acclimatization. The worms with normal motility were selected for the experiment. Petridishes

Table V — Results of Antitubercular activity of standard drugs

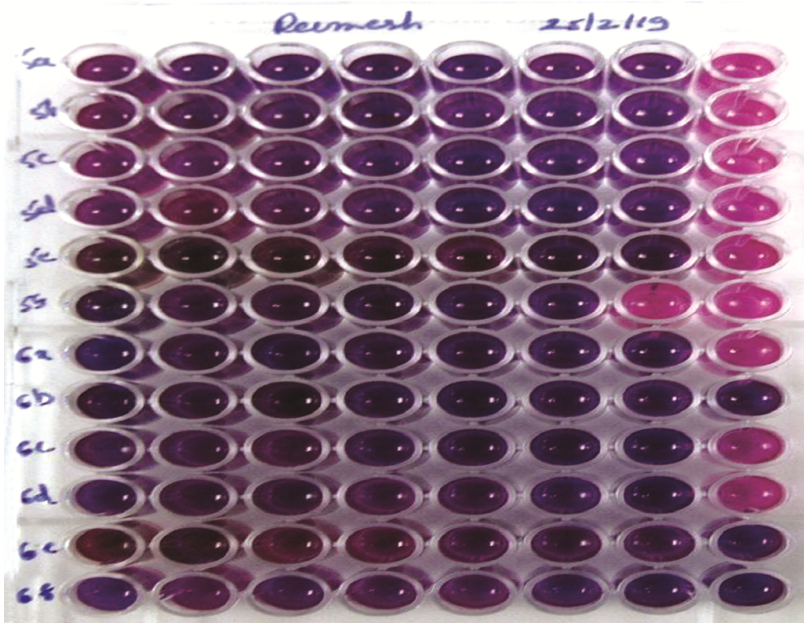


Photograph of Standard Drugs

S. No.	Std.	100 µg/mL	50µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.6 µg/mL	0.8 µg/mL
01	P	S	S	S	S	S	S	R	R
02	C	S	S	S	S	S	S	R	R
03	S	S	S	S	S	S	R	R	R

S=Sensitive R=Resistant

Table VI — Results of antitubercular activity of compounds **5a-f** and **6a-f**



Photograph of compounds **5a-f** and **6a-f**

S. No.	Sample	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.6 µg/mL	0.8 µg/mL
01	**5a**	S	S	S	S	S	S	S	R
02	**5b**	S	S	S	S	S	S	S	R
03	**5c**	S	S	S	S	S	S	S	R
04	**5d**	S	S	S	S	S	S	S	R
05	**5e**	S	S	S	S	S	S	S	R
06	**5f**	S	S	S	S	S	S	R	R
07	**6a**	S	S	S	S	S	S	S	R
08	**6b**	S	S	S	S	S	S	S	S
09	**6c**	S	S	S	S	S	S	S	R
10	**6d**	S	S	S	S	S	S	S	R
11	**6e**	S	S	S	S	S	S	S	S
12	**6f**	S	S	S	S	S	S	S	S

of equal size were selected and in each petridish 25 mg of the test compounds in 0.1% Tween-80 suspension were placed and the volume is made upto 25 mL with 6% dextrose solution. In another petridish 25 mL of 0.1% Tween-80 prepared in 6% dextrose solution was placed, which served as control. Albendazole suspended in 6% dextrose solution was placed which served as standard. In each petridish

Table VII — Results of Anthelmintic activity of compounds **5a-f** and **6a-f**

Compd	Mean paralyzing time (min)	Mean death time (min)
Albendazole	33	46
5% dextrose solution	–	–
5a	141	160
5b	123	153
5c	130	170
5d	151	182
5e	146	192
5f	149	198
6a	125	164
6b	119	155
6c	123	181
6d	134	162
6e	159	195
6f	141	180

four worms were placed. The time taken by each worm for paralysis and for the death was noted by placing the worms in water maintained at 50^0C. The results obtained are tabulated in the Table VII.

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