

## Synthesis and antitubercular, antiviral and anticancer activity of 3-(3-mercaptoalkyl-7H-[1,2,4]triazolo[3,4-*b*][1,3,4]- thiadiazin-6-yl)chromen-2-one and its derivatives

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3-(3-Mercaptoalkyl-7H-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)-chromen-2-one **3** have been prepared by the condensation of 3-(2-bromoacetyl)coumarin **1** and 4-amino-5-mercaptoalkyl-4H-[1,2,4]triazole-3-thiol **2** in anhydrous ethanol. Similarly the 7,8-benzo analogues of **1** on reaction with **2** resulted in the formation of 7,8-benzo derivatives of **3s-x**. Reaction of **3a** with phenacyl chloride in anhydrous ethanol gave corresponding 3-(3-(2-oxo-2-phenylethyl-sulphanylmethyl)-7H-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)chromen-2-one **4**. The 7,8-benzo analogues of **3s-t** on treatment with phenacyl chloride in anhydrous ethanol under reflux gave **4g** and **4h**. The newly synthesized compounds are characterized on the basis of elemental analysis, IR, <sup>1</sup>H NMR and mass spectral data. Some of the compounds are screened for their antitubercular, antiviral and anticancer activities.

**Keywords:** Benzopyran-2-one, thiadiazine, 1,2,4-triazole, antitubercular, antiviral, anticancer.

Coumarin derivatives have been found to exhibit various remarkable activities such as fluorescent dyes<sup>1</sup>, CNS depressants<sup>2</sup>, antitumor agents<sup>3</sup>, HIV proliferator<sup>4</sup> and as powerful anticoagulents<sup>5</sup>. Various 1,2,4-triazoles and N-bridged heterocycles derived from them are found to be associated with diverse pharmacological activity<sup>6-11</sup>. The 1,2,4-triazole nucleus has recently been incorporated into a wide variety of therapeutically interesting drugs including H<sub>1</sub>/H<sub>2</sub> histamine receptor blockers, choline esterase activity agents, CNS stimulants, antianxiety agents and sedatives<sup>12</sup>.

Prompted by the above observations and in continuation of our search for biologically active nitrogen and sulfur containing heterocycles<sup>13-15</sup>. It was decided to synthesize title compounds because many derivatives of 2H-1,3,5-thiadiazines have shown to be animal growth compounds<sup>16</sup>. They were also shown to possess varied biological activities like antimicrobial<sup>17</sup>, antitubercular<sup>18</sup>.

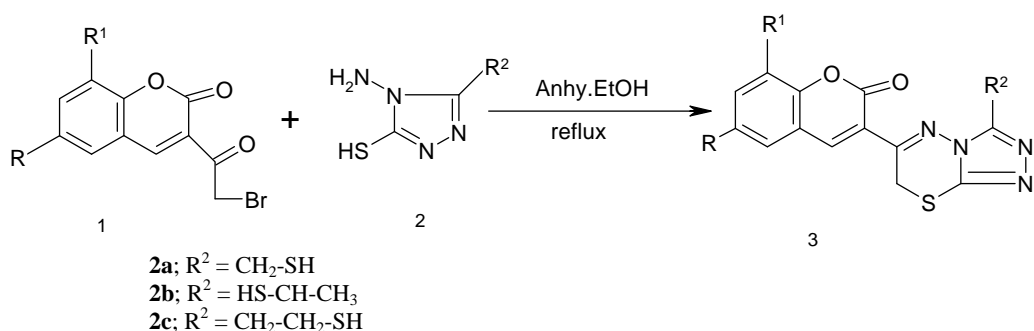
Synthesis of 3-(3-mercaptoalkyl-7H-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-6-yl)chromen-2-one **3** derivatives has been achieved by the condensation of 3-(2-bromoacetyl)coumarin **1** with 4-amino-5-mercaptoalkyl-4H-[1,2,4]triazole-3-thiol **2** in anhydrous ethanol under reflux for 3 to 4 hr (**Scheme I**). The 3-

(3-mercaptoalkyl-7H-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-6-yl)-chromen-2-one **3a** displayed strong absorption bands due to C=N and lactone carbonyl of coumarin at 1608 and 1722 cm<sup>-1</sup>. The <sup>1</sup>H NMR spectrum of **3a** exhibited a characteristic singlet for -CH<sub>2</sub>- of thiadiazine ring at δ 4.26. The acidic -SH proton appeared at δ 2.50. The remaining protons were observed in the usual regions.

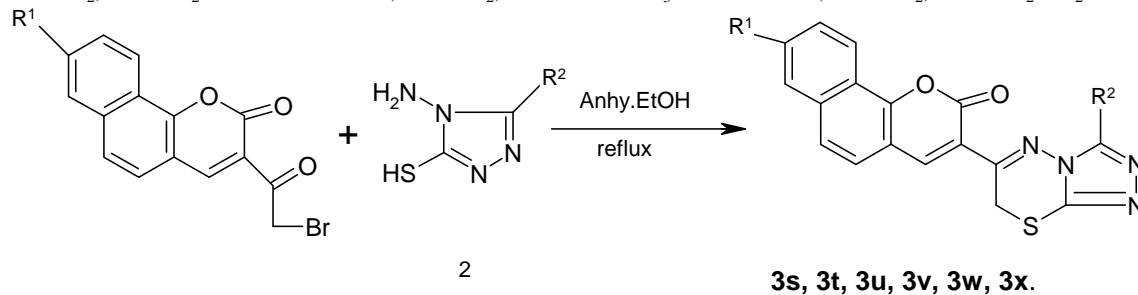
The compounds **4a-f** have been obtained by the reaction of various 3-(3-mercaptomethyl-7H-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-6-yl)chromen-2-one **3a** with phenacyl chloride in anhydrous ethanol. Similarly 7,8-benzo analogues of **4g** and **4h** have been prepared **Scheme II**. The <sup>1</sup>H NMR spectrum of **4a** exhibited a characteristic singlet for -CH<sub>2</sub>-S-CO- at δ 3.7 while the -S-CH<sub>2</sub>- of the thiadiazine ring appeared as singlet at δ 4.20. The triazole attached -CH<sub>2</sub>-S- appeared as singlet at δ 4.7. The remaining protons were observed in the usual regions.

### Experimental Section

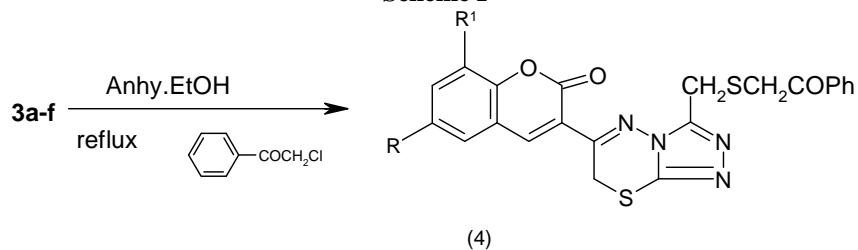
All melting points were recorded on cintex melting point apparatus and are uncorrected. The purity of the compounds was checked by TLC. IR spectra were recorded on a Perkin-Elmer spectrum GX series FT-IR spectrophotometer. The <sup>1</sup>H NMR spectra on a



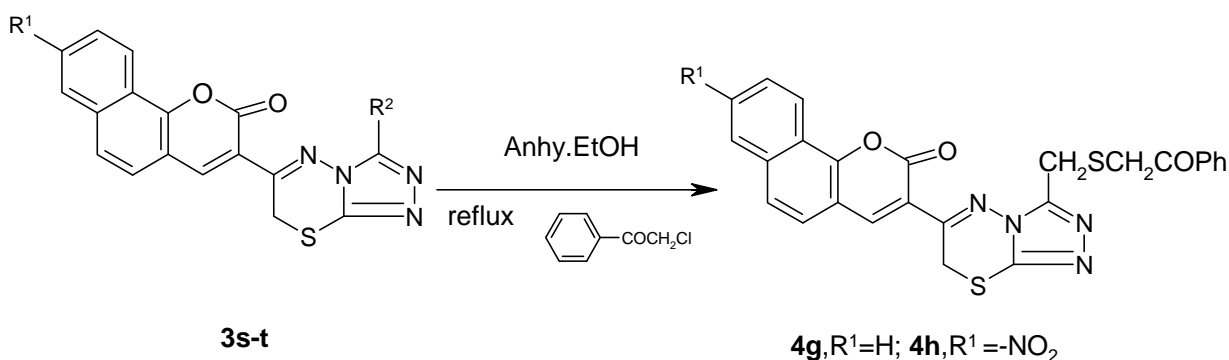
**3a;**  $R = R^1 = \text{H}, R^2 = \text{-CH}_2\text{-SH}$       **3g;**  $R = R^1 = \text{H}, R^2 = \text{HS-CH-CH}_3$       **3m;**  $R = R^1 = \text{H}, R^2 = \text{-CH}_2\text{-CH}_2\text{-SH}$   
**3b;**  $R^1 = \text{OCH}_3, R = \text{H}, R^2 = \text{-CH}_2\text{-SH}$       **3h;**  $R^1 = \text{OCH}_3, R = \text{H}, R^2 = \text{HS-CH-CH}_3$       **3n;**  $R^1 = \text{OCH}_3, R = \text{H}, R^2 = \text{-CH}_2\text{CH}_2\text{SH}$   
**3c;**  $R = \text{Cl}, R^1 = \text{H}, R^2 = \text{-CH}_2\text{-SH}$       **3i;**  $R = \text{Cl}, R^1 = \text{H}, R^2 = \text{HS-CH-CH}_3$       **3o;**  $R = \text{Cl}, R^1 = \text{H}, R^2 = \text{-CH}_2\text{-CH}_2\text{-SH}$   
**3d;**  $R = R^1 = \text{Cl}, R^2 = \text{-CH}_2\text{-SH}$       **3j;**  $R = R^1 = \text{Cl}, R^2 = \text{HS-CH-CH}_3$       **3p;**  $R = R^1 = \text{Cl}, R^2 = \text{-CH}_2\text{-CH}_2\text{-SH}$   
**3e;**  $R = \text{Br}, R^1 = \text{H}, R^2 = \text{-CH}_2\text{-SH}$       **3k;**  $R = \text{Br}, R^1 = \text{H}, R^2 = \text{HS-CH-CH}_3$       **3q;**  $R = \text{Br}, R^1 = \text{H}, R^2 = \text{-CH}_2\text{-CH}_2\text{-SH}$   
**3f;**  $R = R^1 = \text{Br}, R^2 = \text{-CH}_2\text{-SH}$       **3l;**  $R = R^1 = \text{Br}, R^2 = \text{HS-CH-CH}_3$       **3r;**  $R = R^1 = \text{Br}, R^2 = \text{-CH}_2\text{-CH}_2\text{-SH}$   
**3s;**  $R^1 = \text{H}, R^2 = \text{-CH}_2\text{-SH}$       **3u;**  $R^1 = \text{H}, R^2 = \text{HS-CH-CH}_3$       **3w;**  $R^1 = \text{H}, R^2 = \text{-CH}_2\text{-CH}_2\text{-SH}$   
**3t;**  $R^1 = \text{NO}_2, R^2 = \text{-CH}_2\text{-SH}$       **3v;**  $R^1 = \text{NO}_2, R^2 = \text{HS-CH-CH}_3$       **3x;**  $R^1 = \text{NO}_2, R^2 = \text{-CH}_2\text{-CH}_2\text{-SH}$



Scheme I



**4a;**  $R = R^1 = \text{H}$       **4d;**  $R = R^1 = \text{Cl}$   
**4b;**  $R^1 = \text{OCH}_3, R = \text{H}$       **4e;**  $R = \text{Br}, R^1 = \text{H}$   
**4c;**  $R = \text{Cl}, R^1 = \text{H}$       **4f;**  $R = R^1 = \text{Br}$



Scheme II

Varian dpx 200 MHz spectrometer using TMS as internal standard (chemical shifts in  $\delta$ , ppm) and mass spectra on a Jeol-JMS-300 spectrometer at 70 eV.

The various 3-(2-bromoacetyl)coumarins were prepared according to our earlier procedure<sup>19</sup>. 4-Amino-5-mercaptomethyl-4*H*-[1,2,4]triazole-3-thiol has been prepared by the condensation of thiocarbonylhydrazide with mercaptoacetic acid<sup>20-22</sup>.

### Preparation of 4-amino-5-(1-mercaptoethyl)-4*H*-[1,2,4]triazole-3-thiol **2b**

#### General Procedure

A mixture of thiocarbonylhydrazide (0.01 mole) and 2-mercapto propionic acid (0.01 mole) was refluxed for 3 hr on a heating mantle. The reaction-mixture was cooled and was filtered, washed with water and recrystallised from methanol to give **2b**. m.p. 95-97°C.

**4-Amino-5-(1-mercaptoethyl)-4*H*-[1,2,4]triazole-3-thiol **2b**:** IR (KBr): 1612 (C=N), 2541 (-SH) and 3267 (-NH<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  1.59 (d, 3H, -CH<sub>3</sub>), 2.51 (s, 1H, alkyl SH), 3.33 (s, 1H, SH, D<sub>2</sub>O exchangeable), 4.21 (q, 1H, -CH), 5.57 (s, 2H, NH<sub>2</sub>, D<sub>2</sub>O exchangeable); MS: *m/z* 176.

**Preparation of 4-amino-5-(2-mercapto-ethyl)-4*H*-[1,2,4]triazole-3-thiol **2c**.** It has been prepared by the condensation of thiocarbonylhydrazide with excess 3-mercaptopropionic acid as per procedure described in the literature<sup>23-26</sup>.

### Preparation of 3-(3-mercapto-alkyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazin-6-yl)chromen-2-one **3**. General procedure

A mixture of 3-(2-bromoacetyl)coumarin **1a-f** (1.33g, 0.005 mole) and 4-amino-5-mercaptoalkyl-4*H*-[1,2,4]triazole-3-thiol **2a-c** (0.740g, 0.005 mole) in anhydrous ethanol (10 mL) was refluxed for about 3-4 hr. The solid separated was collected by filtration dried and recrystallised from methanol to give **3a** (Table I). All the other compounds (**3b-x**) were prepared similar procedure.

**3-(3-Mercapto-methyl-7*H*-[1,2,4]triazolo[3,4-*b*]-[1,3,4]thiadiazin-6-yl)chromen-2-one **3a**:** IR (KBr): 1608 (C=N), 1722 (lactone-C=O) and 2451(-SH); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  2.50 (s, 1H, SH), 4.03 (s, 2H, -CH<sub>2</sub>-SH), 4.26 (s, 2H, -S-CH<sub>2</sub>- of thiadiazine ring), 7.46-7.55 (m, 4*H*, Ar-H), 8.66 (s, 1H, C<sub>4</sub> of coumarin); MS: *m/z* 330.

**3-(3-Mercapto-methyl-7*H*-[1,2,4]triazolo[3,4-*b*]-[1,3,4]thiadiazin-6-yl)-8-methoxy-chromen-2-one**

**3b:** IR (KBr): 1610 (C=N) and 1720 (lactone C=O), 2535 (-SH); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  1.70 (s, 1H, SH with D<sub>2</sub>O exchangeable), 2.73 (s, 2H of -CH<sub>2</sub>-), 3.9 (s, 5H, 2H of -CH<sub>2</sub>- and 3H of OCH<sub>3</sub>), 7.20-7.26 (m, 3H, Ar-H) and 8.48 (s, 1H, C<sub>4</sub> of Coumarin); MS: *m/z* 360.

**3-[3-(1-Mercapto-ethyl)-7*H*-[1, 2, 4]triazolo[3, 4-*b*][1,3,4]thiadiazin-6-yl)-chromen-2-one **3g**:** IR (KBr): 1603 (C=N), 1724 (lactone -C=O) and 2545 (-SH); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  1.55 (s, 1H, SH), 1.66 (d, 3H, CH<sub>3</sub>), 4.0-4.2 (m, 3H, 1H of -CH- and 2H of -S-CH<sub>2</sub>-), 7.1-7.8 (m, 4*H*, Ar-H), 8.50 (s, 1H, C<sub>4</sub> of coumarin); MS: *m/z* 344.

**3-[3-(2-Mercapto-ethyl)-7*H*-[1, 2, 4]triazolo[3, 4-*b*][1,3,4]thiadiazin-6-yl)-chromen-2-one **3m**:** IR (KBr): 1606 (C=N), 1720 (lactone C=O) and 2363 (-SH); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  1.08 (s, 1H, SH, D<sub>2</sub>O exchangeable), 2.95 (t, 2H, -CH<sub>2</sub>-), 3.15 (t, 2H, -CH<sub>2</sub>-S- of side chain), 4.26 (s, 2H, -S-CH<sub>2</sub>- of thiadiazine ring), 7.4-7.8 (m, 4*H*, Ar-H), 8.5 (s, 1H, C<sub>4</sub> of coumarin); MS: *m/z* 344.

**Preparation of 3-[3-(2-oxo-2-phenyl-ethylsulfanylmethyl)-7*H*-[1,2,4] triazolo [3,4-*b*][1,3,4]thiadiazin-6-yl]chromen-2-one **4a**, General procedure.** A mixture of **3**-(3-mercaptomethyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl)chromen-2-one. **3a** (0.001 mole) and phenacyl chloride (0.001 mole) in anhydrous ethanol (10 mL) was refluxed for about 3-4 hr. The solid separated was collected by filtration, washed with ethanol, dried and recrystallized from methanol to give **4a** (Table I). All the other compounds **4b-h** were prepared similar procedure.

**3-(3-(2-Oxo-2-phenyl-ethylsulfanylmethyl)-7*H*-[1,2,4]triazolo[3,4-*b*]-[1,3,4]thiadiazin-6-yl]chromen-2-one **4a**.** IR (KBr): 1606 (-C=N), 1690 (Ketone C=O) and 1720 (lactone C=O); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  3.7 (s, 2H, -SCH<sub>2</sub>-CO-), 4.2 (s, 2H, -SCH<sub>2</sub>- of thiadiazine ring), 4.7 (s, 2H, -S-CH<sub>2</sub>-), 7.3-7.95 (m, 9H, Ar-H), 8.50 (s, 1H, C<sub>4</sub> of coumarin); MS: *m/z* 448.

### Biological evaluation

**Antitubercular activity.** Primary antitubercular screening is conducted at 6.25  $\mu$ g/mL (or molar equivalent of highest molecular weight compound in a series of congeners) against *Mycobacterium tuberculosis* H<sub>37</sub> R<sub>v</sub> (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay<sup>27</sup> (MABA). Compounds effecting <90% inhibition in the primary screen (i.e., MIC > 6.25  $\mu$ g/mL) are not generally

**Table I** — Analytical Data of Compounds **3a-x** and **4a-h**

Compd	M.p. (°C)	Yield (%)	Mol. Formula (Mol. wt.)	Found (Calcd)%			Compd	M.p. (°C)	Yield (%)	Mol. Formula (Mol. wt.)	Found (Calcd)%		
				C	H	N					C	H	N
<b>3a</b>	220-22	82	C <sub>14</sub> H <sub>10</sub> N <sub>4</sub> S <sub>2</sub> O <sub>2</sub> (330)	59.90 (50.92)	3.03 (3.05)	16.92 (16.96)	<b>3q</b>	194-96	86	C <sub>15</sub> H <sub>11</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Br (423)	42.51 (42.56)	2.60 (2.62)	13.21 (13.24)
<b>3b</b>	138-40	80	C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> O <sub>3</sub> S <sub>2</sub> (360)	49.94 (49.99)	3.30 (3.36)	15.45 (15.50)	<b>3r</b>	210-12	80	C <sub>15</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Br <sub>2</sub> (502)	35.84 (35.87)	2.00 (2.02)	11.13 (11.16)
<b>3c</b>	250-52	84	C <sub>14</sub> H <sub>9</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Cl (364)	46.04 (46.09)	2.44 (2.49)	15.34 (15.36)	<b>3s</b>	170-72	88	C <sub>18</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> (380)	56.80 (56.83)	3.15 (3.18)	14.70 (14.73)
<b>3d</b>	258-60	86	C <sub>14</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Cl <sub>2</sub> (399)	42.10 (42.11)	2.00 (2.02)	14.00 (14.03)	<b>3t</b>	175-77	84	C <sub>18</sub> H <sub>11</sub> N <sub>5</sub> O <sub>4</sub> S <sub>2</sub> (425)	50.80 (50.82)	2.58 (2.61)	16.44 (16.46)
<b>3e</b>	182-84	90	C <sub>14</sub> H <sub>9</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Br (409)	41.06 (41.09)	2.20 (2.22)	13.65 (13.69)	<b>3u</b>	265-67	90	C <sub>19</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> (394)	57.80 (57.87)	3.54 (3.58)	14.18 (14.20)
<b>3f</b>	188-90	92	C <sub>14</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Br <sub>2</sub> (488)	34.40 (34.45)	1.61 (1.64)	11.44 (11.48)	<b>3v</b>	>280	81	C <sub>16</sub> H <sub>13</sub> N <sub>5</sub> O <sub>5</sub> S <sub>2</sub> (439)	51.90 (51.93)	2.94 (2.98)	15.90 (15.94)
<b>3g</b>	188-90	86	C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> (344)	52.31 (52.34)	3.48 (3.51)	16.25 (16.27)	<b>3w</b>	234-36	84	C <sub>19</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> (394)	57.81 (57.85)	3.54 (3.58)	14.18 (14.20)
<b>3h</b>	202-04	85	C <sub>16</sub> H <sub>14</sub> N <sub>4</sub> O <sub>3</sub> S <sub>2</sub> (374)	51.30 (51.32)	3.74 (3.77)	14.94 (14.96)	<b>3x</b>	243-45	88	C <sub>19</sub> H <sub>13</sub> N <sub>5</sub> O <sub>4</sub> S <sub>2</sub> (439)	51.90 (51.93)	2.94 (2.98)	15.90 (15.94)
<b>3i</b>	176-78	80	C <sub>15</sub> H <sub>11</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Cl (378)	47.50 (47.55)	2.90 (2.93)	14.76 (14.79)	<b>4a</b>	115-17	90	C <sub>22</sub> H <sub>16</sub> N <sub>4</sub> O <sub>3</sub> S <sub>2</sub> (448)	58.89 (58.91)	3.57 (3.60)	12.44 (12.49)
<b>3j</b>	184-86	88	C <sub>15</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Cl <sub>2</sub> (412)	43.54 (43.59)	2.40 (2.44)	13.54 (13.56)	<b>4b</b>	198-200	92	C <sub>23</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub> S <sub>2</sub> (478)	57.70 (57.73)	3.75 (3.79)	11.68 (11.71)
<b>3k</b>	215-17	89	C <sub>15</sub> H <sub>11</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Br (423)	42.51 (42.56)	2.60 (2.62)	13.20 (13.24)	<b>4c</b>	204-06	90	C <sub>22</sub> H <sub>15</sub> N <sub>4</sub> O <sub>3</sub> S <sub>2</sub> Cl (482)	54.71 (54.74)	3.10 (3.13)	11.56 (11.60)
<b>3l</b>	224-26	92	C <sub>15</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Br <sub>2</sub> (502)	35.84 (35.87)	2.00 (2.01)	11.12 (11.16)	<b>4d</b>	215-17	88	C <sub>22</sub> H <sub>14</sub> N <sub>4</sub> O <sub>3</sub> S <sub>2</sub> Cl <sub>2</sub> (517)	51.04 (51.07)	2.70 (2.73)	10.81 (10.83)
<b>3m</b>	165-67	80	C <sub>15</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> (344)	52.39 (52.41)	3.48 (3.51)	16.25 (16.27)	<b>4e</b>	210-12	86	C <sub>22</sub> H <sub>15</sub> N <sub>4</sub> O <sub>3</sub> S <sub>2</sub> Br (527)	50.07 (50.10)	2.84 (2.87)	10.60 (10.62)
<b>3n</b>	135-37	82	C <sub>16</sub> H <sub>14</sub> N <sub>4</sub> O <sub>3</sub> S <sub>2</sub> (374)	51.30 (51.32)	3.74 (3.77)	14.94 (14.96)	<b>4f</b>	225-27	90	C <sub>22</sub> H <sub>14</sub> N <sub>4</sub> O <sub>3</sub> S <sub>2</sub> Br <sub>2</sub> (606)	43.54 (43.58)	2.30 (2.33)	9.22 (9.24)
<b>3o</b>	166-68	78	C <sub>15</sub> H <sub>11</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Cl (378)	47.51 (47.55)	2.90 (2.93)	14.75 (14.79)	<b>4g</b>	194-96	82	C <sub>26</sub> H <sub>18</sub> N <sub>4</sub> O <sub>3</sub> S <sub>2</sub> (498)	62.60 (62.64)	3.60 (3.64)	11.20 (11.24)
<b>3p</b>	174-76	82	C <sub>15</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> Cl <sub>2</sub> (412)	43.54 (43.59)	2.40 (2.44)	13.54 (13.56)	<b>4h</b>	202-04	84	C <sub>26</sub> H <sub>17</sub> N <sub>5</sub> O <sub>5</sub> S <sub>2</sub> (543)	57.40 (57.45)	3.12 (3.15)	12.86 (12.88)

The compounds **3a-x** and **4a-h** were recrystallized from methanol

evaluated further. From the **Table II** it is evident that none of the compounds **3a-c**, **3e-f**, **3s** and **3t** exhibited antitubercular activity.

**Antiviral activity.** The antiviral activities<sup>28</sup> of **4a-h** were also determined against HSV-1, HSV-2, VV, VSV and HSV-1, TK<sup>-</sup> strains in HEL cell cultures. The compounds did not exhibit an appreciable antiviral activity (i.e., minimal antiviral by effective concentration  $\geq 5$  fold lower than minimal cytotoxic concentration). Similarly, they did not show any specific activity against other viruses (e.g., VSV, Coxsackie virus B<sub>4</sub> and respiratory syncytial virus in HeLa cell cultures. Also, the compounds did not show

**Table II** — Antitubercular activity of compounds (**3a-c**, **3e-f**, **3s** and **3t**)

Compd	Assay	MIC ( $\mu$ g/mL)	% Inhibition	Activity
<b>3a</b>	Alamar	> 6.25	0	--
<b>3b</b>	Alamar	> 6.25	0	--
<b>3c</b>	Alamar	> 6.25	0	--
<b>3e</b>	Alamar	> 6.25	0	--
<b>3f</b>	Alamar	> 6.25	6	--
<b>3s</b>	Alamar	> 6.25	4	--
<b>3t</b>	Alamar	> 6.25	4	--

**Table III** — Cytotoxicity and antiviral activity of compounds. HeLa cell cultures **4a-h**

Comp.	Minimum cytotoxic concentration <sup>a</sup> (μg/mL)	Minimum inhibitory concentration <sup>b</sup> (μg/mL)				
		<i>Herpes simplex</i> virus-1 (KOS)	<i>Herpes simplex</i> virus-2 (G)	<i>Vaccinia</i> virus	<i>Vesicular stomatitis</i> virus	<i>Herpes simplex</i> virus-1 TK KOS ACV <sup>r</sup>
<b>4a</b>	80	> 16	> 16	> 16	> 16	> 16
<b>4b</b>	80	> 16	> 16	> 16	> 16	> 16
<b>4c</b>	80	> 16	> 16	> 16	> 16	> 16
<b>4d</b>	≥ 16	> 16	> 16	> 16	> 16	> 16
<b>4e</b>	≥ 16	> 16	> 16	> 16	> 16	> 16
<b>4f</b>	≥ 16	> 16	> 16	> 16	> 16	> 16
<b>4g</b>	80	> 16	> 16	> 16	> 16	> 16
<b>4h</b>	≥ 16	> 16	> 16	> 16	> 16	> 16
Brivudin	≥ 400	0.0256	400	16	> 400	> 400
Ribavirin	> 400	> 400	> 400	240	> 400	> 400
Acyclovir	> 400	0.0768	0.0768	> 400	> 400	48
Ganciclovir	> 100	0.0038	0.0192	> 100	> 100	0.48

**Table IV** — Cytotoxicity and antiviral activity of compounds HeLa cell cultures **4a-h**

Compd	Minimum cytotoxic concentration <sup>a</sup> (μg/mL)	Minimum inhibitory concentration <sup>b</sup> (μg/mL)		
		<i>Vesicular stantitis</i> virus	<i>Coxsackie</i> virus B <sub>4</sub>	Respiratory syncytial virus
<b>4a</b>	80	> 16	> 16	> 16
<b>4b</b>	400	> 80	> 80	> 80
<b>4c</b>	400	> 80	> 80	> 80
<b>4d</b>	80	> 16	> 16	> 16
<b>4e</b>	400	> 80	> 80	> 80
<b>4f</b>	400	> 80	> 80	> 80
<b>4g</b>	400	> 80	> 80	> 80
<b>4h</b>	≥ 80	> 80	> 80	> 80
Brivudin	≥ 400	> 400	> 400	> 400
(S)-DHPA	≥ 400	> 400	> 400	> 400
Ribavirin	≥ 400	48	400	16

<sup>a</sup>Required to cause a microscopically detectable alteration of normal cell morphology.<sup>b</sup>Required to reduce virus-induced cytopathogenicity by 50%**Table V** — Cytotoxicity and antiviral activity of compounds Vero cell cultures (**4a-h**)

Compd	Minimum cytotoxic concentration <sup>a</sup> (μg/mL)	<i>Parainfluenza</i> -3 virus	Minimum inhibitory concentration <sup>b</sup> (μg/mL)			
			<i>Reovirus</i> -1	<i>Sindbis</i> virus	<i>Coxsackie</i> virus B <sub>4</sub>	<i>Punta Toro</i> virus
<b>4a</b>	≥ 80	> 80	> 80	> 80	> 80	> 80
<b>4b</b>	80	> 16	> 16	> 16	> 16	> 16
<b>4c</b>	≥ 80	> 80	> 80	> 80	> 80	> 80
<b>4d</b>	≥ 80	> 80	> 80	> 80	> 80	> 80
<b>4e</b>	≥ 80	> 80	> 80	> 80	> 80	> 80
<b>4f</b>	≥ 80	> 80	> 80	> 80	> 80	> 80
<b>4g</b>	≥ 80	> 80	> 80	> 80	> 80	> 80
<b>4h</b>	80	> 16	> 16	> 16	> 16	> 16
Brivudin	> 400	> 400	> 400	> 400	> 400	> 400
(S)-DHPA	> 400	> 400	> 400	> 400	> 400	> 400
Ribavirin	> 400	48	48	400	> 400	48

<sup>a</sup>Required to cause a microscopically detectable alteration of normal cell morphology.<sup>b</sup>Required to reduce virus-induced cytopathogenicity by 50%.

any specific activity against other viruses like *Parainfluenza*, *Reovirus*-1, *Sindbis virus*, *Coxsackie virus* B<sub>4</sub> and *Punta Toro virus* in Vero cell cultures **Tables III, IV** and **V**. Compounds **3a-c**, **3e-f**, **3s** and

**3t** were tested for their biological activities in various tumor cell lines<sup>29-31</sup>. The **Table VI** indicates the inhibitory effects on the growth of a variety of tumor cell virus, L 1210, Molt 4/C8 and CEM. When the

**Table VI**— Inhibitory effects of compounds. The proliferation of murine leukaemia cells (L 1210/0) and human T-lymphocyte cells (Molt 4/C8, CEM/0) (**3a-c**, **3e-f**, **3s** and **3t**)

Compd	IC <sub>50</sub> (µg/mL) <sup>a</sup>		
	L 1210/0	Molt 4/C8	CEM/0
<b>3a</b>	58 ± 10	52 ± 6	25 ± 5
<b>3b</b>	73 ± 20	69 ± 16	52 ± 16
<b>3c</b>	73 ± 5	87 ± 4	46 ± 18
<b>3e</b>	76 ± 21	73 ± 12	34 ± 25
<b>3f</b>	108 ± 9	95 ± 15	69 ± 26
<b>3s</b>	61 ± 9	70 ± 8	17 ± 4
<b>3t</b>	17 ± 0	19 ± 1	12 ± 1

<sup>a</sup>50% inhibitory concentration.

compounds were tested for their cytotoxic by measuring the IC<sub>50</sub> values in the proliferation of murine leukemia cells (L<sub>12</sub> 10%) and human T-lymphocyte (Molt 4/L<sub>8</sub>, CEM/0) cells. None of the compounds showed IC<sub>50</sub> value less than 10 µg/mL on only one of the cell lines tested. **3t** is some what relatively having cytotoxic properties below 20 µg/mL on all the tested cells. In general these compounds are not having any cytotoxic activity.

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### References

- 1 Raue R & Brack A, *Belg*, **1963**, 621380; *Chem Abstr*, 58, **1963**, 11506a.
- 2 Moffett R B, *J Med Chem*, 7, **1964**, 446.
- 3 Raev L, Voinov E, Ivanov I & Popov D, *Pharmazie*, 45, **1990**, 696; *Chem Abstr*, 114, **1990**, 74711b.
- 4 Kun E & Aurelian L, *US Pat*, **1991**, 412783; *Chem Abstr*, 115, **1991**, 97071t.
- 5 Bohdem S & Elzbieta B, *Pol PL*, **1986**, 136525 *Chem Abstr*, 113, **1990**, 40455k.
- 6 Walser A, Flyman T & Musan C, *J Het Chem*, 28, **1991**, 1121.
- 7 Hirota T, Sajaki K, Yumamota H & Nakayama T, *J Het Chem*, 28, **1991**, 257.
- 8 Kane J M, Barton B M, Dudley M W, Sorenson S M & Stueger M A, *J Med Chem*, 33, **1990**, 2772.
- 9 Bardbury R H & Rivert J E, *J Med Chem*, 34, **1991**, 151.
- 10 Kumamoto T, Toyooka K, Nishida M, Kuwahara H, Yoshiyuki Y, Kawada J & Kubota S, *Chem Pharm Bull*, 38, **1990**, 2595.
- 11 Ashour P F A & Almazora S A H, *Farmaco*, 45, **1990**, 1207.
- 12 Heindel N D & Rcid J R, *J Het Chem*, **1980**, 1087.
- 13 Rajeswar Rao V, Mohan Rao G, Ravi Kumar V & Aditya Vardhan V, *Phos Sulf and Silicon*, 113, **1996**, 47.
- 14 Ravinder P, Rajeswar Rao V & Padmanabha Rao T V, *Collec Czch Chem Commun*, 53, **1988**, 326.
- 15 Aditya Vardhan V & Rajeswar Rao V, *Indian J Chem*, 36B, **1997**, 1085.
- 16 Haenel H, *Bibl Nutr Dieta*, 9, **1967**, 18; *Chem Abst*, 68, **1968**, 10788f.
- 17 Noesler G H & Schnegelberger H, *Ger Pat*, 1 284, 042 **1968**; *Chem Abstr*, 70, **1969**, 90742r.
- 18 Tartler G, Weuffen W & Froehling P, *Arch Eptl Veterinaarmed*, 19, **1965**, 9; *Chem Abtr*, 66, **1967**, 17801x.
- 19 Rajeswar Rao V & Padmanabha Rao T V, *Indian J Chem*, 25B, **1986**, 413.
- 20 Denton D A & Suschitzky H, *J Chem Soc*, **1963**, 4741.
- 21 Sandstrom J, *Acta Chem Scand*, 15, **1961**, 1295.
- 22 Dhaka K S, Mohan J, Chandha V K & Pujari H K, *Indian J Chem*, 12, **1974**, 287.
- 23 Thomas G, Tahilaramani & Dobholkar D A, *Indian J Chem*, 7, **1969**, 959.
- 24 Audrieth L F, Scott E S & Kipper P S, *J Org Chem*, 19, **1954**, 733.
- 25 Bayer H & Kroger E F, *Ann*, 637, **1960**, 135.
- 26 Reaid J R & Heindak N D, *J Het Chem*, 13, **1976**, 925.
- 27 Collins L & Microplate Almar S G, *Antimicrob Agents Chemother*, 41, **1997**, 1004.
- 28 Andrei G, Snoec K R, Reymen D, Liesnard C, Goubau P, Desmyter J & De Clereq E, *Eur J Clin Microbiol Infect Dis*, 14, **1995**, 318.
- 29 De Clereq E, Balzarini J, Torrence P F, Merters M P, Schmidt C L, Sugar D, Barr P J, Jones A S, Verhelst G & Walker R T, *Mol Pharmacol*, 19, **1981**, 321.
- 30 Balzarini J, Karlsson A, Wang C, Bohman K, Harska J, Votruba A, Fridland A A, Van Acrchot P, Her dewijn & De Clereq E, *J Biol Chem*, 268, **1993**, 24591.
- 31 Balzarini J, Bohmon C & De Clereq E, *J Biol Chem*, 268, **1993**, 6332.