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# Antiarrhythmic Activities of Some Newly Synthesized Tricyclic and Tetracyclic Thienopyridine Derivatives

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## Abstract

3,5-Bis(4-chlorobenzylidene)-1-ethylpiperidin-4-one (**1b**) was condensed with malononitrile or cyanothioacetamide to yield pyranopyridine **2** and thiopyridopyridine **3b**, respectively. Treatment of compound **3b** with methyl iodide or ethyl chloroacetate in the presence of a base catalyst gave the corresponding compounds **4** and **5**. Compound **3b** was reacted with 2-chloro-*N*-arylacetamide derivatives to yield compounds **7a,b**, which were reacted with benzoyl chloride or sodium nitrite to give the corresponding tetracyclic compounds **8a,b** and **9a,b**, respectively. Compound **2** was treated with acetic anhydride or formic acid to yield the corresponding *N*-acetylpyranopyridine **10** and pyranopyrimidine **11**. Treatment of compound **2** with triethyl *ortho*-formate gave compound **12**, which was cyclized with hydrazine hydrate to give *N*-aminopyrimidine **13**. Some of the synthesized compounds showed high antiarrhythmic activities comparable with Procaine amide and Lidocaine as positive controls.

## Keywords

Pyridinethione • Thienopyridine • Thienopyrimidine • Thienotriazine • Pyranopyrimidine • Antiarrhythmic agents

## Introduction

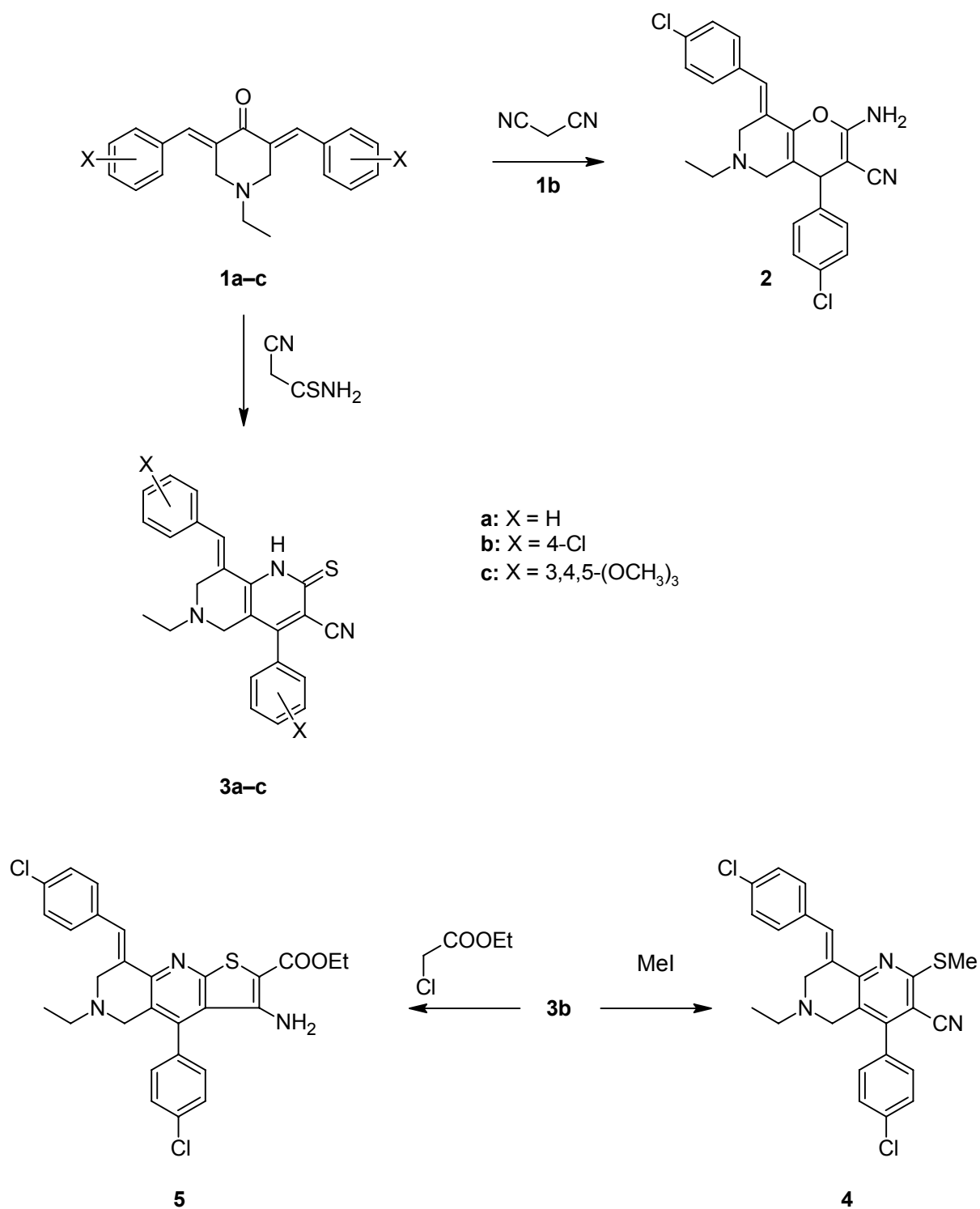
The search for new heterocyclic compounds and novel methods for their synthesis is a major topic in contemporary organic synthesis. As part of our program in this area, we have synthesized some pyridine and thiazolopyrimidine derivatives of biological interest as anticancer activity [1–5]. On the other hand, thienopyrimidine and thioxopyrimidine derivatives have promising biological [6, 7] and inhibitor of VEGFR-2 kinase activity [8, 9]. Recently, some new pyridine, pyrimidine and their derivatives have been synthesized and used as analgesic, anticonvulsant and anti-parkinsonian agents [10–15]. In addition, *Atheral et al* [16] have synthesized a novel series of thieno[2,3-*d*]pyrimidines which can be used in combating fungi in plant and inhibited the growth of cancer cells [17, 18]. In continuation of our previous work we synthesized some new tricyclic and tetracyclic heterocyclic compounds containing tetrahydropyridopyridine nucleus and tested their antiarrhythmic activities.

## Results and Discussion

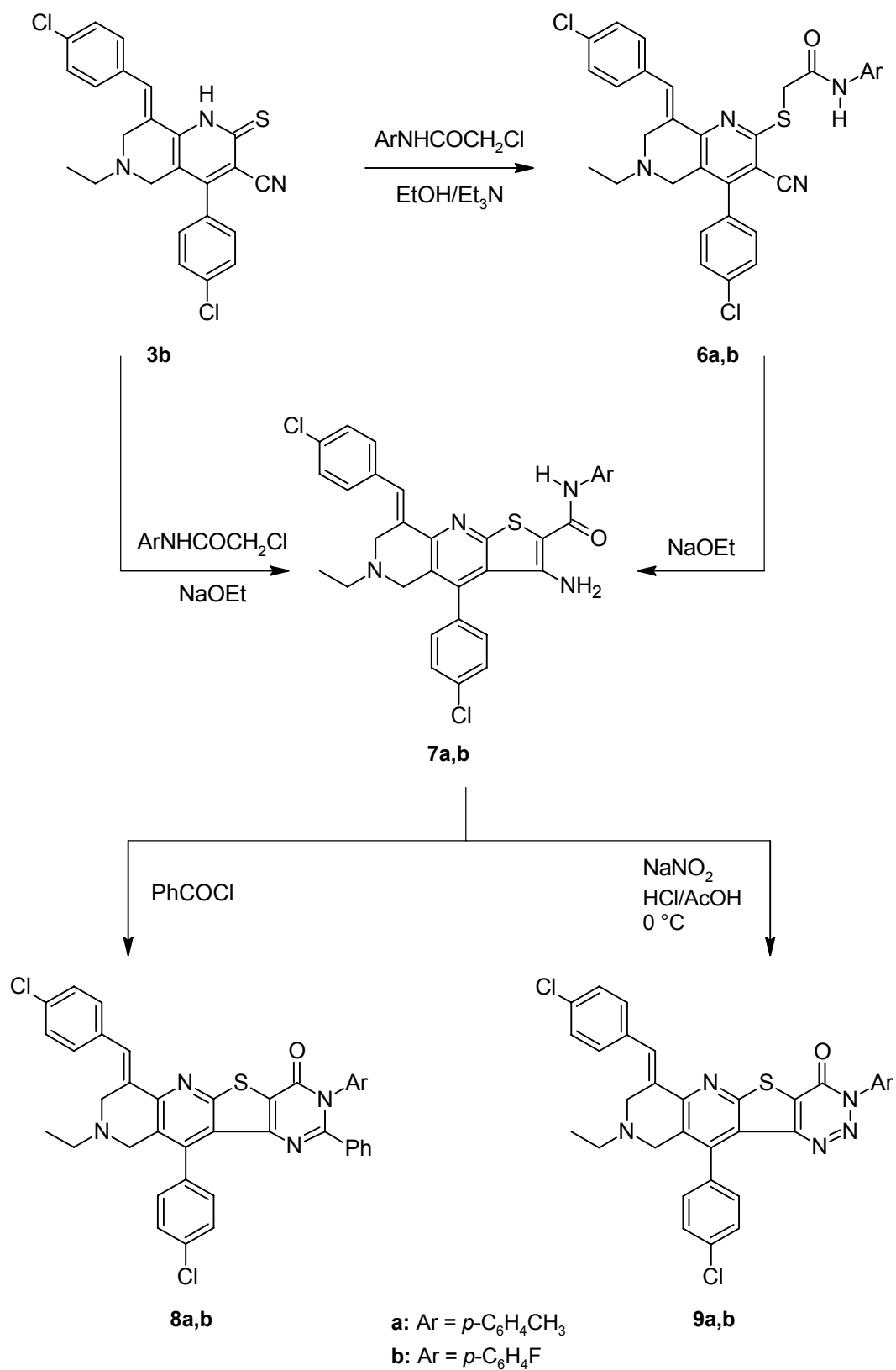
3,5-Bis(benzylidene)-1-ethylpiperidin-4-ones **1a–c** and pyranopyridine derivative **2** were used as starting materials and prepared according to the published method [3]. Compounds **1a–c** were reacted with cyanothioacetamide in refluxing ethanol containing drops of Et<sub>3</sub>N to give pyridinethione derivatives **3a–c**, which were reacted with methyl iodide in sodium ethoxide solution with stirring at room temperature to yield the methylmercaptopyridine derivative **4**. Similarly, compound **3b** was reacted with ethyl chloroacetate in refluxing ethanol in the presence of drops of Et<sub>3</sub>N as a catalyst to give the thienopyridine derivative **5** (Sch. 1).

Compound **3b** was reacted with 2-chloro-*N*-arylacetamide derivatives namely, 2-chloro-*N*-(*p*-tolyl)acetamide or 2-chloro-*N*-(4-fluorophenyl)acetamide in refluxing ethanol containing drops of Et<sub>3</sub>N to yield 2-(*N*-aryl)-carboxamidomethylthiopyridine derivatives **6a,b**, which were cyclized by boiling in sodium ethoxide solution to afford the corresponding 2-(*N*-aryl)-carboxamidomethyl-thienopyridine derivatives **7a,b**. The latter compound **7a,b** were also obtained directly from the reaction of compound **3b** with 2-chloro-*N*-arylacetamide derivatives in refluxing sodium ethoxide solution. Fusion of compounds **7a,b** with an excess of benzoyl chloride gave pyrido[thieno[3,2-*d*]pyrimidin-4-one derivatives **8a,b**. Moreover, treatment of compounds **7a,b** in HCl/AcOH mixture with sodium nitrite solution at 0 °C affording thieno[3,2-*d*][1,2,3]triazin-4-one derivatives **9a,b** (Sch. 2).

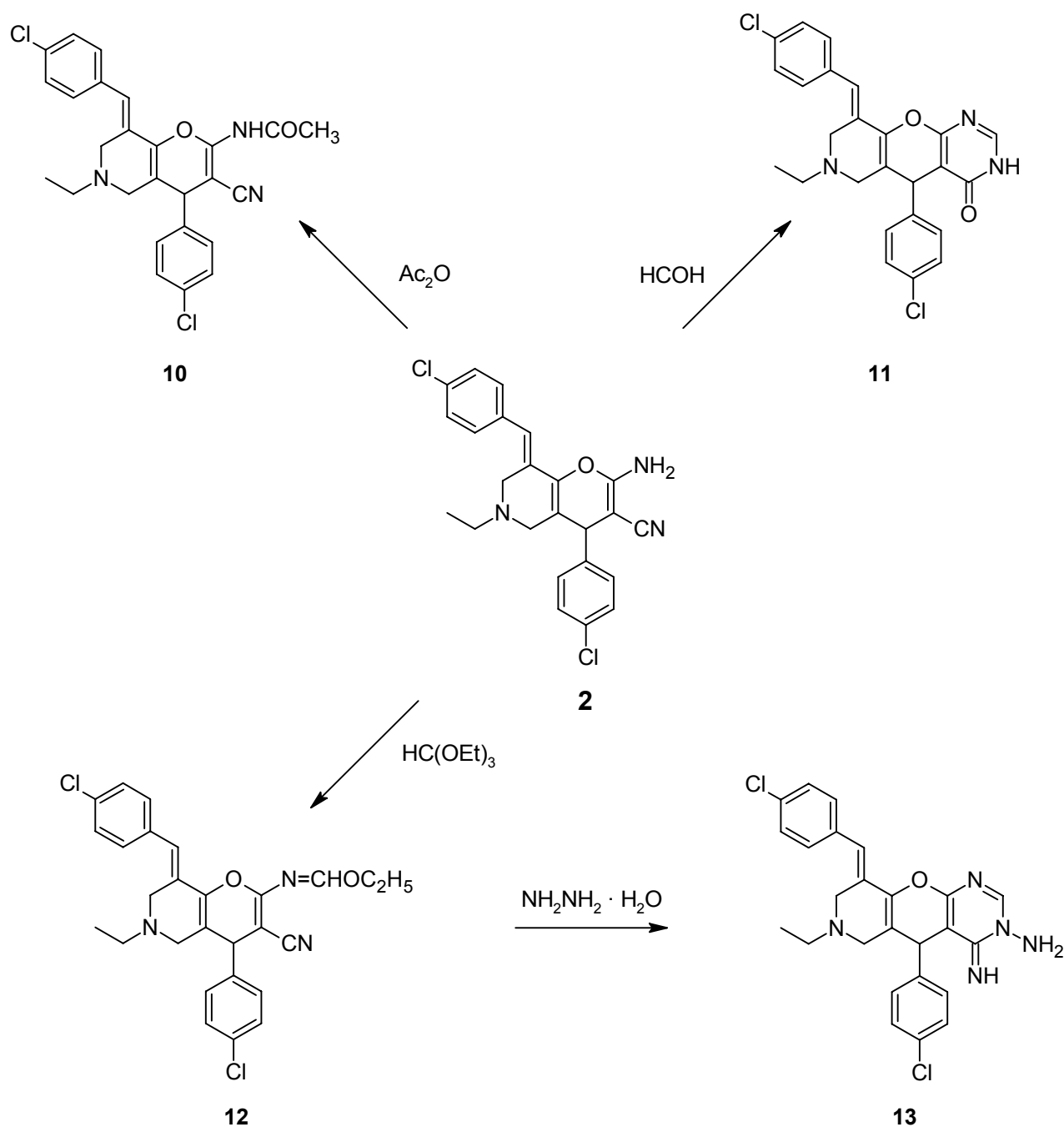
Compound **2** was reacted with acetic anhydride or formic acid to yield *N*-acetylpyranopyridine **10** and pyranopyrimidine derivative **11**, respectively. Compound **2** was condensed with triethyl *ortho*-formate in refluxing acetic anhydride to give the corresponding ethoxymethylidenamino derivative **12**, which was cyclized with hydrazine hydrate in ethanol at room temperature with stirring to give *N*-aminopyrimidine derivative **13** (Sch. 3).



Sch. 1.



Sch. 2.



Sch. 3.

### Pharmacological Screening

The newly synthesized compounds were pharmacologically screened for their Anti-arrhythmic potency (Table 1).

Procaine amide, 5 mg/kg iv and lidocaine 5 mg/kg i.v. led to an increase in  $\text{LD}_{100}$  by 65%, which corresponds to a  $\text{LD}_{100}$  of approximately 9  $\mu\text{g}/100 \text{ mg}$ .

From Table 1, Compounds **6a**, **3c**, **12** and **13** displayed nearly equal anti-arrhythmic activities as procaine amide and lidocaine. Compounds **1b**, **4**, **5**, **7b**, **8a**, **8b**, **9a**, and **9b** are more active than procaine amide and lidocaine, they are arranged in descending manner. Also, **2**, **3a**, **3b**, **6b**, **7a**, **10** and **11** showed anti-arrhythmic activities less than procaine and lidocaine, they are arranged in descending manner.

**Tab. 1.** Anti-arrhythmic activities of the newly synthesized compounds

Compound in (5 mg/kg)	Percentage Increase in $LD_{100}$
1	$76 \pm 0.08$
2b	$54 \pm 0.05$
3a	$48 \pm 0.05$
3b	$41 \pm 0.05$
3c	$67 \pm 0.07$
4	$79 \pm 0.07$
5	$77 \pm 0.09$
6a	$65 \pm 0.06$
6b	$45 \pm 0.05$
7a	$47 \pm 0.04$
7b	$77 \pm 0.08$
8a	$75 \pm 0.08$
8b	$86 \pm 0.09$
9a	$72 \pm 0.07$
9b	$78 \pm 0.10$
10	$37 \pm 0.05$
11	$55 \pm 0.06$
12	$69 \pm 0.07$
13	$68 \pm 0.07$

All data were significantly different from the normal control value at  $P \leq 0.05$

### Structural Activity Relationship (SAR)

- The naphthyridine moiety and a high degree of aromaticity is essential for anti-arrhythmic activity.
- Hetero-aromaticity increases the anti-arrhythmic activity
- Fused ring systems give anti-arrhythmic activity but to a lower extent.

### Experimental

All melting points were taken on a Electrothermal IA 9000 series digital melting point apparatus. Elemental analytical data (in accordance with the calculated values) were obtained from the microanalytical unit, Cairo University, Cairo, Egypt. The IR spectra (KBr) were recorded on a Pye Unicam SP-1000 spectrophotometer. The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded at 270 MHz and 64.5 MHz, respectively, on a Varian EM-360

Spectrometer using TMS as an internal standard. The mass spectra were performed using a VC 2AB-3F spectrometer (70 eV). All reactions were followed by TLC (silica gel, aluminium sheets 60 F<sub>254</sub>, Merck).

**(8E)-4-aryl-8-(arylmethylidene)-6-ethyl-2-thioxo-1,2,5,6,7,8-hexahydro-1,6-naphthyridine-3-carbonitriles (3a–c)**

A mixture of compound **1a–c** (0.01 mole), cyanothioacetamide (0.10 g, 0.01 mole) and few drops of Et<sub>3</sub>N in ethanol (100 ml) was refluxed for 6 h. The reaction mixture was poured onto cold water and acidified with HCl to pH 3, the orange solid formed was filtered off, washed with water, dried and recrystallized to give **3a–c**.

**(8E)-8-Benzylidene-6-ethyl-4-phenyl-2-thioxo-1,2,5,6,7,8-hexahydro-1,6-naphthyridine-3-carbonitrile (3a)**

Yield (55%), M.p. 205 °C (Ethanol); IR (KBr)  $\nu$  = 3345 (NH), 2212 (CN) cm<sup>-1</sup>; MS m/z (%): 383 [M<sup>+</sup>] base peak (100) corresponding to the molecular formula C<sub>24</sub>H<sub>21</sub>N<sub>3</sub>S, 354 (79). Analysis for C<sub>24</sub>H<sub>21</sub>N<sub>3</sub>S (383.51): Calc. C, 75.16; H, 5.52; N, 10.96; S, 8.36, Found C, 75.12; H, 5.56; N, 11.02; S, 8.26.

**(8E)-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-6-ethyl-2-thioxo-1,2,5,6,7,8-hexahydro-1,6-naphthyridine-3-carbonitrile (3b)**

Yield (50%), M.p. 228 °C (EtOH); IR (KBr)  $\nu$  = 3320 (NH), 2208 (CN) cm<sup>-1</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  = 0.9 (t, 3H, CH<sub>3</sub>), 2.40 (q, 2H, CH<sub>2</sub>), 3.33, 3.42 (2s, 4H, 2CH<sub>2</sub>), 7.30–7.80 (m, 9H, ArH + benzylic proton), 7.95 (s, 1H, NH, exchangeable with D<sub>2</sub>O); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  = 13.35 (CH<sub>3</sub>), 50.55, 51.45, 58.90 (3 CH<sub>2</sub>), 125.30, 135.25 (C=C), 103.25, 114.55, 145.50, 153.62 (Pyridine-C), 117.95 (CN), 125.50, 126.75, 128.55, 129.15, 128.30, 129.10, 132.57, 133.50 (Ph-C), 165.25 (C=S); MS m/z (%): 451 [M<sup>+</sup>] base peak (100) corresponding to the molecular formula C<sub>24</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>3</sub>S, 453 [M+2] (80), 455 [M+4] (30), 326 (79). Analysis for C<sub>24</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>3</sub>S (452.40): Calc. C, 63.72; H, 4.23; Cl, 15.67; N, 9.29; S, 7.09, Found C, 63.69; H, 4.20; Cl, 15.63; N, 9.32; S, 7.12.

**(8E)-6-Ethyl-2-thioxo-4-(3,4,5-trimethoxyphenyl)-8-(3,4,5-trimethoxybenzylidene)-1,2,5,6,7,8-hexahydro-1,6-naphthyridine-3-carbonitrile (3c)**

Yield (70%), M.p. 255 °C (Ethanol); IR (KBr)  $\nu$  = 3358 (NH), 2220 (CN) cm<sup>-1</sup>; MS m/z (%): 563 [M<sup>+</sup>] base peak (100) corresponding to the molecular formula C<sub>30</sub>H<sub>33</sub>N<sub>3</sub>SO<sub>6</sub>, 543 (80), 394 (75). Analysis for C<sub>30</sub>H<sub>33</sub>N<sub>3</sub>SO<sub>6</sub> (563.66): Calc. C, 63.92; H, 5.90; N, 7.45; S, 5.69, Found C, 63.88; H, 5.94; N, 7.48; S, 5.73

**(8E)-8-(4-Chlorobenzylidene)-4-(4-chlorophenyl)-6-ethyl-2-(methylsulfanyl)-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile (4)**

A mixture of compound **3b** (0.45 g, 0.001 mole) and methyl iodide 10% excess was suspended in sodium ethoxide solution (10 mg sodium metal in 50 ml ethanol) and stirred for 1h. at room temperature. The reaction mixture was poured onto cold water, the obtained solid was filtered off, dried and recrystallized to afford **4**. Yield (50%), M.p. 150 °C (Ethanol) IR (KBr)  $\nu$  = 2217 (CN) cm<sup>-1</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.10 (t, 3H, CH<sub>3</sub>), 2.37 (q, 2H, CH<sub>2</sub>), 2.70 (s, 3H, CH<sub>3</sub>), 3.20–3.35 (m, 4H, 2CH<sub>2</sub>) 7.20–7.95 (m, 9H, ArH + benzylic proton); MS m/z (%): 466 [M<sup>+</sup>] (85) corresponding to the molecular formula C<sub>25</sub>H<sub>21</sub>Cl<sub>2</sub>N<sub>3</sub>S,

468  $[M^+ + 2]$  (base peak 100), 337 (68). Analysis for  $C_{25}H_{21}Cl_2N_3S$  (466.43): Calc. C, 64.38; H, 4.54; Cl, 15.20; N, 9.01; S, 6.87, Found C, 64.32; H, 4.58; Cl, 15.15; N, 9.06; S, 6.83.

**Ethyl (8E)-3-amino-4-(4-chlorophenyl)-8-(4-chlorobenzylidene)-6-ethyl-5,6,7,8-tetrahydrothieno[2,3-b][1,6]naphthyridine-2-carboxylate (5)**

To a mixture of compound **3b** (0.45g, 0.001 mole) and ethyl chloroacetate (0.14g, 0.001 mole) in ethanol (50 ml), few drops of  $Et_3N$  were added then the reaction mixture was refluxed for 4h. The solid formed was filtered off, dried and recrystallized to give **5**. Yield (35%), M.p. 238 °C (Ethanol), IR (KBr)  $\nu$  = 3410, 3430 ( $NH_2$ ), 1738 (CO)  $cm^{-1}$ ;  $^1H$ -NMR (DMSO- $d_6$ )  $\delta$ : 1.05, 1.10 (2t, 6H,  $2CH_3$ ), 2.46 (q, 2H,  $CH_2$ ), 3.29-3.35 (m, 4H,  $2CH_2$ ), 4.05 (q, 2H,  $CH_2$ ), 4.55 (s, 2H,  $NH_2$ ), 7.50–7.90 (m, 9H, ArH + benzylic proton); MS  $m/z$  (%): 538  $[M^+]$  base peak (100) corresponding to the molecular formula  $C_{28}H_{25}Cl_2N_3SO_2$ , 412 (86). Analysis for  $C_{28}H_{25}Cl_2N_3SO_2$  (538.49): Calc. C, 62.45; H, 4.68; Cl, 13.17; N, 7.80; S, 5.95, Found C, 62.41; H, 4.62; Cl, 13.13; N, 7.83; S, 5.91.

**2-((8E)-4-(4-Chlorophenyl)-8-(4-chlorobenzyliden)-3-cyano-6-ethyl-5,6,7,8-tetrahydro-1,6-naphthyridin-2-yl)sulfanyl)-N-arylacetamides (6a,b)**

*Method A:*

To a mixture of compound **3b** (0.45g, 0.001 mole) and anhydrous sodium acetate (2g) in ethanol (50 ml), 2-chloro-*N*-arylacetamide, namely, 2-chloro-*N*-(*p*-tolyl)acetamide or 2-chloro-*N*-(4-fluorophenyl)acetamide (0.001 mole) was added. The reaction mixture was refluxed for 2h then cooled and poured onto cold water. The solid formed was filtered off, dried and recrystallized to give **6a,b** respectively.

*Method B:*

To a mixture of compound **3b** (0.45g, 0.001 mole) and 2-chloro-*N*-arylacetamide namely, 2-chloro-*N*-(*p*-tolyl)acetamide or 2-chloro-*N*-(4-fluorophenyl)acetamide (0.001 mole) in ethanol (50 ml), few drops of  $Et_3N$  were added then the reaction mixture was refluxed for 3h. The solid formed was filtered off, dried and recrystallized to give **6a,b** respectively.

**2-((8E)-4-(4-Chlorophenyl)-8-(4-chlorobenzylidene)-3-cyano-6-ethyl-5,6,7,8-tetrahydro-1,6-naphthyridin-2-yl)sulfanyl)-N-(4-methylphenyl)acetamide (6a)**

Yield (55%) [A], (75%) [B], M.p. 218 °C (Dioxan), IR (KBr)  $\nu$  = 3294 (NH), 2215 (CN) and 1662 (CO)  $cm^{-1}$ ;  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 0.98 (t, 3H,  $CH_3$ ), 2.27 (s, 3H,  $CH_3$ ), 2.50 (q, 2H,  $CH_2$ ), 3.40, 3.70 (2d, 4H,  $2CH_2$ ), 4.10 (s, 2H,  $CH_2$ ) 7.10–7.83 (m, 13H, ArH + benzylic proton), 8.20 (s, 1H, NH, exchangeable with  $D_2O$ ); MS  $m/z$  (%): 599  $[M^+]$  (55) corresponding to the molecular formula  $C_{33}H_{28}Cl_2N_4SO$ , 449 base peak (100). Analysis for  $C_{33}H_{28}Cl_2N_4SO$  (599.58): Calc. C, 66.11; H, 4.71; Cl, 11.83; N, 9.34; S, 5.35, Found C, 66.15; H, 4.76; Cl, 11.86; N, 9.30; S, 5.38

**2-((8E)-4-(4-Chlorophenyl)-8-(4-chlorobenzylidene)-3-cyano-6-ethyl-5,6,7,8-tetrahydro-1,6-naphthyridin-2-yl)sulfanyl)-N-(4-fluorophenyl)acetamide (6b)**

Yield (60%) [A], (78%) [B], M.p. 205 °C (Dioxan), IR (KBr)  $\nu$  = 3315 (NH), 2210 (CN) and 1655 (CO)  $cm^{-1}$ .  $^1H$ -NMR ( $CDCl_3$ )  $\delta$ : 1.05 (t, 3H,  $CH_3$ ), 2.35 (q, 2H,  $CH_2$ ), 3.35, 3.60 (2d, 4H,  $2CH_2$ ), 4.21 (s, 2H,  $CH_2$ ), 7.15–7.90 (m, 13H, ArH + benzylic proton), 8.32 (s, 1H, NH, exchangeable with  $D_2O$ );  $^{13}C$ -NMR ( $CDCl_3$ )  $\delta$  = 12.75 ( $CH_3$ ), 41.25, 49.55, 52.15, 55.85



(4CH<sub>2</sub>), 127.20, 134.50 (C=C), 106.25, 126.10, 152.35, 161.42, 161.85 (Pyridine-C), 116.75 (CN), 116.50, 125.30, 136.15, 155.25, 126.23, 127.80, 128.45, 129.25, 134.55, 136.15 (Ph-C), 167.85 (C=O); MS m/z (%): 603 [M<sup>+</sup>] (100) base peak corresponding to the molecular formula C<sub>32</sub>H<sub>25</sub>Cl<sub>2</sub>FN<sub>4</sub>SO. Analysis for C<sub>32</sub>H<sub>25</sub>Cl<sub>2</sub>FN<sub>4</sub>SO (603.54): Calc. C, 63.68; H, 4.18; Cl, 11.75; N, 9.28; S, 5.31, Found C, 63.72; H, 4.22; Cl, 11.80; N, 9.32; S, 5.35

**(8E)-3-Amino-4-(4-Chlorophenyl)-8-(4-chlorobenzylidene)-6-ethyl-N-aryl-5,6,7,8-tetrahydrothieno[2,3-b][1,6]naphthyridine-2-carboxamides (7a,b)**

*Method A:*

A mixture of compound **6a,b** (0.001 mole) and 2-chloro-*N*-arylacetamide namely, 2-chloro-*N*-(*p*-tolyl)acetamide or 2-chloro-*N*-(4-fluorophenyl)acetamide (0.001 mole) was suspended in sodium ethoxide solution (10 mg sodium metal in 50 ml ethanol) and refluxed for 2h. On cooling, the product precipitated was filtered off and recrystallized to afford **7a,b** respectively.

*Method B:*

A mixture of compound **3b** (0.45g, 0.001 mole) and 2-chloro-*N*-arylacetamide namely, 2-chloro-*N*-(*p*-tolyl)acetamide or 2-chloro-*N*-(4-fluorophenyl)acetamide (0.001 mole) in sodium ethoxide solution (10 mg sodium metal in 50 ml ethanol) was refluxed for 5 h. The reaction mixture was cooled, poured onto cold water and the solid formed was filtered off and recrystallized to afford **7a,b** respectively.

**(8E)-3-Amino-4-(4-chlorophenyl)-8-(4-chlorobenzylidene)-6-ethyl-N-(4-methylphenyl)-5,6,7,8-tetrahydrothieno[2,3-b][1,6]naphthyridine-2-carboxamide (7a)**

Yield (80%) [A], (45%) [B], M.p. 172 °C (Ethanol), IR (KBr)  $\nu$  = 3483, 3399 (NH<sub>2</sub>), and 1645 (CO) cm<sup>-1</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 0.98 (t, 3H, CH<sub>3</sub>), 2.24 (s, 3H, CH<sub>3</sub>), 2.35 (q, 2H, CH<sub>2</sub>), 3.35, 3.82 (2d, 4H, 2CH<sub>2</sub>), 5.67 (s, 2H, NH<sub>2</sub>, exchangeable with D<sub>2</sub>O); 7.10–7.80 (m, 13H, ArH + benzylic proton), 8.13 (s, 1H, NH, exchangeable with D<sub>2</sub>O); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  = 12.65, 20.66 (2CH<sub>3</sub>), 51.31, 53.55, 59.90 (3CH<sub>2</sub>), 128.25, 137.65 (C=C), 119.15, 124.10, 146.50, 155.62, 156.77 (Pyridine-C), 128.75, 129.65, 129.77, 131.05, 132.35, 132.75, 133.55, 134.05, 135.76, 163.80 (Ph-C, Thiophene-C), 169.25 (C=O); MS m/z (%): 599 [M<sup>+</sup>] (30) corresponding to the molecular formula C<sub>33</sub>H<sub>28</sub>Cl<sub>2</sub>N<sub>4</sub>SO, 570 (25), 509 base peak (100). Analysis for C<sub>33</sub>H<sub>28</sub>Cl<sub>2</sub>N<sub>4</sub>SO (599.58): Calc. C, 66.11; H, 4.71; Cl, 11.83; N, 9.34; S, 5.35, Found C, 66.15; H, 4.74; Cl, 11.86; N, 9.37; S, 5.31.

**(8E)-3-Amino-4-(4-chlorophenyl)-8-(4-chlorobenzylidene)-6-ethyl-N-(4-fluorophenyl)-5,6,7,8-tetrahydrothieno[2,3-b][1,6]naphthyridine-2-carboxamide (7b)**

Yield (75%) [A], (50%) [B], M.p. 152 °C (Ethanol), IR (KBr)  $\nu$  = 3450, 3366 (NH<sub>2</sub>), and 1655 (CO) cm<sup>-1</sup>. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.05 (t, 3H, CH<sub>3</sub>), 2.55 (q, 2H, CH<sub>2</sub>), 3.40, 3.70 (2d, 4H, 2CH<sub>2</sub>), 5.67 (s, 2H, NH<sub>2</sub>, exchangeable with D<sub>2</sub>O); 7.10–7.80 (m, 13H, ArH + benzylic proton), 8.18 (s, 1H, NH, exchangeable with D<sub>2</sub>O); MS m/z (%): 602 [M<sup>+</sup>] (30) corresponding to the molecular formula C<sub>32</sub>H<sub>25</sub>Cl<sub>2</sub>FN<sub>4</sub>SO, 442 (25), 111 base peak (100). Analysis for C<sub>32</sub>H<sub>25</sub>Cl<sub>2</sub>FN<sub>4</sub>SO (603.54): Calc. C, 63.68; H, 4.18; Cl, 11.75; N, 9.28; S, 5.31, Found C, 63.73; H, 4.22; Cl, 11.71; N, 9.32; S, 5.35.

**(7E)-11-(4-Chlorophenyl)-7-(4-chlorobenzylidene)-9-ethyl-2,3-diphenyl-7,8,9,10-tetrahydropyrimido[4',5':4,5]thieno[2,3-b][1,6]naphthyridin-4(3H)-ones (8a,b)**

A mixture of compound **7a,b** (0.001 mole) and excess benzoyl chloride (6 ml) was refluxed for 2 h. Excess of benzoyl chloride was extracted several times with pet. ether (60–80) and the residue was recrystallized to give **8a,b**.

**(7E)-11-(4-Chlorophenyl)-7-(4-chlorobenzylidene)-9-ethyl-3-(4-methylphenyl)-2-phenyl-7,8,9,10-tetrahydropyrimido[4',5':4,5]thieno[2,3-b][1,6]naphthyridin-4(3H)-one (8a)**

Yield (65%), M.p. 205 °C (Ethanol), IR (KBr)  $\nu$  = 1648 (CO)  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 1.05 (t, 3H,  $\text{CH}_3$ ), 2.13 (s, 3H,  $\text{CH}_3$ ), 2.45 (q, 2H,  $\text{CH}_2$ ), 3.65–3.85 (m, 4H, 2 $\text{CH}_2$ ), 6.95–7.77 (m, 18H, ArH + benzylic proton), MS  $m/z$  (%): 685 [ $\text{M}^+$ ] (44) corresponding to the molecular formula  $\text{C}_{40}\text{H}_{30}\text{Cl}_2\text{N}_4\text{SO}$ , 559 (35). Analysis for  $\text{C}_{40}\text{H}_{30}\text{Cl}_2\text{N}_4\text{SO}$  (685.67): Calc. C, 70.07; H, 4.41; Cl, 10.34; N, 8.17; S, 4.68, Found C, 70.13; H, 4.45; Cl, 10.38; N, 8.23; S, 4.62.

**(7E)-11-(4-Chlorophenyl)-7-(4-chlorobenzylidene)-9-ethyl-3-(4-fluorophenyl)-2-phenyl-7,8,9,10-tetrahydropyrimido[4',5':4,5]thieno[2,3-b][1,6]naphthyridin-4(3H)-one (8b)**

Yield (60%), M.p. 192 °C (Ethanol), IR (KBr)  $\nu$  = 1645 (CO)  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 1.08 (t, 3H,  $\text{CH}_3$ ), 2.35 (q, 2H,  $\text{CH}_2$ ), 3.53–3.77 (m, 4H, 2 $\text{CH}_2$ ), 7.10–7.85 (m, 18H, ArH + benzylic proton);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  = 12.55 ( $\text{CH}_3$ ), 53.50, 56.35, 60.40 (3  $\text{CH}_2$ ), 127.60, 136.50 (C=C), 117.25, 124.30, 126.23, 127.80, 129.45, 133.50, 135.35, 137.20 (Ph-C), 148.55, 156.32, 158.53 (Pyridine-C), 147.35, 162.10, 165.34 (Pyrimidine-C), 167.25 (C=O); MS  $m/z$  (%): 689 [ $\text{M}^+$ ] (44) corresponding to the molecular formula  $\text{C}_{39}\text{H}_{27}\text{Cl}_2\text{FN}_4\text{SO}$ , 559 (35). Analysis for  $\text{C}_{39}\text{H}_{27}\text{Cl}_2\text{FN}_4\text{SO}$  (689.63): Calc. C, 67.92; H, 3.95; Cl, 10.28; N, 8.12; S, 4.65, Found C, 67.96; H, 4.41; Cl, 10.22; N, 8.27; S, 4.65.

**(7E)-11-(4-Chlorophenyl)-7-(4-chlorobenzylidene)-9-ethyl-3-phenyl-7,8,9,10-tetrahydro[1,2,3]triazino[4',5':4,5]thieno[2,3-b][1,6]naphthyridin-4(3H)-ones (9a,b)**

To a mixture of compound **7a,b** (0.001 mole) in conc. hydrochloric acid (5 ml) and glacial acetic acid (5 ml), sodium nitrite solution (7 ml 10%, 0.001mole) was added at 0 °C during 5 minutes with stirring. After half hour, the reaction mixture was poured onto water, the solid thus precipitated was collected, dried and recrystallized to give **9a,b**.

**(7E)-11-(4-Chlorophenyl)-7-(4-chlorobenzylidene)-9-ethyl-3-(4-methylphenyl)-7,8,9,10-tetrahydro[1,2,3]triazino[4',5':4,5]thieno[2,3-b][1,6]naphthyridin-4(3H)-one (9a)**

Yield (85%), M.p. 216 °C (Ethanol), IR (KBr)  $\nu$  = 1668 (CO)  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 1.07 (t, 3H,  $\text{CH}_3$ ), 2.42 (s, 3H,  $\text{CH}_3$ ), 3.01 (q, 2H,  $\text{CH}_2$ ), 3.75, 4.12 (2d, 4H, 2 $\text{CH}_2$ ), 7.20–7.85 (m, 13H, ArH + benzylic proton);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  = 13.25, 22.35 (2 $\text{CH}_3$ ), 49.20, 52.95, 57.70 (3 $\text{CH}_2$ ), 129.80, 138.30 (C=C), 122.15, 128.45, 127.80, 129.40, 133.50, 134.65, 135.35, 138.40 (Ph-C), 147.55, 155.45, 157.25 (Pyridine-C), 139.15, 152.35 (Pyrimidine-C), 168.65 (C=O); MS  $m/z$  (%): 609 [ $\text{M}^+$ ] base peak (100) corresponding to the molecular formula  $\text{C}_{33}\text{H}_{25}\text{Cl}_2\text{N}_5\text{SO}$ , 580 (75). Analysis for  $\text{C}_{33}\text{H}_{25}\text{Cl}_2\text{N}_5\text{SO}$  (609.11) Calc. C, 65.01; H, 4.14; Cl, 11.48; N, 11.49; S, 5.25, Found C, 65.06; H, 4.18; Cl, 11.53; N, 11.52; S, 5.28.

**(7E)-11-(4-Chlorophenyl)-7-(4-chlorobenzylidene)-9-ethyl-3-(4-fluorophenyl)-7,8,9,10-tetrahydro[1,2,3]triazino[4',5':4,5]thieno[2,3-b][1,6]naphthyridin-4(3H)-one (9b)**

Yield (80%), M.p. 227 °C (Ethanol), IR (KBr)  $\nu$  = 1655 (CO)  $\text{cm}^{-1}$ .  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 1.25 (t, 3H,  $\text{CH}_3$ ), 3.01(q, 2H,  $\text{CH}_2$ ), 3.88, 4.25 (2d, 4H,  $2\text{CH}_2$ ), 7.20–7.85 (m, 13H, ArH + benzylic proton), MS  $m/z$  (%): 613 [ $\text{M}^+$ ] (35) corresponding to the molecular formula  $\text{C}_{32}\text{H}_{22}\text{Cl}_2\text{FN}_5\text{SO}$ , 585 base peak (100), 458 (85). Analysis for  $\text{C}_{32}\text{H}_{22}\text{Cl}_2\text{FN}_5\text{SO}$  (613.11): Calc. C, 62.63; H, 4.18; Cl, 11.41 N, 11.42; S, 5.21, Found C, 62.67; H, 4.23; Cl, 11.46 N, 11.45; S, 5.26.

**N-[(8E)-4-(4-Chlorophenyl)-8-(4-chlorobenzylidene)-3-cyano-6-ethyl-5,6,7,8-tetrahydro-4H-pyrano[3,2-c]pyridin-2-yl]acetamide (10)**

A mixture of compound **2** (0.01 mole) in acetic anhydride (10 ml) was refluxed for 1 h. After cooling the reaction mixture was poured onto cold water, the solid formed was filtered off, dried and recrystallized to give **10**. Yield (65%), M.p. 205 °C (Ethanol), IR (KBr)  $\nu$  = 2195 (CN), 1679 (CO)  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ )  $\delta$ : 1.25 (t, 3H,  $\text{CH}_3$ ), 2.10 (s, 3H,  $\text{CH}_3$ ), 3.01(q, 2H,  $\text{CH}_2$ ), 3.27, 3.60 (2d, 4H,  $2\text{CH}_2$ ), 3.95 (s, 1H, pyran proton), 7.10–7.85 (m, 9H, ArH + benzylic proton) and 8.24 (s, 1H, NH, exchangeable with  $\text{D}_2\text{O}$ ), MS  $m/z$  (%): 479 [ $\text{M}^+$ ] (22) corresponding to the molecular formula  $\text{C}_{26}\text{H}_{23}\text{Cl}_2\text{N}_3\text{O}_2$ , 436 base peak (100), 393 (55). Analysis for  $\text{C}_{26}\text{H}_{23}\text{Cl}_2\text{N}_3\text{O}_2$  (479.11): Calc. C, 65.12; H, 4.84; Cl, 14.60; N, 8.77, Found C, 65.17; H, 4.80; Cl, 14.65; N, 8.71.

**(9E)-5-(4-Chlorophenyl)-9-(4-chlorobenzylidene)-7-ethyl-3,5,6,7,8,9-hexahydro-4H-pyrido[3',4':5,6]pyrano[2,3-d]pyrimidin-4-one (11)**

A mixture of compound **2** (0.01 mole) in formic acid (10 ml) was refluxed for 1 hr. After cooling the reaction mixture was poured onto cold water, the solid formed was filtered off, dried and recrystallized to give **11**. Yield (75%), M.p. 183 °C (Ethanol), IR (KBr)  $\nu$  = 1669 (CO)  $\text{cm}^{-1}$ ,  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ )  $\delta$ : 1.25 (t, 3H,  $\text{CH}_3$ ), 3.01 (q, 2H,  $\text{CH}_2$ ), 3.35, 3.60 (2d, 4H,  $2\text{CH}_2$ ), 4.10 (s, 1H, pyran proton) 7.20–7.65 (m, 9H, ArH + benzylic proton), 7.95 (s, 1H, pyrimidine proton), 8.25 (s, 1H, NH, exchangeable with  $\text{D}_2\text{O}$ ), MS  $m/z$  (%): 465 [ $\text{M}^+$ ] (50) corresponding to the molecular formula  $\text{C}_{25}\text{H}_{21}\text{Cl}_2\text{N}_3\text{O}_2$ , 436 base peak (100), 393 (55). Analysis for  $\text{C}_{25}\text{H}_{21}\text{Cl}_2\text{N}_3\text{O}_2$  (465.10): Calc. C, 64.50; H, 4.55; Cl, 15.04; N, 9.03, Found C, 64.55; H, 4.51; Cl, 15.09; N, 9.07.

**Ethyl [(8E)-4-(4-chlorophenyl)-8-(4-chlorobenzylidene)-3-cyano-6-ethyl-5,6,7,8-tetrahydro-4H-pyrano[3,2-c]pyridin-2-yl]imidofornate (12)**

A mixture of compound **2** (0.01 mole), triethyl *ortho*-formate (0.01 mole) and acetic anhydride (20 ml) was refluxed for 6 h. The solvent was removed under vacuum, the residue obtained was solidified by ether and recrystallized to give **12**. Yield (75%), M.p. 155 °C (Ethanol), IR (KBr)  $\nu$  = 2980 (CH), 2218 (CN), 1630 (C=N)  $\text{cm}^{-1}$ ,  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ )  $\delta$ : 1.10, 1.25 (2t, 6H,  $2\text{CH}_3$ ), 3.01(q, 2H,  $\text{CH}_2$ ), 3.57, 3.80 (2d, 4H,  $2\text{CH}_2$ ), 3.95 (q, 2H,  $\text{CH}_2$ ), 4.15 (s, 1H, pyran proton), 7.10–7.85 (m, 10H, ArH + benzylic proton + CH); MS  $m/z$  (%): 492 [ $\text{M}^+$ ] (85) corresponding to the molecular formula  $\text{C}_{27}\text{H}_{25}\text{Cl}_2\text{N}_3\text{O}_2$ , 393 (100) base peak. Analysis for  $\text{C}_{27}\text{H}_{25}\text{Cl}_2\text{N}_3\text{O}_2$  (393.13): Calc. C, 65.70; H, 5.11; Cl, 14.18; N, 8.52, Found C, 65.75; H, 5.14; Cl, 14.22; N, 8.56

**(9E)-5-(4-Chlorophenyl)-9-(4-chlorobenzylidene)-7-ethyl-4-imino-6,7,8,9-tetrahydro-4H-pyrido[3',4':5,6]pyrano[2,3-d]pyrimidin-3(5H)-amine (13)**

A mixture of compound **12** (0.01 mole) and hydrazine hydrate (99%, 5ml) in ethanol (50 ml) was stirred at room temperature for 1 h. the solid obtained was filtered off and recrystallized to give **13**. Yield (55%), M.p. 179 °C (EtOH), IR (KBr)  $\nu$  = 3316, 3335 (NH<sub>2</sub>), 1635 (C=N) cm<sup>-1</sup>, <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>)  $\delta$ : 0.97 (t, 3H, CH<sub>3</sub>), 2.72 (q, 2H, CH<sub>2</sub>), 3.75-4.18 (m, 4H, 2CH<sub>2</sub>), 4.26 (s, 1H, pyran proton), 5.65 (s, 2H, NH<sub>2</sub>, exchangeable by D<sub>2</sub>O), 7.30–7.95 (m, 9H, ArH + benzylic proton) and 8.15 (s, 1H, pyrimidine proton), MS  $m/z$  (%): 479 [M<sup>+</sup>] (83) corresponding to the molecular formula C<sub>25</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>5</sub>O, 463 base peak (100), 436 (90). Analysis for C<sub>25</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>5</sub>O (479.13): Calc. C, 62.61; H, 4.84; Cl, 14.60; N, 14.61, Found C, 62.66; H, 4.80; Cl, 14.64; N, 14.65.

**Pharmacological Assay**

**Anti-arrhythmic Activity [19–24].**

*Purpose and Rational*

The plant alkaloid aconitine persistently activates sodium channel. Infusion of aconitine in the anesthetized rat causes ventricular arrhythmias. Drugs considered to have anti-arrhythmic properties can be tested in aconitine-intoxicated rats.

*Procedure*

Male Ivanovas rats weighing 300–350g are used. The animals are anesthetized by intra peritoneal injection of 1.25 g/kg urethane: 5 mg/kg aconitine dissolved in 0.1 N HNO<sub>3</sub> is administered by continuous infusion into the saphenous vein of 0.1ml/min and the ECG in lead II is recorded every 30 seconds. The test compound is injected IV at a screening dose of 3 mg/kg 5min before the start of the aconitine infusion, 24 animals are used per compound.

Animals were obtained from the animal house colony of the National Research Center, Cairo, Egypt. The animals were allowed free access to water and were kept on a constant standard diet. All animal experiments were carried out in compliance with the institutional guidelines on the use of living animals.

*Evaluation*

The anti-arrhythmic effect of a test compound is measured by the amount of aconitine/100g animal.

(Duration of infusion) which induces:

Ventricular extra systoles.

Ventricular tachycardia.

Ventricular fibrillation.

Higher doses of aconitine in the treated group as compared to an untreated control group are an indication of anti-arrhythmic activity. Statistical significance between the groups is assessed by the student's T-test.

## Authors' Statements

### Competing Interests

The authors declare no conflict of interest.

### Animal Rights

The institutional and (inter)national guide for the care and use of laboratory animals was followed. See the experimental part for details.

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