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Synthesis and Reactions of Some Substituted Heterocyclic Systems as Anti-arrhythmic Agents

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Summary. A series of substituted heterocyclic systems were prepared from N^1-[4-(2-thienylmethylene)phenyl]-5-chloro-2-methoxybenzamide, which was prepared from the corresponding 5-chloroanisic acid (2-methoxy-4-chlorobenzoic acid) as starting material. Condensation of the thienylmethylene derivative with guanidine hydrochloride, urea, or thiourea afforded the aminopyrimidine, pyrimidinone, and thioxopyrimidine derivatives. The latter was condensed with chloroacetic acid to yield a thiazolopyrimidine, which was condensed with 2-thiophenealdehyde to yield the arylmethylene derivative, however, it was also prepared directly from thiopyrimidine by the action of chloroacetic acid, 2-thiophenealdehyde, and anhydrous sodium acetate. Treating of the thienylmethylene derivative with phenylhydrazine or hydrazine hydrate in dioxane afforded *N*-phenylpyrazoline and a pyrazoline, which was reacted with acetyl chloride in dioxane affording the *N*-acetyl analogue. The thienylmethylene derivative was reacted with malononitrile or ethyl cyanoacetate in the presence of ammonium acetate to yield the corresponding cyanoaminopyridine and cyanopyrimidone derivatives. Also, it was reacted with hydroxylamine hydrochloride in pyridine to give the oxime derivative, which was cyclized with acetic anhydride. On the other hand, condensation of the thienylmethylene derivative with ethyl cyanoacetate in the presence of sodium ethoxide or cyanothioacetamide gave the cyanopyrane and pyridine thione derivative, which was treated with ethyl chloroacetate affording the ethyl carboxylate derivative. The pharmacological screening showed that many of these compounds have good anti-arrhythmic activity and low toxicity.

Keywords. 5-Chloroanisic acid; 2-Thiophenealdehyde; Thioxopyrimidine; Thiazolopyrimidine; Anti-arrhythmic activity.

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Introduction

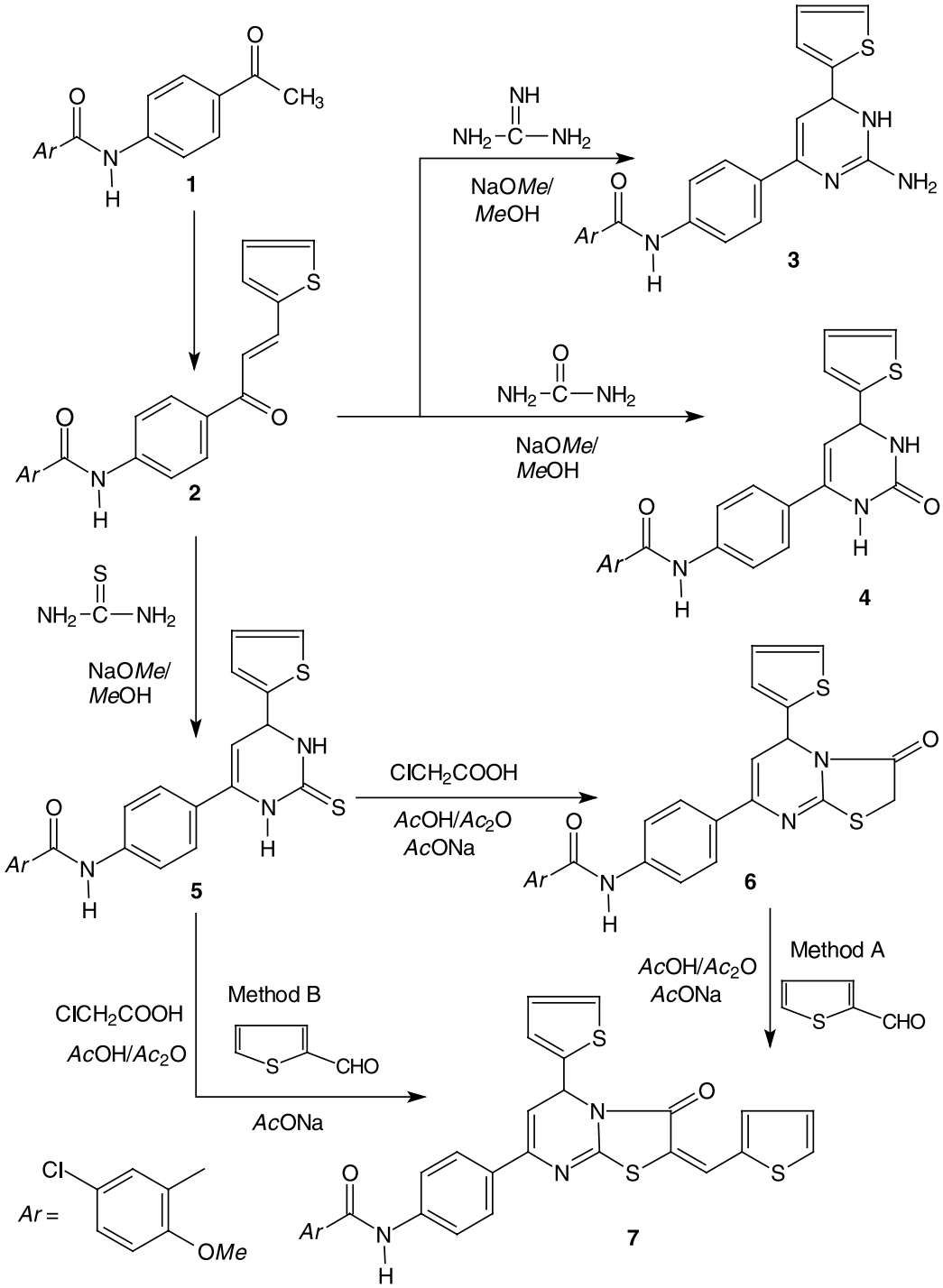
In previous work our group has reported that certain substituted pyridines and their chiral macrocyclic derivatives have antidepressant, antimicrobial, anticancer, analgesic, and anticonvulsant activities [1–7]. It has been reported that substituted heterocyclic derivatives act as anti-inflammatory [8, 9] and anticancer agents [10–12] and they are also used as antimicrobial agents, in particular, their 3-substituted derivatives [13–15]. Recently, some new substituted pyrimidine and thiazolopyrimidine derivatives have been synthesized, which exhibit analgetic, anti-inflammatory, antiparkinsonian, and androgenic-anabolic activities [16, 17]. On the other hand, substituted heterocyclic derivatives showed promising biological activities [18–20]. In view of these observations and in continuation of our previous work in heterocyclic chemistry, we synthesized some new heterocyclic compounds containing pyridone, pyridinethione, pyrazoline, and pyrimidine rings and tested their anti-arrhythmic activities.

Results and Discussion

Synthesis

The N^1-[4-(2-thienylmethylene)phenyl]-5-chloro-2-methoxybenzamide (**2**) was synthesized from treating of N^1-(4-acetylphenyl)-5-chloro-2-methoxybenzamide (**1**) with 2-thiophenealdehyde. Condensation of **2** with diamino reagents, namely, guanidine

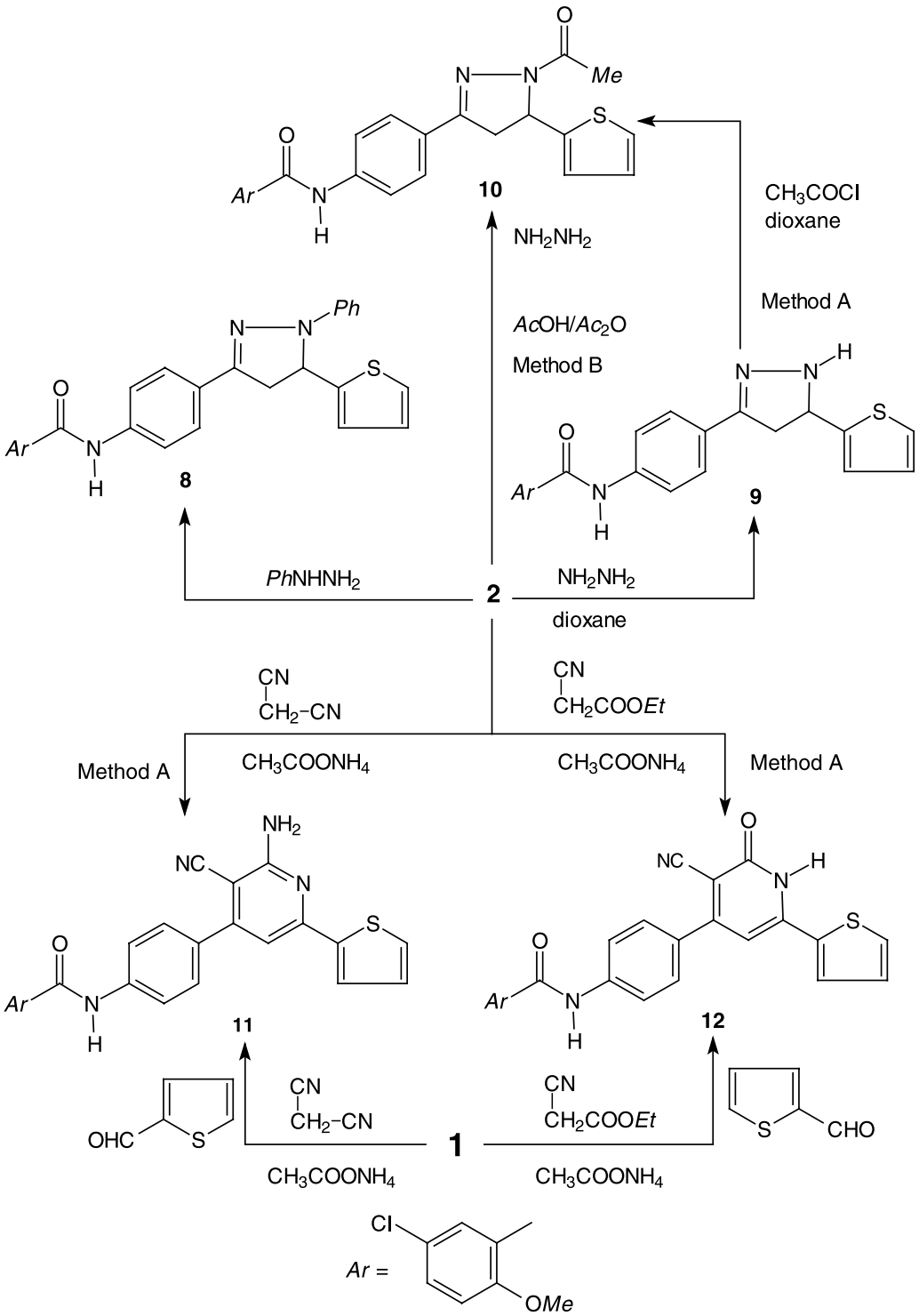
hydrochloride, urea, or thiourea in methanolic sodium methoxide afforded the corresponding aminopyrimidine **3**, pyrimidinone **4**, and thioxopyrimidine **5**. Also, **5** was condensed with chloroacetic acid in a mixture of acetic acid/acetic anhydride in the presence of anhydrous sodium acetate to yield the corresponding thiazolopyrimidine **6**, which was condensed with 2-thiophenealdehyde in the presence of anhydrous sodium acetate and a glacial acetic acid/acetic anhydride mixture to yield arylmethylene derivative **7**. However, the latter compound was also prepared directly from **5** by the action of chloroace-



Scheme 1

tic acid, 2-thiophenealdehyde, and anhydrous sodium acetate in the presence of an acetic acid/acetic anhydride mixture (Scheme 1).

Cyclocondensation of **2** with phenylhydrazine gave *N*-phenylpyrazoline **8**, but treatment with hydrazine hydrate in refluxing dioxane afforded the pyrazoline **9**, which was treated with acetyl chloride in dioxane to yield the 1-acetylpyrazoline **10**, which was also prepared directly from **2** by the action of hydrazine hydrate in the presence of an acetic acid/acetic anhydride



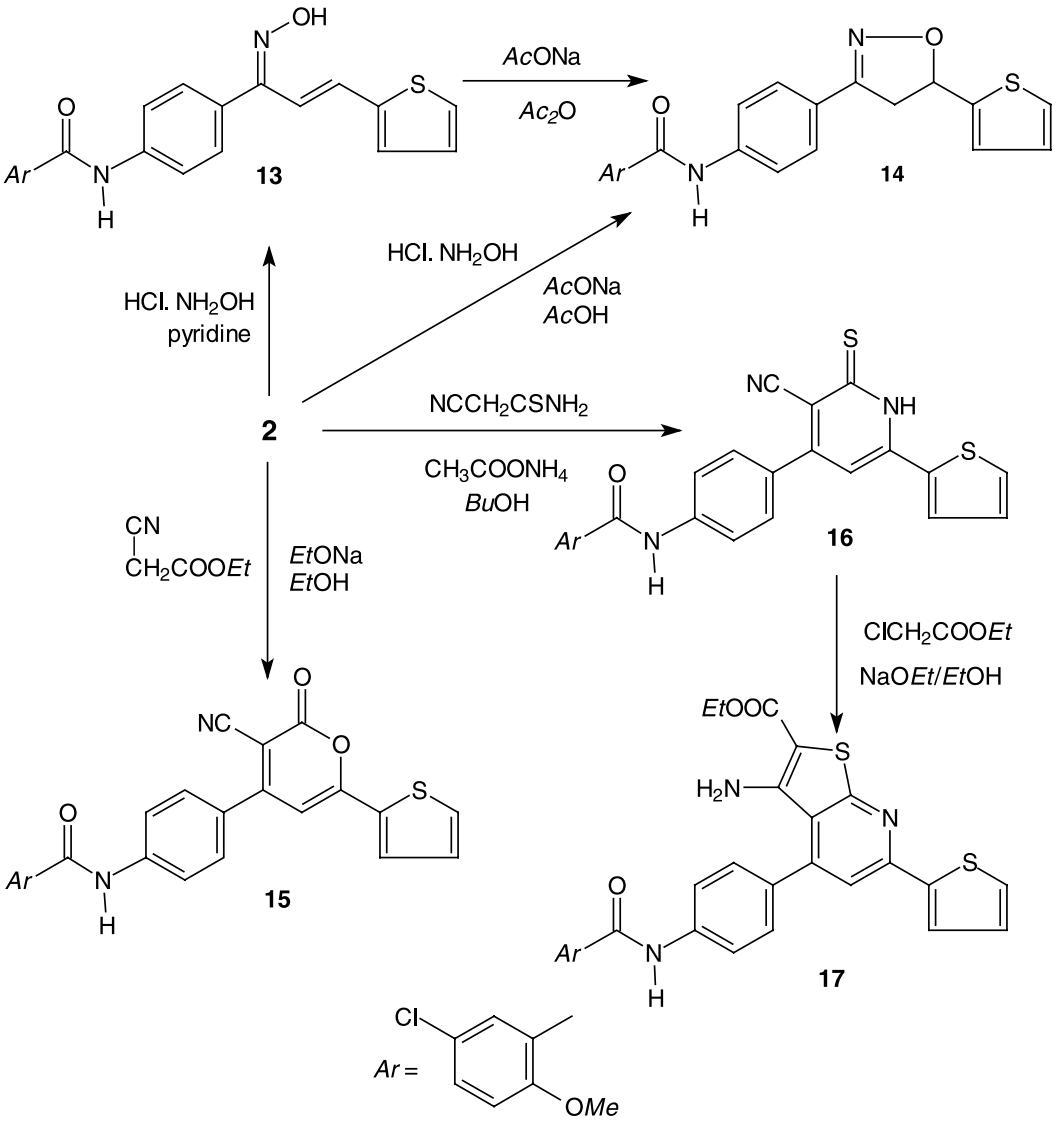
Scheme 2

mixture. While, condensation of **2** with malononitrile or ethyl cyanoacetate in the presence of ammonium acetate in ethanol gave cyanoaminopyridine **11** and cyanopyridone **12**. A one-step synthesis of **11** and **12** could be achieved by condensation of **1** with 2-thiophenealdehyde and malononitrile or ethyl cyanoacetate in the presence of ammonium acetate (Scheme 2).

Condensation of **2** with hydroxylamine hydrochloride in pyridine afforded the corresponding 3-β-(2-thienyl)acryloylpyridine oxime **13**. The latter was cyclized with refluxing acetic anhydride to the oxazole **14**, which was also prepared directly from **2** by reaction with hydroxylamine hydrochloride in the presence of anhydrous sodium acetate in refluxing acetic acid. Condensation of **2** with ethyl cyanoacetate in ethanol in the presence of sodium ethoxide gave cyanopyridone **15**. But, **2** was condensed with cyanothioacetamide in the presence of ammonium acetate in *n*-butanol to yield pyridinethione **16**, which was treated with ethyl chloroacetate in the presence of *Et*ONa to give the ethyl-3-aminothieno[2,3-*b*]pyridine-2-carboxylate (**17**) (Scheme 3).

Pharmacological Screening

Procaine amide, 5 mg/kg i.v. and lidocaine 5 mg/kg i.v. led to an increase in LD_{100} by 65%, which corresponds to a LD_{100} of approximately 9 μg/100 mg. All the tested compounds showed potent activities and the degree of potency in descending order is **10**, **9**, **8**, **14**, **17**, **7**, **6**, (**4** and **14**), (**3**, **5**, and **13**), **16**, **12**, and **11** (*cf.* Table 1).



Scheme 3

Table 1. Anti-arrhythmic activities of the newly synthesized compounds

Compound in (5 mg/kg)	Percentage increase in LD_{100}/%
3	75 ± 0.091
4	76 ± 0.081
5	75 ± 0.071
6	81 ± 0.063
7	83 ± 0.064
8	93 ± 0.071
9	94 ± 0.061
10	95 ± 0.082
11	71 ± 0.093
12	73 ± 0.090
13	75 ± 0.089
14	92 ± 0.091
15	76 ± 0.090
16	74 ± 0.091
17	85 ± 0.099

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

Table 2. Acute toxicity (LD_{50}) of the synthesized compounds

Compound no.	LD_{50} mg/kg
3	341 ± 0.19
4	454 ± 0.18
5	561 ± 0.21
6	741 ± 0.11
7	842 ± 0.13
8	951 ± 0.17
9	743 ± 0.18
10	681 ± 0.11
11	727 ± 0.11
12	751 ± 0.19
13	762 ± 0.19
14	734 ± 0.19
15	772 ± 0.18
16	709 ± 0.17
17	753 ± 0.18

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

Structural Activity Relationship (SAR)

- The five membered ring derivatives (namely pyrazolines and isoxazoles) showed more potent anti-arrhythmic activities than the six membered ring derivatives (namely pyridines, pyrimidines, and pyrans).
- Hetero-aromaticity increases the anti-arrhythmic activity.
- Fused ring systems give anti-arhythmic activity but to a lower extent.

Determination of Acute Toxicity (LD_{50})

The LD_{50} was determined by using rats. They were injected with different increasing doses of the synthesized compounds. The dose that killed 50% of the animals (*cf.* Table 2) was calculated according to *Austen* and *Brocklehurst* [21].

Experimental

All melting points were taken on Electrothermal IA 9000 series digital melting point apparatus. Elemental analytical data (in accord with the calculated values) were obtained from the microanalytical unit, Cairo University, Cairo, Egypt. The IR spectra (KBr) were recorded on a Shimadzu CVT-04 spectrophotometer. The ^{1}H NMR spectra were measured with Jeol 270 MHz in *DMSO*-d_6 or $CDCl_3$. The chemical shifts were recorded in δ (ppm) relative to *TMS*. The mass spectra were obtained using a Varian MAT CH-5 spectrometer (70 eV). All reactions were followed by TLC (silica gel, aluminum sheets 60 F_{254}, Merck).

N^1-[4-(2-Thienylmethylene)phenyl]-5-chloro-2-methoxybenzamide (**2**, $C_{21}H_{16}NO_3ClS$)
A mixture of 0.303 g acetyl derivative **1** (1 mmol), 0.112 g 2-thiophenealdehyde (1 mmol) in 20 cm^3 absolute ethanol, and 0.5 cm^3 piperdine was refluxed for 30 min. The reaction mixture was left overnight at room temperature, the obtained solid was filtered off, and crystallized to give 0.26 g **2** (66%). Mp 258–259°C (*Me*OH); IR (film): $\bar{\nu} = 3336$–3230 (NH), 1692, 1680 (2 CO) cm^{-1}; ^{1}H NMR ($CDCl_3$): $\delta = 3.56$ (s, OCH_3), 6.90 (d, CH-arylidene), 7.12–7.82 (m, *Ar*–H + CH-arylidene), 10.64 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 398$ (M^+, 22) and at 267 (100, base peak).

*Substituted Pyrimidine Derivatives **3**, **4**, and **5***
A solution of 0.398 g **2** (1 mmol), 1.2 mmol diamino reagents, namely, guanidine hydrochloride, urea, or thiourea and ~0.1 g sodium methoxide (1.5 mmol) in 25 cm^3 absolute methanol was refluxed for 2–4 h. The reaction mixture was evaporated to dryness under reduced pressure, dried, and crystallized to give 0.3 g **3** (72%), 0.29 g **4** (65%), and 0.39 g **5** (86%).

N^1-{4-[2-Amino-6-(2-thienyl)-4-pyrimidinyl]phenyl}-5-chloro-2-methoxybenzamide (**3**, $C_{22}H_{19}ClN_4O_2S$)
Mp 198–199°C (*Ac*OH); IR (film): $\bar{\nu} = 3455$–3210 (NH, NH_2), 1694 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$): $\delta = 3.57$ (s, OCH_3), 5.20 (d, Ha-pyrimidine), 5.44 (s, NH_2, exchangeable with D_2O), 6.75–7.94 (m, *Ar*–H + Hb-pyrimidine), 10.44 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 439$ (M^+, 6) and 198 (100, base peak).

N^1-{4-[6-(2-Thienyl)-2-oxo-4-pyrimidinyl]phenyl}-5-chloro-2-methoxybenzamide (**4**, $C_{22}H_{18}ClN_3O_3S$)
Mp 205–207°C (*Ac*OH); IR (film): $\bar{\nu} = 3455$–3215 (NH), 1695 (CO), 1226 (CS) cm^{-1}; ^{1}H NMR ($CDCl_3$): $\delta = 3.55$ (s, OCH_3), 5.19 (d, Ha-pyrimidine), 7.16–8.05 (m, *Ar*–H + Hb-pyrimidine), 8.40 and 8.52 (2s, 2NH, exchangeable with

D_2O), 10.58 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 440$ (M^+, 100, base peak).

N^1-{4-[6-(2-Thienyl)-2-thioxo-4-pyrimidinyl]phenyl}-5-chloro-2-methoxybenzamide (**5**, $C_{22}H_{18}ClN_3O_2S_2$)
Mp 218–220°C (*Ac*OH); IR (film): $\bar{\nu} = 3465$–3218 (NH), 1696 (CO), 1228 (CS) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 3.56$ (s, OCH_3), 5.18 (d, Ha-pyrimidine), 7.05–8.00 (m, *Ar*–H + Hb-pyrimidine), 8.44 and 8.55 (2s, 2NH, exchangeable with D_2O), 10.62 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 456$ (M^+, 100, base peak).

7-{4-[4-(5-Chloro-2-methoxybenzoyl)amino]phenyl}-3-oxo-5-(2-thienyl)-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine (**6**, $C_{24}H_{18}ClN_3O_3S_2$)
A mixture of 0.456 g **5** (1 mmol) and 0.1 g chloroacetic acid (1 mmol) was dissolved in 40 cm^3 of a mixture of *Ac*OH: Ac_2O (1:3) in the presence 1.5 g anhydrous sodium acetate, and was refluxed for 6 h. The reaction mixture was cooled and poured onto cold water with stirring, the formed solid was filtered off, and crystallized to give 0.40 g **6** (82%). Mp 192–194°C (*Et*OH); IR (film): $\bar{\nu} = 3358$–3310 (NH), 1732 (CO), 1692 (CONH) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 3.56$ (s, OCH_3), 3.70 (s, CH_2-thiazole), 5.54 (d, Ha, pyrimidine), 7.22–7.66 (m, *Ar*–H + Hb-pyrimidine), 10.35 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 56.22$ (OCH_3), 164.85 ($CONH_2$), 115.65, 119.7, 121.50, 126.05, 126.7, 128.55, 132.5, 133.21, 135.05, 156.84 (*Ph*-C), 45.65, 114.04, 142.10, 162.75 (pyrimidinyl-C), 123.56, 126.70, 126.90, 139.40 (thionyl-C), 171.45 (CO, thiazole), 30.86 (CH_2, thiazole) ppm; MS (EI, 70 eV): $m/z = 496$ (M^+, 24) and 230 (base peak, 100).

7-{4-[4-(5-Chloro-2-methoxybenzoyl)amino]phenyl}-2-(2-thienylmethylene)-3-oxo-5-(2-thienyl)-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine arylidine (**7**, $C_{29}H_{20}ClN_3O_3S_3$)
Method A: A mixture of 0.456 g **5** (1 mmol), 0.1 g chloroacetic acid (1 mmol), 1.5 g anhydrous sodium acetate in 40 cm^3 of a mixture of *Ac*OH:Ac_2O (1:3) and 0.112 g 2-thiophenealdehyde (1 mmol) was refluxed for 6 h. The reaction mixture was cooled and poured onto ice-water, the obtained solid was collected by filtration, and crystallized to give 0.40 g **7** (68%). Mp 210–212°C (*Ac*OH); IR (film): $\bar{\nu} = 3365$–3315 (NH), 1708 (CO, this shift to lower frequency is due to conjugation with the exocyclic double bond), 1688 (CONH) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 3.55$ (s, OCH_3), 5.54 (d, Ha, pyrimidine), 7.28–7.72 (m, *Ar*–H + Hb-pyrimidine + benzylic proton), 10.22 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 56.08$ (OCH_3), 164.88 ($CONH_2$), 115.62, 119.65, 121.45, 126.10, 126.72, 128.54, 132.56, 133.24, 135.15, 156.80 (*Ph*–C), 45.60, 114.12, 142.15, 162.95 (pyrimidinyl-C), 123.52, 126.46, 126.86, 127.00, 128.10, 130.05, 137.66, 139.35 (thionyl-C), 165.90 (CO, thiazole), 121.66 (CH-arylidine) ppm; MS (EI, 70 eV): $m/z = 590$ (M^+, 100, base peak).

Method B: A mixture of 0.496 g **6** (1 mmol) and 0.112 g 2-thiophenealdehyde (1 mmol) in 40 cm^3 of a mixture of *Ac*OH: Ac_2O (1:3) was refluxed for 5 h, allowed to cool, then poured onto water, the solid formed was collected by filtration, and crystallized to yield 0.44 g **7** (75%), as identified by its mp, mixed m.p., and R_f value on TLC by comparison with an authentic sample from method A.

N^1-{4-[1-Phenyl-5-(2-thienyl)-4,5-dihydro-1H,3-pyrazolyl]-phenyl}-5-chloro-2-methoxybenzamide (**8**, $C_{27}H_{22}ClN_3O_2S$)
A solution of 0.398 g **2** (1 mmol) and 0.108 g phenyl hydrazine (1.5 mmol) in 15 cm^3 absolute ethanol was refluxed for 5 h. The reaction mixture was poured onto ice, the obtained solid was collect by filtration, dried, and crystallized to give 0.41 g **8** (82%). Mp 212°C (*Bu*OH); yield 62%; IR (film): $\bar{\nu} = 3358$–3155 (NH), 1695 (CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta =$ 1.96–2.18 (d, CH_2-pyrazoline), 3.56 (s, OCH_3), 3.92 (m, CH-pyrazoline), 7.10–7.94 (m, *Ar*–H), 10.55 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 488$ (M^+, 100, base peak).

N^1-{4-[5-(2-Thienyl)-4,5-dihydro-1H-3-pyrazolyl]phenyl}-5-chloro-2-methoxybenzamide (**9**, $C_{21}H_{18}ClN_3O_2S$)
A solution of 0.398 g **2** (1 mmol) and 0.4 cm^3 hydrazine hydrate (8 mmol) in 20 cm^3 dioxane was refluxed for 2 h. The solvent was evaporated under reduced pressure, the residue was washed with *n*-hexane, and crystallized to give 0.3 g **9** (72%). Mp 198–200°C (*Et*OH); IR (film): $\bar{\nu} = 3445$–3295 (NH), 1698 (CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 2.12$–2.25 (d, CH_2-pyrazoline), 3.54 (s, OCH_3), 3.85 (m, CH-pyrazoline), 6.94 (s, NH, exchangeable with D_2O), 7.25–7.95 (m, *Ar*–H), 10.54 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 56.18$ (OCH_3), 165.05 ($CONH_2$), 45.70, 62.64, 156.00 (pyrazoline-C), 115.68, 118.57, 122.95, 128.34, 129.36, 131.34, 133.80, 133.92, 139.38, 141.51, 147.92, 148.10 (*Ar*–C) ppm; MS (EI, 70 eV): $m/z = 412$ (M^+, 36) and 314 (100, base peak).

N^1-{4-[1-Acetyl-5-(2-thienyl)-4,5-dihydro-1H-3-pyrazolyl]-phenyl}-5-chloro-2-methoxybenzamide (**10**, $C_{23}H_{20}ClN_3O_3S$)
Method A: A mixture of 0.412 g **9** (1 mmol) and ~0.1 g acetyl chloride (1 mmol) in 30 cm^3 dioxane was stirred at room temperature for 5 h. The reaction mixture was evaporated under reduced pressure, the product was extracted with dichloromethane, washed with aqueous sodium bicarbonate, dried over anhydrous Na_2SO_4, evaporated under reduced pressure, and crystallized to give 0.26 g **10** (58%). Mp 264–266°C (*Et*OH); IR (film): $\bar{\nu} = 3336$–3150 (NH), 1722, 1698 (2 CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 1.82$ (s, CH_3), 2.05–2.21 (m, CH_2-pyrazoline), 3.55 (s, OCH_3), 3.86 (m, CH-pyrazoline), 7.23–7.96 (m, *Ar*–H), 10.42 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 454$ (M^+, 188) and 278 (100, base peak).

Method B: A mixture of 0.398 g **2** (1 mmol) and 0.4 cm^3 hydrazine hydrate (8 mmol) in 40 cm^3 of a mixture of *Ac*OH:Ac_2O (3:1) was refluxed for 3 h, allowed to cool, and then poured onto water. The obtained solid was filtered off and crystallized to give 0.36 g **10** (74%).

N^1-{4-[6-Amino-5-cyano-2-(2-thienyl)-4-pyridyl]phenyl}-5-chloro-2-methoxybenzamide (**11**, $C_{24}H_{17}ClN_4O_2S$)
Method A: A solution of 0.398 g **2** (1 mmol), ~0.1 g malononitril (1.2 mmol), and 0.616 g ammonium acetate (8 mmol) in 25 cm^3 *n*-butanol was refluxed for 3 h. After cooling, the precipitate was filtered off, dried, and crystallized to give 0.4 g **11**

(88%). Mp 186–188°C (acetone/*Me*OH); IR (film): $\bar{\nu}$ = 3462–3254 (NH, NH_2), 2222 (CN), 1698 (CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.54$ (s, OCH_3), 5.46 (s, NH_2, exchangeable with D_2O), 7.12–7.95 (m, *Ar*–H), 10.58 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 56.22$ (OCH_3), 116.15 (CN), 164.70 (CONH), 111.42, 137.40, 145.05, 163.74, 176.05 (pyridine-C), 111.64, 113.08, 117.85, 125.65, 127.44, 129.00, 130.21, 134.15, 141.12, 141.84, 143.18, 154.70 (*Ar*–C) ppm; MS (EI, 70 eV): $m/z = 461$ (M^+, 100, base peak).

Method B: A mixture of 0.303 g **1** (1 mmol), ~0.10 g malononitrile (1 mmol), and 0.616 g ammonium acetate (8 mmol) in 20 cm^3 *n*-butanol was refluxed for 5 h. After cooling, the formed product was collected by filtration, dried, and crystallized to give 0.35 g **11** (75%).

N^1-{4-[3-Cyano-2-oxo-6-(2-thienyl)-1,2-dihydro-4-pyridinyl]-phenyl}-5-chloro-2-methoxy-benzamide (**12**, $C_{24}H_{16}ClN_3O_3S$)
Method A: A solution of 0.398 g **2** (1 mmol), 0.13 cm^3 ethyl cyanoacetate (1.2 mmol), and 0.616 g ammonium acetate (8 mmol) in 20 cm^3 *n*-butanol was refluxed for 2 h. The formed precipitate after cooling was filtered off, dried and crystallized to give 0.36 g **12** (79%). Mp 158–160°C (*Ac*OH); IR (film): $\bar{\nu}$ = 3450–2678 (NH), 2220 (CN), 1694, 1676 (2 CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.56$ (s, OCH_3), 7.16–7.98 (m, *Ar*–H), 8.70 (s, NH exchangeable with D_2O), 10.54 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 462$ (M^+, 22) and 238 (100, base peak).

Method B: A mixture of 0.303 g **1** (1 mmol), ~0.13 g ethyl cyanoacetate malononitrile (1 mmol), and 0.6 g ammonium acetate (8 mmol) in 20 cm^3 *n*-butanol was refluxed for 4 h. After cooling, the formed product was collected by filtration, dried, and crystallized to give 0.35 g **12** (75%).

N^1-{4-[(2-Thienyl)acryloylphenyl] oxime}-5-chloro-2-methoxy-benzamide (**13**, $C_{21}H_{17}ClN_2O_3S$)
A mixture of 0.398 g **2** (1 mmol) and ~0.1 g $NH_2OH \cdot HCl$ (1 mmol) in 30 cm^3 dry pyridine was refluxed for 6 h. The reaction mixture was cooled, poured into ice-water, and neutralized with 1 *N* HCl. The obtained solid was collected by filtration, dried (under vacuum), and crystallized to give 0.26 g (64%) **13**. Mp 236–238°C (*Ac*OH); IR (film): $\bar{\nu}$ = 3536–3265 (OH, NH), 1678 (C=N), 1610 (C=C) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 2.46$ (s, OH, exchangeable with D_2O), 3.56 (s, OCH_3), 6.36 (d, $J = 14.55$ Hz, CH-olefinic), 6.76 (d, $J = 14.60$ Hz, CH-olefinic), 7.14–7.98 (m, *Ar*–H), 10.55 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z = 413 (M^+, 100, base peak).

N^1-{4-[5-(2-Thienyl)oxazolyl]phenyl}-5-chloro-2-methoxy-benzamide (**14**, $C_{21}H_{17}ClN_2O_3S$)
Method A: A solution of 0.413 g **13** (1 mmol) in 50 cm^3 acetic anhydride was refluxed for 10 h. After cooling, the reaction mixture was poured into ice-water, the obtained solid was filtered off, washed with water, dried (under vacuum), and crystallized to give 0.27 g (65%) **14**. Mp 211–213°C (*Et*OH); IR (KBr): $\bar{\nu}$ = 3348–3235 (NH), 1666 (C=N), 1608–1600 (C=C) cm^{-1}; ^1H NMR (*DMSO*-d_6): δ = 1.6–1.9 (m, CH_2-oxazole), 3.54 (s, OCH_3), 4.2 (m, CH-oxazole), 7.10–7.96 (m, *Ar*–H), 10.57 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 413$ (M^+, 100, base peak).

Method B: A mixture of 0.398 g **2** (1 mmol), ~0.1 g $NH_2OH \cdot HCl$ (1 mmol), and 0.082 g anhydrous sodium acetate (1 mmol) in 30 cm^3 glacial acetic acid was refluxed for 6 h. The reaction mixture was cooled, poured into ice-water, the obtained solid was collected by filtration, washed with water, dried (under vacuum), and crystallized to give 0.24 g (58%) **14** as identified by mp and TLC in comparison with an authentic sample.

N^1-{4-[3-Cyano-2-oxo-6-(2-thienyl)-2H-4-pyranyl]phenyl}-5-chloro-2-methoxybenzamide (**15**, $C_{24}H_{15}ClN_2O_4S$)
A solution of 0.398 g **2** (1 mmol), 0.13 cm^3 ethyl cyanoacetate (1.2 mmol), and 68 mg sodium ethoxide (1 mmol) in 20 cm^3 absolute ethanol was refluxed for 2 h. The reaction mixture was evaporated under reduced pressure, the residue was solidified with *n*-hexane, the obtained solid was filtered off, and crystallized to give 0.36 g **15** (77%). Mp 276–278°C (*Me*OH); IR (film): $\bar{\nu}$ = 3425–3250 (NH), 2226 (CN), 1718, 1697 (2 CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.54$ (s, OCH_3), 7.05 (s, pyrane-H), 7.08–7.95 (m, *Ar*–H), 10.49 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 56.36$ (OCH_3), 117.84 (CN), 163.72 (CONH), 106.05, 131.10, 145.15, 154.72, 157.76 (pyrane-C), 114.70, 116.46, 117.78, 128.25, 129.45, 129.80, 133.88, 134.10, 139.88, 144.92, 144.96, 153.15 (*Ar*–C) ppm; MS (EI, 70 eV): $m/z = 463$ (M^+, 46) and 352 (100, base peak).

N^1-{4-[3-Cyano-2-thioxo-6-(2-thienyl)-1,2-dihydro-4-pyridinyl]phenyl}-5-chloro-2-methoxy-benzamide (**16**, $C_{24}H_{16}ClN_3O_2S_2$)
A solution of 0.398 g **2** (1 mmol), 0.12 g cyanothioacetamide (1.2 mmol), and 0.616 g ammonium acetate (8 mmol) in 25 cm^3 *n*-butanol was refluxed for 3 h. After cooling the precipitated was filtered off, dried, and crystallized to give 0.36 g **16** (76%). Mp 245–247°C (*Et*OH); IR (film): $\bar{\nu}$ = 3455–3258 (NH), 2222 (CN), 1695 (CO), 1240 (CS) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.55$ (s, OCH_3); 4.54 (s, CSNH, exchangeable with D_2O), 7.15–8.10 (m, *Ar*–H), 10.61 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 56.18$ (OCH_3), 117.76 (CN), 164.90 (CONH), 112.88, 138.94, 145.37, 163.76, 171.54 (pyridine-C), 112.92, 118.12, 126.84, 127.79, 129.72, 129.89, 134.10, 134.20, 140.91, 141.21, 145.38, 153.12 (*Ar*–C) ppm; MS (EI, 70 eV): $m/z = 478$ (M^+, 16) and 230 (100, base peak).

Ethyl 3-amino-4-{4-[(5-chloro-2-methoxybenzoyl)amino]-phenyl}-6-(2-thienyl)thieno[2,3-b]-pyridine-2-carboxylate (**17**, $C_{28}H_{22}N_3O_4ClS_2$)
A solution of 0.478 g **16** (1 mmol), 0.122 g ethyl chloroacetate (1 mmol), and 0.68 g sodium ethoxide (10 mmol) in 10 cm^3 ethanol was refluxed for 4 h. The reaction mixture was evaporated under reduced pressure, the obtained solid was filtered off, dried, and crystallized to give 0.33 g **17** (58%). Mp 296–298°C (*Me*OH); IR (film): $\bar{\nu}$ = 3492–3268 (NH, NH_2), 1732, 1696 (2 CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 1.13$ (t, CH_3), 3.57

(s, OCH_3), 4.52 (q, OCH_2), 5.73 (s, NH_2, exchangeable with D_2O), 7.10–7.98 (m, *Ar*–H), 10.62 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 564$ (M^+, 25) and 528 (100, base peak).

Pharmacological Assay

Anti-arrhythmic Activity [22–27]

Animals obtained from the animal house colony of the National Research Center, Cairo, Egypt. All animals were allowed free access to water and kept on a constant standard diet.

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

Nineteen groups of male Ivanovas rats weighing 300–350 g are used, each group of 12 animals. The first group represents the control group and received no drug, while the second received aconitine and groups 3–17 received the tested newly synthesized agents, group 18 received the standard reference drug procaine amide, while group 19 received the second standard reference drug lidocaine.

The animals are anesthetized by intra peritoneal injection of 1.25 g/kg urethane: 5 mg/kg aconitine dissolved in 0.1 *N* nitric acid is adminstered by continuous infusion into the saphenous vein of 0.1 cm^3/min and the ECG in lead II is recorded every 30 sec. The test compound is injected i.v. at a screening dose of 3 mg/kg 5 min before the start of the aconitine infusion, 24 animals are used per compound.

Evaluation

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

Duration of infusion which induces, ventricular extra systoles, ventricular tachycardia, and ventricular fibrillation. Higher doses of aconitine in the treated group as compared to an untreated control group are an indication of anti-arrhythmic activity. The statistical significance between the groups is assessed by the Student's T-test.

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