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Synthesis, Reactions, and Anti-arrhythmic activity of Substituted Heterocyclic Systems Using 5-Chloroanisic Acid as Starting Material

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Summary. A series of substituted heterocyclic systems were prepared from *N*1-[4-(4-fluorocinnamoyl)phenyl]-5-chloro-2-methoxybenzamide, which was prepared from the corresponding 5-chloroanisic acid (2-methoxy-4-chlorobenzoic acid) as starting material. Treating of the cinnamoyl derivative with hydrazine hydrate in dioxane afforded a pyrazoline, which was reacted with morpholine and paraformaldehyde to give the *N*-substituted pyrazoline. Acylation of pyrazoline with acetyl chloride in dioxane afforded the *N*-acetyl analogue. Also, the cinamoyl derivative was reacted with methylhydrazine, phenylhydrazine, or ethyl cyanoacetate to yield the corresponding *N*-methyl-, *N*-phenylpyrazoline, pyrane, and pyridone derivatives. Condensation of the cinnamoyl derivative with cyanothioacetamide gave the pyridinethione derivative, which was treated with ethyl chloroacetate affording the ethyl carboxylate derivative. Also, it was reacted with malononitrile or ethyl acetoacetae to give the cyano amino analougues and ethyl carboxylate, which was reacted with methylhydrazine to give the (indazolyl)phenyl derivative. On the other hand, reaction of cinnamoyl derivative with acetyl acetone afforded the cyclohexenyl derivative, which was reacted with hydrazine hydrate to give the [methylindazolyl]phenyl derivative. Condensation of the cinnamoyl derivative with guanidine hydrochloride or thiourea afforded the aminopyrimidine derivative and thioxopyrimidine. 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. The pharmacological screening showed that many of these compounds have good anti-arrhythmic activity and low toxicity.

Keywords. 5-Chloroanisic acid; 4-Fluorobenzaldehyde; Thioxopyrimidine; Thiazolopyrimidine; Anti-arrhythmic activity.

Introduction

In previous work we have reported on the substituted heterocyclic derivatives as analgesic, anticonvulsant, and antiandrogenic agents [1–4] and demonstrated their antimicrobial activity [5–8]. On the other hand, cyanopyridone and cyanopyridine derivatives are promising antimicrobial agents [9, 10] and display anticancer activities [11–15]. Some of the chiral heterocyclic compounds containing a pyridine moiety have been reported as anticancer and anti-inflammatory agents [16, 17]. In addition, the heterocyclic nitrogen derivatives exhibited a general ionophoric potency for divalent cations [18] and are used as novel thiocyanate-selective membrane sensors [19]. Recently, we reported that certain substituted heterocyclic compounds exhibited antiparkinsonian, anti-inflammatory, antimicrobial, and anticonvulsant activities [20–24]. In

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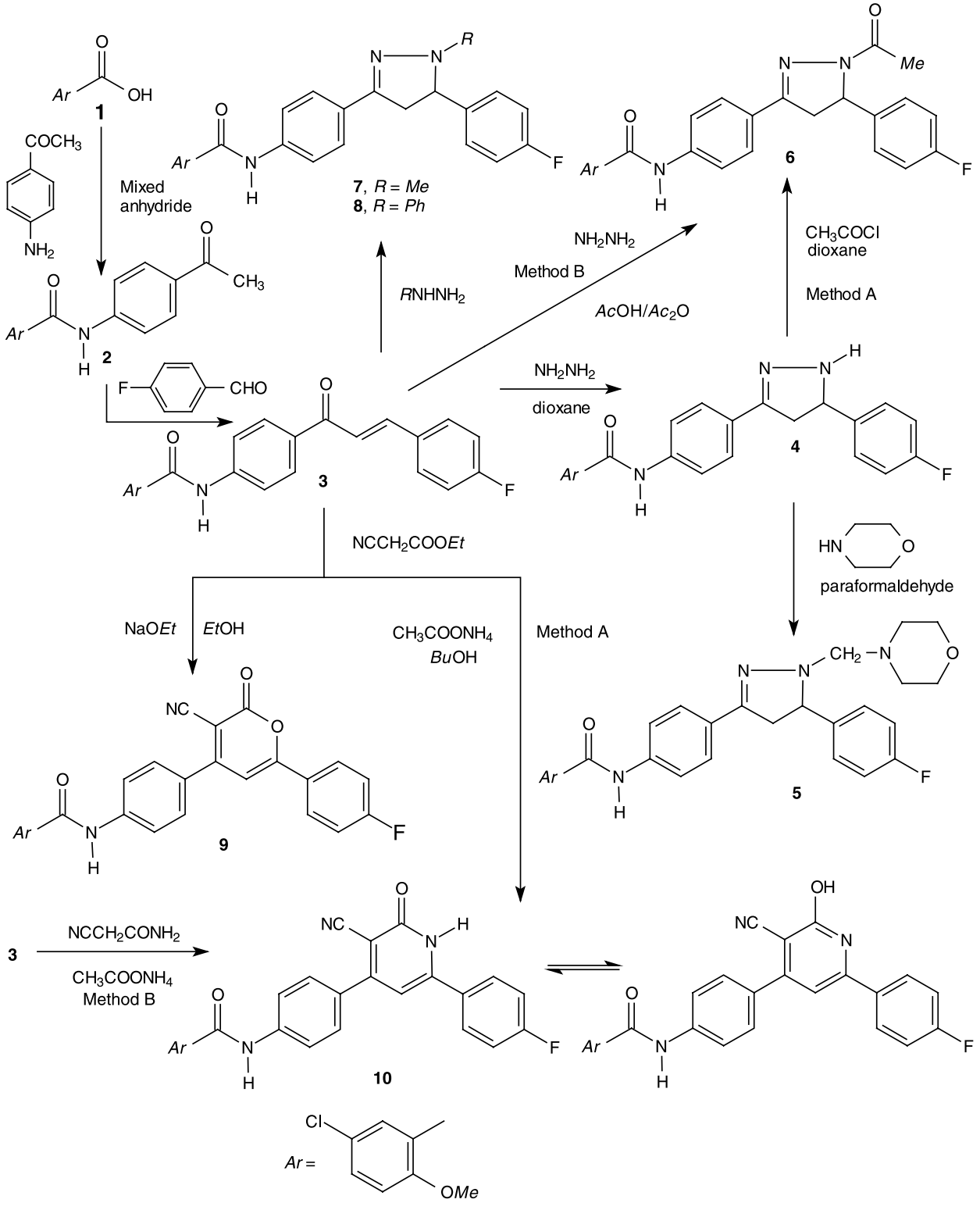
view of these observations and in continuation of our previous work in heterocyclic chemistry, we synthesized some new heterocyclic compounds containing pyridone, pyridinethione, pyrazoline, and pyrimidine rings and tested their anti-arrhythmic activities.

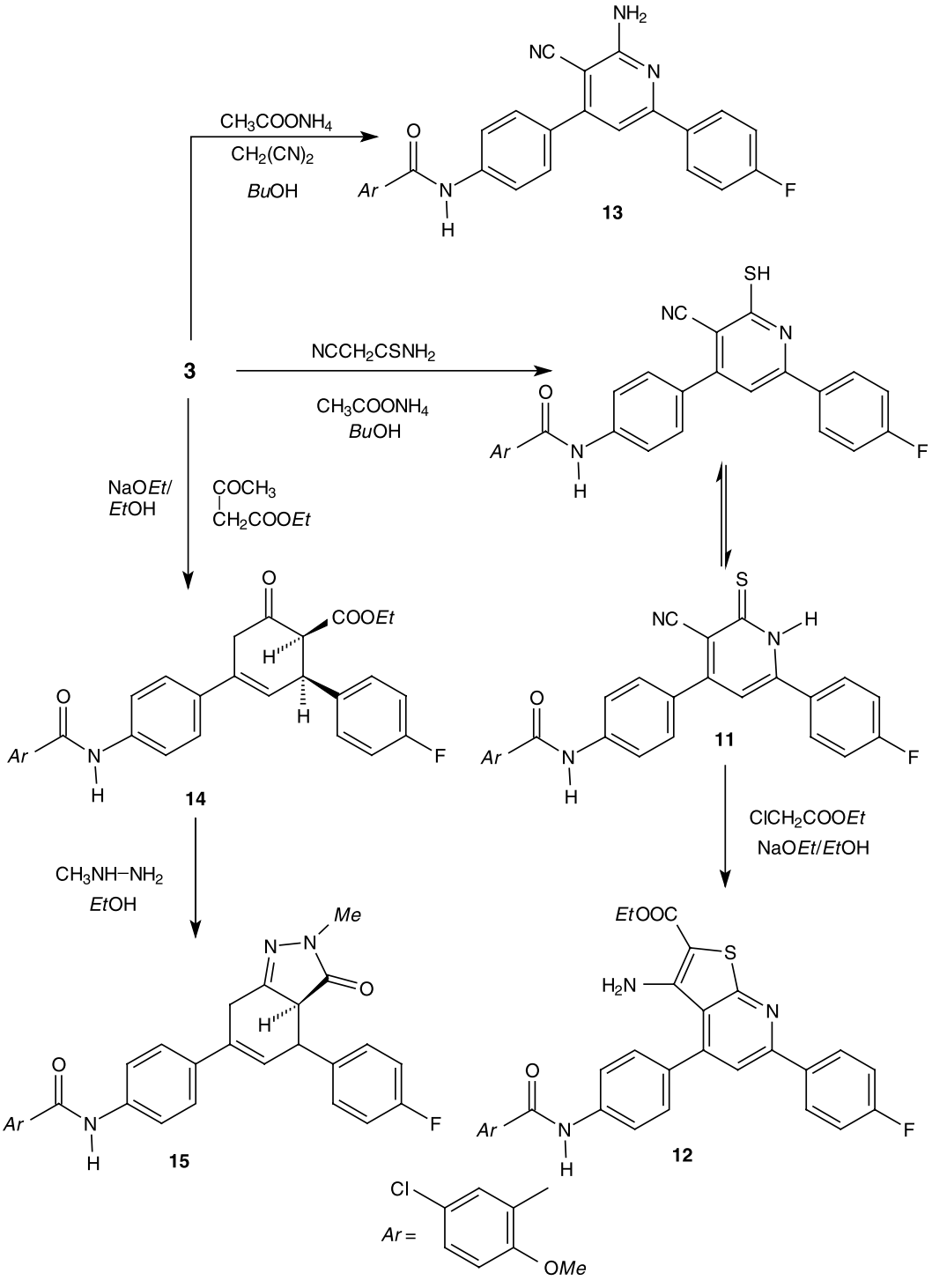


Scheme 1

Results and Discussion

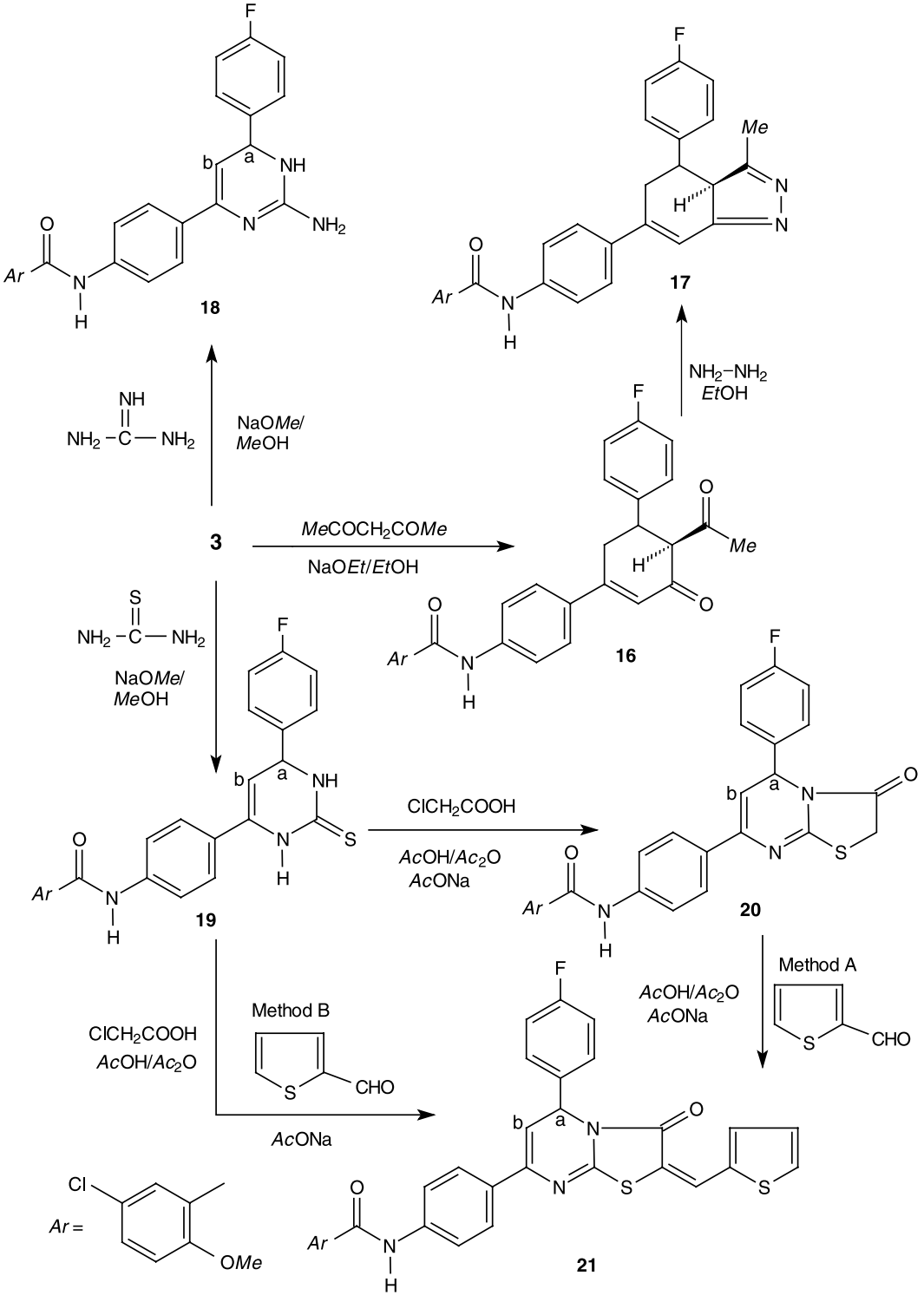
Synthesis

The starting material **3** was synthesized from 5-chloroanisic acid (**1**) and *p*-aminoacetophenone according to the mixed anhydride technique [16] providing **2**, followed by treating with 4-fluorobenzaldehyde in ethanolic piperidine to afford *N*1-[4-(4-fluorocinnamoyl)phenyl]-5-chloro-2-methoxybenzamide **3**. Cyclocondensation of **3** with hydrazine hydrate in



Scheme 2

refluxing dioxane afforded the pyrazoline **4**, which was treated with morpholine in the presence of paraformaldehyde to give the *N*-morpholinomethylpyrazoline **5**. But **4** was reacted with acetyl chloride in dioxane to yield the 1-acetylpyrazoline **6**, which was also prepared directly from **3** by the action of



Scheme 3

hydrazine hydrate in the presence of an acetic acid/acetic anhydride mixture. Also, **3** was treated with methylhydrazine or phenylhydrazine to yield *N*-methylpyrazoline **7** and *N*-phenylpyrazoline **8**. Condensation of **3** with ethyl cyanoacetate in the presence of sodium ethoxide in ethanol gave cyanopyrane **9**, but in the presence of ammonium acetate in *n*-butanol afforded cyanopyridone **10**, which was prepared also from **3** with cyanoacetamide under the same conditions (Scheme 1).

Condensation of **3** with cyanothioacetamide in the presence of ammonium acetate in *n*-butanol yielded pyridinethione **11**, which was treated with ethyl chloroacetate in the presence of *Et*ONa to give ethyl-3-aminothieno[2,3-*b*]pyridine-2-carboxylate derivative **12**. But, **3** was condensed with malononitrile in refluxing *n*-butanol in the presence of ammonium acetate, which gave the cyanoaminopyridine **13**. In addition, **3** was reacted with ethyl acetoacetae in the presence of *Et*ONa to yield ethyl 3-cyclohexene-1-carboxylate **14**, which was then reacted with methylhydrazine in refluxing ethanol to afford the *N*-methylpyrazolone **15** (Scheme 2).

On the other hand, reaction of **3** with acetylacetone in the presence of *Et*ONa afforded 4-acetyl-3-oxo-1-cyclohexene **16**, which was treated with hydrazine hydrate in ethanol to give the 3-methylpyrazoline derivative **17**. Condensation of **3** with diamino reagents, namely, guanidine hydrochloride or thiourea in methanolic *Me*ONa afforded the corresponding aminopyrimidine **18** and thioxopyrimidine **19**. Also, **19** 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 **20**, 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 **21**. However, the latter compound was also prepared directly from **19** by the action of chloroacetic acid, 2-thiophenealdehyde, and anhydrous sodium acetate in the presence of acetic acid/acetic anhydride mixture (Scheme 3).

Pharmacological Screening

Procaine amide, 5 mg/kg iv, and lidocaine, 5 mg/kg iv, led to an increase in LD_{100} by 65%, which corresponds to a LD_{100} of approximately 9 μg/100 mg.

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

Compound in (5 mg/kg)	Percentage increase in LD_{100}/%
3	76 ± 0.081
4	77 ± 0.092
5	no effect
6	48 ± 0.046
7	65 ± 0.063
8	67 ± 0.066
9	72 ± 0.072
10	54 ± 0.050
11	no effect
12	77 ± 0.077
13	no effect
14	79 ± 0.071
15	no effect
16	47 ± 0.041
17	37 ± 0.051
18	75 ± 0.081
19	86 ± 0.091
20	45 ± 0.051
21	78 ± 0.099

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

From Table 1, compounds **5**, **11**, **13**, and **15** showed no anti-arrhythmic activities but compounds **7** and **8** displayed nearly equal anti-arrhythmic activities as procaine amide and lidocaine. Compounds **3**, **4**, **9**, **10**, **12**, **14**, **19**, **21**, and **18** are more active than procaine amide and lidocaine, they arranged in descending manner. Also, **6**, **10**, **16**, **20**, and **17** showed anti-arrhythmic activities less than procaine and lidocaine, they arranged in descending manner.

Structural Activity Relationship (SAR)

- The benzylidene 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-arrythmic 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 was calculated according to *Austen* and *Brocklehurst* [25].

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

Compound no.	LD_{50}/mg kg^{-1}
3	242.52 ± 0.27
4	297.76 ± 0.25
5	294.33 ± 0.15
6	298.85 ± 0.19
7	238.87 ± 0.17
8	298.67 ± 0.22
9	317.99 ± 0.11
10	387.49 ± 0.19
11	309.29 ± 0.18
12	309.99 ± 0.22
13	374.33 ± 0.27
14	274.37 ± 0.29
15	285.89 ± 0.23
16	242.29 ± 0.29
17	338.44 ± 0.18
18	226.62 ± 0.19
19	278.22 ± 0.27
20	276.01 ± 0.19
21	298.22 ± 0.12

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

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 recorded at 270 MHz on a Varian EM-360 spectrometer using *TMS* as an internal standard. The mass spectra were performed using a Varian MAT CH-5 spectrometer (70 eV). All reactions were followed by TLC (silica gel, aluminum sheets 60 F_{254}, Merck).

N1-[4-(4-Fluorocinnamoyl)phenyl]-5-chloro-2-methoxybenzamide (**3**, $C_{23}H_{17}FClNO_3$)
A mixture of 0.303 g acetyl derivative **2** (1 mmol), 0.124 g 4-fluorobenzaldehyde (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 0.35 g **3** (87%). Mp 268–269°C (*Et*OH); IR (film): $\bar{\nu}$ = 3340–3240 (NH), 1698, 1679 (2CO) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 3.57 (s, OCH_3), 6.91 (d, CH-arylidene), 7.23–7.78 (m, *Ar*–H + CH-arylidene), 10.66 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): δ = 57.10 (OCH_3), 164.98, 188.96 (2C=O), 129.10, 155.20 (C=C), 133.79, 127.90, 134.60, 128.70, 115.22, 159.50, 139.90, 117.40, 129.10, 142.70, 136.70, 130.0, 124.18, 148.90 (*Ar*–C) ppm; MS (EI, 70 eV): m/z (%) = 410 [M$^+$, 12] and at 268 (100, base peak).

N1-{4-[5-(4-Fluorophenyl)-4,5-dihydro-1H-3-pyrazolyl]phenyl}-5-chloro-2-methoxybenzamide (**4**, $C_{23}H_{19}FClN_3O_2$)
A solution of 0.41 g **3** (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 **4** (71%). Mp 201–202°C (*Me*OH); IR (film): $\bar{\nu}$ = 3435–3285 (NH), 1698 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 2.15–2.24 (d, CH_2-pyrazoline), 3.52 (s, OCH_3), 3.86 (m, CH-pyrazoline), 6.95 (s, NH, exchangeable with D_2O), 7.28–7.94 (m, *Ar*–H), 10.55 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): δ = 56.19 (OCH_3), 165.10 ($CONH_2$), 45.71, 62.65, 156.10 (pyrazoline-C), 115.70, 118.60, 123.0, 128.40, 128.90, 129.30, 131.30, 133.81, 133.90, 139.40, 141.50, 147.90, 148.01, 161.65 (*Ar*–C) ppm; MS (EI, 70 eV): m/z (%) = 424 [M$^+$, 16] and at 328 (100, base peak).

N1-{4-[N-Morpholinomethyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-3-pyrazolyl]phenyl}-5-chloro-2-methoxybenzamide (**5**, $C_{28}H_{28}FClN_4O_3$)
A mixture of 0.424 g **4** (1 mmol), ~0.1 g morpholine (1 mmol), and 0.2 g paraformaldehyde in 30 cm^3 absolute ethanol was refluxed for 2 h. The reaction mixture was evaporated under reduced pressure, dried, and crystallized to give 0.29 g **5** (56%). Mp 118–120°C (*Me*OH); IR (film): $\bar{\nu}$ = 3386–3156 (NH), 1692 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 2.05–2.20 (d, CH_2-pyrazoline), 2.40–2.45 (m, CH_2-morpholine), 3.31–3.35 (m, CH_2-morpholine), 3.56 (s, OCH_3), 3.81 (s, N–CH_2–N), 3.88 (m, CH-pyrazoline), 7.43–8.17 (m, *Ar*–H), 10.66 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z (%) = 523 [M$^+$, 22] and at 119 (100, base peak).

N1-{4-[1-Acetyl-5-(4-fluorophenyl)-4,5-dihydro-1H-3-pyrazolyl]phenyl}-5-chloro-2-methoxybenzamide (**6**, $C_{25}H_{21}FClN_3O_3$)
Method A: A mixture of 0.424 g **4** (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 $MgSO_4$, evaporated under reduced pressure, and crystallized to give 0.37 g **6** (80%). Mp 266–268°C (*Me*OH); IR (film): $\bar{\nu}$ = 3320–3100 (NH), 1718, 1694 (2CO) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 1.85 (s, CH_3), 2.10–2.18 (m, CH_2-pyrazoline), 3.53 (s, OCH_3), 3.85 (m, CH-pyrazoline), 7.26–7.98 (m, *Ar*–H), 10.46 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z (%) = 466 [M$^+$, 8] and at 280 (100, base peak).

Method B: A mixture of 0.41 g **3** (1 mmol) and 0.4 cm^3 hydrazine hydrate (8 mmol) in 40 cm^3 *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 0.34 g **6** (72%).

*N1-{4-[1-Substituted-5-(4-fluorophenyl)-4,5-dihydro-1H-3-pyrazolyl]phenyl}-5-chloro-2-methoxybenzamide **7** and **8***
General procedure: A solution of 0.41 g **3** (1 mmol) and methylhydrazine or phenylhydrazine (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 0.25 g **7** (58%) and 0.31 g **8** (62%).

N1-{4-[1-Methyl-5-(4-fluorophenyl)-4,5-dihydro-1H-3-pyrazolyl]phenyl}-5-chloro-2-methoxybenzamide
(**7**, $C_{24}H_{21}FClN_3O_2$)
Mp 273–275°C (*Me*OH); IR (film): $\bar{\nu} = 3360–3105$ (NH), 1699 (CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 2.10–2.25$ (d, CH_2-pyrazoline), 2.57 (s, N–CH_3), 3.54 (s, OCH_3), 3.87 (m, CH-pyrazoline), 7.13–7.96 (m, *Ar*–H), 10.60 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 36.60$ (N–CH_3), 55.20 (OCH_3), 164.91 (CONH), 43.20, 67.69, 155.10 (pyrazoline-C), 115.24, 117.10, 118.90, 120.14, 127.90, 128.40, 128.80, 129.59, 131.09, 133.70, 135.10, 139.10, 139.70, 158.10 (*Ar*–C) ppm; MS (EI, 70 eV): m/z (%) = 438 [M^+, 16] and at 186 (100, base peak).

N1-{4-[1-Phenyl-5-(4-fluorophenyl)-4,5-dihydro-1H-3-pyrazolyl]phenyl}-5-chloro-2-methoxybenzamide
(**8**, $C_{29}H_{23}FClN_3O_2$)
Mp 218°C (*Me*OH); IR (film): $\bar{\nu} = 3350–3145$ (NH), 1692 (CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 1.99–2.16$ (d, CH_2-pyrazoline), 3.56 (s, OCH_3), 3.91 (m, CH-pyrazoline), 7.10–7.98 (m, *Ar*–H), 10.58 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z (%) = 500 [M^+, 100, base peak].

N1-{4-[3-Cyano-2-oxo-6-(4-fluorophenyl)-2H-4-pyranyl]-phenyl}-5-chloro-2-methoxybenzamide (**9**, $C_{26}H_{16}ClFN_2O_4$)
A solution of 0.41 g **3** (1 mmol), 0.13 cm^3 ethyl cyanoacetate (1.2 mmol), and 68 mg of 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 0.32 g **9** (67%). Mp 267–269°C (*Me*OH/toluene); IR (film): $\bar{\nu} = 3405–2900$ (NH), 2226 (CN), 1715, 1698 (2CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.53$ (s, OCH_3), 7.01 (s, pyrane-H), 7.12–7.96 (m, *Ar*–H), 10.58 (s, NH, exchangeable with D_2O) ppm; ^{13}C-NMR ($CDCl_3$): $\delta = 56.38$ (OCH_3), 117.88 (CN), 163.78 (CONH), 106.08, 131.08, 145.10, 154.70, 157.78 (pyrane-C), 114.73, 116.50, 117.88, 128.15, 129.47, 129.84, 130.25, 133.90, 134.09, 139.90, 144.91, 144.98, 153.17, 166.60 (*Ar*–C) ppm; MS (EI, 70 eV): m/z (%) = 475 [M^+, 32] and at 351 (100, base peak).

N1-{4-[3-Cyano-2-oxo-6-(4-fluorophenyl)-1,2-dihydro-4-pyridinyl]phenyl}-5-chloro-2-methoxybenzamide
(**10**, $C_{26}H_{17}FClN_3O_3$)
Method A: A solution of 0.41 g **3** (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 0.32 g **10** (68%). Mp 258–260°C (*Et*OH); IR (film): $\bar{\nu} = 3452–2685$ (NH), 2218 (CN), 1696, 1678 (2CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.55$ (s, OCH_3), 7.22–8.12 (m, *Ar*–H + CH-pyridine), 8.72 (s, NH exchangeable with D_2O), 10.55 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z (%) = 474 [M^+, 22] and at 238 (100, base peak).

Method B: A solution of 0.41 g **3** (1 mmol), 0.1 g cyanoacetamide (1.2 mmol), and 0.616 g ammonium acetate (8 mmol) in 20 cm^3 *n*-butanol was refluxed for 2 h. After cooling, the solid formed was filtered off, dried, and crystallized to give 0.34 g **10** (72%).

N1-{4-[3-Cyano-2-thioxo-6-(4-fluorophenyl)-1,2-dihydro-4-pyridinyl]phenyl}-5-chloro-2-methoxybenzamide
(**11**, $C_{26}H_{17}ClFN_3O_2S$)
A solution of 0.41 g **3** (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 0.45 g **11** (92%). Mp 148–150°C (toluene); IR (film): $\bar{\nu} = 3460–3250$ (NH), 2219 (CN), 1697 (CO), 1230 (CS) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.59$ (s, OCH_3), 4.56 (s, CSNH, exchangeable with D_2O), 7.15–8.11 (m, *Ar*–H + CH-pyridinethione), 10.64 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 56.19$ (OCH_3), 117.80 (CN), 164.92 (CONH), 112.86, 138.90, 145.36, 163.75, 171.50 (pyridine-C), 112.82, 112.90, 118.10, 126.80, 127.77, 129.70, 129.87, 134.09, 134.18, 140.90, 141.20, 145.36, 153.10, 163.75 (*Ar*–C) ppm; MS (EI, 70 eV): m/z (%) = 490 [M^+, 65] and at 229 (100, base peak).

Ethyl 3-amino-4-{4-[(5-chloro-2-methoxybenzoyl)amino]-phenyl}-6-(4-fluorophenyl)thieno[2,3-b]pyridine-2-carboxylate (**12**, $C_{30}H_{23}FClN_3O_4S$)
A solution of 0.5 g **11** (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 0.4 g **12** (70%). Mp 301–303°C (*Me*OH/methylacetate); IR (film): $\bar{\nu} = 3490–3265$ (NH, NH_2), 1735, 1693 (2CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 1.10$ (t, CH_3), 3.62 (s, OCH_3), 4.51 (q, OCH_2), 5.75 (s, NH_2, exchangeable with D_2O), 7.12–7.97 (m, *Ar*–H + CH-pyridine), 10.66 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z (%) = 576 [M^+, 15] and at 530 (100, base peak).

N1-{4-[6-Amino-5-cyano-2-(4-fluorophenyl)-4-pyridyl)-phenyl]}-5-chloro-2-methoxybenzamide (**13**, $C_{26}H_{18}ClN_4O_2$)
A solution of 0.41 g **3** (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 0.34 g **13** (72%). Mp 228–230°C (acetone/*Me*OH); IR (film): $\bar{\nu} = 3460–3250$ (NH, NH_2), 2223 (CN), 1694 (CO) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.51$ (s, OCH_3), 5.45 (s, NH_2, exchangeable with D_2O), 6.96–7.97 (m, *Ar*–H + CH-pyridine), 10.64 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 56.19$ (OCH_3), 116.10 (CN), 164.75 (CONH), 111.50, 137.43, 145.10, 163.80, 176.11 (pyridine-C), 111.68, 113.10, 117.90, 125.60, 127.55, 129.10, 130.25, 134.10, 134.17, 141.10, 141.80, 143.20, 154.74, 163.88 (*Ar*–C) ppm; MS (EI, 70 eV): m/z (%) = 473 [M^+, 100, base peak].

Ethyl 4-{4-[(5-chloro-2-methoxybenzoyl)amino]phenyl}-2-oxo-6(S)-(4-fluorophenyl)-3-cyclohexene-1(S)-carboxylate (**14**, $C_{29}H_{25}FClNO_5$)

A solution of 0.41 g **3** (1 mmol), 0.15 cm^3 ethyl acetoacetate (1.2 mmol), and ~0.1 g sodium ethoxide (1.5 mmol) in 25 cm^3 absolute ethanol was refluxed for 3 h. The reaction mixture was evaporated under reduced pressure, the residue was crystallized to give 0.48 g **14** (92%). Mp 164–166°C (*Et*OH); IR (film): $\bar{\nu}$ = 33100–2980 (NH), 1728, 1718, 1697 (3CO) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 1.05 (t, CH_3), 2.49 (m, CH_2-cyclohexene), 3.52 (s, OCH_3), 3.78 (d, CH-cyclohexene), 3.88 (m, CH-cyclohexene), 4.18 (m, OCH_2), 6.57 (s, CH-ene), 7.10–7.96 (m, *Ar*–H), 10.72 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z (%) = 522 [M^+, 7] and at 279 (100, base peak).

N1-[4-(3-Oxo-4-(4-fluorophenyl)-3,3a(S),4(S),5-tetrahydro-2-methyl-2H-6-indazolyl)phenyl]-5-chloro-2-methoxybenzamide (**15**, $C_{28}H_{23}FClN_3O_3$)

A solution of 0.522 g **14** (1 mmol) and 0.46 g methylhydrazine (1 mmol) in 10 cm^3 absolute ethanol was refluxed for 1.5 h. The reaction mixture was left overnight at room temperature, the obtained solid was filtered off and crystallized to give 0.24 g **15** (48%). Mp 154–156°C (*Et*OH); IR (film): $\bar{\nu}$ = 3375–3205 (NH), 1715, 1694 (2CO) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 2.54 (s, CH_3), 2.70 (m, CH_2-indazolyl), 3.14 (d, CH-indazolyl), 3.52 (d, CH-indazolyl), 3.62 (s, OCH_3), 6.78 (s, CH-indazolyl), 7.21–8.05 (m, *Ar*–H), 10.48 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z (%) = 504 [M^+, 100, base peak].

N1-{4(R)-[4-Acetyl-3-oxo-5(R)-(4-fluorophenyl)-1-cyclohexenyl]phenyl}-5-chloro-2-methoxybenzamide (**16**, $C_{28}H_{23}FClNO_4$)

A solution of 0.41 g **3** (1 mmol), 0.12 cm^3 acetyl acetone (1.2 mmol) and ~0.1 mg sodium ethoxide (1.5 mmol) in 10 cm^3 absolute ethanol was refluxed for 2 h. The reaction mixture was evaporated under reduced pressure, the residue was crystallized to give 0.46 g **16** (94%). Mp 213–215°C (acetone/*Me*OH); IR (film): $\bar{\nu}$ = 3380–3190 (NH), 1728, 1710, 1699 (3CO) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 1.84 (s, $COCH_3$), 2.67 (m, CH_2-cyclohexene), 3.54 (s, OCH_3), 3.76 (m, CH-cyclohexene), 3.83 (m, CH-cyclohexene), 6.84 (s, CH-cyclohexene), 7.12–7.89 (m, *Ar*–H), 10.66 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): δ = 29.70 (CH_3), 56.91 (OCH_3), 163.40 (CONH), 36.46, 40.49, 68.10, 132.25, 159.68, 198.31 (cyclohexenone-C), 207.11 (CO), 113.20, 117.33, 117.80, 124.89, 126.91, 129.0, 130.28, 134.80, 135.60, 142.60, 142.70, 151.60, 154.70, 164.11 (*Ar*–C) ppm; MS (EI, 70 eV): m/z (%) = 492 [M^+, 20] and at 381 (100, base peak).

N1-{4-[3-Methyl-4-(4-fluorophenyl)-4,5-dihydro-3a(R)H-6-indazolyl]phenyl}-5-chloro-2-methoxybenzamide (**17**, $C_{28}H_{23}FClN_3O_2$)

A solution of 0.5 g **16** (1 mmol) and 0.4 cm^3 hydrazine hydrate (8 mmol) in 5 cm^3 absolute ethanol was refluxed for 3 h. The separated solid was filtered off and crystallized to give 0.42 g **17** (87%). Mp 163–165°C (*Et*OH); IR (film): $\bar{\nu}$ = 3340–3180 (NH), 1697 (CO, amide) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 1.98 (s, CH_3), 3.28 (m, CH_2-indazolyl), 3.58 (s, OCH_3), 3.79 (m, CH-indazolyl), 4.09 (m, CH-indazolyl), 6.78 (s, CH-indazolyl), 7.12–7.89 (m, *Ar*–H), 10.65 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR ($CDCl_3$): δ = 17.40 (CH_3), 56.17 (OCH_3), 163.16 (CONH), 36.61, 39.31, 51.11, 134.18, 143.60, 144.80, 154.10 (indazolyl-C), 113.18, 117.56, 118.17, 126.70, 129.35, 130.18, 130.38, 134.18, 138.16, 142.16, 143.21, 154.57, 154.81, 164.28 (*Ar*–C) ppm; MS (EI, 70 eV): m/z (%) = 488 [M^+, 14] and at 251 (100, base peak).

*Substituted Pyrimidine Derivatives **18** and **19***

A solution of 0.41 g **3** (1 mmol), diamino reagents, namely, guanidine hydrochloride or thiourea (1.2 mmol) 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 0.41 g **18** (91%) and 0.30 g **19** (65%).

N1-{4-[2-Amino-6-(4-fluorophenyl)-4-pyrimidinyl]phenyl]}-5-chloro-2-methoxybenzamide (**18**, $C_{24}H_{20}FClN_4O_2$)

Mp 210–212°C (*Ac*O*Me*); IR (film): $\bar{\nu}$ = 3450–3200 (NH, NH_2), 1696 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 3.53 (s, OCH_3), 5.18 (d, Ha-pyrimidine), 5.46 (s, NH_2, exchangeable with D_2O), 6.98–8.04 (m, *Ar*–H + Hb-pyrimidine), 8.40 (s, NH, exchangeable with D_2O), 10.42 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z = 451 [M^+, 12] and at 200 (100, base peak).

N1-{4-[6-(4-Fluorophenyl)-2-thioxo-4-pyrimidinyl]phenyl]}-5-chloro-2-methoxybenzamide (**19**, $C_{24}H_{19}FClN_3O_2S$)

Mp 230–232°C (*Ac*O*Me*); IR (film): $\bar{\nu}$ = 3460–3210 (NH), 1697 (CO), 1225 (CS) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 3.58 (s, OCH_3), 5.22 (d, Ha-pyrimidine), 7.24–8.31 (m, *Ar*–H + Hb-pyrimidine), 8.42 and 8.54 (2s, 2NH, exchangeable with D_2O), 10.65 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z (%) = 468 [M^+, 100, base peak].

7-{4-[4-(5-Chloro-2-methoxybenzoyl)amino]phenyl}-3-oxo-5-(4-fluorophenyl)-2,3-dihydro-5H-thiazolo[3,2-a]-pyrimidine (**20**, $C_{26}H_{19}FClN_3O_3S$)

A mixture of 0.468 g **19** (1 mmol) and 0.1 g chloroacetic acid (1 mmol) was dissolved in 40 cm^3 *Ac*OH/Ac_2O (1/3) in the presence of 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 0.35 g (68%) **20**. Mp 188–190°C (*Ac*OH/H_2O); IR (film): $\bar{\nu}$ = 3355–3295 (NH), 1735, 1698 (2CO) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): δ = 3.53 (s, OCH_3), 3.72 (s, CH_2-thiazole), 5.52 (d, Ha, pyrimidine), 7.26–7.50 (m, *Ar*–H + Hb-pyrimidine), 10.25

(s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z (%) = 508 [M^+, 10] and at 228 (base peak, 100).

7-{4-[4-(5-Chloro-2-methoxybenzoyl)amino]phenyl}-2-(2-thienylmethylene)-3-oxo-5-(4-fluorophenyl)-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine (**21**, $C_{31}H_{21}FClN_3O_3S_2$)

Method A: A mixture of 0.468 g **19** (1 mmol), 0.1 g chloroacetic acid (1 mmol), and 1.5 g anhydrous sodium acetate in 40 cm^3 *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 0.5 g (84%) **21**. Mp 212–214°C (*Ac*OH/H_2O); IR (film): $\bar{\nu}$ = 3355–3325 (NH), 1716, 1694 (2CO) cm^{-1}; ^1H NMR (*DMSO*-d_6): δ = 3.58 (s, OCH_3), 5.56 (d, Ha, pyrimidine), 7.32–7.65 (m, *Ar*–H + Hb-pyrimidine + benzylic proton + thiophen-H), 10.15 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z (%) = 602 [M^+, 100, base peak].

Method B: A mixture of 0.5 g **20** (1 mmol) and 0.112 g 2-thiophenealdehyde (1 mmol) in 40 cm^3 *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 0.42 g (72%) **21**, as identified by its mp, mixed mp, and R_f value on TLC by comparison with authentic sample from method A.

Pharmacological Assay

Anti-arrhythmic Activity [26–31]

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 300–350 g 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_3 is administered 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 IV 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.
- 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.

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