

Synthesis, Reactions, and Anti-inflammatory Activity of Heterocyclic Systems Fused to a Thiophene Moiety Using Citrazinic Acid As Synthone

Abd El-Galil E. Amr^{1,*}, Nermien M. Sabry¹, and Mohamed M. Abdulla²

¹ National Research Center, Applied Organic Chemistry Department, Cairo, Dokki, Egypt

² Research Units, Hi-Care Pharmaceutical Co., Cairo, Egypt

Received January 3, 2007; accepted January 10, 2007; published online May 11, 2007

© Springer-Verlag 2007

Summary. A series of pyridines, pyrimidinones, and oxazinones were synthesized as anti-inflammatory agents using citrazinic acid (2,6-dihydroxyisonicotinic acid) as a starting material. Acryloyl pyridine was treated with cyanothioacetamide to give cyano pyridine-thione, which was reacted with ethyl chloroacetate to yield the corresponding amino ester. The ester was hydrolysed to the sodium salt, which was treated with acetic anhydride to afford 2-methyloxazinone, which was treated with ammonium acetate to afford 2-methylpyrimidinone followed by methylation with methyl iodide to yield 2,3-dimethylpyrimidinone. In addition, the oxazinone derivative was reacted with aniline or hydrazine hydrate to give 3-phenyl- or 3-aminopyrimidinones. The latter reacted with thiophene-2-carboxaldehyde or phenylisothiocyanate to afford *Schiff's* bases or thiosemicarbazides. 3-Aminopyrimidinone was treated with phthalic anhydride or 1,2,4,5-benzenetetracarboxylic acid dianhydride or toluene-3,5-diisocyanate to afford the corresponding imide, *bis*-imide, and *bis*-semicarbazide derivatives. The pharmacological screening showed that many of these compounds have good anti-inflammatory activity comparable to Prednisolone[®] as reference drug.

Keywords. Citrazinic acid; Oxazinone; Pyrimidinone; Anti-inflammatory; Prednisolone[®].

Introduction

In our previous work we have found that certain substituted pyridines and their derivatives show antimicrobial and pharmacological properties [1–5]

and antitumor activities [6, 7]. In addition, the biological and analgesic activities of many heterocyclic compounds containing a sulfur atom have been reviewed [8–11]. On the other hand, thienopyrimidine and thioxopyrimidine derivatives have promising biological [12, 13] and anticancer activities [14, 15]. Recently, some new pyridine, pyrimidine and their derivatives have been synthesized and used as analgesic, anticonvulsant, and anti-parkinsonian agents [16–22]. In view of these observations and in continuation of our previous work in pyridine chemistry, we synthesized some new heterocyclic compounds containing the thiophene ring fused with a pyridine, oxazinone, or pyrimidinone nucleus and tested their anti-inflammatory activity.

Results and Discussion

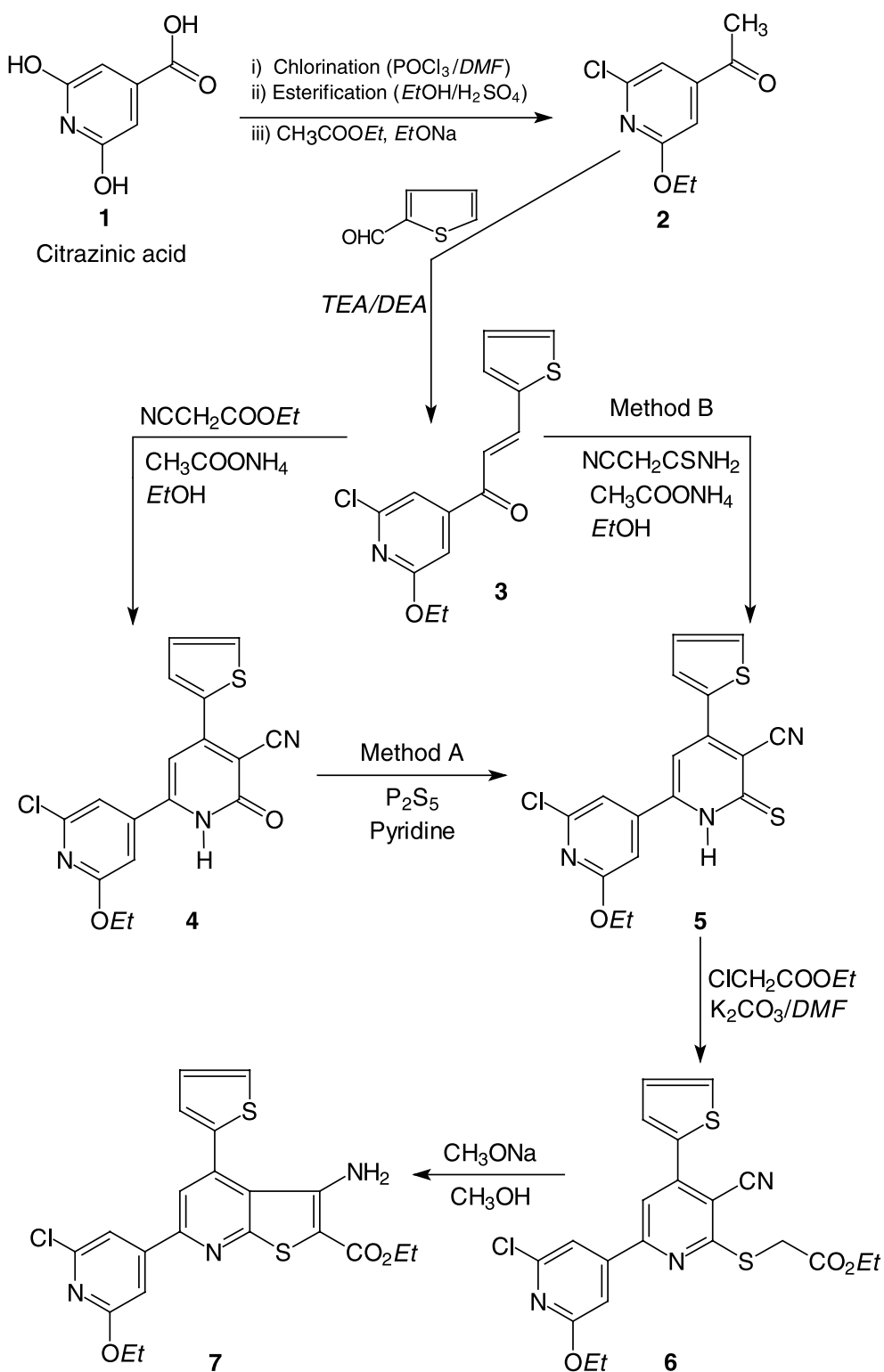
Synthesis

The starting materials **3** and **4** were prepared from citrazinic acid (2,6-dihydroxyisonicotinic acid, **1**) via the corresponding 2-chloro-6-ethoxy-4-acetylpyridine (**2**) according to literature methods [1, 23]. Thionation of **4** to the corresponding thione derivative **5** was achieved by the action of P₂S₅ in dry pyridine (method A), which was prepared directly from **3** with cyanothioacetamide in the presence of

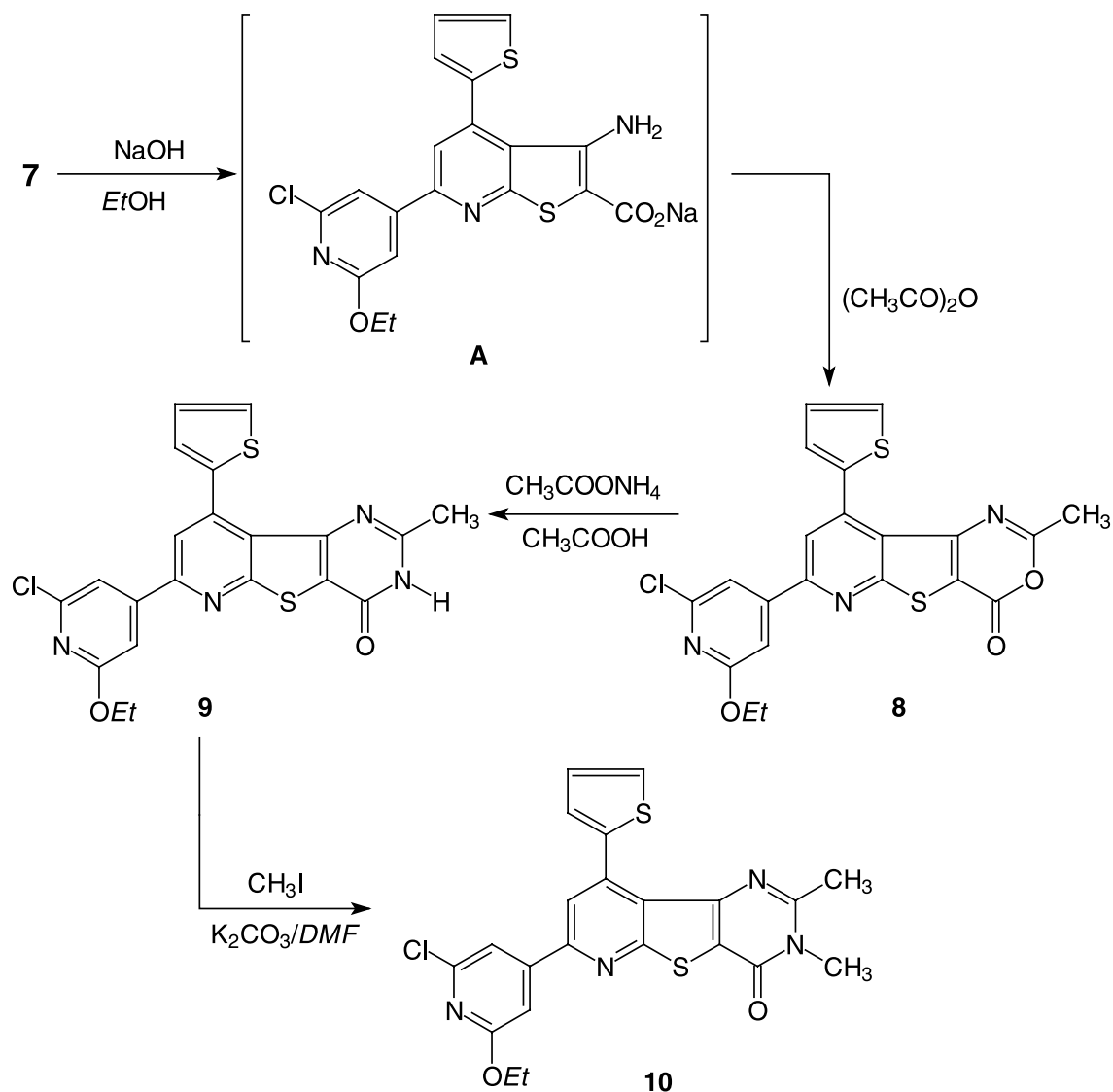
* Corresponding author. E-mail: aamr1963@yahoo.com

ammonium acetate in refluxing ethanol (method B). Condensation of **5** with ethyl chloroacetate in the presence of anhydrous K_2CO_3 gave the ethyl ester

derivative **6**, which was cyclized by sodium methoxide in methanol to give the amino ester derivative **7** (Scheme 1). The IR spectra of **7** showed the absence



Scheme 1



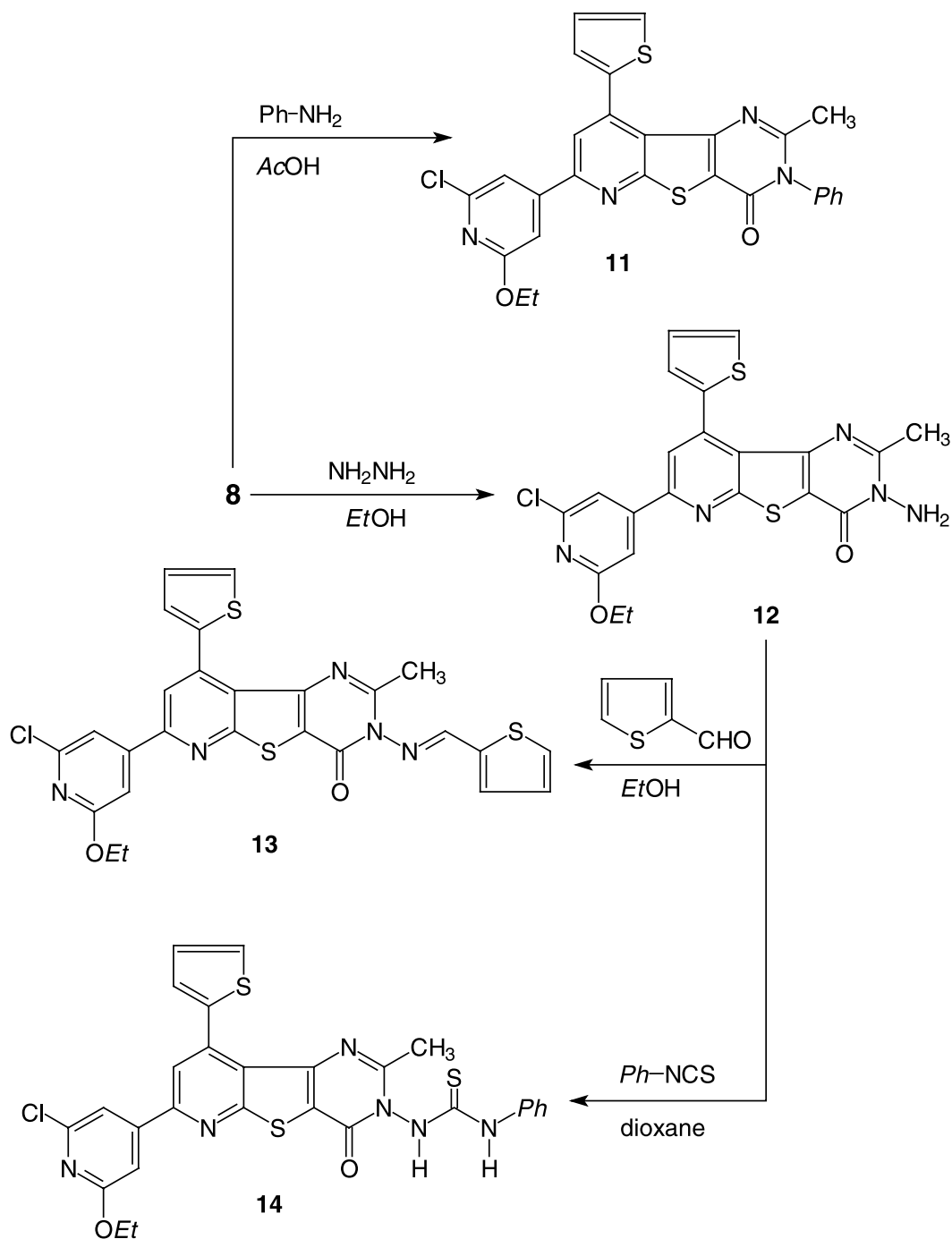
of $\bar{\nu}$ ($C\equiv N$) for **6** and the presence of broad band corresponding to $\bar{\nu}$ (NH_2).

Compound **7** was hydrolyzed by refluxing with ethanolic NaOH solution to the corresponding sodium salt **A**, which was treated with refluxing acetic anhydride to give the oxazinone derivative **8**. Reaction of **8** with ammonium acetate in refluxing acetic acid afforded the corresponding pyrimidinone derivative **9**, which was treated with methyl iodide in *N,N*-dimethylformamide in the presence of anhydrous K_2CO_3 to yield the 3-methyl-pyrimidinone derivative **10** (Scheme 2).

Similarly, reaction of oxazinone **8** with aniline in acetic acid or with hydrazine hydrate in ethanol

under reflux afforded the 3-phenyl- and 3-amino-pyrimidines **11** and **12**. Condensation of **12** with thiophene-2-carboxaldehyde in refluxing ethanol containing a few drops of piperidine yielded the corresponding *Schiff's* base **13**. Also, **12** was treated with phenylisothiocyanate in refluxing dioxane to give the thiosemicarbazide **14** (Scheme 3).

On the other hand, reaction of **12** with phthalic anhydride or 1,2,4,5-benzenetetracarboxylic acid dianhydride in refluxing acetic acid afforded the corresponding imide **15** and bis-imide **16**. Upon refluxing with toluene-3,5-diisocyanate in dioxane it afforded the bis-semicarbazide **17** (Scheme 4).



Scheme 3

Pharmacological Screening

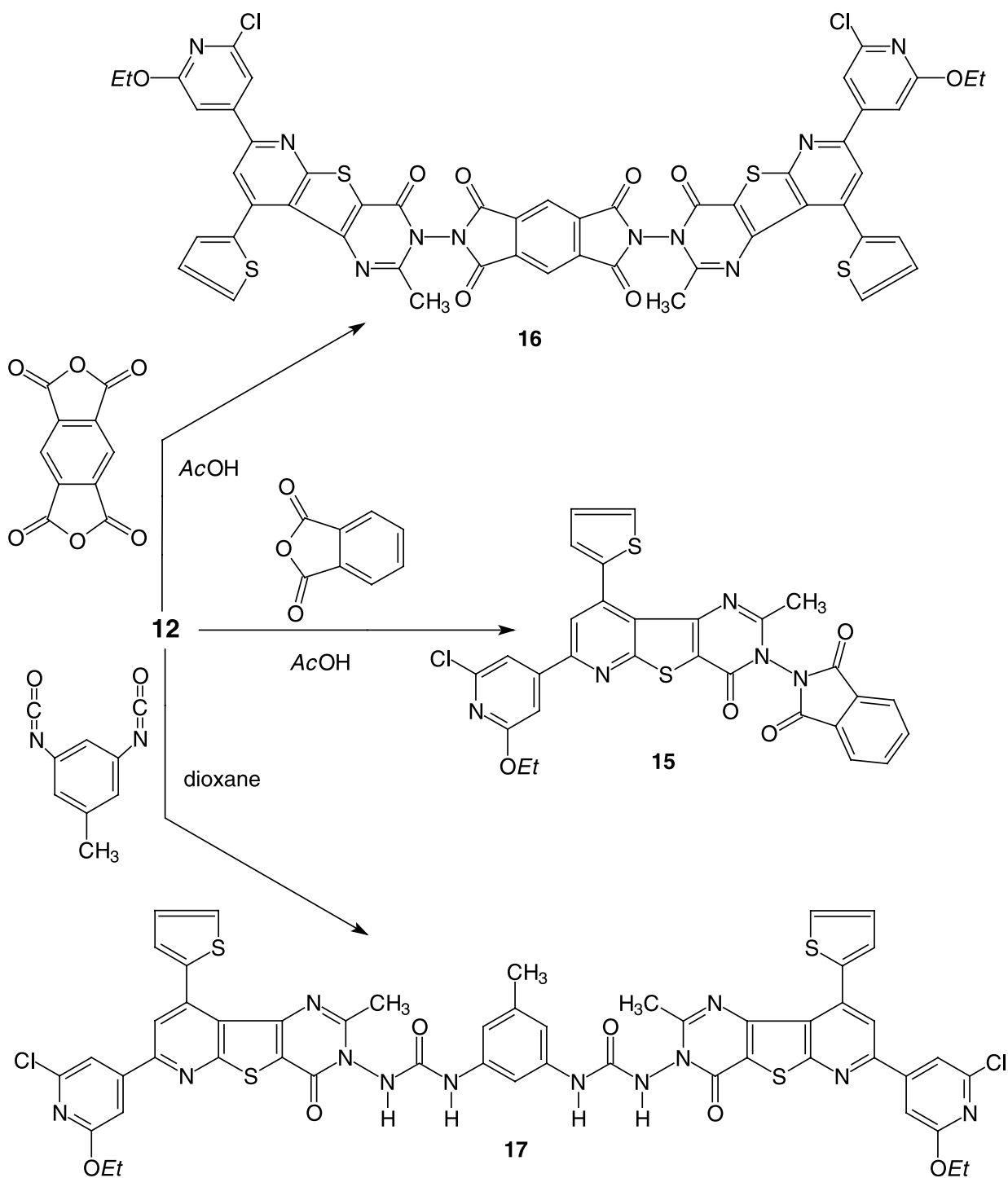
Anti-inflammatory Potency

Initially the acute toxicity of the compounds was assayed determining their LD_{50} . Interestingly, all the synthesized compounds and starting materials were less toxic than the reference drug (Table 1).

Then the newly synthesized compounds were pharmacologically screened for their anti-inflammatory potency using male albino rats (Tables 2 and 3).

Purpose and Rational

For the determination of the antiphlogistic potency of the synthesized compounds, two standard



Scheme 4

tests were realized at 25 and 50 mg/kg body weight of the rats, namely the protection against carrageenan-induced edema according to *Winter et al.* [24] and the inhibition of plasma PGE₂. The latter is known as a good confirming indicator for the carrageenan-induced rat paw edema [25]. Re-

garding the protection against carrageenan-induced edema, four compounds, namely **9**, **10**, **12**, and **14**, were found to be more potent (120%) than Prednisolone[®]. For these compounds, a similar activity profile was realized for the inhibition of plasma PGE₂.

Table 1. Acute toxicity LD_{50} of the synthesized and starting compounds

Compound no.	$LD_{50}/\text{mg kg}^{-1}$
Prednisolone [®]	1.618
1	1.780
2	1.965
3	1.875
4	2.350
5	3.715
6	2.500
7	1.800
8	1.882
9	2.970
10	2.680
11	2.615
12	2.751
13	1.980
14	1.920
15	4.186
16	2.872
17	2.264

Table 2. Anti-inflammatory potency of the synthesized compounds (protection against carrageenan-induced edema)

Compound no.	Dose/ mg kg^{-1}	% Protection against carrageenan-induced edema
Prednisolone [®]	25	81.00
	50	93.00
5	25	–
	50	38.28
6	25	–
	50	58.26
7	25	–
	50	36.49
8	25	–
	50	42.38
9	25	88.54
	50	97.15
10	25	86.25
	50	97.05
11	25	47.50
	50	51.60
12	25	86.84
	50	99.41
13	25	65.80
	50	88.16
14	25	85.52
	50	96.10
15	25	75.38
	50	85.24
16	25	–
	50	64.10
17	25	62.90
	50	64.80

Table 3. Anti-inflammatory potency of the synthesized compounds (inhibition of plasma PGE2)

Compound no.	Dose/ mg kg^{-1}	% Inhibition of plasma PGE2
Prednisolone [®]	25	77.00
	50	91.00
5	25	–
	50	40.98
6	25	–
	50	54.46
7	25	–
	50	38.50
8	25	–
	50	66.80
9	25	94.65
	50	96.58
10	25	82.65
	50	93.63
11	25	55.76
	50	65.00
12	25	93.30
	50	98.10
13	25	68.12
	50	59.73
14	25	80.36
	50	93.45
15	25	70.08
	50	82.75
16	25	–
	50	50.68
17	25	54.88
	50	66.98

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 Pye Unicam SP-1000 spectrophotometer. The ¹H NMR spectra were recorded at 270 MHz on Varian EM-360 Spectrometer using TMS as an internal standard. The Central Services Laboratory, Cairo University, Egypt. The mass spectra were performed using VG 2AB-3F spectrometer (70 eV). All reactions were followed by TLC (silica gel, aluminum sheets 60 F₂₅₄, Merck). Starting materials **2–4** were prepared from citrazinic acid **1** according to published procedures [1, 23].

2-Chloro-6-ethoxy-4-[3-cyano-4-(2-thienyl)-2-thioxopyridin-6-yl]pyridine (**5**, C₁₇H₁₂CIN₃OS₂)

Method A: A mixture of 0.357 g **4** (1 mmol) and 2.23 g P₂S₅ (10 mmol) in 50 cm³ dry pyridine was heated under reflux for 6 h with stirring. The reaction mixture was cooled, and then poured into ice, the separated solid was collected by filtration,

washed with H₂O, dried under vacuum, and crystallized to afford 0.24 g (65%) **5**.

Method B: A mixture of 0.3 g **3** (1 mmol), 0.10 g ethyl cyanothioacetamide (1 mmol) and 0.6 g ammonium acetate (8 mmol) in 30 cm³ absolute ethanol was refluxed for 5 h. After cooling, the formed product was collected by filtration, washed with ethanol, dried and crystallized to give 0.28 g (76%) **5**. Mp 214–217°C (AcOH); IR (film): $\bar{\nu}$ = 3365 (NH), 2224 (C≡N), 1235 (C=S) cm⁻¹; ¹H NMR (270 MHz, DMSO-d₆): δ = 1.35 (t, CH₃), 3.85 (q, CH₂), 7.15–7.25 (m, 3 thiophene-H), 8.22–8.27 (m, 2 pyr-H), 8.58 (s, pyr-5'-H), 9.24 (s, NH, exchangeable with D₂O) ppm; MS (EI, 70 eV): m/z (%) = 374 [M⁺, 12] and at 267 [100, base peak].

2-Chloro-6-ethoxy-4-[3-cyano-2-ethylthioglycolate-4-(2-thienyl)pyridin-6-yl]pyridine (6, C₂₁H₁₈ClN₃O₃S₂)

To a mixture of 0.373 g **5** (1 mmol) and 0.18 g anhydrous K₂CO₃ (1 mmol) in 25 cm³ *N*-dimethylformamide was stirred at room temperature for 2 h, 0.18 g ethyl chloroacetate (1.5 mmol) was added with stirring. The reaction mixture was heated at 60°C for 2 h and after cooling poured into ice. The solid formed was collected by filtration and crystallized to afford 0.3 g (68%) **6**. Mp 191–193°C (dioxane); IR (film): $\bar{\nu}$ = 2219 (C≡N), 1736 (C=O, ester) cm⁻¹; ¹H NMR (270 MHz, DMSO-d₆): δ = 1.32, 1.38 (2t, 2CH₃), 3.85, 3.98 (2q, 2CH₂), 4.70 (s, S–CH₂), 6.95–7.24 (m, 3 thiophene-H), 8.15–8.18 (m, 2 pyr-H), 8.60 (s, pyr-5'-H) ppm; MS (EI, 70 eV): m/z (%) = 460 [M⁺, 8] and at 414 [100, base peak].

2-Chloro-6-ethoxy-4-[3-amino-2-carbethoxy-4-(2-thienyl)thieno[2,3-*b*]pyridin-6-yl]pyridine (7, C₂₁H₁₈ClN₃O₃S₂)

A mixture of 0.459 g **6** (1 mmol) in 20 cm³ sodium methoxide solution (2%) was refluxed for 1 h on a water bath at 70°C with stirring. The reaction mixture was evaporated under reduced pressure, the obtained residue was dissolved in CH₂Cl₂, washed with H₂O, 10 cm³ 1 N HCl and then H₂O. The solvent was dried over anhydrous CaCl₂, evaporated under reduced pressure, and the product was crystallized to afford 0.38 g (82%) **7**. Mp 206–208°C (EtOH); IR (film): $\bar{\nu}$ = 3442–3310 (NH₂), 1742 (C=O, ester) cm⁻¹; ¹H NMR (270 MHz, DMSO-d₆): δ = 1.32, 1.38 (2t, 2CH₃), 3.84, 3.96 (2q, 2CH₂), 4.32 (brs, NH₂, exchangeable with D₂O), 7.10–7.25 (m, 3 thiophene-H), 8.18–8.25 (m, 2 pyr-H), 8.62 (s, pyr-5'-H) ppm; MS (EI, 70 eV): m/z (%) = 460 [M⁺, 4] and at 334 [100, base peak].

2-Chloro-6-ethoxy-4-[2-methyl-4-oxo-9-(2-thienyl)pyrido[3,2-*d*]thieno[3,2-*d*]oxazin-7-yl]pyridine (8, C₂₁H₁₄ClN₃O₃S₂)

A mixture of 0.459 g **7** (1 mmol) in 100 cm³ ethanolic NaOH (5%) was heated under reflux for 4 h. The solvent was evaporated under reduced pressure, the obtained sodium salt [A] was dissolved in 100 cm³ acetic anhydride and refluxed for 6 h. The reaction mixture was concentrated and allowed to cool. The obtained solid was collected and crystallized to afford 0.34 g (74%) **8**. Mp 149–151°C (AcOH/H₂O); IR (film): $\bar{\nu}$ = 1735 (C=O) cm⁻¹; ¹H NMR (270 MHz, DMSO-d₆): δ = 1.15 (s, CH₃), 1.32 (t, CH₃), 3.85 (q, CH₂), 6.98–7.25

(m, 3 thiophene-H), 8.20–8.26 (m, 2 pyr-H), 8.55 (s, pyr-5'-H) ppm; MS (EI, 70 eV): m/z (%) = 456 [M⁺, 22] and at 353 [100, base peak].

2-Chloro-6-ethoxy-4-[2-methyl-4-oxo-9-(2-thienyl)-3,4-dihydropyrido[3,2-*d*]thieno[3,2-*d*]pyrimidin-7-yl]pyridine (9, C₂₁H₁₅ClN₄O₂S₂)

A mixture of 0.455 g **8** (1 mmol) and 0.6 g ammonium acetate (8 mmol) in 100 cm³ glacial acetic acid was refluxed for 6 h. The reaction mixture was concentrated under reduced pressure, then poured into H₂O, and the solid formed was collected by filtration and crystallized to afford 0.30 g (70%) **9**. Mp 165–167°C (EtOH/H₂O); IR (film): $\bar{\nu}$ = 3420 (NH), 1669 (C=O) cm⁻¹; ¹H NMR (270 MHz, DMSO-d₆): δ = 1.10 (s, CH₃), 1.35 (t, CH₃), 3.84 (q, CH₂), 7.05–7.24 (m, 3 thiophene-H), 8.12–8.21 (m, 2 pyr-H), 8.62 (s, pyr-5'-H), 8.35 (s, NH exchangeable with D₂O) ppm; MS (EI, 70 eV): m/z (%) = 455 [M⁺, 42] and at 298 [100, base peak].

2-Chloro-6-ethoxy-4-[2,3-dimethyl-4-oxo-9-(2-thienyl)-3,4-dihydropyrido[3,2-*d*]thieno[3,2-*d*]pyrimidin-7-yl]pyridine (10, C₂₂H₁₇ClN₄O₂S₂)

A solution of 0.454 g **9** (1 mmol) in 20 cm³ DMF was stirred with 0.19 g anhydrous K₂CO₃ (1 mmol) for 10 min at room temperature, then 0.28 g methyl iodide (2 mmol) in 5 cm³ DMF were added. The reaction mixture was heated at 60°C for 4 h, after cooling poured into H₂O, and the precipitate was filtered off and crystallized to afford 0.3 g (62%) **10**. Mp 197–199°C (DMF/H₂O); IR (film): $\bar{\nu}$ = 1672 (C=O) cm⁻¹; ¹H NMR (270 MHz, DMSO-d₆): δ = 1.05 (s, CH₃), 1.37 (t, CH₃), 2.48 (s, N–CH₃), 3.90 (q, CH₂), 6.96–7.15 (m, 3 thiophene-H), 8.10–8.17 (m, 2 pyr-H), 8.42 (s, pyr-5'-H) ppm; MS (EI, 70 eV): m/z (%) = 469 [M⁺, 32] and at 242 [100, base peak].

2-Chloro-6-ethoxy-4-[2-methyl-4-oxo-3-phenyl-9-(2-thienyl)-3,4-dihydropyrido[3,2-*d*]thieno[3,2-*d*]pyrimidin-7-yl]pyridine (11, C₂₇H₁₉ClN₄O₂S₂)

A mixture of 0.456 g **8** (1 mmol) and ~0.1 g aniline (1 mmol) in 50 cm³ glacial acetic acid was heated under reflux for 6 h. The reaction mixture was concentrated, poured onto ice, and the formed solid was filtered off and crystallized to afford 0.4 g (75%) **11**. Mp 156–158°C (MeOH/H₂O); IR (film): $\bar{\nu}$ = 1679 (C=O) cm⁻¹; ¹H NMR (270 MHz, DMSO-d₆): δ = 1.15 (s, CH₃), 1.33 (t, CH₃), 3.88 (q, CH₂), 6.90–7.60 (m, 8 phenyl-H and thiophene-H), 8.22–8.25 (m, 2 pyr-H), 8.52 (s, pyr-5'-H) ppm; MS (EI, 70 eV): m/z (%) = 531 [M⁺, 16] and at 201 [100, base peak].

2-Chloro-6-ethoxy-4-[3-amino-2-methyl-4-oxo-9-(2-thienyl)-3,4-dihydropyrido[3,2-*d*]thieno[3,2-*d*]pyrimidin-7-yl]pyridine (12, C₂₁H₁₆ClN₅O₂S₂)

A mixture of 0.454 g **8** (1 mmol) and 0.4 cm³ hydrazine hydrate (8 mmol) in 100 cm³ absolute ethanol was refluxed for 4 h. After cooling the solid formed was collected and crystallized to afford 0.3 g (64%) **12**. Mp > 250°C (AcOH/H₂O); IR (film): $\bar{\nu}$ = 3365–3300 (NH₂), 1670 (C=O) cm⁻¹;

^1H NMR (270 MHz, DMSO-d_6): $\delta = 1.12$ (s, CH_3), 1.34 (t, CH_3), 3.85 (q, CH_2), 4.35 (brs, NH_2 , exchangeable with D_2O), 7.00–7.25 (m, 3 thiophene-H), 8.16–8.26 (m, 2 pyr-H), 8.56 (s, pyr-5'-H) ppm; MS (EI, 70 eV): m/z (%) = 470 [M^+ , 100, base peak].

2-Chloro-6-ethoxy-4-[2-methyl-4-oxo-3-(2-thienylmethylidene)amino]-9-(2-thienyl)-3,4-dihydropyrido[30,20:4,5]thieno[3,2-d]pyrimidin-7-yl]pyridine (**13**, $\text{C}_{26}\text{H}_{18}\text{ClN}_5\text{O}_2\text{S}_3$)

A mixture of 0.469 g **12** (1 mmol) and 0.12 g thiophene-2-carbaldehyde (1 mmol) in 25 cm^3 absolute ethanol was refluxed for 6 h. The obtained solid was filtered off, washed with ethanol, and crystallized to afford 0.48 g (86%) **13**. Mp > 250°C ($\text{EtOH}/\text{H}_2\text{O}$); IR (film): $\bar{\nu} = 1688$ (C=O), 1660 (C=N) cm^{-1} ; ^1H NMR (270 MHz, DMSO-d_6): $\delta = 1.16$ (s, CH_3), 1.38 (t, CH_3), 3.92 (q, CH_2), 7.10–7.45 (m, 7 thiophene-H + CH=N), 8.14–8.20 (m, 2 pyr-H), 8.48 (s, pyr-5'-H) ppm; MS (EI, 70 eV): m/z (%) = 564 [M^+ , 10] and at 164 [100, base peak].

2-Chloro-6-ethoxy-4-[2-methyl-4-oxo-3-(2-phenylthiosemicarbazido)amino]-9-(2-thienyl)-3,4-dihydropyrido[30,20:4,5]thieno[3,2-d]pyrimidin-7-yl]pyridine (**14**, $\text{C}_{28}\text{H}_{21}\text{ClN}_6\text{O}_2\text{S}_3$)

A mixture of 0.469 g **12** (1 mmol) and 0.14 g phenyl isothiocyanate (1 mmol) in 50 cm^3 dry dioxane containing 2 cm^3 triethylamine was heated under reflux for 10 h. The solvent was evaporated under reduced pressure, the obtained residue was solidified with *n*-hexane. The obtained solid was filtered off, washed with diethyl ether, dried and crystallized to afford 0.4 g (65%) **14**. Mp 142–144°C (AcOH); IR (film): $\bar{\nu} = 3336$ –3250 (NH), 1678 (C=O), 1232 (C=S) cm^{-1} ; ^1H NMR (270 MHz, DMSO-d_6): $\delta = 1.14$ (s, CH_3), 1.34 (t, CH_3), 3.88 (q, CH_2), 4.15–4.25 (bs, 2 NH-CS, exchangeable with D_2O), 6.94–7.55 (m, 8 thiophene-H and phenyl-H), 8.06–8.16 (m, 2 pyr-H), 8.48 (s, pyr-5'-H) ppm; MS (EI, 70 eV): m/z (%) = 605 [M^+ , 6] and at 467 [100, base peak].

Synthesis of **15** and **16**

A mixture of 1.10 g **12** (2 mmol) and 0.29 g phthalic anhydride (2 mmol) or 0.22 g 1,2,4,5-benzenetetracarboxylic acid dianhydride (1 mmol) was refluxed in 50 cm^3 glacial acetic acid for 6 h. The reaction mixture was cooled, the obtained product was collected by filtration, dried under vacuum, and crystallized to afford 0.5 g (84%) **15** and 0.98 g (88%) **16**.

2-Chloro-6-ethoxy-4-[2-methyl-4-oxo-3-(phthalimido)-9-(2-thienyl)-3,4-dihydropyrido[30,20:4,5]thieno[3,2-d]pyrimidin-7-yl]pyridine (**15**, $\text{C}_{29}\text{H}_{18}\text{ClN}_5\text{O}_4\text{S}_2$)

Mp > 300°C (DMF); IR (film): $\bar{\nu} = 1678$, 1682 (C=O) cm^{-1} ; ^1H NMR (270 MHz, DMSO-d_6): $\delta = 1.12$ (s, CH_3), 1.36 (t, CH_3), 3.82 (q, CH_2), 7.15–3.68 (m, 7 thiophene-H and phenyl-H), 8.14–8.16 (m, 2 pyr-H), 8.52 (s, pyr-5'-H) ppm; MS (EI, 70 eV): m/z (%) = 600 [M^+ , 16] and at 315 [100, base peak].

1,2,4,5-Bis-[2-chloro-6-ethoxy-4-[2-methyl-4-oxo-3-(phthalimido)-9-(2-thienyl)-3,4-dihydropyrido[30,20:4,5]thieno[3,2-d]pyrimidin-7-yl]pyridine]phthalimide (**16**, $\text{C}_{52}\text{H}_{30}\text{Cl}_2\text{N}_{10}\text{O}_8\text{S}_4$)

Mp > 300°C (DMF); IR (film): $\bar{\nu} = 1668$, 1676 (C=O) cm^{-1} ; ^1H NMR (270 MHz, DMSO-d_6): $\delta = 1.10$ (s, 2 CH_3), 1.35 (t, 2 CH_3), 3.88 (q, 2 CH_2), 7.05–7.55 (m, 8 thiophene-H and phenyl-H), 8.25–8.34 (m, 4 pyr-H), 8.58 (s, 2 pyr-5'-H) ppm; MS (EI, 70 eV): m/z (%) = 1122 [M^+ , 6] and at 380 [100, base peak].

3,5-Bis-[2-chloro-6-ethoxy-4-[2-methyl-4-oxo-3-(phenysemicarbazido)-9-(2-thienyl)-3,4-dihydropyrido[30,20:4,5]thieno[3,2-d]pyrimidin-7-yl]pyridine]toluene (**17**, $\text{C}_{51}\text{H}_{38}\text{Cl}_2\text{N}_{12}\text{O}_6\text{S}_4$)

A mixture of 1.10 g **12** (2 mmol) and 0.174 g toluene-3,5-diisocyanate (1 mmol) in 50 cm^3 dry dioxane containing 2 cm^3 TEA was refluxed for 12 h. The solvent was evaporated under reduced pressure and the oily product was triturated with *n*-hexane and petroleum ether (40–60°C). The obtained solid was filtered off, dried under vacuum and crystallized to afford 0.62 g (56%) **17**. Mp > 300°C (AcOH); IR (film): $\bar{\nu} = 3346$ –3298 (NH), 1680 (C=O) cm^{-1} ; ^1H NMR (270 MHz, DMSO-d_6): $\delta = 1.08$ (s, 2 CH_3), 1.35 (t, 2 CH_3), 2.25 (s, CH_3), 3.85 (q, 2 CH_2), 7.05–7.56 (m, 9 thiophene-H and phenyl-H), 8.15–8.26 (m, 4 pyr-H), 8.54 (s, 2 pyr-5'-H), 8.65 (brs, 4 NHCO, exchangeable with D_2O) ppm; MS (EI, 70 eV): m/z (%) = 1114 [M^+ , 14] and at 176 [100, base peak].

Pharmacological Screening

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 animal was calculated according to Austen *et al.* [26].

Anti-inflammatory Activity

Carrageenan-Induced Edema (Rats Paw Test)

Groups of adult male albino rats (150–180 g), each of eight animals were orally dosed with tested compounds at a dose level of 25–50 mg/kg one hour before carrageenan challenge. All animals were obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. Foot paw edema was induced by subplantar injection of 0.05 cm^3 of a 1% suspension of carrageenan in saline into the plantar tissue of one hind paw. An equal volume of saline was injected to the other hand paw and served as control. Four hours after drug administration the animals were decapitated, blood was collected, and the paws were rapidly excised. The average weight of edema was examined for the treated as well as the control group and the percentage inhibition of weight of edema was also evaluated. Prednisolone[®] (5 mg/kg) was employed as standard reference against which the tested compounds were compared.

Estimation of Plasma Prostaglandin E2 (PGE2)

Heparinized blood samples were collected from rats ($n = 8$), plasma was separated by centrifugation at 12000 g for 2 min at

40°C, immediately frozen, and stored at 20°C until use. The design correlate EIA prostaglandin E2 (PGE2) kit (Aldrich, Steinheim, Germany) is a competitive immuno assay for the quantitative determination of PGE2 in biological fluids. The kit uses a monoclonal antibody to PGE2 to bind, in a competitive manner, the PGE2 in the sample after a simultaneous incubation at room temperature. The excess reagents were washed away and the substrate was added, after a short incubation time the enzyme reaction was stopped, and the yellow color generated was read on a microplate reader DYNATech, MR 5000 at 405 nm (Dynatech Industries Inc., McLean, VA, USA). The intensity of the bound yellow color is inversely proportional to the concentration of PGE2 in either standard or samples.

References

- [1] Attia A, Abdel-Salam OI, Abo-Ghaliya MH, Amr AE (1995) *Egypt J Chem* **38**: 543
- [2] Amr AE, Ashraf MM, Salwa FM, Nagla AA, Hammam AG (2006) *Bioorg Med Chem* **14**: 5481
- [3] Amr AE, Abdel-Latif NA, Abdalla MM (2006) *Acta Pharm* **56**: 203
- [4] Amr AE, Abdalla MM (2006) *Bioorg Med Chem* **14**: 4341
- [5] Abo-Ghaliya MH, Amr AE (2004) *Amino Acids* **26**: 283
- [6] Amr AE, Abdel-Salam OI, Attia A, Stibor I (1999) *Collect Czech Chem Commun* **64**: 288
- [7] Attia A, Abdel-Salam OI, Amr AE (2000) *Egypt J Chem* **43**: 297
- [8] Brana MF, Castellano JM, Moran M, Perez de Vega MJ, Qian XD, Romerdahl CA, Keihauer G (1995) *Eur J Med Chem* **30**: 235
- [9] Chakrabarti JK, Horsman L, Hotten TM, Pullar IA, Tupper DE, Wright FC (1980) *J Med Chem* **23**: 878
- [10] Ram VJ, Pandey HK, Vlietinck AJ (1981) *J Heterocycl Chem* **18**: 1277
- [11] Fahmy HH, El-Eraqy W (2001) *Arch Pharm Res* **24**: 171
- [12] DeClercq E (1986) *Anticancer Res* **6**: 549
- [13] DeClercq E (1986) *J Med Chem* **29**: 156
- [14] Hammam AG, Sharaf M, Abdel-Hafez NA (2001) *Ind J Chem* **40B**: 213
- [15] Hammam AG, Fahmy AFM, Amr AE, Mohamed AM (2003) *Ind J Chem* **42B**: 1985
- [16] Amr AE, Hegab MI, Ibrahiem AA, Abdulla MM (2003) *Monatsh Chem* **134**: 1395
- [17] Nehad AA, Amr AE, Alhusain AI (2006) *Monatsh Chem* (Accepted 23-11-2006)
- [18] Amr AE, Abdel-Latif NA, Abdalla MM (2006) *Bioorg Med Chem* **14**: 273
- [19] Amr AE, Sayed HH, Abdulla MM (2005) *Arch Pharm Chem Life Sci* **338**: 433
- [20] Amr AE (2005) *Z Naturforsch* **60b**: 90
- [21] Amr AE, Abou-Galia MH, Abdulla MM (2006) *Z Naturforsch* **61b**: 1335
- [22] Amr AE, Abdulla MM (2006) *Arch Pharm Chem Life Sci* **339**: 88
- [23] Amr AE (2000) *Ind J Heterocycl Chem* **10**: 49
- [24] Winter CA, Risely EA, Nuss GW (1962) *Proc Soc Exp Bio Med* **111**: 541
- [25] Herrmann F, Lindemann A, Gamss J, Mertelsmann R (1999) *Eur J Immunol* **20**: 2513
- [26] Austen KF, Brocklehurst WE (1961) *J Exp Med* **113**: 521