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Anti-inflammatory activity of heterocyclic systems using abietic acid as starting material

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Abstract A series of pyridines, pyrimidinone, oxazinones, and their derivatives were synthesized as anti-inflammatory agents using abietic acid (7-isopropyl-1,4*a*-dimethyl-1,2,3,4,4*a*,4*b*,5,6,10,10*a*-decahydrophenanthrene-1-carboxylic acid) as the starting material. The arylidiene derivative was treated with cyanothioacetamide to give cyano pyridine-thione, which was reacted with ethyl chloroacetate to yield the corresponding cyano ester. The ester was hydrolysed to the sodium salt, which was reacted 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 thiosemicarbazide derivatives. The pharmacological screening showed that many of these compounds have good anti-inflammatory activity comparable to Prednisolone® as reference drug.

Keywords Abietic acid; Oxazinone; Pyrimidinone; Anti-inflammatory; Prednisolone®.

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Introduction

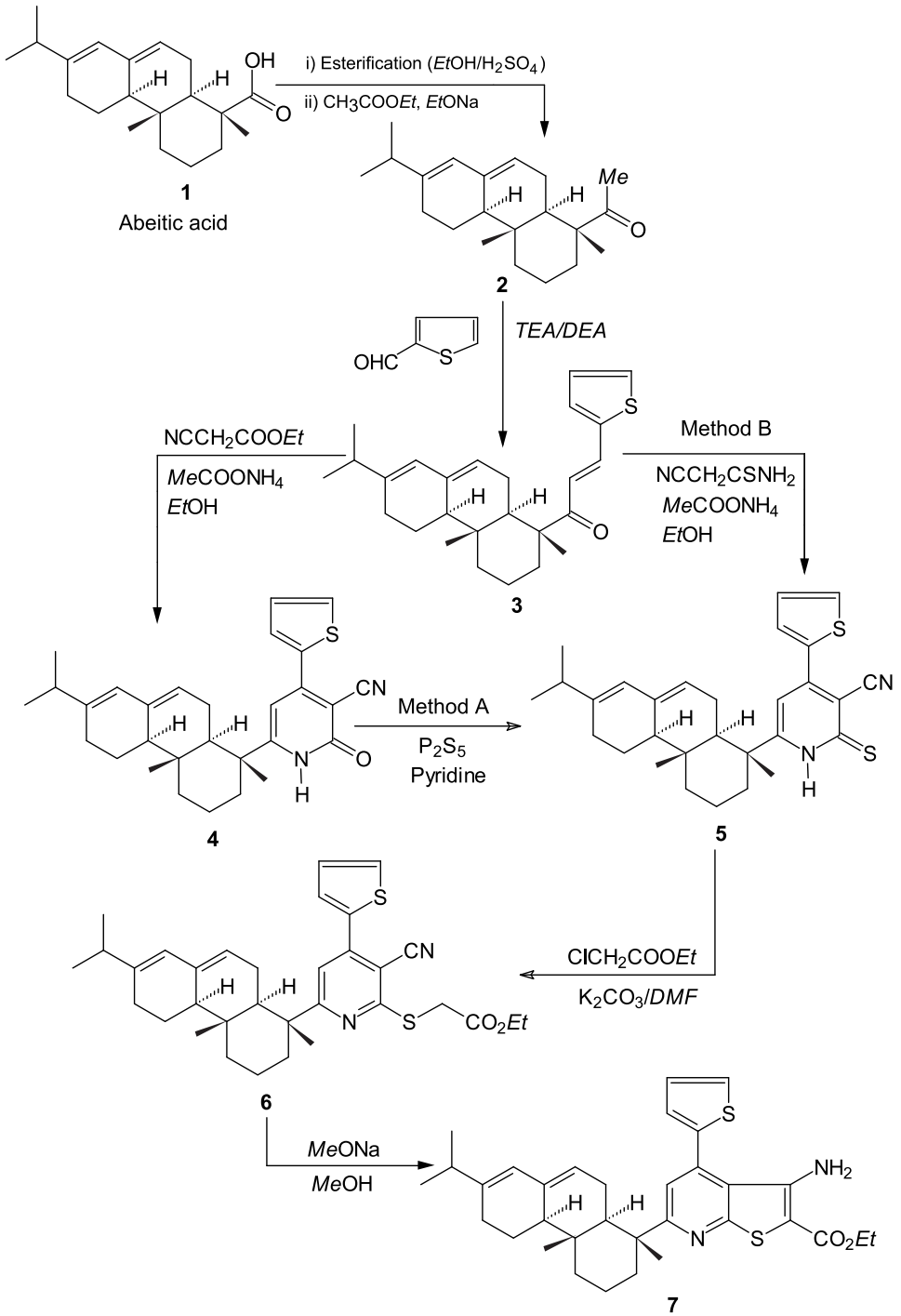
In our previous work we have found that certain substituted pyridines and their derivatives show antimicrobial and pharmacological properties [1–5] and antitumor activities [6, 7]. In addition, the biological and analgesic activities of many heterocyclic compounds containing a sulfur atom have been reviewed [8–11]. On the other hand, thienopyrimidine and thioxopyrimidine derivatives have promising biological [12, 13] and anticancer activities [14, 15]. Recently, some new pyridines, pyrimidines, and their derivatives have been synthesized and used as analgesic, anticonvulsant and antiparkinsonian 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 abietic acid (7-isopropyl-1,4*a*-dimethyl-1,2,3,4,4*a*,4*b*,5,6,10,10*a*-decahydrophenanthrene-1-carboxylic acid, **1**) *via* the corresponding acid 1-(7-isopropyl-1,4*a*-dimethyl-1,2,3,4,4*a*,4*b*,5,6,10,10*a*-decahydrophenanthrene-1-yl)ethanone (**2**) according to

literature methods [1, 23]. Thionation of **4** to the corresponding thione derivative **5** was achieved by the action of P_2S_5 in dry pyridine (method A), which was prepared directly from **3** with cyanothioacetamide in the presence of 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

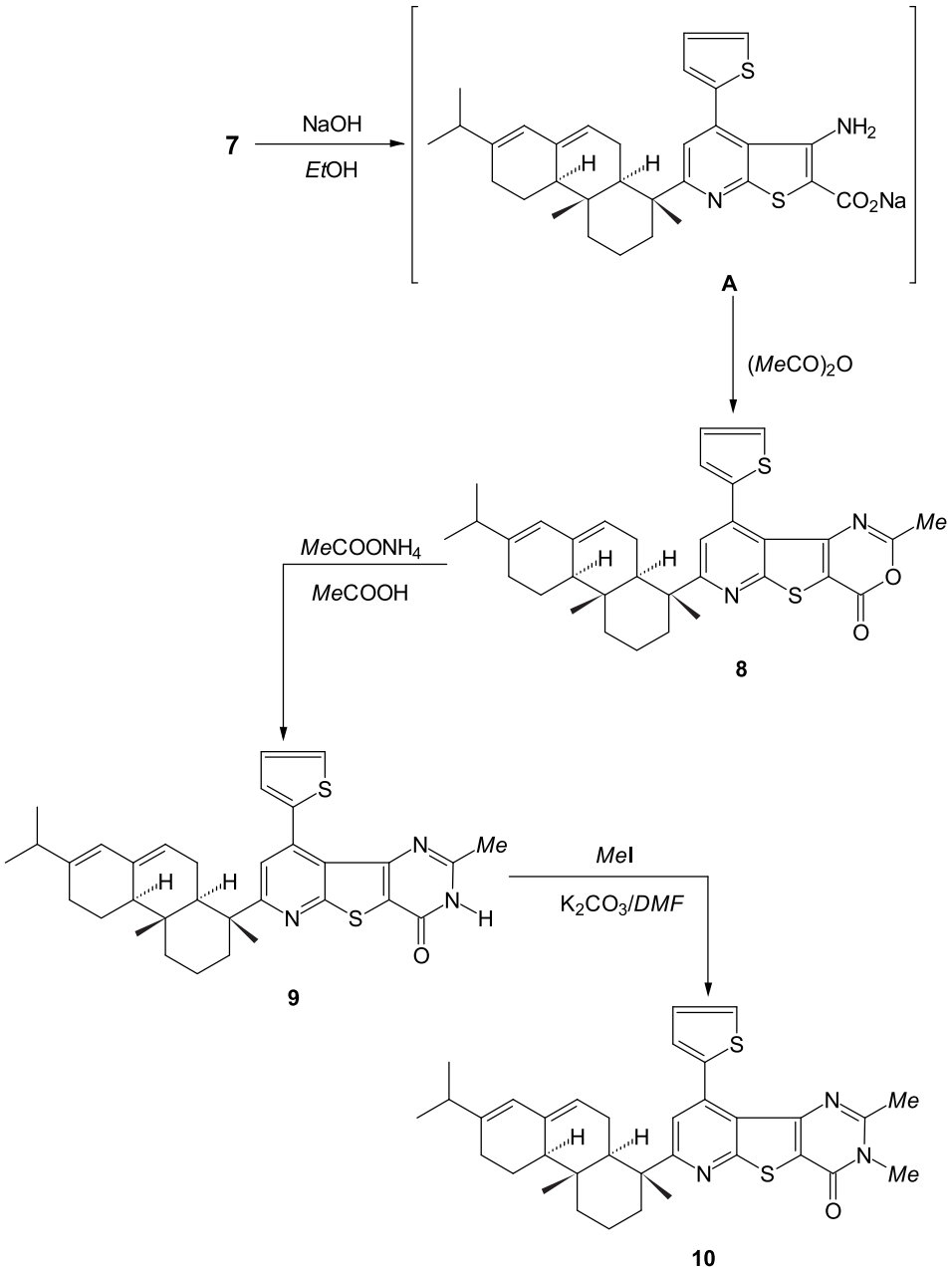


Scheme 1

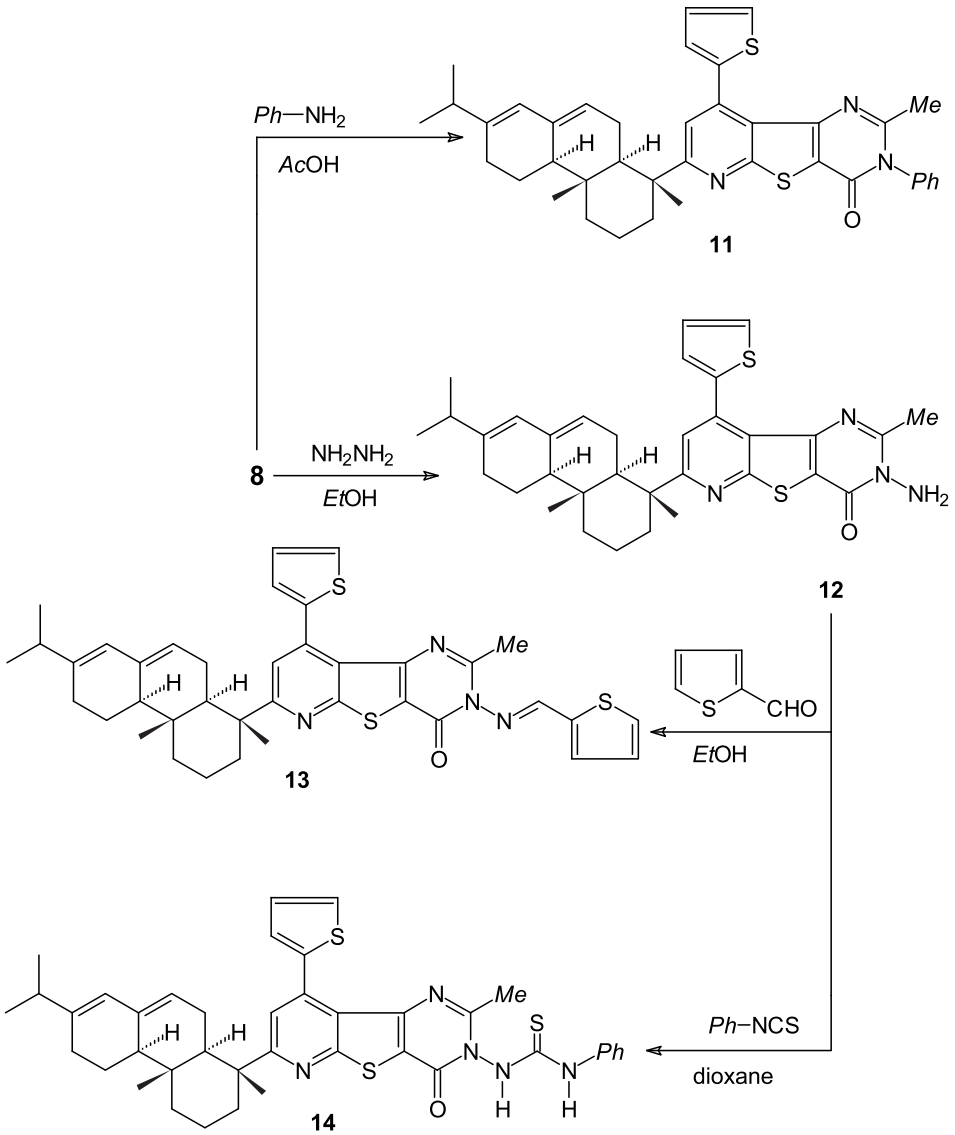
spectra of **7** showed the absence of $\bar{\nu}$ (C≡N) for **6** and the presence of a broad band corresponding to $\bar{\nu}$ (NH_2).

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-aminopyrimidines **11** and **12**. Condensation of **12** with thiophene-2-carboxaldehyde in refluxing ethanol containing a few drops of pipridine yielded the corresponding *Schiff*'s base **13**. Also, **12** was treated with phenylisothiocyanate in re-



Scheme 2



Scheme 3

fluxing dioxane to give the thiosemicarbazide **14** (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 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 carragenan-induced rat paw edema [25]. Regarding the protection against carrageenan-induced edema, all the tested compounds showed potent anti-inflammatory activities. The order of activity in descending manner is **9**, **10**, **11**, **12**, **13**, **14**, **8**, **7**, **6**, **5**, **4**, **3**, and **2**.

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

Compound no.	LD_{50}/mg kg^{-1}
Prednisolone®	1.618
1	2.758
2	2.987
3	2.984
4	2.121
5	2.764
6	3.984
7	3.6745
8	3.694
9	3.987
10	3.987
11	3.932
12	2.987
13	2.342
14	3.256

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

Compound no.	Dose/ mg kg^{-1}	Protection against carrageenan® induced edema/%
Prednisilone®	25	81.00[a]
	50	93.00[b]
1	25	80.00[c]
	50	93.00[a]
2	25	80.80[c]
	50	84.85[c]
3	25	81.18[b]
	50	87.45[c]
4	25	83.19[a]
	50	88.45[b]
5	25	85.88[c]
	50	90.19[c]
6	25	87.56[c]
	50	91.19[a]
7	25	88.12[c]
	50	93.18[b]
8	25	90.90[c]
	50	94.12[b]
9	25	96.43[c]
	50	99.68[c]
10	25	95.12[b]
	50	98.13[b]
11	25	94.13[c]
	50	98.00[a]
12	25	93.00[a]
	50	97.44[b]
13	25	92.16[b]
	50	96.17[b]
14	25	91.25[b]
	50	95.19[b]

[a] $P<0.05$; [b] $P<0.01$; [c] $P<0.001$

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

Compound no.	Dose/ mg kg^{-1}	Inhibition of plasma PGE2/%
Prednislone®	25	77.00[c]
	50	91.00[c]
1	25	67.00[a]
	50	79.13[b]
2	25	68.78[a]
	50	80.65[b]
3	25	69.50[c]
	50	81.66[c]
4	25	70.55[b]
	50	82.98[a]
5	25	71.16[c]
	50	83.19[c]
6	25	72.19[a]
	50	84.56[b]
7	25	73.12[b]
	50	84.25[a]
8	25	75.12[c]
	50	85.18[c]
9	25	83.78[a]
	50	91.18[b]
10	25	82.16[c]
	50	90.14[c]
11	25	80.14[b]
	50	90.00[b]
12	25	79.24[a]
	50	88.19[a]
13	25	77.14[c]
	50	87.14[c]
14	25	76.12[c]
	50	86.12[a]

[a] $P<0.05$; [b] $P<0.01$; [c] $P<0.001$

Structural activity relationship (SAR)

1. As the degree of poly heterocyclic fused system increases the anti-inflammatory activity increases.
2. The triazafluorene system is more active than thae diaza one.
3. Free NH on the pyrmidine nucleus is more active than substituted ones (the order of activity of N-sustituents is methyl, phenyl, amino, thienyl-methene, and thiourea).
4. S-substitution increases the anti-inflammatory activities.
5. The thion derivative increase anti-inflammatory than carbonyl function.

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. Optical relations were measured with an Atago Polax-D polarimeter. The IR spectra (KBr) were recorded on a Pye Unicam SP-1000 spectrophotometer. The ^{1}H NMR spectra were recorded at 270 MHz on Varian EM-360 Spectrometer using *TMS* as an internal standard at 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 F_{254}, Merck). Starting materials **2–4** were prepared from abietic acid **1** according to published procedures [1, 23].

1-(7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)ethanone (**2**, $C_{21}H_{32}O$)
Mp 274–276°C (*Ac*OH); $[\alpha]_D^{25} = -16 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 1$, *Me*OH); IR (film): $\bar{\nu} = 1638$ (C=C), 1787 (C=O), cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.11$ (d, 2CH_3), 1.16 (s, CH_3), 1.23 (m, CH_2), 1.29 (s, CH_3), 1.36 (t, CH_2), 1.44 (t, CH_2), 1.63 (t, CH_2), 1.76 (s, CH), 1.92 (d, CH_2), 1.96 (t, CH), 2.0 (t, CH_2), 2.22 (s, CH_3), 2.52 (m, CH), 5.57 (d, olefinic), 5.77 (d, olefinic) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 16.5$, 17.8, 18.0, 20.1, 20.2 (4C), 23.8, 28.1, 29.9, 31.0, 31.6, 33.8, 34.0, 41.1, 47.1, 47.8, 116.8 (olefin), 124.7 (olefin), 145.3 (olefin), 152.3 (olefin), 212.0 (C=O) ppm; MS (EI, 70 eV): $m/z = 300$ [M$^+$, 95] and at 167 [100, base peak].

1-(7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)-3-thien-2-ylpropenone
(**3**, $C_{26}H_{34}OS$)
Mp 266–267°C (*Ac*OH); $[\alpha]_D^{25} = -67 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 1$, *Me*OH); IR (film): $\bar{\nu} = 1634$ (C=C), 1791 (enone) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.13$ (d, 2CH_3),1.18 (s, CH_3), 1.23 (m, CH_2), 1.27 (s, CH_3), 1.37 (t, CH_2), 1.44 (t, CH_2), 1.64 (t, CH_2), 1.78 (s, CH), 1.92 (d, CH_2), 1.96 (t, CH), 2.0 (t, CH_2), 2.52 (m, CH), 5.57 (d, olefinic), 5.77 (d, olefinic), 6.63 (s, 1H-enone), 7.58 (s, 1H-enone), 7.31–7.66 (m, thiophene) ppm; MS (EI, 70 eV): $m/z = 394$ [M$^+$, 100, base peak].

6-(7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)-2-oxo-4-thien-2-yl-1,2-dihydropyridine-3-carbonitrile (**4**, $C_{29}H_{34}N_2OS$)
A mixture of 0.394 g **3** (1 mmol), 0.95 g ethyl cyanoacetate (1 mmol) and 0.6 g ammonium acetate (8 mmol) in 30 cm^3 absolute ethanol was refluxed for 5 h. After cooling, the formed product was collected by filtration, washed with ethanol, dried and crystallized to give 0.41 g (90%) **5**. Mp 234–237°C (*Ac*OH); $[\alpha]_D^{25} = -28$ 10^{-1} deg cm^2 g^{-1} ($c = 1$, *Me*OH); IR (film): $\bar{\nu} = 3371$ (NH), 2225 (C≡N), 1638 (amide) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.12$ (d, 2CH_3), 1.18 (s, CH_3), 1.25 (m, CH_2), 1.29 (s, CH_3), 1.35 (t, CH_2), 1.45 (t, CH_2), 1.65 (t, CH_2), 1.75 (s, CH), 1.92 (d, CH_2), 1.95 (t, CH), 2.05 (t, CH_2), 2.55 (m, CH), 5.55 (d, olefinic), 5.75 (d, olefinic), 7.15–7.25 (m, thiophene-H), 8.55 (s, pyr-5′-H), 9.55 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 17.11$, 18.0, 20.12, 20.56, 21.10, 23.18, 28.11, 29.91, 31.11, 32.11, 33.81, 34.8, 41.11, 47.11, 48.2, 108.0, 110.2, 116.8 (olefin), 117.2 (CN), 124.7 (olefin), 126, 127.23, 130, 136, 137, 145.3 (olefin), 152.3 (olefin), 162.9 (CO), 170.0 ppm; MS (EI, 70 eV): $m/z = 458$ [M$^+$, 100, base peak].

6-(7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)-4-thien-2-yl-2-thioxo-1,2-dihydropyridine-3-carbonitrile (**5**, $C_{29}H_{34}N_2S_2$)
Method A: A mixture of 0.458 g **4** (1 mmol) and 2.23 g P_2S_5 (10 mmol) in 50 cm^3 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 H_2O, dried under vacuum, and crystallized to afford 0.35 g (75%) **5**.

Method B: A mixture of 0.4 g **3** (1 mmol), 0.10 g ethyl cyanothioacetamide (1 mmol) and 0.6 g ammonium acetate (8 mmol) in 30 cm^3 absolute ethanol was refluxed for 5 h. After cooling, the formed product was collected by filtration, washed with ethanol, dried and crystallized to give 0.38 g (81%) **5**. Mp 234–237°C (*Ac*OH); $[\alpha]_D^{25} = -41 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 1$, *Me*OH); IR (film): $\bar{\nu} = 3368$ (NH), 2227 (C≡N), 1238 (C=S) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.11$ (d, 2CH_3), 1.16 (s, CH_3), 1.23 (m, CH_2), 1.29 (s, CH_3), 1.36 (t, CH_2), 1.44 (t, CH_2), 1.63 (t, CH_2), 1.76 (s, CH), 1.92 (d, CH_2), 1.96 (t, CH), 2.0 (t, CH_2), 2.52 (m, CH), 5.57 (d, olefinic), 5.77 (d, olefinic), 7.15–7.25 (m, thiophene-H), 8.58 (s, pyr-5′-H), 9.24 (s, NH, exchangeable with D_2O) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 17.80$, 18.00, 20.20, 20.66, 21.10, 23.80, 28.10, 29.90, 31.00, 32.20, 33.80, 35.10, 41.10, 47.10, 48.20, 108.00, 110.20, 116.80 (olefin), 117.20 (CN), 124.70 (olefin), 126.00, 127.23, 130.00, 136.00, 137.00, 145.30 (olefin), 152.30 (olefin), 170.00, 186.60 (thione) ppm; MS (EI, 70 eV): $m/z = 474$ [M$^+$, 15] and at 367 [100, base peak].

(3-Cyano-6-(7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)-4-thien-2-yl-1,2-dihydropyridine-2-ylsulfanyl}acetic acid ethyl ester
(**6**, $C_{33}H_{40}N_2O_2S_2$)
To a mixture of 0.474 g **5** (1 mmol) and 0.18 g anhydrous K_2CO_3 (1 mmol) in 25 cm^3 *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 0.34 g **6** (65%). Mp 191–193°C (dioxane); $[\alpha]_D^{25} = -49 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 1$, *Me*OH); IR (film): $\bar{\nu} = 2218$ (C≡N), 1737 (C=O, ester) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.13$ (d, 2CH_3), 1.17 (s, CH_3), 1.22 (m, CH_2), 1.28 (s, CH_3), 1.35 (t, CH_2), 1.38 (t, CH_3), 1.43 (t, CH_2), 1.61 (t, CH_2), 1.77 (s, CH), 1.93 (d, CH_2), 1.94 (t, CH), 1.96 (t, CH_2), 2.55 (m, CH), 4.55 (q, CH_2), 5.58 (d, olefinic), 4.70 (s, S–CH_2), 5.78 (d, olefinic), 6.95–7.24 (m, thiophene-H), 8.60 (s, pyr-5′-H) ppm; MS (EI, 70 eV): $m/z = 560$ [M$^+$, 18] and at 524 [100, base peak].

3-Amino-6-(7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,-10,10a-decahydrophenanthrene-1-yl)-4-thien-2-yl-thieno[2,3-b]pyridine-2-carboxylic acid ethyl ester
(**7**, $C_{33}H_{40}N_2O_2S_2$)
A mixture of 0.562 g **6** (1 mmol) in 20 cm^3 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 CH_2Cl_2, washed with H_2O, 10 cm^3 1 *N* HCl and then H_2O. The solvent was dried over anhydrous $CaCl_2$, evaporated under reduced pressure, and the product was crystallized to afford 0.53 g (92%) **7**. Mp 308°C (*Et*OH); $[\alpha]_D^{25} = -37 \times 10^{-1}$ deg $cm^2\,g^{-1}$ ($c = 1$, *Me*OH); IR (film): $\bar{\nu} =$ 3444–3310 (NH_2), 1742 (C=O, ester) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.13$ (d, $2CH_3$), 1.17 (s, CH_3), 1.21 (m, CH_2), 1.31 (s, CH_3), 1.36 (t, CH_2), 1.42 (t, CH_3), 1.48 (t, CH_2), 1.67 (t, CH_2), 1.78 (s, CH), 1.92 (d, CH_2), 1.96 (t, CH), 2.0 (t, CH_2), 2.58 (m, CH), 3.4 (CH_2), 4.32 (brs, NH_2, exchangeable with D_2O), 5.57 (d, olefinic), 5.77 (d, olefinic), 7.10–7.25 (m, thiophene-H), 8.62 (s, pyr-5′-H) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 13.6$, 17.8, 19.0, 20.2, 20.8, 21.21, 23.8, 28.1, 29.9, 31.0, 32.1, 33.8, 34.0, 41.1, 47.1, 48.6, 59.1, 116.8 (olefin), 122.8, 124.7 (olefin), 125.3, 127.16, 128.2, 129.1, 134.0, 136.0, 138.5, 142.6, 144.1, 145.3 (olefin), 152.3 (olefin), 161.0 (CO), 163.9 ppm; MS (EI, 70 eV): $m/z = 560$ [M^+, 4] and at 334 [100, base peak].

2-(7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)-6-methyl-4-thien-2-yl-7-oxa-9-thia-1,5-diazafluoren-8-one (**8**, $C_{33}H_{36}N_2O_2S_2$)

A mixture of 0.55 g **7** (1 mmol) in 100 cm^3 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 cm^3 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 0.39 g (70%) **8**. Mp 215–217°C (*Ac*OH/H_2O); $[\alpha]_D^{25} = -34 \times 10^{-1}$ deg $cm^2\,g^{-1}$ ($c = 1$, *Me*OH); IR (film): $\bar{\nu} = 1736$ (C=O) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.10$ (d, $2CH_3$), 1.14 (s, CH_3), 1.25 (s, CH_2), 1.29 (s, CH_3), 1.36 (m, CH_2), 1.38 (s, CH_3), 1.44 (t, CH_2), 1.63 (t, CH_2), 1.76 (s, CH), 1.92 (d, CH_2), 1.96 (t, CH), 2.09 (t, CH_2), 2.56 (m, CH), 5.57 (d, olefinic), 5.77 (d, olefinic), 6.98–7.25 (m, thiophene-H), 8.20–8.26, 8.55 (s, pyr-5′-H) ppm; MS (EI, 70 eV): $m/z = 556$ [M^+, 22] and at 353 [100, base peak].

2-(7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)-6-methyl-4-thien-2-yl-7H-9-thia-1,5,7-triazafluoren-8-one (**9**, $C_{33}H_{37}N_3OS_2$)

A mixture of 0.55 g **8** (1 mmol) and 0.6 g ammonium acetate (8 mmol) in 100 cm^3 glacial acetic acid was refluxed for 6 h. The reaction mixture was concentrated under reduced pressure, then poured into H_2O, and the solid formed was collected by filtration and crystallized to afford 0.39 g (70%) **9**. Mp 216–218°C (*Et*OH/H_2O); $[\alpha]_D^{25} = -13\ 10^{-1}$ deg $cm^2\,g^{-1}$ ($c = 1$, *Me*OH); IR (film): $\bar{\nu} = 3428$ (NH), 1670 (C=O) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.11$ (d, $2CH_3$), 1.14 (s, CH_3), 1.23 (m, CH_2), 1.29 (s, CH_3), 1.36 (t, CH_2), 1.40 (s, CH_3), 1.44 (t, CH_2), 1.61 (t, CH_2), 1.77 (s, CH), 1.92 (d, CH_2), 1.96 (t, CH), 2.0 (t, CH_2), 2.51 (m, CH), 5.53 (d, olefinic), 5.77 (d, olefinic), 7.1–7.25 (m, thiophene-H), 8.61 (s, pyr-5′-H), 8.39 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 555$ [M^+, 38] and at 211 [100, base peak].

2-(7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)-6,7-dimethyl-4-thien-2-yl-7H-9-thia-1,5,7-triazafluoren-8-one (**10**, $C_{34}H_{39}N_3OS_2$)

A solution of 0.55 g **9** (1 mmol) in 20 cm^3 *DMF* was stirred with 0.19 g anhydrous K_2CO_3 (1 mmol) for 10 min at room temperature, then 0.28 g methyl iodide (2 mmol) in 5 cm^3 *DMF* were added. The reaction mixture was heated at 60°C for 4 h, after cooling poured into H_2O, and the precipitate was filtered off and crystallized to afford 0.35 g (62%) **10**. Mp 197–199°C (*DMF*/H_2O); $[\alpha]_D^{25} = -81 \times 10^{-1}$ deg $cm^2\,g^{-1}$ ($c = 1$, *Me*OH); IR (film): $\bar{\nu} = 1668$ (C=O) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.10$ (d, $2CH_3$), 1.14 (s, CH_3), 1.23 (m, CH_2), 1.27 (s, CH_3), 1.36 (t, CH_2), 1.41 (s, CH_3), 1.44 (t, CH_2), 1.63 (t, CH_2), 1.76 (s, CH), 1.90 (d, CH_2), 1.96 (t, CH), 1.98 (t, CH_2), 2.44 m, (CH), 2.48 (s, N–CH_3), 5.51 (d, olefinic), 5.79 (d, olefinic), 6.96–7.15 (m, thiophene-H), 8.0 (s, pyr-5′-H) ppm; MS (EI, 70 eV): $m/z = 569$ [M^+, 32] and at 504[100, base peak].

2-(7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)-6-methyl-7-phenyl-4-thien-2-yl-7H-9-thia-1,5,7-triazafluoren-8-one (**11**, $C_{39}H_{41}N_3OS_2$)

A mixture of 0.55 g **8** (1 mmol) and ~0.1 g aniline (1 mmol) in 50 cm^3 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 0.53 g (85%) 11. Mp 312–314°C (*Me*OH/H_2O); $[\alpha]_D^{25} = -26 \times 10^{-1}$ deg $cm^2\,g^{-1}$ ($c = 1$, *Me*OH); IR (film): $\bar{\nu} = 1678$ (C=O) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.10$ (d, $2CH_3$), 1.13 (s, CH_3), 1.19 (s, CH_3), 1.23 (m, CH_2), 1.31 (s, CH_3), 1.36 (t, CH_2), 1.44 (t, CH_2), 1.60 (t, CH_2), 1.74 (s, CH), 1.90 (d, CH_2), 1.96 (t, CH), 1.98 (t, CH_3), 2.50 (m, CH), 5.59 (d, olefinic), 5.81 (d, olefinic), 6.90–7.33 (m, 5 phenyl-H and thiophene-H), 8.42 (s, pyr-5′-H) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 16.9$, 17.7, 18.8, 20.22 20.7, 21.12, 23.7, 28.2, 29.8, 31.0, 32.1, 33.7, 34.1, 41.2, 47.1, 48.20, 116.9 (olefin), 120.2, 120.5, 121.0, 122.8, 124.6 (olefin), 125.3 (2C–Ar), 127.5 (2C–Ar), 128.7, 129.0, 136.0, 137.1, 138.2, 144.1, 142.6, 145.8 (olefin), 146, 152.2 (olefin), 163.9, 164.4, 165 ppm; MS (EI, 70 eV): $m/z = 631$ [M^+, 100, base peak].

2-(7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)-6-methyl-4-thien-2-yl-[(thien-2-ylmethylene)amino]-7H-9-thia-1,5,7-triazafluoren-8-one (**12**, $C_{33}H_{38}N_4OS_2$)

A mixture of 0.454 g **8** (1 mmol) and 0.4 cm^3 hydrazine hydrate (8 mmol) in 100 cm^3 absolute ethanol was refluxed for 4 h. After cooling the solid formed was collected and crystallized to afford 0.49 g (87%) **12**. Mp 350°C (*Ac*OH/H_2O); $[\alpha]_D^{25} = -39 \times 10^{-1}$ deg $cm^2\,g^{-1}$ ($c = 1$, *Me*OH); IR (film): $\bar{\nu} =$ 3373–3300 (NH_2), 1678 (C=O) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.10$ (d, $2CH_3$),1.13 (s, CH_3), 1.18 (s, CH_3), 1.23 (m, CH_2), 1.29 (s, CH_3), 1.36 (t, CH_2), 1.48 (t, CH_2), 1.61 (t, CH_2), 1.74 (s, CH), 1.90 (d, CH_2), 1.95 (t, CH), 1.99 (t, CH_2), 2.50 (m, CH), 4.35 (brs, NH_2, exchangeable with D_2O), 5.63 (d, olefinic), 5.71 (d, olefinic), 7.00–7.25 (m, thiophene-H), 8.56 (s, pyr-5′-H) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 14.4$,

17.1, 18.0, 20.3, 20.7, 21.1, 23.8, 28.2, 30.0, 31.0, 32.6, 33.8, 34.1, 41.1, 47.1, 48.2, 116.7 (olefin), 120.1, 122.8, 124.7 (olefin), 125.3, 127.15, 128.6, 136.1, 137.17, 142.6, 144.1, 145.7 (olefin), 146.8, 152.7 (olefin), 163.9, 164.5, 167.8 ppm; MS (EI, 70 eV): $m/z = 570$ [M^+, 58] and at 312 [100, base peak].

7-Amino-2-(7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)-6-methyl-4-thien-2-yl-7H-9-thia-1,5,7-triazafluoren-8-one (**13**, $C_{38}H_{40}N_4OS_3$)

A mixture of 0.57 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 0.63 g (96%) **13**. Mp 289°C (*Et*OH/H_2O); $[\alpha]_D^{25} = -28 \times 10^{-1}$ deg $cm^2\,g^{-1}$ ($c = 1$, *Me*OH); IR (film): $\bar{\nu} = 1688$ (C=O), 1660 (C=N) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.10$ (d, $2CH_3$), 1.13 (s, CH_3),1.19 (s, CH_3), 1.23 (m, CH_2), 1.27 (s, CH_3), 1.37 (t, CH_2), 1.43 (t, CH_2), 1.61 (t, CH_2), 1.78 (s, CH), 1.90 (d, CH_2), 1.94 (t, CH), 1.96 (t, CH_2), 2.50 (m, CH), 5.60 (d, olefinic), 5.80 (d, olefinic), 7.10–7.40 (m, thiophene-H + CH=N), 8.50 (s, pyr-5′-H) ppm; MS (EI, 70 eV): $m/z = 664$ [M^+, 100, base peak].

1-[2-(7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene-1-yl)-6-methyl-4-thien-2-yl-8H-9-thia-1,5,7-triazafluoren-7-yl]-3-phenyl-thiourea
(**14**, $C_{40}H_{43}N_5OS_3$)

A mixture of 0.57 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 0.53 g (75%) **14**. Mp 213–214°C (*Ac*OH); $[\alpha]_D^{25} = -89 \times 10^{-1}$ deg $cm^2\,g^{-1}$ ($c = 1$, *Me*OH); IR (film): $\bar{\nu} = 3331$–3258 (NH), 1675 (C=O), 1242 (C=S) cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 1.11$ (d, $2CH_3$), 1.16 (s, CH_3), 1.19 (s, CH_3), 1.23 (m, CH_2), 1.29 (s, CH_3), 1.36 (t, CH_2), 1.44 (t, CH_2), 1.63 (t, CH_2), 1.76 (s, CH), 1.92 (d, CH_2), 1.96 (t, CH), 2.0 (t, CH_2), 2.52 (m, CH), 4.17–4.27 (bs, 2 NH–CS, exchangeable with D_2O), 5.57 (d, olefinic), 5.77 (d, olefinic), 6.97–7.57 (m, thiophene-H and phenyl-H), 8.47 (s, pyr-5′-H) ppm; MS (EI, 70 eV): $m/z = 705$ [M^+, 39] and at 546 [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 12,000 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.

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