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ORIGINAL PAPER

Synthesis and pharmacological activity of some synthesized polyazacyclopenta[*a*]naphthalenes and pyrazolo[3,4-*b*]pyridines using 2-amino-6-methyl-4-phenylnicotinonitrile as synthon

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Abstract A series of pyridines, pyrimidines, and their derivatives were synthesized using 2-amino-6-methyl-4-phenylnicotinonitrile as starting material. Thirteen new heterocyclics containing a pyridine ring were thus prepared. Pharmacological screening showed that many of these compounds have good anti-inflammatory and analgesic activity comparable with those of diclofenac potassium and valdecoxib as reference drugs. Assignment of the structures of the new compounds was based on chemical and spectroscopic evidence. Synthesis, spectroscopic data, and pharmacological properties of the compounds are reported in detail.

Keywords 2-Amino-6-methyl-4-phenylnicotinonitrile · Polyazacyclopenta[*a*]naphthalenes · Pyrazolopyridines · Anti-inflammatory and analgesic activity

Introduction

In previous work we prepared some substituted pyridines and their derivatives [1–6]. In addition, the biological and analgesic activity of many heterocyclic compounds containing a pyridine moiety have been reviewed [7–9]. On the other hand, pyrimidine derivatives have promising biological [10, 11] and anticancer activity [12, 13]. Recently, some new pyridine and pyrimidine derivatives have been synthesized and tested for androgenic–anabolic, antihypertensive, and antiparkinsonian activity [14–16]. In view of these observations and in continuation of our previous work in heterocyclic chemistry, we synthesized some new heterocyclic compounds containing the pyridine moiety fused with other heterocyclic nuclei and tested their pharmacological activity.

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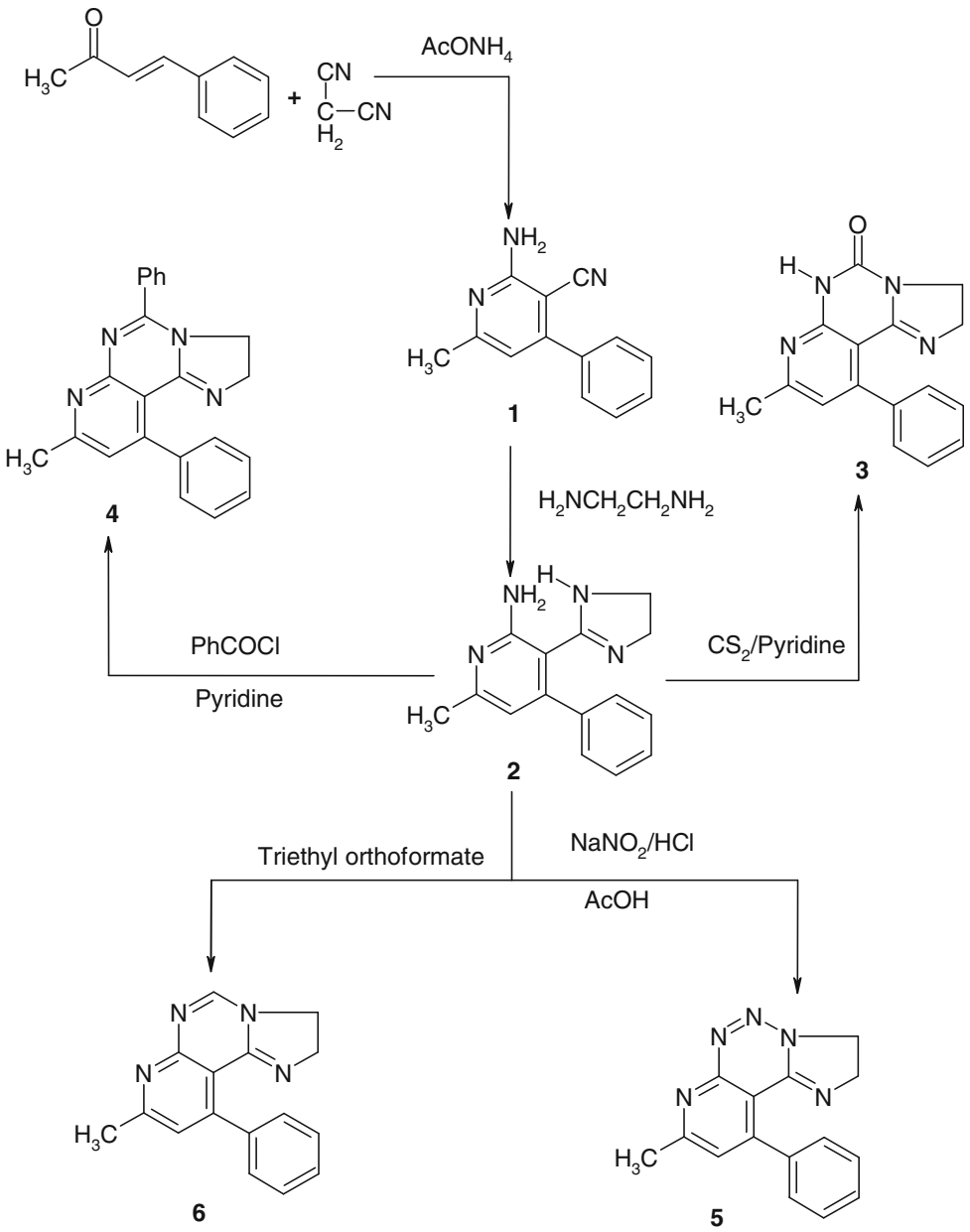
Results and discussion

Chemistry

In this paper we report the synthesis and a preliminary pharmacological activity screening of several pyridine derivatives based on 2-amino-6-methyl-4-phenylnicotinonitrile (**1**), which was prepared from 4-phenyl-but-3-en-2-one and malononitrile in the presence of ammonium acetate according to Ref. [9]. Reaction of **1** and 1,2-ethylenediamine in the presence of carbon disulfide produced imidazoloaminopyridine **2**, which was heated under reflux with carbon disulfide or benzoyl chloride in pyridine to yield the corresponding tetraazacyclopenta[*a*]naphthalenethione **3** and tetraazacyclopenta[*a*]naphthalene **4**. Compound **2** was treated with sodium nitrite and hydrochloric acid in acetic acid to give pentaazacyclopenta[*a*]naphthalene **5**. When it was heated under reflux in triethylorthoformate, it afforded the corresponding tetraazacyclopenta[*a*]naphthalenethione **6** (Scheme 1).

Reaction of **1** with hydroxylamine hydrochloride in the presence of anhydrous sodium acetate in glacial acetic acid gave the corresponding pyrazolopyridine **7**, which was treated with acetylacetone or ethyl acetoacetate in acidic medium to afford the corresponding trimethyltetraazafluorene **8** and dimethyltetraazafluorenone **9**. Heating of **7** under reflux with phenyl isothiocyanate in pyridine or chloroacetyl chloride in dimethylformamide gave the corresponding pyrazolopyridine phenylthiourea **10** and

Scheme 1



tetraazacyclopentaindenone **11**. Condensation of **7** with 4-nitrobenzaldehyde or cinnamaldehyde in ethanol under reflux in the presence of piperidine as catalyst afforded the corresponding the Schiff bases **12** and **13** (Scheme 2).

Pharmacological screening

All animals were obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. Initially, the acute toxicity of the compounds was assayed by determining their LD_{50}. Interestingly, all the synthesized compounds were less toxic than valdecoxib (Table 1). The newly synthesized compounds were then screened pharmacologically for their anti-inflammatory and analgesic activities.

Anti-inflammatory activity

Purpose and rationale

For determination of the antiphlogistic potency of the synthesized compounds, two standard tests were conducted at doses 2.5 and 5 mg/kg body weight of the rats, namely, protection against carrageenan-induced edema according to Winter et al. [17] and inhibition of plasma PGE2. The latter is known as a good confirming indicator for carrageenan-induced rat paw edema [18]. Regarding protection against

Scheme 2

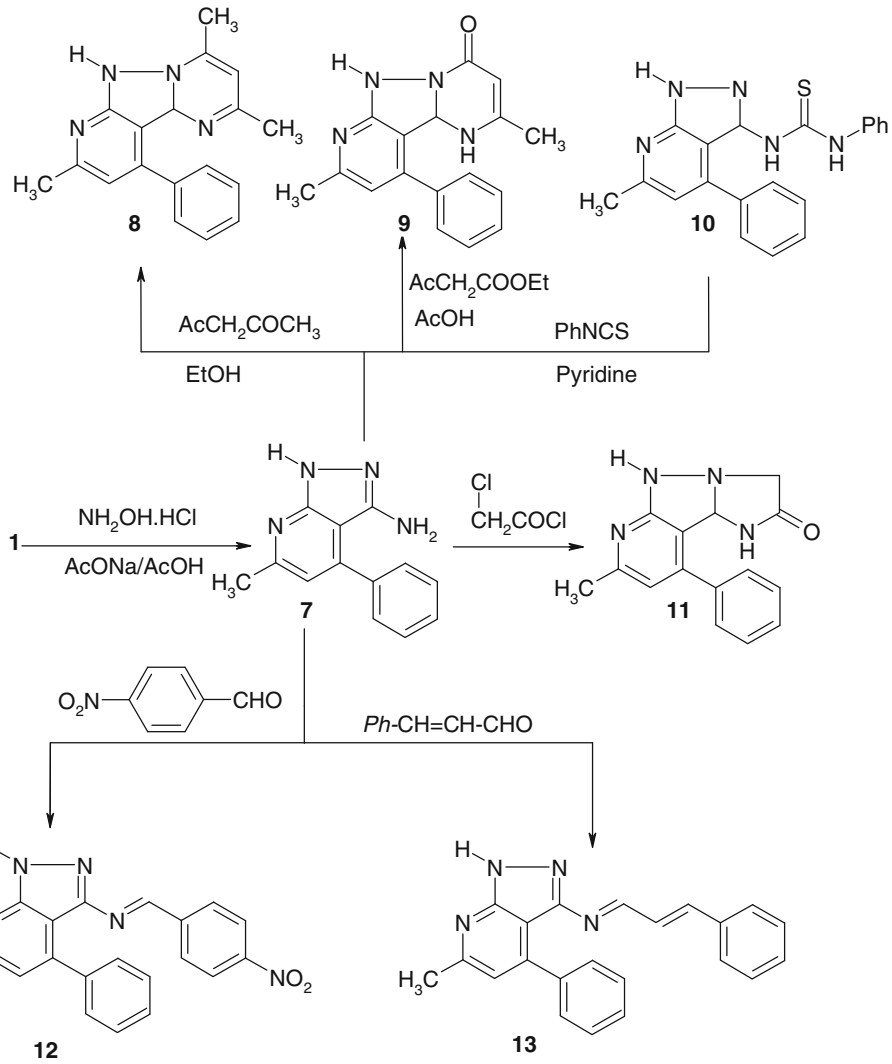


Table 1 Acute toxicity (LD_{50}) of the synthesized compounds

Compound no.	LD_{50}/mg kg^{-1}
1	2,213.78 ± 0.19
2	2,100.89 ± 0.17
3	1,456.09 ± 0.12
4	1,249.87 ± 0.12
5	2,134.89 ± 0.10
6	1,720.00 ± 0.11
7	2,112.55 ± 0.11
8	1,620.14 ± 0.24
9	2,612.56 ± 0.16
10	2,213.87 ± 0.18
11	1,272.87 ± 0.17
13	1,345.98 ± 0.14
Valdecoxib®	1,180.01 ± 0.23

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

carrageenan-induced edema, all tested compounds were found to be more potent than diclofenac potassium. For these compounds, a similar activity profile was obtained for the inhibition of plasma PGE2. Concerning anti-inflammatory activity, the descending order of activity is **7**, **2**, **10**, **1**, **3**, **11**, **6**, **5**, **9**, **4**, **13**, and **8**. Compounds **7**, **2**, **10**, **1**, and **3** are the most active (Table 2).

Analgesic activity

All the compounds tested had analgesic activity in a hot-plate assay (Table 3). Interestingly, the analgesic activity of all the compounds **1–10**, **12**, and **13** was more potent than that of valdecoxib as reference drug (Table 3) and, compared with valdecoxib, after 120 min these analgesic activities were increased. Compounds **7**, **10**, **2**, **1**, **3**, **12**, **6**, **5**, **9**, **4**, **13**, and **8** are arranged in descending order of analgesic potency. Compound **7** was three times more active than valdecoxib, and compounds **1**, **10**, **2**, and **3** were twice as active as valdecoxib after 2 h.

Table 2 Anti-inflammatory activity of some newly synthesized compounds

Groups	Dose/mg kg^{-1}	Protection against edema/%	Inhibition of plasma PGE2/%
1	2.5	96.45 ± 0.052	79.26 ± 0.040
	5.0	97.89 ± 0.042	81.48 ± 0.052
2	2.5	98.12 ± 0.077	83.14 ± 0.040
	5.0	98.09 ± 0.063	85.05 ± 0.030
3	2.5	92.90 ± 0.073	74.70 ± 0.040
	5.0	97.76 ± 0.062	79.05 ± 0.060
4	2.5	82.10 ± 0.077	58.00 ± 0.030
	5.0	93.00 ± 0.060	73.55 ± 0.040
5	2.5	80.94 ± 0.074	62.10 ± 0.040
	5.0	96.00 ± 0.062	78.87 ± 0.030
6	2.5	85.06 ± 0.055	60.13 ± 0.040
	5.0	96.10 ± 0.066	76.10 ± 0.040
7	2.5	89.88 ± 0.091	62.15 ± 0.030
	5.0	99.34 ± 0.080	85.15 ± 0.070
8	2.5	78.80 ± 0.090	56.56 ± 0.050
	5.0	91.94 ± 0.060	75.05 ± 0.040
9	2.5	91.10 ± 0.064	72.43 ± 0.048
	5.0	95.18 ± 0.075	77.55 ± 0.040
10	2.5	90.26 ± 0.034	61.10 ± 0.050
	5.0	98.55 ± 0.035	82.05 ± 0.048
11	2.5	86.14 ± 0.053	58.42 ± 0.050
	5.0	98.16 ± 0.052	80.08 ± 0.054
13	2.5	79.28 ± 0.081	56.91 ± 0.040
	5.0	92.90 ± 0.098	76.10 ± 0.041
Diclofenac potassium®	2.5	70.14 ± 0.061	53.05 ± 0.040
	5.0	76.23 ± 0.082	70.05 ± 0.051

All results were significantly different from the standard and normal control value at $P \leq 0.05$

Table 3 Analgesic activity some newly synthesized compounds

Compound	Activity compared with that of valdecoxib after time:					
	10 min	20 min	30 min	60 min	90 min	120 min
1	0.60 ± 0.02	0.60 ± 0.05	0.89 ± 0.03	1.00 ± 0.01	1.18 ± 0.10	2.34 ± 0.12
2	0.65 ± 0.02	0.75 ± 0.07	0.80 ± 0.06	1.15 ± 0.15	1.43 ± 0.12	2.45 ± 0.14
3	0.54 ± 0.01	0.55 ± 0.03	0.86 ± 0.05	0.88 ± 0.08	1.12 ± 0.12	2.22 ± 0.21
4	0.46 ± 0.01	0.46 ± 0.03	0.52 ± 0.04	0.64 ± 0.06	0.80 ± 0.08	1.30 ± 0.08
5	0.46 ± 0.02	0.46 ± 0.03	0.60 ± 0.04	0.74 ± 0.07	0.99 ± 0.09	1.58 ± 0.04
6	0.46 ± 0.01	0.48 ± 0.04	0.61 ± 0.05	0.76 ± 0.07	0.99 ± 0.09	1.63 ± 0.06
7	0.73 ± 0.02	0.84 ± 0.07	0.97 ± 0.08	1.20 ± 0.12	1.63 ± 0.16	3.55 ± 0.13
8	0.41 ± 0.01	0.42 ± 0.03	0.45 ± 0.04	0.58 ± 0.05	0.89 ± 0.08	1.11 ± 0.07
9	0.48 ± 0.01	0.48 ± 0.03	0.56 ± 0.05	0.67 ± 0.09	0.87 ± 0.09	1.47 ± 0.14
10	0.66 ± 0.01	0.62 ± 0.06	0.96 ± 0.07	1.00 ± 0.01	1.24 ± 0.12	2.56 ± 0.11
12	0.50 ± 0.01	0.54 ± 0.05	0.81 ± 0.06	0.85 ± 0.08	1.11 ± 0.11	1.89 ± 0.08
13	0.42 ± 0.01	0.42 ± 0.04	0.50 ± 0.05	0.61 ± 0.06	0.97 ± 0.09	1.28 ± 0.16
Valdecoxib	1.00	1.00	1.00	1.00	1.00	1.00

All results were significantly different from the standard and normal control value at $P \leq 0.05$

Structure–activity relationship

The pyridine nucleus and poly nitrogen atoms in the molecule are essential for the anti-inflammatory and analgesic activity. The fused ring at positions 2 and 3 with the substituents at positions 4 and 6 increases the activity.

Experimental

Chemistry

Melting points were determined in open glass capillaries using an Electrothermal IA 9000 digital melting-point apparatus. Elemental analyses were performed with an Elementar Vario EL by the Microanalytical Unit, National Research Center, Cairo Egypt and were found to be within ±0.4% of the theoretical values. Infrared spectra were recorded on Carl Zeiss model UR 10 spectrophotometer using the KBr disc technique. ^{1}H NMR spectra were recorded on Varian Gemini 270 MHz spectrometer (DMSO-d_6) and chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as internal standard. Mass spectra were measured using a Finnigan SSQ 7000 mass spectrometer. Follow up of the reactions and checking of the purity of the compounds was performed by TLC on silica gel-precoated aluminum sheets (type 60 F_{254}; Merck, Darmstadt, Germany).

2-Amino-6-methyl-4-phenylnicotinonitrile (**1**, $C_{13}H_{11}N_3$)
A mixture of 0.29 g 4-phenylbut-3-en-2-one (2 mmol), 0.13 g malononitrile (2 mmol), and 0.3 g ammonium acetate (4 mmol) in 30 cm^3 absolute ethanol was heated under reflux for 5 h. After cooling, the product formed was collected by filtration, washed with ethanol, dried, and crystallized to give 0.31 g **1** (75%). Mp 140–142 °C (EtOH); IR (film): $\bar{\nu}$ = 3,341–3,223 (NH_2), 2,209 (C≡N), 1,604 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 2.52 (s, CH_3), 4.50 (s, NH_2, exchangeable with D_2O), 7.10–7.48 (m, *Ar*-H and pyridyl-H) ppm; MS (EI, 70 eV): *m*/*z* = 209 (M^+, 100, as a base peak).

3-(4,5-Dihydro-1H-imidazol-2-yl)-6-methyl-4-phenylpyridin-2-ylamine (**2**, $C_{15}H_{16}N_4$)
To a mixture of 1.05 g **1** (5 mmol) and 7.5 cm^3 ethylenediamine, 0.70 cm^3 carbon disulfide was added dropwise with stirring. The reaction mixture was heated under reflux for 4 h. After cooling, it was poured on to ice-water. The solid formed was isolated by filtration, washed with water, dried, and crystallized to give 1.40 g **2** (80%). Mp 160–161 °C (EtOH); IR (film): $\bar{\nu}$ = 3,336–3,211 (NH, NH_2), 1,605 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 1.80–1.78 and 2.71–2.68 (m, 2 CH_2), 2.48 (s, CH_3), 3.54 (s, NH_2, exchangeable with D_2O), 7.00–7.45 (m, *Ar*-H), 7.52 (s, pyridyl-H), 8.01 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): *m*/*z* = 352 (M^+, 12) and at 219 (100, base peak).

7-Methyl-9-phenyl-2,5-dihydro-3H-1,3a,5,6-tetraazacyclopenta[a]naphthalene-4-thione (**3**, $C_{16}H_{14}N_4S$)
A mixture of 1.26 g **2** (5 mmol) and 15 cm^3 carbon disulfide in 50 cm^3 dry pyridine was heated under reflux for 10 h. The solid obtained was isolated by filtration while still hot, washed with water, dried, and crystallized to give 1.03 g **3** (70%). Mp 172–174 °C (dioxane–H_2O); IR (film): $\bar{\nu}$ = 3,331–3,213 (NH), 1,600 (C=N), 1,247 (C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 1.25–1.22 and 3.80–3.78 (2 m, 2 CH_2), 2.48 (s, CH_3), 7.05–7.40 (m, *Ar*-H), 7.50 (s, pyridyl-H), 8.75 (s, NH, exchangeable with D_2O) ppm; MS (EI, 70 eV): *m*/*z* = 294 (M^+, 6) and at 279 (100, base peak).

7-Methyl-4,9-diphenyl-2,3-dihydro-1,3a,5,6-tetraazacyclopenta[a]naphthalene (**4**, $C_{22}H_{18}N_4$)
A mixture of 1.26 g **2** (5 mmol) and 0.7 g benzoyl chloride (5 mmol) in 20 cm^3 dry pyridine was heated under reflux for 6 h. The reaction mixture was cooled, poured on to water, and the solid formed was isolated by filtration, washed with water, dried, and crystallized to give 1.0 g **4** (60%). Mp 128–130 °C (AcOH); IR (film): $\bar{\nu}$ = 3,070 (CH-Ar), 1,610 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 1.90–1.88 and 2.76–2.74 (2 m, 2 CH_2), 2.46 (s, CH_3), 7.18–7.46 (m, *Ar*-H), 7.55 (s, pyridyl-H) ppm; MS (EI, 70 eV): *m*/*z* = 338 (M^+, 16) and at 246 (100, base peak).

7-Methyl-9-phenyl-2,3-dihydro-1,3a,4,5,6-pentaazacyclopenta[a]naphthalene (**5**, $C_{15}H_{13}N_5$)
To a cold solution of 1.26 g **2** (5 mmol) in 15 cm^3 hydrochloric acid and 15 cm^3 acetic acid, 2 g sodium nitrite in 15 cm^3 water was added with stirring. After complete addition the reaction mixture was stirred at room temperature for 2 h. The solid product obtained was isolated by filtration, washed with water, and crystallized to give 0.8 g **5** (60%). Mp 185-183 °C (dioxane); IR (film): $\bar{\nu}$ = 3,115 (CH-Ar), 1,608 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 1.88–1.86 and 2.70–2.68 (2 m, 2 CH_2), 2.46 (s, CH_3), 7.15–7.42 (m, *Ar*-H), 7.52 (s, pyridyl-H) ppm; MS (EI, 70 eV): *m*/*z* = 263 (M^+, 8) and at 248 (100, base peak).

7-Methyl-9-phenyl-2,3-dihydro-1,3a,5,6-tetraazacyclopenta[a]naphthalene (**6**, $C_{16}H_{14}N_4$)
A mixture of 1.26 g **2** (5 mmol) and 15 cm^3 triethylorthoformate was heated under reflux for 3 h. After cooling, the reaction mixture was poured on to cold water. The solid precipitate was isolated by filtration, washed with water, dried, and crystallized to give 0.65 g **6** (50%). Mp 240–242 °C (dioxane); IR (film): $\bar{\nu}$ = 3,100 (CH-Ar), 1,604 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 1.90–1.89 and 2.73–2.70 (2 m, 2 CH_2), 2.45 (s, CH_3), 7.17–7.46 (m, *Ar*-H and

pyrimidinyl-H), 7.50 (s, pyridyl-H) ppm; MS (EI, 70 eV): $m/z = 262$ (M^+, 4) and at 170 (100, base peak).

6-Methyl-4-phenyl-1H-pyrazolo[3,4-b]pyridin-3-ylamine (**7**, $C_{13}H_{12}N_4$)

A mixture of 1.05 g **1** (5 mmol), 0.35 g hydroxylamine hydrochloride (5 mmol), and 2 g anhydrous sodium acetate in 30 cm^3 glacial acetic acid was heated under reflux for 5 h. The reaction mixture was left overnight at room temperature and then poured on to water. The solid precipitate was isolated by filtration, washed with water, and crystallized to give 0.85 g **7** (70%). Mp 188–190 °C (EtOH); IR (film): $\bar{\nu}$ = 3,450–3,320 (NH, NH_2), 1,610 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.50$ (s, CH_3), 6.80 (s, NH_2 exchangeable with D_2O), 7.30–7.50 (m, *Ar*-H), 7.52 (s, pyridyl-H), 8.62 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 224$ (M^+, 12) and at 193 (100, base peak).

2,6,8-Trimethyl-4-phenyl-4b,9-dihydro-1,5,8a,9-tetraazafluorene (**8**, $C_{18}H_{18}N_4$)

To a mixture of 1.12 g **7** (5 mmol) and 0.5 cm^3 acetylacetone (5 mmol) in 25 cm^3 ethanol, a few drops of acetic acid were added. The reaction mixture was heated under reflux for 6 h, concentrated under reduced pressure, and allowed to cool. The solid formed was isolated by filtration, and crystallized to give 0.87 g **8** (60%). Mp 120–122 °C (EtOH); IR (film): $\bar{\nu}$ = 3,344 (NH), 1,605 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 1.30$, 1.90 and 2.50 (3 s, $3CH_3$), 3.34 (s, CH), 6.97–7.32 (m, *Ar*-H and CH-pyrimidine), 7.52 (s, pyridyl-H), 10.51 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 290$ (M^+, 8) and at 168 (100, base peak).

2,6-Dimethyl-4-phenyl-4b,9-dihydro-5H-1,5,8a,9-tetraazafluoren-8-one (**9**, $C_{17}H_{16}N_4O$)

A mixture of 1.12 g **7** (5 mmol) and 0.65 cm^3 ethyl acetoacetate (5 mmol) in 20 cm^3 glacial acetic acid was heated under reflux for 5 h. The solid product that formed after cooling was collected by filtration, dried, and crystallized to give 0.73 g **9** (50%). Mp 135–137 °C (EtOH); IR (film): $\bar{\nu}$ = 3,343–3,216 (NH), 1,660 (C=O), 161 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 1.70$ and 2.52 (2 s, $2CH_3$), 5.05 and 6.0 (2 s, 2CH), 7.10–7.40 (m, *Ar*-H), 7.49 (s, pyridyl-H), 9.50 and 10.42 (2 s, 2NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 292$ (M^+, 8) and at 234 (100, base peak).

1-(6-Methyl-4-phenyl-1H-pyrazolo[3,4-b]pyridin-3-yl)-3-phenylthiourea (**10**, $C_{20}H_{17}N_5S$)

A mixture of 1.12 g **7** (5 mmol) and 0.66 cm^3 phenyl isothiocyanate (5 mmol) in 30 cm^3 pyridine was heated under reflux for 3 h. The precipitate that formed was isolated by filtration, dried, and crystallized to give 1.1 g **10** (60%). Mp 105–107 °C (dioxane); IR (film): $\bar{\nu}$ = 3,330–3,219 (NH), 1,598 (C=N), 1,252 (C=S) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.44$ (s, CH_3), 7.03–7.42 (m, *Ar*-H), 7.51 (s, pyridyl-H), 8.69, 8.81 and 10.5 (3 s, 3NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 359$ (M^+, 12) and at 252 (100, base peak).

6-Methyl-4-phenyl-3a,8-dihydro-3H-3,7,8,8a-tetraazacyclopenta[a]inden-2-one (**11**, $C_{15}H_{14}N_4O$)

A mixture of 2.24 g **7** (10 mmol) and 1.13 g chloroacetyl chloride (10 mmol) in 15 cm^3 dimethylformamide with a few drops of piperidine was heated under reflux for 6 h. The reaction mixture was poured on to ice–water, and the solid which separated was crystallized to give 1.7 g **11** (65%). Mp 172–174 °C (DMF–EtOH); IR (film): $\bar{\nu}$ = 3,350–3,260 (NH), 1,675 (C=O), 1,602 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.54$ (s, CH_3), 3.35 and 3.42 (2d, CH_2), 5.85 (s, CH), 7.05–7.46 (m, *Ar*-H), 7.45 (s, pyridyl-H), 9.45 and 10.36 (2 s, 2NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 266$ (M^+, 8) and at 194 (100, base peak).

*Synthesis of the Schiff bases **12** and **13***

A suspension of 1.12 g **7** (5 mmol) and 4-nitrobenzaldehyde or cinnamaldehyde (5 mmol) in 30 cm^3 ethanol containing a few drops of piperidine was heated under reflux for 6 h. The reaction mixture was triturated with cold water, and the solid product was isolated by filtration, dried, and crystallized to give 0.98 g **12** (55%) and 1.0 g **13** (60%).

4-Nitrobenzylidene-(6-methyl-4-phenyl-1H-pyrazolo[3,4-b]pyridin-3-yl)-amine (**12**, $C_{20}H_{15}N_5O_2$)

Mp 110–112 °C (dioxane); IR (film): $\bar{\nu}$ = 3,410 (NH), 1,612 (C=N), 1545 (NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.48$ (s, CH_3), 6.85–7.35 (m, *Ar*-H), 7.50 (s, pyridyl-H), 8.15 (s, CH=N), 10.42 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 357$ (M^+, 18) and at 208 (100, base peak).

(7-Methyl-4-phenyl-1H-pyrazolo[3,4-b]pyridin-3-yl)-(3-phenylallylidene)amine (**13**, $C_{22}H_{18}N_4$)

Mp 150–152 °C (DMF–EtOH); IR (film): $\bar{\nu}$ = 3,398 (NH), 1,660 (C=C), 1,608 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): $\delta = 2.50$ (s, CH_3), 3.33 and 3.56 (2d, 2CH), 6.74-7.38 (m, *Ar*-H), 7.52 (s, pyridyl-H), 8.14 (d, CH = N), 10.25 (s, NH exchangeable with D_2O) ppm; MS (EI, 70 eV): $m/z = 338$ (M^+, 6) and at 235 (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 animals was calculated according to Austen et al. [19] (Table 1).

Anti-inflammatory activity

Carrageenan-induced edema (rat's paw test)

Groups of adult male albino rats (150–180 g), each of eight animals, were orally dosed with tested compounds at a dose of 2.5–5 mg/kg one hour before the carrageenan challenge. Foot paw edema was induced by sub-plantar injection of 0.05 cm^3 of a 1% suspension of carrageenan in saline solution into the plantar tissue of one hind paw. An equal volume of saline was injected to the other hind 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 and control groups, and the percentage inhibition of the weight of the edema was evaluated. Diclofenac potassium (5 mg/kg) was employed as standard reference to which the tested compounds were compared (Table 2).

Estimation of plasma prostaglandin E2 (PGE2)

Heparinized blood samples were collected from rats ($n = 8$), plasma was separated by centrifugation at 12,000g 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 immunoassay for quantitative analysis 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 simultaneous incubation at room temperature. 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, McLean, VA, USA). The intensity of the bound yellow color is inversely proportional to the concentration of PGE2 in either standard or sample.

Analgesic activity

Sixty Webster mice of both sexes weighting 20–25 g were divided into ten groups. One group was kept as control (received saline), the second group received vehicle (gum acacia), the third received valdecoxib as reference drug, and the other groups received the test compounds (s.c. administration; dose 5 mg/kg). Mice were dropped gently into a dry glass beaker of 1 dm^3 capacity maintained at 55.0–55.5 °C. Normal reaction time in seconds for all animals was determined 10, 20, 30, 45, 60, 90, and 120 min after administration of the compound. The reaction time was the time from the instant the mouse reached the hot beaker until the animals licked its feet or jumped out of the beaker [20]. The potencies relative to that of valdecoxib were determined (Table 3).

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