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Synthesis and Pharmacological Activity of Some New Thieno[2,3-*d*]pyrimidine and Pyrimidopyrazolotheienopyrimidine Derivatives

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Abstract: A series of fused pyrimidopyrazolotheinopyrimidine derivatives were synthesized using 2-(1-methyl-1*H*-indol-3-ylmethyl)]-4-methyl-5-ethoxycarbonyl-6-mercaptopyrimidine (1) as a starting material. Initially the acute toxicity of the compounds was assayed via the determination of their LD_{50}. All compounds were interestingly less toxic than the reference drug (Prednisolone®).The pharmacological screening showed that many of these compounds have good anti-inflammatory and analgesic activities comparable to Prednisolone® and Valdecoxib as reference drugs. The structure assignments of the new compounds based on chemical and spectroscopic evidence. The detailed synthesis, spectroscopic data and pharmacological properties are reported.

Key words: Thienopyrimidines, Pyrazolothienopyrimidines, Pyrimidopyrazolothienopyrimidine, Analgesic and Anti-inflammatory activities

INTRODUCTION

Pyrimidines and fused pyrimidines, being an integral part of *DNA* and *RNA* in it, play an essential role in several biological processes and have considerable chemical and pharmacological importance, particularly, the pyrimidine ring can be found in nucleoside antibiotics, antibacterials, cardio-vascular as well as agro chemical and veterin products [1-9]. In addition, many of these derivatives were found to possess a variety of pronounced activities such as antimalarial [10-12], antimicrobial [13-18], anti-inflammatory [19-23], anti-allergic [24,25]. Recently, some new substituted pyrimidine derivatives have been synthesized, which exhibit analgesic, anti-inflammatory, antiparkinsonian and androgenic-anabolic activities [26-31]. In view of these observations and as continuation of our previous work on pyrimidine chemistry, we synthesized some new compounds containing pyrimidine and pyrazolo-pyrimidothienopyrimidine moieties and tested their antiinflammatory and analgesic activities in comparison to Prednisolone® and Valdecoxib as reference drugs.

MATERIALS AND METHODS

Melting points were determined on an Electrothermal IA 9000 SERIES digital melting point apparatus (Electrothermal, Essex, U.K.) and are uncorrected. Elemental analyses were performed in the Microanalytical Unit, National Research Centre, Cairo Egypt and were found within ±0.4% of the theoretical values. The IR spectra (KBr) were recorded on a FT IR-8201 PC Spectrophotometer. The ^{1}H-NMR spectra were measured with Jeol 270 MHz (Japan) in DMSO-d_6. The chemical shifts were recorded (δ, ppm) relative to TMS. The Mass spectra were run at 70 eV with a Finnigan SSQ 7000 spectrometer (Thermo-Instrument system incorporation, USA). All reactions were followed by *TLC* (silica gel, aluminium sheets 60 F_{254}, Merck).

2-(1-Methyl-1H-indol-3-ylmethyl)]-6-hydroxy-7-methylthieno[2,3-d]pyrimidine-5-carbonitrile (2): A mixture of compound 1 (0.34 g, 1 mmol), chloroacetonitrile (0.08 g, 1 mmol) in absolute ethanol (30 ml) in the presence of few drops of piperidine was refluxed for 4h.

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The obtained solid product was collected by filtration, dried and crystallized to give 2. Yield 72%, m.p. 195-197°C (EtOH); IR (Kbr, cm^{-1}): 3520 (OH) and 2218 (CN); ^{1}H NMR (DMSO-d_6) δ: 2.19 (s, 3H, CH_3), 2.36 (s, 3H, NCH_3), 3.42 (s, 2H, CH_2), 7.16-7.45 (m, 5H, Ar-H) and 12.40 (s, 1H, OH, exchangeable with D_2O); MS *m/z* (%): 334 (M^+, 16) corresponding to the molecular formula $C_{18}H_{14}N_4OS$ and base peak at 235 (100).

2-(1-Methyl-1H-indol-3-ylmethyl)]-6-chloro-7-methylthieno[2,3-d] pyrimidine-5-carbonitrile (3): A solution of 2 (0.33 g, 1 mmol) in phosphurus oxychloride (30 ml) was refluxed for 4 h. The reaction mixture was poured into water, the separated solid was filtered off, washed with water, dried and crystallized to give 3. Yield 68%, m.p. 281-282°C (EtOH); IR (KBr, cm^{-1}): 2220 (C≡N); ^{1}H NMR (DMSO-d_6) δ: 2.15 (s, 3H, CH_3), 2.43 (s, 3H, NCH_3), 3.29 (s, 2H, CH_2), 6.81-7.32 (m, 5H, Ar-H); MS *m/z* (%): 352 (M^+, 18) corresponding to the molecular formula $C_{18}H_{13}ClN_4S$ and base peak at 266 (100).

2-(1-Methyl-1H-indol-3-ylmethyl)]-5-amino-7H-8-methylpyrazolo[3,4:4,5]thieno[2,3-d]pyrimidine (4): A mixture of 3 (0.35 g, 1 mmol) and hydrazine hydrate (1 ml) in acetic acid (30 ml) was refluxed for 3 h. The reaction mixture was evaporated under reduced pressure, the residue was solidified with diethyl ether, filtered off, dried and and crystallized to give 4. Yield 60%, m.p. > 300°C (EtOH); IR (KBr, cm^{-1}): 3410-3235 (NH, NH_2); ^{1}H NMR (DMSO-d_6) δ: 2.23 (s, 3H, CH_3), 2.61 (s, 3H, NCH_3), 3.42 (s, 2H, CH_2), 6.23 (s, 2H, NH_2, exchangeable with D_2O), 6.82-7.43 (m, 5H, Ar-H), 9.56 (s, 1H, NH, exchangeable with D_2O); MS *m/z* (%): 348 (M^+, 23) corresponding to the molecular formula $C_{18}H_{16}N_6S$ and base peak at 291 (100).

Synthesis of Thieno[2,3-d]pyrimidines (5-9)

General Method: A mixture of 4 (0.35 g, 1 mmol) and active methylene reagents, namely, acetyl acetone, ethyl acetoacetate, ethyl cyanoacetate, diethyl malonate or malononitrile (1 mmol) in polyphosphoric acid (30 ml) was refluxed for 4 h. The reaction mixture was poured into water, the separated solid was collected by filtration, washed with water, dried and crystallized from the proper solvent to give 5-9, respectively.

2-(1-Methyl-1H-indol-3-ylmethyl)]-6,8,10-trimethyl-pyrimidino[2``,3``:5,1]pyrazolo[3`,4`:4,5]-thieno[2,3-d]pyrimidine (5): Yield 60%, m.p. 215-217°C (EtOH); IR (KBr, cm^{-1}): 1616-1480 (C=N, C=C); ^{1}H NMR (DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 2.41 (s, 3H, NCH_3), 2.63 (s, 3H, CH_3), 3.10 (s, 3H, CH_3), 3.62 (s, 2H, CH_2), 7.16-7.45 (m, 6H, Ar-H); MS *m/z* (%): 412 (M^+, 100) corresponding to the molecular formula $C_{23}H_{20}N_6S$ and as base peak.

2-(1-Methyl-1H-indol-3-ylmethyl)]-6,10-dimethyl-8-hydroxy-pyrimidino[2``,3``:5,1]pyrazolo [3`,4`:4,5] thieno[2,3-d]pyrimidine (6): Yield 65%, m.p. 285-287°C (MeOH); IR (KBr, cm^{-1}): 3510-3360 (OH); ^{1}H NMR (DMSO-d_6) δ: 2.16 (s, 3H, CH_3), 2.25 (s, 3H, NCH_3), 2.71 (s, 3H, CH_3), 3.52 (s, 2H, CH_2), 6.93-7.61 (m, 6H, Ar-H) and 11.36 (s, 1H, OH, exchangeable with D_2O); MS *m/z* (%): 414 (M^+, 19) corresponding to the molecular formula $C_{22}H_{18}N_6OS$ and base peak at 267 (100).

2-(1-Methyl-1H-indol-3-ylmethyl)]-5H-8-amino-10-methyl-pyrimidino[2``,3``:5,1]pyrazolo[3`,4`:4,5]thieno[2,3-d]pyrimidine-6-one (7): Yield 42%, m.p. > 300°C (EtOH); IR (Kbr, cm^{-1}): 3386-3215 (NH, NH_2), 1690 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.15 (s, 3H, CH_3), 2.64 (s, 3H, NCH_3), 3.51 (s, 2H, CH_2), 6.92-7.51 (m, 6H, Ar-H), 9.21 (s, 2H, NH_2, exchangeable with D_2O), 11.42 (s, 1H, NH, exchangeable with D_2O); MS *m/z* (%): 415 (M^+, 30) corresponding to the molecular formula $C_{21}H_{17}N_7OS$ and base peak at 271 (100).

2-(1-Methyl-1H-indol-3-ylmethyl)]-5H-8-hydroxy-10-methyl-pyrimidino[2``,3``:5,1]pyrazolo-[3`,4`:4,5]thieno[2,3-d]pyrimidine-6-one (8): Yield 53%, m.p. > 300°C (EtOH); IR (KBr, cm^{-1}): 3540-3390 (OH), 3320-3240 (NH), 1702 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.18 (s, 3H, CH_3), 2.42 (s, 3H, NCH_3), 3.63 (s, 2H, CH_2), 6.43 (s, 1H, NH, exchangeable with D_2O), 6.92-7.51 (m, 6H, Ar-H), 11.30 (s, 1H, OH, exchangeable with D_2O); MS *m/z* (%): 416 (M^+, 42) corresponding to the molecular formula $C_{21}H_{16}N_6O_2S$ and base peak at 235 (100).

2-(1-Methyl-1H-indol-3-ylmethyl)]-6,8-diamino-10-methyl-pyrimidino[2``,3``:5,1]pyrazolo[3`,4`:4,5]thieno[2,3-d]pyrimidine (9): Yield 56%, m.p. 237-239°C (EtOH); IR (KBr, cm^{-1}): 3340-3215 (NH_2); ^{1}H NMR (DMSO-d_6) δ: 2.09 (s, 3H, CH_3), 2.23 (s, 3H, NCH_3), 3.56 (s, 2H, CH_2), 6.92-7.51 (m, 6H, Ar-H), 9.73 (s, 2H, NH_2, exchangeable with D_2O), 10.72 (s, 2H, NH_2, exchangeable with D_2O); MS *m/z* (%): 414 (M^+, 100) corresponding to the molecular formula $C_{21}H_{18}N_8S$ and as base peak.

Synthesis of imidazolo[2``,3``:5,1]pyrazolo[3`,4`:4,5] thieno[2,3-d]pyrimidines (10 and 11): A mixture of compound 4 (0.35 g, 1 mmol) and chloroacetyl chloride and/or oxalyl chloride (1 mmol) in DMF (20 mL) and few drops of piperidine was refluxed for 6 h. After cooling,

the reaction mixture was poured into ice-water; the separated solid was collected and crystallized from appropriate solvent to give 10 and 11,respectively.

2-(1-Methyl-1H-indol-3-ylmethyl)]-5,7-dihydro-9-methyl-imidazolo[2``,3``:5,1]pyrazolo[3`,4`: 4,5]thieno[2,3-d]pyrimidin-6-one (10): Yield 62%, m.p. 243-245°C (DMF/EtOH); IR (KBr, cm^{-1}): 3294 (NH), 1680 (C=O); ^1H NMR (DMSO-d_6) δ: 2.18 (s, 3H, CH_3), 2.53 (s, 3H, NCH_3), 3.54 (s, 2H, CH_2), 3.72 (s, 2H, CH_2), 7.21-7.74 (m, 5H, Ar-H), 11.31 (s, H, NH, exchangeable with D_2O); MS *m/z* (%): 388 (M^+, 8) corresponding to the molecular formula $C_{20}H_{16}N_6OS$ and base peak at 331 (100).

2-(1-Methyl-1H-indol-3-ylmethyl)]-5H-9-methyl-imidazolo[2``,3``:5,1]pyrazolo[3`,4`:4,5]thieno-[2,3-d]pyrimidin-6,7-dione (11): Yield 54%, m.p. 268-270°C (DMF/EtOH); IR (KBr, cm^{-1}): 3342 (NH), 1702 (C=O), 1680 (C=O); ^1H NMR (DMSO-d_6) δ: 2.16 (s, 3H, CH_3), 2.50 (s, 3H, NCH_3), 3.24 (s, 2H, CH_2), 6.91-7.45 (m, 5H, Ar-H), 10.46 (s, H, NH, exchangeable with D_2O); MS *m/z* (%): 402 (M^+, 46) corresponding to the molecular formula $C_{20}H_{14}N_6O_2S$ and base peak at 267 (100).

2-(1-Methyl-1H-indol-3-ylmethyl)]-8-amino-6-phenyl-10-methyl-pyrimidino[2``,3``:5,1]pyrazolo[3`,4`:4,5] thieno[2,3-d]pyrimidine (12): A mixture of compound 4 (0.35 g, 1 mmol) and benzoyl acetonitrile (1 mmol) in ethanol (30 mL) and few drops of piperidine was refluxed for 8 h. The solid product was collected and crystallized to give 12. Yield 51%, m.p. > 300 °C (MeOH); IR (KBr, cm^{-1}): 1620-1435 (C=N, C=C); ^1HNMR (DMSO-d_6) δ: 2.21 (s, 3H, CH_3), 2.43 (s, 3H, NCH_3), 3.27 (s, 2H, CH_2), 7.21-7.85 (m, 11H, Ar-H), 10.31 (s, 2H, NH_2, exchangeable with D_2O); MS *m/z* (%): 475 (M^+, 100) corresponding to the molecular formula $C_{27}H_{21}N_7S$ and as base peak.

2-(1-Methyl-1H-indol-3-ylmethyl)]-8-phenyl-10-methyl-pyrimidino[2``,3``:5,1]pyrazolo[3`,4`:4,5] thieno[2,3-d]pyrimidine (13): A mixture of compound 4 (0.35 g, 1 mmol) and cinnamaldehyde (0.13 g, 1 mmol) in acetic acid (30 mL) was refluxed for 5 h. The solid product was collected and crystallized to give 13. Yield 58%, m.p. 257-259 °C (AcOH); IR (KBr, cm^{-1}): 1612, 1495 (C=N, C=C); ^1H NMR (DMSO-d_6) δ: 2.20 (s, 3H, CH_3), 2.38 (s, 3H, NCH_3), 3.61 (s, 2H, CH_2), 6.89-7.63 (m, 12H, Ar-H); MS *m/z* (%): 460 (M^+, 22) corresponding to the molecular formula $C_{27}H_{20}N_6S$ and base peak at 235 (100).

2-(1-Methyl-1H-indol-3-ylmethyl)]-5H-10-methyl-pyrimidino[2``,3``:5,1]pyrazolo[3`,4`:4,5]-thieno[2,3-d]pyrimidine-8-one (14): A mixture of compound 4 (0.35 g, 1 mmol) and ethyl acrylate (0.11 g, 1 mmol) in DMF (30 mL) and few drops of piperidine was refluxed for 7 h. The solid product was collected and crystallized to give 14. Yield 60%, m.p. 238-239 °C (AcOH); IR (KBr, cm^{-1}): 3310-3256 (NH), 1692 (C=O); ^1HNMR (DMSO-d_6) δ: 2.11 (s, 3H, CH_3), 2.43 (s, 3H, NCH_3), 3.29 (s, 2H, CH_2), 6.25 (s, H, NH, exchangeable with D_2O), 7.21-7.83 (m, 7H, Ar-H); MS *m/z* (%): 400 (M^+, 32) corresponding to the molecular formula $C_{21}H_{16}N_6OS$ and base peak at 303 (100).

Synthesis of Pyrimidino Derivatives 15-18

General Method: A mixture of compound 4 (0.35 g, 1 mmol) and benzylidine derivatives namely: benzylidine acetophenone, benzoyl benzylidine acetonitrile, benzylidine malononitrile and/or ethyl benzylidine cyanoacetate (1 mmol) in DMF (20 mL) in presence of few drops of piperidine was refluxed for 4 h. The solid product was collected and crystallized from appropriate solvent to give 15-18, respectively.

2-(1-Methyl-1H-indol-3-ylmethyl)]-6,8-diphenyl-10-methyl-pyrimidino[2``,3``:5,1]pyrazolo-[3`,4`:4,5] thieno[2,3-d]pyrimidine (15): Yield 55%, m.p.291-293°C (DMF/EtOH); IR (KBr, cm^{-1}): 1620, 1430 (C=N and C=C); ^1H NMR (DMSO-d_6) δ: 2.11 (s, 3H, CH_3), 2.32 (s, 3H, NCH_3), 3.54 (s, 2H, CH_2), 6.83-7.92 (m, 16H, Ar-H); MS *m/z* (%): 536 (M^+, 15) corresponding to the molecular formula $C_{33}H_{24}N_6S$ and base peak at 433 (100).

2-(1-Methyl-1H-indol-3-ylmethyl)]-8-amino-7-benzoyl-6-phenyl-10-methyl-pyrimidino[2``,3``:-5,1] pyrazolo[3`,4`:4,5] thieno[2,3-d]pyrimidine (16): Yield 58%, m.p. > 300°C (EtOH); IR (KBr, cm^{-1}): 3320-3215 (NH_2), 1720 (CO); ^1HNMR (DMSO-d_6) δ: 2.12 (s, 3H, CH_3), 2.38 (s, 3H, NCH_3), 3.27 (s, 2H, CH_2), 6.92-7.75 (m, 15H, Ar-H), 12.30 (s, 2H, NH_2, exchangeable with D_2O); MS *m/z* (%): 579 (M^+, 14) corresponding to the molecular formula $C_{34}H_{25}N_7OS$ and base peak at 305 (100).

2-(1-Methyl-1H-indol-3-ylmethyl)]-8-amino-6-phenyl-10-methyl-pyrimidino[2``,3``:5,1]pyrazolo-[3`,4`:4,5] thieno[2,3-d]pyrimidine-7-carbonitrile (17): Yield 49%, m.p. 275-277°C (DMF/EtOH); IR (KBr, cm^{-1}): 3416-3235 (NH_2), 2225 (CN); ^1HNMR (DMSO-d_6) δ: 2.24 (s, 3H, CH_3), 2.53 (s, 3H, NCH_3), 3.70 (s, 2H, CH_2), 6.82-7.45 (m, 10H, Ar-H), 10.45 (s, 2H, NH_2, exchangeable with D_2O); MS *m/z* (%): 500 (M^+, 100) corresponding to the molecular formula $C_{28}H_{20}N_8S$ and as base peak.

2-(1-Methyl-1H-indol-3-ylmethyl)]-8-amino-7-ethoxycarbonyl-6-phenyl-10-methyl-pyrimidino-[2``,3``:5,1]pyrazolo[3`,4`:4,5]thieno[2,3-d]pyrimidine (18): Yield 51%, m.p. > 300°C (EtOH); IR (KBr, cm^{-1}): 3325-3240 (NH_2), 1715 (C=O); ^1H NMR (DMSO-d_6) δ: 1.38 (s, 3H, CH_3), 2.09 (t, 3H, CH_3), 2.31 (s, 3H, NCH_3), 3.34 (s, 2H, CH_2), 3.56 (q, 2H, CH_2), 7.01-7.65 (m, 10H, Ar-H), 9.56 (s, 2H, NH_2, exchangeable with D_2O); MS *m/z* (%): 547 (M^+, 23) corresponding to the molecular formula $C_{30}H_{25}N_7O_2S$ and base peak at 312 (100).

Pharmacological Screening

Determination of Acute Toxicity (LD_{50}): The *LD_{50}* for compounds were determined by injected different gradual increased doses of the tested compounds to adult male albino rats, then calculating the dose corresponding to 50% animal death, according to Austen *et al.* [32] (Table 1).

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. 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 (Table 2).

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

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

Compound N°	LD_{50} [mg/kg]
3	2.068±0.012
4	2.478±0.013
5	2.100±0.012
6	2.150±0.013
7	1.820±0.011
8	1.914±0.012
9	2.561±0.011
10	2.705±0.011
12	2.681±0.012
14	1.815±0.011
16	1.750±0.011
18	2.111±0.013
Prednisolone®	1.618±0.016

Table 2: Anti-inflammatory activities of some new synthesized compounds

Compound N°	Dose [mg/kg]	Protection against carrageenan-induced edema [%]*	Inhibition of plasma PGE2 [%]*
3	25	87.16±0.058	84.61±0.110
	50	98.41±0.072	95.16±0.120
4	25	93.65±0.080	78.62±0.096
	50	95.10±0.076	82.66±0.087
5	25	92.12±0.066	77.41±0.088
	50	93.15±0.075	81.56±0.086
6	25	91.58±0.090	92.33±0.087
	50	97.16±0.082	95.55±0.110
7	25	90.16±0.077	81.16±0.088
	50	96.18±0.075	91.62±0.100
8	25	63.86±0.065	53.11±0.088
	50	84.10±0.067	73.82±0.079
9	25	55.70±0.069	50.99±0.100
	50	74.13±0.074	71.00±0.098
10	25	65.80±0.076	48.16±0.082
	50	87.16±0.081	79.77±0.079
12	25	93.44±0.086	89.44±0.085
	50	96.18±0.083	92.96±0.094
14	25	-	-
	50	38.14±0.054	31.16±0.076
16	25	55.22±0.055	43.18±0.088
	50	67.15±0.068	62.13±0.078
18	25	76.12±0.068	72.13±0.120
	50	75.24±0.080	73.54±0.100
Prednisolone®	25	81.0±0.100	77.0±0.084
	50	93.0±0.082	91.0±0.087

* The doses tested were 25, 50 mg and carryout three determinations for each dose

Table 3: Analgesic Activities of some new synthesized compounds

Comp. No.	Comparative analgesic potency to Diclofenac potassium after time in minutes			
	10 min	30 min	60 min	120 min
3	0.46±0.01	0.51±0.04	0.68±0.06	1.30±0.08
4	0.42±0.01	0.46±0.04	0.56±0.05	1.11±0.07
5	0.74±0.03	0.98±0.09	1.23±0.11	2.20±0.20
6	0.46±0.01	0.62±0.06	0.76±0.07	1.63±0.06
7	0.44±0.01	0.51±0.05	0.60±0.06	1.28±0.16
8	0.50±0.01	0.80±0.07	0.86±0.08	1.89±0.08
9	0.46±0.02	0.58±0.05	0.73±0.07	1.58±0.04
10	0.64±0.01	0.96±0.08	1.01±0.01	2.15±0.18
12	0.54±0.01	0.85±0.05	0.90±0.08	2.22±0.21
14	0.65±0.02	0.80±0.07	1.12±0.14	2.40±0.14
16	0.46±0.01	0.55±0.05	0.67±0.09	1.44±0.14
18	0.60±0.02	0.92±0.03	1.00±0.01	2.34±0.12
Valdecoxib®	1.00	1.00	1.00	1.00

All results were significantly different from the standard and normal control value at P = 0.05

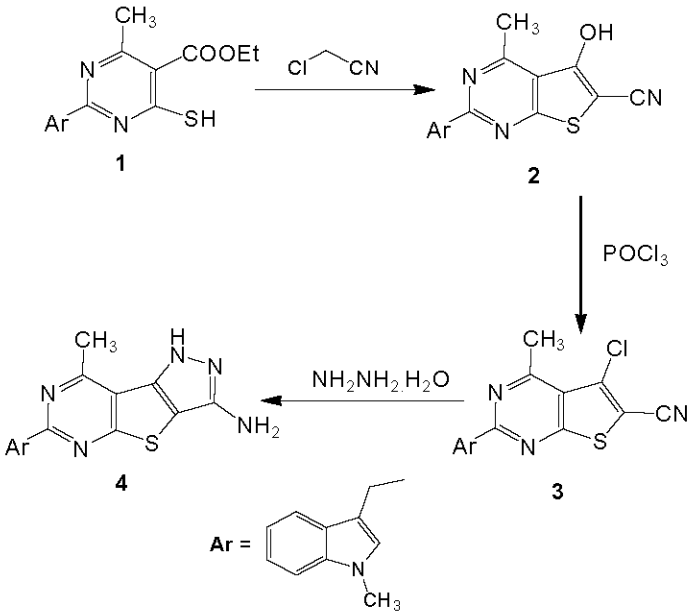
DYNATech, MR 5000 at 405 nm (Dynatech Industries Inc., McLean, VA, USA). The intensity of the bound yellow color is inversely proportional to the concentration of PGE2 in either standard or samples.

Analgesic Activity: Sixty *Webster* mice of both sexes weighting 20-25 g were divided into 10 groups. One group was kept as control (received saline), the second group received vehicle (Gum acacia) and the third one received Valdecoxib® as a reference drug, whereas the other groups received the test compounds (SC administration). Mice were dropped gently in a dry glass beaker of 1 dm^3 capacity maintained at 55-55.5°C. Normal reaction time in seconds for all animals was determined at time intervals of 10, 20, 30, 45, 60, 90 and 120 minutes. This is the interval extending from the instant the mouse reaches the hot beaker till the animals licks its feet or jump out of the beaker (dose 5 mg/kg) [33]. The relative potencies to Valdecoxib® were determined (Table 3).

RESULTS AND DISCUSSION

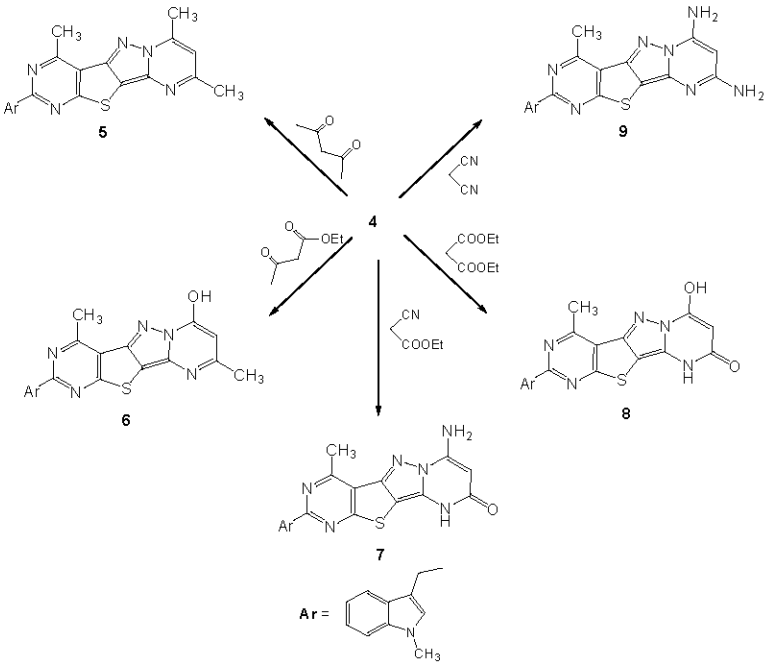
Chemistry: 2-(1-Methyl-1*H*-indol-3-ylmethyl)]-4-methyl-5-ethoxycarbonyl-6-mercaptopyrimidine (1) was prepared according to Ouf *et al.* [34] and used as starting material. The reaction of compound 1 with chloroacetonitrile produced the corresponding thienopyrimidine derivative 2, which was treated with phosphurus oxychloride to afford compound 3, then cyclized by treating with hydrazine hydrate to pyraz olothienopyrimidine 4 (Scheme 1).

Reaction of 4 with active methylene reagents namely, acetyl acetone, ethyl acetoacetate, ethyl cyanoacetate, diethyl malonate or malononitrile in refluxing polyphosphuric acid afforded the corresponding pyrimidopyrazolothieno[2,3-*d*]pyrimidine derivatives 5-9, respectively (Scheme 2).



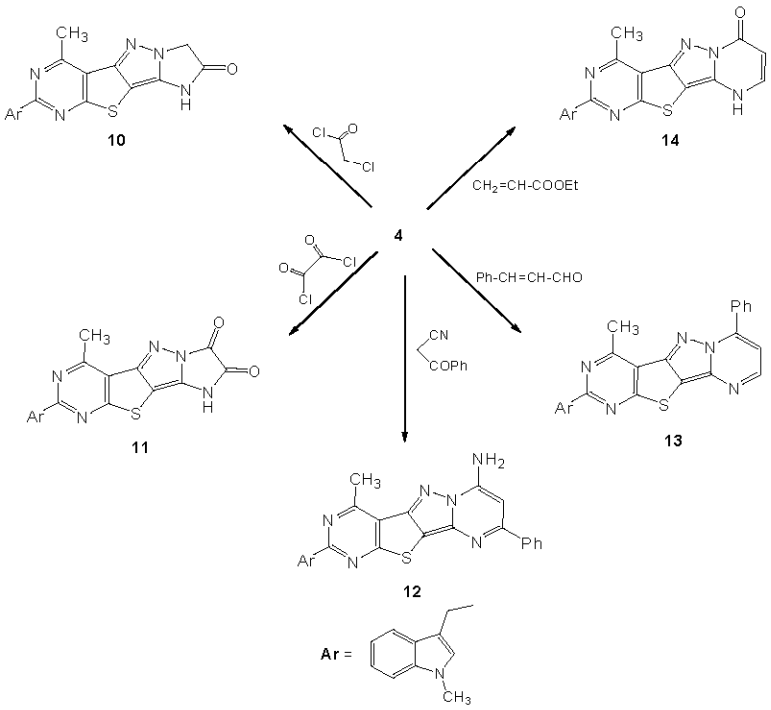
Scheme 1

Scheme 1:



Scheme 2

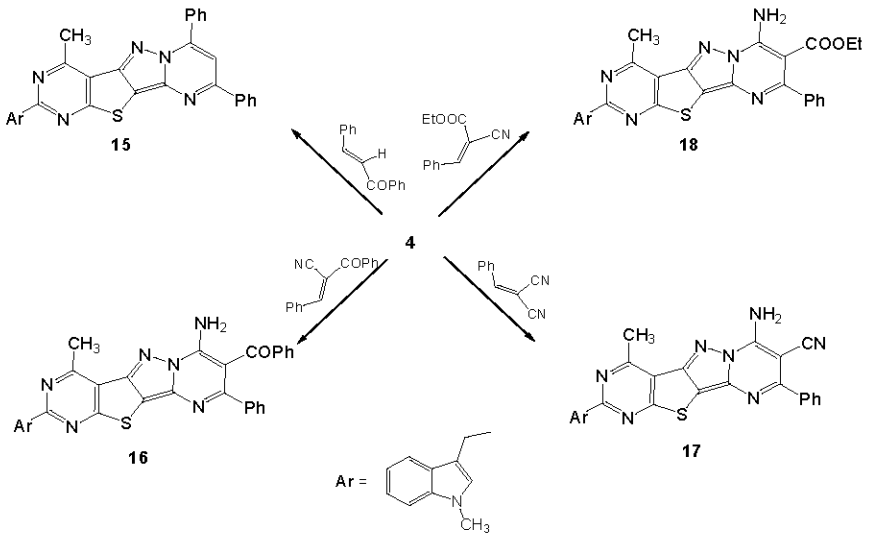
Scheme 2:



Scheme 3

Scheme 3:

Treatment of 4 with chloroacetyl chloride or oxalyl chloride in refluxing DMF in the presence of piperidine as a catalyst afforded the corresponding imidazolopyrazolothieno[2,3-*d*]pyrimidine derivatives 10 and 11, respectively. But, 4 was reacted with benzoyl acetonitrile, cinnamaldehyde or ethyl acrylate to afford pyrimidinopyrazolothieno[2,3-*d*]pyrimidine derivatives 12-14, respectively (Scheme 3).



Scheme 4:

Scheme 4

In addition, compound 4 was reacted with benzylidine derivatives namely, benzoyl benzylidine, benzoyl benzylidine acetonitrile, benzylidine malononitrile or ethyl benzylidine cyanoacetate in refluxing DMF in the presence of few drops of piperidine to yield the corresponding thieno[2,3-*d*]-pyrimidine derivatives 15-18, respectively (Scheme 4).

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 via the determination of their LD_{50}. All compounds were interestingly less toxic than the reference drug (Prednisolone®) (Table 1). Then the newly synthesized compounds were screened pharmacologically for their anti-inflammatory and analgesic activities using male albino rats (Tables 2 and 3).

Anti-Inflammatory Activity

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 rat body weight namely, the protection against Carrageenan® induced edema according Winter *et al.* [35] and the inhibition of plasma PGE2. The later is known as a good confirming indicator for the Carrageenan® induced rat paw edema [36]. Regarding the protection against Carrageenan® induced edema, eight compounds namely 3, 4, 5, 6, 7 and 12 were found more potent than Prednisolone®. Where, their protection percentage against carrageenan induced edema at two dose levels 25 and 50 mg/kg are 87.16±0.06/98.41±0.07, 93.65±0.08/95.10±0.08, 92.12±0.07/93.15±0.08, 91.58±0.10/97.16±0.08, 90.16±0.08/96.18±0.08 and 93.44±0.09/96.18±0.08,respectively(Prednisolone®81/93). On the other hand, the inhibition of plasma PGE2 for the compounds 3, 6, 7 and 12 were found more potent than Prednisolone® at two tested doses levels 25 and 50 mg/kg. The inhibition percentage for the latter compounds were found as: 84.61±0.11/95.16± 0.12, 92.33±0.09/95.55±0.11, 81.16±0.09/91.62±0.100 and 89.44±0.09/92.96±0.09, respectively.

Analgesic Activity: All tested compounds exhibited analgesic activity in a hot plate assay. Interestingly, the analgesic activities of the all the compounds were more potent than Valdecoxib® as a reference drug (Table 2). Compounds 14, 18, 12, 5, 10, 8, 6, 9, 16, 3, 7 and 4 are arranged in descending order of analgesic potency. Compounds 14, 18, 12, 5 and 10 showed more twice times the activity of Valdecoxib® after two hours.

Structure Activity Relationship (SAR): The pyrazolo[3`,4`:4,5] thieno[2,3-d]pyrimidine nucleus essential for both the antiinflammatory and analgesic activities. As nucleophilicity of the substitution increase the activities. As the aromaticity increases with minimal steric hindrance, the activities increase.

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