European Journal of Medicinal Chemistry 44 (2009) 4787–4792

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Analgesic, anticonvulsant and anti-inflammatory activities of some synthesized benzodiazipine, triazolopyrimidine and bis-imide derivatives

Said A. Said [a], Abd El-Galil E. Amr [b,*], Nermien M. Sabry [b], Mohamed M. Abdalla [c]

[a] Department of Chemistry, Faculty of Science, Zagazige University, Zagazige, Egypt
[b] Applied Organic Chemistry Department, National Research Center, El-Tahrir St., Dokki, Cairo, Egypt
[c] Research Unit, Univet Pharmaceutical Co., Egypt

ARTICLE INFO

Article history:
Received 29 January 2009
Received in revised form
30 June 2009
Accepted 16 July 2009
Available online 21 July 2009

Keywords:
4-Phenyl-but-3-en-2-one
Triazolopyrimidines derivatives
Pharmacological screening

ABSTRACT

A series of diazipine, pyrimidine, fused triazolopyrimidine and imide derivatives were newly synthesized using 4-phenyl-but-3-en-2-one **1** as a starting material and compounds **2** and **9** are intermediates. Initially the acute toxicity of the compounds was assayed via the determination of their LD_{50}. All the compounds were interestingly less toxic than the reference drug. The pharmacological screening showed that many of these obtained compounds have good analgesic, anticonvulsant and anti-inflammatory activities comparable to Valdecoxib®, Carbamazepine® and Predensilone® as reference drugs. Regarding the protection against Carrageenan® induced edema, five compounds were found more potent than Prednisolone®. On the other hand, in searching for COX-2 inhibitor, the inhibition of plasma PGE2 for the compounds were determined and four compounds were found more potent than Prednisolone®. The detailed synthesis, spectroscopic data, pharmacological screening and acute toxicity LD_{50} for the synthesized compounds were reported.

1. Introduction

In previous work we prepared certain substituted pyridines, pyrimidines derivatives and their pharmacological screening [1–8]. 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 [9–17]. Pyrimidines present an interesting group of compounds many of which possess wide-spread pharmacological properties such as analgesic, antiarrhythmic, and anticancer activities [18–20]. Recently, some new substituted pyrimidine derivatives have been synthesized, which exhibit analgesic, anti-inflammatory, antiparkinsonian, and androgenic-anabolic activities [21–26]. In view of these observations and as continuation of our previous work on pyrimidine chemistry, we synthesized some new compounds containing pyrimidine nuclei, and tested their analgesic, anticonvulsant and anti-inflammatory activities in comparison to Valdecoxib®, Carbamazepine® and Predensilone® as reference drugs.

2. Results and discussion

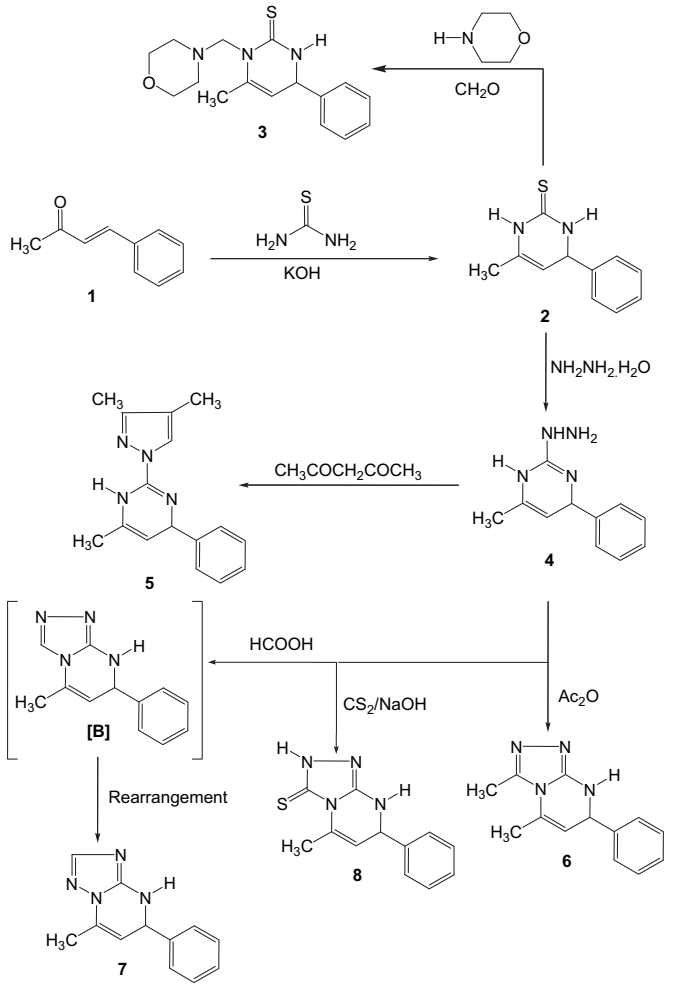
2.1. Chemistry

4-Phenyl-but-3-en-2-one **1** was synthesized according to the reported procedure [27]. The reaction of compound **1** with thiourea in the presence of ethanolic potassium hydroxide yielded the corresponding thioxopyrimidine **2** [28], which was reacted with morpholine in the presence of formaldehyde or hydrazine hydrate in ethanolic potassium hydroxide to afford the corresponding 3-morpholinomethyl- and 2-hydrazino-pyrimidine derivatives **3** and **4**, respectively. The hydrazine derivative **4** was reacted with acetylacetone to give 2-(dimethylpyrazolo)pyrimidine derivative **5**. When it was treated with acetic anhydride or formic acid or carbon disulfide to afford the corresponding fused triazolopyrimidine derivatives **6**–**8**, respectively (Scheme 1). Compound **7** was isolated from **5** via the intermediate **B** according to the Dimroth rearrangement [29] to the more thermodynamically stable product. The structures of the synthesized compounds were assigned on the bases of its spectral data and elemental analysis (cf. Section 4).

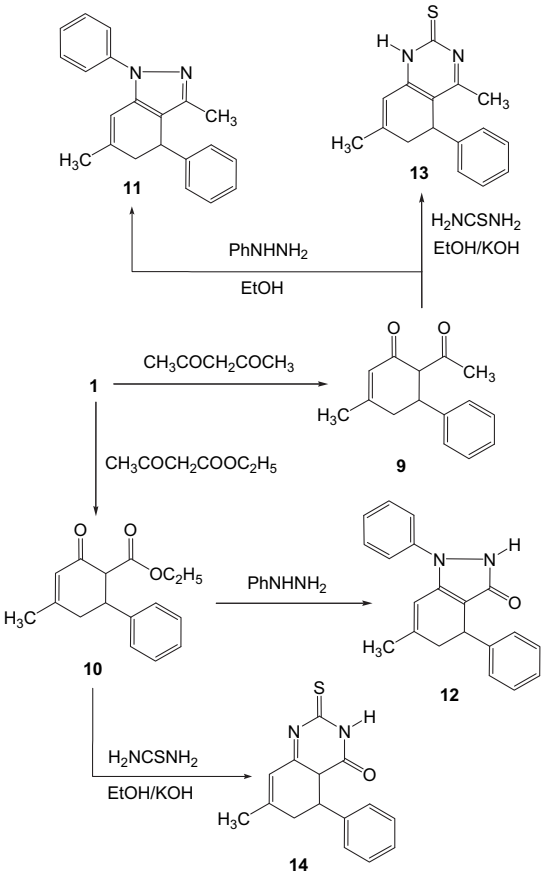
Cycloaddition of **1** with dicarbonyl derivatives, namely, acetylacetone or ethyl acetoacetate in the presence of sodium ethoxide as a catalyst afforded the corresponding substituted cyclohexenone derivatives **9** [30] and **10**, respectively. The reaction of **9** or **10** with phenylhydrazine in refluxing ethanol afforded the corresponding

* Corresponding author. Fax: +202 33370 931.
E-mail address: aamr1963@yahoo.com (A.E.-GalilE. Amr).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.07.013



Scheme 1.



Scheme 2.

N-phenylpyrazolo derivatives **11** and **12**, respectively. While, condensation of **9** or **10** with thiourea in refluxing alcoholic potassium hydroxide afforded the corresponding substituted thioxopyrimidine derivatives **13** and **14**, respectively (Scheme 2). The structures of the synthesized compounds were assigned on the bases of its spectral data and elemental analysis (cf. Section 4).

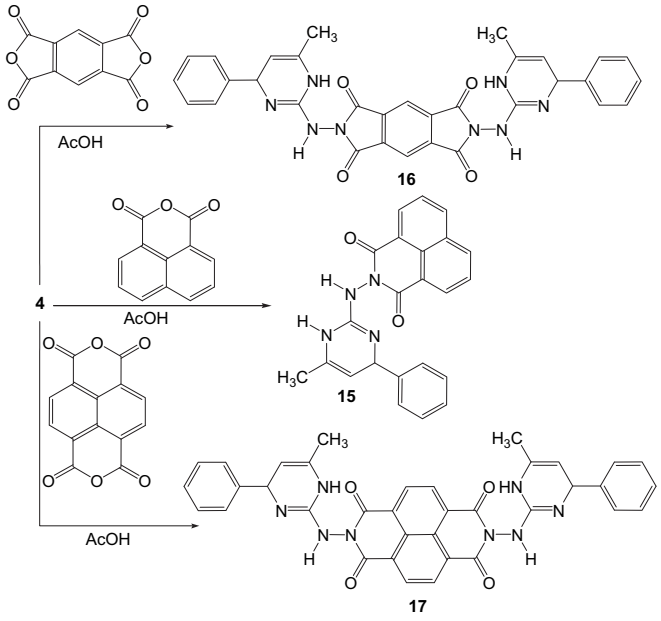
Finally, condensation of **6** with acid anhydrides, namely, 1,8-naphthalene dicarboxylic acid anhydride, 1,2,4,5-benzenetertracarboxylic acid dianhydride or 1,4,5,8-naphthalene tertracarboxylic acid dianhydride in refluxing glacial acetic acid afforded the corresponding imide **15** and bis-imide derivatives **16** and **17**, respectively (Scheme 3). The structures of the synthesized compounds were assigned on the bases of its spectral data and elemental analysis (cf. Section 4).

2.2. Pharmacological screening

The tested three pharmacological properties namely, analgesic, anticonvulsant, and anti-inflammatory all have despite their different biological receptors a neurological basis. Nien representative compounds **2**, **3**, **7**, **8**, **10**, **12**, **14**, **15**, and **17** were studied with respect to these properties. 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).

2.2.1. Analgesic activity

All the tested compounds exhibited analgesic activities in a hot plate assay (Table 2). The most potent are compounds **2** and **14** showing higher activities than that of Valdecoxib by nearly



Scheme 3.

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

Compound no.	LD_{50}/mg kg^{-1}
Prednisolone®	1.618
1	1.985
2	1.805
3	1.688
4	1.878
5	1.790
6	1.970
7	1.751
8	1.800
9	1.715
10	1.735
11	1.920
12	1.860
13	1.872
14	1.882
15	1.850
16	1.680
17	1.780

130–150%. Also, the analgesic activities of **10**, **15**, **13**, **12**, **3**, **17**, and **7** approached those of Valdecoxib, and showed 60–90% activity as compared to Valdecoxib) (100%) (Table 2).

2.2.2. Anticonvulsant activity

Antagonism against yohimbine-induced clonic seizures in mice is considered to be a predictive model of potential anticonvulsant and GABA-mimetic [31]. Compounds **8**, **12**, and **15** are devoid of anticonvulsant activity in the yohimbine-induced clonic seizures assay, in which they provide no protection against yohimbine-induced clonic seizures. Compounds **2** and **10** showed interesting anticonvulsant activities. Their relative potencies to Carbamazepine (1.0) are 1.0 and 0.68, respectively. Compounds **3**, **7**, **14**, and **17** are more potent than Carbamazepine where their relative potencies are 2.30, 2.51, 2.04, and 1.80, respectively (Table 3). ED_{50} was estimated via determining the dose, which protected 5% of the tested animals against the convulsant induced by yohimbine.

2.2.3. Anti-inflammatory activity

2.2.3.1. Purpose and rational. For the determination of the anti-phlogistic 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. [32] and the inhibition of plasma PGE2. The latter is known as a good confirming indicator for the carrageenan-induced rat paw edema [33]. Regarding the protection against carrageenan-induced edema, four compounds, namely **2**, **3**, **7**, and **15** were found to be more potent than Prednisolone®. For these compounds, a similar activity profile was realized for the inhibition of plasma PGE2 (Tables 4 and 5).

3. Conclusion

In conclusion, several new substituted pyrimidine and fused triazolopyrimidine derivatives displaying potential analgesic, anticonvulsant and anti-inflammatory activities were synthesized from 4-phenyl-but-3-en-2-one **1** in few steps and good yield. Particularly, compounds **2** and **14** showing higher activities than that of Valdecoxib as analgesic agents and also compounds **3**, **7**, **14**, and **17** are more potent than Carbamazepine as anticonvulsant agents. While, four compounds, namely **2**, **3**, **7**, and **15**, were found to be more potent than Prednisolone® as anti-inflammatory agents.

4. Experimental

4.1. Chemistry

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus. Elemental analyses were performed on Elementar, Vario EL, Microanalytical Unit, National Research Center, Cairo, Egypt and were found within ±0.4% of the theoretical values. Infrared spectra were recorded on Carlzeise Spectrophotometer 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 the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. The mass spectra were measured using a Finnigan SSQ 7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-precoated aluminum sheets (Type 60 F_{254}, Merck, Darmstadt, Germany).

*4.1.1. 6-Methyl-1-morpholin-4-ylmethyl-4-phenyl-3,4-dihydro-1H-pyrimidine-2-thione (**3**)*

A mixture of compound **2** (0.2 g, 1 mmol), morpholine (~0.1 g, 1 mmol) and formaldehyde (0.1 g, ~3 mmol) in 30 ml ethanol was refluxed for 3 h. The reaction mixture was concentrated under reduced pressure, the separated solid was collected by filtration and crystallized from methanol to give compound **3** in pure form (65% yield); m.p. 148–150 °C; IR (KBr) ν_{max}: 3395 (NH), 3049 (aromatic CH), 2914 (aliphatic CH), 1560 (C=C), 1245 (C=S) cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6): δ 1.71 (s, 3H, CH_3), 2.38 (t, 4H, 2 CH_2-N), 3.55 (t, 4H, 2CH_2–O), 4.65 (s, 2H, N–CH_2–N), 4.15 (d, 1H, CH-pyrimidine), 6.80–7.40 (m, 6H, Ar-H + CH-pyrimidine), 8.82 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ19.82 (CH_3), 51.15, 66.30 (4CH_2), 67.10 (N–CH_2–N), 57.05, 98.65, 144.85 (pyramid-C), 174.85 (C=S), 125.80, 126.55, 128.42, 142.20 (Ph–C);

Table 2
Analgesic activities of selected compounds in a hot plate assay.

Compound	Analgesic activity related to Valdecoxib after						
	10 min ± SE	20 min ± SE	30 min ± SE	45 min ± SE	60 min ± SE	90 min ± SE	120 min ± SE
Valdecoxib	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.01
2	1.30 ± 0.19	1.41 ± 0.13	1.40 ± 0.28	1.39 ± 0.31	1.41 ± 0.19	1.39 ± 0.11	1.40 ± 0.28
3	0.60 ± 0.013	0.62 ± 0.011	0.72 ± 0.012	0.74 ± 0.018	0.76 ± 0.011	0.76 ± 0.011	0.76 ± 0.013
7	0.61 ± 0.010	0.62 ± 0.016	0.72 ± 0.013	0.72 ± 0.018	0.73 ± 0.018	0.76 ± 0.016	0.76 ± 0.012
8	0.76 ± 0.012	0.83 ± 0.014	0.82 ± 0.012	0.85 ± 0.015	0.86 ± 0.018	0.83 ± 0.012	0.83 ± 0.019
10	0.86 ± 0.010	0.87 ± 0.010	0.88 ± 0.011	0.90 ± 0.016	0.90 ± 0.015	0.92 ± 0.015	0.90 ± 0.017
12	0.80 ± 0.014	0.88 ± 0.015	0.90 ± 0.017	0.93 ± 0.021	0.94 ± 0.028	0.92 ± 0.018	0.93 ± 0.024
14	0.97 ± 0.013	0.98 ± 0.015	1.40 ± 0.140	1.49 ± 0.180	1.50 ± 0.320	1.50 ± 0.300	1.40 ± 0.420
15	0.65 ± 0.012	0.60 ± 0.012	0.82 ± 0.012	0.84 ± 0.015	0.85 ± 0.020	0.85 ± 0.016	0.86 ± 0.017
17	0.88 ± 0.011	0.90 ± 0.010	0.93 ± 0.016	0.88 ± 0.019	0.83 ± 0.019	0.79 ± 0.016	0.64 ± 0.011

Table 3
Anticonvulsant activities of selected compounds (as ED_{50} values) needed to antagonize yohimbine-induced clonic seizure and compared to the anticonvulsant activity of Carbamazepine.

Compound	ED_{50} [mg/kg]/±SE	Relative potency compared to Carbamazepine ± SE
Control	0	0
Carbamazepine	28 ± 0.30	1.0 ± 0.01
2	32 ± 0.34	1.0 ± 0.0091
3	13 ± 0.12	2.30 ± 0.019
7	10 ± 0.11	2.51 ± 0.021
8	No protection	No protection
10	50 ± 0.39	0.68 ± 0.008
12	No protection	No protection
14	15 ± 0.12	2.04 ± 0.023
15	No protection	No protection
17	15 ± 0.13	1.80 ± 0.0178

MS *m*/*z* (%): $M^+ + 1$, 304 (8), 265 (12), 149 (18), 111 (25), 82 (100). Analysis calculated for $C_{16}H_{21}N_3OS$ (303.43): C, 63.34; H, 6.98; N, 13.85; S, 10.57. Found: C, 63.26; H, 6.92; N, 13.80; S, 10.52.

*4.1.2. 6-(Methyl-4-phenyl-1,4-dihydro-pyrimidin-2-yl)-hydrazine (**4**)*

A mixture of **2** (0.2 g, 1 mmol) and hydrazine hydrate (0.4 ml, 8 mmol) in ethanolic KOH (30 ml, 2%) was heated under reflux for 5 h. The reaction mixture was cooled and the precipitated product was filtered off, dried, and crystallized from methanol to give compound **4** (70% yield); mp. 162–163 °C; IR (KBr) ν_{max}: 3500–3368 (NH, NH_2), 3028 (aromatic CH), 2914 (aliphatic CH), 1652 (C═N), 1548 (C═C) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.78 (s, 3H, CH_3), 2.65 (s, 2H, NH_2 exchangeable with D_2O), 4.05 (d, 1H, CH-pyrimidine), 6.80–7.50 (m, 6H, Ar-H + CH-pyrimidine), 8.20 and 8.80 (2s, 2H, 2 NH exchangeable with D_2O); MS *m*/*z* (%): M^+, 202 (8), 170 (12), 157 (18), 131 (100), 91 (80). Analysis calculated for $C_{11}H_{14}N_4$ (202.26): C, 65.32; H, 6.98; N, 27.70. Found: C, 65.27; H, 6.93; N, 27.65.

*4.1.3. 2-(3,4-Dimethylpyrazol-1-yl)-6-methyl-4-phenyl-1,4-dihydropyrimidine (**5**)*

To a solution of compound **4** (0.2 g, 1 mmol) in methanol (50 ml), acetylacetone (0.1 g, 1 mmol) was added. The reaction mixture was refluxed for 5 h, then the obtained solid was filtered off, dried and crystallized from ethanol to give **5** in 65% yield; m.p. 228–230 °C; IR (KBr) ν_{max}: 3405 (NH), 3015 (aromatic CH), 2924 (aliphatic CH), 1652 (C═N), 1558 (C═C) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.90, 2.44 and 2.48 (3s, 9H, 3 CH_3), 3.95 (d, 1H, CH-pyrimidine), 6.75 (d, 1H, CH-pyrimidine), 7.00–7.40 (m, 5H, Ar-H), 7.80 (s, 1H, CH-pyrazole), 10.45 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 11.50, 14.50, 21.05 ($3CH_3$), 56.85, 97.55, 147.20, 162.84 (pyramid-C), 114.65, 128.10, 139.65 (pyrazole-C), 125.66, 127.12, 128.85, 141.55 (Ph-C); MS *m*/*z* (%): M^+, 268 (2), $M^+ - 2$, 266 (7), 250 (6), 215 (25), 185 (12), 149 (100), 134 (15), 91 (28). Analysis calculated for $C_{16}H_{18}N_4$ (266.35): C, 72.15; H, 6.81; N, 21.04. Found: C, 72.06; H, 6.78; N, 20.98.

Table 4
Anti-inflammatory potency of the synthesized compounds (protection against Carragenan® induced edema).

Compound no.	Dose/mg kg^{-1}	% Protection against Carrageenan® induced edema
Predensilone®	25	81.00
	50	92.00
2	25	84.52
	50	96.10
3	25	85.84
	50	98.41
7	25	85.25
	50	96.05
8	25	–
	50	42.38
10	25	–
	50	60.26
12	25	63.90
	50	64.80
14	25	74.38
	50	85.24
15	25	87.80
	50	93.15
17	25	48.50
	50	51.60

Table 5
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
	50	91.00
2	25	78.36
	50	93.45
3	25	90.30
	50	978.10
7	25	80.65
	50	94.63
8	25	–
	50	66.80
10	25	–
	50	54.46
12	25	54.88
	50	66.98
14	25	72.08
	50	82.75
15	25	88.65
	50	95.62
17	25	58.76
	50	65.00

*4.1.4. Synthesis of fused triazolopyrimidine derivatives **6** and **7***

A solution of **4** (0.2 g, 1 mmol) in acetic anhydride or formic acid (50 ml) was heated under reflux for 5 h. The reaction mixture was concentrated under reduced pressure, the solid obtained was filtered off, washed with water, dried and crystallized from ethanol to give triazolopyrimidine **6** and **7**, respectively.

*4.1.4.1. 3,5-Dimethyl-7-phenyl-7,8-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine (**6**).* Yield: 65%; m.p. 252–254 °C. IR (KBr) ν_{max}: 3426 (NH), 3028 (aromatic CH), 2912 (aliphatic CH), 1652 (C═N), 1600 (C═C) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.70 and 2.49 (2s, 6H, 2 CH_3), 3.98 (d, 1H, CH-pyrimidine), 6.65 (d, 1H, CH-pyrimidine), 6.97–7.51 (m, 5H, Ar-H), 10.49 (s, 1H, NH exchangeable with D_2O); MS *m*/*z* (%): M^+, 226 (18), 211 (32), 196 (82), 168 (100), 142 (15), 134 (10), 91 (28). Analysis calculated for $C_{13}H_{14}N_4$ (226.28): C, 69.00; H, 6.24; N, 24.76. Found: C, 68.95; H, 6.20; N, 24.71.

*4.1.4.2. 7-Methyl-5-phenyl-4,5-dihydro-[1,2,4]triazolo[1,5-a]pyrimidine (**7**).* Yield 60%; m.p. 250–252 °C. IR (KBr) ν_{max}: 3402 (NH), 3026 (aromatic CH), 2912 (aliphatic CH), 1660 (C═N), 1596 (C═C) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.90 (s, 3H, CH_3), 3.95 (d, 1H, CH-pyrimidine), 6.80–7.48 (m, 6H, Ar-H + CH-pyrimidine), 7.52 (s, 1H, CH-triazole), 8.45 (s, 1H, NH exchangeable with D_2O); MS *m*/*z* (%): M^+, 212 (28), 196 (18), 168 (85), 156 (14), 91 (82), 77 (100). Analysis calculated for $C_{12}H_{12}N_4$ (212.26): C, 67.91; H, 5.70; N, 26.40. Found: C, 67.86; H, 5.65; N, 26.34.

*4.1.5. 5-Methyl-7-phenyl-7,8-dihydro-2H-[1,2,4]triazolo[4,3-a]pyrimidine-3-thione (**8**)*

A mixture of **4** (0.2 g, 1 mmol) and carbon disulphide (1 ml) in ethanolic sodium hydroxide (0.4 g in ethanol 15 ml) was refluxed

on a water bath for 10 h. The reaction mixture was evaporated under reduced pressure, the residue was dissolved in water, the alkaline solution was filtered off. The clear filtrate was acidified with acetic acid, the obtained precipitate was collected by filtration, dried and crystallized from ethanol to give compound **8**. Yield: 75%; m.p. 144–146 °C; IR (KBr) ν_{max}: 3415 and 3396 (2 NH), 3025 (aromatic CH), 2918 (aliphatic CH), 1656 (C=N), 1565 (C=C), 1248 (C=S) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.72 (s, 3H, CH_3), 4.20 (d, 1H, CH-pyrimidine), 6.82–7.70 (m, 6H, Ar–H + CH-pyrimidine), 8.80 and 9.90 (2s, 2H, 2 NH exchangeable with D_2O); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 20.80 (CH_3), 52.95, 98.15, 144.75, 153.88 (pyramid-C), 152.10 (C=S), 126.55, 126.95, 127.80, 142.10 (Ph-C); MS *m/z* (%): $M^+ - 1$, 243 (12), 215 (65), 200 (82), 148 (35), 91 (100). Analysis calculated for $C_{12}H_{12}N_4S$ (244.32): C, 58.99; H, 4.95; N, 22.93; S, 13.12. Found: C, 58.95; H, 4.91; N, 22.89; S, 13.08.

*4.1.6. Synthesis of 4-methyl-2-oxo-6-phenyl-cyclohex-3-enecarboxylic acid ethyl ester (**10**)*

A solution of ethyl acetoacetate (10 mmol) in sodium ethoxide solution (0.3 g sodium metal in 140 ml absolute ethanol) was stirred at r.t. for 1 h. The chalcone **1** (12 mmol) was added to the above solution with stirring. The reaction mixture was refluxed for 3 h, poured onto cold hydrochloric acid. The obtained solid was filtered off, washed with water, dried, and crystallized from methanol to give **10**, in yield 60%; mp. 95–97 °C (MeOH); IR (KBr) ν_{max}: 3026 (aromatic CH), 2912 (aliphatic CH), 1735 (C=O, ester), 1709 (C=O), 1580 (C=C) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.23 and 1.85 (2s, 6H, 2 CH_3), 2.41 (t, 2H, CH_2), 3.30–3.43 (m, 1H, CH), 3.56 (t, 1H, CH), 4.15 (q, 2H, CH_2), 6.25 (s, 1H, CH), 7.00–7.43 (m, 5H, Ar-H); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 14.45, 22.46 (2CH_3), 59.75 (CH_2), 25.35, 34.25, 62.78, 124.65, 156.66 (cyclohexene-C), 169.88, 196.85 (2C=O), 125.62, 126.95, 128.35, 145.50 (Ph-C); MS *m/z* (%): $M^+ - 2$, 256 (10), 241 (22), 218 (42), 181 (36), 149 (68), 90 (100). Analysis calculated for $C_{16}H_{18}O_3$ (258.32): C, 74.40; H, 7.02. Found: C, 74.35; H, 6.95.

*4.1.7. Synthesis of dimethyl-diphenyl-indazole derivatives **11** and **12***

A mixture of **9** or **10** (1 mmol) and phenylhydrazine (0.11 g, 1 mmol) in 20 ml ethanol was refluxed for 6 h. The reaction mixture was evaporated under reduced pressure, the residue was triturated with ethyl ether. The obtained solid was filtered off, dried and crystallized from ethanol to give **11** and **12**, respectively.

*4.1.7.1. 3,6-Dimethyl-1,4-diphenyl-4,5-dihydro-1*H*-indazole (**11**).* Yield 70%; m.p. 150–152 °C. IR (KBr) ν_{max}: 3033 (aromatic CH), 2913 (aliphatic CH), 1660 (C=N), 1602 (C=C) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.65 and 2.20 (2s, 6H, 2 CH_3), 2.38 (d, 2H, CH_2), 3.46 (t, 1H, CH), 6.28 (s, 1H, CH), 6.95–7.65 (m, 10H, Ar-H); MS *m/z* (%): $M^+ - 1$, 299 (3), 283 (21), 212 (30), 149 (100), 134 (18), 91 (45). Analysis calculated for $C_{21}H_{20}N_2$ (300.41): C, 83.96; H, 6.71; N, 9.33. Found: C, 83.90; H, 6.66; N, 9.28.

*4.1.7.2. 6-Methyl-1,4-diphenyl-1,2,4,5-tetrahydro-indazol-3-one (**12**).* Yield 70%; mp. 132–134 °C. IR (KBr) ν_{max}: 3380 (NH), 3032 (aromatic CH), 2916 (aliphatic CH), 1665 (C=O), 1654 (C=N), 1560 (C=C) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.66 (s, 3H, CH_3), 2.40 (d, 2H, CH_2), 3.48 (t, 1H, CH), 6.24 (s, 1H, CH), 6.98–7.45 (m, 10H, Ar-H), 8.42 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 22.85 (CH_3), 25.48, 42.25, 106.55, 115.65, 144.95, 149.50 (cyclohexene-C), 156.88 (C=O), 112.20, 118.85, 125.90, 127.15, 128.15, 128.45, 135.60, 142.50 (2Ph-C); MS *m/z* (%): $M^+ + 2$, 304 (4), 284 (15), 256 (12), 213 (5), 185 (8), 149 (18), 97 (25), 82 (100). Analysis calculated for $C_{20}H_{18}N_2O$ (302.38): C, 79.44; H, 6.00; N, 9.26. Found: C, 79.40; H, 5.95; N, 9.2.

*4.1.8. Synthesis of substituted quinazolines **13** and **14***

Thiourea (~0.1 g, 1 mmol) was added to **9** or **10** (1 mmol) in 100 ml ethanolic potassium hydroxide (2%). The reaction mixture was refluxed for 6 h, and then poured gradually with stirring into cold water. The solid formed was filtered off, washed with H_2O, and crystallized from ethanol to give **13** and **14**.

*4.1.8.1. 4,7-Dimethyl-5-phenyl-5,6-dihydro-1*H*-quinazoline-2-thione (**13**).* Yield 65%; m.p. 174–176 °C. IR (KBr) ν_{max}: 3375 (NH), 3015 (aromatic CH), 2910 (aliphatic CH), 1658 (C=N), 1555 (C=C), 1250 (C=S) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.21 and 1.70 (2s, 6H, 2 CH_3), 2.49 (t, 2H, CH_2), 3.30 (t, 1H, CH), 7.19–7.40 (m, 6H, Ar-H + CH), 8.20 (s, 1H, NH exchangeable with D_2O); MS *m/z* (%): M^+, 268 (12), 224 (100), 210 (24), 196 (10), 170 (22), 92 (16). Analysis calculated for $C_{16}H_{16}N_2S$ (268.38): C, 71.61; H, 6.01; N, 10.44; S, 11.95. Found: C, 71.56; H, 5.94; N, 10.39; S, 11.90.

*4.1.8.2. 7-Methyl-5-phenyl-2-thioxo-2,4a,5,6-tetrahydro-3*H*-quinazolin-4-one (**14**).* Yield 70%; mp. 202–204 °C. IR (KBr) ν_{max}: 3444 (NH), 3026 (aromatic CH), 2904 (aliphatic CH), 1706 (C=O), 1654 (C=N), 1596 (C=C), 1245 (C=S) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.72 (s, 3H, CH_3), 2.42 (d, 2H, CH_2), 2.75 (d, 1H, CH), 3.25 (m, 1H, CH), 6.28 (s, 1H, CH), 6.98–7.40 (m, 5H, Ar-H), 8.45 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 22.56 (CH_3), 28.55, 35.05, 46.58, 105.95, 152.66, 163.60 (cyclohexene-C), 170.99 (C=O), 185.05 (C=S), 125.65, 126.65, 128.10, 147.50 (Ph-C); MS *m/z* (%): M^+ 270 (28), 255 (10), 241 (12), 212 (35), 149 (100), 134 (22), 91 (45). Analysis calculated for $C_{15}H_{14}N_2OS$ (270.36): C, 66.64; H, 5.22; N, 10.36; S, 11.86. Found: C, 66.60; H, 5.18; N, 10.3; S, 11.80.

*4.1.9. Synthesis of imid **15** and bis-imids **16** and **17***

A mixture of **4** (0.4 g, 2 mmol) and acid anhydride, namely, 1,8-naphthalene dicarboxylic anhydride (2 mmol), 1,2,4,5-benzenetertracarboxylic acid dianhydride (1 mmol) or 1,4,5,8-naphthalene tertracarboxylic acid dianhydride (1 mmol) in 50 ml glacial acetic acid was heated under reflux for 6 h. The reaction mixture was concentrated under reduced pressure, the residue was solidified with ether, filtered off and crystallized from the proper solvents to yield **15**–**17**, respectively.

*4.1.9.1. 2-(6-Methyl-4-phenyl-1,4-dihydro-pyrimidin-2-ylamino)-benzo[*de*]isoquinoline-1,3-dione (**15**).* Yield 78%; mp. 240–242 °C (dioxane); IR (KBr) ν_{max}: 3450–3330 (2 NH), 3024 (aromatic CH), 2910 (aliphatic CH), 1722 (2 C=O), 1655 (C=N), 1560 (C=C) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.74 (s, 3H, CH_3), 4.15 (d, 1H, CH-pyrimidine), 6.85–7.38 (m, 6H, Ar-H + CH-pyrimidine), 7.55–7.90 (m, 6H, naphthyl-H), 8.18 and 8.76 (2s, 2H, 2 NH exchangeable with D_2O); MS *m/z* (%): $M^+ + 2$ 384 (19), M^+, 382 (8), 368 (14), 231 (85), 153 (80), 125 (60), 97 (100). Analysis calculated for $C_{23}H_{18}N_4O_2$ (382.43): C, 72.24; H, 4.74; N, 14.65. Found: C, 72.18; H, 4.70; N, 14.60.

*4.1.9.2. 2,7-Bis-(6-methyl-4-phenyl-1,4-dihydro-pyrimidin-2-ylamino)-benzo[*lmn*][3,8]phena-nthroline-1,3,6,8-tetraone (**16**).* Yield 60%; m.p. >250 °C (AcOH/H_2O). IR (KBr) ν_{max}: 3520–3380 (4 NH), 3035 (aromatic CH), 2918 (aliphatic CH), 1722, 1724 (4 C=O), 1665 (C=N), 1570 (C=C) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.72 (s, 6H, 2 CH_3), 4.18 (d, 2H, 2 CH-pyrimidine), 6.90–7.42 (m, 12H, 10 Ar-H + 2 CH-pyrimidine), 7.88 (s, 2H, Ar-H), 8.22 and 8.82 (2s, 4H, 4 NH exchangeable with D_2O); MS *m/z* (%): M^+, 586 (4), 552 (8), 384 (25), 355 (10), 231 (100), 153 (55), 97 (64). Analysis calculated for $C_{32}H_{26}N_8O_4$ (586.62): C, 65.52; H, 4.47; N, 19.10. Found: C, 65.48; H, 4.42; N, 19.02.

*4.1.9.3. 2,6-Bis-(6-methyl-4-phenyl-1,4-dihydro-pyrimidin-2-ylamino)pyrrolo[3,4-*f*]isoindole-1,3,5,7-tetraone (**17**).* Yield 65%; m.p.

>250 °C (acetic acid/water). IR (KBr) ν_{max}: 3535–3382 (4 NH), 3032 (aromatic CH), 2924 (aliphatic CH), 1718, 1720 (4 C=O), 1662 (C=N), 1565 (C=C) cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6): δ 1.68 (s, 6H, 2 CH_3), 4.22 (d, 2H, 2 CH-pyrimidine), 6.86–7.44 (m, 12H, 10 Ar-H + 2 CH-pyrimidine), 7.95 (d, 4H, naphthyl-H), 8.26 and 8.78 (2s, 4H, 4 NH exchangeable with D_2O); MS m/z (%): $M^+ + 1$, 637 (10), 621 (8), 552 (20), 478 (12), 384 (15), 292 (25), 231 (45), 153 (76), 91 (100). Analysis calculated for $C_{36}H_{28}N_8O_4$ (636.68): C, 67.92; H, 4.43; N, 17.60. Found: C, 67.88; H, 4.38; N, 17.55.

4.2. Pharmacological screening

All animals were obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. The acute toxicity (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. [34].

4.2.1. 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 tested compounds (SC administration). Mice were dropped gently in a dry glass beaker of one-liter 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 min. 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) [35] relative potencies to that of Valdecoxib were determined (Table 2).

4.2.2. Anticonvulsant activity

Male Webster mice (20–30 g) were individually placed in clear plastic cylinder and the tested compounds were administrated intra-peritoneally (5 mg/kg), 30 min prior to a dose of 45 mg/kg of yohimbine-HCl. The animals were observed for onset and number of clonic seizures [36] (Table 3). Evaluation of ED_{50} values for compounds with 95% confidence limits were calculated for the antagonism of yohimbine-induced clonic seizure according to Austen et al. [34].

4.2.3. Anti-inflammatory activity

4.2.3.1. 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 1 h before carrageenan challenge. Foot paw edema was induced by subplantar injection of 0.05 ml 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.

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.

References

[1] A.E. Amr, A.A. Nehad, M.M. Abdalla, Bioorg. Med. Chem. 14 (2006) 373–380.
[2] A.E. Amr, M.M. Abdalla, Bioorg. Med. Chem. 14 (2006) 4341–4352.
[3] A.E. Amr, M.S. Nermien, M.M. Abdalla, F.A. Bakr, Eur. J. Med. Chem. 44 (2009) 725–735.
[4] A.E. Amr, A.A. Korany, M.M. Abdalla, Eur. J. Med. Chem. 44 (2009) 901–909.
[5] A.E. Amr, M.H. Abo-Ghalia, M.M. Abdalla, Arch. Pharm. Chem. Life Sci. 340 (2007) 304–309.
[6] M.F. Issa, A.E. Amr, M.S. Nermien, M.M. Abdalla, Arch. Pharm. Chem. Life Sci. 341 (2008) 174–180.
[7] A.F. El-Farargy, M.M. Hamad, S.A. Said, A.F.S. Ahmed, G.M. El-Gendy, Pak. J. Sci. Ind. Res. 35 (1992) 19–28.
[8] M.M. Hamad, S.A. Said, Y.M. El-Ekyabi, Monatsh. Chem. 127 (1996) 549–555.
[9] J. Clark, M.S. Shohhet, D. Korakas, G. Varvounis, J. Heterocycl. Chem. 30 (1993) 1065–1072.
[10] I.Y. Kogowwra, N.N. Yimatsusita, J.K. Pfkador, Eur. J. Med. Chem. 28 (1993) 769–781.
[11] B. Tozkoparan, M. Ertan, P. Kelicen, R. Demirdamar, Farmaco 54 (1999) 588–593.
[12] J. Quiroga, B. Insuasty, S. Craz, P. Herrandez, A. Bolafios, R. Moreno, A. Hormoza, R.H. De Almeidas, J. Heterocycl. Chem. 35 (1998) 1333–1338.
[13] M. Santagati, M. Modica, A. Santagati, F. Russo, S. Spampinato, Pharmazie 51 (1996) 7–15.
[14] V.K. Ahluwalia, M. Chopra, R. Chandra, J. Chem. Res (s) (2000) 162–163.
[15] L.V.G. Nargund, V.V. Badiger, S.U. Yarnal, Eur. J. Med. Chem. 29 (1994) 245–247.
[16] M. Vanlaar, E. Volerts, M. Verbaten, Psychopharmacology 154 (2001) 189–197.
[17] K. Danel, E.B. Pedersen, C. Nielsen, J. Med. Chem. 41 (1998) 191–198.
[18] A.A. Nehad, M.S. Nermien, M.M. Ashraf, M.M. Abdulla, Monatsh. Chem. 138 (2007) 715–721.
[19] F.A.S. Ahmed, M.M. Abdulla, A.E. Amr, A.H. Azza, Monatsh. Chem. 138 (2007) 1019–1027.
[20] A.G. Hammam, A.F.M. Fahmy, A.E. Amr, A.M. Mohamed, Indian J. Chem. 42B (2003) 1985–1993.
[21] A.E. Amr, M.I. Hegab, A.A. Ibrahim, M.M. Abdalah, Monatsh. Chem. 134 (2003) 1395–1409.
[22] A.E. Amr, M.M. Abdulla, Indian J. Heterocycl. Chem. 12 (2002) 129–134.
[23] A.A. Nehad, A.E. Amr, A.I. Alhusien, Monatsh. Chem. 138 (2007) 559–567.
[24] A.E. Amr, M.S. Nermien, M.M. Abdulla, Monatsh. Chem. 138 (2007) 699–707.
[25] A.E. Amr, M.M. Ashraf, F.M. Salwa, A.A. Nagla, A.G. Hammam, Bioorg. Med. Chem. 14 (2006) 5481–5488.
[26] A.E. Amr, H.H. Sayed, M.M. Abdulla, Arch. Pharm. Chem. Life Sci. 338 (2005) 433–440.
[27] A.A. Nehad, Scientia Pharm. 74 (2005) 193–216.
[28] M.A. Sammour, S. Soliman, U.A.R.J. Chem. 13 (1970) 7–24.
[29] A.E. Rashad, O.A. Heikal, A.O.H. El-Nezhawy, F.M.E. Abdel-Megeid, Heteroatom Chem. 16 (2005) 226–234.
[30] C.D. Mudaliar, K.R. Nivalkar, S.H. Mashraqui, Org. Prep. Proc. Int. 29 (1997) 584–587.
[31] J. Litchfield, F.J. Wilcoxon, Pharmacol. Exp. Ther. 96 (1949) 99–113.
[32] C.A. Winter, E.A. Risely, G.W. Nuss, Proc. Soc. Exp. Bio. Med. 111 (1962) 541–545.
[33] F. Herrmann, A. Lindemann, J. Gamss, R. Mertelsmann, Eur. J. Immunol. 20 (1999) 2513–2517.
[34] K.F. Austen, W.E. Brocklehurst, J. Exp. Med. 113 (1961) 521–525.
[35] A. Tgolsen, G.H. Rofland, O.G. Berge, K. Hole, J. Pharmacol. Exp. Ther. 25 (1991) 241–250.
[36] R. Dunm, S. Fielding, Drug Dev. Res. 10 (1987) 177–188.