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Original article

Non-carboxylic analogues of arylpropionic acids: Synthesis, anti-inflammatory activity and ulcerogenic potential

Kamel A. Metwally [a,*], Shada H. Yaseen [a], El-Sayed M. Lashine [a], Hassan M. El-Fayomi [b], Mohamed E. El-Sadek [a]

[a] *Department of Medicinal Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt*
[b] *Department of Pharmacology, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt*

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Abstract

Two series of 1,2,4-triazoles and 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles derived from three selected arylpropionic acids namely, ibuprofen, flurbiprofen and naproxen, were synthesized and evaluated for anti-inflammatory activity and ulcerogenic potential. All the tested compounds exhibited anti-inflammatory activity comparable to that of hydrocortisone. Compared to ibuprofen, however, all the tested compounds displayed more potent anti-inflammatory activity. Compounds tested for ulcerogenicity showed no or minimal ulcerogenic effect compared to indomethacin.

Keywords: Arylpropionic acids; Anti-inflammatory; Ulcerogenicity

1. Introduction

In the early 1990s, it was discovered that the cyclooxygenase enzyme (COX) which is the key enzyme in the biosynthesis of prostaglandins from arachidonic acid, exists as two isoforms, one constitutive (COX-1) and the other inducible (COX-2) [1]. COX-1 is constitutively expressed and provides cytoprotection in the GIT while COX-2 is inducible and mediates inflammation [2–4]. The traditional NSAIDs in current use non-selectively inhibit COX-1 and COX-2. In fact, most NSAIDs show greater selectivity for COX-1 than COX-2 [5]. Consequently, long-term therapy with non-selective NSAIDs may cause appreciable GI irritation, bleeding and ulceration [6]. These clinical shortcomings comprise a major challenge confronting medicinal chemists to develop safer agents that spare COX-1 and subsequently its gastric cytoprotective role. In other words, the development of selective COX-2 inhibitors is instrumental for combating inflammation without the harmful side effects of the presently available NSAIDs.

The discovery of COX-2 provided the rationale for the design of drugs devoid of GI disorders while retaining clinical efficacy as anti-inflammatory agents. Understanding the relationship between chemical structure and enzyme activity is crucial in the design of new COX-2 selective inhibitors. COX-1 and COX-2 have similar structures yet a few differences do exist. Of particular importance, substitution at position 523 between isoleucine (in COX-1) and the relatively smaller valine (in COX-2) creates extra space in the active site of COX-2 recognized as the COX-2 side pocket at which celecoxib, rofecoxib and related drugs appear to act. Additional exchange of valine/isoleucine at position 434 further increases the effective size of the active site channel by enhancing the mobility of side chains within the side pocket. The final result of these differences is about 20% increase in the active site of COX-2 compared to COX-1. In the light of these findings, it could be speculated that arylpropionic acids act non-selectively probably because of their relatively small

* Corresponding author. Tel.: +20 5523 08021; fax: +20 5523 03266.
E-mail address: kametwally@hotmail.com (K.A. Metwally).

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size tolerated by both COX-1 and COX-2. It appears that bulkier structures are more likely to confer selectivity to COX-2 due to its wider active site [7–9].

It has been reported that modification of the carboxyl function of representative NSAIDs results in retained anti-inflammatory activity with reduced ulcerogenic potential [10–14]. In addition, 1,2,4-triazoles [15–23] particularly 1,2,4-triazole-5-thiols and their condensed heterocyclic derivatives [24–28] particularly 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles have been reported to possess anti-inflammatory activity. In the present investigation, we were interested in replacing the carboxyl function of three well-known arylpropionic acids, namely ibuprofen, flurbiprofen and naproxen, by selected bulkier moieties with the aim of improving the safety profile of these agents while retaining anti-inflammatory activity.

2. Chemistry

Following reported procedures [29–32], heating ammonium thiocyanate and benzoyl chloride in dry acetone afforded benzoylisothiocyanate which was treated with substituted anilines in dry acetone to give *N*-benzoyl-*N*′-arylthioureas (**1a–h**) as illustrated in Scheme 1. The resulting *N*-benzoyl-*N*′-arylthioureas were cleaved by means of aqueous NaOH to yield the corresponding arylthioureas (**2a–h**) which were subsequently methylated using methyl iodide in methanol or acetone to afford the requisite aryl-*S*-methylisothiouronium iodides (**3a–h**) in good yields.

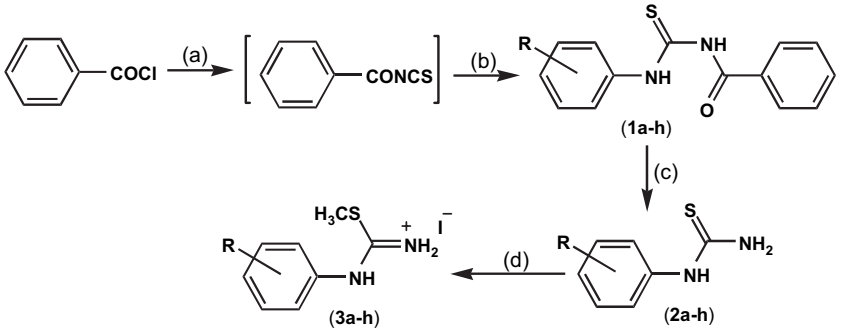
On the other hand, the required hydrazides were prepared according to reported procedures [33–35]. The arylpropionic acid was esterified with ethanol in the presence of sulphuric acid to give the corresponding esters (**4a–c**). Reaction of the resulting esters with hydrazine hydrate in ethanol produced the corresponding acid hydrazides (**5a–c**) which were refluxed with aryl-*S*-methylisothiouronium iodides (**3a–h**) in dry pyridine to afford the target 6-arylamino-4*H*-1,2,4-triazoles (**6a–x**) as shown in Scheme 2. Physical constants of (**6a–x**) are given in Table 1.

The chemical structure of the target triazoles was confirmed by IR, ^{1}H NMR and mass spectral data and microanalysis. IR spectra showed the appearance of characteristic absorption bands at ~ 3200 and ~ 3300 cm^{-1} corresponding to the triazole NH groups. ^{1}H NMR spectra of selected triazoles revealed the presence of two downfield D_2O-exchangeable singlets characteristic of the two NH groups. The arylamino NH group showed absorption in the range of δ 8.75–9.38 whereas the absorption peak of the triazole NH group appeared more downfield in the range of δ 12.81–13.10 consistent with ^{1}H NMR spectra of similar triazoles reported in the literature [36]. Furthermore, mass fragmentation pattern of compound (**6a**) was consistent with that reported for similar 5-arylamino-1,2,4-triazoles [36].

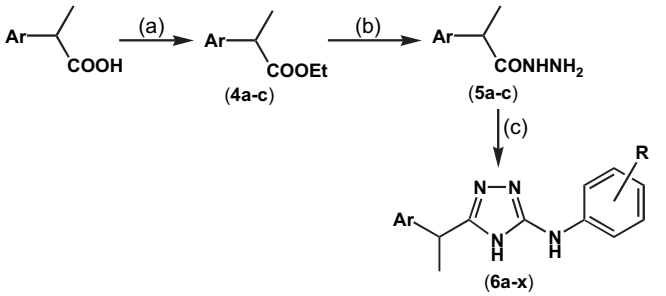
The synthetically accessible 4-amino-5-mercapto-3-substituted *s*-triazoles (**8a–c**) were utilized as key intermediates for the synthesis of the target 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles (**9a–c**) and (**10a–f**). The Reid and Heindel procedure [37] was adopted to prepare these intermediates. The acid hydrazides (**5a–c**) were allowed to react with carbon disulphide in the presence of KOH at room temperature to afford the corresponding aroyldithiocarbazates as the potassium salts (**7a–c**) which were subsequently converted to the required 4-amino-5-mercapto-*s*-triazoles (**8a–c**) through direct hydrazinolysis with hydrazine hydrate in ethanol as outlined in Scheme 3. Physical constants of (**8a–c**) are shown in Table 2.

The structure of the synthesized 4-amino-5-mercapto-1,2,4-triazoles (**8a–c**) was confirmed by ^{1}H NMR spectral data and microanalysis. ^{1}H NMR spectra showed a downfield D_2O-exchangeable singlet at δ 13.53 attributed to the thiol group while the amino group appeared at δ 5.39 as a singlet that was also exchangeable by D_2O. Reaction of the intermediate 4-amino-5-mercapto-1,2,4-triazoles (**8a–c**) with carbon disulphide in ethanolic KOH under reflux conditions afforded the target 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole-6-thiols (**9a–c**) in good yields (Table 3). ^{1}H NMR spectral data of compound (**9b**) showed a characteristic D_2O-exchangeable singlet at δ 13.61 corresponding to the 6-thiol group.

Finally, the target 6-aryl-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles (**10a–f**) were obtained in low yields through treatment of 4-amino-5-mercapto-1,2,4-triazoles (**8a**,**b**) with benzoyl chloride or *p*-substituted derivatives in phosphorous oxychloride as illustrated in Scheme 3. Our attempts to improve the yield as well as attempts to obtain these particular products from naproxen hydrazide were unsuccessful. ^{1}H NMR spectra revealed the absence of D_2O-exchangeable protons and all the target compounds were microanalyzed satisfactorily. In addition, mass fragmentation pattern of compound (**10d**) was



Scheme 1. Reagents and conditions: (a) NH_4SCN, acetone, reflux. (b) Substituted anilines, acetone, reflux. (c) Aqueous NaOH, reflux. (d) CH_3I, acetone or methanol, room temperature.



Scheme 2. Reagents and conditions: (a) EtOH, H_2SO_4, reflux. (b) $NH_2NH_2 \cdot H_2O$, EtOH, reflux. (c) (**3a–h**), pyridine, reflux.

consistent with that reported for similar 6-aryl-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles [38]. Physical constants of (**10a–f**) are summarized in Table 4.

3. Results and discussion

The anti-inflammatory activity of the target compounds (**6c**), (**6d**), (**6k**), (**6l**), (**6s**), (**6t**), (**9a**) and (**10f**) was evaluated by applying the carrageenan-induced rat paw edema bioassay in rats following the method of Winter et al. [39]. Ibuprofen was used as a reference substance in the assay. For comparison purposes, hydrocortisone was additionally used as a second reference standard representing steroidal anti-inflammatory agents. The obtained pharmacological results revealed that the 1,2,4-triazoles (**6c**), (**6d**), (**6k**), (**6l**), (**6s**) and (**6t**) are more active than the 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles represented by (**9a**) and (**10f**) as shown in Table 5. All the tested compounds, however, were found more potent than ibuprofen. In comparison to hydrocortisone, all the test compounds showed comparable anti-inflammatory activity (Figs. 1–4). The triazole (**6s**) displayed the highest anti-inflammatory activity among the set of compounds tested in the present study.

Test compounds that exhibited the most potent anti-inflammatory activity namely, the triazoles (**6d**), (**6k**), (**6l**) and (**6s**) were further evaluated for their ulcerogenic potential in rats [40]. In general, the tested compounds showed a better GI safety profile (0–33.3% ulceration) compared to indomethacin as illustrated in Table 6.

4. Conclusion

It could be concluded that replacement of the carboxyl function of arylalkanoic acids by certain bulkier moieties generally improves their pharmacological profile. It was interesting to note that all the non-carboxylic test compounds were found to have anti-inflammatory activity greater than that of ibuprofen. More interestingly, some of the test compounds were more potent than hydrocortisone. In addition, compounds tested for ulcerogenicity showed no or minimal ulcerogenic effects in rats.

5. Experimental protocols

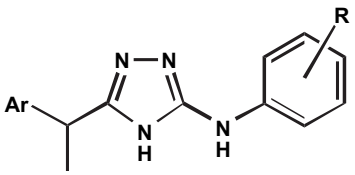
5.1. Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded on a Bruker FT-IR spectrophotometer using KBr pellets. ^{1}H NMR spectra were recorded on a Varian-Gemini 200 MHz spectrometer in DMSO-d_6. Chemical shifts were expressed in parts per million with tetramethylsilane (TMS) as an internal standard. MS spectra were measured with Shimadzu GC MS-QP instrument. Elemental analyses (C, H, N) were performed at the National Research Centre, Cairo, Egypt. Compounds (**1a–h**) [29,31,32], (**2a–h**) [29], (**3a–h**) [30], (**4a–c**) [33–35] and (**5a–c**) [33–35] were synthesized according to literature procedures.

5.1.1. 3-Arylamino-5-[1-(4-isobutylphenyl)ethyl]-(4H)-1,2,4-triazoles (*6a–h*)

A mixture of the appropriate aryl-*S*-methylisothiouronium iodide (**3a–h**; 10 mmol) and 2-methyl-2-(4-isobutylphenyl) acetic acid hydrazide (**5a**; 10 mmol) in pyridine (10 mL) was heated at reflux for 2 h. The cooled mixture was poured into crushed ice and extracted with CH_2Cl_2 (2 × 50 mL). The organic extract was washed with 5% $NaHCO_3$, brine and water, dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude products were purified by recrystallization from DMF/H_2O. IR of compound (**6a**) (KBr, cm^{-1}): 3296, 3199 (NH). ^{1}H NMR of compound (**6a**) (DMSO-d_6) δ: 0.82 (d, 6H, $J = 6.6$ Hz, $2CH_3$), 1.56 (d, 3H, $J = 7.2$ Hz, CH_3), 1.74–1.83 (m, 1H, CH), 2.38 (d, 2H, $J = 7.2$ Hz, CH_2), 4.16 (q, 1H, $J = 7.2$ Hz, CH), 6.74–6.77 (m, 1H, Ar–H), 7.07 (d, 2H, J = 7.8 Hz, Ar–H), 7.15–7.25 (m, 4H, Ar–H), 7.47 (d, 2H, $J = 7.5$ Hz, Ar–H), 9.02 (s, 1H, NH), 12.92 (s, 1H, NH). MS of compound (**6a**): (*m*/*z*) 320 (M^+, base peak). ^{1}H NMR of compound (**6b**) (DMSO-d_6) δ: 0.82 (d, 6H, $J = 6.6$ Hz, $2CH_3$), 1.57 (d, 3H, $J = 7.2$ Hz, CH_3), 1.82–1.86 (m, 1H, CH), 2.38 (d, 2H, $J = 7.2$ Hz, CH_2), 4.15 (q, 1H, CH), 7.08 (d, 2H, $J = 7.8$ Hz, Ar–H), 7.19–7.23 (m, 4H, Ar–H), 7.50 (d, 2H, $J = 9.0$ Hz, Ar–H), 9.25 (s, 1H, NH, D_2O exchangeable), 12.98 (s, 1H, NH, D_2O exchangeable). ^{1}H NMR of compound (**6f**) (DMSO-d_6) δ: 0.83 (d, 6H, $J = 6.6$ Hz, $2CH_3$), 1.57 (d, 3H, $J = 6.9$ Hz, CH_3), 1.76–1.81 (m, 1H, CH), 2.38 (d, 2H, $J = 7.2$ Hz, CH_2), 4.16 (q, 1H, CH), 6.99–7.10 (m, 4H, Ar–H), 7.19 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.47–7.52 (m, 2H, Ar–H), 9.06 (s, 1H, NH, D_2O exchangeable), 12.91 (s, 1H, NH, D_2O exchangeable). ^{1}H NMR of compound (**6g**) (DMSO-d_6) δ: 0.82 (d, 6H, $J = 6.6$ Hz, $2CH_3$), 1.56 (d, 3H, $J = 7.2$ Hz, CH_3), 1.77–1.82 (m, 1H, CH), 2.19 (s, 3H, CH_3), 2.38 (d, 2H, $J = 6.9$ Hz, CH_2), 4.18 (q, 1H, $J = 7.2$ Hz, CH), 6.96 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.07 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.19 (d, 2H, $J = 7.8$ Hz, Ar–H), 7.37 (d, 2H, $J = 8.4$ Hz, Ar–H), 8.86 (s, 1H, NH), 12.85 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 20.00, 20.25, 21.94 (2C), 29.30, 36.81,

Table 1
Physical constants of 3-arylamino-5-(1-substituted-ethyl)-4*H*-1,2,4-triazoles (**6a–x**)



Compound	R	Ar	Yield (%)	M.P. (°C)	Formula	Analysis (%)	
						Calcd	Found
6a	H	4-Isobutylphenyl	75	162–165	$C_{20}H_{24}N_4$	C = 74.97	75.29
						H = 7.55	8.05
						N = 17.48	17.27
6b	4-Cl	4-Isobutylphenyl	71	182–184	$C_{20}H_{23}ClN_4$	C = 67.69	67.32
						H = 6.53	6.88
						N = 15.79	15.54
6c	3-Cl	4-Isobutylphenyl	72	152–153	$C_{20}H_{23}ClN_4$	C = 67.69	68.02
						H = 6.53	6.55
						N = 15.79	16.03
6d	4-Br	4-Isobutylphenyl	68	186–188	$C_{20}H_{23}BrN_4$	C = 60.16	60.15
						H = 5.81	5.92
						N = 14.03	14.03
6e	3-Br	4-Isobutylphenyl	70	137–138	$C_{20}H_{23}BrN_4$	C = 60.16	60.06
						H = 5.81	5.81
						N = 14.03	14.23
6f	4-F	4-Isobutylphenyl	67	169–171	$C_{20}H_{23}FN_4$	C = 70.98	70.56
						H = 6.85	7.06
						N = 16.56	16.09
6g	4-CH_3	4-Isobutylphenyl	73	186–188	$C_{21}H_{26}N_4$	C = 75.41	75.65
						H = 7.84	8.13
						N = 16.75	16.59
6h	4-OCH_3	4-Isobutylphenyl	72	180–182	$C_{21}H_{26}N_4O$	C = 71.97	71.55
						H = 7.48	7.70
						N = 15.99	15.60
6i	H	2-Fluorobiphenyl	75	201–202	$C_{22}H_{19}FN_4$	C = 73.72	73.78
						H = 5.34	5.18
						N = 15.63	15.28
6j	4-Cl	2-Fluorobiphenyl	71	219–220	$C_{22}H_{18}ClFN_4$	C = 67.26	67.17
						H = 4.62	4.59
						N = 14.26	14.15
6k	3-Cl	2-Fluorobiphenyl	72	138–140	$C_{22}H_{18}ClFN_4$	C = 67.26	67.06
						H = 4.62	4.64
						N = 14.26	14.42
6l	4-Br	2-Fluorobiphenyl	68	222–224	$C_{22}H_{18}BrFN_4$	C = 60.42	60.62
						H = 4.15	4.35
						N = 12.81	13.00
6m	3-Br	2-Fluorobiphenyl	70	156–157	$C_{22}H_{18}BrFN_4$	C = 60.42	60.43
						H = 4.15	4.68
						N = 12.81	12.76
6n	4-F	2-Fluorobiphenyl	67	183–184	$C_{22}H_{18}F_2N_4$	C = 70.20	69.87
						H = 4.82	4.65
						N = 14.88	15.18
6o	4-CH_3	2-Fluorobiphenyl	73	177–179	$C_{23}H_{21}FN_4$	C = 74.17	74.04
						H = 5.68	5.96
						N = 15.04	14.54
6p	4-OCH_3	2-Fluorobiphenyl	72	167–169	$C_{23}H_{21}FN_4O$	C = 71.12	71.00
						H = 5.45	5.61
						N = 14.42	14.02
6q	H	6-Methoxynaphthyl	75	213–215	$C_{21}H_{20}N_4O$	C = 73.23	73.50
						H = 5.85	5.71
						N = 16.27	16.03
6r	4-Cl	6-Methoxynaphthyl	71	217–218	$C_{21}H_{19}ClN_4O$	C = 66.58	66.71
						H = 5.05	5.15
						N = 14.79	14.74

(*continued on next page*)

Table 1 (*continued*)

Compound	R	Ar	Yield (%)	M.P. (°C)	Formula	Analysis (%)	
						Calcd	Found
6s	3-Cl	6-Methoxynaphthyl	72	194–195	$C_{21}H_{19}ClN_4O$	C = 66.58	66.41
						H = 5.05	5.41
						N = 14.79	14.77
6t	4-Br	6-Methoxynaphthyl	68	229–230	$C_{21}H_{19}BrN_4O$	C = 59.59	59.69
						H = 4.52	4.12
						N = 13.24	13.04
6u	3-Br	6-Methoxynaphthyl	70	139–140	$C_{21}H_{19}BrN_4O$	C = 59.59	59.90
						H = 4.52	4.94
						N = 13.24	13.24
6v	4-F	6-Methoxynaphthyl	67	180–182	$C_{21}H_{19}FN_4O$	C = 69.60	70.00
						H = 5.28	5.57
						N = 15.46	15.58
6w	4-CH_3	6-Methoxynaphthyl	73	190–191	$C_{22}H_{22}N_4O$	C = 73.72	73.85
						H = 6.19	6.63
						N = 15.63	15.19
6x	4-OCH_3	6-Methoxynaphthyl	72	217–218	$C_{22}H_{22}N_4O_2$	C = 70.57	70.37
						H = 5.92	6.26
						N = 14.96	15.46

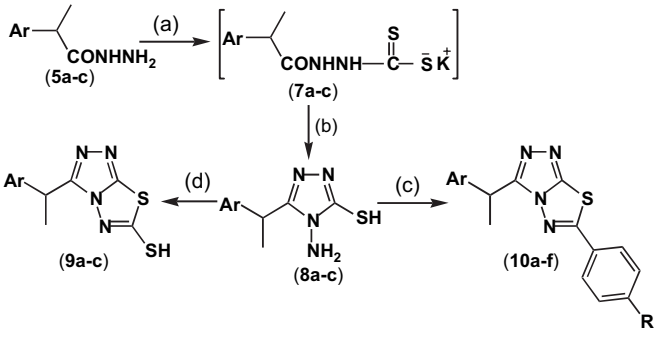
44.06, 115.60 (2C), 115.70, 120.44, 126.66 (2C), 127.20, 128.70 (2C), 128.72 (2C), 139.05, 139.55, 140.50. ^{1}H NMR of compound (**6h**) (DMSO-d_6) δ: 0.83 (d, 6H, $J = 6.6$ Hz, $2CH_3$), 1.55 (d, 3H, $J = 7.2$ Hz, CH_3), 1.74–1.81 (m, 1H, CH), 2.38 (d, 2H, $J = 7.2$ Hz, CH_2), 3.74 (s, 3H, OCH_3), 4.14 (q, 1H, $J = 7.2$ Hz, CH), 6.78 (d, 2H, $J = 9.0$ Hz, Ar–H), 7.07 (d, 2H, $J = 7.8$ Hz, Ar–H), 7.19 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.39 (d, 2H, $J = 8.7$ Hz, Ar–H), 8.75 (s, 1H, NH), 12.81 (s, 1H, NH).

*5.1.2. 3-Arylamino-5-[1-(2-fluorobiphenyl)ethyl]-(4H)-1,2,4-triazoles (**6i–p**)*

A mixture of the appropriate aryl-*S*-methylisothiouronium iodide (**3a–h**; 11 mmol) and 2-methyl-2-(2-fluorobiphenyl) acetic acid hydrazide (**5b**; 10 mmol) in pyridine (10 mL) was heated at reflux for 2 h. The cooled mixture was poured into crushed ice and the resulting precipitate was collected by filtration. The crude product was purified by recrystallization from ethanol. IR of compound (**6j**) (KBr, cm^{-1}): 3315, 3194 (NH). ^{1}H NMR of compound (**6i**) (DMSO-d_6) δ: 1.63 (d, 3H, $J = 7.2$ Hz, CH_3), 4.30 (q, 1H, $J = 7.2$ Hz, CH), 6.75 (m, 1H, Ar–H), 7.18–7.27 (m, 4H, Ar–H), 7.40–7.50 (complex m, 8H, Ar–H), 8.98 (s, 1H, NH), 12.98 (s, 1H, NH). ^{1}H NMR of compound (**6j**) (DMSO-d_6) δ: 1.63 (d, 3H, $J = 7.2$ Hz, CH_3), 4.30 (q, 1H, $J = 6.9$ Hz, CH), 7.22–7.43 (m, 4H, Ar–H), 7.46–7.54 (complex m, 8H, Ar–H), 9.24 (s, 1H, NH), 13.06 (s, 1H, NH). ^{1}H NMR of compound (**6l**) (DMSO-d_6) δ: 1.64 (d, 3H, $J = 6.9$ Hz, CH_3), 4.31 (q, 1H, $J = 6.6$ Hz, CH), 7.22–7.53 (complex m, 12H, Ar–H), 9.31 (s, 1H, NH, D_2O exchangeable), 13.10 (s, 1H, NH, D_2O exchangeable). ^{1}H NMR of compound (**6n**) (DMSO-d_6) δ: 1.63 (d, 3H, $J = 7.2$ Hz, CH_3), 4.30 (q, 1H, $J = 7.2$ Hz, CH), 7.00–7.06 (m, 2H, Ar–H), 7.22–7.28 (m, 2H, Ar–H), 7.38–7.53 (complex m, 8H, Ar–H), 9.09 (s, 1H, NH), 13.02 (s, 1H). ^{1}H NMR of compound (**6p**) (DMSO-d_6) δ: 1.62 (d, 3H, $J = 6.6$ Hz, CH_3), 3.77 (s, 3H, CH_3), 4.25 (q, 1H, CH), 6.78–6.90 (m, 3H, Ar–H), 7.22–7.53 (complex m, 9H, Ar–H), 9.38 (s, 1H, NH, D_2O exchangeable), 12.95 (s, 1H, NH, D_2O exchangeable).

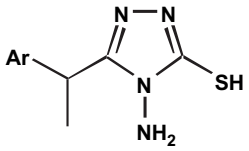
*5.1.3. 3-Arylamino-5-[1-(6-methoxy-2-naphthyl)ethyl]-(4H)-1,2,4-triazoles (**6q–x**)*

A mixture of the appropriate aryl-*S*-methylisothiouronium iodide (**3a–h**; 10 mmol) and 2-methyl-2-(6-methoxy-2-naphthyl) acetic acid hydrazide (**5c**; 10 mmol) in pyridine (10 mL) was heated at reflux for 3 h. The reaction mixture was cooled to room temperature, poured into crushed ice and the resulting precipitate was filtered, washed with 50% cold aqueous MeOH and dried. The crude product was purified by silica gel column chromatography using CH_2Cl_2/MeOH (95:5) as an eluent followed by recrystallization from DMF/EtOH. IR of compound (**6s**) (KBr, cm^{-1}): 3313, 3194 (NH). ^{1}H NMR of compound (**6q**) (DMSO-d_6) δ: 1.66 (d, 3H, $J = 6.9$ Hz, CH_3), 3.85 (s, 3H, OCH_3), 4.33 (q, 1H, $J = 6.6$ Hz, CH), 6.74–6.77 (m, 1H, Ar–H), 7.12–7.20 (m, 3H, Ar–H), 7.26 (s, 1H, Ar–H), 7.41 (d, 1H, $J = 8.4$ Hz,



Scheme 3. Reagents and conditions: (a) CS_2, KOH, EtOH, room temperature. (b) $NH_2NH_2 \cdot H_2O$, reflux. (c) Benzoyl chloride or 4-substituted derivatives, $POCl_3$, reflux. (d) CS_2, KOH, EtOH, reflux.

Table 2
Physical constants of the 5-(1-substituted-ethyl)-4-amino-4*H*-1,2,4-triazole-3-thiols (**8a–c**)



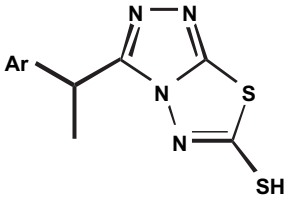
Compound	Ar	Yield (%)	M.P. (°C)	Formula	Analysis (%)	
					Calcd	Found
8a	4-Isobutylphenyl	78	146–148	$C_{14}H_{20}N_4S$	C = 60.84	60.75
					H = 7.29	7.33
					N = 20.27	19.89
8b	2-Fluorobiphenyl	80	169–170	$C_{16}H_{15}FN_4S$	C = 61.13	61.44
					H = 4.81	4.67
					N = 17.82	17.55
8c	6-Methoxynaphthyl	75	150–152	$C_{15}H_{16}N_4OS$	C = 59.98	60.25
					H = 5.37	5.90
					N = 18.65	18.33

Ar–H), 7.47 (d, 2H, $J = 7.8$ Hz, Ar–H), 7.71 (s, 1H, Ar–H), 7.75 (d, 1H, $J = 2.1$ Hz, Ar–H), 7.78 (d, 1H, $J = 2.7$ Hz, Ar–H), 9.03 (s, 1H, NH), 12.97 (s, 1H, NH). ^{1}H NMR of compound (**6s**) (DMSO-d_6) δ: 1.67 (d, 3H, $J = 7.2$ Hz, CH_3), 3.85 (s, 3H, CH_3), 4.36 (q, 1H, $J = 7.2$ Hz, CH), 6.76 (d, 1H, $J = 7.5$ Hz, Ar–H), 7.12–7.17 (m, 2H, Ar–H), 7.19 (d, 1H, $J = 2.4$ Hz, Ar–H), 7.35 (d, 1H, $J = 7.8$ Hz, Ar–H), 7.41 (d, 1H, $J = 8.4$ Hz, Ar–H), 7.68 (d, 1H, $J = 8.4$ Hz, Ar–H), 7.76 (s, 1H, Ar–H), 7.78 (d, 2H, $J = 2.1$ Hz, Ar–H), 9.37 (s, 1H, NH), 13.09 (s, 1H, NH). ^{1}H NMR of compound (**6w**) (DMSO-d_6) δ: 1.66 (d, 3H, $J = 6.6$ Hz, CH_3), 2.19 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 4.35 (q, 1H, CH), 6.97 (d, 2H, $J = 7.50$ Hz, Ar–H), 7.14 (d, 1H, $J = 7.8$ Hz, Ar–H), 7.25 (s, 1H, Ar–H), 7.38–7.40 (m, 3H, Ar–H), 7.70–7.77 (m, 3H, Ar–H), 8.89 (s, 1H, NH, D_2O exchangeable), 12.90 (s, 1H, NH, D_2O exchangeable). ^{1}H NMR of compound (**6x**) (DMSO-d_6) δ: 1.66 (d, 3H, $J = 6.9$ Hz, CH_3), 3.67 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 4.32 (q, 1H, $J = 7.2$ Hz, CH), 6.78 (d, 2H, $J = 9.0$ Hz, Ar–H), 7.12–7.16 (d, 1H, $J = 8.7$ Hz, Ar–H), 7.27 (s, 1H, Ar–H), 7.42 (d, 3H, $J = 8.7$ Hz, Ar–H), 7.71–7.79 (m, 3H, Ar–H), 8.78 (s, 1H, NH, D_2O exchangeable), 12.87 (s, 1H, NH, D_2O exchangeable).

*5.1.4. 4-Amino-5-(1-substituted-ethyl)-4H-1,2,4-triazole-3-thiols (**8a–c**)*

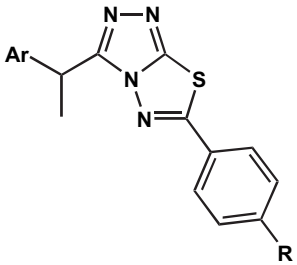
To an ice-cooled mixture of the appropriate acid hydrazide (**5a–c**; 10 mmol) and potassium hydroxide (0.84 g; 15 mmol) in absolute ethanol (20 mL), carbon disulphide (12 mL; 198 mmol) was added in a dropwise manner. After the addition was complete, absolute ethanol (15 mL) was added and

Table 3
Physical constants of the 3-(1-substituted-ethyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole-6-thiols (**9a–c**)



Compound	Ar	Yield (%)	M.P. (°C)	Formula	Analysis (%)	
					Calcd	Found
9a	4-Isobutylphenyl	70	198–199	$C_{15}H_{18}N_4S_2$	C = 56.57	56.77
					H = 5.70	5.74
					N = 17.59	18.13
9b	2-Fluorobiphenyl	72	201–202	$C_{17}H_{13}FN_4S_2$	C = 57.29	57.37
					H = 3.68	3.77
					N = 15.72	16.01
9c	6-Methoxynaphthyl	68	212–213	$C_{16}H_{14}N_4OS_2$	C = 56.12	56.16
					H = 4.12	3.98
					N = 16.36	16.25

Table 4
Physical constants of the 6-aryl-3-(1-substituted-ethyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles (**10a–f**)



Compound	R	Ar	Yield (%)	M.P. (°C)	Formula	Analysis (%)	
						Calcd	Found
10a	H	4-Isobutylphenyl	31	144–145	$C_{21}H_{22}N_4S$	C = 69.58	69.17
						H = 6.12	6.65
						N = 15.46	15.77
10b	Cl	4-Isobutylphenyl	28	168–170	$C_{21}H_{21}ClN_4S$	C = 63.54	63.56
						H = 5.33	5.47
						N = 14.11	14.20
10c	CH_3	4-Isobutylphenyl	37	140–142	$C_{22}H_{24}N_4S$	C = 70.18	69.89
						H = 6.42	6.20
						N = 14.88	14.52
10d	H	2-Fluorobiphenyl	29	209–210	$C_{23}H_{17}FN_4S$	C = 68.98	68.72
						H = 4.28	3.99
						N = 13.99	14.00
10e	Cl	2-Fluorobiphenyl	23	179–180	$C_{23}H_{16}ClFN_4S$	C = 63.52	63.16
						H = 3.71	3.71
						N = 12.88	12.88
10f	CH_3	2-Fluorobiphenyl	18	194–196	$C_{24}H_{19}FN_4S$	C = 69.55	69.24
						H = 4.62	4.88
						N = 13.52	13.33

the reaction mixture was allowed to stir at room temperature for 16 h. After addition of dry ether (50 mL), the obtained product was treated with hydrazine hydrate (99%; 20 mmol) and water (4 mL) and heated at reflux for 30 min. The reaction mixture was then diluted with water (10 mL) and neutralized to litmus with concentrated hydrochloric acid. The separated solid was filtered, washed with cold water and recrystallized from ethanol. ^{1}H NMR of compound (**8a**) (DMSO-d_6) δ: 0.83 (d, 6H, $J = 6.6$ Hz, $2CH_3$), 1.61 (d, 3H, $J = 7.2$ Hz, CH_3), 1.75–1.82 (m, 1H, CH), 2.38 (d, 2H, $J = 7.2$ Hz, CH_2), 4.30 (q, 1H, $J = 6.9$ Hz, CH), 5.39 (s, 2H, NH_2, D_2O exchangeable), 7.07 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.15 (d, 2H, $J = 8.1$ Hz, Ar–H), 13.53 (s, 1H, SH, D_2O exchangeable). The crude products were used without further characterization in the synthesis of the next compounds.

*5.1.5. 3-(1-Substituted-ethyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole-6-thiols (**9a–c**)*

The appropriate 3-(1-substituted-ethyl)-4-amino-4*H*-1,2,4-triazole (**8a–c**; 10 mmol) was dissolved in a solution of potassium hydroxide (0.56 g; 10 mmol) in absolute ethanol (25 mL). Carbon disulphide (15 mL; 248 mmol) was then added and the mixture was heated at reflux for 18 h. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in 10% potassium hydroxide solution and filtered. The cold filtrate was acidified with concentrated hydrochloric acid and the separated solid was filtered, washed with water and recrystallized from aqueous ethanol. ^{1}H NMR of compound (**9b**) (DMSO-d_6) δ: 1.57 (d, 3H, $J = 7.2$ Hz, CH_3), 4.45 (q, 1H, $J = 7.5$ Hz, CH), 7.17–7.53 (complex m, 8H, Ar–H), 13.61 (s, 1H, SH, D_2O exchangeable).

*5.1.6. 6-Aryl-3-(1-substituted-ethyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles (**10a–f**)*

Benzoyl chloride (0.28 g, 2 mmol) was added to a solution of the appropriate 3-(1-substituted-ethyl)-4-amino-4*H*-1,2,4-triazole-5-thiol (**8a**,**b**) in phosphorous oxychloride (10 mL) and the mixture was heated at reflux for 4–7 h. The excess of

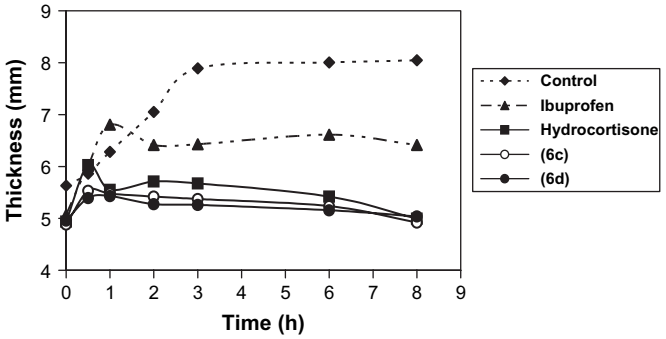


Fig. 1. Anti-inflammatory activity of test compounds (**6c**) and (**6d**).

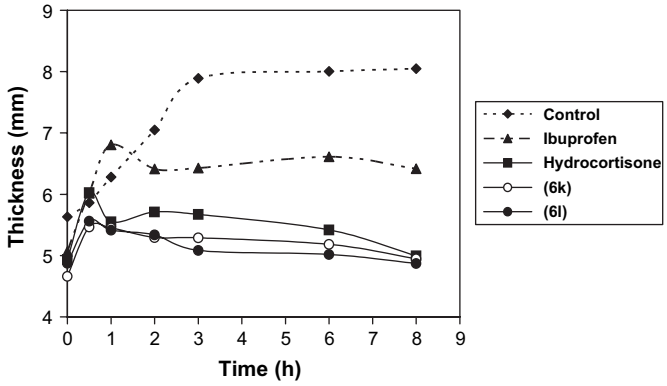


Fig. 2. Anti-inflammatory activity of test compounds (**6k**) and (**6l**).

phosphorous oxychloride was removed under reduced pressure, ice water was added and the precipitate was filtered, washed with 20% $NaHCO_3$ solution and water. The crude product was recrystallized from ethanol to give a white solid. ^{1}H NMR of compound (**10b**) (DMSO-d_6) δ: 0.78 (d, 6H, $J = 6.6$ Hz, $2CH_3$), 1.73 (d, 4H, $J = 7.2$ Hz, CH, CH_3), 2.34 (d, 2H, $J = 7.2$ Hz, CH_2), 4.64 (q, 1H, $J = 7.5$ Hz, CH), 7.07 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.25 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.61 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.86 (d, 2H, $J = 8.7$ Hz, Ar–H). ^{1}H NMR of compound (**10d**) (DMSO-d_6) δ: 1.82 (d, 3H, $J = 6.9$ Hz, CH_3), 4.81 (q, 1H, $J = 7.2$ Hz, CH), 7.31–7.50 (complex m, 8H, Ar–H), 7.60–7.66 (m, 3H, Ar–H), 7.90 (dd, 2H, $J = 1.8$, 1.5 Hz, Ar–H). MS of compound (**10d**): (*m*/*z*) 400 (M^+, 62.1%), 121 (base peak). ^{1}H NMR of compound (**10f**) (DMSO-d_6) δ: 1.81 (d, 3H, $J = 6.9$ Hz, CH_3), 2.38 (s, 3H, CH_3), 4.90 (q, 1H, $J = 6.6$ Hz, CH), 7.30–7.52 (complex m, 10H, Ar–H), 7.79 (d, 2H, $J = 7.5$ Hz, Ar–H).

5.2. Anti-inflammatory activity

Adult male albino rats weighing 150–200 g were used in this study. Rats were obtained from the animal house of the National Research Centre, Dokky, Cairo. The animals were kept for one week in the animal house of the Faculty of Pharmacy, Zagazig University under 12 h day and night cycle, for accommodation with free access to food and water ad libitum.

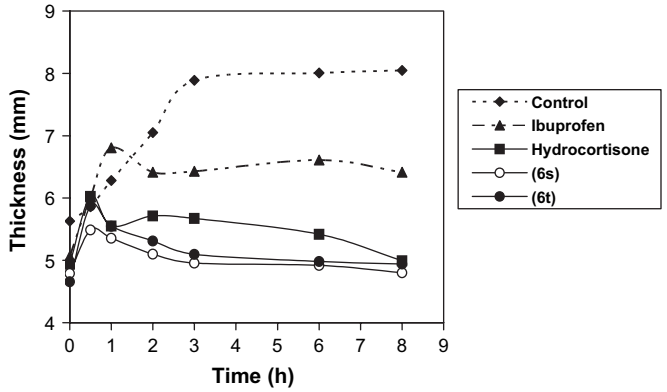


Fig. 3. Anti-inflammatory activity of test compounds (**6s**) and (**6t**).

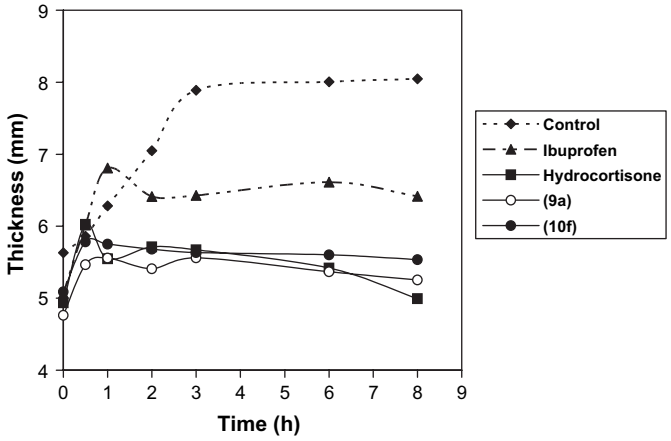


Fig. 4. Anti-inflammatory activity of test compounds (**9a**) and (**10f**).

The animals were randomly divided into 11 groups, six rats each. Inflammation was induced in the right hind paw of rats by injection of 0.05 mL of 1% carrageenan solution, dissolved in normal saline, into the sub-plantar region of the hind paw [39]. Ibuprofen and the test compounds were dissolved in DMSO and were injected subcutaneously in a dose of 30 mg/kg body weight. Hydrocortisone was used in a dose of 200 mg/kg body weight as subcutaneous injection. Control rats received subcutaneous injection of DMSO. The hind paw volume was measured for each rat before, and 0.5, 1.5, 2, 3, 6 and 8 h after the carrageenan injection. The mean of the induced paw thickness was calculated together with the standard error of the mean. The total area under the curve (AUC), representing the thickness of the edema, and the time in hours, were calculated by the trapezoidal method.

5.3. Ulcerogenicity

Male albino rats weighing 150–200 g were fasted for 12 h prior to the administration of the compounds. Water was given ad libitum. The animals were divided into groups, each of six animals. Control group received 1% gum acacia orally. Other

Table 5
The anti-inflammatory activity of the test compounds

Compound	AUC (% h)		
	Mean ± S.E.	% Effect	% Change
Control	59.95 ± 2.21	100.00	0.00
Hydrocortisone	44 ± 0.72*	73.39	−26.61
Ibuprofen	51.6 ± 0.94•,*	86.07	−13.93
6c	42.48 ± 0.61•,*	70.86	−29.14
6d	41.68 ± 0.63**,•,*	69.52	−30.48
6k	41.76 ± 0.45**,•,*	69.65	−30.35
6l	40.98 ± 0.91**,•,*	68.36	−31.64
6s	40.07 ± 0.79**,•,*	66.08	−33.16
6t	41.77 ± 0.99•,*	69.67	−30.33
9a	43.23 ± 1.2•,*	72.10	−27.90
10f	44.97 ± 1.81•,*	75.00	−25.00

*Significantly different from control group at $P < 0.05$.
•Significantly different from ibuprofen group at $P < 0.05$.
**Significantly different from hydrocortisone group at $P < 0.05$.

Table 6
Ulcerogenic effects of the test compounds

Compound	Control	Indomethacin	**6d**	**6k**	**6l**	**6s**
% Ulceration	0.0	100	33.3	0.0	0.0	0.0

groups received indomethacin or the test compounds orally in two equal doses, 30 mg/kg each at 0 and 12 h for three successive days. Animals were sacrificed by diethyl ether 6 h after the last dose and the stomach was separated. An opening at the greater curvature was made and the stomach was washed with cooled saline and inspected with a 3× magnifying lens for any evidence of hyperemia, haemorrhage or ulcer [40]. The percentage ulceration for each group was calculated as follows:

$$\%\,\text{Ulceration} = \frac{\text{number of animals bearing ulcer in a group}}{\text{total number of animals in the same group}} \times 100$$

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References

[1] T. Hla, K. Neilson, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 7384–7388.
[2] L. Mernett, A. Kalgutkar, Trends Pharmacol. Sci. 20 (1999) 465–469.
[3] G. Dannhardt, W. Kiefer, Eur. J. Med. Chem. 36 (2001) 109–126.
[4] C. Almansa, J. Alfon, A. de Arriba, F. Cavalcanti, I. Escamilla, L. Gomez, A. Miralles, R. Soliva, J. Bartroli, E. Carceller, M. Merlos, J. Garcia-Rafanell, J. Med. Chem. 46 (2003) 3463–3475.
[5] L. Jackson, C. Hawkey, Exp. Opin. Invest. Drugs 8 (1999) 963–971.
[6] M. Allison, A. Howatson, C. Torrance, F. Lee, R. Russell, N. Engl. J. Med. 327 (1992) 749–754.
[7] C. Hawkey, Best Pract. Res. Clin. Gastroenterol. 15 (2001) 801–820.
[8] R. Kurumbail, A. Stevens, J. Gierse, J. McDonald, R. Stegeman, J. Pak, D. Gildehaus, J. Miyashiro, T. Penning, K. Seibert, C. Iskason, W. Stallings, Nature 384 (1996) 644–648.
[9] C. Luong, A. Miller, J. Barnett, J. Chow, C. Ramesha, M. Browner, Nat. Struct. Biol. 3 (1996) 927–933.
[10] H. Akgun, B. Tozkoparan, M. Ertan, F. Aksu, S. Inan, Arzneimittelforschung 46 (1996) 891–894.
[11] V. Shanbhag, A. Crider, R. Gokhale, A. Harpalani, R. Dick, J. Pharm. Sci. 81 (1992) 149–154.
[12] A. Kalgutkar, A. Marnett, B. Crews, R. Remmel, L. Marnett, J. Med. Chem. 43 (2000) 2860–2870.
[13] M. Duflos, M. Nourrisson, J. Brelet, J. Courant, G. Le Baut, N. Grimaud, J. Petit, Eur. J. Med. Chem. 36 (2001) 545–553.
[14] A. Kalgutkar, B. Crews, S. Rowlinson, C. Garner, K. Seibert, L. Marnett, Science 280 (1998) 1268–1270.
[15] L. Labanauskas, V. Kalcas, E. Udrenaite, P. Gaidelis, A. Brukstus, V. Dauksas, Pharmazie 56 (2001) 617–619.
[16] L. Labanauskas, E. Udrenaite, P. Gaidelis, A. Brukstus, Farmaco 59 (2004) 255–259.
[17] M. Mullican, M. Wilson, D. Connor, C. Kostlan, R. Schrier, R. Dyer, J. Med. Chem. 36 (1993) 1090–1099.
[18] D. Boschelli, D. Connor, D. Bornemeier, R. Dyer, J. Kennedy, P. Kuipers, G. Okonkwo, D. Schrier, C. Wright, J. Med. Chem. 36 (1993) 1802–1810.
[19] A. Varvaresou, T. Siatra-Papastaikoudi, A. Dalla Tsotinis, A. Tsantili-Kakoulidou, A. Vamvakides, Farmaco 53 (1998) 320–326.
[20] A. El-Emam, T. Ibrahim, Arzneimittelforschung 41 (1991) 1260–1268.
[21] G. Mazzone, R. Pignatello, S. Mazzone, A. Panico, F. Barbera, T. Catti, S. Chiechio, R. Arrigo-Reina, C. Castorina, A. Russo, Farmaco 47 (1992) 149–169.
[22] G. Mekuskiene, P. Gaidelis, P. Vainelavicius, Pharmazie 53 (1998) 94–96.
[23] B. Tozkoparan, E. Kupeli, E. Yesilada, S. Isik, M. Ozalp, M. Ertan, Arzneimittelforschung 55 (2005) 533–540.
[24] H. Fahmy, G. Soliman, Arch. Pharm. Res. 34 (2001) 180–189.
[25] B. Tozkoparan, E. Aktay, E. Yesilada, M. Ertan, Arzneimittelforschung 51 (2001) 470–477.
[26] B. Tozkoparan, G. Kilcigil, R. Ertan, M. Ertan, P. Kelicen, R. Demirdamar, Arzneimittelforschung 49 (1999) 1006–1011.
[27] B. Tozkoparan, N. Gokhan, E. Kupeli, E. Yesilada, M. Ertan, Arzneimittelforschung 54 (2004) 35–41.
[28] B. Berk, E. Aktay, E. Yesilada, M. Ertan, Pharmazie 56 (2001) 613–616.
[29] C. Rasmussen, F. Villani, L. Weaner, B. Reynolds, A. Hood, L. Hecker, S. Nortey, A. Hanslin, M. Costanzo, E. Powell, A. Molinari, Synthesis (1988) 456–459.
[30] C. Rasmussen, F. Villani, B. Reynolds, J. Plampin, A. Hood, L. Hecker, S. Nortey, A. Hanslin, M. Costanzo, R. Howse, A. Molinari, Synthesis (1988) 460–466.
[31] A. Rasschaert, G. Benoy, J. van Besouw, British Patent 1,099,767, 1968.
[32] E. Schroepl, R. Pohloudek-Fabini, Pharmazie 23 (1968) 484–490.
[33] M. El-Sadek, L. Abdel-Aziz, K. Metwally, Zagazig J. Pharm. Sci. 5 (1996) 29–35.
[34] M. Ebeid, M. Al-Ashamawi, S. Abbas, M. Abu Kull, Egypt J. Pharm. Sci. 30 (1989) 389–398.
[35] M. Amir, A. Oberoi, S. Alam, Indian J. Chem. Sect. B: Org. Chem. Incl. Med. Chem. 33 (1999) 237–239.
[36] S. Demirayak, K. Benkli, K. Guven, Pharm. Acta Helv. 72 (1998) 285–290.
[37] J. Reid, N. Heindel, J. Heterocycl. Chem. 13 (1976) 925–932.
[38] Z. Zhang, S. Xiao-Wen, Heterocycles 48 (1998) 561–584.
[39] C. Winter, E. Risley, G. Nuss, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547.
[40] M. Abouzeit-Har, T. Verimer, J. Long, Pharmazie 37 (1982) 593–595.