European Journal of Medicinal Chemistry 45 (2010) 482–491

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Novel substituted and fused pyrrolizine derivatives: Synthesis, anti-inflammatory and ulcerogenecity studies

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ARTICLE INFO

Article history:
Received 6 August 2009
Received in revised form
15 October 2009
Accepted 20 October 2009
Available online 25 October 2009

Keywords:
Pyrrolizines
Pyrimidopyrrolizines
Anti-inflammatory activity
Analgesic activity
Ulcerogenicity

ABSTRACT

Synthesis of several substituted pyrrolizines **10a–f**, **11a–f**, **13a–c**, pyrimidopyrrolizines **14a–c**, **15a–c**, and pyrrolizinopyrimidoisoindoles **12a–c** was discussed. The starting compounds 6-amino-7-cyano-*N*-(4-(un)substitutedphenyl)-2,3-dihydro-1*H*-pyrrolizine-5-carboxamides **9a–c** were reacted with different aldehydes, acid chlorides, and acid anhydrides to give the target compounds. The structures of the new compounds were characterized by spectral and elemental analyses. All compounds were tested for their anti-inflammatory activity using the carrageenan-induced rat paw oedema model and exhibited weak to good activities compared to ketorolac as the reference drug. Also, analgesic activity of selected compounds, which are the most active in the anti-inflammatory screening, was measured using the acetic acid-induced writhing model; revealing activities comparable to or higher than ketorolac. Ulcer indices for the most active compounds were calculated and some compounds showed no or minimal ulcerogenic effect compared to ketorolac.

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1. Introduction

Inflammation is a normal and essential response to any noxious stimulus, which threatens the host and may vary from a localized response to a more generalized one [1]. The inflammatory process is designed to provide a rapid mechanism by which the host can respond to the invasion of foreign materials and return to homeostatic equilibrium. Acute inflammation is mediated by the release of autacoids such as histamine, serotonin, bradykinin, prostaglandins and leukotrienes [2]. On the other hand, the chronic inflammatory process involves the release of diverse mediators, as interleukins, interferon and tumor necrosis factor α (TNF-α), a cytokine that plays a major role in this kind of inflammatory process and whose production is associated with some inflammatory diseases such as rheumatoid arthritis, Crohn's disease and others [2].

Non-steroidal anti-inflammatory drugs (NSAIDs), which are widely used for reducing pain and swelling associated with inflammation, represent a research area of continuous and ever-growing development [3]. NSAIDs cause several serious adverse effects; the most important one is gastric injury that might later cause gastric ulceration and renal injury [4]. Therefore, there is a continuous attempt to produce anti-inflammatory drugs that are devoid of classical NSAID toxicity, especially gastrointestinal injury.

Pyrrolizine ring constitutes a skeleton for many natural and synthetic compounds of diverse biological activities [5–7]. Licofelone (ML3000) **1** is an anti-inflammatory drug with potent activity in various animal experimental models for acute and chronic inflammation. It is a balanced inhibitor of 5-lipoxygenase (5-LOX) and cyclooxygenase-1 and -2 (COX-1 and COX-2). In addition, it has antiplatelet, and anti-bronchoconstrictive activity with excellent gastrointestinal safety [8,9]. Ketorolac **2** is another important pyrrolizine derivative that is a nonselective inhibitor of COX enzymes that may play an important role in the treatment of sever neuropathic pain (Fig. 1) [10]. Other effective pyrrolizine derivatives **3** and **4** lacking the free carboxylic group characteristic for most NSAIDs are reported (Fig. 1) [11].

Prompted by these findings, and aiming to discover novel and potent anti-inflammatory agents that might be devoid of the gastrointestinal side effects, the pyrrolizine ring, and specifically, the *N*-(4-(un)substitutedphenyl)-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide moiety was used as the core heterocyclic scaffold of our target compounds (Fig. 2). The design concept of this scaffold lies on its structural similarity to ketorolac **2**, both having a pyrrolizine ring system bearing an out-of-plane phenyl ring at position 5. Our target compounds differ in that the phenyl ring at position 5 is attached to the pyrrolizine nucleus through an amide link and in that they lack the carboxyl group at position1. Structure extension

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doi:10.1016/j.ejmech.2009.10.031

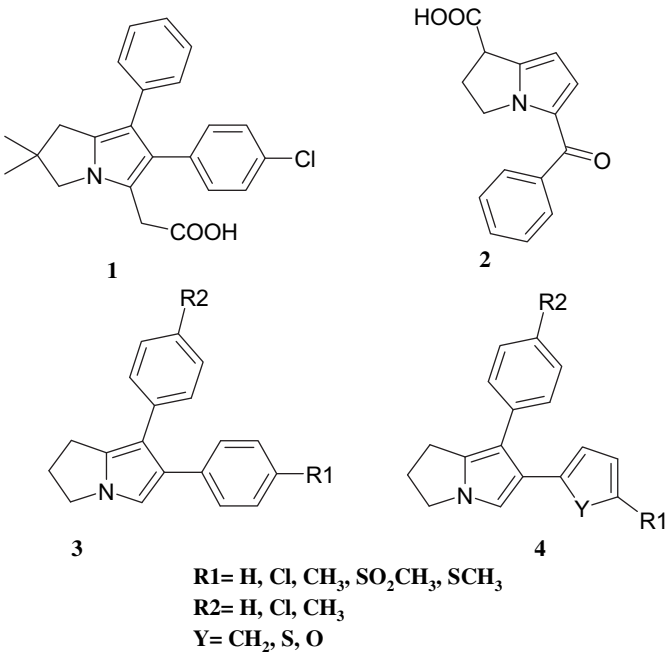


Fig. 1. Examples of pyrrolizine compounds with anti-inflammatory activity.

has been made through introduction of additional substitutions at positions 6 and 7 of the pyrrolizine ring, which have been modified to evolve our target compounds (Fig. 2). Accordingly, the designed compounds fall into two main groups, the first includes the substituted pyrrolizine derivatives having in the 6 position different benzylidineamino **10a–f**, phenylpropionylamino **11a–f** and isoindolyl groups **13a–c** (general formula **A**). The amide derivatives **11a–c** and **11d–f** can be viewed as hybrid molecules between our pyrrolizine scaffold, and ibuprofen and ketoprofen structures, respectively, hoping to have synergistic action. The second group of compounds comprises the fused pyrrolizine derivatives, (general formula **B**) exemplified by the pyrrolizinopyrimidoisoindole compounds **12a–c**, which can be viewed as the restrained analogues of compounds **13a–c**, and the pyrimidopyrrolizines **14a–c** and **15a–c**.

2. Results and discussion

2.1. Chemistry

As shown in Scheme 1, compounds **9a–f** were prepared from the reaction of 2-pyrrolidin-2-ylidinemalononitrile **8** with the corresponding α-chloroacetanilides **6a–f**. Compounds **9a–c** served as the starting materials for other target compounds, where condensation

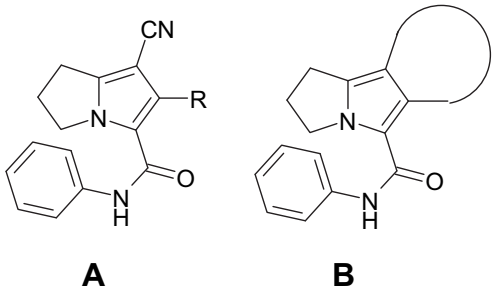


Fig. 2. General structures of the target compounds.

with the appropriate aldehydes in absolute ethanol in the presence of glacial acetic acid gave the benzylidine compounds **10a–f**, and their reaction with the appropriate acid chlorides, usually prepared in situ via the action of thionyl chloride on the corresponding acids, furnished the amide derivatives **11a–f**.

According to Scheme 2, the reaction of **9a–c** with phthalic anhydride in glacial acetic acid yielded, unexpectedly, two products as revealed by the TLC of the reaction mixture. Removal of the reaction solvent under vacuum left out the residue containing the two products which were successfully separated by fractional crystallization from acetone whereby the pyrrolizinopyrimidoisoindoles **12a–c** were obtained from the acetone-insoluble fraction and the 6-isoindolyl derivatives **13a–c** were isolated from the acetone-soluble fraction, as later confirmed by the spectral and elemental analyses. The major products are **13a–c**. The main clear difference between the two products was in their IR spectra which revealed the presence of a cyano absorption band in the range 2226–2222 cm^{-1} for compounds **13a–c** and the absence of this band in the IR spectra of compounds **12a–c**.

The target compounds **14a–c** and **15a–c** were obtained from the reaction of compounds **9a–c** with maleic anhydride and succinic anhydride, respectively. The synthesized compounds were characterized by spectral and elemental analyses. Evidence for cyclization and formation of the fused pyrimidinone ring was drawn from IR spectra that showed OH carboxylic absorption band at about 2550 cm^{-1} together with the disappearance of the characteristic cyano band. Also, ^1H NMR spectra showed 3 exchangeable protons in the range of 9.60–11.70 ppm assigned for 2NH protons and one COOH proton.

The proposed mechanism for the formation of products **12–15a–c** is demonstrated in Figs. 3 and 4 [12,13].

2.2. Pharmacological screening

2.2.1. Anti-inflammatory activity

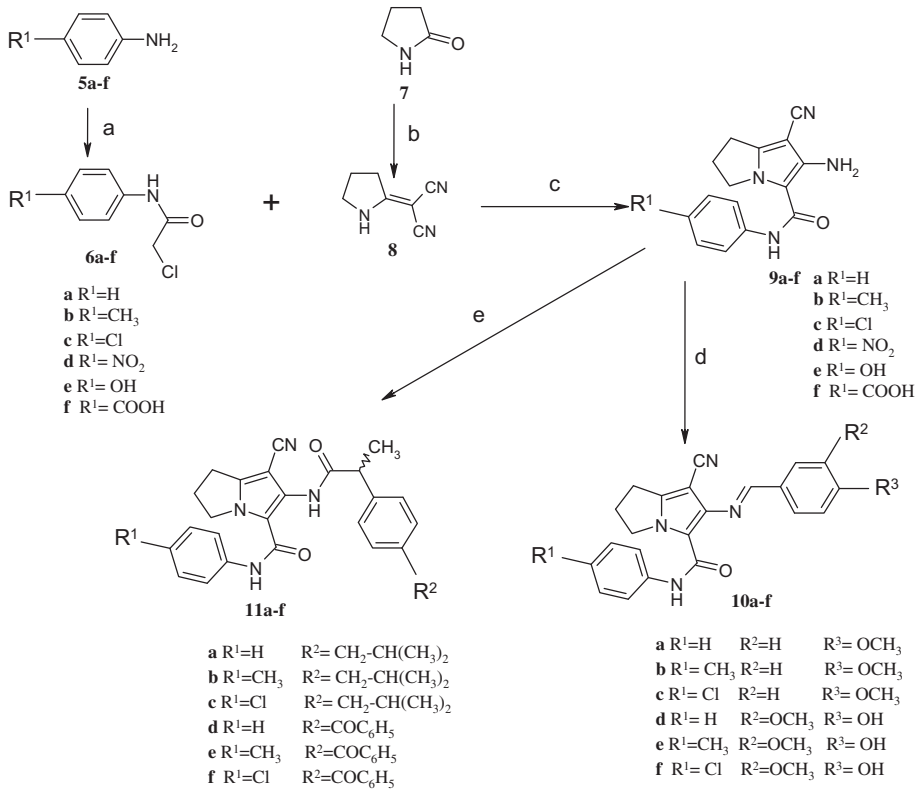
Evaluation of anti-inflammatory activity of the synthesized compounds was performed using the carrageenan-induced rat paw oedema model using ketorolac as the reference drug [14]. Mean changes in paw oedema thickness of animals pretreated with the tested compounds after 1, 2, 3, and 4 h. from induction of inflammation was measured and shown in Table 1 together with the inhibition percent of oedema by the tested compounds.

The 6-amino-7-cyano-*N*-(un)substitutedphenyl-5-carboxamides **9a–f** showed moderate activities compared to ketorolac. Regarding the effect of the electronic nature of the *para*-substituent on the activity, results revealed that compounds with electron withdrawing groups (Cl, NO_2, COOH) **9c, 9d** and **9f** exhibited higher activities than the unsubstituted compound **9a** which in turn possessed higher activity than compounds with electron donating groups (CH_3, OH) **9b** and **9e**. This relation between the electronic nature of the substituent and activity holds true in most of the other series.

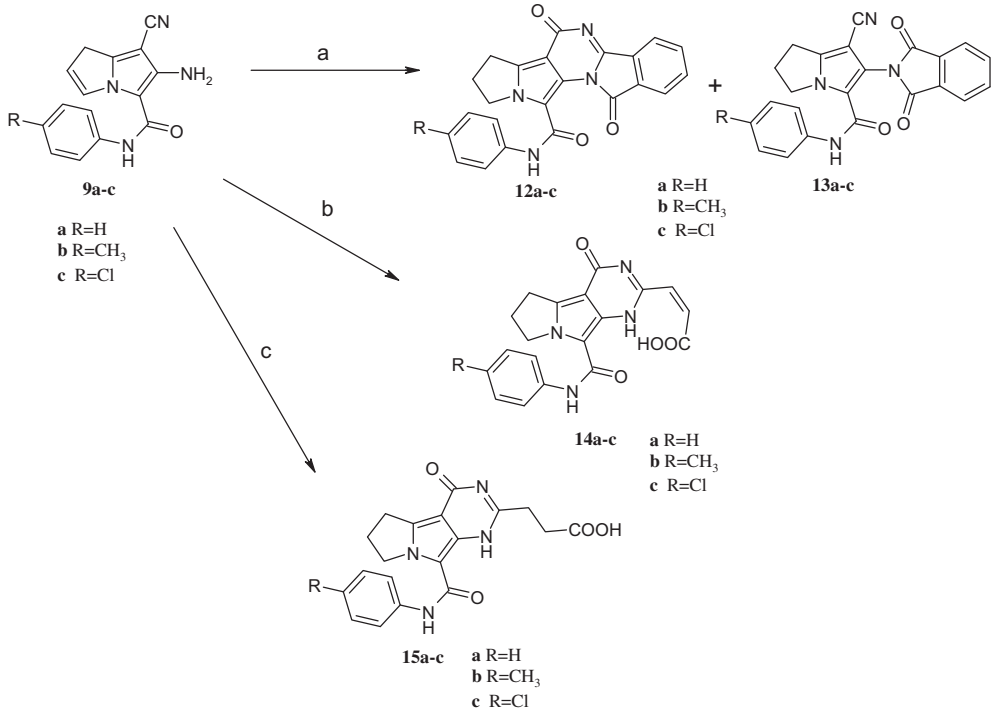
The benzylidene amino derivatives **10a–f** exhibited weak to moderate activities relative to ketorolac. Within this series, it could be observed that the nature and position of the substituent on the benzylidene ring had minor effect on activity.

As for the amide derivatives **11a–f**, which can be viewed as hybrid molecules bearing the ibuprofen and ketoprofen moieties, it is noteworthy that they showed activities slightly lower than that of ketorolac but having the advantage of being less ulcerogenic as will be shown later.

Interestingly, activities of the 6-isoindolyl derivatives **13a–c** were much lower than that of ketorolac while their restrained analogues **12a–c** were much higher. Another important



Scheme 1. Reagents and solvents: a: $ClCH_2COCl$, glacial acetic acid, sodium acetate; b: dimethylsulfate in benzene then malononitrile; c: dry acetone, anhydrous K_2CO_3; d: ArCHO, ethanol, glacial acetic acid; e: ArCOCl, dry benzene.



Scheme 2. Reagents and solvents: a: phthalic anhydride, glacial acetic acid; b: maleic anhydride glacial acetic acid; c: succinic anhydride, glacial acetic acid.

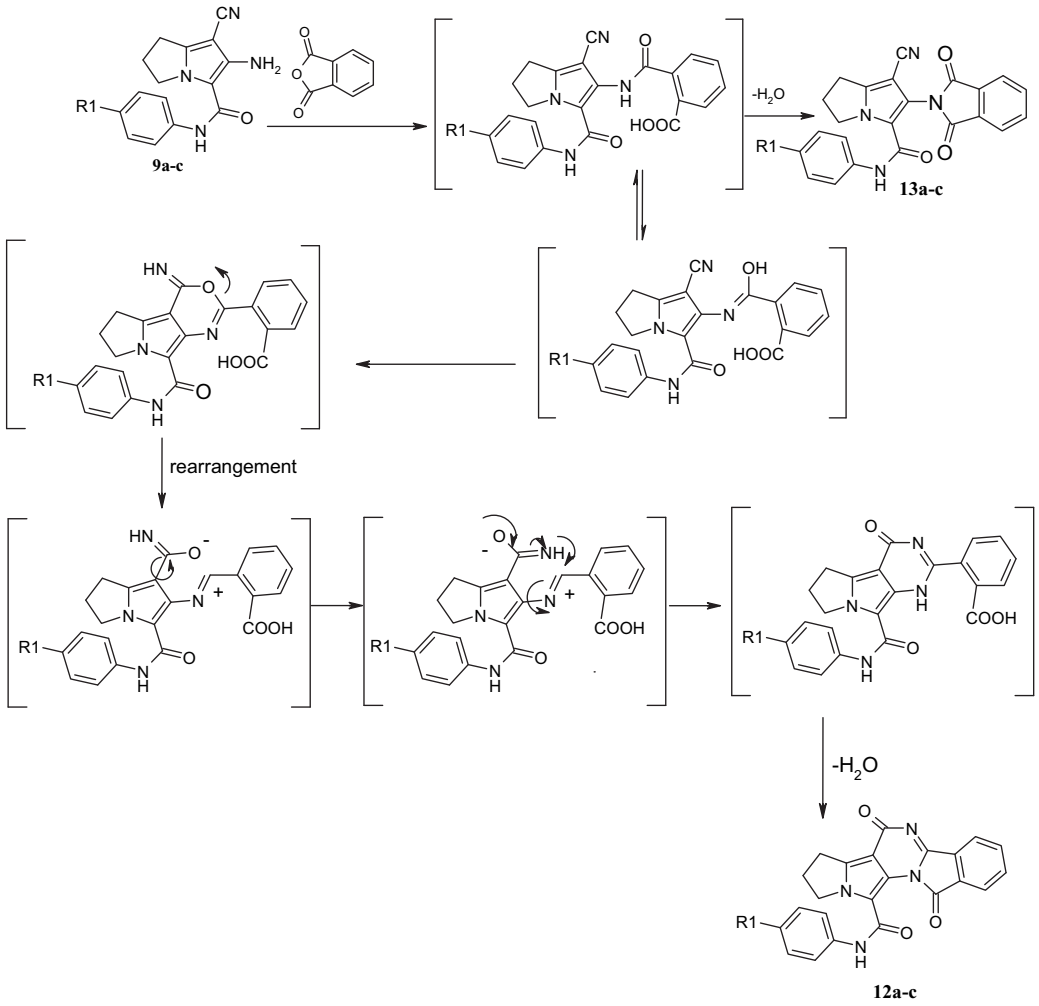


Fig. 3. The proposed reaction mechanism of the formation of compounds **12a–c** and **13a–c**.

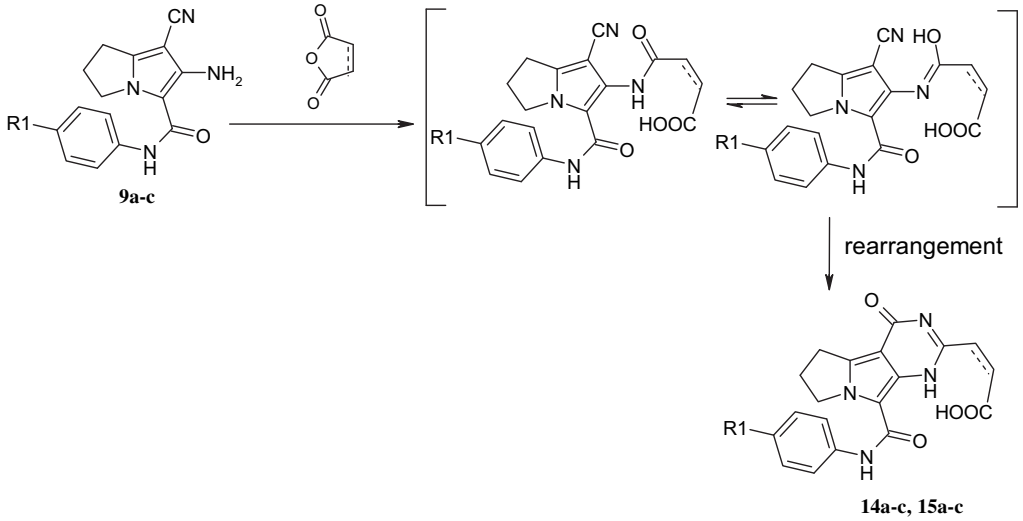


Fig. 4. The proposed reaction mechanism of the formation of compounds **14a–c** and **15a–c**.

Table 1
Oedema thickness and inhibition percent of control, ketorolac and tested compounds.

Comp.	Oedema thickness($\times 10^{-2}$ mm) ± SEM (Inhibition%)			
	1h	2h	3h	4h
Control	140.8 ± 1.39	155.6 ± 2.80	167.4 ± 2.09	195.6 ± 3.08
Ketorolac	41.6 ± 2.02 (70.46)	40.2 ± 1.62 (74.17)	41.2 ± 2.06 (75.39)	40.4 ± 1.54 (79.35)
9a	112.6 ± 1.03 (20.03)	116.2 ± 0.86 (25.32)	112.8 ± 1.59 (32.62)	121.2 ± 1.66 (38.04)
9b	121.2 ± 2.27 (13.92)	123.6 ± 1.6 (20.57)	124.4 ± 1.36 (25.69)	130.2 ± 1.16 (33.44)
9c	103.6 ± 2.25 (26.42)	101.0 ± 2.17 (35.09)	101.4 ± 2.86 (39.43)	111.0 ± 1.76 (43.25)
9d	108.4 ± 2.68 (23.01)	113.8 ± 2.58 (26.86)	107.2 ± 3.22 (31.11)	116.6 ± 3.08 (40.39)
9e	121.8 ± 1.02 (13.49)	128.4 ± 1.72 (17.48)	135.8 ± 2.04 (18.88)	149.2 ± 2.44 (23.72)
9f	100.2 ± 1.86 (28.84)	102.6 ± 2.32 (34.06)	93.4 ± 2.16 (44.21)	100.6 ± 3.06 (48.57)
10a	117.6 ± 1.72 (16.48)	112.4 ± 2.44 (27.76)	113.2 ± 2.85 (32.38)	126.6 ± 2.23 (35.28)
10b	109.2 ± 2.65 (22.44)	108.4 ± 3.16 (30.33)	109.4 ± 3.14 (34.65)	110.6 ± 2.25 (43.46)
10c	96.2 ± 2.08 (31.68)	94.4 ± 2.27 (39.33)	95.2 ± 2.42 (43.13)	97.8 ± 3.01 (50.00)
10d	99.2 ± 1.56 (29.55)	102.8 ± 2.3 (34.32)	106.4 ± 1.86 (36.44)	122.4 ± 1.44 (37.42)
10e	112.6 ± 2.93 (20.03)	116.2 ± 2.06 (25.32)	117.6 ± 2.4 (29.75)	128.0 ± 2.97 (34.56)
10f	91.6 ± 1.91 (34.94)	90.6 ± 2.62 (41.77)	90.2 ± 2.42 (46.12)	96.6 ± 4.37 (50.61)
11a	98.8 ± 3.07 (29.83)	99.6 ± 3.75 (35.99)	75.2 ± 2.01 (55.08)	80.8 ± 1.66 (58.69)
11b	89.8 ± 2.22 (36.22)	88.2 ± 3.65 (43.32)	72.6 ± 2.73 (56.63)	75.4 ± 1.96 (61.45)
11c	85.6 ± 2.18 (39.20)	87.8 ± 2.63 (43.57)	66.2 ± 1.77 (60.45)	71.2 ± 1.77 (63.60)
11d	114.6 ± 1.63 (18.61)	108.4 ± 3.39 (30.33)	100.6 ± 1.47 (39.91)	92.8 ± 2.42 (52.56)
11e	103.2 ± 2.78 (26.71)	104.4 ± 3.92 (32.91)	92.4 ± 2.84 (44.80)	86.4 ± 2.2 (55.83)
11f	108.6 ± 3.61 (22.87)	103.4 ± 2.77 (33.56)	93.4 ± 2.25 (44.21)	92.4 ± 2.4 (52.76)
12a	57.6 ± 1.91 (59.09)	70.6 ± 2.29 (54.63)	85.2 ± 2.06 (49.10)	106 ± 2.88 (45.81)
12b	64.8 ± 1.66 (53.98)	73.6 ± 2.11 (52.70)	83.6 ± 2.11 (50.06)	109.8 ± 2.18 (43.87)
12c	54.4 ± 1.69 (61.36)	62.4 ± 2.23 (59.90)	80.4 ± 1.69 (51.7)	101.4 ± 1.63 (48.16)
13a	119.6 ± 1.33 (15.06)	130.4 ± 2.36 (16.20)	131.8 ± 2.13 (21.27)	152.2 ± 3.02 (22.19)
13b	129.2 ± 2.75 (8.24)	139.4 ± 2.71 (10.41)	135.8 ± 1.56 (18.88)	159.8 ± 3.22 (18.30)
13c	118.2 ± 2.54 (16.05)	127.8 ± 2.92 (17.87)	130.4 ± 1.96 (22.10)	146.8 ± 2.08 (24.95)
14a	64.6 ± 1.96 (54.12)	68.4 ± 2.06 (56.04)	70.4 ± 2.14 (57.95)	65.2 ± 2.62 (66.67)
14b	68.6 ± 2.2 (51.28)	71.6 ± 2.42 (53.98)	76.2 ± 2.62 (54.48)	73.6 ± 3.01 (62.37)
14c	61.2 ± 1.59 (56.53)	63.6 ± 2.32 (59.13)	61.8 ± 1.66 (63.08)	59.4 ± 2.62 (69.63)
15a	76.2 ± 1.07 (45.88)	76.4 ± 3.04 (50.90)	76.8 ± 2.31 (54.12)	75.4 ± 2.38 (61.45)
15b	82.2 ± 2.08 (41.62)	78.8 ± 2.63 (49.36)	80.4 ± 2.04 (51.97)	86.2 ± 2.44 (55.93)
15c	71.6 ± 2.14 (49.15)	66.4 ± 2.64 (57.33)	66.8 ± 2.18 (60.10)	73.6 ± 2.48 (62.37)

Data analyzed by one way ANOVA, (n = 5), all compounds were significantly different from control and from ketorolac using Student's *t*-test, $P < 0.05$.

observation is the unexpected decline in activity of compounds **12a–c** with time deviating from all other screened compounds.

On the other hand, the pyrimidopyrrolizine compounds **14a–c** and **15a–c** exhibited anti-inflammatory activities comparable to that of ketorolac. The double bond in the side chain of compounds **14a–c**, which may confer some restriction in the free rotation of the carboxyl group, had no effect on the activities of compounds **14a–c** compared to their saturated derivatives **15a–c**.

In conclusion, it could be seen that the most active compounds were **11a–f**, **14a–c** and **15a–c** possessing, in average, 75% the activity of ketorolac.

2.2.2. Analgesic activity

The most potent drugs in the anti-inflammatory screening **11a–c**,**e**, **14a–c** and **15a**,**c** were further tested for their analgesic activity using the acetic acid-induced writhing test in mice [15–18] using ketorolac as a reference drug. All tested compounds, except **15c**, possessed good analgesic activity comparable to or higher than ketorolac. The most active compound was **15a**. (Table 2)

2.2.3. Acute ulcerogenicity study

Ulcerogenic effect of derivatives **11a–f**, **14a–c** and **15a–c** with best overall profile in animal efficacy model was evaluated for gastric ulcerogenic potential in rats (Table 3) [19,20]. When compared to ketorolac, compounds **11a**, **11c** and **11d** did not cause any gastric ulceration at the same dose level while compounds **11b**, **11e** and **11f** exhibited only little gastric ulceration; about 30% that of ketorolac. On the other hand, compounds **14a–c** and **15a–c** showed higher gastric ulceration than ketorolac. These results

Table 2
Effects of the tested compounds on acetic acid-induced abdominal writhing in mice.

	Writhing reflex ± SEM	Inhibition%
Control	76.83 ± 1.08[a]	–
Ketorolac	4.60 ± 0.50	94.01
11a	3.00 ± 0.95	96.09
11b	3.40 ± 0.51	95.57
11c	2.20 ± 0.86[a]	97.13
11e	6.60 ± 0.98	91.40
14a	3.80 ± 0.58	95.05
14b	5.00 ± 0.71	93.49
14c	1.80 ± 0.37[a]	97.65
15a	0.40 ± 0.24[a]	99.47
15c	10.00 ± 0.71[a]	86.98

Data analyzed by one way ANOVA, ($n = 5$), all compounds were significantly different from control.

[a] Statistically significant from ketorolac using Student's *t*-test, $P < 0.05$.

Table 3
Ulcer index of the most active compounds.

	%Incidence/10	Average no of ulcer	Average severity	Ulcer index
Control	0	0	0	0
Ketorolac	8	1.6	2	11.6
11a	0	0	0	0
11b	2	0.2	1	3.2
11c	0	0	0	0
11d	0	0	0	0
11e	2	0.6	1	3.6
11f	2	0.6	1	3.6
14a	10	5.8	1.66	17.46
14b	10	6.8	1.5	18.3
14c	10	5.2	1.89	17.09
15a	8	7	1.66	16.66
15b	10	6.2	1.71	17.91
15c	8	5.8	1.86	15.66

revealed the advantageously high gastric tolerance to compounds **11a–f** compared to ketorolac.

3. Conclusions

Various substituted pyrrolizines and pyrimido-fused pyrrolizines were synthesized and screened for anti-inflammatory and ulcerogenic potential. Hybrid compounds **11a–f**, which bear the ibuprofen and ketoprofen moieties, and the pyrimido-fused pyrrolzine derivatives **14a–c** and **15a–c**, exhibited the highest anti-inflammatory activity. Despite their slightly lower activity, compared to ketorolac, compounds **11a–f** achieved the advantage of being much less ulcerogenic as revealed from the results of acute ulcerogenicity studies. Furthermore, analgesic activity performed on selected compounds with the higher anti-inflammatory profile showed promising analgesic activity. Combining the analgesic activity with the ulcerogenic potential of the tested compounds, it could be revealed that the most active and, at the same time, safest compound was **11c**.

4. Experimental protocols

4.1. Chemistry

Melting points were uncorrected and were carried out by open capillary tube method using IA 9100MK-Digital Melting Point Apparatus. Microanalyses were carried out at the microanalytical Center, Faculty of Science, Cairo University. Infrared spectra were made on Bruker Vector 22 (Japan) infrared spectrophotometers and were expressed in wavenumber (cm^{-1}) using potassium bromide disc. The proton magnetic resonance ^1H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 300 MHz and Bruker APX400 spectrometer at 400 MHz in the specified solvent. Chemical shifts were reported on the δ scale and were related to that of the solvent and J values are given in Hz. ^{13}C NMR spectra were obtained on a Bruker APX400 at 100 MHz. Mass spectra were recorded on Fennigan MAT, SSQ 7000, Mass spectrometer, at 70 eV (EI) and Waters Micromass Q-Tof Micro mass spectrometer (ESI). All mass spectra were recorded in EI mode unless otherwise stated. Compounds **6a** [21], **6b**, **6c** [22], **6d** [23,24], **6e** [25], **6f** [26], **8** [27] and **9a–c** [28] were prepared according to the reported procedures.

4.1.1. General method for preparation of compounds (*9d–f*)

A mixture of 2-pyrrolidin-2-ylidenemalononitrile **8** (1 g, 7.5 mmol), the appropriate acetanilide **6d–f** (7.5 mmol) and anhydrous potassium carbonate (1.04 g, 7.5 mmol) in dry acetone (50 ml) was stirred under reflux for 24 h. The mixture was filtered, concentrated and left to cool, whereby crystals were formed, collected, dried and recrystallized from ethanol–acetone.

*4.1.1.1. 6-Amino-7-cyano-N-(4-nitrophenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide(**9d**).* Yellow crystals, m.p. 235–237 °C, yield 55%. IR υ_{max}/cm^{-1} 3360, 3290 (NHs), 3074, 3049 (CH aromatic) 2993–2847 (CH aliphatic), 2220 (CN), 1673 (CO), 1602 (NH), 1559 (C=C). ^1H NMR (DMSO-d_6-400 MHz): δ 2.50 (m, 2H, CH_2-2), 2.99 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.30 (t, 2H, $J = 7.2$ Hz, CH_2-3), 4.78 (s, 2H, NH_2), 7.88–8.37 (m, 4H, aromatic protons), and 9.80 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 19.69 (C-1), 36.39 (C-2), 50.62 (C-3), 78.28 (C-7), 116.28 (CN), 118.06 (C-6), 119.77 (C-2′, -6′), 124.07 (C-5), 129.28 (C-3′, -5′), 139.06 (C-7a), 146.28 (C-1′), 164.55 (C-4′), 172.53 (CO). Anal. Calcd. for $C_{15}H_{13}N_5O_3$ (311.30): C, 57.87; H, 4.21; N, 22.50. Found: C, 57.63; H, 4.01; N, 22.99.

*4.1.1.2. 6-Amino-7-cyano-N-(4-hydroxyphenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**9e**).* White crystals, m.p. 187–188 °C, yield 51%. IR υ_{max}/cm^{-1} 3391 (OH), 3266 (NHs), 3071 (CH aromatic), 2925 (CH aliphatic), 2218 (CN), 1671 (COs), 1606 (NH), 1545–1512 (C=C). ^1H NMR (DMSO-d_6-400 MHz): δ 2.51 (m, 2H, CH_2-2), 2.89 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.22 (t, 2H, $J = 7.2$ Hz, CH_2-3), 4.62 (s, 2H, NH_2), 5.47 (s, 1H, OH), 6.85–7.58 (m, 4H, aromatic protons), 10.01 (s, 1H, NH). MS: m/z(%): 282 (M^+, 37), 250 (10), 174 (100), 146 (57), 147 (63), 119 (55), 92 (27), 66 (62). Anal. Calcd. for $C_{15}H_{14}N_4O_2$ (282.30): C, 63.82; H, 5.00; N, 19.85. Found: C, 64.10; H, 4.87; N, 20.16.

*4.1.1.3. 4-[(6-Amino-7-cyano-2,3-dihydro-1H-pyrrolizine-5-carbonyl)amino]benzoic acid (**9f**).* White crystals, m.p. 245–248 °C, yield 59%. IR υ_{max}/cm^{-1} 3347 (OH), 3285 (NHs), 3018 (CH aromatic), 2968 (CH aliphatic), 2848–2561 (broad band OH) 2224 (CN), 1685 (CO), 1604 (NH), 1535 (C=C). ^1H NMR (DMSO-d_6-400 MHz): δ 2.40 (m, 2H, CH_2-2), 2.89 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.22 (t, 2H, $J = 7.2$ Hz, CH_2-3), 4.30 (s, 2H, NH_2), 7.71–7.92 (m, 4H, aromatic protons), 9.50 and 10.65 (2 s, 2H, NH and COOH). MS: m/z(%): 310 (M^+, 32), 281 (4), 252 (4), 214 (7.2), 196 (2.5), 174 (100), 146 (44), 119 (32), 92 (33), 65 (59). Anal. Calcd. for $C_{16}H_{14}N_4O_3$ (310.31): C, 61.93; H, 4.55; N, 18.06. Found: C, 61.70; H, 4.49; N, 18.08.

4.1.2. General method for preparation of compounds (*10a–f*)

A mixture of the carboxamide **9a–c** (3.75 mmol), the appropriate aldehyde (3.75 mmol) and glacial acetic acid (0.5 ml) in absolute ethanol (20 ml) was refluxed for 4 h. The reaction mixture was concentrated, set aside to cool, whereby crystals were formed, collected and recrystallized from ethanol.

*4.1.2.1. (EZ) 7-Cyano-6-[(4-methoxybenzylidene)amino]-N-phenyl-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**10a**).* Yellow crystals, m.p. 203–205 °C, yield 84%. IR υ_{max}/cm^{-1} 3229 (NH), 3056, 3030 (CH aromatic) 2979–2947 (CH aliphatic), 2206 (CN), 1674 (CO), 1598 (NH), 1543 (C=C). ^1H NMR ($CDCl_3$-300 MHz): δ 2.56 (m, 2H, CH_2-2), 3.04 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.92 (s, 3H, OCH_3), 4.54 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.03–7.39 (m, 5H, 5 aromatic protons), 7.66 and 7.90 (2 d, 4H, $J = 8.7$ Hz, *para*-substituted aromatic H), 9.10 (s, 1H, N=CH) and 10.70 (s, 1H, NH, which disappeared on deuteration). MS: m/z(%): 384 (M^+, 48), 355 (6), 293 (24), 292 (100), 277 (14), 264 (7), 249 (11), 135 (24). $C_{23}H_{20}N_4O_2$ (384.43): C, 71.86; H, 5.24; N, 14.57. Found: C, 71.80; H, 4.83; N, 14.40.

*4.1.2.2. (EZ) 7-Cyano-6-[(4-methoxybenzylidene)amino]-N-(4-tolyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**10b**).* Yellow crystals, m.p. 209–211 °C, yield 87%. IR υ_{max}/cm^{-1} 3219 (NH), 3015 (CH aromatic), 2958–2918 (CH aliphatic), 2213 (CN), 1663 (CO), 1595 (NH), 1542 (C=C). ^1H NMR ($CDCl_3$-300 MHz): δ 2.34 (s, 3H, CH_3), 2.55 (m, 2H, CH_2-2), 3.04 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.92 (s, 3H, OCH_3), 4.52 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.04, 7.95 (2d, 4H, $J = 8.7$ Hz, *para*-substituted aromatic H), 7.17 and 7.57 (2d, 4H, $J = 8.4$ Hz, para-substituted aromatic H), 9.03 (s, 1H, N=CH) and 10.69 (s, 1H, NH, which disappeared on deuteration). Anal. Calcd. for $C_{24}H_{22}N_4O_2$ (398.46): C, 72.34; H, 5.57; N, 14.06. Found: C, 72.42; H, 5.26; N, 14.16.

*4.1.2.3. (EZ) N-(4-Chlorophenyl)-7-cyano-6-[(4-methoxybenzylidene amino)]-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**10c**).* Yellow crystals, m.p. 213–216 °C, yield 81%. IR υ_{max}/cm^{-1} 3224 (NH), 3060 (CH aromatic) 2981–2939 (CH aliphatic), 2211 (CN), 1665 (CO), 1597 (NH), 1542 (C=C). ^1H NMR ($CDCl_3$-300 MHz): δ 2.54 (m, 2H, CH_2-2), 3.01 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.91 (s, 3H, OCH_3), 4.48 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.02, 7.87 (2d, 4H, $J = 7.5$, aromatic H), 7.29 (d, 2H, $J = 6.9$ Hz, aromatic H), 7.59 (d, 2H, $J = 6.9$ Hz, aromatic H), 9.02 (s, 1H, N=CH) and 10.75 (s, 1H, NH, which disappears on deuteration).

$C_{23}H_{19}ClN_4O_2$ (418.88): C, 63.52; H, 4.40; N, 12.88. Found: C, 63.81; H, 4.29; N, 12.69.

*4.1.2.4. (EZ) 7-Cyano-6-[(4-hydroxy-3-methoxybenzylidene)amino]-N-phenyl-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**10d**).* Yellow crystals, m.p. 233–236 °C, yield 84%. IR υ_{max}/cm^{-1} 3444 (OH), 3234 (NH), 3069, 3040 (CH aromatic) 2995–2843 (CH aliphatic), 2211 (CN), 1672 (CO), 1595 (NH), 1512 (C=C). ^{1}H NMR ($CDCl_3$-300 MHz): δ 2.55 (m, 2H, CH_2-2), 3.03 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.98 (s, 3H, OCH_3), 4.52 (t, 2H, $J = 7.2$ Hz, CH_2-3), 6.16 (s, 1H, OH, which disappeared on deuteration), 7.03–7.67 (m, 8H, aromatic H), 9.05 (s, 1H, N=CH) and 10.71 (s, 1H, NH, which disappeared on deuteration). ^{13}C NMR (DMSO-d_6): δ 24.19 (C-1), 25.16 (C-2), 50.18 (C-3), 55.88 (CH_3), 76.72 (C-7), 100.00 (CN), 109.71 (C-6), 116.00 (C-2″), 116.31 (C-5″), 119..21 (C-2′, -6′), 123.88 (C-6″), 125.63 (C-4′), 126.80 (C-1″), 129.35 (C-3′, -5′), 138.64 (C-5), 140.03 (C-1′), 148.75 (C-7a), 148.87 (C-4″) 152.06 (C-3″), 157.92 (CH=N), 160.00 (CO). MS *m/z*(%): 401 ($M^+ + 1$, 51), 400 (M^+, 62), 308 (100), 293 (9), 277 (23), 250 (46), 184 (6), 157 (7). Anal. Calcd. for $C_{23}H_{20}N_4O_3$ (400.43): C,68.99; H, 5.03; N, 13.99. Found: C, 68.95; H, 4.89; N, 13.80.

*4.1.2.5. (EZ) 7-Cyano-6-[(4-hydroxy-3-methoxybenzylidene)amino]-N-(4-tolyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**10e**).* Yellow crystals, m.p. 245–246 °C, yield 89%. IR υ_{max}/cm^{-1} 3324 (OH), 3224 (NH), 3050 (CH aromatic), 3000–2854 (CH aliphatic), 2217 (CN), 1661 (CO), 1599 (NH), 1545–1516 (C=C). ^{1}H NMR ($CDCl_3$-300 MHz): δ 2.34 (s, 3H, CH_3), 2.56 (m, 2H, CH_2-2), 3.05 (t, 2H, $J = 7.5$ Hz, CH_2-7), 4.01 (s, 3H, OCH_3), 4.53 (t, 2H, $J = 7.2$ Hz, CH_2-3), 6.17 (s, H, OH, which disappeared on deuteration), 7.04–7.61 (m, 7H, aromatic protons), 9.00 (s, H, N=CH) and 10.69 (s, H, NH, which disappeared on deuteration). Anal. Calcd. for $C_{24}H_{22}N_4O_3$ (414.46): C, 69.55; H, 5.35; N, 13.52. Found: C, 69.63; H, 5.31; N, 13.68.

*4.1.2.6. (EZ) N-(4-Chlorophenyl)-7-cyano-6-[(4-hydroxy-3-methoxybenzylidene)amino]-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**10f**).* Yellow crystals, m.p. 255–258 °C, yield 84%. IR υ_{max}/cm^{-1} 3318 (OH), 3232 (NH), 3067 (CH aromatic) 2943–2846 (CH aliphatic), 2210 (CN), 1669 (CO), 1613 (NH), 1583–1546 (C=C). ^{1}H NMR ($CDCl_3$-300 MHz): δ 2.58 (m, 2H, CH_2-2), 3.07 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.00 (s, 3H, OCH_3), 4.53 (t, 2H, $J = 7.2$ Hz, CH_2-3), 6.09 (s, H, OH, which disappeared on deuteration), 7.06–7.64 (m, 7H, aromatic protons), 9.06 (s, H, N=CH) and 10.76 (s, H, NH, disappeared on deuteration). Anal. Calcd. for $C_{23}H_{19}ClN_4O_3$ (434.87): C, 63.52; H, 4.40; N, 12.88. Found: C, 63.81; H, 4.29; N, 12.69.

*4.1.3. General method for preparation of compounds (**11a–f**)*

The corresponding acid (3.75 mmol) and thionyl chloride (1 ml) were heated on water bath for one hour, the excess thionyl chloride was distilled under vacuum and the residue was dissolved in 20 ml dry benzene. The appropriate compound **9a–c** (3.75 mmol) was added and the reaction mixture was stirred for one hour then left to stand for 48 h at room temperature. The precipitate formed was filtered, washed with water and hot ethanol, and recrystallized from ethanol-acetone.

*4.1.3.1. (RS) 7-Cyano-6-[2-(4-isobutylphenyl)propionylamino]-N-phenyl-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**11a**).* White crystals, m.p. 189–191 °C, yield 61%. IR υ_{max}/cm^{-1} 3284, 3209 (NHs), 3097, 3044 (CH aromatic), 2991–2866 (CH aliphatic), 2211 (CN), 1701 (COs), 1592 (NH and C=C). ^{1}H NMR (DMSO-d_6-400 MHz): δ 0.82 (d, 6H, $J = 6.3$ Hz, $(CH_3)_2$CH), 1.44 (d, 3H, $J = 7.2$ Hz, CH_3CHCO), 1.76 (m, 1H, $(CH_3)_2$CH), 2.37–2.51 (m, 4H, CH_2-2, CH_2CH), 2.98 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.91 (q, 1H, $J = 7.2$ Hz, CHCO), 4.26 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.00–7.58 (m, 9H, aromatic protons), 9.33 (s, 1H, NHCOCH, which disappeared on deuteration) and 10.22 (s, 1H, CONH–Ph, which disappeared on deuteration). MS (ESI): *m/z*: 454 (M^+), 453 (M-1), 389, 274, 227, 174. Anal. Calcd. for $C_{28}H_{30}N_4O_2$ (454.56): C, 73.98; H, 6.65; N, 12.33. Found: C, 73.71; H, 6.27; N, 12.67.

*4.1.3.2. (RS) 7-Cyano-6-[2-(4-isobutylphenyl)propionylamino]-N-(4-tolyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**11b**).* White crystals, m.p. 195–197 °C, yield 57%. IR υ_{max}/cm^{-1} 3281, 3203 (NHs), 3074, 3017 (CH aromatic), 2954–2866 (CH aliphatic), 2214 (CN), 1703, 1663 (COs), 1593 (NH), 1540–1514 (C=C). ^{1}H NMR (DMSO-d_6-400 MHz): δ 0.82 (d, 6H, $J = 6.3$ Hz, $(CH_3)_2$CH), 1.44 (d, 3H, $J = 7.2$ Hz, CH_3CHCO), 1.76 (m, 1H, $(CH_3)_2$CH), 2.25 (s, 3H, CH_3 tolyl), 2.36–2.51 (m, 4H, CH_2-2, CH_2CH), 2.97 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.92 (q, 1H, $J = 7.2$ Hz, CHCO), 4.25 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.01–7.71 (m, 8H, aromatic proton), 9.25 (s, H, NHCOCH, which disappeared on deuteration) and 10.17 (s, H, CONH–Ph, which disappeared on deuteration). ^{13}C-NMR (DMSO-d_6): δ 18.45 (CH_3CHCO), 20.38 (C-1), 22.10 ($(CH_3)_2$CH), 24.27 (CH_3–Ph), 29.47 (C-2), 35.90 (CH$(CH_3)_2$), 44.39(CHCO), 49.21(C-3), 58.99 (CH_2CH), 84.14 (C-7), 114.33 (CN), 119.32 (C-2′, -6′), 120.42 (C-5), 126.97 (C-2", -6"), 128.89 (C-3", -5"), 129.11 (C-3′, -5′), 132.53 (C-1"), 135.54 (C-4′), 138.02 (C-7a), 145.75 (C-1′), 156.97 (C-6), 163.74 (C-4"), 171.98 (CONHPh), 174.71 (COCH). Anal. Calcd. for $C_{29}H_{32}N_4O_2$ (468.59): C, 74.33; H, 6.88; N, 11.96. Found: C, 74.33; H, 7.06; N, 11.99.

*4.1.3.3. (RS) N-(4-Chlorophenyl)-7-cyano-6-[2-(4-isobutylphenyl) propionylamino]-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**11c**).* White crystals, m.p. 205–207 °C, yield 55%. IR υ_{max}/cm^{-1} 3404, 3260 (NHs), 3096, 3049, 3011 (CH aromatic), 2953–2869 (CH aliphatic), 2222 (CN), 1663 (COs), 1597 (NH), 1539–1519 (C=C). ^{1}H NMR (DMSO-d_6-300 MHz): δ 0.81 (d, 6H, $J = 6.3$, $(CH_3)_2$CH), 1.44 (d, 3H, $J = 7.2$, CH_3CHCO), 1.74 (m, 1H, $(CH_3)_2$CH), 2.34 (d, 2H, $J = 6.9$, $CHCH_2$), 2.50 (m, 2H, CH_2-2), 2.98 (t, 2H, $J = 7.5$, CH_2-1), 3.87 (q, 1H, $J = 7.2$ Hz, CHCO), 4.24 (t, 2H, $J = 7.2$, CH_2-3), 6.98–7.39 (m, 8H, aromatic protons), 9.45 (s, 1H, NHCOCH, which disappeared on deuteration) and 10.13 (s, 1H, CONH–Ph, which disappeared on deuteration) which disappeared on deuteration. Anal. Calcd. for $C_{28}H_{29}ClN_4O_2$ (489.01): C, 68.77; H, 5.98; N, 11.46. Found: C, 68.93; H, 5.72; N, 11.22.

*4.1.3.4. (RS) 6-[2-(3-Benzoylphenyl)propionylamino]-7-cyano-N-phenyl-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**11d**).* White crystals, m.p. 192–194 °C, yield 64%. IR υ_{max}/cm^{-1} 3207, 3135 (NHs), 3031 (CH aromatic) 2998–2878 (CH aliphatic), 2220 (CN), 1662 (COs), 1602 (NH), 1560 (C=C). ^{1}H NMR (DMSO-d_6-300 MHz): δ 1.53 (d, 3H, $J = 6.6$ Hz, CH_3CHCO), 2.50 (m, 2H, CH_2-2), 3.03 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.13 (q, 1H, $J = 6.6$ Hz, CHCO), 4.31 (t, 2H, $J = 6.6$ Hz, CH_2-3), 7.08–7.84 (m, 14H, aromatic protons), 9.26 (s, 1H, NHCOCH, which disappeared on deuteration) and 10.24 (s, 1H, CONH–Ph, which disappeared on deuteration). MS (ESI): *m/z*: 501 ($M^+ - 1$), 412, 267, 240, 159. Anal. Calcd. for $C_{31}H_{26}N_4O_3$ (502.56): C, 74.09; H, 5.21; N, 11.15. Found: C, 74.07; H, 5.03; N, 11.00.

*4.1.3.5. (RS) 6-[2-(3-Benzoylphenyl)propionylamino]-7-cyano-N-(4-tolyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**11e**).* White crystals, m.p. 197–199 °C, yield 61%. IR υ_{max}/cm^{-1} 3405, 3266 (NHs), 3058, 3027(CH aromatic) 2975–2875 (CH aliphatic), 2222 (CN), 1659 (COs), 1599 (NH), 1562–1517 (C=C). ^{1}H NMR ($CDCl_3$-300 MHz): δ 1.67 (d, 3H, $J = 6.6$ Hz, CH_3CHCO), 2.26 (s, 3H, tolyl CH_3), 2.50 (m, 2H, CH_2-2), 2.95 (t, 2H, $J = 7.5$ Hz, CH_2-1), 3.96 (q, 1H, $J = 6.6$ Hz, CHCO), 4.33 (t, 2H, $J = 6.6$ Hz, CH_2-3), 7.02–7.85 (m, 13H, aromatic protons), 8.03 (s, 1H, NHCOCH, which disappeared on deuteration) and 9.13 (s, 1H, CONH–Ph, which disappeared on

deuteration). Anal. Calcd. for $C_{32}H_{28}N_4O_3$, (516.59): C, 74.40; H, 5.46; N, 10.85. Found: C, 74.29; H, 5.19; N, 10.62.

*4.1.3.6. (RS) 6-[2-(3-Benzoylphenyl)propionylamino]-N-(4-chlorophenyl)-7-cyano-2,37-dihydro-1H-pyrrolizine-5-carboxamide (**11f**).* White crystals, m.p. 234–6 °C, yield 54%. IR υ_{max}/cm^{-1} 3287, 3210 (NHs), 3099, 3045 (CH aromatic) 2991–2805 (CH aliphatic), 2211 (CN), 1702 (COs), 1591 (NH), 1559 (C=C). ^1H NMR (DMSO-d_6-300 MHz): δ 1.54 (d, 3H, $J = 6.6$ Hz, $\underline{CH_3}CHCO$), 2.51 (m, 2H, CH_2-2), 3.02 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.10 (q, 1H, $J = 6.6$ Hz, $\underline{CH}CO$), 4.32 (t, 2H, $J = 6.6$ Hz, CH_2-3), 7.05–7.81 (m, 13H, aromatic protons), 9.33 (s, 1H, $\underline{NH}COCH$, which disappeared on deuteration) and 10.26 (s, 1H, $CO\underline{NH}$–Ph, which disappeared on deuteration). Anal. Calcd. for $C_{31}H_{25}ClN_4O_3$ (537.01): C, 69.33; H, 4.69; N, 10.43. Found: C, 69.19; H, 4.58; N, 10.60.

*4.1.4. General method for preparation of compounds (**12a–c** and **13a–c**)*

A mixture of the carboxamide derivatives **9a–c** (3.75 mmol) and phythalic anhydride (0.56 g, 3.75 mmol) in glacial acetic acid (20 ml) was refluxed for 3 h, the solution was evaporated under reduced pressure, and the product was washed with water and dried. The crude powder was dissolved in acetone, the insoluble product was filtered and washed with hot acetone, the residue was recrystallized from ethanol to give compounds **12a–c**. The acetone filtrate and washings were evaporated till dryness to leave out compounds **13a–c** which were recrystallized from acetone–water.

*4.1.4.1. 7,13-Dioxo-N-phenyl-2,3,7,13-tetrahydro-1H-pyrrolizino[1′,2′:5,6]pyrimido[2,1-a] isoindole-5-carboxamide (**12a**).* Yellow powder, m.p. 340–343 °C, yield 9%. IR υ_{max}/cm^{-1} 3303 (NH), 3058, 3027 (CH aromatic), 2955–2915 (CH aliphatic), 1772, 1703, 1667 (COs), 1610 (NH), 1548 (C=C). ^1H NMR (DMSO-d_6-300 MHz): δ 2.56 (m, 2H, CH_2-2), 3.11 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.45 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.14–8.19 (m, 9H, aromatic H), 10.00 (s, 1H, NH, which disappeared on deuteration). MS: *m/z* (%): 397 ($M^+ + 1$, 10), 396 (M^+, 29), 304 (70), 209 (8), 130 (58), 102 (61), 91 (26), 65 (100). Anal. Calcd. for $C_{23}H_{16}N_4O_3$ (396.40): C, 69.69; H, 4.07; N, 14.13. Found: C, 69.89; H, 4.02; N, 13.86.

*4.1.4.2. 7,13-Dioxo-N-(4-tolyl)-2,3,7,13-tetrahydro-1H-pyrrolizino[1′,2′:5,6]pyrimido[2,1-a]iso- indole-5-carboxamide (**12b**).* Yellow powder, m.p. 339–342 °C, yield 11%. IR υ_{max}/cm^{-1} 3311 (NH), 3063 (CH aromatic), 2989–2920 (CH aliphatic), 1774, 1703, 1666 (COs), 1609 (NH), 1545–1507 (C=C). ^1H NMR (DMSO-d_6-300 MHz): δ 2.30 (s, 3H, CH_3-tolyl), 2.50 (m, 2H, CH_2-2), 2.93 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.27 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.19–7.93 (m, 8H, aromatic protons), 9.71 (s, 1H, CONH, which disappeared on deuteration). Anal. Calcd. for $C_{24}H_{18}N_4O_3$ (410.42): C, 70.23; H, 4.42; N, 13.65. Found: C, 70.72; H, 4.03; N, 13.96.

*4.1.4.3. N-(4-Chlorophenyl)-7,13-dioxo—2,3,7,13-tetrahydro-1H-pyrrolizino[1′,2′:5,6]pyrimido [2,1-a]isoindole-5-carboxamide (**12c**).* Yellow powder, m.p. 365–369 °C, yield 7%. IR υ_{max}/cm^{-1} 3313 (NH), 3069 (CH aromatic), 2926–2857 (CH aliphatic), 1776, 1709, 1670 (COs), 1616 (NH), 1545 (C=C). ^1H NMR (DMSO-d_6-300 MHz): δ 2.50 (m, 2H, CH_2-2), 3.05 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.37 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.40–8.13 (m, 8H, aromatic protons), 9.96 (s, 1H, CONH, which disappeared on deuteration). Anal. Calcd. for $C_{23}H_{15}ClN_4O_3$ (430.84): C, 64.12; H, 3.51; N, 13.00. Found: C, 64.01; H, 3.42; N, 13.54.

*4.1.4.4. 7-Cyano- 6-(1,3-dioxoisoindolin-2-yl)-N-phenyl-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**13a**).* White crystals, m.p. 243–246 °C, yield 76%. IR υ_{max}/cm^{-1} 3316 (NH), 3065, 3020 (CH aromatic), 2994–2962(CH aliphatic), 2222 (CN), 1782, 1715, 1675 (COs), 1598 (NH), 1534 (C=C). ^1H NMR (DMSO-300 MHz): δ 2.51 (m, 2H, CH_2-2), 3.09 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.37 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.03–7.44 (m, 5H, aromatic protons), 7.91–8.01 (m, 4H, aromatic protons) and 9.93 (s, 1H, NH, which disappeared on deuteration). ^{13}C NMR (DMSO-d_6): 24.95 (C-1), 25.92 (C-2), 49.84 (C-3), 84.71 (C-7), 114.57 (CN), 120.06 (C-6), 120.40 (C-4′), 121.22 (C-2′, -6′), 124.28 (C-4″, -7″), 124.44 (C-5), 129.00 (C-3′, -5′), 131.78 (C-5″, -6″), 135.56 (C-3a″, -7a″), 138.70 (C-1′), 146.71 (C-7a), 157.81 (CONH), 166.18 (2 CO, C-1″, -3″). MS: *m/z*(%): 396 (M^+, 14), 304 (100), 276 (6), 248 (6), 221 (3), 192 (3), 130 (8), 65 (16). Anal. Calcd. for $C_{23}H_{16}N_4O_3$ (396.40): C, 69.69; H, 4.07; N, 14.13. Found: C, 69.23; H, 3.75; N, 14.43.

*4.1.4.5. 7-Cyano-6-(1,3-dioxoisoindoinl-2-yl)-N-(4-tolyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**13b**).* White crystals, m.p. 271–273 °C, yield 81%. IR υ_{max}/cm^{-1} 3360 (NH), 3030 (CH aromatic), 2922–2855 (CH aliphatic), 2226 (CN), 1781, 1713, 1665 (COs), 1593 (NH), 1568–1519 (C=C).^1H NMR (DMSO-d_6-300 MHz): δ 2.22 (s, 3H, CH_3), 2.51 (m, 2H, CH_2-2), 3.09 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.36 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.05–7.29 (m, 4H, aromatic H), 7.91–8.01 (m, 4H, aromatic protons of isoindolyl ring) and 9.83 (s, 1H, NH, which disappeared on deuteration). Anal. Calcd. for $C_{24}H_{18}N_4O_3$ (410.42): C, 70.23; H, 4.42; N, 13.65. Found: C, 70.22; H, 4.01; N, 13.35.

*4.1.4.6. N-(4-Chlorophenyl)-7-cyano-6-(1,3-dioxoisoindolin-2-yl)-2,3-dihydro-1H- pyrrolizine-5-carboxamide (**13c**).* White crystals, m.p. 260–263 °C, yield 74%. IR υ_{max}/cm^{-1} 3245 (NH), 3067, 3032 (CH aromatic), 2952 (CH aliphatic), 2226 (CN), 1787, 1727, 1669 (COs), 1640 (NH), 1598–1521 (C=C). ^1H NMR ($CDCl_3$-300 MHz): δ 2.59 (m, 2H, CH_2-2), 3.09 (t, 2H, $J = 7.5$ Hz, CH_2-1), 4.41 (t, 2H, $J = 7.2$ Hz, CH_2-3), 7.24–7.42 (m, 4H, aromatic H), 7.84–8.02 (m, 4H, aromatic protons) and 8.65 (s, 1H, NH, which disappeared on deuteration). Anal. Calcd. for $C_{23}H_{15}ClN_4O_3$ (430.84): C, 64.12; H, 3.51; N, 13.00. Found: C, 63.96; H, 3.74; N, 13.15.

*4.1.5. General method for preparation of compounds (**14a–c**)*

A mixture of compounds **9a–c** (3.75 mmol) and maleic anhydride (0.37 g, 3.75 mmol) in 20 ml glacial acetic anhydride was refluxed for 3 h, the solution was evaporated under reduced pressure, and the product was washed with water and recrystallized from ethanol/acetone.

*4.1.5.1. (EZ) 3-(4-Oxo-9-(phenylcarbamoyl)-4,5,6,7-tetrahydro-1H-pyrimido[5,4-a]pyrrolizin-2-yl) acrylic acid (**14a**).* Yellow crystals, m.p. 300–302 °C, yield 64%. IR υ_{max}/cm^{-1} 3446, 3294 (NH), 3081, 3017 (CH aromatic) 2955–2918 (CH aliphatic), 2855 (broad band, OH), 1757, 1701, 1670 (COs), 1601 (NH), 1548 (C=C). ^1H NMR (DMSO-d_6-300 MHz): δ 2.51 (m, 2H, CH_2-6), 3.06 (t, 2H, $J = 7.5$ Hz, CH_2-5), 4.43 (t, 2H, $J = 7.2$ Hz, CH_2-7), 6.83–7.74 (m, 7H, 5 aromatic protons + 2 vinylic protons $\underline{CH}$=$\underline{CH}$COOH), 9.92, 10.16 and 11.96 (3s, 3H, 2 NHs and COOH, which disappeared on deuteration). MS (ESI): *m/z*: 363 ($M^+ - 1$), 305, 280, 218, 203, 175, 162, 150, 115. Anal. Calcd. for $C_{19}H_{16}N_4O_4$ (364.35): C, 62.63; H, 4.43; N, 15.38. Found: C, 62.50; H, 4.30; N, 15.45.

*4.1.5.2. (EZ) 3-(4-Oxo-9-(4-tolylcarbamoyl)-4,5,6,7-tetrahydro-1H-pyrimido[5,4-a]pyrrolizin-2-yl) acrylic acid (**14b**).* Yellow crystals, m.p. 334–336 °C, yield 67%. IR υ_{max}/cm^{-1} 3308, 3135 (NH), 3080 (CH aromatic), 2921 (CH aliphatic), 2560 (OH), 1700, 1657 (COs), 1603, (NH) 1545–1514 (C=C). ^1H NMR (DMSO-d_6-300 MHz): δ 2.28 (s, 3H, CH_3), 2.50 (m, 2H, CH_2-6), 3.00 (t, 2H, $J = 7.5$ Hz, CH_2-5), 4.27 (t, 2H, $J = 7.2$ Hz, CH_2-7), 7.10–7.61 (m, 6H, 4 aromatic protons + 2 vinylic protons $\underline{CH}$=$\underline{CH}$COOH), 9.68, 10.14 and 11.67 (3s, 3H, 2 NHs and COOH, which disappeared on deuteration). Anal. Calcd. for

$C_{20}H_{18}N_4O_4$ (378.38): C, 63.48; H, 4.79; N, 14.81. Found: C, 63.37; H, 5.20; N, 15.35.

*4.1.5.3. (EZ) 3-(9-(4-Chlorophenylcarbamoyl)-4-oxo-4,5,6,7-tetrahydro-1H-pyrimido[5,4-a] pyrrolizin-2-yl)acrylic acid (**14c**).* Yellow crystals, m.p. 332–334 °C, yield 65%. IR υ_{max}/cm^{-1} 3440, 3300, 3258 (NHs), 3082 (CH aromatic) 2964–2916 (CH aliphatic), 2550 (OH), 1765–1667 (COs), 1601 (NH), 1546 (C=C). ^{1}H NMR (DMSO-d_6-300 MHz): δ 2.51 (m, 2H, CH_2-6), 2.99 (t, 2H, $J = 7.5$ Hz, CH_2-5), 4.44 (t, 2H, $J = 7.2$ Hz, CH_2-7), 6.93–7.75 (m, 6H, 4 aromatic protons and 2 vinylic protons CH=CHCOOH), 9.97, 10.18 and 11.85 (3s, 3H, 2 NHs and COOH, which disappeared on deuteration). Anal. Calcd. for $C_{19}H_{15}ClN_4O_4$ (398.80): C, 57.22; H, 3.79; N, 14.05. Found: C, 57.54; H, 3.84; N, 13.80.

*4.1.6. General method for preparation of compounds (**15a–c**)*

The target compounds **15a–c** were prepared from compounds **9a–c** using the same method adopted for the preparation of compound **14a–c** using succinic anhydride (0.38 g, 3.75 mmol).

*4.1.6.1. 3-(4-Oxo-9-(phenylcarbamoyl)-3,4,5,6-tetrahydro-1H-pyrimido[5,4-a]pyrrolizin-2-yl) propanoic acid (**15a**).* Buff crystals, m.p. 271–274 °C, yield 69%. IR υ_{max}/cm^{-1} 3435, 3291, 3198 (NHs), 3090 (CH aromatic), 2973–2865 (CH aliphatic), 2593 (OH), 1723–1649 (COs), 1618 (NH), 1560 (C=C). ^{1}H NMR (DMSO-d_6-300 MHz): δ 2.51 (m, 2H, CH_2-6), 2.76 (t, 2H, $J = 6$ Hz, CH_2CH_2COOH) + 2.87 (t, 2H, $J = 6$ Hz, CH_2COOH), 3.08 (t, 2H, $J = 7.5$ Hz, CH_2-5), 4.44 (t, 2H, $J = 7.2$ Hz, CH_2-7), 7.06–7.72 (m, 5H, aromatic protons), 9.78, 10.22, 11.69 (3s, 3H, two NHs and COOH, which disappeared on deuteration). ^{13}C NMR (DMSO-d_6): δ 24.89 (C-5′), 25.89 (C-6′), 29.01 (C-2), 29.92 (C-3), 49.32 (C-7), 101.71 (C-4a′), 110.96 (C-9a′), 119.32 (C-2″, -6″), 123.36 (C-4″), 129.32 (C-3″, -5″), 139.38 (C-9′), 139.64 (C-1″), 142.31 (C-4b′), 157.02 (C-2′), 158.52 (CONH), 158.94 (COOH), 173.94 (C-4′). MS: m/z(%): 366 (M^+, 7), 349 (6), 256 (45), 228 (68), 201 (5), 120 (11), 92 (34), 65 (100). Anal. Calcd. for $C_{19}H_{18}N_4O_4$ (366.37): C, 62.29; H, 4.95; N, 15.29. Found: C, 62.79; H, 4.55; N, 15.62.

*4.1.6.2. 3-(4-Oxo-9-(4-tolylcarbamoyl)-3,4,5,6-tetrahydro-1H-pyrimido[5,4-a]pyrrolizine-2-yl) propanoic acid (**15b**).* Buff crystals, m.p. 280–283 °C, yield 73%. IR υ_{max}/cm^{-1} 3448, 3292, 3205 (NHs), 3082 (CH aromatic), 2963–2862 (CH aliphatic), 2594 broad band (OH), 1790–1648 (COs), 1616 (NH), 1553–1516 (C=C). ^{1}H NMR (DMSO-d_6-300 MHz): δ 2.27 (s, 3H, CH_3), 2.49 (m, 2H, CH_2-6), 2.78 (t, 2H, $J = 6$ Hz, CH_2CH_2COOH), 2.85 (t, 2H, $J = 6$ Hz, CH_2COOH), 3.03 (t, 2H, $J = 7.5$ Hz, CH_2-5), 4.39 (t, 2H, $J = 7.2$ Hz, CH_2-7), 7.12–7.60 (m, 4H, aromatic protons), 10.12, 11.69 and 12.25 (3 br s, 3H, two NHs and COOH, which disappeared on deuteration). ^{13}C NMR (DMSO-d_6): δ 20.42 (CH_3), 24.35(C-5′), 25.39 (C-6′), 28.49(C-2, C-3), 49.28(C-7′), 83.75(C-4a′), 113.88(C-9a′), 119.54 (C-4″), 120.49 (C-9′),120.72 (C-2″, -6″), 128.89(C-3″, -5″), 132.98 (C-1″), 135.70(C-4b′), 146.04(C-2′), 156.99(CONH),, 175.51(COOH, C-4′). MS: m/z(%): 380 (M^+, 13), 362 (39), 274 (25), 256 (100). Anal. Calcd. for $C_{20}H_{20}N_4O_4$ (380.40): C, 63.15; H, 5.30; N, 14.73. Found: C, 63.14; H, 5.07; N, 15.11.

*4.1.6.3. 3-(9-(4-Chlorophenylcarbamoyl)-4-oxo-3,4,5,6-tetrahydro-1H-pyrimido[5,4-a] pyrrolizin-2-yl)propanoic acid (**15c**).* Buff crystals, m.p. 290–293 °C, yield 72%. IR υ_{max}/cm^{-1} 3400, 3284, 3247 (NHs), 3062 (CH aromatic), 2922 (CH aliphatic), 1787, 1671 (COs), 1616, 1598 (NH, C=C), 1487, 1440 (C–N, C–O), 827, 783 (C–Cl). ^{1}H NMR (DMSO-d_6-300 MHz): δ 2.50 (m, 2H, CH_2-6), 2.77 (t, 2H, $J = 6$ Hz, CH_2CH_2COOH), 2.89 (t, 2H, $J = 6$ Hz, CH_2COOH), 3.05 (t, 2H, $J = 7.5$ Hz, CH_2-5), 4.40 (t, 2H, $J = 7.2$ Hz, CH_2-7), 7.34–7.73 (m, 4H, aromatic protons), 10.22, 10.35 and 11.62 (3 br s, 3H, two NHs and COOH, which disappeared on deuteration). Anal. Calcd. for. $C_{19}H_{17}ClN_4O_4$ (400.82): C, 56.93; H, 4.28; N, 13.98. Found: C, 57.14; H, 4.25; N, 14.40.

4.2. Pharmacological screening

4.2.1. Anti-inflammatory activity [14]

Adult albino rats of both sexes weighing between 120–150 g were used. Rats were uniformly hydrated by giving 3 ml water/rat through gastric inoculation to reduce variability to oedema response. Animals were divided into groups each of five animals. The control group was given saline solution containing few drops of Tween 80. Ketorolac tromethamine (39.23 mmol/kg) was administrated as standard drug for comparison and compounds under examination (39.23 mmol/kg) were suspended in distilled water by the aid of few drops of Tween 80 and were given intraperitoneally one hour before induction of inflammation. Induction of inflammation was performed by S.C. injection of 50 μl of 1% carrageenan-sodium gel (Sigma–Aldrich, USA), into the subplantar region of the right hind paw. The dorso-ventral diameter (thickness) of the right and left hind paws of each rat was measured using a pair of dial thickness gauge calipers accurate to 0.0001 cm 1 h, 2 h, 3 h and 4 h after induction of inflammation. The left hind paw diameter served as a control for the degree of inflammation in the right hind paw. The percentage of anti-inflammatory activity (% inhibition of inflammation) was calculated according to the following equation

$$\%\ \text{inhibition} = (1 - L_t/L_c) \times 100$$

L_t is the mean increase in paw thickness in rats treated with the tested compounds.

L_c is the mean increase in paw thickness in control group.

Data were analyzed by SPSS statistical package version 10. Results are presented in Table 1.

4.2.2. Analgesic activity [15–18]

Analgesic activity was measured using the acetic acid-induced writhing test in mice. Five albino mice of either sex (18–22 g) were used in each group. One hour after intraperitoneal administration of a suspension of the tested compound or ketorolac (39.23 mmol/kg), each mouse was injected with 0.3 ml of 1% acetic acid solution intraperitoneally. Starting 5 min after the acetic acid injection, the number of mascular contractions in each mouse was counted for a period of 30 min. A significant reduction in the number of writhings by any test compound as compared to control animals was considered as a positive analgesic response. Percentage inhibition was calculated using the following formula, where n is the average number of writhings in control group and n' is the average number of writhings in treated group.

$$\%\ \text{Inhibition} = [(n - n')/n] \times 100$$

Results are tabulated in Table 2.

4.2.3. Acute ulcerogenicity studies [19,20]

Adult albino rats of both sexes weighing between 120–150 g were used. Animals were divided into groups each of five animals. Rats were fasted 20 h before drug administration. The tested compounds and ketorolac were given orally in a dose of 39.23 mmol/kg suspended in 1% Tween while one group received vehicle (1% Tween). Rats were fasted for two hours, allowed to feed for 2 h then fasted for another 20 h. Rats were given another two doses in the second and third days. In the fourth day, rats were sacrificed, the stomach removed, opened along with the greater curvature and rinsed with 0.9% saline. The number of mucosal damage (red spots) was counted and their severity (ulcerogenic

severity) was graded from 0 to 4 according to the following score assignment:

	Score		Score
Normal (No injury)	0	Slight injury	3
Latent small red spot	1	Severe injury	4
Wide red spot	2		

The following figures were calculated:

- % Incidence/10 = [number of rats showing ulcer of any grade divided by total number of rats in the group × 100]/10.
- Average number of ulcers: number of ulcers in the group/total number of rats in the group.
- Average severity: Σ [each ulcer multiplied by its score of severity]/number of ulcers in the group.

Ulcer index = the sum of the 3 figures
Results are tabulated in Table 3.

Acknowledgements

The authors are grateful to Dr. Gamal Ahmed El-Sherbeny Mostafa, lecturer of Pharmacology, Faculty of Pharmacy, Beni-Suef University for his kind help in performing the pharmacological screening.

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