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Synthesis and biological evaluation of new 3-substituted indole derivatives as potential anti-inflammatory and analgesic agents

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Abstract—Treatment of 3-cyanoacetyl indole **1** with the diazonium salts of 3-phenyl-5-aminopyrazole and 2-aminobenzimidazole afforded the corresponding hydrazones **4** and **5**. 3-Cyanoacetyl indole reacted with phenylisothiocyanate to give the corresponding thioacetanilide derivative **7**. Treatment of **7** with hydrazonoyl chlorides afforded the corresponding 1,3,4-thiadiazole derivatives **8a–f** and **9**. Also, the thioacetanilide reacted with α-haloketones to afford thiophene derivatives **10a,b** (tenidap analogues), or thiazolidin-4-one derivative **11**. The newly synthesized compounds were found to possess potential anti-inflammatory and analgesic activities.

1. Introduction

Indomethacin and tenidap are non-steroidal anti-inflammatory drugs (NSAIDs) (Fig. 1) and have been shown to exert anti-inflammatory effects.[1,2] Tenidap is an inhibitor of prostaglandin[3] interleukin-1[4] production in the body used for the treatment of rheumatoid arthritis and osteoarthritis. It also inhibits both enzymes cyclooxygenase and 5-lipoxygenase,[5] which convert arachidonic acid into prostaglandin and leukotrienes[3] and exhibit superior activity compared to indomethacin. Synthetic approaches based on NSAIDs have been taken with the aim of improving their profile[6–8] where the action of NSAID is in lowering the prostaglandin production through inhibition of cyclooxygenase (COX). Indole, the potent basic pharmacodynamic nucleus, has been reported to possess a wide variety of biological properties viz., anti-inflammatory,[9–11] anticonvulsant,[12] cardiovascular[13] and antibacterial.[14] In addition, the indole nucleus is incorporated in various natural products such as alkaloids.[15–20] Furthermore, substitution of heterocyclic moiety at 3-position of the indole ring obviously influenced the anti-inflammatory activity.[21] Besides these, pyrazole derivatives were found to possess anti-inflammatory[22] and antimicrobial[23] activities. Also, 1,3,4-thiadiazoles were reported as highly anti-inflammatory,[24] antimicrobial[25] and anticonvulsant[26] agents. Encouraged by the above observations and considering the interesting pharmacological profile of tenidap, we synthesized new 3-substituted indoles incorporating an extra heterocyclic ring: pyrazole; benzimidazole; 1,3,4-thiadiazoles; thiophenes (tenidap analogues) and thiazolidinone as promising anti-inflammatory and analgesic agents.

The biological activity and structure–activity relationship (SAR) of the newly synthesized compounds were evaluated compared to indomethacin and were found to possess potent anti-inflammatory and analgesic activities.

2. Results and discussion

2.1. Chemistry

In continuation of the previous work on the synthesis of indole alkaloid derivatives,[27] we report here

Keywords: Indole; 1,3,4-Thiadiazoles; Thiophenes; Anti-inflammatory and tenidap.
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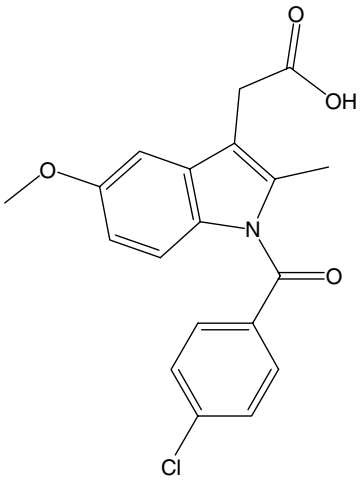
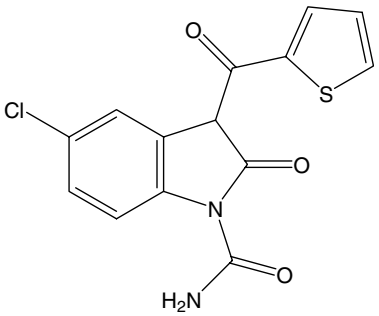


Indomethacin



Tenidap

Figure 1.

synthesis of some new 3-substituted indole derivatives. The reactivity of 3-cyanoacetyl indole **1** towards some heterocyclic diazonium salts such as 3-phenyl-5-amino-1*H*-pyrazole diazonium salt **2** and 2-amino-1*H*-benzimidazole diazonium salt **3** was investigated. Thus, 3-cyanoacetyl indole **1** was found to couple smoothly with the diazonium salts **2** and **3** to afford the corresponding hydrazones **4** and **5**, respectively (Scheme 1). The structure of compound **4** was established on the basis of its elemental analysis and spectral data. Its ^{1}H NMR spectrum revealed singlet signal at δ 7.5 characteristic of the pyrazole proton (H-4) which was confirmed by ^{13}C NMR and HSQC experiments at δ 107.18. The structure of compound **5** was established on the basis of its elemental analysis and spectral data. ^{1}H NMR spectrum of compound **5** revealed a singlet signals at δ 12.01 and 12.71 characteristic for NH groups. In addition, the mass spectrum revealed the peak at *m*/*z* 328 corresponding to its molecular ion (see Section 4).

All attempts to perform intramolecular cyclization of hydrazones **4** and **5** to the closed form by prolonged heating in pyridine were unsuccessful and the starting materials **4** and **5** were recovered unaffected (Scheme 1).

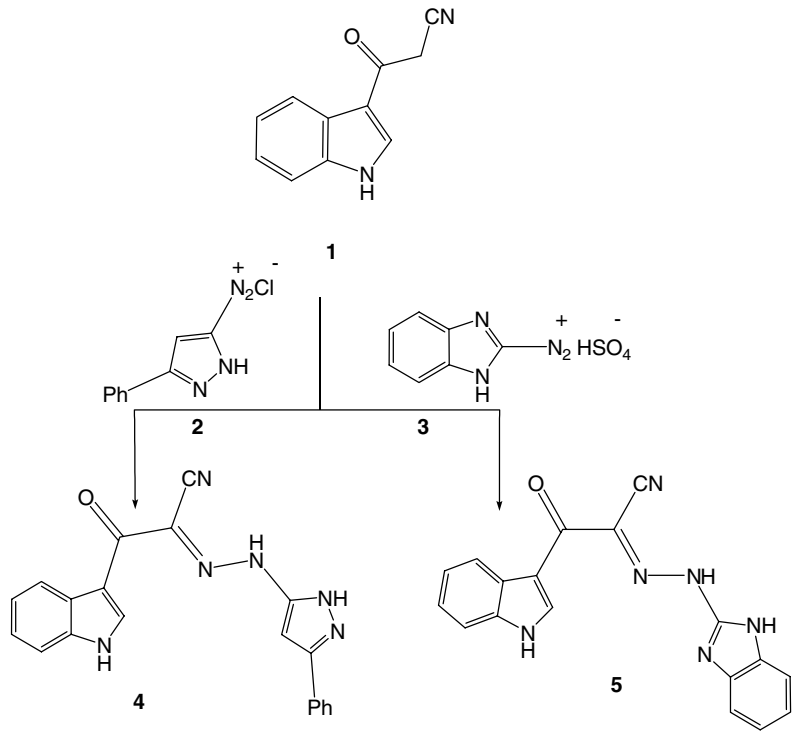
Treatment of compound **1** with phenylisothiocyanate and potassium hydroxide in dimethylformamide at room temperature afforded the potassium salt intermediate **6** which was converted into the corresponding thioacetanilide derivative **7** upon treatment with 10% hydrochloric acid. The thioacetanilide **7** reacted with hydrazonoyl chlorides **12a**–**f** in refluxing ethanol and in the presence of triethylamine to afford, in each case, only 1,3,4-thiadiazole isolable product. Elemental analyses and spectral data of the reaction products were in complete agreement with those of the corresponding 1,3,4-thiadiazole structures **8a**–**f** (Scheme 2). For example, the ^{1}H NMR spectrum of compound **8f** revealed a triplet and quartet signals at δ 1.35 and 4.43 due to methyl and methylene of the ester group, respectively, which was confirmed by ^{13}C NMR and HSQC experiments at δ 13.86 and 63.12, respectively. In addition to AA′ XX′ system signals at δ 7.68 and 7.78 corresponding to *p*-substituted phenyl (129.34 and 129.80), respectively, and two C=O at δ 165.10 and 179.17 in ^{13}C NMR spectra (Fig. 2).

Moreover, the structure of compounds **8a**–**f** was further supported by an independent synthesis from the treatment of the potassium salt intermediate **6** with 1-chloro-1-(2-phenylhydrazono)propan-2-one and/or ethyl 2-chloro-(2-phenylhydrazone)acetate **12a**–**f** at room temperature (Scheme 2).

Also, compound **7** reacted similarly with 1-[chloro-(phenyl)methylene]-2-phenylhydrazine **13** to afford 3-(1*H*-indole-3-yl)-2-(3,5diphenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)-3-oxopropanenitrile **9** based on its elemental analysis and spectral data. It has characteristic absorption peaks in IR spectrum at 3223, 2191 and 1569 cm^{-1} due to NH, CN and carbonyl groups, respectively. In addition, the mass spectrum revealed a peak at *m*/*z* 420 corresponding to its molecular ion (see Section 4).

Next, compound **7** reacted with phenacyl bromide and with α-chloroacetone in refluxing ethanol and in the presence of a catalytic amount of triethylamine to give the corresponding 3-(3-indolyl)thiophene derivatives **10a**,**b** which were considered as analogues of tenidap, as depicted in Scheme 3.

Compound **10a** showed characteristic peaks at 3223, 2190 and 1704 cm^{-1} due to NH, CN and C=O groups, respectively. IR spectrum and its ^{1}H NMR spectrum revealed a singlet signal at δ 1.90 characteristic of the $COCH_3$ protons and singlet signal at δ 7.01 characteristic of the NHPh group. As well a peak at *m*/*z* 357 corresponds to its molecular ion in the mass spectrum. Similarly compound **10b** showed characteristic peaks at 3277, 2201 and 1687 cm^{-}1 due to NH, CN and C=O groups, respectively (in its IR spectrum), and its ^{1}H



Scheme 1.

NMR spectrum revealed singlet at δ 7.76 characteristic of the NHPh group. In addition, the peak at *m*/*z* 419 corresponds to its molecular ion in its mass spectrum.

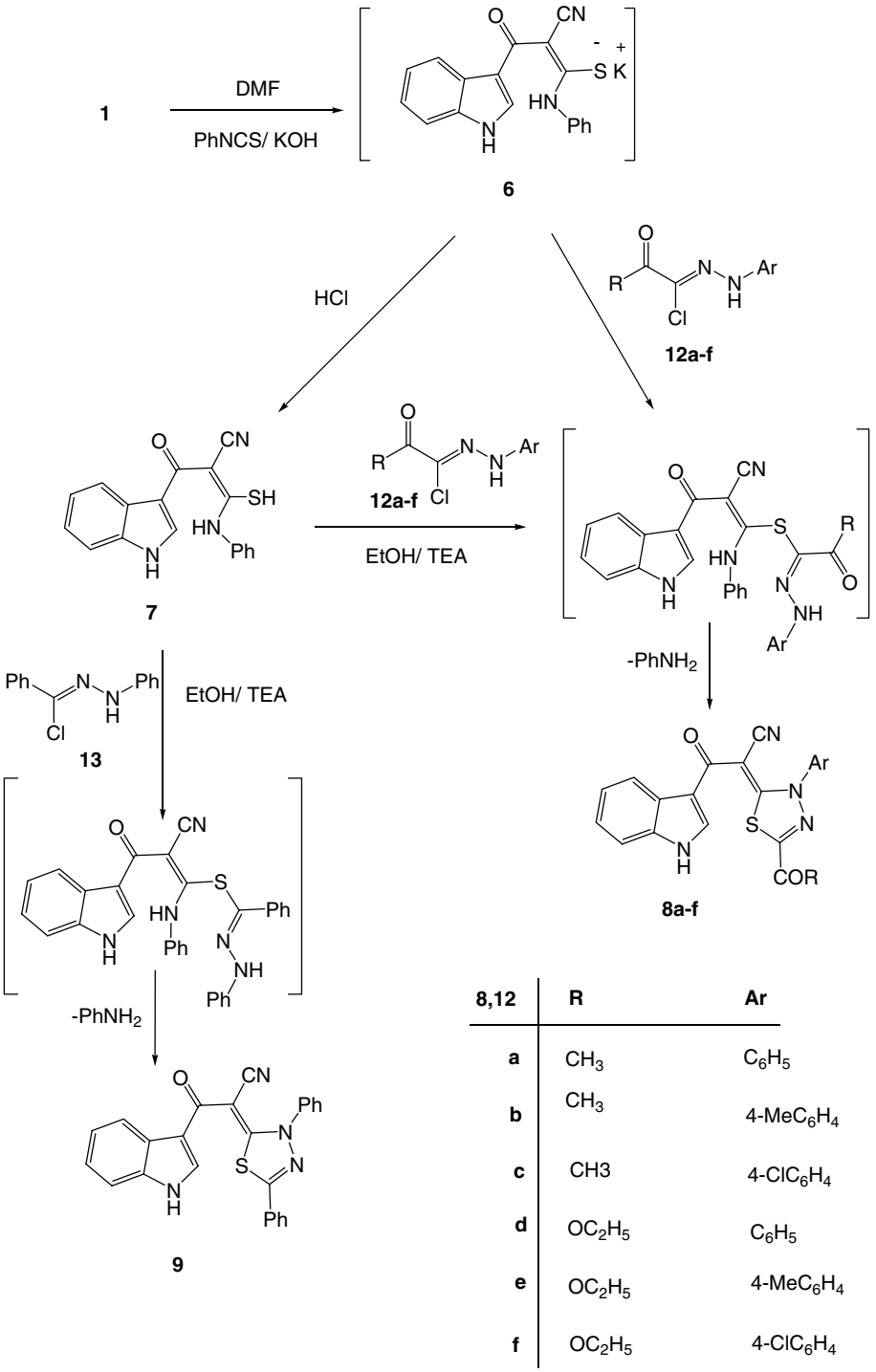
While, thioacetanilide derivative **7** reacted similarly with ethyl chloroacetate under the same reaction conditions to give the corresponding thiazolidin-4-one derivative **11**. The structure of compound **11** was established on the basis of its elemental analysis and spectral data. Its IR spectrum showed the presence of absorption peaks at 3304, 2198, 1717 and 1616 cm^{-1} due to NH, CN and two C=O groups, respectively, and its ^1H NMR spectrum revealed a singlet signal at δ 4.01 characteristic of the methylene protons of thiazolidinone ring. In addition, the mass spectrum revealed a peak at *m*/*z* 359 corresponding to its molecular ion (see Section 4).

2.2. Pharmacology

2.2.1. Anti-inflammatory activity. The activity of the newly synthesized compounds compared to indomethacin as a reference was measured before and 4 h after carrageenan injection. Percent of the oedema inhibition was calculated as regards saline control group and potency was calculated as regards the percentage of the change of the indomethacin and tested compounds, as depicted in Table 1a and Table 1b. All the tested compounds showed a reasonable inhibition of oedema size ranging between 5.5% for compound **8d** and 44.4% for compound **10a** and 29.9% for indomethacin. Hydrazone **5**, which comes from the diazonium salt of 2-aminobenzimidazole **3**, was found to be more potent than hydrazone **4**, which comes from the diazonium salt of 3-phenyl-5-aminopyrazole **2**. As shown in Table 1, the 3-(3-indolyl)thiophene derivative **10a** was the most potent anti-inflammatory compound which was considered as analogue of tenidap. The thioacetanilide **7** showed good activity in comparison with the other tested compounds (28%). Also, 1,3,4-thiadiazole derivative **8b** was found to be more potent than indomethacin. However, thiazolidin-4-one derivative **11** showed the least inhibitory effect (7.7%).

From the structure–activity relationship (SAR) viewpoint, the anti-inflammatory activity of the 5-acetyl 3-(3-indolyl)thiophene derivative **10a** was found to be the highest one. 5-Acetyl-1,3,4-thiadiazole derivative of substituted *p*-methylphenyl **8b** was more potent anti-inflammatory compound than *p*-chlorophenyl derivative **8c** (CH_3 > Cl). The anti-inflammation effect of the thiazolidine ester derivatives **8e**,**f** is less than that of its acetyl derivatives. In addition, the chlorinated ester derivative of 1,3,4-thiadiazole system **8f** was found to be slightly more effective than its non-chlorinated one **8e**. Also 1,3,4-thiadiazole with biphenyl groups **9** showed good activity (31%).

2.2.2. Analgesic activity. Next, the analgesic activity (antinociceptive) of the synthesized 3-indole derivatives was also investigated. It was assessed by two different models: the acetic acid-induced writhing test and the hot-plate test. Chemical models of nociception, by using acetic acid-induced writhing (visceral pain), and thermal model by using hot-plate test (thermal pain). Collier et al.[36] proposed that acetic acid acts indirectly by



8,12	R	Ar
a	CH_3	C_6H_5
b	CH_3	4-MeC_6H_4
c	CH3	4-ClC_6H_4
d	OC_2H_5	C_6H_5
e	OC_2H_5	4-MeC_6H_4
f	OC_2H_5	4-ClC_6H_4

Scheme 2.

inducing the release of endogenous mediators which stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs ($NSAID_S$) and opioids. While the hot-plate model of analgesia measures nociception induced by central mechanisms. Some of the 3-indole derivatives exhibited analgesic effects as shown in Table 2 and Table 3. Compared with the control, the potency of the tested compounds **11** and **8c** was found to be the highest (Table 3). Hydrazone **5**, which comes from the diazonium salt of 2-aminobenzimidazole **3**, was found to be less potent than hydrazone **4**, which comes from the diazonium salt of 3-phenyl-5-aminopyrazole **2**. According to the structure–activity relationship (SAR) it is clear that the thiazolidin-4-one ring system is more active than both the 1,3,4-thiadiazole ring and hydrazone moiety. The reactivity of PhCO > CH_3CO group in 3-(3-indolyl)thiophene derivatives **10a**,**b**. Among the same ring system (1,3,4-thiadiazole derivatives), we noticed

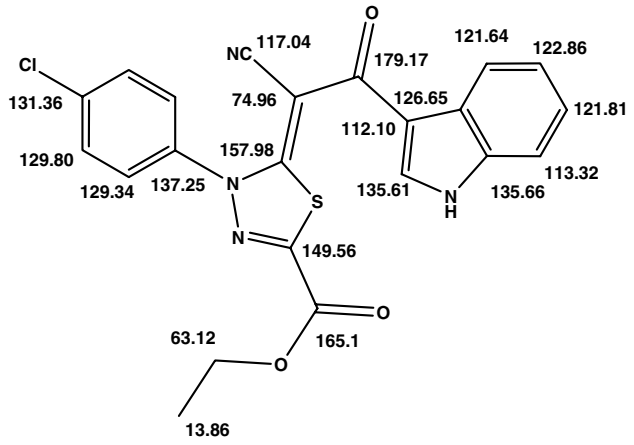
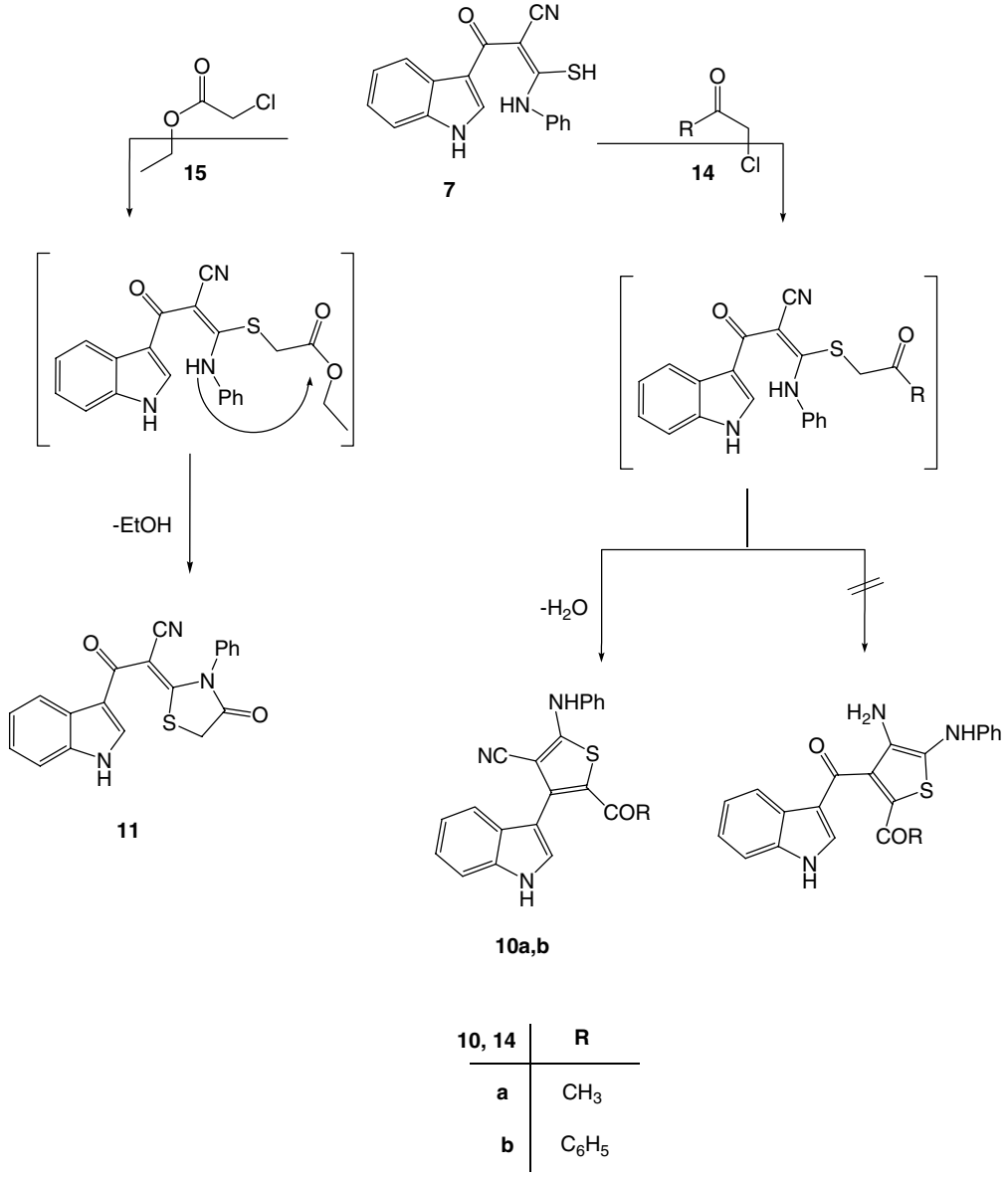


Figure 2. Complete ^{13}C assignment of compound **8f**.

that the chlorinated ketone derivatives are more effective than their non-chlorinated analogues (Table 3).

3. Conclusions

In conclusion, simple and convenient methods for the synthesis of the new anti-inflammatory 3-indole heterocyclic derivatives starting from 3-cyanoacetyl indole were demonstrated. In this approach cyanoacetyl group at the 3-position of indole is used as a precursor to construct the appropriately substituted hydrazones, 1,3,4-thiadiazole derivatives, thiophene derivatives as tenidap analogues, and thiazolidin-4-one derivative. The anti-inflammatory, analgesic activities and structure–activity relationship (SAR) of the newly synthesized compounds were provided. 3-(3-Indolyl)thiophene derivative **10a**,



10, 14	R
a	CH_3
b	C_6H_5

Scheme 3.

Table 1a. Inhibitory effect of some new 3-indole derivatives on carrageenan-induced oedema of the hind paw in mice

Compound	Dose (mg/kg)	Oedema		Oedema (%) (X ± SE)	Oedema inhibition (%)	Potency
		Zero min (basal)	4 h oedema (cm) (% increase)			
Control	1 mL saline	0.23 ± 0.006	0.46 ± 0.01	109.1 ± 6.3	—	—
IND	35	0.26 ± 0.03	0.39 ± 0.03	76.5 ± 3.6*	−29.9	1
4	70	0.21 ± 0.003	0.41 ± 0.2	94.8 ± 5.3	−13.1	0.4
5	70	0.20 ± 0.002	0.37 ± 0.01	81.3 ± 4.8*	−25.48	0.9
7	70	0.22 ± 0.002	0.38 ± 0.03	78.3 ± 4.1*	−28	0.9
8b	70	0.22 ± 0.004	0.37 ± 0.02	73.4 ± 4.4*	−32.7	1.1
8c	70	0.22 ± 0.001	0.42 ± 0.01	92.7 ± 5.1*	−15	0.5
8e	70	0.22 ± 0.009	0.43 ± 0.01	96.4 ± 3.9	−11.5	0.4
8f	70	0.20 ± 0.004	0.38 ± 0.009	88.3 ± 3.2*	−19.1	0.6
10a	70	0.20 ± 0.09	0.32 ± 0.004	60.7 ± 4.9*	−44.4	1.5
11	70	0.20 ± 0.004	0.41 ± 0.01	100.7 ± 4.1	−7.7	0.3

Data represent mean values ± SE of six mice per group and the percent changes versus basal (zero min) values and 4 h post-carrageenan injection. Data were analyzed using one-way ANOVA and Duncan's multiple comparison test $^{*}P < 0.05$.
Percent oedema inhibition was calculated as regards saline control group.
Potency was calculated as regards the percentage change of the indomethacin treated group.
IND, indomethacin.

Table 1b. Inhibitory effect of some new 3-indole derivatives on carrageenan-induced oedema of the hind paw in mice

Compound	Dose (mg/kg)	Oedema		Oedema (%) (X ± SE)	Oedema inhibition (%)	Potency
		Zero min (basal)	4 h oedema (cm) (% increase)			
Control	1 mL saline	0.21 ± 0.006	0.43 ± 0.01	103.8 ± 4.6	—	—
IND	35	0.23 ± 0.02	0.37 ± 0.04	65.2 ± 2.8*	−36.79	1
8a	70	0.23 ± 0.03	0.41 ± 0.03	81.1 ± 5.1*	−21.87	0.6
8d	70	0.22 ± 0.01	0.44 ± 0.02	98.07 ± 4.2	−5.5	0.2
9	70	0.24 ± 0.002	0.40 ± 0.01	71.58 ± 2.4*	−31.04	0.9
10b	70	0.22 ± 0.009	0.42 ± 0.03	89.02 ± 2.9*	−14.24	0.4

Data represent mean values ± SE of six mice per group and the percent changes versus basal (zero min) values and 4 h post-carrageenan injection. Data were analyzed using one-way ANOVA and Duncan's multiple comparison test $^{*}P < 0.05$.
Percent oedema inhibition was calculated as regards saline control group.
Potency was calculated as regards the percentage change of the indomethacin treated group.
IND, indomethacin.

Table 2. Analgesic effect of oral administration of some new 3-indole derivatives on visceral pain by using writhing test in mice

Compound	Dose (mg/kg)	Number of writhing/30 min (X ± SE)	Change (%)	Potency
Saline	1 mL	83.5 ± 3.6	—	—
Indomethacin	35	16 ± 1.2*	−80.7	1
4	70	31.5 ± 2.3*	−62.5	0.77
5	70	30.75 ± 1.8*	−63.2	0.78
7	70	30 ± 2.1*	−64.1	0.79
8a	70	23.75 ± 1.5*	−71.6	0.88
8b	70	35.2 ± 2.9*	−57.8	0.7
8c	70	45.3 ± 3.5*	−45.7	0.57
8d	70	32 ± 1.5*	−61.7	0.76
8e	70	18.75 ± 1.3*	−77.5	0.96
8f	70	20 ± 1.4*	−76	0.9
9	70	54 ± 3.5*	−35.3	0.4
10a	70	26.25 ± 2.2*	−68.6	0.85
10b	70	27.75 ± 1.3*	−66.8	0.8
11	70	32.6 ± 2.8*	−60.9	0.75

Data represent mean values ± SE of six mice per group and percentage inhibition of number of writhing/30 min. Statistical comparison of the difference between saline control group and treated groups was done by one-way ANOVA and Duncan's multiple comparison test $^{*}P < 0.05$.
Potency was calculated as regards the percentage change of the indomethacine.

analogue of tenidap, was the most potent anti-inflammatory compound. According to this study, we could point out that the 3-substituted indole derivatives played a very important role as anti-inflammatory and analgesic agents and considering the interesting pharmacological profile of tenidap. These observations may provide some predictions in order to design further promising 3-indole compounds.

Table 3. Analgesic effect of oral administration of some new 3-indole derivatives on thermal pain by using hot-plat test in mice

Compound	Dose (mg/kg)	Latency (S)		Change (%)	Potency
		Basal (X ± SE)	1 h (X ± SE)		
Saline	1 mL	11.3 ± 0.8	12 ± 0.9	6.1	—
Indomethacin	35	11.5 ± 0.6	14.1 ± 1*	22.6	1
4	70	11.3 ± 1.1	14.5 ± 1.2*	28.3	1.3
5	70	12.8 ± 0.9	13.9 ± 0.8	8.6	0.4
7	70	12 ± 0.7	14.9 ± 1.1*	24.2	1.1
8a	70	12.2 ± 0.6	12.5 ± 0.8	2.4	0.1
8b	70	11.9 ± 0.5	14.4 ± 0.9*	21	0.9
8c	70	12.5 ± 0.9	21.2 ± 1.5*	69.6	3.1
8d	70	12.5 ± 1.1	13.7 ± 0.7	9.6	0.4
8e	70	11.4 ± 0.5	12.7 ± 0.9	11.4	0.5
8f	70	11.1 ± 0.7	12.2 ± 1	9.9	0.4
9	70	11 ± 0.5	14 ± 0.6*	27.3	1.2
10a	70	12.5 ± 1.1	13.9 ± 1.1	11.2	0.5
10b	70	11 ± 0.6	15.7 ± 1.3*	42.7	1.9
11	70	11.5 ± 0.9	20.3 ± 1.6*	76.5	3.4

Data represent mean values ± SE of six mice per group, shown at the basal (zero time) and 1 value for each group (saline, indomethacin and tested compounds). Statistical comparisons between basal (pre-drug values) and post-drug values.
Data were analyzed using one-way ANOVA and Duncan's multiple comparison test $^{*}P < 0.05$.
Percentage change was calculated from basal (pre-drug) values and post-drug values.
Potency was calculated as regards the percentage change of the indomethacin.

4. Experimental

4.1. Chemistry

4.1.1. General. Melting points were determined on a Gallenkamp melting point apparatus and the values are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer. ^{1}H NMR spectra were recorded on a Jeol EX-270 MHz spectrometer using DMSO-d_6 as solvent and TMS as the internal standard, ^{13}C NMR and 2D NMR spectra were recorded on a varian Mercury VX 300 NMR using DMSO-d_6 as solvent and TMS as an internal standard. Mass spectra were recorded on a Finnigan mat. SSQ-7000 GC-MS spectrometer. Microanalyses were performed at the Microanalytical Center of Cairo University. 3-Cyanoacetyl indole **1**,[28] hydrazonoyl chlorides **12a–c**,[29] **12d–f**[30] and **13**[31] and phenacyl bromides **14b**[32] were prepared as reported in the literature.

4.1.2. Reaction of the 3-cyanoacetyl indole (1) with diazonium salts of 3-phenyl-5-aminopyrazole (2) and 2-aminobenzimidazole (3): general procedure. To a stirred cold solution of 3-cyanoacetyl indole **1** (0.37 g, 2 mmol) in pyridine (25 mL) was added the diazonium salt **2** or **3** (2 mmol) portionwise over a period of 30 min. The reaction mixture was kept in an icebox overnight and then diluted with water. The solid that precipitated was filtered off, washed with water and dried. Recrystallization from DMF gave the corresponding hydrazones **4** or **5**, respectively.

4.1.2.1. 2-(2-(3-Phenyl-1*H*-pyrazol-5-yl)hydrazono)-3-(1*H*-indole-3-yl)-3-oxopro-panenitrile (4). In 0.58 g (82%) yield; mp > 300 °C; IR (KBr) ν_{max} 3178 (NH), 3172 (NH), 2226 (CN), 1658 (C=O), 1596 (C=N), 1228 cm^{-1}; ^{1}H NMR: δ 7.34 (m, 2H, H-5, H-6), 7.49 (s, 1H, NH), 7.50 (s, 1H, H-4, pyrazole), 7.54 and 8.12 (m, 5H, Ph), 7.65 (d, 1H, H-7), 7.77 (d, 1H, H-4), 8.1 (s, 1H, NH), 8.67 (s, 1H, H-2), 12.65 (br s, 1H, NH); ^{13}C NMR: δ 107.18, 109.90, 122.86, 127.25, 130.98, 133.11, 135.07, 136.77, 139.15, 140.54, 144.54, 146.66, 147.86, 160.58, 167.82. EIMS *m*/*z* (%) 336 ($M^{+}-18$, 100), 310 (21), 166 (43), 139 (25), 116 (11), 77 (54), 51 (48). Anal. Calcd for $C_{20}H_{14}N_6O$: C, 67.79; H, 3.89; N, 23.72; O, 4.87. Found: C, 67.69; H, 3.93; N, 23.68; O, 4.97.

4.1.2.2. 2-(2-(1*H*-Benzo[*d*]imidazol-2-yl)hydrazono)-3-(1*H*-indole-3-yl)-3-oxopro-panenitrile (5). In 0.51 g (78%) yield; mp > 300 °C; IR (KBr) ν_{max} 3448 (NH), 3226 (NH), 2221 (CN), 1704 (C=O), 1626 (C=N), 1228 cm^{-1}; ^{1}H NMR: δ 7.19 (m, 2H, H-5, H-6), 7.42 (m, 4H, benzoimidazol), 7.5 (d, 1H, H-7), 8.32 (d, 1H, H-4), 8.56 (s, 1H, H-2), 12.01 (s, 1H, NH), 12.71 (br s, 1H, NH); ^{13}C NMR: δ 111.19, 111.92, 113.02, 113.77, 121.50, 121.65, 122.70, 122.98, 123.98, 123.22, 126.68, 130.64, 135.91, 136.27, 157.39, 181.02. EIMS *m*/*z* (%) 328 (M^{+}, 85), 311 (45), 164 (18), 144 (100), 116 (75), 89 (65), 63 (25). Anal. Calcd for $C_{18}H_{12}N_6O$: C, 65.85; H, 3.68; N, 25.60; O, 4.87. Found: C, 65.73; H, 3.88; N, 25.59; O, 4.91.

4.1.3. Synthesis of the thioacetanilide derivative (7). To a stirred solution of potassium hydroxide (0.6 g, 10 mmol) in dimethylformamide (50 mL), 3-cyanoacetyl indole **1** (1.8 g, 10 mmol) was added. After stirring for 30 min, phenylisothiocyanate (1.4 g, 10 mmol) was added to the resulting mixture. The stirring was continued for further 6 h then poured over crushed ice containing hydrochloric acid. The solid product was filtered off, washed with water, dried and finally recrystallized from DMF/H_2O to afford 3-(1*H*-indole-3-yl)-2-(mercapto(phenylamino)methylene)-3-oxopropanenitrile **7** as a orange

powder in 2.74 g (86%) yield; mp 190–193 °C; IR (KBr) ν_{max} 3329 (NH), 3245 (NH), 2196 (CN), 1704 (C=O), 1626 (C=N) cm^{-1}; ^{1}H NMR: δ 7.16 (m, 5H, Ph), 7.26 (m, 2H, H-5, H-6), 7.75 (d, 1H, H-7), 8.17 (d, 1H, H-4), 8.64 (s, 1H, H-2), 11.88 (s, 1H, NH), 14.25 (s, 1H, SH); MS *m/z* (%) 319 (M$^+$, 34), 166 (49), 144 (100), 139 (15), 116 (19), 77 (67). Anal. Calcd for $C_{18}H_{13}N_3OS$: C, 67.69; H, 4.10; N, 13.16; O, 5.01; S, 10.04. Found: C, 67.23; H, 4.05; N, 13.34; S, 10.15.

4.1.4. Reaction of the thioacetanilide derivative 7 with hydrazonoyl chlorides. To a solution of the thioacetanilide derivative **7** (0.64 g, 2 mmol) in absolute ethanol (20 mL), the appropriate hydrazonoyl chlorides **12a–f** or **13** (2 mmol) were added, in the presence of triethylamine (0.3 mL). The reaction mixture was refluxed for 1 h and then allowed to cool. The formed solid product was filtered off, washed with ethanol and recrystallized from EtOH/DMF to afford the corresponding thiadiazole derivatives **8a–f** and **9**.

4.1.4.1. 2-(5-Acetyl-3-phenyl-1,3,4-thiadiazol-2(3*H*)-lidene)3-(1*H*-indole-3-yl)-3-oxopropa-nenitrile (8a). Orange-yellow (0.53 g, 69%); mp 270–273 °C; IR (KBr) ν_{max} 3220 (NH), 2198 (CN), 1687 (C=O), 1574 (C=N) cm^{-1}; ^{1}H NMR: δ 2.3 (s, 3H, $COCH_3$) 7.19 (m, 5H, Ph), 7.28 (m, 2H, H-5, H-6), 7.86 (d, 1H, H-7), 8.35 (d, 1H, H-4), 8.61 (s, 1H, H-2), 12.02 (s, 1H, NH); MS *m/z* (%) 386 (M$^+$, 38), 343 (49), 309 (25), 266 (15), 144 (100), 77 (67). Anal. Calcd for $C_{21}H_{14}N_4O_2S$: C, 65.27; H, 3.65; N, 14.50; O, 8.28; S, 8.30. Found: C, 65.12; H, 3.54; N, 14.56; O, 8.31; S, 8.35.

4.1.4.2. 2-(5-Acetyl-3-*p*-tolyl-1,3,4-thiadiazol-2(3*H*)-lidene)3-(1*H*-indole-3-yl)-3-oxopropa-nenitrile (8b). Yellow needles (0.61 g, 76%); mp > 300 °C; IR (KBr) ν_{max} 3222 (NH), 2197 (CN), 1676 (C=O), 1567 (C=N) cm^{-1}; ^{1}H NMR: δ 2.44 (s, 3H, $COCH_3$), 2.5 (s, 3H, *p*-CH_3–Ph), 7.20 (m, 2H, H-5, H-6), 7.44 (d, 2H, J = 7.5 Hz), 7.60 (d, 2H, J = 7.5 Hz), 7.50 (d, 1H, H-7), 8.25 (d, 1H, H-4), 8.33 (s, 1H, H-2), 11.88 (s, 1H, NH); MS *m/z* (%) 400 (M$^+$, 54), 357 (35), 309 (71), 268 (15), 144 (100), 77 (67). Anal. Calcd for $C_{22}H_{16}N_4O_2S$: C, 65.98; H, 4.03; N, 13.99; O, 7.99; S, 8.01. Found: C, 65.76; H, 4.11; N, 13.87; O, 8.05; S, 8.12.

4.1.4.3. 2-(5-Acetyl-3-4-chlorophenyl-1,3,4-thiadiazol-2(3*H*)-lidene)3-(1*H*-indole-3-yl)-3-oxopropanenitrile (8c). Yellow (0.65 g, 77%); mp 283 °C; IR (KBr) ν_{max} 3271 (NH), 2192 (CN), 1679 (C=O), 1570 (C=N) cm^{-1}; ^{1}H NMR: δ 2.47 (s, 3H, $COCH_3$), 7.19 (m, 2H, H-5, H-6), 7.41 (d, 2H, J = 7.8 Hz), 7.63 (d, 2H, J = 7.8 Hz), 7.68 (d, 1H, H-7), 8.35 (d, 1H, H-4), 8.61 (s, 1H, H-2), 11.93 (s, 1H, NH); MS *m/z* (%) 420 (M$^+$, 34), 384 (12), 377 (41), 268 (55), 144 (100), 77 (37). Anal. Calcd for $C_{21}H_{13}ClN_4O_2S$: C, 59.93; H, 3.11; Cl, 8.42; N, 13.31; O, 7.60; S, 7.62. Found: C, 59.79; H, 3.17; Cl, 8.33; N, 13.39; O, 7.67; S, 7.56.

4.1.4.4. Ethyl 5-(1-cyano-2-(1*H*-indol-3-yl)-2-oxoethylidene)-4,5-dihydro-4-phenyl-1,3,4-thiadiazole-2-carboxylate (8d). Yellow needles (0.62 g, 75%); mp 260–261 °C; IR (KBr) ν_{max} 3229 (NH), 2195 (CN), 1684 (C=O), 1571 (C=N) cm^{-1}; ^{1}H NMR: δ 1.36 (t, 3H, CH_3, J = 7.2 Hz), 4.45 (q, 2H, CH_2, J = 7.2 Hz), 7.19 (m, 5H, Ph), 7.23 (m, 2H, H-5, H-6), 7.81 (d, 1H, H-7), 8.32 (d, 1H, H-4), 8.46 (s, 1H, H-2), 11.87 (s, 1H, NH); MS *m/z* (%) 416 (M$^+$, 51), 323 (19), 273 (25), 144 (100). Anal. Calcd for $C_{22}H_{16}N_4O_3S$: C, 63.45; H, 3.87; N, 13.45; O, 11.53; S, 7.70. Found: C, 63.49; H, 3.66; N, 13.39; O, 11.58; S, 7.64.

4.1.4.5. Ethyl 5-(1-cyano-2-(1*H*-indol-3-yl)-2-oxoethylidene)-4,5-dihydro-*p*-tolyl-1,3,4-thiadiazole-2-carboxylate (8e). Yellow-orange (0.61 g, 71%); mp 246–248 °C; IR (KBr) ν_{max} 3389 (NH), 2196 (CN), 1708 (C=O), 1571 (C=N) cm^{-1}; ^{1}H NMR: δ 1.35 (t, 3H, CH_3, J = 7.2 Hz), 2.43 (s, 3H, *p*-CH_3–Ph), 4.43 (q, 2H, CH_2, J = 7.2 Hz), 7.21 (m, 2H, H-5, H-6), 7.34 (d, 2H, J = 7.8 Hz), 7.52 (d, 2H, J = 7.8 Hz), 7.78 (d, 1H, H-7), 8.25 (d, 1H, H-4), 8.33 (s, 1H, H-2), 11.89 (s, 1H, NH); MS *m/z* (%) 430 (M$^+$, 21), 339 (39), 266 (65), 144 (100). Anal. Calcd for $C_{23}H_{18}N_4O_3S$: C, 64.17; H, 4.21; N, 13.01; O, 11.15; S, 7.45. Found: C, 64.39; H, 3.99; N, 13.11; O, 11.68; S, 7.54.

4.1.4.6. Ethyl 5-(1-cyano-2-(1*H*-indol-3-yl)-2-oxoethylidene)-4,5-dihydro-4-chlorophenyl-1,3,4-thiadiazole-2-carboxylate (8f). Yellow (0.67 g, 74%); mp 289–291 °C; IR (KBr) ν_{max} 3272 (NH), 2195 (CN), 1735 (C=O), 1570 (C=N) cm^{-1}; ^{1}H NMR: δ 1.35 (t, 3H, CH_3, J = 7.2 Hz), 4.43 (q, 2H, CH_2, J = 7.2 Hz), 7.20 (m, 2H, H-5, H-6), 7.68 (d, 2H, J = 7.8 Hz), 7.78 (d, 2H, J = 7.8 Hz), 7.48 (d, 1H, H-7), 8.26 (d, 1H, H-4), 8.35 (s, 1H, H-2), 11.93 (s, 1H, NH); ^{13}C NMR: δ 13.86, 63.12, 74.95, 112.11, 113.32, 117.04, 121.64, 121.80, 122.86, 126.70, 129.34, 129.80, 131.36, 135.61, 135.66, 137.25, 149.56, 157.98, 165.1, 179.17; MS *m/z* (%) 450 (M$^+$, 21), 338 (55), 265 (65), 144 (100). Anal. Calcd for $C_{22}H_{15}ClN_4O_3S$: C, 58.60; H, 3.35; Cl, 7.86; N, 12.43; O, 10.65; S, 7.11. Found: C, 58.44; H, 3.38; Cl, 7.86; N, 12.53; O, 10.71; S, 7.07.

4.1.4.7. 3-(1*H*-Indole-3-yl)-2-(3.5-diphenyl-1,3,4-thiadiazol-2(3*H*)- lidene)-3-oxopropane-nitrile (9). Orange-yellow (0.53 g, 69%); mp 286–289 °C; IR (KBr) ν_{max} 3223 (NH), 2191 (CN), 1589 (C=O), 1564 (C=N) cm^{-1}; ^{1}H NMR: δ 7.19–7.27 (m, 10H, 2Ph), 7.31 (m, 2H, H-5, H-6), 7.76 (d, 1H, H-7), 8.31 (d, 1H, H-4), 8.44 (s, 1H, H-2), 11.92 (s, 1H, NH); MS *m/z* (%) 420 (M$^+$, 28), 343 (41), 266 (19), 144 (100), 77 (67). Anal. Calcd for $C_{25}H_{16}N_4OS$: C, 71.41; H, 3.84; N, 13.32; O, 3.80; S, 7.63. Found: C, 71.36; H, 3.75; N, 13.34; O, 3.76; S, 7.61.

4.1.5. Reaction of thioacetanilide derivative 7 with α-haloketones: general procedure. To a solution of the thioacetanilide derivative **7** (0.64 g, 2 mmol) in ethanol (20 mL), the appropriate α-haloketone, chloroacetone **14a**, phenacyl bromide **14b** or ethyl chloroacetate **15** (2 mmol) were added. Triethylamine (0.2 mL) was added dropwise and the reaction mixture was refluxed for 1 h then allowed to cool. The formed solid product was filtered off, washed with ethanol and recrystallized from EtOH/DMF to afford the corresponding thiophene

derivatives **10a**,**b** and thiazolidin-4-one derivative **11**, respectively.

4.1.5.1. 5-Acetyl-4-(1*H*-indole-3-yl)-2-(phynylamino)thiophene-3-carbonitrile (10a). Yellow (0.53 g, 74%); mp > 300 °C; IR (KBr) ν_{max} 3223 (NH), 2190 (CN), 1704 (C=O), 1574 (C=N) cm^{-}1; ^{1}H NMR: δ 1.90 (s, 3H, $COCH_3$), 7.01 (s, 1H, NH), 7.18 (m, 2H, H-5, H-6), 7.46 (d, 1H, H-7), 7.42-7.52 (m, 5H, Ph), 8.23 (d, 1H, H-4), 8.26 (s, 1H, H-2), 11.60 (s, 1H, NH); MS *m*/*z* (%) 357 (M^+, 14), 280 (46), 265 (56), 239 (25), 224 (100), 77 (67). Anal. Calcd for $C_{21}H_{15}N_3OS$: C, 70.57; H, 4.23; N, 11.76; O, 4.48; S, 8.97. Found: C, 70.43; H, 4.27; N, 11.68; O, 4.49; S, 8.86.

4.1.5.2. 5-Benzoyl-4-(1*H*-indole-3-yl)-2-(phynylamino)-thiophene-3-carbonitrile (10b). Yellow (0.55 g, 66%); mp > 300 °C; IR (KBr) ν_{max} 3277 (NH), 2201 (CN), 1687 (C=O), 1574 (C=N) cm^{-}1; ^{1}H NMR: δ 7.23 (m, 2H, H-5, H-6), 7.40–7.62 (m, 10H, 2Ph), 7.76 (s, 1H, NH), 7.80 (d, 1H, H-7), 8.30 (d, 1H, H-4), 8.56 (s, 1H, H-2), 12.02 (s, 1H, NH); MS *m*/*z* (%) 419 (M^+, 14), 280 (46), 265 (56), 239 (25), 224 (100), 77 (67). Anal. Calcd for $C_{26}H_{17}N_3OS$: C, 74.44; H, 4.08; N, 10.02; O, 3.81; S, 7.64. Found: C, 74.49; H, 4.03; N, 10.11; O, 3.75; S, 7.61.

4.1.5.3. 3-(1*H*-Indole-3-yl)-2-(4-oxo-3-phenylthiazolidin-2-ylidene)-3-oxopropanenitrile (11). Yellow (0.55 g, 66%); mp >300 °C; IR (KBr) ν_{max} 3304 (NH), 2198 (CN), 1717 (C=O), 1616 (C=N) cm^{-}1; ^{1}H NMR: δ 4.01 (s, 2H, CH_2) 7.20 (m, 2H, H-5, H-6), 7.46-7.52 (m, 5H, Ph), 7.54 (d, 1H, H-7), 8.20 (d, 1H, H-4), 8.25 (s, 1H, H-2), 11.90 (s, 1H, NH); MS *m*/*z* (%) 359 (M^+, 24), 266 (12), 144 (100), 77 (16). Anal. Calcd for $C_{20}H_{13}N_3O_2S$: C, 66.84; H, 3.65; N, 11.69; O, 8.90; S, 8.92. Found: C, 74.49; H, 4.03; N, 10.11; O, 3.75; S, 7.61.

4.2. Pharmacology

4.2.1. Animals. Swiss mice (weighing 20–30 g) purchased from the animal house of National Research Centre were used. The animals were housed in standard metal cages in an air-conditioned room at 22 ± 3 °C, 55 ± 5% humidity and provided with standard laboratory diet and water ad libitum. Experiments were performed between 9 am and 3 pm. Group of six mice was used for each experiment. All experimental procedures were conducted in accordance with the Guide for Care and Use of Laboratory Animals and in accordance with the Local Animal Care and Use Committee. The distilled water was used as a vehicle for all tested compounds and 5% sodium bicarbonate for indomethacin.

4.2.2. Anti-inflammatory activity. Anti-inflammatory activity in acute model was carried out according to Winter et al.[33] and Obkowicz et al.[34] Mice were divided into 15 groups each of six. They received orally saline as control, tested compounds (70 mg/kg) and indomethacin (35 mg/kg), according to Rani et al. and Souza et al.[38,40] after induction of oedema by subplanter injection of 50 μL of 1% carrageenan (Sigma, USA) in saline into the pad of right paw. The difference in hind footpad thickness was measured before and 4 h after carrageenan injection. The oedema was expressed as a percentage of change from the control group (pre-drug) values. The dose of the tested compounds and indomethacin was based on the rat dose converted to that of mice according to Paget and Barnes.[39]

In anti-inflammatory study, the percentage of oedema inhibition was calculated from the mean effect in the control and treated animals according to the following equation:

$$\%\ \text{Oedema inhibition} = \{[(\%\ \text{oedema formation of control group} - \%\ \text{oedema formation of treated group})] / [\%\ \text{oedema formation of control group}] \times 100\}$$

While the potency was calculated as regards indomethacin treated group according to the following equation:

$$\text{Potency} = \{\%\ \text{oedema inhibition of treated group}\} / \{\%\ \text{oedema inhibition of indomethacin treated group}\}$$

4.2.3. Antinociceptive activity. This activity was determined by measuring the responses of animals to the Koster test and hot-plate test.

4.2.3.1. Koster test. Acetic acid-induced writhing in mice was performed according to the Koster test.[35,36] The mice were divided into 15 groups and received all the tested compounds at dose of 70 mg/kg and indomethacin at dose of 35 mg/kg. After 60 min interval, the mice received 0.6% acetic acid ip (0.2 mL/mice). The number of writhings in 30 min period was counted and evaluated.

4.2.3.2. Hot-plate test. Hot-plate test was conducted according to Eddy and Leimback[37] using an electronically controlled hot-plate (Ugo Basile, Italy) adjusted at 52 ± 0.1 °C and the cut-off time was 60 s. Fifteen groups of mice each of six were used. The mice were divided and received the same doses of tested compounds and indomethacin as mentioned before. The time elapsed until either paw licking or jumping occurs is recorded before and 1 h after oral administration.

4.2.4. Statistical analysis. Data are expressed as means ± SE. In anti-inflammatory study data are expressed as means ± SE. The results of carrageenan-induced paw oedema are expressed as percentage of change to the control (pre-drug) values. Differences between vehicle control and treatment groups were tested using one-way ANOVA followed by multiple comparisons by the Duncan's multiple range test. A probability value <0.05 was considered statistically significant.

References and notes

1. Flower, R. J.; Moncada, S.; Vane, J. R. *Goodman and Gilman's the Pharmacological Basis of Therapeutics*, 7th ed.; MacMillan: New York, 1985, pp 695.

2. Moore, P. F.; Larson, D. L.; Otterness, I. G.; Weissman, A.; Kadin, S. B.; Sweeney, F. J.; Eskara, J. D.; Nagahisa, A.; Sakakibara, M.; Carty, T. J. *Inflamm. Res.* **1996**, *45*, 54.
3. Catry, T. J.; Showell, H. J.; Losee, L. D.; Kadin, S. B. *Arthritis Rheum.* **1988**, *31*, S89.
4. McDonald, B.; Loose, L.; Rosenwasser, L. *J. Arthritis Rheum.* **1988**, *31*, S17.
5. Moilanen, E.; Alanco, J.; Asmawi, M. Z.; Vepaatalo, H. *Eicosanoids* **1988**, *7*, 35.
6. Wallace, J. L. *Can. J. Physiol. Pharmacol.* **1994**, *72*, 1493.
7. Mosti, L.; Menozzi, G.; Fossa, P.; Filippelli, W.; Gessi, S.; Rinaldi, B.; Falcone, G. *Arzmeimittelforschung* **2000**, *50*, 963.
8. Schindler, R.; Hoefgen, N.; Heinecke, K.; Poppe, H.; Szelenyi, I. *Pharmazie* **2000**, *55*, 857.
9. Misra, U.; Hitkari, A.; Saxena, A. K.; Gurtu, S.; Shanker, K. *Eur. J. Med. Chem.* **1996**, *31*, 629.
10. Andreani, A.; Rambaldi, M.; Locatelli, A.; Pifferi, G. *Eur. J. Med. Chem.* **1994**, *29*, 903.
11. Ebeid Mohamad, Y.; Lashine Sayed, M.; El-Adl Sobhy, M.; Abou Kull Mansour, E. *Zagazig J. Pharm. Sci.* **1994**, *3*, 40.
12. El-Gendy Adel, A.; Abdou Naida, A.; Sarhan El-Taher, Z.; El-Banna Hosny, A. *Alexandria J. Pharm. Sci.* **1993**, *7*, 99.
13. Kumar, A.; Saxena, K. K.; Gurtu, S.; Sinha, J. N.; Shanker, K. *Indian Drugs* **1986**, *24*, 1.
14. Dandia, A.; Sehgal, V.; Singh, P. *Indian J. Chem.* **1993**, *32B*, 1288.
15. Sakemi, S.; Sun, H. H. *J. Org. Chem.* **1991**, *56*, 4304.
16. (a) Kawasaki, Y.; Yamashita, M.; Otha, S. *J. Chem. Soc., Chem. Commun.* **1994**, 2085; (b) Kawasaki, Y.; Yamashita, M.; Otha, S. *Chem. Pharm. Bull.* **1996**, *44*, 1831.
17. (a) Bartik, K.; Braekman, J. C.; Daloze, D.; Stoller, C.; Huysecom, J.; Vandevyver, G.; Ottinger, R. *Can. J. Chem.* **1987**, *65*, 2118; (b) Tsuji, S.; Rinehart, K. L.; Gunasekera, S. P.; Kashman, Y.; Cross, S. S.; Lui, M. S.; Pomponi, S. A.; Diaz, M. C. *J. Org. Chem.* **1988**, *53*, 5446; (c) Kawasaki, Y.; Katsuma, H.; Nakayama, Y.; Yamashita, M.; Otha, S. *Heterocycles* **1998**, *48*, 1887.
18. Sun, H. H.; Sakemi, J. *J. Org. Chem.* **1991**, *56*, 4307.
19. Vervoort, H. C.; Richards-Gross, S. E.; Fenical, W.; Lee, A. Y.; Clardy, J. *J. Org. Chem.* **1997**, *62*, 1486.
20. Takahashi, S.; Matsunaga, T.; Hasegawa, C.; Saito, H.; Fujita, D.; Kiuchi, F.; Tsuda, Y. *Chem. Pharm. Bull.* **1998**, *46*, 1527.
21. Verma, M.; Tripathi, M.; Saxena, A. K.; Shanker, K. *Eur. J. Med. Chem.* **1994**, *29*, 941.
22. (a) Bekhit, A. A.; Ashour, H. M. A.; Guemei, A. A. *Arch. Pharm.* **2005**, *338*, 167; (b) Selvam, C.; Jachak, S. M.; Thilagavathi, R.; Chakraborti, A. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1793.
23. Bekhit, A. A.; Abdel-aziem, T. *Bioorg. Med. Chem.* **2004**, *12*, 1935.
24. (a) Labanauskas, L.; Kalcas, V.; Udrenaite, E.; Gaidelis, P.; Brukstus, A.; Dauksas, V. *Pharmazie* **2001**, *56*, 617; (b) Schenone, S.; Bruno, O.; Ranise, A.; Bondavalli, F.; Filippelli, W.; Falcone, G.; Giordano, L.; Vitelli, M. R. *Bioorg. Med. Chem.* **2001**, *9*, 2149; (c) Mullican, M. D.; Wilson, M. W.; Conner, D. T.; Kostlan, C. R.; Schrier, D. J.; Dyer, R. D. *J. Med. Chem.* **1993**, *36*, 1090.
25. Dogan, H. N.; Duran, A.; Rollas, S.; Sener, G.; Uysal, M. K.; Gulen, D. *Bioorg. Med. Chem.* **2002**, *10*, 2893.
26. (a) Ilies, M. A.; Masereel, B.; Rolin, S.; Scozzafava, A.; Campeanu, G.; Cimpeanu, V.; Supuran, C. T. *Bioorg. Med. Chem.* **2004**, *12*, 2717; (b) Archana Srivastava, V. K.; Kumar, A. *Eur. J. Med. Chem.* **2002**, *37*, 873.
27. Radwan, M. A. A.; El-Sherbiny, M. *Bioorg. Med. Chem* **2007**, *15*, 1206.
28. Slatt, J.; Romero, I.; Bergman, J. *Synthesis* **2004**, 2760.
29. Dieckmann, W.; Platz, O. *Chem. Ber.* **1906**, *38*, 2989.
30. Hegarty, A. F.; Cashoman, M. P.; Scoti, F. L. *Chem. Commun.* **1971**, *13*, 884.
31. Wolkoff, P. *Can. J. Chem.* **1975**, *53*, 1333.
32. Cowper, R. M.; Davidson, L. H. *Org. Syn., Coll.* **1943**, *II*, 840.
33. Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544.
34. Obukowicz, M. G.; Welsch, D. J.; Salsgiver, W. J.; Martin-Berger; Chinn, K. S.; Duffin, K. L. *J. Pharmacol. Exp. Ther.* **1998**, *287*, 157.
35. Koster, R.; Anderson, M.; De Beer, E. J. *Fed. Proc.* **1959**, *18*, 412.
36. Collier, H. D. J.; Dinnin, L. C.; Johnson, C. A.; Schneider, C. *Br. J. Pharmacol.* **1968**, *32*, 295.
37. Eddy, N. B.; Leimback, D. *J. Pharmacol. Exp. Ther.* **1953**, *107*, 385.
38. Rani, P.; Srivastava, V. K.; Kumar, A. *Eur. J. Med. Chem.* **2004**, *39*, 449–452.
39. Paget, G. E.; Barnes, J. M. *Evaluation of Drug Activities Pharmacometics*; Academic Press: London and New York, 1964, 1–135.
40. Souza, S. M.; Aquino, L. C.; Bandeira, M. A.; Nobre, M. E.; Viana, G. S. *Phytother. Res.* **2007**, *21*, 220.