DOI 10.1002/ardp.200400959 Arch. Pharm. Chem. Life Sci. 2005, 338, 378–384

Synthesis of Some Floctafenine Derivatives of Expected Anti-inflammatory/Analgesic Activity

Gehan Hegazy Hegazy[a], Azza Taher[b], Asmaa Ahmed El-Zaher[a]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt
[b] Department of Organic Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt

New derivatives related to floctafenine were synthesized and representative examples were screened for their anti-inflammatory and analgesic activities. All compounds tested were found to exhibit anti-inflammatory and analgesic activities and some were more potent than the references, floctafenine and indomethacin, in carragenan-induced rat's paw edema. None of the tested compounds showed an ulcerogenic effect on the *p*-benzoquinone-induced writhing test in mice.

Keywords: Floctafenine; Esters; Antipyrine; Anti-inflammatory/Analgesic activity

Received: November 15, 2004; Accepted: June 2, 2005

Introduction

Although there are many nonsteroidal anti-inflammatory drugs (NSAIDs) on the market, there is still a need for new research focusing on these drugs due to their serious side effects including gastric toxicity and kidney damage [1]. Most NSAIDs act through the inhibition of cyclooxygenase producing their side effects [2, 3]. Therefore, investigations on new anti-inflammatory agents devoid of serious side effects are still a challenge and the goal of many researchers. Studies on floctafenine, one of the clinically used anti-inflammatory agents [4, 5] and a relatively weak cyclooxygenase (COX) inhibitor, indicate that it can be used as a valid alternative for many patients who show adverse effects to other NSAIDs [6, 7]. Floctafenine is primarily recommended for the short term treatment of moderately to severe pain [6]. In recent work [8], certain modifications were carried out on a clinically related anti-inflammatory drug, glafenine and some of the new derivatives exhibited even more activity than the parent drug in the models used. Application of some modifications to the related anti-inflammatory drug, floctafenine, were carried out keeping in mind that the anthranilic acid ring should not be coplanar with the quinoline ring for better binding at the hypothetical receptor site and that the quinoline ring must be in ortho-position to the anthranilic acid [9]. Modifications involve only the glyceryl ester which is not important for activity. The analogues were synthesized in reactions in which the glyceryl ester moiety is degraded to the corresponding aldehyde, replaced by other esters as *n*-butyl, 2-metyl propyl, and isopropyl esters or converted to hydrazone derivatives.

Correspondence: Gehan Hegazy Hegazy, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt, Phone: +20 2 382-1041, Fax: +20 2 362-8426, e-mail: elzaher_a@yahoo.com

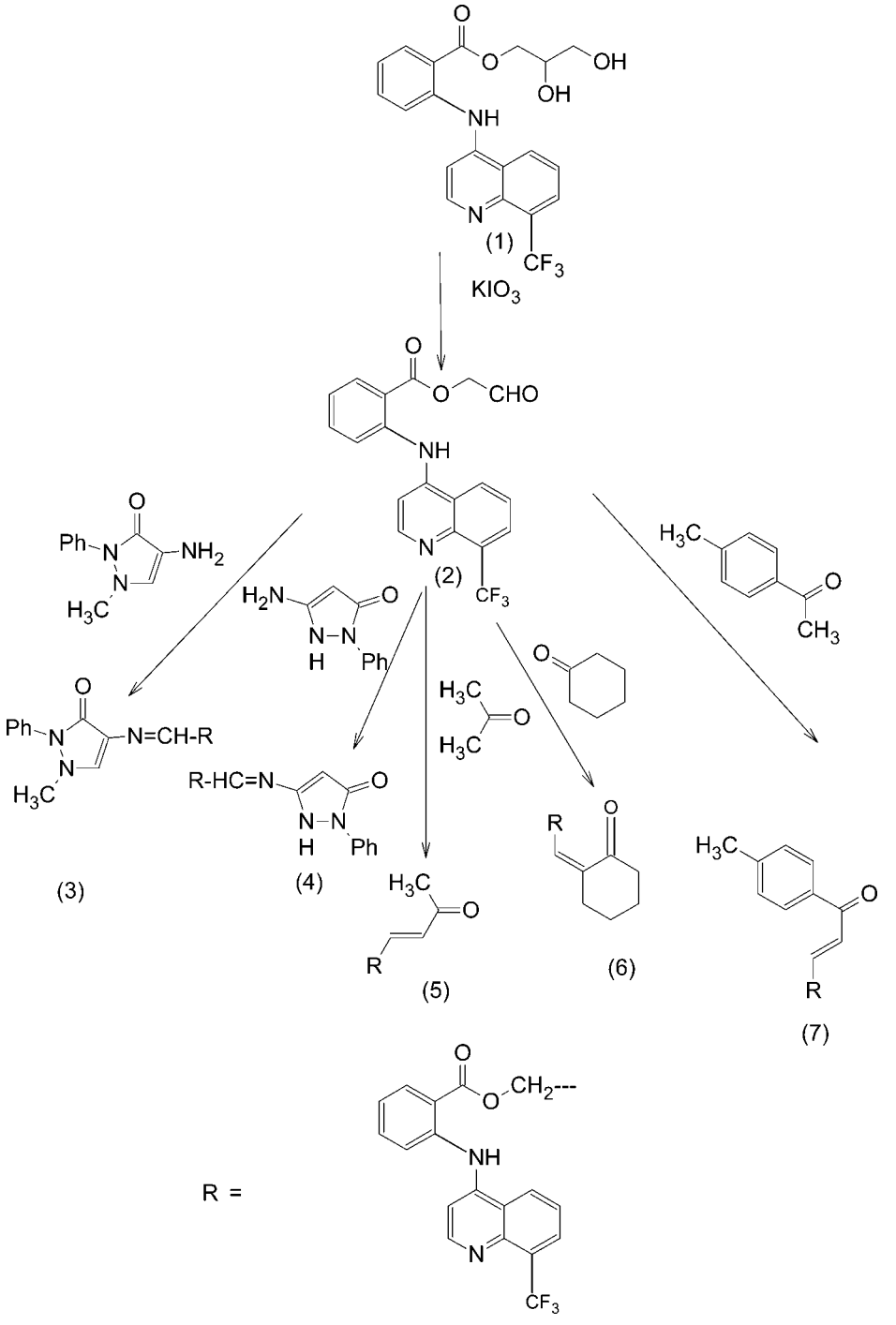
Aryl pyrazole derivatives including celecoxib, a market launched anti-inflammatory drug which could selectively inhibit COXII [10–12], encouraged us to carry out another reaction in which a pyrazole nucleus was attached to the anthranilic acid moiety via condensation of an aminopyrazole and the terminal aldehyde group of floctafenine. Keeping this purpose in mind, our studies have focused on floctafenine derivatives to develop more potent and safer profile NSAIDs potentially without gastric side effects.

Results and discussion

Chemistry

The synthetic pathways utilized to prepare the target compounds are illustrated in Schemes 1-3. Floctafenine **1** was treated with potassium periodate to give aldehyde derivative **2** which was reacted with aminopyrazolones, namely 4-amino-1-methyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one and 5-amino-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one, to afford compounds **3** and **4**, respectively. The reaction between compound **2** and different ketones, i.e. acetone, cyclohexanone, and *p*-methylacetophenone yielded compounds **5**–**7** (Scheme 1).

Compound **1** was hydrolyzed with sodium hydroxide to give floctafenic acid **8a** [13], which upon reacting with thionyl chloride gave the intermediate acid chloride derivative **8b** (Scheme 2). This intermediate was reacted without isolation with different alcohols namely *n*-butanol, isobutanol, and isopropanol to give compounds **9**–**11**. Hydrazinolysis of **1** with hydrazine hydrate 99% gave the hydrazide derivative **12** which condenses with *p*-fluorobenzaldehyde, anisaldehyde, and vanillin to give **13**–**15** (Scheme 3). In conclusion, a small library was synthesized.



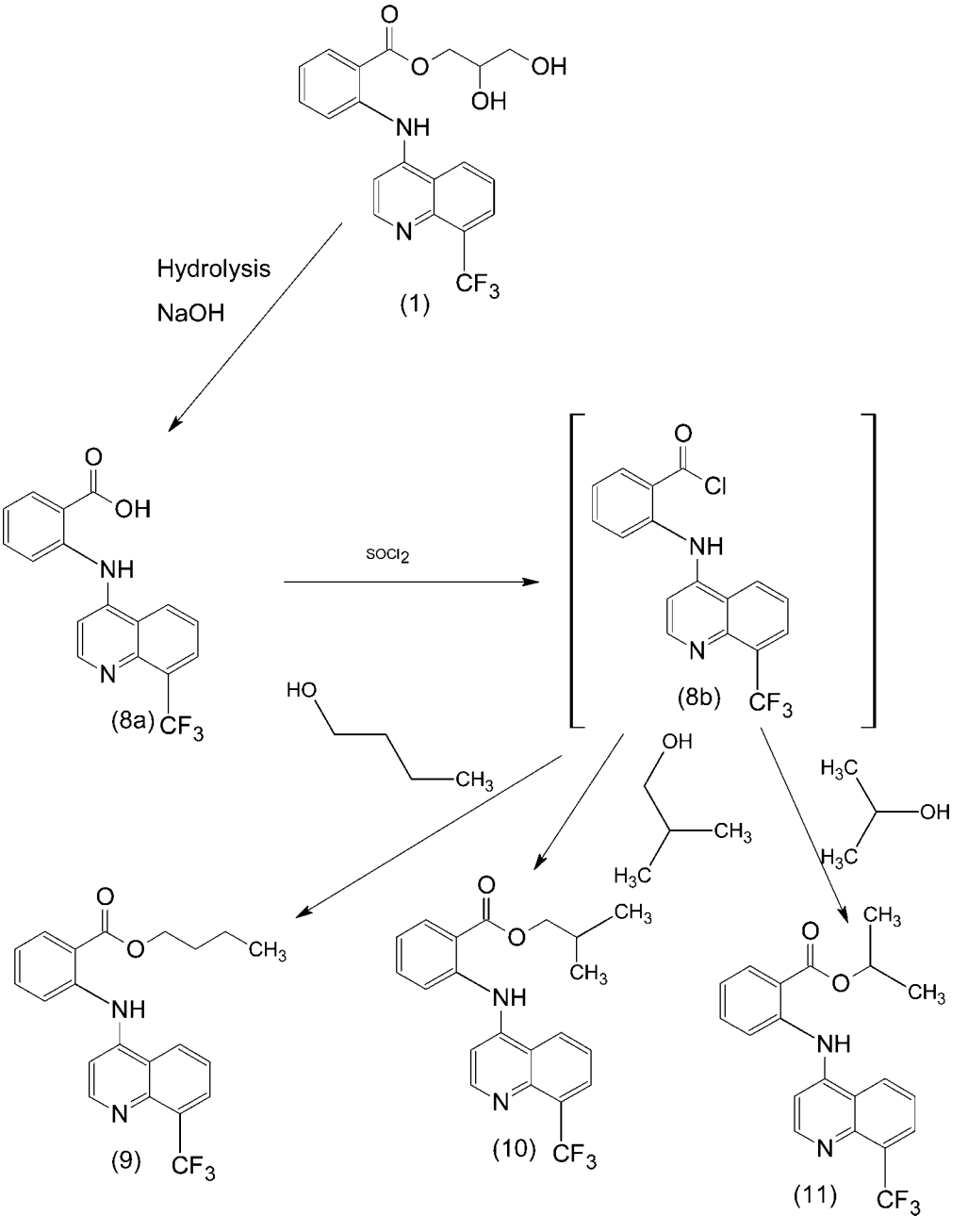
Scheme 1. Synthesis of compounds **2-7**.

Pharmacology

Anti-inflammatory activities of the compounds were assessed by utilizing carragenan-induced rat's paw edema model [14] while analgesic activities were investigated by *p*-benzoquinone-induced writhing test [15]. Since floctafenine is very effective in some acute inflammation but it is distinctly less active in carragenan-induced edema [16] indomethacin was included in rat's paw edema model.

Evaluation of anti-inflammatory activity

The inhibitory activity of the studied newly synthesized compounds on carragenan-induced rat's paw edema was de-



Scheme 2. Synthesis of compounds **9**-**11**.

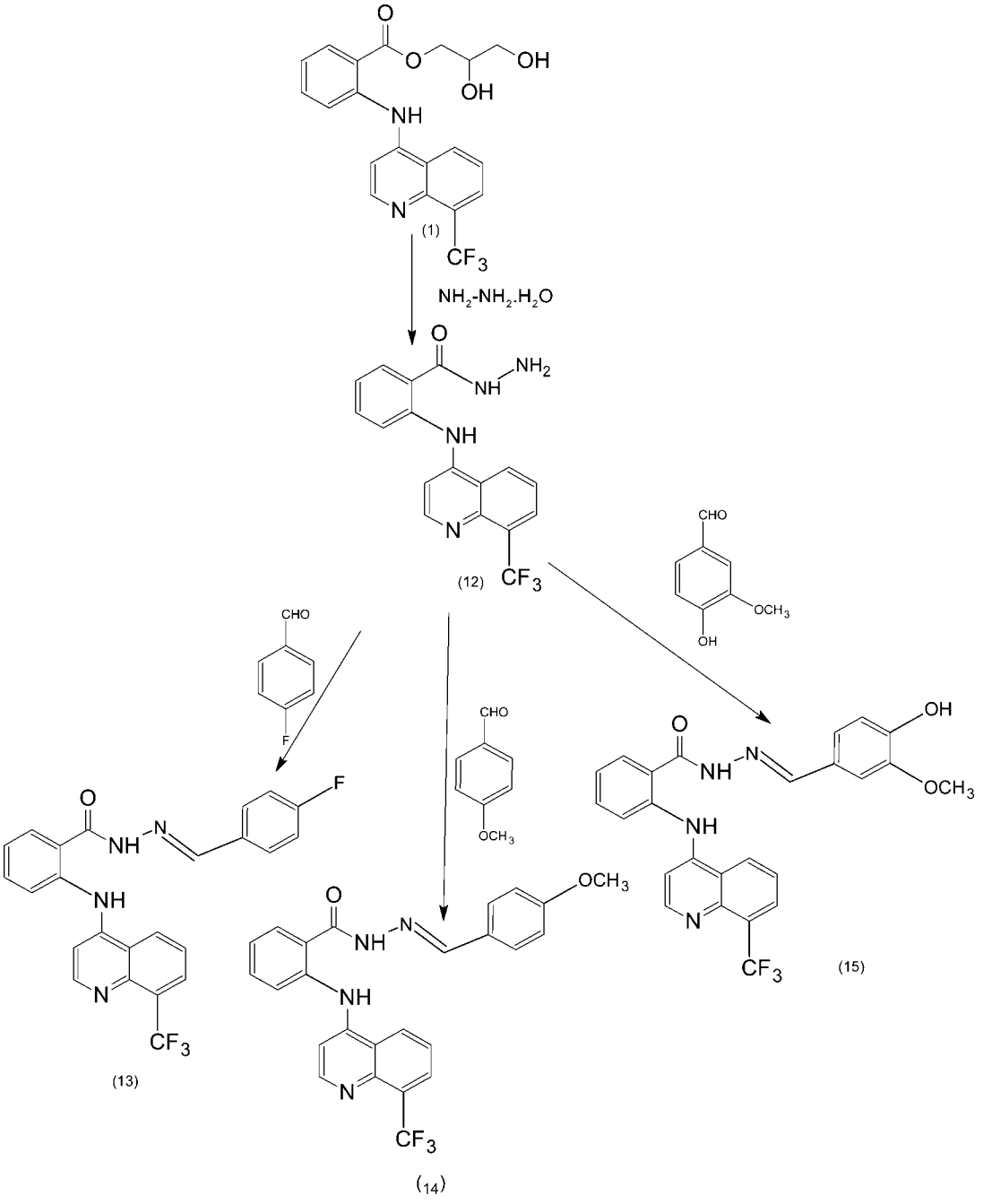
termined according to the method of Winter et al. [14] testing the ability of the synthesized compounds to suppress the development of edema in the right hind paw induced by sub-planter injection of carragenan.

Results of testing the anti-inflammatory activity

As shown in Table 1, compounds **5, 9, 10,** and **11** as well as indomethacin showed a significant activity, the percent protection of edemas ranged from 90,29%−72,13%, while compounds **4**, **12**, and **14** had no significant anti-inflammatory activity compared to indomethacin.

Evaluation of analgesic activity

Potential analgesic activity of the new compounds was evaluated following a modification of the *p*-benzoquinone-induced writhing in mice [15]. In this test, both central and peripheral analgesics are detected. The test, therefore, has



Scheme 3. Synthesis of compounds **12-15**.

been used by many investigators and can be recommended as a simple screening method. In this method pain is induced by injecting irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior, which is called writhing. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

Results of analgesic activity test

As shown in Table 2, compounds **5**, **9**, **11**, and **15** showed a significant analgesic activity compared to the untreated control group. The percent inhibition of writhing ranged from 85% with compound **15** to 68% with compound **9**, with no significant difference from floctafenine (66%).

Table 1. Effects of compounds on carragenan-induced rat's paw edema model and ulcerogenic effect.

Compound	Increase in weight of paw edema ± SEM [%]	Protection [%]	Gastric-ulcerogenic effect
Control	60.59 ± 0.48	–	–
Indomethacin	13.87 ± 0.32	76.95	5/6
Floctafenine	53.12 ± 0.12	12.32	2/6
4	52.26 ± 0.14	13.75	0/6
5	5.88 ± 0.15†	90.29	0/6
6	26.98 ± 0.08	55.47	0/6
9	12.38 ± 0.08†	79.57	0/6
10	16.89 ± 0.07†	72.13	0/6
11	10.58 ± 0.21†	82.54	0/6
12	46.67 ± 0.18	22.98	0/6
14	45.21 ± 0.13	25.39	0/6
15	32.54 ± 0.15	46.30	0/6

† Significantly different from control group at $p < 0.05$ (One-way ANOVA followed by Tukey-Kramer multiple comparison test).

Table 2. Effect of compounds on *p*-benzoquinone-induced writhing.

Compound	Number of writhes (mean ± SEM)	Inhibition of writhing [%]
Control	24.25 ± 0.48	–
Floctafenine	8.25 ± 4.87†	65.98
5	5.00 ± 3.11†	79.38
6	10.75 ± 3.50	55.67
9	7.75 ± 3.47†	68.04
11	7.50 ± 2.75†	69.07
15	3.75 ± 1.25†	84.54

† Significantly different from control group at $p < 0.05$ (One-way ANOVA followed by Tukey-Kramer multiple comparison test).

Compound **6** had no significant analgesic action though statistically comparable to floctafenine. On the other hand, compounds **4**, **10**, **12**, and **14** were found to be inactive in this test.

Evaluation of ulcerogenic effect

The risk of gastrointestinal ulceration, bleeding and even perforation with nonsteroidal anti-inflammatory drug therapy is well known. The mechanisms by which these drugs cause gastrointestinal irritation are complex. Deleterious effects may result from local actions, which cause injuries to the submucosal capillaries with subsequent necrosis and bleeding, or from inhibition of the formation of protective prostaglandins. Gastric irritation properties of orally administrated compounds were evaluated.

Results of the ulcerogenic side effect test

None of the compounds tested showed an ulcerogenic effect compared to untreated control (Table 1). This indicates that the new compounds may exert their anti-inflammatory effect via mechanisms that have no adverse impact on the gastric mucosa.

Conclusion

As expected, the prepared compounds showed marked anti-inflammatory activity. Compound **5** showed the highest anti-inflammatory activity in this series. Compounds **14**, **15** showed moderate anti-inflammatory activity. Replacement of glyceryl esters by *n*-butyl, isobutyl, and isopropyl esters as shown in compounds **9**–**11** increase the anti-inflammatory activities indicating that the ester function imparts more potent anti-inflammatory activity rather than the hydrazone function. The type of esters did not significantly affect the activity. Compound **15** showed moderate anti-inflammatory activity but showed a strong analgesic effect. Compounds **5**, **9**, and **11** showed high anti-inflammatory activity and strong analgesic effect. In contrast to the anti-inflammatory activity, the analgesic activity increases in hydrazone derivatives. The glyceryl ester moiety can be replaced by other esters without largely affecting the activity, moreover, in some cases it may lead to an increase in activity. Glyceryl esters may be replaced by 2–4 carbon skeletons or even by aromatic spacer as in compound **15** without a drastic change in activity. Fortunately, none of the tested compounds showed ulcerogenic effects.

Experimental

Chemistry

All chemicals were obtained from Aldrich (Steinheim, Germany) or Merck Chemical Co. (Darmstadt, Germany). Melting points were determined on electrothermal Graffin apparatus (London, UK) and are uncorrected. Microanalyses were carried out at the microanalytical center, Cairo University, and are within ±0.4% unless otherwise stated. IR spectra were determined using potassium bromide discs on Shimadzu IR-435 spectrometer Shimadzu, Kyoto, Japan). ^{1}H-NMR spectra were made on Joel NMR Varian Gemini 200 MHz spectrometer (Jeol, Tokyo, Japan; Varian, Palo Alto, CA, USA); chemical shifts (δ) are given in parts per million (ppm) down field from TMS as the internal standard. Mass spectra were recorded on Hewlett Packard 5988 spectrometer at 70ev (Hewlett-Packard, Palo Alto, CA, USA). Progress of the reactions were monitored by TLC using precoated aluminum sheets silica gel Merck 60 F 254 using benzene: acetone 8:2 or chloroformmethanol 9.5:0.5 and were visualized by UV lamp.

2-Oxoethyl 2-{[8-(trifluoromethyl)quinolin-4-yl]amino}benzoate ***2***

To a solution of **1** (4.06 g, 0.01 mol) in1% acetic acid, potassium periodate (2.76 g, 0.01 mol) was added with stirring for 15 min. The precipitate formed was filtered and washed with water, crystallized from ethanol (Table 3). IR: **2**: 3300 (NH), 1680 (CO). ^{1}H-NMR

Table 3. Molecular formula, yields, and melting points of the synthesized compounds.

Compound	Mol. formula (Mol. weight)	Yield [%]	M.P. [°C]
2	$C_{19}H_{13}F_3N_2O_3$ (374.31)	95	150
3	$C_{29}H_{22}F_3N_5O_3$ (545.51)	71	226
4	$C_{28}H_{20}F_3N_5O_3$ (531.00)	72	145
5	$C_{22}H_{17}F_3N_2O_3$ (414.37)	90	165
6	$C_{25}H_{21}F_3N_2O_3$ (454.44)	71	175
7	$C_{28}H_{21}F_3N_2O_3$ (490.47)	70	130
9	$C_{21}H_{19}F_3N_2O_2$ (388.38)	79	216
10	$C_{21}H_{19}F_3N_2O_2$ (388.38)	44	261
11	$C_{20}H_{17}F_3N_2O_2$ (374.35)	36	245
12	$C_{17}H_{13}F_3N_4O$ (346.31)	80	165
13	$C_{24}H_{16}F_4N_4O$ (452.40)	60	140
14	$C_{25}H_{19}F_3N_4O_2$ (464.43)	66	162
15	$C_{25}H_{19}F_3N_4O_3$ (480.43)	76	120

(DMSO): **2**: 4.08 (d, 2H, -CH_2), 6.16 (t, 1H, CHO) exchanged by D_2O, 7.25−8.72 (m, 9H, aromatic), 9.96 (s, 1H, NH) exchanged by D_2O.

*2-[(1-Methyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)imino]-ethyl 2-{8-(trifluoromethyl)quinolin-4-yl]amino}benzoate (**3**) and 2-[(5-Oxo-1-phenyl-2,5-dihydro-1H-pyrazol-3-yl)imino]ethyl 2-{[8-(trifluoromethyl)quinolin-4-yl]amino}benzoate **4***

A mixture of **2** (3.74 g, 0.01 mol) and the appropriate amine (0.01 mol) were kept under reflux in absolute ethanol in the presence of one drop glacial acetic acid for 5 h. At the end of the reaction time, the precipitate formed was filtered and crystallized from aqueous ethanol (Table 3). IR: **3**: 3400 (NH), 1660 (CO). ^{1}H-NMR (DMSO): **3**: 1.21 (s, 3H, CH_3 -N), 3.95 (d, 2H, -OCH_2), 7.08−8.68 (m, 16H, aromatic, azomethane, CH- of pyrazole ring), 10.18 (s, 1H, NH) exchanged by D_2O. IR: **4**: 3300(NH), 1680(CO). ^{1}H-NMR (DMSO): **4**: 5.00 (d, 2H, O-CH_2), 7.00−8.60 (m, 16H, aromatic, azomethane, CH of pyrazole ring), 10.00 (s, 2H, 2(NH)) exchanged by D_2O.

*Spacing allyl 2-{[8-(trifluoromethyl)quinolin-4-yl]amino}benzoate **5−7***

A mixture of **1** (4.06 g, 0.01 mol) and the corresponding ketone (0.01 mol) in sodium ethoxide solution (0.46 g, 0.02 mol) was refluxed for 4 h. The reaction mixture was acidified with 10% hydrochloric acid and cooled. The precipitate formed was filtered and crystallized from ethanol (Table 3). IR: **5**: 3400 (NH), 1680 (CO), 1620 (CO). ^{1}H-NMR (DMSO): **5**: 2.53 (s, 3H, CH_3), 4.14 (d, 2H, O-CH_2), 6.81 (d, 2H, CH=CH), 7.48−8.91, (m, 9H, aromatic), 11.15 (s, 1H, NH) exchanged by D_2O. ^{1}H-NMR (DMSO): **6**: 0.82−1.9 (m, 8H, cyclohexyl), 4.42 (d, 2H, O-CH_2), 7.02−8.72 (m, 10H, -CH= and aromatic), 13.6 (s, 1H, NH) exchanged by D_2O. M^+ M/Z: **7**: 491.2 (1%). ^{1}H-NMR (DMSO):**7**: 2.51 (d, 3H, CH_3), 4.26 (d, 2H, O-CH_2), 7.22−8.72 (m, 15H, aromatic and olefinic protons CH=CH), 10.6 (s, 1H, NH) exchanged by D_2O.

*Alkyl 2-{[8-(trifluoromethyl)quinolin-4-yl]amino}benzoate **9−11***

A mixture of **8a** (3.32 g, 0.01 mol), thionyl chloride (15 mL) and one drop dimethylformamide was refluxed for 2 h. The excess thionyl chloride was distilled under reduced pressure using dry benzene. The appropriate alcohol (0.01 mol) was added and refluxed in dichloromethan and one drop of triethylamine for 2 h. The precipitate formed was filtered off and crystallized from ethanol/ether (Table 3). M^+ M/Z: **9**: 388.19 (37%). ^{1}H-NMR (DMSO): **9**: 0.6 (t, 3H, CH_3), 0.78 (m, 2H, CH_2- CH_3), 1.33 (m, 2H, CH_2-CH_2-CH_3), 4.10 (t, 2H, -O-CH_2), 6.69−8.95 (m, 9H, aromatic), 11.19 (s, 1H, NH) exchanged by D_2O. ^{1}HNMR (DMSO):**10**: 2.77−2.88 [d of d, 6H, 2(CH_3)], 4.6 (m, 1H, -CH), 6.5 (d, 2H, -O-CH_2), 7.54−8.36 (m, 9H, aromatic), 11.30 (s, 1H, NH) exchanged by D_2O. ^{1}H-NMR (DMSO):**11**: 3.72 [d, 6H, 2(CH_3)], 6.25 (m, 1H, CH-O), 7.62−8.33 (m, 9H, aromatic), 11.22 (s, 1H, NH) exchanged by D_2O.

*2-{[8-Ttrifluoromethyl)quinolin-4-yl]amino}benzohydrazide **12***

A mixture of **1** (8.12 g, 0.02 mol) and hydrazine hydrate (1.35 g ,0.03 mol) in absolute ethanol (50 mL) and glacial acetic acid (one drop) was refluxed for 12 h. The solvent was distilled under reduced pressure and the residue formed was crystallized from aqueous ethanol (Table 3). ^{1}H-NMR (DMSO):**12**: 4.43 (s, 2H, NH_2) exchanged by D_2O, 7.18−8.69 (m, 9H, aromatic), 8.93(s, 1H, O=C-NH) exchanged by D_2O, 10.82 (s, 1H, -NH) exchanged by D_2O.

*2-{[8-(Trifluoromethyl)quinolin-4-yl]amino}substituted methylene benzohydrazide **13−15***

A mixture of **12** (3.46 g, 0.01 mol) and the corresponding aldehyde (0.01 mol) was refluxed in absolute ethanol (20 mL) and one drop of glacial acetic acid for 4 h. The reaction was then poured on ice/water and the precipitate formed was filtered off and crystallized from aqueous ethanol (Table 3). IR: **13**: 3400, 3300 (NH and O=C-NH), 1680 (CO). ^{1}H-NMR (DMSO): **13**: 6.95−8.66 (m, 13H, aromatic), 9.88 (s, 1H, N=CH-), 10.31(s, 1H, NH-C=O) exchanged by D_2O, 11.9 (s, 1H, NH-) exchanged by D_2O. ^{1}H-NMR (DMSO): **14**: 4.23 (s, 3H, O-CH_3), 6.98−8.67 (m, 13H, aromatic), 9.88 (s, 1H, N=CH-), 10.34 (s, 1H, NH-C=O) exchanged by D_2O, 11.92 (s, 1H, NH-) exchanged by D_2O. IR: **15**: 3300−3500 (NH, OH), 1680 (CO). ^{1}H-NMR (DMSO):**15**: 3.85 (s, 3H, O-CH_3), 6.92−8.57 (m, 12H, aromatic), 9.78 (s, 1H, N=CH-), 10.00 (s, 2H, OH, NH-C=O) exchanged by D_2O, 11.91 (s, 1H, NH-) exchanged by D_2O.

Pharmacology

Carragenan- induced rat's paw edema

Groups of adult male albino rats (150−200 g) of six animals each were given the tested compounds orally at a dose level of 5g/kg one

hour before carragenan injection. Rat paw edema was induced by sub-planter injections of 0.05 ml of 1% suspension of carragenan in saline into the planter tissue of one paw. An equal volume of saline was injected into the other paw and served as control. Four hours after drug administration the animals were decapitated and the paws were rapidly excised. The average weight of edema was estimated for the treated group as well as for control group and the percentage inhibition of the weight of edema was also evaluated. Indomethacin and floctafenine were employed as standards against which the tested compounds were compared. Results are shown in Table 1.

p-Benzoquinone-induced writhing test

Mice of either sex weighing 20−25 g, purchased from the Research Institute of Ophthalmology (Giza, Egypt) were used. During the experimentation period, the animals were housed in wire-mesh cages and kept under conventional laboratory conditions at the animal facility of the Faculty of Pharmacy, Cairo University. Animals were fed standard pellet diet (obtained from El-Nasr Chemical Company, Cairo, Egypt) and allowed free access to water. Writhing reaction was induced by intraperitoneal (i.p.) injection of aliquots of 0.25 ml of *p*-benzoquinone 0.02% suspension in 1% carboxymethylcellulose. Test animals were injected with the standard (floctafenine) or the test compounds in a dose of 50 mg/kg i.p., 30 minutes prior to *p*-benzoquinone administration. Seven groups of four animals each were used; the first received *p*-benzoquinone only and served as control. The other six groups were injected with a single dose of either floctafenine or one of the five tested compounds prior to *p*-benzoquinone administration. Mice were placed individually into glass beakers and five minutes were allowed to elapse before they were observed for a period of 10 min and the number of writhes was recorded for each animal. The percent inhibition of writhe in each group was calculated. The effect of the tested compounds were compared to control and standard by ordinary one-way ANOVA followed by Tukey-Kramer multiple comparison test, with $p < 0.05$ considered significant. Results are shown in Table 2.

Gastric ulcerogenic effects

Groups of six male albino rats with an average weight between 150 and 175 g were used. They were starved 48 h (water and *libitum*) prior to drug administration. The test drugs **5**, **9**, **11**, and **15** were administrated orally in 10 mL/kg as aqueous solution or suspension. Doses chosen were those proved active in the anti-inflammatory tests. The animals were sacrificed seven hours post drug or vehicle. Stomachs were removed and placed on saline- soaked filter paper until inspection. A longitudinal incision along the greater curvature was made with fine scissors. The stomach was inverted over the index finger and the presence or absence of gastric irritation was determined. The presence of a single or multiple lesions (erosion, ulcer or perforation) was considered to be positive. The number of ulcers and the occurrence of hyperemia were recorded. Results are shown in Table 1.

Statistical analysis of data

Data obtained from the animal experiments were expressed as the mean standard error (±SEM). Statistical differences between the treatments and the control were tested by ANOVA test. Data with $p < 0.05$ value was considered to be significant.

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