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Synthesis of novel 4-substituted-7-trifluoromethylquinoline derivatives with nitric oxide releasing properties and their evaluation as analgesic and anti-inflammatory agents

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Abstract—Six derivatives of the general formula 2- or 4-(7-trifluoromethylquinolin-4-ylamino) benzoic acid *N′*-(nitrooxyacetyl or propionyl) hydrazide and an oxime of the formula 1-[4-(7-trifluoromethylquinolin-4-ylamino)phenyl]ethanone oxime were synthesized and tested for their in vivo anti-inflammatory, analgesic, and ulcerogenic properties, as well as their in vitro nitric oxide release ability. Compound 2-(7-trifluoromethylquinolin-4-ylamino)benzoic acid *N′*-(2-nitrooxy propionyl)hydrazide **12** showed an anti-inflammatory activity comparable to that of indomethacin in the carrageenan-induced rat paw edema test, and equipotency to glafenine in the acetic acid mice induced writhing model at 100 mg/kg p.o., respectively. All the final compounds showed no tendency to induce stomach ulceration in rats; nitric oxide seems to contribute to their excellent safety profile.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs to reduce pain, inflammation, and fever. However, their use is limited by their significant side effects upon the stomach and the kidney. Their side effects as well as their therapeutic actions are related to their ability to inhibit cyclooxygenase enzymes involved in the first step of the arachidonic acid cascade.[1,2] In addition, the damaging effect of some NSAIDs upon the stomach and intestine is in part due to their acidic, nature, as with indomethacin, ibuprofen, diclofenac, naproxene, aspirin, etc.[3] Although basic NSAIDs such as glafenine and floctafenine are expected to be devoid of the primary insult effect, their damaging effect upon the stomach and kidney is still prominent as they inhibited prostaglandin biosynthesis as strongly as indomethacin.[4,5]

Recent strategies adopted to minimize the side effects of NSAIDs include the use of the dual LOX/COX inhibitors, the use of selective COX-2 inhibitors, and the use of hybrid molecules made up of non-selective or selective COX inhibitors together with a nitric oxide releasing function.[6–8] Recent data revealed serious cardiovascular side effects to selective COX-2 inhibitors.[7,9] In addition, such drugs only minimize the development of new gastric ulcers but do not affect the existing ones.[10] The strategy involving the use of hybrid molecules made up of non-selective COX inhibitors together with a nitric oxide donating moiety constitutes one of the most promising approaches, because nitric oxide supports several endogenous GIT defense mechanisms, including increase in mucus, bicarbonate secretions, increase in mucosal blood flow, and inhibition of the activation of proinflammatory cells. Moreover, because of the beneficial cardiovascular effects of NO, such drugs are expected to be devoid of the potential adverse cardiovascular effects associated with the use of selective COX-2 inhibitors.[8,11] Among those NO-NSAIDs that came into clinical trials are nitroaspirin, nitronaproxene, nitroketoprofen, nitroibuprofen, etc. Among the nitric oxide donors adopted to prove the validity of this principle are furoxans, oximes, hydrazides, and organic nitrates.[12–14] However, the long-term safety profile of such emerging classes of compounds is still to be determined.

In view of the above facts, herein we report the synthesis, anti-inflammatory, analgesic, and ulcerogenic properties as well as the nitric oxide releasing characteristics

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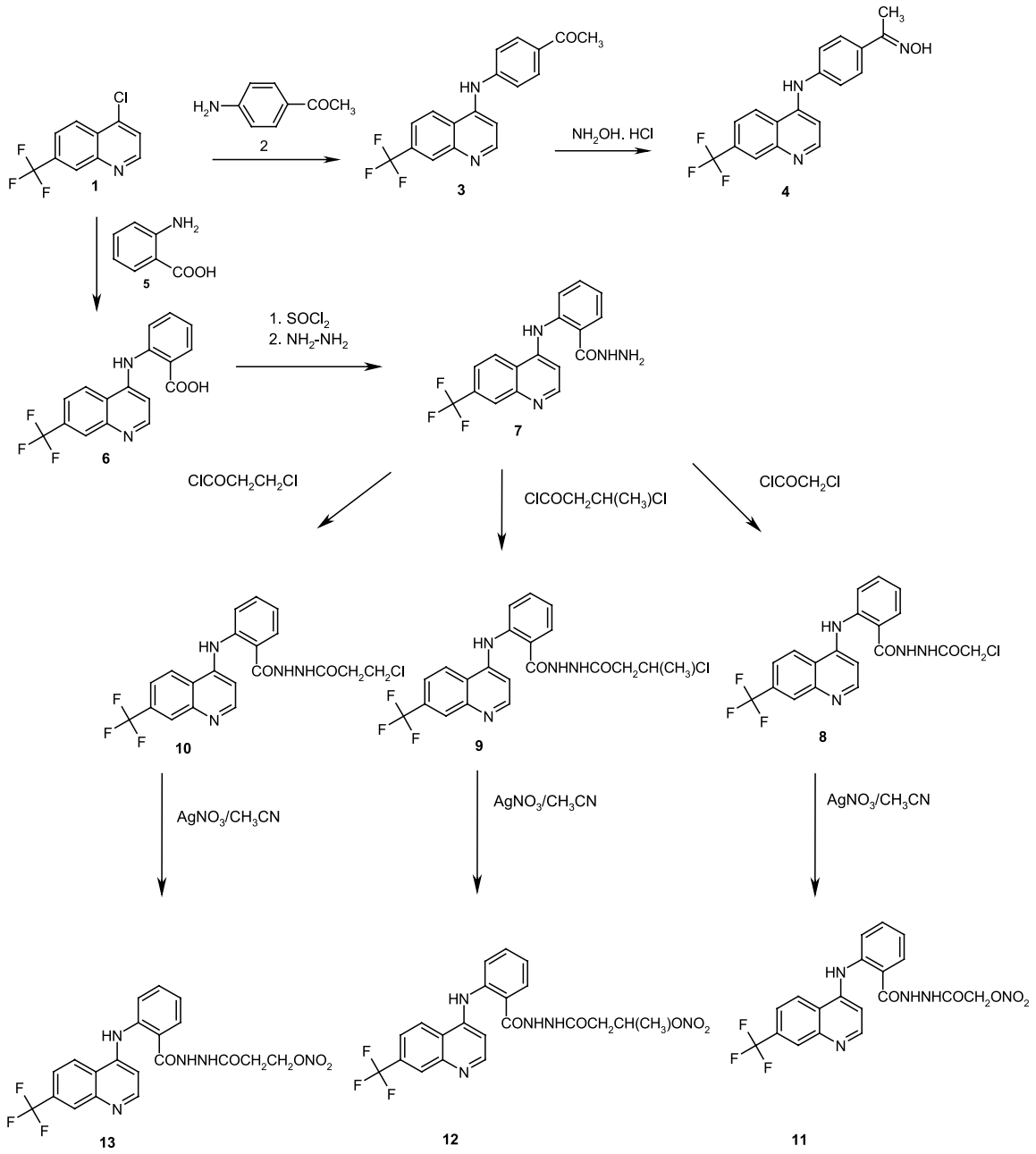
of hybrid molecules structurally related to basic NSAIDs, and incorporating organic nitrates and oximes as the nitric oxide donating fragment.

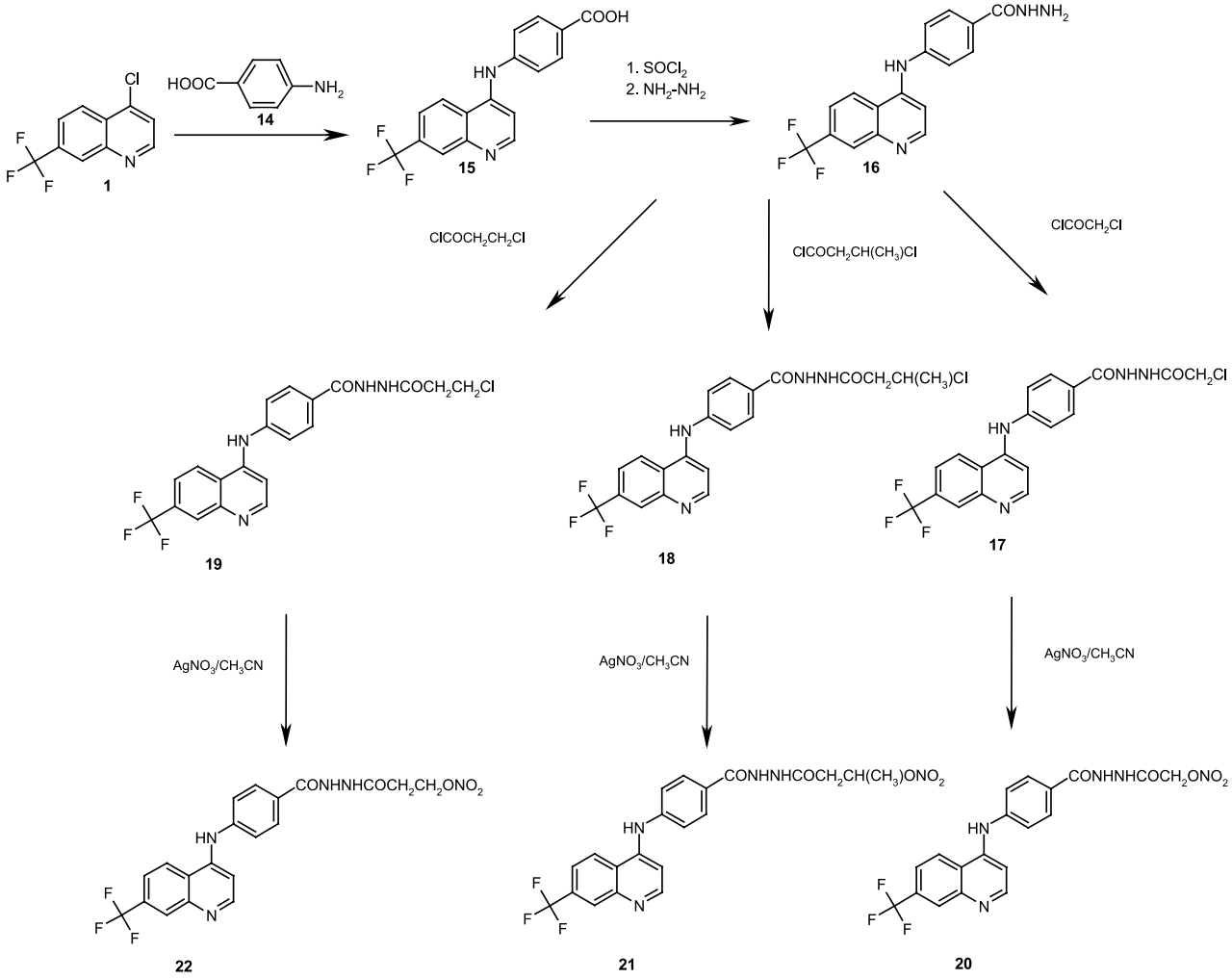
2. Chemistry

The synthesis of the final compounds **4**, **11**–**13**, and **20**–**22** is outlined in Schemes 1 and 2. Briefly, 4-chloro-7-trifluoromethylquinoline was reacted with 4-aminoacetophenone, anthranilic acid, and *p*-aminobenzoic acid to give the corresponding anilino derivatives **3**, **6**, and **15,** respectively. Condensation of **3** with hydroxylamine hydrochloride gave the corresponding oxime **4**. Meanwhile, reaction of **6** and **15** with thionyl chloride followed by hydrazine hydrate yielded the corresponding hydrazides **7** and **16**, respectively. The reaction of these hydrazides with the appropriate acid chloride, namely acetyl chloride, 2-chloropropionyl chloride, and 3-chloropropionyl chloride, gave the corresponding haloalkyl derivatives **8**–**10** and **17**–**19**. The terminal chloro function is then converted to the target nitrate derivatives with $AgNO_3$ and acetonitrile.

To evaluate the thiol-induced nitric oxide generation, compounds **4**, **11**–**13**, and **20**–**22** were dissolved in phosphate buffer (pH 7.4), methanol, and H_2O mixture at 37 °C for 1 h in the presence of 1:5 molar ratio of cysteine, the produced nitrite which is a convenient index of nitric oxide production trend was determined by



Scheme 1.



Scheme 2.

Griess reagent.[15,16] These compounds were unable to generate nitrite under the same conditions at pH 1.

3. Results and discussion

Clinically used, basic NSAIDs such as glafenine and fluctafenine are 4-substituted quinoline derivatives with a 7-chloro or 8-trifluoromethyl substituent on the quinoline moiety, respectively. However, their damaging effect upon the stomach and kidney is still prominent as they inhibited prostaglandin biosynthesis as strongly as indomethacin.[4,5] Organic nitrates and oximes are among the moieties known for their nitric oxide releasing properties.[12–14] So, we designed our compounds as hybrids of 4-substituted-7-trifluoromethylquinolines to ensure the activity together with organic nitrates or oximes as the NO releasing moiety to minimize their side effects.

The final compounds **4**, **11**–**13**, and **20**–**22** and the two haloalkyl derivatives **9** and **19** were tested for their in vivo anti-inflammatory action in the carrageenan-induced rat paw edema assay.[17] Rat paw edema was induced by subplantar injections of 0.1 ml of (1%) suspension of carrageenan in saline into one paw, an equal volume of saline was injected into the other paw and served as a control. After three hours, the average percentage increase in foot edema weight was estimated and served as a control. Treated groups received the appropriate test compound or the positive controls orally one hour prior to carrageenan injection. The edema reduction in treated animals, if any, was expressed as the percentage inhibition of the edema weight relative to that of untreated group. Glafenine and indomethacin were employed as positive controls. The results are shown in Table 1. In general, all the final compounds showed potential anti-inflammatory action comparable or slightly lower than that of glafenine and indomethacin, with the oxime being among the least active.

There was no preferential pattern for the anthranilic acid derivatives **11**–**13** relative to their *p*-aminobenzoic acid corresponding analogues **20**–**22**. The positioning of the nitrate function relative to the terminal hydrazone carbonyl and the length of the alkyl spacer does not seem crucial for the relative potency. The chloroalkyl derivatives **9** and **19** were less active than their nitrooxy derivatives **19** and **22**; this indicates that the nitrate moiety may contribute to the anti-inflammatory action of the finals.

Table 1. Analgesic, anti-inflammatory, ulcerogenic, and nitric oxide releasing properties of the finals and reference compounds

Compound	Dose per os (mg/kg)	Anti-inflammatory activity after 240 min % increase in weight of edema ± SEM (% inhibition)[a]	Analgesic activity number of stretchings ± SEM (% inhibition)[a]	Lesion index in mm	% NO release[b]
Control	—	69.3 ± 0.1	24.3 ± 1.8	—[c]	—
4	100	37.3 ± 0.1 (46.2)	20.5 ± 1.5 (15.6)	0[d]	0.5
9	100	36.8 ± 0.1 (46.9)	12.0 ± 1.5 (50.6)	2	—
11	100	30.3 ±0.1 (56.3)	11.3 ± 1.8 (53.5)	0	0.47
12	100	29.8 ± 0.1 (57.0)	11.8 ± 2.8 (51.4)	0	0.60
13	100	33.5 ± 0.1 (51.7)	12.3 ± 1.4 (49.4)	0	0.60
19	100	39.6 ± 0.1 (42.9)	17.0 ± 1.3 (30.0)	1	—
20	100	37.10 ± 0.1 (46.5)	16.8 ± 1.4 (30.9)	0	0.54
21	100	30.4 ± 0.1 (56.1)	15.0 ± 0.9 (38.3)	0	0.55
22	100	33.0 ± 0.1 (52.4)	15.5 ± 1.8 (36.2)	1	0.32
Glafenine	100	34.6 ± 0.1 (50.1)	11.8 ± 1.1 (51.4)	6.5 ± 1.3	—
Indomethacin	10	30.1 ± 0.1 (56.7)	—	25 ± 2.1	—

[a] Test was performed with at least 6 animals/compound; $P < 0.05$.
[b] Percentage of NO released ($n = 2$) relative to a theoretical maximum release of 1 mol NO/mol of test compound; determined by Griess reagent in the presence of 5 mM L-cysteine, at pH 7.4.
[c] Not tested.
[d] Determined on the basis of the lesion greatest length in mm; expressed as the mean ± SEM, $P < 0.05$.

For a comparative bioassay, the ED_{50}'s of compound **12**, glafenine, and indomethacin were determined. The dose–response curves were constructed using doses at equal logarithmic dose intervals, viz., 3, 10, 30, 100, and 300 mg/kg p.o. of glafenine and compound **12**; and doses of 0.3, 1, 3, and 10 mg/kg p.o. of indomethacin in the anti-inflammation model. The ED_{50}'s were determined graphically. The resultant ED_{50}'s of indomethacin, glafenine, and compound **12** were 7.5, 98, and 83 mg/kg p.o., respectively.

The potential analgesic activity of the new compounds was evaluated in mice by injecting acetic acid into the peritoneal cavity as the pain inducer.[18] The animals react with a characteristic stretching behavior, which is called writhing. The analgesic activity of a compound was assessed by measuring the inhibition of writhing in treated animals (5 min after acetic acid injection and for 10 min) relative to the writhing in untreated group. Glafenine was used as a positive control. The anthranilic acid derivatives with the nitrate function **11**–**13** showed comparable analgesic profile to glafenine. The oxime derivative **4** and the *p*-aminobenzoic acid corresponding analogues **20**–**22** were inferior to them. The chloroalkyls **9** and **19** were nearly as active as their nitrooxy analogues **12** and **22** in the analgesia test. The ED_{50}'s of glafenine and compound **12** were determined in the analgesia model utilizing the same doses from the anti-inflammation model, the resultant ED_{50}'s were equal (90 mg/kg p.o.) for both.

For the gastric mucosal ulcerogenicity, the rats from the anti-inflammatory test were sacrified; their stomachs were removed, opened, and examined under a microscope for the presence of visible hemorrhagic lesions. Interestingly, all the animals that administered nitrate containing compounds and the oxime were nearly totally devoid of such lesions; this indicates the excellent safety profile to such class of compounds. The ulcer indices of compounds **9** and **19** were better than that of glafenine but less than those of their nitrooxy derivatives **19** and **22**. This indicates that nitric oxide is contributing to the safety profile of this class of compounds.

The nitric oxide releasing properties of such class of compounds were assessed in phosphate buffer, pH 7.4, in the presence of L-cysteine, relative to nitric oxide released from standard sodium nitrite solution. Both the oxime **4** and the nitrate containing molecules **11**–**13** and **20**–**22** were found to release NO (Table 1). However, all the finals were not able to release NO at pH 1. Compounds **9** and **19** did not release any nitric oxide under the given conditions.

It is worthy to mention that NO-Aspirin does not produce nitrite at pH 1 and it is scarcely hydrolyzed in the gastric lumen, thus, it is more likely that the gastro protective action of NO is mediated systemically.[8,16] In addition, it seems that the absence of a free carboxyl functional group contributes to this pattern.

In conclusion, the use of hybrid molecules containing nitric oxide releasing moieties looks as a promising approach to improve the safety of NSAIDs without altering their effectiveness.

4. Experimental

4.1. Chemistry

Melting points were determined on the Electrothermal Melting Point apparatus and were uncorrected. Infrared spectra were recorded on the Shimadzu-470 infrared spectrometer. ^{1}H NMR spectra were recorded in DMSO-d_6 on Varian XL-200 MHz spectrometers (chemical shifts are given in parts per million (PPM) downfield from TMS). Elemental analyses (C, H, N) were performed by the Microanalytical Unit, Faculty of Science, Cairo University; the values were found to be within ±0.4% of the theoretical ones, unless otherwise indicated.

Mass spectra were made on Hewlett Packard GC–MS, model 5890, series II. Intermediates **3**,[19] **6**,[20] and **15**[21] were prepared by reported procedures. All the new compounds were crystallized from ethanol.

4.1.1. 1-[4-(7-Trifluoromethylquinolin-4-ylamino)phenyl]ethanone oxime (4). A mixture of an equimolar amount of **3** (5 mmol) and hydroxylamine hydrochloride in ethanol (20 ml) was refluxed for 3 h and left to cool. The separated solid was filtered, washed with dilute ammonia solution and water, dried, and crystallized.

Yield 90%; mp 328–330 °C; IR (KBr, cm^{-1}): 3300, 3170; ^1H NMR (DMSO-d_6): 2.15 (s, 3H, CH_3), 7.10–8.75 (m, 9H, aromatic), 9.80 (br s, 1H, OH, exchangeable), 11.20 (s, 1H, NH, exchangeable); Anal. ($C_{18}H_{14}F_3N_3O$) C, H, N; EI-MS: *m*/*z* 345 (M^+).

4.2. General procedure for the preparation of compounds 7 and 16

A mixture of (5 g, 15 mmol) of **6** or **15** and excess of thionyl chloride was refluxed for 4 h, excess thionyl chloride was removed under reduced pressure, and the residue was refluxed with excess hydrazine hydrate in alcohol (30 ml) for 6 h. The mixture was left to cool and then evaporated under reduced pressure. The residue obtained was filtered, washed with water, dried, and crystallized.

4.2.1. 2-(7-Trifluoromethylquinolin-4-ylamino) benzoic acid hydrazide (7). Yield 95%; mp 233–235 °C; IR (KBr, cm^{-1}): 3200, 1710; ^1H NMR (DMSO-d_6): 4.43 (br s, 2H, NH_2, exchangeable), 7.18–8.70 (m, 9H, aromatic), 8.94 (br s, 1H, NH exchangeable), 10.8 (br s, 1H, NH exchangeable); Anal. ($C_{17}H_{13}F_3N_4O$) C, H, N.

4.2.2. 4-(7-Trifluoromethylquinolin-4-ylamino) benzoic acid hydrazide (16). Yield 90%; mp 210–212 °C; IR (KBr, cm^{-1}): 3250, 1720; ^1H NMR (DMSO-d_6): 4.10 (br s, 2H, NH_2, exchangeable), 7.10–8.77 (m, 9H, aromatic), 9.02 (br s, 1H, NH exchangeable), 10.60 (br s, 1H, NH exchangeable); Anal. ($C_{17}H_{13}F_3N_4O$) C, H, N.

4.3. General procedure for the preparation of compounds 8–10 and 17–19

A mixture of (0.35 g, 1 mmol) of **7** or **16** was refluxed with (1.2 mmol) of the appropriate acid chloride, viz., acetyl chloride, 2-chloropropionyl chloride, and 3-chloropropionyl chloride in dry benzene (15 ml) and in the presence of triethylamine (0.1 ml) for 10 h. The solution was evaporated to dryness; the residue obtained was filtered, washed with water, and crystallized.

4.3.1. 2-(7-Trifluoromethylquinolin-4-ylamino) benzoic acid *N*′-(2-chloroacetyl)hydrazide (8). Yield 43%; mp 235–237 °C; IR (KBr, cm^{-1}): 3500, 1720, 1690; ^1H NMR (DMSO-d_6): 3.80 (br s, 1H, NH, exchangeable), 4.21 (s, 2H, CH_2Cl), 7.16–8.65 (m, 9H, aromatic), 10.38–10.82 (br m, 2H, NHs, exchangeable); Anal. ($C_{19}H_{14}ClF_3N_4O_2$) C, H, N; EI-MS: *m*/*z* 422 (M^+) and 424 (M^++2).

4.3.2. 2-(7-Trifluoromethylquinolin-4-ylamino)benzoic acid *N*′-(2-chloropropionyl)hydrazide (9). Yield 36%; mp 205–207 °C; IR (KBr, cm^{-1}): 3500, 1716, 1700; ^1H NMR (DMSO-d_6): 1.62–1.66 (d, 3H, CH–CH_3), 3.45 (br s, 1H, NH, exchangeable), 4.66–4.70 (q, 1H, *CH*–CH_3), 7.15–8.97 (m, 9H, aromatic), 10.48 (s, 1H, NH, exchangeable), 10.68 (s, 1H, NH, exchangeable); Anal. ($C_{20}H_{16}ClF_3N_4O_2$) C, H, N.

4.3.3. 2-(7-Trifluoromethylquinolin-4-ylamino)benzoic acid *N*′-(3-chloropropionyl)hydrazide (10). Yield 33%; mp 116–118 °C; IR (KBr, cm^{-1}): 3300, 1723, 1700; ^1H NMR (DMSO-d_6): 2.70–2.76 (t, 2H, CO–CH_2–), 3.50 (br s, 1H, NH, exchangeable), 3.87–3.87 (t, 2H, CH_2Cl), 7.10–9.20 (m, 9H, aromatic), 10.15 (s, 1H, NH, exchangeable), 10.60 (br s, 1H, NH, exchangeable); Anal. ($C_{20}H_{16}ClF_3N_4O_2$) C, H, N; EI-MS: *m*/*z* 436 (M^+) and 438 (M^++2).

4.3.4. 4-(7-Trifluoromethylquinolin-4-ylamino)benzoic acid *N*′-(2-chloroacetyl)hydrazide (17). Yield 70%; mp 190–192 °C; IR (KBr, cm^{-1}): 3300, 1730, 1710; ^1H NMR (DMSO-d_6): 3.61 (br s, 1H, NH, exchangeable), 4.21 (s, 2H, CH_2Cl), 7.11–9.10 (m, 9H, aromatic), 10.45 (s, 1H, NH exchangeable), 10.62 (s, 1H, NH, exchangeable); Anal. ($C_{19}H_{14}ClF_3N_4O_2$) C, H, N.

4.3.5. 4-(7-Trifluoromethylquinolin-4-ylamino)benzoic acid *N*′-(2-chloropropionyl)hydrazide (18). Yield 61%; mp 310–312 °C; IR (KBr, cm^{-1}): 3450, 1732, 1717; ^1H NMR (DMSO-d_6): 1.61–1.64 (d, 3H, CH–CH_3), 3.45 (br s, 1H, NH, exchangeable), 4.60–4.65 (q, 1H, *CH*–CH_3), 7.19–8.95 (m, 9H, aromatic), 10.50 (s, 1H, NH, exchangeable), 10.70 (s, 1H, NH, exchangeable); Anal. ($C_{20}H_{16}ClF_3N_4O_2$) C, H, N.

4.3.6. 4-(7-Trifluoromethylquinolin-4-ylamino)benzoic acid *N*′-(3-chloropropionyl) hydrazide (19). Yield 80%; mp 217–218 °C; IR (KBr, cm^{-1}): 3320, 1730, 1710; ^1H NMR (DMSO-d_6): 2.70–2.76 (t, 2H, CO–CH_2–), 3.50 (br s, 1H, NH, exchangeable), 3.92–3.95 (t, 2H, CH_2Cl), 7.15–8.83 (m, 9H, aromatic), 10.22 (s, 1H, NH, exchangeable), 10.57 (br s, 1H, NH, exchangeable); Anal. ($C_{20}H_{16}ClF_3N_4O_2$) C, H, N.

4.4. General procedure for the preparation of compounds 11–13 and 20–22

A solution of the appropriate chloroalkyl derivative **8**–**10** and **17**–**19** (1.8 mmol) in dry acetonitrile (2 ml) was treated portionwise with a solution of $AgNO_3$ (0.34 g, 2 mmol) in dry acetonitrile (5 ml) and the whole mixture was stirred at room temperature for 3 h. The mixture was then filtered, evaporated to dryness, and the residue was crystallized from absolute ethanol.

4.4.1. 2-(7-Trifluoromethylquinolin-4-ylamino)benzoic acid *N*′-(2-nitrooxyacetyl)hydrazide (11). Yield 33%; mp 200–202 °C; IR (KBr, cm^{-1}): 3124, 1733, 1710; ^1H NMR (DMSO-d_6): 4.20 (s, 2H, CH_2), 7.70–8.69 (m,

11H, aromatic and 2 NHs, exchangeable), 10.30 (br s, 1H, NH, exchangeable); Anal. ($C_{19}H_{14}F_3N_5O_5$) C, H, N; EI-MS: *m/z* 450 (M^++1).

4.4.2. 2-(7-Trifluoromethyl-quinolin-4-ylamino)benzoic acid *N*′-(2-nitrooxypropionyl)hydrazide (12). Yield 30%; mp 119–120 °C; IR (KBr, cm^{-1}): 3150, 1720, 1698; ^1H NMR (DMSO-d_6): 1.56–1.59 (d, 3H, CH_3), 3.50 (br s, 1H, NH, exchangeable), 4.56–4.60 (q, 1H, *CH*–CH_3), 6.80–7.80 (m, 9H, aromatic), 10.40 (s, 1H, NH, exchangeable), 10.60 (s, 1H, NH, exchangeable); Anal. ($C_{20}H_{16}F_3N_5O_5$) C, H, N.

4.4.3. 2-(7-Trifluoromethylquinolin-4-ylamino)benzoic acid *N*′-(3-nitrooxypropionyl)hydrazide (13). Yield 54%; mp 202–203 °C; IR (KBr, cm^{-1}): 3100, 1731, 1717; ^1H NMR (DMSO-d_6): 2.71–2.74 (t, 2H, CO-CH_2), 3.05–3.08 (t, 2H, CH_2–O), 4.10 (br s, 1H, NH, exchangeable), 7.14–8.81 (m, 9H, aromatic), 10.12 (s, 1H, NH, exchangeable), 10.61 (br s, 1H, NH, exchangeable); Anal. ($C_{20}H_{16}F_3N_5O_5$) C, H, N.

4.4.4. 4-(7-Trifluoromethylquinolin-4-ylamino)benzoic acid *N*′-(2-nitrooxyacetyl)hydrazide (20). Yield 80%; mp 104–106 °C; IR (KBr, cm^{-1}): 3350, 1710, 1683; ^1H NMR (DMSO-d_6): 4.10 (s, 2H, CH_2), 7.50–8.9 (m, 11H, aromatic and 2 NHs, exchangeable), 12.00 (br s, 1H, NH, exchangeable); Anal. ($C_{19}H_{14}F_3N_5O_5$) C, H, N.

4.4.5. 4-(7-Trifluoromethylquinolin-4-ylamino)benzoic acid *N*′-(2-nitrooxypropionyl)hydrazide (21). Yield 52%; mp 141–142 °C; IR (KBr, cm^{-1}): 3150, 1730, 1712; ^1H NMR (DMSO-d_6): 1.61–1.63 (d, 3H, CH_3), 3.50 (br s, 1H, NH, exchangeable), 4.64–4.66 (q, 1H, *CH*–CH_3), 7.10–8.93 (m, 9H, aromatic), 10.40 (s, 1H, NH, exchangeable), 10.63 (s, 1H, NH, exchangeable); Anal. ($C_{20}H_{16}F_3N_5O_5$) C, H, N.

4.4.6. 4-(7-Trifluoromethylquinolin-4-ylamino)benzoic acid *N*′-(3-nitrooxypropionyl)hydrazide (22). Yield 85%; mp 233–235 °C; IR (KBr, cm^{-1}): 3180, 1720, 1700; ^1H NMR (DMSO-d_6): 2.72–2.76 (t, 2H, CO–CH_2), 3.08–3.10 (t, 2H, CH_2–O), 3.83 (m, 1H, NH, exchangeable), 7.10–9.05 (m, 9H, aromatic), 10.13 (s, 1H, NH, exchangeable), 10.53 (br s, 1H, NH, exchangeable); Anal. ($C_{20}H_{16}F_3N_5O_5$) C, H, N.

4.5. Pharmacology

All the tested compounds were initially dissolved in DMSO, then diluted with 1% CMC, and the final concentration of DMSO was 5%. Each compound was prepared immediately before use and given intragastrically.

4.5.1. Anti-inflammatory activity and gastrotoxicity.[14,17] *Carrageenan rat paw edema*. The procedure employed was a modification of the method of Winter et al.[17] Groups of adult male albino rats (150–180 g), 6 animals per group, were deprived of food but not water 24 h before the experiment. The tested compounds were given orally at a dose level of 100 mg/kg, glafenine (100 mg/kg) or indomethacin (10 mg/kg) one hour before carrageenan injection. A control group of animals received carrageenan without any medication. Rat paw edema was induced by subplantar injection of 0.1 ml of 1% suspension of carrageenan in saline into one paw. An equal volume of saline was injected into the other paw of each animal. Treated animals received a dose of 100 mg/kg of the appropriate compound 1 h before carrgeenan.

Three hours after carrageenan, 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 evaluated and statistically analyzed.

The stomachs of the sacrificed animals from the rat paw test were removed, opened along the lesser curvature and examined under a microscope for the presence of macroscopically visible lesions. The number of lesions in each animal, if any, was counted. Gastric lesion score is expressed in millimeters of lesion and calculated by summing the length of all lesions in a given stomach.

ED_{50}'s determination: as in the single dose test, but the dose response curves were constructed using doses of 3, 10, 30, 100, and 300 mg/kg p.o. of compound **12** and **glafenine** and doses of 0.3, 1, 3, and 10 mg/kg p.o. of indomethacin. The average response to each dose was calculated and the ED_{50}'s were determined graphically.

4.5.2. Analgesic activity.[18] *Acetic acid writhing test*. This test was performed according to a modification of the method of Manoury et al.[18] Mice of either sex weighing 20–25 g were housed in wire-mesh cages and kept under conventional laboratory conditions. Animals were fasted overnight with water ad libitum.

The test compounds or the positive control (glafenine) were given orally to the animals (6 animals/group) at a dose level of 100 mg/kg. One hour after treatment, each animal received an ip injection of 10 ml/kg of 0.6% acetic acid in water. A control group of animals received the appropriate dose of acetic acid without any medication. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The number of writhing movements was counted 5 min after acetic acid injection and for 10 min. The number of writhes was recorded for each animal. The percent inhibition of writhe in each group was calculated. The effect of the test compounds was compared with that of control and standard, and analyzed statistically.

ED_{50}'s were determined as in the single dose test and utilizing doses of of 3, 10, 30, 100, and 300 mg/kg p.o. of compound **12** and glafenine. The average response to each dose was calculated and the ED_{50}'s were determined graphically.

Detection of nitrite. A solution of the appropriate compound (20 μL) in dimethylsulfoxide (DMSO) was added to 2 mL of 1:1 v/v mixture either of 50 mM phosphate buffer (pH 7.4) or of an HCl solution (pH 1) with

MeOH, containing 5× of 10^{-4} M L-cysteine. The final concentration of drug was 10^{-4} M. After 1 h at 37 °C, 1 mL of the reaction mixture was treated with 250 μL of Griess reagent [sulfanilamide (4 g), *N*-naphthylethylenediamine dihydrochloride (0.2 g), 85% phosphoric acid (10 mL) in distilled water (final volume: 100 mL)]. After 10 min at room temperature, the absorbance was measured at 540 nm. Sodium nitrite standard solutions (10–80 nmol/mL) were used to construct the calibration curve.

The results were expressed as the percentage of NO released ($n = 2$) relative to a theoretical maximum release of 1 mol NO/mol of test compound.

References and notes

1. Insel, P. A. Analgesic-antipyretic and antiinflammatory agents and drugs employed in the treatment of gout. In Hardman, J. G., Limbird, L. E., Molinoff, P. B., Ruddon, R. W., Gilman, A. G., Eds., 9th ed.; The Pharmacological Basis of Therapeutics; McGraw-Hill: New York, 1996, p 617.
2. Wolfe, M. N.; Lichtestein, D. R.; Singh, G. *N. Engl. J. Med.* **1999**, *128*, 1121.
3. Cena, C.; Lolli, M. L.; Lazzarato, L.; Guaita, E.; Morini, G.; Coruzzi, G.; McElroy, S. P.; Megson, I. L.; Fruttero, R.; Gasco, A. *J. Med. Chem.* **2003**, *46*, 747.
4. Lequesne, M.; Fannius, J.; Reginster, J. Y.; Verdickt, W.; du Laurier, M. V. *Revue du rhumatisme* **1997**, *64*, 327.
5. Van Kolfschoten, A. A.; Zandberg, P.; Jager, L. P.; Van Noordwijk, J. *Toxicol. Appl. Pharmacol.* **1983**, *69*, 37.
6. Bias, P.; Buchner, A.; Klesser, B.; Laufer, S. *Am. J. Gastroenterol.* **2004**, *99*, 611.
7. Doggrell, S. A. *Expert Opin. Pharmacother.* **2005**, *6*, 347.
8. Velazquez, C.; Rao, P. N. P.; McDonald, R.; Knaus, E. E. *Bioorg. Med. Chem.* **2005**, *13*, 2749.
9. Dogne, J. M.; Supuran, C. T.; Pratico, D. *J. Med. Chem.* **2005**, *48*, 2251.
10. Hunt, R. H.; Barkun, A. N.; Baron, D.; Bombardier, C.; Bursey, F. R.; Marshall, J. R.; Morgan, D. G.; Pare, P.; Thomson, A. B.; Whittaker, J. S. *Can. J. Gastroenterol.* **2002**, *16*, 231.
11. Muscara, M. N.; McKnight, W.; Del Soldato, P.; Wallace, J. L. *Pharmacol. Lett.* **1998**, *62*, 235.
12. Mann, N. S. *Gastroenterology* **2003**, *125*, 1918.
13. Keeble, J. E.; Moore, P. K. *Br. J. Pharmacol.* **2002**, *137*, 295.
14. Rehse, K.; Brehme, F. *Arch. Pharm. Pharm. Med. Chem.* **2000**, *333*, 157.
15. Sorba, G.; Medana, C.; Fruttero, R.; Cena, C.; Di Stilo, A.; Galli, U.; Gasco, A. *J. Med. Chem.* **1997**, *40*, 463.
16. Lolli, M. L.; Cena, C.; Medana, C.; Lazzarato, L.; Morini, G.; Coruzzi, G.; Manarini, S.; Fruttero, R.; Gasco, A. *J. Med. Chem.* **2001**, *44*, 3463.
17. Winter, C. A.; Risley, E. A.; Nuss, G. W. *J. Pharmacol. Exp. Ther.* **1963**, *141*, 369.
18. Manoury, P. M.; Dumas, A. P.; Najer, H. *J. Med. Chem.* **1979**, *22*, 554.
19. Abadi, A. H.; Al-Khamees, H. A. *Arch. Pharm. Pharm. Med. Chem.* **1998**, *331*, 319.
20. Allais, A.; Rousseau, G.; Meier, J.; Nomine, G.; Peterfalvi, M.; Deraedt, R.; Chifflot, L.; Benzoni, J.; Fournex, R. *Chim. Therap.* **1973**, *8*, 154.
21. McCall, J. M.; TenBrink, R. E.; Kamdar, B. V.; Skaletzky, L. L.; Perricone, S. C.; Piper, R. C.; Delehanty, P. J. *J. Med. Chem.* **1986**, *29*, 133.