Bioorganic & Medicinal Chemistry 16 (2008) 9443–9449

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Site specific chemical delivery of NSAIDs to inflamed joints: Synthesis, biological activity and γ-imaging studies of quaternary ammonium salts of tropinol esters of some NSAIDs or their active metabolites

Mange Ram Yadav [a,*], Vijay P. Pawar [a], Sejal M. Marvaniya [a], Parmeshwari K. Halen [a], Rajani Giridhar [a], Anil K. Mishra [b]

[a] Pharmacy Department, Faculty of Technology & Engineering, Kalabhavan, The M.S. University of Baroda, Vadodara 390 001, India
[b] Institute of Nuclear Medicine and Allied Sciences, Brig. S.K. Mazumdar Road, New Delhi 110 054, India

ARTICLE INFO

Article history:
Received 2 August 2008
Revised 16 September 2008
Accepted 17 September 2008
Available online 20 September 2008

Keywords:
Tropinol
NSAIDs
Anti-inflammatory activity
γ-Imaging studies
Site specific delivery

ABSTRACT

Quaternized tropinol ester derivatives of some commonly used non-steroidal anti-inflammatory drugs (NSAIDs) or their active metabolites, were prepared and studied for their anti-inflammatory activity in a chronic inflammation model and for inflamed tissue tropism. The quaternized esters were radiolabeled with ^{99m}Technetium (^{99m}Tc) and their selective localization in the inflamed tissue was traced using scintigraphy. In the chronic arthritis rodent model, most of the quaternized esters exhibited anti-inflammatory effect comparable to their respective parent drugs. In the γ-imaging studies only the quaternary derivatives exhibited selective accumulation into the inflamed tissue unlike the parent NSAIDs or the unquaternized tropinol esters. This work is a step ahead in the direction of use of quaternary ammonium ester derivatives for site specific chemical delivery of commonly used NSAIDs to the inflamed tissues to minimize their GIT side effect or other systemic toxicities.

1. Introduction

NSAIDs are among the most widely used prescription drugs, for the treatment of pain and inflammation, particularly arthritis.[1] As inhibitors of COX isozymes, traditional NSAIDs relieve the signs and symptoms of inflammation by decreasing prostaglandin (PG) production but may cause serious gastrointestinal (GI) and renal damage, especially with the long term use.[2,3] To improve the GI tolerance of NSAIDs by masking the free carboxylic group temporarily, numerous ester and amide prodrugs of NSAIDs have been designed and evaluated in the past.[4–6] However, it is still not possible to avoid the systemic side effects of the parent drugs by the prodrug approach.

Classical NSAIDs inhibit COX-1 and COX-2 isozymes to varying extent.[7] The differential tissue distribution of COX-1 and COX-2 provided a rationale for the development of selective COX-2 inhibitors as anti-inflammatory and analgesic agents that lacked the GI and hematological liabilities exhibited by currently marketed NSAIDs.[8] Highly selective COX-2 inhibitors have been developed and marketed as promising gastroprotective agents.[9] Despite the initial enthusiasm surrounding selective COX-2 inhibitors, questions remain regarding their ultimate benefit and safety, since in certain circumstances COX-2 may be important to homeostasis.[10] Rofecoxib was withdrawn[11] worldwide because of evidence of increased cardiovascular risk in selected, high dose patients that may reflect rofecoxib's greater COX-2 selectivity.[12] This voluntary withdrawal of rofecoxib from the market by Merck and Co. raised the question of cardiovascular safety of the entire class of COX-2 inhibitors.[13]

An alternative way to reduce the adverse reactions is to carry the drugs selectively to their target tissue. Pharmacokinetic studies on ^{14}C-labeled acetylcholinesterase reactivators containing a quaternary ammonium (QA) moiety demonstrated a rapid and intensive concentration of these compounds in cartilaginous tissues after intramuscular injection.[14,15] It was demonstrated on cultured chondrocytes that high specificity for cartilage resulted for QA compounds from ionic interactions between the QA entity of the reactivator and the anionic sites (carboxylate and sulfate ester groups) of proteoglycans, a major component of cartilage.[16] This property has been used to target NSAIDs toward cartilage. New potential anti-inflammatory drugs have been synthesized by binding pharmacologically active structures to QA salts. These new molecules could concentrate in the cartilaginous tissue and thus allow a significant decrease of the effective dose and therefore an attenuation of adverse effects such as GI toxicity.[17] A study on quaternization of oxicams suggested that a QA group linked to an NSAID could help to increase the drug concentration in joints and permit a decrease in the administered dose to diminish the

* Corresponding author. Tel.: +91 0265 2434187; fax: +91 0265 2418927.
E-mail address: mryadav11@yahoo.co.in (M.R. Yadav).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.09.050

side effects of these drugs.[17] In a study on biodistribution of ^{14}C-labeled QA–glucosamine conjugates it was shown that introduction of QA-moiety on D-glucosamine allowed the molecule to be carried more selectively and intensively to cartilaginous tissues soon after injection.[18]

To optimize the therapeutic efficacy of the existing NSAIDs, a program was initiated in this Department. Under this program aminoalcohol ester derivatives of carboxyl group-bearing NSAIDs were prepared and evaluated for their potency and GI protecting efficiency[19–24] in comparison to the parent drugs. All the ester derivatives exhibited equal or higher anti-inflammatory activity than the parent NSAIDs except for the tropinol esters, in the acute model of inflammation. Another important observation made during these studies, regarding the tropinol ester derivatives, was their resistance toward enzymatic hydrolysis in simulated experiments.

The observation[14–18] that QA compounds are selectively and intensivey concentrated in the cartilaginous tissue prompted us to study the tropinol esters of some NSAIDs after quaternization, for their localization into the inflamed tissues and their anti-inflammatory activity in a chronic model. Tropinol esters specifically were chosen for the study due to their higher stability in the in vitro experiments. Although, these derivatives did not exhibit anti-inflammatory activity in the acute model for a period of 3 h but, it was hypothesized that these derivatives would be slowly hydrolyzed chemically in the blood, releasing the parent NSAIDs for exhibiting their inherent anti-inflammatory activity.

In this paper we report the synthesis of QA derivatives of tropinol esters of indomethacin (**1**), ketoprofen (**2**), biphenylacetic acid (**3,** an active metabolite of fenbufen), flurbiprofen (**4**), ibuprofen (**5**), 6-methoxy-2-napthylacetic acid (**6**) (6-MNA, an active metabolite of nabumetone) and naproxen (**7**); their tagging to technetium-99m (^{99m}Tc) and tissue localization studies, and their efficacy as anti-inflammatory agents in a chronic inflammation model, adjuvant arthritis.

2. Results and discussion

2.1. Syntheses

Indomethacin (**1**) was reacted with tropinol (**8**) in presence of dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) to obtain the ester (**9**) as an oily product. The ester (**9**) was quaternized with methyl iodide to obtain the desired quaternary derivative (**16**). The other products (**17–22**) (Scheme 1) were obtained in a similar way by reacting their respective esters[21,22,24] (**10**–**15**) with methyl iodide. All the compounds were characterized by their spectral and elemental data.

2.2. γ-Imaging studies

In order to check the site specific concentration of these quaternary derivatives (**16–22**) it was planned to tag these derivatives with radioactive ^{99m}Tc and study their site specific localization tendency in the inflamed tissue. For such a study to give some meaningful output it is essential that the compounds should have high binding affinity for the radioactive complexing agent and the complexed derivatives (^{99m}Tc–QA) should be stable enough in serum to last for a period of about 4 hours or more.

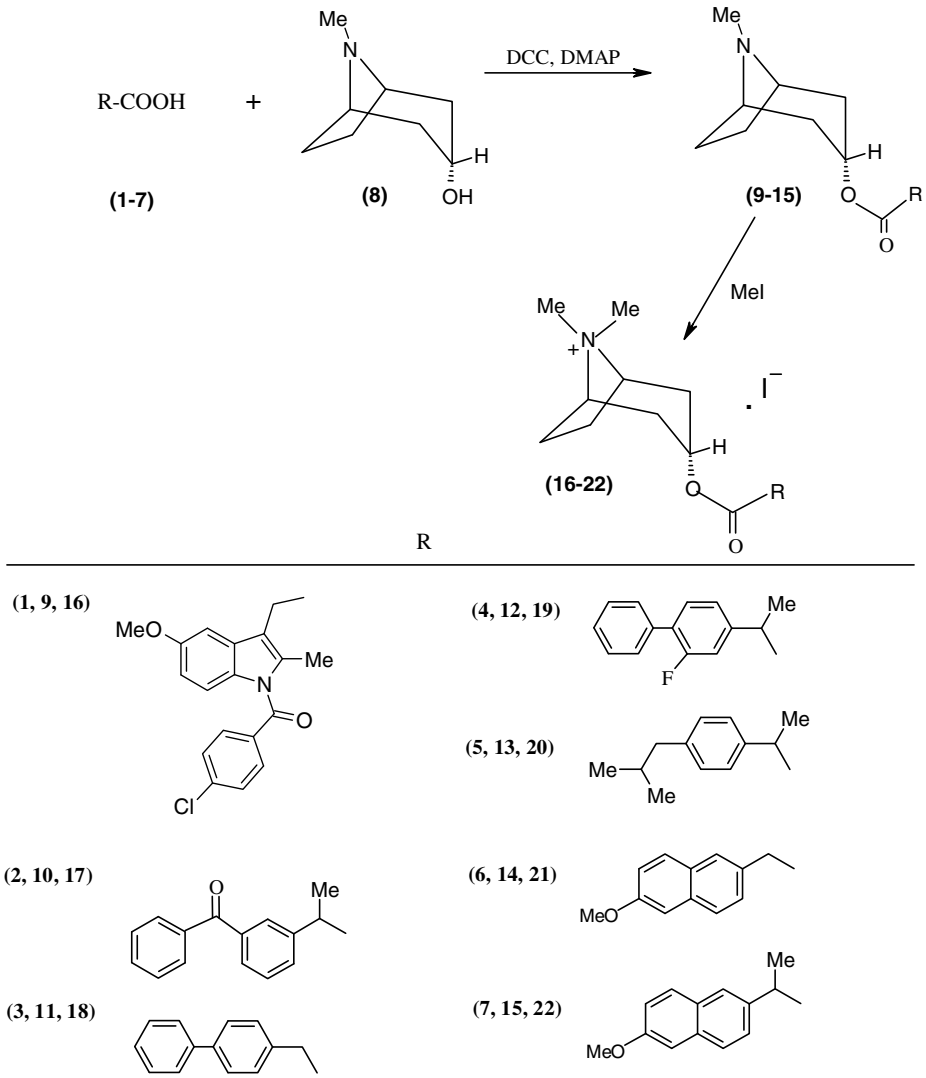
Radiolabeling of the compounds (**16–22**) with ^{99m}Tc was carried out by direct labeling method.[25] Technetium, available as pertechnetate (TcO_4^-) with +7 oxidation state does not complex with ligands by direct addition. For this to occur, it has to be reduced to +4 oxidation state using stannous chloride in acidic medium. For determining the ideal conditions for highest degree of complexation of the compounds (**16–22**) with ^{99m}Tc, experiments were performed under different set of conditions by varying parameters like quantity of stannous chloride used for reduction at pH 4.0, change of pH and incubation period of the ligands and the metal ions in the medium. pH 7.0 and incubation period of 30 min were found out to be optimum to afford highest radiolabeling efficiency (Tables 1 and 2). All the radiolabeled complexes (^{99m}Tc–QA) were found to be stable enough for 6 h in serum and normal saline (Table 3).

γ-Imaging studies were planned to be carried out to study the localization pattern of the ^{99m}Tc–QA conjugates in inflamed tissues of the animal model. Carrageenan was used as a phlogistic agent for producing inflammation in one of the hind paws of rat. The conjugates (^{99m}Tc–QA) were administered intravenously and imaging studies were performed at different time intervals. The parent NSAIDs (**1–7**) were also labeled with ^{99m}Tc and used as one set of controls in the imaging studies. Two tertiary amine derivatives (**11, 12**) were also labeled with ^{99m}Tc in similar way and used as another set of controls. These studies clearly revealed significant differences between the ^{99m}Tc–QA conjugates and the parent drugs (**1–7**) for their localization behavior in the inflamed tissue (Fig. 1). None of the parent NSAIDs (**1–7**) showed affinity for the inflamed joint tissue whereas, all the quaternized compounds (**16–22**) selectively localized in the inflamed areas. The two tertiary amines (**11** and **12**) also did not show any preferential localization behavior. The images also revealed a preferential distribution of the ^{99m}Tc–QA conjugates in liver and kidney.

2.3. Anti-inflammatory activity

The tropinol esters (**9–15**) did not exhibit any anti-inflammatory activity in the carrageenan-induced rat paw edema model[20–24] which is considered to be a model for acute inflammatory conditions. In the kinetic studies, these esters were found to be resistant to hydrolysis in plasma and buffers (pH 2.0 and 7.4) up to 8 h. The absence of anti-inflammatory activity in the acute inflammation model could be due to the non-conversion of these esters (**9–15**) into their parent NSAIDs in a duration of 3 h (the time period for which% inhibition of inflammation was recorded in the carrageenan-induced inflamed model). Considering that the ester derivatives (**16–22**) ultimately would be chemically hydrolyzed in due course of time, liberating the parent NSAIDs, it was planned to evaluate their anti-inflammatory potential in a chronic inflammation model. Adjuvant arthritis, an accepted and well established standard model[25] was chosen for this purpose. The parent NSAIDs (**1–7**) were used as standards for comparing the anti-inflammatory activity of the quaternized ester derivatives (**16–22**), which were administered on equivalent molar doses. Animals were dosed daily with parent drugs (**1–7**) and the derivatives (**16–22**) separately from day 1 to day 12. The phlogistic agent (*Mycobacterium butyricum*) suspended in heavy paraffin oil was injected into the subplantar region of the hind paws of the rats only on day 1. Paw volumes of all the test animals and controls were measured on days 3, 7, 14 and 21.

On days 3 and 7, the derivatives (**16–22**) showed lesser inhibition than the parent NSAIDs (**1–7**). On day 14 all the derivatives exhibited comparable anti-inflammatory activity to the parent NSAIDs except for derivatives (**17** and **18**). On day 21, all the quaternary ammonium ester derivatives exhibited significantly higher anti-inflammatory activity than the parent drugs except for derivative **17** (Table 4). The percent inhibition of inflammation exhibited by the parent drugs (**1–7**) declined on 21st day as compared to that on 14th day whereas, the percent inhibition of inflammation by the derivatives increased on 21st day in comparison to that observed on 14th day. This could be due to the slow chemical



Scheme 1.

Table 1
Effect of pH on the percent binding of parent NSAIDs and their derivatives with ^{99m}Tc

Compound	pH					
	5.5	6.0	6.5	7.0	7.5	8.0
% Radiolabeling						
1	77.15	83.23	96.01	96.37	95.87	93.12
2	79.96	89.56	90.97	91.48	91.30	90.56
3	75.71	91.50	93.50	96.78	94.67	91.75
4	75.96	82.56	93.06	95.22	94.12	90.11
5	71.45	79.96	94.76	95.71	94.96	91.07
6	70.05	79.66	95.06	95.05	94.12	92.12
7	69.05	75.74	90.08	93.95	92.98	87.50
16	67.34	78.50	90.79	92.90	91.44	87.16
17	61.58	72.26	85.30	86.42	86.01	84.36
18	79.06	89.34	95.98	96.24	96.66	90.40
19	76.90	86.56	96.59	97.61	95.46	91.47
20	62.21	74.50	92.01	93.86	91.76	87.63
21	69.70	77.70	91.40	91.56	90.23	86.67

Table 2
Effect of incubation time on the percent binding of compounds

Compound	Time (min)					
	0	5	10	20	30	40
% Radiolabeling						
1	66.56	87.15	93.15	96.12	96.38	96.40
2	60.66	81.56	86.79	91.37	91.93	91.95
3	66.12	84.23	89.73	96.72	97.28	97.15
4	68.51	86.16	90.97	95.20	96.01	96.20
5	71.56	86.55	88.93	94.98	95.0	95.96
6	63.61	81.56	91.50	94.52	94.59	94.56
7	70.07	87.85	90.26	94.01	94.30	94.60
16	62.16	82.12	85.24	92.60	92.71	92.70
17	60.05	82.15	85.26	87.57	88.05	87.99
18	66.15	83.70	88.20	94.57	94.63	94.12
19	61.01	81.56	87.56	97.46	97.63	97.58
20	61.32	68.36	85.23	93.06	93.10	93.08
21	61.34	80.66	87.77	91.96	92.05	92.70
22	62.57	80.61	84.96	92.98	92.99	92.95

hydrolysis of the ester derivatives (**16–22**) in the body to release the parent NSAIDs (**1–7**) which exhibited their normal anti-inflammatory activity. Concentration of the parent NSAIDs (**1–7**) must have fallen down on day 21 due to their metabolic inactivation as the last dosing was performed on day 12.

3. Conclusions

Cartilage targeting strategy for the conventional NSAIDs has been explored for reducing their local and systemic side effects.

Table 3
Stability studies of radiolabeled complexes in normal saline and human serum

Compound	% Radiolabeling efficiency (saline)					% Radiolabeling efficiency (serum)				
	0.25 h	02 h	04 h	06 h	24 h	0.25 h	02 h	04 h	06 h	24 h
1	96.37	95.60	93.4	89.37	84.55	95.39	94.60	90.40	89.32	74.50
2	93.95	92.38	85.95	85.47	81.95	92.95	92.38	85.95	83.47	74.95
3	96.78	96.44	96.07	92.82	84.27	95.89	92.44	89.07	86.89	74.27
4	91.48	89.61	88.82	81.65	81.18	90.48	89.61	88.82	86.65	72.18
5	95.7	94.04	90.56	87.89	81.19	94.70	93.04	90.56	86.89	78.19
6	95.05	94.66	94.09	90.90	82.65	94.55	91.66	88.09	87.96	72.69
7	95.22	94.27	91.89	88.23	82.52	93.22	93.20	91.89	88.23	79.52
16	92.90	92.01	86.94	83.54	81.56	91.90	91.01	88.94	83.54	80.51
17	86.42	85.22	81.88	76.11	76.19	87.42	85.22	80.88	76.19	75.19
18	96.24	94.92	92.81	86.18	82.97	93.24	92.92	91.81	86.18	82.03
19	97.61	97.35	97.35	95.16	93.17	96.61	96.35	96.35	95.16	91.37
20	93.86	93.38	92.70	88.52	86.75	92.81	92.38	91.70	86.52	84.75
21	91.56	89.69	89.95	85.65	81.08	90.56	89.09	88.95	85.65	80.08
22	93.18	93.24	91.78	91.30	85.53	92.08	92.00	91.78	91.30	85.53

The carboxylic group-bearing NSAIDs were derivatized into quaternary tropinol esters. These esters showed the tendency for selective accumulation into the inflamed tissues and exhibited anti-inflammatory activity comparable to their parent drugs in chronic inflammation model. Selective concentration of these ester derivatives into the tissue in demand could also allow reduction in their doses. It could be concluded that site specific delivery of these quaternary ester derivatives could prove to be an effective strategy for long term therapy of inflammatory conditions like arthritis with these well known time tested NSAIDs inflicting GIT and systemic side effects to the minimum extent.

4. Experimental

Parent drug indomethacin (**1**) was obtained as gift sample from M/s Ranbaxy Laboratories, Gurgaon, India. Tropinol was obtained by the basic hydrolysis (methanol/potassium hydroxide) of atropine. Lambda (Type IV) carrageenan, Freund's adjuvant and all the other chemicals were purchased from Sigma–Aldrich. Distilled water was used in preparation of buffer solutions. Anhydrous sodium sulfate was used as drying agent. Melting points were taken in open capillaries and are uncorrected. ^{1}H NMR spectra were recorded on a 300/400 MHz instrument. The IR spectra were recorded on Shimadzu-8300 FT-IR using KBr disc. Absorbance was measured on a Shimadzu UV-1601 spectrophotometer. Plethysmometer (Eugo-Basil, Italy) was used for measuring the rat paw volume. Scintigraphic studies were performed using SPECT Gamma camera (LC-75-005, Diacam, Siemens, USA). The protocol for the animal experiments performed was approved by the IAEC (Institutional Animal Ethics Committee) as registered under CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Govt. of India.

4.1. Synthesis

4.1.1. Synthesis of tropinol ester (9) of indomethacin (1)

Parent drug (**1**) (3.91 mmol) was dissolved in anhydrous dichloromethane (25 ml). The solution was cooled in ice bath, dicyclohexylcarbodiimide (DCC, 0.9 g, 4.36 mmol) and dimethylaminopyridine (DMAP, 0.06 g, 0.49 mmol) was added to the above solution and the reaction mixture stirred for 30 min. A solution of tropine (0.55 g, 3.91 mmol) in anhydrous dichloromethane (10 ml) was added drop-wise with stirring to the above chilled reaction mixture. The stirring was continued for 16 h at RT. The reaction mixture was filtered to remove DCU. The filtrate was diluted with dichloromethane (50 ml) and stirred with dilute acetic acid (10%, 10 ml) to convert unreacted DCC to dicyclohexylurea (DCU). Organic layer was separated and washed with dilute ammonia solution (2 × 10 ml) to remove any unreacted acid and then washed with chilled water. The organic layer was separated, dried, and evaporated to get the ester (8-dimethyl-8-azabicyclo[3,2,1]octan-3-yl) 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate (**9**) as an oily product.

4.1.2. 8-Methyl-8-azabicyclo[3,2,1]octan-3-yl 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-acetate methiodide (16)

The oily tropinol ester (**9**) obtained above was refluxed with methyl iodide (0.8 ml) in diisopropyl ether (20 ml) for half an hour. Ether was removed under vacuum and the residue triturated with ethyl acetate to remove impurities. The crude product was dried and crystallized from methanol to afford the quaternized product (**16**); 71% yield, mp 228–30 °C; IR(KBr, cm^{-1}): 1728, 1686, 1313, 1225, 1151, 1070, 1010 and 752; PMR (DMSO-d_6): δ (ppm) 7.65–7.67 (d, 2H), 7.52–7.55 (d, 2H), 6.94–6.95 (d, 1H), 6.85–6.87 (d, 1H), 6.66–6.69 (dd, 1H), 5.13–5.16 (t, 1H), 4.02 (s, 2H), 3.83 (s, 3H), 3.75 (s, 2H), 3.30 (s, 3H), 3.17 (s, 3H), 2.59–2.68 (m, 2H), 2.40 (s, 3H), 2.16–2.18 (m, 2H), 1.84–1.99 (m, 4H). Anal. Calcd for $C_{28}H_{32}N_2O_4ClI$ (622.92): C 53.98, H 5.17, N 4.49; found: N 4.36%.

4.1.3. 8-Methyl-8-azabicyclo[3,2,1]octan-3-yl 2-(3-benzoylphenyl)propionate methiodide (17)

The ester (**10**)[24] was reacted with methyl iodide as described under compound (**16**) to obtain the methiodide (**17**), 85% yield, mp 211–13 °C; IR(KBr, cm^{-1}): 1728, 1661, 1576, 1448, 1319, 1286, 1207, 117, 1159, 1043, 719, and 704; PMR (DMSO-d_6): δ (ppm) 7.44–7.81 (m, 9H), 5.05–5.08 (t, 1H), 4.01–4.05 (m, 2H), 3.73–3.81 (m, 1H), 3.28 (s, 3H), 3.14 (s, 3H), 2.52–2.60 (m, 2H), 1.97–2.07 (m, 2H), 1.65–1.87 (m, 3H), 1.54–1.56 (d, 3H), 1.39–1.45 (m, 1H). Anal. Calcd for $C_{25}H_{30}NO_3I$ (519.40): C 57.80, H 5.82, N 2.69; found: C 57.62, H 5.76, N 2.48%.

4.1.4. 8-Methyl-8-azabicyclo[3,2,1]octan-3-yl 4-biphenylacetate methiodide (18)

Prepared from ester (**11**)[23] as described under compound (**16**): 90% yield, mp 248–53 °C; IR(KBr, cm^{-1}): 1730, 1489, 1175, 1156, 1045, 1005, 750 and 650; PMR (DMSO-d_6): δ (ppm) 7.31–7.60 (m, 9H), 5.13–5.16 (t, 1H), 3.69 (s, 2H), 3.28 (s, 3H), 3.12 (s, 3H), 2.72–2.81 (m, 2H), 2.23–2.31 (m, 2H), 1.66–2.17 (m, 4H). Anal. Calcd for $C_{23}H_{28}NO_2I$ (477.38): C 57.86, H 5.91, N 2.93; found: C 57.55, H 6.22, N 2.76%.

4.1.5. 8-Methyl-8-azabicyclo[3,2,1]octan-3-yl 2-(2-fluoro-4-biphenyl)propionate methiodide (19)

Prepared form ester (**12**)[23] as described under compound (**16**). 87% yield, mp 235–38 °C; IR(KBr, cm^{-1}): 1728, 1661, 1574, 1485,

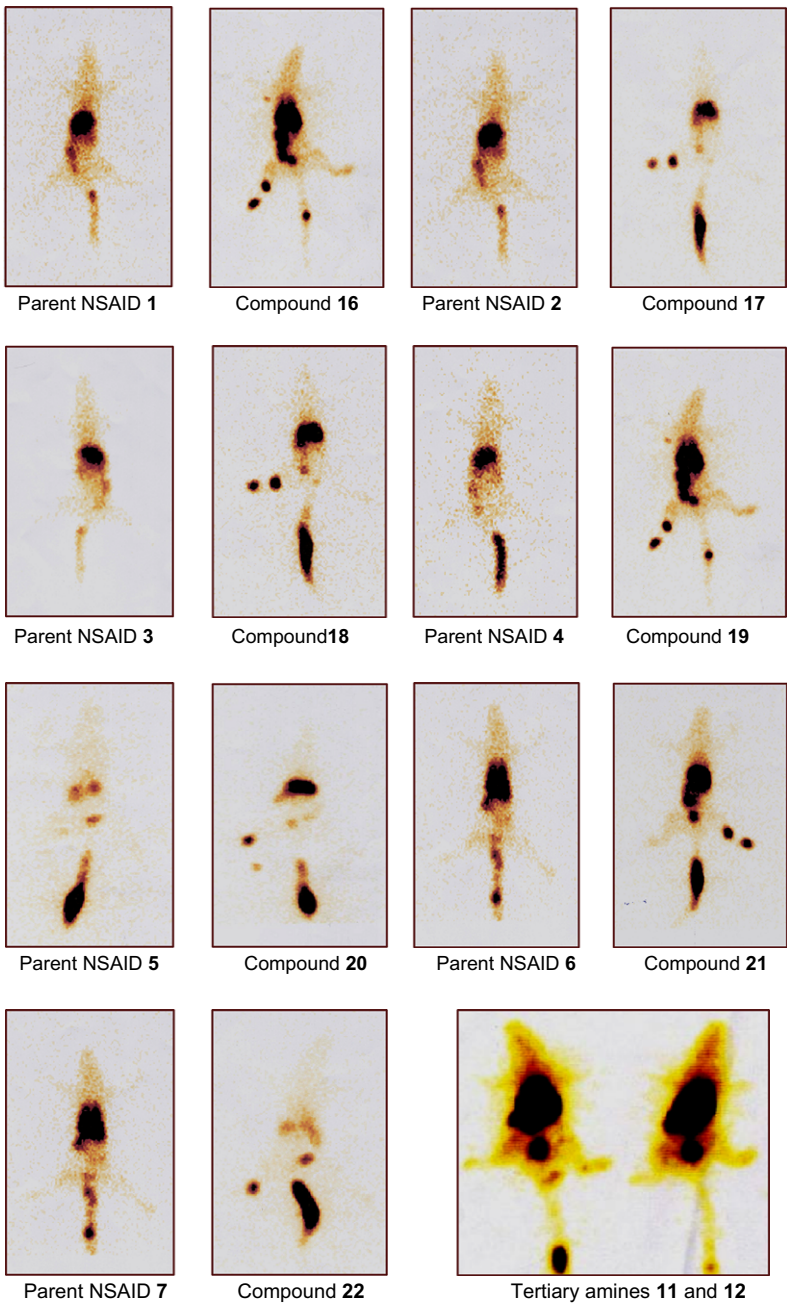


Figure 1. Gamma images of compounds **1**–**7** and **16**–**22** in arthritis model in rats.

1456, 1418, 1159, 1043, 1006, 935, 914, 768 and 698; PMR (DMSO-d_6): δ (ppm) 7.08–715 (m, 2H), 7.39–7.54 (m, 6H), 5.13–5.16 (t, 1H), 4.18–4.28 (m, 2H), 3.70–3.79 (m, 1H), 3.32 (s, 3H), 3.21 (s, 3H), 2.75–2.82 (m, 2H), 2.25–2.30 (m, 2H), 1.78–2.09 (m, 4H), 1.53–1.58 (d, 3H). Anal. Calcd for $C_{24}H_{29}NO_2FI$ (509.39): C 56.59, H 5.73, N 2.74; found: C 56.44, H 5.96, N 2.51%.

4.1.6. 8-Methyl-8-azabicyclo[3,2,1]octan-3-yl 2-(4-isobutylphenyl)propionate methiodide (20)

The ester (**13**)[24] was reacted as described under compound (**16**) to obtain the desired product (**20**). 83% yield, mp 270–72 °C; IR(KBr, cm^{-1}): 1732, 1508, 1456, 1202, 1184, 1163, 1050, 1005; PMR ($CDCl_3$): δ (ppm) 7.10–7.15 (dd, 4H), 5.12–5.15 (t, 1H), 4.26–4.28 (m, 1H), 4.09–4.11 (m, 1H), 3.64–3.70 (q, 1H), 3.49 (s, 3H), 3.32 (s, 3H), 2.58–2.74 (m, 2H), 2.44–2.46 (d, 2H), 1.99–2.21 (m, 2H), 1.69–1.90 (m, 4H), 1.48–1.50 (d, 3H), 1.36–1.44 (m, 1H), 0.88 (d, 6H). Anal. Calcd for $C_{22}H_{34}NO_2I$ (471.42): C 56.05, H 7.26, N 2.97; found: C 56.27, H 7.06, N 3.22%.

4.1.7. 8-Methyl-8-azabicyclo[3,2,1]octan-3-yl 6-methoxy-2-naphthylacetate methiodide (21)

The ester (**14**)[21] was reacted as described under compound (**16**) to obtain the product (**21**). 78% yield, mp 276–78 °C; IR(KBr, cm^{-1}): 1732, 1632, 1607, 1481, 1429, 1391, 1337, 1267, 1205, 1159, 1132, 1030, 851 and 820; PMR (DMSO-d_6): δ (ppm) 7.63–7.72 (m, 2H), 7.32–7.35 (dd, 1H), 7.12–7.18 (m, 2H), 5.10–5.15 (t, 1H), 4.03–4.06 (m, 1H), 3.92 (s, 3H), 3.82–3.86 (m, 3H), 3.28 (s, 3H), 3.11 (s, 3H), 2.49–2.65 (m, 2H), 1.98–2.08 (m, 2H), 1.76–1.90 (m, 2H), 1.63–1.68 (m, 1H), 1.32–1.40 (m, 1H). Anal. Calcd for $C_{22}H_{28}NO_3I$ (481.36): C 54.89, H 5.86, N 2.90; found: N 2.48%.

Table 4
Percent anti-inflammatory activity of compounds (Freund's adjuvant arthritis model)

Compound (parent derivative)	Dose (mg/kg)	% Inhibition of paw edema on days			
		3rd	7th	14th	21st
1	0.81	37.4 ± 1.56	46.50 ± 1.45	54.60 ± 3.13	49.2 ± 2.05
16	1.56	23.9 ± 1.79	40.54 ± 1.41	56.35 ± 2.26	55.97 ± 2.78*
2	0.85	35.10 ± 3.30	43.9 ± 3.20	54.4 ± 2.26	55.03 ± 2.56
17	1.80	17.03 ± 1.62	29.96 ± 1.10	39.75 ± 2.10	44.82 ± 1.54*
3	0.85	37.40 ± 1.56	46.47 ± 1.44	54.60 ± 3.78	48.5 ± 2.36
18	2.00	22.31 ± 3.50	36.86 ± 1.92	47.71 ± 1.58	53.51 ± 2.11*
4	2.59	36.52 ± 1.98	44.22 ± 1.82	55.57 ± 2.58	53.07 ± 2.49
19	5.61	25.53 ± 2.33	40.96 ± 2.73	53.62 ± 1.37	60.41 ± 1.40*
5	7.20	35.10 ± 2.93	44.1 ± 2.39	53.82 ± 0.95	53.21 ± 2.42
20	16.44	22.52 ± 3.72	41.5 ± 2.06	53.81 ± 3.00	60.12 ± 2.81*
6	4.27	35.6 ± 3.48	45.5 ± 2.77	56.4 ± 1.75	52.51 ± 1.85
21	10.01	23.05 ± 1.98	40.86 ± 2.08	55.75 ± 2.09	71.29 ± 1.19*
7	5.13	36.30 ± 1.67	46.6 ± 2.51	54.7 ± 2.66	50.20 ± 2.78
22	11.60	23.04 ± 2.48	39.70 ± 1.16	53.46 ± 1.42	58.74 ± 2.82*

Each value represents the mean ± SD ($n = 6$). Significance levels $^{*}p < 0.05$ in comparison to respective parent drug.

4.1.8. 8-Methyl-8-azabicyclo[3,2,1]octan-3-yl 2-(6-methoxy-2-naphthyl)propionate methiodide (22)

The ester (**15**)[21] was reacted with methyl iodide as described under compound (**16**) to obtain the quaternized product (**22**). 82% yield, mp 260–62 °C; IR(KBr, cm^{-1}): 1728, 1634, 1609, 1464, 1265, 1178, 1157, 1080, 1043, 1025 and 800; PMR (DMSO-d_6-$CDCl_3$): δ (ppm) 7.65–7.75 (m, 3H), 7.33–7.35 (dd, 1H), 7.13–7.17 (m, 2H), 5.09–5.19 (t, 1H), 4.05–4.07 (m, 1H), 3.93 (s, 3H), 3.84–3.89 (m, 2H), 3.29 (s, 3H), 3.13 (s, 3H), 2.53–2.70 (m, 2H), 2.01–2.12 (m, 2H), 1.91–1.95 (m, 1H), 1.75–1.85 (m, 1H), 1.65–1.70 (m, 1H), 1.59–1.60 (d, 3H), 1.36–1.46 (m, 1H). Anal. Calcd for $C_{23}H_{30}NO_3I$ (495.39): C 55.76, H 6.10, N 2.82; found: N 2.48%.

4.2. Radiolabeling of the compounds

Radiolabeling of the compounds with reduced ^{99m}Tc was carried out as per the direct labeling method.[25] Sodium pertechnetate (^{99m}Tc) (1.0 ml, 2.0 mci/ml) was mixed with stannous chloride solution (0.1 ml, 1 mg/ml in acetic acid (10%)) and mixed well. The pH was adjusted to 7.0 using sodium bicarbonate solution (0.5 M) and to this mixture, solution of the compounds (1.0 ml, dissolved in DMSO/water (9:1)) was added and incubated for 15 min (37 ± 1 °C). The labeling efficiency/percent labeling was determined by performing ascending instant thin layer chromatography (ITLC) using acetone as the mobile phase. Radiolabeled complex (2–3 μl) was applied at a point 1.0 cm away from one end of an ITLC-SG strip. The strip was developed in acetone and the solvent front was allowed to rise up to 8 cm from the origin. The strip was cut one cm below the solvent front and the radioactivity in each segment was determined by scintillation counting. The free pertechnetate which moved with the solvent ($R_f = 0.9$) and the reduced/hydrolyzed (R/H) technetium along with the labeled complex remaining at the point of application were determined. ITLC was also run in pyridine-acetic acid- water (3:5:1.5 v/v, PAW) system to determine the amount of reduced/hydrolyzed (R/H) ^{99m}Tc (radio-colloids). The R/H ^{99m}Tc remained at the point of application while both the free pertechnetate and the labeled complex moved away with the solvent front in this solvent system (PAW). The difference between the activity for the spots which moved along with the solvent front using acetone from that obtained in the PAW system (at the point of application) gave the net amount of ^{99m}Tc-labeled complex.

The experiment was repeated by varying the conditions one at a time, that is, using different moles of stannous chloride (0.05–0.2 ml), changing the *p*H of the medium (5.5 and 8) and varying the incubation time period (5–40 min) of the reaction mixture. All the compounds were radiolabeled by this procedure.

4.3. In vitro stability studies of ^{99m}Tc-labeled complexes in saline

The in vitro stability of radiolabeled complexes was determined in sodium chloride (0.9%) by ascending thin layer chromatography. The ^{99m}Tc-labeled compound solution (0.1 ml) prepared in saline (0.9%) as described above was mixed with normal saline (1.9 ml) and incubated (37 ± 1 °C). ITLC was performed at different time intervals (0, 2, 4, 6 and 24 h) as described above, in acetone to assess the stability of the complex. Any decrease in percentage of ^{99m}Tc-labeled complex was considered as its degree of degradation.

4.4. In vitro stability studies of ^{99m}Tc-labeled complexes in human serum

The in vitro stability of radiolabelled complexes was tested in human serum. The study was accomplished by incubating an aliquot of 0.1 ml of labeled complex prepared in saline (0.9%) and mixed with human serum (1.9 ml) and incubated (37 ± 1 °C). ITLC was performed at different time intervals (0, 2.0, 4.0, 6.0, and 24 h) as described above, in acetone to assess the stability of the complex. Any decrease in percentage of ^{99m}Tc-labeled complex was considered as its degree of degradation.

4.5. γ-Imaging studies

Sprague–Dawley rats (three in a group) were used for the studies. Imaging was performed using a Single Photon Emission Computerized Tomography gamma camera. Inflammation was induced in each rat by subcutaneous injection of carrageenan (0.1 ml, 1% w/v in normal saline) into the sub-plantar region of the right paw. Three hours later when maximum inflammation was achieved, ^{99m}Tc-labeled complex (0.2 ml) of the QA (**9–22**) was administered through the tail vein. The animals were anaesthetized (ketamine, im), fixed on a board using the adhesive tapes and γ-imaging photographs (Fig. 1) were taken 3 h after injecting the complex through the tail vein.

4.6. Anti-inflammatory activity (Arthritis model)

Sprague–Dawley male rats with an initial body weight of 150–200 g were used. Rats were divided into groups of six each for test

compounds and control group.[26] On day 1, they were injected into the sub-plantar region of the left hind paw with 0.1 ml of complete Freund's adjuvant (6 mg/ml *Mycobacterium butyricum* suspended in heavy paraffin oil). Once a day dosing with the test compounds or the standard (on equivalent molar doses) was started on the same day and continued for 12 days. Paw volumes of both sides was measured plethysmographically. The paw volumes were measured on days 3, 7, 14 and 21. From day 13 to 21, the animals were not dosed with the test compounds or the standards. Parent NSAIDs (**1**–**7**) as standards and QA (**16**–**22**) dissolved in aqueous-DMSO were administered to the animals through tail vein in the doses as given in Table 4. Results (Table 4) are expressed as percentage inhibition of edema formation, calculated by the formula, % Inhibition of paw edema = $(1 - Ed_{test}/Ed_{control}) \times 100$ where Ed_{test} and $Ed_{control}$ are the edema volumes in compound treated and control groups, respectively. The data obtained in the pharmacological experiment was subjected to statistical analysis using the Student's *t* test and the chosen level of significance was $p < 0.05$.

Acknowledgments

The authors gratefully acknowledge the help provided by Mrs. Krishna Chuttani, Mr. Ankur Kaul and Mr. Anil K. Babbar in successful completion of this work.

References and notes

1. Roberts, L. J.; Morrow, J. D. In *The Pharmacological Basis of Therapeutics*; Goodman, L. S., Gilman, A. G., Hardman, J. G., Limbird, A. E., Eds., 10th ed.; McGraw Hill: New York, 2001; pp 687–733.
2. Khanpure, S. P.; Letts, L. G. *The Eicosanoids*; John Wiley & Sons: London, 2004. pp 131–162.
3. Gajraj, N. M. *Anesth. Analg.* **2003**, *96*, 1720.
4. Wallace, J. L.; Reuter, B.; Cicala, C.; McKnigh, T. W.; Grisham, M.; Cirino, G. *Eur. J. Pharmacol.* **1994**, *257*, 249.
5. Tammara, V. K.; Narurkar, M. M.; Crider, A. M.; Khan, M. A. *J. Pharm. Sci.* **1994**, *83*, 644.
6. Mahfouz, N. M.; Omar, F. A.; Aboul-Fadl, T. *Eur. J. Med. Chem.* **1999**, *34*, 551.
7. Warner, T. D.; Giuliano, F.; Vojnovic, I.; Bukara, A.; Mitchell, J. A.; Vane, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7563.
8. Kalgutkar, A. S.; Marnett, A. B.; Crews, B. C.; Remmel, R. P.; Marnett, L. J. *J. Med. Chem.* **2000**, *43*, 2860.
9. Tally, J. J. In *Progress in Medicinal Chemistry*; King, F. D., Oxford, A., Eds.; Elsevier: Amsterdam, 1999; pp 201–234.
10. Hawkey, C. J. *Lancet* **1999**, *353*, 307.
11. Scheen, A. J. *Rev. Med. Liege* **2004**, *59*, 565.
12. Furberg, C. D.; Psaty, B. M.; Firzgerald, G. A. *Circulation* **2005**, *111*, 249.
13. Dogne, J. M.; Supuran, C. T.; Pratico, D. *J. Med. Chem.* **2005**, *48*, 2251.
14. Garrigue, H.; Maurizis, J. C.; Nicolas, C.; Madelmont, J. C.; Godeneche, D.; Hulot, T.; Morge, X.; Demerseman, P.; Sentenac-Roumanou Vegre, A. *Xenobiotica* **1990**, *20*, 699.
15. Garrigue, H.; Maurizis, J. C.; Madelmont, J. C.; Nicolas, C.; Megneil, J. M.; Louvel, A.; Demerseman, P.; Sentenac-Roumanou Vegre, A. *Xenobiotica* **1991**, *21*, 583.
16. Maurizis, J. C.; Ollier, M.; Nicolas, C.; Madelmont, J. C.; Garrigue, H.; Veyre, A. *Biochem. Pharmacol.* **1992**, *44*, 1927.
17. Nicolas, C.; Verny, M.; Giraud, I.; Oller, M.; Rapp, M.; Maurizis, J.; Madelmont, J. C. *J. Med. Chem.* **1999**, *42*, 5235.
18. Giraud, I.; Rapp, M.; Maurizis, J.; Madelmont, J. *Bioconjugate Chem.* **2000**, *11*, 212.
19. Yadav, M. R.; Halen, P. K.; Hemalatha, B.; Chagti, K. K.; Giridhar, R. *ARS Pharm.* **2005**, *46*, 263.
20. Halen, P. K.; Yadav, M. R.; Reshmi, C. S.; Chagti, K. K.; Giridhar, R. *ARS Pharm.* **2006**, *47*, 61.
21. Halen, P. K.; Chagti, K. K.; Giridhar, R.; Yadav, M. R. *Chem. Biodiv.* **2006**, *3*, 1238.
22. Halen, P. K.; Chagti, K. K.; Giridhar, R.; Yadav, M. R. *Med. Chem. Res.* **2007**, *16*, 101.
23. Halen, P. K.; Raval, M. K.; Chagti, K. K.; Giridhar, R.; Yadav, M. R. *Arch. Pharm. Chem. Life Sci. (Weinheim)* **2007**, *340*, 88.
24. Halen, P. K.; Chagti, K. K.; Giridhar, R.; Yadav, M. R. *Chem. Biol. Drug Des.* **2007**, *70*, 450.
25. Saha, G. B. In *Physics and Radiobiology of Nuclear Medicine*; Saha, G. B., Ed., 5th ed.; Springer-Verlag: New York, 1993; pp 100–106.
26. Pearson, C. M.; Wood, F. D. *Arthritis Rheum.* **1959**, *2*, 440.