Tetrahedron 64 (2008) 8642–8645

Contents lists available at ScienceDirect

Tetrahedron

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

Total synthesis and analgesic activity of 6-fluoroindan-1-carboxylic acid

Sharmistha Das [a], Hasina Yasmin [a], M. Mehedi Masud [a], Suvas C. Roy [b], Lutfun Nahar [c], M. Mukhlesur Rahman [d], Simon Gibbons [d], Sitesh C. Bachar [e], Satyajit D. Sarker [c,*]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh
[b] Department of Pharmacy, University of Science & Technology Chittagong, Foy's Lake, Chittagong, Bangladesh
[c] School of Biomedical Sciences, University of Ulster, Cromore Road, Coleraine BT52 1SA, Co. Londonderry, Northern Ireland, UK
[d] Centre for Pharmacognosy and Phytochemistry, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK
[e] Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh

ARTICLE INFO

Article history:
Received 23 April 2008
Received in revised form 19 June 2008
Accepted 3 July 2008
Available online 8 July 2008

Keywords:
3-Fluorophenylcyanoethylacrylate
3-Fluorophenyl succinic acid
6-Fluoro-3-oxo-indan-1-carboxylic acid
6-Fluoroindan-1-carboxylic acid
Analgesic activity

ABSTRACT

6-Fluoroindan-1-carboxylic acid (**4**) was conveniently synthesised from 3-fluorobenzaldehyde in six steps. The structure of this new compound and three other intermediates, 3-fluorophenylcyanoethylacrylate (**1**), 3-fluorophenyl succinic acid (**2**) and 6-fluoro-3-oxo-indan-1-carboxylic acid (**3**) was elucidated by comprehensive spectral data analyses. The analgesic activity of compounds **3** and **4** was assessed by the acetic acid induced writhing in *Swiss albino* mice.

1. Introduction

Compounds containing indan ring systems, especially with a carboxylic acid functionality, possess anti-inflammatory activity. Among indan derivatives 1*H*-indene-3-acetic acid-5-fluoro-2-methyl-1-[4-(methylsulfinyl)-phenyl]methylene (Sulindac) and indan-1,3-dione are well known anti-inflammatory agents.[1] Juby et al.[2] observed significant anti-inflammatory activity among a series of substituted indan-1-carboxylic acids. Moreover, a number of methoxy indan-1-alkanoic acids were synthesised with considerable anti-inflammatory properties.[3] Indan derivatives, with a halo-substituted indanyl group were found to possess analgesic and anti-inflammatory activities.[4–7] It was observed that aromatic halogen substitution could be a reasonable means of increasing the analgesic and anti-inflammatory activities and widening the margin of safety.[7] As part of our quest for new and more effective analgesic and anti-inflammatory agents,[7] we now report on the total synthesis of 6-fluoroindan-1-carboxylic acid (**4**) from 3-fluorobenzaldehyde in six steps, and the assessment of analgesic activity of the intermediate 6-fluoro-3-oxo-indan-1-carboxylic acid (**3**) and the target compound **4** by the acetic acid induced writhing in *Swiss albino* mice.

2. Results and discussion

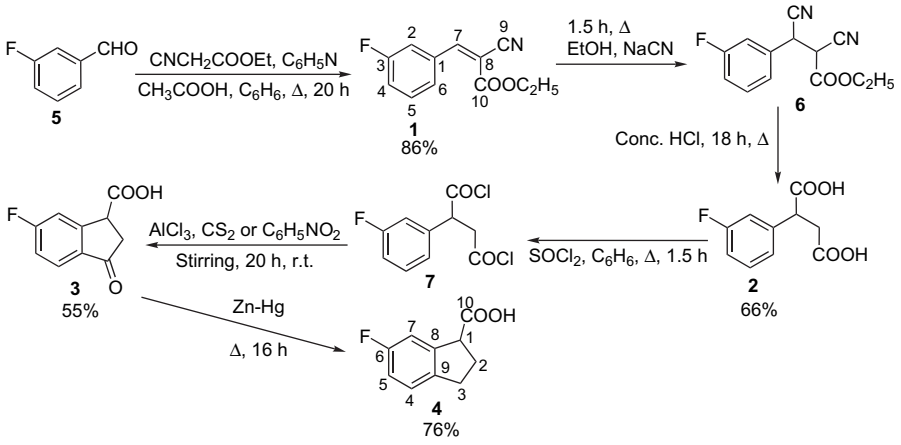
6-Fluoroindan-1-carboxylic acid (**4**) was conveniently synthesised from 3-fluorobenzaldehyde (**5**) in six steps with an overall 24% yield (Scheme 1). The five intermediates were 3-fluorophenylcyanoethylacrylate (**1**), 3-fluorophenyl-α,β-dicyanoethyl propionate (**6**), 3-fluorophenyl succinic acid (**2**), 3-fluorophenyl succinyl chloride (**7**) and 6-fluoro-3-oxo-indan-1-carboxylic acid (**3**). Among these intermediates, compounds **1**–**3** were isolated in pure form. The structures of compounds **1**–**4** were elucidated by spectroscopic means, notably, UV, IR, HRMS and extensive 1D and 2D NMR analyses (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC).

3-Fluorobenzaldehyde (**5**) was condensed with ethyl-cyanoacetate in the presence of pyridine in 1:1 molar ratio using dry benzene as reaction solvent to produce 3-fluorophenyl-cyanoethylacrylate (**1**) with an excellent yield of 86% (Scheme 1). The reaction between compound **1** and sodium cyanide yielded compound 3-fluorophenyl α,β-dicyanoethylpropionate (**6**), which was subjected to acid hydrolysis to obtain 3-fluorophenyl succinic acid (**2**) (yield 66%) (Scheme 1).

The diacid (**2**) was cyclised by Friedel–Crafts acylation reaction to obtain 6-fluoro-3-oxo-indan-1-carboxylic acid (**3**) via the formation of 3-fluorophenyl succinyl chloride (**7**) as a highly moisture sensitive liquid material (Scheme 1). The reaction was carried out in two different solvents, nitrobenzene and CS_2 to compare the yields.

* Corresponding author. Tel.: +44 28 7032 4302; fax: +44 28 7032 4965.
E-mail address: s.sarker@ulster.ac.uk (S.D. Sarker).

0040-4020/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2008.07.010



Scheme 1. Synthesis of compounds **1**–**4**.

It was found that the reaction carried out in CS_2 gave a slightly better yield (55% as opposed to 40%). The 3-oxo-derivative (**3**) was subjected to reduction by Clemmensen reduction to obtain the target compound 6-fluoroindan-1-carboxylic acid (**4**) with a yield of 76% (Scheme 1).

The analgesic activity of compounds **3** and **4** was assessed by the acetic acid induced writhing in *Swiss albino* mice.[8] Since the analgesic and the anti-inflammatory activities of various indan-1-acids and tetrazoles were reported previously[3,9–13] the 6-fluoro-3-oxo-indan-1-carboxylic acid (**3**) and 6-fluoroindan-1-carboxylic acid (**4**) were evaluated for their analgesic activity. The results showed that the writhing induced by acetic acid was significantly reduced by the test compounds in a dose dependent manner (Table 1). 6-Fluoro-3-oxo-indan-1-carboxylic acid (**3**) showed a 19.5% ($p<0.05$) inhibition at the dose of 25 mg/kg body weight and 30.54% ($p<0.005$) inhibition at the dose of 50 mg/kg body weight. 6-Fluoroindan-1-carboxylic acid (**4**) showed 21.56% ($p<0.005$) inhibition at the dose of 25 mg/kg body weight and 35.93% ($p<0.0005$) inhibition at the dose of 50 mg/kg body weight. It is notable that the absence of the ketonic carbonyl functionality at C-3 in **4**, increased the cyclopentane ring flexibility, and contributed to the slight increase in the analgesic activity measured by % inhibition of induced writhing in mice. The analgesic activity of **3** and **4** was comparable to those of the positive controls, e.g., aminopyrine with 47.89% ($p<0.0005$) inhibition at 30 mg/kg body weight, indomethacin with 48.5% ($p<0.0005$) inhibition at 8 mg/kg body weight and diclofenac Na with 62.88% ($p<0.0005$) inhibition at 10 mg/kg body weight. None of the test compounds (**3** and **4**) displayed any significant side effects at test doses. However, the behavioural pattern of the mice was slightly affected, e.g., reduced movement, head down and increased respiration. The present study has demonstrated that the fluoro substitution potentiates the analgesic activity of compounds **3** and **4**, and the 6-fluoroindan-1-carboxylic acid and (**4**), which is a reduced product of **3** possess slightly better analgesic activity than the keto or 3-oxo compound.

The writhing reflex in mice induced by acetic acid is a sensitive procedure to evaluate the potential analgesic activity of drugs. It has been suggested that acetic acid acts by releasing endogenous mediators, which stimulate the nociceptive neurons in mice.[14] Acetic acid is sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of analgesic agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis. Acetic acid is also sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) and to narcotics and other centrally acting drugs.[14–16] Recently it has been found that the nociceptive activity of acetic acid may be due to the release of cytokines, such as TNF-α, interleukin-1β and interleukin-8, by resident peritoneal macrophages and mast cells.[17] Based on this report, it can be assumed that in the present study the antinociceptive action showed by compounds **3** and **4** in the acetic acid induced writhing test might be due to inhibition of the release of TNF-α, interleukin-1β and interleukin-8, by resident peritoneal macrophages and mast cells.

3. Experimental

3.1. General

The chemicals and solvents used in various reactions were purchased from Merck (Germany), BDH (India) or SD Fine Chemicals (India), and used without purification. The melting points were determined by using Adco Melting Point Apparatus and were uncorrected. Thin-layer chromatography was performed using

Table 1
Analgesic activity of 6-fluoro-3-oxo-indan-1-carboxylic acid (**3**) and 6-fluoroindan-1-carboxylic acid (**4**)

Test compounds/controls	Group	Dose (mg/kg body weight)	Total number of writhing	Mean±SD	% Inhibition
6-Fluoro-3-oxo-indan-1-carboxylic acid (**3**)	A	25	31, 29, 16, 18, 24, 17	22.5±5.90	19.15[a]
	B	50	26, 24, 13, 15, 18, 20	19.33±4.61	30.54[b]
6-Fluoroindan-1-carboxylic acid (**4**)	C	25	22, 19, 20, 22, 27, 25	21.83±2.54	21.56[b]
	D	50	19, 15, 23, 18, 12, 20	17.83±3.53	35.93[c]
Aminopyrine	E	30	17, 08, 19, 20, 14, 09	14.50±4.64	47.89[c]
Indomethacin	F	8	12, 14, 18, 12, 13, 20	14.33±3.68	48.50[c]
Diclofenac Na	G	10	07, 14, 14, 05, 10, 12	10.33±3.39	62.88[c]
Saline	H	—	32, 30, 28, 25, 28, 24	27.83±2.73	—

All values are means of six mice.
[a] Probability values (calculated as compared to control using student's *t*-test): <0.05.
[b] Probability values (calculated as compared to control using student's *t*-test): <0.005.
[c] Probability values (calculated as compared to control using student's *t*-test): <0.0005.

Kieselgel 60 F_{254} plates (Merck). The absorption maxima (λ_{max}) of all the newly synthesised compounds were determined in absolute methanol by using Genesis-2 spectrophotometer. By using 8010M FTIR spectrometer, the characteristic absorption bands (ν_{max}) of the newly synthesised compounds were recorded on KBr disk. NMR spectra were recorded in CD_3OD on a Bruker AVANCE 500 MHz NMR spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C) using the residual solvent peaks as internal standard. MS analyses were performed, on a Finnigan MAT95 spectrometer. HMBC spectra were optimised for a long range J_{H-C} of 9 Hz and NOESY experiment was carried out with a mixing time of 0.4 s.

3.2. Synthesis of compounds 1–4

3.2.1. Synthesis of 3-fluorophenylcyanoethyl acrylate (*1*)

3-Fluorophenylcyanoethylacrylate (**1**) was prepared from 3-fluorobenzaldehyde (**5**) (7.45 g, 0.06 mol) by the reaction with ethylcyanoacetate (6.78 g, 0.06 mol) through refluxing in benzene (dry, 60 mL) in presence of pyridine (2 mL) and glacial acetic acid (2 mL) for 20 h (Scheme 1). On completion of the reaction, benzene was removed in vacuo to obtain a solid residue. The residue was treated with concd. HCl (5×2=10 mL) to remove pyridine as salt present in the reaction mixture. Then the reaction mixture was washed repeatedly with water (50×3=150 mL) to remove all water soluble impurities, filtered and recrystallised in acetone–water to obtain compound **1** as a white crystalline solid (yield 11.38 g, 86.64%).

Mp 86–88 °C. UV (MeOH): 280 nm. IR (KBr): 2235 (CN), 1690 (–$COOC_2H_5$ ester) and 1120 (C–F) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6): Table 2. CIMS *m*/*z*: $[M+NH_3]^+$ 237. HR-EIMS *m*/*z*: $[M]^+$ 219.0692 calcd 219.0695 for $C_{12}H_{10}O_2FN$.

3.2.2. Synthesis of 3-fluorophenyl succinic acid (*2*)

Compound **1** (10.95 g, 0.05 mol) was allowed to react with NaCN (2.45 g, 0.05 mol) dissolved in water (10 mL) in presence of 50% alcohol (100 mL) under reflux for 1.5 h (Scheme 1). The reaction mixture was cooled, poured into water (200 mL), concd. HCl (100 mL) was added to it and kept overnight. The biphasic mixture produced was separated and the aqueous part was extracted with chloroform (50×3=150 mL) and added to the biphasic mixture part. The combined chloroform extract was washed with water (50 mL), dried over sodium sulfate and finally concentrated under reduced pressure. 3-Fluorophenyl-α,β-dicyanoethyl propionate (**6**) was obtained as a semisolid mass (10.30 g, yield 84%). Compound **6** was used without further purification or spectral analyses for the synthesis of 3-fluorophenyl succinic acid (**2**). Compound **6** (9.84 g, 0.04 mol) was hydrolysed by refluxing with concd. HCl (11.65 M, 100 mL) for 18 h (Scheme 1). On completion of the reaction, the reaction mixture was cooled and the precipitated white crystals were filtered, washed with water (20×3=60 mL) and recrystallised from hot water to obtain a pure white crystalline solid in 65.5% yield as 3-fluorophenyl succinic acid (**2**) (5.55 g).

Mp 170–172 °C. UV (MeOH): 278 nm. IR (KBr): 1650 (COOH) and 1120 (C–F) cm^{-1}. ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD): Table 2. ESI-MS *m*/*z*: $[M-H]^+$ 211. HR-ESIMS *m*/*z*: $[M-H]^+$ 211.0406 calcd 211.0406 for $C_{10}H_8FO_4$.

3.2.3. Synthesis of 6-fluoro-3-oxo-indan-1-carboxylic acid (*3*)

3-Fluorophenyl succinic acid (**2**) was cyclised to 6-fluoro-3-oxo-indan-1-carboxylic acid (**3**) by Friedel–Crafts acylation reaction using two different methods, one using nitrobenzene and the other using CS_2 as the solvent, to compare the percent yield of 6-fluoro-3-oxo-indan-1-carboxylic acid (**3**). In the Friedel–Crafts acylation reaction using nitrobenzene as a solvent, compound **2** (2.33 g, 0.011 mol) was first converted to acylchloride (**7**) by refluxing it with thionyl chloride (specific gravity 1.631, 2.83 g; 0.024 mol) for 1.5 h in benzene (dry, 25 mL) (Scheme 1). The benzene and excess thionyl chloride were removed in vacuo to obtain 3-fluorophenyl succinyl chloride (**7**) as a liquid. Anhydrous aluminium chloride (8.25 g, 0.06 mol) was poured into the solution of **7** in nitrobenzene (30 mL), and stirred thoroughly for 20 h at room temperature (Scheme 1). The reaction mixture was poured into ice–water mixture (100 mL), and the solid mass thus formed was isolated by evaporation of nitrobenzene with steam and recrystallisation from alcohol–water (30 mL) to give compound 6-fluoro-3-oxo-indan-1-carboxylic acid (**3**) (0.85 g, 40%).

In the Friedel–Crafts acylation reaction using CS_2 as the solvent, compound **2** (2.33 g, 0.011 mol) was converted to acylchloride by refluxing it with thionyl chloride (specific gravity 1.631, 2.830 g; 0.024 mol) for 1.5 h in benzene (dry, 30 mL). The benzene and excess thionyl chloride were removed in vacuo to obtain 3-fluorophenyl succinyl chloride (**7**) as a liquid. Anhydrous aluminium chloride (8.25 g, 0.06 mol) was poured into the solution of **7** in CS_2 (30 mL), and stirred thoroughly for 20 h at room temperature (Scheme 1). The reaction mixture was poured into ice–water mixture (100 mL). The solvent CS_2 was evaporated in a hot water bath (50 °C). After cooling the mixture a precipitate was formed, filtered, washed thoroughly with water and recrystallised from alcohol–water to afford compound 6-fluoro-3-oxo-indan-1-carboxylic acid (**3**) (1.18 g, 55.3%).

White crystalline solid. Mp 162–164 °C. UV (MeOH): 281 nm. IR (KBr): 1680 (>C=O), 1650 (COOH) and 1120 (C–F) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6): Table 2.

Table 2
^1H and ^{13}C NMR data of compounds **1**–**4**

Position	Chemical shifts δ in ppm							
	^1H NMR (coupling constant *J* in Hz)				^{13}C NMR			
	1[a]	**2**[b]	**3**[a]	**4**[b]	**1**[a]	**2**[b]	**3**[a]	**4**[b]
1	—	—	3.94 dd (12.5, 6.5)	3.52 dd (12.0, 8.0)	133.8	141.7	46.4	48.4
2	7.81[c]	7.12 m	2.96 dd (21.5, 12.5), 2.58 dd (21.5, 6.5)	2.54 m, 2.22 m	115.0	115.2	37.2	23.7
3	—	—	—	2.74 m, 2.33 m	161.1	161.2	196.8	29.3
4	7.36 br dt	7.12 m	7.13 d (8.0)	7.11 d (8.0)	120.9	114.3	123.9	124.0
5	7.57 m	7.36 m	7.37 dd (8.0, 2.2)	7.33 dd (8.0, 2.1)	132.4	130.2	112.1	110.9
6	7.79[c]	7.12 m	—	—	128.3	124.4	163.4	163.1
7	8.32 s	2.56 dd (5.0, 17.0)	7.09 d (2.2)	7.04 d (2.1)	154.5	46.5	114.9	112.9
8	—	3.94 dd (5.0, 10.0), 2.96 dd (10.0, 17.0)	—	—	115.2	37.2	141.4	138.4
9	—	—	—	—	107.8	173.6	130.4	132.3
10	—	—	12.44 br s	12.23 br s	162.1	170.3	173.6	174.0
10-C_2H_5	1.38 t (9.0), 4.37 q (9.0)	—	—	—	14.4, 63.4	—	—	—

[a] Spectrum obtained in DMSO-d_6.
[b] Spectrum obtained in CD_3OD.
[c] Overlapped peaks.

CIMS m/z: $[M+NH_3]^+$ 212. HR-ESIMS m/z: $[M+H]^+$ 195.0458 calcd 195.0457 for $C_{10}H_8FO_3$.

*3.2.4. Synthesis of 6-fluoroindan-1-carboxylic acid (**4**)*

6-Fluoroindan-1-carboxylic acid (**4**) was obtained from 6-fluoro-3-oxo-indan-1-carboxylic acid (**3**) by Clemmensen reduction (Scheme 1). Compound **3** (1.94 g, 0.01 mol) was treated with amalgamated zinc (10 g), water (10 mL), concd. HCl (20 mL, 11.65 M) and benzene (30 mL) and refluxed for 16 h. The layers were separated and the aqueous layer was extracted with benzene (20×3=60 mL). The combined benzene layers were washed with water and dried over anhydrous sodium sulfate. After removal of the solvent under reduced pressure, the resulting solid mass was recrystallised from alcohol–water to give 6-fluoroindan-1-carboxylic acid (**4**) (1.36 g, 76%).

White crystalline solid. Mp 128–130 °C. UV (MeOH): 280 nm. IR (KBr): 1650 (COOH) and 1120 (C–F) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6): Table 2. CIMS m/z: $[M+NH_3]^+$ 198. HR-ESIMS m/z: $[M+H]^+$ 181.0665 calcd 181.0665 for $C_{10}H_{10}FO_2$.

3.3. Assessment of analgesic activity

The analgesic activity of compounds **3** and **4**, and the positive controls, aminopyrine (BDH, Germany), indomethacin (BDH, India) and diclofenac Na (BDH, Germany) was studied by acetic acid induced writhing test as described by Vogel and Vogel[8] with little modification.

3.3.1. Animals

Young *Swiss albino* mice aged 4–5 weeks weighed 20–25 g of either sex were used for the assessment of analgesic activity. They were collected from the animal house of the International Center for Diarrheal Diseases and Research' Bangladesh (ICDDR'B), Mohakhali, Dhaka. The mice were kept in groups of six in plastic polyvinyl cages (BIK industries, India) having dimensions of (28×22×13) cm^3. The animals were given standard mice feed delivered by ICDDR'B and water ad libitum. They were kept in the laboratory environment for 7 days maintaining light and dark; were fasted overnight and weighed before the experiment.

3.3.2. Test compounds and positive controls

6-Fluoro-3-oxo-indan-1-carboxlic acid (**3**) and 6-fluoroindan-1-carboxlic acid (**4**) were weighed in 20 mg each and taken into separate graduated test tubes. Compounds were then dissolved in 2 mL of saline solution and a few drops of 0.1 N NaOH in saline. The pH of the solution was adjusted to 7.4±0.2 by drop wise addition of 0.1 N HCl in saline. Then the final volumes of the solutions were adjusted to 10 mL with saline water.

The solutions of the positive controls aminopyrine (BDH, Germany), indomethacin (BDH, India) and diclofenac Na (BDH, Germany) were prepared as follows. Each of these drugs (5 mg) was dissolved separately in 2 mL of saline solution and 2–3 drops of 0.1 N NaOH in saline. The pH of the solution was adjusted to 7.4±0.2 by drop wise addition of 0.1 N HCl in saline. Finally, the volume was adjusted to 6 mL with saline.

3.3.3. Protocol

The mice were randomly divided into eight groups, which consisted of six mice in each group. Groups A and B received the test compound **3**, and groups C and D received the test compound **4**. All test compounds were administered orally with a help of a feeding needle at doses of 25 and 50 mg/kg body weight in the groups, respectively. Mice groups E, F and G received positive controls, aminopyrine 30 mg/kg body weight, diclofenac Na 10 mg/kg body weight and indomethacin 8 mg/kg body weight, respectively. Group H was kept as negative control giving saline solution only. A 40 min interval was allowed to ensure proper absorption of the administered compounds. Then the writhing inducing chemical, acetic acid solution (0.7%, 0.1 mL/10 g), was administered intraperitoneally (ip) to each of the animals of a group. After an interval of 10 min, numbers of writhing were counted for another 10 min. The average percent decrease in writhing was calculated and compared against the control (saline treated) group. Percent inhibition was calculated using the following formula.

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

where, W_c=average writhing counted for control group; W_t=average writhing calculated for individual test group.

Acknowledgements

One of the authors (S.C.B.) is grateful to the Ministry of Science, Information, Communication and Technology, Government of Bangladesh, Dhaka for a grant (No. BTAJOPROMA/Sha-9/B.:ANU.:PRO.:/2007-2008/BS-74/118) to perform some parts of the project. The mass spectral analyses were carried out in the EPSRC National Mass Spectrometry Service Centre, Swansea, and the NMR spectroscopy was performed in the School of Pharmacy, University of London.

References and notes

1. Shen, T. Y. *Angew Chem., Int. Ed. Engl.* **1972**, *11*, 460.
2. Juby, P. F.; Goddwin, W. R.; Hudyma, J. A.; Parttykas, R. A. *J. Med. Chem.* **1972**, *15*, 1297.
3. Mukhopadhya, A.; Lahiri, S. C. *Indian J. Exp. Biol.* **1992**, *30*, 583.
4. Ray, S. M.; Lahiri, S. C. *J. Indian Chem. Soc.* **1990**, *67*, 324.
5. Roy, A.; Gupta, J. K.; Lahiri, S. C. *J. Indian Chem. Soc.* **1983**, *60*, 377.
6. Roy, A.; Lahiri, S. C. *Indian J. Pharmacol* **1985**, *17*, 63.
7. Bachar, S. C.; Lihiri, S. C. *Die Pharmazie* **2004**, *59*, 435.
8. Vogel, H. G.; Vogel, W. H. *Drug Discovery and Evaluation; Pharmacological Assays*; Springer: Berlin, 1997; Vol. 402, 370.
9. Bepary, S.; M. Pharm. Thesis; Department of Pharmacy, University of Dhaka, 1997.
10. Paul, R. K.; M. Pharm. Thesis; Department of Pharmacy, University of Dhaka, 1999.
11. Hasina, Y.; M. Pharm. Thesis; Department of Pharmacy, University of Dhaka, 2000.
12. Roy, A. K.; Ray, A. M.; Gupta, J. K.; Lahiri, S. C. *Indian J. Physiol. Pharmacol.* **1980**, *24*, 369.
13. Mukhopadhyay, A.; Roy, A.; Lahiri, S. C. *J. Indian Chem. Soc.* **1985**, *62*, 690.
14. Collier, H. O.; Kinneen, L. C.; Johnson, C. A.; Schneider, C. *Br. J. Pharmacol.* **1968**, *32*, 295–402.
15. Santos, A. R. S.; Vedana, E. M. A.; Freitas, G. A. G. *Inflammatory Research* **1998**, *47*, 302.
16. Reichert, J. A.; Daughters, R. S.; Rivard, R.; Simone, D. A. *Pain* **2001**, *89*, 221.
17. Ronaldo, A. R.; Mariana, L. V.; Sara, M. T.; Adriana, B. P. P.; Steve, P.; Ferreira, S. H.; Fernando, Q. C. *Eur. J. Pharmacol.* **2000**, *387*, 111.