Total Synthesis and Analgesic Activity of 6-Fluoroindan-1-acetic Acid and its 3-Oxo Derivative

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Abstract: 6-Fluoro-3-oxo-indan-1-acetic acid (**5**) and 6-fluoroindan-1-acetic acid (**6**) were conveniently synthesised from 3-fluorobenzaldehyde in four and five steps, respectively. The structures of these new compounds and two other intermediates, 3-fluorobenzylidine-*bis*-acetoacetate (**2**) and 3-fluoro-β-phenyl glutaric acid (**3**) were elucidated by spectroscopic means, notably, HRMS, 1D and 2D NMR. The analgesic activity of compounds **5** and **6** were assessed by the acetic acid induced writhing in *Swiss albino* mice.

Key Words: 3-fluorobenzylidine-*bis*-acetoacetate, 3-fluoro-β-phenyl glutaric acid, 6-fluoro-3-oxo-indan-1-acetic acid, 6-fluoroindan-1-acetic acid, analgesic activity.

INTRODUCTION

Analgesic and anti-inflammatory drugs that are in use today show considerable variations in potency, incidence of side effects and individual patient responses. Historically, aspirin has been the drug of choice as a general pain-killer because of its better tolerance, low toxicity and low cost. Similarly, ibuprofen is the most commonly used first line analgesic and anti-inflammatory agent as it combines hood efficacy with a low incidence of side effects [1,2]. However, its analgesic efficacies are weaker than those of other non-steroidal anti-inflammatory drugs (NSAIDs). Diclofenac is a popular alternative as a first or second line analgesic. Indomethacin, which apparently has a superior anti-inflammatory property, is associated with a higher incidence of side effects and is often used as a third line agent. Azapropazone produces a similar effect to naproxen, but is associated with a high incidence of gastrointestinal (GI) complications. The most significant adverse effects of NSAIDs are GI bleeding and perforation, which occur in approximately 1% of patients taking NSAIDs for long term [1]. Because of the major limitations of existing NSAIDs it has become necessary to develop more effective and less toxic new analgesic and anti-inflammatory agents.

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Indan derivatives, particularly those with a carboxylic acid functionality, possess anti-inflammatory property [2]. For example, 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 [3]. Recently, significant anti-inflammatory activity among a series of substituted indan-1-carboxylic acids has been reported [4], a number of methoxyindan-1-alkanoic acids have been synthesised with considerable anti-inflammatory properties [5], and indan derivatives with a halo-substituted indanyl group have been found to possess analgesic and anti-inflammatory properties [6-9]. It has been established that aromatic halogen substitution could increase the analgesic and anti-inflammatory potency and widen the margin of safety [9]. As part of our continuing search for new, more effective analgesic and anti-inflammatory agents with little or no side-effects [2, 9], we now report on the total synthesis of 6-fluoroindan-1-acetic acid (**6**) from 3-fluorobenzaldehyde, and the assessment of its analgesic activity along with the intermediate 6-fluoro-3-oxo-indan-1-acetic acid (**5**) using the acetic acid induced writhing in *Swiss albino* mice.

CHEMISTRY AND ANALGESIC ACTIVITY

Synthesis and Spectroscopic Identification of 6-fluoro-3-oxo-indan-1-acetic Acid (5) and 6-fluoro-indan-1-acetic Acid (6)

6-Fluoroindan-1-acetic acid (**6**) was conveniently synthesised from 3-fluorobenzaldehyde (**1**) in five steps with an

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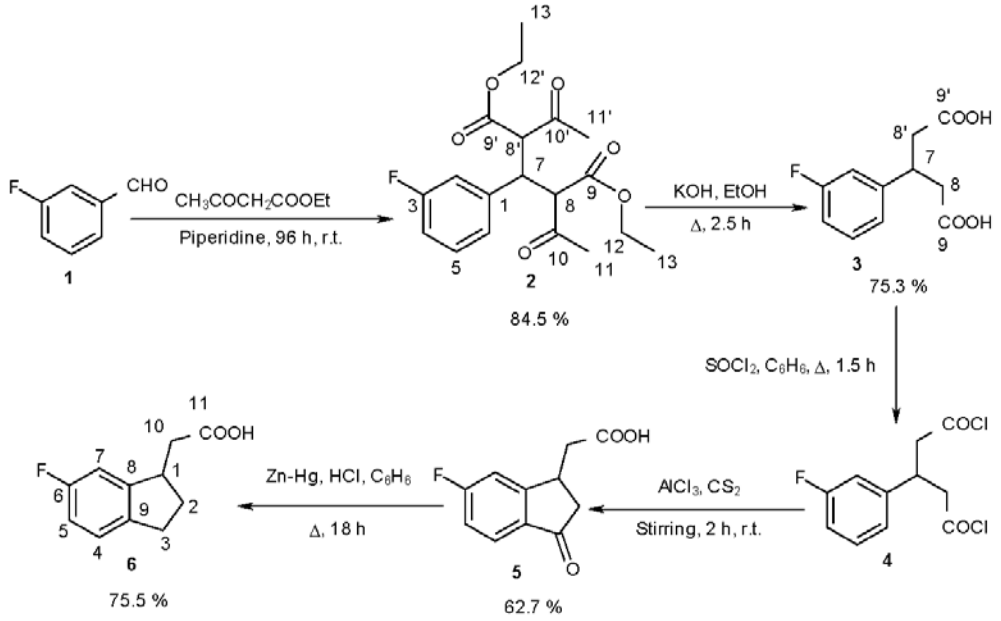
overall 30.1% yield (Scheme **1**). The four intermediates were 3-fluorobenzylidine-*bis*-acetoacetate (**2)**, 3-fluoro-β-phenyl glutaric acid (**3**), 3-fluorophenyl succinyl chloride (**4**) and 6-fluoro-3-oxo-indan-1-acetic acid (**5**). The structures of compounds **2, 3, 5** and **6** were elucidated unambiguously by spectroscopic means, particularly by comprehensive 1D and 2D NMR analyses, e.g. ^{1}H NMR, ^{13}C NMR, ^{13}C DEPT-135, ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC. The UV spectral analyses of **2, 3, 5** and **6** revealed the presence of aromaticity in these molecules. The IR spectra revealed the presence of ester and ketonic carbonyl functionalities and a C-F in **2**; a C-F and a carboxylic acid (COOH) moieties in **3**; a COOH, a ketonic carbonyl and a C-F groups in **5**; and a COOH and a C-F moieties in **6**.

3-Fluorobenzaldehyde (**1**) was condensed with ethylcyanoacetate in the presence of piperidine in 1:2 molar ratio using the Knoevenagel reaction [10] to produce 3-fluorobenzylidine-*bis*-acetoacetate (**2)** with an excellent yield of 84.5% (Scheme **1**). A CI-MS spectrum of **2** revealed the *pseudo*molecular ion $[M+NH_4]^+$ at *m/z* 384, and an HR-EIMS showed the molecular ion $[M]^+$ peak at 366.1479, corresponding to the molecular formula $C_{19}H_{23}FO_6$. In the ^{1}H NMR spectrum of **2** (Table **1**), there were signals for four aromatic methines (δ 7.04, 7.11, 7.17 and 7.30) corresponding to a 1, 3-di-substituted benzene ring system, three other methines (δ 3.02-3.94), two methyls (δ 2.88 / 2.90) assignable to two Me-CO- functionalities, and two ethoxy groups (δ 3.84 / 3.80 and 0.85 / 0.95) representing two -COOEt substructure. The ^{13}C NMR (Table **1**) displayed signals corresponding to 19 carbons including four aromatic methines (δ 114.2, 115.10, 124.1 and 130.8), two aromatic quaternary (δ 139.9 and 161.1), three methines (δ 38.0, 42.6 and 43.0), two ketonic carbonyl carbon (δ199.7 and 199.9), two ester carbonyl (δ169.8 and 169.9), two oxymethylene (δ64.3 and 64.4) and four methyl carbons (δ14.4, 14.4, 14.9 and 15.2). The ^{1}H and ^{13}C NMR spectra confirmed the identity of **2** as 3-fluorobenzylidine-*bis*-acetoacetate.

Compound **2** was alkali hydrolysed to 3-fluoro-β-phenyl glutaric acid (**3**; yield 75.3%) (Scheme **1**). A CI-MS spectrum of **3** revealed the *pseudo*molecular ion $[M+NH_4]^+$ at *m/z* 244, and an HR-EIMS showed the molecular ion $[M]^+$ peak at 226.0642, corresponding to the molecular formula $C_{11}H_{11}FO_4$. In the ^{1}H and ^{13}C NMR spectra (Table **1)**, in addition to the signals corresponding to a 1,3-disubstituted benzene ring as in **2**, there were signals for a methine (δ_H 3.02-3.94, δ_C 36.3), two methylene (δ_H 3.90, 2.88 and 3.94, 2.90, δ_C 37.2 and 37.3), and two carboxylic acid functionalities (δ_C 172.2 and 170.0). All these signals together with 2D NMR data analyses confirmed the identity of compound **3** as 3-fluoro-β-phenyl glutaric acid.

The compound **3** was cyclized to 3-fluoro-3-oxo-indan-acetic acid (**5**; 62.7 %) *via* the formation of 3-fluoro-β-phenyl glutaryl chloride (**4**), a moisture sensitive liquid material (Scheme **1**). Clemmensen reduction of **5** resulted in 6-fluoroindan-1-acetic acid (**6**; 75.5 %). A CI-MS spectrum of **5** revealed the $[M+NH_4]^+$ ion at *m/z* 226, and an HR-EIMS spectrum displayed $[M]^+$ ion at *m/z* 208.0536 confirming the molecular formula $C_{11}H_9FO_3$. In the ^{1}H NMR spectrum of **5** (Table **1**), there were signals corresponding to a 1,3,6-trisubstituted benzene ring (δ 7.07, 7.13 and 7.37), a highly deshielded signal (δ 12.41) for -OH of a carboxylic acid group, a methine (δ 3.04) and two methylene (δ 2.20 and 2.44; 3.92) groups. The ^{13}C NMR (Table **1**) exhibited signals for 11 carbons including three aromatic methines (δ 112.2, 114.9 and 123.9), three aromatic quaternary (δ 130.4, 141.3 and 163.5), a methine (δ 40.4), two methylene (δ 37.2 and 38.9), and acid carbonyl (δ70.0) and a ketonic carbonyl carbon (δ196.9). Thus the identity of the new compound **5** was confirmed as 6-fluoro-3-oxo-indan-1-acetic acid.

A CI-MS spectrum of **6** displayed the *pseudo*molecular ion $[M+NH_4]^+$ at *m/z* 212, and an HR-EIMS displayed the molecular ion $[M]^+$ at *m/z* 194.0743 corresponding to the molecular formula $C_{11}H_{11}FO_2$. In the ^{1}H and ^{13}C NMR spectra of **6** (Table **1**) were similar to those of **5** with the excep-



Scheme 1. Synthesis of compounds **2**-**6**.

Table 1. ^{1}H and ^{13}C NMR Data of Compounds 2, 3, 5 and 6

Position	Chemical Shifts δ in ppm							
	^{1}H NMR (Coupling Constant *J* in Hz)				^{13}C NMR			
	2	**3**	**5**	**6**	**2**	**3**	**5**	**6**
1	-	-	3.04 m	3.00 m	139.9	140.2	40.4	40.6
2	7.04 br d (2.0)	7.01 br d (2.0)	2.88 dd (21.5, 12.5) 2.42 dd (21.5, 6.5)	2.44 m 2.20 m	115.1	115.1	37.2	23.7
3	-	-	-	2.68 m 2.31 m	161.1	161.9	196.9	29.3
4	7.11 dd (8.0, 2.0)	7.12 dd (8.0, 2.0)	7.13 d (8.0)	7.10 d (8.0)	114.2	114.0	123.9	124.0
5	7.30 dd (8.0)	7.32 dd (8.0)	7.37 dd (8.0, 2.2)	7.33 dd (8.0, 2.1)	130.8	130.8	112.0	110.9
6	7.17 dd (8.0, 2.0)	7.16 dd (8.0, 2.0)	-	-	124.1	124.1	163.5	163.1
7	3.02-3.94* s	2.46 m	7.07 d (2.2)	7.04 d (2.1)	38.0	36.3	114.9	112.9
8	3.02-3.94* s	3.90 m 2.88 m	-	-	42.6	37.2	141.3	138.4
8’	3.02-3.94* s	3.94 m 2.90 m	-	-	43.0	37.3	-	-
9	3.02-3.94* s	-	-	-	169.9	172.0	130.4	132.3
9’	3.02-3.94* s	-	-	-	169.8	172.2	-	-
10	-	-	3.92 d (6.8)	3.91 d (6.8)	199.9	-	38.9	39.0
10’	-	-	-	-	199.7		-	-
11	2.88 s	-	12.41 br s	12.41 br s	15.2	-	170.0	171.1
11’	2.90 s	-	-	-	14.9	-	-	-
12	3.84 q (7.0)	-	-	-	64.4	-	-	-
12’	3.80 q (7.0	-	-	-	64.3	-	-	-
13	0.85 t (7.0)	-	-	-	14.4	-	-	-
13’	0.95 t (7.0)	-	-	-	14.4	-	-	-

Spectra obtained in DMSO-d_6

*Overlapped peaks.

tions that there were signals for an additional methylene group (δ_H 2.31 and 2.68, δ_C 29.3), and there were no signal for any ketone carbonyl carbon. All these signals together with 2D NMR data analyses confirmed the identity of the new compound **6** as 6-fluoroindan-1-acetic acid.

Analgesic Activity of 6-fluoro-3-oxo-indan-1-acetic Acid (5) and 6-fluoro-indan-1-acetic Acid (6)

The analgesic activity of compounds **5** and **6** were assessed by the acetic acid induced writhing in *Swiss albino* mice [2, 11], because the analgesic and the anti-inflammatory activity of various indan-1-acids and tetrazoles were reported previously [5, 12-15]. The results of the current study showed that the writhing induced by acetic acid was significantly reduced by the test compounds in a dose dependent manner (Table **2**). While 6-fluoro-3-oxo-indan-1-acetic acid (**5**) showed 23.9 and 32.3% ($p < 0.0005$) inhibitions, respectively, at the doses of 25 and 50 mg/kg-body weight, 6-fluoroindan-1-acetic acid (**6**) exhibited 37.7 and 46.7% ($p < 0.0005$) inhibitions, respectively, at the doses of 25 and 50 mg/kg-body weight. The analgesic activity of **5** and **6** were comparable to those of the positive controls, e.g. aminopyrine with 47.9% ($p < 0.00005$) 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 62.9% ($p < 0.0005$) inhibition at 10 mg/kg body weight. None of the test compounds (**5** and **6**) 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.

Table 2. Analgesic Activity of 6-fluoro-3-oxo-indan-1-acetic Acid (5) and 6-fluoroindal-1-acetic Acid (6)

Test Compounds/Controls	Group	Dose (mg/kg body weight)	Total Number of Writhing	Mean± SD	% Inhibition
6-Fluoro-3-oxo-indan-1-acetic acid (**5**)	A	25	27, 17, 24, 18, 19, 22	21.17±3.53	23.93[a]
	B	50	22, 14, 15, 18, 24, 20	18.83±3.57	32.34[a]
6-Fluoroindal-1-acetic acid (**6**)	C	25	21, 15, 13, 24, 12, 19	17.33±4.35	37.73[a]
	D	50	14, 10, 23, 16, 16, 10	14.83±4.41	46.71[a]
Aminopyrine	E	30	17, 08, 19, 20, 14, 09	14.50±4.64	47.89[a]
Indomethacin	F	8	12, 14, 18, 09, 13, 20	14.33±3.68	48.50[a]
Diclofenac Na	G	10	07, 14, 14, 05, 10, 12	10.33±3.39	62.88[a]
Saline	H	-	32, 30, 28, 25, 28, 24	27.83±2.73	-

[a] Probability values (calculated as compared to control using student's t-test): < 0.0005.

All values are means of six mice.

It is noteworthy that the absence of the ketonic carbonyl functionality at C-3 in **6** increased the cyclopentane ring flexibility, and contributed to the increased analgesic activity of **6** measured by % inhibition of induced writhing in mice. This phenomenon was previously observed with two similar compounds, 6-fluoro-3-oxo-indan-1-carboxylic acid and 6-fluoroindan-1-carboxylic acid [2]. It could also be noted that an increase in carbon number, i.e. acetic acid (-CH_2COOH as in **5** and **6**) instead of a carboxylic acid (-COOH) [2] functionality, appeared to have increased the analgesic potency of such compounds.

The writhing reflex in mice induced by acetic acid is a sensitive procedure to assess the potential analgesic property of drugs. It has been suggested that acetic acid acts by releasing endogenous mediators which stimulate the nociceptive neurons in mice [16]. Acetic acid is sensitive to cyclooxygenase inhibitors and has been used to study the effect of analgesic agents that primarily inhibit the cyclooxygenase involved in prostaglandin synthesis. Acetic acid is also sensitive to NSAIDs and to narcotics and other centrally acting drugs [2, 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]. In the light of this report [17], it is reasonable to assume that the antinociceptive action showed by the compounds **5** and **6** in the acetic acid induced writhing test was probably due to inhibition of the release of TNF-α, interleukin-1β, and interleukin-8, by resident peritoneal macrophages and mast cells.

EXPERIMENTAL SECTION

3.1. General

The chemicals and solvents used in various reactions were purchased from Merck (Germany); BDH **(**India), or SD Fine Chemicals (India). The melting points were determined by using Adco Melting Point Apparatus and were uncorrected. Thin-layer chromatography was performed using 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 DMSO-d_6 on a Bruker AVANCE 500 MHz NMR Spectrometer (500 MHz for ^{1}H 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 optimized for a long range $J_{H\text{-}C}$ of 9 Hz and NOESY experiment was carried out with a mixing time of 0.4 s.

3.2. Synthesis of Compounds 2-6

3.2.1. Synthesis of 3-fluorobenzylidine-bis-acetoacetate (2)

3-Fluorobenzylidine-*bis*-acetoacetate (**2**) was prepared from 3-fluorobenzaldehyde (**1**) (17.3 g; 0.18 mol) by condensation with ethyl acetoacetate (46.8 g; 0.36 mole) in the presence of piperidine (3.5 mL) in anhydrous condition for 96 h at r.t. following the Knoevenagel reaction [10] (Scheme **1**). On completion of the reaction a solid mass was obtained, which was crushed in a mortar and pestle, washed with ether to remove piperidine and filtered. The resulting solid was recrystallised from acetone-water as compound 3-fluorobenzylidine-*bis*-acetoacetate (**2**) (55.67 g; mp. 154-156^o; yield 84.5%).

Crystalline solid, mp. 154-156 ^{o}C. UV (MeOH) λ_{max} in nm: 278. IR (KBr) ν_{max} in cm^{-1}: 1730 (COOEt), 1620 (COMe) and 1115 (C-F). ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6): Table **1**. CI-MS *m/z*: $[M+NH_4]^+$ 384. HR-EIMS *m/z:* $[M]^+$ 366.1478 calcd. 366.1479 for $C_{19}H_{23}FO_6$.

3.2.2. Synthesis of 3-fluoro-β-phenyl Glutaric Acid (3)

Compound **2** (54.9 g, 0.15 mol) was hydrolyzed in presence of 25% alcoholic solution of KOH (50 gm) by refluxing for 2.5 h (Scheme **1**). The alcohol was then distilled off under reduced pressure, diluted with water, washed with chloroform and neutralised by conc. HCl in cold condition with constant stirring. The precipitation thus formed was filtered and recrystallized from alcohol-water to afford 3-fluoro-β-phenyl glutaric acid (**3**) as a crystalline solid (25.53 g; mp. 124-126^o; yield 75.3%).

Crystalline solid, mp. 124-126 °C. UV (MeOH) λ_{max} in nm: 280. IR (KBr) ν_{max} in cm^{-1}: 1690 (COOH) and 1115 (C-F). ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6): Table **1**. CI-MS *m/z*: $[M+NH_4]^+$ 244. HR-EIMS *m/z:* $[M]^+$ 226.0642 calcd. 226.0641 for $C_{11}H_{11}FO_4$.

3.2.3. Synthesis of 3-fluorophenyl Succinyl Chloride (4)

Compound **3** (22.6 g; 0.1 mol) was converted to acyl-chloride (**4**) by refluxing it with thionyl chloride (sp. gr. 1.631; 23.81 g; 0.2 mol) in benzene (dry, 100 mL) as solvent for 1.5 h. The benzene and excess thionyl chloride were removed *in vaccuo* to obtain 3-fluoro-β-phenyl gluteryl chloride **(4)** as a liquid which was used for the next step without further purification or spectroscopic identification (Scheme **1**).

3.2.4. Synthesis of 6-fluoro-3-oxo-indan-1-acetic Acid (5)

Anhydrous aluminium chloride (40.0 g, 0.3 mol) was added portion-wise to the liquid (**4**) in a well stirred condition using CS_2 (100 mL) as a solvent. The reaction mixture was stirred for 2 h at r.t. (Scheme **1**), and then decomposed in ice-water mixture (300 mL). The solvent CS_2 was evaporated in hot water bath. After cooling off the mixture, the precipitates were filtered, washed thoroughly with water and recrystallised from alcohol-water to yield 6-fluoro-3-oxo-indan-1-acetic acid (**5**) (13.0 g; mp. 148-150°; yield 62.68%).

Crystalline solid, mp. 148-150 °C. UV (MeOH) λ_{max} in nm: 280. IR (KBr) ν_{max} in cm^{-1}: 1680 (C=O), 1650 (COOH) and 1115 (C-F). ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6): Table **1**. CI-MS *m/z*: $[M+NH_4]^+$ 226. HR-EIMS *m/z:* $[M]^+$ 208.0534 calcd. 208.0536 for $C_{11}H_9FO_3$.

3.2.5. Synthesis of 6-fluoroindan-1-acetic Acid (6)

6-Fluoroindan-1-acetic acid (**6**) was obtained from 6-fluoro-3-oxo-indan-1-acetic acid (**5**) by Clemmensen reduction (Scheme **1**). Compound **5** (10.4 g; 0.05 mol) was treated with amalgamated zinc (50 g), water (50 mL), conc. HCl (40 mL) and immiscible solvent benzene (60 mL) by refluxing in a water bath till (18 h) the reaction mixture gave no keto test. The reduced product was separated in benzene layer and the aqueous layer was extracted with benzene (20×3 = 60 mL) and combined with the benzene layer, which was washed with water and dried over anhydrous sodium sulphate. After removal of the solvent *in vaccuo*, a brownish oily liquid was obtained. Compound **6** (7.3 g; mp 48-50°; yield 75.5%) was obtained as a crystalline solid (from alcohol water) from this oily liquid.

Crystalline solid, mp. 48-50 °C. UV (MeOH) λ_{max} in nm: 280. IR (KBr) ν_{max} in cm^{-1}: 1650 (COOH) and 1115 (C-F). ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6): Table **1**. CI-MS *m/z*: $[M+NH_4]^+$ 212. HR-EIMS *m/z:* $[M]^+$ 194.0742 calcd. 194.0743 for $C_{11}H_{11}FO_2$.

3.3. Assessment of analgesic activity

The analgesic activity of the compounds **5** and **6**, 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 [11] 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 6 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 seven 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-acetic acid (**5**) and 6-fluoro-indan-1-acetic acid (**6**) were weighed in 20 mg each and taken into separate graduated test tubes. The compounds were then dissolved in 2 mL of saline solution and a few drops of 0.1N NaOH in saline. The pH of the solution was adjusted to 7.4±0.2 by drop wise addition of 0.1N 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 0.1N NaOH in saline. The pH of the solution was adjusted to 7.4±0.2 by drop wise addition of 0.1N 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 6 mice in each group. Groups A and B received the test compound **5,** and groups C and D received the test compound **6**. 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 forty minutes 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 (i.p.) to each of the animals of a group. After an interval of ten minutes 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 (SCB) is grateful to the Ministry of Science, Information, Communication and Technology,

Government of Bangladesh, Dhaka for a grant (No. BTAJO-PROMA/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.

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Received: 27 April, 2009 **Revised: 01 July, 2009** **Accepted: 01 July, 2009**

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