Indian Journal of Chemistry
Vol. 48B, February 2009, pp. 267-272

Synthesis, analgesic and anti-inflammatory activities of bis(indolyl)methanes

Kuppusamy Sujatha[a], Paramasivan T. Perumal[b,*], Doraisamy Muralidharan[b] & Melani Rajendran[c]

[a]Sri Ramachandra College of Pharmacy,
Sri Ramachandra University, Porur, Chennai 600 116, India
[b]Organic Chemistry Division, Central Leather Research Institute, Adyar, Chennai 600 020, India.
E-mail: ptperumal@hotmail.com
[c]Department of Anatomy, Sri Ramachandra University, Porur, Chennai 600 116, India

Received 30 April 2008; accepted (revised) 7 November 2008

A series of bis(indolyl)methanes have been synthesized by stirring a mixture of indole and aldehydes in methanol:water (1:1 v/v) containing catalytic amount of sodium bisulphite at RT. Acute toxicity, analgesic, anti-inflammatory and ulcerogenic activities of the prepared bis(indolyl)methanes are evaluated *in vivo* in comparison to standard drugs (ibuprofen and indomethacin). In acute toxicity study, no mortality is observed in the tested compounds and compound **3c** is found to be safe. All the tested compounds show significant analgesic and anti-inflammatory activity without an ulcerogenic activity. The most active compounds of this series are **3c** and **3d**.

Keywords: Bis(indolyl)methanes, acute toxicity, analgesic, anti-inflammatory, ulcerogenic activity

Inflammation is a response of the tissue to an infection, irritation or foreign substance[1] and is a part of the host defense mechanism. The inflammatory process involves a series of events that can be elicited by numerous stimuli (e.g. infectious agents, ischemia, antigen-antibody interaction and thermal or other physical injuries) [2]. Almost two decades ago, steroids namely prednisolone, dexamethasone, betamethasone, etc. were considered to be the choicest anti-inflammatory drugs. Owing to the several adverse effects caused by either short-term or long-term steroid therapy, these have been more or less replaced by much safer and better-tolerated non-steroidal anti-inflammatory drugs (NSAID). The seriousness and enormous after effects of steroid therapy necessitated an accelerated research towards the development of NSAIDs since the past two decades[3,4]. NSAIDs have been highly useful for treating acute, self-limited inflammatory conditions. The development of NSAIDs has helped in understanding the tissue mechanism of inflammation.

Indoles have been widely identified as a privileged structure or pharmacophore, with representation in over 3000 natural isolates[5] and are known to possess broad spectrum of biological and pharmaceutical activities[6,7]. Indomethacin[8], tenidap[9] are indole derivatives, which were found to possess anti-inflammatory activity with analgesic and antipyretic properties. They inhibit production of eicosanoids like prostacyclin, thromboxanes and prostaglandins by inhibition of cyclo oxygenase (cox) and thereby reduce oedema.

It is generally agreed that the NSAIDs and analgesic drugs available in the market (aspirin, phenylbutazone, oxyphenbutazone, indomethacin, tenidap, ibuprofen and ketoprofen) are highly acidic in nature and suffer from a common drawback of gastro-intestinal toxicity[4]. The search for new therapeutic agents with high margin of safety and freedom from normally associated G.I toxic effects such as ulceration, hemorrhage and perforation has been a priority of pharmacologists and pharmaceutical industries[10]. In particular, bis (indolyl)methanes (BIM) have received much attention in recent years[11]. Such compounds are prone to develop interesting bioactivity and find useful applications as breast cancer preventative[12] and anti-bacterial agents[13]. BIM is the most active cruciferous substance for promoting beneficial estrogen metabolism in women and men[14]. Hong *et al.*[15] and Kedmi *et al.*[16] reported recently the potential beneficial effects of 3,3-biiindolyl methanes on the proliferation and induction of apoptosis in human prostate and breast cancer cells. In the light of these observations, BIM was synthesized with the hope to develop safer and potent analgesic and anti-inflammatory agents without ulcerogenic activity.

Results and Discussion

Chemistry

The synthetic pathway employed in the preparation of BIMs is outlined in **Scheme I**. BIMs were prepared readily by the reaction of substituted aldehydes with indole catalyzed by sodium bisulphite. After completion of the reaction, the reaction-mixture was worked-up and column chromatographed to get pure products **3a-h** in good yields. The structures of the compounds **3a-h** were confirmed by spectral, elemental analyses and comparison with the available literature data[17].

Biological Evaluation

Acute toxicity

The test compounds **3a-h** were tested in mice (500 mg/kg, 1000 mg/kg, and 2000 mg/kg,p.o.) after 14 days of administration for their safety as per OECD guidelines[18]. The compounds did not produce any significant changes in the body weight, food, water intake and other behavioural patterns. There was also no change in the haematological parameters and organ weights of treated animals compared to control. No mortality was observed in the control and compound treated groups. Histopathological examination of organs *viz*. heart, liver, brain, lung, kidney, spleen, stomach, testis and ovary in animals treated with compounds **3c** and **3d** were studied.

Results of the histopathological studies revealed that compound **3c** (500 mg/kg, 1000 mg/kg, 2000 mg/kg), compound **3d** (500 mg/kg) and control did not show any pathological changes. Compound **3d** at a dose of 1000 mg/kg, p.o. produced mild swelling and narrowing of sinusoid in liver, proliferation of epithelial cells lining the tubules, congestion of interstitial vessels and glomeruli in kidney. Compound **3d** at 2000 mg/kg dose level induced congestion and hyperemia of glomeruli, few tubules showing extra cytoplasmic inclusion in varying sizes in kidney and multifocal necrosis in liver. The other vital organs examined did not show any changes.

Analgesic activity

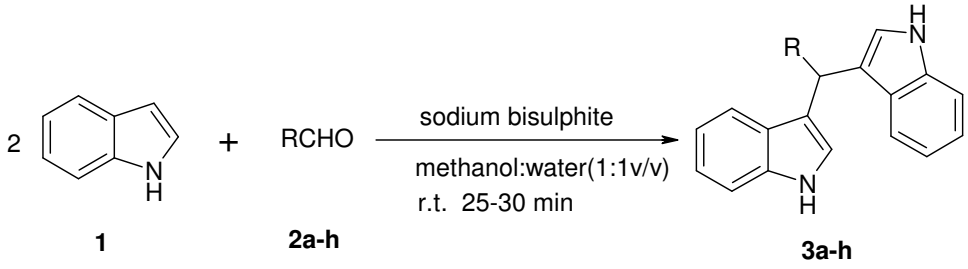
The analgesic activity of the prepared BIMs was determined by the tail immersion method[19, 20]. In this investigation, it has been observed that all the tested BIMs show significant analgesic activity without ulcerogenic liability. Among these, compounds **3b**, **3g** and **3h** (284.4 -306% analgesic activity) showed enhanced activity and comparable to those of ibuprofen, which is used as a reference standard (309% analgesic activity). Compounds **3c** and **3d** (310.5 and 414% activity) were found to be more potent than the standard drug ibuprofen while compounds **3a**, **3e** and **3f** gave moderate results (**Table I** and **Figure 1**).

Anti-inflammatory activity

The anti-inflammatory activity of the prepared bis(indolyl)methanes was determined by the carrageenan induced paw oedema standard method in rats[22]. In this investigation, it has been observed that all the tested BIMs show significant anti-inflammatory activity without ulcerogenic liability. From the obtained data (**Table II** and **Figure 2**), compounds having 2-nitrophenyl **3c**, 3-methoxy 4-hydroxyphenyl **3d** and 3-nitrophenyl substitution **3h** revealed enhanced anti-inflammatory activity. Compounds **3a, 3b**, **3e**, **3f** and **3g** showed moderate activity and the substituent groups have no marked effect on the anti-inflammatory activity.

Ulcerogenic activity

The ulcerogenic liability of the prepared compounds **3a**, **3b**, **3e-h** were determined in Wistar rats following the previously reported standard



3a) R= Hydrogen
3b) R= 4-methylphenyl
3c) R= 2-nitrophenyl
3d) R= 3-methoxy4-hydroxyphenyl
3e) R= 4-chlorophenyl
3f) R= 4-methoxyphenyl
3g) R= phenyl
3h) R= 3-nitrophenyl

Scheme I

Table I—Analgesic activity of BIMs

Compd	Mean Tail withdrawal latencies (sec)± SE					% Analgesic activity				
	0 min	30 min	1 hr	2 hr	3 hr	0 min	30 min	1 hr	2 hr	3 hr
Control	2.4±0.055*	2.38±0.071*	2.3±0.114*	2.4±0.1414*	2.4±0.055*	-	-	-	-	-
3a	3.16±0.07*	5.22 ±0.06*	6.90±0.084*	6.96 ± 0.06*	3.16±0.07*	165.19	218.34	220.25	227.84	165.19
3b	2.34±0.0341*	5.52 ±0.09*	5.54 ±0.14*	6.22 ±0.06*	2.34±0.0341*	235.9	236.75	265.81	305.98	235.9
3c	2.28 ±0.06*	5.36±0.04*	6.36±0.051*	6.74±0.051*	2.28 ± 0.06*	235.09	278.95	295.61	310.52	235.09
3d	2.0 ±0.10*	3.8 ±0.114*	6.0±0.23*	8.36 ± 0.11*	2.0 ±0.10*	190.0	300.0	418.0	414.0	190.0
3e	3.08 ±0.04*	5.58±0.04*	7.20±0.071*	7.38±0.021*	3.08 ± 0.04*	181.16	233.77	239.61	243.50	181.16
3f	3.2±0.071*	5.22 ±0.06*	7.30±0.045*	7.18±0.0735*	3.2 ± 0.071*	163.12	228.12	224.37	233.12	163.12
3g	2.14±0.065*	5.16±0.068*	5.52±0.086*	6.22 ± 0.06*	2.14 ± 0.065*	241.12	257.94	290.65	303.74	241.12
3h	2.3 ±0.071*	6.16±0.087*	7.0 ±0.071*	6.48 ± 0.04*	2.3 ± 0.071*	267.82	304.35	281.74	284.35	267.82
Ibuprofen (standard)	2.44±0.07*	4.56 ±0.04*	5.46±0.0245*	7.26 ± 0.051*	2.44 ± 0.07*	186.88	223.77	297.54	309.02	186.88

Statistical analysis was carried out by using one-way Anova (F-test) followed by Dunnett's t test.
*Significantly different from the control value at $p<0.001$.

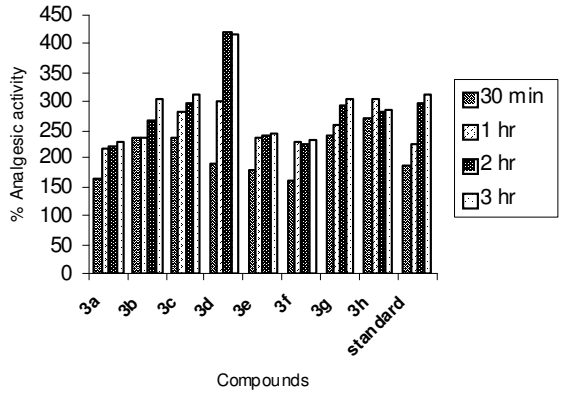


Figure 1 — Analgesic activity of BIMs

method[22]. Histopathological examination of stomach did not reveal presence of any ulcer. The histopathological examination of stomachs of mice treated with compounds **3c** and **3d** did not show any pathological changes in acute toxicity study. Thus besides having the analgesic and anti-inflammatory activity, the compounds were devoid of ulcerogenicity.

Experimental Section

Materials

Melting points were measured in capillary tubes and are uncorrected. Infrared spectra were recorded as solids in KBr pellets on a Perkin-Elmer FTIR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with JEOL 500 MHz high-resolution NMR spectrometer with TMS as internal standard. Mass spectra were obtained using JEOL DX-303 in EI ionization mode at 70 eV. The elemental analyses of the compounds were recorded using ThermoFinnigan FLASH EA 1112 CHNS analyzer.

Typical procedure for the synthesis of bis-indolylmethanes

To a mixture of indoles (2.5 mmole), substituted aldehydes (1.25 mmole) in methanol:water (1:1), sodium bisulphite (20 mole %) was added and stirred at RT for appropriate time. When the reaction was complete, it was extracted thrice with dichloromethane (10 mL). The combined organic layers were dried using anhydrous sodium sulphate, filtered and the solvent evaporated. The crude products were purified by column chromatography over silica gel (ethyl acetate:petroleum ether, 4:6 v/v).

Pharmacology

For acute toxicity and analgesic activity, healthy adult swiss albino mice of either sex weighing 25-30 g were used. For anti-inflammatory and ulcerogenic activity, healthy adult wistar rats of either sex weighing between 150-200 g were used. All the animals were maintained as per the norms of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) and the study protocols were approved by CPCSEA and Institutional Animal Ethics Committee constituted for the purpose.

Acute toxicity study

The study was in accordance with the OECD guidelines[18]. Group of five mice each wase administered with single oral doses of the control (1% CMC) and the test compounds **3a-h** at 500 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight dose levels respectively. Animals were fasted overnight prior to

Table II — Anti-inflammatory activity of the bis(indolyl)methanes

Compd	Mean values (± SE) of oedema Volume at different intervals					Percentage of anti-inflammatory activity at different intervals				
	60 min	120 min	180 min	240 min	300 min	60 min	120 min	180 min	240 min	300 min
Control	1.634± 0.003*	1.874± 0.022*	1.83± 0.005*	1.77± 0.009*	1.74± 0.007*	-	-	-	-	-
3a	1.264± 0.019*	1.198± 0.02*	1.156± 0.005*	1.11± 0.01*	1.03± 0.007*	22.64	36.07	36.97	37.29	40.80
3b	1.296± 0.04*	1.192± 0.013*	1.126± 0.012*	1.048± 0.014*	0.924± 0.003*	20.68	36.39	38.60	40.79	46.89
3c	1.202± 0.03*	1.184± 0.023*	0.956± 0.022*	0.896± 0.013*	0.832± 0.008*	26.44	36.82	47.87	49.38	52.18
3d	1.196± 0.008*	1.158± 0.019*	0.95± 0.017*	0.894± 0.01*	0.82± 0.008*	26.80	38.21	48.20	49.49	52.87
3e	1.328± 0.03*	1.344± 0.026*	1.092± 0.031*	1.01± 0.009*	0.936± 0.005*	18.73	28.28	40.46	42.94	46.21
3f	1.30± 0.01*	1.228± 0.032*	1.152± 0.005*	1.122± 0.01*	1.038± 0.007*	20.44	34.47	37.19	36.61	40.34
3g	1.43± 0.013*	1.206± 0.085*	1.186± 0.009*	1.056± 0.012*	0.966± 0.01*	12.48	36.65	35.33	40.34	44.48
3h	1.348± 0.024*	1.138± 0.019*	0.936± 0.016*	0.904± 0.014*	0.846± 0.014*	17.50	39.27	48.96	48.92	51.38
Indomethacin (standard)	1.14± 0.017*	1.005± 0.023*	0.744± 0.012*	0.4462± 0.023*	0.406± 0.002*	30.23	43.85	59.43	74.79	76.66

Statistical analysis was carried out by using one-way Anova (F-test) followed by Dunnett's t test.
*Significantly different from the control value at $p<0.001$.

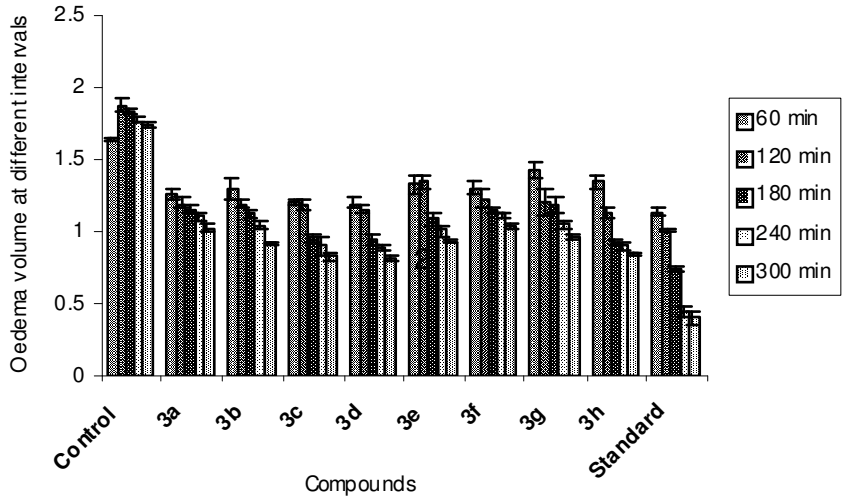


Figure 2 — Anti-inflammatory activity of BIMs

drug administration and weights of the animals were noted. After the administration of test compounds food was withheld for a further 3-4 hr. The animals were observed for a period of 14 days. Cage side observations included, changes in skin and fur, eyes and mucous membrane (nasal), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, defecation), circulatory, central nervous system (ptosis, drowsiness, gait, tremors and convulsion) and behavioural pattern were studied. At the end of the test, surviving animals were weighed and necropsies of animals treated with compounds **3c** and **3d** were

carried out and all gross pathological changes were recorded. Histopathological examination of organs showing evidence of gross pathology in animals surviving at the end of experiment was performed.

Analgesic activity

The analgesic activity of the prepared BIMs was determined by the standard tail immersion method in mouse[19,20]. Albino mice of either sex weighing 25-30 g were divided into 10 groups of five animals each. Mice were placed in individual, conical paper bags, fashioned from a square of stiff paper, folded and stapled to form a cone or pyramid. The animal tail projected from one side. At intervals, the mouse was held so that its tail was totally immersed in a bath at the temperature of 55°C. The time until the typical reaction- a violent jerk of the tail was recorded and noted as the basal reaction time. Normally, a mouse withdraws its tail within 2-4 sec. Any animal failing to respond within 2-4 sec was rejected. The animals were administered p.o with the control (1% sodium carboxy methyl cellulose), test compounds **3a-h** and ibuprofen (standard) at a dose of 100 mg/kg as an aqueous suspension in 1% sodium carboxy methyl cellulose. Time taken for tail withdrawal response was recorded at 30 min, 1 hr, 2 hr and 3 hr after administering the compounds. The cut off time was fixed at 15 sec to prevent injury to the tail. Increase in reaction time (interval for withdrawal of tail by the animal) was considered as an analgesic activity. The percentage analgesic activity was calculated by using the formula, % analgesic activity = $(T_2/T_1)*100$ where T_1 is reaction time before treatment and T_2 is reaction time after treatment. The significant difference between groups was tested by one-way ANOVA followed by Dunnett's t test.

Anti-inflammatory activity

The anti-inflammatory activity was evaluated using carrageenan induced rat hind paw oedema method [21]. The animals fasted for 24 hr were divided into control, standard and test groups each consisting of five rats. The first groups of rats were treated with sodium carboxy methyl cellulose (1% w/v) suspension (control), second group was administered per orally with a dose of 10 mg/kg of indomethacin and the third group was treated with 100 mg/kg of suspension of the test compounds. After 30 min, the animals were injected subcutaneously with 0.1 mL of freshly prepared suspension of carrageenan solution (1% in 0.9% saline) into the sub plantar region of the right hind paw of rats. The volume of hind paw was measured using a Digital plethysmometer LE 7500 (Spain) both in control and in animals treated with standard and test compounds at 60,120, 180, 240 and 300 min after carrageenan challenge. The initial paw volume was measured within 30 sec of the injection. The anti-inflammatory activity was expressed as a percentage inhibition of the inflammation in treated animals in comparison with the control group[23]. Percentage inhibition of oedema = (1-Vt/Vc) 100 where Vc and Vt are the mean relative changes in the volume of paw oedema in the control and drug treated groups respectively. The significant difference between groups were statistically analysed by one-way ANOVA followed by Dunnett's t test.

Ulcerogenic liability

The ulcerogenic liability was determined by the method of Djahanguiri[23]. The animals of group 3 treated with test compounds except compound **3c** and **3d** were sacrificed 8 hr after dosing and the stomach is removed and opened along the greater curvature and washed with 0.9% saline and microscopically examined to assess for severity of histopathological changes if any. The severity of ulcers will be assigned according to the arbitrary scoring system and the ulcer index was calculated[24].

Conclusion

We have described the synthesis, acute toxicity, analgesic, anti-inflammatory and ulcerogenic activity of bis(indolyl)methanes in mice and rats. In acute toxicity study, no mortality was observed in the compound treated groups **3a-h** at 500, 1000 and 2000 mg/kg dose levels. Histopathological examination of vital organs of mice treated with compound **3c** did not show any pathological changes and was found to be safe even at 2000 mg/kg dose level. However, further detailed toxicological investigations are required particularly to elucidate their chronic toxicity. The results obtained clearly indicate that the compounds discussed here showed significant analgesic (227.8-414.0% analgesic activity) and anti-inflammatory activities (40.3-52.9% inhibition of oedema) without an ulcerogenic liability. Compounds **3c** and **3d** appear to be the most active derivatives in our series. These observations may promote the synthesis of more active bis(indolyl) methanes in future. The mechanism involved in the activities is under study.

Acknowledgement

The author (KS) thanks the management and the principal, Sri Ramachandra College of Pharmacy, Sri Ramachandra University for providing necessary facilities for carrying out the pharmacological studies.

References

1 Turner R A, *Screening Methods in Pharmacology*, (Academic Press, New York),1, **1965**, 152.
2 Paul A I, *Analgesic-antipyretic and Anti-inflammatory Agents and Drugs Employed in the Treatment of Gout. In Goodman and Gilmans, The Pharmacological Basis of Therapeutic,* Perry B M & Raymond W R, Eds, (McGraw-Hill, New York), 9th edn, **1996**, 618.
3 Ashutoshkar, *Medicinal Chemistry* (New Age International Publishers, New Delhi), 2nd edn, **2003**, 329.
4 Anupama P & Chaturvedi S C, *Indian J Pharm Sci,* 67, **2005**, , 116.
5 Bell R, Carmeli S & Sar N, *J Nat Prod,* 57, **1994**, 1587.
6 Fukuyama T & Chen X, *J Am Chem Soc,* 116, **1994,** 3125.
7 Sundberg R J, *The Chemistry of Indoles,* (Academic press, New York) **1996**.
8 Kumar A, Sharma A, Nidhi M, Preethi S, Kavita K, Kuldeep K S & Virendra K S, *Indian J Chem,* 43B, **2004,** 1532.
9 Moore P F, Larson D L, Otterness I G, Weissman A, Kadin S B, Sweeney F J, Eskra J D, Nagashisa A & Sakakibara M T, *J Carty Inflamm Res,* 45, **1996**, 54.
10 Mattison N, Trimple A G & Lasagna I, *Clin Pharmacol Ther,* 43, **1998**, 290.
11 Garbe T R, Kobayashi M, Shimizu N, Takesue N, Ozawa M & Yukawa H, J*Nat Prod,* 63*,* **2000,** 596.
12 Michnovicz J J & Bradlow H L, *Proc R Soc Edinburg,* 12, **1989,** 1571.
13 Osawa T & Namiki M, *Tetrahedron Lett*, 24, **1983**, 4719.
14 Zeligs M A, *J Med Food,* 1, **1998,** 67.
15 Hong C, Firestone G L & Bjeldanes L F, *Biochem Pharmacol*, 63, **2002,** 1085.
16 Kedmi M N, Yannai S, Haj A & Fares F A, *Food Chem Toxicol*, 41, **2003,** 745.
17 Nagarajan R & Perumal P T, *Synth Commun,* 32, **2002,** 105.
18 *OECD Guidelines for Testing of Chemicals*, No.401 acute oral toxicity (Paris), **1987**, 1.
19 Vogel G H & Vogel W H, *Drug Discovery and Evaluation*, (Springer Verlag, Berlin), **1998**, 413.
20 Ghosh M N, *Fundamentals of Experimental Pharmacology*, (Scientific Book Agency, Calcutta), 2nd edn, **1984**, 145.
21 Winter C A, Risley E A & Nuss G W, *Proc Soc Exp Biol Med,* 111, **1962,** 544.
22 Djahanguiri S J, *Pharm Pharmacol*, 21, **1969,** 541.
23 Kulkarni S K, *Hand Book of Experimental Pharmacology*, (Vallabh Prakashan), 3rd edn, Delhi, **1999**, 149.
24 Bansal E, Srivastava V K & Kumar A, *Eur J Med Chem,* 36, **2001,** 81.