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Synthesis of unsymmetrical triindolylmethanes as potent analgesic agents

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ABSTRACT

Various unsymmetrical triindolylmethanes were synthesized by multistep approach. In final step the targeted synthesis was carried out by the condensation of two moles of simple indole with previously prepared ethyl 3-formyl-indole-2-caboxylates in presence of antimony trichloride as catalyst. The pharmacological screening of the obtained compounds was proved for their potent analgesic activity.

KEYWORDS

Ethyl indole-2-carboxylates;
Formylation;
Triindolylmethane;
Antimony trichloride;
Analgesic activity.

INTRODUCTION

Indole and its analogs constitute the active class of compounds possessing wide spectrum of biological activities, such as anti-inflammatory[1], anticonvulsant[2], antimicrobial[3-5], cardiovascular[6] and analgesics[7]. In addition, the indole nucleus is incorporated in various natural products such as alkaloids[8]. There are several reports on synthetic and pharmaceutical activities of natural bisindole[9,10] and trisindole alkaloids[11]. Many of the most important bisindolylmethanes and trisindolylmethanes (TIMs) were isolated from various terrestrial and marine natural sources[12] exhibit a range of important biological activities[13]. It was recently discovered that TIMs isolated from bacteria[14] served as bacterial metabolic[15] and cytotoxic agents[16]. Recently various therapeutically useful diindolyl and triindolylmethanes has been developed[17].

In this regard, to improve the essential pharmacophoric features of the Triindolylmethane molecule, we have outlined efficient simple route for the synthesis of unsymmetrical unnatural TIMs derivatives (5a-e) containing strong electron withdrawing groups on different position. The condensation of 3-formyl indole ester and two molar equivalent of indole in presence of antimony trichloride as catalyst in CH_3CN as solvent at 70-80 °C afforded desired products in high yield without much difficulty (Scheme 1) and evaluated for their analgesic activity by acetic acid induced writhing in mice.

EXPERIMENTAL

General

Melting points were determined with a capillary melting point apparatus and are uncorrected. Purity of the compounds were checked by TLC on silica gel and purified by column chromatography using solvent system petroleum ether and ethyl acetate. The IR spectra were recorded on a Nicolet Impact 410 FT-IR Spec-

trophotometer using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker-400 MHz spectrometer in $CDCl_3$ using TMS as an internal standard. The mass spectra were recorded on a GC- 2010 Shimadzu Mass Spectrometer (GC-MS). All solvents and reagents were analytical graded or chemically pure.

Synthesis of ethyl pyruvate phenylhydrazones (2a-e)

The mole equivalent of phenylhydrazine hydrochloride derivatives (1a-e) and ethyl pyruvate was taken in ethyl alcohol and same ratio with slight excess of sodium acetate was added. It was refluxed on water bath for 30 minutes and progress of the reaction was monitored by TLC. Upon completion of reaction, the reaction mixture was added to ice cold water with constant stirring. The solid obtained was filtered, dried and recrystallized with ethanol.

Cyclization of ethyl pyruvate phenylhydrazones to Indole-2-carboxylates (3a-e)

Ethyl pyruvate phenylhydrazone (2a-e) was taken in 10 g of polyphosphoric acid in ethyl alcohol (5ml) and stirred well for proper mixing. The reaction mass was slowly heated to 80-90°C and for about 1 hour. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the reaction mixture was added slowly into ice cold water with constant stirring. The solid thus separated was filtered, washed with water, dried and crude product was recrystalised with ethyl acetate. The derivatives 3b, 3c, 3d, and 3e were obtained by similar procedure and purified by column chromatography (ethyl acetate: pet ether, 4:6) on silica gel (60-120 mesh). The physical measurements of the indole carboxylates were matched with the earlier report[18].

Synthesis of ethyl 3-fomyl-1H-indole-2-carboxylates (4a-e)

Mixture of 1:1 molar ratio of phosphorous oxychloride and *N*,*N*-dimethylformamide were stirred well at 0°C to form imminium salt of *N*, *N*-Dimethyl formamide. Same ratio of ethyl indole-2-carboxylates (3a-e) were added under anhydrous condition in ice bath with constant stirring. The reaction mixture was then refluxed on water bath of about 6 to 7 hours. After the completion of reaction as indicated by TLC, it was poured to ice cold water and neutralized by sodium bicarbonate. The separated product (4a-e) was filtered and recrystalised with methanol.

Synthesis of triindolylmethanes (5a-e)

The mixture contains 1:2 ratios of ethyl 3-fomyl-1H-indole-2-carboxylates (4a-e) and indole was taken in acetonitrile (5 ml) in presence of $SbCl_3$ (20 mol%). The resulting mixture was refluxed on water bath for about 20-30 min. Progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and poured to ice water with constant stirring. The separated solid was filtered. The crude compound was purified by column chromatography technique. The structures of prepared compounds were determined by their physical (TABLE 2) and spectral studies.

5a ($C_{28}H_{22}N_4O_4$)

Orange crystals; IR ν_{max}(KBr) ν cm^{-1}:3392, 2923, 2853, 1705, 1658. ^{1}H NMR ($CDCl_3$) δ ppm (TMS): 9.2 (1H, s), 8.5 (1H, s), 8.15 (1H, dd), 8.0 (2H, s), 7.5 (2H, d), 7.45 (3H, m), 7.2 (3H, t), 7.0 (2H, t), 6.8 (1H, s), 4.5 (2H, q), 2.1 (1H, s), 1.4 (3H, t). ^{13}C NMR($CDCl_3$) δ ppm (TMS): 14, 29, 30, 61, 111.1, 111.8, 119.3, 119. 6, 120.2, 120.9, 122, 123, 127, 136, 238. MS: m/z = 479(M+1).

5b ($C_{28}H_{22}N_4O_4$)

Orange crystals; IR ν_{max}(KBr) ν cm^{-1}:3414, 2924, 2854, 1700, 1622, ^{1}H NMR ($CDCl_3$) δ ppm (TMS): 9.4(1H, s), 8.1 (1H, s), 8.0 (1H, dd), 7.6 (1H, s), 7.5-6.9(10H, m), 6.75 (1H, s), 6.5 (1H, s), 4.3 (2H, q), 2.7 (1H, s), 1.1 (3H, t). ^{13}C NMR($CDCl_3$) δ ppm (TMS): 14, 22, 29, 31, 110, 111, 116, 118, 119, 121, 122, 124, 128, 237. MS: m/z = 479(M+1).

5c ($C_{28}H_{22}N_4O_4$)

Orange crystals; IR ν_{max}(KBr) ν cm^{-1}: 3465, 3408, 3344, 3078, 2943, 2921, 2648, 1656, 1616. ^{1}H NMR ($CDCl_3$) δ ppm (TMS): 10.8 (1H, s), 9.9 (1H, s), 9.4 (1H, s), 8.6 (1H, d), 8.4 (1H, s), 8.2 (1H, t), 8.0 (1H, s), 7.65 (1H, dd), 7.55 (1H, d), 7.4 (2H, m), 7.1 (2H, m), 6.9 (2H, t), 6.75 (1H, s), 4.5 (2H, q), 2.0 (1H, s), 1.4 (3H, t). ^{13}C NMR($CDCl_3$) δ ppm (TMS): 14, 29, 108, 111, 119.3, 119.6, 122.1, 123, 230. MS: m/z = 479(M+1).

5d ($C_{28}H_{22}ClN_3O_2$)

Light pink crystals; IR ν_{max}(KBr) ν cm^{-1}: 3371, 3073, 2924, 2884, 1674, 1651. ^1H NMR ($CDCl_3$) δ ppm (TMS): 8.9 (1H, s), 8 (2H, s), 7.4 (5H, m), 7.1 (3H, m), 7.0 (2H, t), 6.75 (3H, s), 4.5 (2H, q), 2.0 (1H, s), 1.3 (3H, t). ^{13}C NMR($CDCl_3$) δ ppm (TMS): 14, 19, 29, 30, 61, 110, 111, 118, 119.2, 119.8, 120, 121, 123, 124, 136. MS: m/z = 469 (M+1).

5e ($C_{28}H_{22}ClN_3O_2$)

Light pink crystals; IR ν_{max}(KBr) ν cm^{-1}: 3434, 3360, 3057, 2924, 2853, 1708, 1615. ^1H NMR ($CDCl_3$) δ ppm (TMS): 9.2 (1H, m), 9 (1H, s), 7.9 (1H, s), 7.6 (1H, m), 7.35 (3H, d), 7.1 (4H, m), 6.8 (5H, m). 4.2 (2H, q), 2.8 (1H, s), 1.2 (3H, t). ^{13}C NMR($CDCl_3$) δ ppm (TMS):14, 17, 29, 30, 49, 110, 110.8, 118, 119, 121, 122, 124, 136. MS: m/z = 469(M+1).

Pharmacology

Analgesic activity

Swiss albino mice (15-25 g) of either sex were used to assess the analgesic activity. They were housed 3 to 4 per cage at 22±2°C and 12 hr dark/light under controlled environment. Animals were fed standard laboratory food and water was given *ad libitum*. Mice were kept for 7 days in laboratory for habituation. All the experiments were performed in light period, and were conducted according to the CPCSEA regulations; India and the Institutional Animal Ethics Committee (SETCP/IAEC/09-10/11) approved the experimental protocol.

Analgesic property of TIMs was carried out as described by the method based on acetic acid induced writhing in mice[19]. Seven groups of mice (n = 6) were randomly formed. The groups were treated as control (distilled water, *p. o.*) and standard (Aspirin 100 mg/kg *p.o.*) while test groups received suspensions of TIMs (100 mg/kg *p.o.v*) in 0.1% Tween-80 respectively. Acetic acid solution 0.6% v/v (10 ml/ kg) was injected by intraperitoneal route one hour after treatment and number of writhes (i.e. index of pain reaction against chemical stimuli characterized by abdominal muscle contraction together with turning of trunk and extension of hind limbs) was counted over a period of 20 min. Analgesic activity was expressed as percentage of inhibition of writhes with respect to the control group (TABLE 1)

The percentage of protection of the compounds comparing with the activity of standard drug was calculated by using the formula

% Inhibition = [1-V_c/V_t] X 100

Where, V_c = Mean number of writhing in test animals; V_t = Mean number of writhing in control.

TABLE 1 : Inhibition effect of TIMs (100 mg/kg b.w) on acetic acid writhing in mice

Compound no.	R	Mean no. of writhing in 20 mins ± S.E.M		% Protection
		Without the administration of drug (control)	With the administration of drug	
Aspirin	------	24.00 ± 0.577	11.00 ± 0.516	54.16
5a	5-NO_2	21.60± 0.843	17.00 ±0.572	21.29
5b	4-NO_2	24.16 ± 0.394	17.33 ± 1.415	28.26
5c	6-NO_2	19.16 ± 0.394	14.50 ± 0.939	24.32
5d	6-Cl	29.33 ± 5.334	13.50 ± 0.449	53.90
5e	4-Cl	22.83 ± 2.359	11.33 ± 0.598	50.00

RESULTS AND DISCUSSION

The indole-2-carboxylate derivatives 3a-e were prepared via Fischer cyclization of hydrazones using polyphosphoric acid (PPA) as catalyst and ethyl alcohol as co-solvent in excellent yield by adopting our in house developed method[18], and further introduction of formyl group proceeded by Vilsmeier-Haack reaction to furnish intermediates 4a-e. Finally TIMs 5a-e were synthesized by treating 2 moles of indole with 1 mole formyl indole carboxylate (4a-e) in presence of antimony trichloride (20 mole%) in acetonitrile as solvent is as shown in Scheme 1. Since appropriately substituted indole-3-carboxaldehyde are not commercially easily available to get diverse substituted TIMs compounds, in this report we adopted our earlier method of synthesis of various indole-2-carboxylates as key intermediates to produce new TIMs compounds. All the synthesized compounds were characterized by their IR, ^1H NMR, ^{13}C NMR and GC-MS spectral data which displayed the expected patterns.

Free radicals have been demonstrated to be a contributing factor in the tissue injury and modulation of the pain[20]. Any injury or tissue damage is associated with pain and inflammation. Pain is a complex process mediated by many physiological mediators e.g. prostag-

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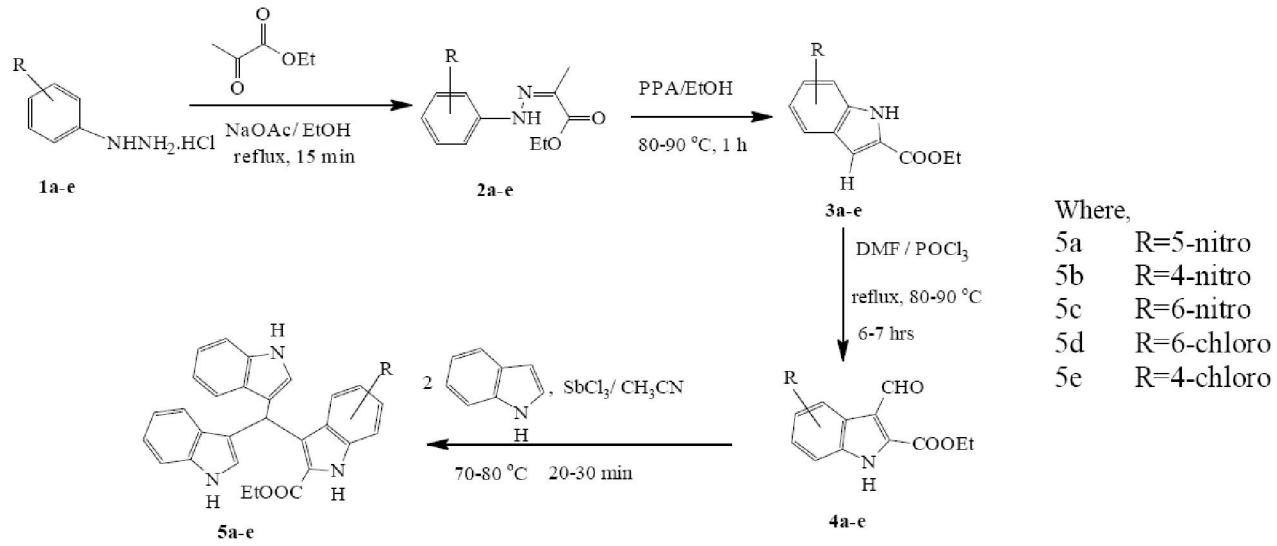
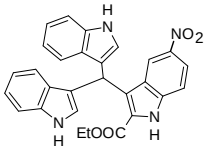
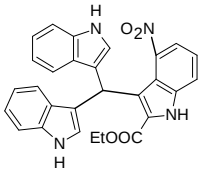
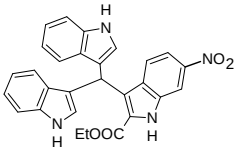
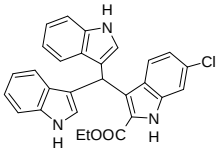
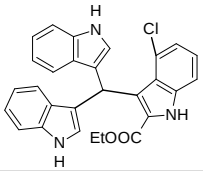


Scheme 1 : Synthesis of triindolylmethanes (5a-e)

landins, bradykinins, substance-p etc. In the acetic acid induced writhing model the constrictions induced by acetic acid in mice results from an acute inflammatory reaction with production of PGE2 and PGF2α in the peritoneal fluid[21,22]. The acetic acid-induced writhing reaction in mice has long been used as a screening tool for the assessment of analgesic or anti-inflammatory properties of new agents, and is described as a typical model for visceral inflammatory pain[23]. Acetic acid produced a painful reaction and acute inflammation in the peritoneal area, which was considered to be a non-selective antinociceptive model, could indirectly induce the release of endogenous mediators such as serotonin, histamine, bradykinin and prostaglandins, stimulated the nociceptive neurons that were sensitive to non-steroidal anti-inflammatory drugs.

Pretreatment with TIMs derivatives 5d and 5e (100 mg/kg b. w) markedly reduced the painful response produced by acetic acid, manifested as writhing at the employed doses. The % protection by these compounds was found to be 53.90% and 50.00% respectively and other compounds showed less activity compared with the activity of standard drug aspirin having percentage of protection value 54.16. Therefore, it is likely that TIMs derivatives 5d and 5e at the tested dose might suppress the formation of these substances or antagonize their action for exerting analgesic activity. The results of analgesic activity of the TIMs are expressed in TABLE 2. One way ANOVA followed by multiple Tukeyís comparison test. Values are presented as the mean ± SE (standard error); n = 6 for all groups, ^{c} $p < 0.001$ as compared to control (without drug) group.

TABLE 2 : Physical data of triindolylmethanes (5a-e)

Compound	Structural formula	Yield[a] (%)	m.p. (^{0}C)
5a		90	202-205
5b		74	208-211
5c		90	236-238
5d		75	256-258
5e		66	198-200

[a]**Isolated yield**

CONCLUSION

In conclusion, the facile synthetic route has been

proposed for the preparation of unsymmetrical triindolylmethanes which showed potent analgesic activity compared to standard aspirin.

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