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Synthesis and Pharmacological Screening of Indan-1-propionic Acids as Anti-hypercholesterolemic Agents

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Non-methoxy and methoxy indan-1-propionic acids have been synthesized from their corresponding benzaldehyde *via* indan-1-acetic acids as intermediate. The chemical structures of the proposed compounds were established based on ^{1}H NMR and IR spectral data. The synthesized compounds were screened for anti-hypercholesterolemic activity in male albino Charles Forster rats at 50 mg/kg dose. Dimethoxy substituted indan-1-propionic acid (compound **3**) showed better activity profile than their homologues but none of them was found in superior to standard drug clofibrate.

Key Words: Indan-1-propionic acids, Normocholesterolemic, Hypercholesterolemic.

INTRODUCTION

Indan ring system has been found to act as an inert carrier, which serves to hold biologically active functional moieties in a stereospecific manner[1]. Several workers[2,3] reported that indan compounds possess significant pharmacological potentialities due to presence of nuclear protons in its ring structure. Indan nuclei were undergone some structural or molecular modifications in our laboratory either by introducing functional groups or ring fusion. Both the modifications resulted many promising pharmacological activities[4-8]. A number of non-methoxy and methoxy substituted indan acids were studied for cholesterol lowering activity but none of them was found in superior to reference standard clofibrate[9]. Further, we synthesized various α-alkyl substituted indan acetic acids and reported the smaller alkyl group ($-CH_3$, $-C_2H_5$) at the α-carbon in the acetic acid moiety exhibited anti-hypercholesterolemic activity[10,11]. In addition, we logically extended the above work to get better anti-hypercholesterolemic activity among trimethoxy substituted indan-1-acetic acids. However, no significant cholesterol lowering activity was shown by trimethoxy indan-1-acetic acids[12]. Keeping the above points in view, the synthesis of non-methoxy and methoxy substituted indan-1-propionic acids with an expectation of better anti-hypercholesterolemic activity are reported. The present communication deals with the synthesis and anti-hypercholesterolemic screening in both normo and hypercholesterolemic animal model.

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EXPERIMENTAL

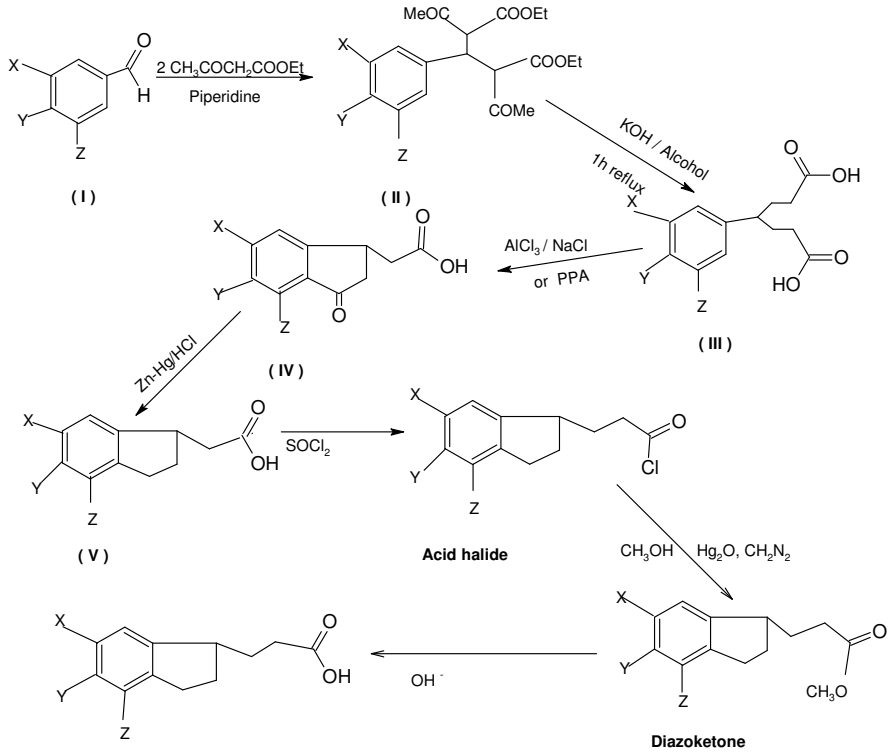
Simple and mono, di, tri-methoxy benzaldehyde (**I**) were used as starting material for synthesis of the respective indan-1-propionic acids. Compound **I** was reacted with two moles of ethyl acetoacetate (EAA) in presence of catalytic amount of piperidine at room temperature to obtain the substituted phenylbisacetoacetate (**II**). Acid hydrolysis of **II** was carried out with 6 N KOH in 50 % ethanol to get the diacid **III**.

Compound **III** was treated with aluminium chloride in the presence of catalytic amounts of sodium chloride when intramolecular Friedel-Craft's cyclization and hence ring closure took place to give the ketonic product **IV**. The cyclization was also tried with polyphosphoric acid but it gave poorer yields of keto product with more impurities[13]. The ketonic group of compound **IV** was finally removed using Clemmensen's reduction to give the corresponding indan-1-acetic acids (**V**)[10,12].

Synthesis of the title compounds was carried out by the following **Scheme-I**. Melting points were determined by Adco capillary apparatus and were uncorrected. TLC was used for checking the purity of the compounds. Identity of the compounds was ascertained by elemental microanalysis and spectral analysis. UV, IR and NMR spectral data were recorded on a Hitachi 200-20 UV-vis spectrophotometer, Perkin-Elmer Infracord 297 spectrophotometer and 400 MHz Brucker Avance II NMR spectrophotometer, respectively. Spectral data of all compounds confirmed well with their proposed structure[14,15].

Preparation of 3-(indan-1'-yl)-propionic acid (1): Indan-1-acetic acid (8.8 g, 0.05 mol) was dissolved in 50 mL dry benzene and allow refluxing under water bath. 5.8 g (0.08 mol) of thionyl chloride was added very slowly to indan-1-acetic acid under anhydrous condition and reflux continues for 1 h. Benzene and excess thionyl chloride was removed by co-distillation. Indan-1-acetyl chloride was collected by distillation under reduce pressure (1.5 mm of Hg) at 120 ºC.

Dry ethereal solution of diazomethane (prepared from 42.6 g of nitrosomethyl urea) was taken up in 1 L round bottom flask fitted with a reflux condenser under anhydrous condition and cooled in an ice-salt mixture. Indan-1-acetyl chloride was taken in 50 mL sodium dried ether and then added slowly to the above ethereal solution under anhydrous condition with constant stirring through magnetic stirrer. This mixture was allowed to stand overnight at room temperature. Ether was then removed at room temperature under reduced pressure. The residual indan-1-acetyl diazoketone was taken up in 400 mL of methanol and a slurry of silver oxide (prepared from 6.8 g $AgNO_3$ and 3.8 g NaOH) in methanol was added in portions at 30-40 ºC and the course of reaction was followed by the nitrogen evolution. The whole mixture was then refluxed at 60-80 ºC for 1 h. The solution was filtered with charcoal in boiling condition and alcoholic solution was concentrated. 100 mL of distilled water was added to the cold residual solution and 3-(indan-1'-yl)-methyl propionate was extracted with 200 mL of ether. Ethereal layer was washed consecutively with dilute acid, alkali and water and then dried over anhydrous Na_2SO_4. Solvent was distilled off and purified by distillation under reduced pressure (2-3 mm of Hg) at 130-135 ºC.



where, X = Y = Z = H, X = OCH_3, Y = Z = H, X = Y = OCH_3, Z = H, X = Y = Z = OCH_3

Scheme-I: Synthesis of non-methoxy and methoxy indan-1-propionic acids

The ester was refluxed with 50 mL of 95 % alcohol and 50 mL of 10 % KOH solution on a steam bath for 3 h. Alcohol was distilled off and washed successively with water and finally with ether. The resulting product 3-(indan-1'-yl)-propionic acid (**1**) was precipitated out from the aqueous solution by neutralization with calculated amount of HCl under ice-cold condition and kept overnight at room temperature. Compound (**1**) was then purified by crystallization from aqueous alcoholic solvent. The yield 7.04 g (78 %) and m.p. 51-53 ºC was recorded. (Anal. calcd. (%) for $C_{12}H_{14}O_2$: C, 75.79, H, 7.37. Found (%): C, 75.52, H, 7.54). λ_{max} (CH_3OH) at 265 nm; IR: OH of COOH at 3390 cm^{-1}, -CH_2-CH_2- at 2942 cm^{-1}, C=O of COOH at 1710 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 2.42 (2H, m, 2'-CH_2); 2.50 (2H, dd, 2-CH_2); 2.62 (2H, dd, 3-CH_2); 2.82 (2H, m, 3'-CH_2); 4.72 (1H, qn,1'-CH); 7.36 (1H, d, 5'-ArH); 7.60 (1H, d, 6'-ArH); 7.68 (1H, s, 4'-ARH); 7.72 (1H, s, 7'-ArH); 9.98 (1H, brs, 1-COOH).

Preparation of 3-(6'-methoxy indan-1'-yl)-propionic acid (2): 8.0 g (0.04 mol) of 6'-methoxy indan-1'-yl acetic acid was reacted with 5.8 g (0.08 mol) of thionyl chloride in presence of dry benzene (50 mL) under refluxing for 45 min in anhydrous condition. Resulting acid chloride was distilled at 142-145 ºC at 1.5 mm of Hg. 6.6 g (0.03 mol) of 6'-methoxy indan-1'-yl chloride was taken in 50 mL of sodium dried

ether which was added slowly in ethereal solution of diazomethane and the reaction procedure was followed as described above.

The resulting diazoketone on hydrolysis and silver catalyzed rearrangement in methanol results in formation of 3-(6'-methoxy indan-1'-yl)-propionate. This upon hydrolysis with a mixture of aqueous alkaline solution with alcohol results in 3-(6'-methoxy indan-1'-yl)-propionic acid. The crude product was crystallized from aqueous-alcohol. Yield of the purified product was 6.24 g (78 %) and m.p. 69-71 ºC. (Anal. calcd. (%) for $C_{13}H_{16}O_3$: C, 70.91, H, 7.27. Found (%): C, 71.24, H, 7.08). λ_{max} (CH_3OH) at 280 nm; IR: OH of COOH at 3380 cm^{-1}, -CH_2-CH_2- at 2945 cm^{-1}, C=O of COOH at 1715 cm^{-1} and C-OCH_3 at 1200 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ): 2.44 (2H, m, 2'-CH_2); 2.52 (2H, dd, 2-CH_2); 2.60 (2H, dd, 3-CH_2); 2.80 (2H, m, 3'-CH_2); 3.68 (3H, s, 6'-OCH_3); 4.70 (1H, qn, 1'-CH); 7.30 (1H, d, 5'-ArH); 7.50 (1H, d, 6'-ArH); 7.60 (1H, s, 4'-ArH); 7.62 (1H, s, 7'-ArH); 9.95 (1H, brs, 1-COOH).

Preparation of 3-(5',6'-dimethoxy indan-1'-yl)-propionic acid (3): 9.44 g (0.04 mol) of 5',6'- dimethoxy indan-1'-yl-acetic acid was reacted with 5.8 g (0.08 mol) of thionyl chloride in 100 mL of dry benzene and refluxed for 0.5 h under anhydrous condition. Excess thionyl chloride was co-distilled out with benzene. This acid halide being extremely thermolabile was used as such without further purification. This halide was dissolved in ether and added in diazomethane under the same reaction procedure as mentioned earlier. After completion of addition the whole mixture was taken in 400 mL methanol and allowed to silver oxide catalyzed hydrolysis and Wolff rearrangement to produce corresponding propionate. The entire extraction and recrystallization were then followed as described earlier to provide the pure compound **3**. Yield 7.08 g (75 %), m.p. 138-139 ºC. (Anal. calcd. (%) for $C_{14}H_{18}O_4$: C, 67.20, H, 7.20. Found (%): C, 67.02, H, 7.26). λ_{max} (CH_3OH) at 283 nm; IR: OH of COOH at 3378 cm^{-1}, -CH_2-CH_2- at 2950 cm^{-1}, C=O of COOH at 1720 cm^{-1} and C-OCH_3 at 1189 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ): 2.48 (2H, m, 2'-CH_2); 2.55 (2H, dd, 2'-CH_2); 2.65 (2H, dd, 3-CH_2); 2.82 (2H, m, 3'-CH_2); 3.70 (3H, s, 6'OCH_3); 3.74 (3H, s, 5'-OCH_3); 4.72 (1H, qn, 1'-CH); 7.32 (1H, d, 5'-ArH); 7.54 (1H, d, 6'-ArH); 7.63 (1H, s, 4'-ArH); 7.65 (1H, s, 7'-ArH); 9.92 (1H, brs, 1-COOH).

Preparation of 3-(4',5',6'-dimethoxy indan-1'-yl)-propionic acid (4): 4',5',6'-trimethoxy indan-1'-acetic acid, 10.72 g (0.04 mol) was taken in 100 mL dry benzene and 5.8 g (0.08 mol) of thionyl chloride under anhydrous condition and allow to refluxed for 0.5 h. Diazomethane reaction and silver oxide catalyzed hydrolysis of the acid halide was carried in the same way as described above to provide ester of propionic acid. The entire extraction and recrystallization were then followed as described earlier to provide the pure compound **4**. Yield of the purified product was 7.5 g (70 %) and m.p. 169-171 ºC. (Anal. calcd. (%) for $C_{14}H_{20}O_5$: C, 62.68, H, 7.46. Found (%): C, 62.88, H, 7.50). λ_{max} (CH_3OH) at 285 nm; IR: OH of COOH at 3382 cm^{-1}, -CH_2-CH_2- at 2953 cm^{-1}, C=O of COOH at 1725 cm^{-1} and C-OCH_3 at 1188 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ): 2.50 (2H, m, 2'-CH_2); 2.58 (2H, dd, 2-CH_2); 2.62(2H, dd, 3-CH_2); 2.83 (2H, m, 3'-CH_2); 3.68 (3H, s, 6'-OCH_3); 3.77 (3H, s, 5'-OCH_3);

3.80 (3H, s, 4'-OCH_3); 4.72 (1H, qn, 1'-CH); 7.30 (1H, d, 5'-ArH); 7.53 (1H, d, 6'-ArH); 7.61 (1H, s, 4'-ArH); 7.62 (1H, s, 7'-ArH); 10.05 (1H, brs, 1-COOH).

Pharmacology: The synthesized compounds were screened for antihypercholesterolemic activity in normo- and hypercholesterolemic animal model.

Normocholesterolemic: Male albino Charles Foster rats weighing 120 ± 10 g were obtained from the animal house of the Department of Pharmaceutical Technology, Division of Medicinal Chemistry, Jadavpur University, Kolkata, India. They were acclimatized to the laboratory environment for at least one week. They were fed standard laboratory diets (Gold Mohur, Hindustan Lever Ltd., Mumbai, India) and clean tap water *ad libitum*. Experimental procedures were also examined and approved by internal ethical committee for animal welfare.

Hypercholesterolemic: Seven week old male albino Charles Foster rats weighing 130 ± 10 were used. High cholesterol diet was prepared from laboratory standard diet by adding 1 % cholesterol and 0.5 % sodium cholate. Animals were fed on high cholesterol diet for 10 days[16].

Administration of sample: Test compounds were dissolved in distilled water and the pH of the solution was adjusted to 7.5. The test drugs and clofibrate (aqueous solution) were then administered orally with the aid of a cannula at a dose of 50 mg/kg body weight for 14 days. Rats in the control group received 0.5 mL the drug-free vehicle.

Blood collection: On the 14th day, tail vein blood was drawn from the overnight fasted animals, respectively. Body weight of the each animal was recorded every day before drug administration. For biochemical study, blood (2-3 mL) was collected in non-heparinized 10 mL test tube and was centrifuged at 1800 rpm at 4 ºC for 10 min and the serum was stored at -20 ºC until analyzed.

Biochemical studies: The biochemical parameters were estimated at 14th day after completion of drug treatment included serum total cholesterol, triglyceride, total liver cholesterol and high-density lipoprotein (HDL). Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) fractions were calculated as: (i) VLDL = triglycerides/5 (ii) LDL = total cholesterol-(HDL cholesterol + VLDL cholesterol), respectively[17]. Biochemical measurements were carried out using biochemical kits (Span, India) and an automated biochemical analyzer (Vitros-250, Johnson and Johnson Co. USA).

Liver cholesterol: At the end of experimental period all animals were sacrificed by ether anesthesia and liver was then removed, washed with saline and dried by soaking with filter paper, total liver weight were measured. Liver samples of 0.1 g weights were homogenized in 0.1 M *tris* HCl buffer at pH 7.4 and the homogenates were extracted with alcohol-acetone (1:1 v/v). The solvent was redissolved with glacial acetic acid for estimation of total liver cholesterol[18].

Statistical analysis: The results are presented as mean ± SD. The results were analyzed statistically by using one-way analysis of variance (ANOVA) followed by Dunnett's-test. p values less than 0.05 were considered as significant. All analysis was performed using SPSS 10.0 statistical software.

RESULTS AND DISCUSSION

Non-methoxy and methoxy indan-1-propionic acids were synthesized from corresponding indan-1-acetic acid by Arndt-Elster reaction. The resulting acid halides were reacted with diazomethane and subsequent silver catalyzed Wolf-rearrangement in methanol to provide diazoketone. The percentage of yield in each case was satisfactory. The title compounds thus prepared were confirmed by elemental, IR and ^{1}H NMR analyzes.

The newly synthesized compounds were subjected to preliminary testing for their anti-hypercholesterolemic activity in comparison with the reference drug, clofibrate. The anti-hypercholesterolemic activity of the test compounds was evaluated by normocholesterolemic and hypercholesterolemic animal model. The normocholesterolemic results (Table-1) show that the test compounds exhibit variable anti-hypercholesterolemic at a dose level of 50 mg/kg. Compound **3** having dimethoxy in the indan nucleus showed significant cholesterol lowering activity. Hypercholesterolemic results presented in Table-2 indicated that compound **3** showed significant antihypercholesterolemic activity than their homologues. It was

TABLE-1
EFFECT OF INDAN-1-PROPIONIC ACIDS ON BODY WEIGHT, SERUM LIPID PROFILES, LIVER CHOLESTEROL AND LIVER WEIGHT IN NORMOCHOLESTEROLEMIC RATS*

Compd. no	Body Wt (gm)	Serum Cholesterol (mg %)	Serum Triglycerides (mg %)	HDL Cholesterol (mg %)	LDL Cholesterol (mg %)	VLDL Cholesterol (mg %)	Liver Cholesterol (mg %)	Liver Wt (gm)/body Wt (gm)
1	128.16 ± 1.94	77.00 ± 1.41	35.83 ± 2.14	26.50 ± 1.52	42.16 ± 2.31	6.83 ± 0.29	2.78 ± 0.15	5.78 ± 0.21
2	128.17 ± 1.95	76.33 ± 1.97[a]	35.66 ± 1.96	28.00 ± 1.78	40.33 ± 1.78	6.70 ± 0.24	2.80 ± 0.21	5.63 ± 0.33
3	127.16 ± 1.94	74.00 ± 0.63[b]	31.83 ± 1.72	30.66 ± 0.81[c]	36.96 ± 1.53[b]	6.37 ± 0.34	2.88 ± 0.28	5.70 ± 0.39
4	126.50 ± 1.88	77.16 ± 2.04	35.16 ± 3.45	27.50 ± 2.07	40.10 ± 3.43	6.60 ± 0.44	2.85 ± 0.18	5.85 ± 0.19
Clofibrate	128.16 ± 2.79	64.33 ± 0.82[c]	29.17 ± 2.40[c]	25.67 ± 1.63	32.83 ± 1.55[c]	5.84 ± 0.48[b]	2.96 ± 0.10	5.98 ± 0.12
Control	128.33 ± 1.86	80.00 ± 1.41	36.17 ± 2.13	26.33 ± 1.63	45.90 ± 3.22	7.23 ± 0.43	2.87 ± 0.17	5.85 ± 0.18

*Rats in the experimental groups were given test compounds and clofibrate at a daily dose of 50 mg/kg for 14 days, whereas those in the control group received an equal volume of pure water. Each value represents the mean ± SD (n = 6). p values were > 0.05 (non-significant) for each the experimental groups compared to the control group, a < 0.05, b < 0.01, c < 0.001.

TABLE-2
EFFECT OF INDAN-1-PROPIONIC ACIDS ON BODY WEIGHT, SERUM LIPID PROFILES, LIVER CHOLESTEROL AND LIVER WEIGHT IN HYPERCHOLESTEROLEMIC RATS*

Compd. no	Body Wt (g)	Serum Cholesterol (mg %)	Serum Triglycerides (mg %)	HDL Cholesterol (mg %)	LDL Cholesterol (mg %)	VLDL Cholesterol (mg %)	Liver Cholesterol (mg %)	Liver Wt (gm)/body Wt (g)
1	136.17 ± 1.72	100.33 ± 2.80	47.16 ± 0.75	30.17 ± 0.75	60.53 ± 1.84	9.20 ± 0.25	2.72 ± 0.17	5.78 ± 0.21
2	136.83 ± 2.64	100.01 ± 2.19	46.70 ± 1.03	31.50 ± 0.84	58.96 ± 2.58	9.00 ± 0.25	2.82 ± 0.21	5.76 ± 0.13
3	136.16 ± 2.04	96.16 ± 1.94[b]	43.67 ± 1.03[c]	31.16 ± 1.32	56.30 ± 3.12[a]	8.70 ± 0.24[b]	2.81 ± 0.21	5.70 ± 0.19
4	135.83 ± 2.93	98.67 ± 2.16	47.83 ± 1.72	29.16 ± 0.98	59.67 ± 1.91	9.32 ± 0.29	2.82 ± 0.18	5.82 ± 0.15
Clofibrate	136.50 ± 1.87	99.34 ± 2.80	42.17 ± 1.33[c]	29.33 ± 0.82	61.54 ± 3.01	8.43 ± 0.26[b]	2.96 ± 0.10	6.08 ± 0.14
Control	136.84 ± 1.17	102.34 ± 2.94	49.17 ± 1.60	29.16 ± 1.17	60.46 ± 1.97	9.70 ± 0.21	2.85 ± 0.11	5.86 ± 0.23
Normal	128.33 ± 1.86	80.00 ± 1.41	36.17 ± 2.13	26.33 ± 1.63	45.90 ± 3.22	7.23 ± 0.43	2.87 ± 0.17	5.85 ± 0.18

*Rats in the experimental groups were given test compounds and clofibrate at a daily dose of 50 mg/kg for 14 days, whereas those in the control group received an equal volume of pure water. Each value represents the mean ± SD (n = 6). p values were > 0.05 (non-significant) for each the experimental groups compared to the control group, a < 0.05, b < 0.01, c < 0.001.

also observed that compound-**3** showed lowering effects of cholesterol, triglyceride, LDL- and VLDL-cholesterol whereas standard drug clofibrate exhibited only lowering effect of triglyceride and VLDL-cholesterol. No change of body weight, liver weight and liver cholesterol were observed in any test agents in both animal models.

From the preliminary pharmacological screening results, it was clear that indiscriminate chain lengthening would not be beneficial for cholesterol lowering activity. Keeping these observation it seemed likely that further modification of chemical structure of the parent compound may improve the cholesterol lowering activity.

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