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Design, Synthesis and Evaluation of Substituted Benzeneacetic Acid Ester derivatives as Potential Antiinflammatory and Analgesic Agents

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ABSTRACT: A series of novel analogs of lead compound α-cyclohexyl-α-hydroxy-benzeneacetic acid-4-(diethylamino)-2-butynyl ester hydrochloride (Lead Compound) with diverse structural modifications were synthesized by reacting 4-diethylamino-2-butynyl acetate with α-cyclohexyl-α-hydroxy-benzeneacetic acid methyl ester and product isolated as hydrochloride salt. All the synthesized compounds were characterized and screened for analgesic and anti-inflammatory activities. Potent analogs were evaluated for their ulcer index. Compounds displayed promising analgesic and anti-inflammatory activity and satisfactory ulcer index as desired, in comparison to the reference standard, based on which definite structure-activity relationship could be established.
KEY WORDS: Anti-inflammatory, Analgesic, Ulcer Index, Diclofenac.

INTRODUCTION

The therapeutic efficacy of currently available non-steroidal anti-inflammatory drugs is significantly limited by associated gastro-intestinal toxicity, which causes a higher incidence of morbidity in long-term NSAID users [1]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain, and fever. Most of the NSAIDs that are available in the market are known to inhibit isoforms of the enzyme cyclooxygenase (COX), a constitutive form, COX-1, and an inducible form, COX-2, to elicit therapeutic effect. However, long-term clinical use of NSAIDs is associated with significant side effects such as gastrointestinal lesions, bleeding, and nephrotoxicity. Diclofenac, Sulindac, Ibuprophen are potent NSAIDs but they have sevier GI side effects. Development of analgesic and anti-inflammatory active drugs with less ulcerogenic side effects is still a distinct dream; therefore, discovery of new and safer anti-inflammatory drugs represents a challenging goal [2–3].

Substituted glycolic acid esters are reported to possess significant anticonvulsant [4, 5], antimicrobial [6, 7], antihelminthic [8, 9], antiallergic [10], antitumor [11], anticancer [12], monoamine oxidase (MAO) inhibitor [13], and CNS activities [14], and, in particular, analgesic as well as anti-inflammatory activities [15–17].

Keeping in view the reported high analgesic and anti-inflammatory activity of the substituted benzeneacetic acid esters in the literature; it was decided to synthesize a series of novel α-cyclohexyl-α-hydroxy-benzeneacetic acid-4-(diethylamino)-2-butynyl ester hydrochloride (Lead Compound) derivatives with diverse structural modifications and evaluate their analgesic and anti-inflammatory activities.

Synthesis of lead compound involves simple condensation of 4-diethylamino-2-butynyl acetate (Intermediate A) prepared in a two step process starting with different propargyl acetate homologs in

the first step followed by condensation with various di-substituted amines and α-cyclohexyl-α-hydroxybenzeneacetic acid methyl ester synthesized by Grignard process involving preparation of cyclohexyl bromide magnesium salt, followed by in situ condensation with different homologs of α-oxo-benzeneacetic acid methyl ester, achieved the targeted structurally diverse analogs of lead compound desired for the study.

The structures of all the novel analogs were established by physical data (Melting range differences), Mass spectra, FTIR and ^{1}H NMR spectroscopy.

The compounds were evaluated for their analgesic and anti-inflammatory potential using Diclofenac sodium as reference standard. Some analogs showed encouraging results for potency which were evaluated for their ulcer index. Based on the results obtained, a definite structure-activity relationship was established.

EXPERIMENTAL

For Synthesis and Characterization of compounds

Synthesis of lead molecule i.e. α-cyclohexyl-α-hydroxy-benzeneacetic acid-4-(diethylamino)-2-butynyl ester hydrochloride involves simple condensation of 4-diethylamino-2-butynyl acetate (Intermediate A) and α-cyclohexyl-α-hydroxybenzeneacetic acid methyl ester **(**Intermediate B) as shown in Figure 1. The standard laboratory synthetic process is presented in following chapters. The diverse analogs of intermediate 4-diethylamino-2-butynyl acetate were synthesized in a two step process by synthesis of different propargyl acetate homologs in the first step and condensation with various substituted amines followed by reaction with different α-cyclohexyl-α-hydroxybenzeneacetic acid methyl ester analogs in the final step; synthesized by Grignard process involving preparation of cyclohexyl magnesium bromide and in situ condensation with respective homologs of α-oxo-benzeneacetic acid methyl ester.

Melting points of the synthesized compounds were determined on Thomas Hoover capillary apparatus and are uncorrected. IR spectra of the synthesized compounds were acquired on Perkin Elmer FTIR Spectrum. ^{1}H NMR, spectra were acquired on Bruker 300MHz NMR spectrometer and mass spectra on Shimadzu Qp-2010 mass spectrometer. Perkin Elmer Clarus 500 GC system and Agilent 1100 HPLC system was used to monitor progress of the reaction and to evaluate purity of compounds. Chemicals and solvents used were procured from Sigma-Aldrich or from Rankem Ltd. The sample of reference standard Diclofenac sodium was obtained from Lake Chemicals, Bangalore.

For pharmacological activity studies

The synthesized compounds were evaluated for analgesic and anti-inflammatory activities. The animals were maintained in colony cages at 25-28^{0}C, relative humidity of 40–50% under 12 h light and dark cycles. All the animals were acclimatized for a week before use. The animals were fed with standard animal feed and water ad libitum. The Institutional Animal Ethics Committee approved the protocol adopted for experimentation with animals.

- **Standard process for preparation of propargyl acetate (2) and its analogs.**

To a precooled solution (<10^{o}C) of propargyl alcohol (200 gm, 3.57 moles) and triethylamine (430.5 gm, 4.26 mol) in dichloromethane (1.0 L), was added acetyl chloride (311.0 gm, 3.96 mol) maintaining temperature less than 15^{o}C. The reaction mass was slowly warmed to 20-25^{o}C and stirred for 0.5 hour. Completion of reaction was confirmed by GC and water was added to the reaction mass. The reaction mass was then stirred for 15 minutes, layers settled and organic layer containing product was separated. The product was isolated by distillation after recovery of dichloromethane solvent. Yield: 318.5 g, 91%.

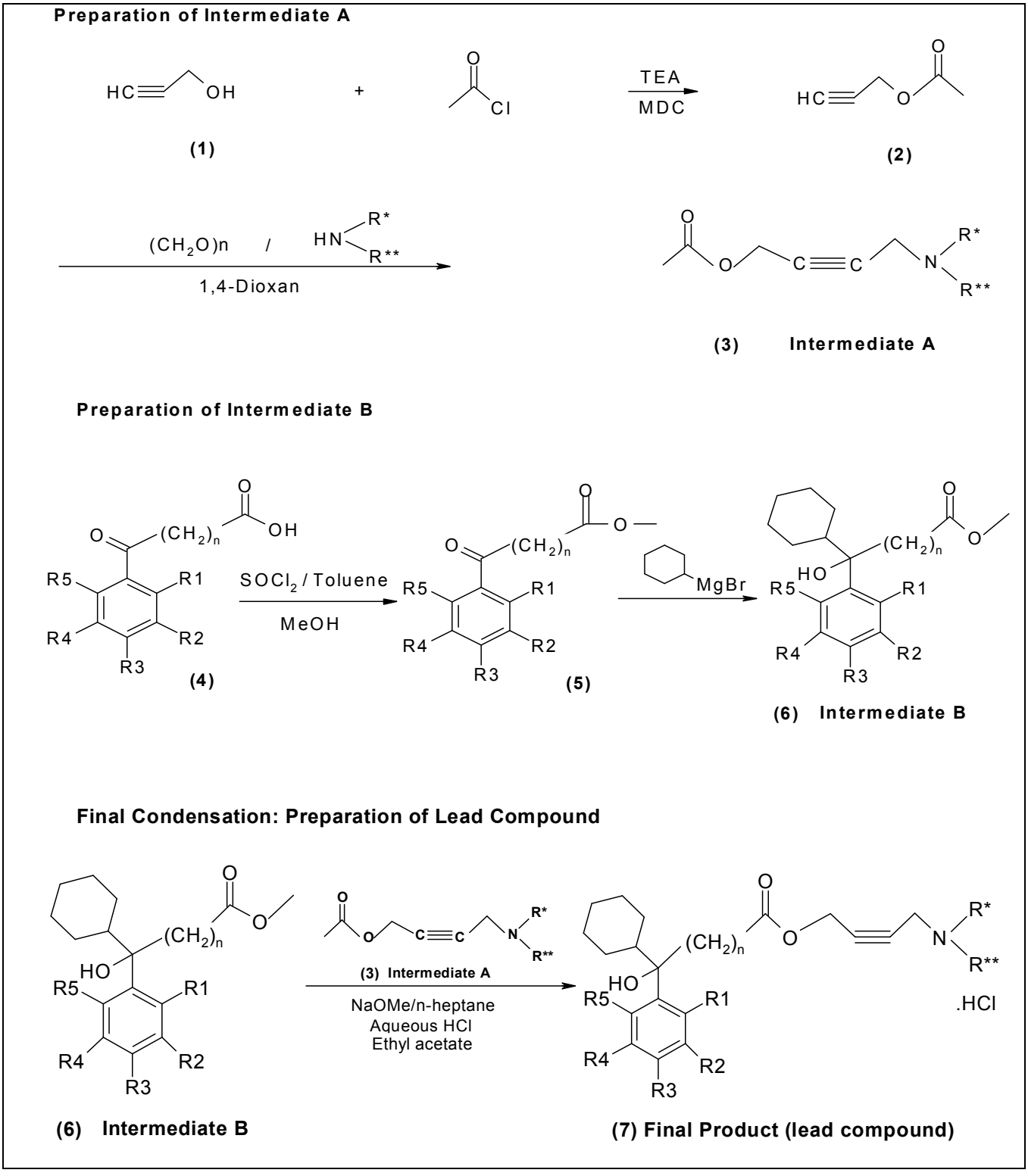
- **Standard process for the preparation of 4-diethylamino-2-butynyl acetate (3) and its analogs.**

To the mixture of *p*-formaldehyde (90 gm, 3.0 mole), diethylamine (210 gm, 2.87 moles) and cuprous chloride (5 gm) in 1,4-dioxane (1050 mL) was added propargyl acetate (250 gm, 2.55 mol) under stirring at 30-35^{o}C. The reaction mass was then heated using an oil bath to 90-95°C and maintained for 1 hour. Completion of reaction was confirmed by GC, cooled to 15^{o}C and filtered on hyflo bed. On recovery of solvent 1,4-dioxane (70 %) crude product was isolated (415.5 gm, 89 %) which was purified by distillation under reduced pressure (1mm, 75-80^{o}C) to get pure product as brownish yellow oil. Yield: 373.5 gm, 80.2 %.

- **Standard process for the preparation of α-oxo-benzeneacetic acid methyl ester (5) and its analogs.**

To the solution of α-oxo-benzeneacetic acid (150 gm, 1.0 mole) and dimethylformamide (1.0 mL) in toluene (450 mL) was added thionyl chloride (178.5gm, 1.5 moles). The solution was heated to 45-50^{o}C and continued for 1 hour. The reaction mass was then subjected to distillation when toluene was recovered (~390 mL) followed by distillation of product under reduced pressure (1 mm, 60^{o}C). The isolated acid chloride product (155 gm, 92 %) was poured in methanol (300 mL) and methyl ester of was isolated by distillation under reduced pressure (1 mm, 98^{o}C) after initial recovery of methanol. Yield: 143.5 gm, 87%.

Figure 1: Reaction scheme for preparation of lead compound and its analogs (7-7j).



Standard process for the preparation of α-cyclohexyl-α-hydroxybenzeneacetic acid methyl ester (6) and its analogs.

Magnesium turnings (15.8 gm) and iodine (200 mg.) were added to tetrahydrofuran (73.0 gm) under nitrogen atmosphere and the mixture was stirred at about 25°C for 0.5 hour. Initially cycloxexyl bromide (4.1 gm, 0.025 mol) was added drop wise followed by tetrahydrofuran (292.0 gm) was added and remaining cyclohexyl bromide (86 gm, 0.53 mol) was added drop wise at 60-70°C. The mixture was stirred at 60-70°C and reaction completion was confirmed by GC. The mixture was cooled to 20-30°C and this Grignard solution was added drop wise to a mixture of α-oxo-benzeneacetic acid methyl ester (82.1 gm, 0.5 mol) and tetrahydrofurane (82.0 mL) at 5-15°C. The reaction mass further stirred for 1 hour and reaction completion was confirmed by HPLC for absence of α-oxo-benzeneacetic acid methyl ester. Tetrahydrofurane was evaporated under reduced pressure at 65-80°C and toluene (150.0 mL) was added. This mixture was then added drop wise at < 35°C to 7N hydrochloric acid (215.0 mL) and allowed to stir for 0.5 hour. The layers were settled, organic layer containing product was separated, cooled to 0-5°C and product isolated by filtration. The product was further purified by crystallization from ethyl acetate. Yield: 80.7 gm, 65%.

Standard process for the preparation of α-cyclohexyl-α-hydroxy-benzeneacetic acid-4-(diethyl amino)-2-butynyl ester hydrochloride (7) and its analogs (7a-7j).

To the solution of α-cyclohexyl-α-hydroxybenzeneacetic acid methyl ester (160 gm, 0.65 mol) in n-heptane (800 mL) was added solution of 4-diethylamino-2-butynyl acetate (130 gm, 0.71 mol) in *n*-heptane (800 mL) followed by sodium methoxide (8.0 gm) at 25-30°C. Heat the reaction mass to 90-95°C when distillation of methyl acetate and n-heptane mixture starts. This is continued further for 3 hours. Reaction completion was confirmed by HPLC and added *n*-heptane (400 mL) followed by water (400 mL). The reaction mass stirred for 10 minutes, layers settled and organic layer containing product was separated and washed further with water (100 mL). The organic layer was then extracted with 10 % hydrochloric acid solution (4 x 100 mL) and combined acidic aqueous layer was subjected to chilling (0 ± 5°C) when product is precipitated as hydrochloride salt. The product slurry stirred for 1 hour at same temperature, filtered and product dried at 40-45°C under reduced pressure (120 mm/Hg). The crude product is purified by crystallization from ethyl acetate to isolate pure product as off white crystalline powder. Yield 218.5 gm, 86.4 %.

Test Protocol: Analgesic activity

All the synthesized compounds were screened for their analgesic activity by reduction in acetic acid induced writhing method [18] on albino mice. Albino mice (avg. wt. 25 g.) were divided in eight groups with five mice each. Each mice was tested for their writhing response to acetic acid (0.3 mL of 0.6 % v/v, i.p.), which was considered as initial writhing counts. After washout period of seven days same mice were fastened for 18 h and used for screening of test and standard compounds. One group was kept as control group and received only water, second group was kept as standard and received diclofenac sodium suspension (1% sodium CMC) at a dose level of 50 mg/Kg p.o., remaining six groups received individual test compounds in suspension (1% sodium CMC) form at a dose level of 50 mg/Kg p.o. After 60 mins each mice was administered 0.3 mL of 0.6 % v/v acetic acid solution intraperitonially and after five minutes writhings were recorded for 15 mins. Difference between initial writhing and after treatment was used for calculation of % activity.

Test Protocol: Anti-inflammatory activity

Inhibition in carageenan induced rat paw edema method developed by Winter [19] was used. Albino rats of either sex (150-200 g) were divided in five groups with five animals in each group. Rats were deprived of food for 12 h prior to experiment. First group was used as control and received only water, second group received diclofenac sodium suspension (1 % sodium CMC) at a dose level of 50 mg/Kg p.o., remaining three groups received individual test compounds in suspension (1 % sodium CMC) form at a dose level of 50 mg/Kg p.o. After an hour carageenan suspension 0.1 mL (1 % w/v in sodium CMC) was injected into sub plantar region of the left hand paw of the rat. Immediately the paw volume was measured using plethismometer (initial paw volume). After 3 h paw volume was again measured. Difference between subsequent reading and initial reading was used for calculation of % reduction.

Test Protocol: Ulcerogenic potential

Gastric ulceration is considered to be main side effect of NSAIDs. So it was important to check ulcerogenic potential of the most potent compound "7", "7i" and "7j".

Albino rats of either sex (160-180 g) were divided into three groups of three rats each. The animals were deprived of food for 12 h before administration of drug. First group was control group and received only water, second group received diclofenac sodium suspension (1 % sodium CMC) at a dose level of 50 mg/Kg p.o. and third group received compounds "7", "7i" and "7j", in suspension (1 % sodium CMC) form

at a dose level of 50 mg/Kg p.o. After six hours animals were sacrificed and stomach was cut opened along the greater curvature and examined for haemmorhages and ulcers [20]. Ulcer index was calculated.

RESULTS AND DISCUSSIONS

Chemistry

Structurally diverse analogs of lead compound were prepared following the synthetic protocol described in experimental section as presented in Table 1. All the novel analogs of lead compound were characterized by Mass (M/Z), FTIR (Table 2) and ^{1}H NMR (Table 3) techniques and supported by physical data (Table 2).

Table 1: Analogs of lead molecule (7-7j)

Product	R1	R2	R3	R4	R5	n	R*	R**
7	H	H	H	H	H	0	$-C_2H_5$	$-C_2H_5$
7a	H	H	H	H	H	2	$-C_2H_5$	$-C_2H_5$
7b	H	H	H	H	H	3	$-C_2H_5$	$-C_2H_5$
7c	H	CH_3	H	H	H	2	$-C_2H_5$	$-C_2H_5$
7d	CH_3	CH_3	H	H	H	2	$-C_2H_5$	$-C_2H_5$
7e	H	H	H	H	H	0	$-CH(CH_3)_2$	$-CH(CH_3)_2$
7f	H	H	H	H	H	0	$-(CH_2)_3-CH_3$	$-(CH_2)_3-CH_3$
7g	H	H	H	H	H	2	$-CH(CH_3)_2$	$-CH(CH_3)_2$
7h	H	H	H	H	H	3	$-CH(CH_3)_2$	$-CH(CH_3)_2$
7i	H	H	H	H	H	2	$-CH_3$	$-CH_3$
7j	H	H	H	H	H	3	$-CH_3$	$-CH_3$

Table 2: M. P., Mass (M/Z) and FTIR spectral data of synthesized compound (7-7j)

Comp.	M.P. ^{0}C	Mass (M/Z)	IR Data
7a	143-144.5	386 (M+H)	-OH stretching (3512 cm^{-1}), aromatic C-H stretching (3095 cm^{-1}), aliphatic C-H stretching (2928 cm^{-1}),-C≡C- stretching (2071 cm^{-1}), -C=O stretching (1743 cm^{-1}), symmetric -C=O stretching (1467 cm^{-1}), asymmetrical -C-O-C and --C-O stretching (1246-1171 cm^{-1}), monosubstituted benzene stretching (two bands at770 cm^{-1} and 698 cm^{-1})
7b	161-162	400 (M+H)	-OH stretching (3318 cm^{-1}), aromatic C-H stretching (3096 cm^{-1}), aliphatic C-H stretching (2992-2845 cm^{-1}), -C≡C- stretching (2069 cm^{-1}), -C=O stretching (1756 cm^{-1}), asymmetrical -C-O-C and --C-O stretching (1246-1133 cm^{-1}), monosubstituted benzene stretching (two bands at 770 cm^{-1} and 695 cm^{-1})
7c	157-158.5	400 (M+H)	-OH stretching (3514 cm^{-1}), -NH stretching (3317 cm^{-1}), aromatic C-H stretching (3096 cm^{-1}), aliphatic C-H stretching (2992-2845 cm^{-1}), -C≡C- stretching (2070 cm^{-1}), -C=O stretching (1745 cm^{-1}), asymmetrical -C-O-C and --C-O

			stretching (1273-1161 cm^{-1}), monosubstituted benzene stretching (two bands at (770 cm^{-1} and 695 cm^{-1}), mata substituted benzene stretching (two bands at704 cm^{-1} and 781 cm^{-1})
7d	177-178.6	413 (M)	-OH stretching (3510 cm^{-1}), aromatic C-H stretching (3095 cm^{-1}), aliphatic C-H stretching (2991-2859 cm^{-1}), -C≡C- stretching (2071 cm^{-1}), -C=O stretching (1744 cm^{-1}), symmetric -C=O stretching (1467 cm^{-1}), asymmetrical -C-O-C and --C-O stretching (1246-1162 cm^{-1}), monosubstituted benzene stretching (two bands at 770 cm^{-1} and 697 cm^{-1}),mata disubstituted benzene stretching(bands at 697 $cm^{-1}$and 809cm^{-1})
7e	121-123	386 (M+H)	-OH stretching (3316 cm^{-1}), aromatic C-H stretching (3096 cm^{-1}), aliphatic C-H stretching (2992-2861 cm^{-1}), -C≡C- stretching (2069 cm^{-1}), -C=O stretching (1745 cm^{-1}), asymmetrical -C-O-C and --C-O stretching (1274-1161 cm^{-1}), monosubstituted benzene stretching (two bands at770 cm^{-1} and 695 cm^{-1})
7f	183-184.7	414 (M+H)	-OH stretching (3510 cm^{-1}), aromatic C-H stretching (3095 cm^{-1}), aliphatic C-H stretching (2928-2860 cm^{-1}), -C≡C- stretching (2069 cm^{-1}), -C=O stretching (1743 cm^{-1}), symmetric -C=O stretching (1464 cm^{-1}), asymmetrical -C-O-C and --C-O stretching (1246-1141 cm^{-1}), monosubstituted benzene stretching (two bands at 771 cm^{-1} and 703 cm^{-1})
7g	191-193	414 (M+H)	-OH stretching (3316 cm^{-1}), aromatic C-H stretching (3096 cm^{-1}), aliphatic C-H stretching (2992-2861cm^{-1}), -C≡C- stretching (2142 cm^{-1}), -C=O stretching (1746 cm^{-1}), asymmetrical -C-O-C and --C-O stretching (1274-1161 cm^{-1}), monosubstituted benzene stretching (two bands at 770 cm^{-1} and 695 cm^{-1})
7h	173-175	427 (M)	-OH stretching (3316 cm^{-1}), aromatic C-H stretching (3096 cm^{-1}), aliphatic C-H stretching (2992-2861cm^{-1}), -C≡C- stretching (2070 cm^{-1}), -C=O stretching (1744 cm^{-1}), asymmetrical -C-O-C and --C-O stretching (1274-1161 cm^{-1}), monosubstituted benzene stretching (two bands at 770 cm^{-1} and 695 cm^{-1})
7i	163-165.3	358 (M+H)	-OH stretching (3512 cm^{-1}), aromatic C-H stretching (3095 cm^{-1}), aliphatic C-H stretching (2928 cm^{-1}),-C≡C- stretching (2071 cm^{-1}), -C=O stretching (1743 cm^{-1}), symmetric -C=O stretching (1467 cm^{-1}), asymmetrical -C-O-C and --C-O stretching (1246-1171 cm^{-1}), monosubstituted benzene stretching (two bands at770 cm^{-1} and 698 cm^{-1})

7j	153-153.4	372 (M+H)	- OH stretching (3317 cm^{-1}), aromatic C-H stretching (3095 cm^{-1}), aliphatic C-H stretching (2992-2860cm^{-1}), -C≡C- stretching (2069 cm^{-1}), -C=O stretching (1744 cm^{-1}), asymmetrical -C-O-C and --C-O stretching (1274-1161 cm^{-1}), monosubstituted benzene stretching (two bands at 770 cm^{-1} and 695 cm^{-1})

Table 3: ^{1}H NMR spectral data of synthesized compound (7-7j)

Comp.	^{1}H NMR (ð)
7	Chemical Shift ð, 1.12-0.90 ppm (m, 6H, -CH_3), 1.41-1.37 ppm (m, 6H, -CH of cyclohexyl),1.57-1.48 (m, 4H, -CH of cyclohexyl),1.75-1.71 (m, 1H, -CH of cyclohexyl), 3.05-2.51 (q, 4H, -CH_2), 3.36 (s, 2H, -CH_2), 4.88 (s, 2H, -CH_2), 5.78 (s, 1H, -OH), 7.38-7.24 (m, 3H, CH-Ar), 7.57-7.55 (m, 2H, CH-Ar)
7a	Chemical Shift ð, 0.98-1.20 ppm (t, 6H, -CH_3), 1.32-1.43 (m, 2H, -CH of cyclohexyl), 1.44-1.52 (m, 8H, -CH of cycloxexyl), 2.10-2.00 (m, 1H, -CH of cyclohexyl), 2.18-2.12 (t, 2H, -CH_2), 2.31-2.25 (t, 2H, -CH_2), 2.60-2.48 (q, 4H, -CH_2), 3.05 (s, 2H, -CH_2), 4.77 (s, 2H, -CH_2), 5.52 (bs, 1H, -OH), 7.35-7.40 (d, 3H, CH-Ar), 7.73-7.52 (m, 2H, CH-Ar)
7b	Chemical Shift ð,1.24-1.10 ppm (t, 6H, -CH_3), 1.36-1.32 (m, 2H, -CH of cyclohexyl), 1.56-1.45 (m, 8H, -CH of cycloxexyl), 1.68-1.73 (m, 4H, -CH_2), 2.02-2.00 (m, 1H, -CH of cyclohexyl), 2.50 (s, 2H, -CH_2), 3.02-2.80 (q, 4H, -CH_2), 4.15 (s, 2H, -CH_2), 4.90 (s, 2H, -CH_2), 5.89 (s, 1H, -OH), 7.25-7.34 (d, 3H, CH-Ar), 7.65-7.55 (m, 2H, CH-Ar)
7c	Chemical Shift ð,1.24-1.19 ppm (t, 6H, -CH_3),1.40-1.36 ppm (m, 2H, -CH of cyclohexyl), 1.56-1.47 (m, 6H, -CH of cyclohexyl),1.66-1.80 ppm (m, 2H, -CH of cyclohexyl), 2.00-1.90 (m, 1H, -CH of cyclohexyl), 2.32-2.31 (t, 2H, -CH_2), 2.55-2.45 (t, 2H, -CH_2), 2.70 (s, 3H, -CH_3-Ar), 2.90-2.75 (m, 4H, -CH_2), 4.10 (s, 2H, -CH_2), 4.87 (s, 2H, -CH_2), 5.90 (s, 1H, -OH), 7.37-7.23 (m, 3H, CH-Ar), 7.56-7.54 (t, 1H, CH-Ar)
7d	Chemical Shift ð, 0.95-1.10 ppm (t, 6H, -CH_3), 1.36-1.23 ppm (m, 2H, -CH of cyclohexyl), 1.36-1.51 (m, 8H, -CH of cyclohexyl), 1.90-1.95 (m, 1H, -CH of cycloxexyl), 2.17-2.10 (t, 2H, -CH_2), 1.19-2.27 (t, 2H, -CH_2), 2.39 (s, 6H, -CH_3-Ar), 2.45-2.57 (q, 4H, -CH_2), 3.10 (s, 2H, -CH_2), 4.73 (s, 2H, -CH_2), 5.85 (bs, 1H, -OH), 6.83-6.95 (m, 3H, -CH-Ar),
7e	Chemical Shift ð, 1.05 (s, 12H, -CH_3), 1.19 (s, 2H, -CH of cycloxexyl), 1.40-1.61 (m, 8H, -CH of cyclohexyl), 2.35-2.12 (m, 1H, -CH of cycloxexyl), 3.00-2.91 (m, 2H, -CH), 3.15 (s, 2H, -CH_2), 4.71 (s, 2H, -CH_2), 6.75 (bs, 1H, -OH), 7.40-7.18 (bd, 5H, CH-Ar)
7f	Chemical Shift ð, 0.96 ppm (s, 6H, -CH_3), 1.25 (s, 2H, -CH of cycloxexyl), 1.36-1.70 (m, 16H, -CH of cyclohexyl, CH_2), 2.38-2.21 (m, 4H, -CH_2), 2.55-2.38 (m, 1H, -CH of cyclohexyl), 3.05 (s, 2H, -CH_2), 4.73 (s, 2H, -CH_2), 6.81 (bs 1H, -OH), 7.30-7.21 (m, 4H, CH-Ar), 7.57-7.22 (m, 1H, CH-Ar)

7g	Chemical Shift ð,1.08 (s, 12H, -CH_3),1.24-1.22 (m, 2H, -CH of cycloxexyl),1.31-1.49 (m, 8H, -CH of cyclohexyl),1.90 (s, 1H, -CH of cycloxexyl), 2.13-2.10 (t, 2H, -CH_2), 2.45-2.30 (t, 2H, -CH_2), 3.01-2.90 (m, 2H, -CH), 3.10 (s, 2H, -CH_2), 4.72 (s, 2H, -CH_2), 5.90 (s, 1H, -OH), 7.40-7.37 (m, 3H, CH-Ar), 7.62-7.57 (t, 2H, CH-Ar)
7h	Chemical Shift ð, 1.15-1.00 (d, 12H, -CH_3), 1.16-1.26 (m, 2H, -CH of cycloxexyl), 1.42-1.58 (m, 2H, -CH of cycloxexyl), 1.60-1.89 (m, 7H, -CH_2, -CH of cyclohexyl), 2.31-2.25 (t, 2H, -CH_2), 2.97 (s, 2H, -CH), 3.10 (s, 2H, -CH_2), 4.75 (s, 2H,-CH_2), 6.91 (bs, 1H, -OH), 7.50-7.25 (m, 3H, CH-Ar), 7.72-7.51 (m, 2H, CH-Ar)
7i	Chemical Shift ð, 1.27-0.90 ppm (m, 2H, -CH of cyclohexyl), 1.45-1.27 (m, 8H, -CH of cycloxexyl),1.90-1.80 (m, 1H, -CH of cyclohexyl), 2.00 (s, 6H, -CH_3), 2.30-2.12 (m, 2H, -CH_2), 2.45-2.40 (t, 2H, --CH_2), 3.05 (s, 2H, -CH_2), 4.77 (s, 2H, -CH_2), 5.50 (s, 1H, -OH), 7.29-7.26 (m, 2H, CH-Ar), 7.38-7.29 (m, 2H, -CH-Ar), 7.67-7.65 (m, 1H, CH-Ar)
7j	Chemical Shift ð, 1.24-1.10 (m, 3H, -CH of cyclohexyl), 1.50-1.25 (m, 7H, -CH of cycloxexyl), 1.54-1.75 (m, 4H, -CH_2), 1.93-1.82 (m, 1H, -CH of cyclohexyl), 2.30-2.20 (t, 2H, -CH_2), 2.27 ppm (s, 6H, -CH_3), 3.05 (s, 2H, -CH_2), 4.71 (s, 2H, -CH_2), 6.81 (bs, 1H, -OH), 7.41-7.22 (m, 3H, -CH-Ar), 7.61-7.72 (m, 2H, -CH-Ar)

Table 4: Results of Analgesic Activity, Anti-inflammatory Activity and Ulcerogenic Index

Compounds	Analgesic Activity	Anti-inflammatory Activity	Ulcer Index
Diclofenac sodium	52.75 ± 3.29	62.16 ± 2.43	0.224 ± 0.028
7 (Lead Compound)	49.16 ± 2.15	59.10 ± 2.75	0.310 ± 0.021
7a	40.36 ± 2.30	NT	NT
7b	39.20 ± 2.75	NT	NT
7c	47.36 ± 1.75	40.30 ± 1.30	NT
7d	45.11 ± 3.67	36.10 ± 2.56	NT
7e	35.33 ± 3.19	NT	NT
7f	40.35 ± 0.80	NT	NT
7g	30.35 ± 1.30	NT	NT
7h	30.40 ± 2.00	NT	NT
7i	50.18 ± 2.30	60.56 ± 3.46	0.290 ± 0.021
7j	51.48 ± 1.40	61.80 ± 2.43	0.198 ± 0.030

Note: *NT- Not Tested

Pharmacology

From the results of analgesic activity described in Table 4, it is observed that the lead compound "7" has exhibited promising analgesic activity but the potency is noted less in compounds "7a" and "7b" where an ethyl and propyl linker is incorporated prior to carbonyl carbon, (n = 2 and 3 respectively). The analgesic potency is seen enhanced with aromatic proton substitution by electron donating alkyl groups on the phenyl ring as in the compound "7c" and "7d"

that is lower against reference standard but higher within the group (>45).

Replacing two ethyl groups on amino nitrogen by isopropyl and butyl respectively in compounds "7e" and "7f" showed no benefits over potency, as seen from the table, compounds showed no encouraging results. The compounds "7g' and "7h" with two isopropyl groups on amino nitrogen and respective linker (n=2 and 3) prior to carbonyl carbon has also shown diminished potency and can be claimed as inactive. Promising results are obtained for compounds "7i" and "7j" where ethyl groups are replaced with two methyl groups on amino nitrogen and linker prior to carbonyl carbon (n= 2 and 3 respectively). These compounds are found equipotent to Diclofenac sodium.

In conclusion the lead compound "7" and compounds "7c", "7d", "7i" and "7j" have shown promising results of analgesic activity and can be evaluated further for anti-inflammatory potential.

Results of anti-inflammatory activity presented in Table 4, indicate that lead compound "7" have shown slightly reduced potency than the reference standard Diclofenac sodium. The compounds "7c" and "7d" have shown low anti-inflammatory potency contradicting their analgesic activity results. These compounds can be claimed as inactive. It's encouraging to see that compounds "7i" and "7j" have maintained consistency of results by exhibiting promising anti-inflammatory activity as shown for analgesic potency. The levels of potency were well comparable to reference standard Diclofenac sodium and are recommended for evaluation of Ulcerogenic potential.

Ulcerogenic index of compounds as in Table 4, "7" and "7i" was found to be higher than the standard drug while results for compound "7j" are promising with index found significantly less than Diclofenac sodium.

CONCLUSION

Based on test results, a definite structure-activity relationship could be established. The compounds where two ethyl groups on amino nitrogen are replaced with isopropyl or butyl have shown reduced potency but replacing ethyl with methyl has led compound potent. The compounds where ethyl or propyl linker is incorporated (n=2 or 3) have not exhibited promising results except when two methyl groups are replaced on amino nitrogen.

Within the limits of our study described in this paper, compound "7j" is claimed as a promising candidate with desired analgesic, anti-inflammatory potency and with lower ulcerogenic index which suggest its less GI side effects. The compound is recommended for further evaluation of its formulation potential.

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