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Original Research Paper

NOVEL FUSED SYSTEM ANTI-INFLAMMATORY AGENTS WITH LOW ULCEROGENECITY PROFILE

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ABSTRACT

A main objective of the present research study is to synthesize series of novel fused 8-substituted 3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one derivatives and to evaluate them for their anti-inflammatory and ulcerogenic effect. The stated compounds were synthesized with the initial reaction of various aromatic aldehydes and N-acetylglycine in acetic anhydride and anhydrous sodium acetate by Erlenmeyer-Azlactone synthesis to give oxazol-5(4H)-one derivatives which on reaction with substituted phenylhydrazine in dry benzene gave 1H-imidazol-5(4H)-one derivatives and lastly condensation with chloroacetamide in N,N-dimethylformamide gave stated compounds. All synthesized compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, LC-MS and HPTLC etc and are well supported by spectroscopic data. The anti-inflammatory and ulcerogenic activities were compared with standard drug indomethacin and synthesized compounds were found to be less ulcerogenic as compared with standard drug. Compounds belonging to series 7 are more active than series 5 and former are less ulcerogenic than standard drug.

Keywords: NSAID's, Erlenmeyer azlactone synthesis, Anti-inflammatory, Ulcerogenecity.

INTRODUCTION

Literature survey revealed that imidazole and triazine heterocycles are important building blocks for development of novel medicinal, pharmaceutical and agricultural agents. Most classes of heterocyclic medicinal compounds contain imidazole ring in their chemical structure such as antioxidant agents[1], anticancer agents[2], antifungal[3], antibacterial[4], antiprotozoal.[5] Coming to the triazine nucleus, it has very importance because of its effectiveness in most of chemical compounds such as drugs, polymers, resins, agricultures and optics.[6-10] Triazine based complexes of copper has superoxide radical scavenging effect in pathological cascade.[11] Various novel fused 1,2,4-triazine aryl analogues antitumor effect[12], ethanol induced antistress effect in mouse brain[13], and adenosine receptor antagonist.[14] The literature survey revealed that when dynamically active 2 hetocycles couples with each other, new molecule development occurs and which can display synergistic effect on biological systems. This encouraged us to synthesize imidazole ring clamped with triazine ring could give entrance to novel anti-inflammatory agents.

Therefore an attempt has been made to synthesize newer 4,8-disubstituted-3,4-dihydro-6-methyl-imidazo[1,5-b][1,2,4]triazin-2(8H)-one derivatives as antioxidants. It is now world wide known that some of selective COX2 inhibitors like rofecoxib are already withdrawn from the market due to their unexpected CVS adverse effects[15], hypersensitivity and higher blood pressure.[16] So development of NSAID's with safety profile is still a challenge for pharmaceutical field. Classical NSAID's like aspirin, indomethacin are non-selective with respect to COX1 and COX2. Aspirin is more selective for COX1 than COX2 and selective inhibition of COX1 reduces production of PGE2 and PGI2 which results in gastrointestinal ulcerations and ulcerogenic effect.[17] So it is clear that neither selective COX2 inhibition nor Selective COX1 inhibition is beneficial. This led to way for development of the balanced COX1/COX2 inhibitor which may produce less or no ulcerogenic effect and may be devoid of fatal CVS effects.

MATERIALS AND METHODS

General

Animal experiments are performed as per OECD guidelines and all protocols were sanctioned by IAEC having number CPCSEA/IAEC/INV/31/2012. All reagents were used as purchased from E. Merck and used without further purification. Melting points were determined by using a Remi digital melting point determination apparatus and are uncorrected. Purity of compounds were checked by high performance thin-layer chromatography (HPTLC) and was performed on CAMAG twin with applicator Linomat-IV and plate specifications are Merck precoated silica gel 60 F_{254} with 0.2 mm thickness. Spectroscopic data were recorded by using FT-IR (Shimadzu spectrophotometer 8400 using KBr), ^{1}H NMR (Varian Mercury 400, Model- Unity AS400, serial- S0121719, frequency 400 MHz using DMSO as a solvent and tetramethylsilane (TMS) as an internal standard and chemical shifts were expressed as δ values in ppm), ^{13}C NMR (INOVA-300 with 75 MHz frequency DMSO as a solvent and tetramethylsilane (TMS) as an internal standard), LC-MS (Benchtop Agilent 1100 series LC–MSD (Agilent Technologies, Waldbronn, Germany), Column: C18, preparation on ODS (octadecylsilica) Hypersil column (Agilent Technologies), Flow-rate was 0.25 mL/min to 0.50 mL/min). Anti-inflammatory activity was performed using digital Plethysmometer (Panlab LE 7500) and carrageenan induced rat hind paw edema method. Ulcerogenic studies were performed using a method reported by Cioli.

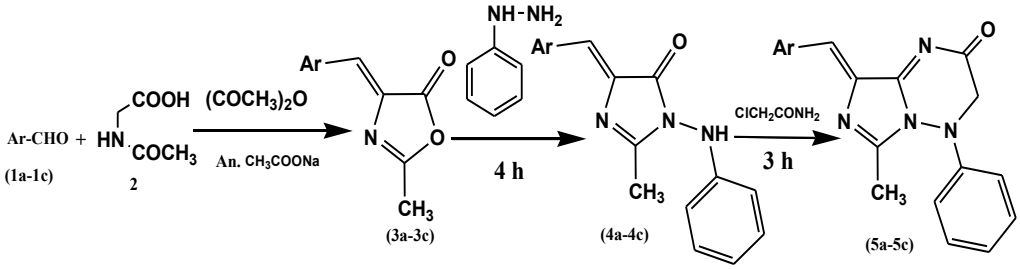
Experimental

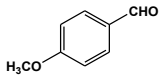
a. Typical procedure for synthesis of compounds (3a-3c) by Erlenmeyer-Azlactone synthesis [18]:

Warm a mixture of 29 g (0.25 mol) of N-acetylglycine, 37.5 ml (0.37 mol) of aromatic aldehydes (1a-1c), 15 gm (0.183 mol) of anhydrous sodium acetate and 59 mL (0.62 mol) of acetic anhydride in 500 mL flask equipped with a reflux condenser, on water bath with occasional shaking until solution is complete (10-20 min). Boil the resulting solution for 1 h, cool and leave in a refrigerator overnight. Stir the solid mass of yellow crystals with 60 mL of cold water, transfer to a Buchner funnel and wash well with cold water. Wash with a little ether. Crystallized from carbon tetrachloride and used for next step of synthesis.

b. Typical procedure for synthesis of compounds (4a-4c), (6a-6c):

A solution of 3a-3c (6 mmole) in dry benzene (30 mL) and phenylhydrazine, 2,4-dinitro phenylhydrazine, 4-fluoarophenylhydrazine (5 mmole) was heated under reflux for 4 h. Then the mixture was poured upon water. The precipitated solid was filtered off, dried and crystallized from ethanol to get the desired compounds.



a. 
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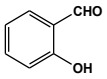
b. 
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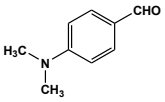
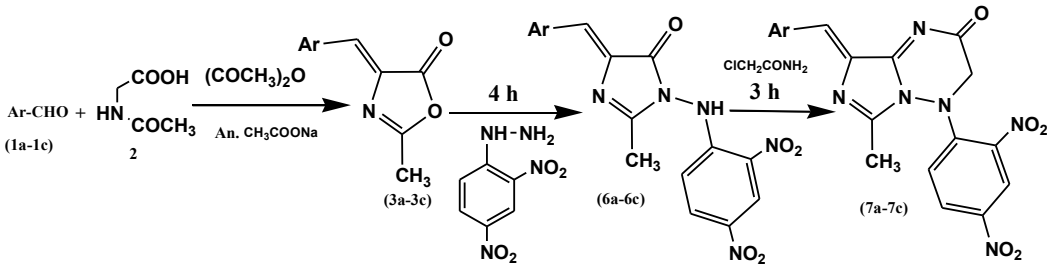
c. 
4-dimethylaminobenzaldehyde

Scheme: 1

c. *Typical procedure for synthesis of compounds (5a-5c), (7a-7c):*

A solution of 4a-4c, 6a-6c (8 mmole) and chloroacetamide (8 mmole) was refluxed for 3 h in boiling N, N-dimethylformamide (30 mL). Then the mixture was poured into water. The precipitated solid was filtered off, dried and crystallized from ethanol to get the desired compounds.



Scheme: 2

Acute Toxicity Studies[19]

Swiss albino mice of either sex weighing 20–25 g were used for acute toxicity and analgesic activity. Animals were housed under standard environmental conditions of temperature (24 ± 1°C) and relative humidity of 30-70 %. A 12:12 hr light dark cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) constituted in accordance with the guidelines of the committee for the purpose of control and supervision of experiment on animals (CPCSEA), government of India. The acute toxicity test aims at establishing the therapeutic index, i.e. the ratio between pharmacologically effective dose and the lethal dose on the same strain and species (LD_{50}/ED_{50}). The greater is the therapeutic index; the drug will be safer. Healthy adult albino mice (20–25 g) of either sex, starved overnight were subjected to acute toxicity studies as per guidelines (AOT No. 425) suggested by Organization for Economic Co-operation and Development (OECD) 2001. The mice were observed continuously for 2 hr for behavioral, neurological and autonomic profiles for any lethality or death for next 48 hr. Based on the results obtained from this study the doses for further pharmacological studies were fixed. The synthesized compounds did not produce any toxicity (morbidity and mortality) up to dose level of 1000 mg/kg body weight for all three series. So, we arbitrarily selected 100 mg/kg as the dose of the test compounds and 20 mg/kg as the dose of the standard compound (indomethacin) for anti-inflammatory activity.

Anti-Inflammatory Activity[20]

Anti-inflammatory activity of compounds (5a-5c, 7a-7c) was determined by observing specific suppression of signs of typical inflammatory reaction induced experimentally in laboratory animals. To mimic the clinical condition, anti-inflammatory activity testing was carried out using carrageenan induced rat paw edema method.

Preparation of Solution:

0.1 % of NaCl in Non-deionized distilled water containing 15 drops of triton/liter of solution.

Preparation and Administration of Doses:

The suspensions of test and standard drug (indomethacin) were prepared in 2 % gum acacia mucilage. The test compounds were administered orally using gastric feeding tube. The dose of 1 ml/100 gm body weight of all test materials was

given to the rats as per the procedure explained below.

Procedure:

Wister rats of either sex weighing 175 to 200 g were used in the anti-inflammatory. Rats were divided into groups of six (mean weight 185 g) each. They were starved overnight with water *ad libitum* prior to the day of the experiment. Control group received 0.5 ml of 2 % gum acacia mucilage, orally, while standard group received 100 mg/kg body weight of indomethacin and test groups received 100 mg/kg of test compounds of sample. All the doses were given by oral route. One hr after drug administration, 0.1 ml of 1 % carrageenan in normal saline was injected into the sub plantar region of left leg paw. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema volume was measured with the help of Digital Plethysmometer LE 7500 by solution displacement method. The reading was taken at zero hr immediately after carrageenan injection and then at 0, 1, 2, 3, 4 & 5 hr. The difference between 0 hr and subsequent readings was taken as actual edema volume.

The percentage inhibition of edema in the various treated groups was calculated by using the formula

% Inhibition = Control - Test/ Control x 100

The same procedure was repeated on rats of another group of six each (mean weight 185 g) for each series of test compounds using indomethacin 100 mg/kg as reference standard.

Statistical analysis:

The anti-inflammatory activity data obtained was analyzed by one-way ANOVA followed by Dunnett's test. The results were expressed as mean ± standard error of mean (SEM) for each group, $p<0.05$ were considered as statistically significant.

Ulcerogenecity Studies[21]:

Gastrointestinal side effects constitute the most frequent of all the adverse reactions of NSAIDs. Hence it was considered necessary to investigate the ulcerogenic activity of synthesized compounds.

Methodology:

Wister rats of either sex weighing 175 to 200 g were used in the ulcerogenecity study. Acute ulcerogenesis test was performed according to Cioli *et al.* Albino rats of either sex were divided into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after oral administration of test compounds (100 mg/kg for all the compounds) and standard compounds indomethacin (100 mg/kg) as well as celecoxib (10 mg/kg). Control rats received 0.5 ml of 2 % gum acacia mucilage as vehicle. Rats were fasted for 24 hr before dosing, with water *ad libitum*. After the drug treatment, the rats were fed normal diet for 17 h and then sacrificed by ether inhalation. The stomach was cut along the greater curvature and washed with distilled water and cleaned gently by dipping in saline. The mucosal damage was examined by means of a magnifying glass and was assessed according to the following scoring system:

0.5 Redness, 1.0 Spot ulcers, 1.5 Hemorrhagic streaks, 2.0 Ulcers >3 mm, but ≤5 mm, 3.0 Ulcers >5 mm.

The difference between the mean score of each treated group and the mean score of control group was regarded as severity index of gastric mucosal damage. Histopathological studies were performed using stomach specimens which were removed and transferred into 10 % formalin solution. The samples were then subjected for further microscopic examination.

RESULTS

All the results of study are shown in tables and figures. Results of anti-inflammatory activity are shown in table 5 and it reveals that compound 7b has minimum paw volume amongst all compounds except standard drug. The results are shown in table 6. Histopathological report showed from figure 1 indicates gross evidence of tiny pin head hemorrhages at multiple places for indomethacin. The test compounds 7b do not show such visible hemorrhagic spots. Microscopic examination of indomethacin showed tiny mucosal ulcerations with fresh hemorrhages. Sections from 7b showed congested blood vessels. However there is no evidence of

ulcerations or hemorrhages in the sections. So section from 7b were within normal limits.

DISCUSSION AND CONCLUSION

Total 6 final compounds and total 15 compounds including intermediates were synthesized by initiating a reaction between aromatic aldehydes (1a-1c) and N-acetyl glycine (2) to get 4-substituted-2-methyloxazol-5(4H)-one which on condensation with phenylhydrazine derivatives for 4h in second step and with chloracetamide for 3h in third step gave final proposed compounds. The novelty of the final compounds was confirmed through databases search facility of STN international. All compounds including intermediate were characterized by physicochemical parameters such as MP, HPTLC (R_f value) and spectroscopic methods like IR, H-NMR, C-NMR, LC-MS etc and all compounds showed best correlation with the same. From table 5, it is clear that compounds belonging to series 7 are more active as anti-inflammatory agents than that of series 5, this may be because of presence of electron withdrawing nitro group in series 7 compounds. A decreasing order of average % inhibition for anti-inflammatory activity for series 7 compounds is 7b>7a>7c and for series 5 compounds is 5b>5a>5c. This may be due to +I effect of hydroxyl group present in compound 7b & 5b. Amongst series 7, compound 7b is most active with average % inhibition of 16.02 and amongst series 5, compounds 5b is most active with average % inhibition of 12.70 , while for standard drug, indomethacin, it was 33.71. While comparing ulcerogenecity effect, compound 7b showed less ulcer index than standard drug indomethacin, this may be because of presence of nitro group in series 7 compounds which may nitric oxide (NO) donating ability. Tested compound might have donated NO which in turn modulate various physiological functions in the digestive system and have resulted in gastric protective action.[22] So the lead compounds synthesized can definitely serve as template or lead for further development anti-inflammatory drugs with low ulcerogenecity profile. In future cardioprotective effects of these compounds are taken into consideration.

Table 1: Physical data of intermediate compounds of series 3, 4 & 6

Code	Chemical Name	Mol. Form.	Mol. Wt.	MP (°C)	% Yield
3a	4-(3-methoxybenzylidene)-2-methyloxazol-5(4H)-one	$C_{12}H_{11}NO_3$	217.22	195-197	88
3b	4-(2-hydroxybenzylidene)-2-methyloxazol-5(4H)-one	$C_{11}H_9NO_3$	203.19	305-307	89
3c	4-[4-(dimethylamino)benzylidene]-2-methyloxazol-5(4H)-one	$C_{13}H_{14}N_2O_2$	230.26	82-85	82
4a	5-(3-methoxybenzylidene)-2-methyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one	$C_{16}H_{17}N_3O_2$	307.34	176-178	83
4b	5-(2-hydroxybenzylidene)-2-methyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one	$C_{17}H_{15}N_3O_2$	293.31	90-92	72
4c	5-[4-(dimethylamino)benzylidene]-2-methyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one	$C_{19}H_{20}N_4O$	320.38	109-111	69
6a	1-(2,4-dinitrophenylamino)-4-(4-methoxybenzylidene)-2-methyl-1H-imidazol-5(4H)-one	$C_{18}H_{15}N_5O_6$	397.34	223-224	53
6b	1-(2,4-dinitrophenylamino)-4-(2-hydroxybenzylidene)-2-methyl-1H-imidazol-5(4H)-one	$C_{17}H_{13}N_5O_6$	383.31	190-193	67
6c	1-(2,4-dinitrophenylamino)-4(4-(dimethylamino)benzylidene)-2-methyl-1H-imidazol-5(4H)-one	$C_{19}H_{18}N_6O_5$	410.38	87-89	59

Table 2: Physical data of final compounds of series 5 & 7

Code	Chemical Name	Mol. Form.	Mol. Wt.	MP (°C)	% Yield
5a	8-(3-methoxybenzylidene)-3,4-dihydro-6-methyl-4-phenylimidazo[1,5-b][1,2,4]triazin-2(8H)-one	$C_{20}H_{18}N_4O_2$	346.38	120-122	90
5b	8-(2-hydroxybenzylidene)-3,4-dihydro-6-methyl-4-phenylimidazo[1,5-b][1,2,4]triazin-2(8H)-one	$C_{19}H_{16}N_4O_2$	332.35	82-83	79
5c	8-[4-(dimethylamino)benzylidene]-3,4-dihydro-6-methyl-4-phenylimidazo[1,5-b][1,2,4]triazin-2(8H)-one	$C_{21}H_{21}N_5O$	359.42	75-77	81
7a	8-(4-methoxybenzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one	$C_{20}H_{16}N_6O_6$	436.37	188-189	61
7b	8-(2-hydroxybenzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one	$C_{19}H_{14}N_6O_6$	422.35	82-83	89
7c	8-(4-chlorobenzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one	$C_{21}H_{19}N_7O_5$	449.41	210-211	56

Table 3: Spectral interpretation of intermediate compounds of series 3, 4 & 6

Code	Structure	IR (KBr, ν_{max}, cm^{-1})	^1H NMR (δ, ppm, DMSO-d6, 400 MHz)	^{13}C NMR (δ, ppm, DMSO-d6, 75 MHz)	LC-MS (m/z): $[M^+ +1]$
3a	4-OCH_3-C_6H_4	881 (C-H bend), 1170 (C-O str), 1300 (C-N str), 1509 (C=C str), 1680 (C=N str), 1772 (C=O str), 2902 (CH_3 str), 3068 (C-H str)	2.34 (s, 1H, CH_3), 3.73 (s, 1H, OCH_3), 6.75 (d, 1H, ArH), 6.81 (s, 1H, ArH), 6.86 (d, 1H, ArH), 7.10 (t, 1H, ArH), 7.64 (s, 1H, CH).	--	--
3b	2-OH-C_6H_4	855 (C-H bend), 1294 (C-O str), 1355 (C-N str), 1520 (C=C str), 1605 (C=N str), 1671 (C=O str), 2970 (CH_3 str), 3041 (C-H str), 3324 (O-H str)	1.42 (s, 1H, CH_3), 6.68 (s, 1H, CH), 6.71 (d, 1H, ArH), 6.77 (t, 1H, ArH), 6.97 (t, 1H, ArH), 7.13 (d, 1H, ArH), 11.32 (s, 1H, OH)	--	--
3c	4-$N(CH_3)_2$-C_6H_4	802 (C-H bend), 1263 (C=C str), 1544 (C=N str), 1637 (C=N str), 1759 (C=O str), 2967 (CH_3 str), 3077 (C-H str)	2.25 (s, 1H, CH_3), 2.85 (s, 6H, 2 X CH_3), 6.58 (s, 1H, CH), 7.14 (d, 2H, ArH), 7.65 (d, 2H, ArH)	--	--
4a	4-OCH_3-C_6H_4	851 (C-H bend), 1334 (C-N str), 1519 (C=C str), 1634 (C=N str), 1723 (C=O str), 3032 (CH_3 str), 3134 (C-H str), 3485 (N-H str)	2.34 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 6.65 (d, 1H, ArH), 6.66 (t, 1H, ArH), 6.71 (d, 2H, ArH), 6.81 (s, 1H, ArH), 6.86 (d, 1H, ArH), 7.10 (t, 1H, ArH), 7.19 (t, 2H, ArH), 7.56 (s, 1H, CH), 7.94 (s, 1H, NH)	20.87, 55.09, 108.04, 112.67, 113.02, 113.85, 118.45, 119.54, 129.63, 129.73, 130.40, 137.54, 144.76, 161.89, 166.43	308.92
4b	2-OH-C_6H_4	884 (C-H bend), 1133 (C-O str), 1231 (C-N str), 1617 (C=C str),	2.13 (s, 3H, CH_3), 6.21 (s, 1H, NH), 6.32 (s, 1H, CH), 6.64 (d, 3H, ArH), 6.74 (t,	21.31, 108.55, 113.31, 115.86, 116.12, 119.21, 121.23, 127.81, 129.65,	294.37

		1637 (C=N str), 1747 (C=O str), 2971 (CH_3 str), 3128 (C-H str), 3431 (N-H str), 3674 (O-H str)	2H, ArH), 6.96 (t, 1H, ArH), 7.17 (d, 1H, ArH), 7.19 (t, 2H, ArH), 11.71 (s, 1H, OH)	130.61, 144.72, 151.81, 158.91, 166.13	
4c	4-$N(CH_3)_2$-C_6H_4	889 (C-H bend), 1341 (C-N str), 1519 (C=C str), 1631 (C=N str), 1752 (C=O str), 2969 (CH_3 str), 3069 (C-H str), 3431 (N-H str)	2.24 (s, 3H, CH_3), 2.85 (s, 6H, 2 × CH_3), 6.29 (s, 1H, NH), 6.55 (d, 2H, ArH), 6.63 (d, 2H, ArH), 6.69 (s, 1H, CH), 6.71 (d, 2H, ArH), 7.15 (t, 1H, ArH)	20.93, 40.87, 108.98, 113.93, 114.65, 119.45, 124.65, 127.35, 129.46, 130.76, 144.24, 149.67, 151.87, 166.13	321.77
6a	4-OCH_3-C_6H_4	887 (C-H bend), 1239 (C-O str), 1387 (C-N str), 1612 (C=C str), 1627 (C=N str), 1745 (C=O str), 2971 (CH_3 str), 3067 (C-H str), 3410 (N-H str)	2.36 (s, 3H, CH_3), 6.31 (s, 1H, CH), 6.76 (d, 2H, ArH), 6.81 (s, 1H, NH), 6.82 (t, 1H, ArH), 7.14 (t, 2H, ArH), 7.23 (d, 1H, ArH), 8.52 (d, 1H, ArH), 9.04 (s, 1H, ArH)	21.31, 108.55, 113.31, 115.86, 116.12, 119.21, 121.23, 127.81, 129.65, 130.61, 144.72, 151.81, 158.91, 166.13	397.34
6b	2-OH-C_6H_4	849 (C-H bend), 1261 (C-O str), 1324 (C-N str), 1672 (C=C str), 1672 (C=N str), 1763 (C=O str), 2966 (CH_3 str), 3041 (C-H str), 3476 (N-H str), 3623 (OH str)	2.54 (s, 3H, CH_3), 6.43 (s, 1H, CH), 6.64 (s, 2H, NH), 6.69 (d, 1H, ArH), 6.74 (t, 1H, ArH), 6.94 (t, 1H, ArH), 7.18 (d, 1H, ArH), 7.21 (d, 1H, ArH), 8.51 (d, 1H, ArH), 9.03 (s, 1H, ArH), 11.72 (s, 1H, ArH)	20.93, 40.87, 108.98, 113.93, 114.65, 119.45, 124.65, 127.35, 129.46, 130.76, 144.24, 149.67, 151.87, 166.13	383.31
6c	4-$N(CH_3)_2$-C_6H_4	861 (C-H bend), 1306 (C-N str), 1548 (C=C str), 1635 (C=N str), 1764 (C=O str), 2979 (CH_3 str), 3031 (C-H str), 3410 (N-H str)	2.39 (s, 3H, CH_3), 2.88 (s, 6H, 2 × CH_3), 6.19 (s, 1H, NH), 6.34 (s, 1H, CH), 6.55 (d, 2H, ArH), 7.11 (d, 2H, ArH), 7.18 (d, 1H, ArH), 8.64 (d, 1H, ArH), 9.02 (d, 1H, ArH)	20.76, 108.87, 113.24, 119.35, 127.89, 128.78, 129.65, 130.76, 133.23, 134.21, 144.87, 151.09, 166.12	410.38

Table 4: Spectral interpretation of final compounds of series 5 & 7

Code	Structure	IR (KBr, ν_{max}, cm^{-1})	^1H NMR (δ, ppm, DMSO-d6, 400 MHz)	^{13}C NMR (δ, ppm, DMSO-d6, 75 MHz)	LC-MS (m/z): $[M^+ +1]$
5a	4-OCH_3-C_6H_4	876 (C-H bend), 1365 (C-N str), 1523 (C=C str), 1633 (C=N str), 1745 (C=O str), 2872 (=CH_2 str, sym), 2902 (=CH_2 str, asym), 3054 (CH_3 str), 3121 (C-H str)	2.34 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 4.17 (s, 2H, CH_2), 6.62 (s, 1H, CH), 6.66 (d, 1H, ArH), 6.67 (d, 2H, ArH), 6.72 (t, 1H, ArH), 6.80 (s, 1H, ArH), 6.85 (d,1H, ArH), 7.11 (t, 1H, ArH), 7.19 (t, 1H, ArH)	21.30, 56.65, 62.40, 102.62, 113.65, 119.65, 123.26, 126.86, 127.70, 128.26, 128.62, 129.43, 136.20, 138.26, 144.70, 151.42, 164.86, 200.56	347.64
5b	2-OH-C_6H_4	885 (C-H bend), 1110 (C-O str), 1223 (C-N str), 1617 (C=C str), 1631 (C=N str), 1764 (C=O str), 2928 (=CH_2 str, sym), 2851 (=CH_2 str, asym), 3074 (CH_3 str), 3164 (C-H str), 3613 (O-H str)	2.82 (s, 3H, CH_3), 4.23(s, 2H, CH_2), 6.64 (d, 3H, ArH), 6.78 (t, 1H, ArH), 6.89 (t, 1H, CH & 2H, ArH), 6.93 (t, 1H, ArH), 7.18 (d,1H, ArH), 7.22 (d, 1H, ArH), 11.72 (s, 1H, ArH)	21.38, 62.55, 102.66, 113.21, 116.12, 115.81, 119.23, 121.33, 127.11, 127.98, 129.21, 129.99, 144.72, 151.44, 158.31, 164.56, 200.21	333.16

5c	4-N(CH_3)$_2$-C_6H_4	878 (C-H bend), 1358 (C-N str), 1528 (C=C str), 1628 (C=N str), 1767 (C=O str), 2931 (=CH_2 str, sym), 2847 (=CH_2 str, asym), 3036 (CH_3 str), 3127 (C-H str), 3431 (N-H str)	2.67 (s, 3H, CH_3), 2.82 (s, 6H, 2 × CH_3), 4.14 (s, 3H, CH_2), 6.57 (d, 2H, ArH), 6.67 (s, 1H, CH), 6.68 (d, 2H, ArH), 6.72 (t, 1H, ArH), 7.13 (d, 2H, ArH), 7.19 (t, 2H, ArH)	21.76, 40.78, 62.87, 102.76, 113.98, 114.45, 119.23, 124.73, 127.00, 127.96, 129.67, 144.72, 148.93, 151.27, 164.29, 200.37	360.28
7a	4-OCH_3-C_6H_4	884 (C-H bend), 1231 (C-O str), 1376 (C-N str), 1616 (C=C str), 1644 (C=N str), 1753 (C=O str), 2915 (=CH_2 str, sym), 2951 (=CH_2 str, asym), 2979 (CH_3 str), 3057 (C-H str)	2.57 (s, 3H, CH_3), 4.19 (s, 2H, CH_2), 6.66 (s, 1H, CH), 6.73 (d, 2H, ArH), 6.82 (t, 1H, ArH), 7.11 (t, 2H, ArH), 7.22 (d, 1H, ArH), 8.51 (d, 1H, ArH), 9.03 (s, 1H, ArH)	21.03, 55.00, 62.04, 110.00, 117.98, 118.95, 119.98, 123.00, 129.05, 130.98, 131.00, 144.81, 151.87, 155.98, 164.92, 200.09	436.37
7b	2-OH-C_6H_4	861 (C-H bend), 1274 (C-O str), 1372 (C-N str), 1561 (C=C str), 1692 (C=N str), 1762 (C=O str), 2837 (=CH_2 str, sym), 2915 (=CH_2 str, asym), 2972 (CH_3 str), 3061 (C-H str), 3634 (OH str)	2.34 (s, 3H, CH_3), 4.18 (s, 2H, CH_2), 6.67 (d, 1H, ArH), 6.77 (t, 1H, ArH), 6.81 (s, 1H, CH), 6.92 (t, 1H, , ArH), 7.17 (d, 1H, ArH), 7.21 (d, 1H, ArH), 8.51 (d, 1H, ArH), 9.04 (s, 1H, ArH), 11.79 (s, 1H, OH)	20.65, 61.44, 102.75, 116.98, 117.54, 119.21, 121.24, 127.11, 127.89, 129.88, 132.65, 139.98, 143.23, 144.28, 158.87, 166.43, 164.56, 200.45	422.35
7c	4-N(CH_3)$_2$-C_6H_4	756 (C-Cl str), 816 (C-H bend), 1265 (C-N str), 1529 (C=C str), 1662 (C=N str), 1765 (C=O str), 2845 (=CH_2 str, sym), 2931 (=CH_2 str, asym), 2967 (CH_3 str), 3087 (C-H str)	2.34 (s, 3H, CH_3), 4.19 (s, 2H, CH_2), 6.63 (s, 1H, CH_3), 7.18 (d, 1H, ArH), 7.22 (d, 2H, ArH), 7.23 (d, 2H, ArH), 8.52 (d, 1H, ArH), 9.03 (s, 1H, ArH)	21.39, 61.98, 102.76, 115.14, 119.23, 127.00, 127.89, 128.12, 129.23, 132.87, 133.76, 134.23, 139.27, 143.98, 144.98, 164.23, 200.12	440.79

Table 5: Anti-inflammatory activity [paw volume (mL) & % inhibition] of final series 5 & 7

Code	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2
Control	0.56	NA	1.81	NA	2.31	NA	1.37	NA	1.66	NA	1.25	NA
5a	0.52	7.14	1.59	12.15	2.06	10.82	1.24	9.48	1.47	11.44	1.56	9.30
5b	0.51	7.14	1.59	12.15	1.94	16.01	1.15	**16.05**	1.47	13.85	1.53	11.04
5c	0.54	3.57	1.6	11.60	2.07	10.39	1.25	8.75	1.49	10.24	1.57	8.70
7a	0.51	8.92	1.56	13.81	1.97	14.71	1.14	**16.70**	1.4	15.66	1.49	13.37
7b	0.51	10.71	1.58	12.70	1.98	14.28	1.14	16.70	1.42	14.45	1.25	**27.32**
7c	0.51	8.92	1.57	13.25	2.02	12.54	1.14	16.70	1.48	13.85	1.51	12.20
Std.	0.49	12.50	0.94	48.06	0.98	57.57	1.1	19.70	1.14	31.32	1.15	33.14

n=6, significant at the level of $^{**}p<0.0001$ (indicated by bold digits) as compared to control.
A1, B1, C1, D1, E1, F1: Paw volume in mL, A2, B2, C3, D2, E2, F2: % inhibition. NA: not applicable.

Table 6: Ulcerogenic effects of 7b in comparison with indomethacin

Compound Code	Dose (mg/kg, p.o)	Ulcer Index Mean ± SEM
Indomethacin	100	2.526 ± 0.27
7b	100	0.896± 0.45
Data analyzed by one way ANOVA followed by Dunnett's test (n = 6)		

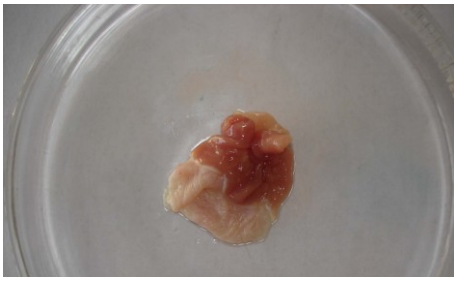



Figure1: Ulcerogenic effect of indomethacin and 7b respectively

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