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Synthesis, antimicrobial and anti-inflammatory activity of some novel benzimidazoles analogs

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ABSTRACT

*A series of novel benzimidazole analogs **B1-B10** were synthesized by condensation of various 2-aryl/alkylbenzimidazole with 2-[4-(2-methypropyl) phenyl] propanoyl chloride obtained from 2-[4-(2-methylpropyl) phenyl] propanoic acid and thionyl chloride. All the synthesized compounds were screened for in vitro antimicrobial activities against Bacillus substilis, Staphylococcus aureus, Escherichia coli, Asperigilius niger and Candida albicans. Out of all prepared analogs compound **B2** and **B4** exhibits excellent antibacterial and anti fungal activity against Escherichia coli and Candida albicans respectively. In addition, the in vivo anti-inflammatory activity of the synthesized compounds was determined using the carrageenin-induced paw oedema method in rats. The substituted benzimidazole derivatives **B2**, **B3** and **B4** show significant anti-inflammatory activity with reference to standard drug diclofenac sodium. The structures of the newly synthesized compounds were elucidated by using IR, ^{1}H-NMR.*

Keywords: Benzimidazole, Anti-inflammatory activity, Antimicrobial activity, Diclofenac sodium.

INTRODUCTION

Inflammation is the complex biological response of vascular tissues against aggressive agents such as pathogens, irritants, or damaged cells which results in formation of protein rich exudates. It is a dynamic process and can be classified as either acute or chronic. Acute inflammation is the exudation of plasma proteins and fluids and the emigration of leukocytes. Chronic inflammation is inflammation of prolonged duration in which tissue destruction, active inflammation and attempts at repair are proceeding simultaneously [1]. At present, although diverse classes of compounds were synthesized which posses anti-inflammatory activities includes non-steroidal anti-inflammatory drugs (NSAIDS), steroidal anti-inflammatory agents or synthetic forms of natural cortisol (glucocorticoids), pharmaceutical biologics and many more. Although the drug treatment has been improved to same extent but yet, it is still the challenge for the pharmaceutical chemist to explore the more effective, potent, less toxic therapeutic agents to treat as well as reduce the signs and symptoms of acute inflammation and chronic inflammatory diseases [2].

Among different types of NSAIDs used, imidazole and fused imidazole with six-membered rings occupy central position among the compounds which are used as analgesic and anti-inflammatory agents. Benzimidazole is a heterocyclic aromatic organic compound, bicyclic in nature and consists of the fusion of benzene and imidazole ring. It possesses many pharmacological properties ranging from anti-inflammatory [3-4], antitumuor [5-6], antimicrobial [7-9], anticonvulsant [10-11], antihelmentic [12-13], antiviral [14] etc. The most prominent

benzimidazole compound in nature is N-ribosyldimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin B12.

In addition, it is well known that bacterial infections often produce pain and inflammation. In normal practice, two groups of agents (chemotherapeutic and anti-inflammatory) are prescribed simultaneously to treat bacterial infections with inflammatory disorders. Unfortunately, none of the drugs possesses these three activities in a single component. Therefore, our aim is to find a compound having dual effect both antimicrobial and anti-inflammatory activities. In this direction, the aforesaid numerous pharmacological activities of substituted benzimidazoles prompted us to study some novel benzimidazole analogs with comparable antibacterial and anti-inflammatory potencies.

MATERIALS AND METHODS

2.1. Chemistry

All melting points were determined by open capillary tube method and are uncorrected. IR spectra recorded on Perkin Elmer RX1 spectrophotometer using KBr pellets and are expressed in cm^{-1}. The ^1H NMR spectra were recorded on Brucker 300 MHz spectrometer in ($CDCl_3$) using TMS as an internal reference and chemical shifts is measured in δ ppm. The progress of the reaction was monitored by TLC using 0.2 mm thickness aluminium sheet precoated with silica gel Merck 60F 254 and visualization was done using iodine/UV lamp for detection of the spots. Chloroform: methanol (9:1) solvent system was used for the determination of R_f value.

2.2. General procedure for the synthesis of title compounds

To materialize the proposed scheme we synthesized 2-[4-(2-methylpropyl) phenyl]-1-[2 aryl/alkyl benzimidazole-1-yl] propan-1-one analogs **(B1-B10)** by treating 2-Aryl/alkyl benzimidazole with 2-[4-(2-methypropyl) phenyl] propanoyl chloride. The synthetic strategy has been explored to obtain the title compounds in excellent yield as shown in **Scheme 1**.

2.2.1. Procedure for the synthesis of 2-Aryl benzimidazole

A mixture of o-phenylenediamine (0.01 mol) and aromatic acids (0.01 mol) in orthophosphoric acid (20 ml) was refluxed for 6-7h. The completion of the reaction was monitored by TLC. After the completion, the reaction mixture was poured into a beaker containing crushed ice. Then 10% ammonium hydroxide solution was added to the reaction mixture with constant stirring until just alkaline. The solid was separated by filtration, dried and re-crystallized from alcohol to afford 2-aryl benzimidazole.

2.2.2. Procedure for the synthesis of 2-Alkyl benzimidazole

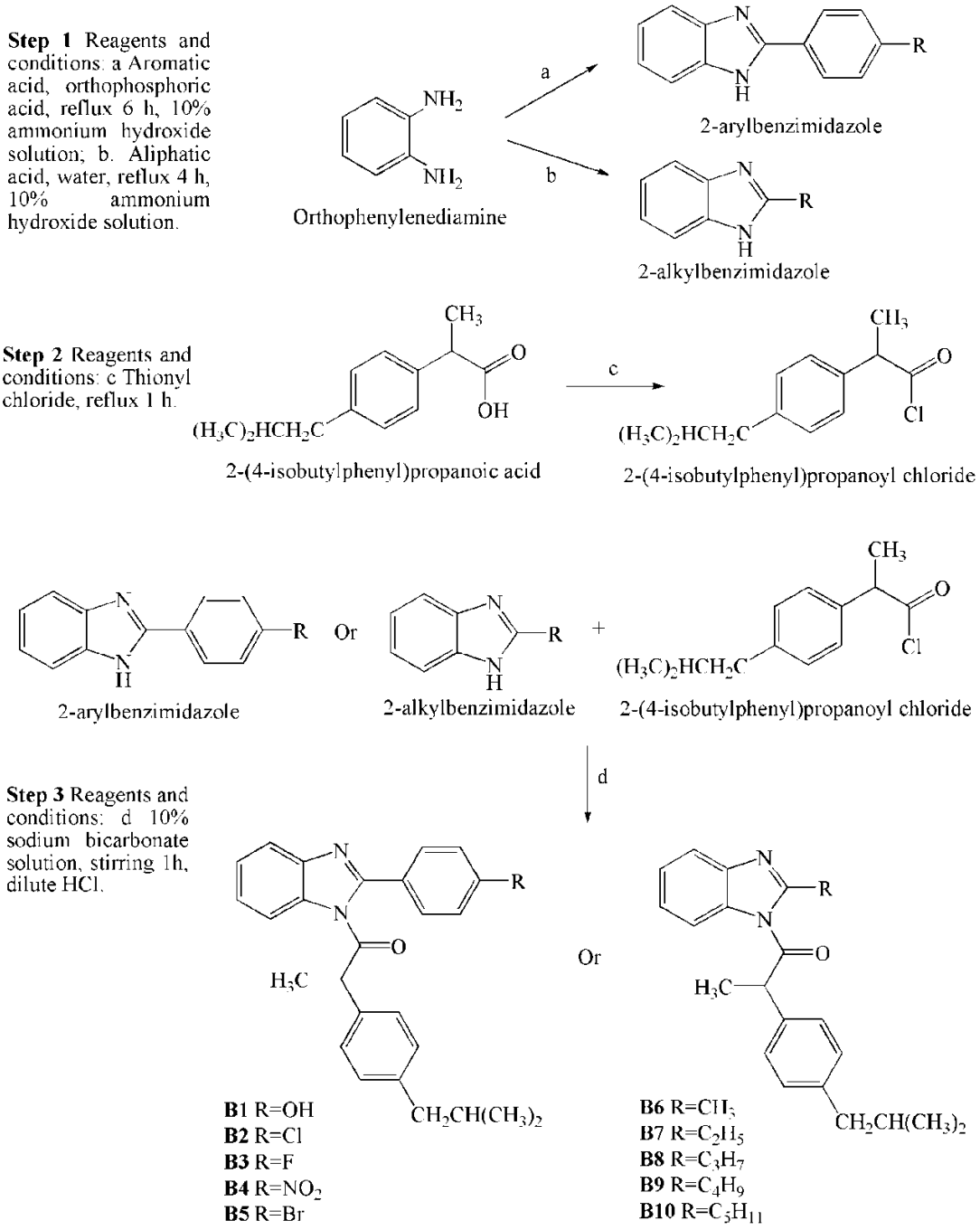
A mixture of o-phenylenediamine (0.01 mol) and aliphatic acids (0.03 mol) in water (20 ml) was refluxed for 4-5h. The completion of the reaction was monitored by TLC. After the completion, the reaction mixture was allowed to cool and basified with 10% ammonium hydroxide solution with constant stirring. The solid was separated by filtration, dried and re-crystallized from alcohol to afford 2-alkyl benzimidazole.

2.2.3. Procedure for the synthesis of 2-[4-(2-methypropyl) phenyl] propanoyl chloride

A mixture of 2-[4-(2-methylpropyl) phenyl] propanoic acid (0.01 mol, 2g) and re-distilled thionyl chloride (0.01 mol, 1.1 ml) was refluxed for about an hour. During refluxing, the flask was shacked from time to time to ensure uniform mixing. After the completion, the reaction mixture was allowed to cool. The solid was separated by filtration and dried to afford 2-[4-(2-methypropyl) phenyl] propanoyl chloride.

2.2.4. Procedure for the synthesis of 2-[4-(2-methylpropyl) phenyl]-1-[2 aryl/alkyl benzimidazole-1-yl] propan-1-one (B1-B10)

In a solution of aryl/alkyl benzimidazole (0.01 mol) in 10 ml of 10% sodium bicarbonate solution added 2-[4-(2-methylpropyl) phenyl] propanoyl chloride (0.02 mol) and then the reaction mixture was shaken vigorously for 1 hour in a Stoppard test tube. The stopper was removed from time to time since carbon dioxide is evolved during the reaction. When the odour of the solution was disappeared, acidified the solution with dilute hydrochloric acid to congo red. The solid was separated by filtration and dried to afford 2-[4-(2-methylpropyl) phenyl]-1-[2 aryl/alkyl benzimidazole-1-yl] propan-1-one (**Scheme 1**).



Scheme 1: Synthesis of 2-[4-(2-methylpropyl) phenyl]-1-[2 aryl/alkyl benzimidazole-1-yl] propan-1-one analogs

2.2.4.1. 1-(2-(4-hydroxyphenyl)-1H-benzo[d]imidazol-1-yl)-2-(4-isobutylphenyl)propan-1-one **(B1):** Molecular formula: $C_{26}H_{26}N_2O_2$; Molecular weight: 398.49; R_f Value 0.75; mp 210°C; Yield 70%; IR (KBr): 3417 (-OH), 1721 (C=O), 1269 (C-N=), 2956 (C-H *str*); ^{1}HNMR ($CDCl_3$) δ: 0.94 (s, 9H, CH_3), 2.47 (s, 1H, CH), 1.52 (s, 1H, CH), 2.49 (s, 2H, CH_2), 5.2 (s, 1H, Ar-OH), 7.12-7.26 (m, 12H, Ar-H).

2.2.4.2. 1-(2-(4-chlorophenyl)-1H-benzo[d]imidazol-1-yl)-2-(4-isobutylphenyl)propan-1-one **(B2):** Molecular formula: $C_{26}H_{25}ClN_2O$; Molecular weight: 416.17; R_f Value 0.65; mp 260°C; Yield 75%; IR (KBr): 761 (C-Cl), 2954 (Aliphatic C-H *str*), 1488 (Aromatic CH bend), 1688 (C=O).

2.2.4.3. 1-(2-(4-fluorophenyl)-1H-benzo[d]imidazol-1-yl)-2-(4-isobutylphenyl)propan-1-one **(B3):** Molecular formula: $C_{26}H_{25}FN_2O$; Molecular weight: 400.48; R_f Value 0.80; mp 220°C; Yield 73%; IR (KBr): 1317 (C-F), 2918 (Aliphatic CH), 1457 (C-H bend), 1381 (C-N), 1721 (C=O), 1457 (Aromatic C=C).

2.2.4.4. 2-(4-isobutylphenyl)-1-(2-(4-nitrophenyl)-1H-benzo[d]imidazol-1-yl)propan-1-one **(B4):** Molecular formula: $C_{26}H_{25}N_3O_3$; Molecular weight: 427.49; R_f Value 0.75; mp 239°C; Yield 70%; IR (KBr): 1353 (NO_2), 2918 (Aliphatic C-H), 2954 (Aromatic C-H *str*), 1704 (C=O), 1353 (C-N).

2.2.4.5. 1-(2-(4-bromophenyl)-1H-benzo[d]imidazol-1-yl)-2-(4-isobutylphenyl)propan-1-one **(B5):** Molecular formula: $C_{26}H_{25}BrN_2O$; Molecular weight: 461.39; R_f Value 0.85; mp 225°C; Yield 65%; IR (KBr): 588 (-Br), 1720 (C=O), 2869 (C-H str), 1268 (C-N=); ^{1}HNMR ($CDCl_3$) δ: 7.11-7.25 (m,12H, Ar-H), 2.48 (s, 2H,CH_2), 0.93 (s, 9H, CH_3), 2.46 (s, 1H,CH), 1.51 (s, 1H,CH).

2.2.4.6. 2-(4-isobutylphenyl)-1-(2-methyl-1H-benzo[d]imidazol-1-yl)propan-1-one **(B6):** Molecular formula: $C_{21}H_{24}N_2O$; Molecular weight: 320.42; R_f Value 0.64; mp 199°C; Yield 72%; IR (KBr): 1267 (C-N), 1663 (C=N), 3256 (Free N-H).

2.2.4.7. 1-(2-ethyl-1H-benzo[d]imidazol-1-yl)-2-(4-isobutylphenyl)propan-1-one **(B7):** Molecular formula: $C_{22}H_{26}N_2O$; Molecular weight: 334.45; R_f Value 0.65; mp 210°C; Yield 65%; IR (KBr): C=O 1665, Aliphatic alkanes (C-H) *str* 2952 , Aromatic alkanes (C-H) *str* 3014 , C-H bend 1381, C-N 1381.

2.2.4.8. 2-(4-isobutylphenyl)-1-(2-propyl-1H-benzo[d]imidazol-1-yl)propan-1-one **(B8):** Molecular formula: $C_{23}H_{28}N_2O$; Molecular weight: 348.48; R_f Value 0.70; mp 225°C; Yield 75%; IR (KBr): 1720 (C=O), 1268 (C-N), 2955 (Aliphatic CH_3).

2.2.4.9. 1-(2-butyl-1H-benzo[d]imidazol-1-yl)-2-(4-isobutylphenyl)propan-1-one **(B9):** Molecular formula: $C_{24}H_{30}N_2O$; Molecular weight: 362.50; R_f Value 0.65; mp 195°C; Yield 70%; IR (KBr): 2951 (Aliphatic C-H *str*), 3025 (Aromatic C-H *str*), 1381 (C-N), 1650 (C=N), 1381 (CH_3 bend).

2.2.4.10. 2-(4-isobutylphenyl)-1-(2-pentyl-1H-benzo[d]imidazol-1-yl)propan-1-one **(B10):** Molecular formula: $C_{25}H_{32}N_2O$; Molecular weight: 376.53; R_f Value 0.75; mp 205°C; Yield 64%; IR (KBr): 2950 (Aliphatic CH), 3254 (Aromatic CH str), 1365 (C-N), 1658 (C=O); ^{1}HNMR ($CDCl_3$) δ:0.9 (s, 9H, -CH_3), 7.04-7.14 (m, 8H, Ar-H), 2.48 (s, 2H,-CH_2), 2.50 (s, 1H,CH), 1.56 (s, 1H,-CH), 1.51 (m, 8H,-CH_2).

2.3. Biological activity

2.3.1. Antimicrobial activity

All the prepared analogs were evaluated for *in vitro* antimicrobial activity against different bacterial (Gram positive and Gram negative) and fungal strains by tube dilution method. Nutrient agar medium and sabourard dextrose agar with an incubation of 24 h at 37°C were taken for antibacterial and antifungal activity respectively. The growth in the tubes was observed visually for turbidity and inhibition was determined by the absence of growth. MIC was determined by the lowest concentration of sample that prevented the development of turbidity. From the observed values of MIC, the intermediate concentrations between MIC values were prepared and the accurate MIC values were determined. The antibacterial and antifungal potential of prepared analogs were compared with standard drug ciprofloxacin and fluconazole respectively as depicted in **Table 1**.

2.3.2. Anti-inflammatory activity

Adult male wistar rats (100–150g) from the Animal House of Guru Jambheshwar University, Hisar, were used throughout the work. They were kept under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into twelve groups of six rats each. Test compounds (**B1-B10**) and standard drug Diclofenac sodium were suspended in DMSO, which was used as a vehicle for the control group. The rats were dosed with test drugs orally (100 mg/kg body weight) including the reference standard (20mg/kg) with help of oral catheter. After 30 minutes drug administration, 50 µl of 1% *w/v* carrageenan solution in saline (0.9%)

was injected in the sub plantar region of the left hind paw of control as well as standard and test groups. The volume of increased paw edema was determined digitally by the caliper after injection at 30, 60, 120, 240 minutes interval. The percentage protection against inflammation was calculated as follows: (1–Vd)/Vc × 100, where Vc is the increase in paw volume in the absence of the test compound (control) and Vd is the increase of paw volume after injection of the test compound.

2.3.3. Statistical analysis

The Data were expressed as means ± SEM. Significant differences between the control and the treated groups were obtained using ANOVA followed by Dunnett's test. The differences in results were considered significant when $P < 0.05$, as compared to control.

RESULTS AND DISCUSSION

3.1. Chemistry

The synthetic pathway leading to the title compounds is given in **Scheme 1**. 2-aryl/alkyl benzimidaoles prepared from o-phenylenediamine with aromatic or aliphatic acids reacted with 2-[4-(2-methypropyl) phenyl] propanoyl chloride prepared by treating 2-[4-(2-methylpropyl) phenyl] propanoic acid with thionyl chloride, affords title 2-[4-(2-methylpropyl) phenyl]-1-[2 aryl/alkyl benzimidazole-1-yl] propan-1-one compounds **B1-B10**. The purity and structures of all the synthesized compounds have been elucidated on the basis of their spectral data including IR and ^{1}H NMR.

3.2. Anti microbial activity

All the synthesized compounds were evaluated for *in vitro* antimicrobial activity against two Gram positive bacterial strains *i.e. Staphylococus aureus* (MTCC 3160) and *Bacilius substilis* (MTCC 2063), one Gram negative bacteria *i.e. E. coli* (MTCC 40) and two fungal strains *i.e. Candida albicans (MTCC 227)* and *Asperigilius niger (MTCC 8189)* by tube dilution method. All the prepared analogs show good to moderate antibacterial and antifungal activity with respect to standard drugs ciprofloxacin and fluconazole respectively. Three compounds namely **B2**, **B3** and **B4** shows excellent antibacterial activity against *Escherichia coli* with the MIC value 1.56 µ/ml. In addition, compound **B2** and **B4** also exhibit excellent anti fungal activity against *Candida albicans* with the MIC value of 1.56 µml. The antimicrobial activity of the test compounds is depicted in **Table 1**.

Table 1. Results of antimicrobial bioassay of test compounds

Compound	*Bacillus substilis* (MTCC 2063)	*Staphylococcus Aureus* (MTCC 3160)	*Escherichia coli* (MTCC 40)	*Asperigilius niger* (MTCC 8189)	*Candida albicans* (MTCC 227)
B1	3.12	6.25	6.25	12.5	6.25
B2	3.12	3.12	1.56	3.12	1.56
B3	3.12	6.25	1.56	3.12	3.12
B4	3.12	3.12	1.56	3.12	1.56
B5	6.25	3.12	3.12	6.25	6.25
B6	12.5	6.25	6.25	12.5	12.5
B7	12.5	12.5	6.25	6.25	6.25
B8	6.25	12.5	12.5	6.25	6.25
B9	6.25	6.25	6.25	12.5	6.25
B10	12.5	12.5	12.5	12.5	12.5
Ciprofloxacin	1.56	1.56	1.56	-	-
Fluconazole	-	-	-	1.56	1.56

3.3. Anti-inflammatory Activity:

The *in-vivo* anti-inflammatory activity was studied using carrageenan-induced rat paw edema model. The anti-inflammatory activity of the test compounds was compared with standard drug Diclofenac sodium as depicted in **Table 2**. The test and standard drug produced significant inhibition of paw edema as compared to control. Out of all prepared analogs **B2, B3** and **B4** exhibit significant anti-inflammatory activity with percentage inhibition 31%, 21% and 27 % respectively, after 4 hours more activity than standard drug Diclofenac sodium. In addition, compounds **B5, B1** and **B8** showed moderate anti-inflammatory activity with percentage inhibition of 22%, 15% and 14%respectively.

Table 2: Anti-inflammatory Activity of the synthesized compounds, Paw volume in ml, Mean±SEM (% inhibition of paw edema ± S.D)

Compound	30 min	1 hr	2 hr	4 hr
Control	1.512±0.040	1.491±0.027	1.474±0.026	1.475±0.029
Standard	1.301±0.005 (14±.014)	0.951±0.008 (37±0.021)	0.882±0.009 (41±0.022)	0.825±0.004 (45±0.011)
B1	1.341±0.010 (12±0.02)	1.311±0.004 (13±0.011)	1.312±0.009 (11±0.023)	1.258±0.071 (15±0.042)
B2	1.272±0.020 (16±0.050)	1.216±0.055 (19±0.136)	1.143±0.054 (23±0.133)	1.032±0.055 (31±0.136)
B3	1.288±0.020 (15±0.049)	1.255±0.035 (16±0.087)	1.351±0.019 (12±0.046)	1.173±0.392 (21±0.096)
B4	1.261±0.024 (17±0.060)	1.208±0.037 (19±0.091)	1.197±0.021 (20±0.052)	1.081±0.042 (27±0.104)
B5	1.291±0.028 (15±0.070)	1.221±0.026 (19±0.064)	1.126±0.053 (24±0.123)	1.163±0.056 (22±0.137)
B6	1.372±0.030 (10±0.073)	1.343±0.023 (10±0.058)	1.325±0.031 (11±0.077)	1.315±0.021 (11±0.052)
B7	1.371±0.037 (10±0.082)	1.345±0.032 (11±0.073)	1.312±0.029 (11±0.071)	1.326±0.024 (11±0.060)
B8	1.362±0.030 (10±0.074)	1.337±0.018 (11±0.045)	1.318±0.056 (11±0.139)	1.273±0.041 (14±0.101)
B9	1.374±0.040 (10±0.098)	1.312±0.029 (13±0.073)	1.312±0.032 (11±0.079)	1.321±0.018 (11±0.046)
B10	1.376±0.039 (10±0.096)	1.342±0.035 (11±0.087)	1.321±0.027 (11±0.066)	1.323±0.030 (11±0.075)

n=6 animal in each group; the observations are mean±SEM, P<0.05, when compared with control (ANOVA followed by Dunnett's test).

CONCLUSION

The present investigation describes synthesis of some novel benzimidazole analogs with comparable antibacterial and anti-inflammatory potencies, obtained by treating 2-Aryl/alkyl benzimidazole with 2-[4-(2-methypropyl) phenyl] propanoyl chloride. All spectral data (IR and ^{1}HNMR) were in accordance with assumed structures. Compounds **B2**, **B3** and **B4** showed remarkable reduction in inflammation as compared with the standard drug diclofenc sodium, after 4 h of carrageenan administration.

Moreover, Compounds **B2** and **B4** also showed excellent antibacterial and antifungal activity against as compared with the standard ciprofloxacin and fluconazole respectively. The antimicrobial and anti-inflammatory activity of the prepared analogs may be due to the presence of electron withdrawing group, since the activity decreased in those compounds which possess aryl chain substituted by the alkyl groups. The promising results gave us scope for further work in this area. It has been felt necessary from the results obtained that there is a need for further advanced studies, at least on the few of the test compounds which are found to be superior.

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