RESEARCH PAPER

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Synthesis, Biological Evaluation and Qsar Analysis of some new Derivatives of Ketoprofen and Flurbiprofen

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New derivatives of ketoprofen and flurbiprofen were synthesized by Mannich reaction. The Mannich base amides of ketoprofen and flurbiprofen were synthesized using different amines. These were then evaluated for analgesic, anti-inflammatory, ulcerogenic activities and quantitative structure-activity relationship was analysed. Some of the derivatives were found to be potent with practically no ulcerogenic activity.

KETOPROFEN, α-(3-benzoyl phenyl) propionic acid and flurbiprofen 2-Fluro-α-Methyl-4-biphenyl acetic acid, two non-steroidal anti-inflammatory drugs have been in clinical use since two decades. As with other NSAIDS, these two drugs suffer from gastrointestinal complications ranging from mild dyspepsia, gastric discomfort and heart burn, to nausea, vomiting and gastric bleeding[1]. It was thought worthwhile to synthesize some derivatives of ketoporfen and flurbiprofen by blocking the carboxylic acid moiety to give compounds with encouraging pharmacological activities with less ulcerogenic index[2-4].

EXPERIMENTAL

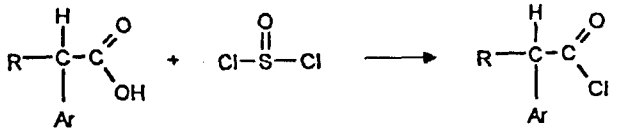
The melting points were determined by open capillary method and are uncorrected. The purity of compounds were ascertained by TLC on silica gel G using hexane:chloroform:glacial acetic acid (9:3:0.5) solvent system. Infrared spectra of the compounds were recorded in potassium bromide phase using Perkin Elmer model-841 spectrophotometer. The NMR spectra of the compounds were recorded on varian EM-360-L (60 MHZ) instrument using TMS as the internal standard. Mass spectroscopy was done on Jeol D-300 model. Nitrogen, carbon, and hydrogen estimation was done on Carlo-Erba 1108 Heraeus instrument. The QSAR analysis of the compounds were done by the software cerius2 version 1.6.

*For Correspondence

Synthesis

Synthesis of Mannich Bases, of amide of ketoprofen and flurbiprofen was carried out in three steps :

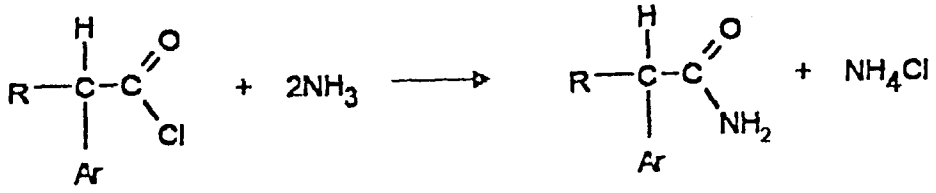
Step 1 : 2-(3-benzoyl phenyl)-2 methyl acetyl chloride (KAC) and 2- Fluoro-α-Methyl acetyl chloride (FAC).



Acid NSAID Acid Chloride

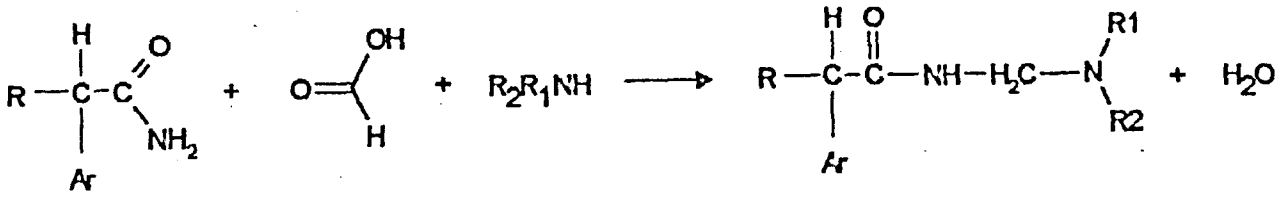
To prepare KAC/FAC, ketoprofen/flurbiprofen (0.05 M) was dissolved in minimum amount of chloroform and freshly distilled thionyl chloride (0.05 M ± 30% excess, 5 ml) was slowly added to it. The mixture was refluxed for 12 hours at 55-60° with continuous stirring on a magnetic stirrer. The excess thionyl chloride and chloroform was removed under reduced pressure to give the product (KAC/FAC) which was dried under vacuum.

Step 2 : 2-(3-benzoyl phenyl)-2 methyl acetamide (KAM) and 2-Fluro-α Methyl-4-biphenyl acetamide (FAM)



Acid Chloride Amide

Step 3 : Mannich bases (K1-K8 and F1-F8) :



Amide Formaldehyde Secondary Amine Mannich Base

To prepare KAM/FAM, chilled ammonium hydroxide was added in excess (50 ml) (5%) to crude ketoprofen acid chloride (KAC)/flurbiprofen acid chloride (FAC) prepared earlier. By maintaining low temperature, vigorous reaction take place. After the reaction subsided, crude amide was filtered and recrystallized from boiling water.

About 1 mole KAM/FAM, 2 moles of powdered paraformaldehyde and 1 mole of respective amine were placed in a 250 ml round flask. Absolute alcohol (40 ml) was then added to it. The contents were refluxed for 8-12 hours and was allowed to cool in a 500 ml wide mouthed conical flask. About 200 ml of acetone was slowly added to precipitate the compounds. The compounds were recrystallized from acetone/ethanol mixture.

The physical data (i.e. molecular formula, melting point, yield, C, H and N estimations) of the compounds are given in Table 1 and Table 2.

The synthesized compounds (K1-K8) showed characteristic IR band at 3510-3350 (N-H) str.), 1700-1650 (c=o str.), 1550-1450 (-NH bond of amide). The compounds gave NMR signals at δ 1.25 (d, 3H) (-CH_3), δ 2.76 (q, 1H) (CH_3-CH-), δ 7.6 (m, 9H) (Ar-H), δ 3.5 (S, 2h) (-NH-CH_2), δ 8.65 (t, 1H) (-CONH). The mass signals of the compounds were m/e 352, 378, 350, 446, 338, 311, 452 and 394.

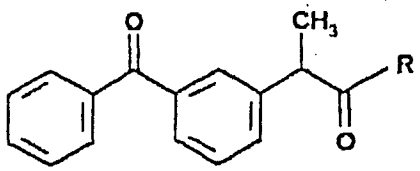
F1-F8 showed IR band at 3510-3350 (N-H str.), 1700-1650 (c=o str.) 1550-1450 (-NH bond of amide). The compounds gave NMR singles at δ 1.25 (d, 3H) (-CH_3), δ 2.76 (q, 1H) (CH_3-CH-), δ 7.6 (M, 9H) (-NH-CH_2), δ 8.65 (t, 1H) (-CONH-). The mass signals of the compounds were m/e 342, 368, 340, 436, 328, 301, 452, 384.

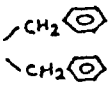
Analgesic Activity :

The analgesic activity of synthesized compounds (K1-K8, F1-F8) was determined by tail flick method[5] using an analgesiometer, (Inco. Ambala, India). Male albino rats weighing between 150-200 g were distributed into test and standard group comprising of six animals each. The screening was done at room temperature (35±1°). The compounds and standard (i.e. ketoprofen and flurbiprofen respectively) were orally administered at doses equimolar to standard.

The tail flick response was evoked by placing rat tail over a wire element heated electrically. The intensity of heat was initially adjusted (current 3.0 A) so that base line tail flick latency averaged between 2-4 seconds, in all animals. Cut off time in the absence of a response was 15 seconds in order to avoid injury to tail. First control latency was noted for each group, then after administration of test

Table 1 : Physical Data for synthesized compounds (k1-K8)



Compound	R	Molecular Formula	M.P. (°C)	Yeild	Nitrogen Found (Calc.)	Carbon Found (Calc.)	Hydrogen Found (Calc.)
K1	-NH-CH_2-N 	$C_{21}H_{24}N_2O_3$	179-181	69.6	7.72 (8.23)	73.59 (8.23)	6.54 (7.05)
K2	-NH-CH_2-CH_2-CH_2-N 	$C_{24}H_{30}N_2O_2$	190-192	69.9	6.96 (7.40)	75.57 (76.19)	7.72 (7.93)
K3	-NH-CH_2-N 	$C_{22}H_{26}N_2O_2$	168-170	67.3	7.71 (8.24)	77.22 (77.64)	7.12 (7.64)
K4	-NH-CH_2-N 	$C_{29}H_{30}N_2O_2$	209.211	65.0	6.74 (6.27)	77.64 (78.02)	7.96 (8.52)
K5	-NH_2-CH_2-N-(C_2H_5)$_2$	$C_{21}H_{26}N_2O_2$	138-140	61.4	7.56 (8.18)	73.26 (73.68)	6.52 (7.60)
K6	-NH-CH_2-N-(CH_2)$_2$	$C_{19}H_{22}N_2O_2$	128-130	65.5	8.42 (8.91)	72.11 (72.61)	6.98 (7.00)
K7	-NH-CH_2-N 	$C_{31}H_{30}N_2O_2$	216-218	72.6	7.44 (6.06)	79.92 (80.52)	5.96 (6.49)
K8	NH-CH_2-N-(C_4H_9)$_2$	$C_{25}H_{34}N_2O_2$	149-151	71.3	6.52 (7.10)	75.66 (76.14)	7.94 (8.62)

*Uncorrected

and standard compounds, tail latency of each group was noted after every 15 minutes upto 2 hours. (Table 3).

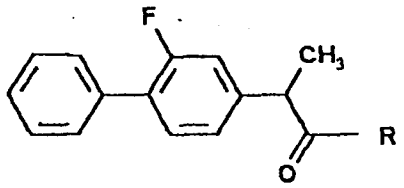
Antiinflammatory Activity :

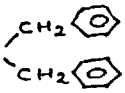
Suspension of test compounds (K1-K8, F1-F8) were prepared in distilled water using 2% gum acacia. In all cases, control received the same quantity of gum acacia. Anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema method of winter *et al*[6]. Albino rats of either sex weighing between 200-300 g were randomly distributed in control and experimental group of six animals. At 0 h the test compounds and standard were administered orally at doses equimolar to standard. One hour after this treatment, edema was induced in the hind paw of the rat by injection of 0.1 ml of 1% carageenan in distilled water into planter tissue of the paw. The initial paw volume was measured using a plethysmometer within 30 second of the injection. The relative increase in paw edema was found by remeasuring the paw volume after 3 h of carageenan injection (Table 3).

Ulcerogenic Activity :

The ulcerogenic activity was determined by the method of Hitchens et al[7]., taking ketoprofen and flurbiprofen

Table 2: Physical Data for synthesized compounds (F1-F8)



Compound	R	Molecular	M.P. (°C)	Yeild	Nitrogen	Carbon
Hydrogen	Formula			Found (Calc.)	Found (Calc.)	Found (Calc.)
F1	-NH-CH_2-N 	$C_{20}H_{22}N_2O_2F$	189-191	75.6	7.58 (8.09)	68.85 (69.36)	6.11 (6.64)
F2	-NH-CH_2-CH_2-CH_2-N 	$C_{22}H_{29}N_2OF$	199-201	72.1	6.98 (7.60)	74.98 (75.00)	7.21 (7.93)
F3	-NH-CH_2-N 	$C_{21}H_{25}N_2OF$	176-178	69.1	7.72 (8.23)	73.50 (74.11)	7.12 (7.64)
F4	-NH-CH_2-N 	$C_{28}H_{37}N_2OF$	220-222	66.8	5.91 (6.42)	76.42 (77.06)	7.96 (8.48)
F5	-NH_2-CH_2-N-$(C_2H_5)_2$	$C_{20}H_{25}N_2OF$	159-161	68.5	7.99 (8.53)	72.66 (73.17)	6.90 (7.62)
F6	-NH-CH_2-N-$(CH_2)_2$	$C_{18}H_{21}N_2OF$	136.138	71.2	8.81 (9.33)	71.96 (72.00)	6.49 (7.00)
F7	-NH-CH_2-N 	$C_{30}H_{29}N_2OF$	236.238	73.8	5.67 (6.19)	78.94 (79.64)	5.93 (6.41)
F8	NH-CH_2-N-$(C_4H_2)_2$	$C_{24}H_{33}N_2OF$	169.171	70.2	6.78 (7.29)	74.31 (75.00)	7.06 (8.59)

*Uncorrected

respectively as standard. The test compounds (K1-K8, F1-F8) and standard were administered orally, as a suspension of 2% gum acacia at a constant volume.

Male albino rats weighing between 200-300 g were starved for 18 h but water was given *ad libitum*. They were divided into groups of six and were fed the test and standard compounds orally. The control were fed only 2% gum acacia suspension. Approximately 5 h after drug administration the rats were sacrificed and the stomachs were removed and fixed in 10% formalin. Each stomach clamped with hemostat at the oesophagal and pyloric ends and inflated with 8-10 ml of air. After 2-5 minutes, the stomach were opened and number of lesions were examined by means of 2x2 binocular magnifier. All ulcers> 0.5 nm were counted and per compound average number of ulcers were determined (Table 3).

Quantitative Structure activity Relationship Analysis

Various parameters (Table 4) were calculated for the ketoprofen and flurbiprofen derivatives using the software carious2, version 1.6[9]. Then the parameters were subjected to stepwise multiple parameter linear regression analysis. The following equations were obtained for analgesic, anti-inflammatory and ulcerogenic activity for ketoprofen and flurbiprofen derivatives.

Table 3 : Analgesic, Anti-inflammatory and Ulcerogenic Activity of Synthesized Compounds

Compound	Analgesic activity Mean Test Latency Sec.+S.E. (after 15 min.)	Anti inflammatory activity % Inhibition of Edema at 3 hours	Ulcerogenic activity Ulcerogenic Index
Ketoprofen	10.2 ± 0.55	84.37	2.50
K1	9.4 ± 0.25	71.37	2.75
K2	11.2 ± 0.42	81.25	1.50
K3	8.4 ± 0.27	79.37	0.03
K4	11.2 ± 0.41	90.52	0.03
K5	10.8 ± 0.55	76.37	1.87
K6	9.4 ± 0.25	76.34	0.38
K7	13.4 ± 0.45	73.75	2.25
K8	10.6 ± 0.28	84.37	0.19
Flurbiprofen	6.6 ± 0.28	72.00	1.50
F1	7.4 ± 0.45	80.00	1.18
F2	11.0 ± 0.35	65.00	0.19
F3	11.8 ± 0.55	65.62	1.88
F4	6.0 ± 0.50	67.50	0.03
F5	7.6 ± 0.77	91.25	0.03
F6	6.5 ± 0.27	85.62	0.19
F7	5.6 ± 0.45	70.65	0.18
F8	6.2 ± 0.55	90.00	0.88

A) QSAR for ketoprofen derivatives

1) QSAR equation for analgesic activity

Analgesic activity =-0.1825 (0.3786) Apol +11.53 (1.558)

n=8, r=o.6567, T-test = 2.134, std=2.2728

2) QSAR equation for anti-inflammatory activity. No significant equations were obtained for anti-inflammatory activity.

3) QSAR equation for ulcerogenic activity

Ulcerogenic activity =0.2523 (0.2827) density +0.1217 (1.1632)

n=8, r=0.7989, T-test=3.2538, std=0.304

B) QSAR for flurbiprofen derivatives

1) QSAR equation for analgesic activity

Analgesic activity = - 0.1837 (0.5533) density +10.6418 (2.7835)

n=8, r=0.8256, T-test=3.872, std=0.598

Table 4 : parameters calculated for QSAR Analysis of synthesized compounds

Compound	Molecular Volume	Density	log P	Molecular Refractivity	Area	Sum of Atomic Polarizibil-ities	Molecula-r Weight	Dipole moment
Ketoprofen	214.710	1.184	1.669	70.584	313.500	10778.99	254.284	5.610
K1	314.221	1.121	0.830	97.407	434.413	14070.98	352.432	7.525
K2	363.125	1.042	2.339	109.862	478.818	15265.96	378.513	3.876
K3	330.019	1.062	1.279	100.831	427.924	14239.44	350.459	4.567
K4	436.885	1.022	4.389	129.324	569.399	17691.50	446.631	3.550
K5	319.668	1.058	1.089	99.548	451.355	13866.94	338.448	2.997
K6	286.148	1.091	0.029	90.517	412.871	12840.42	310.395	2.881
K7	379.346	1.145	3.559	130.874	525.305	19097.53	436.536	5.583
K8	390.914	1.009	3.029	117.610	520.830	15919.98	394.556	6.512
Flurbiprofen	203.156	1.202	3.479	42.297	284.787	9923.86	244.264	8.536
F1	302.599	1.131	0.909	69.120	412.891	13215.96	342.412	8.072
F2	350.498	1.051	4.119	81.575	458.425	14410.94	368.493	10.047
F3	314.657	1.082	3.059	72.544	420.659	13384.42	340.439	7.099
F4	426.430	1.024	6.619	101.037	544.393	16836.50	436.611	8.055
F5	306.394	1.071	2.869	71.261	398.719	13011.92	328.429	6.479
F6	272.311	1.103	1.809	62.230	360.216	11985.40	300.375	6.935
F7	367.954	1.153	5.339	102.587	496.129	18242.72	424.518	6.510
F8	378.893	1.015	4.989	89.323	476.694	15064.96	384.536	3.610

2) QSAR equation for anti-inflammatory activity

Anti-inflammatory activity = -3.9957 (1.6606) dipole +105.2766 (8.3551)

n=8, r=0.7628, T-test= 2.4061, std=3.158

3) QSAR equation for ulcerogenic activity no significant correlationships were obtained for ulcerogenic activity.

RESULTS AND DISCUSSION

Some Mannich base amides of ketoprofen and flurbiprofen were synthesized using different amines and subjected to physical, chemical and spectral analysis. These compounds were then subjected to the evaluation of their analgesic, anti-inflammatory, ulcerogenic activity using standard procedures and thereafter QSAR analysis was performed.

Compounds K2, K4, K5, K7 and K8 exhibited analgesic activity comparable or superior to ketoprofen. Similarly in flurbiprofen series almost all compounds exhibited analgesic activity comparable or superior to flurbiprofen.

All the compounds showed anti-inflammatory activity in the ketoprofen series and one compound, K4 was found to be superior to ketoprofen and in flurbiprofen series four

compounds, F1, F5, F6 were found to be superior to flurbiprofen.

In compounds K3, K4, K6 and K8 the ulcerogenic index was decreased drastically as compared with ketoprofen and F2, F4, F5 F6 and F7 a drastic decrease in ulcerogenic index can be observed as compared with flurbiprofen.

The QSAR analysis of the compounds were done by taking eight parameters. In the ketoprofen series the ulcerogenic activity correlates well with the density of the molecules. As the density increases, the ulcerogenic activity also increases. This is probably due to decrease in absorption and increase in residence time in GI tract. In the flurbiprofen series, the analgesic activity correlates quite well with density. Anti-inflammatory activity has little correlation with the dipole movement. Ulcerogenic activity has no significant correlationships with any parameter.

Thus the study shows that this chemical modification leads to slight increases in anti-inflammatory and analgesic activity but most importantly leads to a significant decrease in ulcerogenic index. The QSAR analysis done for the compounds may be useful for further development in this series of compounds.

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