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EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF THE NOVEL DERIVATIVES OF 1, 3, 4-OXADIAZOLE IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

The synthesis of novel compound libraries along with screening is a rapid and effective approach for the discovery of potential chemical agents, and it becomes an important method in pharmaceutical chemistry research.We present the potential use of the heterocyclic 1,3,4-oxadiazole and Morpholine rings in The design and synthesis of new derivatives of5-[4-(morpholin-4-yl) phenyl]-1,3,4-oxadiazol-2-ol reaction scheme-SMRB5 synthesized derivatives were established by the combined practice of IR, NMR and Mass spectroscopy. Further these synthesized derivatives were subjected to s *in-vitro* and *in-vivo* for anti-inflammatory and analgesic activities and represented graphically.

INTRODUCTION

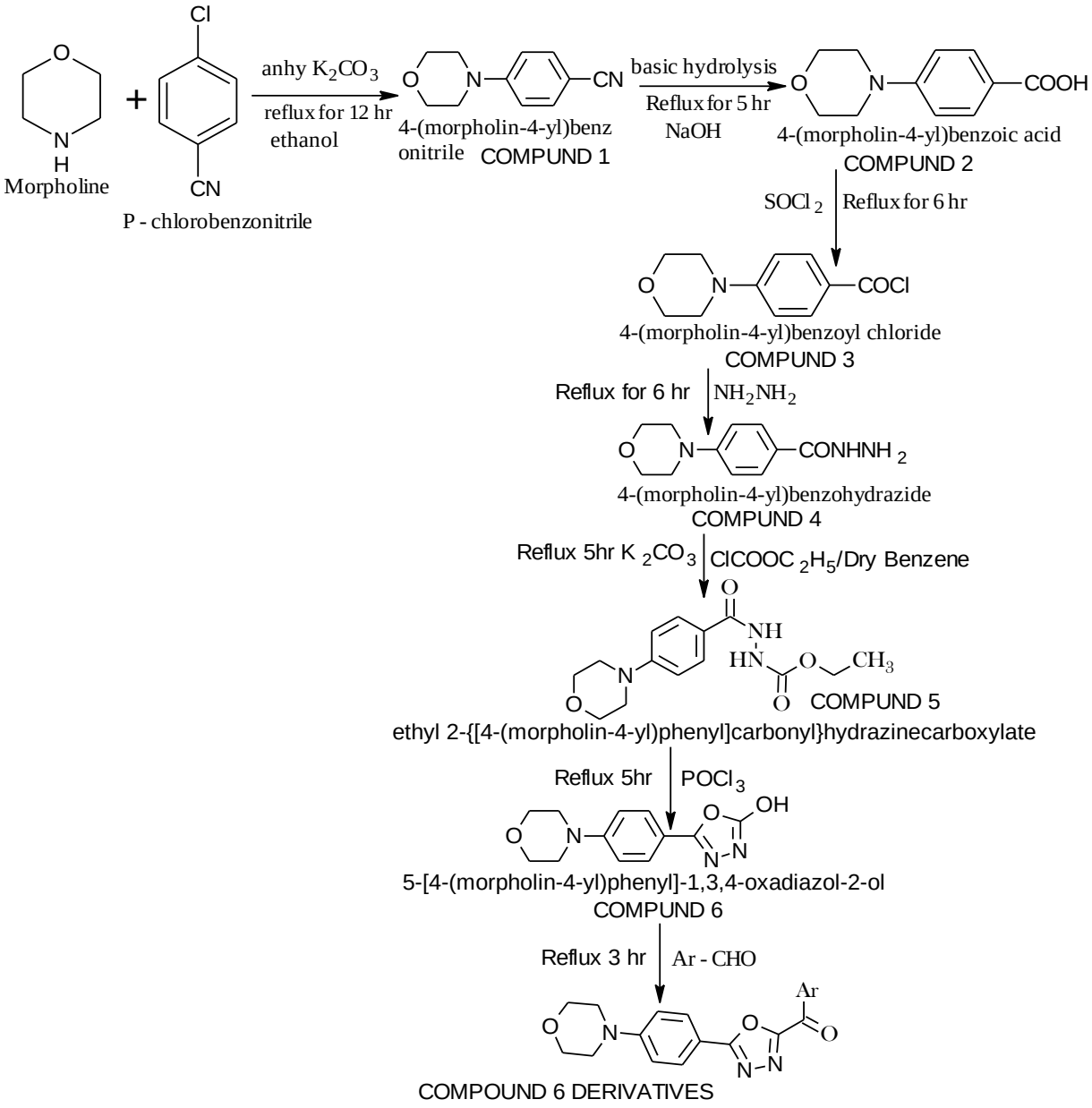
Oxadiazoles are the heterocyclic compounds containing one oxygen and two nitrogen atoms in a five membered ring possessing a diversity of useful biological effects. Nitrogen heterocycles play an important role in the drug discovery scenario. The nitrogenated cores commonly occur as fragments in the structure of most drugs with varied ring sizes, We describe herein the main synthetic approaches of the 1,3,4-oxadiazoles and congeners highlighting the structures that found utility in medicinal and biological chemistry The literature covering the 1,3,4-oxadiazole compounds is very vast and an exhaustive description of all their synthetic methods and applications exceeds the limits imposed for such a review. We will concentrate, thus, on the design and synthesis of new derivatives of5-[4-(morpholin-4-yl) phenyl]-1, 3, 4-oxadiazol-2-ol reaction scheme-SMRB5 synthesized Derivatives were established by the combined practice of IR, NMR and Mass spectroscopy. Further these synthesized derivatives were subjected to s *in-vitro* and *in-vivo* for anti-inflammatory and analgesic activities and represented graphically [1-5].

B) RESEARCH METHODOLOGY:

Method of Preparation of Synthesis of ethyl 2-{[4-(morpholin-4-yl) phenyl] carbonyl} hydrazine carboxylate (SMRB5-5)

A mixture of (4-morpholin-4-yl) benzohydrazide (compound 4) (0.0015 mol) and ethylchloroformate (0.0015 mol) and anhydrous potassium carbonate (5g) were refluxed in dry benzene (15 mL) for 15 h. The reaction mixture was filtered from the potassium salts and the filtrate was concentrated under reduced pressure. The residual resinous mass solidified after standing over night. The resulting solid (compound 5) was obtained from ethanol as colorless needles.

Scheme: SMRB5[5-8]:



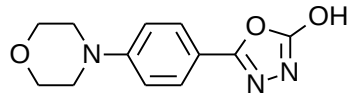
Scheme: 5-[4-(morpholin-4-yl) phenyl]-1,3,4-oxadiazol-2-ol (SMRB5-6).

A mixture of ethyl 2-{[4-(morpholin-4-yl) phenyl] carbonyl} hydrazine carboxylate(compound 5) (0.001 mol) and $POCl_3$ (3 mL) was refluxed gently for 3 h. The reaction mixture was cooled and poured into ice water. The aqueous solution was treated with sodium carbonate solution (10%) until alkaline. The resulting solid (compound 6) was collected washed with water and crystallized from aqueous ethanol as microscopic needles.

Method of Preparation of Derivatives of 5-[4-(morpholin-4-yl) phenyl]-1,3,4-oxadiazol-2-ol. (SMRB5-6A-6N)

A mixture of substituted Aromatic aldehydes (1 mmol), 5-[4-(morpholin-4-yl) phenyl]-1,3,4-oxadiazol-2-ol. (SMRB5-6) (1 mmol) in ethanol were refluxed for 3-4 h. As the reaction progresses, the Derivatives of 5-[4-(morpholin-4-yl) phenyl]-1,3,4-oxadiazol-2-ol. (SMRB5-6) separates out as a solid product in the reaction mixture and was collected by simple filtration. The product obtained so was dried and purified by

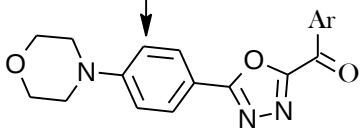
recrystallisation from hot ethyl acetate/hexane.The purity of the product **(SMRB5-6A-6N)** was confirmedby a single spot on TLC plate using methanol: carbon tetrachloride (8:2, v/v) as solvent system.Shown in table No.1. (SMRB5-6F): 3190 cm^{-1}N-H stretch of 2^o amine, 3110 cm^{-1}aromatic C-H stretch, 2940 cm^{-1}aliphatic C-H stretch, 1670 cm^{-1}C = O stretch, 1600 cm^{-1}C = N stretch, 1500 cm^{-1}N=O stretch. H^1NMR spectrum of compound {5-[4 -(morpholin-4-yl) phenyl]- 1,3,4- oxadiazol-2-yl}(3 nitrophenyl) methanone (SMRB5-6F) 3.528 δ 8H, -N$(CH_2)_2$ Morpholine(d), 7.44-7.90 δ 8H, Ar-H (m)8.032 δ 1H, -N^+ Proton (s) M^+ Peaks (Mass Peak)at m/z 379 and Base Peak is 335 (SMRB5-6J): 3380 cm^{-1}O-H stretch, 3210 cm^{-1}N-H stretch of 2^o amine, 3160 cm^{-1} aromatic C-H stretch, 2930 cm^{-1}aliphatic C-H stretch, 2490 cm^{-1}-OCH_3 stretch, 1610 cm^{-1}C = O stretch, 1550 cm^{-1}C = N stretch. 2.465 δ 3H, -CH_3 (s), 3.692 δ 8H, -N (CH2)2 Morpholine(d), 5.514 δ 1H, -OH (s) 7.477-7.97 δ 8H, Ar-H (m). M^+ Peaks (Mass Peak) at m/z 381 and Base Peak is 335



5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-ol

COMPUND 6

Reflux 3 hr | Ar - CHO



COMPOUND 6 DERIVATIVES

Scheme: Derivatives of 5-[4-(morpholin-4-yl) phenyl]-1,3,4-oxadiazol-2-ol.

(SMRB5-6A-6N)

Table No.1: Derivatives of 5-[4-(morpholin-4-yl) phenyl]-1, 3, 4-oxadiazol-2-ol. (SMRB5-6)

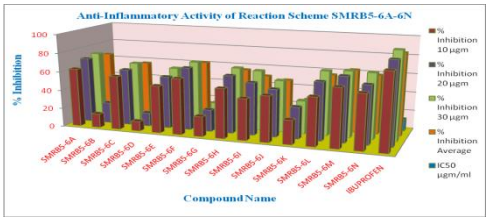
Sl. No	Product Code	Name of -ArCHO	Name of Derivatives of SMRB5-6
1	SMRB5-6A	Benzaldehyde	{5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}(phenyl)methanone
2	SMRB5-6B	4- Fluorobenzaldehyde	(4-fluorophenyl){5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}methanone
3	SMRB5-6C	3- Methoxy Benzaldehyde	(3-methoxyphenyl){5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}methanone
4	SMRB5-6D	2- Chlorobenzaldehyde	(2-chlorophenyl){5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}methanone
5	SMRB5-6E	4- Methoxy Benzaldehyde	(4-methoxyphenyl){5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}methanone
6	SMRB5-6F	3- Nitrobenzaldehyde	{5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}(3-nitrophenyl)methanone
7	SMRB5-6G	4- Chlorobenzaldehyde	(4-chlorophenyl){5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}methanone
8	SMRB5-6H	3-Methoxy 4-Hydoxy Benzaldehyde	(4-hydroxy-3-methoxyphenyl){5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}methanone
9	SMRB5-6I	4- Hydroxy Benzaldehyde	(4-hydroxyphenyl){5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}methanone
10	SMRB5-6J	3-Hydroxy 4-Methoxy Benzaldehyde	(3-hydroxy-4-methoxyphenyl){5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}methanone
11	SMRB5-6K	4- Bromobenzaldehyde	(4-bromophenyl){5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}methanone
12	SMRB5-6L	2- Hydroxy Benzaldehyde	(2-hydroxyphenyl){5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}methanone
13	SMRB5-6M	4- Nitrobenzaldehyde	{5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}(4-nitrophenyl)methanone
14	SMRB5-6N	2-Nitrobenzaldehyde	{5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}(2-nitrophenyl)methanone

In-Vitro Anti-Inflammatory

In-vitro Anti-inflammatory activity was carried out using Bovine Serum albumin denaturation method. All the title compounds (SMRB5-6A-6N) were screened for anti-inflammatory activity. The results of the anti-inflammatory activity of the compounds are shown in the table no 2 and graph 1. SMRB5-6A, SMRB5-6F and SMRB5-6M showed good activity. Whereas compounds SMRB5-6C, SMRB5-6I and SMRB5-6N showed mild activity and remaining compounds showed poor anti-inflammatory activity.[9-11]

Table No 2*: In-vitro* anti-inflammatory activity of derivatives of 5-[4-(morpholin-4-yl) phenyl]-1, 3, 4-oxadiazol-2-ol (SMRB5-6A-6N)

Comp code	% Inhibition				IC_{50} µgm/ml
	10 µgm	20 µgm	30 µgm	Average	
SMRB5-6A	62.53	70.19	72.53	68.41	13.80
SMRB5-6B	14.63	22.16	25.45	20.74	19.63
SMRB5-6C	57.41	60.58	64.14	60.71	14.53
SMRB5-6D	10.26	14.35	17.40	14.00	21.32
SMRB5-6E	50.30	55.74	61.29	55.77	16.10
SMRB5-6F	59.55	66.85	69.38	65.26	14.08
SMRB5-6G	21.23	23.25	26.10	23.52	18.50
SMRB5-6H	52.46	61.55	65.73	59.91	15.16
SMRB5-6I	43.68	55.70	64.10	54.49	15.70
SMRB5-6J	48.45	50.44	55.24	51.37	16.35
SMRB5-6K	25.39	33.50	35.26	31.38	17.10
SMRB5-6L	50.64	61.35	68.35	60.11	15.30
SMRB5-6M	61.77	68.50	69.85	66.70	13.85
SMRB5-6N	57.54	61.40	69.30	62.74	15.10
IBUPROFEN	80.65	87.47	93.45	87.19	12.57



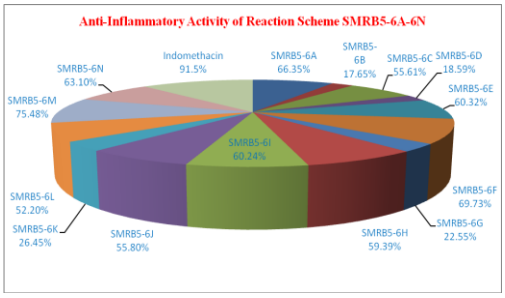
Graph No 1: *In-vitro* anti-inflammatory Activity of derivatives of 5-[4-(morpholin-4-yl) phenyl]-1, 3, 4-oxadiazol-2-ol (SMRB5-6A-6N)

***In-vivo* Anti-Inflammatory Evaluation:** Anti-inflammatory activity was evaluated by carrageenan induced paw edema test using groups of albino rats weighing 100-120 g each and 6 rats per group[12-14]. The result of the anti-inflammatory screening at the endof four hours after the administration of carrageenan showed that compounds SMRB5-6A to SMRB5-6N exhibitededema reduction of 17.65 to 75.48%, in comparison to standard Indomethacin, which showed anedema reduction of about 64.65%.Interestingly, compounds with aromatic aldehydes substitution at position C-2 of the oxadiazole moiety (SMRB5-6A, SMRB5-6F and SMRB5-6M) in the present series exhibited significant anti-inflammatory efficacy ranging from 17.65 to 75.48% edema reduction shown in the table no 3 and graph 2.

Table No 3: *In- vivo* anti-inflammatory activity of the test compounds of derivatives of 5-[4-(morpholin-4-yl) phenyl]-1, 3, 4-oxadiazol-2-ol (SMRB5-6A-6N)at 25 mg/kg by carrageenan induced paw edema method.

Sl. No	Compound	% Protection
1	SMRB5-6A	66.35±0.50***
2	SMRB5-6B	17.65±1.46***
3	SMRB5-6C	55.61±1.35***
4	SMRB5-6D	18.59±0.69***
5	SMRB5-6E	60.32±1.50***
6	SMRB5-6F	69.73±1.10***
7	SMRB5-6G	22.55±1.20***
8	SMRB5-6H	59.39±1.45***
9	SMRB5-6I	60.24±0.65***
10	SMRB5-6J	55.80±0.30***
11	SMRB5-6K	26.45±1.05***
12	SMRB5-6L	52.20±0.60***
13	SMRB5-6M	75.48±0.35***
14	SMRB5-6N	63.10±0.80***
15	Indomethacin	91.50±0.75***

Results are expressed as the mean values from three independent experiments ± SEM.
Data was analysed by Dunnett's test n=3: (***) equals P≤0.0001



Graph No 2: *In-vivo*anti-inflammatory activity of the test compounds of derivatives of 5-[4-(morpholin-4-yl) phenyl]-1,3,4-oxadiazol-2-ol (SMRB5-6A-6N)at 25 mg/kg bycarrageenan induced paw edema method**.**

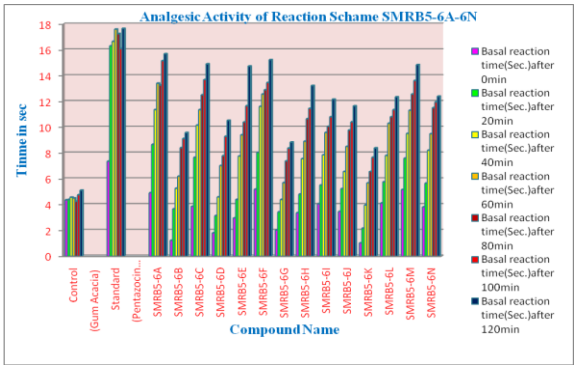
***In-vivo* Analgesic Evaluation**

Analgesic activity was carried out by Eddy's hot plate method. Six groups of albino mice of either sex each comprising of four animals. The derivatives of 5-[4-(morpholin-4-yl) phenyl]-1, 3, 4-oxadiazol-2-ol (SMRB5-6A-6N) have shown a significant Analgesic activity. Results are tabulated in table No 4 and Graph No 3. The compounds SMRB5-6A and SMRB5-6F have shown potent Analgesic activity. The compounds SMRB5-6C, SMRB5-6E and SMRB5-6M showed a moderate analgesic activity. The other compound also showed a significant analgesic activity till 120 minutes[15].

Table No 4: Data showing analgesic activity of derivatives of 5-[4-(morpholin-4-yl) phenyl]-1,3,4-oxadiazol-2-ol (SMRB5-6A-6N)

Sl. No	Compound	Basal reaction time(Sec.)after						
		0min	20min	40min	60min	80min	100min	120min
1	**Control (Gum Acacia)**	4.35±0.30	4.38±0.5	4.56±0.35	4.49±0.65	4.24±0.40	4.75±0.45	5.10±0.25
2	**Standard (Pentazocin 10mg/kg)**	7.35±1.08	16.30±0.25	16.65±1.20	17.60±0.55	17.25±1.50	16.10±0.78	17.65±0.85
3	SMRB5-6A	4.90±0.35	8.66±1.25	11.35±0.58	13.40±1.20	13.25±0.64	15.15±0.45	15.69±0.40
4	SMRB5-6B	1.21±1.30	3.65±0.55	5.25±1.20	6.19±0.48	8.40±1.29	9.11±1.25	9.57±1.50
5	SMRB5-6C	3.85±1.55	7.65±1.45	10.15±1.23	11.35±0.35	12.50±0.45	13.68±1.76	14.90±0.35
6	SMRB5-6D	1.79±1.49	3.13±1.25	4.58±0.65	7.01±1.30	7.78±1.80	9.28±0.45	10.52±0.46
7	SMRB5-6E	2.95±0.25	4.38±0.66	7.75±1.25	9.40±1.17	10.39±1.45	11.65±1.27	14.73±1.35
8	SMRB5-6F	5.18±0.52	8.03±1.45	11.60±1.15	12.56±1.75	12.89±0.55	13.46±1.37	15.23±1.78
9	SMRB5-6G	2.01±1.40	3.42±1.32	4.38±0.33	5.69±1.28	7.37±0.55	8.37±1.46	8.83±1.58
10	SMRB5-6H	3.35±1.24	4.80±0.57	7.55±1.46	8.90±1.30	10.65±1.43	11.45±1.50	13.22±0.19
11	SMRB5-6I	4.05±1.02	5.50±0.65	7.85±0.76	9.58±0.48	10.06±0.46	10.80±0.75	12.16±1.09
12	SMRB5-6J	3.45±0.40	5.22±1.06	6.55±1.46	8.50±1.48	9.77±1.75	10.40±0.20	11.64±0.50
13	SMRB5-6K	1.01±1.48	2.15±1.30	3.95±1.46	5.67±0.85	6.55±1.39	7.66±1.54	8.38±0.80
14	SMRB5-6L	4.10±0.45	5.75±1.68	7.80±1.84	10.30±1.25	10.80±0.50	11.35±1.17	12.34±0.62
15	SMRB5-6M	5.15±1.45	7.59±1.65	9.50±0.68	11.30±1.45	12.56±1.29	13.61±0.28	14.84±1.42
16	SMRB5-6N	3.80±0.36	5.65±1.80	8.20±1.30	9.48±1.45	11.50±1.35	11.95±0.55	12.39±0.25

Dose 20, 25 mg/kg for analgesic activity,
Mean ± SEM, n=4



Graph No 3: Data showing analgesic activity of derivatives of 5-[4-(morpholin-4-yl) phenyl]-1, 3, 4-oxadiazol-2-ol (SMRB5-6A-6N)

CONCLUSION

Compounds reported were derivatives of reaction scheme-SMRB5; they were obtained in high purity with good yield. The FTIR studies show peeks at 1550-1710 cm^{-1} C=O stretch proves formation of derivatives of corresponding structure (SMRB5-6) and these derivatives will be tested for their biological activities.H^1 NMR spectrum data and mass spectra of synthesized derivatives compounds of Scheme SMRB5-6 analysis prove that resultant compound. The results of the anti-inflammatory activity of the compounds are shown in the table no 2 and graph 1. SMRB5-6A, SMRB5-6F and SMRB5-6M showed good activity. Whereas compounds SMRB5-6C, SMRB5-6I and SMRB5-6N showed mild activity and remaining compounds showed poor anti-inflammatory activity.

The statistical analysis of the anti-inflammatory data by Dunnett's test revealed that compounds SMRB5-6A, SMRB5-6F and SMRB5-6M exhibited significant anti-inflammatory activity compared to control shown in Table no 3 and Graph 2.

Analgesic activity results are tabulated in table No 4 and Graph No 3. The compounds SMRB5-6A and SMRB5-6F have shown potent Analgesic activity. The compounds SMRB5-6C, SMRB5-6E and SMRB5-6M showed a moderate analgesic activity. The other compound also showed a significant analgesic activity till 120 minutes.

Conflict of Interest: -Nil-

Financial Support: Self

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