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SYNTHESIS AND BIOLOGICAL SCREENING OF A NOVEL CLASS OF POTENTIAL ANTI-INFLAMMATORY AGENTS

Somashekhar M.[1]* and R. B. Kotnal[1]

Department of Pharmaceutical Chemistry, BLDEA's SSM College of Pharmacy and Research Centre Vijaypur-586103, Karnataka.

***Corresponding Author: Somashekhar M.**

Department of Pharmaceutical Chemistry, BLDEA's SSM College of Pharmacy and Research Centre Vijaypur-586103, Karnataka.

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ABSTRACT

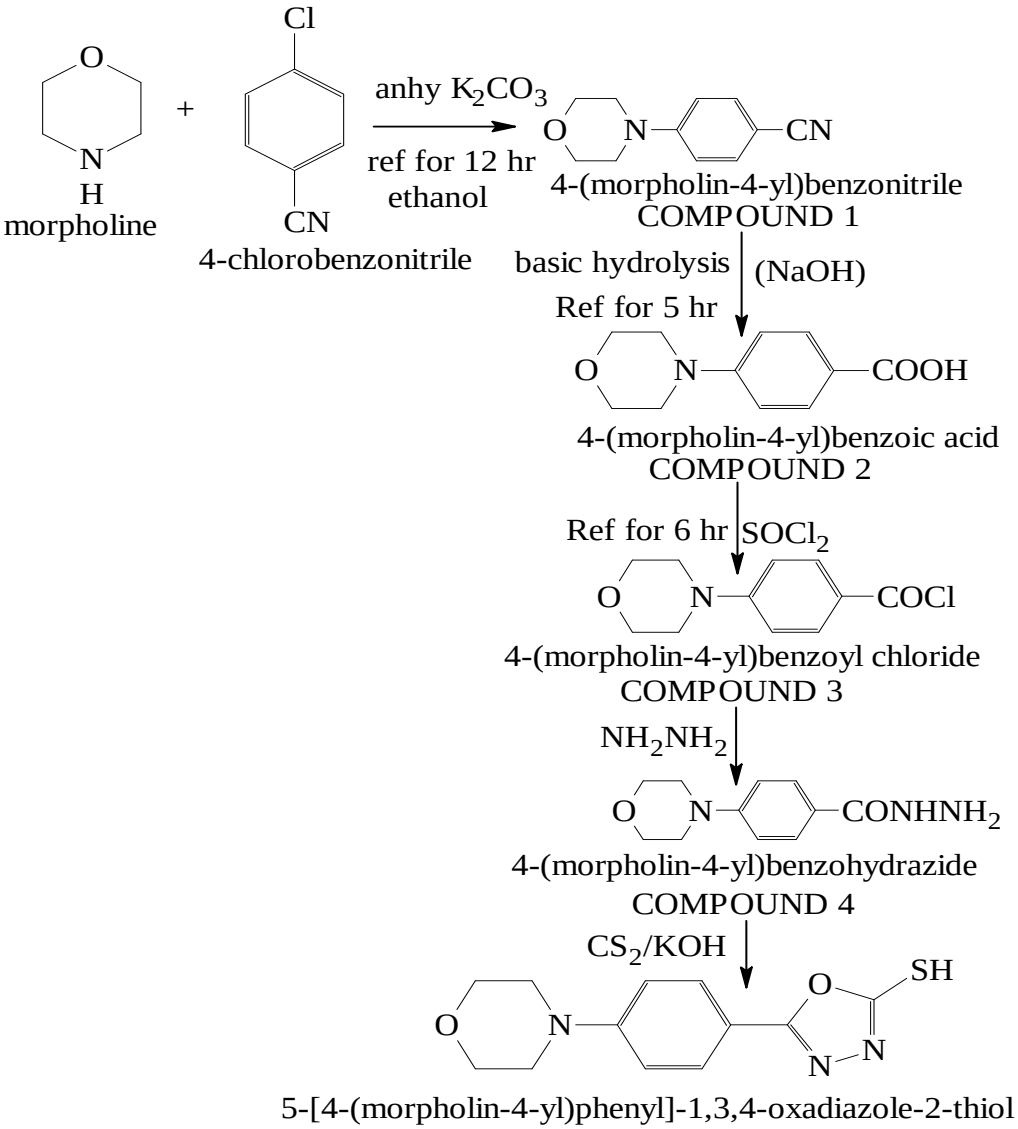
A series of novel 5[4-Morpholin-4-yl-Phenyl)-1, 3, 4-oxadiazole 2-thiol were synthesized by the morpholie and 4-chloro-benzonitrile on reflux gives 4-(morpholine-4-yl) benzonitrile. This upon treating with sodium hydroxide gives 4-(morpholin-4-yl) benzoic acid. Then this upon treating with thionyl chloride gives 4-(morpholin-4-yl) benzoyl chloride. This on further treatment with hydrazine-hydrate gives 4-(morpholin-4-yl) benzohydrazide which is treated for cylcisation with carbon disulphide in presence of potassium hydroxide gives resultant compound 5[4-Morpholin-4-yl-Phenyl)-1,3,4-oxadiazole 2-thiol then it is treated with various substituted to form 1, 3, 4-oxadiazole derivatives. Hydrazides were synthesized so as to increase intracellular concentration and so as to try and decrease the resistant developed due to decrease intracellular concentration of the drug. These synthesized compounds were subjected to preliminary biological evaluation. The characterization of synthesized compounds was identified on the basis of IR, 1HNMR, Mass spectroscopy. The compounds have been evaluated for anti-inflammatory activity.

KEYWORDS: Morpholine, P-Chlorobenzonitrile, 1, 3, 4-Oxadiazole, Anti-inflammatory activity.

INTRODUCTION

1,3,4-Oxadiazoles are unicyclic ring system which found to have diverse chemical reactivity and broad spectrum of biological activity. 1,3,4-oxadiazole considered as simple five membered Heterocyclic molecule possessing one oxygen and two nitrogen atoms at C-1, C-3 and C-4 respectively. Although they have been known from long ago to be biologically active, their varied biological features are still of great scientific interest. 1,3,4-oxadizole exhibited a wide range of biological activities which includes antimicrobial, anti-tubercular, anti-allergic, anticonvulsant, hypoglycemic, vasodilator, anti-inflammatory, analgesic, anthelmintic, anticancer, antiviral, antioxidant, hemolytic, anti-proliferative activities etc. The 1,3,4-oxadiazole undergoes number of reactions including electrophilic substitution, nucleophilic substitution, thermal and photochemical reactions. In drug discovery and development, a number of compounds containing an oxadiazole moiety are in late stage clinical trials, including Zibotentan as an anticancer agent and Ataluren for the treatment of cystic fibrosis. So far, one oxadiazole containing compound, Raltegravir, an antiretroviral drug for the treatment of HIV infection, has been launched onto the marketplace. Given below is a brief account of various alterations conducted on 1,3,4-oxadiazole ring and their associated biological activities.[1-4]

Scheme: SMRB2[5-10]



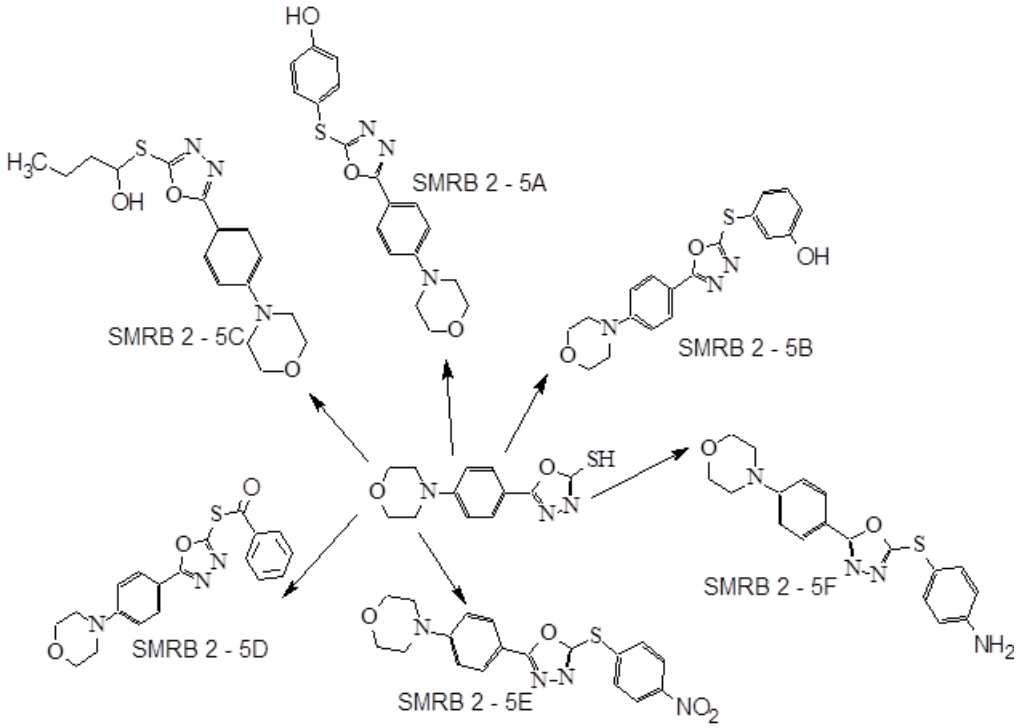
Scheme: 5[4-Morpholin-4-yl-Phenyl)-1,3,4-oxadiazole 2-thiol. (SMRB2-5).

MATERIAL AND METHODS

Derivatives of 5[4-Morpholin-4-yl-Phenyl)-1,3,4-oxadiazole 2-thiol. (SMRB2-5)

Method of Preparation of Derivatives of 5[4-Morpholin-4-yl-Phenyl)-1,3,4-oxadiazole 2-thiol.(SMRB2-5A-5F)

A Mixture of 5[4-Morpholin-4-yl-Phenyl)-1,3,4-oxadiazole 2-thiol.(0.03mole) and substituted halides (0.03mole) in ethanol 30ml and was added KOH 0.39gm the reaction mixture was refluxed and monitored through TLC for every two hours. After cooling overnight at room temperature and dried under vacuum to obtain title compound. The crude product was recrystalized from aqueous ethanol, Physical data presented in table No.2 and 3.



Scheme: Derivatives of 5[4-Morpholin-4-yl-Phenyl)-1,3,4-oxadiazole 2-thiol. (SMRB2-5).

Table No. 1: Derivatives of 5[4-Morpholin-4-yl-Phenyl)-1,3,4-oxadiazole 2-thiol. (SMRB2-5).

Sl.No	Product Code	Name of -RX	Name of Derivatives of SMRB2-5
1	SMRB2-5A	Mono chloro benzene	4-({5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}sulfanyl)phenol
2	SMRB2-5B	3-Bromo benzene	3-({5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}sulfanyl)phenol
3	SMRB2-5C	Chloro butanol	1-({5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}sulfanyl)butan-1-ol
4	SMRB2-5D	Benzoyl chloride	*S*-{5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl} benzenecarbothioate
5	SMRB2-5E	1-chlor 4-nitro benzene	4-(4-{5-[(4-nitrophenyl)sulfanyl]-1,3,4-oxadiazol-2-yl}phenyl)morpholine
6	SMRB2-5F	4-Chloro anilline	4-({5-[4-(morpholin-4-yl)phenyl]-1,3,4-oxadiazol-2-yl}sulfanyl)aniline

Table No. 2: Physical properties of compound SMRB2-5A, SMRB2-5B, SMRB2-5C and SMRB2-5D.

Sl/No	Parameter	SMRB2-5A	SMRB2-5B	SMRB2-5C	SMRB2-5D
1	Molecular Formula	$C_{18}H_{17}N_3O_3S$	$C_{18}H_{17}N_3O_3S$	$C_{16}H_{21}N_3O_3S$	$C_{19}H_{17}N_3O_3S$
2	Molecular weight	355.41	355.41	335.42	367.42
3	**T**heoretical yield	1.34gm	1.34gm	1.27gm	1.39gm
4	Practical yield	0.68gm	1.12gm	0.89gm	1.05gm
5	**%** yield	50.74 %	83.58 %	70.07 %	75.53 %
6	Melting point	166-167^{0}C	198-199^{0}C	192-193^{0}C	193-194^{0}C
7	Recrystallization Solvent	Ethanol	Ethanol	Ethanol	Ethanol
8	Solvent for TLC	Cyclohexane:Ethylacetate 1:1	Cyclohexane:Ethylacetate 1:1	Cyclohexane:Ethylacetate 1:1	Cyclohexane:Ethylacetate 1:1
9	R_f Value	0.84	0.82	0.90	0.73

Table No. 3: Physicochemical properties of compounds SMRB2-5E, and SMRB2-5F.

Sl.no	Parameter	SMRB2-5E	SMRB2-5F
1	Molecular Formula	$C_{18}H_{16}N_4O_4S$	$C_{18}H_{18}N_4O_2S$
2	Molecular weight	384.41	354.43
3	**T**heoretical yield	1.46gm	1.34gm
4	Practical yield	1.23gm	1.00gm
5	**%** yield	84.24%	74.62%
6	Melting point	190-192^{0}C	201-204^{0}C
7	Recrystallization Solvent	Ethanol	Ethanol
8	TLC	Cyclohexane:Ethylacetate 1:1	Cyclohexane:Ethylacetate 1:1
9	RF Value	0.92	0.84

DISCUSSION

FTIR spectrum of compound 1-({5-[4-(morpholin-4-yl) phenyl]-1,3,4-oxadiazol-2-yl}sulfanyl) butan-1-ol (SMRB2-5C): 3180 cm^{-1} N-H Stretch of 2^{o} amine, 3050 cm^{-1} aromatic C-H stretch, 2970 cm^{-1} aliphatic C-H stretch, 1680 cm^{-1} C = O stretch, 1575 cm^{-1} C = N stretch, 1150 cm^{-1} C-S stretch. H^{1}NMR δ Value (ppm) 1.67 –NH, 2.34 -$N(CH_2)_2$ Morpholine, 2.51 -CH_3 (3H), 7.36 Ar-H, 7.93-CONH, 3.9-CH_2.

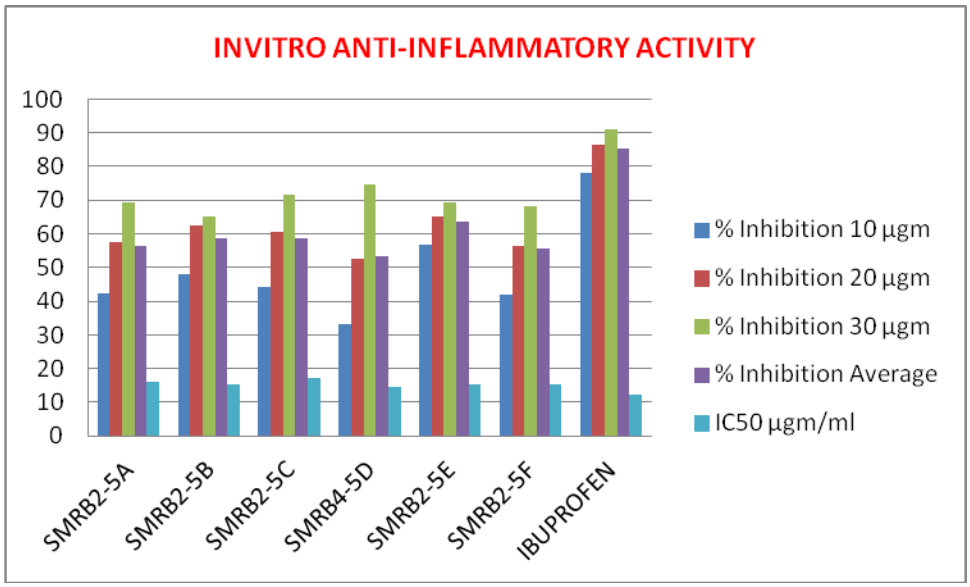
IN-VITRO ANTI-INFLAMMATORY

In-vitro Anti-inflammatory activity the synthesized compounds were screened for in-vitro anti-inflammatory activity by inhibition of bovine serum albumin denaturation method. The test compounds were dissolved in minimum amount of dimethyl sulphoxide (DMSO) and diluted with phosphate buffer (0.2M, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1 ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 270 ±10 C for 15 min. Denaturation was induced by keeping the reaction mixture at 600 ± 10 C in a water bath for 10 min. After cooling the turbidity was measured at 660 nm. (Shimadzu Spectrometer). Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average was taken. The percentage of inhibition is calculated from the following formula. The standard solution was also prepared as similar to that of the test solution. Ibuprofen was used as a standard. % Inhibition = 100(1 - Vt/Vc).

Where, Vt = Drug absorbance of triplicate average
Vc = Control absorbance of triplicate average

Table No 4: In-vitro anti-inflammatory Activity.

Comp code	% Inhibition				IC_{50} µgm/ml
	10 µgm	20 µgm	30 µgm	Average	
SMRB2-5A	42.15	57.39	69.33	56.29	16.23
SMRB2-5B	48.13	62.66	65.21	58.66	15.34
SMRB2-5C	44.21	60.45	71.44	58.70	17.13
SMRB4-5D	33.16	52.63	74.83	53.54	14.67
SMRB2-5E	56.60	65.33	69.43	63.78	15.38
SMRB2-5F	42.10	56.38	68.33	55.60	15.28
IBUPROFEN	78.18	86.36	91.12	85.22	12.16



Graph 1: In-vitro anti-inflammatory Activity.

INVIVO 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, the animals were injected with 0.1 mL of carrageenan (1% solution in normal saline) in the plantar tissue of the right hind paw. The first group received only 0.5% carboxymethylcellulose (CMC) orally and served as untreated control. The test groups received compounds suspended in 0.5% CMC orally at a dose of 25 mg kg-1 one hour prior to carrageenan injection. While the positive control group received 25 mg kg-1 Indomethacin suspended in 0.5% CMC, orally one hour before carrageenan injection. Four hours after carrageenan administration, the paw volumes (mL) were measured using the mercury displacement technique with the help of a plethysmograph. The percent inhibition of

paw edema was calculated by using the following formula.

$$\%\text{Inhibition} = \left(\frac{a-x}{b-y}\right) \times 100$$

Where, **x** is the mean paw volume of rats in the test group before the administration of carrageenan and/or test compounds or reference drug, **a** is the mean paw volume of rats after the administration of carrageenan in the test group (reference drug/ test compound treated), **b** is the mean paw volume of rats after the administration of carrageenan in the control group, **y** is the mean paw volume of rats before the administration of carrageenan in the control group. The mean percent inhibition of Indomethacin and tested compounds at 25 mg kg^{-1} concentrations was compared with control using the repeated measures ANOVA with Dunnett's test.

Table No 5: In vivo anti-inflammatory activity of the test compounds at 25 mg/kg by carrageenan induced paw edema method.

Sl. No	Compound	% Protection
1	SMRB2-5A	48.12±0.65***
2	SMRB2-5B	60.45±1.02***
3	SMRB2-5C	58.32±0.43***
4	SMRB4-5D	55.29±1.16***
5	SMRB2-5E	68.12±2.30***
6	SMRB2-5F	52.23±1.80***
8	Indomethacin	60.30±1.46***

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.

CONCLUSION

Compounds reported were derivatives of reaction scheme-SMRB2; they were obtained in high purity with good yield. The FTIR studies show peeks at 1030-1275 cm^{-1} C-S stretch proves formation of derivatives of corresponding structure (SMRB2-5) and H^1 NMR spectrum data and mass spectra of synthesized derivatives compounds of Scheme SMRB2 analysis proves that resultant compound and further carried out for biological activities. These derivatives tested for their biological activities. Synthesized compounds were screened for their In-vitro and In-vivo anti-inflammatory activity. The results of the In vitro anti-inflammatory activity of the compounds are shown in the table No. 4 and graph No 1. SMRB2-5B, SMRB2-5C and SMRB2-5E showed good activity. The statistical analysis of the In vivo anti-inflammatory data by Dunnett's test shown in Table No. 5 and revealed that compounds SMRB2-5A-SMRB2-5F exhibited significant anti-inflammatory activity compared to control. The percentage inhibition of inflammation by the test compounds at dose of 25mg/kg at the end of four hours time intervals are expressed as m ± SEM.

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