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Research Article

SYNTHESIS and EVALUATION of ANTI ULCEROGENIC STUDIES of SOME NOVEL 1,3,4-OXADIAZOLE and 3-MERCAPTO-1,2,4-TRIAZOLE

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ABSTRACT
Synthesis of novel 1,3,4-oxadiazole and 3-mercapto-1,2,4-triazole derivatives of diclofenac acid along with their derivatives has been done. The entire synthesized compounds were characterized by UV, IR and ^{1}HNMR spectroscopy. The Synthetic compound **4c** showed reduction in ulcerogenic activity (1.23 ± 0.35), and compounds **4d, 4f, 6a, 6c** and **6e** at 10mg/kg therapeutic dose on stomach was negligible compared to drug Diclofenac potassium at the same dose levels
KEYWORDS: 1, 3, 4-oxadiazole and 3-mercapto-1,2,4-triazole derivatives, diclofenac acid, Antiulcer activity.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain and inflammation, particularly for different types of arthritis. Among the most popular NSAIDs worth mentioning is diclofenac potassium, which is approved in more than 120 countries across the globe since its introduction, 28 years ago, and is ranked 30thamong the top 200 drugs with respect to new prescriptions. Diclofenac (marketed as Flector patch, Voltaren, Voltarol, Voltfast, Votalin, Diclac, Diclon, Dicloflex, Diclogem (available in India), Diclofenacum, Difen, Difene, Cataflam, Naklofen, Pennsaid, Panamor, Rhumalgan, Modifenac, Abitren, Olfen, Voveran, Arthrotec, Dedolor, Deflamat, Vetagesic, Topac and Zolterol, with various drug dose combinations). The name is derived from its chemical name of 2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid.

In general, NSAIDs exhibit a similar pattern of adverse effects on the gastrointestinal tract including nausea, vomiting, and diarrhea. However, the most serious and detrimental adverse effect attributed to the prolonged use of NSAIDs is the development of gastric ulceration. The ulcerogenic properties of NSAIDs stem from the fact that they are organic acids, which can irritate the gastric mucosa, and also from their inhibitory effects on prostaglandin biosynthesis. Prostaglandins are the natural stimulatory agents for mucin secretion. The latter is carbohydrate polymer, normally produced by the stomach, and acts as an endogenous cytoprotective substance against the digestive effects of trypsin and hydrochloric acid. Accordingly, by inhibiting prostaglandin synthesis, mucin secretion will be indirectly reduced and an increased risk of ulceration arises. The enzyme COX has 2 subtypes: COX-1 and COX-2. The former exists throughout the biological system including in the stomach, while the second (COX-2) is much less abundant in the stomach. This discovery prompted investigators and researchers to develop selective COX-2 inhibitors to minimize the ulcerogenic potential of NSAIDs. Two major drugs were produced by this approach: celecoxib , and rofecoxib. The medicinal chemistry of the 2 drugs indicates high lipophilic characteristics with an acidic functionality represented by the sulfonamido group in celecoxib, or the bio-isosteric group methylsulfone in rofecoxib. The subtype-COX-2 enzyme has a selective binding area for

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Table 1: Physical data of synthetic compounds

S.No	Code	Molecular Formula	Molecular Weight	Color	Nature	% Yield
1	**1**	$C_{14}H_{11}Cl_2NO_2$	296.15	White	Powder	97.61
2	**2**	$C_{16}H_{15}Cl_2NO_2$	324.2	Pale orange	Powder	94.13
3	**3**	$C_{14}H_{13}Cl_2N_3O$	310.18	Pale Yellow	Powder	89.58
4	**4a**	$C_{21}H_{14}Cl_3N_3O$	430.71	Arsenic	Powder	58.63
5	**4b**	$C_{22}H_{17}Cl_2N_3O_2$	426.3	Dark violet	powder	70.98
6	**4c**	$C_{23}H_{19}Cl_2N_3O_3$	456.32	Maroon	powder	81.22
7	**4d**	$C_{21}H_{14}Cl_2FN_3O$	414.26	Ash	powder	87.42
8	**4e**	$C_{21}H_{14}Cl_2FN_3O$	414.26	Ash	Powder	66.28
9	**4f**	$C_{22}H_{17}Cl_2N_3O$	410.3	Arsenic	Powder	79.54
10	**4g**	$C_{21}H_{14}BrCl_2N_3O$	475.17	British raising	powder	64.72
11	**5**	$C_{15}H_{11}Cl_2N_3OS$	352.24	Pale yellow	powder	77.19
12	**6a**	$C_{20}H_{15}Cl_2N_5S$	428.34	Yellowish brown	powder	81.71
13	**6b**	$C_{20}H_{15}Cl_2N_5S$	428.34	Yellowish brown	powder	76.34
14	**6c**	$C_{21}H_{16}Cl_2N_4OS$	443.35	Yellowish brown	powder	76.18
15	**6d**	$C_{21}H_{16}Cl_2N_4OS$	443.35	Reddish brown	powder	61.84
16	**6e**	$C_{25}H_{18}Cl_2N_4S$	477.41	Brick red	Powder	89.55
17	**6f**	$C_{18}H_{13}Cl_2N_5S_2$	434.37	Brown	Powder	56.73

the sulfone group while the subtype-COX-1 lacks such an area.

They are also well known to have Anti inflammatory[1,2], analgesic[3,4] , antihelmentic[5], antiviral[6,7], antibacterial[8,9], antifungal[10,11], antiulcer[12,13], insecticidal[14], anticonvulsant[15,16], lipid per oxidation[17,18], anti tuberculosis[19], hypolipidemic[20], antidiabetic[21], antimitotic[22], neurotoxicity[23] and antidepressant[24] activities. Syed Mohammad Ashhad Halim, et al., had reported the synthesis of two organizing complexes, trimethyltin and diphenyltin with diclofenac sodium as ligand, Structure elucidation of the complexes prepared was carried out by infrared, multi nuclear magnetic resonance and mass spectroscopy. The spectral data suggest that trimethyltin diclofenate is four coordinate tetrahedral monomer while diphenyltin bis (diclofenate) retained its hexa coordinated octahedral geometry in solution. The biological activity of these two complexes proved to be powerful biocides.

MATERIALS AND METHODS

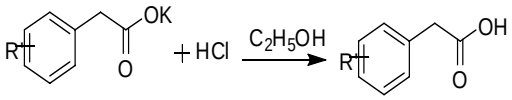
Experimental

All the chemicals are analytical grade and were purified by the established methods. Melting points and were determined by open capillary tubes method purity and homogeneity of the compounds was routinely determined by thin layer chromatography on glass plates using silica gel G as absorbent and solvent system. Benzene: Ethyl acetate: Methanol (8.5:1.4:0.1). Spots were visualized by iodine vapor by irradiation with UV light.^{1}HNMRspectra was recorded on Bruker Ultra shield (300MHZ) spectrometer using DMSO (TMS as internal standard).

Synthesis of various substituted 2-aryl 5h-1,3,4 oxadiazoles and 4,5-diaryl 3-mercapto-1,2,4-triazoles

Synthesis of substituted Benzyl Acetic Acid

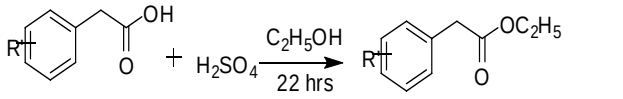
Diclofenac potassium (0.101 mol) was dissolved in ethanol (2.5 mol); to this solution conc.H_2SO_4 was added drop wise to hydrolyze the salt to acid. The acid obtained was filtered, dried mp 153–155 º C.



Substituted Benzyl Potassium Acetate — Substitiuted Benzyl Acetic Acid

Synthesis of Substituted Benzo Ethyl Ester

2-[(2, 6-dichloroanilino) phenyl] acetic acid (0.05 mol) was dissolved in absolute ethanol (10 ml) conc. H_2S0_4 (1ml) was added and the reaction mixture was refluxed for 22 hrs. Reaction mixture gave on processing ethyl ester (**2**). The solid obtained was washed with sodium bicarbonate solution (10% 50 ml) and recrystallized from methanol.

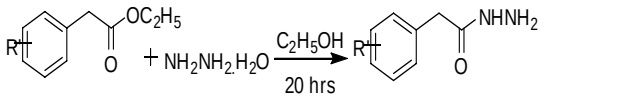


substituted Benzyl Acetic Acid — Substituted Benzo Ethyl Ester

Synthesis of Substituted Benz Etho hydrazide

Compound **2** (0.01 mol) and hydrazine hydrate (0.02 mol) were refluxed in absolute ethanol (50 ml) for 20 hrs. The mixture was concentrated, cooled and

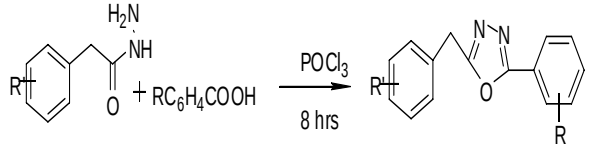
poured in ice cold water. The solid thus separated out was filtered, dried and recrystallized from ethanol.



Substituted Benzo Ethyl Ester — Substituted Benz Etho hydrazide

Synthesis of various Substituted 2-aryl 5H-1, 3, 4-oxadiazoles

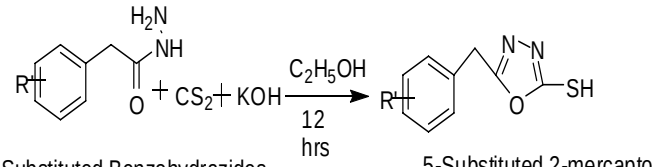
Compound **3** (0.001 mol) and appropriate aromatic acid (0.001 mol) was dissolved in phosphorus oxychloride and refluxed for 8 hrs. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid thus separated out was filtered, washed with water, dried and recrystallized from ethanol.



Substituted Benz Etho hydrazide — Substituted 2-Aryl 5H-1,3,4-oxadiazoles

Synthesis of 5-Substituted 2-mercapto-1,3,4-oxadiazoles

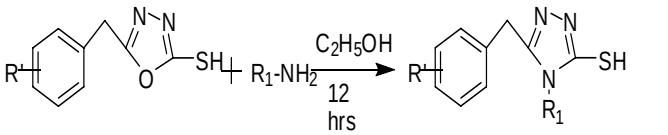
A mixture of **3** (0.005 mol), KOH (0.005 mol) and carbon disulphide (5 ml) in ethanol (50 ml) was refluxed on a steam bath for 12 h. The solution was then concentrated, cooled and acidified with dilute HCl. The solid mass that separated out was filtered, washed with ethanol, dried and recrystallized from ethanol.



Substituted Benzohydrazides — 5-Substituted 2-mercapto 1,3,4-oxadiazole

Synthesis of various 4,5-disubstituted 3-Mercapto-1,2,4-triazoles

To a solution of corresponding compounds **5** (0.01 mol) in a round bottom flask added (0.03mol) of various substituted aromatic primary amines in ethanol. Refluxed for 6hrs the product obtained was 4,5-disubstituted -3-Mercapto-1,2,4-triazoles.

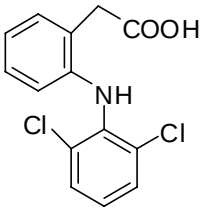


Substituted 2-mercapto 1,3,4-oxadiazole — 4,5-disubstituted-3-Mercapto 1,2,4-triazoles

Spectral analysis[25,26]

COMPOUND: - 1

2-(2-(2,6-Dichloro phenyl amino) phenyl) acetic acid

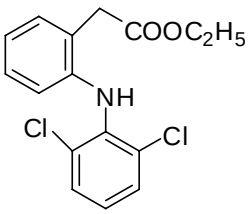


IR Spectral Data

3252 (N-H stretching),3069 (C-H stretching,Aromatic) 2969 (C-H stretching (Aliphatic)), 1580 (C=C stretching),2366 (O-H stretching (Acid)),1276 (O-C stretching (Aliphatic)), 1093 (C-Cl (Aromatic).

COMPOUND: - 2

Ethyl 2-(2,6-dichloro phenyl amino) phenyl) acetate

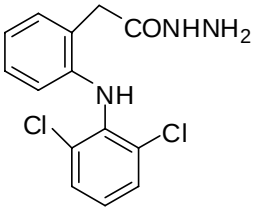


IR Spectral Data

3296 (N-H stretching),1731(C=O stretching (Ester)),1359 (C-N stretching (Aromaticamine), 1580(C=C stretching(Aromatic), 783 (C-H Bending (Aromatic), 1239(C-O stretching(Ester), 668(N-H Bend).

COMPOUND: - 3

2-(2-(2,6-dichloro phenyl amino) phenyl) acetohydrazide



IR Spectral Data

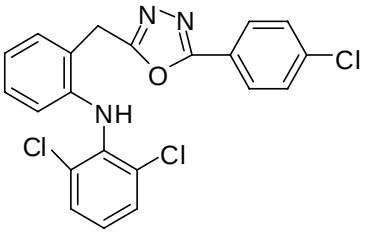
3326 (N-H stretching),1638(C=O stretching (Ester)),1357 (C-N stretching (Aromaticamine), 1503(C=C stretching(Aromatic), 1087(C-Cl (Aromatic), 773(N-H Bend)

NMR Spectral Data

3.66(Methyl protons), 7.34-7.36(Dichloro phenyl protons), 6.89-7.0(Aromatic protons), 7.5(Secondary NH), 4.0(Aromatic C-NH).

COMPOUND: - 4a

N-(2,6-dichlorophenyl)-2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)benzenamine

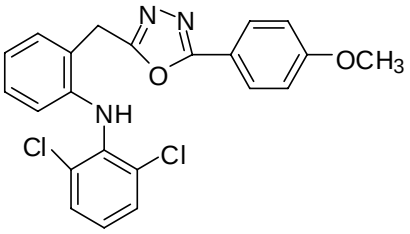


IR Spectral Data

3429 (N-H stretching), 1604 (C=C stretching (Aromatic) 1656 (C=N stretching)1011 (C-O stretching (Aromatic) 1091(C-Cl (Aromatic) 2925 (C-H Stretching (Aromatic)

COMPOUND: - 4b

2,6-dichloro-*N*-(2-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2- yl)methyl)phenyl)benzenamine

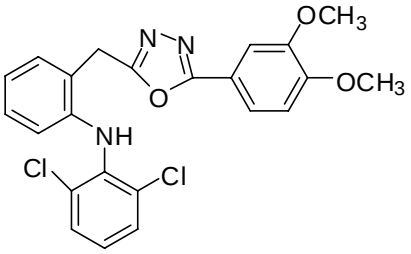


IR Spectral Data

3428 (N-H stretching), 1467 (C=C stretching (Aromatic), 1609 (C=N stretching)C-O stretching (Aromatic) ,1019(C-Cl (Aromatic) ,2925 (C-H Stretching (Aromatic).

COMPOUND: - 4c

***N*-(2,6-dichlorophenyl)-2-((5-(3,4-methoxyphenyl)-1,3,4-oxadiazol-2- yl) methyl)benzenamine**



IR Spectral Data

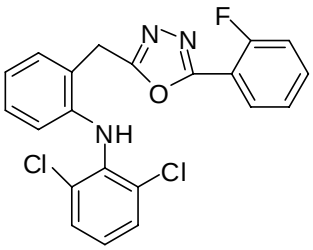
3429 (N-H stretching), 1467 (C=C stretching (Aromatic), 1602 (C=N stretching) C-O stretching (Aromatic), 1022 C-Cl (Aromatic), 2928 (C-H Stretching (Aromatic).

NMR Spectral Data

3.75 (Methyl protons), 7.49-7.59 (Dichloro phenyl protons), 7.26-7.35 (Dimethoxy phenyl protons), 6.94(1 Benzene), 4.01 (Aromatic C-NH)

COMPOUND: - 4d

***N*-(2,6-dichlorophenyl)-2-((5-(2-flurophenyl)-1,3,4-oxadiazol-2- yl)methyl)benzenamine**

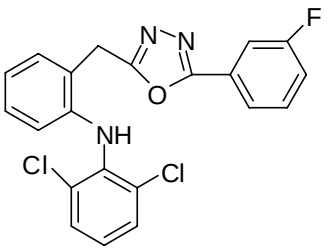


IR Spectral Data

3427 (N-H stretching), 1442 (C=C stretching (Aromatic), 1612 (C=N stretching),1225(C-O stretching (Aromatic) ,1030 (C-Cl (Aromatic) ,3074 (C-H Stretching (Aromatic), 1105(C-F (Aromatic).

COMPOUND: - 4e

***N*-(2,6-dichlorophenyl)-2-((5-(3-flurophenyl)-1,3,4-oxadiazol-2- yl)methyl)benzenamine**

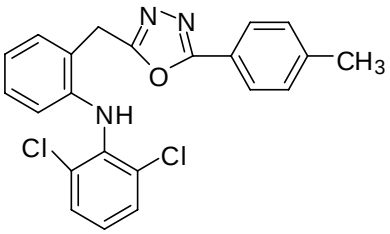


IR Spectral Data

3402 (N-H stretching), 1564 (C=C stretching (Aromatic), 1610 (C=N stretching),1269(C-O stretching (Aromatic) ,1097 (C-Cl (Aromatic) ,3076 (C-H Stretching (Aromatic), 1199(C-F (Aromatic).

COMPOUND: - 4f

***N*-(2,6-dichlorophenyl)-2-((5-*p*-tolyl-1,3,4-oxadiazol-2 yl)methyl)benzenamine**

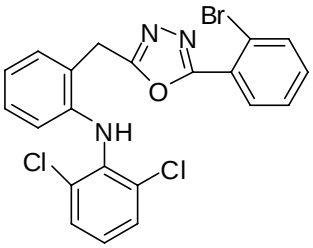


IR Spectral Data

3404 (N-H stretching), 1468 (C=C stretching (Aromatic), 1610 (C=N stretching), 1181(C-O stretching (Aromatic) ,1098(C-Cl (Aromatic) ,2925 (C-H Stretching (Aromatic), 1105(C-F (Aromatic).

COMPOUND: - 4g

2-((5-(2-bromophenyl)-1,3,4-oxadiazol-2-yl)methyl-*N*-(2,6- dichlorophenyl)benzenamine

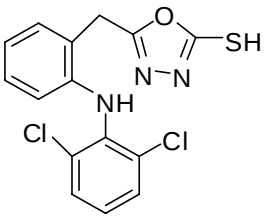


IR Spectral Data

3426 (N-H stretching), 1467 (C=C stretching (Aromatic), 1608 (C=N stretching), 1195 (C-O stretching (Aromatic), 1077 (C-Cl (Aromatic) ,2925 (C-H Stretching (Aromatic), 787(C-Br (Aromatic).

COMPOUND: - 5

5-(2-(2,6-dichloro phenyl amino)benzyl)-1,3,4-oxadiazole-2-thiol



IR Spectral Data

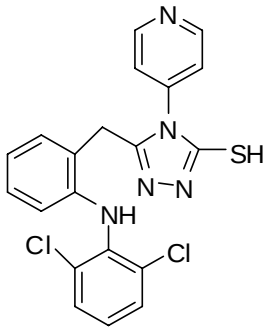
3376 (N-H stretching), 2366 (SH stretching), 1617(C=N stretching),1303 (C-O Stretching (Aromatic), 1061(C-Cl (Aromatic), 2930(C-H Stretching (Aromatic).

NMR Spectral Data

3.67 (Aromatic C-SH), 7.30-7.35(Dichloro phenyl protons),3.83(Methyl protons), 6.99(1 Benzene), 4.1 (Aromatic C-NH).

COMPOUND: - 6a

5-(2-(2,6-dichloro phenyl amino)benzyl-4-(pyridin-4-yl-4*H*-1,2,4-triazole-3- thiol

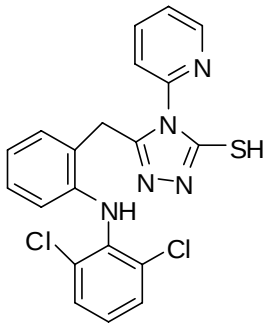


IR Spectral Data

3431 (N-H stretching), 1599 (C=C stretching (Aromatic) ,1645 (C=N stretching),2535 (SH stretching), 989(C-Cl (Aromatic)),3163 (C-H Stretching (Aromatic) ,3305 (O-H stretching (Phenol),1335 (C-N Stretching).

COMPOUND: - 6b

5-(2-(2,6-dichloro phenyl amino)benzyl-4-(pyridin-2-yl-4*H*-1,2,4-triazole-3-thiol

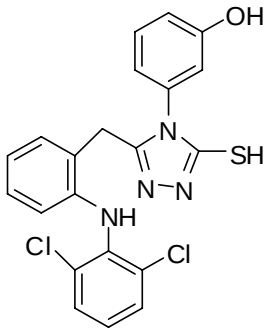


IR Spectral Data

3299 (N-H stretching), 1579 (C=C stretching (Aromatic) ,1647 (C=N stretching),2367 (SH stretching),1270 (C-O Stretching (Aromatic) ,3305 (O-H stretching (Phenol),1420 (C-N Stretching).

COMPOUND: - 6c

3-(3-(2-(2,6-dichloro phenyl amino)benzyl)-5-mercapto-4*H*-1,2,4-triazole4- yl)phenol

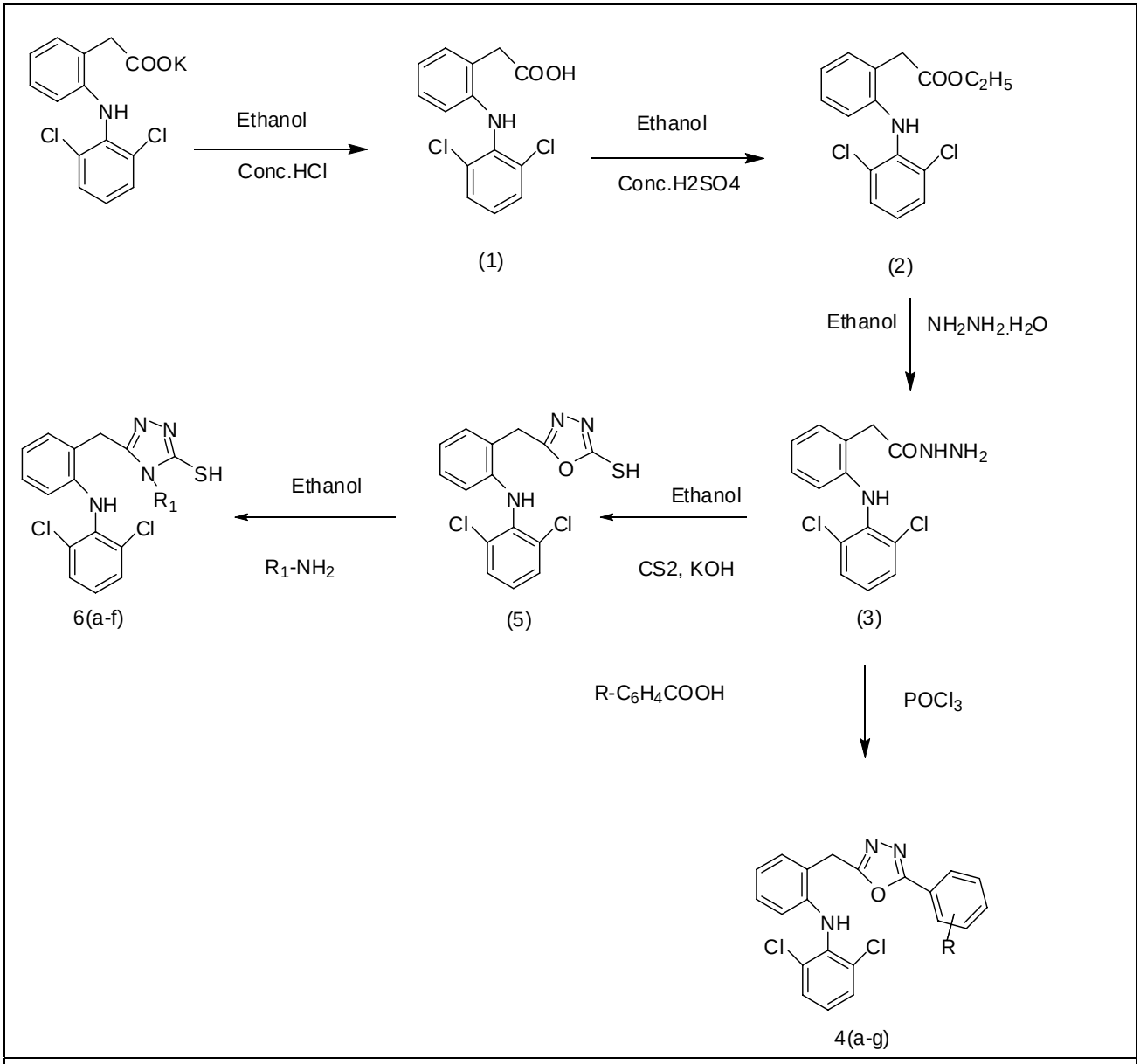


IR Spectral Data

3360 (N-H stretching), 1598 (C=C stretching (Aromatic) ,1656 (C=N stretching),2609 (SH stretching),1257 (C-O Stretching (Aromatic) ,3296 (O-H stretching (Phenol),1302 (C-N Stretching).

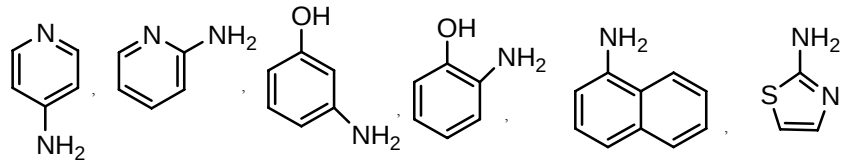
COMPOUND: - 6d

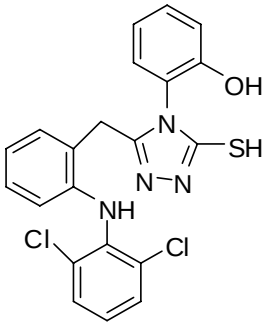
2-(3-(2-(2,6-dichloro phenyl amino)benzyl)-5-mercapto-4*H*-1,2,4-triazole4- yl)phenol



R = 4-Cl, 4-OCH_3, 3,4-OCH_3, 2-F, 3-F, p-CH_3, 2-Br

R_1 =





IR Spectral Data

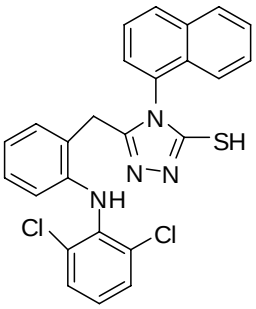
3376 (N-H stretching), 1510 (C=C stretching (Aromatic) ,1602 (C=N stretching),2588 (SH stretching),1270 (C-O Stretching (Aromatic) ,3305 (O-H stretching (Phenol),1405 (C-N Stretching).

NMR Spectral Data

3.71 (Aromatic C-SH),4.81 (Aromatic C-OH),3.81(Methyl protons),7.27-7.35 (Benzene),4.1 (Aromatic C-NH).

COMPOUND: - 6e

5-(2-(2,6-dichloro phenyl amino)benzyl)-4-(naphthalen-1-yl)-4*H*-1,2,4-Triazole-3-thiol



COMPOUND: - 6f
5-(2-(2,6-dichloro phenyl amino)benzyl)-4-(thiazol-2-yl)-4*H*-1,2,4-triazole 3-thiol

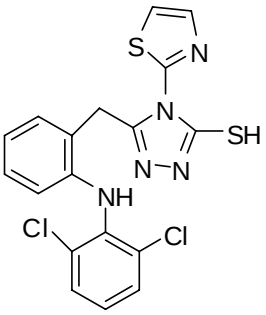


TABLE 2:Physical data of synthetic compounds

S.No	Code	Solubility					Melting Point(°c)	Rf value
		CHCl3	DMF	DMSO	Me-OH	Et-OH		
1	**1**	+	+	+	+	+	150-154	0.55
2	**2**	+	+	+	+	+	128-131	0.60
3	**3**	+	+	+	+	+	136-140	0.70
4	**4a**	+	+	~ +	~ +	+	150-153	0.53
5	**4b**	+	+	+	-	+	143-148	0.78
6	**4c**	+	+	~ +	+	+	127-130	0.75
7	**4d**	+	+	+	~ +	+	145-148	0.80
8	**4e**	+	+	+	+	+	136-140	0.69
9	**4f**	+	+	+	+	+	123-127	0.60
10	**4g**	+	+	+	+	+	126-130	0.65
11	**5**	+	+	+	+	+	124-128	0.71
12	**6a**	-	+	+	+	~ +	122-125	0.67
13	**6b**	+	+	+	+	+	142-145	0.57
14	**6c**	-	+	+	+	~ +	133-137	0.51
15	**6d**	+	+	+	+	+	138-142	0.69
16	**6e**	+	+	+	+	+	176-180	0.80
17	**6f**	+	+	+	+	+	92-95	0.77

Where + → soluble, - → insoluble, ~ +→ slightly soluble

IR Spectral Data
3338 (N-H stretching), 1525 (C=C stretching (Aromatic) ,1596 (C=N stretching),2365 (SH stretching),2965 (C-H Stretching (Aromatic), 1332 (C-N Stretching).

IR Spectral Data
3266 (N-H stretching),1517 (C=C stretching (Aromatic), 1618 (C=N stretching),2364(SH stretching),1033 C-Cl (Aromatic).

Pharmacological studies

Acute ulcerogenecity studies[27,28,29,30,31,32]

The compounds, which showed anti-inflammatory activity comparable to that of the standard drug diclofenac and also showed high analgesic activity, were screened for their ulcerogenic activity.

Requirements:

Animal : Albino rat (150-200g)

Drugs and chemicals: Ether, Diclofinac potassium (standard) , Carboxy methyl cellulose (1%w/v), DMSO, Saline solution, Distilled water
Test compounds: 4c, 4d, 4f, 6a, 6c, 6e

Albino rats were divided into 8 groups of six animals in each group. Potential for ulcerogenecity was evaluated after p.o. administration of test or standard

TABLE No.3: Ulcerogenic effects of synthesized compounds in comparison with Diclofenac potassium

Compound code	Dose (mg/kg, p.o)	Ratio of ulcerated animals	Ulcer index (mean±SE)
Control (normal saline)	10ml/kg orally	Nil	0.00±0.00
Diclofenac potassium (std)	10mg/kg.I.P	6/6	2.64±0.41
4c	10mg/kg in 0.5ml DMSO	4/6	1.23±0.35
4d	10mg/kg in 0.5ml DMSO	1/6	0.32±0.12
4f	10mg/kg in 0.5ml DMSO	2/6	0.42±0.06
6a	10mg/kg in 0.5ml DMSO	1/6	0.30±0.08
6c	10mg/kg in 0.5ml DMSO	1/6	0.41±0.11
6e	10mg/kg in 0.5ml DMSO	2/6	0.52±0.16

Data are expressed as mean ± S.E.M., data analyzed by one way ANOVA followed by Newman's Keul's multiple range test to determine the significance of the difference between the standard group and rats treated with the test compounds. The differences in results were considered significant at $P < 0.01$.

compounds at 10mg/kg in therapeutic doses. Control rats received 0.5 ml of DMSO as vehicle. Animals were fasted for 24 h before dosing, with water ad libitum. In order to induce prominent ulcers, after the drug treatment, the rats were ligand with pyloric end for 4 hr and then sacrificed by ether inhalation. The animals were sacrificed and dissected along the greater curvature of the stomach. And the stomach specimen were washed with distilled water and cleaned gently by dipping in saline. The mucosal damage was examined by means of a magnifying glass (fig.1). For each stomach, the mucosal damage was assessed according to the following scoring system:

Score Assignment

0.0 Normal (No injury, bleeding, and latent injury).
0.5 Latent injury or widespread bleeding.
1.0 Slight injury (2 to 3 dotted lines).
2.0 Severe injury (continuous lined injury or 5 to 6 dotted injuries).
3.0 Very severe injury (several continuous lined injuries).
4.0 Widespread lined injury or widened injury.

The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.

RESULTS AND DISCUSSION

Acute ulcerogenecity studies:

Close inspection of the results obtained by ulcerogenecity studies indicate that ulcerogenic activity of various organized compounds ranging from 0.30 ± 0.08 to 1.23 ± 0.35, whereas the standard drug diclofenac potassium showed high severity index of 2.64 ± 0.41. The Synthetic compound **4c** showed reduction in ulcerogenic activity (1.23 ± 0.35), and compounds **4d, 4f, 6a, 6c** and **6e** at 10mg/kg therapeutic dose on stomach was negligible compared to drug Diclofenac potassium at the same dose levels **(Table No.5)**. Hence it can be said that gastro intestinal tolerance to these compounds is better than that of Diclofenac potassium. The results of potential for ulcerogenecity studies by the synthesized compounds are tabulated in the **Table No.5**. Gastric ulcers after ulcer induction by control, standard and synthesized compounds in rats are shown in **fig.1**

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