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IJPBS |Volume 4| Issue 4|OCT-DEC|2014|120-125

Research Article
Pharmaceutical Sciences

SYNTHESIS, CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITY OF 5-SUBSTITUTED PHENYL-N-(6-(PROPYLTHIO)-1H- BENZO [D] IMIDAZOL-2-YL)-1, 3, 4-OXADIAZOL-2-AMINES

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ABSTRACT

A series of 5-substituted phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amines were prepared by treating substituted 2-benzylidene- N- (6- (propylthio)- 1H-benzo[d]imidazol-2-yl) hydrazine carboxamides with Chloramine T. The newly synthesized derivatives were screened against anti-inflammatory activity using COX kit. The compounds showed dose dependent activity. The compounds which showed better in-vitro activity tested for in-vivo activity using Carrageenan rat paw edema method.

KEY WORDS

benzimidazole, oxadiazole, carrageenan, rat paw edema method.

INTRODUCTION

Numerous compounds bearing oxadiazole ring are known to possess important of pharmacological activities such as antimicrobial[1-3], antifungal4[-5], antitubercular6, and anti-inflammatory[7-9] agents. Our group has been working on development of new series of oxadiazolemoieties with anti-inflammatory activity.This manuscript reports the synthesis and anti-inflammaory activity of aforementioned compounds by COX activity by TMMD assay method and rat paw edema method.

MATERIALS AND METHODS

Melting points (mp) were determined in open capillaries, using Toshniwal melting point apparatus, expressed in ^{o}C and are uncorrected. The IR spectra of the compounds were recorded on thermo Nicolet Nexus 670S series, FT-IR spectrometer using KBr disc. ^{1}H NMR was scanned on Avance-400 MHz instrument. Chemical shifts are expressed in d (ppm) relative to TMS as an internal standard using DMSO-d6 as solvent. Mass spectra were recorded on a LC-MSD-Trap-SL. The purity of the compounds was checked on silica gel-coated aluminum sheets (Merck, 1.005554, silica gel HF254–361, Type 60, 0.25 mm, Darmstadt, Germany) by thin-layer chromatography (TLC). TLC was performed on silica gel G for TLC (Merck) and spots were visualized by iodine vapor or by irradiation with ultraviolet light (short wave length, 254 nm). Column chromatography was performed by using sisco's silica gel for column chromatography (60–120 mesh).

EXPERIMENTAL METHODS

1. Synthesis of N- (6- (propylthio) -1H-benzo[d]imidazol-2-yl) hydrazine carboxamide (II):

Methyl (6-(propylthio)-1H-benzo[d]imidazol-2-yl) carbamate (I)(0.01mol)wasrefluxedhydrazine hydrate 99% (0.2mole) in 20 ml of methanol for 2 hrs. The solvent was evaporated and poured onto ice cold water and collected the product by filtration dried and purified with methanol.

2. Synthesis of 2-benzylidene- N-(6- (propylthio)-1H-benzo [d] imidazol-2-yl) hydrazine carboxamide (V):

A mixture N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl) hydrazine carboxamide (II) (0.01mol) and appropriate aromatic aldehyde (0.01mol) in absolute ethanol (15ml), in the presence of catalytic amount of glacial acetic acid (3 drops) and the reaction mixture was refluxed for 6-7 hr. The reaction mixture was allowed to cool to room temperature and then poured onto crushed ice. The precipitated compound was filtered and washed with water and recryastllized from absolute ethanol.

Synthesis of 5-substituted phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amines (VI):

2-benzylidene-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl) hydrazine carboxamide (0.01mol) was refluxed with 0.1mol of ChloramineT in absolute ethanol for about 1hr. It was allowed to cool to room temperature. That resultant was extracted with ethyl acetate. The product was recryastallized with ethyl acetate.

Characterization:

5-phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amine:IR (KBr, cm^{-1}): 2970.21 (Ar-CH), 3346.77 (NH), 2960.0 (ArC-H), C=O (1637.19)'.^{1}H NMR (DMSO-$d_6$300MHz)δ:0.99(t,3H), 1.3-1.4(m,2H), 1.5 1.6(m,2H), 7.5(d,1H), 7.6(t,1H), 7.78(d,1H), 7.9(s,1H),12(s, NH).Mass spectrum of the compound exhibited molecular ion (M+1) peak at m/z 354.

Anti-inflammatory activity:

***In vitro* Anti-inflammatory activity**

The synthesized compounds were evaluated for their *in vitro* anti-inflammatory activity by TMPD assay method[10]. This assay is based on chromogenic assay based on oxidation of N, N, N', N,-tetra methyl-p-phenylenediamine (TMPD) during the reduction of prostaglandinH_2 by COX-2 enzyme. This measures the peroxides component of cyclooxygenases. The peroxide activity is assayed calorimetrically by monitoring the appearance of oxidized N,N,N',N,-tetramathyl-p-phenylenediamine (TMPD) at 590nm.The final volume of the assay was 220µl I All the wells Background wells contains 160µl of assay buffer and 10µl of heme and 10µl of enzyme. The inhibitor wells contain150µl of assay buffer and 10µl of heme, 10µl of enzyme and 10µl of inhibitor. The plate was shaken for a few seconds and incubated for five minutes at 25^0C.Then 20µl of colorimetric substance, 20µl of arachidonic acid were added. The plate was again shaken for a few seconds and incubated for five minutes at 25^0C.Then the absorbance was noted at 590nm using plate reader.

***In vivo* Anti-inflammatory activity**

Anti-inflammatory activity was assessed by the method described by Winter et.al [11]. Rats were divided into three groups (control, test compounds and standard drug) of six animals each. The standard Diclofenac sodium (100mg/kg dose) and synthesized compounds under study (IVB,C,E,Fand H) were administered orally to all rats. After 30minutes a freshly prepared suspension of carrageenan (1% in 0.9%, saline 0.5ml) was injected under the subplanter tissues of the right hind paw of each rat. The edema volumes of the injected paw were measured at 1st, 2nd, 3rd and 4thhour.The difference between the paw volumes of treated

animals were compared with that of the control group and the mean oedema volume was calculated. From the data obtained mean volume of oedema ± SEM (Standard Error Mean) and percentage reduction in oedema were calculated. Percentage reduction or inhibition in oedema volume calculated by using the formula.

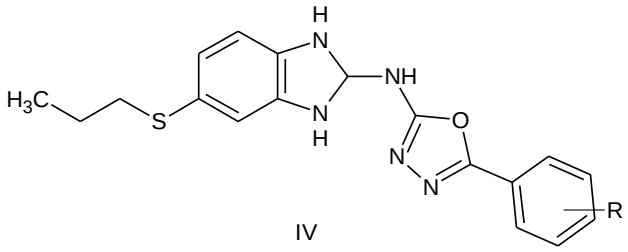
Persentage of inhibition of oedema=1-Vt/Vc*100

Where Vt and Vc are volumes of oedema in test compound/standard drug treated and control group respectively.

RESULTS

5-substituted phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amines (VI a-j) on COX-1 and COX-2 was presented in the table 2 and figure Among the compounds of the series, IVC(R=4-Cl) was active with an IC_{50} value 2.011 for COX-1 and compounds IVB(R=4-OH) and IVF(R=2-furfuryl) were next in order. Compound IVE (R=N $(CH_3)_2$) was active with an IC_{50} values 20.85 for COX-2and compounds IVH(R=4-OCH_3) was next in order. Among the compounds of the series, no compound showed significant action on both the enzymes. The compounds IVC(R=4-Cl), IVB(R=4-OH), IVF(R=2-furfuryl), IVH(R=4-OCH_3) and IVE (R=N$(CH_3)_2$) tested for their *in-vivo* anti-inflammatory activity using Carrageenan induced rat paw edema method and represented in table3.Among the five compounds, IVB(R=4-OH) showed good activity.

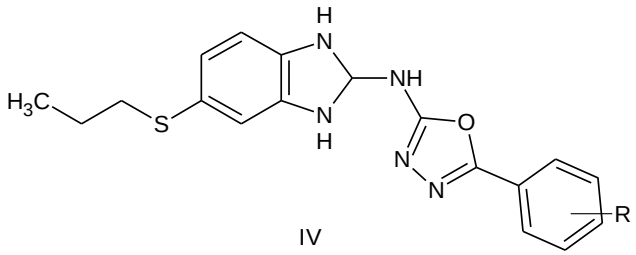
TABLE 1: Physical data of 5-substituted phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amines:



IV

S.no.	Compound	R	Molecular formula	Molecular weight	Melting point (°C)	Percentage yield
1	IVa	H	$C_{18}H_{19}N_5OS$	353.44	142-143	72
2	IVb	4- OH	$C_{18}H_{19}N_5O_2S$	369.44	150-151	75
3	IVc	4-Cl	$C_{18}H_{18}ClN_5OS$	387.89	153-154	73
4	IVd	4-NO_2	$C_{18}H_{18}N_6O_2S$	382.44	158-160	72
5	IVe	$N(CH_3)_2$	$C_{20}H_{24}N_6OS$	396.51	162-163	73
6	IVf	2-furfuryl	$C_{23}H_{27}N_7OS$	421.56	163-164	75
7	IVg	4-F	$C_{18}H_{18}FN_5OS$	371.43	145-146	76
8	IVh	4- OCH_3	$C_{19}H_{21}N_5O_2S$	383.47	149-151	74
9	IVi	2-OH	$C_{18}H_{19}N_5O_2S$	369.44	151-152	75

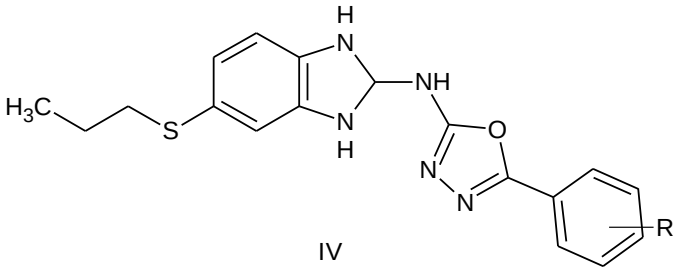
TABLE 2: Anti-inflammatory activity data of 5-substituted phenyl-N-(6-(propylthio)-1H benzo [d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amines:



IV

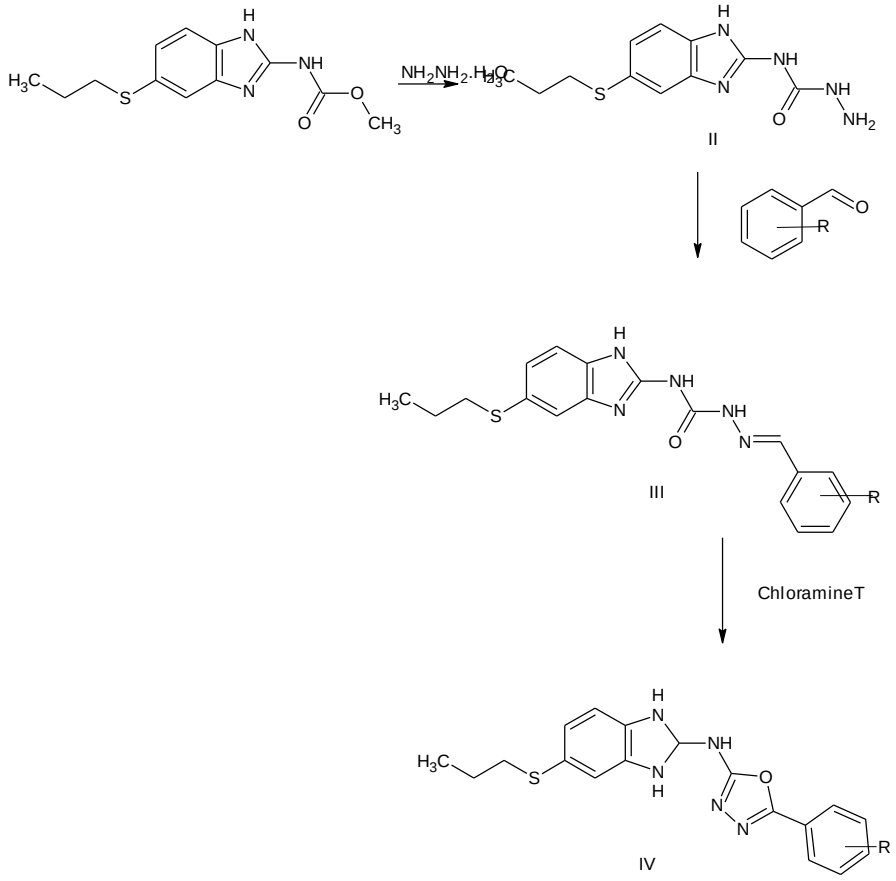
S.No.	Compound	R	IC_{50}(µg/ml)	
			COX-1	COX-2
1	A	H	3.67	95.36
2	B	4- OH	3.233	96.32
3	C	4-Cl	2.011	86.84
4	D	4-NO_2	4.294	91.88
5	E	$N(CH_3)_2$	5.014	20.85
6	F	2-furfuryl	3.358	89.72
7	G	4-F	3.589	91.25
8	H	4- OCH_3	4.298	24.23
9	I	2-OH	6.55	76.61
10	J	Cinnamaldehyde	5.234	96.35

Table3: *In-vivo* anti-inflammatory activity data of 5-phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amine (IV a-j):



IV

S.No	Compound	R	Percentage inhibition of rat paw edema			
			1hr	2hr	3hr	4hr
1	IVE	$N(CH_3)_2$	0.375±0.055902	0.275±0.055902	0.25±0.070711	0.195±0.026926
2	IVB	4- OH	0.275±0.055902	0.235±0.055902	0.2±0.079057	0.077632±0.055902
3	IVH	4-OCH3	0.375±0.055902	0.2925±0.04603	0.235±0.036401	0.2±0.033912
4	IVC	4-Cl	0.025±0.50	0.195±0.035	0.1525±0.0488	0.1075±0.034911
5	IVF	2-furfuryl	0.225±0.018028	0.24±0.05244	0.1975±0.9875	0.1625±0.023848
6	Std.	Diclofenac Sodium	0.029±0.036	0.0207±0.090	0.0212±0.069	0.0216±0.56



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