Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 4| Issue 3|JUL-SEPT|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

K.Blessi Priyanka ,B.Shobarani, G.Sammaiah

University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Andhra Pradesh, India.

*Corresponding Author Email: blessipriyanka@gmail.com

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], antifungal[4-5], antitubercular[6], anti-inflammatory[7-9] agents. Our group has been working on development of new series of oxadiazole moieties with anti-inflammatory activity. This manuscript reports the synthesis and anti-inflammatory 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 (III):

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.

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

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 recryastllized with ethyl acetate.

Characterization:

5-phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amine (IVA):

IR (KBr, cm^{-1}): 2970.21 (Ar-CH), 3346.77(NH), 2960.0 (Ar C-H), C=O (1637.19).

Mass spectrum of the compound exhibited molecular ion (M+1) peak at m/z 354.

^{1}H NMR (DMSO-d300MHz) δ:0.9 (t,3H,CH3),1.5(m,2H,-CH2),2.9(t,2H,s-CH2),Ar-6.8(d, 2H),7.5 (t,3H), 7.6(d,2H), 7.8 (s,1H), 12 (s, NH).

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,-tetramethyl-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 contain 150µl 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 25ºC.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^{0}C.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, F and 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 sub planter tissues of the right hind paw of each rat. The edema volumes of the injected paw were

measured at 1st, 2nd. 3rd and 4th hour. 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. The Percentage reduction or inhibition in oedema volume was calculated by using the formula.

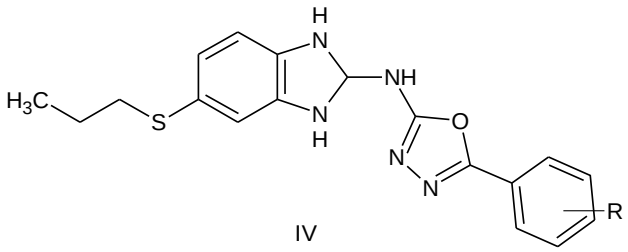
Percentage of inhibition of oedema=1-V_t / V_c *100

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

RESULTS AND DISCUSSION

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. Among them, IVC (R=4-Cl) was active on Cox-1 enzyme. IVE(R=N $(CH3)_{2)}$ was active on COX-2 enzyme. 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$(CH_3)_2$) were tested for their *in-vivo* anti-inflammatory activity using Carrageenan induced rat paw edema method and represented in table 3.Among the five compounds, IVC(R=4-Cl) showed good activity.

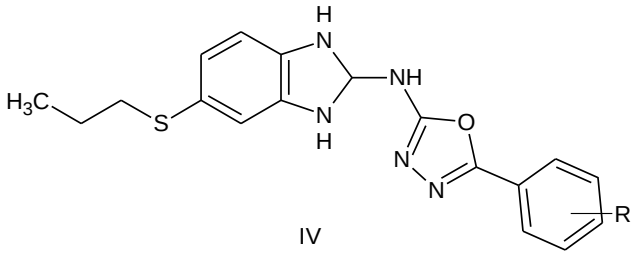
TABLE 1: Physical data of 5-substituted phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amines:



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

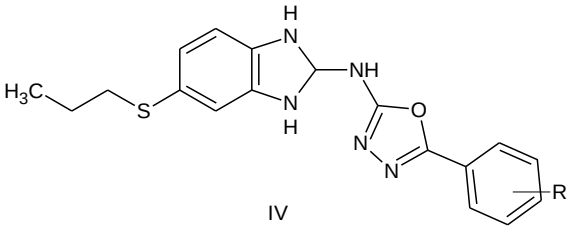
TABLE 2: Anti-inflammatory activity data of 5-substituted phenyl-N-(6-(propylthio)-1H benzo [d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amines:



IV

S.No.	Compound	R	IC_{50}(µg/ml)	
			COX-1	COX-2
1	IV_a	H	3.67	95.36
2	IV_b	4- OH	3.23	96.32
3	IV_c	4-Cl	2.01	86.84
4	IV_d	4-NO_2	4.29	91.88
5	IV_e	4-$N(CH_3)_2$	5.01	20.85
6	IV_f	2-furfuryl	3.35	89.72
7	IV_g	4-F	3.59	91.25
8	IV_h	4- OCH_3	4.29	24.23
9	IV_i	2-OH	6.55	76.61
10	IV_j	2-CHCHCHO	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):

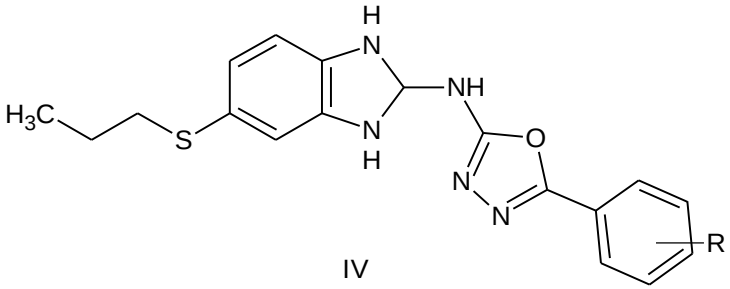


IV

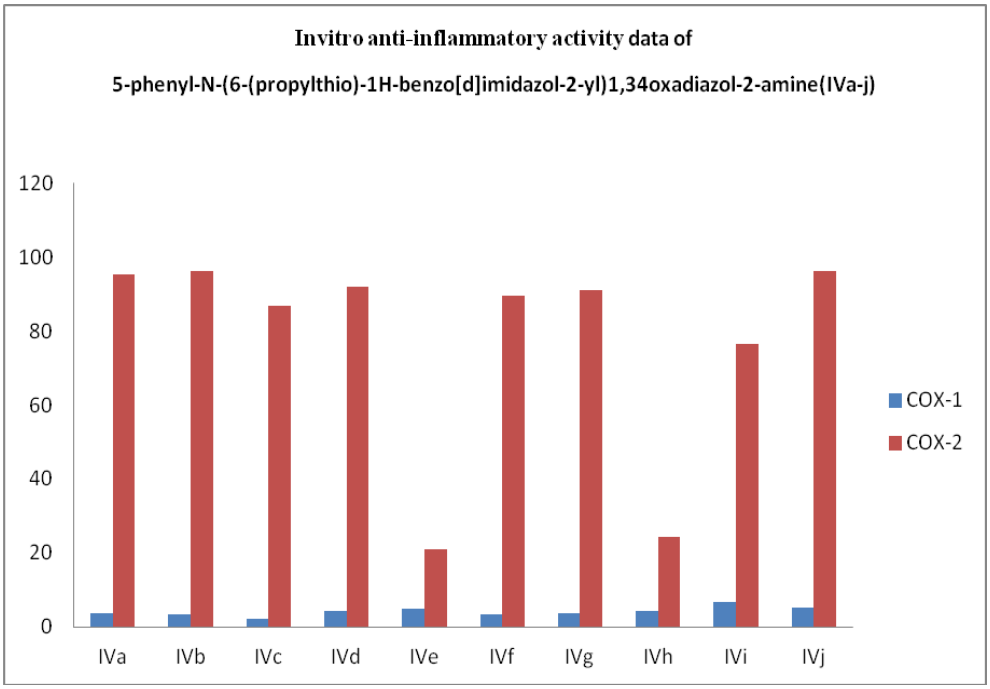
S.No	Compound	R	Percentage inhibition of rat paw edema			
			1hr	2hr	3hr	4hr
1	IVE	$N(CH_3)_2$	0.375±0.055902	0.275±0.055902	0.25±0.070711	0.195±0.026926
2	IVB	4- OH	0.275±0.055902	0.235±0.055902	0.2±0.079057	0.077632±0.055902
3	IVH	4-OCH3	0.375±0.055902	0.2925±0.04603	0.235±0.036401	0.2±0.033912
4	IVC	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

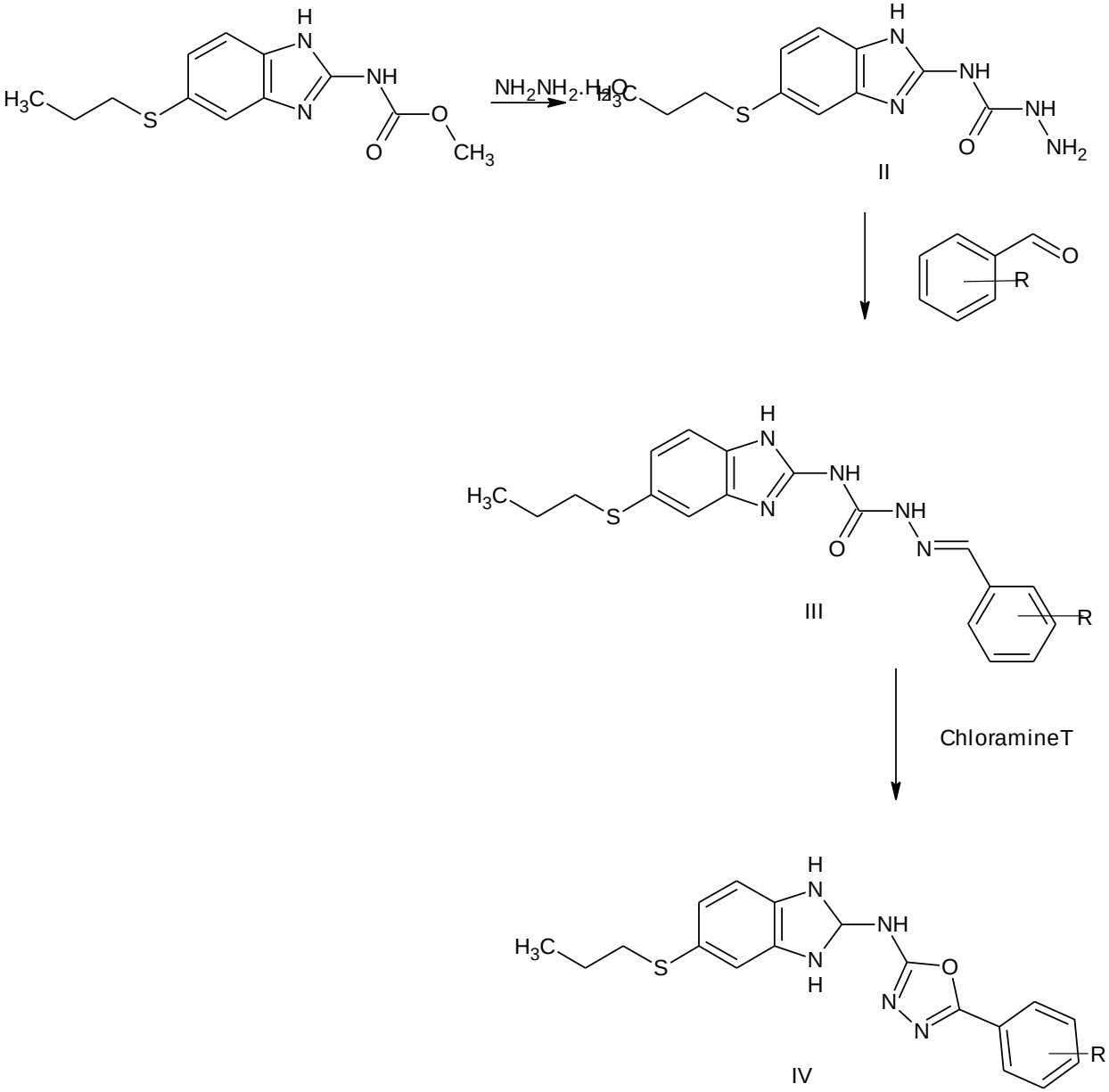
Figure-1:Anti-inflammatory activity data of 5-substituted phenyl-N-(6-(propylthio)-1H benzo [d] imidazol-2-yl) -1, 3, 4-oxadiazol-2-amines (IVa-j):



IV



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



R=H,4-OH,2-OH,4-Cl,4-NO_2,$N(CH_3)_2$,4-OCH_3,CH=CHCHO,2-furfuryl

REFERENCES

1. Gulay Sahin,Erhan Palska,Melike ekizoglu, Meral Ozalp,Synthesis and antimicrobial activity of some 1,3,4-oxadiazole derivatives, *Il Farmaco,(2002),57*,539-542.
2. Mohammed Afroz Bakht, M.Shahar Yar,Sami Gaber Abdel-Hamid, Saleh I.Al Qasoumi, Abdul Samad, Molecular properties prediction, synthesis and antimicrobial activity of some newer oxadiazole derivatives, *Europian Journal of Medicinal Chemistry,(2010),45*,5862-5869.

3. Marina Ishii, Salomao Doria Jorge,Alex Alfredo de Oliverira, Fanny palace-Berl, leda Yuriko Sonehara, Kerly Fernada Mesquita Pasqualoto ,Leoberto Costa Tavares, Synthesis, molecular modeling and preliminary biological evaluation of a set of 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-ixadiazole as potential antibacterial, anti- Trypanosoma cruzi and antifungal agents, *Biorganic & Medicinal Chemistry*.
4. Quiong Chen,Xiao-Lei Zhu,Li-Li Jiang,Zu-Ming Liu,Guang-Fu Yang, Synthesis, antifungal activity and CoMFA analysis of novel 1,2,3-triazino[1,5-a]pyrimidine derivatives, *Europian Journal of Medicinal Chemistry,(2008),43*,595-603.
5. Weiming Xu,Song Yang,Pinaki Bhadury,Jiag He,Ming He,Lili Gao,Deyu Hu,Baoan Song, Synthesis and bioactivity of novel sulfone derivatives containing 2,4-dichlorophenyl substituted 1,3,4-oxadiazole/thiadiazole moiety as chitinase inhibitors, *Pesticide Bichemistry and Physiology,(2011),101*,6-15.
6. Jignsh P.Raval,Tarunkumar N.Akhaja,dhaval M.Jaspara, Kruti N.Myangar, Nilesh H.Patel Synthesis and *in vitro* antibacterial activity of new oxoethylthio-1,3,4-oxadiazole derivatives ,*Journal of Saudi Chemical Society*,(2011),
7. FA Omar,N M Mahfouz,M A Rahman, Synthesis and anti-inflammatory activity of some 1,3,4-oxadiazole derivatives, *Europian Journal of Medicinal Chemistry,(1996),31*,819-825.
8. VirginijaJakabkiene,MildaMalvinaBurbuliene,GiedruteMekuskiene,EmilijaUdrenaite,Povilas Gaidelis, Polivas Vainilavicius, Synthesis and anti-inflammatory activity of 5-(6-methyl-2-substituted 4-pyrimidinyloxymethyl)-1,3,4-oxadiazole-2-thiones and their 3-morpholinomethyl derivatives, *Il Farmaco,(2003),58,*323-328.
9. Shashikant V.Bhandari, Kailash G.Bothara, Mayush K.Raut,Ajit A.Patil, Anikat P.Sarkateand Vinod J.Mokale, Design, synthesis and valuation of Anti-inflammatory, Analgsic and Ulcrognicity studis of Novel S-Substituted phnacyl-1,3,4-oxadiazol-2-thiol abd Schiff bass of Diclofnac acid as Nonulcrogenic Derivatives, *Bioorganic &Medicinal Chemistry,(2008),16*,1822-1831.
10. R. J. Kulmacz, W.E.M. Lands, *Prostaglandins (1983)*, *25,*531-540.
11. C. A. Winter , E. A. Risley, G. W. Nuss, *Experimental Biological Medicine (1962), 111*, 544-547.

***Corresponding Author:**
G.Sammaiah
Assistant Professor,
University College of Pharmaceutical Sciences,
Kakatiya University,
Warangal.