Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Special Issue [1]2022 : 805-811

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

In-Vitro Evaluation of Anti- Inflammatory Activity of Newly Synthesized 1-([1,3,4- Oxadiazino [6,5-b] substituted indol-2-yl methyl) benzenes

B. Padma[1], T. Sri Ramya[1], T. Sunitha[2], *G. Sammaiah[1]
[1]Department of Pharmaceutical Chemistry, Univeristy College of Pharmaceutical Sciences, KakatiyaUniveristy, Warangal
[2]Department of Pharmacology, Univeristy College of Pharmaceutical Sciences, KakatiyaUniveristy, Warangal
Corresponding Author: g.sammaiah@gmail.com

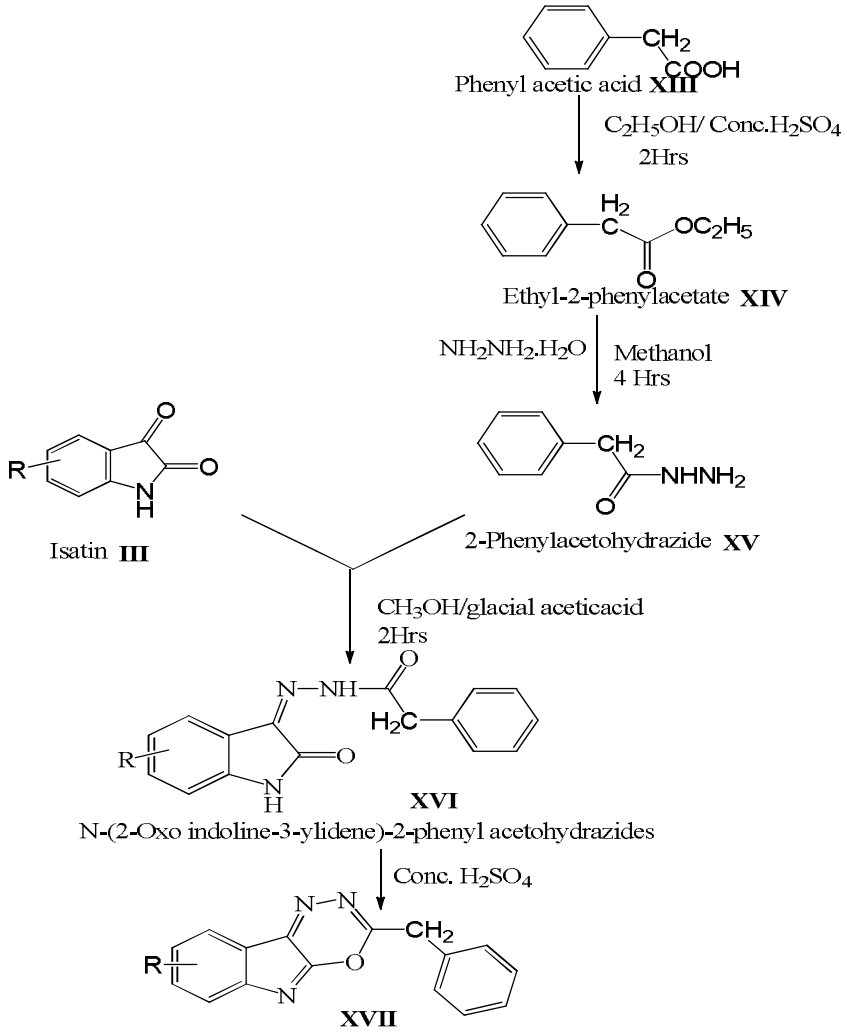
ABSTRACT
A novel series of 1-([1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes was synthesised in good yields and characterised by IR, NMR, and mass spectrum studies in the current work. Cayman's Colorimetric COX (ovine) Inhibitor Screening Assay Kit was used to test compounds for ant-inflammatory action in vitro. The anti-inflammatory activity of all the compounds was observed with IC_{50} values in the range of 38.42 to 84.78 µg/ml. Among the tested compounds XVIIb (R=5-Cl, IC_{50} 38.42 µg/ml), XVIIc (R=7-Cl, IC_{50} 40.41 µg/ml), XVIIh (R=5-Br IC_{50} 45.84 µg/ml), exhibited potent anti-inflammatory activity. Remaining compounds showed mild to moderate anti-inflammatory activity. The activity of all the test compounds was compared with standard drug Indomethacin that showed an IC_{50} value of 0.93 µg/ml for anti-inflammatory activity. The tested compounds with halogen (R=Cl, Br) substituted derivatives exhibited more in vitro anti-inflammatory activity.
Keywords: *Oxadiazino, in-vitro anti-inflammatory activity, halogen, Cayman's kit, Colorimetric screening*

Received 22.02.2022 Revised 21.03.2022 Accepted 11.04.2022

INTRODUCTION
The widespread usage of isatin and its derivatives as important intermediates in chemical synthesis has led to their widespread utilisation. Many alkaloids and medicines, as well as dyes, insecticides, and analytical reagents, contain isatin. Antimicrobial [1], antiviral, antimycobacterial, anti-inflammatory [2] and anticonvulsant [3] actions have also been documented for some of their derivatives. As a result, this discipline is becoming increasingly important, leading to the development of novel isatins. It was thought useful to synthesise several new biologically powerful isatins with the goal of screening for anti-inflammatory action, given the wide range of uses. Analytical and spectral (IR, 1HNMR, and Mass) data were used to purify and characterise the produced compounds.

MATERIAL AND METHODS
The chemicals and solvents (SD fine) were obtained from Hanamkonda's local wendors of ALM chemicals. The IR spectra were collected on a Bruker FT-IR using the KBr pellet method (percent transmittance, wave number in cm-1). The proton nuclear magnetic resonance (1H NMR) spectra were measured using a Bruker Avance II 400 NMR (400 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. DMSO-d6 was used as the solvent unless otherwise noted. An ABI Perkin–Elmer Sciex API-150 mass spectrometer with electrospray ionisation was used to assess the relative intensity of each ion peak, and the results were expressed as a percentage. Melting points were determined without correction using Thomas Hoover melting point apparatus. Thin-layer chromatography (TLC) on silica gel 60 F254 plates was used to monitor the reactions.. All of the compounds utilised were of the AR classification (Sigma-Aldrich, Hi-media). The titled compounds were created as part of a literature review.

Chemistry
The novel 1-(1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes have been synthesized by procedures established by Sammaiah [3], following Scheme-III and characterized by IR,NMR and Mass data. Physical data of new compounds are presented in Table-1.



Scheme-III

General Procedure for the synthesis of 1-(1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes

Synthesis of Ethyl-2-phenyl acetate (XIV)

In a round bottomed flask, phenyl acetic acid (XIII, 0.01 mol) and ethyl alcohol (0.02 mol) were combined (250 ml). The reaction mixture was heated for two hours on a water bath with reflux in the presence of concentrated sulphuric acid (1-2 drops). TLC was used to verify the reaction. Filtration was used to separate the compound, which was then rinsed with cold water and dried. The resulting product was then purified using ethanol recrystallization [5-7].

Synthesis of 2-Phenyl acetohydrazide (XV) In an RB flask, a combination of ethyl-2-phenyl acetate (XIV, 0.01mol) and hydrazine hydrate (0.02 mol) was dissolved in a little amount of alcohol. For 3-4 hours, the reaction mixture was refluxed in a water bath. After the solvent was removed, the chemical was recrystallized from methanol, yielding a colourless crystalline solid.(m.p. 210-212^{0}C).

Synthesis of N-(2-Oxo indoline-3-ylidene)-2-phenyl acetohydrazides (XVI)

2-Phenyl acetohydrazide (XV, 0.01 mol) was cooked in alcohol for 2 hours at reflux with a suitable isatin (III, 0.01 mol). The solvent was concentrated, and the isolated product was filtered and purified using ethanol 7-9 recrystallization. Table 1 shows the physical properties of the novel compounds.

Synthesis of 1-(1,3,4-Oxadiazino [6,5-b] substituted indol-2-yl methyl) benzenes (XVII)

A pure compound of N-(2-Oxo indoline-3-ylidene)-2-phenyl acetohydrazide **(XVI, 0.01mol)** was treated with 5 ml of concentrated sulphuric acid and kept aside for overnight. The resulting product was separated and excess sulphuric acid was neutralized by sodium bicarbonate solution. The new compound was purified by recrystallization from aqueous ethanol. Adopting the above procedures, different 1-(1,3,4-Oxadiazino [6,5-b] substituted indol-2-yl methyl) benzenes were synthesized, identified and

characterized by TLC, IR, NMR and Mass data. The physical and spectral data of compounds (XVIIa-XVIIn) is depicted in Table 1.

Spectral characterization data of the compound **1-(1,3,4-Oxadiazino [6,5-b]-5-bromo-indol-2-yl methyl) benzenes (XVIIh, R=5-Br)**

Infrared spectrum (KBr, Figure-1) has exhibited absorption characteristics of: 3095.81(C-H, Aromatic), 2912.92 (C-H Aliphatic), 667.13 (C-Br), 1017 .95((C-O) Cm^{-1}, respectively.

^{1}HNMR spectrum (DMSO, Figure-2) showed characteristic proton signals at: 4.30 (s, 2H,-Benzyl)**,** 6.82-6.90 (m, 5H, Aromatic), 7.62-7.74 (m, 3H, Aromatic) δ, ppm, respectively.

^{13}CNMRspectrum (DMSO, Figure-3)showed characteristic signals at: 164.677,160.674, 158.078, 141.725, 131.955, 130.446, 128.929, 123.378, 122.628, 116.378, 112.390, 109.718, 108.078, 39.016, 38.716 δ, ppm, respectively.

Mass spectrum (Figure-4) of the compound exhibited its molecular ion (M^{+}) at m/z 324.

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY BY *IN VITRO* METHOD:

The anti-inflammatory activity of the produced compounds was tested in vitro using the TMPD assay technique[4]. The chromogenic assay is based on the oxidation of N,N,N',N,-tetra methyl-p-phenylenediamine (TMPD) by the COX-2 enzyme during prostaglandinH2 reduction. The peroxides component of cyclooxygenases is measured here. The appearance of oxidised N,N,N',N,-tetramathyl-p-phenylenediamine (TMPD) at 590nm is used to determine the peroxide activity. The assay's ultimate volume was 220 l. Background wells contain 160 litres of assay buffer, 10 litres of heme, and 10 litres of enzyme. 150 l of assay buffer, 10 l of heme, 10 l of enzyme, and 10 l of inhibitor are in the inhibitor wells. The plate was shook for a few seconds before being incubated at 25^{o}C for five minutes. Then 20 litres of colorimetric material and 20 litres of arachidonic acid were added to the mixture. The dish was shaken again for a few seconds before being incubated at 25^{o}C for five minutes. The absorbance was then measured using a plate reader at 590nm [9-11].

Principle

The activity of the COX–2 enzyme was evaluated using a chromogenic test based on the oxidation of N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) during the reduction of prostaglandin G2 to prostaglandin H2 by the COX–2 enzyme. The Colorimetric COX inhibitor screening assay measures the peroxidase component of cyclooxygenases. The peroxidase activity was measured using a visible spectrophotometer set to 590 nm.[12].

Procedure

Three wells were added to the background wells with 160 l of Assay Buffer and 10 l of heme. 150 l of Assay Buffer, 10 l of heme, and 10 l of Enzyme are added to the three wells (COX-2). Inhibitor Wells - Three wells were filled with 150 l of Assay Buffer, 10 l of heme, and 10 l of COX-2 Enzyme. In the inhibitor wells, 10 l of inhibitor was added, and 10 l of solvent was added to the 100 percent Initial Activity wells and background wells (whatever solvent the inhibitor was dissolved in). Before being incubated at 25°C for five minutes, the plate was gently shaken for a few seconds. Add 20 l of the colorimetric substrate solution to all of the wells that were used. All of the wells that were used received 20 litres of arachidonic acid. In all of the wells, the ultimate volume of the assay was 220 l. The plate was gently shook for a few seconds before being incubated at 25°C for five minutes. A plate reader was used to measure the absorbance at 590 nm [13-16].

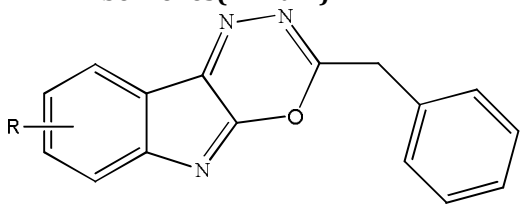
Calculation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of the 100\% initial activity sample} - \text{Absorbance of the inhibitor sample}}{\text{Absorbance of the 100\% initial activity sample}} \times 100$$

RESULTS AND DISCUSSION

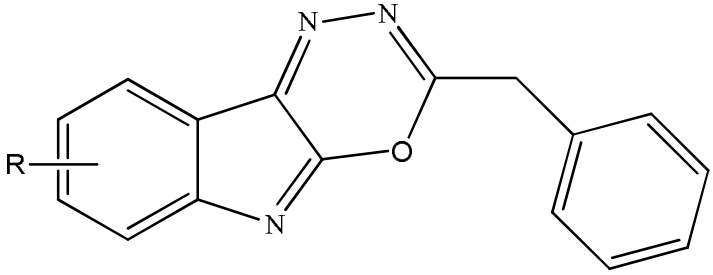
The results of spectral analyses were mentioned in Table-1(Physical data) and Figures 1-4 (Spectra).The *in vitro* anti-inflammatory activity data of 1-(1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes (Scheme-III, XVIIa-XVIIn) are showed in Table-2 and Figure-5. The anti-inflammatory activity of all the compounds was observed with IC_{50} values in the range of 38.42 to 84.78 µg/ml. Among the tested compounds XVIIb (R=5-Cl, IC_{50} 38.42 µg/ml), XVIIc (R=7-Cl, IC_{50} 40.41 µg/ml), XVIIh (R=5-Br IC_{50} 45.84 µg/ml), exhibited potent anti-inflammatory activity. Compounds XVIIf (R=5-F IC_{50} 59.76 µg/ml), was next in the order of anti-inflammatory activity. Remaining compounds showed mild to moderate anti-inflammatory activity.

Table-1. Physical data of 1-([1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes(XVIIa-n)

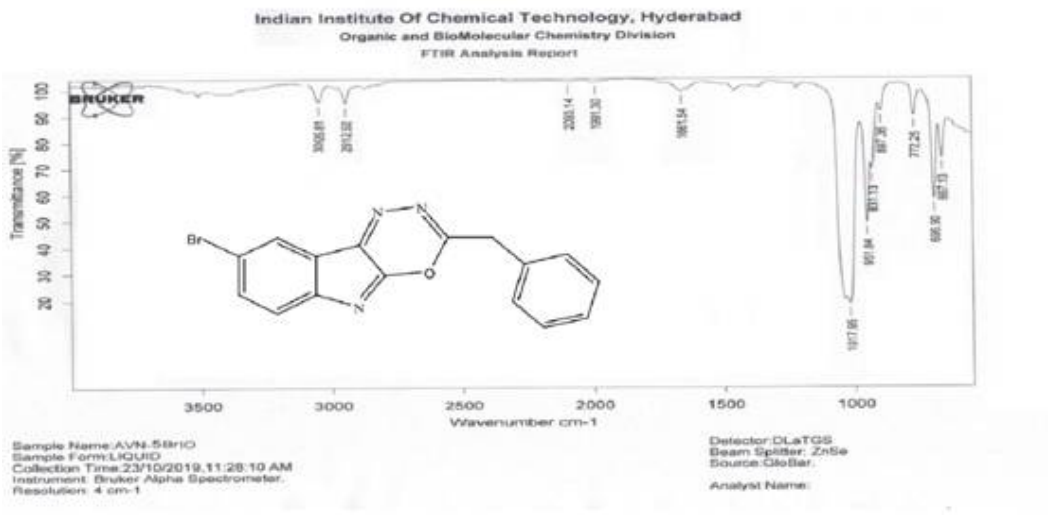


S.No	Compound	Substituent (R)	Mol.Formula	Mol. Wt.	m.p (°C)	% Yield
1	XVIIa	H	$C_{15}H_9N_3O$	247	216-218	58
2	XVIIb	5-Cl	$C_{15}H_8N_3OCl$	281	242-244	68
3	XVIIc	7-Cl	$C_{15}H_8N_3OCl$	281	242-244	70
4	XVIId	5-CH_3	$C_{16}H_{11}N_3O$	261	225-227	62
5	XVIIe	7-CH_3	$C_{16}H_{11}N_3O$	261	225-227	63
6	XVIIf	5-F	$C_{15}H_8N_3OF$	265	302-304	82
7	XVIIg	7-F	$C_{15}H_8N_3OF$	265	302-304	81
8	XVIIh	5-Br	$C_{15}H_8N_3OBr$	324	284-286	88
9	XVIIi	5-NO_2	$C_{15}H_8N_4O_3$	292	298-300	76
10	XVIIj	7-NO_2	$C_{15}H_8N_4O_3$	292	298-300	76
11	XVIIk	5-OH	$C_{15}H_9N_3O_2$	263	243-245	83
12	XVIIl	7-OH	$C_{15}H_9N_3O_2$	263	243-245	83
13	XVIIm	5-COOH	$C_{16}H_9N_3O_3$	291	238-240	75
14	XVIIn	5-$COOC_2H_5$	$C_{18}H_{13}N_3O_3$	319	225-227	57

Table 2 *In vitro* anti-inflammatory activity of 1-([1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes(XVIIa-n)

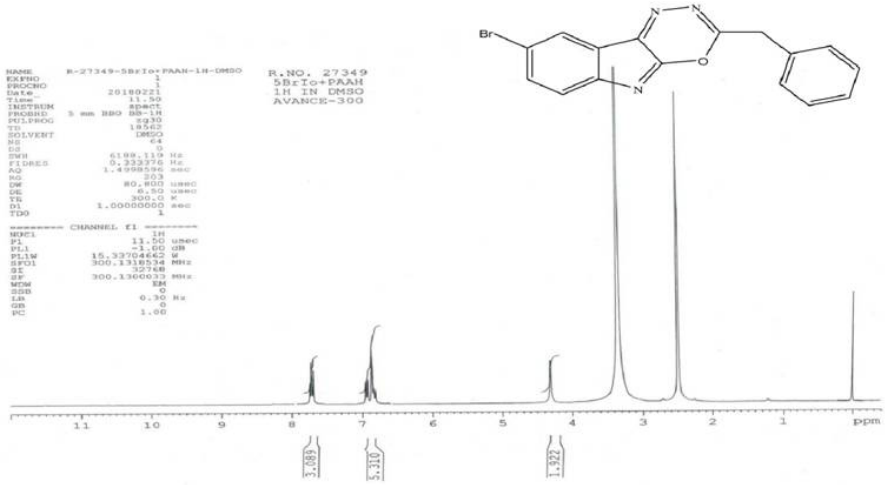


S.No	Compound	R	IC_{50} (µg/ml)
1	XVIIa	H	75.43
2	XVIIb	5-Cl	38.42
3	XVIIc	7-Cl	40.71
4	XVIId	5-CH_3	65.28
5	XVIIe	7-CH_3	63.56
6	XVIIf	5-F	59.76
7	XVIIg	7-F	62.52
8	XVIIh	5-Br	45.84
9	XVIIi	5-NO_2	74.48
10	XVIIj	7-NO_2	73.54
11	XVIIk	5-OH	84.78
12	XVIIl	7-OH	72.27
13	XVIIm	5-COOH	68.24
14	XVIIn	5-$COOC_2H_5$	63.87
15	**Standard**	**Indomethacin**	0.93



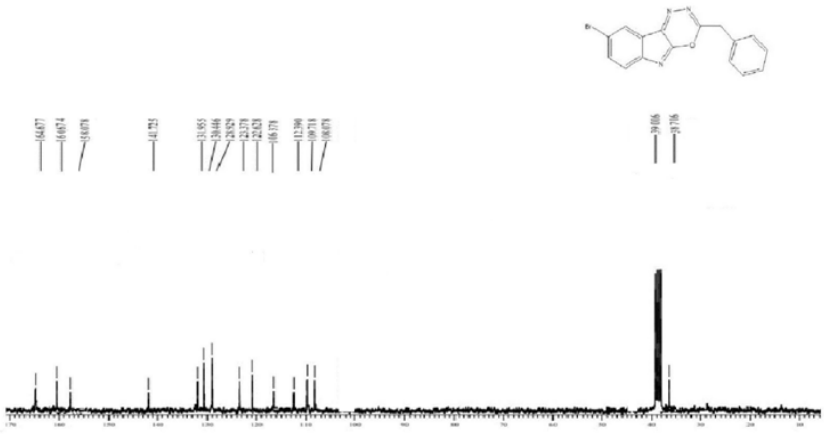
IR spectrum of 1-(1,3,4-Oxadiazino [6,5-b]-5-bromo-indol-2-yl methyl) benzenes (XVIIh, R=5-Br)

Figure 1. IR spectrum of the 1-(1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes



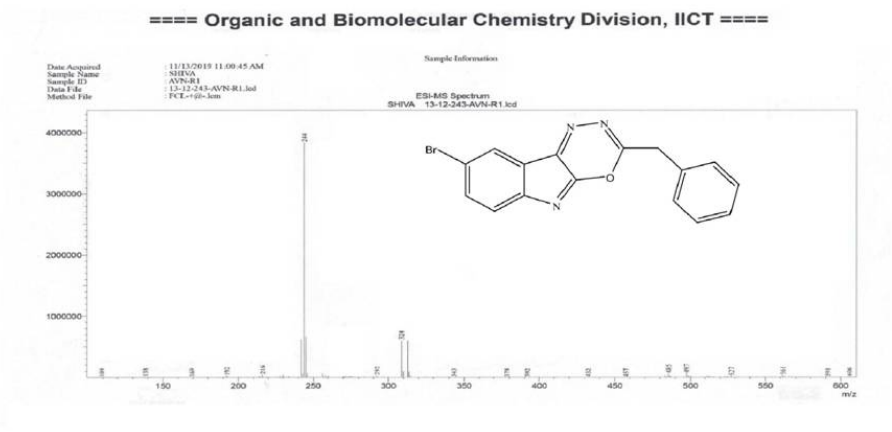
^{1}HNMR spectrum of 1-(1,3,4-Oxadiazino [6,5-b]-5-bromo-indol-2-yl methyl) benzenes (XVIIh, R=5-Br)

Figure 2. ^{1}HNMR spectrum of the 1-(1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes



^{13}CNMR spectrum of 1-(1,3,4-Oxadiazino [6,5-b]-5-bromo-indol-2-yl methyl) benzenes (XVIIh, R=5-Br)

Figure 3. ^{13}CNMR spectrum of the 1-(1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes



Mass spectrum of 1-(1,3,4-Oxadiazino [6,5-b]-5-bromo-indol-2-yl methyl) benzenes (XVIIh, R=5-Br)

Figure 4. Mass spectrum of the 1-(1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes

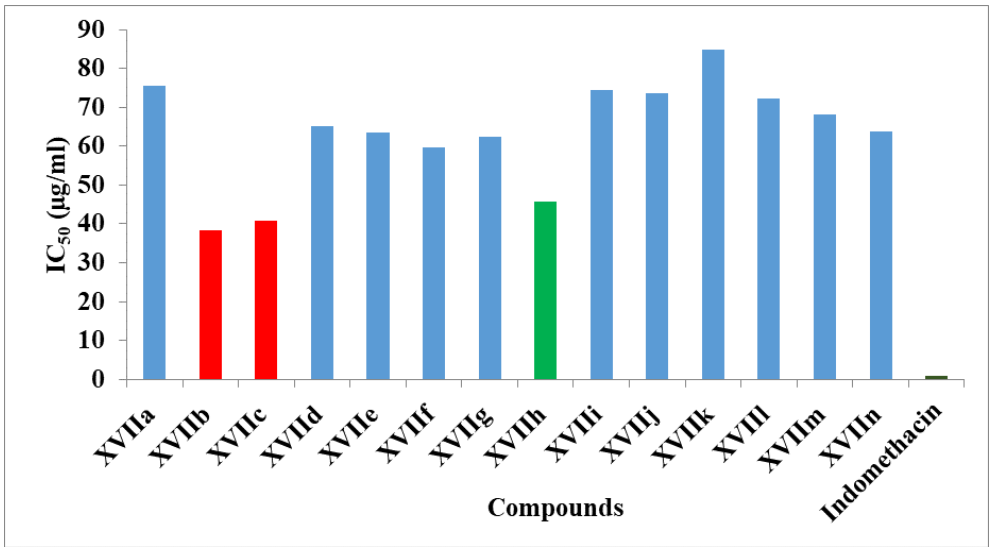


Figure 5. *In vitro*anti-inflammatory activity data of 1-(1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes (XVIIa-XVIIn).

CONCLUSIONS

A series of 1-(1,3,4- Oxadiazino[6,5-b] substituted indol-2-yl methyl) benzenes have been synthesized as shown in Scheme-III. The synthesized compounds were subjected to invitro anti-inflammatory activity. Amongst the compounds tested, substituents with an electron withdrawing group on the aromatic ring showing significant activity than the other substituted compounds.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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CITATION OF THIS ARTICLE

B. Padma, T. Sri Ramya, T. Sunitha, G. Sammaiah. *In-Vitro* Evaluation of Anti- Inflammatory Activity of Newly Synthesized 1-([1,3,4- Oxadiazino [6,5-b] substituted indol-2-yl methyl) benzenes. Bull. Env.Pharmacol. Life Sci., Spl Issue [1] 2022 :805-811