IJBPAS, November, Special Issue, 2021, 10(11): 282-288
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DESIGN, SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF ISATIN DERIVATIVES

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Received 19th July 2021; Revised 20th Aug. 2021; Accepted 29th Sept. 2021; Available online 1st Nov. 2021

https://doi.org/10.31032/IJBPAS/2021/10.11.1024

ABSTRACT

A series of new Isatin derivatives have been designed, synthesized and characterized by IR, ^{1}HNMR, and mass spectral data. The synthesized compounds were screened for *in vitro* anti-inflammatory activity by colorimetric COX (ovine) inhibitor screening assay kit. IC_{50} values of these compounds were determined. Among the series, compounds **XIIc, XIIb, XIIf, XIIg** and **XIIh** exhibited more potent anti-inflammatory activity. Remaining compounds showed mild to moderate anti-inflammatory activity. Results were compared with standard drug Indomethacin. Compound substituted with halogen derivatives are more active than others, but when compared with Indomethacin these are less active.

Keywords: Isatin, Oxadiazine, Synthesis, Anti-inflammatory activity, Cyclooxygenase

INTRODUCTION

Isatin and their derivatives have been associated with various pharmacological and biological and properties **[1]**. The synthesis of a large number of isatin derivatives have been described to obtain biologically potent compounds. Many such compounds have been found to be promising. They showed broad spectrum of biological activity like antimicrobial, antiinflammatory, anticancer, anticonvulsant, anti-oxidant, antifungal and antimycobacterial **[2-8]**. In view of these properties it has been considered worthwhile to synthesize some new biologically potent isatins with an aim to screen for anti-inflammatory activity. The

synthesized compounds has been purified and characterized with the help of their analytical and spectral (IR, ^{1}HNMR & Mass) data.

MATERIALS AND METHODS

All reagents and solvents of laboratory grade were used for synthesis. The melting points were determined in open capillaries using Toshnwal melting point apparatus. Infra-red spectra of the compounds were recorded in KBr pellet using Shimadzu FTIR-8700 spectrometer, ^{1}H NMR spectra on omega-500 MHz spectrometer using TMS as internal standard and mass spectra by the direct inlet method on VG micromass 7070 H spectrometer operating at 70 ev.

The new 3-[4-(2,5-dimethyl amino-1H pyrrol-1-yl-phenyl) [1,3,4]-oxadiazino [6,5-b] substituted indole have been synthesized by following Scheme-I and Characterized by IR, ^{1}HNMR and Mass data. Physical data of new compounds are presented in **Table 1**.

General Procedure for the synthesis of 3-[4-(2,5-dimethyl amino-1H pyrrol-1-yl-phenyl) [1,3,4]-oxadiazino [6,5-b] substituted indole

Synthesis of ethyl 4-(2,5-dimethyl-1H-pyrrol-1-yl) benzoate (IX)

A mixture of ethyl-4-aminobenzoate (VIII, 0.01 mol) and hexane-2,5-dione (II, 0.01 mol) were taken into a RB flask and dissolved in minimum amount of ethyl acetate (10-15 ml). The reaction mixture was refluxed at 120^{0}C for 5-6hrs, progress of the reaction was monitored by TLC. The reaction mixture was cooled and extracted with ether (3 times with 20ml) and washed with 10% citric acid (3 × 20ml) and then with water (20ml). The organic layer was concentrated under reduced pressure to get brown liquid which solidifies at room temperature. The compound obtained was recrystallized with ethyl acetate.

Synthesis of 4-(2,5-dimethyl-1H-pyrrol-1-yl) benzo hydrazide (X)

A mixture of 1:5 ratio of ethyl 4-(2,5-dimethyl-1H-pyrrol-1-yl) benzoate (IX) and hydrazine hydrate, were transferred in to a RB flask and dissolved in minimum quantity of methanol and was refluxed for about 2 hours. The solvent was removed by distillation. The residue is triturated with crushed ice. The product, thus obtained was filtered, washed with distilled water and recrystallized from appropriate solvent.

Synthesis of 4-(2,5-dimethyl-1H-pyrrol-1-yl)-N-(2-oxoindolin-3-ylidene) benzo hydrazides (XI)

4-(2,5-dimethyl-1H-pyrrol-1-yl) benzo hydrazide (V, 0.01 mol) was condensed with isatin (VI, 0.01mol) in methanol and traces of glacial acetic acid

for about 5-6 hours to get their respective 4-(2,5-dimethyl -1H-pyrrol-1-yl) -N'- (2-oxoindolin-3-ylidene) benzohydrazides. The resulting products was filtered and purified by recrystallization method.

Synthesis of 4-(2,5-dimethyl-1H-pyrrol-1-yl-phenyl)-[1,3,4-Oxadiazino [6,5-b] substituted indoles (XII)

A pure compound of 4-(2,5-dimethyl -1H-pyrrol-1-yl) -N'- (2-oxoindolin-3-ylidene) benzo hydrazides. (XI, 0.01 mol) was treated with concentrated sulphuric acid and kept aside for 24 hrs. The resulting products was triturated with crushes ice and filtered and washed with distilled water. The new compound was purified by recrystallization from aqueous ethanol.

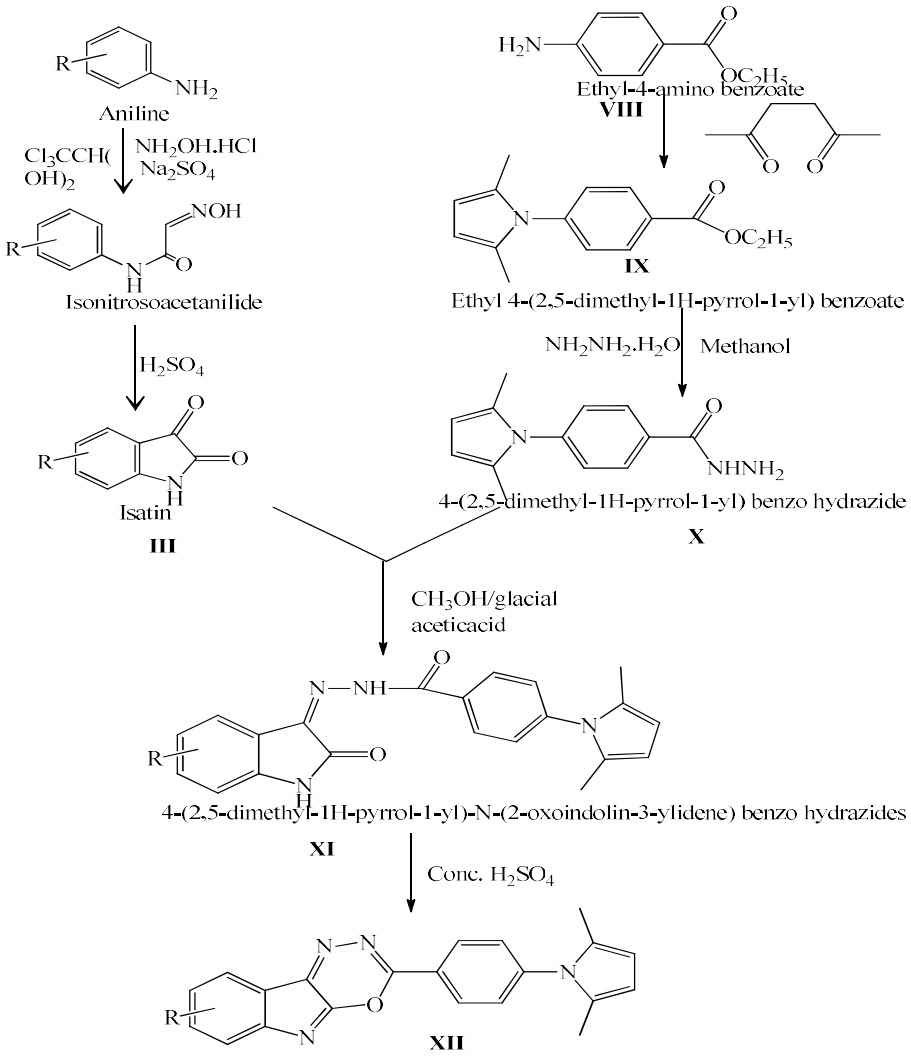
Spectral Characterization data

4-(2,5-Dimethyl-1H-pyrrol-1-yl-phenyl)-[1,3,4]-oxadiazino [6,5-b]-substituted indoles N'-(5-bromo-2-oxoindolin-3-ylidene)-4-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazide (XI)

Infrared spectrum (KBr) has exhibited absorption characteristics of: 3232 (NH), 3071 (Ar C-H), 2976 (Aliphatic -CH), 1727 (C=O), 722 (C-Br) Cm^{-1}, respectively.^{1}HNMR spectrum (DMSO) showed characteristic proton signals at: 2.10 (s,6H, CH_3, Pyrrole), 5.96 – 5.98(d, 2H, Pyrrole), 6.92-6.97(d,1H, Aromatic), &.7.3-7.4(d,2H, Aromatic), 7.46-7.50 (d,1H, Aromatic), 7.97(s,1H,Aromatic), 8.14- 8.18(d,2H, Aromatic), 10.90 (s,1H, CONH), 14.24 (s,1H, NH lactam). δ, ppm, respectively. Mass spectrum of the compound exhibited its molecular ion (M^+) at m/z 437.

Table 1: Physical data of 3-[4-(2,5-dimethyl amino-1H pyrrol-1-yl-phenyl) [1,3,4]-oxadiazino [6,5-b] indoles (XIIa-XIIn)

S. No.	Compound	Substituent (R)	Mol.Formula	Mol. Wt.	m.p (^{o}C)	% Yield
1	XIIa	H	$C_{21}H_{16}N_4O$	340	216-218	45
2	XIIb	5-Cl	$C_{21}H_{15}N_4OCl$	374	244-246	86
3	XIIc	7-Cl	$C_{21}H_{15}N_4OCl$	374	244-246	84
4	XIId	5-CH_3	$C_{22}H_{18}N_4O$	354	232-234	81
5	XIIe	7-CH_3	$C_{22}H_{18}N_4O$	354	232-234	79
6	XIIf	5-F	$C_{21}H_{15}N_4OF$	358	248-250	78
7	XIIg	7-F	$C_{21}H_{15}N_4OF$	358	248-250	75
8	XIIh	5-Br	$C_{21}H_{15}N_4OBr$	418	284-286	92
9	XIIi	5-NO_2	$C_{21}H_{15}N_5O_3$	385	265-267	68
10	XIIj	7-NO_2	$C_{21}H_{15}N_5O_3$	385	265-267	72
11	XIIk	5-OH	$C_{21}H_{16}N_4O_2$	356	221-223	74
12	XIIl	7-OH	$C_{21}H_{16}N_4O_2$	356	221-233	74
13	XIIm	5-COOH	$C_{22}H_{16}N_4O_3$	384	248-250	69
14	XIIn	5-$COOC_2H_5$	$C_{22}H_{20}N_4O_3$	412	243-245	58



4-(2,5-dimethyl-1H-pyrrol-1-yl-phenyl)-[1,3,4-Oxadiazino [6,5-b] substituted indole

Scheme-I

4-(2,5-Dimethyl-1H-pyrrol-1-yl-phenyl)-[1,3,4]-oxadiazino [6,5-b] indole (XIIa, R=H)

Infrared spectrum (KBr) has exhibited absorption characteristics of: 3089.25(C-H, Aromatic), 2912.92 (C-H Aliphatic), 1665.83, (-C=N), 1018.62 (C-O) Cm^{-1}, respectively. ^{1}HNMR spectrum (DMSO) showed characteristic proton signals at: 2.14 (s, 6H, CH_3, Pyrrole), 5.60-5.62 (d, 2H,-Pyrrol),7.15-7.30 (m, 4H, Aromatic), 7.60-7.90 (d, 4H, Aromatic) δ, ppm, respectively. Mass spectrum of the compound exhibited its molecular ion (M^+) at m/z 340. Therefore, based on the above specified spectral data, the compound could be characterized as 4-(2,5-Dimethyl-1H-pyrrol-1-yl-phenyl)-[1,3,4]-oxadiazino [6,5-b] indole (XIIa, R=H).

4-(2,5-Dimethyl-1H-pyrrol-1-yl-phenyl)-[1,3,4]-oxadiazino [6,5-b]-5-bromoindole (XIIh, R=Br)

Infrared spectrum (KBr) has exhibited absorption characteristics of: 3051.50 (C-H, Aromatic), 2956.35 (C-H Aliphatic), 1644.31, (-C=N), 1082.84 (C-O), 673.35 (C-Br) Cm^{-1}, respectively. ^{1}HNMR spectrum (DMSO) showed characteristic proton signals at: 2.20 (s, 6H, CH_3, Pyrrole), 5.52-5.58 (d, 2H,-Pyrrol),7.26-7.40 (m, 3H, Aromatic), 7.64-7.92 (d, 4H, Aromatic) δ, ppm, respectively. Mass spectrum of the compound exhibited its molecular ion (M^+) at m/z 418. Therefore, based on the above specified spectral data, the compound could be characterized as 4-(2,5-Dimethyl-1H-pyrrol-1-yl-phenyl)-[1,3,4]-oxadiazino [6,5-b]5-bromoindole (XIIh, R=Br).

Anti-Inflammatory Activity

The newly synthesized compounds were screened for *In vitro* anti-inflammatory activity **[9, 10]**. The assay was performed using colorimetric COX(ovine) inhibitor screening assay kit (Cyaman Chemical, MI, USA).The colorimetric COX(ovine) inhibitor screening assay utilizes the peroxidase activity of ovine cyclooxygenase to oxidize the colorimetric substrate N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD). Enzyme assays were run in 220µl volumes. The mixture in background wells,100%initial activity well and inhibitor wells were prepared according to instructions provided in the kit and pre incubated for five minutes at 250C .The reaction was initiated by addition of 20µl of TMPD solution followed by 10µl of arachidonic acid in all the wells. The assay mixture was shaked and incubated at 250C for 5 min. The enzyme activity was measured as an increase in absorbance at 590nm.

RESULTS AND DISCUSSION

The *In vitro* anti-inflammatory activity data of 4-(2,5-Dimethyl amino-1H pyrrol-1-yl-phenyl) [1,3,4]-oxadiazino [6,5-b] indoles are presented in **Table 2 and Figure 1**. The data reveals that the anti-inflammatory activity was observed in the range of 32.19 to 84.42 (IC_{50}). Compounds XIIb (R=5-Cl), XIIc (R=7-Cl), XIIf (R=5-F), XIIg (R=7-F), XIIh (R=5-Br), XVIIb, XVIIc (R=5-Cl, 7-Cl), XVIIh (R=5-Br), are considered to possess more potent anti-inflammatory activity among the series. Remaining compounds exhibited mild anti-inflammatory activity. Thus the results showed that synthesized compounds possess anti-inflammatory activity. It was observed that the test compounds with electron withdrawing groups (halogens) on the aromatic ring favors anti-inflammatory activity.

Table 2: *In vitro* anti-inflammatory activity data of 4-(2,5-Dimethyl amino-1H pyrrol-1-yl-phenyl) [1,3,4]-oxadiazino [6,5-b] indoles (XIIa-XIIn)

S. No.	Compound	R	IC_{50} (µg/ml)
1	XIIa	H	72.23
2	XIIb	5-Cl	34.33
3	XIIc	7-Cl	32.19
4	XIId	5-CH_3	74.23
5	XIIe	7-CH_3	81.24
6	XIIf	5-F	45.21
7	XIIg	7-F	47.16
8	XIIh	5-Br	49.23
9	XIIi	5-NO_2	76.17
10	XIIj	7-NO_2	71.15
11	XIIk	5-OH	80.18
12	XIIl	7-OH	84.42
13	XIIm	5-COOH	70.42
14	XIIn	5-$COOC_2H_5$	83.42
15	Standard	Indomethacin	0.93

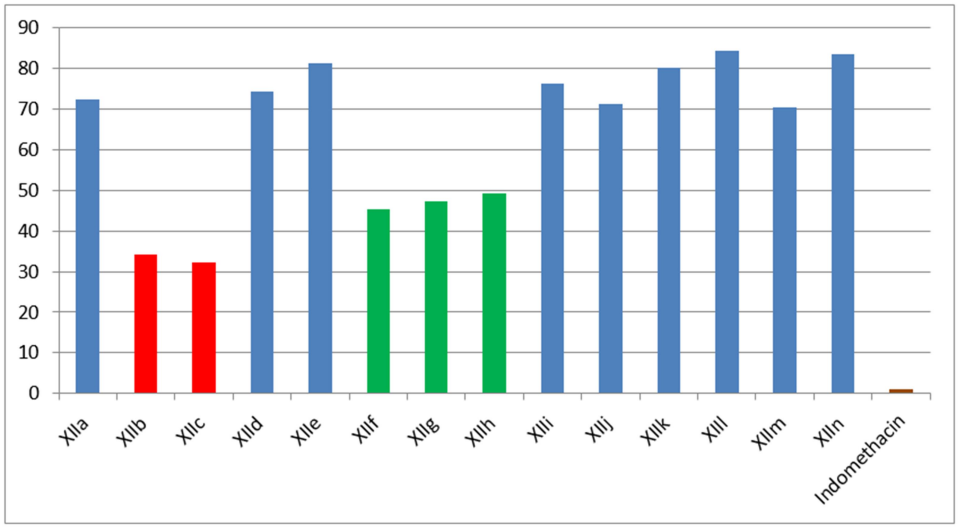


Figure 1: *In vitro* anti-inflammatory activity data of 4-(2,5-Dimethyl amino-1H pyrrol-1-yl-phenyl) [1,3,4]-oxadiazino [6,5-b] indoles (XIIa-XIIn)

CONCLUSIONS

A series of as 3-[4-(2,5-dimethyl amino-1H pyrrol-1-yl-phenyl) [1,3,4]-oxadiazino [6,5-b] substituted indole has been synthesized and subjected to anti-inflammatory activity. Amongst the compounds tested substituent with an electron withdrawing group on the aromatic ring showing significant activity than the other substituted compounds.

Acknowledgement

Authors are highly thankful to University College of Pharmaceutical Sciences, Kakatiya University for providing research facilities in the institute.

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