INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS

VOL.6, ISSUE 11, 2018; 604 - 609; http://ijpda.com; ISSN: 2348-8948

Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF *ISOINDOLINE-1, 3-DIONE* DERIVATIVES

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Date Received: 23rd October 2018; Date accepted: 9th November; Date Published: 30th November 2018

Abstract

A series of *phthalimide* derivatives were synthesized and evaluated for their analgesic and invitro anti-inflammatory activity. The target compounds were obtained by condensation of *N-hydroxymethylphthalimide* with the *substituted triazole*. The structures of the synthesized derivatives were confirmed by means of IR, 1H-NMR spectral data. The analgesic activity was determined by acetic acid induced writhings in mice and Invitro anti-inflammatory activity was evaluated using thermally induced protein denaturation technique. The results revealed the importance of the combination of *triazole* and *phthalimide* moieties as a promising analgesic and anti-inflammatory candidate.

Keywords: *Phthalimide*, Analgesic activity, Invitro anti-inflammatory activity, Protein denaturation technique.

Introduction

Pain can be categorized according to several variables, including its duration such as acute, convalescent and chronic. Secondly depending upon the pathophysiologic mechanisms such as physiologic, nociceptive and neuropathic and thirdly on its clinical context like postsurgical, malignancy related, neuropathic and degenerative. Inflammation can be defined as a defensive but exaggerated local tissue reaction in response to exogenous or endogenous insult. It is complex phenomenon, comprising of biochemical as well as immunological factors. It is recognized by the following symptoms: Calor (Heat), Rubor (Redness), Tumours (Swellings), Dolor (Pain). Drugs presently used for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. *hydrocortisone*. NSAIDs which include both selective and nonselective cyclooxygenase (COX) inhibitors are most widely used for the treatments of pain. The aim of present work to attach the substituted *1,2,4-triazole* residue to isoindoline-1,3-dione in order to find new pharmacologically active molecule. Thus synthesis of *isoindoline-1,3-dione* derivatives has been achieved and evaluated for their analgesic and in-vitro anti-inflammatory activity.

Materials and methods:

Experimental -

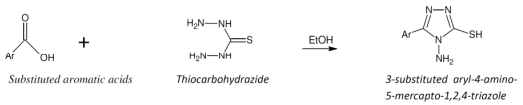
Step I: General procedure for synthesis of *N-hydroxymethylphthalimide*

The mixture of *phthalimide* (0.1mole) and 37 % w/w formaldehyde in water was taken in 250 ml of round bottom flask and refluxed for 4 hours. *N-hydroxymethylphthalimide* precipitated as white crystals, product was filtered off and purified by recrystallization from hot benzene and dried. m. p. 140^{0}C Yield: 14 g (80%)

Step II: Synthesis of *3-substituted aryl-4-amino-5-mercapto-1,2,4 triazole* (2a -2j)

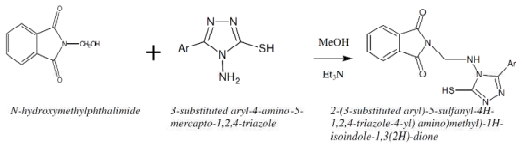
Reflux mixture of *thiocarbohydrazide* and aromatic acid in 1:1 ratio in presence of ethanol for 4 hrs. The resulting solution was allowed to stand overnight in a capped round bottom flask during

which time product crystallized as a coarse off white crust on the sides of the flask.



Step III: Synthesis of *2-(3-substituted aryl)-5-sulfanyl-4H-1,2,4-trizole-4-yl) amino)methyl)-1H-isoindole-1,3(2H)-dione*. (3a-3j)

An equimolar mixture of *N-hydroxymethylphthalimide* and *3-substituted aryl-4-amino-5-mercapto-1,2,4-triazole* (2a-2j) in 20 ml methanol along with few drops of *triethylamine* was refluxed on water bath for 6-7 hours. The solids separated were filtered, dried and recrystallized from ethanol.



Biological screening:

Analgesic activity

Albino mice of either sex having weight range 20-25 gm were selected for study. The test compounds 3a-3j and control were administered orally to mice and Standard (*diclofenac sodium* 5 mg/kg body weight) was injected intraperitonally. 0.6% acetic acid solution (10 ml/kg) was injected intraperitonally after the administration of synthesized compounds and standard. The number of writhing in each mouse was observed for 10 min period starting 20 min after injection of acetic acid. Analgesic activity was expressed as percentage of inhibition of number of writhing, when compared with the control group.

Percentage analgesic activity of compounds was calculated using following formula,

% Analgesic activity = (n – n′/n) x 100

Where, n = Mean number of writhes of control group and

n′ = Mean number of writhes of test group.

The analgesic activity of all synthesized compounds is reported in table no 5.

***In-vitro* anti-inflammatory activity:**

All the compounds were subjected to in-vitro activity by protein denaturation technique in various concentrations i.e. 10, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 ug⁄ml.

The standard drug and test compounds (3a-3j) were diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.5%. Stock solutions of 1000µg/ml of all test compounds were prepared. From these stock solutions different concentrations of 10 to 500 µg/ml were prepared by using phosphate buffer as a solvent, test solution containing different concentrations of drug was mixed with 1 ml of 1 mM albumin solution in phosphate buffer and incubated at 27° ± 1°C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60° ± 10 °C in water bath for 10 min. After cooling the turbidity was measured at 660 nm on UV-Visible Spectrophotometer. Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The *diclofenac* was used as standard drug.

% inhibition of denaturation = 100 × (1-A2/A1)

Where, A1 = Absorption of control sample

A2 = Absorption of test sample

The result of the anti-inflammatory activity of all the synthesized compounds is reported in table no 6.

Spectral Characterization

IR interpretation –

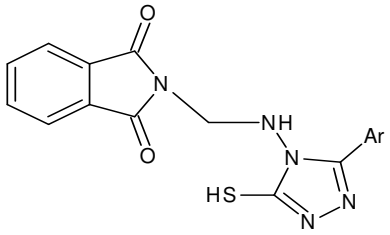
The IR sprectal analysis was carried on JASCO FTIR 4100 spectrophotometer by using KBr. The ^{1}H NMR spectra of synthesized compound where recorded at department of chemistry, University of Pune, at Pune on varian NMR 300 Mhz spectrophotometer using TMS as an internal standard and DMS as solvent.

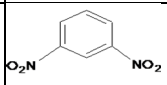
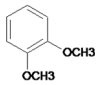
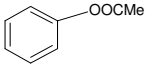
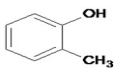
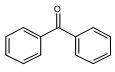
Analgesic Activity –

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) and conducted according to the guidelines for the use and care of experimental animals.

Result and discusson -

Table No-1- Physical properties of synthesized compounds (3a-3j)



Compound Code	Ar	Molecular formula	Molecular weight	Melting point (oC)	Rf Value	Yield (%)
3a		$C_{17}H_{11}O_6N_7S$	441	130 – 132	0.36	64.01
3b		$C_{17}H_{12}O_4N_6S$	396	190 – 192	0.38	62.90
3c		$C_{19}H_{17}O_4N_6S$	411	145 – 147	0.87	52.43
3d		$C_{19}H_{15}O_4N_5S$	409	175 – 178	0.41	59.18
3e		$C_{18}H_{15}O_3N_5S$	381	162 – 164	0.44	64.03
3f		$C_{16}H_{12}O_2N_6S$	352	177 - 179	0.8	64.22
3g		$C_{17}H_{13}O_4N_5S$	383	164 – 166	0.30	75.54
3h		$C_{17}H_{13}O_3N_5S$	367	182 – 184	0.34	55.50
3i		$C_{21}H_{21}O_2N_5S$	407	180 – 182	0.83	62.13
3j		$C_{24}H_{17}O_3N_5S$	455	202 – 204	0.53	69.11

All the synthesized compounds were subjected to analgesic activity at a 25 mg/kg body weight dose using acetic acid writhing method in mice. All the synthesized compound showed significant analgesic activity as compared to the standard diclophenac. The result of the analgesic activity is presented below.

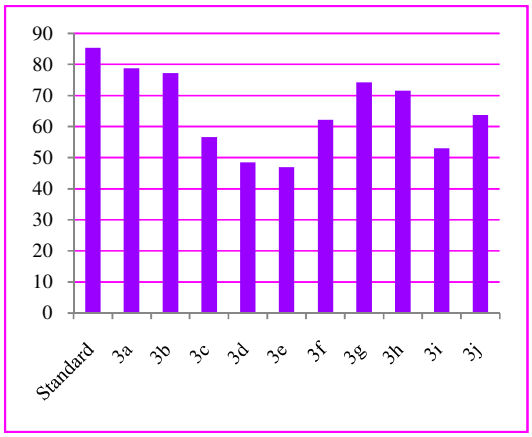
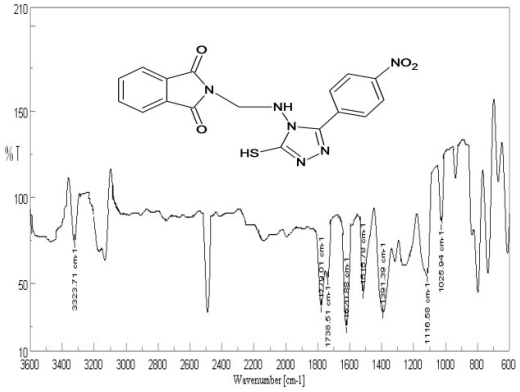


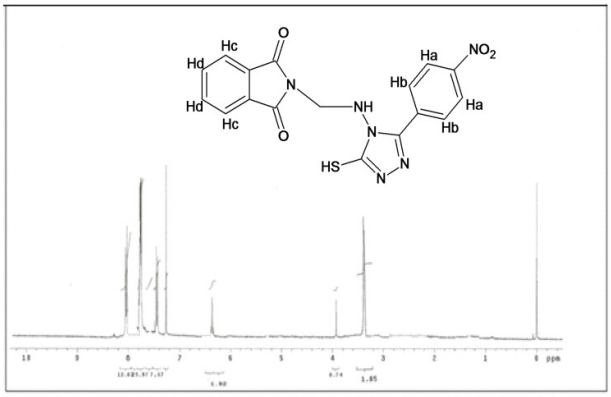
Table no- 4 Graphical representation of Analgesic activity of data *2-(3-substituted aryl)-5-sulfanyl-4H-1,2,4-trizole- 4-yl) amino)methyl)-1H-isoindole-1,3(2H)-dione*. (3a-3j)

Table No-2 - FT-IR spectrum interpretation of *2-({[3-(4-nitrophenyl)-5-sulfanyl-4H-1,2,4-triazole-4-yl]amino}methyl)-1H-isoindole-1,3(2H)-dione (*Fig-1)



Sr. No	Frequency	Functional group
1	1735.51	Imide Carbonyl group
2	3323.71	Sec.N-H
3	1025.94	N-H
4	1620.88	C=N
5	2500	S-H
6	3108.74	C-H
7	1515.78, 1391.49	NO_2
8	1116.58	C-N (Aliphatic)
9	1248.89	C-N (Aromatic)

Table No-3 - H^1 NMR spectrum of *2-({[3-(4-nitrophenyl)-5-sulfanyl-4H-1,2,4-triazole-4-yl]amino)methyl)-1H-isoindole-1,3(2H)-dione*. (Fig-2)



Sr. No	Functional Group	δ ppm
1	S. 1H. NH	6.4
2	S, 2H, CH_2	3.9
3	S, 1H, SH	3.4
4	m, 7H, Ar-H Phenyl	7.2 – 8.0
	(Ar-CH H_a)	7.5
	(Ar-CH H_b)	7.2
	(Ar-CH H_e)	8.0
	(Ar-CH H_d)	7.8

Table no- 5 - Analgesic activity of data *2-(3-substituted aryl)-5-sulfanyl-4H-1,2,4-trizole-4-yl) amino)methyl)-1H-isoindole-1,3(2H)-dione*. (3a-3j)

Group	Dose	No. of writhes	Percentage protection
Control	0.1ml/10gm	33±0.9661	-
Standard	5mg/kg	4.83±0.6009**	85.36
3a	25mg/kg	7±0.5774**	78.78
3b	25mg/kg	7.5±0.7638**	77.27
3c	25mg/kg	14.5±07638.**	56.60
3d	25mg/kg	17±0.9661**	48.48
3e	25mg/kg	17.5±0.9961**	46.96
3f	25mg/kg	12.5±0.8446**	62.12
3g	25mg/kg	8.5±0.7638**	74.24
3h	25mg/kg	10±0.9309**	69.69
3i	25mg/kg	15.5±0.7638**	53.03
3j	25mg/kg	12±1.065**	63.63

***p≤0.01 represent the significant difference when compared with control group.*
Data expressed as Mean ± SEM. n=6
Data was analyzed by one-way ANNOVA followed by Dunnett's test.
Amongst all the synthesized compounds 3a,3b and 3g exhibited excellent analgesic activity with percent protection 78.78, 77.27 and 74.24 respectively as compared to standards.
Compound 3h, 3j and 3f showed good analgesic activity as compared to standard with percent protection 69.69, 63.63 and 62.12 respectively.
Compounds 3c and 3i showed moderate activity as compared to standard.
While remaining compounds 3d, 3e shows very less activity.

Anti-inflammatory Activity –

Table No – 6 - The inhibitory effect of different concentration of test compounds and standard drug.

Concentration (µg/ml)	% Inhibition										
	Std	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j
10	18	26	24	13	15	14	19	12	21	21	19
50	24	32	29	16	19	12	27	23	32	28	22
100	29	38	32	21	22	21	36	29	36	31	28
150	34	46	38	28	26	25	44	32	39	36	37
200	42	54	42	29	32	30	48	39	42	42	43
250	55	62	45	32	38	36	49	48	46	45	47
300	59	68	48	38	42	39	51	49	48	48	49
350	65	70	51	41	46	44	56	55	52	51	54
400	72	73	56	48	48	47	59	68	58	53	59
450	76	79	68	58	55	53	61	71	65	55	62
500	89	83	78	61	56	55	62	74	68	59	64
IC_{50}	242	189.6	285.6	403.5	406.4	423.1	289.4	287.5	95.5	348.4	309.8

IC_{50}: Concentration of test drug needed to inhibit albumin denaturation by 50%
All the synthesized derivatives were screened for in-vitro anti-inflammatory activity by protein denaturation technique.

Amongst all the synthesized compounds 3a, 3b and 3g exhibited excellent anti-inflammatory activity.showed at dose of 500µg/ml(83%, 78%, 74 % inhibition). Compounds 3h, 3j and 3f inhibit denaturation of protein by 68 %, 64 % and 62% respectively. It means these compounds possess good anti-inflammatory activity. Componds 3c and 3i exhibited moderate anti-inflammatory activity. The remaining compounds were found to devoid of any inhibition of albumin denaturation when compared with standard diclofenac.

Conclusion:

In the present study new series of *2-(3-substituted*

aryl)-5-sulfanyl-4H-1,2,4-trizole- 4-yl) amino)methyl)-1H-isoindole-1,3(2H)-dione have been synthesized from combination of *N-hydroxymethylphthalimide* and of *3-substituted aryl-4-amino-5-mercapto-1,2,4-triazole. Substituted 1,2,4-triazole* were prepared by reacting the aromatic acid with *thiocarbohydrazide*. All the synthesized compounds were screened for analgesic and anti-inflammatory activity.

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