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SYNTHESIS, CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITY OF VARIOUS ISATIN DERIVATIVES

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ABSTRACT

Some new 5-mercapto-2-(3-(4-fluorophenylimino)-1-methyl indole-2-one)-1, 3, 4-0xadiazole has been synthesized from isatin& different p-substitute aniline. In the next step, I (a-h) on treatment with ethyl chloroacetate in anhydrous K_2 CO_3 and dry acetone furnished [3(4-phenylimino)-2oxo]-1-indole-ethylacetate II(a-h) which on reaction with hydrazine hydrate in methanol gave [3-(4-substituted phenylimino)-2-oxo]-1-indole acetylhydrazide III(a-h).In the last step, a solution of compounds III(a-h) in ethanolic KOH on treatment with CS_2 gave title compounds 5-mercapto-2-[3-(4-substitutedphenylimino)-1-methyl-indol-2-0ne]-1,3,4-oxadiazole IV(a-h) under microwave irradiation at a power of 140 watts. MW irradiation led to higher yields in much less time than that by conventional method. The newly synthesized compounds were characterized on the basis of elemental analysis, IR, H NMR, and mass spectra. All the synthesized isatin derivatives have been investigated for their anti-inflammatory, antibacterial & antifungal activity.

Key Words: Microwave, Conventional, Synthesis, Isatin, 1.3.4-oxadiazole, Antibacterial activity, Anti-inflammatory activity.

INTRODUCTION

The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating disease. Most of this activity is directed to new natural or synthetic organic compounds. Inorganic compounds continue to be in therapy, eg. Trace elements in nutritional therapy, antacids and radiopharmaceuticals, but organic molecules with increasingly specific pharmalogical activities are clearly dominant. Development of organic compounds has grown beyond traditional synthetic methods. It now includes the exciting new field of biotechnology using the cells biochemistry to synthesize new compounds. Technique ranging from recombinant DNA and site directed mutagenesis to fusion of cell lines have greatly broadened the possibilities for new entities that treat disease [1].

Generation of different heterocyclic derivative has created a boom in the field of medicinal chemistry. Different heterocyclic compounds have shown various biological activities such as antibacterial, antifungal, antiviral, anti-inflammatory and anti-tubular and thus can be used as a lead in the discovery of new drug molecule. An important aspect of medicinal chemistry is to establish a relationship between chemical structure and biological activities. Hundreds of thousands of new drugs are prepared annually throughout the word. Many of them are entered in pharmacological screening to determine whether they have useful biological activity or not. This process of random screening resulting in the identification of new lead compounds. The most serious problem in designing of more efficacious drugs is how to minimize its toxicity and maximize its pharmacological action [2, 3].

Isatin (1H-indole-2, 3-Dione) is a synthetically versatile substrate, where it can be used for the synthesis of a large variety of heterocyclic compounds, such as indoles and quinolines, and as a raw material for drug synthesis. Isatin has also been found in mammalian tissues, and its function as a modulator of biochemical processes

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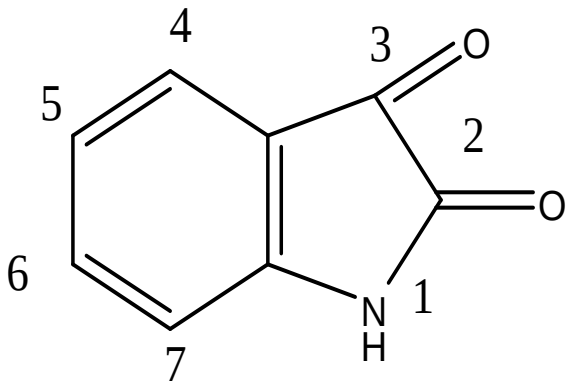
has been the subject of several discussions. The advances in the use of isatin for organic synthesis during the last twenty-five years, as well as a survey of its biological and pharmacological properties are reported in this review and in the accompanying supplementary information [4].

It is evident from literature that isatin derivatives are known to be associated with broad spectrum of biological activity like anti-inflammatory(1), antibacterial(2), antifungal(3) analgesic(4). In view of these facts and as a continuation of our work in the laboratory, prompted us to synthesize some new 5-mercapto-2-(3-(4-fluorophenyl imino)-1-methyl indole-2-one)-1,3,4-oxadiazole..All the synthesized compounds were screened for their in vitro antibactial and antifungal activity. Their chemical structures were confirmed by Infrared, 1H-Nuclear Magnetic Resonance data and elemental analysis. Antimicrobial evaluation was performed by the agar diffusion method against four pathogenic bacteria and two pathogenic fungi. Anti-inflammatory activity was tested by carrageen-induced rat paw edema and compounds were evaluated for analgesic action by acetic acid-induced writhing method [5].

I. a. Synthesis of isatins

Isatin (1H-indole-2, 3-dione, Figure 1) was first obtained by Erdman and Laurent in1841 as a product from the oxidation of indigo by nitric and chromic acids.

Figure 1



The synthetic versatility of isatin has led to the extensive use of this compound in organic synthesis. Three reviews have been published regarding the chemistry of this compound: the first by Sumpter, in 1954, a second by Popp in 1975 and the third on the utility of isatin as a precursor for the synthesis of other heterocyclic compounds3. The synthetic versatility of isatin has stemmed from the interest in the biological and pharmacological properties of its derivatives. These properties are more fully detailed in the supplementary material. In nature, isatin is found in plants of the genus Isatis, in Calanthe discolor LINDL. And in *Couroupitaguianensis*Aubl, and has also been found as a component of the secretion from the parotid gland of Bufofrogs, and in humans as it is a metabolic derivative ofadrenaline8. Substituted isatins are also found in plants, for example the melosatin alkaloids (methoxyphenylpentylisatins) obtained from the Caribbean tumorigenic plant *Melochiatomentosa* as well as from fungi: 6-(3'-methylbuten-2yl) isatin was isolated from *Streptomyces albus* and 5-(3'-methylbuten-2'-yl) isatin from *Chaetomiumglobosum*. Isatin has also been found to be a component of coal tar [6].

I. b. Inflammation

Inflammation is a physiological process in response to tissue damage resulting from microbial pathogen infection, chemical irritation, and/or wounding. The relation between inflammation and atherosclerosis,, diabetes cancer, arthritis and Alzheimer' disease has been well substantiated. The functioning of the immune system is finely balanced by the activities of proinflammatory and anti-inflammatory mediators or cytokines, prostaglandins, and free radicals direct or indirect effect on the pathophysiology of diseases. Chronic inflammation develops from unresolved symptomatic acute inflammation with or without any clinical manifestations. This may activate macrophages and lymphocytes which release inflammatory mediators and also result in excessive formation of reactive oxygen and nitrogen species that damage DNA and cell membranes [7].

Inflammatory cell release prostaglandins with concomitant increase in the expression of key enzyme cyclooxygenase which in turn can activate several transcription factors including NF-αB. Inflammation activates a variety of inflammatory cells, which induce and activate oxidant generating enzymes like NADPH oxidase, xanthine oxidase, myeloperoxidase etc., which produce superoxide anion and other reactive nitrogen species like nitric oxide through activation of inducible nitric oxide synthase (iNOS) [8,9].

Free radicals play major role in persistence of inflammation. During process of inflammation, phagocytes secrete chemically reactive oxidants, radicals, and electrophilic compounds that bring about the elimination of infectious agents. These host tissue. Many drugs of plants origin having antioxidant activity have been reported to have anti-inflammatory activity [10, 11].

Figure 1. Development Of Chronic Inflammation:

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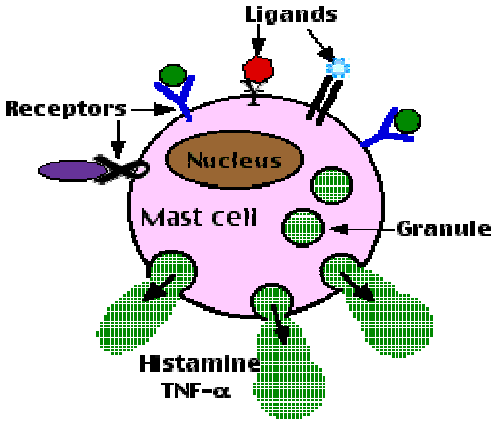

II. RESULT AND DISCUSSION

II .a. Chemistry

Indole-2,3-dione (isatin) and 4-substituted aniline is taken in of ethanol in presence of of glacial acetic acid and then kept in oven for 5min after that cooling and crystals are separated out then filtered after that recrystallized from ethanol, and yellow needles like product(a) obtained. Mixture of product (a) ethylchloroacetate and potassium carbonate in dry acetone will placed in a RBF microwave oven at 10 min, then mixture will cooled after that resulting solid filtered and then dried, after that recrystallized from methanol and yellow compound (b) product obtained. Mixture of product (b) and hydrazine hydrate in methanol in microwave irradiation at 140 watts. After that the reaction mixture powdered into over crushed ice and the solids are collect and wash with water then dry after that recrystallized from ethanol. Yellow colour solid (c) product obtained. Mixture of product(c) is dissolved in ethanolic KOH then cooled at 0 ^{0}C.Product (c) and CS2 (4) ml will add. Reaction mixture subjected to microwave irradiation. small volume of water add and its neutralized with 1N HCl after that resulting solid will reacted with ethyl acetate. The organic layer will dried over Na2SO4.Filtered and concentrated under reduced pressure, and product (d) obtained.

II.b. Materials and methods

All starting materials were from different manufactured company like (SD. Fine chemicals, SDFCL, Lobachem etc.) And all the materials used without further purification all reactions were monitored by thin- layer-chromatography using TLC sheet coated with silica gel GF254 spots were visualized with UV light.

III. Experimental protocols

III. a. Chemistry

Reagents and chemicals were from sd.fine chemicals, SDFCL, Lobachemof highest purity available, and were used without further purification. All the synthesized 3 phenyl iodole derivatives produced and purified in laboratory as described earlier. Melting points are recorded in open capillary one ended tubes and are uncorrected. The IR spectra (KBr) were recorded on a SHIMADZU FTIR-8300, spectrophotometer. The 1H-NMR spectra were recorded on a Bruker Advance-400 MHz spectrometer.

III. a. a. Synthesis of 3-(4-substitutedphenyl imino)-2-indole-2-one

Indole-2,3-dione(isatin)(7.35gm)+4-fluoroaniline(5-6ml) is taken in 100 ml of ethanol in presence of 5-6 ml of glacial acetic acid and then kept in oven for 5min after that cooling and crystals are separated out then filtered after that recrystallized from ethanol, and yellow needles like product(a) obtained.

III. a. b. Synthesis of 3-(4-substitutedphenyl imino)-2-oxa-1-indole ethylacetate

Mixture of product (a) 12gm, ethylchloroacetate (6.1 ml) and potassium carbonate 11 gm in dry acetone will placed in a RBF microwave oven at 10 min, then mixture will cooled after that resulting solid filtered and then dried, after that recrystallized from methanol and yellow compound (b) product obtained.

III. a. c. Synthesis of 3-(4-substitudphenyl imino)-2-oxa-1-indole acetyl hydrazide

Mixture of product (b) 16.5gm is treated with hydrazine hydrate 3ml in methanol 50 ml in microwave irradiation at 140 watts. After that the reaction mixture powdered into over crushed ice and the solids are collect and wash with water then dry after that recrystallized from ethanol. Yellow color solid (c) product obtained.

III. a. d.Synthesis of 5-mercapto-2-[3-(4-substitutedphenylimino)-1-methyl indole-2-one]-1, 3, 4-oxadiazole

Mixture of product(c) is dissolved in ethanolic KOH 75 ml then cooled at 0 C.Product (c) and CS2 (4) ml will added. Reaction mixture subjected to microwave irradiation. Small volume of water add and its neutralized with 1N HCl after that resulting solid will reacted with ethyl acetate. The organic layer will dried over Na2SO4.Filtered and concentrated under reduced pressure, and product (d) obtained

Sk .a.Synthesis of 5-mercapto-2-[3-(4-fluorophenylimino)-1-methyl indole-2-one]-1,3,4-oxadiazole

Yield 64%, mp 192-194 ^{0}C, IR (KBr) v max in cm^{-1} 1670(C=O), 1236(C-F), 1592(C=N), (C-H), 1592 1600(C=C).^{1}H-NMR (CH; 7.76, 7.81, 7.26, 7.22), Solubility-DMSO, H_2O.Rf-0.713.

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Sk.b.Synthesis of 5-mercapto-2-[3-(4-chlorophenylimino)-1-methyl indole-2-one]-1,3,4-oxadiazole .
Yield 59%, mp 202-205 ^{0}C, IR (KBr) v max in cm^{-1} 1732(C=O), 1588 (C=N), 2945 (C-H), 1597(C=C) 722 (C-Cl); H-NMR (CH; 7.81, 7.76, 7.50, 7.22), solubility-DMSO, $CHCl_3$. Rf-0.652.

Sk. c.Synthesis of 5-mercapto-2-[3-(4-bromophenylimino)-1-methyl indole-2-one]-1,3,4-oxadiazole.
Yield 57%, mp 204-206 ^{0}C, IR (KBr) v max in cm^{-1} - 2565(S-H), 715 (C-S), 3450 (N-N), 1655 (C=O) 585 (C-Br); H-NMR (CH; 7.86, 7.81, 7.21, 7.23), solubility-DMSO, water. Rf -0.712.

Sk. d.Synthesis of 5-mercapto-2-[3-(4-hydroxyphenylimino)-1-methyl indole-2-one]-1, 3, 4-oxadiazole.
Yield 61%, mp 207-209 ^{0}C, IR (KBr) v max in cm^{-1} 3415 (N=N), 3300 (N-H), 1678(C=O), 2876(C-H) 1529 (C-OH);^{1}H-NMR (CH; 7.86, 7.50, 6.99, 7.81), solubility-DMSO,H_2O,$CHCl_3$. Rf 0.743.

Sk. e.Synthesis of 5-mercapto-2-[3-(4-methylphenylimino)-1-methyl indole-2-one]-1,3,4-oxadiazole.
Yield 58%, mp 207-209 ^{0}C, IR (KBr) v max in cm $^{-1}$ 2890(C-H), 1622(C=N), 1336 (C-N) 2590(S-H), 3081 (C-CH_3); ^{1}H-NMR (7.81, 7.23, 7.22), Solubility-DMSO,H_2O, Rf-o.743.

Sk. f.Synthesis of 5-mercapto-2-[3-(4-methoxyphenylimino)-1-methyl indole-2-one]-1, 3, 4-oxadiazole.
Yield 62%, mp 199-202 ^{0}C, IR (KBr) v max in cm $^{-1}$ 2820(C-H), 1728(>C=O), 1336 (C-N) 2586(S-H) 2873(C-OCH_3); 1H-NMR (7.21, 7.50, 7.22), Solubility-DMSO,H_2O, Rf-0.712.

Sk. g.Synthesis of 5-mercapto-2-[3-(4-nitrophenylimino)-1-methyl indole-2-one]-1, 3,4-oxadiazole.
Yield 55%, mp 203-205 ^{0}C, IR (KBr) v max in cm^{-1} 1732(C=O), 1634 (C=N), 2945 (C-H), 1597(C=C) 1345 (C-NO_2); 1H-NMR (CH; 7.81, 7.76, 7.86, 7.23), solubility-DMSO, $CHCl_3$. Rf-0.612.

Sk.h.Synthesis of 5-mercapto-2-[3-(4-aminophenylimino)-1-methyl indole-2-one]-1, 3, 4-oxadiazole.
Yield 57%, mp 209-211 ^{0}C, IR (KBr) v max in cm^{-1} - 2565(S-H), 715 (C-S), 3450 (N-N), 1655 (C=O) 1581 (C-NH_2); ^{1}H-NMR (CH; 7.86, 7.81, 7.21, 7.23), solubility-DMSO, water. Rf -0.702.

Anti-inflammatory activity

Carrageenan-induced paw oedema test: 25 animals were grouped into five (a-f) consisting of five animals each. Groups a and b were treated with 0.5 ml of distilled water and 10 mg/kg body weight of indomethacin respectively while groups c, d and e were administered with the test at 50, 100 and 200 mg/kg body weigh respectively. 0.1 ml of 1% carrageenan solution was injected in to the sub plantar region of the right hind paw of the rats, 1 h after the administration of distilled water, indomethacin and test. The paw volume was measured with a micrometers crew gauge (SMC-20326, Sterling Manufacturing Company, AmbalaCantt, India.) at 1, 2, 4 and 6 h after administration of the drug and the test. The difference between the left and right hind paw volumes (indicating the degree of inflammation) was determined in comparison to the control animals. The percentage inhibition of inflammation of the test and there reference drug was calculated using the expression. Percentage inhibition of inflammation = (X - Y/X) x 100where X was the average degree of inflammation of the control and Y was the average degree of inflammation of the test/ indomethacin.

Table No 2. Physico chemical properties of different Isatin derivatives

S.No	Compound	R	Mol.Formula	M.Wt (g)	Yield (%)	M.P(^{0}C)	Rf value
1	Ska	-F	$C_{17}H_{11}O_2N_4F$	322	64%	192-194	0.713
2	Skb	-Cl	$C_{17}H_{11}O_2N_4Cl$	339	59.5%	202-205	0.652
3	Skc	-Br	$C_{17}H_{11}O_2N_4Br$	383	57%	204-206	0.712
4	Sk d	-OH	$C_{17}H_{12}O_3N_4$	320	61.5%	207-209	0.743
5	Sk e	-CH_3	$C_{18}H_{14}O_2N_4$	318	58%	201-203	0.618
6	Sk f	-OCH_3	$C_{18}H_{14}O_3N_4$	334	62%	199-202	0.712
7	Sk g	-NO_2	$C_{17}H_{11}O_4N_5$	349	55%	203-205	0.612
8	Sk h	-NH_2	$C_{17}H_{13}O_2N_5$	319	57%	209-211	0.702

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Table No 3. Anti-inflammatory activity of compounds

Compd	mean values of**Oedemavalume**		Anti-inflammatory activity**(%inhibition±SEM)**	
	3h	**4h**	**3h**	**4h**
Control	1.752±0.073	1.53±0.082	---	---
Indomethacin	0.52±0.027	0.45±0.027	38.27±1.36	39.32±1.37
Sk a	1.08±0'045	0.98±0.045	38.31±1.38	40.69±1.22
Sk b	1.07±0.038	0.98±0.045	40.78±1.82	40.98±1.22
Sk c	0.88±0.032	0.73±0.036	52.00±1.68	52.90±1.39
Sk d	0.86±0.026	0.78±0.032	52.54±1.24	54.02±1.24
Sk e	0.34±0.032	0.24±0.022	82.46±1.66	83.42±1.42
Sk f	0.36±0.036	0.32±0.076	76.56±1.54	80.43±1.62
Sk g	0.76±0.036	0.45±0.027	40.78±1.82	39.82±1.44
Sk h	0.48±0.036	0.51±0.092	76.56±1.34	73.54±1.39

Table no4. Biological activities of the compound Sk_{a-h} (500 µg/ml)Zone of inhibition (mm)

Compounds	**R**	**Antimicrobial activity (500 µg/ml)**			**Anti fungal activity(500 µg/ml)**	
		S.aureus	**B.subtilis**	**E.coli**	**C.albicans**	**A.niger**
Ska	F	24	21	24	16	NS
Skb	Cl	22	26	20	22	NS
Skc	Br	31	28	25	23	27
Skd	OH	25	20	24	18	NS
Ske	CH3	26	26	21	22	NS
Skf	OCH3	32	23	22	20	27
Skg	NO3	12	20	26	23	NS
Skh	NH2	20	22	24	28	26

CONCLUSION

In conclusion, we have synthesized some Isatin derivatives (Sk a-h) and evaluated these compounds for their inhibitionofanti-inflammatory activities. Most of them demonstrated a broad spectrum of Anti microbial activities. The simple Isatin derivatives Sk e, Sk f were concluded as most potent derivatives in all the cases.

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