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PHARMACOLOGICAL SCREENING OF NEW ISATIN-3-[N^2-HETERYL-2-THIOACETYL] HYDRAZONES

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ABSTRACT

Twenty different Isatin-3-[N^2-heteryl-2-thioacetyl] hydrazones prepared earlier were screened for antimicrobial, antimuscarinic and H_1-antihistaminic activities, by standard methods. Results revealed that four test compounds a, b, c and d possessing benzimidazolyl substituent could exhibit good H_1-antihistaminic activity, in relation to standard. Two of the test compounds, h and i showed highest antibacterial activity than the Ampicillin towards *Staphylococcus aureus*. Compounds are showed lower antimuscarinic as well as antifungal activities.

Keywords: Hydrazones, Antimicrobial, antimuscarinic and H_1-antihistaminic.

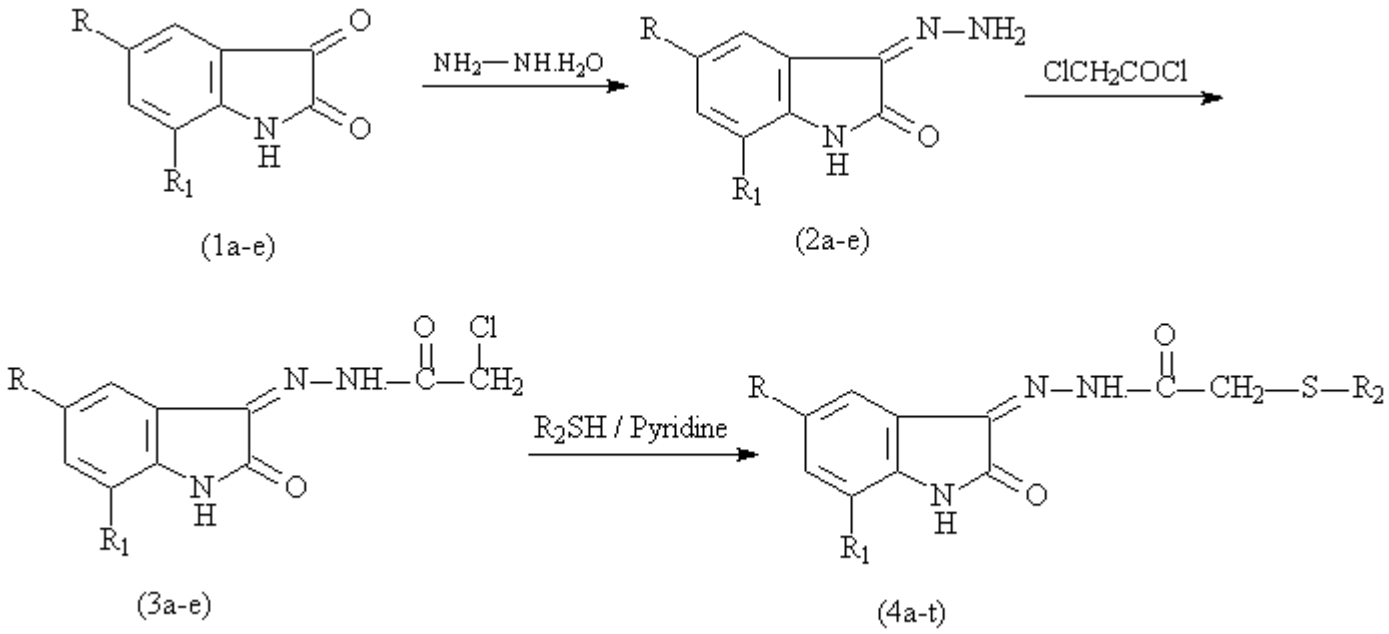
1. Introduction:

It is evident from literature that isatins posses a variety of biological and pharmacological properties such as analgesic [1], anti-inflammatory [2], antimicrobial [3], and MAO-inhibitory [4] activities. It could be noted from literature that some isatin such as 5-bromoisatin, N-methylisatin, isatin itself and isatin-3-oxime stimulated the contractions of isolated guineapig ileum. All these four compounds also blocked acetylcholine-stimulated contractions of the guinea pig- ileum [5]. It is also known from the literature that benzimidazole, imidazole, oxadiazole and thiadiazole moieties are pharmacologically prominent. In view of this some isatin derivatives containing the above heterocyclic systems were prepared and screened for H1-antihistaminic, antimuscaranic, antibacterial, and antifungal activities. The title compounds were screened for H1-antihistaminic activity by the

guineapig-ileum method, antimuscaranic activity by the rat jejunum method and evaluated for antibacterial and antifungal activity by the cup plate method. The results of the investigation are presented in this communication.

2. Investigations, results and discussion:

Scheme-1:



Legends to figure-1

a: R=H, R_1=H, R_2=$C_7H_5N_2$(benzimidazole)

b: R=CH_3, R_1=H, R_2=$C_7H_5N_2$(benzimidazole)

c: R=Cl, R_1=H, R_2=$C_7H_5N_2$(benzimidazole)

d: R=Br, R_1=H, R_2=$C_7H_5N_2$(benzimidazole)

e: R=H, R_1=CH_3, R_2=$C_7H_5N_2$(benzimidazole)

f: R=H, R_1=H, R_2=$C_{15}H_{11}N_2$(4,5-diphenylimidazole)

g: R=CH_3, R_1=H, R_2= $C_{15}H_{11}N_2$(4,5-diphenylimidazole)

h: R=Cl, R_1=H, R_2= $C_{15}H_{11}N_2$(4,5-diphenylimidazole)

i: R=Br, R_1=H, R_2= $C_{15}H_{11}N_2$(4,5-diphenylimidazole)

j: R=H, R_1=CH_3, R_2= $C_{15}H_{11}N_2$(4,5-diphenylimidazole)

k: R=H, R_1=H, R_2=$C_8H_5N_2O$(5-phenyl-1,3,4-oxadiazole)

l: R=CH_3, R_1=H, R_2= $C_8H_5N_2O$(5-phenyl-1,3,4-oxadiazole)

m: R=Cl, R_1=H, R_2= $C_8H_5N_2O$(5-phenyl-1,3,4-oxadiazole)

n: R=Br, R_1=H, R_2= $C_8H_5N_2O$(5-phenyl-1,3,4-oxadiazole)

o: R=H, R_1=CH_3, R_2= $C_8H_5N_2O$(5-phenyl-1,3,4-oxadiazole)

p: R=H, R_1=H, R_2=$C_8H_5N_2S$(5-phenyl-1,3,4-thiadiazole)

q: R=CH_3, R_1=H, R_2= $C_8H_5N_2S$(5-phenyl-1,3,4-thiadiazole)

r: R=Cl, R_1=H, R_2= $C_8H_5N_2S$(5-phenyl-1,3,4-thiadiazole)

s: R=Br, R_1=H, R_2= $C_8H_5N_2S$(5-phenyl-1,3,4-thiadiazole)

t: R=H, R_1=CH_3, R_2= $C_8H_5N_2S$(5-phenyl-1,3,4-thiadiazole)

The structure target compounds are depicted in Figure-1. Isatin hydrazones were obtained from an appropriate isatin in alcohol along with dropwise addition of hydrazine hydrate [6]. Isatin-3-[N^2-(chloroacetyl)] hydrazone were synthesized by refluxing isatin hydrazone with chloro acetyl chloride in dry benzene under anhydrous conditions using calcium chloride guard tube for 2h [7]. Isatin-3-[N^2-(heteryl-2-thioacetyl)] hydrazones (a – t) were synthesized by refluxing Isatin-3-[N^2-(chloroacetyl)] hydrazone with an appropriate heteryl-2-thione (Benzimidazole-2-thione; 4,5-diphenyl imidazole-2-thione; 5-phenyl-1,3,4-oxadiazole-2-thione, and 5-phenyl-1,3,4,-thiadiazole-2-thione) in dry pyridine for 30 min [8]. All the newly synthesized compounds were characterized by physical, spectral (IR, PMR) and elemental analysis. Antibacterial and antifungal screening was carried out using the dilution agar technique [9,10]. Test organisms **Bacteria**: *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli, Bacillus macarances.* **Fungi**:*Pencillium minioluteum, Fusarium solani.* Evaluation of antimicrobial activity as follows.

Since the compounds were poorly water soluble, they were dissolved in propylene glycol. In order to ensure that the solvent had no effect on bacterial growth, an inoculated control test was performed with only propylene glycol at the same dilutions used in our experiment and found inactive in culture media. The compound suspensions were

added at the desired concentration into nutrient agar medium for bacteria and potato-dextrose agar medium for fungi. After solidification, 1µl of the final suspension of 10^8bacteria or 10^5 fungi/ml were applied with a multipoint inoculator. Cultures were incubated for 24h at 37^{o}C for bacteria and 48 h for fungi. Ampicillin and clotrimazole were used as reference compounds. The zone of inhibition of the compounds that completely inhibited growth was considered and expressed in cm. The zone of inhibition was the mean of three measurements. Results of the compounds of antibacterial and antifungal were presented in Table-1. The title compounds were screened for H_1-antihistaminic activity on guinea pig ileum and antimuscarinic activity on rat jejunum by standard methods [11]. Then the IC50 of all the test compounds were recorded and compared with that of the standard drugs, results are presented in Table-2.

Table-1: Antibacterial and antifungal activity of isatin-3-[N^2-heteryl-2-thioacetyl] hydrazones:

Compound*	B.subtilis	B.macerences	E.coli	S.aureus	P.minioleuteum	F.solani
a	6	6	10	6	14	15
B	7	7	9	7	16	12
C	12	8	11	12	12	13
d	14	9	9	12	14	12
e	6	6	8	8	15	10
F	8	8	10	14	12	7
G	10	9	10	14	12	10
H	12	10	9	20	12	12
I	14	11	13	22	14	10
J	8	12	9	14	12	8
K	4	-	6	6	8	-
L	6	-	8	8	10	-
M	6	-	8	9	14	-
N	6	-	12	9	12	-
O	6	-	14	11	10	-
P	12	-	6	9	11	10
Q	9	-	12	8	18	14
R	8	-	11	15	15	9
S	-	-	-	-	16	12
T	-	-	-	-	12	7
Ampicillin*	19**	18**	15**	18**	----------	-------
Clotrimazle*					20**	22**

*10 µg/disk
**Zone of inhibition in cm - not reported.

Table 2: H1-antihistaminic and antimuscarinic activity of Isatin-3-[N2-heteryl-2-thioacetyl]hydrozones.

Compound	H1-antihistaminic*	Antimuscarinic*
a	476.6	not
b	506.7	1013
c	450	535.5
d	451	1239.6
e	720	500
f	1000	Not
g	Not	Not
h	1875	Not
i	1500	Not
j	not	523.1
k	Not	800
l	Not	not
m	Not	not
n	Not	800
o	Not	870
p	Not	not
q	Not	752.2
r	Not	not
s	Not	575.77
t	Not	not
Pheniramine maleate	720	--
Atropine sulfate	--	30

*Dose at which 50% inhibition observed IC_{50}(micrograms)

not=not reported

The compounds with benzimidazolyl substituent a, b, c,and d showed 33.94%, 29.73%, 37.5%, and 37.1% respectively showed higher H_1-antihistaminic activity as compared to pheniramine maleate, 4,5-diphenylimidazole substituent h and i showed 11.11% and 22.22% showed highest antibacterial activity than the Ampicillin towards *S.aureus.* Compounds are showed lower antimuscarinic and antifungal activities as compared to Atropine sulphate and Clotrimazole respectively. The compounds with benzimidazolyl substituent have been found to be

comparatively more potent. The chloro and bromo substituents on indole nucleus of the compounds have enhanced H_1-antihistaminic, and antimicrobial activities.

3. Experimental

3.1. Pharmacology.

3.1.1. H_1-antihistaminic activity

The antihistaminic activity of the test compounds was assayed by the gunea-pig ileum method [11]. A submaximal dose (10μg) of agonist was selected and the response of the tissue to this dose in presence of increasing concentrations (logarithmic doses) of the test compounds, was observed. Pheniramine maleate was employed as the standard and the results are presented in table-2.

3.1.2. Antimuscarinic activity

The antimuscaranic activity of the test compounds was assayed by the rat jejunum method [11]. A submaximal dose (30μg) of agonist was selected and remaining procedure is same as adopted in H_1-antihistaminic activity on guinea pig-ileum. Atropine sulphate was used as standard and the results are presented in table-2.

3.1.3. Antibacterial activity

Antibacterial activity of the test compounds was assayed against two gram positive bacteria Viz., *Bacillus subtilis* and *Bacillus macerences*; and two gram negative bacteria Viz., *Escherichia coli* and *Staphylococcus aureus* by using dilution agar method [9]. Ampicillin a broad spectrum antibiotic was used as standard and results are presented in table-1.

3.1.4. Antifungal activity

The test compounds were assayed for their antifungal activity against two phytopathogenic fungi Viz., *Pencillium minioluteum* and *Fusarium solani* by using potato dextrose agar medium and remaining procedure same as antibacterial activity(dilution agar method)[10]. Clotrimazole was used as standard and results are given in table-1.

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