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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME NEW N4-SUBSTITUTED ISATIN THIOSEMICARBAZONES

Praneeth Kumar A[1*] and Uma sankar K[2]

[1]*SVS Group of Institutions, School of Pharmacy, Bheemaram, Warangal, Telangana.506015.*
[2] *Vaagdevi College of Pharmacy, Warangal, Telangana.506009.*

***Corresponding Author Email:** praneethsvsip@gmail.com

ABSTRACT

Isatin thiosemicarbazones possess many biological activities from antibacterial Isatin reacts with methyl hydrazino carbodithioate and forms respective Schiff's base. This Schiff's base of Isatin on reaction with primary (or) secondary amines forms N4-substituted isatin thiosemicarbazones of respective amines. These compounds screened for antibacterial and antifungal activity. These compounds show modest antibacterial and antifungal activity. Among the nine synthesized compounds IIIg shows good activity against the Bacillus subtilis and Staphylococcus aureus. IIIc shows good activity against Bacillus subtilis and Bacillus magati , further more IIIb shows good activity against the Klebsella pneumonia and E.coli.

KEY WORDS

Isatin, thiosemicarbazones, antibacterial, antifungal activity

INTRODUCTION

Isatin thiosemicarbazones show wide variety of medicinal activities including antibacterial (1), antiviral (2), antineoplastic (3), antiprotozoal (4), antiinflammatory (5) and anticonvulsion(6) activities. By observing these activities we chose the work on the Isatin thiosemicarbazones.

Chemistry

Methyl-1-hydrazino carbodithioate is prepared by using appropriate method.(7) Methyl 1- hydrazino carbodithioate reacts with the Isatin in the presence sulfuric acid yeilds the Methyl-2-(2-oxo-1, 2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbodithioate. This compound on reaction with amines (or) amino acids give respective compounds.Physical characterization and chemical characterization also done to the compounds.

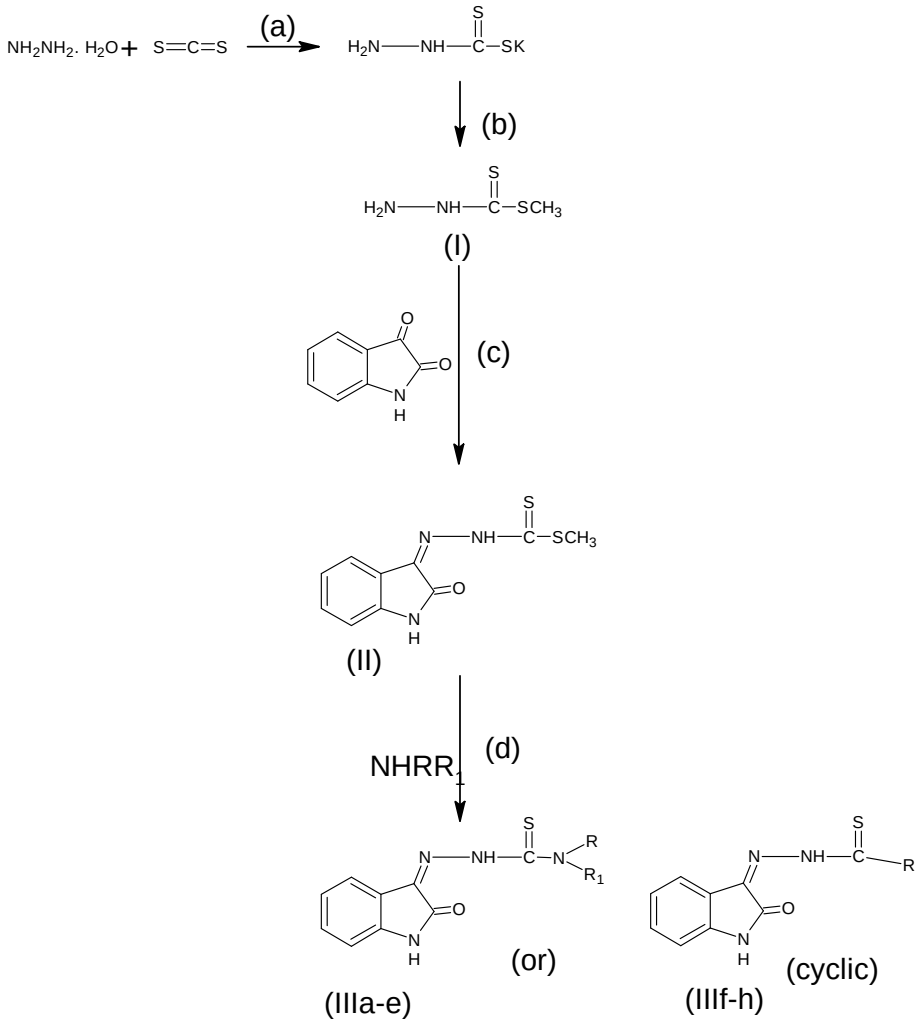
***Synthesis of Methyl-2-(2-oxo-1, 2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbodithioate* (8) (II)**

Mixture of Isatin (0.1m) and Methyl hydrazinocarbodithioate (0.1m) in Methanol are refluxed and sulfuric acid is added as catalyst the reaction was monitered by TLC. After completion of reaction add the mixture to ice cold water and separate the solid by filteration and the solid was washed with water and dried and recrystallized with ethanol. Yield: 60%; m.p: 215^{o}C

Synthesis of N4-substituted Isatin thiosemicarbazones (IIIa-i) (8)

Methyl-2-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)-1-hydrazinecarbodithioate(0.1m) and amines(0.1m) are refluxed using ethanol as solvent the reaction is checked for the evolution of mercapto methane(CH3SH) by using moistened filter paper with sodium nitroprusside which changes into the pink color. Reaction time were about 24-48 hrs and the reaction is monitered by TLC. The excess of solvent present in the reaction mixture was evaporated under vacuum and the solid was collected and further purified by washing with ethanol.

SCHEME

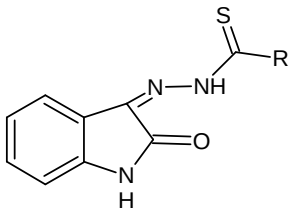


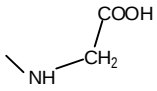
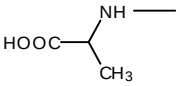
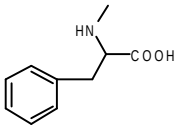
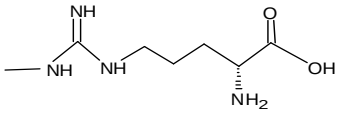
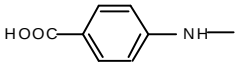
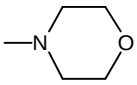
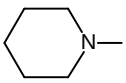
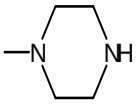
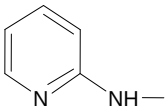
(a) KOH, 10^0C, 3hrs stirring; (b) CH_3I, 2hrs, 10^0C Stirring; (c) CH_3OH, Conc.H_2SO_4, Reflux 5 hrs; d) Ethanol, Reflux, 72hrs – 84hrs.

Biological Evaluation

Anti bacterial Activity:

The synthesized compounds are screened for the anti bacterial activity. Their activity showed on *Escherassia coli* (NCIM-2068), *Klebsella pneumonia*, *Staphylococcus aureus* (NCIM-2076), *Bacillus subtilis* (NCIM-2921), and their zone of inhibition on different strains at 300µg/ml is measured by using cup-plate method in Nutrient agar media.



code	R =	yield in (%)	Chem. form.	M.P. (^{o}C)	Mol.wt
IIIa		54	$C_{11}H_{12}N_4O_3S$	220-225	278.28
IIIb		40	$C_{12}H_{14}N_4O_3S$	235-241	292.31
IIIc		47	$C_{18}H_{18}N_4O_3S$	240-245	368.4
IIId		41	$C_{15}H_{19}N_7O_3S$	225-229	377.42
IIIe		34	$C_{16}H_{14}N_4O_3S$	215-224	340.36
IIIf		48	$C_{13}H_{16}N_4O_2S$	185-190	290.34
IIIg		40	$C_{14}H_{18}N_4OS$	220-225	288.35
IIIh		41	$C_{13}H_{17}N_5OS$	230-235	289.35
IIIi		43	$C_{14}H_{13}N_5OS$	240-250	297.33

Spectral Data of compounds:

Compound - I

IR spectrum (KBr, $cm^{-1)}$ I: 3154.74 (H-N stretch), 3263.19 (N-H stretch), 1154.68 (C=S stretch),714.81(C-S stretch), 2978.48(C-H stretch).

Compound - II

IR spectrum (KBr, cm^{-1}) II: 3466.08 (NH- stretch), 1145.72 (C=S stretch), 788.89 (C-S stretch), 2804.50 (CH_3 stretch), 1658.78 (C=N stretch).

Compound-IIIa

IR spectrum (KBr, cm^{-1}): 3433.29 (NH stretch), 3055.22 (aromatic C-H stretch), 2885.79 (C-H stretch inCH_2), 2678.39 (OH stretch in COOH), 1617.24 (C=N stretch), 1167.15 (C=S stretch).

1H-NMR spectrum (DMSO-d_6, δ ppm) 13.4(s, 1H, -NHCO lactam), 10.8(s, 1H, OH), 9.2 (s, 1H, NH-C=S), 6.8-7.4(m, 4H, Ar-H), 5.3(t, 1H, N<u>H</u>-CH_2), 2.4(d, 2H, CH_2)

(EI-MS): 279(M^++1)

Compound-IIIb

IR spectrum (KBr, cm^{-1}): 3098.11(aromatic C-H stretch), 3230.81(NH stretch), 2914.40 (aliphatic C-H stretch), 2654.41(OH stretch in COOH), 1706.70(C=O stretch), 1639.47 (C=N stretch imine), 1158.13 (C=S stretch).

1H-NMR spectrum (DMSO-d_6, δ ppm): 13.5(s, 1H, -NHCO lactam), 10.7(s, 1H, OH), 9.15 (s, 1H, NH-C=S), 6.9 -7.7(m, 4H, Ar-H), 4.2(d, 1H, N<u>H</u>-CH), 3.1(d, 3H, CH_3), 1.3(m,1H, CH).

(EI-MS): 292 (M^+)

Compound-IIIc

IR spectrum (KBr, cm^{-1}): 3228.84 (NH stretch), 3099.61(aromatic C-H stretch), 2918.30(C-H stretch inCH_2), 2671.41(OH stretch in COOH), 1620.21(C=N stretch imine), 1153.43(C=S stretch).

1H-NMR spectrum (DMSO-d_6, δ ppm): 13.5(s, 1H, -NHCO lactam), 10.9(s, 1H, OH), 9.2 (s, 1H, NH-C=S), 6.9-7.5 (m, 9H, Ar-H), 5.9 (d, 1H, N<u>H</u>-CH), 3.9(m, 1H, CH), 2.2(d, 2H, CH_2)

(EI-MS): 369(M^++1)

Compound-IIId

IR spectrum (KBr, cm^{-1}): 1230.58(C=S stretch), 3313.71(N-H stretch), 1620.21(C=N strech), 3196.05(NH stretch in amides), 1701.22(-C=O stretch in amides), 2854.65(OH stretch in COOH), 3094.17(CH stretch in aromatic), 2924.09(CH stretch in aliphatic).

Compound-IIIe

IR spectrum (KBr, cm^{-1}): 3238.82(NH stretch), 3092.23(aromatic C-H stretch), 2854.39(OH stretch in COOH), 1620.21(C=N stretch imine), 1082.07(C=S stretch).

1H-NMR spectrum (DMSO-d_6, δ ppm): 13.9(s, 1H, NHCO lactam), 10.8(s, 1H, OH),9.2(s ,1H ,NH-C=S), 6.8-7.6 (m, 8H, Ar-H), 5.9(s, 1H, NH-Ar).

(EI-MS): 341(M^++1)

Compound-IIIf

IR spectrum (KBr, cm^{-1}): 1124.50(aliphatic C=S stretch), 1087.85(C-O stretch), 1618.28 (imine C=N stretch), 1222.87(C-O stretch), 3153.61(-CONH stretch), 1670.35(C=O stretch), 3053.15(CH aromatic stretch), 3431.36(-NH sstretch).

1H-NMR spectrum (DMSO, δ ppm): 10.5(s, 1H, -NHCO lactam), 8.0(s, 1H, NH-C=S), 6.8-7.4(m, 4H, Ar-H), 2.9(t, 4H, CH_2), 4.4(t, 4H, CH_2).

(EI-MS): 290(M^+).

Compound-IIIg

IR spectrum (KBr, cm^{-1}): 1165.00(aliphatic C=S stretch), 3077.10(aromatic CH stretch), 1616.35(C=N stretch), 1103.28(C-N stretch), 3410.15(N-H stretch), 2933.73(C-H stretch aliphatic).

1H-NMR spectrum (DMSO-d_6, δ ppm): 13.3(s, 1H, NHCO), 6.7-7.5(m, 4H, ArH), 1.6(t, 4H, CH_2), 7.9 (s, 1H, NH-C=S), 1.3(m, 4H, CH_2), 2.31H (m, 2H, CH_2)

(EI-MS): 289 (M^++1)

Compound-IIIh

IR spectrum (KBr, cm^{-1}): 1169.52(aliphatic C=S stretch), 3079.18(aromatic CH stretch), 1616.35(C=N stretch), 1112.42(C-N stretch), 3410.15(N-H stretch), 2933.73(C-H stretch aliphatic).

1H-NMR spectrum (DMSO-d_6, δ ppm): 11.5(s, 1H, NHCO), 6.8-7.4(m, 4H, ArH),8.0(s, 1H, NH-C=S), 1.3(m, 4H, CH_2), 2.31H (m, 4H, CH_2), 3.5 (m, 1H, NH)

(EI-MS): 291(M^++2)

Zone of Inhibition of the antibacterial activity of compounds at 300µg/ml

Name of the amine from the compound synthesized	B.subtilis in mm	B.magati in mm	K.pneumonia in mm	St.aureus in mm	E.coli in mm
glycine(IIIa)	N.A	4.5	N.A	N.A	5.0
alanine(IIIb)	6.0	5.0	7.0	5.0	N.A
Phenylalanine (IIIc)	8.0	9.0	6.0	6.0	5.0
arginine(IIId)	5.0	N.A	N.A	5.5	N.A
PABA(IIIe)	4.0	N.A	4.0	7.0	5.5
morpholine(IIIf)	N.A	N.A	6.0	4.0	N.A
piperadine(IIIg)	8.5	6.0	5.0	9.0	7.0
piperazine(IIIh)	7.0	5.0	N.A	7.0	N.A
aminopyridine (IIIi)	9.0	5.0	7.0	8.0	9.0
Ciprofloxacin (100µg/ml)	12.0	12.0	11.0	13.0	12.0

Anti fungal activity:

The synthesized compounds are screened for the anti fungal activity. Their activity showed on Candida albicans, A.niger,Alternaria, Drosellaria and Culvernaria. Their zone of inhibition on different strains at 300µg/ml is measured by using cup-plate method in Sabouraud dextrose agar medium.

Zone of Inhibition of the antifungal activity of compounds at 300µg/ml

Name of compound	C.albicans in mm	A.niger in mm	Alternaria in mm	Drosellaria in mm	Curvlarneria in mm
glycine(IIIa)	N.A	N.A	4.5	N.A	6.0
alanine(IIIb)	5.0	4.0	4.5	N.A	7.0
Phenylalanine (IIIc)	4.5	5.0	8.0	4.5	6.0
Arginine(IIId)	N.A	4.0	N.A	N.A	6.0
PABA(IIIe)	4.5	4.0	6.0	N.A	5.0
Morpholine(IIIf)	N.A	3.5	5.0	N.A	4.5
Piperadine(IIIg)	5.5	6.0	6.0	4.0	5.5
Piperazine(IIIh)	N.A	N.A	3.5	5.0	4.5
2-Aminopyridine(IIIi)	N.A	4.0	4.0	5.0	N.A
Flucanazole (300µg/ml)	10.0	9.0	10.0	10.0	9.0

RESULTS AND DISCUSSION

All the compounds are synthesized by above prescribed scheme and they are analyzed by I.R, 1H-NMR and EI-MS methods. All the synthesized compounds are screened for antibacterial activity. Compound IIIc shows good activity against the Bacillus species, Moderate activity against the E.coli, K.pneumonia and St.aureus; Compound IIIi shows good activity against the E.coli, B.subtilis,St. aureus nad low activity against the B.magati. Compound IIIg shows good activity against the St.aureus, E.coli and B.subtilis and low activity against theB.magati and K.pneumonia.

All other compounds show low anti bacterial activity compared to the standard ciprofloxacin at 100 µg/ml.

All the synthesized compounds also screened for antifungal activity among them compound IIIc shows good activity against the alternaria and Culvernaria. Compound IIIg shows good activity against the A.niger,alternaria and Culvernaria.compound IIIe active against the Alternaria.

CONCLUSION

The isatin thiosemicarbazones were synthesized by using appropriate synthetic methods and they were evaluated for antimicrobial activities at 300µg/ml concentration, among the Compound **IIIg** is active against *St. aureus, B. subtilis, E. Coli,*. Compound **IIIc** is active against the *Alternaria, Culvarnaria* . **III b**. All the compounds show modest anti microbial activities. Further investigation should be needed to know other activities of the compounds.

REFERENCES

1. Varma,R.S., and Nobles,W.L., "Synthesis and Antiviral and Antibacterial Activity of Certain N-Dialkylaminomethylisatin β-Thiosemicarbazones" *J. Med. Chem.*, 10:510-515, (1967).
2. Bauer,D.J., and Sadler, P.W.,"The structure-activity relationships of theantiviral chemotherapeuticactivity of Isatin β-thiosemicarbazone" *Brit. J. Pharmacol.*,15: 101, (1960).
3. Petit,G.R. and Settepani., J.A., "Antineoplastic agents VI. Mannich Base Nitrogen Mustards (Part B)" J*. Org. Chem*, 27:1714, (1962).
4. Idan Chiyanzu, Elizabeth Hansell, Jiri Gut, Philip J. Rosenthal, James H. Mc Kerrow and Kelly Chibale., "Synthesis and Evaluation of Isatins and Thiosemicarbazone Derivatives against Cruzain, Falcipain-2 and Rhodesain" , *Bioorg. Med. Chem. Lett*. 13:3527–3530,(2003).
5. Vijey Aanandhi Muthukumar, Shiny George,and Venkatachalam Vaidhyalingam "Synthesis and Pharmacological Evaluation of 1-(1-((Substituted)methyl)-5-methyl-2-oxoindolin-3-ylidene)-4-(substituted pyridin-2-yl) thiosemicarbazide" *Biol. Pharm. Bull,* 31(7):1461-1464, (2008).
6. Seshaiah Krishnan Sridhar , Surendra N. Pandeya , James P. Stables , Atmakuru Ramesh., "Anticonvulsant activity of hydrazones, Schiff and Mannich bases of isatin derivatives" *Eur. j. pharm. Sciences,*16:129–132,(2002).
7. Joseph F.Bartosevich, T.Scott Griffin,Daniel L.Klayman, Carl J.Mason. "2-acetyl pyridine thiosemicarbazones, a new class of potential antimalarial agents" *J.Med.Chem,* 22:855-862,(1979).
8. Humayun pervez, Mohammad s.Iqbal, Muhammad younas tahir, Muhammad Iqbal Choudary, Faiz-ul-hassannsim,and Khalid mohammed khan., "In vitro cytotoxic, antibacterial, antifungal and urease inhibitoryactivities of some N^4- substituted isatin-3-thiosemicarbazones" *J. Enz .Inhib. Med .Chem*. 23(6):848–854, (2008).

***Corresponding Author:**
Praneeth Kumar A*
Email: praneethsvsip@gmail.com