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Synthesis, Characterisation and Screening of Anthelmintic Activity of Some Novel Schiff Bases

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ABSTRACT

Some novel N,N-Dithiocarbohydrazones or Schiff bases 3(a-f) have been synthesised from thiocarbohydrazide by introducing different aromatic aldehydes and ketones along with various substituted isatins. The compounds have been characterised by IR, 1H NMR, 13NMR and Mass spectral techniques and screened for anthelmentic activity by using earthworms. Compounds 3a, 3b, 3d showed potent activity when compared to to Albendazole as standard.

Keywords: Thiocarbohydrazide, Dithiocarbohydrazide, Anthelmintic activity, Albendazole.

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INTRODUCTION

Sciff bases [1-4] of heterocyclic compounds are interesting class of organic compounds possess a wide spectrum of biological activities such as antibacterial, antitubercular, antiinflammatory, anthelmintic, antiviral and antioxidant activity.

Thiocarbohydrazide [5-8] is the closest structural analogue of thiosemicarbazide, derivatives of which are recommended as effective antitubercular and antiviral preparations. Thiocarbohydrazides of the aromatic series exhibit high antiviral and antimicrobial activity [9-10].

Isatin moiety shows biological activities like antibacterial, cytotoxicity, antiinflammatory and analgesic, anti HIV, antitubercular, antiviral activities [14-17].

In the view of these observations, we planned to synthesise some novel schiff bases and to eveluate for anthelmentic activity.

EXPERIMENTAL

Melting points were determined on micro-controler based melting point apparatus CL 725/726 and were uncorrected. The chemicals and reagents used in the project were of AR and LR grade, procured from Aldrich, Hi- media, Lancaster, Loba Merck, NR chem, Rolex, SD-Fine chem Ltd, Sigma. Purity of the compounds was checked by thin layer chromatography using silica gel G as stationary phase and various combinations of n-hexane: ethyl acetate as mobile phase. The spots resolved were visualized by using iodine chamber and UV chamber**:** The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400-4000 cm^{-1} Using KBr pellets and values are reported in cm^{-1} and the spectra were interpreted. ^{1}H-NMR spectra were recorded on DPX-200 MHz NMR spectrometer using DMSO-d_6 and chemical shifts (δ) are reported in parts per million down field from internal reference Tetramethylsilane (TMS) and the Spectra were interpreted. **:** ^{13}C-NMR spectra were recorded on DPX-200 MHz NMR spectrometer using DMSO-d_6 and chemical shifts (δ) are reported in parts per million (ppm) downfield from internal reference Tetramethylsilane (TMS) and the Spectra were interpreted. Mass spectra were recorded on Mass spectrophotometer (model Shimadzu) by LC- MS and the spectra were interpreted.

Step I: Synthesis of Thiocarbohydrazide [10-12]:

Carbon disulphide (13ml, 0.22mol) was added drop wise under stirring to a mixture of 85% hydrazine hydrate (24ml) and water (75ml), then stirring was continued for 30min at room temperature. The reaction temperature was then rapidly raised to 100-110^{0}c. After refluxing for 2hrs, the reaction mixture was cooled in an ice-bath and filtered. The residue was washed with ethanol and followed by ether, then recrystallized from water to afford pure

thiocarbohydrazide crystals. (Lit m.p:170-172^{0}C). The compound is as white crystalline powder (needles) and the yield was about 78%.

Step II: Synthesis of monothiocarbohydrazones (Schiff Bases) (2a-f)

A mixture of Thiocarbohydrazide (0.01mol) and Substituted aromatic aldehyde (0.01mol) dissolved in (30ml) and 5ml glacial acetic acid was added and refluxed for 2-3hrs. The progress of the reaction was monitored by TLC. After completion of the reaction mixture was cooled and kept in refrigerator for overnight. The resultant solid was filtered, dried and recrystallized from suitable solvent to afford compounds. (Lit m.p: 2a=198^{0}C).

Synthesis of 5-Methyl Isatin [18-24]:

Step 1: Synthesis of isonitrosoaceto-P-toluidine from P-toluidine:

9 gm of Chloral hydrate was taken into the round bottom flask and dissolved in 120 ml water. To that 13 gm of sodium sulphate, a solution of 5.4 gm of p-toluidine in 30 ml of water containing 5.12 gm of concentrated hydrochloric acid (4.34 ml) to dissolve the amine and solution of 11 gm of hydroxylamine hydrochloride in 50 ml of water were added. Flask was then heated vigorously until the reaction was completed. Later, the solution containing beaker was cooled in running water followed by the filtration of reminder crystallized product with suction pump and air dried.

Step 2: Synthesis of 5-Methyl Isatin from isonitrosoaceto- P-toluidine:

18.4 gm of concentrated sulphuric acid (10.0 ml) was warmed to 50^{0}C and 2.5 gm of dry isonitrosoaceto-p-toluidine was added in such a rate so as to keep the temperature between 60-70^{0}C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly after the addition of isonitroso compound was finished. The solution was heated to 80^{0}C and kept at this temperature for about 10 min to complete the reaction. Then the reaction mixture was cooled to room temperature and poured into ten times its volume of cracked ice. After standing for 90 mint, the final product was filtered with suction pump followed by washing with cold water to remove sulphuric acid and dried in air.m.p:135-137^{0}C.(Lit m.p: 136-138^{0}C)

Synthesis of N-Benzyl indole 2, 3- dione (N-Benzyl Isatin):

In the round bottomed flask take indole-2,3-dione (Isatin) 0.8gm (3.37mM) and equimolar quantity of benzyl chloride i.e. 6.5ml (3.7mM), mix with 20ml of DMF and to this mixture add 2gm of K_2CO_3. After gentle mixing, reflux for 2 hr, cool and pour to 100 ml of ice cold water. The resultant orange red ppt. was collected and washed with water dried and recrystallized from acetonitrile m.p:134-136^{0}C (Lit m.p:133-134^{0}C).

Synthesis of N-Methyl Isatin derivative:

5 gm of isatin was dissolved in dilute sodium hydroxide solution then 0.62 ml of Di-methyl sulphate was added. Then whole contents were refluxed in water bath for approximately 50 min. After refluxing, the mixture was poured into beaker and cooled in ice. Then the content was evaporated on water bath and dried.m.p:132-134^{0}C (Lit m.p:134^{0}C).

Step III: Synthesis of N, N- Dithiocarbohydrazones (Schiff Bases) 3(a-f):

Equimolar quantities (0.01mol) of Isatin or substituted Isatin and monothiocarbohydrazones (**2a-2f**) were dissolved in warm ethanol and glacial acetic acid (1:1%, 30ml). The reaction mixture was refluxed for 3hrs and then kept in refizerator for overnight. The resultant solid was filtered, dried and recrystallized from suitable solvent to afford compounds.

1-((1H-indol-3-yl) methylene)-5-(2-oxoindolin-3-ylidene) thiocarbonohydrazide:(3a)

Mol. Formula : $C_{18}H_{14}N_6OS$, Mol. Weight : 362.41 g mol^{-1} , Solubility : Methanol, Melting Point : 184-186^{0}C, TLC solvent: n-Hexane: Ethyl Acetate (4:3), R_f values: 0.428, % Yield : 80% (Conventional).

Spectral data:

IR Cm^{-1} (KBr): 3226(-NH *Str*), 3138 (-NH *Str*, lactam), 3070(C-H *Str*, Ar), 1705(C=O *Str*), 1609(C=N *Str*), 1248 (C=S *Str*).

^{1}H-NMR (DMSO δ ppm): 14.0 (1H, -NH), 12.2 (1H, -NH), 11.7 (1H, -NH), 11.3(1H, -NH), 8.67(1H, =CH-), 8.47(1H, N=CH), 6.95-7.9(9H, Ar-H).

^{13}C-NMR (DMSO δppm): 173.9 (C=S), 162.4(C=O), 143.5 (C=N), 142.1 (CH=N), 137.1 (Ar-CH), 136-100(Ar-C).

Mass (EI-MS): 363(M+1, 100%), 385(M+Na, 80%).

1-(5-methyl-2-oxoindolin-3-ylidene)-5-((thiophen-2-yl)methylene) thiocarbonohydrazide.(3b)

Mol.Formula:$C_{15}H_{13}N_5OS_2$, Mol.Weight:343.43 g mol^{-1} , Solubility: Ethanol, Melting Point: 152-156 ^{0}C, TLC solvent : n-Hexane: Ethyl Acetate (2:1), R_f values : 0.545,

% Yield :77% (Conventional) .

Spectral data:

IR Cm^{-1} (KBr): 3150 (-NH *Str*), 1652 (C=O *Str*), 1606 (C=N *Str*), 1583 (HC=N *Str*), 1480 (-CH_3 *Str*), 1372 (C=S *Str*), 1211 (C-S *Str*), 769 (-CH *Str*, benzene).

^{1}H-NMR (DMSO δ ppm): 12.2 (1H, -NH), 11.2 (1H, -NH), 10.1 (1H, -NH), 8.04 (1H, -CH=N), 8.2-7.8 (6H, Ar-H), 2.34 (3H, -CH_3).

^{13}C-NMR (DMSO δppm): 179.3(C=S), 165.8(C=O), 136-148(2C=N), 124-119 (10C=C), 25.2 (C-H).

Mass (EI-MS): 344 (M+1, 100%), 366 (M+Na).

1-(4-hydroxybenzylidene)-5-(1-methyl-2-oxoindolin-3-ylidene) thiocarbonohydrazide.(3c)

Mol. Formula: $C_{17}H_{15}N_5O_2S$, Mol. Weight :353.40 g mol^{-1} Solubility:Methanol/Ethanol Melting Point :242-245^{0}C, TLC solvent : n-Hexane: Ethyl Acetate (1:1) R_f values : 0.542, % Yield :78% (Conventional).

Spectral data:

IR Cm^{-1} (KBr): 3052(OH *Str*), 3211(-NH *Str*), 1647(C=O *Str*), 1552(HC=N *Str*), 1310(C=S *Str*).
^{1}H-NMR (DMSO δ ppm): 13.5(1H, -NH), 12.8(1H, -NH), 8.54(1H, -CH=NH), 7.81-6.43(8H, Ar-H), 5.35(1H,-OH), 3.44(3H, $-CH_3$).
^{13}C- NMR (DMSO δppm): 175.8(C=S), 170.2(C=O), 142.6-138.3(2C=N), 124-118(12C=C), 30.4(C-H).
Mass (EI-MS): 354 (M+1, 100%), 376 (M+Na).

1-(4-chlorobenzylidene)-5-(5-methyl-2-oxoindolin-3-ylidene) thiocarbonohydrazide.(3d)

Mol. Formula :$C_{17}H_{14}ClN_5OS$, Mol. Weight : 371.84 g mol^{-1} , Solubility :Methanol/Ethanol, Melting Point :235-238 ^{0}C, TLC solvent :n-Hexane: Ethyl Acetate (5:3), R_f values : 0.782, % Yield :64% (Conventional) .

Spectral data:

IR Cm^{-1} (KBr): 3116 (-NH *Str*), 1709 (C=O *Str*), 1660 (C=N *Str*), 1592 (HC=N *Str*), 1492 ($-CH_3$ *Str*), 12619 (C=S*Str*), 708 (C-Cl *Str*).
^{1}H- NMR (DMSO δ ppm): 12.8(1H, -NH), 12.2 (1H, -NH), 11.7 (1H, Indole NH), 8.54 (1H, -CH=N), 8.41-7-02 (7H, Ar-H), 2.34 (3H, $-CH_3$).
^{13}C-NMR (DMSO δppm): 173.9 (C=S), 170.45 (C=O), 140-148 (2C=N), 119-135 (12C=C), 22.2 (1C-H).

1-(1-benzyl-2-oxoindolin-3-ylidene)-5-benzylidene thiocarbonohydrazide.(3e)

Mol. Formula: $C_{23}H_{19}N_5OS$,Mol. Weight: 413.49 g mol^{-1} ,Solubility :Methanol/Ethanol, Melting Point :242-245^{0}C, TLC solvent : n-Hexane: Ethyl Acetate (1:1), R_f values: 0.582, % Yield :74% (Conventional) .

Spectral data:

IR Cm^{-1} (KBr): 3219(–NH *Str*), 1697(C=O *Str*), 1655(C=N *Str*), 1583 (HC=N *Str*), 146 2(-CH *Str*), 1238 (C=S *Str*).
^{1}H- NMR (DMSO δ ppm): 12.7(1H, -NH), 12 (1H, -NH), 8.54 (1H, -CH=N), 7.86-7.23 (14H, Ar-H), 4.94 (2H, $-CH_2$-).
^{13}C-NMR (DMSO δppm): 174.3 (C=S), 162.6 (C=O), 128-143 (C=N), 121.3-142.3 (18C=C), 25.4 (1C-C).
Mass (EI-MS): 414(M+1, 100%), 436 (M+Na).

1-(3, 4, 5-trimethoxybenzylidene)-5-(5-methyl-2-oxoindolin-3-ylidene) thiocarbonohydrazide.(3f)

Mol. Formula:$C_{20}H_{21}N_5O_4S$,Mol.Weight :427.48 g mol^{-1} Solubility: Ethanol, Melting Point :152-154 ^{0}C,TLC solvent n-Hexane: Ethyl Acetate (1:1)
R_f values :0.739, % Yield : 52% (Conventional).

Spectral data:

IR Cm^{-1} (KBr): 3231(-NH *Str*), 1721(C=O *Str*), 1655(C=N *Str*), 1577(HC=N *Str*), 14859(CH_3*Str*), 1285(C=S *Str*), 1206(C-O *Str*).
^{1}H-NMR (DMSO δ ppm): 13.2(1H, -NH), 12.8(1H, -NH), 10.2(1H, Indole NH), 8.54(1H, -CH=N), 8.21-6.64(5H, Ar-H), 3.83(9H,-OCH_3), 2.34(3H, -CH_3),
^{13}C-NMR (DMSO δppm): 186.3(C=S), 172(C=O), 136.142(2C=N), 108-156.3(12C=C), 56-60(3O-C).
Mass (EI-MS): 427.48 (M+1, 100%), 41 (M+Na).

ANTHELMENTIC ACTIVITY [25-27]

Anthelmentics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. The World Health Organization estimates that a staggering 2 billion people harbour parasitic worm infections.

Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Also of importance is the infection of domestic pets. Indeed, the companion animal market is a major economic consideration for animal health companies undertaking drug discovery programmes.

Despite the prevalence of parasitic worms, anthelmentic drug discovery is the poor relation of the pharmaceutical industry. The simple reason is that the nations which suffer most from these tropical diseases have little money to invest in drug discovery or therapy. It comes as no surprise therefore that the drugs available for human treatment were first developed as veterinary medicines.

Procedure:

The synthesized compounds are screened for anthelmentic activity by using Earth worms. Six earthworms of nearly equal size were placed in standard drug solution and test compound's solutions at room temperature. Normal saline used as control. The standard drug and test compounds were dissolved in minimum quantity of dimethyl sulfoxide (DMSO) and adjusted the volume up to 10 ml with normal saline solution to get the concentration of 0.1% w/v, 0.2 % w/v and 0.5% w/v. Albendazole was used as a standard drug.

The compounds were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time. To ascertain the death of the motionless worms were frequently applied with external stimuli, which stimulate and induce movement in the worms, if alive. The mean lethal time and paralysis time of the earthworms for different test compounds and standard drug are tabulated.

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RESULTS AND DISCUSSION

Chemistry:

Heterocyclic systems containing endo-and exocylic sulphur atom show a wide spectrum of potential applications. The synthesis of all Compounds were carried out as depicted in Scheme-I. Novel Schiff bases were obtained from condensation of thiocarbohydrazide with substituted aldehyde to give the monothiocarbohydrazones. Then followed by Schiff base reaction with isatin and substituted isatins in presence of acidic medium to gives dithiocarbohydrazones.

The resulting compounds were purified by Column Chromatography using mobile phase as n-Hexane: Ethyl Acetate. All derivatives were recrystallization using Ethanol and Methanol. The new Schiff bases derivatives were synthesized by using appropriate synthetic methods that are mentioned in experimental section. The synthesized thiocarbohydrazide derivatives were characterized by both physical and spectral data like ^{1}H-NMR, ^{13}C-NMR, FT-IR. Newly synthesized compounds were evaluated for and anthelmintic activities.

The Synthesized Novel Schiff bases were Characterized from the Spectral analysis it is revealed that, IR Shows absence of C=O peak for isatin and absence of doublet peak for $-NH_2$ O only Singlet peak around 3100-3300cm^{-1} for -NH is observed for all compounds.^{1}H-NMR Shows presence of –NH peaks at around 10.0-14.0ppm. From ^{13}C-NMR absence of C=O peak for isatin and presence of C=S around 170-180.

All the derivatives showing Characteristic base peak for M+1 peak .And the mass Spectra of all the synthesized compounds obeying the nitrogen rule.

Anthelmintic activity:

The synthesized compounds (3a-3f) were evaluated for anthelminthic activity on Indian earthworms (*Pheretima posthuma*). All compounds showed anthelmenthic activity is shown in table. Among the compounds tested all the compounds were showed significant paralytic time of earthworms, compared to standard drug albendazole at 0.1%, 0.2% and 0.5% concentrations of compounds.

A closer inspiration of data from this table indicated that compound 3a, 3b having more activity and compounds 3d, 3e and 3f showed moderate activity. After all, the synthesized compounds in overall estimation confirm the better activity against *pheretima posthuma.*

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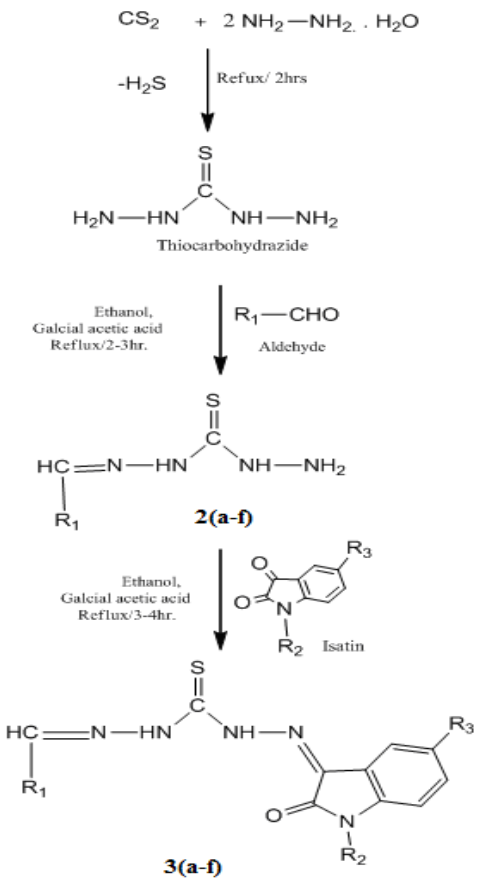
SCHEME-I



3a: R1= , R2=H, R3=H

3b: R1= , R2=H, R3=-CH3

3c: R1= , R2=-CH3, R3=H

3d: R1= , R2= H, R3=-CH3

3e: R1=  ,R2=-CH2C6H5, R3=H

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3f: R1= , R2=H, R3=-CH3.

Table 1: Antihelmintic activity of novel Schiff bases

S.No.	Name	Time in minutes					
		For paralysis			For death		
		% Concentration			% Concentration		
		0.1	0.2	0.5	0.1	0.2	0.5
1	Control	-	-	-	-	-	-
2	Albendazole	15	12	8	44	34	26
3	3a	20	16	12*	56	38	33
4	3b	17*	14	11*	48	32	28*
5	3c	32	24	22	68	55	48
6	3d	20	18	14	52	44	38
7	3e	34	24	20	68	63	58
8	3f	20	18	15	50	47	41

CONCLUSION

The objective of the present work was to synthesize, purify, characterize and evaluate the biological activity of newly synthesized structural analogs of Thiocarbohydrazones derivatives. The yield of the synthesized compound was found to be in the range from 52-82 %. All these molecules were characterized by FTIR, H^1NMR and mass spectral analysis along with physical data. The functional groups in the title compounds were indicated by their IR spectra. The number of protons in the compounds were confirmed by their ^{1}H NMR spectra. The structure of title compounds were confirmed by their Mass Spectra.

Among all the compounds tested on earthworms, all compounds were showed significant paralytic and death time of earthworms, compared to the standard drug albendazole at 0.1%, 0.2% and 0.5% concentrations of compounds. The synthesized compounds (3a-3f) were also screened for anthelmintic activity (by Albendazole as standard reference). The compound (3a, 3b) (11 minutes for paralysis time) has shown highest activity.

In conclusion, the present study highlights the importance of Schiff bases derivatives having various heterocyclic moiety features responsible for the antibacterial, activities and may serve as a lead molecule for further modification to obtain clinically useful novel entities.

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