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Synthesis, Characterisation and Antibacterial Activity of Some Indole Derivatives and Their Inclusion Complexes with β-Cyclodextrin

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ABSTRACT

The condensation product of isatin with semicarbazide(IstscabH), thiosemicarbazide (IsttscabH) and thiocarbohydrazide (BisttcabH) form inclusion complexes with β-cyclodextrin (β-cydx=$C_{42}H_{70}O_{35}$), in 1:1 molar ratio having composition [β-cydx-IstscabH/IsttscabH] and [(β-cydx-BisttcabH)]. The inclusion complexes have been charactised from the studies of IR, UV, NMR and molecular weight determination. The inclusion products are thermally more stable than β-cyclodextrin (240°C). The IR, UV and NMR spectra of isatin derivatives are affected on forming inclusion complexes with β-cyclodextrin ring. The inclusion products as well as isatin derivatives show ionic nature at room temperature in DMF solution. The antibacterial activity of inclusion products are larger than simple Schiff bases.

Keywords: Inclusion complexes, β-cyclodextrin, Isatin Schiff base, Semicarbazone, Thiosemicarbazone, Thiocarbohydrazone antibacterial screening.

INTRODUCTION

β-Cyclodextrin is an oligosaccharide containing seven D-glucose units forming a cyclic ring. The seven D-glucose units joined through 1,4-alpha linkages in such a way as to form a ring, a chain bracelet each link of which in pyranose hexagon. Mendez *et al.,*[1] studies the formation of amphiphilic click cluster of β-cyclodextrin with DNA and effect of structural modification of DNA complexes and their delivery properties. The diameter of β-cyclodextrin ring is about twice the ring of 18-crown-6 and its hole (4.5Å across) is twice broad. The hole of cyclic ring of β-cyclodextrin is expected to entrap a small or linear molecule to form a inclusion complexes. The interaction products of β-cyclodextrin with bioactive molecules may be physiological interest and we have motivated us to

synthesised some of the new products with these Schiff bases. Isatin (indole-2,3 dione) derivatives that possess wide spectrum of medicinal activities like antibacterial[2], antiviral[3], antihelmintic[4], herbicidal[5], fungicidal[6] and antitubercostatic[7] properties. Isatin and indole were formerly extracted from natural products and these were reported to possess immense medicinal activities[8-14]. The Schiff bases of isatin (indole-2,3 dione) with semicarbazone, thiosemicarbazone and thiocarbohydrazide are expected to be get interrupted into β-cyclodextrin hole to form inclusion complexes and these may possess physiological activity. The hydrazide and hydrazine derivatives of isatin and substituted isatin possess pronounced anticonvulsant activities[15-21]. A number of Schiff bases of isatin derivatives have also been used as neurotoxic agent[17]. The epilepsy is a well known neurological disorder characterized by unprovoked seizure of convulsion and thus efforts have directed for its drug development. The isatinsemicarbazones have also been used as an excellent anticonvulsant drug[18-19]. The compound 3-(3',4'-dihydro-2-methymarcapto-4-oxoquinozoline-3'-yl) iminoisatin has been proved to be most active anticonvulsant molecule possessing neurotoxicity[20]. A number of new N-methyl/N-acetyl-5-substituted isatin-3-semicarbazones have been reported to possess anticonvulsant activity by Pandeya and Raja[21]. Since last decades a number of isatin derivatives have been shown to possess potential hypnotic[22], antibacterial[23-24], monoamine oxidaze (MAO) inhibitory[25] and antioxidant[26] properties. However much attention has been focused on chemistry of isatin derivatives due to its biological potentiality like anticancer, anti-protozoa, cytotoxic, their presence in hippocampus cerebellum brain and DNA cleavage[16].

Isatin derivatives have been found in some mammalian tissues where their function is as modulator of biochemical processes[26]. Isatin has been reported as "Tribulin" and identified as a selective inhibitor of monoamine oxidaze (MAO)[25]. A new series of 5-hydroxyisatin derivatives has been synthesized by hydroxylation of aromatic ring in isatin and these has been shown to possess anti-anxiety effect[5]. These immense medicinal activities of isatin derivatives motivated us to study the inclusion complexes of Schiff bases with β-cyclodextrin(β-cyclod), Bisisatinyl thiocarbohydrazide (BisttcabH), isatinylthiosemicarbazone (IsttscabH) and isatinylsemicarbazone (IstscabH). Therefore, in the present paper, we report the synthesis and characterisation of inclusion complexes of IstscabH, IsttscabH and BisttcabH with β-cyclodextrin and investigated bio activity of some bacteria namely *Bacillus subtilis*, *Staphylococcus* and *P. mirabilis*.

EXPERIMENTAL

METHODS AND MATERIAL

β-Cyclodextrin and isatin were obtained from Sigma Aldrich and semicarbazide, thiosemicarbazide, thiocarbohydrazide used were either BDH or E Merck and was extra pure reagents. Solvents used were obtained from BDH. The result of C, H, N, S were obtained from IIT Patna using vario MICRO V-3 Elemental analyser. The IR spectra were recorded on Shimadzu IR Spectrophotometer in KBr disc in the range of 400 to 600 cm^{-1}. All UV-Visible spectra were recorded in ethanol or dimethylformamide (DMF) in the range of 200-800 nm using a Shimadzu Spectrophotometer No. 2000 at IIT Patna. Similarly, ^{1}H NMR and ^{13}C NMR were recorded in DMSO on Bruker 400 MHz NMR spectrophotometer at IIT Patna. The molecular weight of inclusion product determined by Rast method in Camphor[27]. Bacterial screen were performed in the Department of Biochemistry, Patna University, Patna.

Preparation of Isatin Derivatives

Isatinderivatives, isatinylsemicarbazone, (IstscabH), isatinylthiosemicarbazone (IsttscabH) and bisisatinylthiocarbohydrazone (BisttcabH) were prepared by condensing isatin in stoichiometric proportion with semicarbazide, thiosemicarbazide and thiocarbohydrazide in aqueous ethanol containing a few drops of acetic acid at reflux temperature.

Procedure

0.01 Millimole of isatin in 30 mL ethanol and 0.01millimole of semicarbazide (or thiosemicarbazide hydrochloride) dissolved in 40-50 mL hot aqueous ethanol containing 1 mL of acetic acid were mixed together and refluxed on a steam bath for 40-50 min when desired product separated as bright yellow or orange yellow products. The products were cooled and collected on buckner funnel, washed with cold aqueous ethanol and dried over in a desiccator. The dried products were crystallised from hot

ethanol and analysed. The analytical results and melting point of products are recorded in Table 1. The bisisatinylthiocarbohydrazide were prepared as above taking millimole of isatin and 0.01 millimole of thiocarbohydrazide in hot aqueous ethanol 50 mL containing 1 mL acetic acid. The product was separated as orange yellow compound and recrystallized with ethanol-dioxane mixture.

Table 1: Analytical results and physical data of isatin derivatives and their inclusion complexes with β-cyclodextrin

S. No.	Compound & Formula	Elemental Analysis, Found (Cal) (%)				Moleculalar weight	Decomposition
		C	H	N	S	Found (cal) in(g)	Temperature in (°C)
1	IstscabH	52.71	4.12	27.28		204.31	278°C
	($C_9H_8N_4O_2$)	(52.93)	(3.95)	(27.44)			
2	IsttscabH	48.73	3.81	25.19	14.31	220.31	237°C
	($C_9H_8N_4SO$)	(49.06)	(3.66)	(25.44)	(14.55)		
3	BisttcabH	55.81	3.41	22.92	8.91	364.57	302°C
	($C_{17}H_{12}N_6O_2S$)	(56.04)	(3.28)	(23.08)	(8.79)		
4	[β-cydxIstscabH]	45.41	5.71	4.38		1312.21	287-290°C
	($C_{51}H_{78}N_4O_{37}$)	(45.74)	(5.83)	4.18		(1339.29)	
5	[β-cydxIsttscabH]	45.01	5.93	4.01	2.18	1332.31	252-273°C
	($C_{51}H_{78}N_4SO_{36}$)	(45.2)	(5.76)	(4.14)	2.36	(1355.35)	
6	[β-cydxBisttcabH]	47.01	5.68	5.43	2.01	1461.41	295-297°C
	($C_{59}H_{82}N_6O_{37}S$)	(47.26)	(5.47)	(5.61)	(2.14)	(1499.55)	

Preparation of Inclusion Complexes of Isatin Derivatives with β-Cyclodextrin

0.001 millimole of β-cyclodextrin was dissolved in 100 mL aqueous ethanol (1:1) and treated with 0.012 millimole of appropriate isatin derivatives (IstscabH, IsttscabH or BisttcabH) dissolved in 100 mL hot aqueous ethanol (75%). The mixed solution were refluxed for two hours at steam bath and cooled at room temperature when some unreacted ligand separated which was removed by filtration and filtrates were left in air for slow evaporation at room temperature. After 3 to 4 days cream yellow product separated when the volume of mixed solution was about 20-25 mL (1/8 of original volume). The products were collected on a buckner funnel and washed with cold aqueous ethanol and dried in a desiccator over $CaCl_2$. The molecular weight of ligands and inclusion products were determined in Camphor by Rast method[27]. The analytical results C, H, N, S percentage, molecular weight and melting point of products are given in Table 1. The IR, electronic absorption spectra and ^1H NMR as well as ^{13}C NMR spectrum of isatin derivatives and their inclusion complexes were recorded and results are shown in Table 2, 3, 4.

RESULTS AND DISCUSSION

The elemental analysis C,H,N, S of inclusion products of isatin derivatives (IstscabH, IsttscabH and BisttcabH) with β-cyclodextrin ($C_{42}H_{70}O_{35}$), (β-Cydx) correspond to compositions β-cydx-Istscabh ($C_{51}H_{78}N_4O_{37}$), β-cydx-Isttscabh ($C_{51}H_{78}N_4OS$) and β-cydx-BisttcabH ($C_{59}H_{82}N_6O_{36}S$). These products are insoluble in cold water but dissolve slightly in boiling water. These products are soluble in DMF and DMSO without decomposition. The molecular weight of inclusion complexes determined by Rast method in Camphor and these approximately correspond to calculated molecular weight which supports the formation of inclusion product. The melting point of isatin derivatives and their inclusion products with β-cyclodextrin show wide variation in their physical properties from each other. β-Cyclodextrin in general on heating started decomposing with melting above 200°C but inclusion complexes are stable below 240°C. The inclusion complexes of β-cydx-IstscabH and β-cydx-IsttscabH decompose slowly at 285°C and 290°C respectively. The electrical conductance values of isatin derivatives and their inclusion complexes were measured qualitatively at room temperature in DMF solution. All Schiff bases and their inclusion products show electrical conductivity in the range 70-72 ohm^{-1} mol^{-1} cm^2 indicating that in these Schiff bases as well as inclusion products. The Schiff bases NH protons get ionised in DMF solution. The ionic behaviour of NH or tautomeric SH (Thione-thiol) proton has also been supported by ^1H NMR spectra of isatin derivatives[28].

Infrared Spectra

The IR spectrum (Figure-IR_1) of IstscabH shows $\nu(NH_2)$ + ν(NH) vibrations[31-32] as broad band between 3286-3156 cm^{-1}. The ν(CO) carbomide

band of isatin ring and carbohydrazone ν(CO) are observed as very strong band at 1715 and 1693 cm^{-1} respectively[32]. The hydrazine diazene ν(C=N) and benzene ring ν(C=C) bands are also located as very strong band at 1603 cm^{-1}. The ν(NH_2) and ν(NH) could be assigned to a strong bands located at 1471 cm^{-1} and 1451 cm^{-1}. The ν(C-N), ν(N-N) and benzene ring skeletal bands[33] can be assigned to IR bands at 1391, 1350, 1197, 1163 and 1109 cm^{-1}. The isatin ring NH, C-H and semicarbanzone part NH as well as NH_2 deformation vibration of IstscabH can be assigned to IR bands at 1052, 951, 787, 749 and 664.6 cm^{-1}. The IR spectrum of its inclusion complex (Figure-IR_2) with β-cyclodextrin retains most of its IR vibrations but ν(NH), ν(NH_2) and ν(CO) of IstscabH observed as at wave number 3350-3151 cm^{-1} and 1691 cm^{-1} respectively indicating that hydrogen bonds of NH_2 of free ligand is cleaved in the inclusion complexes[31]. The ν(CO) to shifted to lower wave number due to hydrogen bonding of hydroxy (OH) of β-cyclodextrin. The IR spectrum of Isatinylthiosemicarbazone (IsttscabH) (Figure-IR_3) shows ν(C=O) of isatin part at 1727 cm^{-1} and diazine ν(C=N) band at 1615 cm^{-1}. The ring skeletal vibrations of IsttscabH are observed at 1593 cm^{-1}. The ν(C=S) is observed at 1460, 1331, 1322 and 1201 cm^{-1} as strong and sharp band. The IR spectrum of IsttscabH in inclusion complex (Figure-IR_4) showed some changed in its vibrations but much of IR vibration at 1026 cm^{-1} are retained without major change. The inclusion products of β-cyclod-IsttscabH shows as number of new bonds due to inclusion of β-cyclod. The ν(CO) is shifted to lower vibration and observed at 1672 cm^{-1} due to hydrogen bonding but ν(C=S) vibration is shifted to higher wave number and observed at 1128 cm^{-1} as very strong band. These changes in IR vibration supported inclusion of IsttscabH with β-cyclodextrin. The IR spectrum of bis(isatinyl)thio-carbohydrazone (BisttcabH) (Figure-IR_5) shows strong ν(C=O) vibration of (NH-CO) group of isatin ring at 1687 cm^{-1} indicating strong hydrogen bonding but in its inclusion complex of β-cyclodextrin, it is shifted to 1737 cm^{-1} and partial at 1687 cm^{-1} (Figure-IR_6). The ν(C=N) of BisttcabH is located at 1617 cm^{-1} is observed at 1618 cm^{-1} in inclusion complex. The ν(NH) of BisttcabH and its inclusion product observed at 1492 cm^{-1}. A number of IR vibrations of BisttcabH are retained in its inclusions complexes. The extent of hydrogen bonding of free BisttcabH is cleaved and observed with reduced intensity in its inclusion complexes. The shift of bands in IR spectrum of isatin derivatives in its inclusion complexes support the inclusion between interacting molecules without chemical reaction.

Table 2: Diagonostic ir bands (in cm^{-1}) of schiff bases and their inclusion products with their assignment

S.No	Compound & Formula	ν(NH_2)+ν(NH) +ν(OH)	ν(C=O)	ν(C°N) azine	δ(NH)	ν(C=S)+ ν(C–O)	δ(C-H) and ring deformation band
1	IstscabH ($C_9H_8N_4O_2$)	3286 m, 3156 m	1715 vs, 1693 vs	1603 st	1471 st	1052 st	787 m, 741 m
2	[(β-cyclod) (IstscabH)] ($C_{51}H_{78}N_4O_{37}$)	3350 m, 3151 m	1710 st, 1691 vs	1599 st	1452 st	1044 st	780 m, 750 m
3	IsttscabH ($C_9H_8N_4SO$)	3354 m, 3216 m	1727 st	1615 st	1460 st	1026 m	768 m, 734 m
4	[(β-cyclod) (IsttscabH)] ($C_{51}H_{78}N_4SO_{36}$)	3518 m, 3335 m 3216 m, 3164 m	1672 st	1622 st 1593 m	1463 st	1128 st	794 m, 759 m 741 m
5	BisIsttcabH ($C_{17}H_{12}N_6O_2S$)	3292 m, 3264 m 2971 m	1687 st	1617	1492	1155 vs	788, 765, 670
6	[(β-cyclod) (BisIsttscabH)] ($C_{59}H_{82}N_6O_{37}S$)	3294, 3246, 2972, 2803	1737	1618	1492, 1460	1202, 1013	825, 788, 753, 660

m = medium, st = strong, vs = very strong

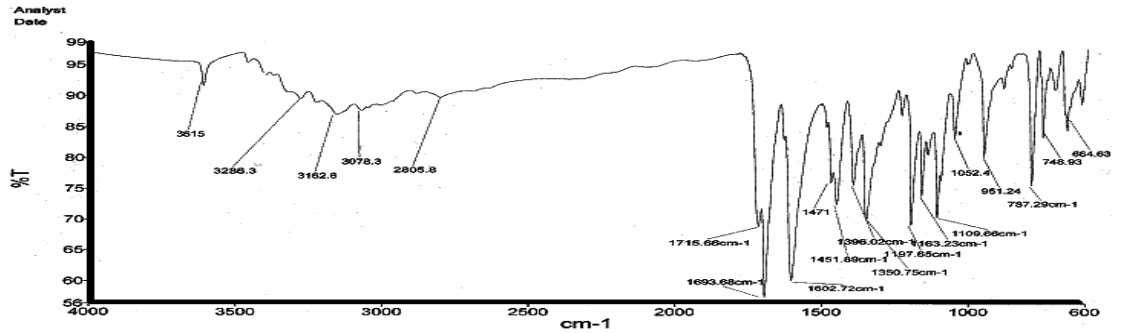


Fig. 1. IR_1(IstscabH), ($C_9H_8N_4O_2$)

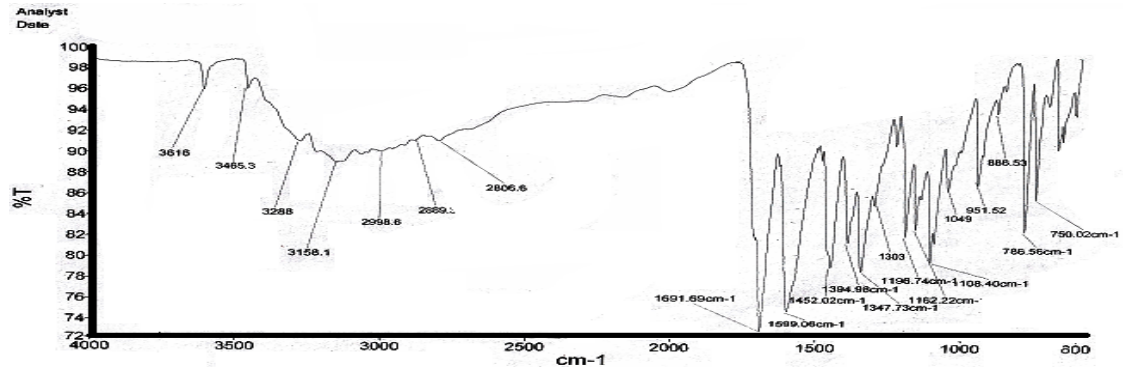


Fig. 2. IR_2(IstscabH) ($C_9H_8N_4O_2$)

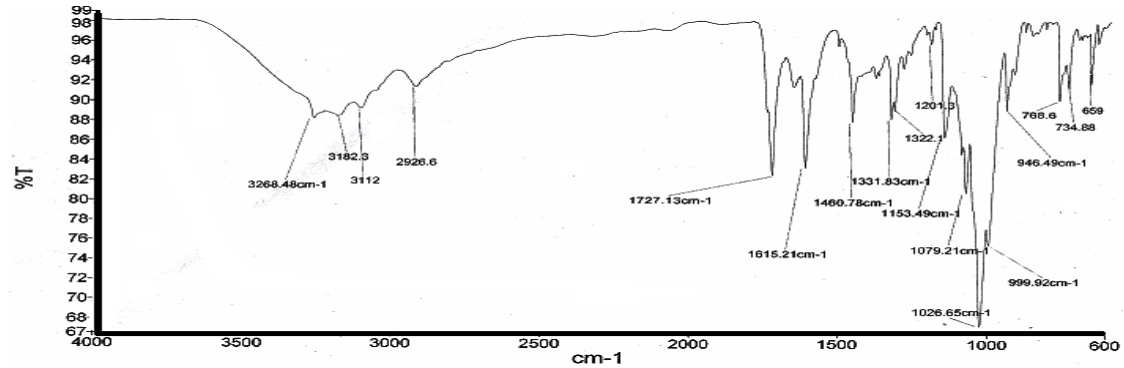


Fig. 3. IR_3(IsttscabH), ($C_9H_8N_4OS$)

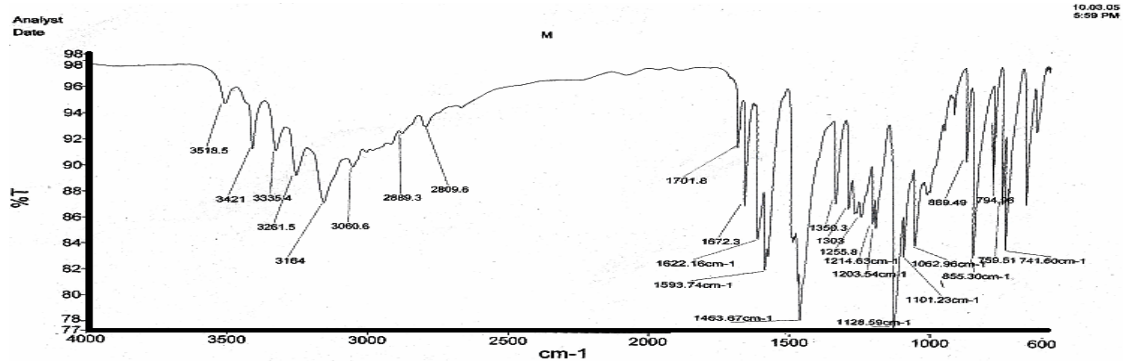


Fig. 4. IR_4 (IsttscabH) ($C_9H_8N_4OS$)

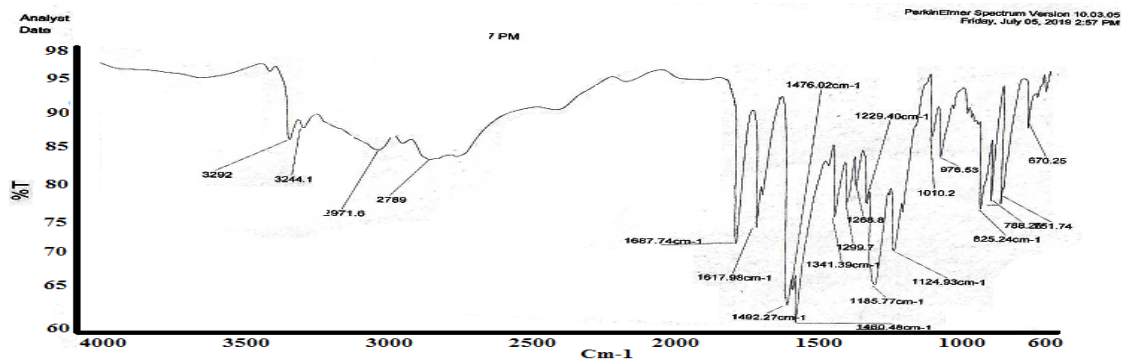


Fig. 5. IR_5(BisttcabH) ($C_{17}H_{12}N_6O_2S$)

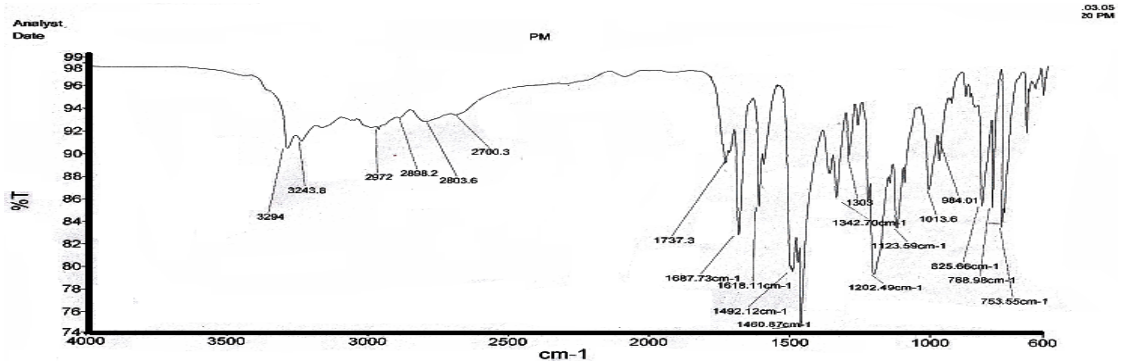


Fig. 6. IR_6(IsttscabH) ($C_9H_9N_5OS$)

Table 3: Electronic absorption bands (in nm) of istscabh/isttscabh/bisttcabh and their inclusion complexes with β-cyclodextrin with assignment

S. No.	Compound & Formula	Transition in nm			
		$\sigma\rightarrow\sigma^*$	$\pi\rightarrow\pi^*$	$n\rightarrow\pi^*$	CT
1	IstscabH ($C_9H_8N_4O_2$)	204, 206 209	239 m, 246 m 315 m	330 m	
2	IsttscabH ($C_9H_8N_4SO$)	202, 206, 210	240 m, 268 m	360 sh	364 st
3	BisttcabH ($C_{17}H_{12}N_6O_2S$)	206, 210	245 m	275 w	360 st
4	[(β-cyclod)(IstscabH)] ($C_{51}H_{78}N_4O_{37}$)	205, 207, 210	238 m, 245 m	349 m	
5	[(β-cyclod)(IsttscabH)] ($C_{51}H_{78}N_4SO_{36}$)	202, 204 206	236 m, 254 m	286 w	370-380 st
6	[(β-cyclod)(BisttcabH)] ($C_{59}H_{82}N_6O_{37}S$)	202, 212, 218	245 m	280 w	350-380 st

CT = Charge Transfer, m = medium, st = strong, w = weak, sh = shoulder

Table 4: ^{1}H NMR and ^{13}C NMR signals of schiff bases and their inclusion products

Compound	^{1}H NMR signals in ppm	^{13}C NMR Signals in ppm
Isatinylsemicarbazone (IstscabH)	δ = 3.44 (NH_2) proton, δ = 6.89, 7.61 benzene (CH) proton, δ = 10.79, 10.72, 11.10, 11.75 ring (NH) and (C=NH) proton	δ = 112.57-142.37 phenyl (C-H) carbon, δ = 155.52, 156.45 (CO) carbon, δ = 163.17, 165.30 ring (CO), (C=N) carbon
[(β-cyclod)(IstscabH)]	δ = 3.31-3.47 (N-H) proton β-cyclod, δ = 6.91-7.61 benzene (CH) proton, δ = 5.12-5.23 (OH) proton of β-cyclod, δ = 10.71, 11.11 and 12.12 ring (NH) and carbazone (NH) proton signal	δ = 116.51-143.37 benzen ring ^{13}C, δ =38.22-40.57 multiplete β-cyclod carbon, δ = 161.32, 167.15 carbazone (C=O) carbon δ = 168.21-170.31, 172.21 (C=N) isatin ring (C=O) carbon
Isatinylthiosemicarbazone (IsttscabH)	δ = 3.32, 4.84 (NH_2) proton, δ = 6.91-8.66 phenyl ring (CH) proton, δ = 11.18, 12.48 thioamide and ring (NH) proton	δ = 102.70-142.80 phenyl ring carbon, δ =39.31-40.56 multiplete β-cyclod C, d = 163.09, 179.17 (C=O) and (C=S) carbon
[(β-cyclod)(IsttscabH)]	δ = 3.28, 4.18 (NH_2) proton, δ = 6.92-8.58 ring (C-H) proton, δ = 5.21-5.46 hydroxy (OH) proton of β-cyclod, δ = 11.31-12.52 thioamide ring (NH) proton	δ = 110.36-143.37 phenyl ring carbon, δ = 164.13, 175.23, 189.13 (C=N), (CO), (C=S) carbon

Electronic Spectra Studies

The electronic absorption spectrum of isatinylsemicarbazone (IstscabH) (Figure-UV_1) in ethanol observed at 204, 206 and 209 nm assignable to $\sigma\rightarrow\sigma^*$ transition. The strong band at 239, 246 and 315 nm are assigned to $\pi\rightarrow\pi^*$ transitions for isatin ring and amide (NH-CO) group. The broad and weak absorption band at 330 nm is assigned as $n\rightarrow\pi^*$ transition[29]. The inclusion complex displays electronic transition located at 205, 207, 210, 238, 245 and 349 nm. The $n\rightarrow\pi^*$ transition of IstscabH is shifted to higher wave number and observed at 349 nm in inclusion complexes. The shift in the electronic spectrum of isatinylthiosemicarbazone(Isttscabh) (Figure-UV_2) in ethanol shows $\sigma\rightarrow\sigma^*$ transition at 202, 206 and 210 nm and $\pi\rightarrow\pi^*$ band at 240, 268 nm. The strong band at 364 nm is assigned to charge transfer transition of (>C=S) group[30]. The medium band at 268 nm is attributed to $n\rightarrow\pi^*$ transition. The inclusion complex of IsttscabH with β-cyclodextrin shows $\sigma\rightarrow\sigma^*$ transition at 202,204, 206, and 212 nm and $\pi\rightarrow\pi^*$ transition at 236 and 254 nm. The medium band at 286 nm is attributed to $n\rightarrow\pi^*$ transition. The charge transfer band near 370-380 nm in inclusion complex is very broad. The change pattern of electronic absorption band supported the inclusion of IsttscabH in β-cyclodextrin. The electronic absorption band of BisttcabH (Figure-UV_3) shows strong absorption band at 206, 210 nm assigned to $\sigma\rightarrow\sigma^*$ transition and 245 nm band as $\pi\rightarrow\pi^*$ transition. The weak absorption band at 275 nm in BisttcabH is tentatively attributed to $n\rightarrow\pi^*$ transition. The strong and broad absorption at 360 nm is assigned to charge transfer transition involving thioamide group (HNC=S). The inclusion product of BisttcabH displays a number of $\sigma\rightarrow\sigma^*$ transition at 202, 212 and 218 nm. The charge transfer transition of BisttcabH was observed as very broad band between 350-380 nm indicating association and formation of inclusion complex.

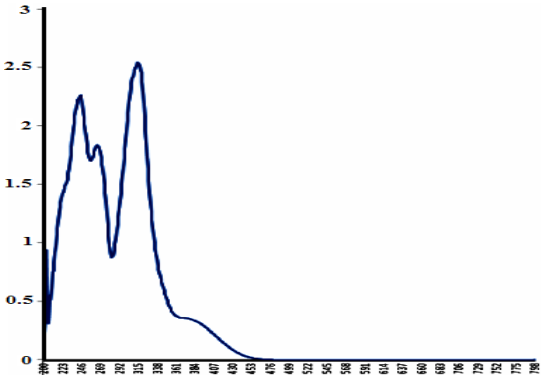


Fig. 7. UV_1(IstscabH) ($C_9H_8N_4O_2$)

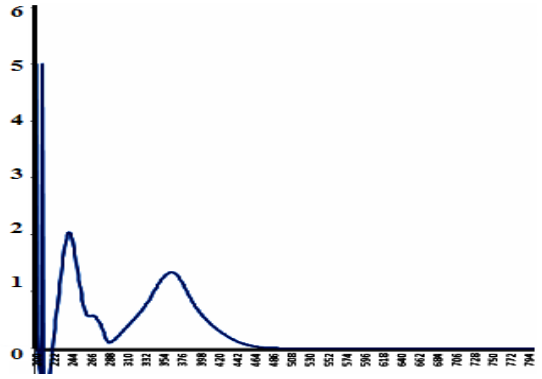


Fig. 8. UV_2(IsttscabH) ($C_9H_8N_4OS$)

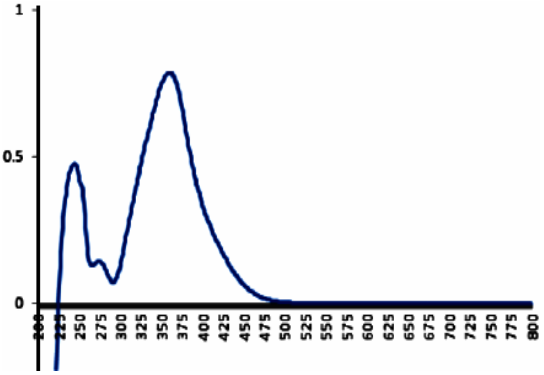


Fig. 9. UV_3 (BisIsttscabH) ($C_{17}H_{12}N_6O_2S$)

NMR Spectra

The proton (H) and ^{13}C NMR spectra of some representative products are shown in (Figure-^1H NMR_1, ^{13}C NMR_1, ^1H NMR_2, ^{13}C NMR_2). The NMR spectrum of isatinylsemicarbozone (IstscabH) shows NH_2 proton signals at δ=3.44 ppm and phenyl ring proton signals as multiplete between δ=6.89 and 7.61 ppm (Figure-^1H NMR_1). The isatinyl (NH) and semicarbozone (–NH–) proton signals are observed at 10.19, 10.72, 11.1 and 11.75 ppm suggested acidic nature of NH. The acidic nature may be due to keto-enol tautomerism of (HNC=O) group. The inclusion product with β-cyclodextrin shows cyclodextrin CH protons signal at multiplete at δ=3.31-3.47 ppm. The phenyl ring (C-H) protons of isatin ring signals were observed as multiplete between δ=6.89 - 7.89 ppm[29]. A broad signal located at δ=5.12-5.73 ppm is assigned as hydroxyl (OH) proton of β-cyclodextrin. The NH proton signal of indole ring and semicarbazone group amide (CONH) in inclusion product is located at 11.18 and 12.48 ppm suggested that NMR signals isatinylsemicarbazone is affected in inclusion complex. The ^{13}C NMR signals of β-cyclodextrin carbons are observed as multiplete

between δ=38.22-40.57 ppm. The (Figure-^{13}C NMR_1) signals of IstscabH phenyl ring carbon signals are observed as multiplete between δ=112.57-142.37 ppm. The ketoamide (C=O) carbon signals were located at δ=155.52, 156.45 ppm and δ=163.17, 165.30 ppm in spectrum of free IstscabH ^{13}C NMR. These signals are raised to higher ppm values in inclusion complexes (Table 4). The proton NMR spectrum of isatinylthiosemicarbazone (Figure-^1H NMR_2) shows NH_2 proton signals at δ=3.32-4.84 ppm and 5.68-5.73 ppm indicating two different isomeric structure of IsttscabH. The benzene ring proton signals are located between δ=6.91-8.66 ppm as multiplete. The thioamide (N-H) proton signals are observed at δ=11.18 and 12.48 ppm. In ^{13}C NMR spectrum (Figure-^{13}C NMR_2) ^{13}C signals of β-cyclod carbons are observed as multiplete between δ= 39.31-40.56 ppm. The phenyl ring carbon ^{13}C NMR of IsttscabH were observed between δ=102.40-142.80 ppm and carbamide ^{13}C NMR signals at δ=163.09, 179.17 and 181.63 ppm. The proton and ^{13}C NMR spectrum of its inclusion complex shows relatively broad proton NMR signals. Theβ-cyclodextrin CH proton signals are affected due to its association with isatinylthiosemicarbazone on inclusion and observed at δ=2.51 and 2.56 ppm. The ν(OH) signal was observed at δ=3.68-3.72 ppm as board band. The ^{13}C NMR signal of β-cyclodextrin was observed between δ=39.30-68.54 ppm which is higher than free β-cyclodextrin signals. In inclusion complexes, the phenyl ring ^{13}C NMR signals were located between δ=110.36-143.37 ppm these are higher than free IsttscabH suggested the association of inclusion complexes. The ^1H NMR spectrum of bis(isatinyl)thiocarbohydrazone(BisttcabH) shows phenyl ring CH proton signals between δ=6.88-7.55 ppm[29]. The isatin ring NH and thioamide HNCS proton signals were observed at δ=11.02 and 13.34 ppm indicating their acidic nature. The ^{13}C NMR signals of methyl carbon in solvent DMSO were observed at δ=39.27-40.52 ppm. The phenyl ring carbon ^{13}C NMR signals were located between δ=112.57 and δ=149.57 ppm. The ^{13}C NMR signals located at 155.85, 159.82 and 183.85 ppm in spectrum were assigned to (C=S), (C=N) and (CO) carbon atoms respectively. In inclusion complexes these ^{13}C NMR signals of (C=S),(C=N) and (CO) carbon were observed at higher ppm values than free isatinyl derivatives (δ=158.25, 161.32 and 188.63 ppm).

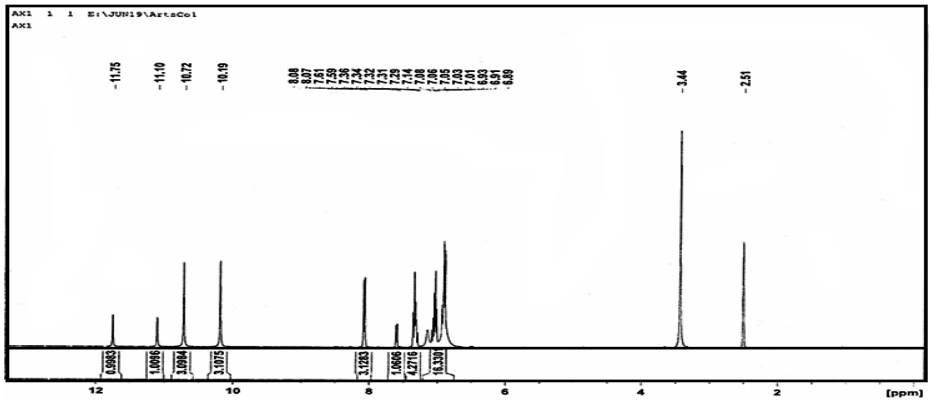


Fig.10. ^1H NMR1(IstscabH) ($C_9H_8N_4O_2$)

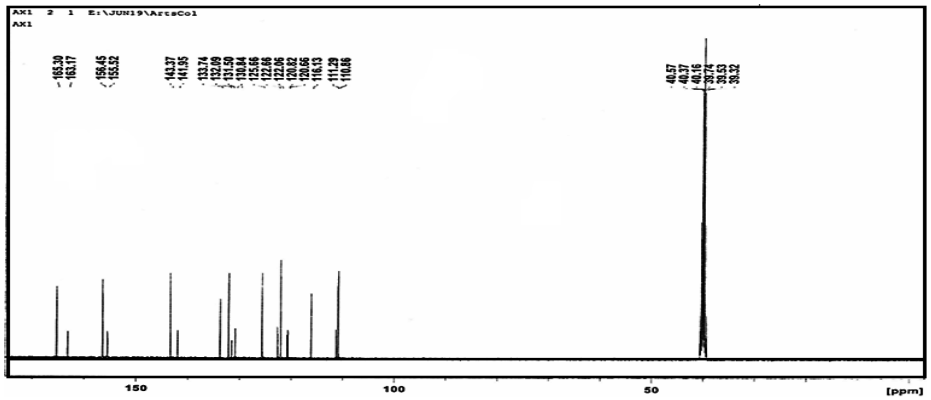


Fig.11. ^{13}C NMR_2(IstscabH) ($C_9H_8N_4O_2$)

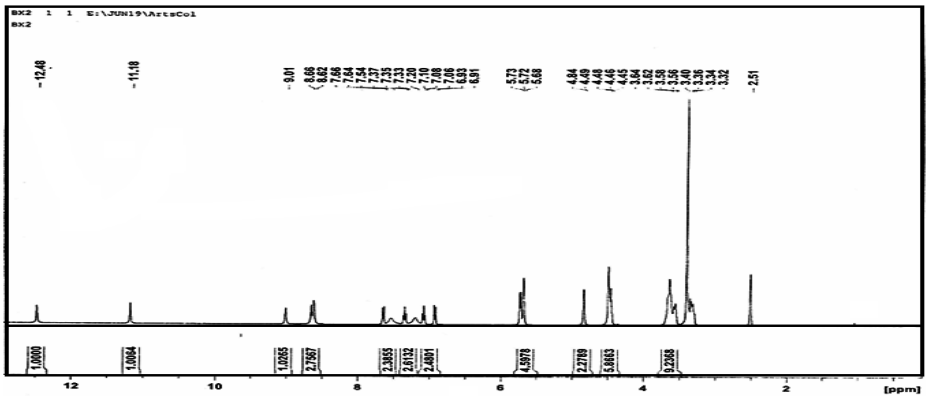


Fig.12. ^{1}H NMR_2 (IsttscabH) ($C_9H_8N_4OS$)

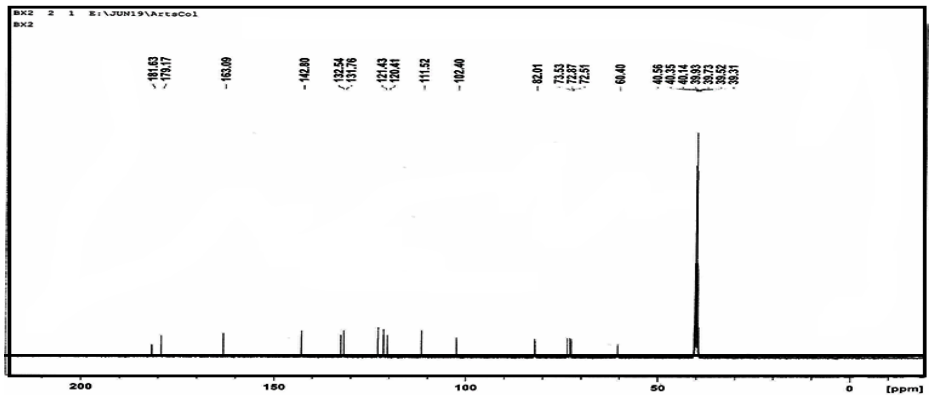


Fig. 13. ^{13}C NMR_2 (IsttscabH) ($C_9H_8N_4OS$)

Antibacterial activity

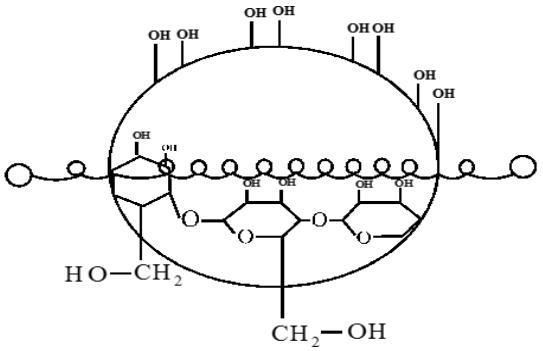
Antibacterial activity of isatin Schiff bases IstscabH, IsttscabH, BisttcabH and their inclusion products with β-cyclodextrin have been screened by growth inhibition technique using petri disc method for bacteria namely *Bacillus subtilis*, *P. mirabilis* and *Straphylococcus aureus* and the results of activities at concentration 100 and 200 ppm using Azithromycin and Ciprofloxacin as standard[34-35] and results are summerized in Table 5. The requisite amount of Schiff bases and inclusion products with β-cyclodextrin were dissolved in DMF to get 100 and 200 μgmL^{-1} (ppm) solution. About 0.5 mL (containing 107 microorganism per mL) of investigated mircroorganism was added to a sterile nutrient agar medium. Just before solidification and then poured on sterile perti discs and allowed to solidify using sterile Cork borer (6 mm in diameter) three holes are made in each disc and then 1 mL of tested inclusion product dissolved in DMF was poured in these holes. Finally the discs were in incubated at 35°C for 24 h and zone of inhibition of growth were measured in mm against each bacteria. A blank containing DMF was measured and the inhibition was practically negligible. It was found that inclusion products showed greater antibacterial activity than Schiff bases. The higher activity of inclusion products with β-cyclodextrin compared to Schiff bases of isatin may be due to decrease in polarizability of inclusion product which enhanced the lipophilicity of product than isatin Schiff bases leading to reduction of permeability of the cells resulting in interference with normal cell process. The results showed that in some cases the activities are comparable to Azithromycin and Ciprofloxacin.

The comparison of IR, UV, ^{1}H NMR and ^{13}C NMR spectral studies of Schiff bases and their inclusion complexes supported that these Schiff bases form stable 1:1 inclusion products and isolated in solid state.

Table 5: antibacterial screening of isatin schiff bases istscabh, isttscabh, bisttcabh and their inclusion products with β-cyclodextrin

Compound	Antibacterial Activity zone of inhibition (mm)					
	Staphylococcus		*P. mirabilis*		*Bacillus Subtilis*	
Concentration →	100 ppm	200 ppm	100 ppm	200 ppm	100 ppm	200 ppm
IstscabH	11	14	10	13	10	13
IsttcabH	9	15	11	14	11	14
BisttcabH	6	13	9	13	11	14
[(β-cyclod) (IstscabH)]	13	20	15	19	12	16
[(β-cyclod) (IsttscabH)]	13	18	14	19	13	18
[(β-cyclod) (BisttcabH)]	14	18	14	18	11	17
Ciprofloxacin	24	25	23	24	23	24
Azithromycin	22	23	23	24	22	23

One the basis of analytical results and spectral studies the following possible structure of expected inclusion complexes of β-cyclodextrin having 1:1 molar ratio with isatinyl () derivatives is shown below.



Where () is inclusion ligand (A). Isatinylsemicarbazone (istscabH), (B) Istalinylthiosemicarbazone (IsttscabH) and (C) Bisisatinyl-thiocarbohydrazone (BisttcabH). The structure is similar to supra molecule.

CONCLUSION

From results of the element analysis, determination of molecular weight, spectral studies and comparison of IR, UV and ^{1}H NMR as well as ^{13}C NMR, it is inferred that isatin derivatives IstscabH, IsttscabH and BisttcabH form 1:1 inclusion complexes with β-cyclodextrin and these products are stable and isolated in solid state. The antibacterial activity of inclusion products are enhanced than simple Schiff bases.

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Conflicts of Interest

The authors declare no conflict of interest.

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