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STUDIES ON INCLUSION COMPLEXES OF SOME 4-ARYLIDENAMINO-5-PHENYL-4H-1, 2, 4-TRIAZOLE-3-THIOLS

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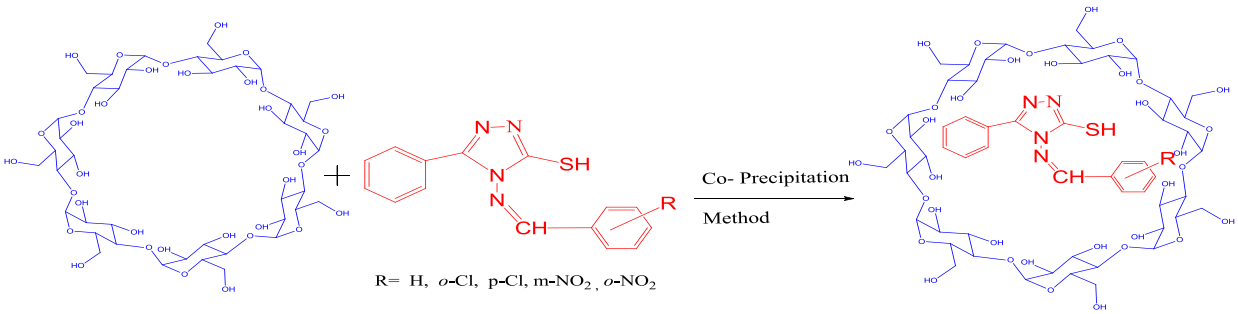
ABSTRACT

The inclusion complexes of some 4-arylidenamino-5-phenyl-4H-1, 2, 4-triazole-3-thiols have been prepared with β-cyclodextrin so as to increase their solubility and bioavailability. The inclusion complexes have been characterized by studying changes in their physical and spectral properties before and after inclusion complexes formation. The thermodynamic stability constant and free energy of activation have been determined to know the stability of inclusion complexes and type of host-guest relation. Finally, the compounds and their inclusion complexes are screened for antibacterial and anthelmintic activities. It is found that inclusion complexes of the newly synthesized compounds have appreciable stability in the cavity of β-cyclodextrin and inclusion complex formation increases their antibacterial and anthelmintic activities significantly as compared to naked compound.



KEYWORDS: Triazole-3-thiol, Inclusion complex. Antibacterial activity, Anthelmintic activity.

INTRODUCTION

The pharmacophore, 1, 2, 4-triazole nucleus has been incorporated into a variety of therapeutically important agents displaying a wide spectrum of pharmacological activities

like antimicrobial[1–5], anticancer[6, 7], antiviral[8], anti-inflammatory[9], analgesic[10], anticonvulsant[11] etc. A number of attempts have been made to improve the activity of these compounds by incorporating different substituents on the triazole nucleus. Triazoles with azomethine group (-CH=N-), are also reported to possess antimicrobial, antiviral and other biological activities[12-14]. But poor solubility of these compounds in polar medium may be a limiting factor for decreasing their absorption leading to inadequate and variable bioavailability. One of the ways to increase solubility of these compounds is to form inclusion complex with a suitable host. The oligosaccharide, β-cyclodextrin is a well-known host molecule that can form inclusion complex with a variety of drug molecules to improve their solubility, stability as well as bioavailability[15-19].

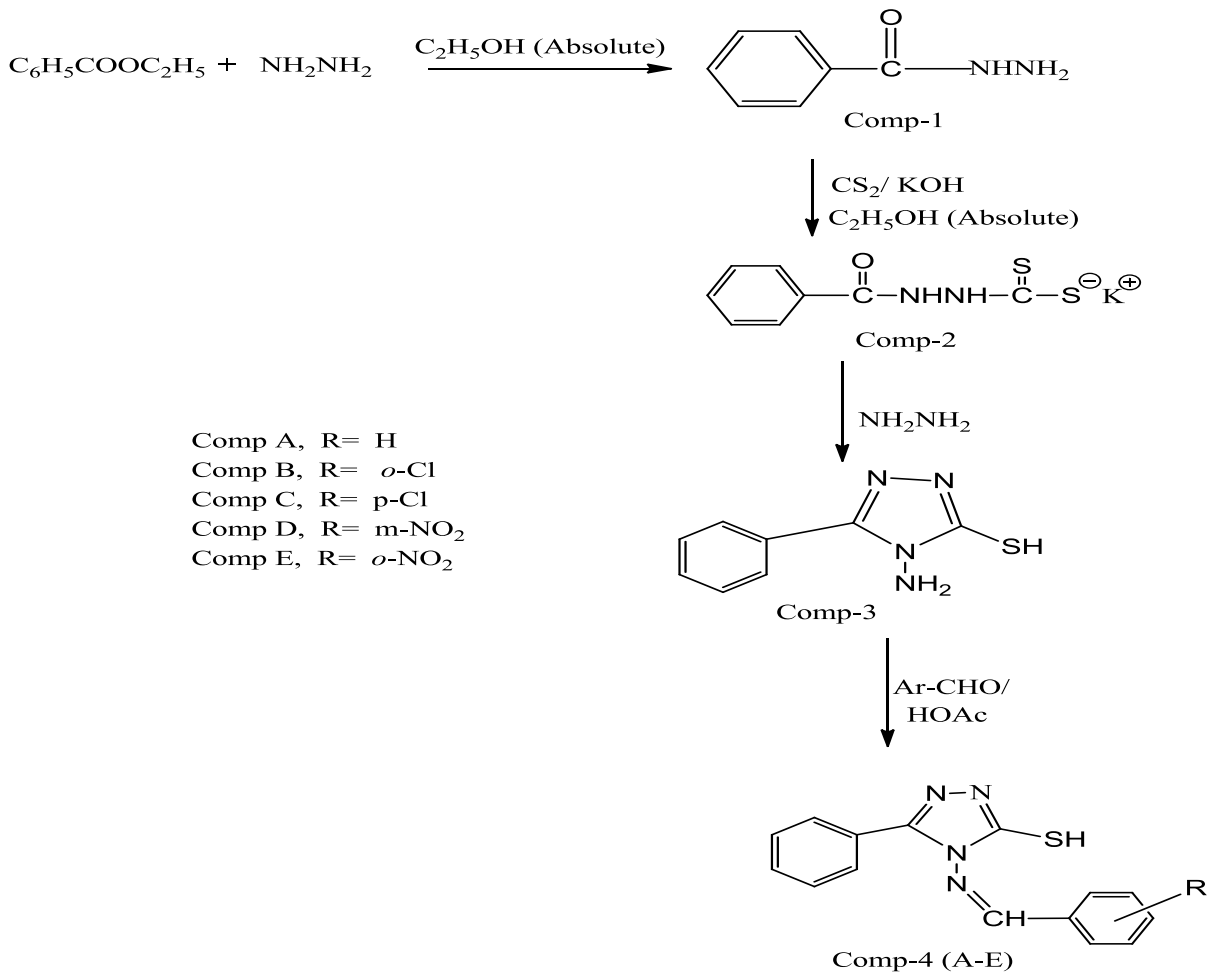
In view of these facts, an attempt has been made to synthesize some 4-arylidenamino -5-phenyl-4H-1, 2, 4-triazole-3-thiol (Schiff bases) in their purest forms and prepare their inclusion complexes with β-cyclodextrin. An attempt has also been made to examine whether inclusion complex formation is thermodynamically allowed and it has any impact on antibacterial and anthelmintic activity of newly synthesized compounds.

EXPERIMENTAL

Material and Methods

All the chemicals used in the present work were procured from local market. Double distilled water was used as solvent. The elemental analysis was performed in a CHN analyzer. Melting points were recorded by open capillary method. Electronic spectra were recorded in Shimadzu UV-1800 spectrophotometer and IR spectra were recorded in KBr pellets in Shimadzu 8400 FT-IR spectrophotometer. ^{1}HNMR spectra were obtained with Brukers spectrophotometer model ultra-shield at 300MHz in DMSO- d_6 solution with TMS as internal standard. Powder X-ray diffraction patterns were recorded using a X'pertPROPANlytical diffractometer. The purity of the newly synthesized compounds was checked by TLC. The synthesis of the titled compounds (4-arylidenamino-5-phenyl-4*H*-1, 2, 4-triazole-3-thiols) was carried out in the following steps as per as per Panda *et al* 2015[20, 21] shown in Scheme -1.

The synthesis of the titled compounds (4-arylidenamino-5-phenyl-4H-1, 2, 4-triazole-3-thiols) was carried out in the following steps as shown in Scheme -I.



Scheme I: Synthesis of the titled compounds

Compound-A: 4-Benzilidenamino -5-phenyl-4H-1, 2, 4-triazole-3-thiol

UV λ_{Max} (nm): 294

IR (KBr) cm^{-1}: 68.27 (N-C-S str), 3034.03, 3099.61, (Ar-H str), 1498.69 (C=C_{str}) 696.38 (C-Sstr), 1350.17 (C-N str).1610.56 (C=N_{str})

NMR (DMSO-d_6): 7.52 -7.90 (m, 10H, Ar-H), 14.25 (s, 1H, SH), 9.70 (s, 1H, N=CH).

Elemental Analysis: Found (Calculated) C; 64.46 (64.26), H; 4.02 (4.31), N; 19.92 (19.98

Inclusion complex of A (I.C.A)

UV λ_{Max} (nm): 270

IR (KBr)cm^{-1} : 937.4 (N-C-S str), 2931.80 (Ar-H str),1409.96 (C=C_{str}), 756.10 (C-S str), 1332.81 (C-N str).1656.85 (C=N_{str}), 3398.57 (OHstr, β-CD), 2931.80 (C-Hstr, β-CD)

NMR (DMSO-d6): 7.32-7.91 (m, 10H, Ar-H), 3.35 (s, 1H, β-CD), 3.43 (s,1H, β-CD), 3.45 (s,1H, β-CD), 3.57 (s,1H, β-CD), 3.59 (s, 1H, β-CD)

Compound-B: 4-[2-Chlorobenzilidenamino] -5-phenyl-4H-1, 2, 4-triazole-3-thiol

UV λ_{Max} (nm): 290

IR (KBr) cm^{-1} : 941.26 (N-C-S str), 3072.60, 3132.70 (Ar-H str), 1502.56 ($C=C_{str}$), 696.30 (C-S str), 1350.17(C-N str).769.60 (C-Clstr), 1610.56($C=N_{str}$)

NMR (DMSO-d_6): 7.49-7.88 (m, 9H, Ar-H), 14.30 (s, 1H, SH), 10.43 (s, 1H, N=CH)

Elemental Analysis: Found (Calculated) C; 57.07 (57.23), H; 3.42(3.52), N; 11.22 (11.26)

Inclusion complex of B (I.C.B)

UV λ_{Max} (nm): 278

IR (KBr) cm^{-1} : 937.40 (N-C-S str), 2931.80, (Ar-H str), 1408.04 ($C=C_{str}$), 1656.85 ($C=N_{str}$),756.10 (C-S str), 1332.81 (C-N str), 792.74 (C-Clstr), 3363.86 (OHstr, β-CD), 2931.80 (C-Hstr, β-CD)

NMR (DMSO-d6): 7.94-7.96 (m, 9H, Ar-H), 3.34 (s, 1H, β-CD), 3.37 (s, 1H, β-CD), 3.54(s, 1H, β-CD), 3.60 (s, 1H, β-CD), 3.62 (s, 1H, β-CD)

Compound-C: 4-[4-Chlorobenzilidenamino] -5-phenyl-4H-1, 2, 4-triazole-3-thiol

UV λ_{Max} (nm): 288

IR (KBr) cm^{-1} : 941.26 (N-C-S str), 3034.03, 3099.61 (Ar-H str), 1502.55 ($C=C_{str}$), 696.30 (C-S str), 1350.17 (C-N str), 769.60 (C-Clstr), 1610.56 ($C=N_{str}$)

NMR (DMSO-d_6): 7.52-7.92 (m, 9H, Ar-H), 14.243 (s, 1H, SH),9.7 65 (s, 1H, N=CH).

Elemental Analysis: Found (Calculated) C; 57.09 (57.23), H; 3.45 (3.52), N; 11.26 (11.26)

Inclusion complex of C (I.C.C)

UV λ_{Max} (nm): 270

IR (KBr)cm^{-1} : 937.40 (N-C-S str), 2931.80 (Ar-H str), 1409.96 ($C=C_{str}$), 705.95 (C-S str), 1348.24 (C-N str), 792.74 (C-Clstr), 1658.78 ($C=N_{str}$), 3367.71 (OHstr, β-CD), 2931.80 (C-Hstr, β-CD)

NMR (DMSO-d6): 7.95-7.98 (m, 9H, Ar-H), 3.58 (s, 1H, β-CD), 3.61 (s, 1H, β-CD), 3.63 (s, 1H, β-CD), 3.65 (s, 1H, β-CD), 3.66 (s, 1H, β-CD)

Compound-D: 4-[3-Nitrobenzilidenamino] -5-phenyl-4H-1, 2, 4-triazole-3-thiol

UV λ_{Max} (nm): 280

IR (KBr) cm^{-1} : 943.19 (N-C-S str), 3032.10, 3088.03 (Ar-H str), 1508.33 ($C=C_{str}$), 675.09 (C-S str), 1350.17 (C-N str), 1537.27, 1350.17 (NO_2), 1608.63 ($C=N_{str}$)

NMR (DMSO-d_6): 7.52-8.33 (m, 9H, Ar-H), 14.34 (s, 1H, SH), 10.023 (s, 1H, N=CH).

Elemental Analysis: Found (Calculated) C; 55.47 (55.37), H; 3.32 (3.41), N; 21.42 (21.53)

Inclusion complex of D (I.C.D)

UV λ_{Max} (nm): 266

IR (KBr)cm^{-1}: 937.40 (N-C-S str), 2933.73 (Ar-H str), 1660.71 ($C=C_{str}$), 705.95 (C-S str), 1361.74 (C-N str), 1409.96 (NO_2), 1660.71 ($C=N_{str}$), 3367.71 (OHstr, β-CD), 2933.73(C-Hstr, β-CD)

NMR (DMSO-d6): 7.94-8.23 (m, 9H, Ar-H), 3.37 (s, 1H, β-CD), 3.54 (s, 1H, β-CD), 3.61 (s, 1H, β-CD), 3.62 (s, 1H, β-CD), 3.65 (s, 1H, β-CD),

Compound-E: 4-[2-Nitrobenzilidenamino] -5-phenyl-4H-1,2,4-triazole-3-thiol

UV λ_{Max} (nm): 295

IR (KBr) cm^{-1} : 943.27 (N-C-S str), 3115.04 (Ar-H str), 696.30 (C-S str), 1498.69 ($C=C_{str}$), 1342.46 (C-N str), 1529.55, 1342.46 (NO_2), 1610($C=N_{str}$)

NMR (DMSO-d_6): 7.53 -8.34 (m, 9H, ArH), 14.304 (s, 1H, SH), 10.499 (s, 1H, N=CH).

Elemental Analysis: Found (Calculated) C; 55.27 (55.37), H; 3.32 (3.41), N; 21.50 (21.53)

Inclusion complex of E (I.C.E)

UV λ_{Max} (nm): 275

IR (KBr)cm^{-1}: 937.40 (N-C-S str), 2933.80 (Ar-H str), 1409.96 ($C=C_{str}$), 704.02 (C-S str), 1361.74 (C-N str), 1409.96 (NO_2), 1658.78 ($C=N_{str}$), 3367.71 (OHstr, β-CD), 2931.80 (C-Hstr, β-CD)

NMR (DMSO-d6): 7.92-8.13 (m, 9H, Ar-H), 3.37 (s, 1H, β-CD), 3.61 (s, 1H, β-CD), 3.63 (s, 1H, β-CD), 3.64 (s,1H, β-CD), 3.66 (s, 1H, β-CD)

Phase Solubility Measurements

The aqueous phase solubility of the compounds at various concentrations of β -cyclodextrin (0-10mM) has been studied by Higuchi-Conner method[22].

Synthesis of inclusion complexes

Co-precipitation method was used for the preparation of inclusion complexes of the compounds with β-cyclodextrin[18, 23].

Study of thermodynamic properties

The thermodynamic stability constants of the complexes were calculated from plot of inverse of change in absorbance versus inverse concentration of β-cyclodextrin using Benesi-Hilderband relation [24].

$1/\Delta A = 1/\Delta\varepsilon + 1/K'. \Delta\varepsilon\,[Guest]_0.[\beta - CD]$

$K'. [Guest]_0 = K$

Where ΔA is change in absorbance, Δε is change in absorption coefficient, K is stability constant, $[Guest]_o$ is the concentration of compound and [β-CD] is the concentration of β-cyclodextrin. The values of K for all the complexes are calculated using the relation

K =Intercept/Slope

The value of ΔG at 298 K was calculated using theequation:

$\Delta G = -RT \ln K$

Evaluation of Anthelmintic activity

The anthelmintic activity was performed on *Pheretima posthuma* (Indian earthworm) as per Panda and Singh (2013)[18] as it has anatomical and physiological resemblance with the intestinal parasites of human beings[25, 26].

Evaluation of Antibacterial activity

The antibacterial activities of compounds were studied as per cup-plate method [27, 28] using bacterial strains of *Escherichia coli* (MTCC 40), *Bacillus subtilis* (MTCC 441), *Staphylococcus aurous*(MTCC 87) and *Proteus vulgaris* (MTCC 426). The results were reported by comparing the zone of inhibition of the test compounds with standard drug (Tetracycline). The results were the mean value of zone of inhibition of three sets measured in millimeter.

RESULTS AND DISCUSSION

Five different 4-arylidenamino -5-phenyl-4H-1, 2, 4-triazole-3-thiols (A, B, C, D and E) have been synthesized as shown in scheme-1 in their purest forms. The inclusion complexes of A, B, C, D and E have been prepared with β-cyclodextrin after determining the optimum concentration of host and guest through aqueous phase solubility study (Figure 1). The structures of the compounds (A, B, C, D and E) and their inclusion complexes have been confirmed from the study of their analytical and spectral characteristics. The elemental composition matches with theoretical data. The IR and ^{1}H NMR data of the compounds confirm the expected structures. The synthesis of inclusion complex of the compounds has

been confirmed from the changes in melting point, colour (Table 1) and spectral characteristics shown above. The higher melting point of the inclusion complexes than their compounds may be attributed to the fact that extra amount of thermal energy is required for the latter to bring it out of β-cyclodextrin cavity[18, 21]. The absorption maxima are shown to undergo a distinct blue shift after their inclusion complex formation. The IR- stretching frequencies due to different bonds undergo downward shift towards lower energy and the peaks become broader, weaker and smoother which may be attributed to the restriction on the compounds for undergoing vibration due to the development of weak interaction like H-bonding, vander-Waal forces and hydrophobic interactions within the cavity. This observation clearly demonstrates transference of the compound from a more protic environment (aqueous media) to a less protic environment (cavity of β-CD). The compound and β-CD interaction leading to inclusion complex formation is further supported by NMR data. It is seen that the NMR signals due to different protons undergo smaller shifts (small shift towards up field in case of compound-A and small shift towards down field in case of other compounds) after their inclusion complex formations which may be due to changes in the microenvironment of the compound after encapsulation. The formation of inclusion complex can be further supported by X-ray diffractometry[29]. The powder X-ray diffractometric pictures of compounds and their inclusion complexes are shown in Figures 3-7. The complexes show all characteristics peaks corresponding to the compounds, but with lower intensity which may be due to reduction in crystallinity. The difference in the XRD spectrum is due to the encapsulation of compound with in β –cyclodextrin cavity resulting in a new crystal structure i.e. inclusion complex.

Table-1: Some physical properties of the synthesized compounds and complexes

Sl No.	Compound/ Complex	Molecular formula	Molecular weight	Colour	M.P. (^{0}C)	Yield (%)
1	Compound- A	$C_{15}H_{12}N_4S$	280.35	Light brown	180-185	73
	I.C.A			white	272-274	75
2	Compound- B	$C_{15}H_{11}ClN_4S$	314.79	yellow	148-150	77
	I.C.B			white	280	77
3	Compound- C	$C_{15}H_{11}ClN_4S$	314.79	Light yellow	165-170	63
	I.C.C			white	273-275	76
4	Compound- D	$C_{15}H_{11}N_5O_2S$	325.35	Yellowish white	185-190	70
	I.C.D			Dull white	282-284	75
5	Compound- E	$C_{15}H_{11}N_5O_2S$	325.35	Pale yellow	170-172	72
	I.C.E			Dull white	282-285	74

Table-2: Thermodynamic stability constant and free energy change of inclusion complexes

Sl.No.	Inclusion complex	Equilibrium Constant (K) in M^{-1}	ΔG (kJ/mol)
1	I.C.A	773.76	-16.478
2	I.C.B	839.04	-16.679
3	I.C.C	802.21	-16.568
4	I.C.D	894.17	-16.837
5	I.C.E	685.37	-16.178

The aqueous phase-solubility diagrams of the compounds with β- cyclodextrin are shown in Figure1. It is seen that aqueous solubility of the compounds increase linearly as a function of the concentration of β- cyclodextrin up to 5th point followed by a decline. This clearly indicates that the concentration at 5th point is the optimum concentration for inclusion complex formation. The plot of inverse absorbance against inverse concentration of β-cyclodextrin gives nearly straight lines with definite slope and intercept for different compounds (Figure 2). The equilibrium constants (K) have been calculated from the slope and intercept[24] and are found to be in the range of 685.37to 894.17 (Table 2). Since all the values are remaining within ideal range[30] complexes formed are quite stable. Further, it is found that the values of all the slopes are less than one indicating the inclusion complexes to have 1:1 stoichiometry[17-21]. Negative values of free energy changes for all the inclusion complexes (Table 2) further suggest that the process of inclusion complex formation is spontaneous and thermodynamically allowed.

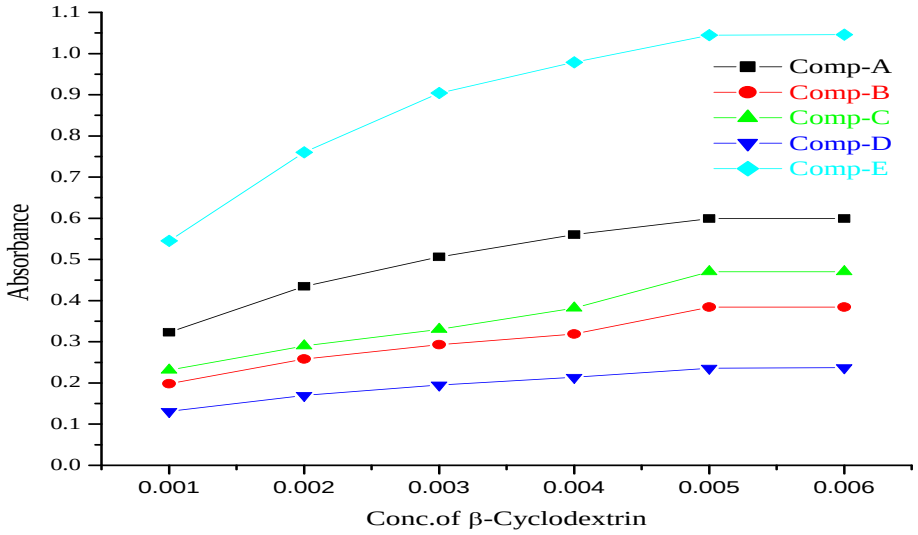


Figure 1: Phase solubility study of the synthesized compounds

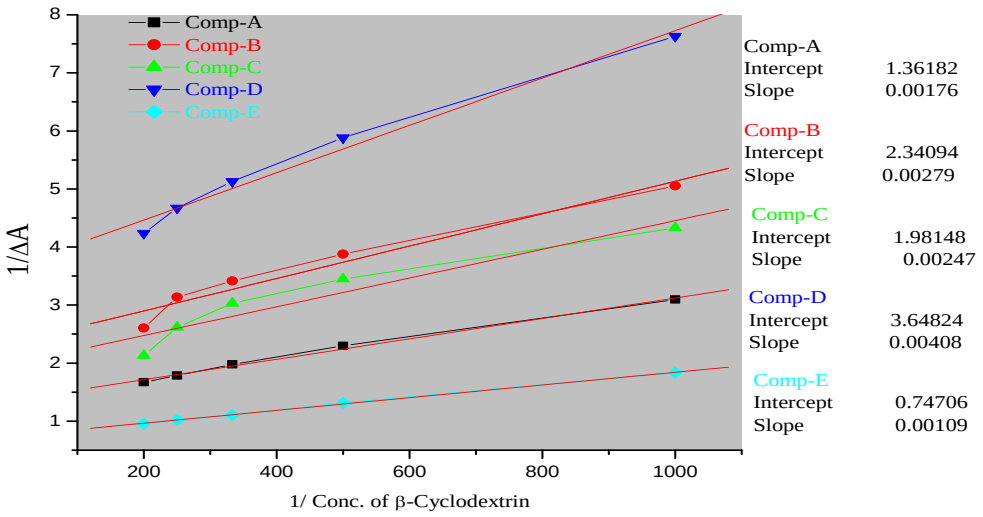


Figure 2: Plot of inverse absorbance against inverse concentration of β- cyclodextrin

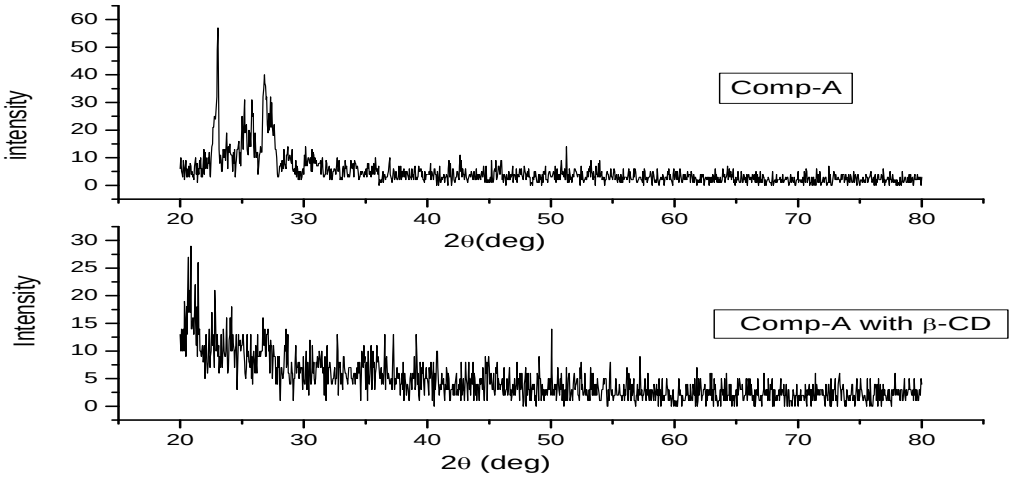


Figure 3: XRD study of the compound A and its inclusion complex with β- cyclodextrin

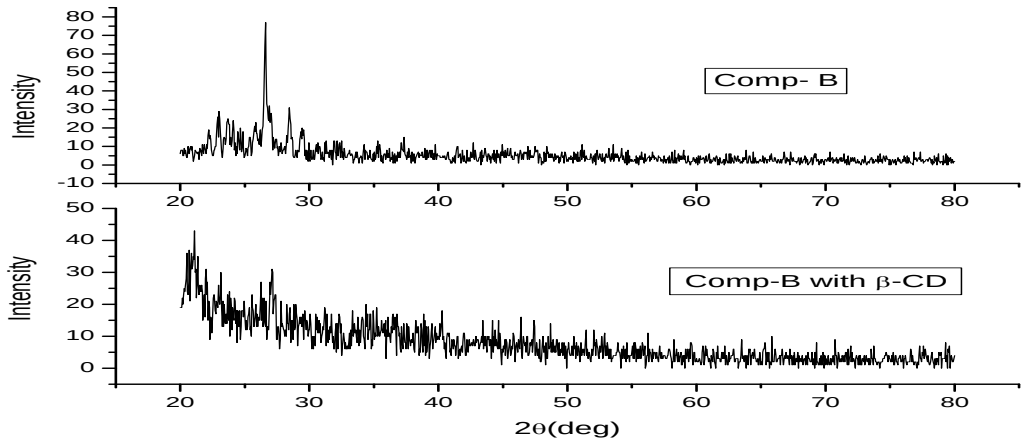


Figure 4: XRD study of the compound-B and its inclusion complex with β- cyclodextrin

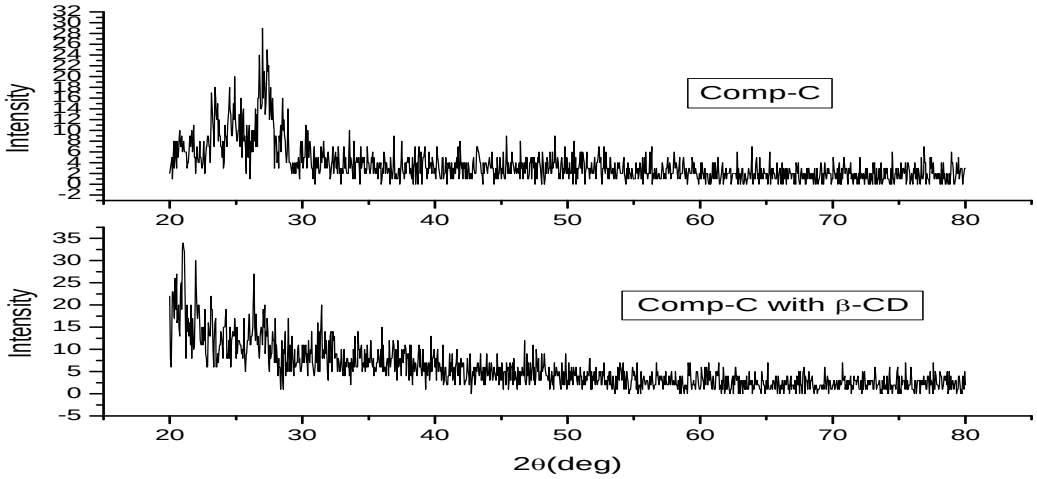


Figure 5: XRD study of the compound-C and its inclusion complex with β- cyclodextrin

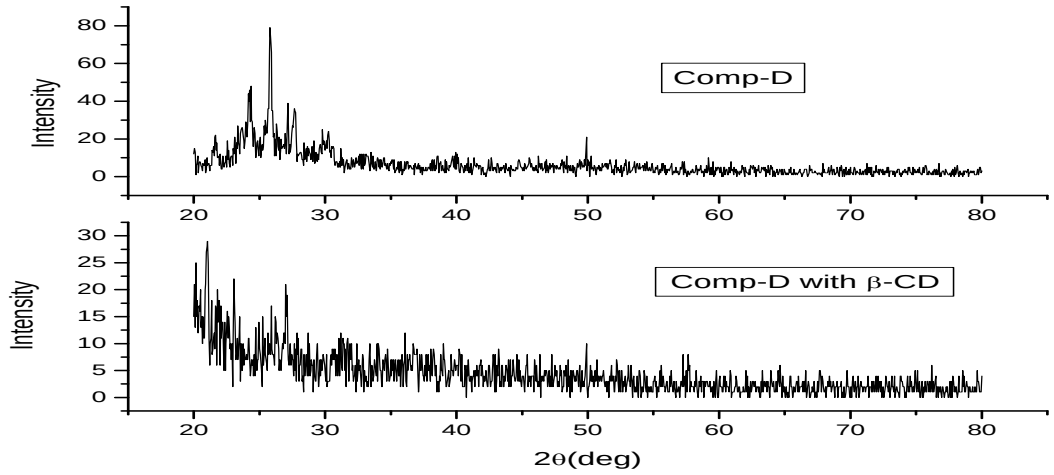


Figure 6: XRD study of the compound-D and its inclusion complex with β- cyclodextrin

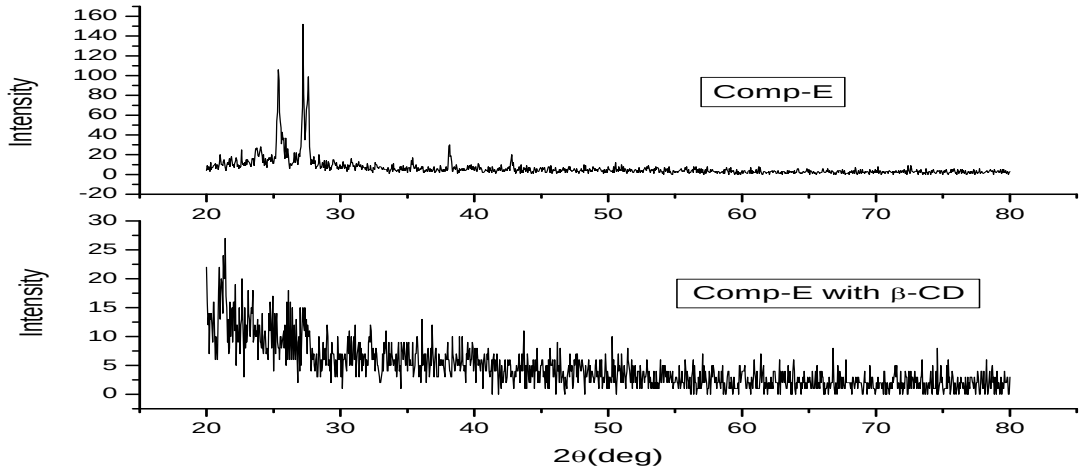


Figure 7: XRD study of the compound-E and its inclusion complex with β- cyclodextrin

The synthesized compounds and their inclusion complexes have been evaluated for their anthelmintic activity by using *Pheretima posthuma* (Indian earthworm) at the concentration of 0.5% w/v (Figure 8). It is seen that both the compounds and their inclusion complexes are capable of causing the paralysis and death of earth worms. However, the inclusion complexes are more efficient in causing the paralysis and death of earth worms as compared to their corresponding compounds. Out all the inclusion complexes, the inclusion complex of compound-D is more efficient in enhancing the anthelmintic activity of the compound.

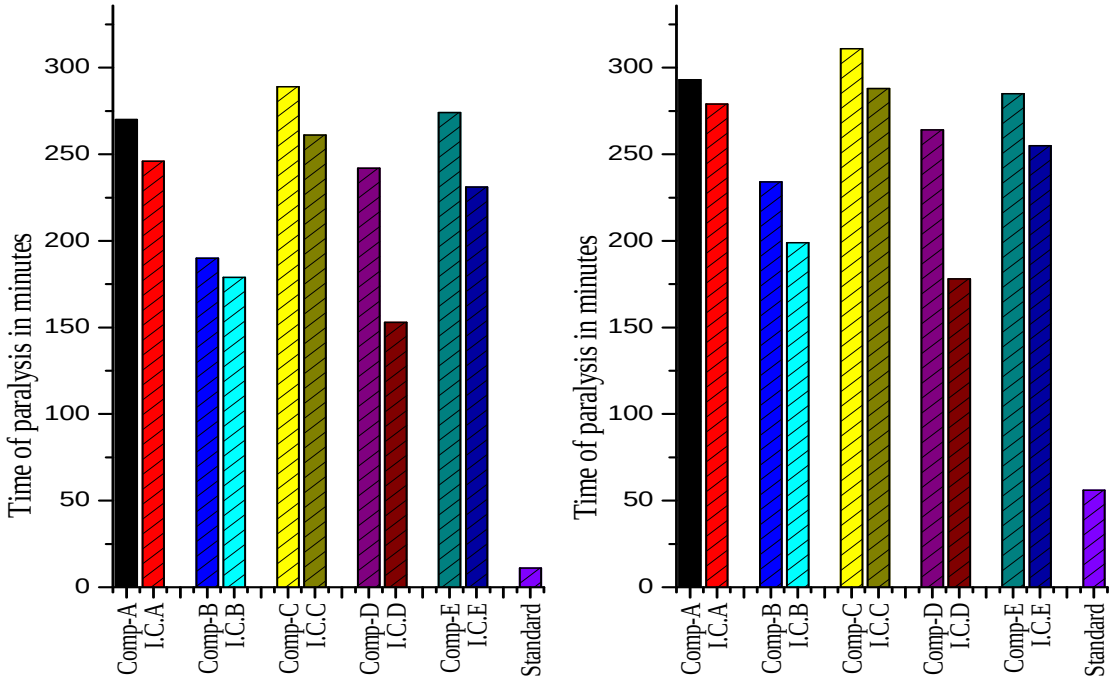


Figure 8: Paralysis and Death study of earthworm with synthesized compounds and their complexes

All the synthesized compounds and their inclusion complexes are screened for their antibacterial activity at a concentration of 500µg/ml using DMSO as a control against *E. coli*, *S. aureus*, B.subtilis and *P. Vulgaris* by cup plate method on nutrient agar media. Tetracycline (20 µg/ml) has been used as standard against all the test organisms. It is found that the inclusion complexes show better zone of inhibition than those of their respective compounds (Figure 9). In case of *E. coli* and *B.subtilis*, the inclusion complex of compound-E is showing highest percentage of enhancement in activity (Table 3). But in case of *S. aureus* and *P. Vulgaris*, the inclusion complex of compound-B is having highest percentage of enhancement in activity. However, the inclusion complexes of other compounds are found

to have moderate to mild activity against the test bacterial strains at the given test concentration. The higher antibacterial and anthelmintic activity of the compounds after inclusion complex formation may be attributed to enhanced solubility of the compounds in the systemic circulation which make them more available to specific tissues.

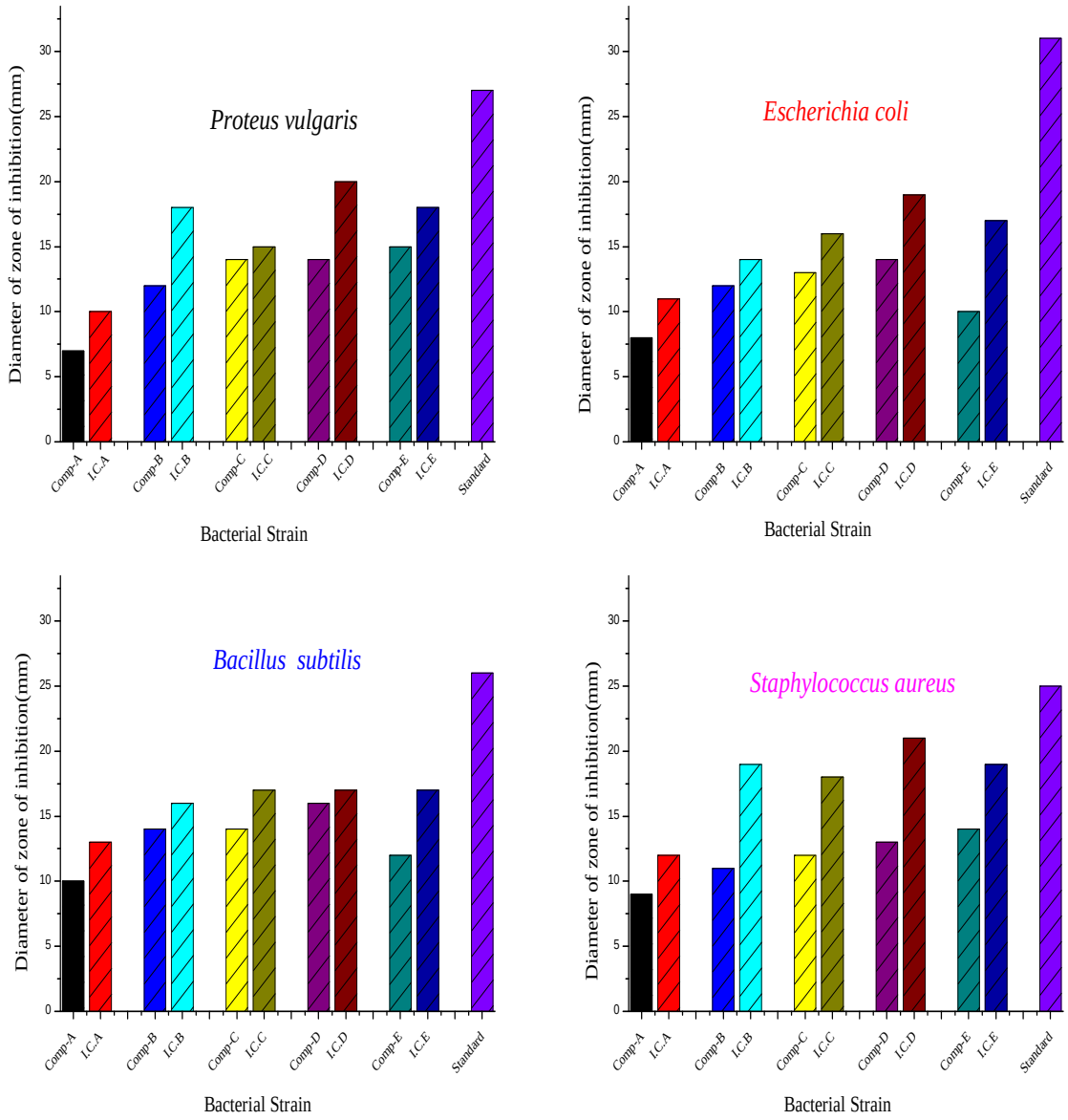


Figure 9: ***In vitro*** **Antibacterial activity of the synthesized compounds and their complexes**

Table-3: Percentage increase in antibacterial activity of the synthesized compounds and their complexes

Sl.No.	Compounds/Complexes	% increase in antibacterial activity			
		E. coli	*B. subtilis*	*S. aureus*	*P.vulgaris*
1	Comp -A	37.5	30	33.33	42.85
	I.C.A				
2	Comp- B	16.66	14.28	72.72	50
	I.C.B				
3	Comp- C	23.07	21.42	50	7.14
	I.C.C				
4	Comp- D	35.71	6.25	61.53	42.86
	I.C.D				
5	Comp-E	70	41.66	35.71	20
	I.C.E				

CONCLUSION

From the results obtained above, it is seen that 4-arylidenamino -5-phenyl-4H-1, 2, 4-triazole-3-thiols show significant antibacterial and anthelmintic activity which are further enhanced after their inclusion complex formation.

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