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Research Article

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Synthesis and antibacterial study of inclusion complexes of 2-(benzothiazolyl-2')-azino-5-arylidene-4-thiazolidinone

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ABSTRACT

A series of 2-(Benzothiazolyl-2')azino-5- arylidene -4-thiazolidinones have been synthesized starting from 2-hydrazinobenzothizole .To enhance the solubility of these synthesized compounds the inclusion complexes were prepared with β-cyclodextrin. The synthesis of compounds and their inclusion complexes have been ascertained from the changes in spectral characteristics and their analytical data. The compounds and their inclusion complexes were screened for their possible antibacterial activity against Escherichia coli and Staphylococcus aureus, by using cup plate method. From the results of their antibacterial study it was observed that the inclusion complexes displayed better antibacterial activity as compared with their respective compounds.

Key words: β-cyclodextrin, *Escherichia*, antibacterial activity, inclusion complexes, cup plate method.

INTRODUCTION

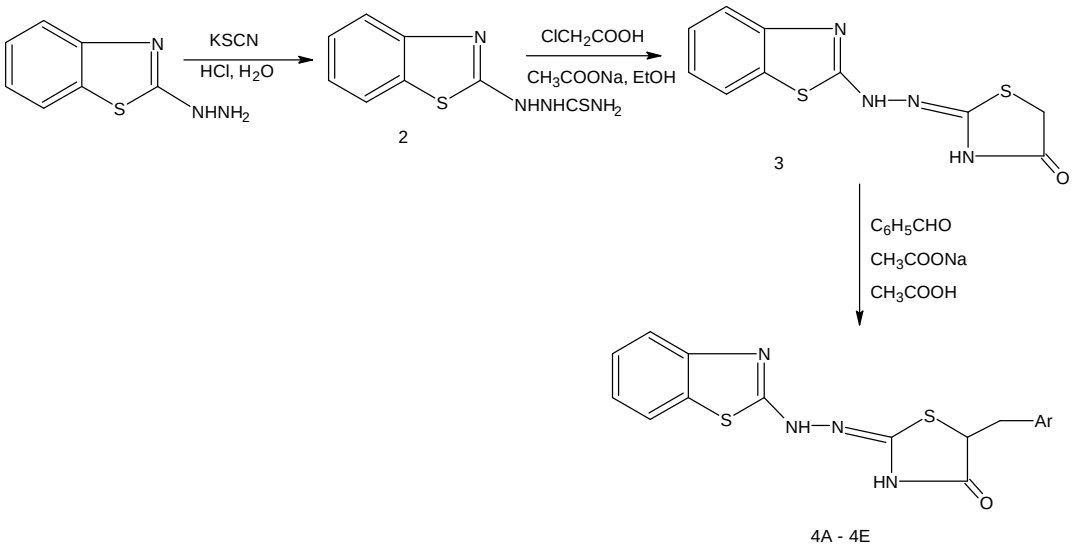
Many heterocyclic structures from synthetic chemistry are reported every year. Some are known frameworks while others are absolutely new. These newly products have been widely investigated on account of their varying biological activities. Some of them have attracted chemists into their total synthesis and the evaluation of their potential as chemotherapeutic agents. Even now, with the complete availability of advanced analytical and spectroscopic techniques, it is possible for structural revisions or assignments are made on the basis of synthetic undertakings. Among them, Thiazolidinone is one of the very important pharmacore. Thiazolidinones are the derivatives of thiazolidine which belong to an important group of heterocyclic compounds containing sulfur and nitrogen in a five member ring. A lot of research work on thiazolidinones has been done in the past. The nucleus is also known as wonder nucleus because it gives out different derivatives with different types of biological activities. Several substituted thiazolidinones have been found to be possessed activities such as antitubercular [1], anti bacterial [2], anti-HIV [3], antiinflammatory [4], antimycobacterial [5], anticonvulsant [6], anti histaminic [7], anticancer [8], antiprotocol [9] and analgesic [10]. Cardiovascular activity is also found to be exhibited by some 4-thiazolidinone derivatives. A novel thiazolidinone herbicide is found to be potent inhibitors of glucose incorporation into cell wall material. In the present work, a series of 2-(Benzothiazolyl-2')azino-5- arylidene -4-thiazolidinones have been synthesized starting from 2-hydrazinobenzothizoles .But Poor solubility of these compounds in the polar medium perhaps a limiting factor reducing bio-accessibility of these compounds and hence lowering their drug efficiency. One of the promising approaches is to encapsulate the drug in the hydrophobic cavity of cyclodextrin[11] and with it to try to increase the bioavailability which may produce better biological activity. Cyclodextrin sare one of the most widely used synthetic model host cavities, which provide a conical cavity for the water insoluble guests to be encapsulated, thereby making them water soluble [12]. Out of all the known cyclodextrins, β-cyclodextrin is usually considered for inclusion complex formation because it is, least toxic and cheaper and easily available in the

market[13]. So, in this present work an attempt has been made to synthesize a number of 2-(benzothiazolyl-2')azino-5- arylidene -4-thiazolidinone derivatives and to prepare their inclusion complexes with β-cyclodextrin. The formation of the compound and their inclusion complexes have been established by the study of their analytical and spectral data. An effort has also been carried out to determine whether inclusion complex formation enhances antibacterial activity of the drug or not.

EXPERIMENTAL SECTION

Apparatus and Materials:
All the chemicals used during the present work are procured from the local market. Double distilled water is used as the solvent for dilution was prepared in the laboratory. The electronic spectra were recorded on Shimadzu UV-1700 Spectrophotometer while IR-spectra were recorded in KBr pellets in 400-4000 cm^{-1} region on a Shimadzu 8400 FTIR Spectrophotometer. Melting points of the synthesized compounds were recorded in open capillary method and are uncorrected. The synthesized compounds and their inclusion complexes were screened for their antibacterial activity against by cup plate method

Synthesis of compounds
The compounds were synthesized as per the method describe by Garnaik et.al [14] (Scheme-I).



Scheme-I

1-(Benzothiazolyl-2')thiosemicarbazide(2):
A mixture of 2-hydrazinobenzothiazole (1.65gm, 10 mmole), potassium thiocyanate (2gm, 20 mmole), conc. HCl (2ml) and water (10ml) were heated slowly under reflux for about three hour. The reaction mixture was cooled, yellow solid separated was filtered and recrystallised from ethanol furnishing white needle. M.P. 141^{o}C, yield-0.7gm (40%), (Found S, 30.20 $C_7H_8N_4S_2$ requires S, 30.10%)

2-(Benzothiazolyl-2')azino-4-thiazolidinone (3):
A mixture of 1-(benzothiazolyl-2')thiosemicarbazide (2.02 gm, 10mmole), monochloroacetic acid (0.9 gm, 10 mmole) and anhydrous sodium acetate (0.2 gm) in absolute ethanol (15 ml) were refluxed for three hour. The excess of solvent was removed and poured into cold water. The solid obtained was filtered, washed with hot water, dried and recrystalised from ethanol, M.P. 158-160^{o}C, yield-1.3 gm (69%), (Found: S, 24.21 $C_{10}H_8N_4OS_2$ requires S, 24.24%).

2-(Benzothiazolyl-2')azino-5-benzylidene-4-thiazolidinone: (4A)
A mixture of 2-(benzothiazolyl-2')azino-4-thiazolidinone (2.18 gm, 10 mmole) benzaldehyde (.9 gm, 10 mmole), fused sodium acetate (1gm) in glacial acetic acid (15ml) was heated under reflux for four hour. The pale yellow solution after cooling to room temperature was poured into ice cold water when yellow solids separated out. The

crude product was filtered, washed with water and recrystalised from ethanol, m.p. 202^{o}C ,yield-2.1 gm (59%). (Found: S, 18.15 $C_{17}H_{12}N_4OS_2$ requires S, 18.18%). Similarly the other compounds of the series (4B-4E) were prepared by using o-chloro benzaldehyde, p-chloro benzaldehyde, p-nitro benzaldehyde and o-hydroxy benzaldehyde respectively.

Synthesis of Inclusion complexes :

The inclusion complexes of the synthesized compounds (4A, 4B, 4C, 4D & 4E) with β-cyclodextrin were prepared as per co-precipitation method[15].The solution of the compounds are prepared in required concentration (0.03mM) and added drop wise to a previously stirred β-cyclodextrin solution. The mixtures are stirred at room temperature for 48 hour and filtered. Then the content is cooled for another 48 hour in a refrigerator. Finally, the precipitate is filtered through G-4 crucible, washed with distilled water and dried in air for 24 hrs.

Aqueous phase solubility study

The aqueous phase solubility of all the compounds have been studied as per Higuchi Connors method [16]. An accurately weight of the compounds were shaken in a rotary flask shaker at room temperature in a series of conical flask for a period of 48 hours till the attainment of equilibrium. The solutions were filtered through Whatmann-42 filter paper and were analyzed on UV-visible spectrophotometer in the range of 200-400nm. The various values of absorbance at λ-max were plotted against different concentrations of β-cyclodextrin. From the phase solubility plots, the thermodynamic stability constant (K_T) of the inclusion complexes are determined using Benesi Hilderbrand relation:

$$\frac{1}{\Delta A} = \frac{1}{\Delta \epsilon} + \frac{1}{K\,[Guest]_o \Delta \epsilon} X \frac{1}{[\beta\text{-}CD]_o}$$

Where ΔA is change in absorbance, Δϵ is change in molar extension coefficient, $[Guest]_o$ is concentration of compound in inclusion complex and $[\beta\text{-}CD]_o$ is molar concentration of β-CD.

Evaluation of Antibacterial activity

The antibacterial activity of compounds was studied as per cup-plate method. [17,18] The solutions of the test compounds were prepared in dimethyl sulphoxide (DMSO) at 500µg/ml. The bacterial strains of *Escherichia coli* (MTCC 40) and *Staphylococcus aureus* (MTCC 87) were inoculated into 100ml of the sterile nutrient broth and incubated at 37±1°C for 24 hours. The density of the bacterial suspension was standardized by McFarland method. Well of uniform diameter (6mm) were made on agar plates, after inoculating them separately with the test organisms aseptically. The drug (500µg/ml) and the test compounds (500µg/ml) were introduced with the help of micropipette and the plates were placed in the refrigerator at 8- 10°C for proper diffusion of drug into the media. After two hour of cold incubation, the Petri plates were transferred to incubator and maintained at 37±2°C for 18-24 hours. Then the Petri plates were observed for zone of inhibition by using venire scale. The results were reported by comparing the zone of inhibition shown by the test compounds with standard drug (Tetracycline). The results were the mean value of zone of inhibition of three sets measured in millimetre and the data were presented in table-II.

RESULTS AND DISCUSSION

Thiazolidinone derivatives have low solubility in polar solvent which may be a limiting factor in reducing their pharmacological activities. The solubility and therapeutic activity of these compounds can be enhanced significantly by forming inclusion complexes with cyclodextrins . The analytical and spectral data of the synthesized compounds and their inclusion complexes are included in Table-I. The formations of the compounds are ascertained from the study of the spectral characteristics and elemental sulphur composition. The IR data and sulphur composition nearly match with the expected values.

The formation of inclusion complexes of the compounds with β-cyclodextrin is confirmed from the changes in melting points of the inclusion complexes with their respective compounds. The melting point of the compounds 4A,4B,4C,4D and 4E are202^{o}C,161^{o}C,135 ^{o}C,145^{o}C and 165^{o}C respectively, whereas the melting point of their corresponding inclusion complexes are208^{o}C,168^{o}C,144^{o}C,156^{o}C and 179^{o}C.respectively. It is due to the fact that extra amount of thermal energy is required to bring the molecules out of the cavity of the β-cyclodextrin.

The formation of colour of the synthesized compounds 4A,4B,4C,4D and 4E are brownish red, deep yellow, dull brown, dull white and light yellow. Similarly the colour of their corresponding inclusion complex are light yellowish, brownish yellow, pale yellow, white and yellow respectively.

In case of IR data of 4A it is seen that the IR frequencies are found to be formed at 667,1282,1595,1728 ,2960 and 3111 cm^{-1} indicating the presence of C-S,C-C,C-N,C=O,C-H, and N-H in the compound as excepected .Similarly the IR data of inclusion complexes of 4A show charectestics absorption at 750,1246,1597,1730,2962 and 3315 cm^{-1} indicating the presence of C-S,C-C,C-N,C=O,C-H and N-H in the compounds. Similarly the IR data of complexes 4B, 4C, 4D and 4E and their inclusion complexes is found to be absorbed at the suitable characteristic frequency. In case of IR data for all compounds, it is seen that the IR frequencies (C=O) undergo downward shift and the peaks become broader, weaker and smoother. But in case of NH stretching vibration, the frequency undergoes a shift towards higher wave number after inclusion complex formation. All these changes noticeably demonstrate transference of compounds into the cavity of β-cyclodextrin and development of weak interaction like H-bonding, Vannder Waals forces, hydrophobic interactions in between the host and guest molecules[19,20].

From the aqueous phase solubility studies, it was found that the aqueous phase solubility plots of the compounds within β-cyclodextrin solution exhibited a linear increase in solubility of these compounds with increasing concentration of β-cyclodextrin. Since the slopes of all the plots were less than unity, the stoichiometry of these complexes may be 1:1. The thermodynamic stability constants (K_T) of inclusion complexes were determined by using Benesi-Hilderband relation. [19] Good linear correlations were obtained for a plot of $1/\Delta A$ verses $[\beta\text{- CD}]_o$ for compounds . The values of K_T for all the complexes were calculated using the relation. K_T = Intercept/Slope .The K_T values of the inclusion complexes of compounds with β- Cyclodextrin were found to be 313,295,276,322 and 215 M-1 respectively (Table 1). The data obtained were within 100 to 1000 M^{-1}(ideal values) indicating appreciable stabilities for the inclusion complexes through host-guest interaction like vannder Waal's force, hydrophobic interaction etc. [20,21] The thermodynamic parameters associated with the interaction of the compound with β-cyclodextrin for 1:1 stoichiometry were calculated by determining stability constant (K_T values) at different temperatures. The K_T values were found to decrease with rise in temperature.

The data obtained from the antibacterial studies conforms that the diameter of the zone of inhibition obtained (Table-II, Fig-1) of the compounds and their corresponding inclusion complexes against two bacterial strains *E. coli* and *S. aureus* visibly recommend that inclusion complex formation increases the antibacterial activities significantly. Among the tested substances the inclusion complex of compound 4D exhibited maximum activity than that of other complexes. This increase of antibacterial activity of the inclusion complexes may be due to the improvement of solubility of the compounds which makes the compounds more bioaccessible to specific tissues leading to increased drug activity.

Table-I: Physical and analytical data of the compounds and their inclusion complexes

Sl. No.	Compound/ complex	Ar.	Colour	M.P. in ^oC	% of yield	K	IR (KBr)
1.	Compound 4A	Phenyl	Brownish red	202	59	-	2960(NH) 1728 (C=O),1741
2	Inclusion complex of comp. 4A		Light yellowish	208	40	313	2963(NH) 1712(C=O),1735
3.	Compound 4B	O-ClPh	Deep yellow	161	65		3037 (NH) 1730(C=O),1724
4.	Inclusion complex of comp. 4B		Brownish yellow	168	41	295	3111 (NH) 1732(C=O),1734
5.	Compound 4C	P-ClPh	Dull brown	135	55		3112 (NH) 1732(C=O),1744
6.	Inclusion complex of comp. 4C		Pale yellow	144	45	276	3159(NH) 1721(C=O), 1736
7.	Compound 4D	P-NO_2Ph	Dull white	145	54		3202(NH) 1708 (C=O),1710
8.	Inclusion complex of comp. 4D		White	156	39	322	3210(NH) 1703 (C=O),1720
9.	Compound 4E	P-OHPh	Light yellow	165	58		3215(NH) 1713(C=O),1712
10	Inclusion complex of comp. 4E		Yellow	179	43	215	3225(NH) 1709 (C=O),1718

Table-II: Antibacterial studies of the compounds and their inclusion complexes

Sl.No.	Compounds/complexes	Diameter of zone of inhibition (mm)	
		E. coli	*S.aureus*
1.	Compound 4A	12	11
2.	Inclusion complex of comp. 4A	14	13
3.	Compound 4B	16	15
4.	Inclusion complex of comp. 4B	18	17
5.	Compound 4C	16	15
6.	Inclusion complex of comp. 4C	21	19
7	Compound 4D	16	14
8	Inclusion complex of comp. 4D	24	22
9	Compound 4E	14	11
10	Inclusion complex of comp. 4E	16	14
11	Control	-	-
12	Standard	28	27

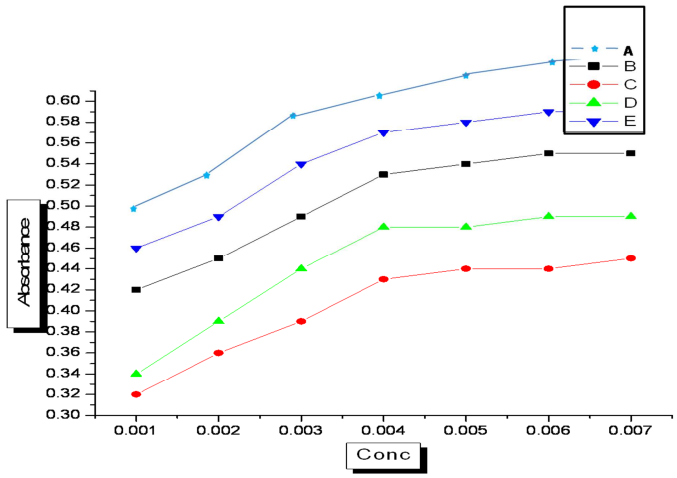


Fig-1: Variation of absorption with concentration

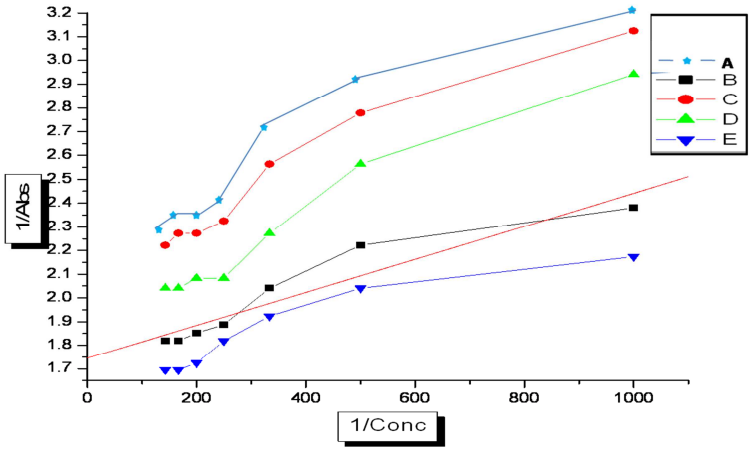


Fig-2: Variation of 1/ absorption with 1/concentration

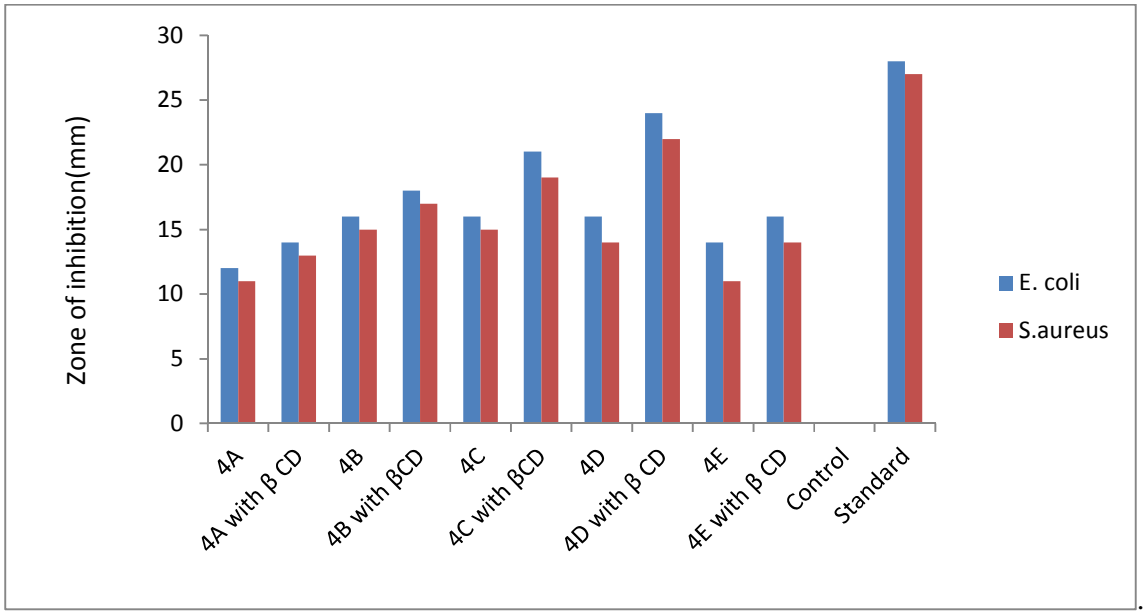


Fig-3: Antibacterial activity of the test substances

CONCLUSION

From the above study, it is observed that the solubility of the synthesized compounds was increased significantly by the formation of inclusion complexes with β-cyclodextrin which can be used as an important technique to increase therapeutic potential of the synthesized drugs. Thiozolidinones and their derivatives show antibacterial activity, which can further be enhanced by forming their inclusion complexes.

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