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A comparative study of inclusion complexes of substituted indole derivatives with β-cyclodextrin

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Some [arylidenamino]-1,3,4-thiadiazino[6,5-b] indoles were synthesized starting from indole-2,3-dione, thiosemicarbazide and aromatic aldehydes with activating and deactivating groups. Inclusion complexes of these compounds were prepared with β-cyclodextrin to increase their solubility and bioaccessibility. Thermodynamic properties like change in free energy, change in enthalpy, change in entropy and stability constant of the inclusion complexes were determined in order to elucidate whether the inclusion complex formation is thermodynamically allowed or not. The compounds and their inclusion complexes were screened against *S. aureus* and *E. coli.* It was found that the antibacterial activities of the compounds significantly increase after inclusion complex formation.

Keywords: Substituted indole, β-cyclodextrin, inclusion complex, antimicrobial activity

INTRODUCTION

Indole and its derivatives are very good pharmacophores exhibiting a wide spectrum of pharmacological activities such as antidepressive, antiinflammatory, fungicidal, bactericidal and tuberculostatic activities [1–4]. Azedinones and thiazolidinones also show excellent antimicrobial activities [5–8]. There are reports that compounds containing indole or substituted indole coupled with azedinone or thiazolidinone units are acting as drugs for treating a number of diseases [7,8]. Since the bioaccessibility of drugs depends upon their solubility, the poor solubility of these compounds in polar medium (water) may be a limiting factor reducing pharmacological activities. The solubility and bioaccessibility of these compounds can be significantly enhanced by forming inclusion complexes with cyclodextrins, non-toxic oligosaccharides [9]. Out of all known cyclodextrins, β-cyclodextrin is usually considered for inclusion complex formation because it is cheap, easily available and highly stable towards heat and oxidation [10, 12].

In the present work an attempt was made to synthesize some 2-[arylidenamino]-1,3,4-thiadiazino[6,5–b]indoles in their purest forms starting from indole-2,3-dione and using aryl aldehydes with activating (p-bromobenzaldehyde) and deactivating (p-nitrobenzaldehyde) groups. The inclusion complexes of the compounds were prepared with β-cyclodextrin as to enhance their solubility in polar medium which may increase the bio-accessibility of the compounds. The formation of compounds and their inclusion complexes were ascertained from elemental analysis data, melting point data and study of spectral characteristics. Thermodynamic properties of the inclusion complexes were also studied to determine the thermodynamic stability of the inclusion complexes and the type of host-guest interaction. The antimicrobial susceptibility of these compounds and their inclusion complexes were also studied.

EXPERIMENTAL

Apparatus and Materials

All chemicals of acceptable purity grade were procured from the local market. Double distilled water used as a solvent was prepared in the laboratory. Electronic spectra were recorded on a Shimadzu UV–1700 spectrophotometer and IR spectra were recorded in KBr pellets on a Shimadzu 8400 FTIR spectrophotometer. Melting points were recorded by the open capillary method.

Synthesis of 2-[arylidenamino]-1, 3, 4-thiadiazino [6,5-b] indoles

Three different 2-[arylidenamino]-1,3,4-thiadiazino [6,5–b] indoles were synthesized starting from indole-2,3-dione (according to Scheme I) through the following intermediate steps [8]:

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i)Synthesis of 3-thiosemicarbazideindole-2-one: a mixture of 2 g of indole-2,3-dione and 1.23 g of thiosemicarbazide in 50 ml of methanol was refluxed for one hour. The completion of the reaction was checked by TLC. The excess of methanol was distilled off. The content was cooled and poured into ice-cold water. It was filtered, washed with water, dried and recrystallised from ethanol to obtain 3-thiosemicarbazideindole-2-one [8].

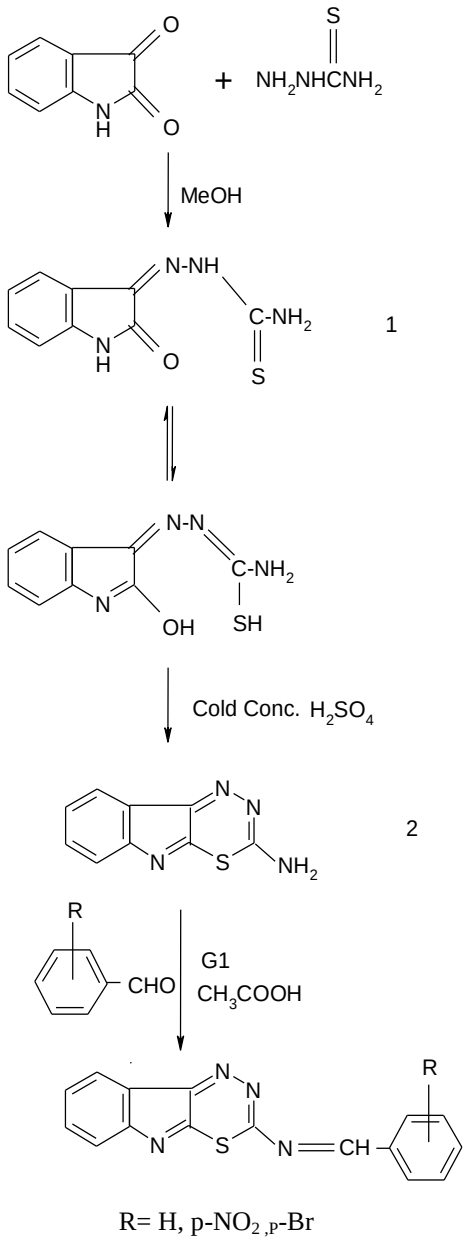
ii) Synthesis of 2-amino-1,3,4-thiadiazino [6,5–b] indole: 3 g of 3-thiosemicarbazideindole-2-one was mixed with a small quantity of cold concentrated H_2SO_4. The reaction mixture was left at room temperature for 16 hours. The reaction mixture was then poured into ice-cold water and neutralized with liquid NH_3 to obtain a solid mass. The latter was filtered through Whatman-42 filter paper. It was washed with water, dried and recrystallised from ethanol to yield 2-amino-1,3,4-thiadiazino [6,5–b] indole.

a) Synthesis of benzylidenamino-1,3,4-thiadiazino [6,5–b] indole (compound I): 1.06 g of benzaldehyde and 2.02 g of 2-amino-1,3,4-thiadiazino [6,5–b] indole were taken in 50 ml of methanol. The mixture was refluxed for 6 hours in presence of glacial acetic acid. The completion of the reaction was checked by TLC and the excess of methanol was distilled off. The refluxed mixture was poured into ice-cold water, filtered, washed with water and dried. The dried mass was recrystallized from ethanol.

b) Synthesis of 2-[4-nitrobenzylidenamino]–1,3,4-thiadiazino [6,5–b] indole (compound II): 1.51 g of p-nitrobenzaldehyde and 2.02 g of 2-amino-1,3,4-thiadiazino [6,5–b] indole were taken in 50 ml of methanol. The mixture was refluxed for 6 hours in presence of glacial acetic acid. The completion of the reaction was checked by TLC and the excess of methanol was distilled off. The refluxed mixture was poured into ice-cold water, filtered, washed with water and dried. The dried mass was crystallized from ethanol.

c) Synthesis of 2-[4-bromobenzylidenamino]-1,3,4-thiadiazino [6,5–b] indole (compound III): 1.87 g of p-bromobenzaldehyde and 2.02 g of 2-Amino-1, 3, 4-thiadiazino [6, 5–b] indole were taken in 50 ml of ethanol. The mixture was refluxed for 6 hours in presence of glacial acetic acid. The completion of the reaction was checked by TLC and the excess of methanol was distilled off. The refluxed mixture was poured into ice-cold water, filtered, washed with water and dried. The dried mass was crystallized from ethanol.

Scheme-I



R= H, p-NO_2 ,p-Br

Phase solubility measurements

The solubility of the compounds at various concentrations in the aqueous phase was checked.

β-cyclodextrin (0–10 mM) was studied by the Higuchi-Corner method [13]. Accurately weighed samples of these compounds were shaken in a rotary flask shaker at room temperature in a series of conical flasks for a period of 48 hours till the attainment of equilibrium. The solutions were filtered through Whatman-42 filter paper and were analyzed in a UV-visible spectrophotometer. The absorbance values at λmax were plotted against different concentrations of β-cyclodextrin.

Synthesis of inclusion complexes

The inclusion complexes of the compounds (I, II and III) with β-cyclodextrin were prepared by the co-precipitation method [14]. Solutions of these compounds of the required concentrations were added dropwise to a β-cyclodextrin solution of the required concentration. The mixtures were stirred for 48 hours and filtered. The filtrate was cooled for 24 hours in a refrigerator. The precipitate obtained was filtered through a G-4 crucible filter, washed with water and dried in air for 24 hours.

Study of thermodynamic properties

The thermodynamic stability constant of the complexes was calculated using the Benesi-Hildebrand relation [15]. The dependence of the stability constant K of each complex on the temperature was calculated. From the slope of the linear plot of ln K *vs*. 1/T, ΔH was obtained. Then ΔS was calculated from van't Hoff's equation

$$\ln K = \Delta H/RT - \Delta S/R$$

The value of ΔG was calculated at 298 K using the equation:

$$\Delta G = -RT \ln K$$

Antibacterial study

The antibacterial activity of the compounds was studied by the cup-plate method. Solutions of the test compounds 500 μg/ml in dimethylsulfoxide (DMSO) were prepared. The bacterial strains were inoculated into 100 ml of the sterile nutrient broth and incubated at 37±1 ^{o}C for 24 hours. The density of the bacterial suspension was standardized by the McFarland method. Wells of uniform diameter (6 mm) were made on agar plates, after separate aseptic inoculation with the test organisms. The standard drug and the test compounds were introduced using micropipettes and the plates were placed in a refrigerator at 8–10ºC for proper diffusion of the drug into the media. After two hours of cold incubation, the petri plates were transferred to the incubator and were maintained at 37±2ºC for 18–24 hours. Then the zone of inhibition in the petri plates was determined using a vernier scale and was compared to that obtained with the standard drug tetracycline. The results were presented as the mean value (mm) of the zone of inhibition of three sets of experiments.

RESULTS AND DISCUSSION

The synthesis of compound I (benzylidenamino-1,3,4 -thiadiazino[6,5–b] indole),

compound II (2-[4-nitrobenzylidenamino]-1,3,4-thiadiazino[6,5–b] indole) and

compound III (2-[4-bromobenzylidenamino]-1,3,4-thiadiazino[6,5–b]indole) were confirmed by elemental analysis and spectral data, as shown in Table 1.

The elemental composition matches the theoretical data. The IR data of compound I show characteristic absorption at 672,1296,1611,1682 and 3141 cm^{-1} indicating the presence of C–S, C–C, N–N, C=N and benzene ring in the compound, as expected. The IR data of compound II show characteristic absorptions at 719, 1301, 1462, 1581, 1701 and 3146 cm^{-1} indicating the presence of C^{-} S, C–C, C–N, N–N, C=N and benzene ring in the compound, as expected. Similarly, the IR data of compound III show characteristic absorptions at 561,719,1527,1591,3022,3051 cm^{-1} indicating the presence of C-Br, C-S, C=N ,N-N, =C-H and benzene ring in the compound. The IR data of the complex I show characteristic absorption at 670,1290,1605,1679 and 3130 cm^{-1} indicating the presence of C-S, C-C, N-N, C=N and benzene ring in the complex. The IR data of the complex II show characteristic absorptions at 712, 1294, 1456, 1573, 1692 and 3135 cm^{-1} indicating the presence of C-S, C-C, C-N, N-N, C=N and benzene ring in the complex. Similarly, the IR data of the complex III show characteristic absorptions at 560,716,1300,1524,1588 and 3048 cm^{-1} indicating the presence of C-Br, C–S, C–C, N–N, C=N and benzene ring in the complex.

The synthesis of inclusion complexes of compound I (benzylidenamino-1,3,4-thiadiazino[6,5-b]indole), compound II (2-[4-nitrobenzylidenamino]-1,3,4-thiadiazino[6,5-b]indole) and compound III (2-[4-bromobenzylidenamino]-1,3,4-thiadiazino[6,5-b]indole) were confirmed by the changes in melting point, colour and spectral characteristics(UV-Vis and IR). The melting points of compounds I, II and III are found to be 224º C, 245º C and 246 º C, respectively, and the melting points of their inclusion complexes are 228º C, 255º C and 249º C (Table 1). The colour of the compounds I, II and III is yellow, while their inclusion complexes are pale yellow, pale yellow and reddish yellow, respectively.

Table 1. Analytical data of compounds with and without inclusion complex

S.No.	Compound/ Complex	Melting Point	Colour	Elemental Analysis (First line - found value, second line - calculated value)					λ max (A^0)	IR (KBr) cm^{-1}
1	Compound I	224	Yellow	C 66.4 66.2	H 3.45 3.44	N 19.4 19.3	S 1.0 1.03	O	3550	672(C-S) 1296(C-C) 1611(N-N) 1682(–C=N) 3141(Ring)
2	Compound I- β- CD	228	Pale Yellow	–	–	–	–	–	3542	670(C-S) 1290(C-C) 1605(N-N) 1679(C=N) 3130()Ring)
3	Compound II	245	Yellow	57.5 57.3	2.7 2.69	20.9 20.9	9.56 9.55	9.55 9.55	3548	719 (C-S) 1301(C-C) 1462 (C-N) 1581(N-N) 1701(–C=N) 3146(Ring)
4	Compound II- β- CD	255	Pale Yellow	–	–	–	–	–	3540	712 (C-S) 1294(C-C) 1456(C-N) 1573(N-N) 1692(-C=N) 3135(Ring)
5	Compound III	246	Yellow	62.8 62.7	3.3 3.26	18.4 18.3	10.5 10.4	5.0 5.2	3552	561(C-Br) 719(C-S) 1301(C-C) 1527(N-N) 1591(C=N) 3051(Ring)
6	Compound III -β- CD	249	Reddish Yellow	–	–	–	–	–	3548	560(C-Br) 716 (C-S) 1300(C-C) 1524(N-N) 1588(C=N) 3418(Ring)

Compound I: benzylidenamino-1,3,4-thiadiazino[6,5-b]indole; Compound II: 2-[4-nitrobenzylidenamino]-1,3,4-thiadiazino[6,5-b]indole; Compound III: 2-[4-bromobenzylidenamino]-1,3,4-thiadiazino[6,5-b]indole.

The absorption maxima of the compounds I, II and III are found at 3550, 3548 and 3552 Aº, while their inclusion complexes have absorption maxima at 3542, 3540 and 3548 Aº, respectively. The higher melting points of the inclusion complexes of the compounds are due to the fact that extra amount of thermal energy is required for the latter to bring it out of the β-cyclodextrin cavity.

It is quite interesting to note that the absorption maxima are shifted towards lower wavelengths after formation of the inclusion complex (Table I). This may be attributed to the transfer of the compound from a more protic environment to a less protic environment in the cavity of β-cyclodextrin, which may be further supported by IR data. The IR stretching frequencies due to different bonds are shifted downward towards low energy and the peaks become broader, weaker and smoother. Such changes in spectral characteristics due to inclusion complex formation may be due to weak interactions like hydrogen bonding, van der Waal's forces, hydrophobic interactions, etc. ,between the guest compound and the host β-cyclodextrin [16–19].

The phase solubility plots of the compounds in β-cyclodextrin solution are shown in Fig. 1. In all cases there is a linear increase in solubility of these compounds with increasing concentration of β-cyclodextrin. Since the slopes of all plots are less than unity, the stoichiometry of these complexes may be written as 1:1[20].

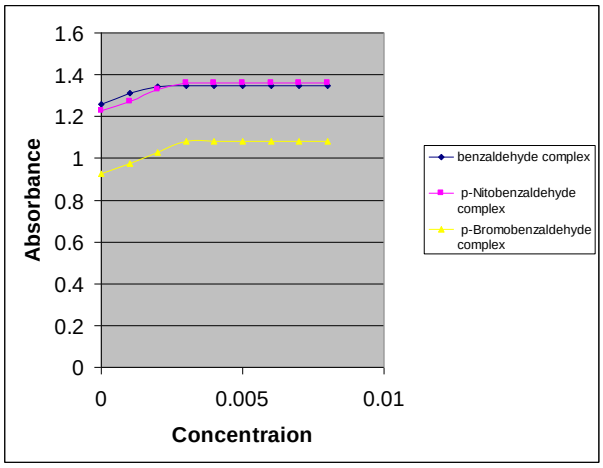


Fig. 1. Phase solubility plot of substituted indole derivatives.

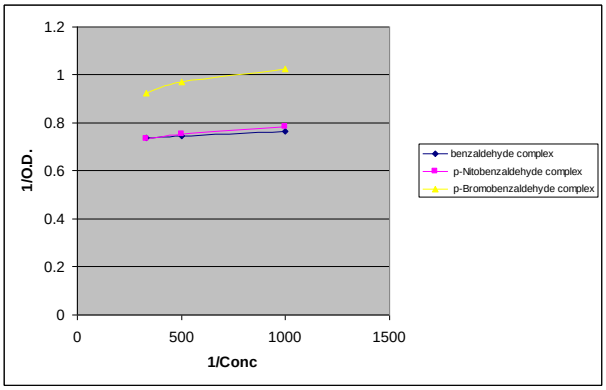


Fig. 2. Plot of 1/ O.D. *vs*. concentration of substituted indole derivatives.

The thermodynamic stability constants (K_T) of the inclusion complexes are determined using the Benesi-Hildebrand relation [15] Good linear correlations are obtained for a plot of $1/\Delta A$ *vs*. $[\beta\text{-CD}]_o$ for the compounds I, II and III (Fig. 2) The values of K_T for all complexes are calculated using the relation

K_T = Intercept/Slope

The K_T values of the inclusion complexes of compounds I, II and III with

β-cyclodextrin are found to be 421, 387 and 413 M^{-1}, respectively (Table 2). The data obtained are within 100 – 1000 M^{-1} (ideal values) indicating appreciable stabilities for the inclusion complexes [20]. The lower values of the stability constants for compounds II and III than for compound I may be related to steric factors (due to the nitro group in compound II and the bromo group in compound III).

The thermodynamic parameters associated with the interaction of the compounds with β-cyclodextrin for 1:1 stochiometry were also calculated by determining the stability constants (K-values) at different temperatures. The K-values were found to decrease with the rise in temperature, as expected for an exothermic process (deencapsulation) [21,22]. The dependence of ln K *vs*. inverse absolute temperature produced linear plots (Fig. 3). From the slopes of the curves, van't Hoff reaction isotherm and van't Hoff equation, the values of ΔG (change in free energy), ΔH (change in enthalpy) and ΔS (change in entropy) were calculated at 298 K (Table 2). As Table 2 shows, ΔG values are negative for all inclusion complexes. These data clearly demonstrate that formation of inclusion complexes of compounds I, II and III with β-cyclodextrin is a spontaneous process. Further it was found that for all three inclusion complexes ΔH values are negative and ΔS values are positive(cf. Table 2). The negative value of the enthalpy

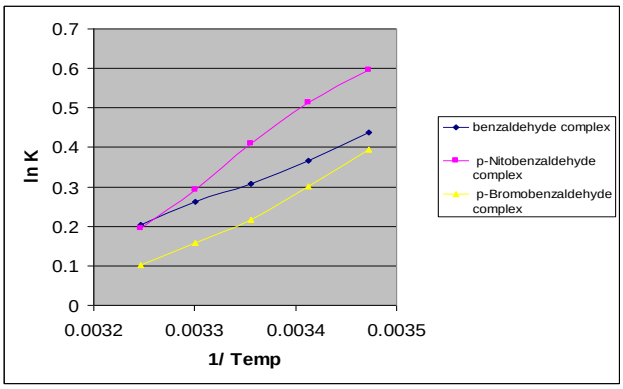


Fig. 3. Plot of ln K vs. 1/T of substituted indole derivatives.

change (ΔH) and the positive value of the entropy change (ΔS) indicate that all three processes of inclusion complex formation are energy allowed and entropy allowed. The smaller value of ΔS for the inclusion complex of compound II may be related to a steric

Table 2: Thermodynamic data of inclusion complexes at 298 K.

Complexes	K M^{-1}	ΔG kJ/mole	ΔH kJ/mole	ΔS J/mole
Compound I- β- CD	421	-14.98	-12.105	9.65
Compound II- β- CD	387	-14.736	-14.934	0.665
Compound III- β- CD	413	-14.932	-13.346	5.321

Compound I: benzylidenamino-1,3,4-thiadiazino[6,5-b]indole. Compound II: 2-[4-nitrobenzylidenamino]-1,3,4-thiadiazino[6,5-b]indole. Compound III: 2-[4-bromobenzylidenamino]-1,3,4-thiadiazino[6,5-b]indole.

factor, which correlates with its lower thermodynamic stability constant (Table 2). The antibacterial activities of the compounds and of their inclusion complexes against *S. aureus* and *E.coli* are shown in Figs. 4A and 4B. Both the compounds and their inclusion complexes are susceptible to the bacteria. However, the inclusion

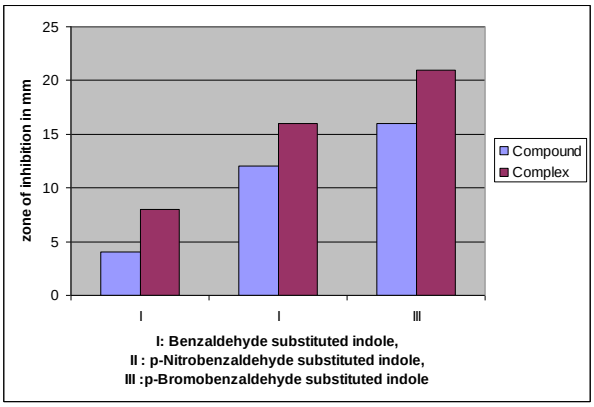


Fig. 4A. Antimicrobial susceptibility test against *S. aureus.*

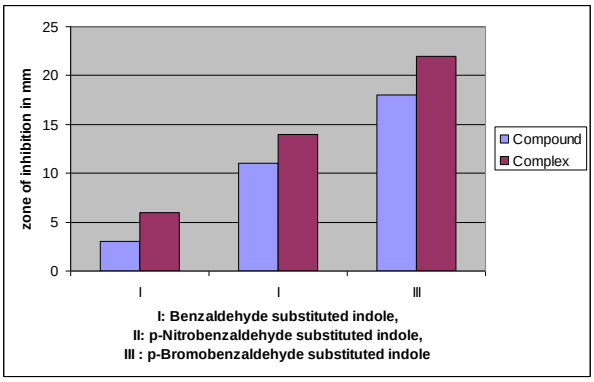


Fig. 4B. Antimicrobial susceptibility test against *E.coli.*

complexes display significantly higher antibacterial activity as compared to their corresponding compounds. This may be attributed to the enhanced solubility of the compounds in the inclusion complexes formed, so that they become more available to specific tissues and display increased antibacterial activity, as earlier suggested [23-26].

CONCLUSION

From the above results and discussion, it is clear that the formation of inclusion complexes of compounds I, II and III is thermodynamically allowed. These complexes can be a very good analytical tool for enhancing the bioaccessibility of the drugs. The study further reveals that the formation of inclusion complexes leads to a significant increase in antibacterial activity.

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СРАВНИТЕЛНО ИЗСЛЕДВАНЕ КОМПЛЕКСИ НА ВКЛЮЧВАНЕ НА ЗАМЕСТЕНИТЕ ИНДОЛ ДЕРИВАТИ С В-ЦИКЛОДЕКСТРИН

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(Резюме)

Някои [арилденамино] -1,3,4-ттиадиазино [6,5-b] индоли са синтезирани като се започне от индол-2,3-дион, тиосемикарбазид и ароматни алдехиди с активиране и деактивиране на групи. Комплекси на включване на тези съединения са получени с бета-циклодекстрин, за да се увеличи тяхната разтворимост и биологичнат им достъпност. Термодинамични свойства, като промяна в свободна енергия, промяна в енталпията, промяна на ентропията и стабилитетна константа на комплекси на включването са определени, за да се изясни дали формирането на комплекси на включване е термодинамично позволено или не. Съединенията и комплексите им на включване бяха проверени спрямо *S. Aureus* и *E. Coli*. Установено бе, че антибактериалниата активност на съединенията се увеличава значително след включването им в образуване на комплекси.