



A Comparative Study of Antioxidant Properties of 2-[Substituted arylidenamino]-1, 3, 4-thiadiazino [6, 5B] Indoles and Their Inclusion Complexes with β -Cyclodextrin

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ABSTRACT

Inclusion complexes of 2-[Arylidenamino]-1, 3, 4-thiadiazino [6, 5b] indoles have been prepared with β -Cyclodextrin for increasing solubility in polar medium. The formation of inclusion complexes has been confirmed from the study of changes in physical and spectral properties of the compounds. The determination of thermodynamic stability constant and other thermodynamic properties indicates that the inclusion complexes are comparatively stable and their formation is thermodynamically allowed. Finally, the compounds and their inclusion complexes are screened for antioxidant activities and it is found that the inclusion complex formation increases the antioxidant activities significantly.

Keywords: Substituted indole, β Cyclodextrin, Inclusion complex, Thermodynamic stability, Antioxidant activity.

INTRODUCTION

Free radicals play an important role in many physiological and pathological activities of living organisms. Any imbalance in the generation and scavenging of free radicals causes diseases. Free radical reactions make significant impact on membrane proteins, enzymes and DNA. [1-3] There are reports that the antioxidants have the ability to scavenge free radicals and reactive oxygen species present in biological systems [4-5] thereby preventing a number of diseases. [6] So for the prevention and treatment of the diseases caused by free radical, it is important to find effective scavengers.

Indole and their derivatives play an important role in biological and medicinal chemistry. They exhibit wide range of pharmacological activities like anti-microbial, antidepressive, anti-inflammatory, anti-fungicidal, antipyretic, antitubercular and antioxidant activities. [7-10] Since the bio-accessibility of a drug depends upon its solubility, one of the factors limiting the pharmacological activities of these compounds may be their poor solubility in polar medium. [11] To overcome this difficulty, an attempt has been made to form the inclusion complex of these compounds with a non-toxic oligosaccharide, β -cyclodextrin. [12-13]

In the present work, inclusion complexes of 2-[arylidenamino]-1, 3, 4-thiadiazino [6, 5b] indoles have been prepared with β -cyclodextrin after synthesizing their pure compounds. The spectral and thermodynamic properties of the compounds and their inclusion complexes have been studied to confirm the inclusion complex formation. Antioxidant activities of these compounds and inclusion complexes are also evaluated to have an idea whether inclusion complex formation is enhancing the antioxidant property of the compounds or not.

MATERIALS AND METHODS

Apparatus and Materials

All the chemicals of acceptable standards are procured from local market. Double distilled water is used as solvent. Electronic spectra are recorded on Shimadzu UV-1700 spectrophotometer and IR spectra are recorded in KBr pellets in Shimadzu 8400 FTIR spectrophotometer. Melting points are recorded by open capillary method.

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

Six different 2-[arylidenamino]-1, 3, 4-thiadiazino [6, 5b] indoles have been synthesized in their purest form starting from indole-2, 3-dione as per the method described by Panda and Tripathy [14-16] given in scheme-I

Phase Solubility Measurements

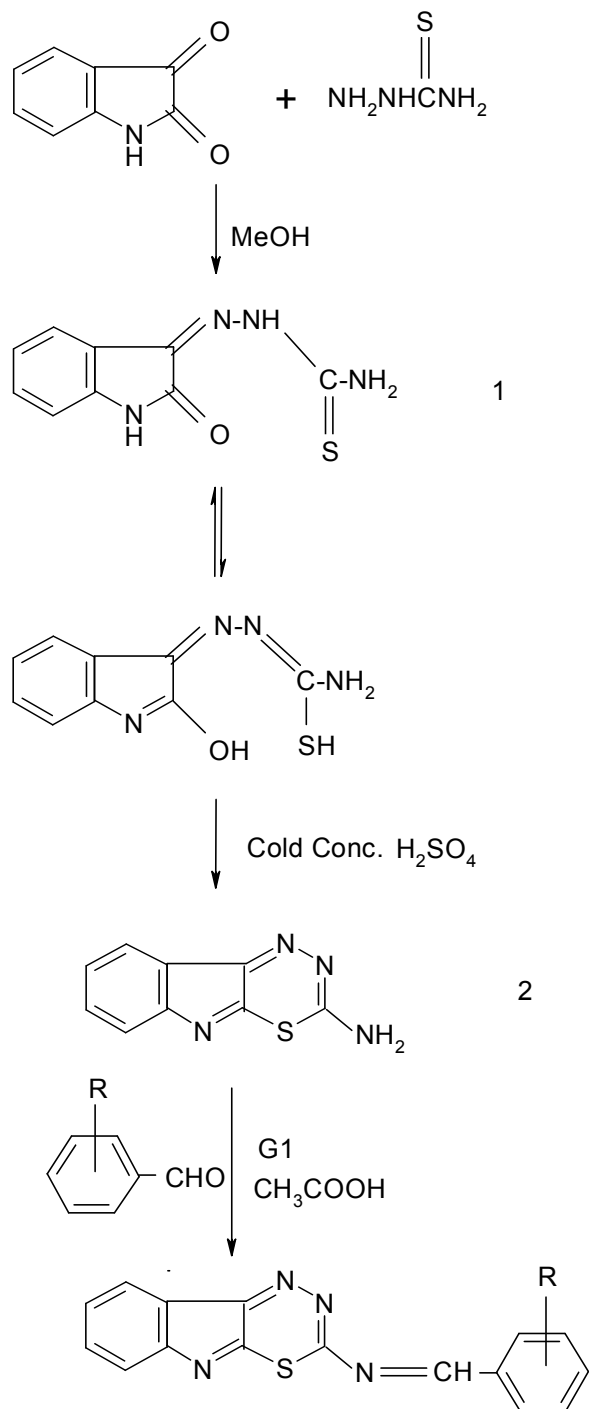
The aqueous phase solubility of the compounds at various concentrations of β -cyclodextrin (0-10mM) has been studied by Higuchi-Corner method. [17] A rotary flash shaker is used

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to shake the accurately weighed sample of these compounds at room temperature in different conical flask for a period of 48 hours till the attainment of equilibrium. Whatmann-42 filter papers are used to filter the solution. These solutions are analyzed in a UV-visible spectrophotometer. The various values of absorbance at λ_{max} are plotted against different concentrations of β -cyclodextrin.

Scheme-I



Where R= H, o-NO₂, o-OH, p-NO₂, p-OCH₃, p-N (CH₃)₂

Synthesis of inclusion complexes

The inclusion complexes of the compounds with β -cyclodextrin have been prepared as per co-precipitation

method.^[18] Proper concentrations of the solutions of these compounds are added drop by drop to β -cyclodextrin solution of the required concentration. Stirring of the solutions is carried out for a period of 48 hours. The stirred solutions are filtered. The filtrates are cooled for 24 hours in refrigerators. The precipitates obtained are filtered, washed with water and dried in open atmosphere for 24 hours.

Study of thermodynamic properties

The stability constant of the complexes K has been calculated with increasing temperature. From the slope and intercept of the linear plot of $\ln K$ vs. $1/T$, ΔH and ΔS are calculated by using vant Hoff's equation

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

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

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

Evaluation of Antioxidant activity

In the present study DPPH (2, 2-Diphenyl-1-picrylhydrazyl) scavenging assay method is used for screening the antioxidant activity of the synthesized compounds as suggested by Tagashira and Ohtake.^[19] Test sample solution is prepared in 100 μ g/ml concentration in ethanolic DPPH. After vortexing, the mixture is incubated for 10 minutes at room temperature. The absorbances of the samples are measured at 517 nm. The activity of the sample is calculated by finding the difference of absorbance between a test sample and a control. Butylated Hydroxyl Toluene (BHT) is used as reference substance

RESULTS AND DISCUSSION

The synthesis of compounds has been confirmed from analytical and spectral data (Table 1). There is a significant change in melting points and UV and IR absorption peak positions before and after the inclusion complex formation as suggested earlier. The phase solubility plots of the compounds in β - cyclodextrin solution (Fig. 1) show that there is a linear increase in solubility of these compounds with increasing concentration of β - cyclodextrin. Since the slopes of all the plots are less than unity the stoichiometry of these complexes may be 1:1.^[20]

The thermodynamic stability constants (K_T) of inclusion complexes are determined by using Benesi-Hilderband relation.^[21] Good linear correlations are obtained for a plot of $1/\Delta A$ verses $[\beta\text{-CD}]_0$ for compounds (Fig. 2). The values of K_T for all the complexes are calculated using the relation

$$K_T = \text{Intercept/Slope}$$

The K_T values of the inclusion complexes of compounds with β - Cyclodextrin are found to be 421, 123, 231, 387, 718.5 and 598 M⁻¹ respectively (Table 2). The data obtained are within 100 to 1000 M⁻¹(ideal values) indicating appreciable stabilities for the inclusion complexes^[22] through host-guest interaction like van der Waal' force, hydrophobic interaction etc.^[23-24] The thermodynamic parameters associated with the interaction of the compound with β -cyclodextrin for 1:1 stoichiometry have also been calculated by determining stability constant (K- values) at different temperatures. The K- values are found to decrease with rise in temperature as expected for an exothermic process (deencapsulation).^[25-26]

The graph of $\ln K$ verses inverse absolute temperature (Fig. 3) produce linear plots from which the value of ΔH , ΔS and ΔG are calculated using van'tHoff equation (Table 2). The determination of thermodynamic parameters suggests that the formation of the entire inclusion complex is thermodynamically allowed.^[27-28]

Table 1: Analytical data of Compounds with and without inclusion complex

S. No.	Compound/ Complex	Melting Point	Colour	λ_{max} (Å)	IR (KBr) cm^{-1}
1.	Compound-I	224	Yellow	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	230	Yellow	3560	719 (C-S),1301(C-C), 1462 (C-N),1581(N-N), 1701(-C=N), 3146(Ring)
4.	Compound-II- β - CD	236	Pale Yellow	3551	717 (C-S),1298(C-C), 1460(C-N),1576(N-N), 1698(-C=N),3138(Ring)
5.	Compound-III	239	Yellow	3540	672 (C-S),1294(C-C), 1611(N-N),1683(-C=N), 3142(Ring)
6.	Compound-III - β - CD	246	Whitish Yellow	3530	669 (C-S),1290(C-C), 1610(N-N),1679(-C=N), 3130(Ring)
7.	Compound-IV	245	Yellow	3548	719 (C-S),1301(C-C), 1462 (C-N),1581(N-N), 1701(-C=N),3146(Ring)
8.	Compound-IV- β - CD	255	Whitish Yellow	3540	712 (C-S),1294(C-C), 1456(C-N),1573(N-N), 1692(-C=N),3135(Ring)
9.	Compound-V	232	Yellow	3556	677(C-S),1213(C-C), 1466(C-N),1575(N-N), 1707(-C=N),3133(Ring)
10.	Compound-V- β - CD	237	Pale Yellow	3550	673(C-S),1210(C-C), 1464(C-N),1570(N-N), 1698(-C=N),3118(Ring)
11.	Compound-VI	216	Yellow	3570	673 (C-S),1302(C-C), 1623(N-N),1734(-C=N), 3174(Ring)
12.	Compound-VI- β - CD	223	Grey Yellow	3562	672 (C-S),1301(C-C), 1620(N-N),1732(-C=N), 3171(Ring)

Table 2: Thermodynamic data of inclusion complexes at 298 K

Complexes	K(M ⁻¹)	ΔG (kJ/MOLE)	ΔH (kJ/MOLE)	ΔS (kJ/MOLE)
Compound-I- β - CD	420.9	-14.98	-12.105	0.00965
Compound-II- β - CD	123.3	-11.824	-12.01	-0.00625
Compound-III- β - CD	231.3	-13.489	-10.36	0.0105
Compound-IV- β - CD	387.3	-14.736	-14.934	-0.00666
Compound-V- β - CD	718.5	-16.2987	-14.470	.00615
Compound-VI- β - CD	598.14	-15.844	-14.852	0.0033326

Compound-I : Benzylidenamino-1, 3, 4-thiadiazino[6, 5b]indole

Compound-II : 2-[2- NitroBenzylidenamino]-1, 3, 4-thiadiazino[6, 5b]indole

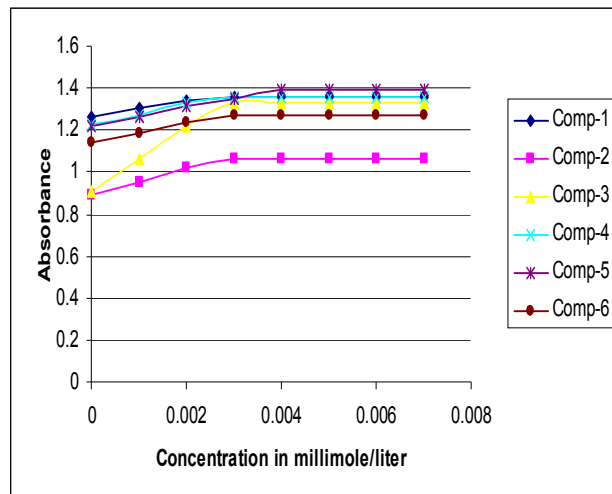
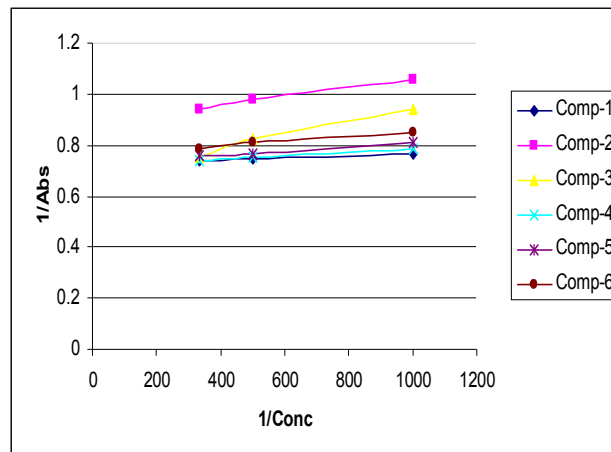
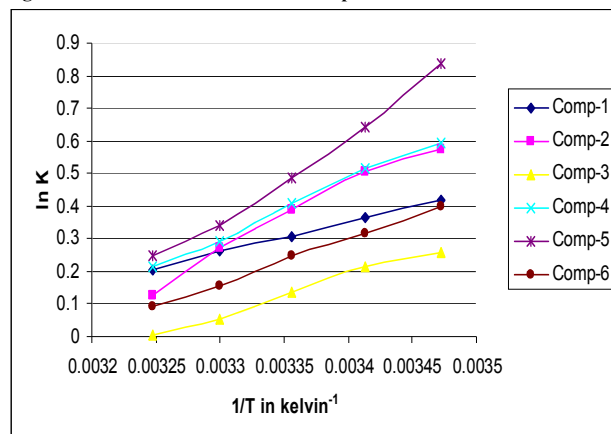
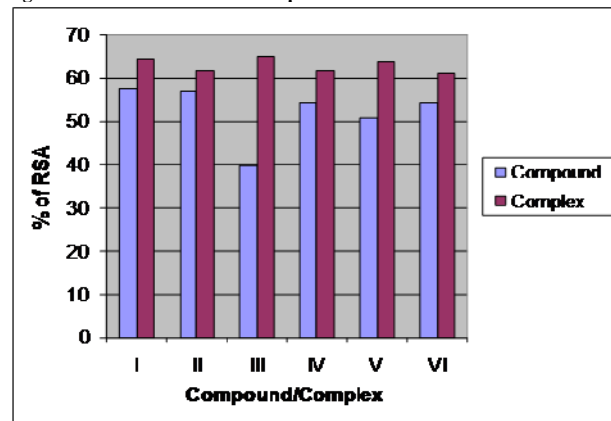
Compound-III : 2-[2- Hydroxy Benzylidenamino]-1, 3, 4-thiadiazino[6, 5b]indole

Compound-IV : 2-[4- NitroBenzylidenamino]-1, 3, 4-thiadiazino[6, 5b]indole

Compound-V : 2-[4- MethoxyBenzylidenamino]-1, 3, 4-thiadiazino[6, 5b]indole

Compound-VI : 2-[2- N,N-dimethyaminoBenzylidenamino]-1, 3, 4-thiadiazino [6, 5b] indole

The antioxidant activities of the compounds and their inclusion complexes are shown in Fig. 4. The radical scavenging activities of the compounds increase significantly after the formation of inclusion complex. This can be correlated to the higher solubility of the compounds due to inclusion complex formation there by increasing the bioaccessibility. Higher the bioaccessibility of the compounds, higher becomes the ability of compounds to trap the reactive oxygen species or free radicals, thereby increasing antioxidant activity of the compounds. ^[29]

**Fig. 1: Plot of Phase Solubility of the compounds****Fig. 2: Plot of 1/O.D Vs. 1/Conc. of complexes****Fig. 3: Plot of ln K vs. 1/T of complexes****Fig. 4: Anti-oxidant activity of Compounds/Complexes**

From the above results and discussion, it is clear that the formation of inclusion complexes of compounds is thermodynamically allowed which can be a very good analytical tool for enhancing the bioaccessibility of the drugs. The study further reveals that the formation of inclusion complex causes a significant increase in antioxidant activity of the compounds.

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