Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2012; 4(3): 191-194

Research Article ISSN 0975-248X

A Comparative Study of Antioxidant Properties of 2-[Substituted arylideamino]-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-fungicidial, 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]

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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

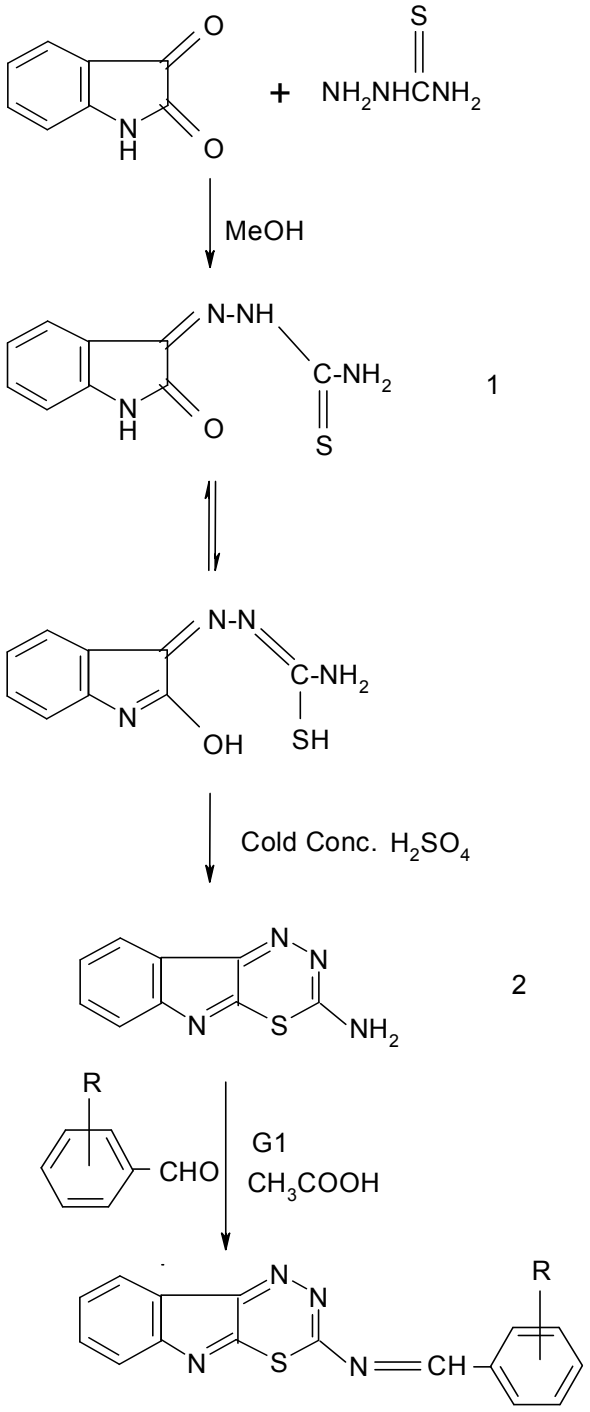
Six different 2-[arylidenamino]-1, 3, 4-thiadiazino [6, 5b] indoles have been synthesized in their purest form starting from indole-2, 3-dione as per the method described by Panda and Tripathy [14-16] given in scheme-I

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

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-NO_2, o-OH, p-NO_2, p-OCH_3, p-N $(CH_3)_2$

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 lnK 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 100µg/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 $[\beta\text{- CD}]_o$ 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 stochiometry 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 (A^0)	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	-.000666
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]

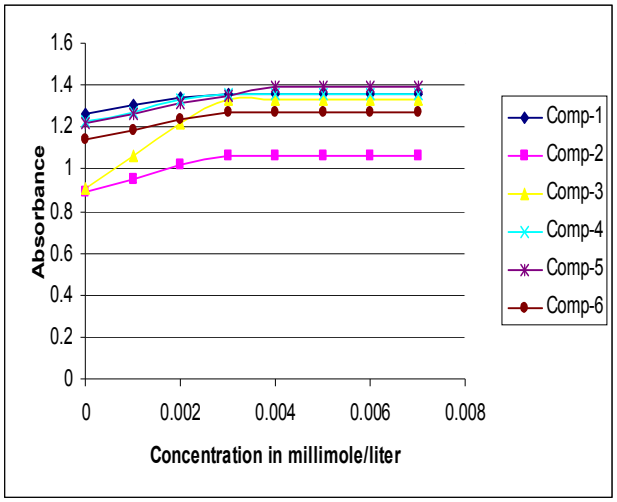


Fig. 1: Plot of Phase Solubility of the compounds

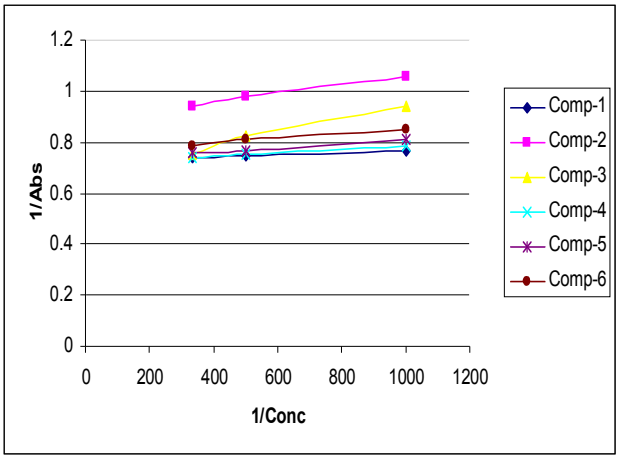


Fig. 2: Plot of 1/O.D Vs. 1/Conc. of complexes

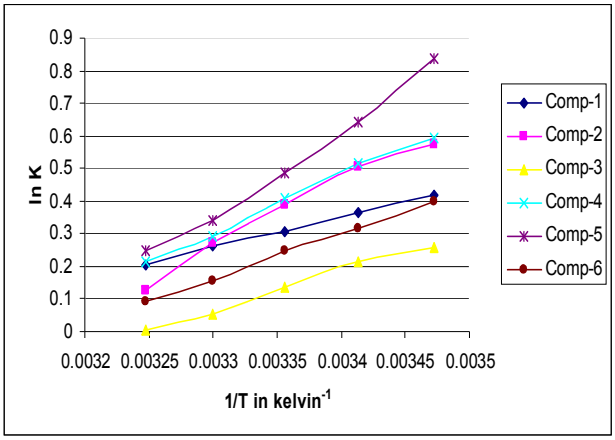


Fig. 3: Plot of ln K vs. 1/T of complexes

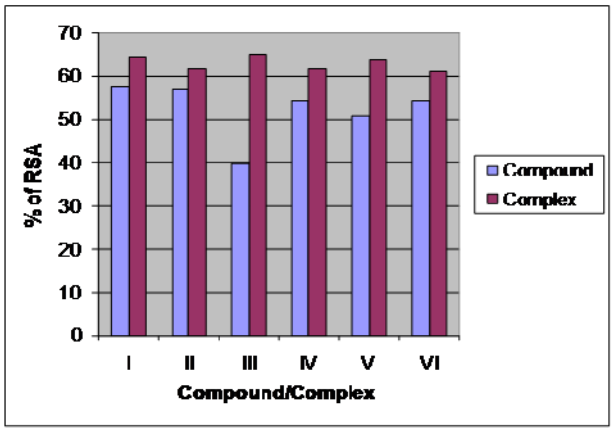


Fig. 4: Anti-oxidant activity of Compounds/Complexes

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.

ACKNOWLEDGEMENT

The authors thank to Dr. U. L. Narayana, Principal, Indira Gandhi Institute of Pharmaceutical science; for carrying out the IR study. Financial assistance from UGC, New Delhi is thankfully acknowledged.

REFERENCES

1. Vandemiale G, Grattaglino I, Altomare E. An update on the role of free radicals and antioxidant defence in human disease. International Journal of Clinical Laboratory Research 1999; 29: 49-55.
2. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidant stress induced cancer. Chemical Biology Interactive 2006; 160: 1-40.
3. Chen G, Wang Y, Hao X, Mu S, Sun Q. Simple Isatin derivatives as free radical scavengers: Synthesis, biological evaluation and structure-activity relationship. Chemistry Central Journal 2011; 5: 1-5.
4. Bankson DD, Kestin M, Rifal N. Role of free radicals in cancer and atherosclerosis. Clinical Lab. Med. 1993; 13: 463-480.
5. Prakash A, Rigelhof F, Miller E. Antioxidant Activity. Cereals Foods World 2000; 45(2): 59-63.
6. Miller HE, Rigelhof F, Marquart L, Prakash A, Kanter M. Antioxidant content of whole grain breakfast cereals, fruits and vegetables. Journal of American Coll. Nutr. 2000; 19(3): 312S-319S.
7. Patil R, Biradar JS. Synthesis and pharmacological evaluation of substituted- 2-triazolo (3, 4-b) (1, 3, 4)-thiadiazoles. Indian Journal of pharmaceutical Science 2001; 63(4): 299-305.
8. Holla BS, Udupa KV. Synthesis and antibacterial activity of nitrofurfuraldehyde as-triazino [5, 6-b] indol-3-ylhydrazones. Journal of Indian Chemical Society 1988; 65(7): 524-525.
9. Panwar H, Verma RS, Srivastava V, Kumar A. Synthesis of some substituted azetidinonyl and thiazolidinonyl-1, 3, 4-thiadiazino[6, 5-b] indoles as prospective antimicrobial agents. Indian Journal of Chemistry 2006; 45B: 2099-2104.
10. Shobha SR, Agaiah B , Sarangpani M. Antioxidant and DNA binding study of 3, 3'-(5, 5'-Methylene)Bis(3-Mercapto-4H-1, 2, 4-Triazol-5, 4-Diyl)Bis(Azan-1-yl-1-ylidene)Diindoline-2-ones. Int. J. Pharm. Technol. 2011; 2: 366-374.
11. Pose-Vilarnovo B, Predomo-Lopez I, Echezarreta-Lopez M. Improvement of water solubility of sulfamethizole through its complexation with β- and hydroxypropyl-β-cyclodextrin: Characterization of the interaction in solution and in solid state. Journal of Pharmaceutical Science 2001; 13(3) : 325-331
12. Belikov VG, Komapantseva EV. Khimiko Farmatsevticheskii Zhurnal. 1990; 24(7): 19.
13. Yano H, Hiramaya F, Arima H. Preparation of prednisolone-appended α-, β- and γ-cyclodextrins: Substitution at secondary hydroxyl groups and <I>in vitro</I> hydrolysis behavior. Journal of. Pharmaceutical. Science 2001; 90(4): 493-503.
14. Panda S, Tripathy JK. Studies on inclusion complexes of substituted indole derivatives with activating and deactivating group. J. Chem. Pharm Res. 2010; 2: 722-732.
15. Panda S, Tripathy JK. Thermodynamic, spectral and antimicrobial properties of inclusion complex of 2-[Benzylidenamino]-1,3,4-thiadiazino[6,5b] indole and 2-[Furfurlidenamino]-1,3,4-thiadiazino[6,5b] indole –a comparative study. Research Journal of Pharmaceutical Technology 2011; 4: 1693-1698.
16. Tripathy JK, Panda S. Thermodynamic and spectral studies of inclusion complexes of substituted indole derivatives with β-cyclodextrin. Asian J. Chem. 2011; 23: 1631-1635.
17. Higuchi T, Connors K. Phase-solubility techniques. Adv. Anal. Chem. Instrum. 1965; 4: 117-212.
18. Panda S, Nayak S S. Inclusion complexes of Acridone and Its Semicarbazone Derivative with β-cyclodextrin-A Thermodynamic, Spectral and Antibacterial Study. Journal of Research in Chemistry 2009; 2(4): 539-543.
19. Tagashira M, Ohtake Y. A new antioxidative 1, 3-benzodioxide from Melissa officinals. Planta Medicinal Journal 1998; 64: 555-558.
20. Mukna AP, Nagarsenkar MS. American Association of Pharmaceutical Science. Pharmaceutical Science Technology 2001; 5(1): 19.
21. Bensi HA, Hilderband JH. A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons. Journal of American Chemical Society 1999; 71: 2703-2707.
22. Szetli J. Molecular entrapment and release properties of drugs by cyclodextrins. Controlled Drug Bio-availability. Vol. 3, Willey Interscience publications, New York**,** 1985, pp: 365.
23. Mohammed KG, Moji CA. Elucidation of solution state complexation in wet granulated Oven Dried Ibuprofen and β-cyclodextrin: FTIR and ^{1}H NMR Studies. Pharmaceutical Development and Technology 2001; 6: 315-324.
24. Nayak S, Panda S, Panda P, Padhy MS. Studies on acridone derivatives with and without inclusion complex formation with β-cyclodextrin. Bulgarian Chemical communication 2010, 42(2): 147-152.
25. Tommasini S, Raneri D, Ficarra R, Calabro ML, Stancanelli R. Improvement in solubility and dissolution rate of flavonoids by complexation with β-cyclodextrin. Journal of pharmaceutical and biomedical Analysis 2004; 35: 379-387.
26. Rajewski RA, Stella VJ. Pharmaceutical applications of cyclodextrins in vivo drug delivery. Journal of Pharmaceutical. Science 1996; 85: 1142-1169.
27. Loukas YL, Vraka V, Gregordias G. Novel non-acidic formulations of haloperidol complexed with β-cyclodextrin derivatives. Journal of Pharmaceutical and Biomedical Analysis 1997; 16: 263-268.
28. Stalin T, Vasantharani P, Shanti B, Sekhar A, Rajendiran N. Inclusion Complexes of trihydroxybenzene with α- and β-cyclodextrin. Indian Journal of Chemistry Sec A 2006; 45: 1113-1120.
29. Astakhova AV, Demina NB. Astakhova AV, Demina NB. Modern drug technologies: Synthesis, characterization and use of inclusion complexes between drugs and cyclodextrins (A Review). Journal of Pharmaceutical Chemistry 2004, 38(2): 105-108.