Studies on inclusion complexes of 2-[Substituted arylideamino]-1, 3, 4-thiadiazino [6,5b]indole with β-Cyclodextrin

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Inclusion complexes of 2-[Arylidenamino]-1,3,4-thiadiazino[6,5b]indoles have been prepared with β-cyclodextrin so as to increase the solubility of these compounds in polar medium which may increase their bioaccessibility. The formation of inclusion complexes is known from the study of changes in physical and spectral properties of the compounds. The stability of the inclusion complexes has been studied by the determination of the thermodynamic parameters like change in free energy, change in enthalpy and change in entropy. Finally compounds and their inclusion complexes are screened for antibacterial and antioxidant activities. It is found that both antibacterial and antioxidant activity of the compounds increase significantly after the inclusion complex formation. In addition, the presence of activating groups on arylidene unit further favours inclusion complex formation and also stabilizes the same.

Keywords: Substituted indole; β Cyclodextrin; Inclusion complex; Antimicrobial activity; Antioxidant activity

1. INTRODUCTION

Indole analogous are very good pharmacophores for designing several chemotherapeutic reagents exhibiting a wide spectrum of pharmacological activities such as antidepressive, anti-inflammatory, anti-fungicidal, anti-bactericidal and anti-tubercular activities [1-4]. Indole and substituted indole on combination with azediones and thiazolidinones show excellent antimicrobial activities [5-8]. 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 [9]. The solubility of these compounds can be enhanced significantly by forming inclusion complexes with a non-toxic oligosaccharide, cyclodextrin. Out of all the known cyclodextrins, β -cyclodextrin is usually considered for inclusion complex formation as it is cheaper, easily available and highly stable towards heat and oxidation [10-12].

In the present work an attempt has been made to prepare inclusion complexes of three 2-[arylidenamino]-1, 3, 4-thiadiazino[6,5b]indoles with β-cyclodextrin after synthesizing their pure compounds and to study their thermodynamic stability and antimicrobial characteristics. . The arylidenamino unit contains two activating groups in two different compounds namely N,N-dimethylamino and methoxy group at para position. The formation of compounds and their inclusion complexes has been ascertained from elemental analysis, melting point data and study of spectral characteristics. Thermodynamic stability of inclusion complexes and the type of interaction in between the host and guest are known from the determination of thermodynamic parameters like change in free energy, change in enthalpy, change in entropy and stability constant. The antimicrobial susceptibility and antioxidant activities of these compounds and their inclusion

complexes are also studied to have an idea whether inclusion complex formation is enhancing the bio accessibility of the drugs or not.

2. EXPERIMENTAL

2.1. Apparatus and Materials

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

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

Three different 2-[arylidenamino]-1, 3, 4-thiadiazino [6,5b] indoles were synthesized starting from indole -2, 3--dione (as per the scheme-I)

i) Synthesis of 3-Thiosemicarbazideindole-2-one:

A mixture of 2gm of indole-2, 3-dione and 1.23gm 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 out. The content was cooled and it was poured into ice cold water. It was filtered, washed with water, dried and recrystallised from ethanol to obtain 3-Thiosemicarbazideindole-2-one.

ii) Synthesis of 2-Amino-1, 3, 4-thiadiazino [6, 5-b] indole:

3gm of 3-Thiosemicarbazide indole-2-one was mixed with small quantity of cold and 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 ammonia to obtain a solid mass. The solid mass was filtered by using Whatmann-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.

iii a) Synthesis of 2-[Benzylidenamino]-1, 3, 4-thiadiazino [6,5b] indole (Compound-I):

1.06gm of benzaldehyde and 2.02gm of 2-Amino-1, 3, 4-thiadiazino [6, 5-b] indole were taken in 50ml 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 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.

iii b) Synthesis of 2-[4-N,N-Dimethylamino benzylidenamino]-1, 3, 4-thiadiazino [6,5b] indole (Compound-II):

1.49 gm of p-N,N-Dimethylaminobenzaldehyde and 2.02gm of 2-Amino-1, 3, 4-thiadiazino [6, 5-b] indole were taken in 50ml of DMF. The mixture was refluxed for 6 hours in presence of glacial acetic acid. The completion of the reaction was checked by TLC and excess of methanol was distilled off. The refluxed mixture was poured into ice-cold water, filtered and washed with water and dried. The dried mass was recrystallized from ethanol.

iii c) Synthesis of 2-[4-Methoxy benzylidenamino]-1, 3, 4-thiadiazino [6,5b] indole (Compound-III):

1.36 gm of p-methoxybenzaldehyde and 2.02gm of 2-amino-1, 3, 4-thiadiazino [6, 5-b] indole were taken in 50ml of DMF. The mixture was refluxed for 6 hours in presence of glacial acetic acid. The completion of the reaction was checked by TLC and excess of methanol was distilled off. The refluxed mixture was poured into ice-cold water, filtered and washed with water and dried. The dried mass was recrystallized from ethanol.

2.3. Phase Solubility Measurements

The aqueous phase solubility of the compound was studied by Higuchi-Corner method at various concentrations of β -cyclodextrin (0-10mM) [13]. Accurately weighed sample of these compounds were shaken in rotary flash 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 these were analyzed in a UV-visible spectrophotometer. The various values of absorbance at λ-max were plotted against different concentrations of β –cyclodextrin.

2.4. Synthesis of inrigclusion complexes

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

2.5. Study of thermodynamic properties

The thermodynamic stability constant of the complexes was calculated using Benesi-Hilderband relation [15]. The stability constant K of each complex was calculated with increasing temperature. From the slope of the linear plot of lnK vs. 1/T, ΔH was calculated. Then ΔS was calculated from vant Hoff's equation

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

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

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

2.5. Evaluation of Antibacterial study

The antibacterial activity of compounds was studied as per cup-plate method. The solutions of the test compounds were prepared in dimethylsulfoxide (DMSO) at 500μg/ml. The bacterial strains were inoculated into 100ml of the sterile nutrient broth and incubated at $37\pm1^{o}C$ for 24 hours. The density of the bacterial suspension was standardized by McFarland method [16]. Well of uniform diameter (6mm) was made on agar plates, after inoculating them separately with the test organisms aseptically. The drug, control and the test compounds 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 hours of cold incubation, the petri plates were transferred to incubator and maintained at $37\pm2^{o}C$ for 24 hours. Then the petri plates were observed for zone of inhibition by using vernier 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 millimeter [17].

2.6. Evaluation of Antioxidant activity

In the present study DPPH (2,2-Diphenyl-1-picrylhydrazyl)scavenging assay method was used for screening the antioxidant activity of the synthesized compounds as suggested by Tagashira and Ohtake [18]. Test sample solution was prepared in 100μg/ml concentration in ethanolic DPPH. After vortexing, the mixture was incubated for 10 minutes at room temperature and the absorbance at 517 nm was measured. The difference in absorbance between a test sample and a control was considered as activity. BHT (Butylated Hydroxyl Toluene) was used as reference substance

3. RESULTS AND DISCUSSION

The structures of compound-I (Benzylidenamino-1, 3, 4-thiadiazino [6,5b] indole), compound-II (2-[4-N,N-Dimethyl benzylidenamino]-1, 3, 4-thiadiazino [6,5b]indole) and compound-III (2-[4-Methoxy benzylidenamino]-1, 3, 4-thiadiazino [6,5b]indole) have been confirmed from analytical and spectral data as shown in Table-1 and Table-2. The elemental composition matches with theoretical data (Table-1) and Infra-Red data indicate the presence of expected bonds and groups in the newly synthesized compounds (Table-2).

Table 1. Analytical data of Compounds with and without inclusion complex.
Compound-I : Benzylidenamino-1,3,4-thiadiazino[6,5b]indole.
Compound-II : 2-[4-N,N-Dimethyl Benzylidenamino]-1,3,4-thiadiazino[6,5b]indole.
Compound-III : 2-[4- Methoxy Benzylidenamino]-1,3,4-thiadiazino[6,5b]indole.

No.	Compound/ Complex	Melting Point	Color	Elemental Analysis (First line indicates finding value & second line indicates calculated value)				
1	Compound-I	224	Yellow	C 66.4 66.2	H 3.45 3.44	N 19.4 19.3	S 11.0 11.03	O
2	Compound-I- β-CD	228	Pale Yellow	-	-	-	-	-
3	Compound-II	216	Yellow	64.7 64.8	4.3 4.5	21.2 21.0	9.5 9.6	-
4	Compound-II- β-CD	223	Grey Yellow	-	-	-	-	-
5	Compound-III	232	Yellow	63.8 63.75	3.8 3.75	17.7 17.5	10.1 10.0	5.0 5.0
6	Compound-III -β-CD	237	Grey Yellow	-	-	-	-	-

Table 2. Spectral data of Compounds with and without inclusion complex.
Compound-I : Benzylidenamino-1,3,4-thiadiazino[6,5b]indole
Compound-II : 2-[4-N,N-Dimethyl Benzylidenamino]-1,3,4-thiadiazino[6,5b]indole
Compound-III : 2-[4- Methoxy Benzylidenamino]-1,3,4-thiadiazino[6,5b]indole

No.	Compound/ Complex	λ_{max} (nm)	IR(KBr) cm^{-1}
1	Compound-I	355.0	672(C-S) 1296(C-C) 1611(N-N) 1682(-C=N) 3141(Ring)
2	Compound-I- β- CD	354.2	670(C-S) 1290(C-C) 1605(N-N) 1679(C=N) 3130()Ring)
3	Compound-II	357.0	673 (C-S) 1302(C-C) 1623(N-N) 1734(-C=N) 3174(Ring)
4	Compound-II- β- CD	356.2	672 (C-S) 1301(C-C) 1620(N-N) 1732(-C=N) 3171(Ring)
5	Compound-III	355.6	677(C-S) 1213(C-C) 1466(C-N) 1575(N-N) 1707(C=N) 3133(Ring)
6	Compound-III -β- CD	355.0	673(C-S) 1210(C-C) 1464(C-N) 1570(N-N) 1698(C=N) 3118(Ring

The inclusion complex formation has been ascertained from significant changes in colour, melting point (Table-1), a blue shift of UV-Visible absorption maximum and shift in Infra-Red signals of characteristic absorption peaks towards lower energy (Table-2). The higher melting point of inclusion complexes than the compounds is due to the fact that extra amount of thermal energy is required for the latter to bring it out of β- cyclodextrin cavity. The shift in UV-Visible absorption maximum and Infra-Red signals of characteristic absorption peaks towards lower energy (Table-2) may be attributed to the transference of the compound from a more protic environment to a less protic environment within the cavity of β- cyclodextrin. Such changes in spectral characteristics due to inclusion complex formation may be attributed to the weak interactions like hydrogen bonding,

van der Waal's forces, hydrophobic interactions etc. between the guest compound and the host [19-20].

The aqueous phase solubility plots of the compounds in β- cyclodextrin solution are shown in Fig. 1. In all the cases, it is seen that there is a linear increase in solubility of these compounds with increasing the concentration of β- cyclodextrin. Since the slopes of all the plots are less than unity the stochiometry of these complexes may be 1:1 [21].
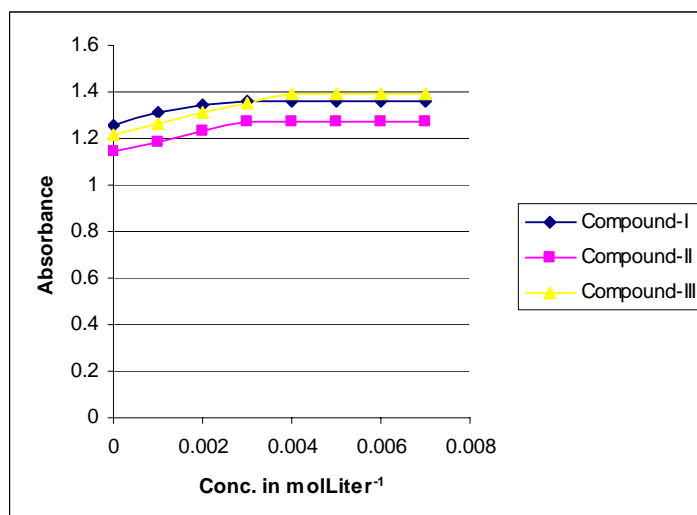

Fig. 1. Phase solubility plot of Compound I, II and III.

The thermodynamic stability constants (K_T) of inclusion complexes are determined by using Benesi-Hilderband relation [15]. Good linear correlations are obtained for plots of $1/\Delta A$ verses [β-CD]$_o$ for compounds I, II and III (Fig. 2). The values of K_T for all the 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 421,598,718 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]. The thermodynamic parameters associated with the interaction of the compounds with β- cyclodextrin for 1:1 stochiometry have also been calculated by determining stability constant (K- values) at different temperatures. The K- values are to found to decrease with rise in temperature as expected for an exothermic process [23-24]. The plots of ln K versus inverse absolute temperature produce linear plots (Fig. 3). From the slopes of the curves, van't Hoff's reaction isotherm and van't Hoff equation, the values of ΔG (change in free energy), ΔH (change in enthalpy) and ΔS (change in entropy) have been calculated at 298 K (Table-2). In Table-2, it is found that ΔG values are negative for all the inclusion complexes. These data clearly demonstrates that formation of inclusion complexes of compounds I, II and III with β- cyclodextrin is a spontaneous process. Further, it is found that in case of all three inclusion complexes, ΔH values are negative and ΔS values are positive (Table-2). The negative value of enthalpy change (ΔH) and positive value of entropy change (ΔS) indicate that all the three inclusion complex formations are energy allowed and entropy allowed processes [25-27]. Comparing the values of thermodynamic stability constants of the inclusion complexes, it is clear that the inclusion complex gets stabilized in presence of activating groups in the arylidene moiety irrespective of steric factor (Table-2).
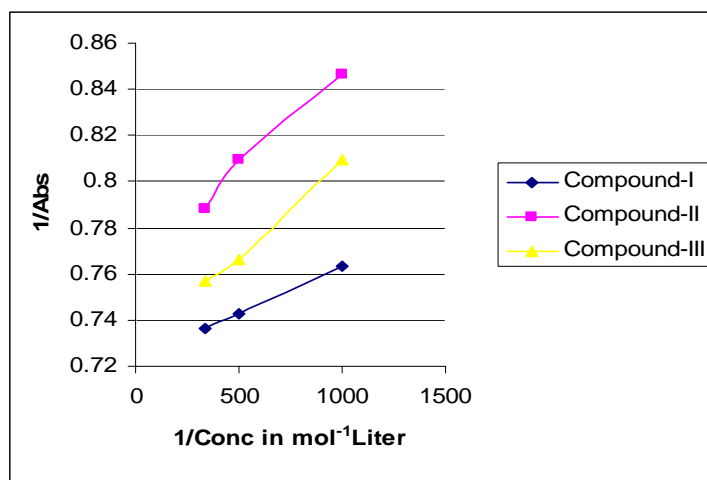
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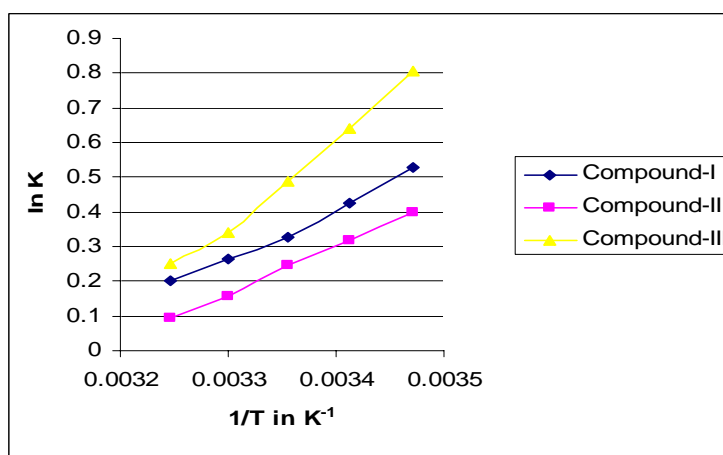**Fig. 2.** Plot of 1/ Conc. vs. 1/Abs. of Compound I, II and III.

Fig. 3. Plot of ln K vs. 1/T of Compound I, II and III.

The antibacterial activities of the compounds and their inclusion complexes against *S.aureus* and *E.coli* are shown in Fig. 4A and 4B. The compounds and their inclusion complexes are susceptible to both the bacteria. However, the inclusion complexes increase the antibacterial activity significantly as compared to their corresponding compounds. This may be attributed to enhanced solubility of the compounds after the inclusion complex formation which becomes more available to specific tissues leading to increased antibacterial activity [16-17].
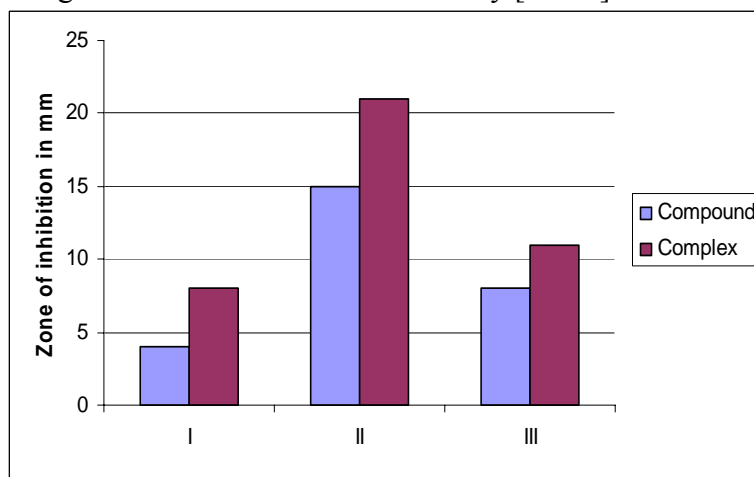
Fig. 4A. Antimicrobial susceptibility test of Compound I, II and III against *S.aureus*.
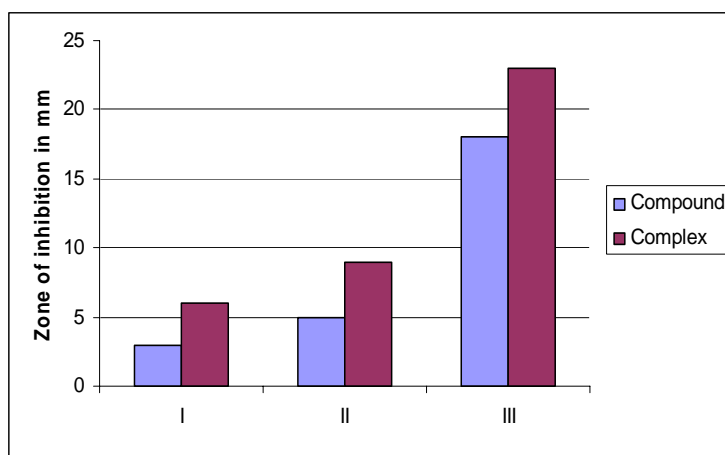
Fig. 4B. Antimicrobial susceptibility test of Compound I, II and III against *E.coli*.

The antioxidant activities of the compounds and their inclusion complexes are shown in Fig. 5. The radical scavenging activities of the compounds increase significantly after the formation of inclusion complex. This can be correlated to the higher stability of the compounds due to inclusion complex formation thereby increasing the bioaccessibility [18].
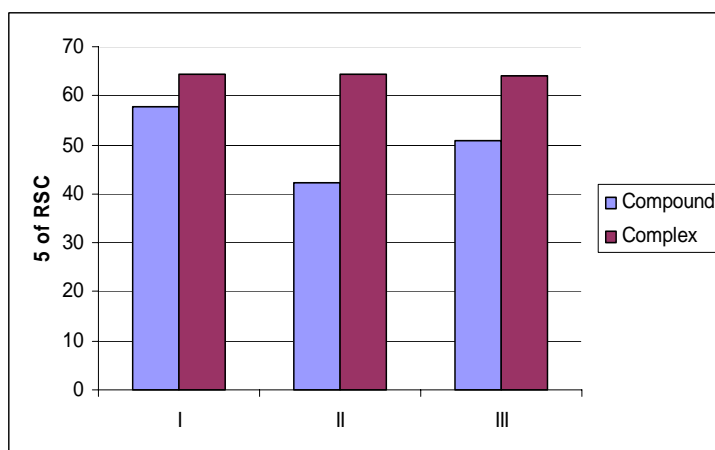

Fig. 5. Anti-Oxidant activity of Compound I, II and III

4. CONCLUSION

From the above results and discussion, it is clear that the formation of inclusion complexes of compound-I, II and III 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 antibacterial activity and antioxidant activity.

ACKNOWLEDGEMENT

Scheme-I

R= H, p-N(CH₃)₂ , p-OCH₃
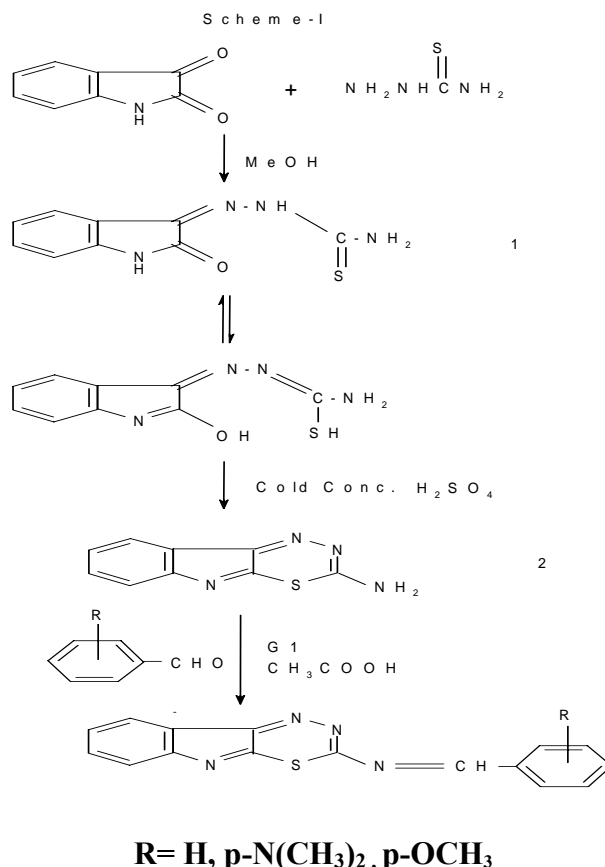
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