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Study of antioxidant properties of 1-nicotinoyl-4-aryl-3-methyl 3a, 4-dihydropyrazole [3, 4c] pyrazoles and their inclusion complexes with β-cyclodextrin

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ABSTRACT

Three different fused pyrazoles with nicotinyl moiety namely 1-nicotinoyl-4-phenyl-3-methyl-3*a*,4-dihydropyrazolo[3,4-*c*]pyrazole,1-Nicotinoyl-4-(4-bromobenzylidene)-3-methyl-3*a*, 4-dihydropyrazolo[3,4-*c*]pyrazoleand1-Nicotinoyl-4-(3-nitrobenzylidene)-3-methyl-3*a*,4-dihydropyrazolo [3,4-*c*]pyrazole have been synthesised and characterised spectroscopycally. To enhance their solubility in polar medium, their inclusion complexes have been prepared with β-cyclodextrin. The formation stability of inclusion complexes has been ascertained from the study of spectral and thermodynamic properties. Finally, the compounds and their inclusion complexes have been screened for antioxidant and anthelmintic activities. It is found that there happens a significant increase in antioxidant activities due to the formation of inclusion complexes.

Key words: Fused pyrazoles, β-cyclodextrin, inclusion complexes, Antioxidant activity.

1. INTRODUCTION

Antioxidant plays a significant role in modulating a number of physiological and pathological activities in all living organisms. [1] Antioxidants have ability to scavenge reactive oxygen species and free radicals thereby preventing a number of diseases [2-5]. So for the prevention and treatment of diseases caused by free radicals or reactive oxygen species, it is important to find effective antioxidants for scavenging such dreaded species.

Pyrazoles and fused pyrazoles exhibit a wide spectrum of biological and pharmacological activities. Some important activities of these compounds include antifungal, antibacterial antidepressant, ant tubercular, insecticidal[6-9] etc. Secondly there are also reports that fused pyrazoles coupled with a nicotinoyl unit, are showing excellent antimicrobial activities [10]. Since the bio accessibility of the compounds depends upon its solubility, one of the factors limiting the pharmacological activities of these compounds may be their poor solubility in polar medium [11]. . The solubility and bio accessibility of these compounds may be enhanced by forming inclusion complex with β- cyclodextrin, nontoxic cheaper oligosaccharides [12-13].

In the present work, an attempt has been made to synthesise some fused bis pyrazoles with nicotinoyl unit such as of 1-Nicotinoyl-4-aryl-3-methyl 3a, 4-dihydropyrazole [3, 4c] pyrazoles in their purest forms and to prepare their inclusion complexes with β- cyclodextrin. The formation of inclusion complexes has been ascertained by the study of the physical and spectral characteristics. Finally antioxidant activities of the compounds and their inclusion complexes are studied to know whether the inclusion complex formation has any impact on their biological activities.

2. MATERIALS AND METHODS

2.1. 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. IR spectra are recorded in KBr pellets in Perkin-Elmer-1800 FT-IR spectrophotometer, and ^1H NMR spectra (DMSO-d_6) are scanned on a DRX-300 (300MHz) spectrophotometer using TMS as internal standard and chemical shifts are expressed in δ, ppm. Purity of synthesized compounds has been checked by elemental analysis and homogeneity has been checked by TLC using silica gel-G, as

adsorbent. Melting points are recorded by open capillary method.

2.2. Synthesis of 1-Nicotinoyl-4-aryl-3-methyl 3a, 4-dihydropyrazole [3, 4c] pyrazoles

The synthesis of the compounds has been done in three steps as shown in scheme-I [10].

2.2.1. Synthesis of 2-nicotinoyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one

A mixture of nicotinic hydrazide (pyridine-3-carbohydrazide)(1.4g,0.01mole)and ethyl acetoacetate(1.3g,0.01mole) is taken in dry ethanol(10mL) and refluxed for 40hr.Excess of solvent is distilled off and the resultant residue is poured on crushed ice to obtain the pale white coloured residue (Compound-I).

IR(KBr):3101(CHstr.,ArH),2948(CHstr.CH3),1687,1654(C=Ostr.), 1600cm-1(C=Nstr.);1HNMR(DMSO-d6):54-8.79(m,4H,Ar-H),4.89(s,2H,CH2),2.26(s,3H,CH3)

2.2.2. Synthesis of 4-Benzylidene-2-nicotinoyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one (A)

Compound-1(0.20g, 0.001mole) is dissolved in a buffer solution of 10ml acetic acid and anhydrous sodium acetate(0 .082g, 0.001mole) and benzaldehyde(0.106g,0.001mole) is added to it. The resultant reaction mixture is refluxed for 12hr, cooled, filtered and poured on crushed ice and kept for sometimes. Solid 4-(bezylidene)-2-nicotinoyl-5-methyl-2, 4-di-hydro-3H-pyrazol-3-one(Compound-A) has been gradually appeared which has been filtered and dried.

Characteristics of compound (A):IR(KBr):3101(C-Hstr.,Ar-H), 2922(C-Hstr.,CH), 1709(C=Ostr.), 1592cm-1(C=Nstr.); 1HNMR(DMSO-*d*6):d7.09-8.01(m,9H,Ar-H),6.22(s,1H,=CH-Ar),2.10(s,3H,CH3).

Similarly, compounds B (4-(4-bromobenzylidene)-2-nicotino-yl-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one) and C(4-(3-nitrobenzylidene)-2-nicotino-yl-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one)have been prepared with some change in reflux time and reaction work-up. Their characteristic spectral data are given below:

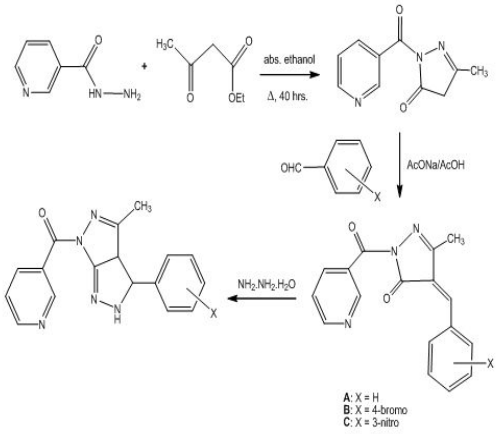
Characteristics of (B): IR(KBr):3093(C-Hstr.,Ar-H),2913(C-Hstr.,CH3),1711(C=Ostr.), 1600(C=Nstr.),738cm-1(C-Br str.); 1HNMR(DMSO-*d*6)d6.94-7.94(m,8H,Ar-H),6.26(s,1H,=CH-Ar),2.12(s,3H,CH3).

Characteristics of (C): IR(KBr):3091(C-Hstr.,Ar-H), 2916(C-Hstr.,CH), 1717(C=Ostr.), 1589cm-1(C=Nstr.); 1HNMR(DMSO-*d*6):d7.07-7.99(m,8H,Ar-H),6.19(s,1H,=CH-Ar), 2.17(s,3H,CH3).

2.2.3. Synthesis of 1-nicotinoyl-4-phenyl-3-methyl-3a,4-dihydropyrazolo[3,4-c] pyrazole(D)

Compound A (0.34g, 0.001mole) and hydrazine hydrate (0.002mole) aretaken in dry ethanol (10mL) and a few drops of acetic acid (as catalyst)is added to it. It is then refluxed for 9hr, concentrated, cooled and poured on crushed ice. The product obtained is washed several times with water and then dried (D).

Similarly compounds E(1-Nicotinoyl-4-(4-bromobenzylidene)-3-methyl-3*a*,4-dihydropyrazolo[3,4-*c*]pyrazole) and F(1-Nicotinoyl-4-(3-nitrobenzylidene)-3-methyl-3*a*,4-dihydropyrazolo[3,4-*c*]pyrazole) are prepared with some change in reaction conditions. Their characteristic spectral and analytical data are given in table1 and 2.



Scheme-I

2.3. Synthesis of inclusion complexes

The inclusion complexes of the compounds with β- cyclodextrin have been prepared as per co-precipitation method [14-18]. 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.

2.4. 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 solutions are prepared in different

concentration (500µg/ml, 100µg/ml and 50µg/ml) in ethanolic DPPH. After vortexing, the mixtures are incubated for 10 minutes at room temperature. The absorbance 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

3. RESULTS AND DISCUSSION

The synthesis of compounds has been confirmed from physical data (Table 1) and spectral data (Table 2). The elemental composition matches with theoretical data (Table-1).The Infra-Red and NMR data indicate the presence of expected bonds and groups in the newly synthesized compounds. The inclusion complex formation has been ascertained from significant changes in colour, melting point (Table-1), a shift in UV-Visible absorption maximum, changes in Infra-Red and NMR signals (Table-2) The higher melting point of inclusion complexes than the compounds may be attributed to the fact that extra amount of thermal energy is required for the latter to bring it out of β-cyclodextrin cavity. The changes in UV-Visible, Infra-Red and NMR signals of characteristic peaks (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 due to the weak interactions like hydrogen bonding, vander Waal's forces, hydrophobic interactions etc. between the guest compound and the host as proposed earlier [17-20].

The antioxidant activities of the compounds and their inclusion complexes at different concentrations are shown in Table-3 and Figure 1, 2 and 3. The radical scavenging activities of the compounds increases significantly after the inclusion complex formation (Table-3 and Figure 1, 2 and 3).This can be attributed to higher solubility of the compounds in polar medium after their inclusion complex formation which enhances their bio accessibility [21]. Higher bio accessibility of the compounds makes them more vulnerable to trap free radicals and/or reactive oxygen species, thereby increasing anti oxidant activity. Further it is seen that there happens an increase in radical scavenging activities of the compounds with an enhancement in their concentration (Figure 1, 2 and 3). This may be due to a direct correlation in between the number of molecules and their radical scavenging activities. Larger the number of molecule, higher becomes their free radical trapping ability [22-26].

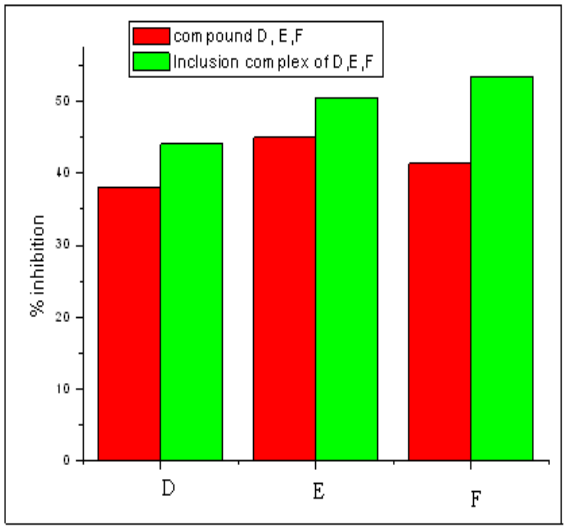


Figure - 1: Percentage of inhibition at 500µg/ml of sample with DPPH

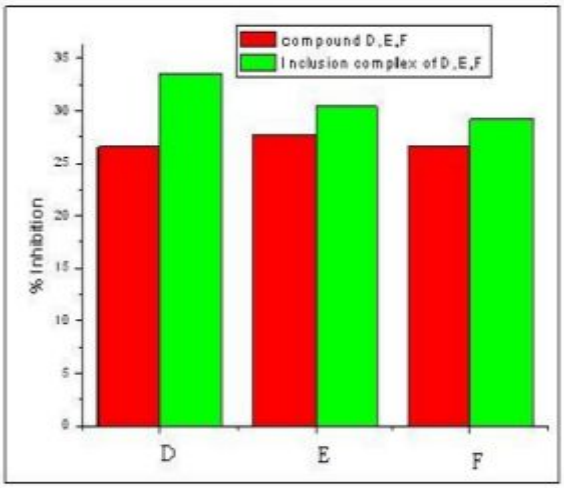


Figure - 2: Percentage of inhibition at 100µg/ml of sample with DPPH

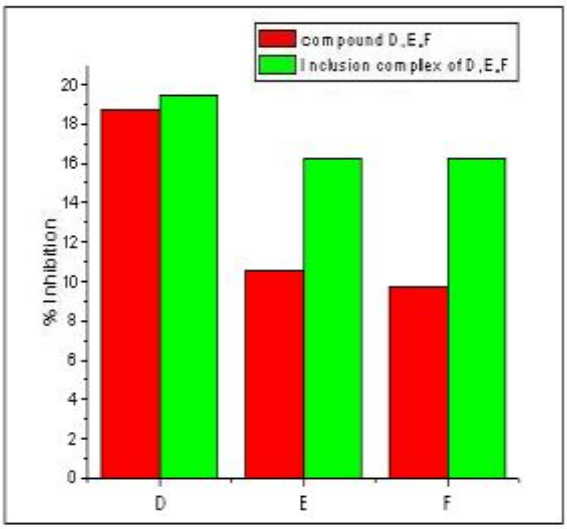


Figure - 3: Percentage of inhibition at 50µg/ml of sample with DPPH

Table - 1: Physical properties of the compounds with and without inclusion complex

Compound/ complex	Colour	Melting Point (°C)	Elemental Analysis (%) (Theoretical) (Experimental)			
			C	H	N	O
Compound- D	Bright white	221	67.1	4.6	23.1	5.2
Compound- D With β-CD	Dull White	273	67.2	4.7	23.0	5.1
Compound- E	Pale yellow	206	52.9	3.6	18.2	4.2
Compound- E With β-CD	Pale white	281	53.0	3.7	18.2	4.1
Compound- F	yellow	220	58.2	4.1	24.0	13.7
Compound- F With β-CD	Pale yellow	288	58.3	4.0	23.9	13.8

Compound-D: 1-Nicotinoyl-4-phenyl-3-methyl-3a,4-dihydropyrazolo[3,4-c]pyrazole.
Compound-E:1-Nicotinoyl-4-(4-bromobenzylidene)-3-methyl-3a,4-dihydropyrazolo [3, 4-c] pyrazole.
Compound-F:1-Nicotinoyl-4-(3-nitrobenzylidene)-3-methyl-3a,4-dihydropyrazolo[3, 4-c] pyrazole.

Table - **2 :** Spectral data of the compounds with and without inclusion complex

Compound/ Complex	UV λ_{Max}(nm)	IR(KBr) cm^{-1}	NMR
Compound- D	262	3392(N-H str), 3019(C-H str, Ar),1650(C=O str), 1541(C=N str)	δ 8.78(s,1H, NH) 7.04-7.86(m,9H,Ar-H), 4.88-4.89(dd,2H,CH-CH),2.14(s,3H,CH_3),1.612(s), 1.427(s), 0.880(t)
Compound- D With β-CD	264	3401(N-H str), 1651(C=O str), 1403(C=N str),	δ 7.264(s,1H, NH), 2.3(d),2.329(s),1.576 (s), 0.859(t)
Compound- E	261	3617, 3385(N-H str),3020(C-H str, Ar),1650(C=O str), 1534(C=N str), 762(C-Br str)	δ 8.601(s,1H, NH) 7.729-7.263(m,8H,Ar-H), 2.352(s,3H,CH_3),1.612(s), 1.427(s), 0.880(t)
Compound- E With β-CD	263	3396(N-H str),3021(C-H str, AR),1648(C=O str), 1534(C=N str), 761(C-Brstr	δ 7.263(s,1H, NH) 7.729-7.263(m,8H,Ar-H), 2.355(s,3H,CH_3),2.329(s),1.571-0.832(s), 0.880(t)
Compound- F	261	3411(N-H str),3021(C-H str, Ar),1646(C=O str), 1530(C=N str)	δ 8.85(s,1H, NH) 7.09-7.87(m,8H,Ar-H),4.80-4.81(dd,2H,CH-CH), 2.05(s,3H,CH3)
Compound- F With β-CD	262	3434(N-H str),3019(C-H str, AR),1650(C=O str), 1529(C=N str)	δ 7.264(s,1H, NH) 7.09-7.87(m,8H,Ar-H),4.181-4.159(dd,2H,CH-CH), 1.574(s,3H,CH_3)

Compound-D: 1-Nicotinoyl-4-phenyl-3-methyl-3a,4-dihydropyrazolo[3,4-c]pyrazole.
Compound-E: 1-Nicotinoyl-4-(4-bromobenzylidene)-3-methyl-3a,4-dihydropyrazolo[3, 4-c] pyrazole.
Compound-F:1-Nicotinoyl-4-(3-nitrobenzylidene)-3-methyl-3a,4-dihydropyrazolo[3,4-c] pyrazole.

Table-3: Anti-oxidant studies of the compounds with and without inclusion complex

Compound	Absorbance(517 nm)		
	Conc.(500µg/ml)	Conc.(100µg/ml)	Conc.(50µg/ml)
Compound-D	0.761	0.903	1.00
Compound- D with β-CD	0.688	0.817	0.991
Compound-E	0.677	0.889	1.10
Compound- E with β-CD	0.609	0.856	1.03
Compound-F	0.621	0.902	1.11
Compound- F with β-CD	0.573	0.871	1.03
Control		1.23	
Standard(ascorbic acid)	0.123	0.316	0.429

% Inhibition = $(A_o-A_1)/A_o \times 100$

where A_o was the absorbance of the control and A_1 was the absorbance of the sample.

Compound- D: 1-Nicotinoyl-4-phenyl-3-methyl-3a,4-dihydropyrazolo[3,4-c]pyrazole.

Compound-E:1-Nicotinoyl-4-(4-bromobenzylidene)-3-methyl-3a,4-dihydropyrazolo[3, 4-c] pyrazole.

Compound- F: 1-Nicotinoyl-4-(3-nitrobenzylidene)-3-methyl-3a,4-dihydropyrazolo[3, 4-c] pyrazole.

4. CONCLUSION

From the above results and discussion, it is clear that the formation of inclusion complexes of compounds (D, E and F) causes a significant increase in antioxidant activity and can be a very good analytical tool for enhancing the bio accessibility of the drugs.

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