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Spectrophotometric determination of 6-(trifluoro methyl) furo [2,3-b] pyridine-2- carbohydrazide derivatives as antioxidants

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ABSTRACT

A novel Antioxidant study of 6-(trifluoro methyl) furo [2, 3-b] pyridine-2- carbohydrazide derivatives, a sensitive spectrophotometric method was employed. The method is based on the reaction of these drugs as n-electron donors with the pi-acceptor 2,3,5,6-tetra chloro 1,4-nenzo quinone. The obtained coloured charge-transfer complex was measured at 440nm. The obtained complexes were confirmed by using the ^{1}HNMR and IR spectral analysis. The proposed procedure could be applied successfully to the determination of the Free radical Scavenging activity of the title compounds. Among the Twelve analogs, V_3 exhibited highest antioxidant activity and very low IC_{50} value. The association constants and standard free energy changes using Benesi-Hildebrand plots were studied.

Key words: Pyridine-2-carbohydrazides, spectrophotometer, bioassay, free energy, antioxidants.

INTRODUCTION

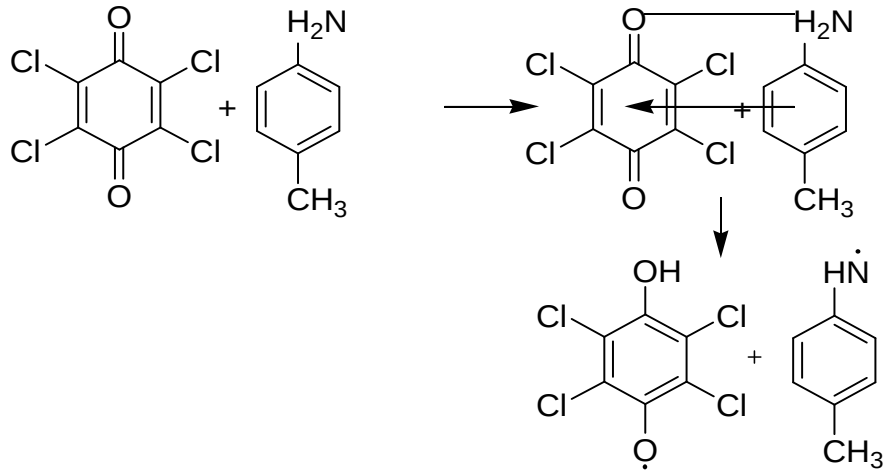
Aromatic heterocyclic compounds are known to form electron donor acceptor complexes with a number of electron acceptors. Although the amines possess considerable donor strength because of their low ionization potentials, these molecules cannot be classified into n-donors or pi-donors due to the lone pair orbital on the nitrogen atom of the amino group entering in conjugation with the pi- orbital of the aromatic ring.

2,3,5,6-tetrachloro-1,4-benzoquinone(choranil) is used for dehydration of hydro aromatic compounds and it is insoluble in water. Chloranil is sensitive to excessive light and heat. It is incompatible with strong oxidizing agents. It is a good electron acceptor.

Intermolecular charge-transfer complexes are formed when electron donor and electron acceptors were interacting. It is a general phenomenon in organic chemistry[1] and Mulliken[2] considered, such complexes arise from a Lewis acid-Lewis base type of interaction, where bond between the components of the complex being postulated to arise from the partial transfer of an electron from the base to the empty orbital of the acceptor. Charge transfer complexes have unique absorption bands in the ultraviolet-visible region. Some of the charge transfer complexes containing chloranil as an acceptor have been reported e.g. Tetra Thio Fulvalene-Chloranil[3], Tetra Thio Fulvalene-imidazole-Chloranil[4], p-phenylenediamine-Chloranil[5], Tetra Methyl-p-Phenylene Diamine-Chloranil[6], Aniline-Chloranil [7], aromatic amines and nitrogen heterocycles-Chloranil[8], N-Aryldithiocarbmates-Chloranil[9], phosphine oxide and tri-n-butylphosphate-Choranil[10] etc.

The molecular interactions between electron donors and acceptors are generally associated with the formation of intensity colored charge transfer complexes, which absorb radiation in the visible region [11]. The spectrophotmetric methods based on these interactions are usually simple and convenient because of the rapid formation of the complexes.

6-(trifluoro methyl) furo[2,3-b] pyridine-2- carbohydrazide derivatives are good n-electron donors and form charge-transfer complexes with pi-acceptors. Pi-acceptors such as 7,78,8-tetracyano-quinodimethane(TCNQ), tetra cyanoethylene(TCNE), 2,3,-dichloro-5,6-dicyano-1,4-benzo Quinone(DDQ), 2,3,5,6-tetrabromo-1,4-benzoquinone (bromanil) and 2,3,5,6-tetrachloro-1,4-benzoquinone(choranil) are known to yield charge-transfer complexes and radical anions with a variety of electron donors[12-13]. The stable 2,3,5,6-tetrachloro-1,4-benzoquinone(chloranil) was formed Charge Transfer complex[14] with the P-toluidine are shown in Scheme.1. It is reported that chloranil forms charge transfer complex with p-toluidine the reaction is shown in Scheme.1.



Scheme.1

Free radical species are known to play important roles in biological systems such as mitochondria, signal transductions, and the immune system. In the human body the free radicals are continuously produced due to the oxygen utilization by the cells of the body. This generates a series of Reactive Oxygen Species (ROS) like superoxide anion (O_2^-) and hydroxyl ($HO^\cdot$) radicals and non- free radical species such as H_2O_2 singlet oxygen (O_2) and nitric oxide (NO).

Natural antioxidants like catalase, superoxide dismutase, and glutathione peroxide are in the body[15]. ROS are highly reactive and can easily react with almost all the biological molecules including DNA, proteins, lipids and lipoproteins [16]. Antioxidants are of great interest because of their involvement in important biological and industrial processes. The main characteristic of an antioxidant is its ability to trap free radical. In general, compounds with antioxidant activity have been found to possess anticancer, anti-cardiovascular, anti-inflammation and many other activities [17-19]. The antioxidants are molecules that are mainly decelerate or prevent the oxidation reaction in vitro by terminating the oxidation of chain reaction [20]. The application of antioxidants in pharmacology is valuable to improve current treatments for diseases.

6-(trifluoro methyl) furo[2,3-b] pyridine-2- carbohydrazide derivatives are chemical agents that exert their principle pharmacological and therapeutic effects by acting at peripheral sites to either enhance or reduce the activity of components of the sympathetic division on autonomic nervous system[21]. These derivatives are used mainly in anticancer and antioxidants. Spectrophotometric methods can be used for the quantitative determination of title compounds.

This study describes simple, direct, sensitive, accurate and precise spectrophotometric methods for the determination of 6-(trifluoro methyl) furo [2,3-b] pyridine-2- carbohydrazide derivatives via reaction with Pi-acceptors in their common dosage forms irrespective of the presence of contaminants or additives.

EXPERIMENTAL SECTION

2.1 Materials

2,3,5,6-tetrachloro-1,4-benzoquinone(chloranil) was purchased from the Avra Synthesis. Pvt. Ltd. L-Ascorbic acid, 1,4-dioxane and Acetone solvents were purchased from the SD Fine- Chem Pvt. Ltd. 6-(trifluoro methyl) furo[2,3-b] pyridine-2- carbohydrazide derivatives was collected from Fluoro Organics Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad.

2.2 Apparatus

Elico SL171-Elect Spectrophotometer with matched 1cm glass cuvettes, Dhona 200D weighing balance, calibrated flasks, beakers, measuring jar was used.

2.3 Preparation of Reagent Solution

Into a 50ml Calibrated flask, 250mg of 2,3,5,6-tetrachloro-1,4-benzoquinone(chloranil) was weighed accurately and dissolved in 2ml of 1,4-dioxane ,and the volume made up to the mark with the same solvent .It was then diluted quantitatively to obtain the suitable concentration.

2.4 Preparation of Standard Stock Solution

Into a 10ml Calibrated flask, 0.5682mM concentration of Ascorbic acid was prepared by weighing 1mg of ascorbic acid accurately and dissolving in 2ml of 1,4- dioxane. The volume was made upto the mark with the same solvent. It was used as standard and by maintaining the standard concentration each time 10ml of 6-(trifluoro methyl) furo[2,3-b] pyridine-2- carbohydrazide analogs solution was prepared.

2.5 General Analytical Procedure

In 10 ml Calibrated flasks, 9ml of 6-(trifluoro methyl) furo[2,3-b] pyridine-2- carbohydrazide analogs solution was placed and then 1ml of the reagent was added . The absorbance of the solution was measured at the wave length of maximum charge transfer bands i.e.at 440nm after the appropriate time interval at room temperature against reagent blank. Absorbance was recorded and percentage of Radical Scavenging Activity [22] was calculated using the following formula.

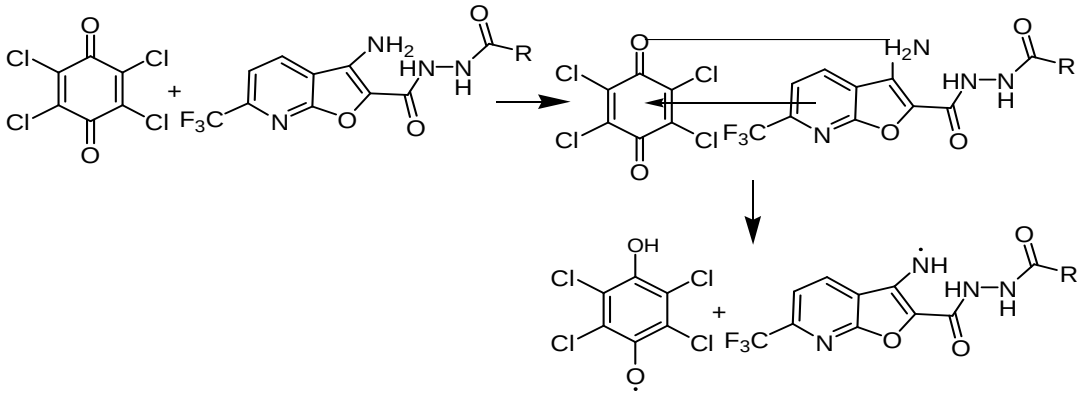
$$\%\text{Radical Scavenging Activity} = \frac{Ab-Aa}{Ab} X100$$

Where Ab=is the absorption of blank sample Aa=is the absorption of the test sample

Sample concentration providing the 50% Radical Scavenging activity is taken as IC_{50} value.

2.6 Reaction with Chloranil

On studying the 6-(trifluoro methyl) furo[2,3-b] pyridine-2- carbohydrazide-chloranil charge transfer complex, the maximum peak was exhibited at 440 nm. Scheme.2 indicates the formation of charge transfer complex. The formed new bond was attributed to an electron transfer complexation reaction between 6-(trifluoro methyl) furo [2,3-b] pyridine-2- carbohydrazide analogs as donor and chloranil as electron acceptor followed by formation of free radicals[23].



Scheme.2

2.7 BIOASSAY

In the present study bioassay of various 6-(trifluoro methyl) furo[2,3-b] pyridine-2- carbohydrazide were determined and expressed as IC_{50} value. Therefore Cecil-Elect Spectrophotometer in which Absorbance of title compounds are noted with time and bioassay was evaluated. The antioxidant activity of the synthesized drugs and the standard was assessed on the basis of the radical scavenging effect of the stable 2,3,5,6-tetrachloro-1,4-benzoquinone(chloranil)free radical activity by modified method.

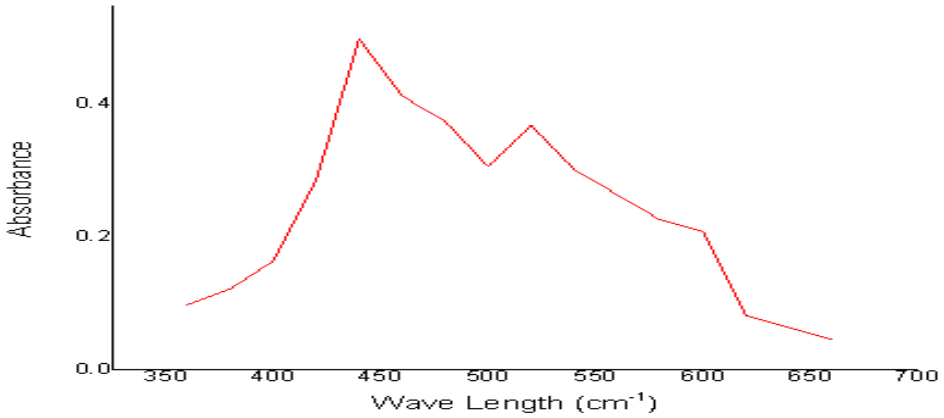


Figure.1 Absorption Spectrum of 3-amino-N'-isobutyryl-6-(trifluoromethyl)furo[2,3-b]pyridine-2-carbohydrazide with Chloranil in 1,4-dioxane. Blank: 1,4-dioxane

2.8 STOICHIOMETRIC STUDY

Jobs method of continuous variation [24] was employed. Master equimolar solutions of each drug with chloranil(80mM) were prepared in 10ml of 1,4-dioxane, and that solution was used to prepare the 40mM,20mM,10mM,5mM,2.5mM concentrations with the same solvent and the Absorbance of the different concentrations was noted down . A series of 10ml portions of master solutions of each drug with the acceptor was made up comprising different complementary proportions(0:10.1:9,2:8,3:7,4:6,5:5,6:4,7:3,8:2,9:1,10:0) in 10ml calibrated flasks. The absorbances of the resulting solutions were measured at the wavelength of maximum absorption after the appropriate time, against reagent blanks. Plots of Time Vs Absorbance and Concentration Vs Absorbance are shown in figure 2 and 3.

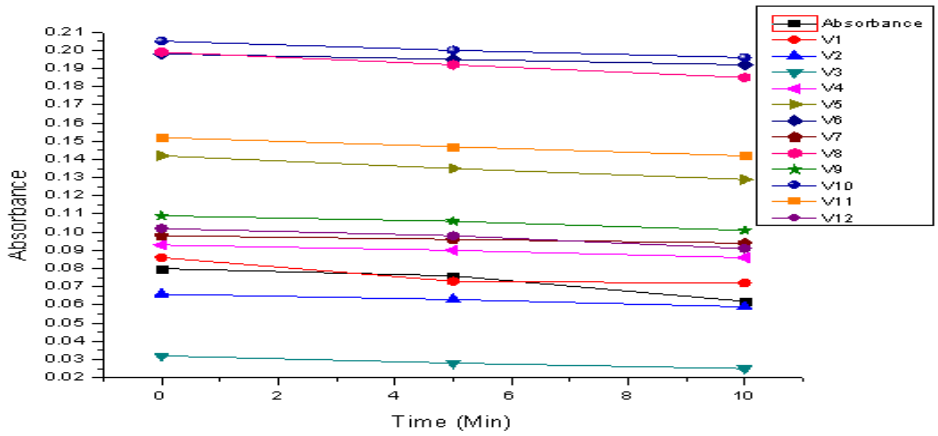


Figure.2

2.9 ASSOCIATION CONSTANTS AND STANDARD FREE ENERGY CHANGES

The association constants were calculated for the interaction of each drug with chloranil complex using Benesi-Hildebrand equation [25].

$[A_0]/A^{AD} = 1/\varepsilon^{AD} + 1/K_c^{AD}.\varepsilon^{AD} \times 1/[Do]$

Where [Ao] and [Do] are the concentrations of the acceptor and donor respectively, A^{AD} is the absorbance of the complex, ε^{AD} is the molar extinction coefficient of the complex and K_c^{AD} is the association constant of the complex. From the above equation, on plotting the values of $[Ao]/A^{AD}$ Versus 1/[Do], Straight lines were obtained .The standard free energy were calculated from the association constants by the following equation[26].

$$\Delta G^0 = -2.303RT\log Kc^{AD}$$

Where ΔG^0 is the free energy change of the complex in Kcal/mole, R is the gas constant [1.987cal/mol kelvin] T is the temperature in kelvin and K_c^{AD} is the association constant of drug-acceptor complex. The high values of association constants are common in n-electron donors where the inter molecular overlap may be considerable.

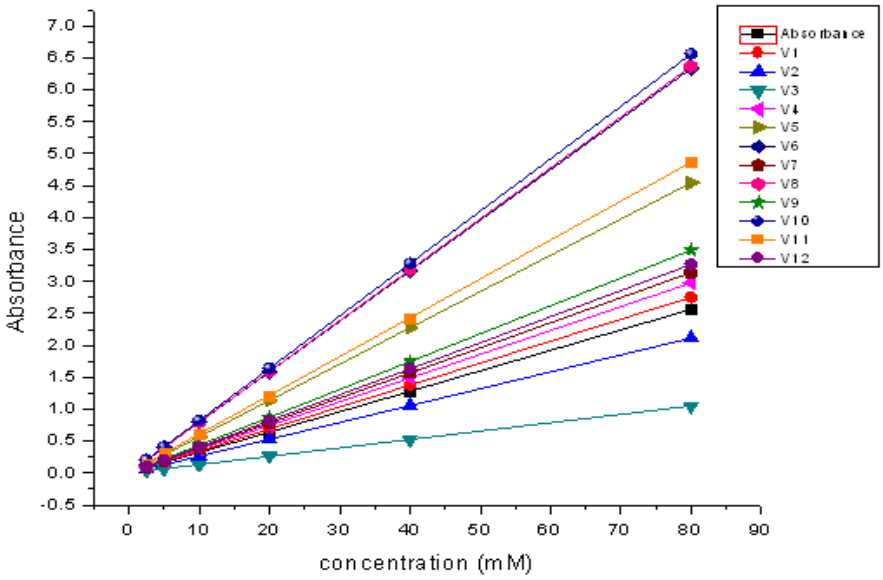


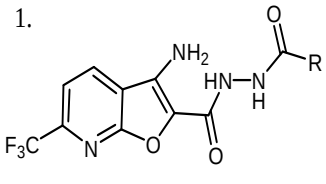
Figure.3

Table 1

COMPD	A^{AD}	ε^{AD}	$K_c^{AD} X10^3$ mol^{-1}	ΔG^0[Kcal/mol]
V1	0.086	34.4	292.63564	-10089.7
V2	0.066	26.4	172.352738	-9149.18
V3	0.033	13.12	42.8637686	-6676.88
V4	0.093	37.2	342.21277	-10367.8
V5	0.142	56.8	797.823829	-11871.7
V6	0.198	79.2	1551.17464	-13053
V7	0.098	39.2	379.999011	-10553.9
V8	0.199	79.6	1566.88264	-13070.9
V9	0.109	43.6	470.092487	-10931.9
V10	0.205	82	1662.79242	-13176.4
V11	0.152	60.8	914.150057	-12113.5
V12	0.102	40.8	411.652406	-10696
Ascorbic acid	0.08	32	253.227163	-9832.76

BASIC STRUCTURES

1.



2.

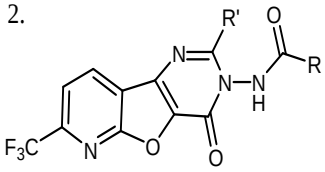
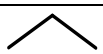
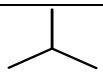
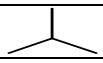
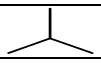


Table 2

Compd	R	R^1	% of Radical Scavenging	IC50value (inug/ml)	Activity
V_1			92.51	114.24	0.942182
V_2			93.54	108.73	0.963651
V_3			97.13	96.52	1.015368
V_4			90.77	103.28	0.985984
V_5			86.15	104.21	0.982103

V_6			80.00	122.16	0.913062
V_7	CH3	CH3	89.95	102.96	0.987331
V_8			79.48	146.56	0.833985
V_9			89.13	121.76	0.914495
V_{10}			79.49	156.54	0.805375
V_{11}			84.92	118.43	0.926538
V_{12}			89.95	120.97	0.917322
Ascorbic acid			91.63	54.57	1.2624

Ascorbic acid is used as standard

RESULTS AND DISCUSSION

The infrared (IR) spectra were determined in a Perkin-Elmer Fourier transform (FDIR spectrum). ^{1}H-NMR spectra were recorded on Varian EM-360 (300MHz mercury plus) spectrometer in DMSO-d_6 or $CDCl_3$ and calibrated using solvent signals [7.25($CDCl_3$) and 2.50(DMSO-d_6)]. All chemical shifts recorded in δ (ppm) using TMS as an internal standard.

CT complex of 3-amino-N'-isobutyryl-6-(trifluoromethyl)furo[2,3-b]pyridine-2-carbohydrazide and chloranil
^{1}H NMR δ (300 MHz, $CDCl_3$): 8.69-8.68 (d, 1H, J, 8.61), 8.23(s,1H), 7.91-7.88(d, 1H, J,7.92), 2.86-2.70 (m, 2H), 2.51-2.27(m, 4H), 2.20(s, 1H), 1.62(m, 8H), 1.61(m, 4H) 1.32-1.29(m, 2H).

CT complex of N-(3-isobutyl-1-oxo-7-(trifluoromethyl) pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2(1H)-yl)-3-methylbutanamide and chloranil
^{1}H NMR δ (300 MHz, $CDCl_3$): 8.69-8.68 (d, 1H, J, 8.61), 8.23(s,1H), 7.91-7.88(d, 1H, J,7.92), 2.86-2.70 (m, 2H), 2.51-2.27(m, 4H), 1.62(m, 8H), 1.32-1.29(m, 2H), 1.12-1.05(m, 13H).

The ^{1}HNMR data of the charge transfer complexes of the 3-amino-N'-isobutyryl-6-(trifluoromethyl)furo[2,3-b]pyridine-2-carbohydrazide, N-(3-isobutyl-1-oxo-7-(trifluoromethyl)pyrido[3',2':4,5]furo[3,2-d]pyrimidin-2(1H)-yl)-3-methylbutanamide was matched with the experimental evidence of the ^{1}HNMR. The δ chemical shifts of all protons of the different groups are exactly matched with charge transfer complexes. So, it is the best evidence for the formation of CT complex.

3.1 IR SPECTRUM OF V_3 AND V_8 COMPLEXES

The IR Spectrum of V_3 and V_8 complexes conformed that the new bond is formed between oxygen atom of the chloranil to the amine functional group of the drugs. The calculated vibrational absorption spectra are in good agreement with the experimental results recorded in the ground state infrared spectra of jet cooled for V_3 ,V_8 CT complexes in the 3100-3700cm^{-1} region[27].In the IR spectrum of CT complex 3426 cm^{-1} (amino internal donor NH toO=C<) and 3553 cm^{-1} (amino free NH).This indicates the formation of >C=O----H-N-Ar CT complex.Moreover,the >C=O , N-H ,stretching band of the intermolecular hydrogen bond of >C=O----H-N-Ar are also drastically red-shifted yet the >C=O,N-H and of >C=O----H-N-Ar are almost unchanged upon electronic excitation to the S_1 state of the hydrogen-bonded CT complexes of V_3,V_8.

6-(trifluoro methyl) furo[2,3-b] pyridine-2- carbohydrazide analogs were tested for antioxidant activity in the present experimental studies. As compared with the standard Ascorbic acid, the title compounds exhibited good antioxidant activity. The proposed procedure has the advantage in that most of the assays were performed in the visible region. The rapid development of colours at room temperature with non-corrosive reagents, the intensity, sensitivity and the stability of colours suggest obvious use of this method for the detection of the Antioxidant studies of the title compounds. Cancer is one of the diseases caused by the free radicals, so the title compounds exhibit good anti cancer and antioxidant activity.

Among the Twelve analogs studied, V_3 exhibited high antioxidant activity, very low IC_{50} value and high percentage of radical scavenging, which was followed by the analogs V_7, V_4 and V_5. The activity was least in V_{10} followed by V_8, both of which had high IC_{50} values of 156.54ug/ml and 146.56 ug/ml respectively.

CONCLUSION

A novel spectrophotometric method for the determination of antioxidant activity of the 6-(trifluoro methyl) furo[2,3-b] pyridine-2- carbohydrazide analogs using chloranil as reagent were studied in the present investigations. The present study, therefore confirms the suitability of chloranil for spectrophotometric analysis of title compound in the micro range. This method could also be applied to the quality control analysis of the investigated compounds.

Acknowledgements
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