SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME BARBITURIC ACID DERIVATIVES VIA SCHIFF´ S BASES

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Abstract

The present work includes the synthesis of different Schiff bases by the reaction of aniline with benzaldehyde and its derivatives. These Schiff bases reacted with phenoxy acid chloride to yield acetanilide derivatives. The synthesis of barbituric acid derivatives has been performed by the reaction of phenoxyacetanilide derivatives with guanidine carbonate and diethyl malonate (DEM) derivatives. Elemental analysis and FT-IR spectroscopy were used to characterize the prepared compounds.

The antibacterial activity of the products were studied in vitro using disc diffusion method against two pathogenic strains of bacteria (*Pseudomonas aeuroginosa and Staphylococcus aureus*) and showed high activity against both types.

تخليق ودراسة الفعالية البايولوجية لبعض احماض الباربيتيورك عن طريق قواعد شف

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الخلاصة

تضمن البحث تحضير قواعد شف مختلفة من تفاعل الانلين مع البنزالديهايد ومشتقاته تمت مفاعلة قواعد شف هذه مع مشتقات كلورايدات حامض الفينوكسي للحصول على مركبات الفينوكسي استنلايدات والتي بدورها تفاعلت مع كربونات الكواندين ثم مع مالونات ثنائي الاثيل للحصول على مشتقات حامض البربتيوريك. تم اعتماد قيم الكربون – الهيدروجين – النيتروجين وأطياف تحول فورير لتشخيص المركبات ألمحضره.

تمت دراسة الفعالية البيولوجية للمركبات ألمحضره تجاه نوعين من البكتريا هما (*Pseudomonas aeuroginosa and Staphylococcus aureus*) وقد أظهرت المركبات ألمحضره فعاليه قويه تجاه كلا النوعين المدروسين.

Introduction

Since 1886 [1] up now, Schiff bases have become increasingly important mainly due to their stability, ease of preparation, structural variability and variety of applications. It has been observed that several Schiff bases show fungicidal [2], anti-inflammatory [3], antibacterial [4], antiviral [5], antioxidant [6], anticancer [7], antibacterial, [8] antifungal, [9] and herbicidal activities [10].

Compounds containing pyrimidine ring play an important role in many biological systems, such as vitamins, several coenzymes and antibiotics where they exist in nucleic acids [11]. Pyrimidine compounds are also used as hypnotic drugs for the nervous system [12]. Barbiturates are a class of drugs that are utilized as anesthetics sleeping agents, and for the treatment of anxiety, epilepsy and other psychiatric disorders. They possess effects on the motor and sensory functions [13, 14]. The complexation of barbituric acid with different metals such as Cr(III), Mn(II), Fe(III), Zn(II) and Cd(II) has been studied and showed high effect on different fungi [15].

Keeping this in view we have now synthesized new barbituric acid derivatives via Schiff bases by facile methodology and tested them for their antibacterial activities.

Experimental

Melting points were recorded using hot stage Gallenkamp melting point apparatus. Infrared spectra were recorded on Shimadzu FTIR-8300 spectrometer as potassium bromide disc or thin films. Thin Layer Chromatography (TLC) was performed on Alumina plates covered with Silica gel layer, and the spots were developed with iodine vapor. Elemental analyses (CHN) were carried out by using Eroea Elemental Anlayzer 3000. All Chemicals were of reagent grade and used without further purification.

1. Synthesis of Schiff Bases 1 (a, b) [16]

In a 100 ml round bottom flask equipped with magnetic stirrer and double surface condenser with calcium chloride guard tube, a mixture of (1.01 g, 0.01 mole) freshly distilled aniline, (0.93 g, 0.01 mole) benzaldehyde, 10 ml of ethanol and one drop of glacial acetic acid was refluxed for 30 min. The mixture was then left to cool in ice bath; a yellowish precipitate separated out. The precipitate was filtered, washed with 2% HCl, followed by water and recrystallized from ethanol.

2. Synthesis of phenoxy acetic acid [17]

To a mixture of (4.7 g, 0.05 mole) chloroacetic acid, and (4.8 g, 0.05 mole) phenol, 25 ml solution of 4.8 mole/L aqueous sodium hydroxide was added slowly with constant stirring. The mixture was stirred for 2 h. till solution turn greenish-yellow. Then the solvent was evaporated till sodium salt precipitated out. The salt was dissolved in water and acidified with conc. HCl. The precipitate was filtered off and recrystallized from ethanol.

3. Synthesis of phenoxy acetyl chloride [17]

A mixture of (0.5 g, 0.003 mole) phenoxy acetic acid and (0.22 ml, 0.003 mole) thionyl chloride was refluxed for 30 min. The excess of thionyl chloride is distilled out. After cooling in ice-bath a viscous liquid was obtained.

4. Synthesis of N-α-(chloro-2,4- substituted phenyl) methyl-N-2-phenoxy cetanilide 2(a, b) [18]

To the crude phenoxy acetyl chloride a solution of Schiff base derivative 1 (a-b) from step 2.1 (1.08 g 0.06 moles), dissolved in 5 ml benzene, was added. The mixture was refluxed for 45 min. After cooling, the precipitate separated out, filtered and washed with 2 % Na_2CO_3, followed by water and recrystallized from (1:1) ethanol-water.

5. Synthesis of N-[α-(2,4-substituted-phenyl -Ń- guanidino)methyl]-N-2-phenoxy acetanilide 3(a, b) [18]

To a mixture of (0.3 g, 0.0007 mole) **2a** in 5 ml absolute ethanol and (0.13 g, 0.0007 mole) guanidine carbonate a solution of (0.0007 mole) anhydrous sodium carbonate dissolved in 5 ml absolute ethanol was added. The reaction mixture was refluxed for 2 h. The solvent was evaporated and the remaining precipitate was Filtered, washed with 2% Na_2CO_3 then with distilled water and recrystallized from (1:1) ethanol-water.

6. Synthesis of N-[α-(2-aminobarbiturat - 2, 4-substituted phenyl) methyl] –N -2-phenoxyacetanilide 4(a, b) [18]

In 100 ml round bottom flask, fitted with a double surface condenser, a mixture of (3 ml, 0.02 mole) Diethylmalonate and 10 ml of sodium ethoxide (0.64 g of dried sodium metal

dissolved in 10 ml absolute ethanol) was stirred for 20 min. A mixture of (8.35 g, 0.02 mole) **3a** in 10 ml absolute ethanol was then added and refluxed for 8 h. After addition of 20 ml of distilled water, the resulting mixture was acidified with 2 ml of concentrated hydrochloric acid. The formed precipitate was filtered, washed with distilled water and recrystallized from (1:1) ethanol-water.

7. Microbiological Tests

In this work, the antibacterial test was performed according to the disc diffusion method [19]. Compounds (3a, 3b, 4a, and 4b) were assayed for their antimicrobial activity *in vitro* against one strain of Gram negative bacteria (*Pseudomonas aeuroginosa)* and one strain of Gram positive bacteria *(Staphylococcus aurous).*

8. Sensitivity Test

The prepared agar and Petri dishes were sterilized by autoclaving for 15 min at 121^{o}C. The agar was surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium, suitably spaced apart holes were made (6 mm in diameter). These holes were filled with (0.02 g) of the prepared compounds dissolved in 1 ml of DMSO as a solvent. The plates were incubated at 37 ^{o}C for 24 hour.

Results and Discussion

1. Chemistry

Schemes 2 and 3 summarized all reactions in this work. The Schiff bases were prepared by the reaction of the primary aromatic amine with different aromatic aldehyde derivatives in absolute ethanol in presence of glacial acetic acid as a catalyst. The purity of the products was permanently examined by TLC. FT-IR spectroscopy was used to characterize the products and intermediates through the whole synthesis. The FT-IR spectra of the formed Schiff bases showed a band in the range of 1610-1627 cm^{-1} which represents the stretching vibration of C=N double bond. Disappearance of two bands (3450 and 3220 cm^{-1}) attributed to stretching vibrations of the amino group in aniline was observed. The disappearance of the C=O absorption band of aldehyde in the range of 1660-1740 cm^{-1} was also detected. These Observations confirm the formation of the Schiff bases. Table 1 shows the physical data and Table 2 the FT-IR spectral data of synthesized compounds 1(a-b).

Phenoxyacetic acid was prepared by the reaction of phenol with monochloroacetic acid in presence of a basic medium (NaOH). FT-IR spectra showed appearance of C=O band for carboxylic acid at about 1700 cm^{-1} and a typical broad strong band at 3400-3350 cm^{-1} attributed to the O-H group. The bands at 1200 cm^{-1} and 1045 cm^{-1} were assigned to the symmetric and asymmetric stretching vibration of C-O-C respectively. Phenoxy acetyl chloride was prepared by the reaction of phenoxyacetic acid with excess of thionyl chloride in the presence of dimethylformamide (DMF) as a catalyst. FT-IR spectra showed a shifting in the position of the stretching vibration of C=O band from 1700 cm^{-1} for the acid to 1748 cm^{-1} for acid chloride. Appearance of C-Cl band at 759 cm^{-1} and a broad strong band at 3381-3250 cm^{-1} attributed to the O-H group suggest a formation of an enol form of the acid chloride.

N-α-(chloro-2,4-substitutedphenyl)methyl-N-2-phenoxyacetanilide was synthesized by the reaction of Schiff bases (1 a-b) with phenoxy acetyl chloride III in dry benzene as a solvent (scheme 2). The FT-IR spectra showed appearance of a lower frequency C=O stretching vibration band at 1662 cm^{-1} for the formed amid group in comparison with the C=O band of the acid chloride (1748 cm^{-1}). The disappearance of C=N absorption band at 1600 cm^{-1} reveals the formation of the amide 2 (a-b).

The values of the elemental analysis (CHN) were in good agreement with the theoretical calculated values (Table 1).

Guanidine is one of the strongest bases (pKa = 13.65) caused by extensive delocalization of the positive charge on the protonated cation [1]. The guanidine derivatives were synthesized by the reaction of guanidine carbonate with benzyl chloride derivatives (2 a, b) in absolute ethanol as a solvent. The reaction mixture was treated with a 2 % solution of sodium carbonate to remove the formed HCl.

The FT-IR spectra showed a band at 1610 cm^{-1} which is attributed to the bending vibration of the amino group. Also the bands at 3338 and 3290 cm^{-1} caused by asymmetric and symmetric stretching vibration band of -NH_2 group respectively and disappearance of C-Cl band at 771 cm^{-1} indicate the replacement of the chloride atom by the Guanidine molecule. Figure 1 shows exemplary the FT-IR spectrum of the compound 3a. The values of the elemental

analysis (CHN) were in good agreement with the calculated values (Table 1).

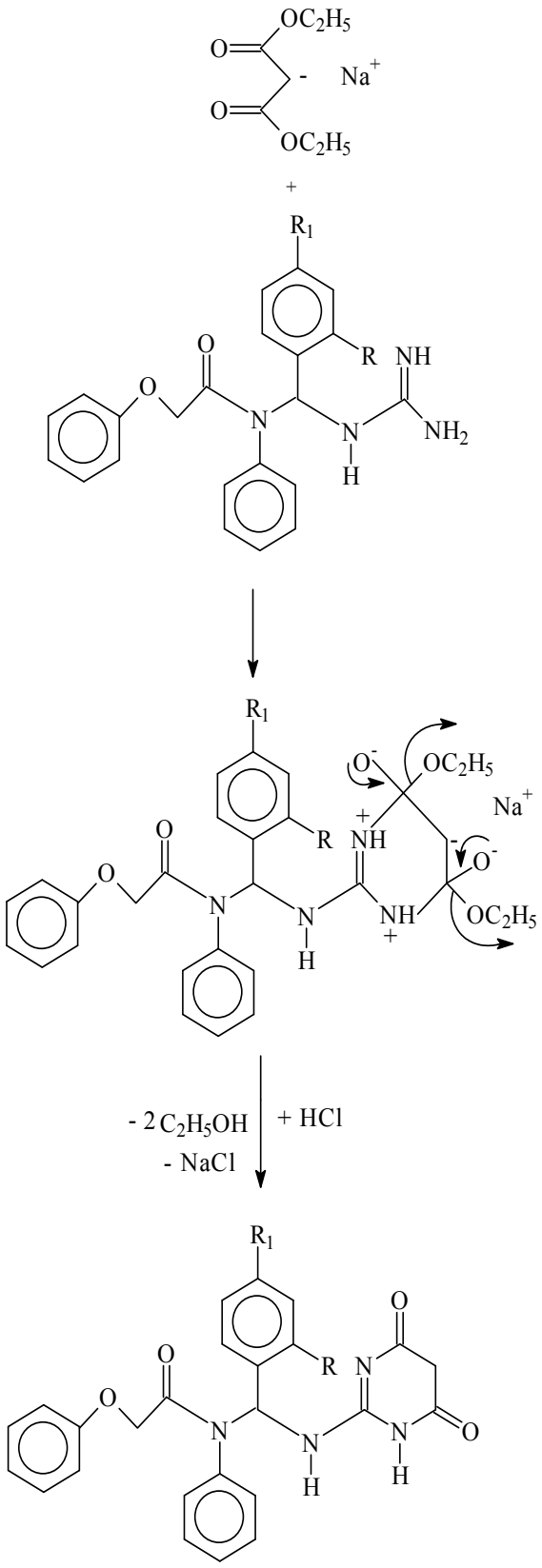
The Guanidine derivatives 3 (a, b) were condensed with diethyl malonate (DEM) under basic conditions to give the corresponding pyrimidine derivatives, which are known as barbituric acids [20]. Diethyl malonate was converted into the sodium malonic ester by the reaction with sodium ethoxide in absolute ethanol to keep DEM in the solution without decomposition. Otherwise, DEM would decompose by heating in neutral or acidic medium into acetic acid, ethanol and CO_2, in a decarboxylation process. Both carbon atoms of the carbonyl groups of DEM can be attacked by a nucleophilic groups (NH_2 and =NH) of the guanidine to form an intermediate, which is stabilized in a tetrahedral mechanism, into the barbituric derivative (scheme 3) [21]. The formation of two ethanol molecules is the driving force for this step.

FT-IR spectra showed, appearance of stretching vibration of C=O band at 1730 cm^{-1}, appearance of N-H band at 3200 cm^{-1}, and disappearance of the asymmetric and symmetric stretching vibrations bands at 3338-3290 cm^{-1} for NH_2 group. The appearance of strong broad band for O-H group at 3480-3400 cm^{-1} indicates the formation of an enol form for the barbituric acid. Figure 2 shows exemplary the FT-IR spectrum of the compound 4a. The elemental analysis (CHN) results of the aimed compounds 4 (a, b) were in a good agreement with the theoretical calculated values.

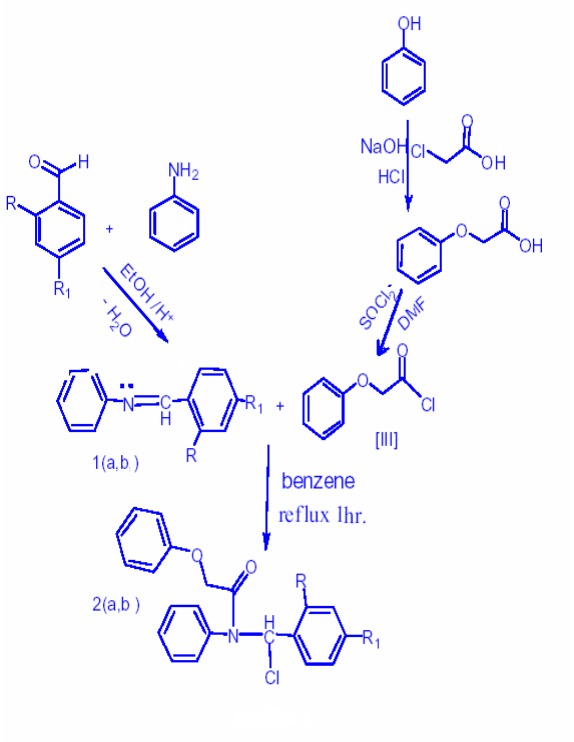
2 Biological activity

The biological activity of the synthesized compounds was determined by measuring the diameter of the empty region around the wall (Inhibition zone). The results of preliminary screening tests are listed in Table 3. From the data presented in table 3, it is obvious that synthesized compounds exhibited high biological activity against both bacteria (*Pseudomonas aeuroginos* and *Staphylococcus aurous).*

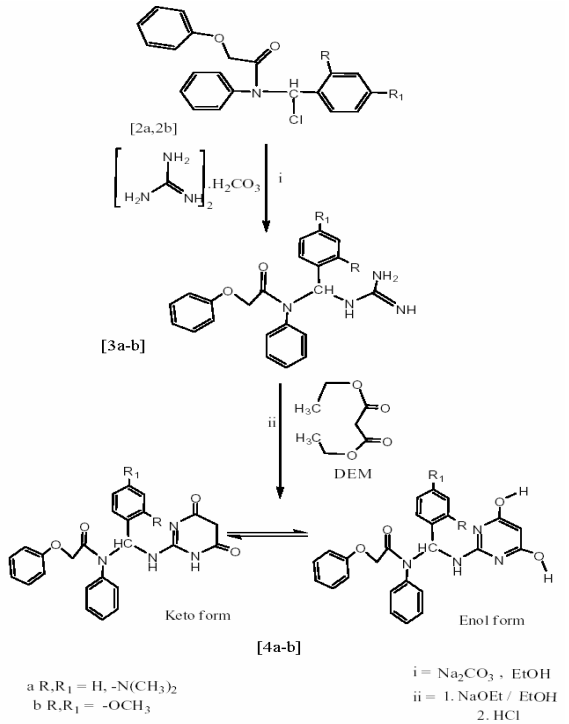
The presence of the OH and NH_2 groups in these derivatives may give rise to their activity. The derivatives with OCH_3 group in combination with OH and NH_2 groups displayed an increased biological activity against both bacteria. This observation promotes us in future work to synthesize barbituric acid derivatives with other groups that can possibly enhance the biological activity.



Scheme 1: Reaction mechanism for the preparation of N- [α-(2-aminobarbiturat-2,4-substituted phenyl) methyl] -N-2- phen-oxyacetanilide 4(a, b).



Scheme 2: Preparation of N-α-(chloro 2,4-substituted phenyl) methyl-N-2-phenoxy-acetanilide 2 (a, b).



Scheme 3: Preparation of N-[α-(2-amino-barbiturat-2,4-substituted phenyl)methyl] -N-2-phen-oxyacetanilide 4 (a, b).

Tables and Figures

Table 1: Physical data and elemental analysis of the synthesized compounds.

Comp. No.	m. p C°	Yield %	Formula	Elemental analysis % (calculated) found		
				C	H	N
1a	150-154	86.6	$C_{15}H_{16}N_2$			
1b	70-73	78.6	$C_{15}H_{15}NO_2$			
2a	93-95	58.0	$C_{23}H_{23}N_2O_2Cl$			
2b	150-155	74.0	$C_{23}H_{22}NO_4Cl$	(67.072) 67.105	(5.346) 5.997	(3.402) 3.302
3a	152-155	46.7	$C_{24}H_{27}N_5O_2$	(69.065) 69.124	6.475) 6.169	(16.787) 17.129
3b	82-85	81.0	$C_{24}H_{26}N_4O_4$	(66.359) 65.406	6.682) 6.530	(12.903) 12.355
4a	75-79	52.2	$C_{27}H_{27}N_5O_4$	(66.804) 66.302	(5.567) 5.734	(14.432) 14.783
4b	89- 92	72.4	$C_{27}H_{26}N_4O_6$	(64.541) 65.218	(5.179) 5.315	(11.155) 11.312

Table 2: IR spectral data for the synthesized compounds in KBr (υ cm^{-1}).

Comp. No.	OH	NH_2 Str.	NH Str.	(CH) aromatc Str.	C=O Str.	C=N Str.	C=C Str.	C-Cl Str.
1a	-	-	-	3080	-	1610	1591 – 1529	-
1b	-	-	-	3100	-	1627	1583 – 1512	-
2a	-	-	-	3100	1649	-	1606-1544	771
2b	-	-	-	3000	1662	-	1593–1519	752
3a	-	3338-3290	3122	3100	1652	1602	1550-1500	-
3b	-	3400-3149	3149	3010	1649	1587	1587-1517	-
4a	3480	-	3200	3000	1730	-	1577	-
4b	3450	-	3200	3000	1737	-	1517	-

Table 3: Antibacterial activities of the synthesized compounds.

Type of Bacteria	Comp. No. 3a	Comp. No. 3b	Comp. No. 4a	Comp. No.4b
Ps. aerugenosa	+++	+++	+ ++	+ ++
Staph aureus	+++	+++	+ ++	+ ++

Not:
- = (0) mm No inhibition = inactive
+ = (1-5) mm = weak activity
++ = (6-10) mm = moderate activity
+++ = (11-15) mm = highest activity

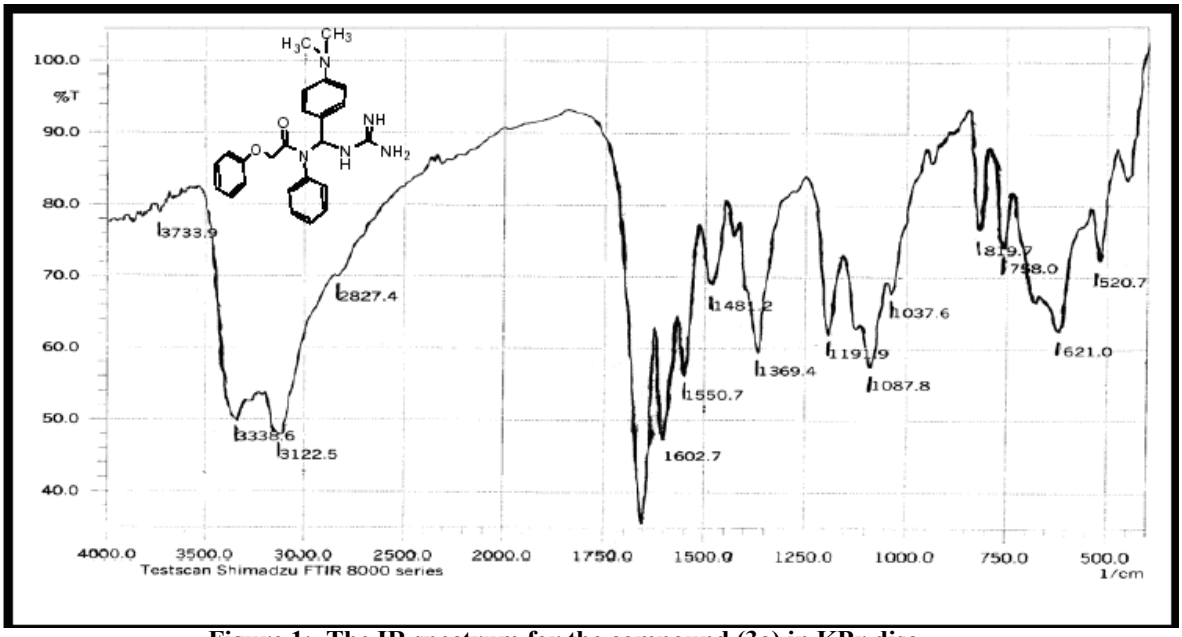


Figure 1: The IR spectrum for the compound (3a) in KBr disc.

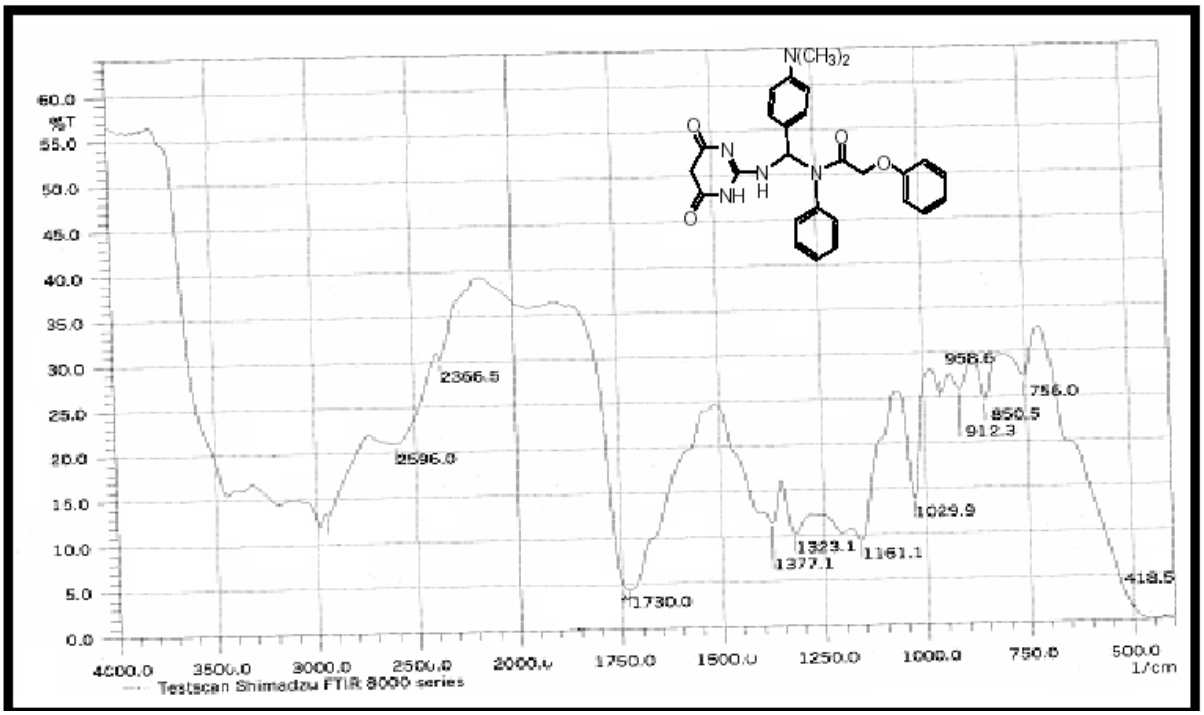


Figure 2: The IR spectrum for the compound (4a) in KBr disc.

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