Synthesis and study of antimicrobial activity of some tetrahydrocarbazole derivatives substituted with NSAID

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Article Info:

Received May 2022
Accepted Jully 2022
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DOI:

Abstract:

New tetrahydrocarbazole derivatives substituted at the heteroatom (N) by non-steroidal anti-inflammatory drug (NSAIDs) were synthesized by reaction of cyclohehexanon (C.H.N.) with phenyl hydrazine (P.H.Z.) to form tetrahydrocarbazole (THCZ), where the latter is reacted with NSAID (Ketoprofen) via amide bond to yield substituted THCZ, compounds chemical structures were verified by: 1H, 13C NMR and FTIR spectroscopy.

Antifungal activity of the synthesized compounds was investigated by docking study and in vitro test to reveal good antifungal activity, but the in vitro test also showed that the compounds have weak to moderate antibacterial activity.

Key words: Tetrahydrocarbazole,non-steroidal anti-inflammatory drug,Molecular Docking, antimicrobial activity.

تخليق ودراسة الفعالية المضادة للمكروبات لبعض مشتقات التتراهايدروكاربازول المعوضة بمضادات الالتهاب غير الستيرويدية.

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الخلاصة:

تم تصنيع مشتقات تتراهايدروكاربازول جديدة معوضه في ذرة الهيتيرو (النيتروجين) بعقار مضاد للالتهاب غير ستيرويدي (NSAIDs) عن طريق تفاعل هكسانون حلقي (C.H.N)مع فينيل هايدرازين (P.H.Z.)لتشكيل رباعي هيدروكربازول (THCZ) ، حيث يتفاعل الأخير مع عقار مضاد للالتهاب غير ستيرويدي (كيتوبروفين) بواسطة تكوين رابطة أمايدية لإنتاج تتراهايدروكاربازول معوض. تم تأكيد التركيب الكيميائي للمركبات عن طريق الرنين النووي المغناطيسي البروتوني واطياف الأشعة تحت الحمراء الدقيق.

تم فحص النشاط المضاد للفطريات للمركبات المحضرة عن طريق دراسة الالتحام الجزيئي والفحص المختبري للكشف عن نشاط مضاد للفطريات جيد، لكن الفحص المختبري أظهر ايضا أن المركبات لها نشاط مضاد للبكتيريا ضعيف إلى متوسط.

الكلمات المفتاحية: تتراهايدروكاربازول، ادوية مضادات الالتهاب غير الستيرويدية ، الالتحام الجزيئي، نشاط مضادات الميكروبات.

Introduction

Antimicrobial resistance is still a public health concern on a worldwide basis. The continual demand for novel antimicrobial treatments that are effective against multidrug-resistant organisms has prompted researchers to engage in a variety of drug development strategies. [1] This research is focused on synthesis of compounds resulting from combination of two biologically active compounds (THCZ and NSAIDS) and studying the antimicrobial activity of these new compounds.

THCZs are an important kind of nitrogen containing aromatic heterocyclic compounds [2], they are ubiquitous in natural products and biologically active compounds [3] with a diverse biological activity [4], exhibit broad-spectrum antifungal activity [5], they are a scaffold of many drugs like frovatriptan (for migraine attack), pirlindole (antidepressant) [6] and ramatroban (immunotherapy for the treatment of COVID-19 disease) [7].

Literature survey showed that compounds with a THCZ skeleton have significant antimicrobial activity [8], anti-Alzheimer [9], hypoglycemic [10], anticancer [11] and exhibit good antifungal activity, such that they might be utilized as a basis for creating new antifungal agents. [12]

NSAIDs are among the most commonly prescribed classes of drugs throughout the world [13]. they are non-antibiotic drugs noted to display antibacterial activity [14], there are observations of antimicrobial activity of some NSAIDs and synergistic activity with other antibiotics such as acetylsalicylic acid and indomethacin (a non-selective-COX inhibitor) [15, 16], also some NSAIDS like diclofenac had inhibitory activity against specific types of bacteria like S. aureus [17]

Antipyretics may suppress virus replication, suppress or stimulate fungal or bacterial growth, change virulence factors expression, affect biofilm production (biofilms refers to structural bacterial colonies encased in a self-produced matrix), influence the adherence, motility and microbe's metabolism, alter the bacterial susceptibility to the antibiotics. They may also be useful in the treatment of biofilm-associated infections, in decreasing virulence factors and in treat resistant microorganisms. [18, 19]

THCZ was prepared by reaction known as Borsche-Drechsel reaction which involves reacting of phenyl hydrazine with cyclohexanone to form cyclohexanone phenylhydrazone which undergoes cyclization under acidic condition with 2-hours reflux to form THCZ [20], NSAID (ketoprofen) was treated with thionyl chloride under 4 hrs. reflux to yield acid chloride of the NSAID [21], Next the products (acid chloride of NSAIDs and THCZ) were refluxed nine hrs. with the presence of triethylamine to yield THCZ substituted at N position by NSAIDS (22) as elucidated in (scheme 1).

Materials and Methods

The IR spectrum was taken on a Tensor 27 Shimizu (japan), Melting point was measured by glass capillary tube on Stuart melting point apparatus (UK), The 1H NMR (499.67 MHz) and 13C NMR (125.66 MHz) spectra were determined by Varian inova (Agilent technologies ,USA) utilizing DMSO as solvent.

C.H.N, dry Benzene (D. Benz.) from ROMIL company (UK), 4-chlorophenylhydrazine of Hyper.com company (China), SOCL2, methanol of CDH company (India) and Diethyl Ether from Alpha Chemicals company (India).

Preparation of the compound 2-(3-benzoylphenyl)-1-(1,2,3,4-tetrahydro-9H-carbazo1-9-y1) propan-1-one (TK).

The compound TK was synthesized by addition of (0.01 mole, 2.7 g) ketoprofen acid chloride in twenty ml D. Benz. and 1 ml triethylamine to (1.7g, 0.01mole) 1,2,3,4-tetrahydrocarbazole in D. Benz. (30 ml) scheme (1), The blend refluxed about nine hrs., the solvent evaporated and

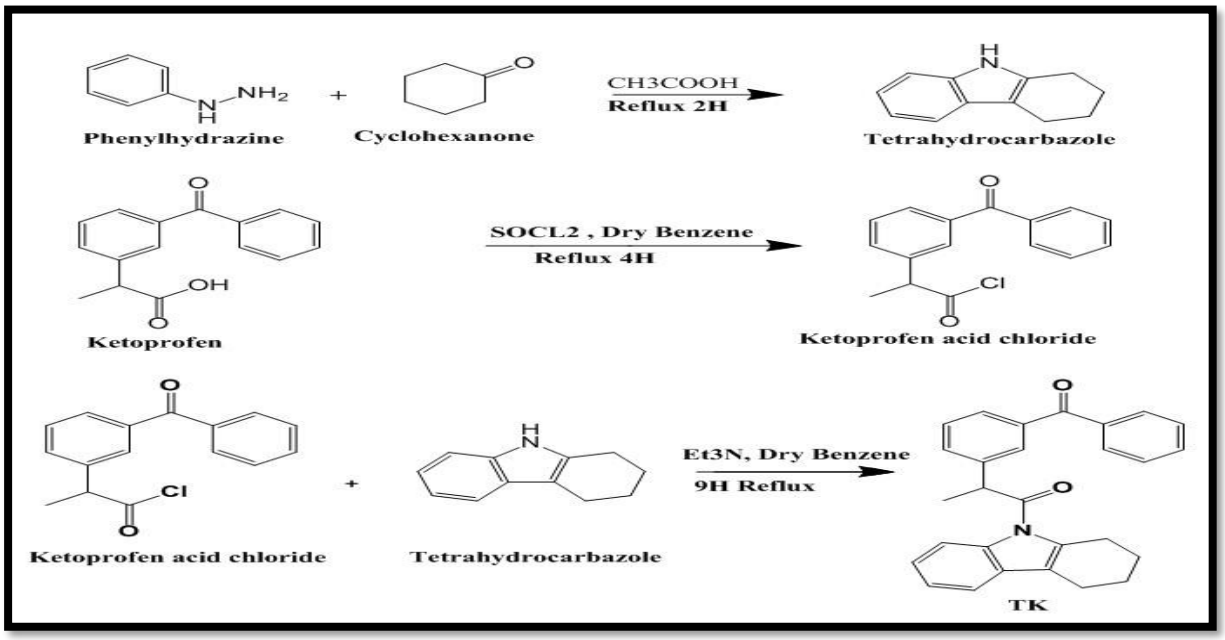
5 percent sod. Bicarb. and water were used to wash the precipitate. [22]

1,2,3,4-THCZ compound was synthesized (scheme 1) by adding (4.9 ml, 0.05 mole) phenylhydrazine throughout 1 hr. to solution of (0.05 mole, 5.21 ml) C.H.N in 16 ml glacial acetic acid with stirring and reflux. Then the blend was refluxed for additional hr. and filtered, the solid crude was then washed with water and 75% methanol before being dried to get 1,2,3,4-THCZ. [23]

Ketoprofen acid chloride was prepared (as in the scheme 1) by adding (0.01 mole, 0.7 ml) SOCl2 to (0.025mole, 6.3 g) ketoprofen in 24 ml D.Benz., blend was refluxed four hrs., dried and ketoprofen acid chloride was produced after washing with 5 mL diethyl ether.

Results and discussion

The procedure of the compounds synthesis was shown in Scheme (1).



Scheme (1): synthesis of the compound TK from THCZ and NSAID.

Identification of the compounds

The prepared compounds identified using lH, 13C NMR and IR spectra.

1H NMR spectroscopy explanation of compound 1,2,3,4-THCZ.

The 1H NMR spectrum of 1,2,3,4-tetrahydrocarbazole Figure (1) displays chemical shifts, the explanations of these spectrum illustrated in Table (1**)**

Table (1) :^{1}H NMR spectroscopy interpretation of compound 1,2,3,4-Tetrahydrocarbazole

Chemical shift	NO. of H	splitting	Explanation
7.03	1	D	Aromatic H (Aro-H)
7.27	1	T	(Aro-H)
7.34	1	T	(Aro-H)
6.96	1	D	(Aro-H)
10.63	1	S	H of Amine
2.74	2	T	Allylic H
1.81	2	Q	H of saturated carbon
1.85	2	Q	H of saturated carbon
2.63	2	T	Allylic H

The ^{13}C NMR spectroscopy interpretation of the compound 1,2,3,4-THCZ.

^{13}C NMR spectrum of 1,2,3,4-Tetrahydrocarbazole Figure (2) displays Chemical shifts, Explanation of these spectrum was illustrated in Table (2)

Table (2) :^{13}C NMR spectroscopy explanation of 1,2,3,4-Tetrahydrocarbazole.

Chemical shift	Explanation
117.53	Aromatic Carbon (Aro-C)
118.41	Aro-C
120.45	Aro-C
110.96	Aro-C
134.83	Aro-C
136.13	Aro-C
23.53	Allylic carbon
23.29	Saturated C
23.39	Saturated C
21.14	Allylic carbon
108.51	Aro-C
127.78	Aro-C

The IR spectrum explanation of 1,2,3,4-THCZ.

IR spectrum of 1,2,3,4-Tetrahydrocarbazole Figure (3) displays bands, explanation of spectrum was illustrated in Table (3).

Table (3): IR spectrum explanation of 1,2,3,4-Tetrahydrocarbazole.

Bands	Interpretation
3385.72	band of sec. amine
3050.23	Aro-H
2923.03	C-H of alkanes
1587.80	Aromatic C=C

The 1H NMR spectrum explanation of T.K. compound.

1H NMR spectrum of TK Figure (4) shows chemical shifts, explanation of the spectrum was illustrated in Table (4)

Table (4): ^{1}H NMR spectrum explanation of TK compound.

Chemical shift	NO. of H	splitting	Explanation
6.91	1	D	Aro-H
6.88	1	T	Aro-H
7.23	1	T	Aro-H
7.55	1	D	Aro-H
2.61	2	T	Allylic H
1.79	2	Q	H of Alkyl
1.83	2	Q	H of Alkyl
2.59	2	T	Allylic H
3.34	1	Q	H next to carbonyl
1.4	3	D	H of Alkyl
7.5	1	D	Aro-H
6.96	1	T	Aro-H
7.65	1	D	Aro-H
7.71	1	S	Aro-H
7.31	2	T	Aro-H
7.3	2	Q	Aro-H
7.59	1	T	Aro-H

The 13C NMR spectrum explanation of T.K. compound.

The 13C NMR spectrum of TK Figure (5) shows chemical shifts, explanation of the spectrum was illustrated in Table (5).

Table (5): ^{13}C NMR spectrum explanation of TK compound.

Chem. shift	Explanation
110.92	Aro-C
110.92	Aro-C
120.42	Aro-C
110.92	Aro-C
127.37	Aro-C
134.80	Aro-C
23.47	Allylic C
23.33	Aliphatic C
23.33	Aliphatic C
23.23	Allylic C
108.47	Aro-C
118.38	Aro-C
176.86	Carbon of Carbonyl group
49.13	Carbon next to Carbonyl group
21.10	Carbon of Alkyl group
125.92	Aro-C
125.92	Aro-C
118.38	Aro-C
117.50	Aro-C
136.07	Aro-C
125.92	Aro-C
197.47	Carbonyl group
134.80	Aro-C
120.42	Aro-C
117.50	Aro-C
118.38	Aro-C

The IR spectrum explanation of T.K. compound.

IR spectrum of TK Figure (6) shows chemical shifts, explanation of the spectrum was illustrated in Table (6)

Table (6): IR spectrum explanation of TK compound.

Band	Explanation
3049.77	Aro-H
2926.83	Asymmetric H of saturated C
2847.63	Symmetric H of saturated C
1760.04	Carbonyl group
1657.92	Carbonyl group
1619.56	Aromatic C=C

The properties of the prepared compounds are illustrated in **Table** (7)

Table (7): properties of the synthesized compounds

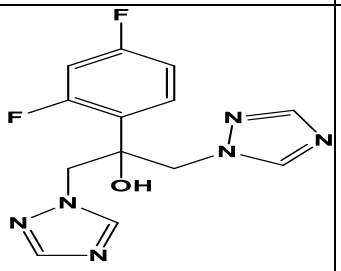
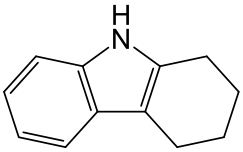
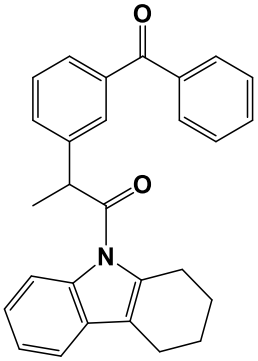
Compounds	Chem. Formula	Mol. weight	Melting Point	Colour
1,2,3,4-Tetrahydrocarbazole	C12H13N	171.24	118-120 °C	Wheat
TK	C28H25NO2	407.5	130-135	Pale yellow

Docking study

Molecular docking experiments were used to investigate the binding modes of such compounds with the target enzyme Sterol 14-demethylase (CYP5l), a cytochrome P450 enzyme needed for sterol syntheses in eukaryotic cells as well as a prevalent target of therapeutic medications used to treat fungal infections. [24]. The RCSB protein data bank's CYP51 (PDB code 5TZ1) receptor was utilized. The synthesized compounds were docked using Genetic Optimization for Ligand Docking (GOLD). GOLD uses to search the conformational space of binding ligands and provides a binding residue score. GOLD scores are used to rank poses. [25].

Table (8) shows the docking scores of the control material (fluconazole) and created compounds (1,2,3,4-THCZ and TK) with fungal *Candida albicans* amino acids (A.A.) and their interactions.

Table (8): Docking score with A.A. of *C. albicans*, PDB: 5TZ1

ligand	Structure	fitness score	A.A (H bond interaction	A.A (Other interaction)
REF. Ligand (fluconazole)		78.58	-	THR 311, HEM 601 (3), LYS 431, HIS 468, ILE 131 (3), TYR 132 (4)
1,2,3,4-tetrahydrocarbazole		-	-	-
TK		108.59	TYR 132	LEU 376 (3), THR 311 (5), GLY 307 (2), ILE 304 (2), TYR 132, HEM 601

Antimicrobial effect

The antibacterial effect was evaluated in vitro versus three gram-negative bacteria (Pseudomonas aeruginosa, E. coli, and Klebsiella pneumoniae) and one gram-positive bacterium (Staphylococcus aureus) and candida albicans which is the most common cause of invasive fungal infection (26), using agar plate (Mueller-Hinton agar for the bacteria, Sabouraud Dextrose for fungi) and dilutions of the synthesized compounds with dimethyl sulfoxide as solvent based on well diffusion assay. For the comparison, antibacterial ciprofloxacin and antifungal fluconazole were employed as reference materials.

Results and discussion

Both docking study (Table 8) and in vitro test (Table 9) confirmed that the synthesized compound TK has potent antifungal activity against candida albicans even higher than the control compound (fluconazole), while the

compound 1,2,3,4-tetrahydrocarbazole showed no antifungal activity in docking study but weak activity in vitro test.

As antibacterial the compound 1,2,3,4-tetrahydrocarbazole showed higher activity than the control compound (ciprofloxacin) in vitro test against *K. pneumoniae*, weak against *P. aeruginosa* and no activity against S. aureus. and *E. coli*.

In vitro test the compound TK showed variable antibacterial activity range between weak to moderate against used bacteria.

The compound TK has potent antifungal activity against candida albicans because it inhibits target enzyme Sterol 14-demethylase (as approved by docking) which is a very important enzyme throughout biology due to its essential role in sterol biosynthesis [27], 1,2,3,4-THCZ was supposed to be active against S. aureus as docking show that it has high fit score with Glutamyl-tRNA(Gln) amidotransferase subunit of S. aureus but the in vitro test showed null activity and this may be due to the fact that docking gives a high probability of effectiveness, but is not completely certain.

Table (9): antimicrobial activity of the synthesized compounds

	ciprofloxac	**fluconaz**	**THCZ**					**TK**				
			stock	**1**	**2**	**3**	**4**	**stock**	**1**	**2**	**3**	**4**
S. aureus.	18		0	0	0	0	0	15	12	10	7	4
P. aeruginosa	20		12	0	0	0	0	14	11	7	0	0
E.coli	23		0	0	0	0	0	19	16	11	0	0
K. pneumoniae	11		14	10	0	0	0	9	3	0	0	0
C. albicans		14	12	11	10	7	4	20	16	14	11	8

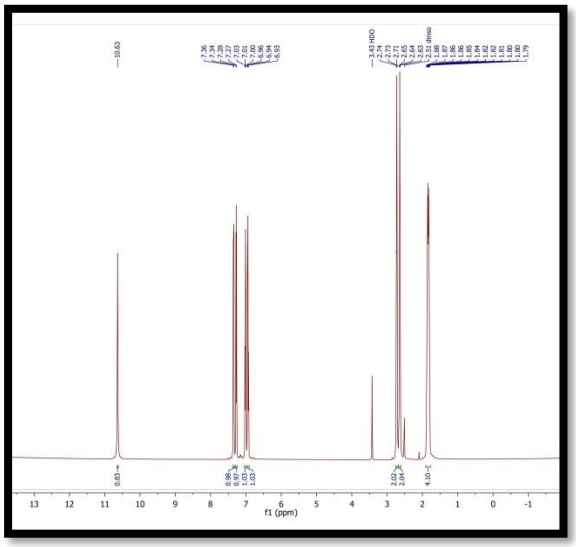


Figure (1): ^{1}H NMR of compound 1,2,3,4-THCZ

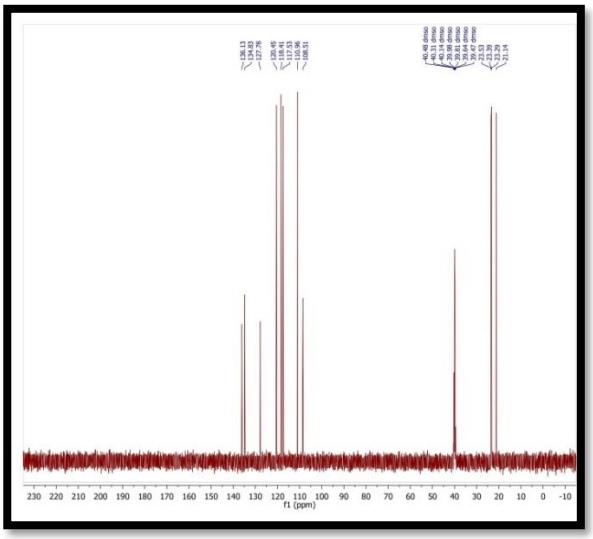


Figure (2) :^{13}C NMR of compound 1,2,3,4-THCZ

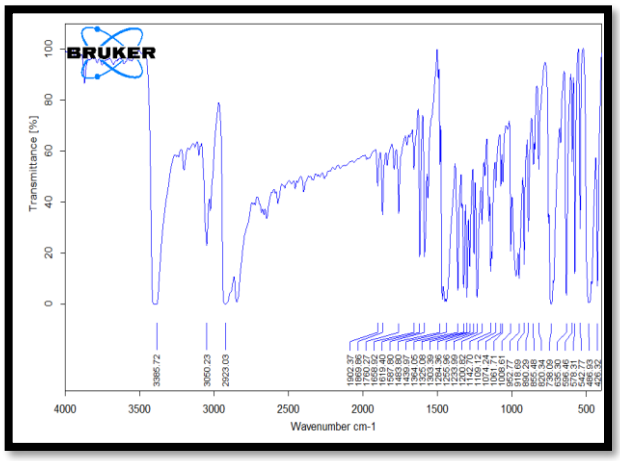


Figure (3): IR Spectroscopy of compound 1,2,3,4-tetrahydrocarbazol

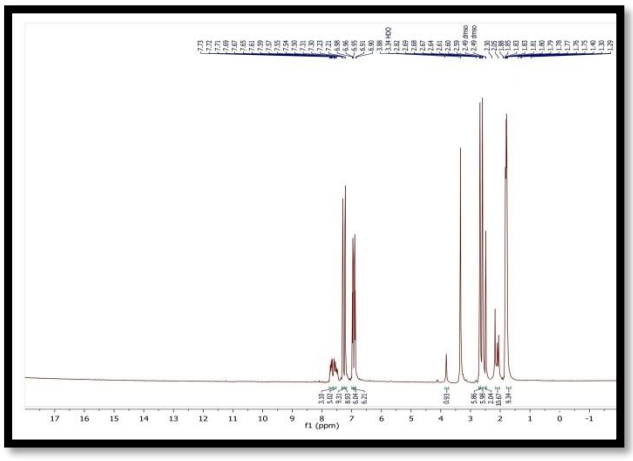


Figure (4): ^{1}H NMR Spectroscopy of compound TK

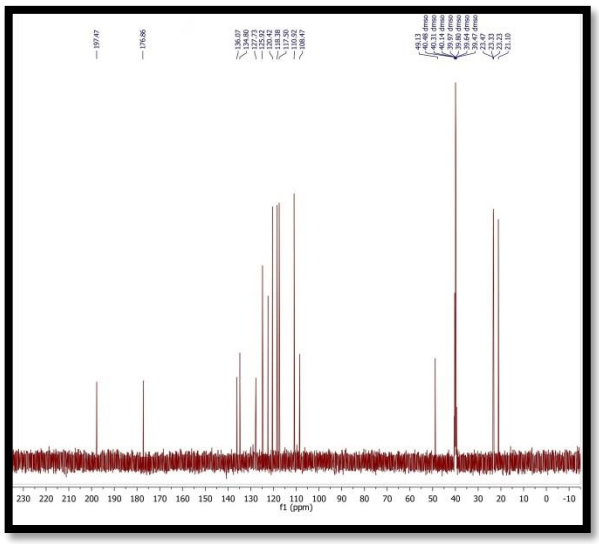


Figure (5) :^{13}C NMR Spectroscopy of compound TK.

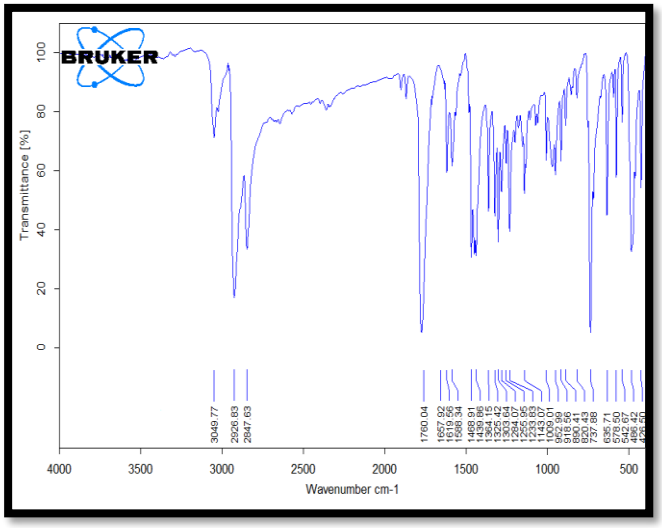


Figure (6): IR Spectroscopy of compound TK.

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