How to Cite:
Ali, H. R., & Naser, A. W. (2022). Synthesis, biological activity and molecular docking study of some new chalcones, pyrazolines and isoxazolines derivatives bearing 1,2,3- triazoline. *International Journal of Health Sciences*, *6*(S6), 76–118. https://doi.org/10.53730/ijhs.v6nS6.9173

Synthesis, biological activity and molecular docking study of some new chalcones, pyrazolines and isoxazolines derivatives bearing 1,2,3- triazoline

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***Abstract*---**In this work a variety of new compounds such as chalcones, pyridine and isooxazoline derivatives has been synthesized. 2-((1-(4-acetylphenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide(1) have been chosen as a starting material. Condensation of compound(1) with aromatic aldehydes namely benzaldehyde, *p*-chloro benzaldehyde, *p*-bromo benzaldehyde, *p*-nitro benzaldehyde, *p*-hydroxy benzaldehyde, *m*-hydroxy benzaldehyde, *p*-N,N-dimethyl benzaldehyde and 2,4-dimethoxy benzaldehyde in the presence of 40% KOH gave chalcone derivatives (2a-h). The cyclization of prepared chalcone derivatives semicarbazide in the presence acetic acid product pyrazoline derivatives(3a-f). Reaction of chalcone derivatives(2a-h) with hydroxylamine hydrochloride in the presence of sodium acetate afforded corresponding isooxazoline derivatives (4a-d). FT-IR, ^{1}HNMR, and ^{13}CNMR were used to characterize the target compounds. The results showed that the target compounds have a good biological activity such as antibacterial and antioxidant. The molecular docking studies of the target 6ul7 with the newly synthesized compounds showed good docking scores with acceptable binding interactions. The present results reveal that the newly synthesized compounds exhibit promising inhibition activity against Escherichia Coli.

***Keywords*---**Chalcone, Pyrazoline, Isoxazoline, Antibacterial, Antioxidants, Molecular Docking.

Introduction

Heterocyclic compounds are compounds that possess complex toroidal component containing atoms in addition to carbon atom. The more common heterogeneous atoms are nitrogen, oxygen and sulfur. The importance of these compounds are several biologically active natural products in Nature[1]. Chalcones are one of the subclasses of flavonoid family , they are open-chain flavonoids which an α,β-unsaturated enone is connected to two aromatic rings[2]. Synthesis of chalcones is important for industries as they are used as intermediates for the synthesis of several heterocyclic compounds[3]. Chalcones can be synthesized by many methods. Generally chalcones were prepared by Claisen-Schmidt condensation of electrophilic substituted benzaldehyde with substituted acetophenone as nucleophile in the presence of bases like NaOH, KOH, $Ba(OH)_2$[4]. Chalcones and their derivatives exhibited biological activities, such as antibacterial[5], antiflammatory[6], antiulcer[7], antidepression[8], antioxidant[9], and anticancer[10]. Pyrazoline is a five-membered heterocyclic compound having two adjacent nitrogen atoms within the ring. In addition to its role as a key precursor for the synthesis of novel organic compounds with medicinal properties[11]. Pyrazoline is an important heterocyclic scaffold which occurs in a number of bioactive compounds and useful synthetic building blocks[12]. Pyrazoline analogues are well known in the area of pharmaceutical research for wide range of biological potential like cytotoxic[13]. Pyrazoline derivatives are important biologically active heterocyclic compounds. These derivatives are the subject of many research studies due to their widespread potential biological activities such as anticancer[14]. Aminopyrine(Analgesic and antipyretic)[15], anticonvulsant[16], antioxidant[17], antimicrobial[18], antiviral[19], neuroprotective activity[20], antidepressant[21], antimalarial[22] and antitrypanosomal[23]. The pyrazoline function is quite stable, and has inspired chemists to utilize this stable fragment in bioactive moieties to synthesize new compounds possessing biological activities[24].
1,2-isoxazolines are oxygen–nitrogen (O,N) heterocycles that are important building blocks for the construction of a variety of compounds with medicinal applications[25]. The isoxazoline derivatives could be utilized in order to regulate the stereo and regiochemistry in natural products synthesis[26]. isoxazolines are key skeletons of several synthetic and naturally occurring pharmacologically active compounds such as antitumor[27], antifungal[28], anticancer[29], antidiabetic[30], antimalarial[31], anti-stress[32], antibacterial[33], antitubulin[34], and antinociceptive activity[35].

Experimental Methods

All chemicals are purchased from Fluka, BDH, and Merck. M. p. is a recorder that uses an electrothermal (m.p) apparatus. The FT-IR spectral data were recorded on a Shimadzu FT-IR8400S spectrophotometer in the Department of Chemistry, College of Science, University of Karbala. ^{1}H-NMR and ^{13}C-NMR spectra are recorded on the central laboratory of Tehran University, 500MHz, using DMSO-d_6 and tetramethylsilane (TMS) as an internal standard.

General Procedure For The Synthesis of Chalcone Derivative (2a-h)[36]

(1g, 0.0027 mole) of compound [2-((1-(4-acetylphenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide] **[1]** was stirred in (10

mL) absolute ethanol with equimolar of some substituted benzaldehydes namely benzaldehyde, *p*-chloro benzaldehyde, *p*-bromo benzaldehyde, *p*-nitro benzaldehyde, *p*-hydroxy benzaldehyde, *m*-hydroxy benzaldehyde, *p*-N,N-dimethyl benzaldehyde and 2,4-dimethoxy benzaldehyde (0.0027 mole), then 40% KOH (10 mL) was added drop wise. The mixture was refluxed for (8-10) h., then it was poured on (50 mL) ice-water, with continuous stirring for 1 h. After the mixture was neutralized with concentrated of hydrochloric acid. The formed precipitate obtained was filtered, washed and recrystallized from ethanol to give chalcones [2a-h].

2-(N-((1-(4-(3-phenylacryloyl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (2a)

Yield 87%, m.p. 108-111°C, FTIR(KBr), υ (cm^{-1}) : 3286(N-H and O-H vib. Coupling) 3059(C-H)Ar, 2924 and 2862(CH_2) 1708(C=O)acid, 1651(C=O)ketone, 1599(C=C), 1529(N=N), 1336(SO_2)Asym, 1168(SO_2)Sym, 1224(C-O)Asym, 1118(C-O)Sym. ^{1}H-NMR(DMSO-d_6), (δppm): 2.43 (d, 2H, C<u>H</u>$_2$ triazoline), 3.67(t, 2H, C<u>H</u>$_2$-NH), 4.77-4.86 (m, 1H, C<u>H</u> triazoline), 6.76-7.00(m,2H,C<u>H</u>=C-<u>H</u>), 7.12-7.99 (m, 14H Ar-H and N<u>H</u>-CH_2), 12.35 (s,1H,-O-H). ^{13}C-NMR (δ ppm): 44.91(<u>C</u>H_2-NH), 54.39(<u>C</u>H_2triazoline), 75.05(<u>C</u>H-N), 119.03(O=C-<u>C</u>=C), 124-145 (<u>C</u>-Ar), 147.78(C=<u>C</u>), 163.92 (O=<u>C</u>-O-H), 195,45(<u>C</u>=O).

2-(N-((1-(4-(3-(4-chlorophenyl)acryloyl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (2b)

Yield 77%, m.p. 112-113°C, FTIR(KBr),υ (cm^{-1}): 3375(O-H), 3302(N-H), 3061(C-H)Ar, 2928 and 2858(CH_2), 1689(C=O)acid, 1633(C=O)ketone, 1599(C=C), 1529(N=N), 1327(SO_2)Asym, 1170(SO_2)Sym, 1230(C-O)Asym, 1120(C-O)sym.

(Z)-2-(N-((1-(4-(3-(4-bromophenyl)acryloyl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (2c)

Yield 80%, m.p. 116-117°C, FTIR(KBr),υ (cm^{-1}): 3373(O-H), 3271(N-H), 3088(C-H)Ar, 2926 and 2858(CH_2) 1681(C=O)acid, 1637(C=O)ketone, 1593(C=C), 1529(N=N), 1325(SO_2)Asym, 1168(SO_2)Sym, 1236(C-O), 1120(C-O)Sym. ^{1}H-NMR(DMSO-d_6): 2.50 (d, 2H, C<u>H</u>$_2$ triazoline), 3.47(t, 2H, C<u>H</u>$_2$-NH), 4.29-4.34 (m, 1H, C<u>H</u> triazoline), 6.31-6.70(m,2H,C<u>H</u>=C-<u>H</u>), 7.76-8.08 (m, 13H Ar-H and N<u>H</u>-CH_2), 12.69 (s,1H,-O-H). ^{13}C-NMR (δ ppm): 45.19(<u>C</u>H_2 -NH), 55.85(<u>C</u>H_2 triazoline), 73.99(<u>C</u>H-N), 119.09(O=C-<u>C</u>=C), 121.08-145.05 (<u>C</u>-Ar), 151.42(C=<u>C</u>), 176.14 (O=<u>C</u>-O-H), 197,13(<u>C</u>=O).

2-(N-((1-(4-(3-(4-nitrophenyl)acryloyl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (2d)

Yield 73%, m.p. 125-127°C, FTIR(KBr),υ (cm^{-1}): 3482(O-H), 3377(N-H), 3076(C-H)Ar, 2929 and 2870(CH_2), 1714(C=O)acid, 1651(C=O)ketone, 1593(C=C), 1516(N=N), 1450(NO_2)Assyn, 1286(NO_2)Sym, 1334(SO_2)Asym, 1168(SO_2)Sym, 1220(C-O)Asym, 1136(C-O) Sym.

2-(N-((1-(4-(3-(4-hydroxyphenyl)acryloyl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (2e)

Yield 76%, m.p. 105-106°C, FTIR(KBr),υ (cm^{-1}): 3363(O-H), 3217(N-H) 3068(C-H)Ar, 2928 and 2858(CH_2), 1718(C=O)acid, 1651(C=O)ketone, 1595(C=C), 1514(N=N), 1332(SO_2)Asym, 1165(SO_2)Sym, 1222(C-O)Asym, 1064(C-O)Sym.

2-(N-((1-(4-(3-(3-hydroxyphenyl)acryloyl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (2f)

Yield 68%, m.p. 118-120°C, FTIR(KBr),υ (cm^{-1}): 3365(O-H), 3267(N-H), 3064(C-H)Ar, 2926 and 2872(CH_2), 1718(C=O)acid, 1651(C=O)ketone, 1595(C=C), 1529(N=N), 1332(SO_2)Asym, 1165(SO_2)Sym, 1273(C-O)Asym, 1122(C-O)Sym.

2-(N-((1-(4-(3-(4-(dimethylamino)phenyl)acryloyl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (2g)

Yield 82%, m.p. 122-124°C, FTIR(KBr),υ (cm^{-1}): 3392(O-H), 3286(N-H), 3036(C-H)Ar, 2922 and 2858(CH_2), 1724(C=O)acid, 1649(C=O)ketone, 1595(C=C), 1525(N=N), 1336(SO_2)Asym, 1180(SO_2)Sym, 1230(C-O)Asym, 1124(C-O)Sym. ^{1}H-NMR: 2.67 (d, 2H, C$\underline{H}_2$ triazoline), 3.03(s, 6H, CH_3), 3.50(t, 2H, C$\underline{H}_2$-NH), 4.91-4.95 (m, 1H, C$\underline{H}$ triazoline), 5.74-5.86(m,2H,C$\underline{H}$=C-$\underline{H}$), 6.10-7.90 (m, 13H Ar-H and N$\underline{H}$-CH_2), 12.76 (s,1H,-O-H). ^{13}C-NMR (δ ppm): 30.01(C$\underline{H}_3$), 42.72(C$\underline{H}_2$-NH), 52.69(C$\underline{H}_2$ triazoline), 71.51(C$\underline{H}$-N), 112.06(O=C-C$\underline{=}$C), 119.09-136.71(C$\underline{-}$Ar), 153.56(C=C$\underline{}$), 176.92 (O=C$\underline{}$-O-H), 196,48(C$\underline{=}$O).

2-(N-((1-(4-(3-(2,4-dimethoxyphenyl)acryloyl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (2h)

Yield 72%, m.p. 128-129°C, FTIR(KBr),υ (cm^{-1}): 3433(O-H), 3371(N-H), 3066(C-H)Ar, 2937 and 2847(CH_2), 1674(C=O)acid, 1649(C=O)ketone, 1600(C=C), 1510(N=N), 1301(SO_2)Asym, 1165(SO_2)Sym, 1213(C-O)Asym, 1114(C-O)Sym

General procedure for the Synthesis of pyrazoline derivatives (3a-f)[37]

To mixture of chalcone compounds [2a-f] (0.01 mole), dissolved in (20 mL) absolute ethanol containing (0.5 mL) acetic acid, (0.01 mole) semicarbazide hydrochloride was added. The mixture was refluxed for 8 h. and left with continuous stirring overnight. After that the mixture was poured into (50 mL) ice water and neutralized by diluted hydrochloric acid. The formed precipitate was filtered, washed and recrystallized from ethanol to give pyrazoline derivatives [3a-f]

2-(N-((1-(4-(1-carbamoyl-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (3a)

Yield 77%, m.p. 131-132°C, FTIR(KBr),υ (cm^{-1}): 3429(NH_2), 3255(O-H), 3070(C-H)Ar, 2928), 1712(C=O)acid and (C=N)pyrazoline vib. Coupling, 1599(C=C), 1529(N=N), 1336(SO_2)Asym, 1170(SO_2)Sym, 1228(C-O)Asym, 1120(C-O)Sym. ^{1}H-NMR: 2.69 (d, 2H, C$\underline{H}_2$ triazoline), 3.01(t, 2H, C$\underline{H}_2$-NH), 3.50 (d, 2H, C$\underline{H}_2$ pyrazoline), 3.88(t, 1H, C$\underline{H}$ pyrazoline), 4.58-4.65 (m, 1H, C$\underline{H}$ triazoline), 6.54(s,2H N$\underline{H}_2$), 7.27-8.11 (m, 14H Ar-H and N$\underline{H}$-CH_2), 11.63(s,1H,-O-H). ^{13}C-NMR (δ ppm): 35.88(C$\underline{H}_2$-NH), 44.07(C$\underline{H}_2$ pyrazoline), 51.77(C$\underline{H}_2$ triazoline), 54.70(C$\underline{H}$ pyrazoline), 75.56(C$\underline{H}$ triazoline), 123.07-146.72(C$\underline{-}$Ar), 152.52(C$\underline{=}$N pyrazoline), 175.21 (O=C$\underline{}$-NH_2), 193,36(O=C$\underline{}$-O-H).

2-(N-((1-(4-(1-carbamoyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (3b)

Yield 85%, m.p. 142-143°C, FTIR(KBr),υ (cm^{-1}): 3485(NH_2), 3362(O-H), 3282(N-H), 3039(C-H)Ar, 2926 and 2856(CH_2), 1681(C=O)acid, 1651(C=O amide and C=N pyrazoline vib. coupling), 1599(C=C), 1521(N=N), 1330(SO_2)Asym, 1168(SO_2)Sym, 1228(C-O)Asym, 1089(C-O)Sym.

2-(N-((1-(4-(5-(4-bromophenyl)-1-carbamoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (3c)

Yield 83%, m.p. 149-150°C, FTIR(KBr),υ (cm^{-1}): 3473(NH_2), 3363(O-H), 3064(C-H)Ar, 2929 and 2862(CH_2) 1693(C=O)amide, 1649(C=O amide and C=N pyrazoline vib. coupling), 1599(C=C), 1525(N=N), 1329(SO_2)Asym, 1168(SO_2)Sym, 1236(O-H)Asym, 1130(O-H)Sym. ^{1}H-NMR: 2.78 (d, 2H, C$\underline{H}_2$ triazoline), 3.51(t, 2H, C$\underline{H}_2$-NH), 3.94(t, 1H, C$\underline{H}$ pyrazoline), 4.26 (d, 2H, C$\underline{H}_2$ pyrazoline), 4.57-4.60 (m, 1H, C$\underline{H}$ triazoline), 6.54(s,2H N$\underline{H}_2$), 7.38-8.16 (m, 13H Ar-H and N$\underline{H}$-CH_2), 12.45(

s,1H,-O-H). ^{13}C-NMR (δ ppm): 42.72(CH_2-NH), 45.11(CH_2 pyrazoline), 51.95(CH_2 triazoline), 55.08(CH pyrazoline), 76.35(CH triazoline), 124.03-144.18(C-Ar), 149.65(C=N pyrazoline), 164.57(O=C-NH_2), 185,47(O=C-O-H).

2-(N-((1-(4-(1-carbamoyl-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (3d)

Yield 72%, m.p. 153-155°C, IR υ (cm^{-1}): 3444(NH_2), 3425(N-H), 3271(O-H), 3064(C-H)Ar, 2927 and 2856(CH_2), 1703(C=O)acid, 1652(C=O)amide and (C=N) pyrazoline vib. Coupling, 1602(C=C), 1515(N=N), 1332(SO_2)Asym, 1164(SO_2)Sym, 1251(C-O)Asym, 1122(νC-O)Sym

2-(N-((1-(4-(1-carbamoyl-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (3e)

Yield 67%, m.p. 136-138°C, FTIR(KBr),υ (cm^{-1}): 3419(NH_2), 3311(O-H), 3254(N-H), 3078(C-H)Ar, 2916 and 2858(CH_2), 1687(C=O)acid, 1583(C=O amide and C=N pyrazoline vib. coupling), 1564(C=C), 1521(N=N), 1386(SO_2)Asym, 1174(SO_2)Sym, 1222(C-O) Asym, 1105(C-O)Sym, . ^{1}H-NMR: 2.33 (d, 2H, CH_2 triazoline), 3.51(t, 2H, CH_2-NH), 3.81 (d, 2H, CH_2 pyrazoline), 4.53(t, 1H, CH pyrazoline), 4.67-4.75 (m, 1H, CH triazoline), 6.56(s,2H NH_2), 6.80-8.14 (m, 13H Ar-H and NH-CH_2), 9.31(s,1H,-O-H), 12.64(s,1H, O=C-O-H) . ^{13}C-NMR (δ ppm): 35.14(CH_2-NH), 44.07(CH_2 pyrazoline), 51.95(CH_2 triazoline), 54.03(CH pyrazoline), 75.30(CH triazoline), 120.03-149.65(C-Ar), 155.51(C=N pyrazoline), 156.56(C-O-H), 171.41(O=C-NH_2), 197,53(O=C-O-H).

2-(N-((1-(4-(1-carbamoyl-5-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (3f)

Yield 72%, m.p. 134-136°C, FTIR(KBr),υ (cm^{-1}): 3439(((NH_2), 3406(O-H),3255(O-H) 3128(C-H)Ar, 2997 and 2862(CH_2), 1728(C=O)acid, 1685(C=O) amide 1653 (C=N) pyrazoline, 1597(C=C), 1554(N=N), 1300(SO_2)Asym, 1170(SO_2)Sym, 1276(C-O)Asym, 1020(C-O)Sym.

General procedure for the Synthesis of isoxazoline derivatives (4a-d)[38]

To A mixture of (0.01 mole) compounds [2a-d], hydroxylamine hydrochloride (0.02 mole) and KOH(0.02 mole) in absolute ethanol (20 mL). The mixture was refluxed for 6-8 h. after cooling to room temperature, the mixture was poured onto ice water, the formed precipitate was filtered and recrystallized from absolute ethanol to give isoxazoline derivatives [4a-d].

2-(N-((1-(4-(5-phenyl-4,5-dihydroisoxazol-3-yl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (4a)

Yield 62%, m.p. 140-141°C, FTIR(KBr),υ (cm^{-1}): 3369(O-H), 3207(N-H), 3059(C-H)Ar, 2926 and 2860(CH_2), 1658(C=O)acid, 1600(C=N), 1519(C=C), 1446(N=N), 1327(νSO_2)Asym, 1163(νSO_2)Sym.

2-(N-((1-(4-(5-(4-chlorophenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (4b)

Yield 76%, m.p. 155-156°C, FTIR(KBr),υ (cm^{-1}): 3383(O-H), 3248(N-H), 3053(C-H)Ar, 2951 and 2883(CH_2), 1714(C=O)acid, 1672(C=N), 1600(C=C), 1523(N=N), 1327(SO_2)Asym, 1168(SO_2)Sym, 1257(C-O)Asym, 1093(C-O). ^{1}H-NMR: 2.79 (d, 2H, CH_2 triazoline), 3.44(t, 2H, CH_2-NH), 3.77 (d, 2H, CH_2 isoxazoline), 4.15(t, 1H, CH isoxazoline), 4.48-4.56 (m, 1H, CH triazoline), 6.54-8.14 (m, 13H Ar-H and NH-

CH_2), 12.24(s,1H,-O-H). ^{13}C-NMR (δ ppm): 43.02(CH_2-NH), 44.81(CH_2 isoxazoline), 54.40(CH_2 triazoline), 73.30(CH isoxazoline), 79.10(CH triazoline), 123.03-147.39(C-Ar), 162.49(C=N isoxazoline), 192,39(O=C-O-H).

2-(N-((1-(4-(5-(4-bromophenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (4c)

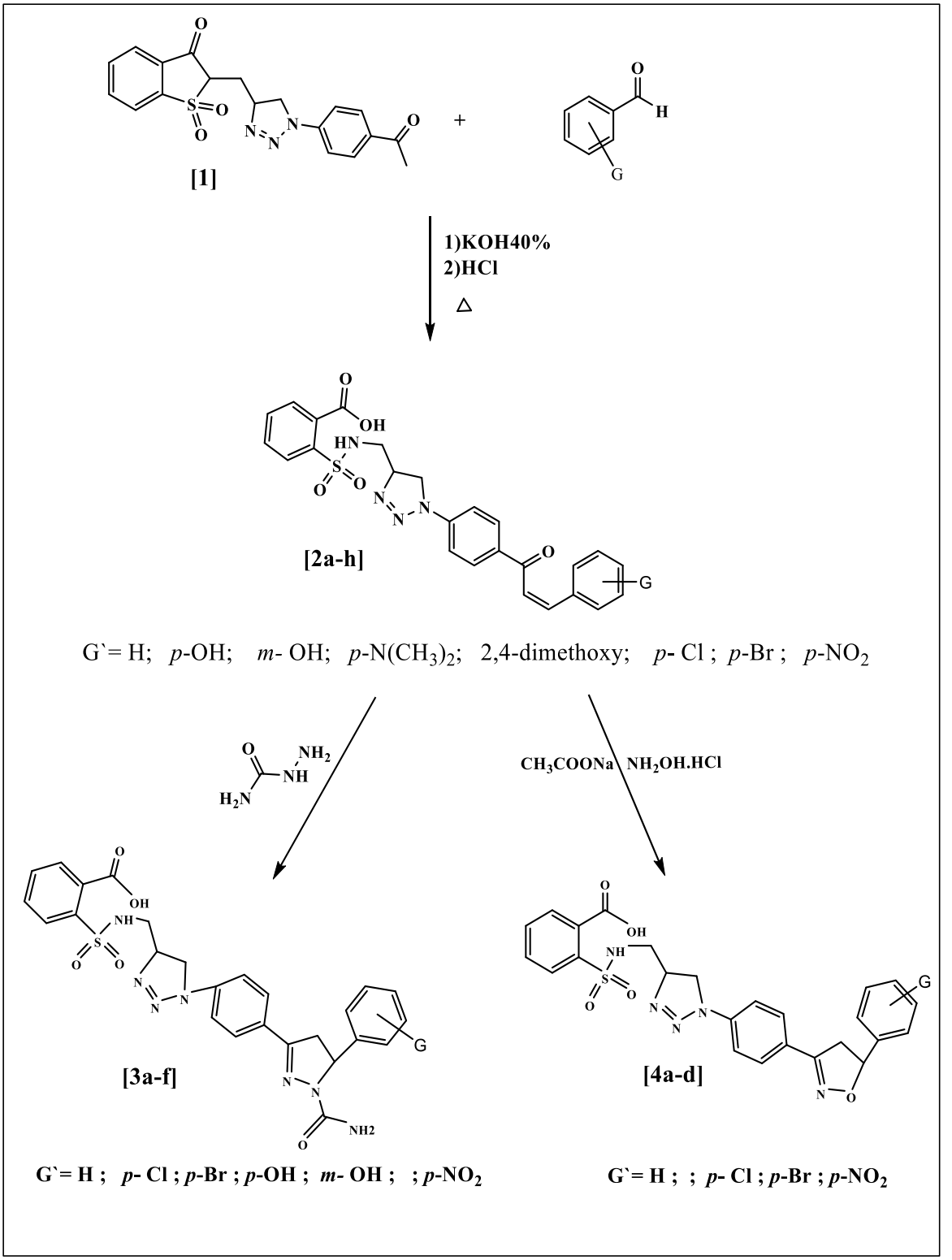
Yield 79%, m.p. 158-160°C, FTIR(KBr),υ (cm^{-1}): 3342(O-H), 3180(N-H)3055(C-H)Ar, 2928 and 2864(CH_2), 1697(C=O)acid, 1672(C=N), 1595(C=C), 1518(N=N), 1319(SO_2)Asym, 1176(SO_2)Sym, 1251(C-O)Asym, 1072(C-O)Sym

2-(N-((1-(4-(5-(4-nitrophenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid (4d)

Yield 81%, m.p. 163-165°C, FTIR(KBr),υ (cm^{-1}): 3381(N-H), 3238(O-H), 3061(C-H)Ar, 2929 and 2864(CH_2), 1708(C=O)acid, 1687(C=N), 1595(C=C), 1516(N=N), 1336(SO_2)Asym, 1166(SO_2)Sym. ^{1}H-NMR: 2.58 (d, 2H, CH_2 triazoline), 3.30(t, 2H, CH_2-NH), 3.56 (d, 2H, CH_2 isoxazoline), 4.17(t, 1H, CH isoxazoline), 4.52-4.61 (m, 1H, CH triazoline), 6.70-8.34 (m, 13H Ar-H and NH-CH_2), 12.52(s,1H,-O-H). ^{13}C-NMR (δ ppm): 51.28(CH_2-NH), 54.70(CH_2 isoxazoline), 61.62(CH_2 triazoline), 71.21(CH isoxazoline), 78.72(CH triazoline), 122.32-140.84(C-Ar), 150.51(C-NO_2) 174.84(C=N isoxazoline), 200,95(O=C-O-H).

Results and Discution

2-((1-(4-acetylphenyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methyl)benzo[d]isothiazol-3(2H)-one 1,1-dioxide(1) as a starting material was prepared via the cyclization of p-acetyl azido benzene with N-allyl saccharin [39]. Chalcone derivatives were synthesized via condensation of benzaldehyde such as benzaldehyde, *p*-bromo benzaldehyde, *p*-chloro benzaldehyde, *p*-hydroxy benzaldehyde, *m*-hydroxy benzaldehyde, *p*-nitro benzaldehyde, 2,4-dimethoxy benzaldehyde and *p*-N,N-dimethyl benzaldehyde in the presence of 40% KOH. The appearance new stretching bands at (1651-1633) and (1600- 1593)cm^{-1} which are due to (C=O) and (C=C) respectively. While ^{1}H-NMR and ^{13}C-NMR showed 5.74 - 7.00 (m,2H,CH=C-H), 12.35- 12.76 (s,1H,-O-H). and 112.06 -119.03(O=C-C=C), 163.92- 176.92 (O=C-O-H), 147.78- 153.56(C=C). The cyclization of prepared chalcones with semicarbazide in the presence of glacial acetic acid[37] and with hydroxylamine hydrochloride in the presence anhydrous sodium acetate[38] afforded the corresponding pyrazolines showed the absence of ν(C=O) ketone group at (1651-1633)cm^{-1} . While the appearance starching bands of ν(C=N) at (1712-1649)cm^{-1} While ^{1}H-NMR and ^{13}C-NMR showed 3.50-4.26(d, 2H, CH_2 pyrazoline), 3.88-4.53 (t, 1H, CH pyrazoline), and 44.07- 45.11 (CH2 pyrazoline), 54.03- 55.08 (CH pyrazoline), 149.65- 152.52(C=N pyrazoline). And isoxazoline derivatives. FT-IR showed disappearance of absorption band of ν(C=O) ketone group at (1651-1633)cm-1 and appearance absorption bands for ν(C=N) at (1687-1658)cm^{-1}. ^{1}H-NMR and ^{13}C-NMR showed the following characteristic signals: 3.56-3.77(d, 2H, CH_2 isoxazoline) and 44.81-54.70(CH_2 isoxazoline), 71.21-73.30(CH isoxazoline), 162.49-174.84(C=N isoxazoline).



Scheme (1). Route synthesized compounds **(2a-h)**, **(3a-f)** and **(4a-d).**

Biological activity

The test was performed according to the disk diffusion method[40] . The prepared compounds were tested against one strain of Gram-positive bacteria (Staphylococcus Aureus), and one Gram-negative bacteria (Escherichia coli). Prepared agar and Petri dishes were sterilized by autoclaving for (15min) at 121 ^{o}C. The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably, spaced apart holes were made all (6mm) in diameter, were filled with 100µl of the prepared compounds (1mg of the compound dissolved in 1ml of DMSO solvent). These plates were incubated at (37^{o}C) for (24hours). The inhibition zones caused by the various compounds on the bacteria were examined. The results of the preliminary screening test are listed in Table (1).

TABLE-1

Antibacterial activity of some prepared compounds and ceftriaxone control drug

Product	***Staphylococcus aureus* (Gram-positive)**	***Escherichia coli* (Gram-negative)**
2d	0	19
2e	0	18
2g	0	17
3b	0	17
3d	0	15
4b	0	14
4d	20	20
Ceftriaxone	12	16
Control	0	0

[Control]: 100µg/mL; Solvent: dimethylsolfoxide

Inhibition Zone: (0) no inhibition; (12-15) moderate; (17-20) strong.

Electrochemical oxidation effect

Electrochemical methods provide high potential for the investigation of antioxidant compounds, assessment of antioxidant capacity, and measurement of the electrochemical index. The devices can be stationary or flow-through and based on cyclic or differential pulse voltammetry as well as potentiostatic analysis. The methods are known for their suitability for food control and monitoring the levels of antioxidant capacity in other biological samples and matrices. The application of electrochemical methods for the analysis of plant and clinical samples concerning the study of their antioxidant properties was studied by different researchers[41]. In this study, some of the synthesized compounds [2d, 2e, 2g, 3b, 3d, 4b, and 4d] have a good antibacterial as shown in Table (1). Some of them were a good antioxidant and medium degree, whereas the others are not suitable for use as an antioxidant. A fabricated and modified glassy carbon electrode (GCE) as a biosensor with mechanical attachment by carbon nanotubes to detect the effect of several compounds [2d, 2e, 2g, 3b, 3d, 4b, and 4d] on mercury ions in blood serum of human health. Cyclic Voltammogram shows the effect of the oxidation current peaks of Hg2+ with blood serum, by modified (GCE), carbon nanotube CNT as a working electrode using Cyclic Voltammogram (CV) method which enhances the oxidation current peak at CV analysis study. Compounds [2g, 2d, 3b, 3d, 4b, and 4d] all prepared compound antioxidant exception compound (2d) considered toxic in therapeutic processes, arrange in terms of their effectiveness

as antioxidant from high antioxidant to low antioxidant respectively, as follows:

2g> 4d > 3b> 4b > 3d> 2d> 2d

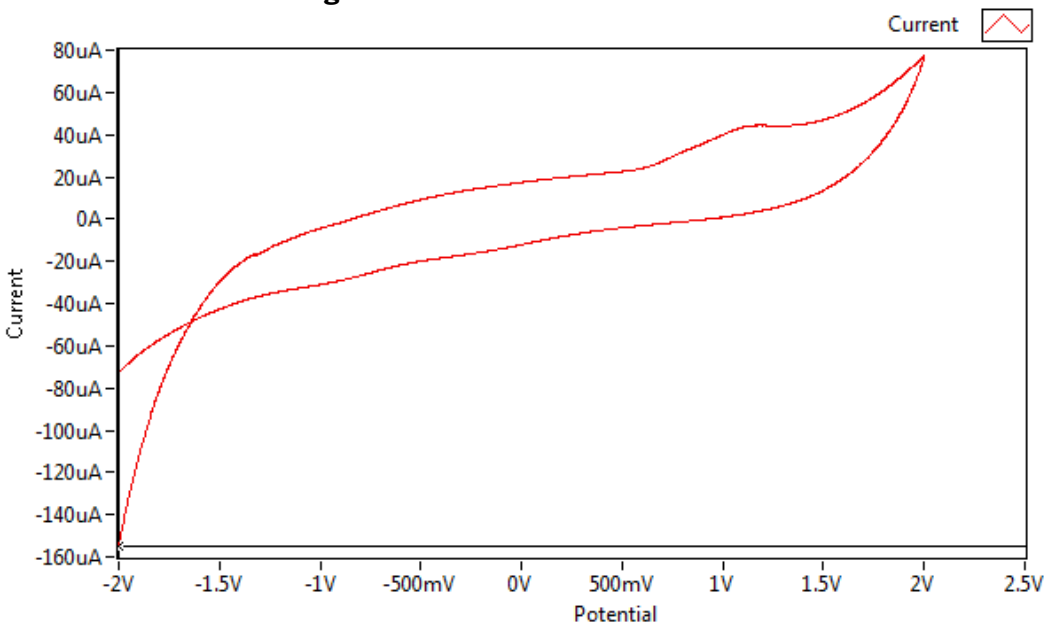


Figure (3). Cyclic voltammogram of compound [2d] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed reduction current peak at (+1)v.

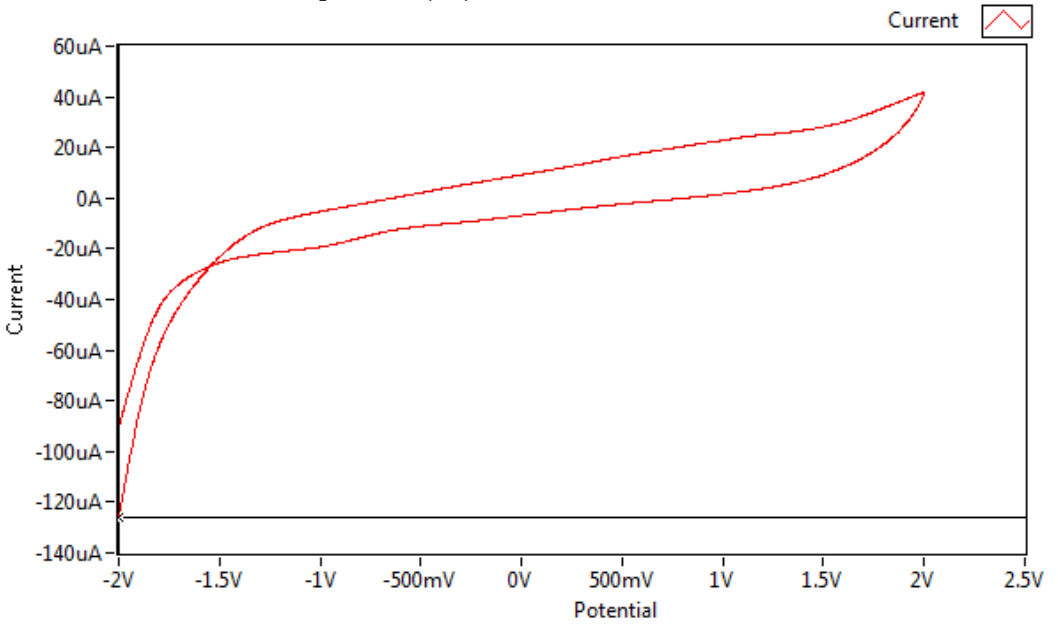


Figure (1). Cyclic voltammogram of compound [2e] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed oxidation current peak at (+1.2)v.

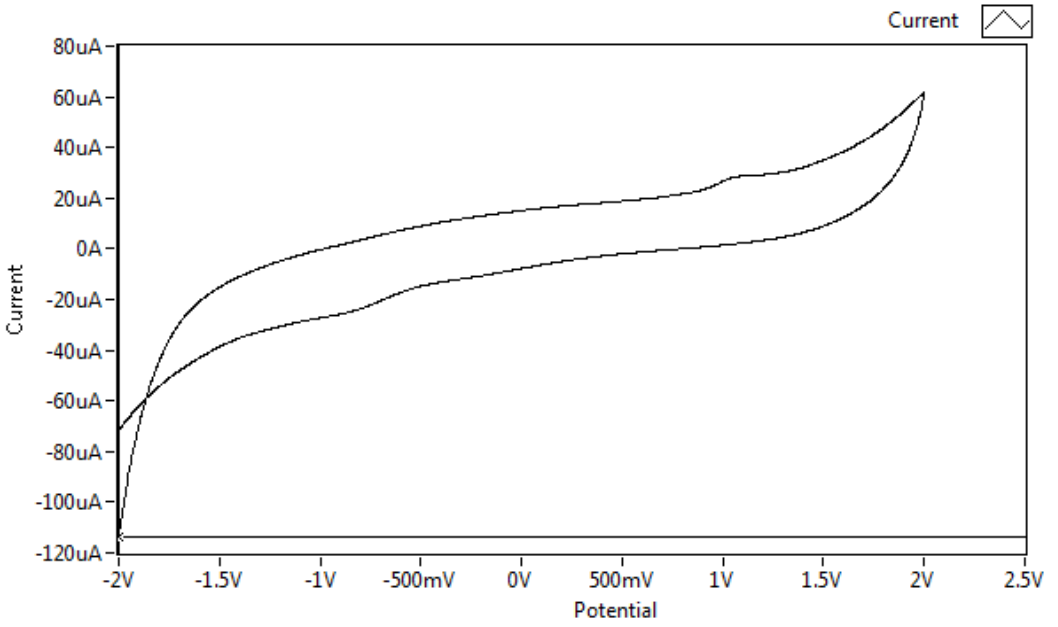


Figure (2). Cyclic voltammogram of compound [2g] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode. Showed two oxidation current peak at (-0.2)v and (+1.2)v.

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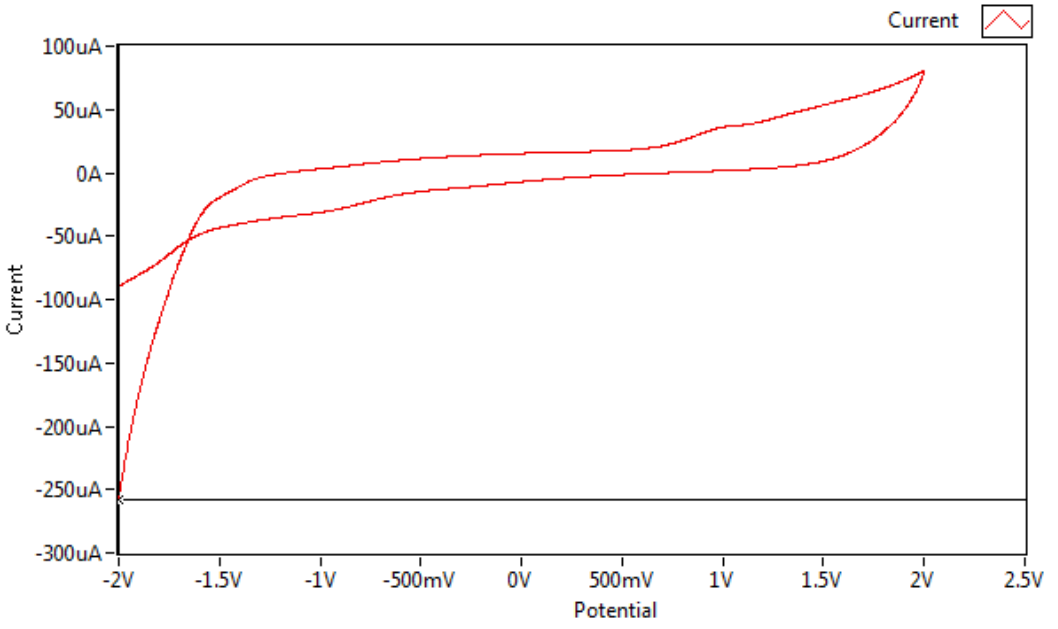

Figure (4). Cyclic voltammogram of compound [3b] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed oxidation current peak at (+1.2) v.

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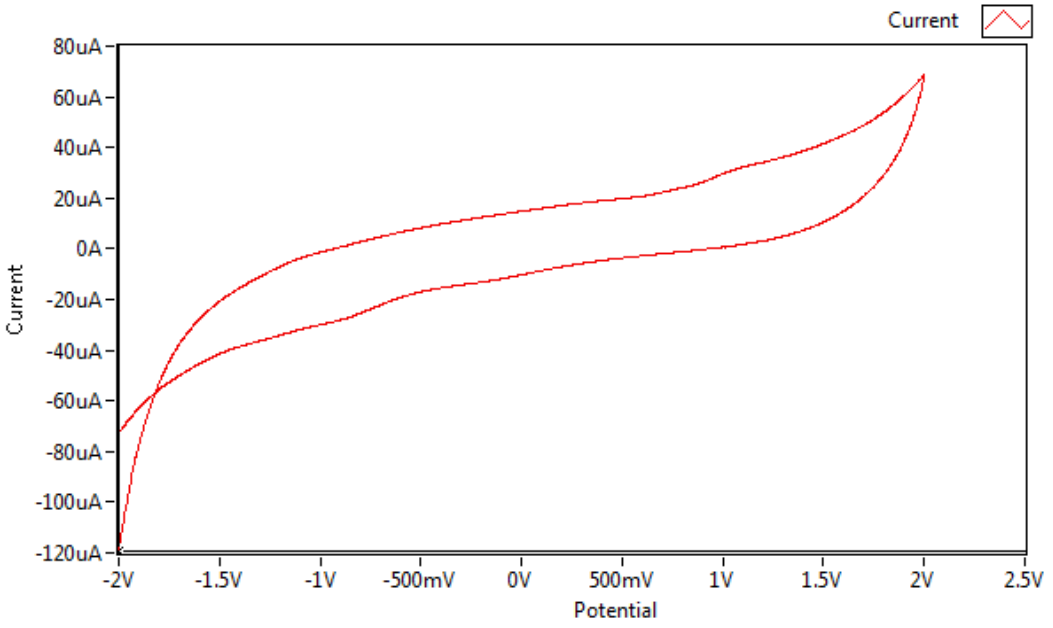

Figure (5). Cyclic voltammogram of compound [3d] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed oxidation current peak at (-0.75)v.

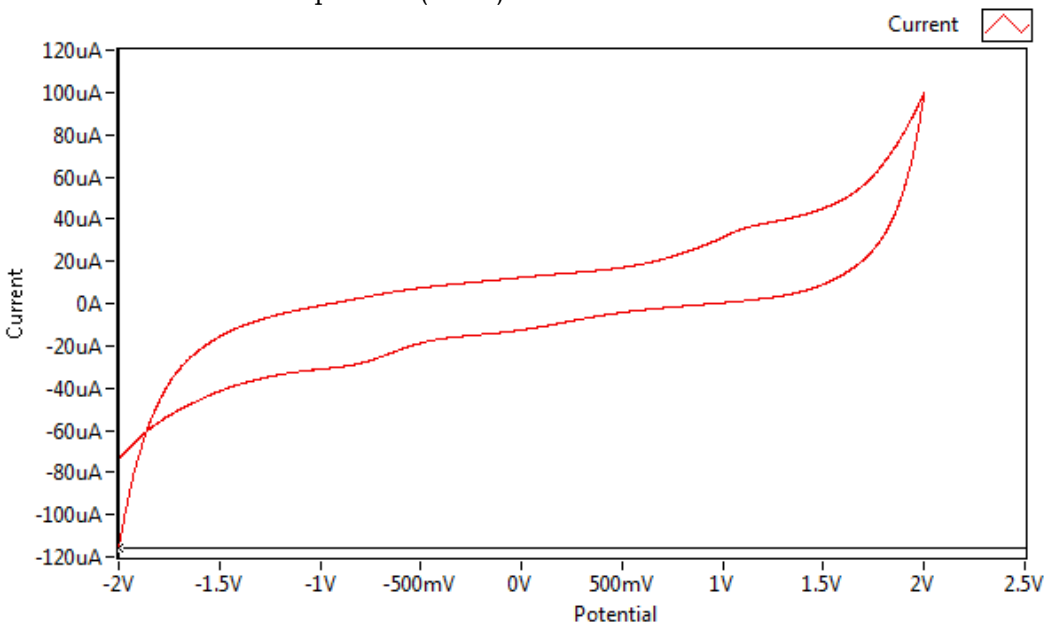

Figure (6). Cyclic voltammogram of compound [4b] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed oxidation current peak at (+1)v.

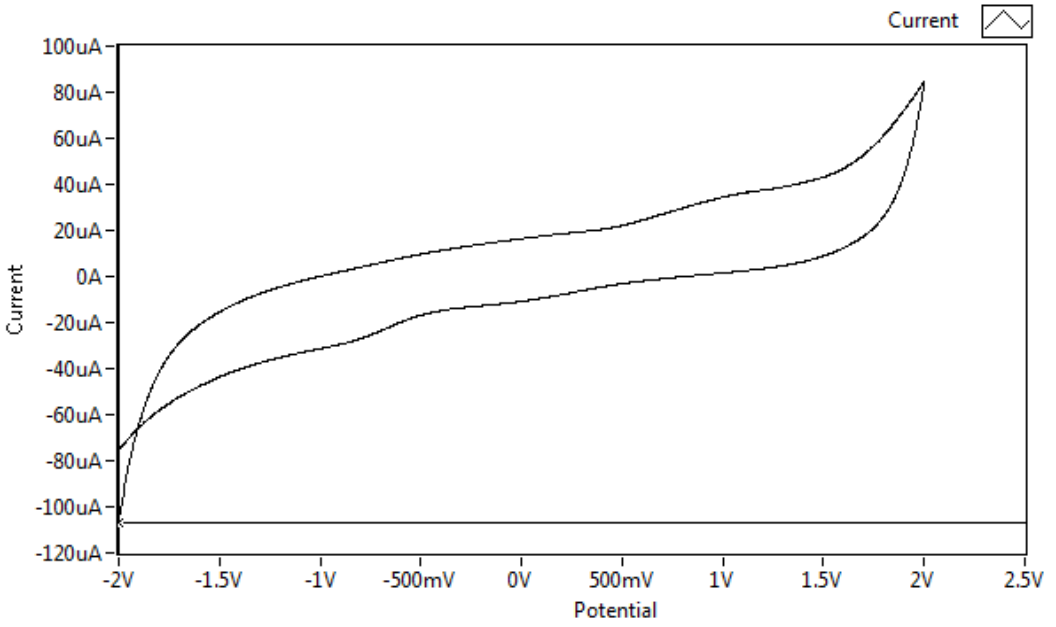

Figure (6). Cyclic voltammogram of compound [4d] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed oxidation current peak at (+1)v.

Molecular docking studies:

Based on the literature, Structure of human ketohexokinase-c complex with fructose, NO_3 and osthole was selected as target for Escherichia Coli. The X-ray crystal structure of 6ul7 (PDB ID: 6ul7 was availed from Protein Data Bank.

The possible binding modes between the ligands and the target protein 6ul7 were loaded in the Pyrex and Biovia discovery studio visualizer is a computer program for predicting protein-ligand interactions. For a given protein and a ligand, Biovia discovery studio visualizer predicts the geometry of the complex as well as an estimate for the strength of binding[27,42].

Preparation of the binding site was done using the Receptor Intelligence of the Receptor Preparation Wizard and this includes a selection of chains, receptor protonation. The active site of the target protein was defined around a radius of 6.50 Å. Biovia discovery studio visualizer uses the constructive incremental build-up algorithm. For validation of the software, the ligands were extracted and re-docked into the active sites. To evaluate the quality of co-crystallized ligands. An RMSD (TABLE 2) value cut-off lesser than 2 Å is considered a good prediction for computed ligand-protein confirmation. The docking scores and the 2D and 3D pose views were generated for further analysis of the interactions and binding affinities of the selected ligands molecules

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TABLE 2. Binding affinity (kcal/mol) of the favorable conformation of series (2a-h)

Compound	Affinity (kcal/mol)
2a	-8.0
2b	-8.1
2c	-8.2
2d	-7.7
2e	-8.5
2f	-6.7
2g	-7.7
2h	-8.0

TABLE 3. Various interactions involved between receptor and compound **2e**

Bond Length (Å)	Type of bond
4.60	Conventional hydrogen bond
7.22	Conventional hydrogen bond
3.73	Conventional hydrogen bond
5.55	Conventional hydrogen bond
7.01	Conventional hydrogen bond
3.37	Conventional hydrogen bond
6.34	Pi-cation -anion
5.89	Pi-cation -anion
5.48	Pi-cation -anion
5.92	Pi-alkyl
5.05	Pi-alkyl
4.67	Pi-alkyl
5.42	Unfavourable donor - accepter
4.23	Unfavourable donor -accepter

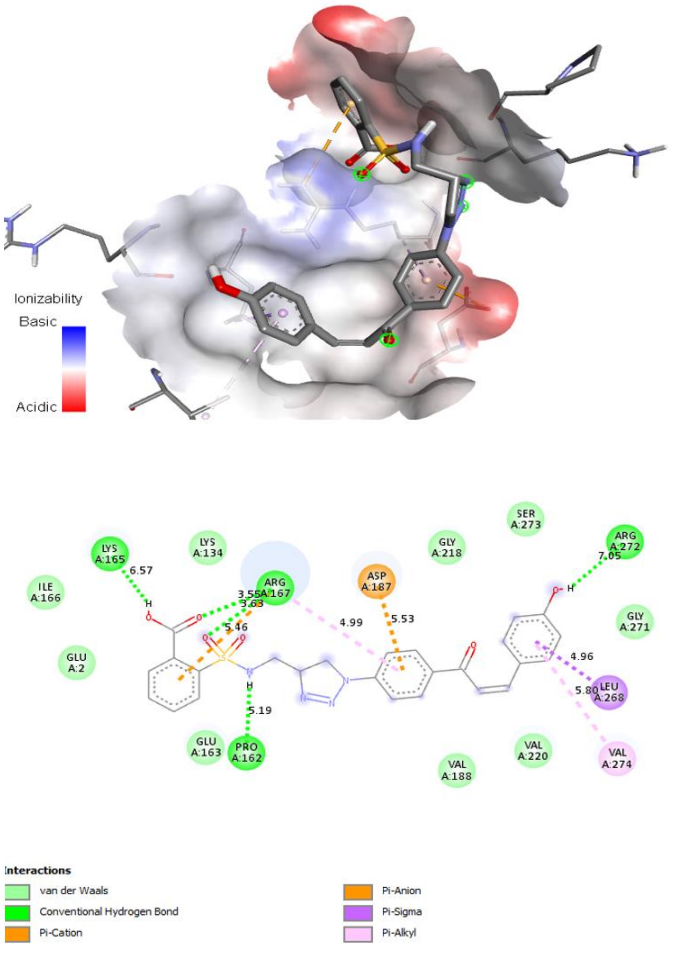


Figure (6) Binding site interaction of structure of compound **2e** 2D and 3D

TABLE 4. Binding affinity (kcal/mol) of the favourable conformation of series **(3a-f)**

Compound	Affinity (kcal/mol)
3a	-7.9
3b	-9.2
3c	-8.4
3d	-8.3
3e	-8.6
3f	-8.3

TABLE 5. Various interactions involved between receptor and compound **3b**

Bond Length (Å)	Type of bond
4.33	Conventional hydrogen bond
5.93	Conventional hydrogen bond
4.93	Conventional hydrogen bond
3.43	Conventional hydrogen bond
4.29	Conventional hydrogen bond

5.75	Conventional hydrogen bond
7.33	Conventional hydrogen bond
5.47	Pi-Cation-anion
5.55	Pi-Cation-anion
5.83	Pi-Cation-anion
3.73	Van der waals
5.13	Pi-Alkyl
4.98	Pi-Alkyl
5.79	Pi-Sigma

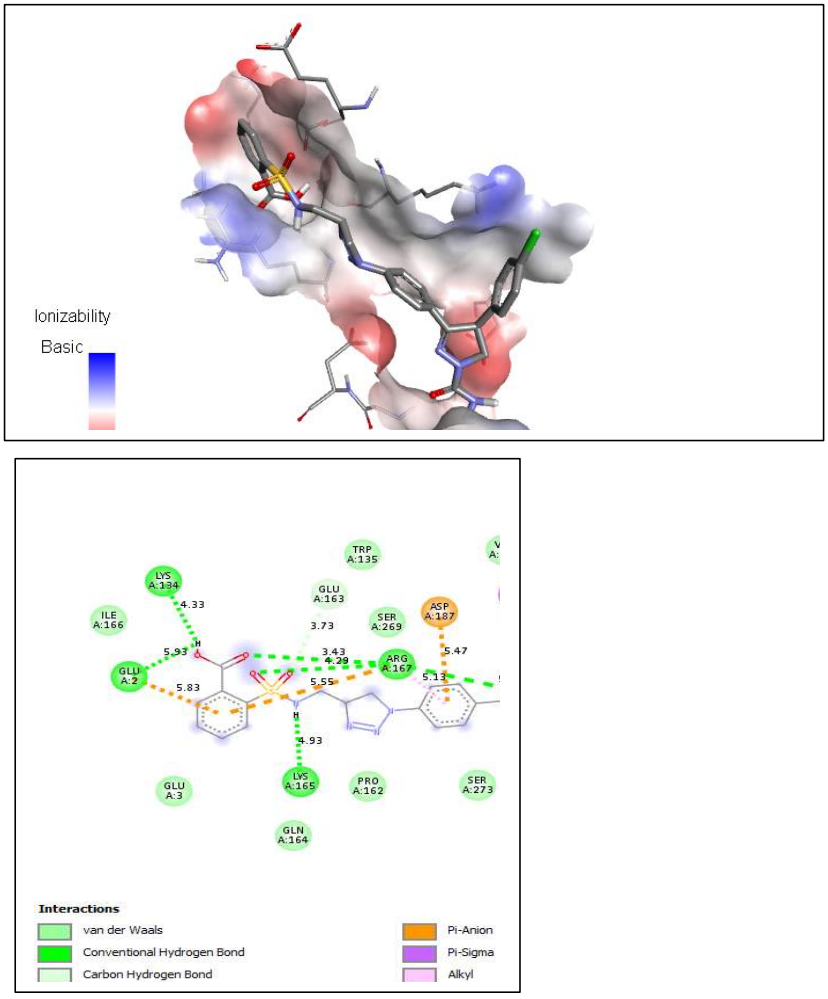


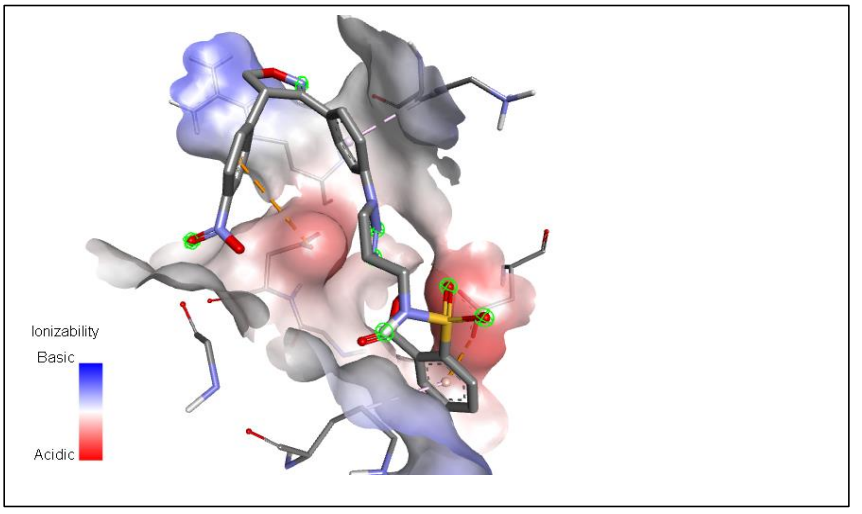
Figure (6) Binding site interaction of structure of compound **3d** 2D and 3D

TABLE 6. Binding affinity (kcal/mol) of the favourable conformation of series **(4a-d)**

4a	-7.7
4b	-7.8
4c	-7.8
4d	-8.0

TABLE 7. Various interactions involved between receptor and compound **4d**

Bond Length (Å)	Type of bond
5.97	Conventional hydrogen bond
6.25	Pi-Cation
5.30	Pi-Pi-Stacked
5.03	Pi-Alkyl
5.91	Pi-Alkyl
4.41	Pi-Alkyl
4.63	Pi-Sigma



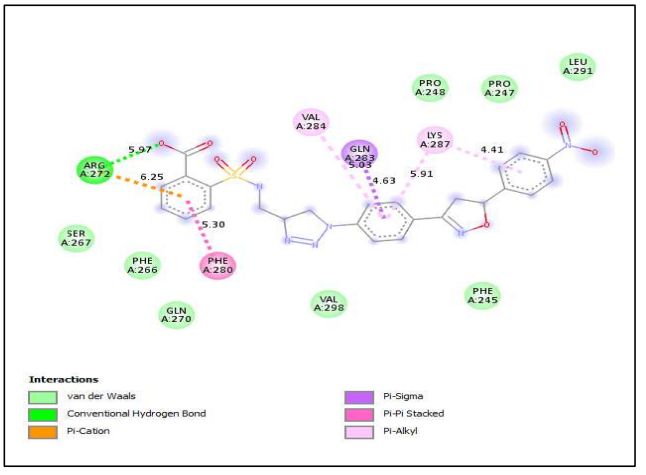


Figure (6) Binding site interaction of structure of compound **4d** 2D and 3D

Conclusion

Three series (2a-h), (3a-f) and (4a-d) synthesized compounds docked to the Escherichia coli putative binding site using Pyrex – virtual Screening Toll and BIOVIO Discovery Studio. All of them have shown promising binding affinity thus could suggest their strong binding interaction. Among them, compounds 2e, 3b and 4d displayed the potential binding affinity more than the others. The synthesized chalcone, pyrazoline and isoxazoline appeared higher effect against Gram-negative bacteria than that of Gram-positive bacteria. Triazolines compound 4d was found to be better activity than Ceftriaxone against Gram-positive bacteria**,** where compounds 2e, 2g, 3b and 4d were found to be better activity than Ceftriaxone against Gram-negative bacteria. Prepared Selected Compounds showed antioxidant activity exception compound 2h showed reduction activity.

Acknowledgment

As the authors of this work, we would love to express our ample thankfulness and gratitude to the Department of Chemistry, College of Science, University of Baghdad and College of Pharmacy, for their magnificent support and providing us with facilities to conduct this research on all accounts. The authors thanks for the University of Tehran, the Faculty of Science, Iran for their significant assistance in ^{1}H NMR and ^{13}CNMR . I extend my heartfelt thanks to Dr. Muhammed Mizher Radhi \ College of Medicine, Middle Technical University, Iraq for assistance in Electrochemical oxidation measurements**.**

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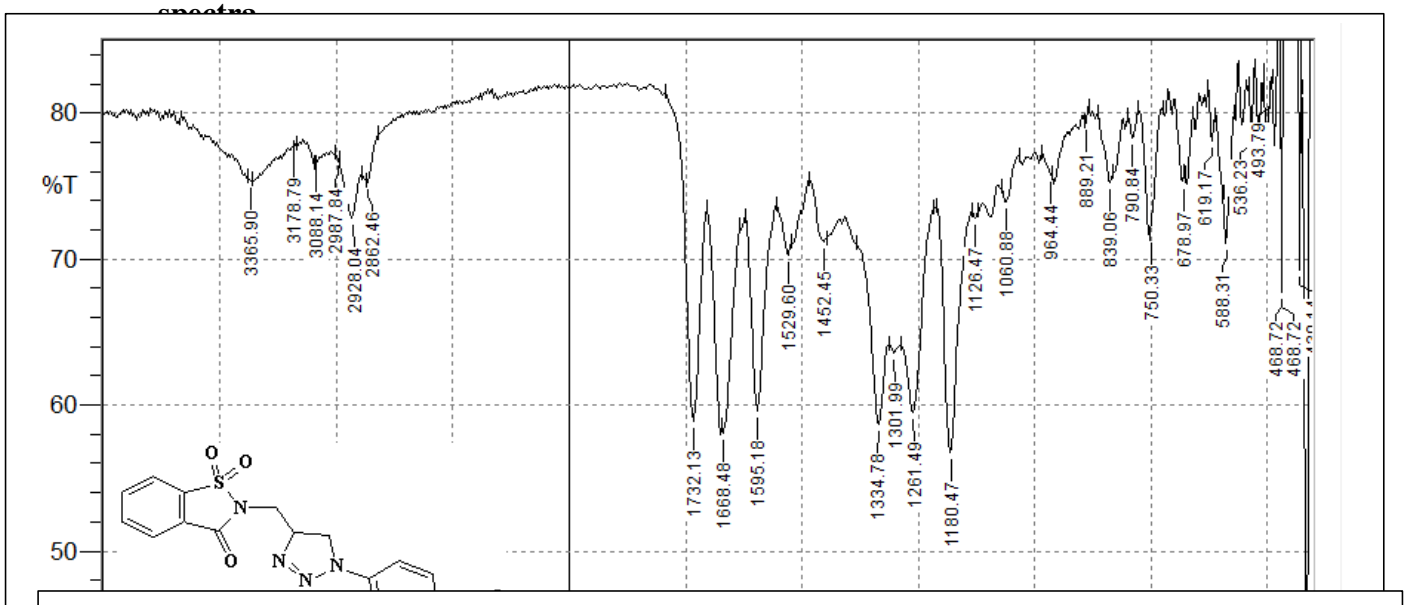
Supporting Information

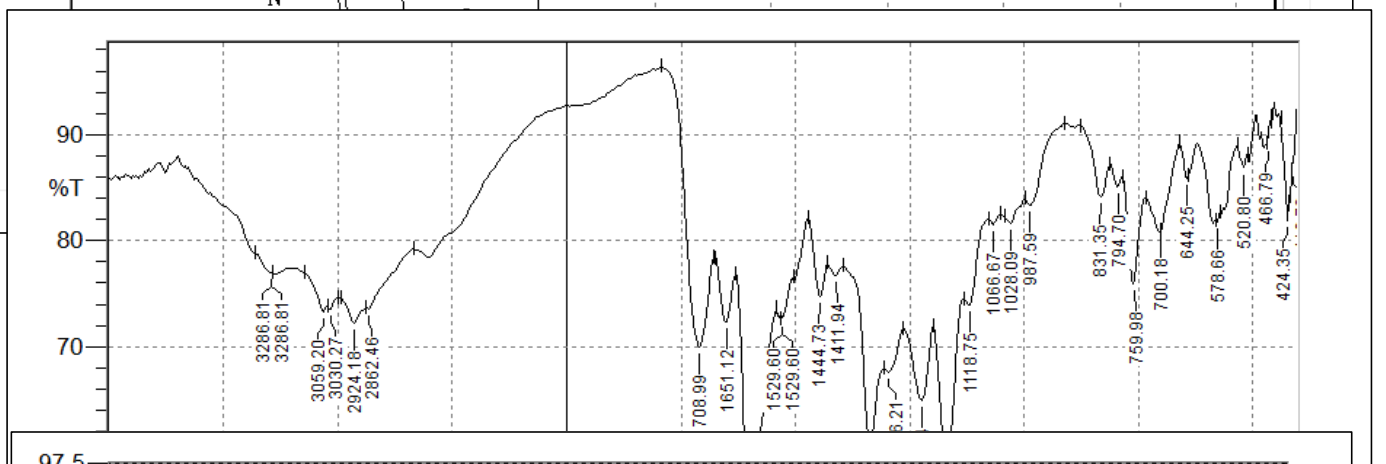
A. Spectroscopic figures

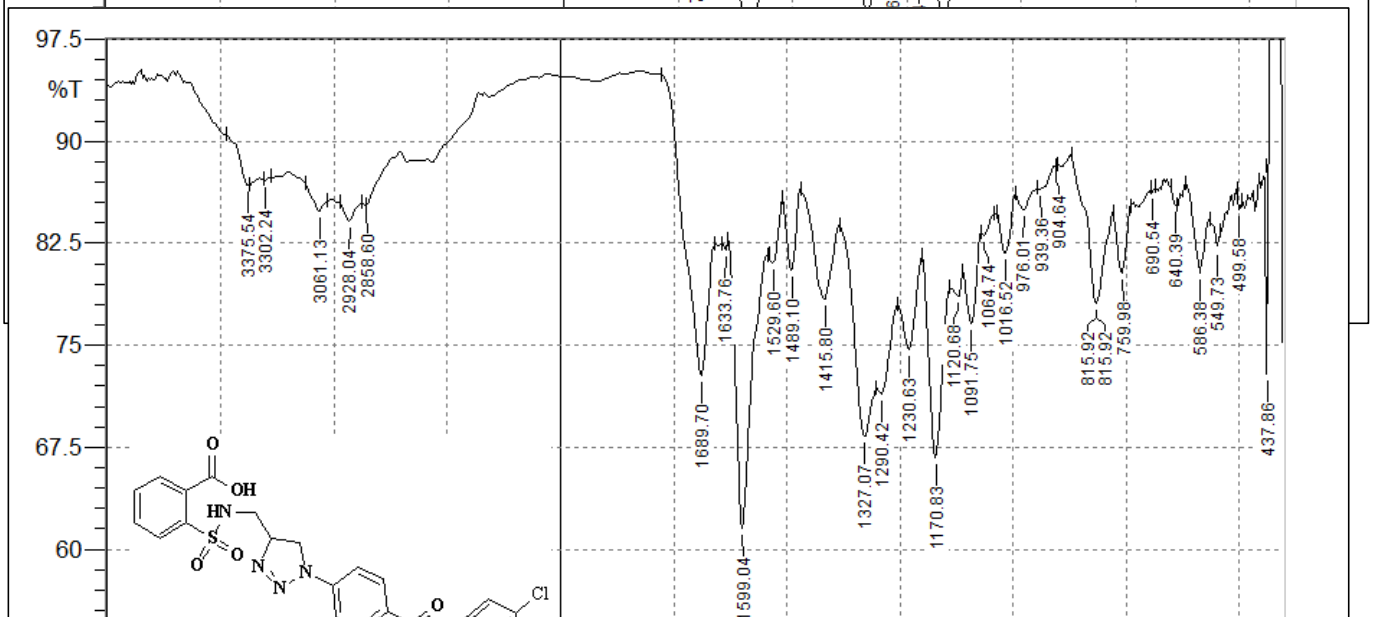
A.1. FT-IR spectra

A.2. ^{1}H-NMR spectra

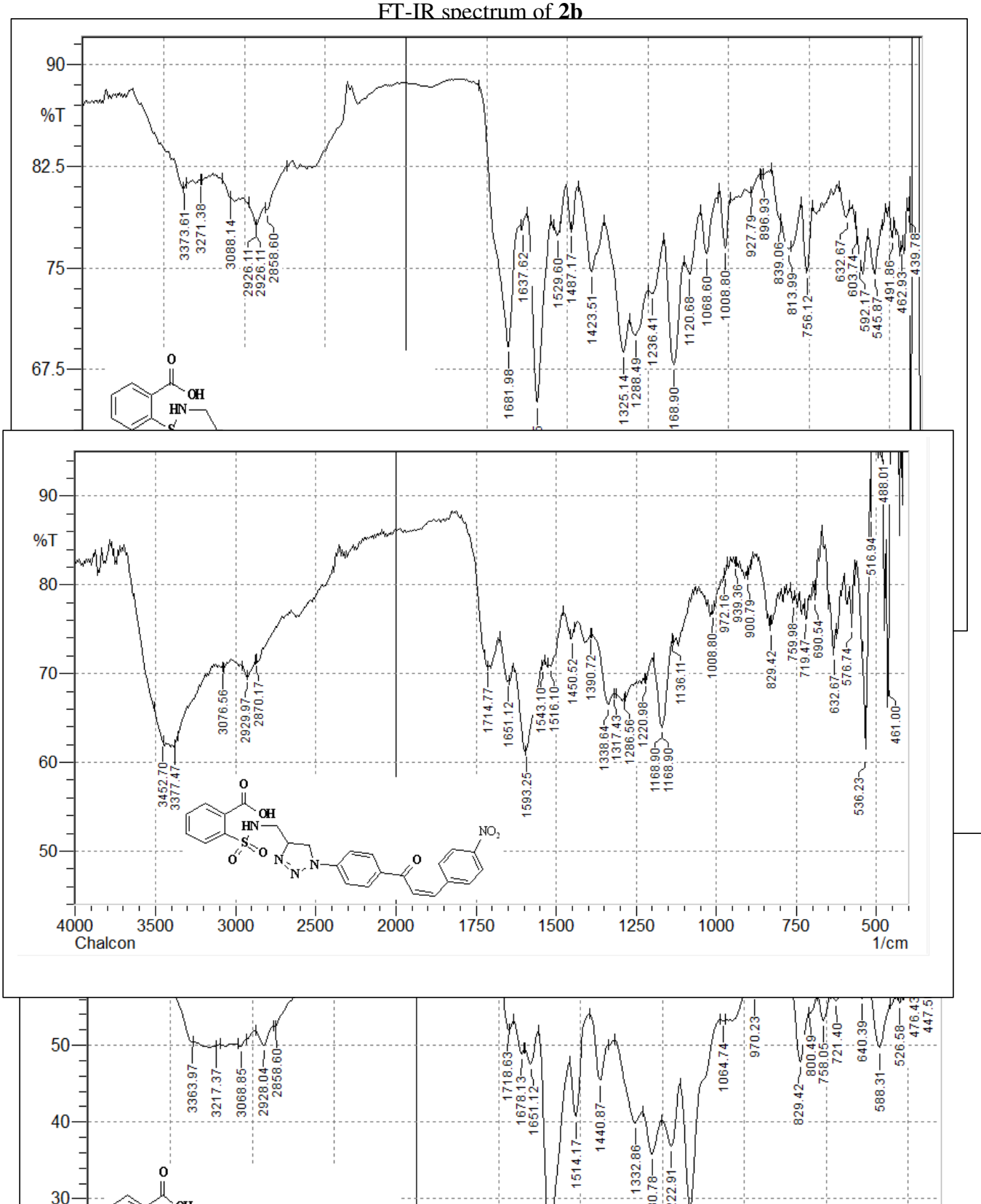
A.3. ^{13}C-NMR spectra



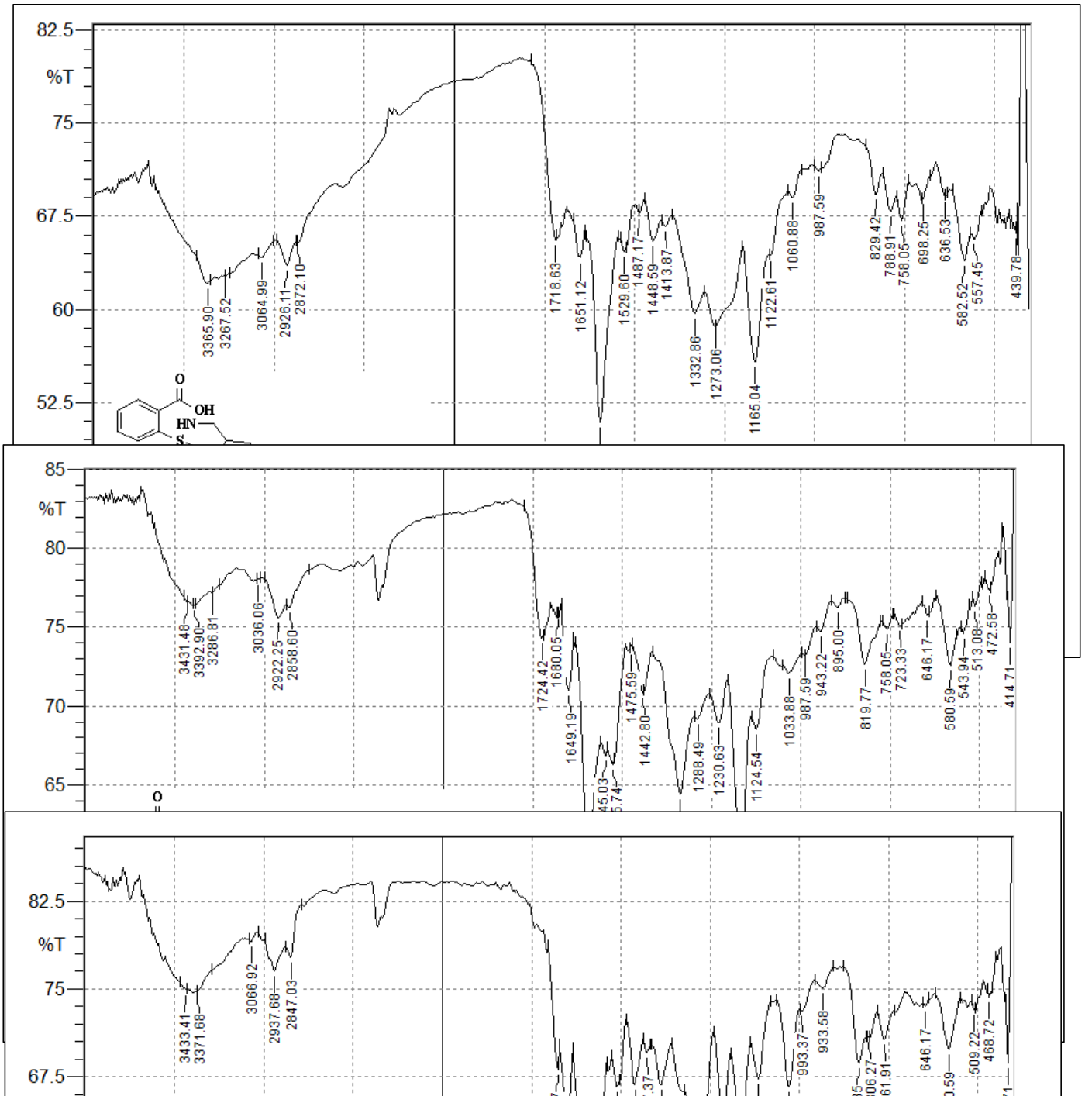




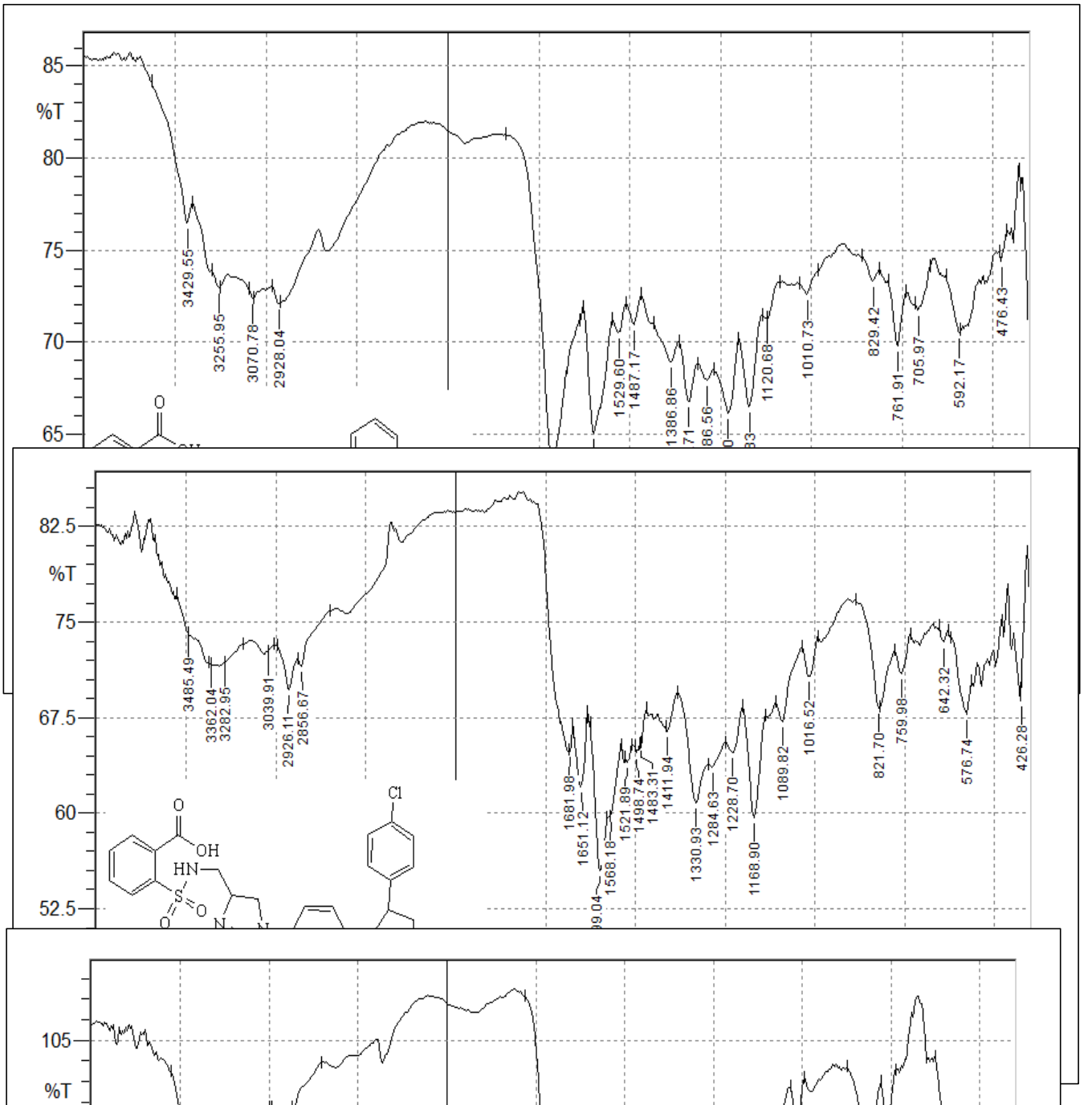
FT-IR spectrum of **2b**



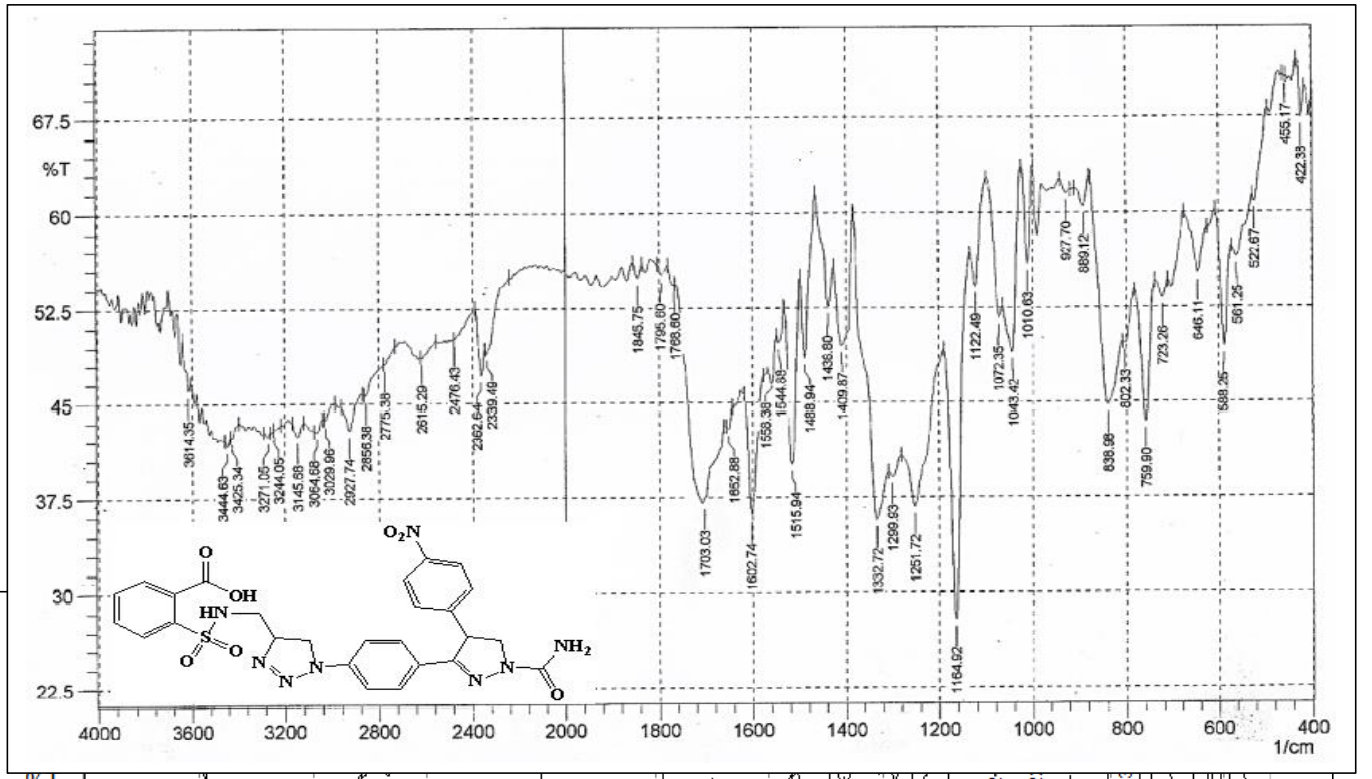
FT-IR spectrum of **2e**

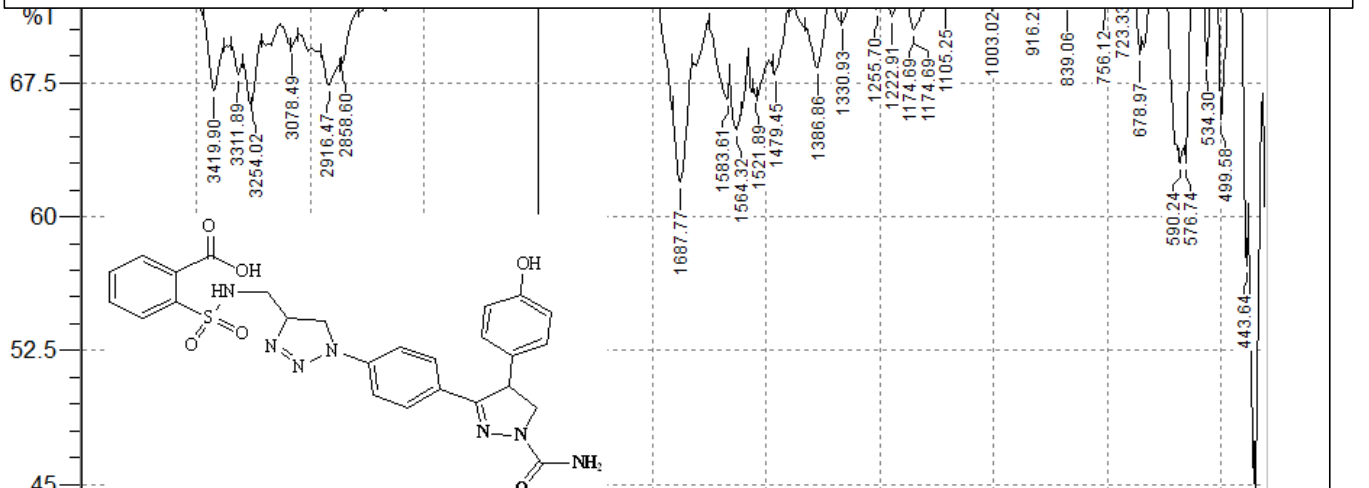


FT-IR spectrum of **2h**



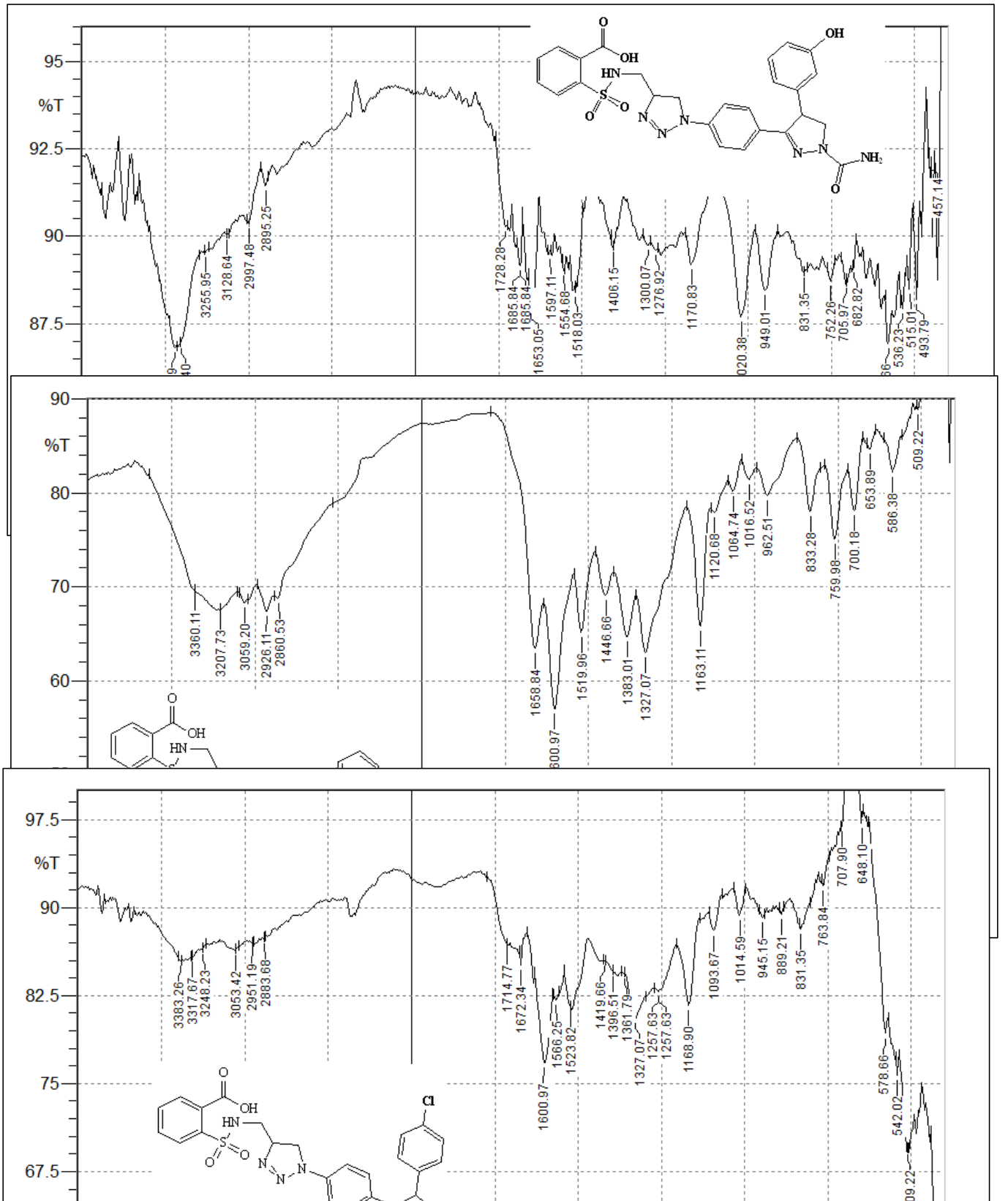
FT-IR spectrum of **3c**



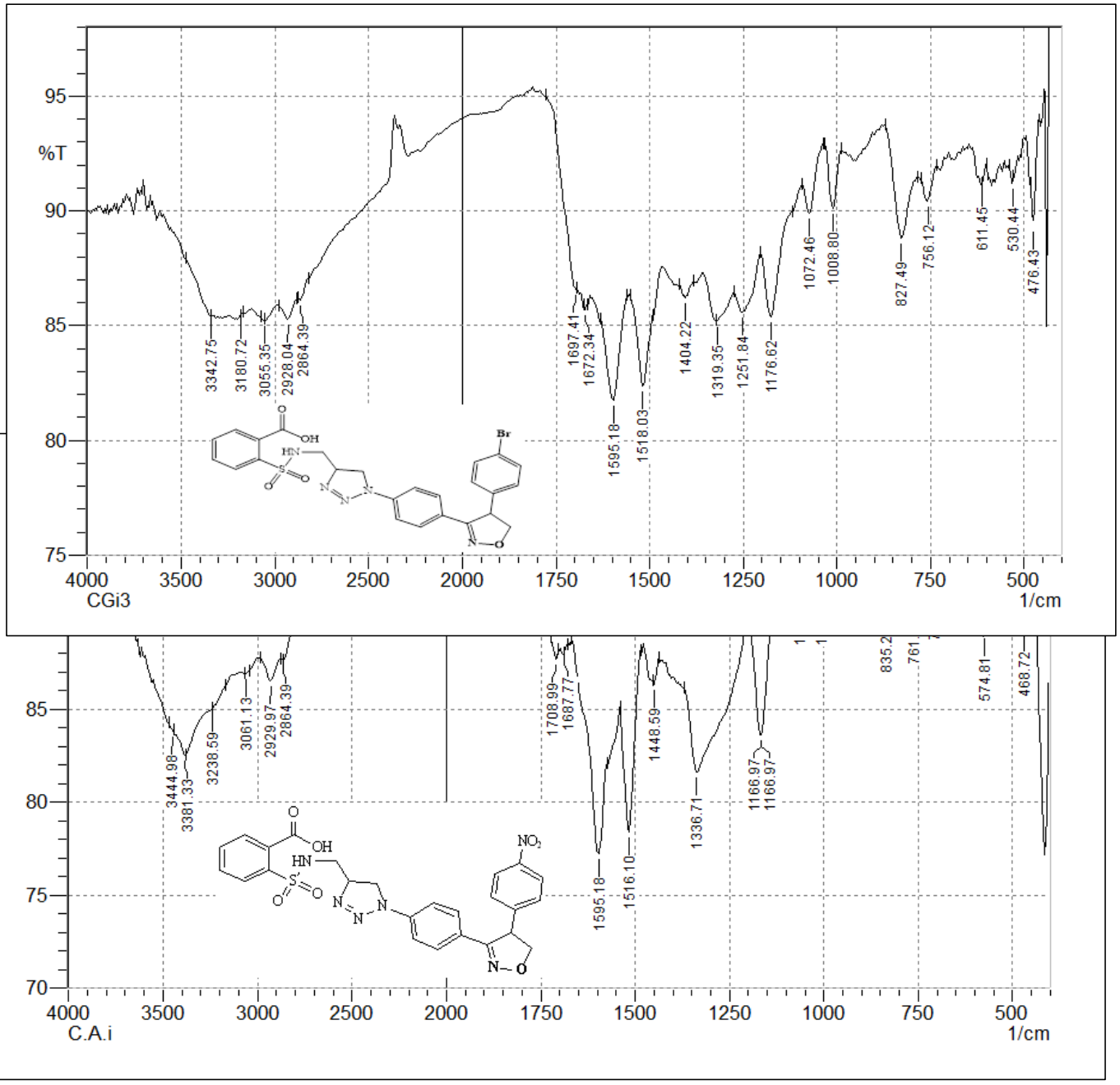


FT-IR spectrum of **3d**

FT-IR spectrum of **3e**

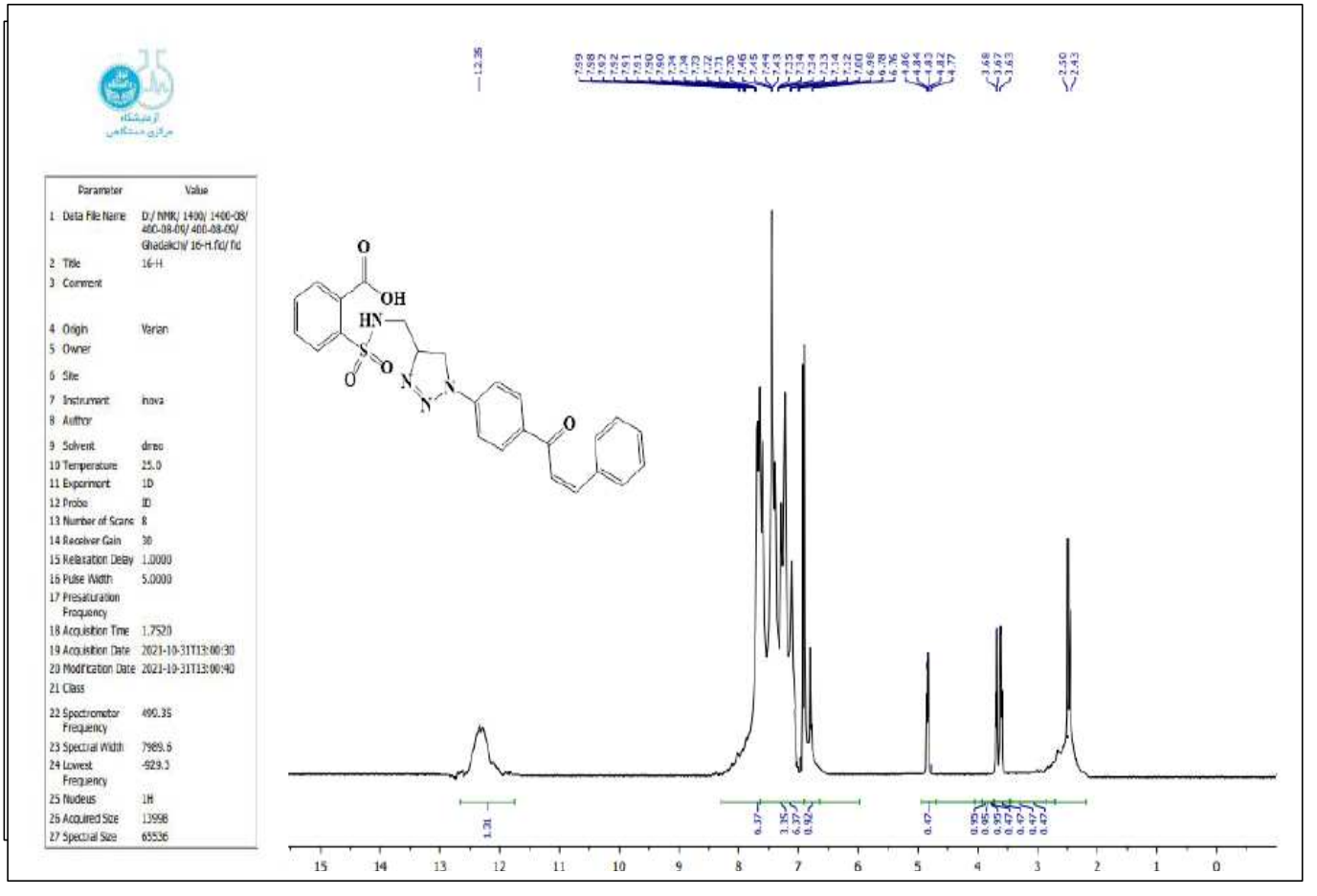


FT-IR spectrum of **4b**



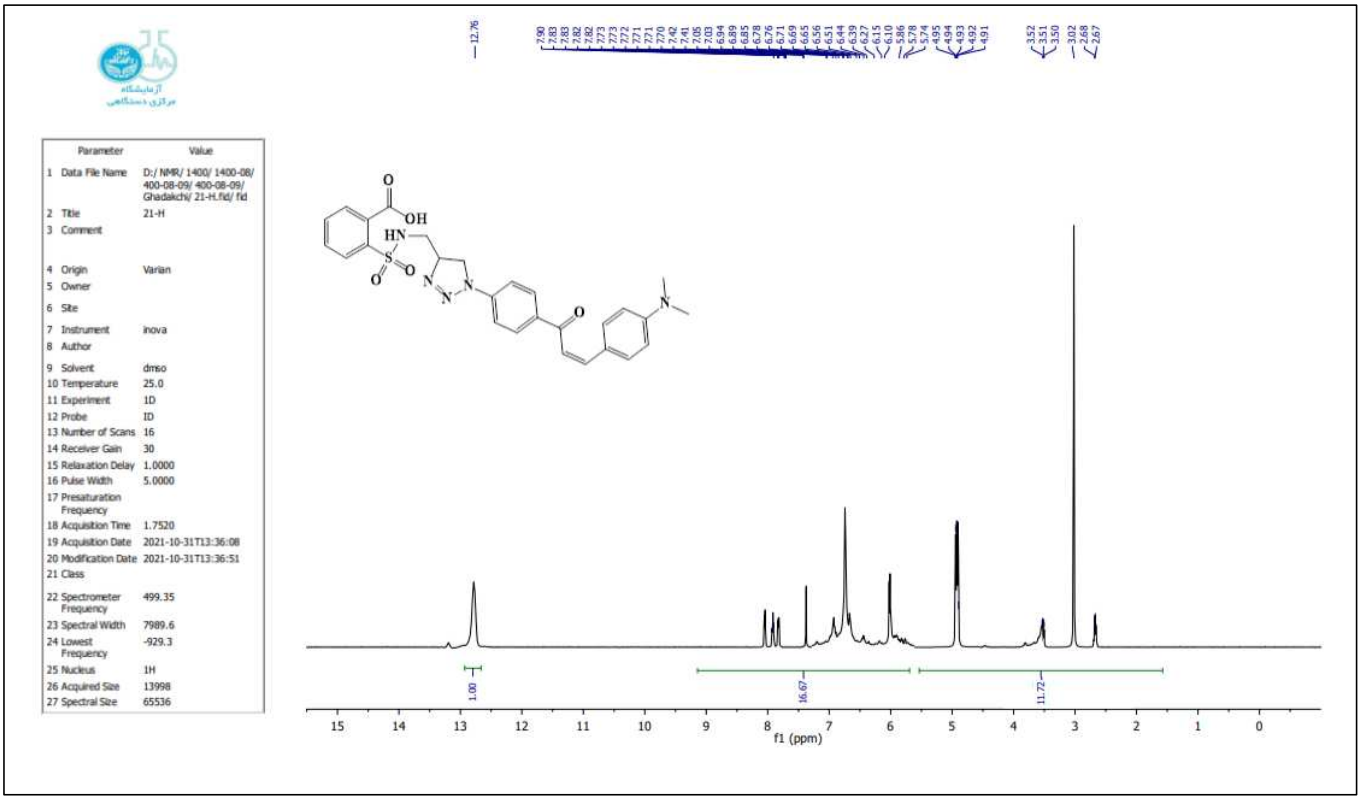
FT-IR spectrum of **4d**

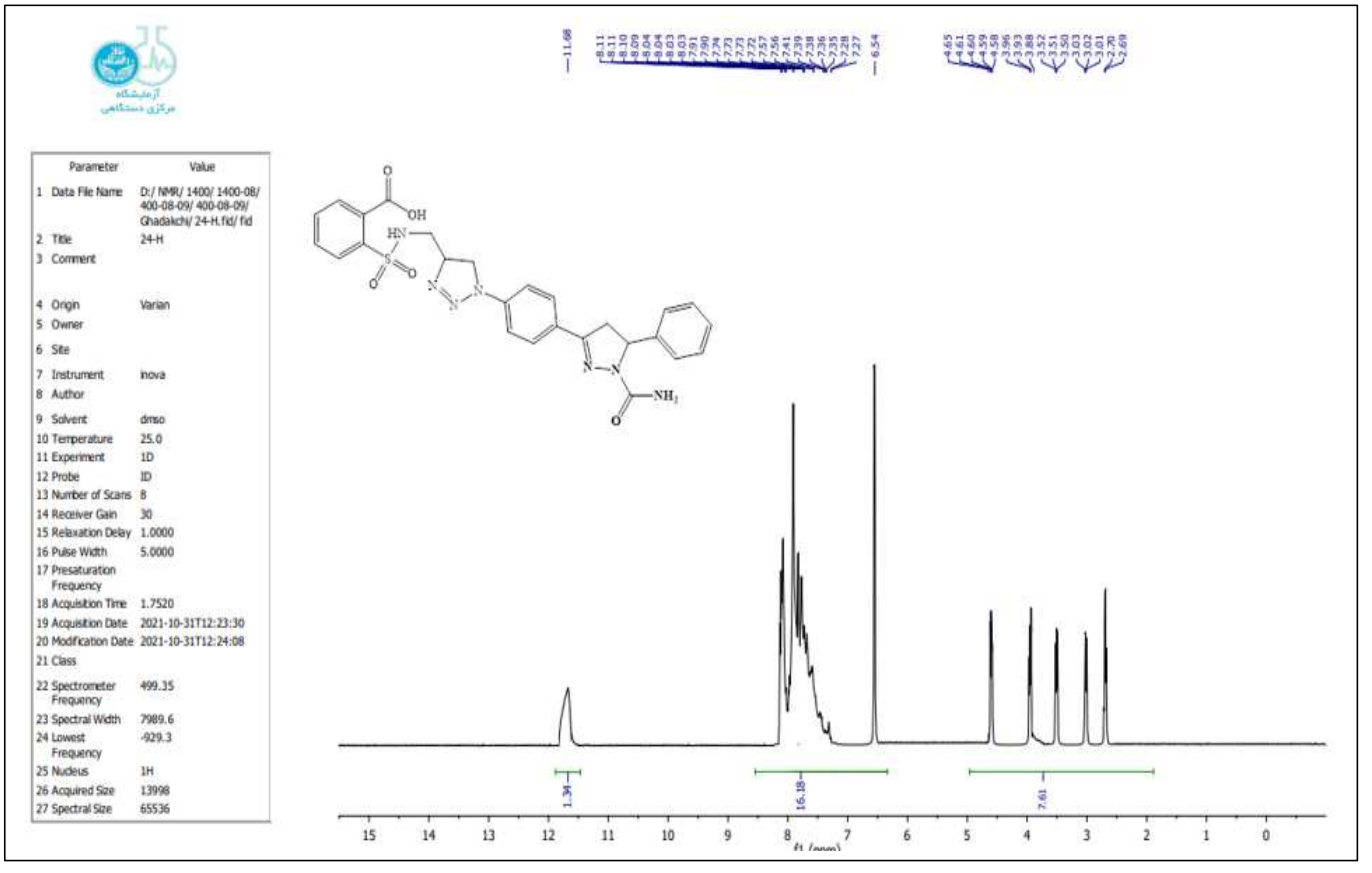
^{1}H NMR spectrum of compound **2a**



^{1}H NMR spectrum of compound **2c**

^{1}H NMR spectrum of compound **2g**





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8	Author	
9	Solvent	dmso
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11	Experiment	1D
12	Probe	ID
13	Number of Scans	8
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16	Pulse Width	5.0000
17	Presaturation Frequency	
18	Acquisition Time	1.7520
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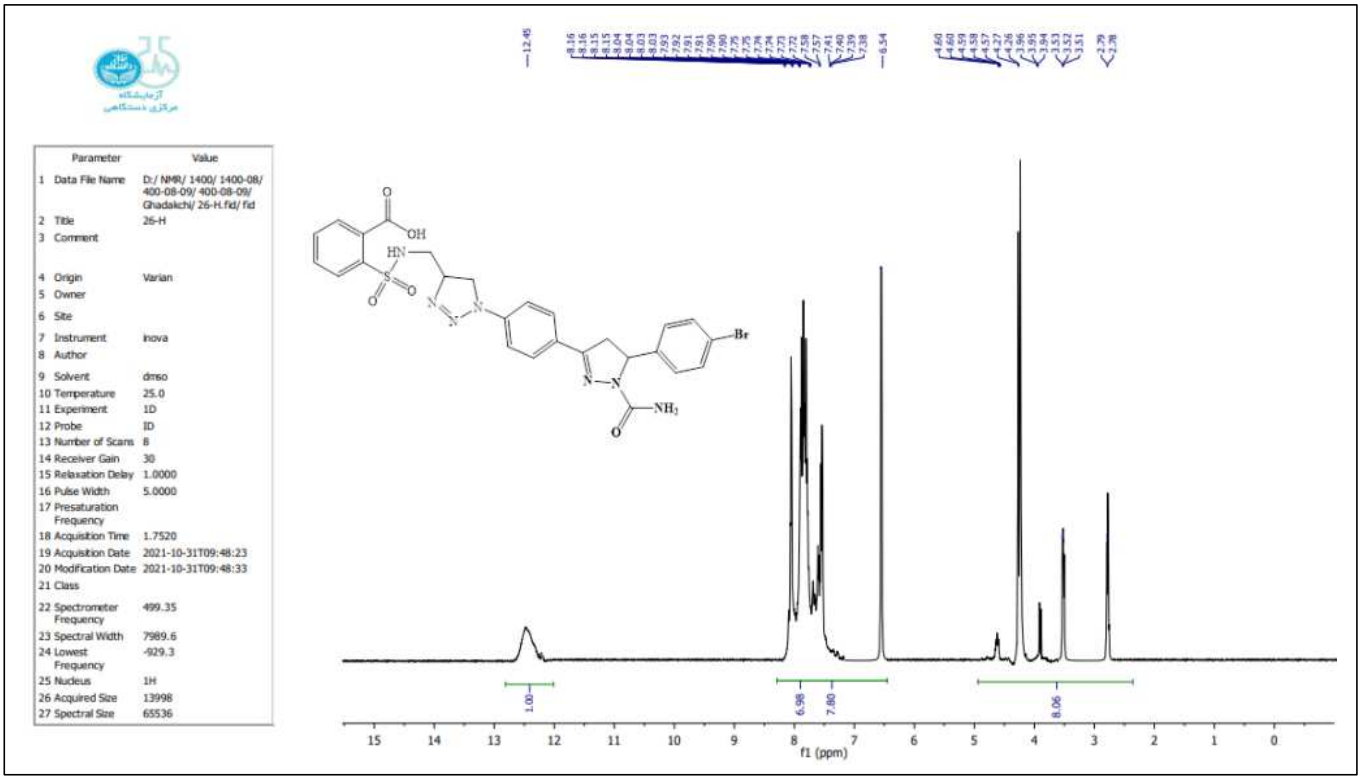
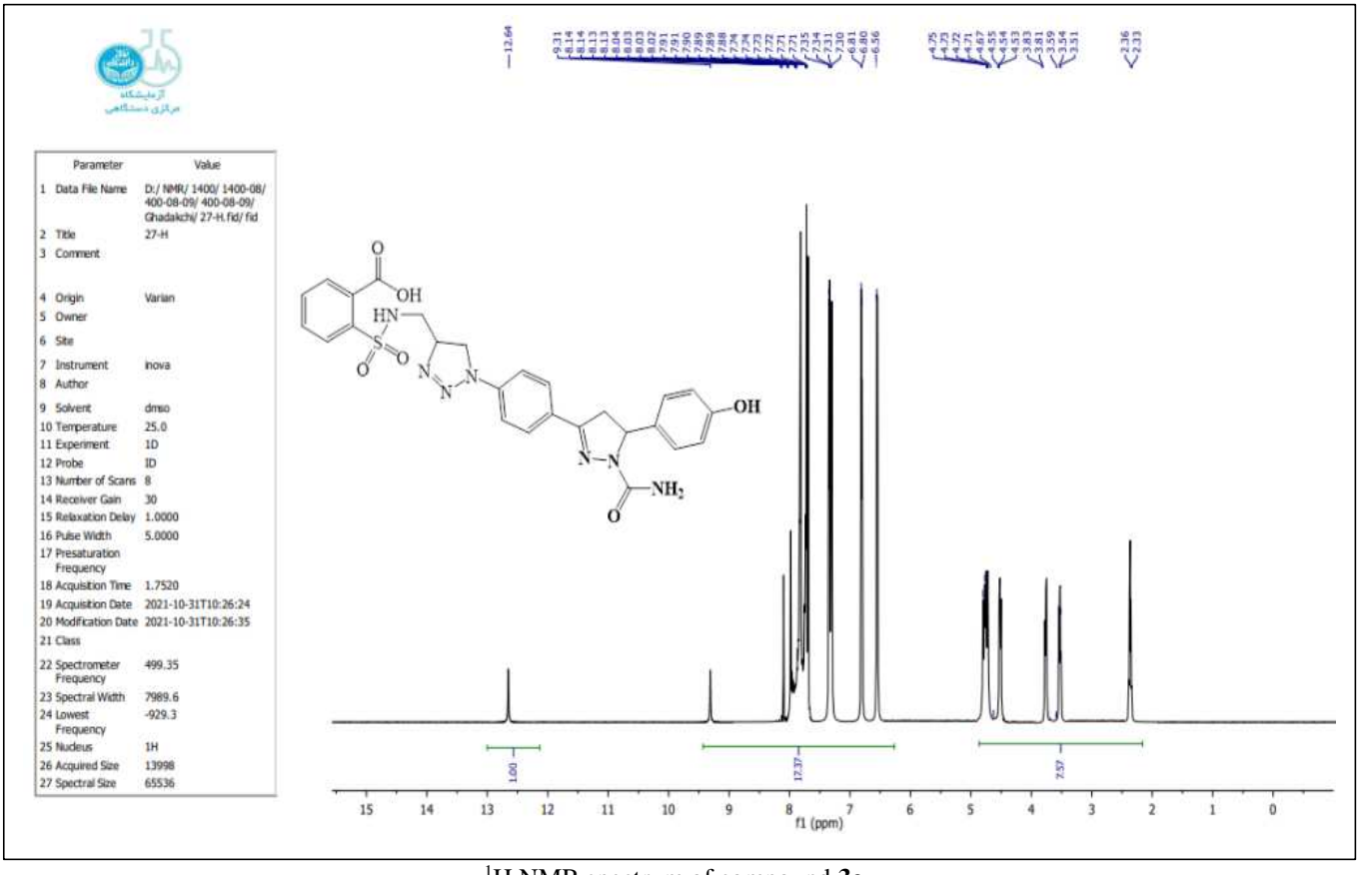
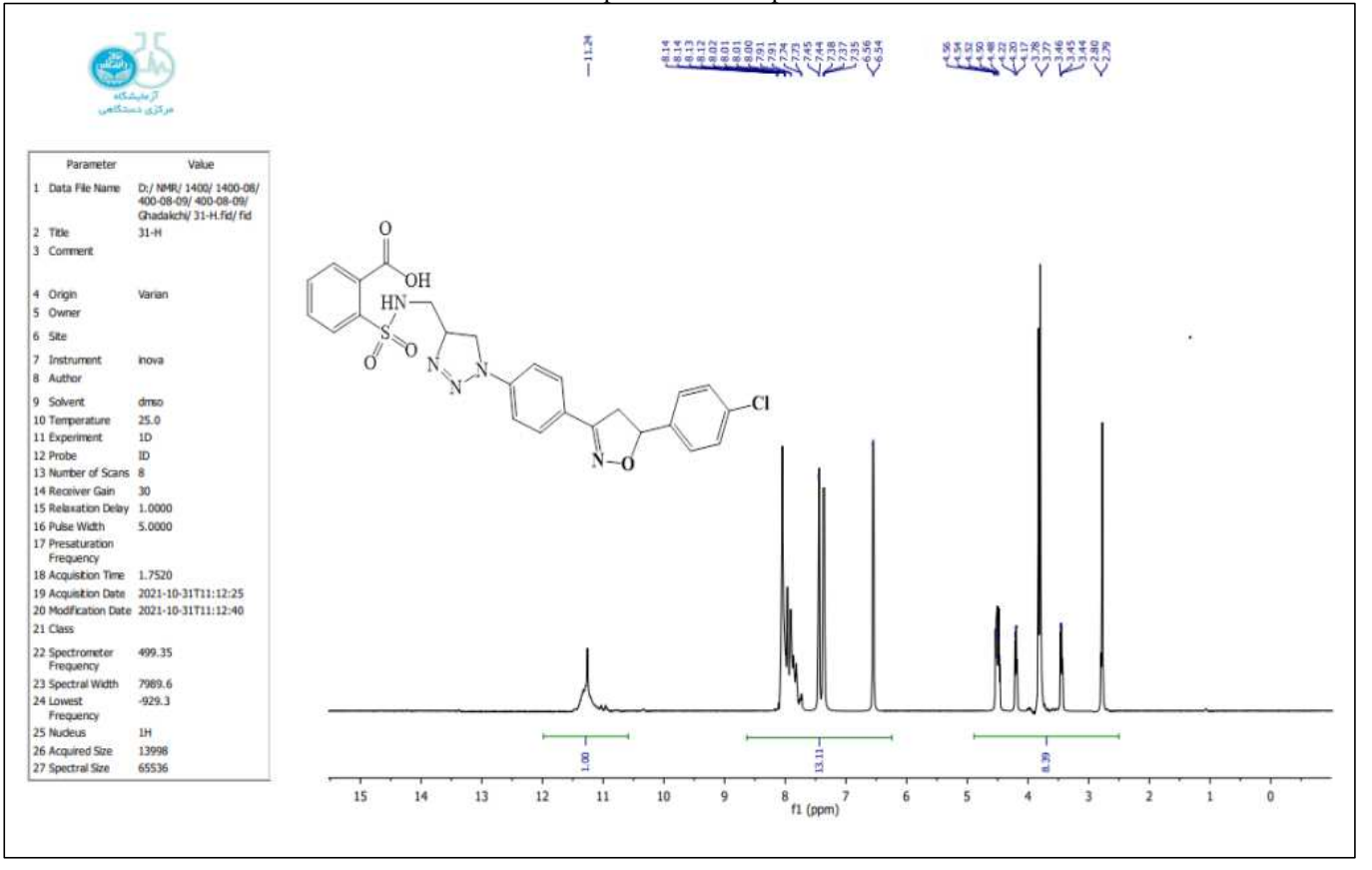
23	Spectral Width	7989.6
24	Lowest Frequency	-929.3
25	Nucleus	1H
26	Acquired Size	13998
27	Spectral Size	65536

^{1}H NMR spectrum of compound **3a**
^{1}H NMR spectrum of compound **3c**

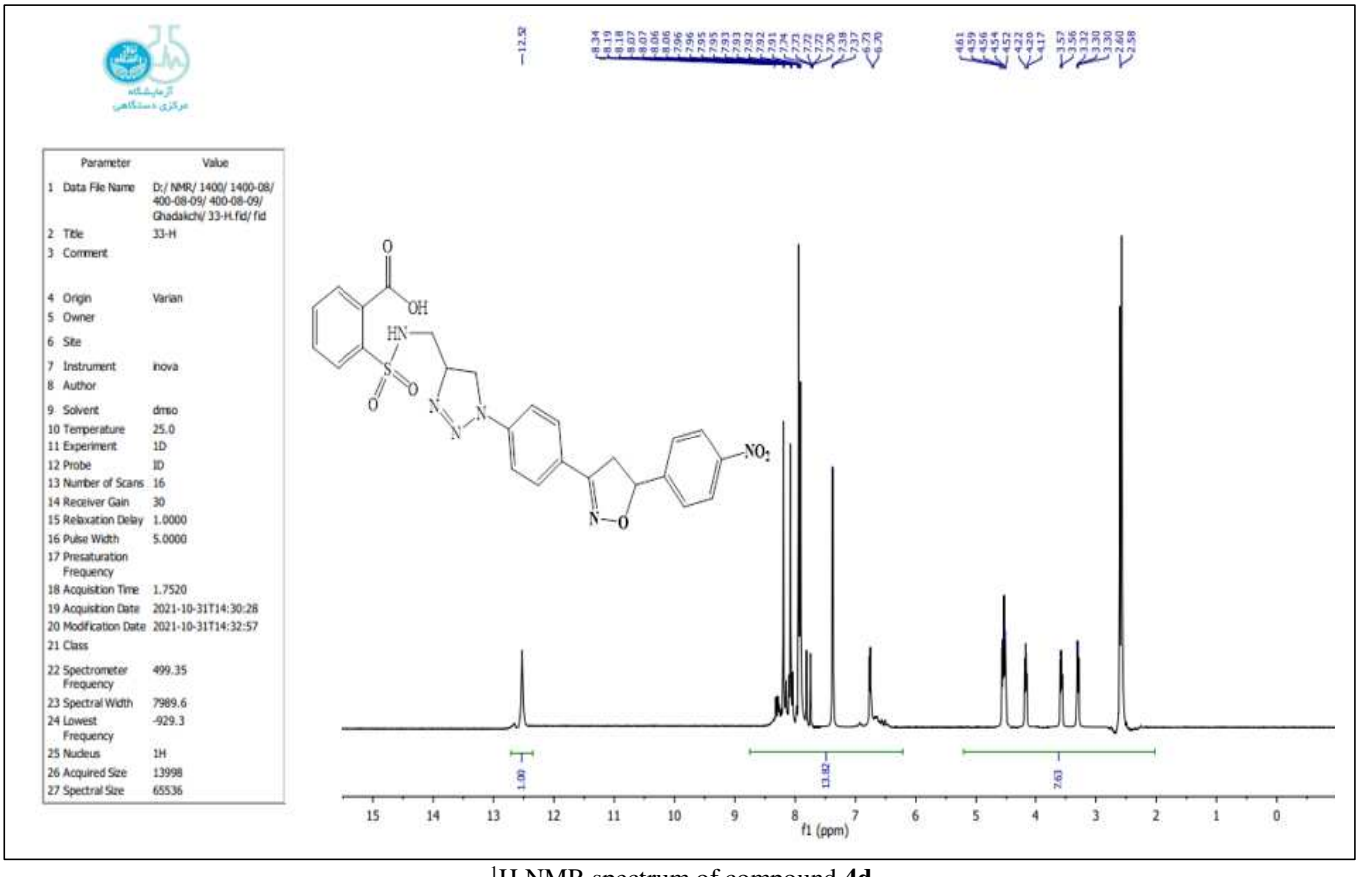




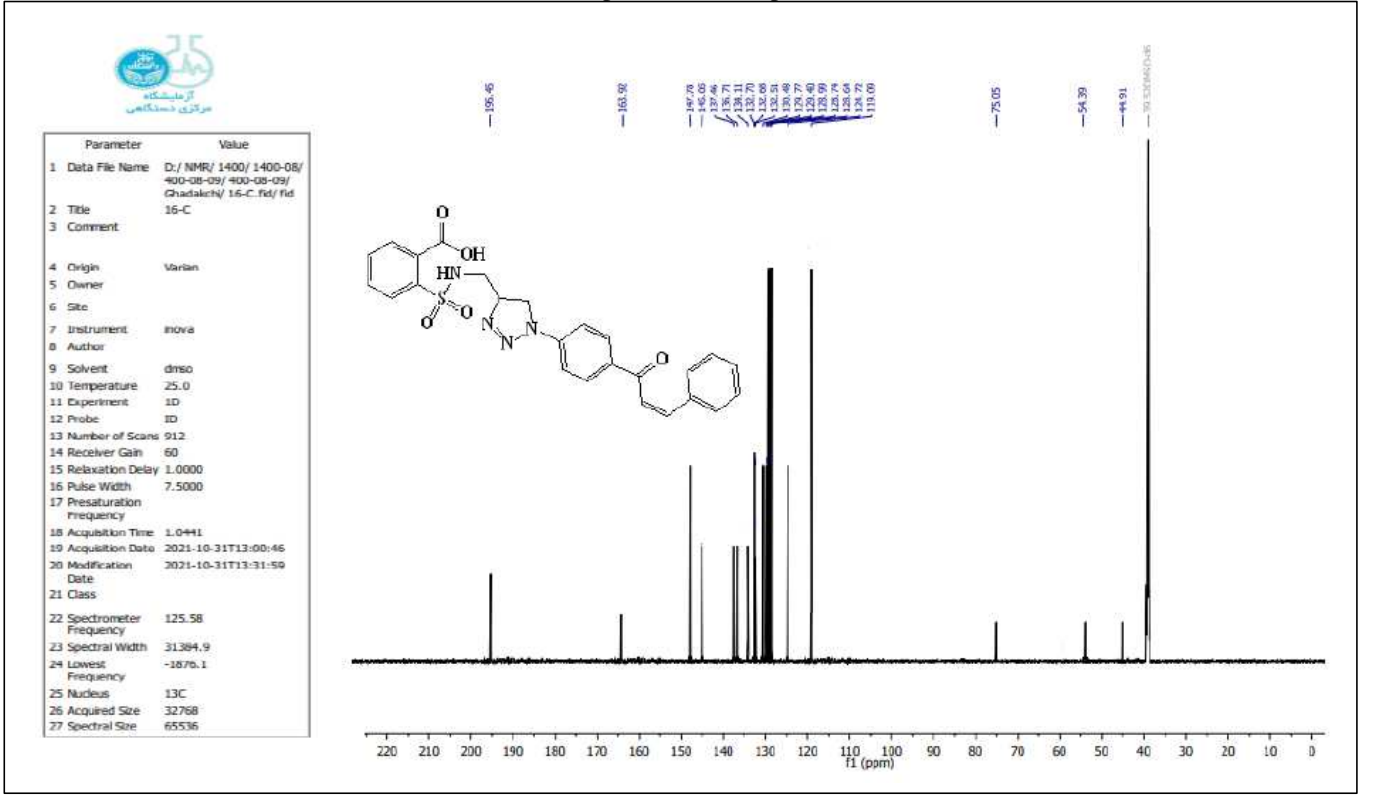
^{1}H NMR spectrum of compound **3e**



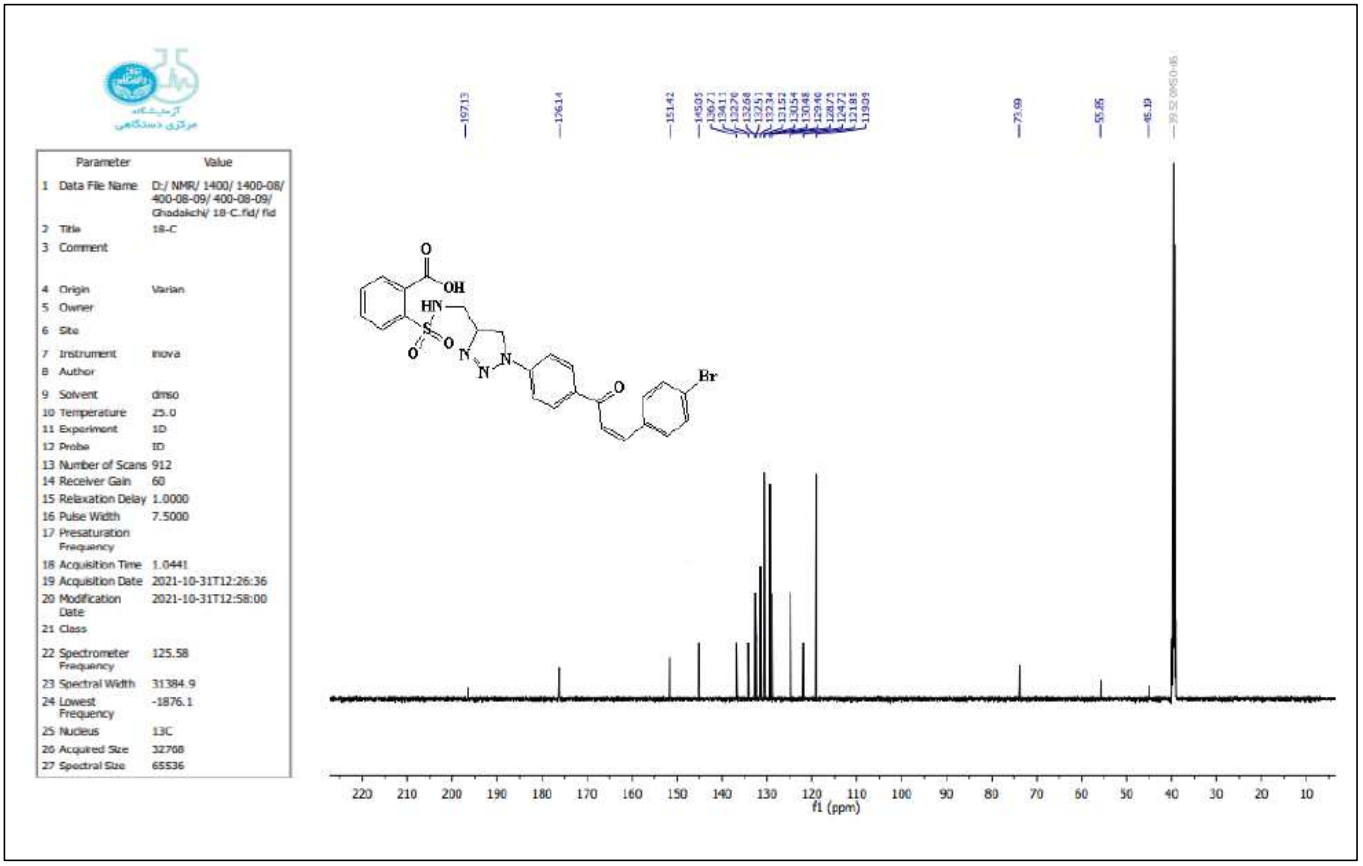
^{1}H NMR spectrum of compound **4b**



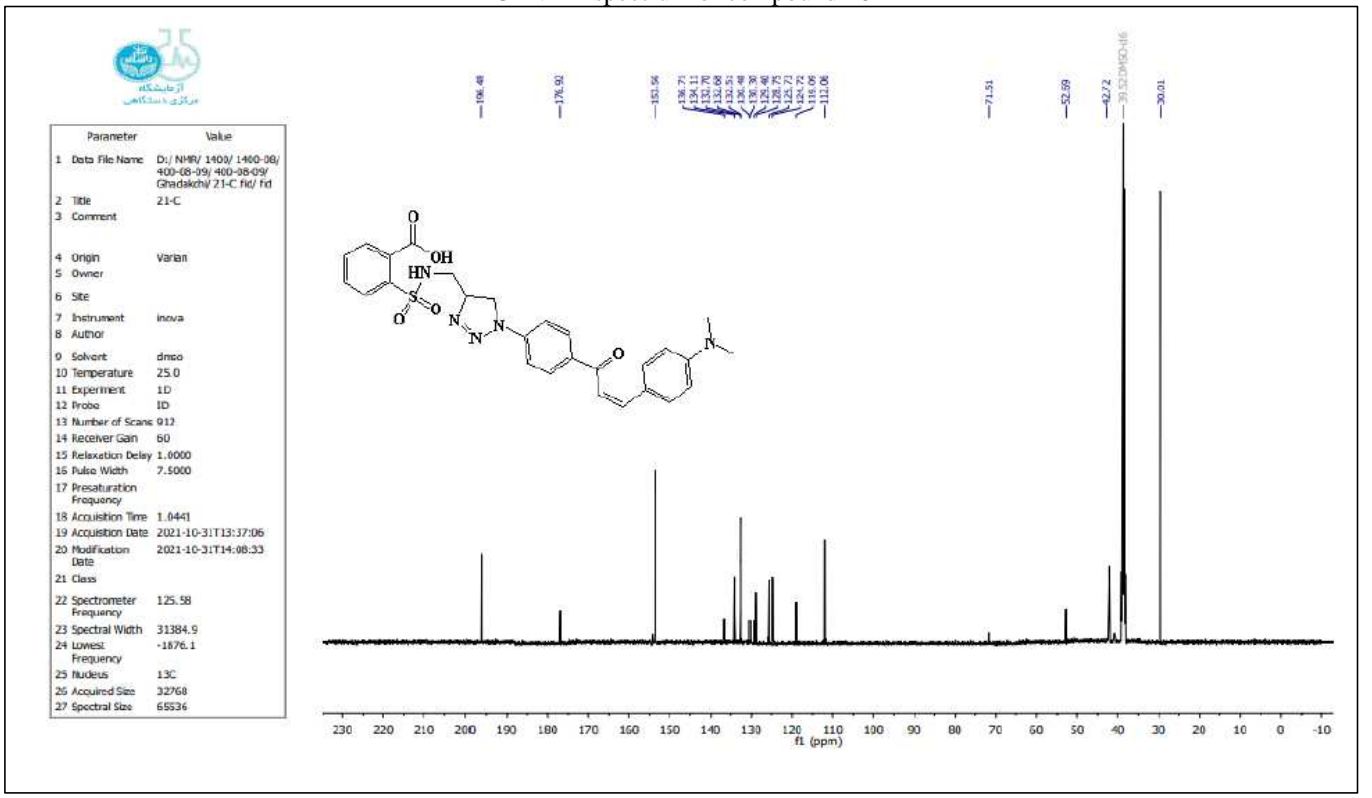
^{1}H NMR spectrum of compound **4d**



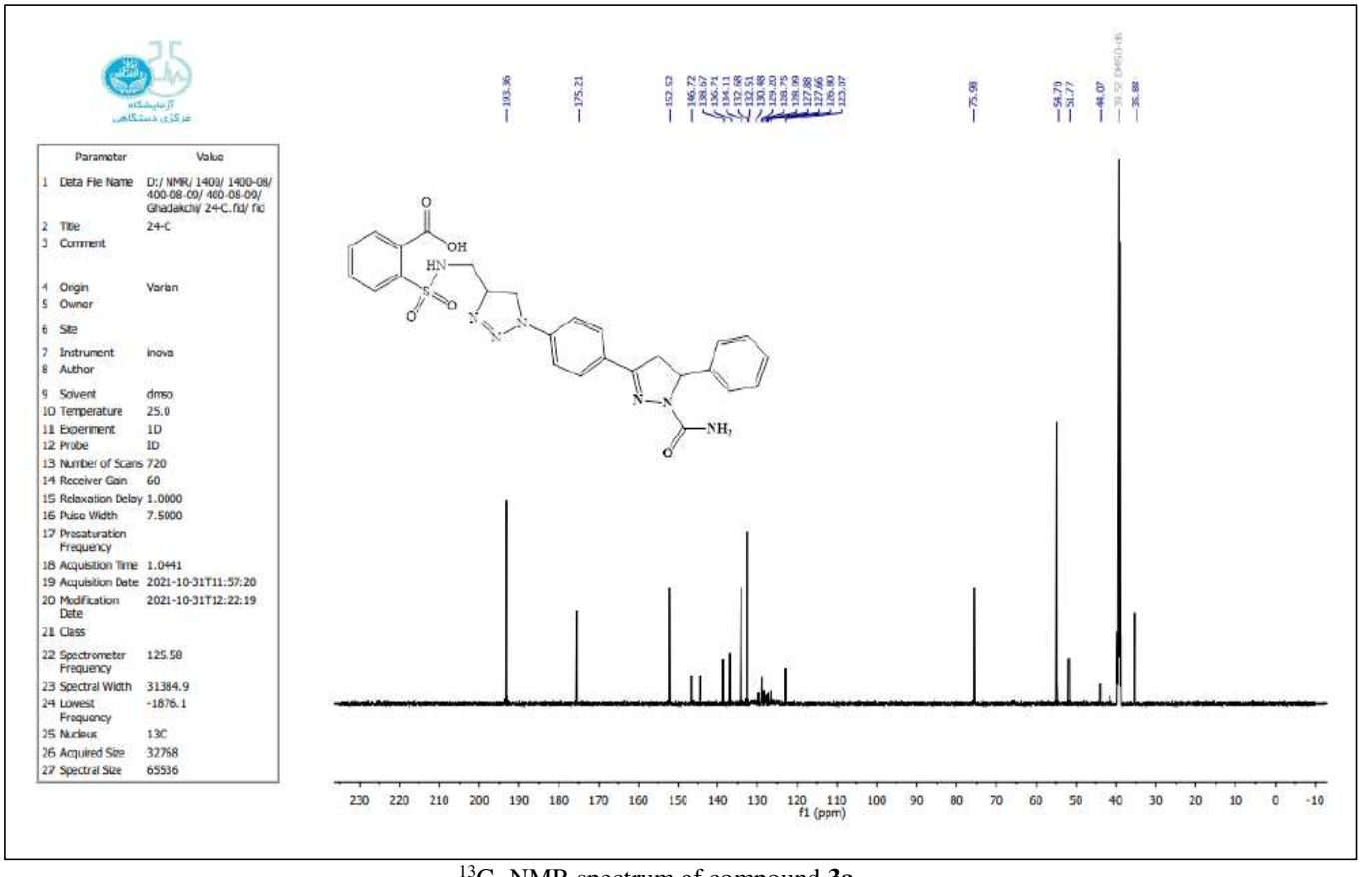
^{13}C NMR spectrum of compound **2a**



^{13}C- NMR spectrum of compound **2c**



^{13}C- NMR spectrum of compound **2g**



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6	Site	
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8	Author	
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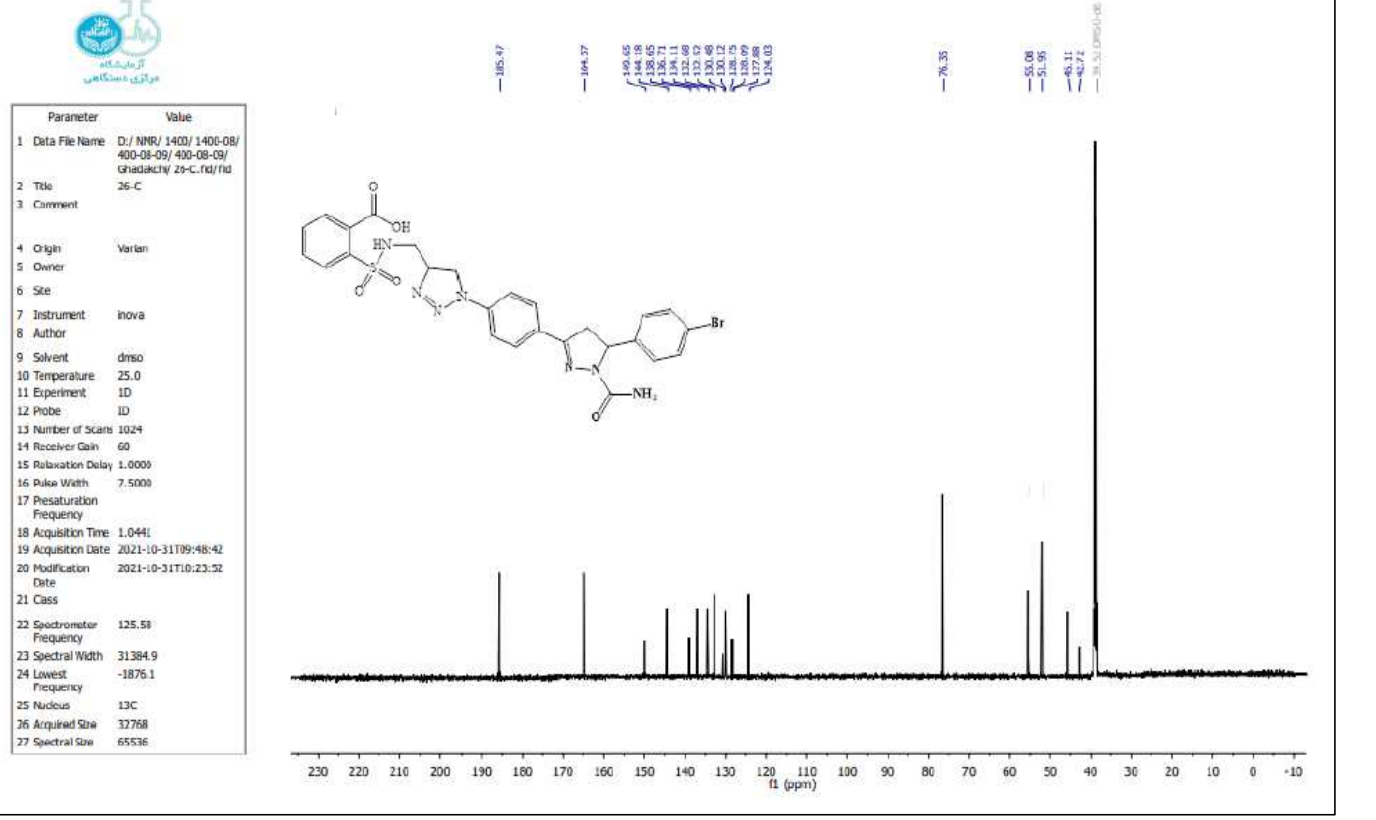
26	Acquired Size	32768
27	Spectral Size	65536

^{13}C- NMR spectrum of compound **3a**



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5	Owner	
6	Site	
7	Instrument	inova
8	Author	
9	Solvent	dmso
10	Temperature	25.0
11	Experiment	1D
12	Probe	ID
13	Number of Scans	1024
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15	Relaxation Delay	1.0000
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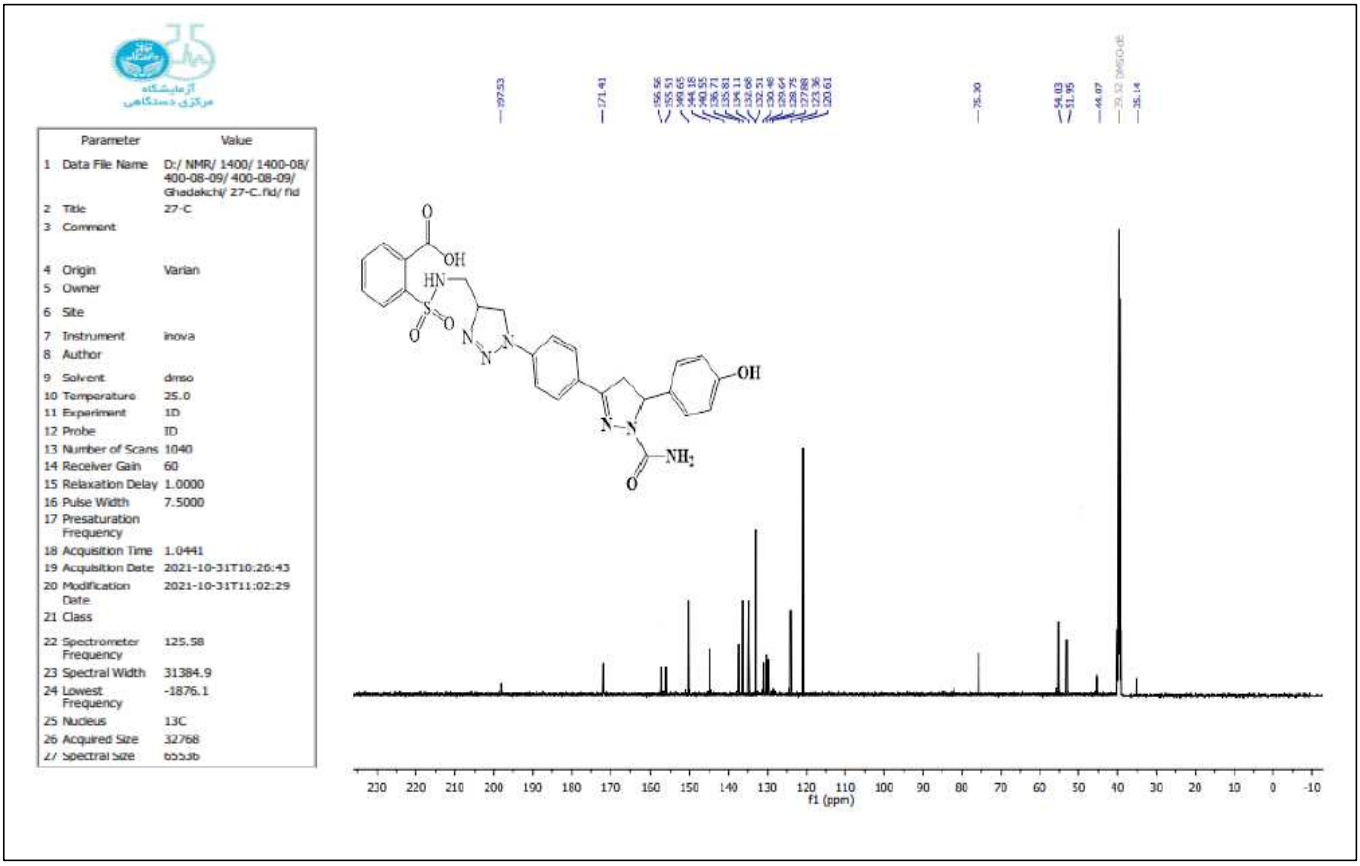
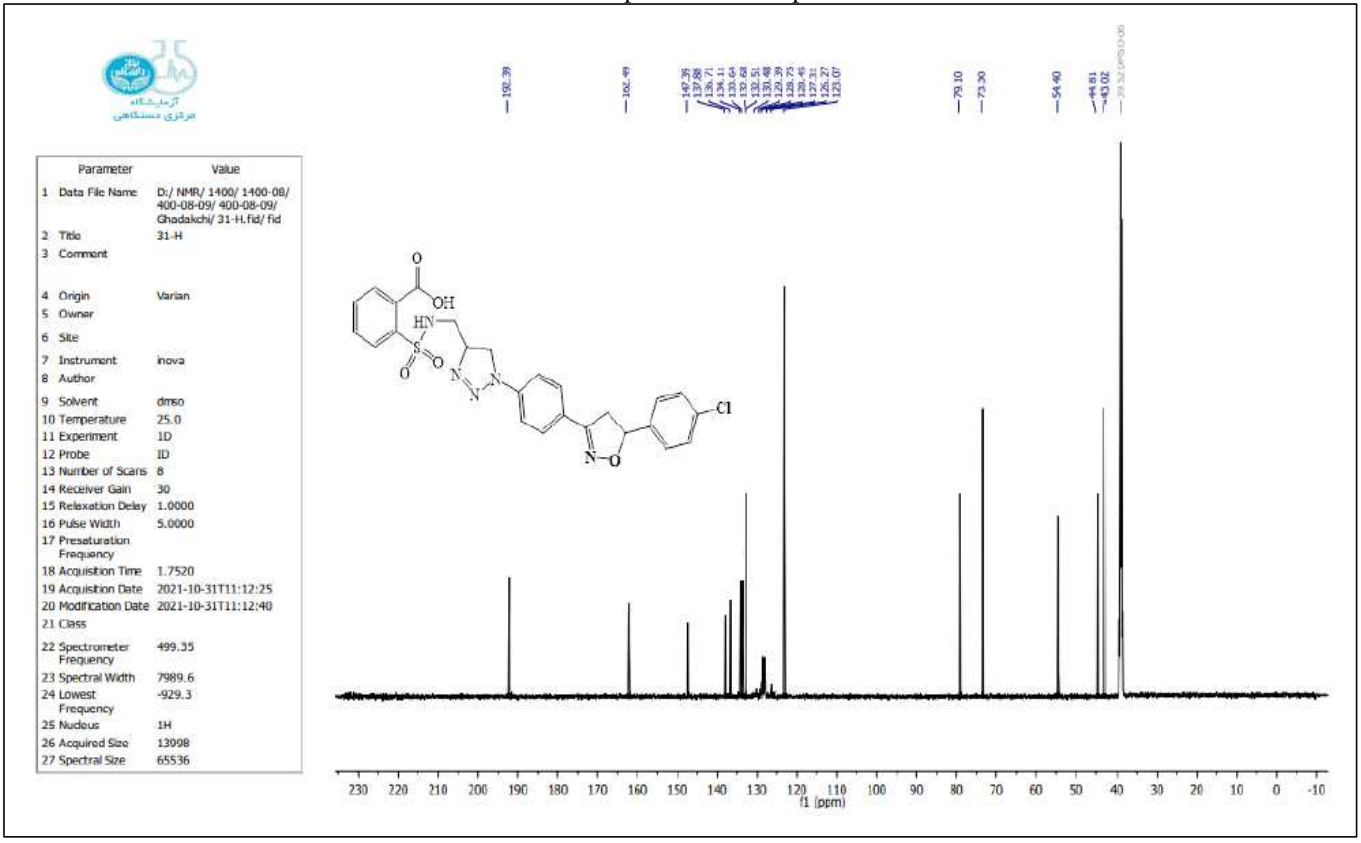
23	Spectral Width	31384.9
24	Lowest Frequency	-1876.1
25	Nucleus	13C
26	Acquired Size	32768
27	Spectral Size	65536

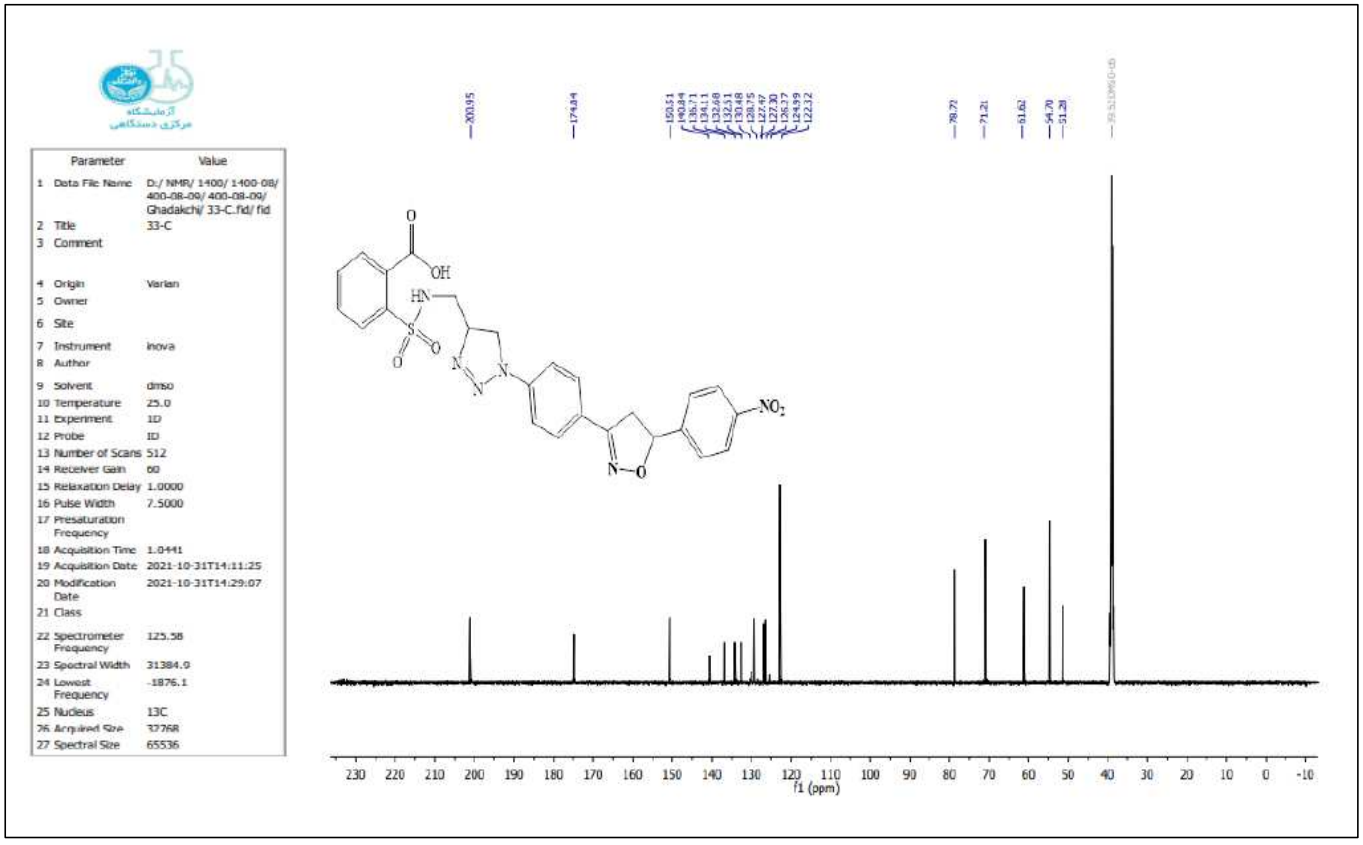
^{13}C- NMR spectrum of compound **3c**



^{13}C- NMR spectrum of compound **3d**



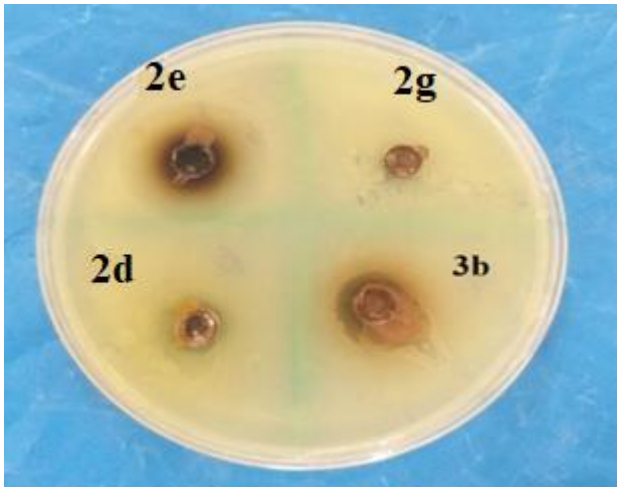
^{13}C- NMR spectrum of compound **4b**

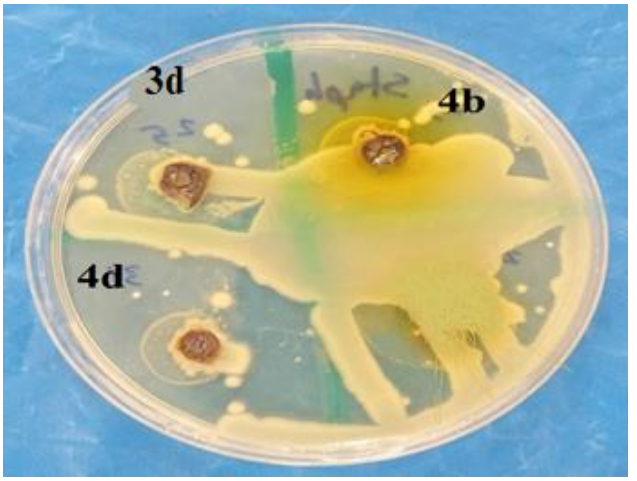


^{13}C- NMR spectrum of compound **4d**

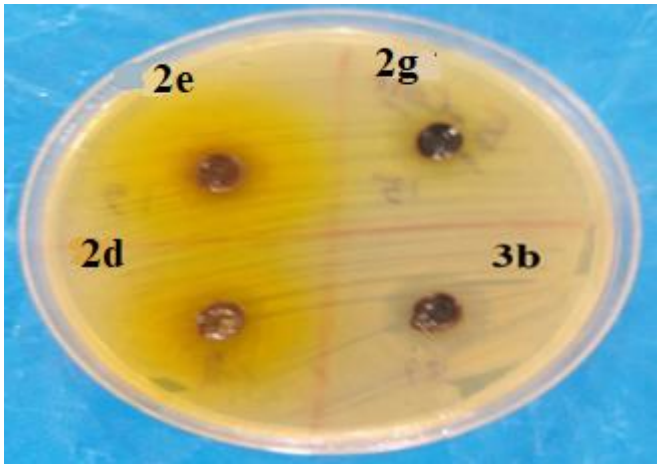
B. Antibacterial photographs

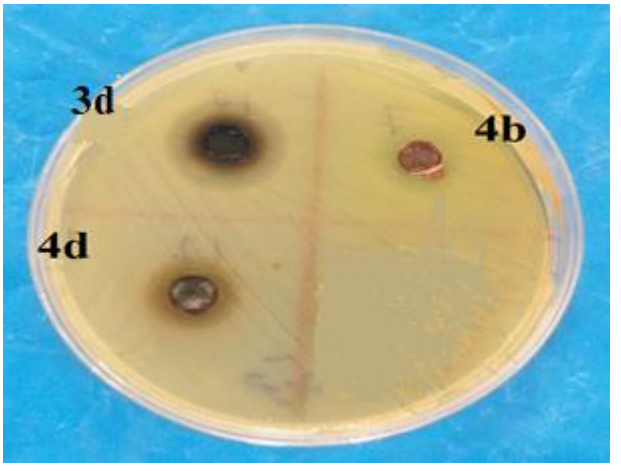
B.1. Antibacterial photographs of Chalcone, pyrazoline and isoxazoline derivatives 2e, 2g, 2d, 3b, 3d, 4b and 4d against Staphylococcus aurous



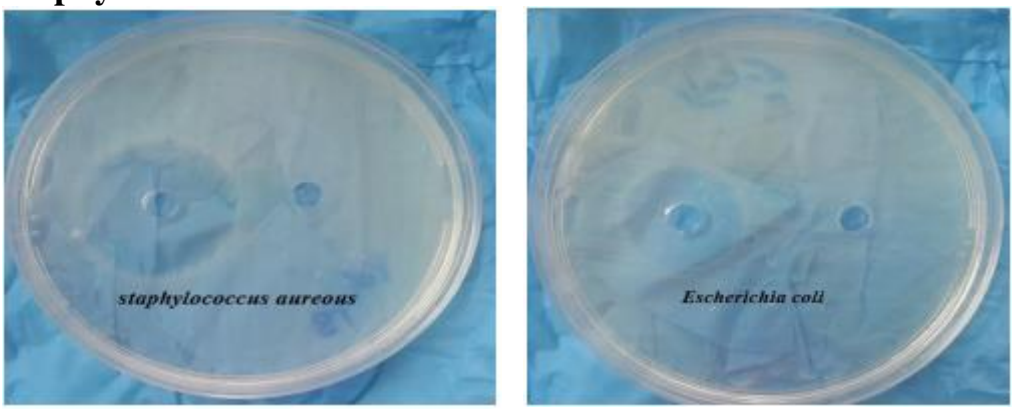


B.2. Antibacterial photographs of Chalcone, pyrazoline and isoxazoline derivatives 2e, 2g, 2d, 3b, 3d, 4b and 4d Escherichia Coli





B.5. Antibacterial photographs of Ceftriaxone against Staphylococcus aureus and Escherichia coli



Antibacterial activity of Ceftriaxone against Staphylococcus aureus and Escherichia coli.