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)

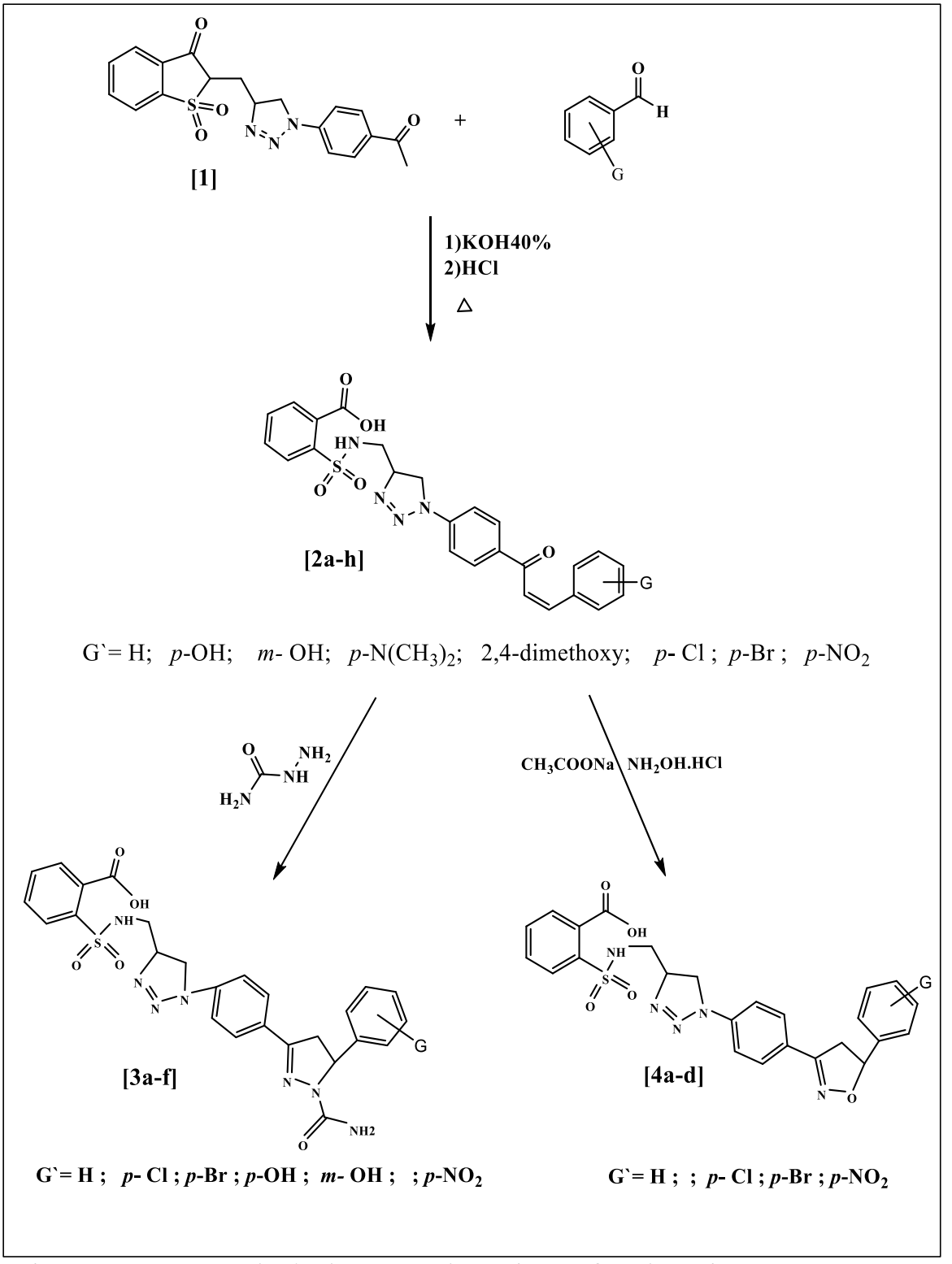
Yield 79%, m.p. 158-160°C, FTIR(KBr),υ (cm^{-1}): 3342(O-H), 3180(N-H)3055(C-H)Ar, 2928 and 2864(CH_2), 1697(C=O)acid, 1672(C=N), 1595(C=C), 1518(N=N), 1319(SO_2)Asym, 1176(SO_2)Sym, 1251(C-O)Asym, 1072(C-O)Sym

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

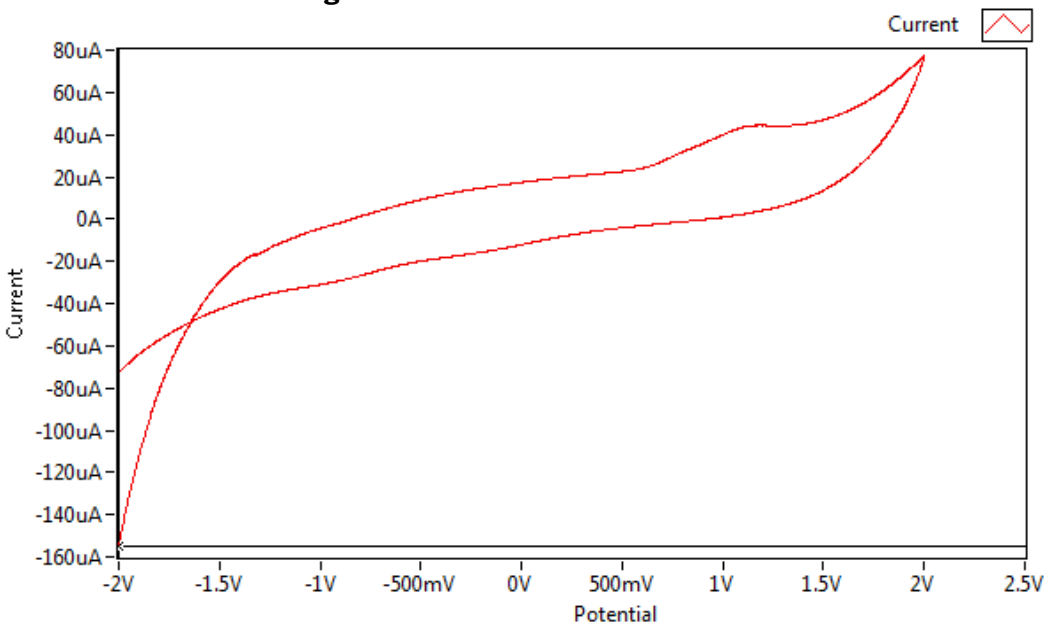


Figure (3). Cyclic voltammogram of compound [2d] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed reduction current peak at (+1)v.

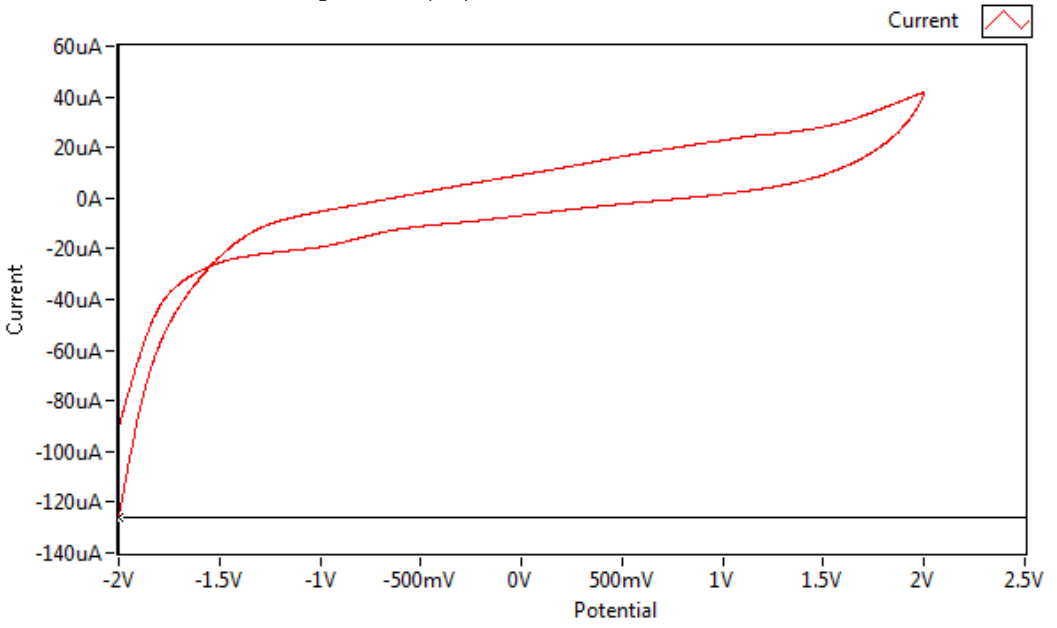


Figure (1). Cyclic voltammogram of compound [2e] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed oxidation current peak at (+1.2)v.

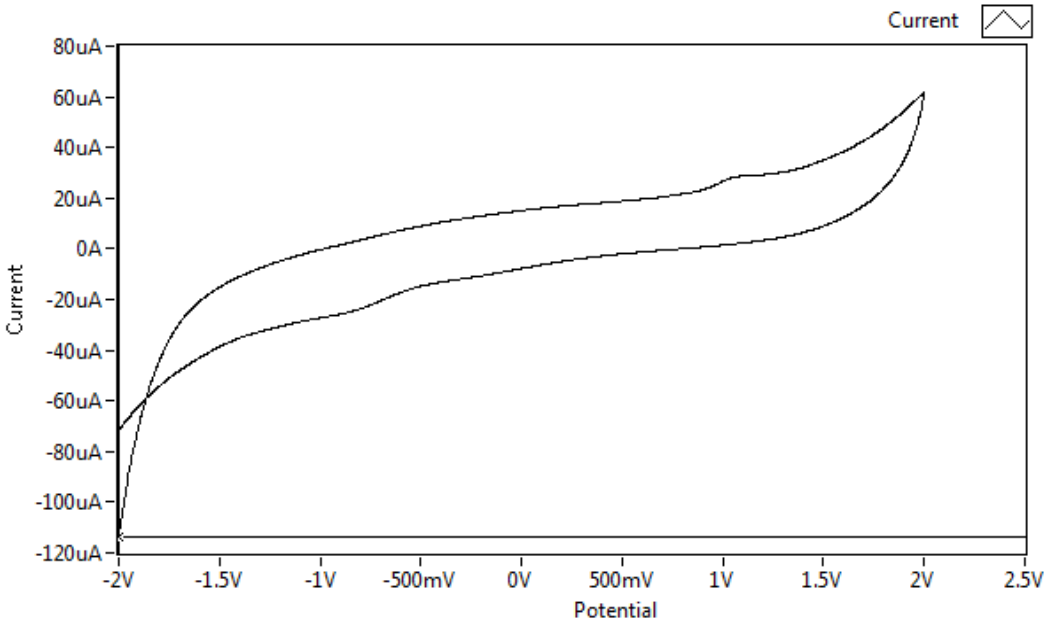


Figure (2). Cyclic voltammogram of compound [2g] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode. Showed two oxidation current peak at (-0.2)v and (+1.2)v.

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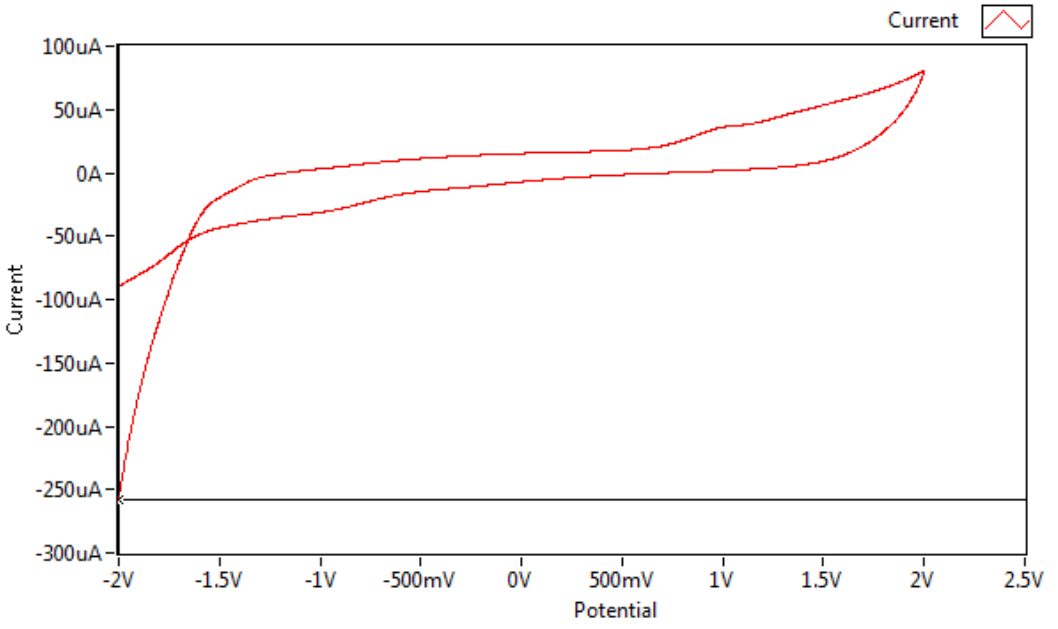

Figure (4). Cyclic voltammogram of compound [3b] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed oxidation current peak at (+1.2) v.

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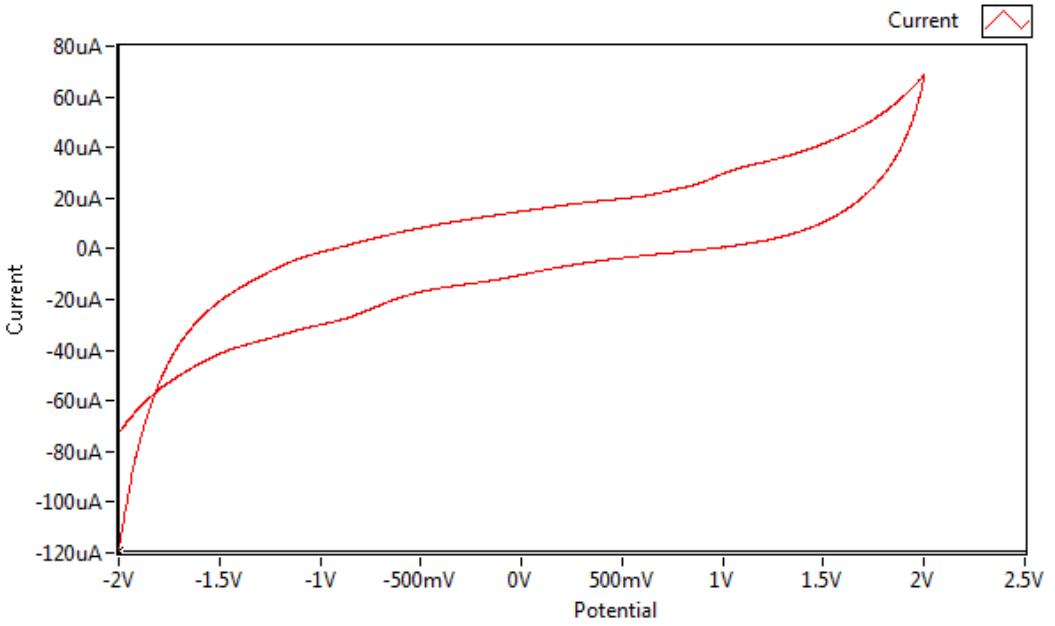

Figure (5). Cyclic voltammogram of compound [3d] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed oxidation current peak at (-0.75)v.

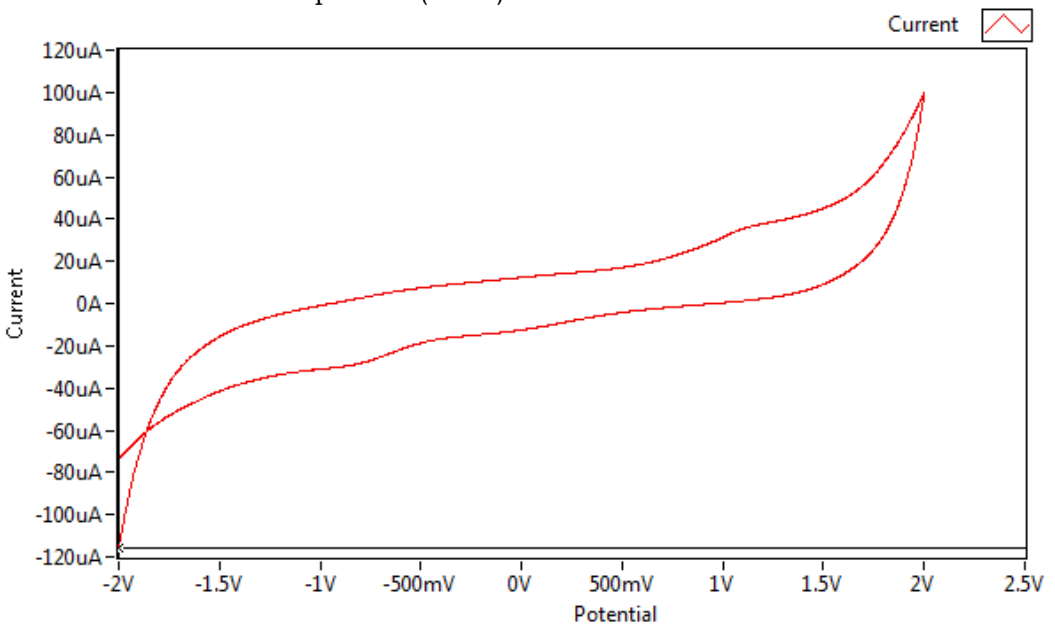

Figure (6). Cyclic voltammogram of compound [4b] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed oxidation current peak at (+1)v.

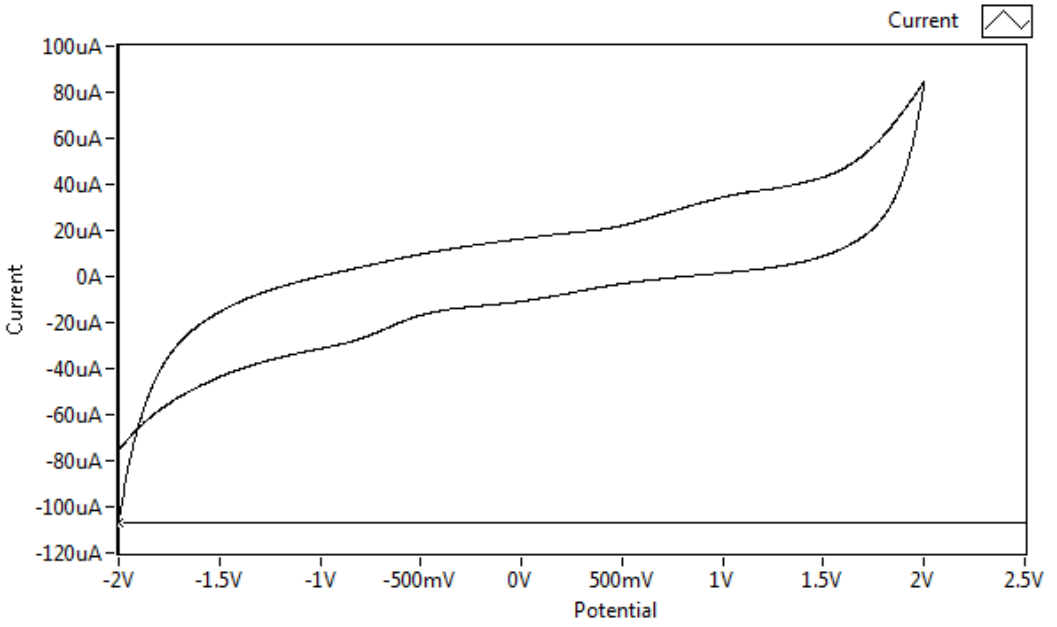

Figure (6). Cyclic voltammogram of compound [4d] at 1000 µ/L concentration in blood serum medium using GCE/CNT and Ag/AgCl as a reference electrode, Showed oxidation current peak at (+1)v.

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

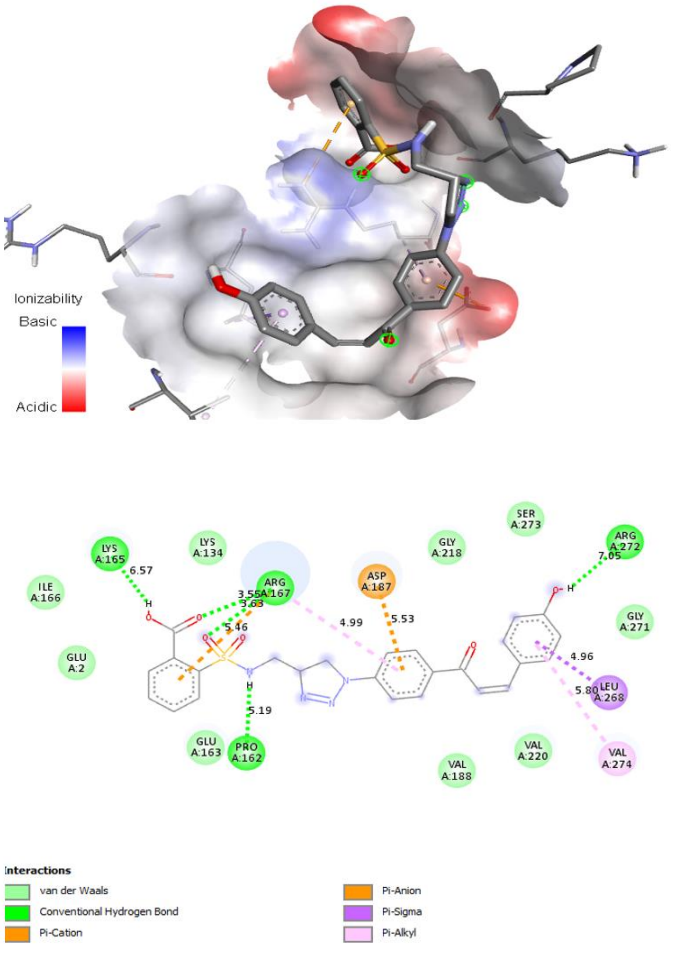


Figure (6) Binding site interaction of structure of compound **2e** 2D and 3D

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

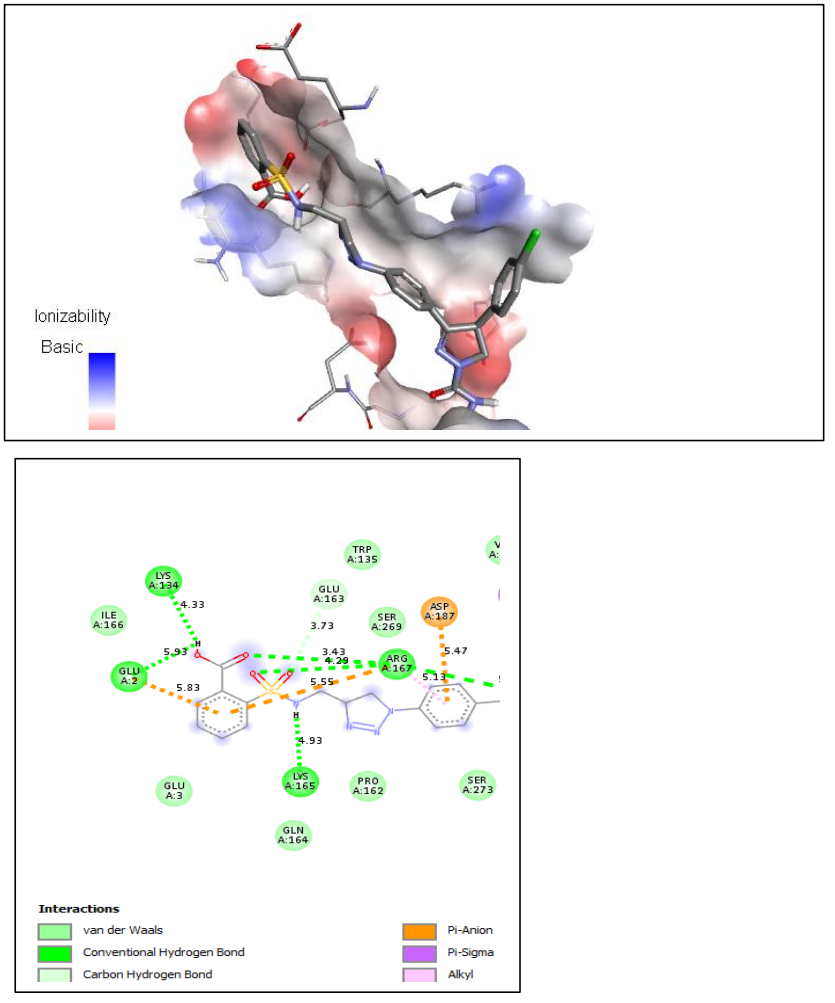


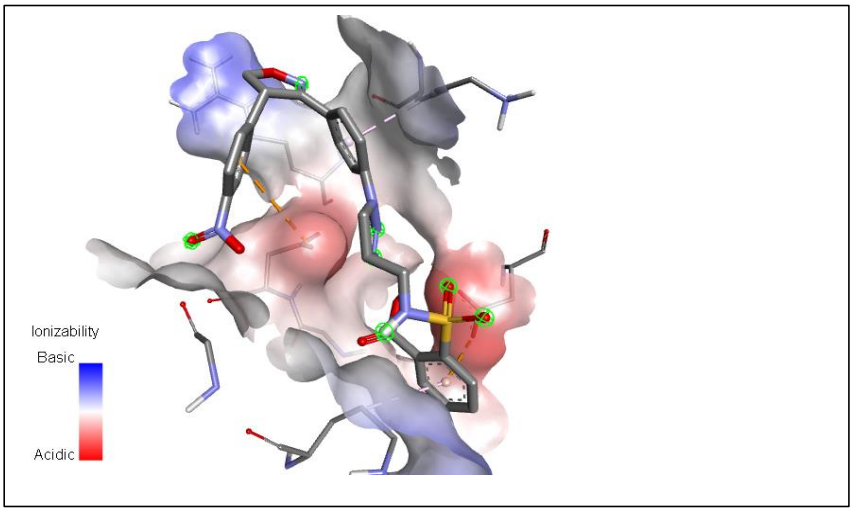
Figure (6) Binding site interaction of structure of compound **3d** 2D and 3D

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



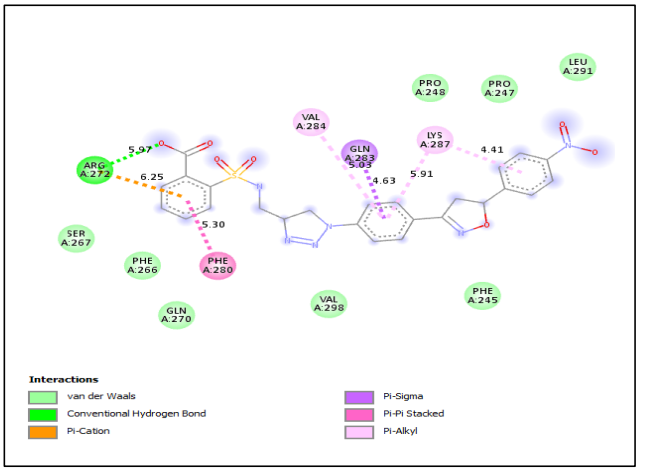


Figure (6) Binding site interaction of structure of compound **4d** 2D and 3D

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**.**

References

1. Saleh SS, AL-Salihi SS, Mohammed IA. Biological activity Study for some heterocyclic compounds and their impact on the gram positive and negative bacteria. *Energy Procedia*. 2019;157:296-306. doi:10.1016/j.egypro.2018.11.194
2. Mirzaei S, Hadizadeh F, Eisvand F, Mosaffa F, Ghodsi R. Synthesis, structure-activity relationship and molecular docking studies of novel quinoline-chalcone hybrids as potential anticancer agents and tubulin inhibitors. *J Mol Struct*. 2020;1202(xxxx):127310. doi:10.1016/j.molstruc.2019.127310
3. Jain S, Kumar S, Lamba BY, Patra J, Mahindroo N. Nanocatalysts: Applications in synthesis of chalcones–a review. *Synth Commun*. 2021;51(1):1-12. doi:10.1080/00397911.2020.1817941
4. Ezhilarasi RM, Jayachandramani N, Mahalakshmi S. A green chemical method for the synthesis of chalcones using Amberlite resin. *Int J Adv Chem Sci Appl*. 2015;3:5-9.
5. Khan SA, Asiri AM, Al-Ghamdi NSM, et al. Microwave assisted synthesis of chalcone and its polycyclic heterocyclic analogues as promising antibacterial agents: In vitro, in silico and DFT studies. *J Mol Struct*. 2019;1190:77-85. doi:10.1016/j.molstruc.2019.04.046
6. Damas L, Rodrigues FMS, Gonzalez ACS, Carrilho RMB, Pineiro M, Pereira MM. Sequential catalytic carbonylation reactions for sustainable synthesis of biologically relevant entities. *J Organomet Chem*. 2020;923:1-7. doi:10.1016/j.jorganchem.2020.121417
7. Pragathi YJ, Veronica D, Anitha K, Rao MVB, Raju RR. Synthesis and biological

evaluation of chalcone derivatives of 1,2,4-thiadiazol-benzo[d]imidazol-2-yl)quinolin-2(1H)-one as anticancer agents. *Chem Data Collect*. 2020;30:1-8. doi:10.1016/j.cdc.2020.100556

8. Higgs J, Wasowski C, Marcos A, et al. Chalcone derivatives: synthesis, in vitro and in vivo evaluation of their anti-anxiety, anti-depression and analgesic effects. *Heliyon*. 2019;5(3). doi:10.1016/j.heliyon.2019.e01376
9. Jin H, Jiang X, Yoo H, et al. Synthesis of Chalcone-Derived Heteroaromatics with Antibacterial Activities. *ChemistrySelect*. 2020;5(40):12421-12424. doi:10.1002/slct.202003397
10. Burmaoglu S, Yilmaz AO, Polat MF, Kaya R, Gulcin İ, Algul O. Synthesis and biological evaluation of novel tris-chalcones as potent carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase and α-glycosidase inhibitors. *Bioorg Chem*. 2019;85:191-197. doi:10.1016/j.bioorg.2018.12.035
11. Varghese B, Al-Busafi SN, Suliman FEO, Al-Kindy SMZ. Synthesis, spectroscopic characterization and photophysics of a novel environmentally sensitive dye 3-naphthyl-1-phenyl-5-(4-carboxyphenyl)-2-pyrazoline. *J Lumin*. 2015;159:9-16. doi:10.1016/j.jlumin.2014.10.045
12. Li Y, Wei L, Wan JP, Wen C. Water-acetic acid mediated chemoselective synthesis of pyrazolines via multimolecular domino reactions of enaminones and sulfonyl hydrazines. *Tetrahedron*. 2017;73(16):2323-2328. doi:10.1016/j.tet.2017.03.019
13. Dofe VS, Sarkate AP, Tiwari S V., et al. Ultrasound assisted synthesis of tetrazole based pyrazolines and isoxazolines as potent anticancer agents via inhibition of tubulin polymerization. *Bioorganic Med Chem Lett*. 2020;30(22):1-8. doi:10.1016/j.bmcl.2020.127592
14. Kumari P, Mishra VS, Narayana C, Khanna A, Chakrabarty A, Sagar R. Publisher Correction: Design and efficient synthesis of pyrazoline and isoxazole bridged indole C-glycoside hybrids as potential anticancer agents (Scientific Reports, (2020), 10, 1, (6660), 10.1038/s41598-020-63377-x). *Sci Rep*. 2020;10(1):1-16. doi:10.1038/s41598-020-67068-5
15. Tanwer N, Kaur R, Rana D, et al. Synthesis and characterization of Pyrazoline derivatives. *J Integr Sci Technol*. 2015;3(2):39-41. http://pubs.iscience.in/journal/index.php/jist/article/view/313
16. Abunada NM, Hassaneen HM, Abu Samaha ASM, Miqdad OA. Synthesis and antimicrobial evaluation of some new pyrazole, pyrazoline and chromeno[3,4-c]pyrazole derivatives. *J Braz Chem Soc*. 2009;20(5):975-987. doi:10.1590/S0103-50532009000500024
17. Revanasiddappa B, Kumar MV, Kumar H. Synthesis and Antidepressant Activity of Pyrazoline Derivatives. *Dhaka Univ J Pharm Sci*. 2020;19(2):179-184. doi:10.3329/dujps.v19i2.50634
18. Salian V V., Narayana B, Sarojini BK, Kumar MS, Sharath Chandra K, Lobo AG. Tailor made biheterocyclic pyrazoline-thiazolidinones as effective inhibitors of Escherichia coli FabH: Design, synthesis and structural studies. *J Mol Struct*. 2019;1192:91-104. doi:10.1016/j.molstruc.2019.04.105
19. Raghuvanshi DS, Verma N, Singh SV, Khare S, Pal A, Negi AS. Synthesis of thymol-based pyrazolines: An effort to perceive novel potent-antimalarials. *Bioorg Chem*. 2019;88(September 2018):102933. doi:10.1016/j.bioorg.2019.102933
20. Nocentini A, Moi D, Balboni G, Salvadori S, Onnis V, Supuran CT. Synthesis and biological evaluation of novel pyrazoline-based aromatic sulfamates with

potent carbonic anhydrase isoforms II, IV and IX inhibitory efficacy. *Bioorg Chem*. 2018;77:633-639. doi:10.1016/j.bioorg.2018.02.021
21. Krishna PR, Prapurna YL. DABCO catalyzed facile synthesis of highly functionalized pyrazolines from Baylis-Hillman acetates and ethyl diazoacetate. *Tetrahedron Lett*. 2010;51(50):6507-6510. doi:10.1016/j.tetlet.2010.10.006
22. Moi D, Nocentini A, Deplano A, Balboni G, Supuran CT, Onnis V. Structure-activity relationship with pyrazoline-based aromatic sulfamates as carbonic anhydrase isoforms I, II, IX and XII inhibitors: Synthesis and biological evaluation. *Eur J Med Chem*. 2019;182:1-11. doi:10.1016/j.ejmech.2019.111638
23. Havrylyuk D, Zimenkovsky B, Karpenko O, Grellier P, Lesyk R. Synthesis of pyrazoline-thiazolidinone hybrids with trypanocidal activity. *Eur J Med Chem*. 2014;85:245-254. doi:10.1016/j.ejmech.2014.07.103
24. Patel VM, Desai KR. Eco-friendly synthesis of fluorine-containing pyrazoline derivatives over potassium carbonate. *Arkivoc*. 2004;1:123-129. doi:10.3998/ark.5550190.0005.111
25. Gaamoussi I, Fichtali I, Tama A Ben, et al. Synthesis, characterization and X-ray structure of glycosyl-1, 2-isoxazoles and glycosyl-1,2-isoxazolines prepared via 1,3-dipolar cycloaddition. *J Mol Struct*. 2013;1048:130-137. doi:10.1016/j.molstruc.2013.05.043
26. Chowdhury P, Das AM, Goswami P. Synthesis of some new steroidal [16α,17α-d]-isoxazolines. *Steroids*. 2005;70(8):494-498. doi:10.1016/j.steroids.2005.01.003
27. Aarjane M, Slassi S, Ghaleb A, Tazi B, Amine A. Synthesis, biological evaluation, molecular docking and in silico ADMET screening studies of novel isoxazoline derivatives from acridone. *Arab J Chem*. 2021;14(4):1-13. doi:10.1016/j.arabjc.2021.103057
28. Basappa, Sadashiva MP, Mantelingu K, Nanjunda Swamy S, Rangappa KS. Solution-phase synthesis of novel Δ2-isoxazoline libraries via 1,3-dipolar cycloaddition and their antifungal properties. *Bioorganic Med Chem*. 2003;11(21):4539-4544. doi:10.1016/j.bmc.2003.08.007
29. Bakht MA, Ansari MJ, Riadi Y, Ajmal N, Ahsan MJ, Yar MS. Physicochemical characterization of benzalkonium chloride and urea based deep eutectic solvent (DES): A novel catalyst for the efficient synthesis of isoxazolines under ultrasonic irradiation. *J Mol Liq*. 2016;224:1249-1255. doi:10.1016/j.molliq.2016.10.105
30. Guirado A, Martiz B, Andreu R, Bautista D. A new and efficient approach to isoxazolines. First synthesis of 3-aryl-5-dichloromethyl-2-isoxazolines. *Tetrahedron*. 2011;67(32):5811-5815. doi:10.1016/j.tet.2011.05.110
31. Dofe VS, Sarkate AP, Tiwari S V., et al. Ultrasound assisted synthesis of tetrazole based pyrazolines and isoxazolines as potent anticancer agents via inhibition of tubulin polymerization. *Bioorganic Med Chem Lett*. 2020;30(22):127592. doi:10.1016/j.bmcl.2020.127592
32. Thari FZ, Tachallait H, El Alaoui NE, et al. Ultrasound-assisted one-pot green synthesis of new N- substituted-5-arylidene-thiazolidine-2,4-dione-isoxazoline derivatives using NaCl/Oxone/Na3PO4 in aqueous media. *Ultrason Sonochem*. 2020;68(May):105222. doi:10.1016/j.ultsonch.2020.105222
33. Krompiec S, Bujak P, Malarz J, et al. An isomerization-1,3-dipolar cycloaddition tandem reaction towards the synthesis of 3-aryl-4-methyl-5-O-

substituted isoxazolines from O-allyl compounds. *Tetrahedron*. 2012;68(30):6018-6031. doi:10.1016/j.tet.2012.05.027

34. Dadiboyena S, Nefzi A. Solid phase synthesis of isoxazole and isoxazoline-carboxamides via [2+3]-dipolar cycloaddition using resin-bound alkynes or alkenes. *Tetrahedron Lett*. 2012;53(16):2096-2099. doi:10.1016/j.tetlet.2012.02.041
35. Karthikeyan K, Veenus Seelan T, Lalitha KG, Perumal PT. Synthesis and antinociceptive activity of pyrazolyl isoxazolines and pyrazolyl isoxazoles. *Bioorganic Med Chem Lett*. 2009;19(13):3370-3373. doi:10.1016/j.bmcl.2009.05.055
36. Salotra R, Utreja D, Sharma P. A Convenient One-Pot Synthesis of Chalcones and Their Derivatives and Their Antimicrobial Activity. *Russ J Org Chem*. 2020;56(12):2207-2211. doi:10.1134/S1070428020120258
37. Ozmen Ozgun D, Gul HI, Yamali C, et al. Synthesis and bioactivities of pyrazoline benzensulfonamides as carbonic anhydrase and acetylcholinesterase inhibitors with low cytotoxicity. *Bioorg Chem*. 2019;84:511-517. doi:10.1016/j.bioorg.2018.12.028
38. Bano S, Alam MS, Javed K, Dudeja M, Das AK, Dhulap A. Synthesis, biological evaluation and molecular docking of some substituted pyrazolines and isoxazolines as potential antimicrobial agents. *Eur J Med Chem*. 2015;95:96-103. doi:10.1016/j.ejmech.2015.03.031
39. Mustafa K. Shneshil*, Ahmed W. Naser, Saadon A. Aowda. Synthesis of some saccharin derivatives containing 1 , 2 , 3-triazoline ring Synthesis and Characterization of New 1 , 2 , 4-Triazole Derivatives Form 2- Naphthol View project prodrug polym ... *Int J ChemTech Res*. 2020;9(9):389-393.
40. Mustafa K. Shneshil*, Ahmed W. Naser SAA. Synthesis of some saccharin derivatives containing 1 , 2 , 3-triazoline ring Synthesis and Characterization of New 1 , 2 , 4-Triazole Derivatives Form 2- Naphthol View project prodrug polym ... *Int J ChemTech Res*. 2016;9(9):389-393.
41. Radhi MM, Abdullah HN, Al-Asadi SA, Al-Mulla EAJ. Electrochemical oxidation effect of ascorbic acid on mercury ions in blood sample using cyclic voltammetry. *Int J Ind Chem*. 2015;6(4):311-316. doi:10.1007/s40090-015-0053-9
42. Singh R, Bansal R. 16,17-N′-(alky/arylsulfonyl)pyrazoline substituted neuroprotective heterosteroids: Synthesis, molecular docking and preclinical efficacy/toxicity studies in rodents. *Steroids*. 2019;148(April):114-124. doi:10.1016/j.steroids.2019.05.002

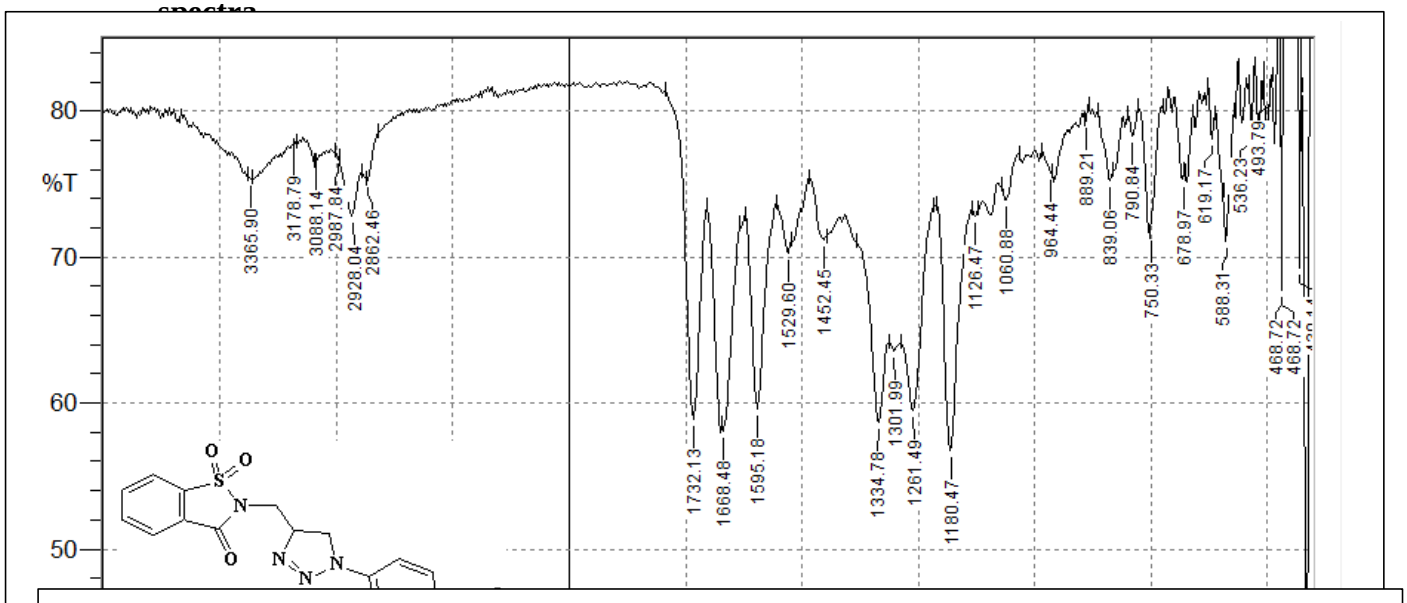
Supporting Information

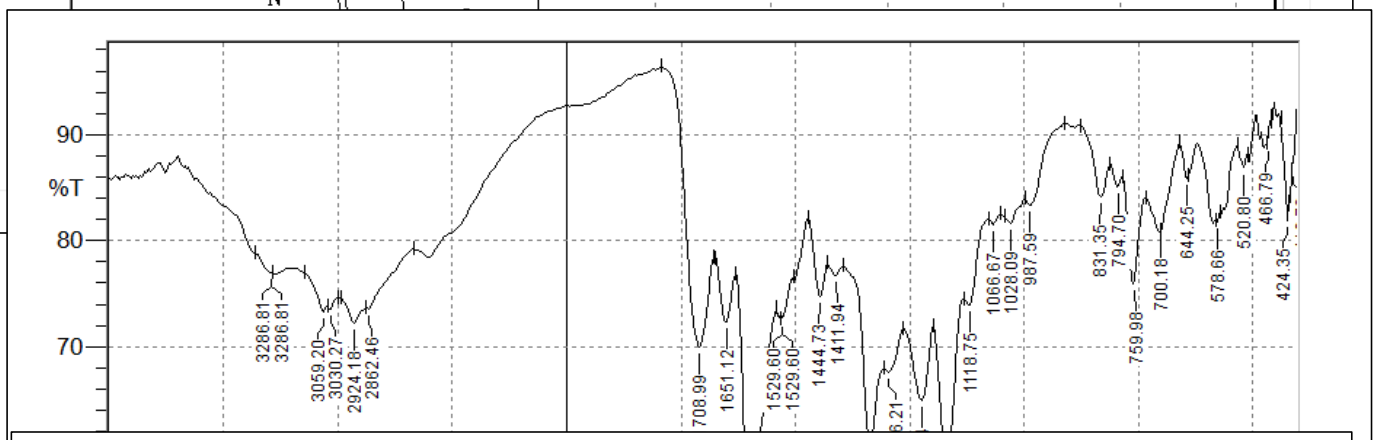
A. Spectroscopic figures

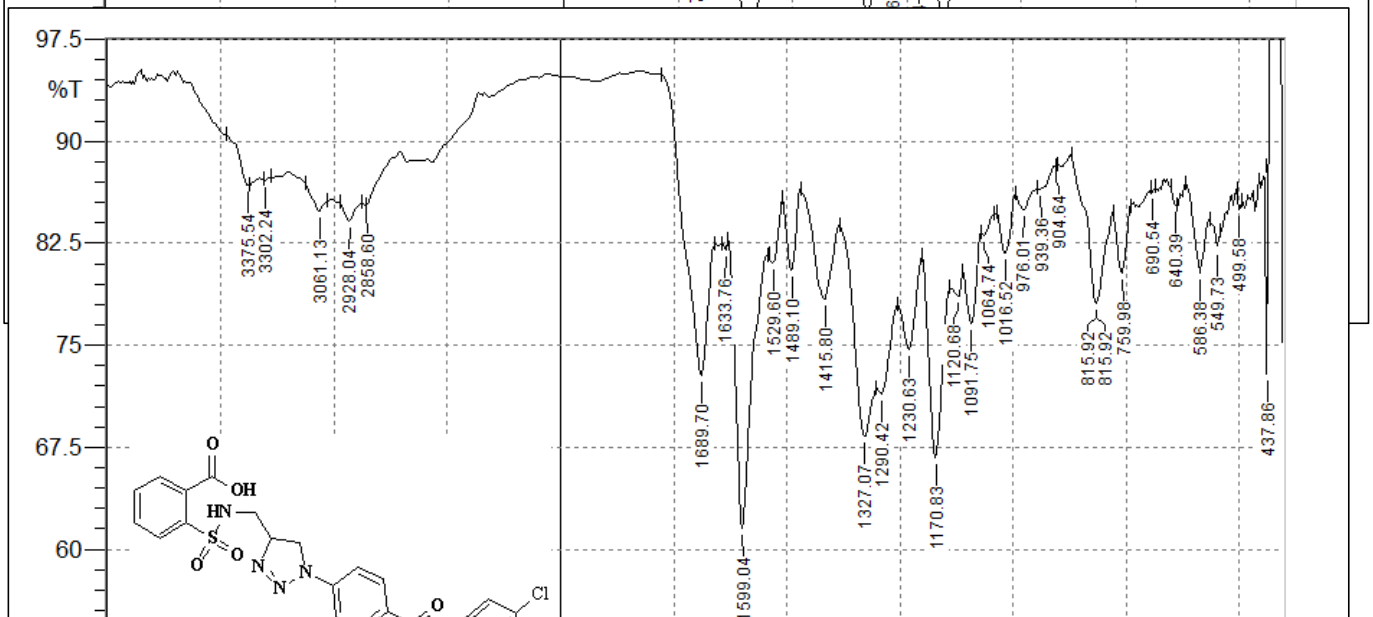
A.1. FT-IR spectra

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

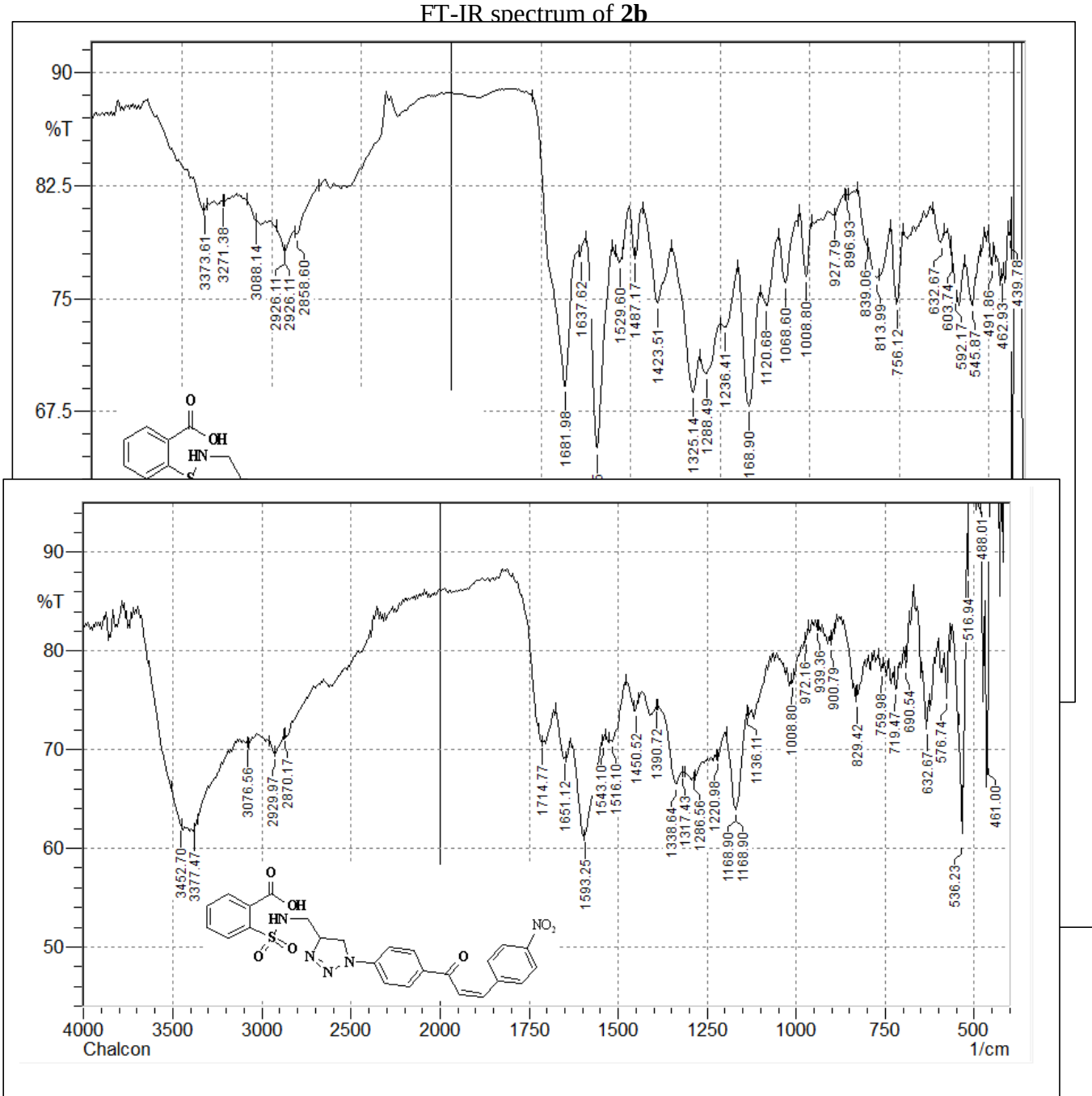
A.3. ^{13}C-NMR spectra



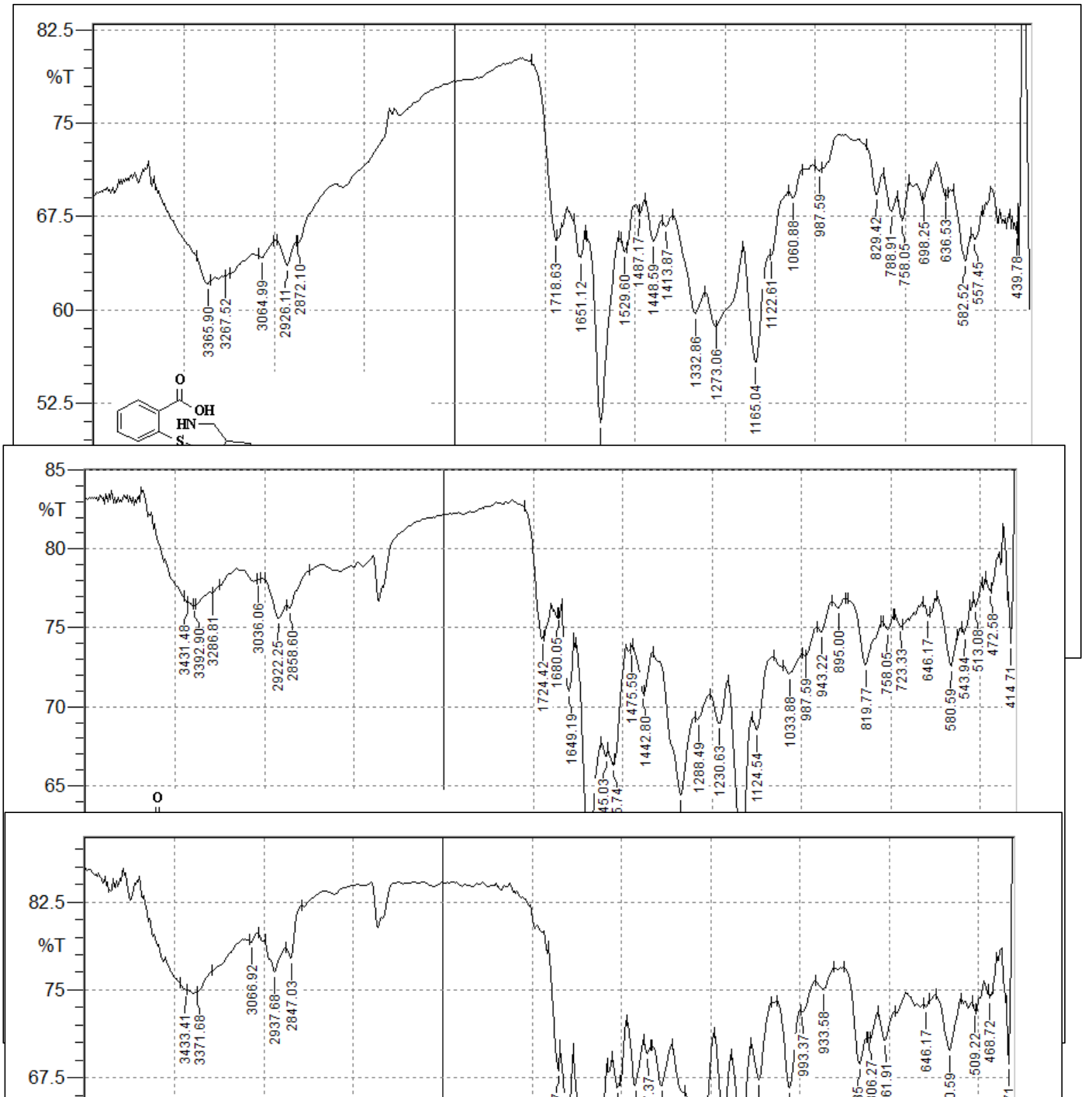




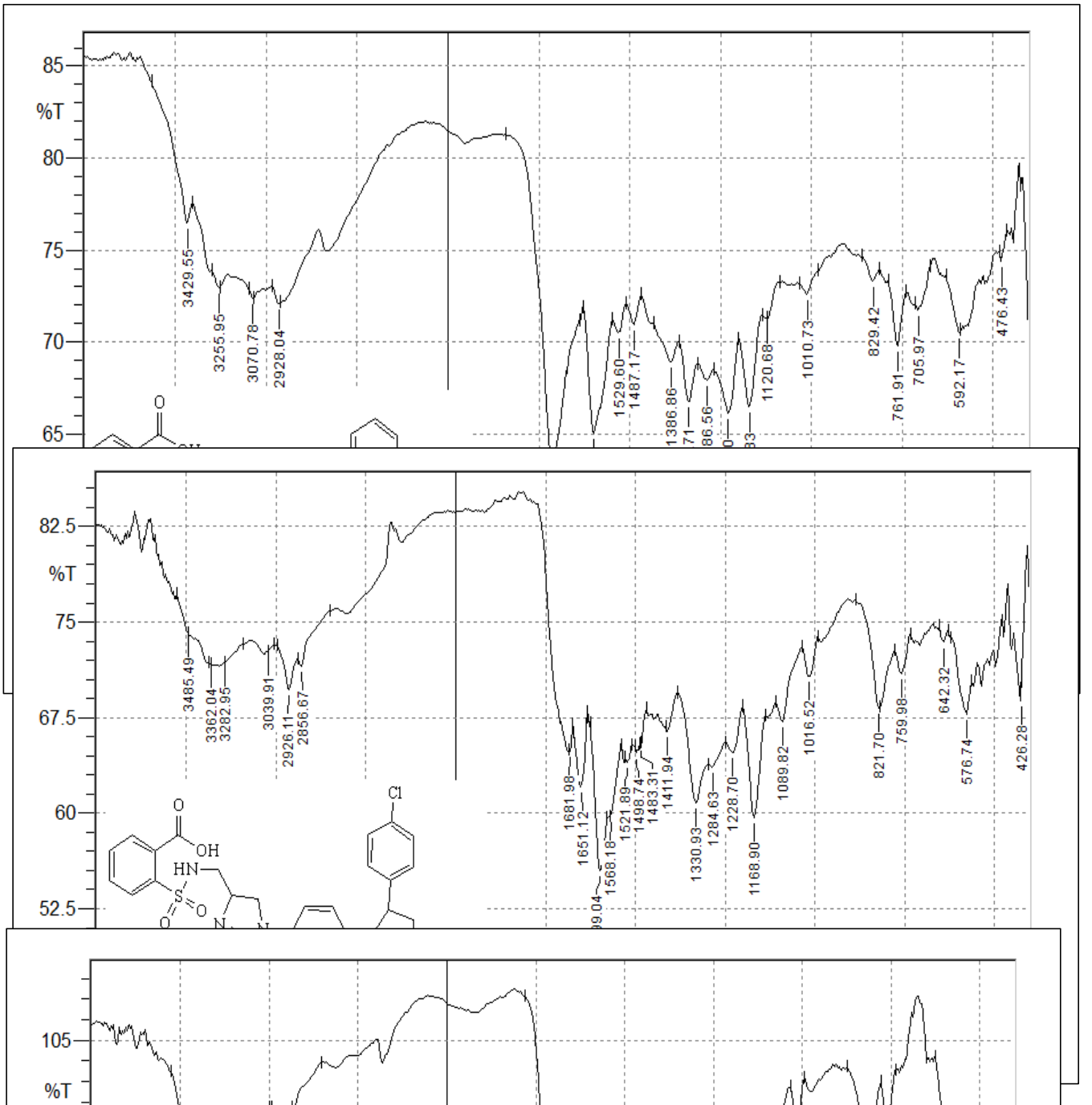
FT-IR spectrum of **2b**



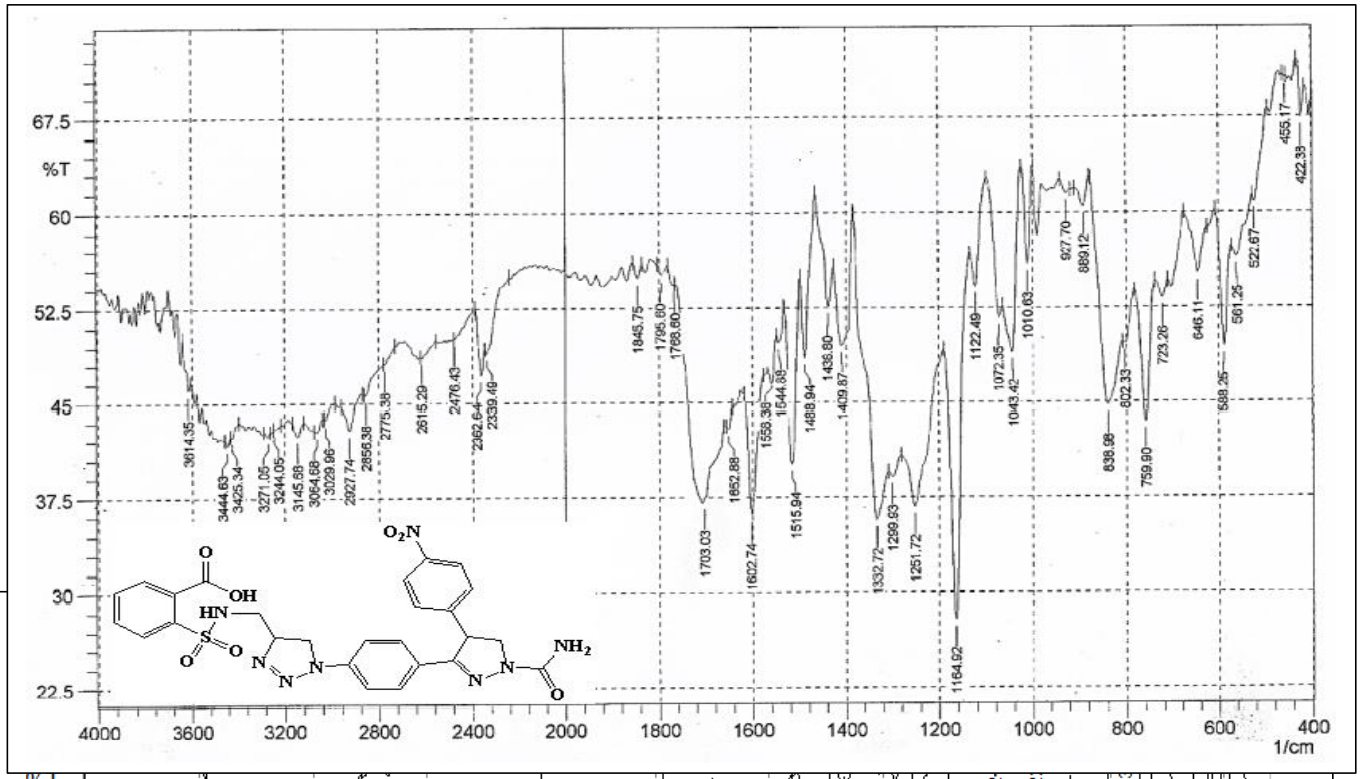
FT-IR spectrum of **2e**

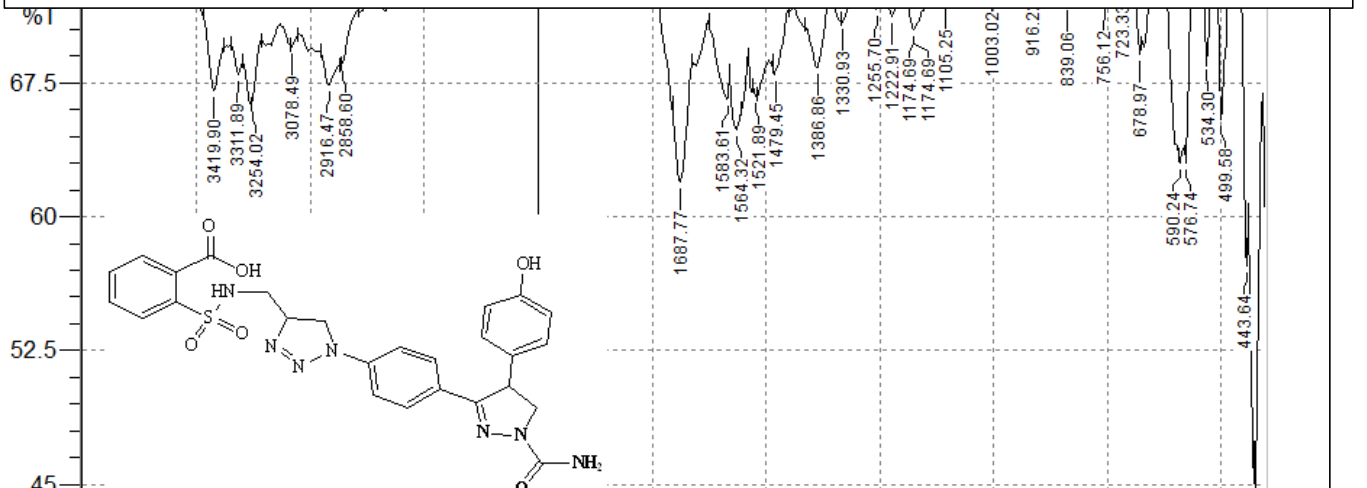


FT-IR spectrum of **2h**



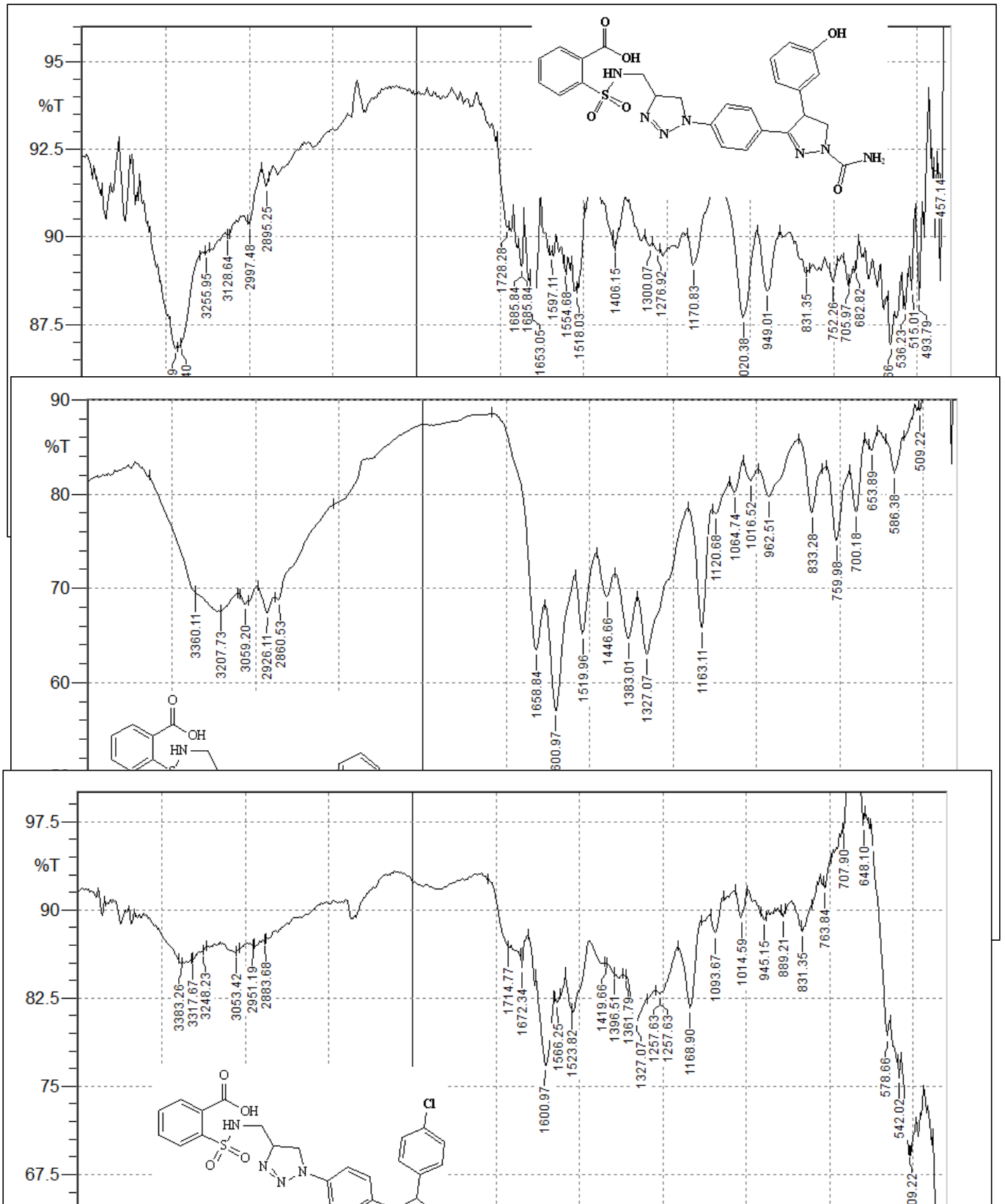
FT-IR spectrum of **3c**



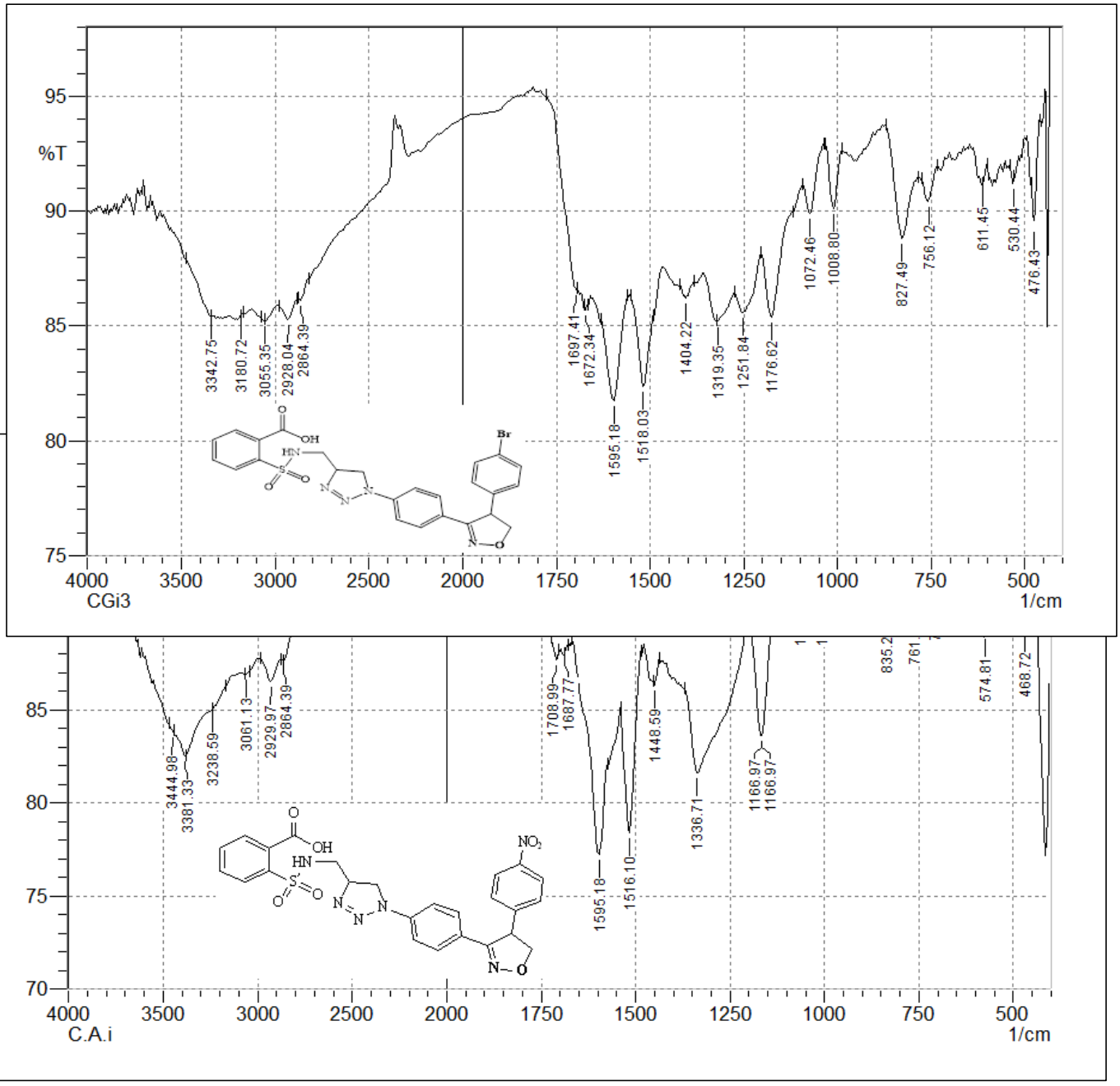


FT-IR spectrum of **3d**

FT-IR spectrum of **3e**

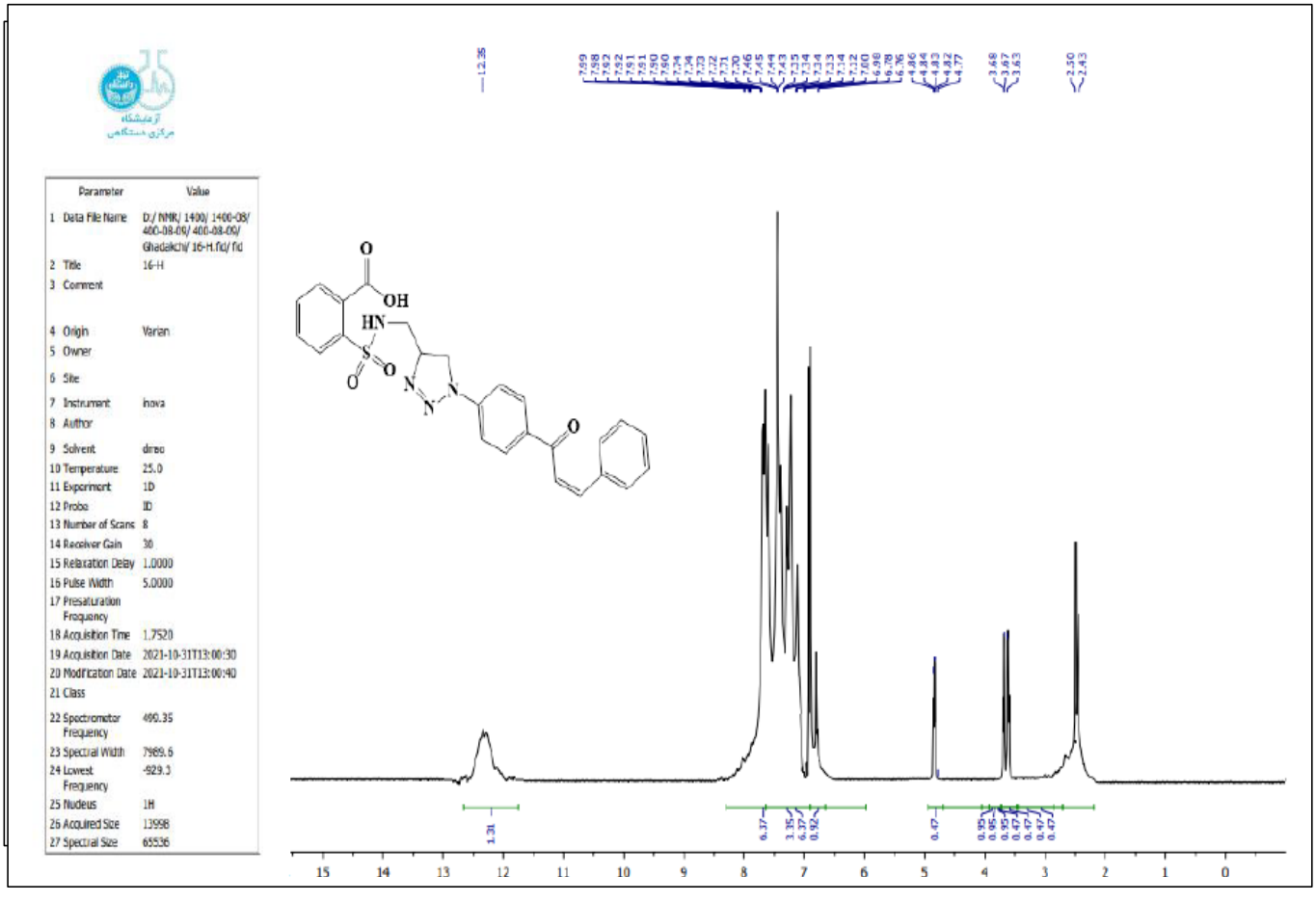


FT-IR spectrum of **4b**



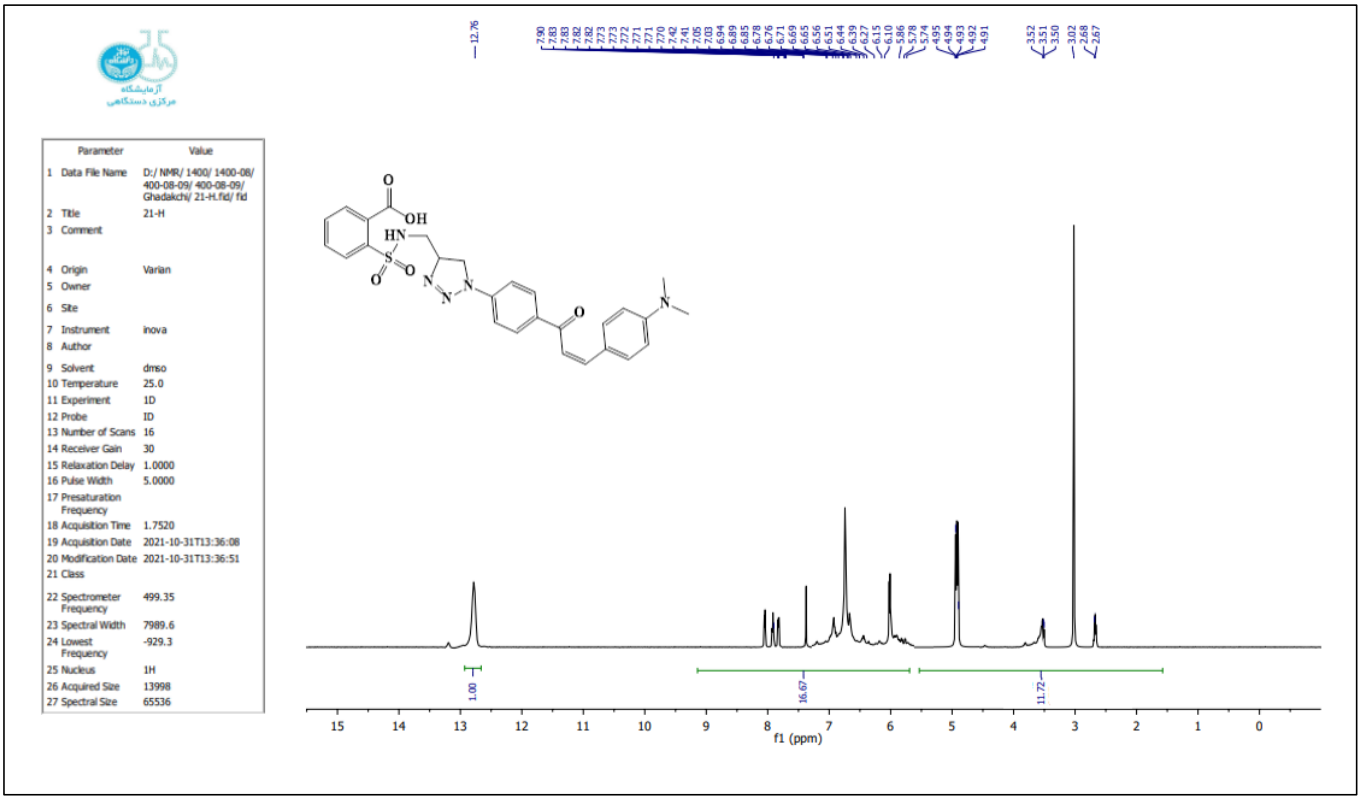
FT-IR spectrum of **4d**

^{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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6	Site	
7	Instrument	inova
8	Author	
9	Solvent	dmso
10	Temperature	25.0
11	Experiment	1D
12	Probe	ID
13	Number of Scans	16
14	Receiver Gain	30
15	Relaxation Delay	1.0000
16	Pulse Width	5.0000
17	Presaturation Frequency	
18	Acquisition Time	1.7520
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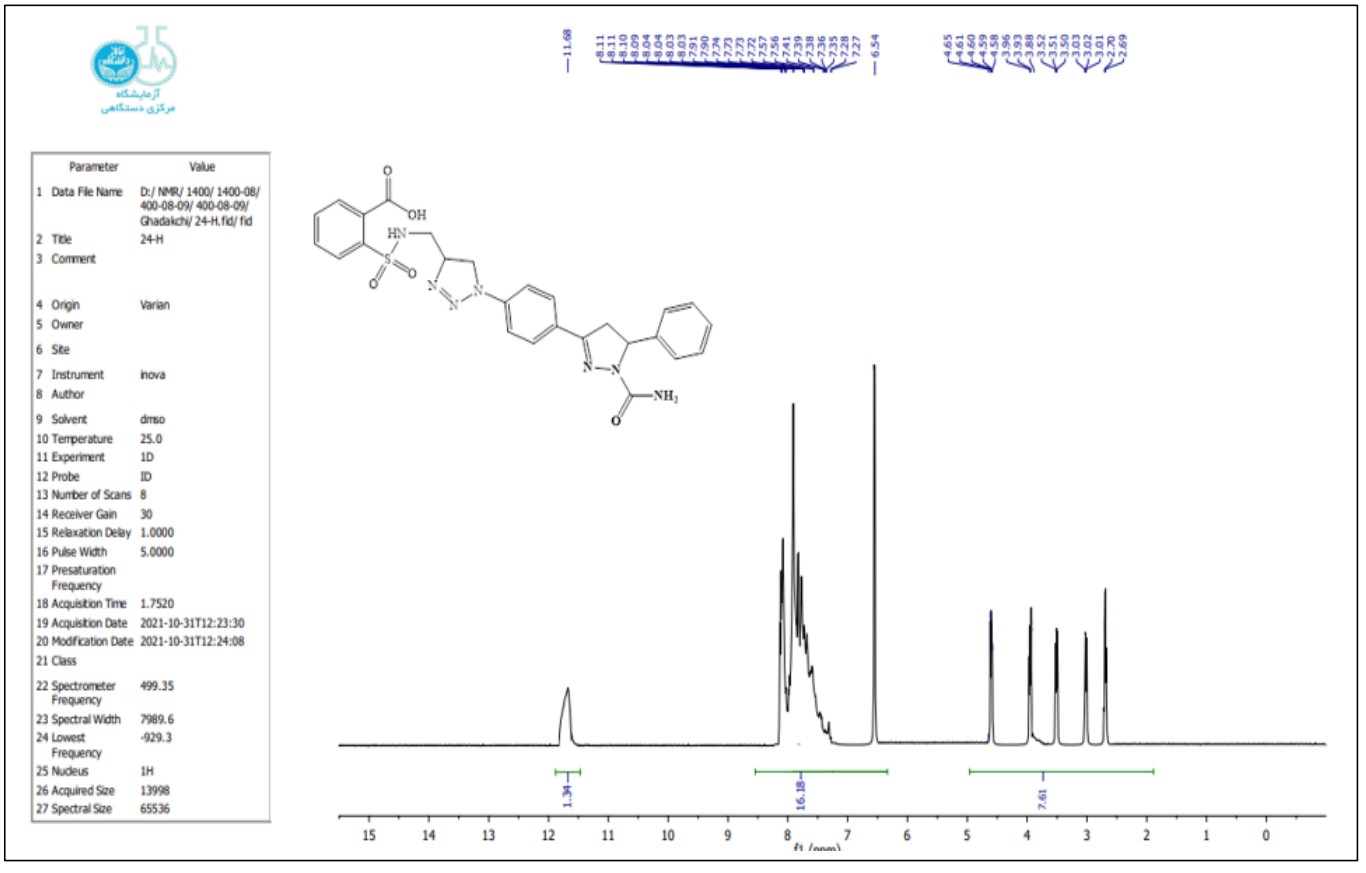
25	Nucleus	1H
26	Acquired Size	13998
27	Spectral Size	65536



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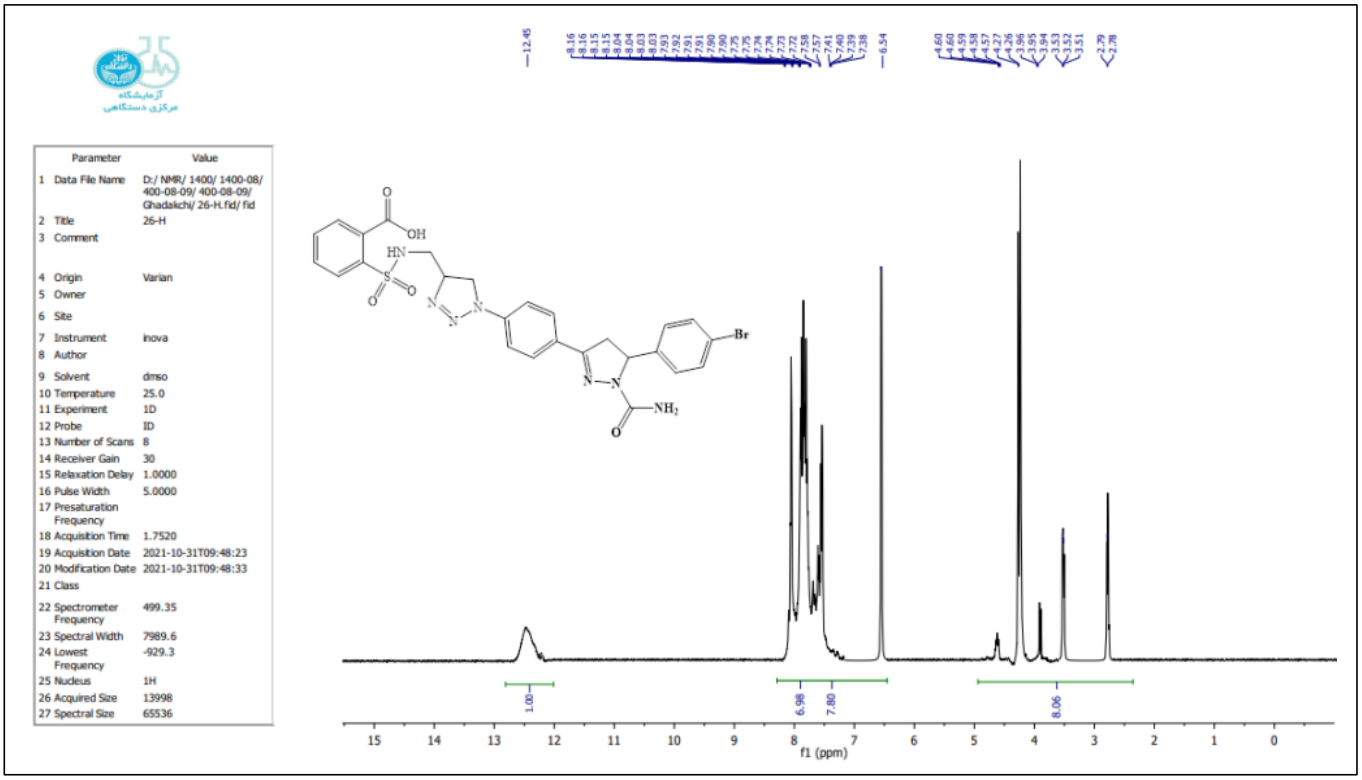
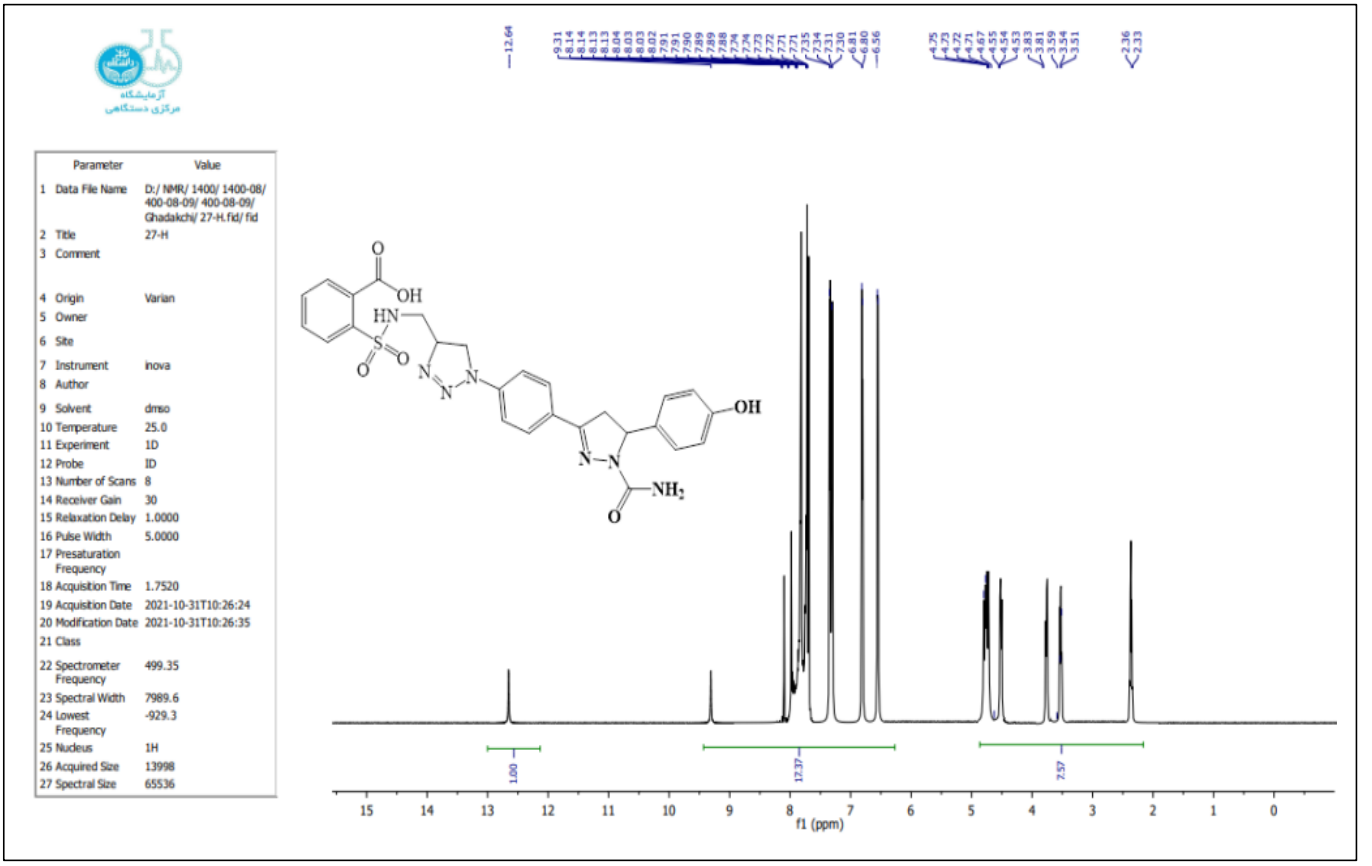
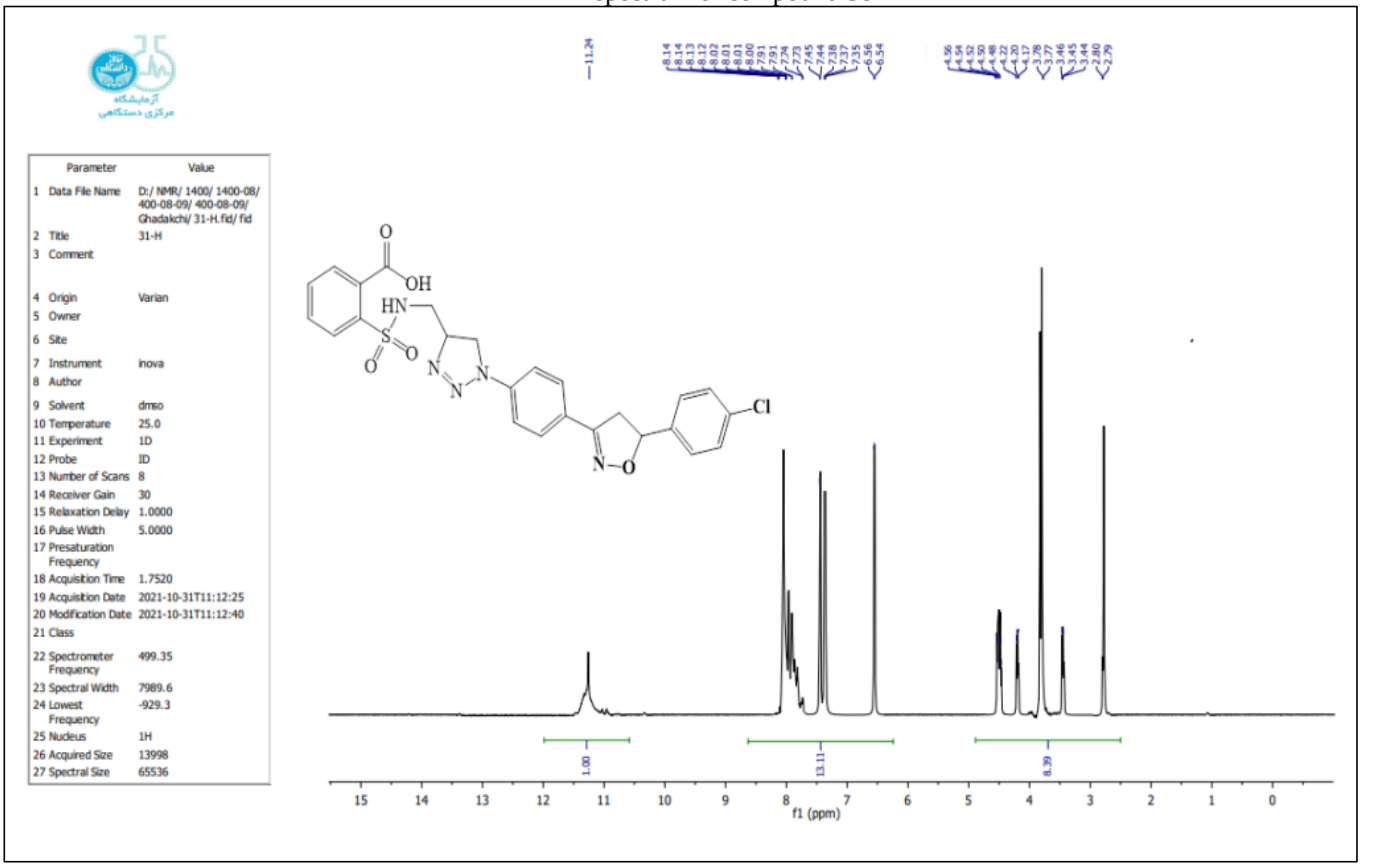
6	Site	
7	Instrument	inova
8	Author	
9	Solvent	dmso
10	Temperature	25.0
11	Experiment	1D
12	Probe	ID
13	Number of Scans	8
14	Receiver Gain	30
15	Relaxation Delay	1.0000
16	Pulse Width	5.0000
17	Presaturation Frequency	
18	Acquisition Time	1.7520
19	Acquisition Date	2021-10-31T12:23:30
20	Modification Date	2021-10-31T12:24:08
21	Class	
22	Spectrometer Frequency	499.35
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**

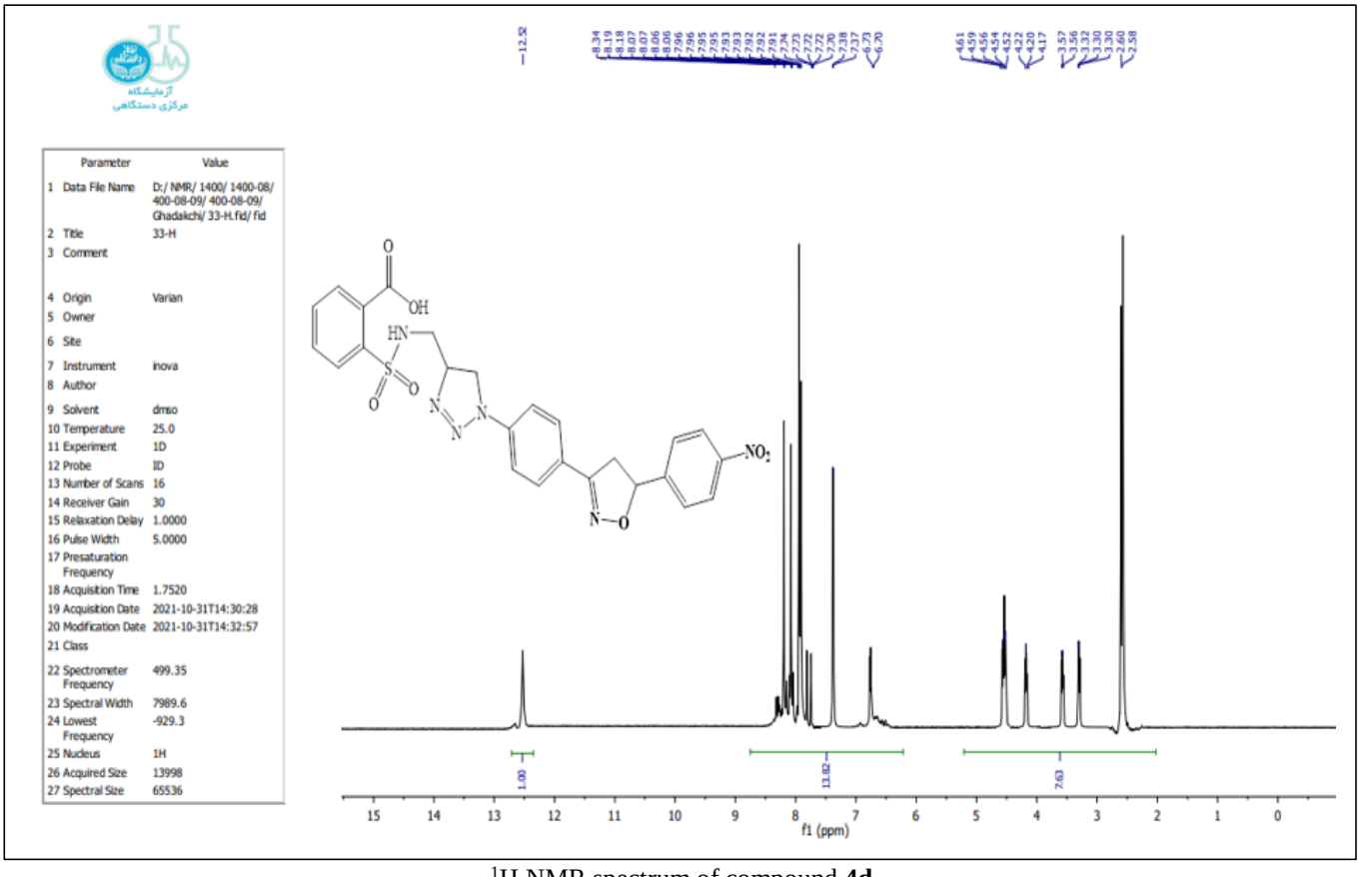




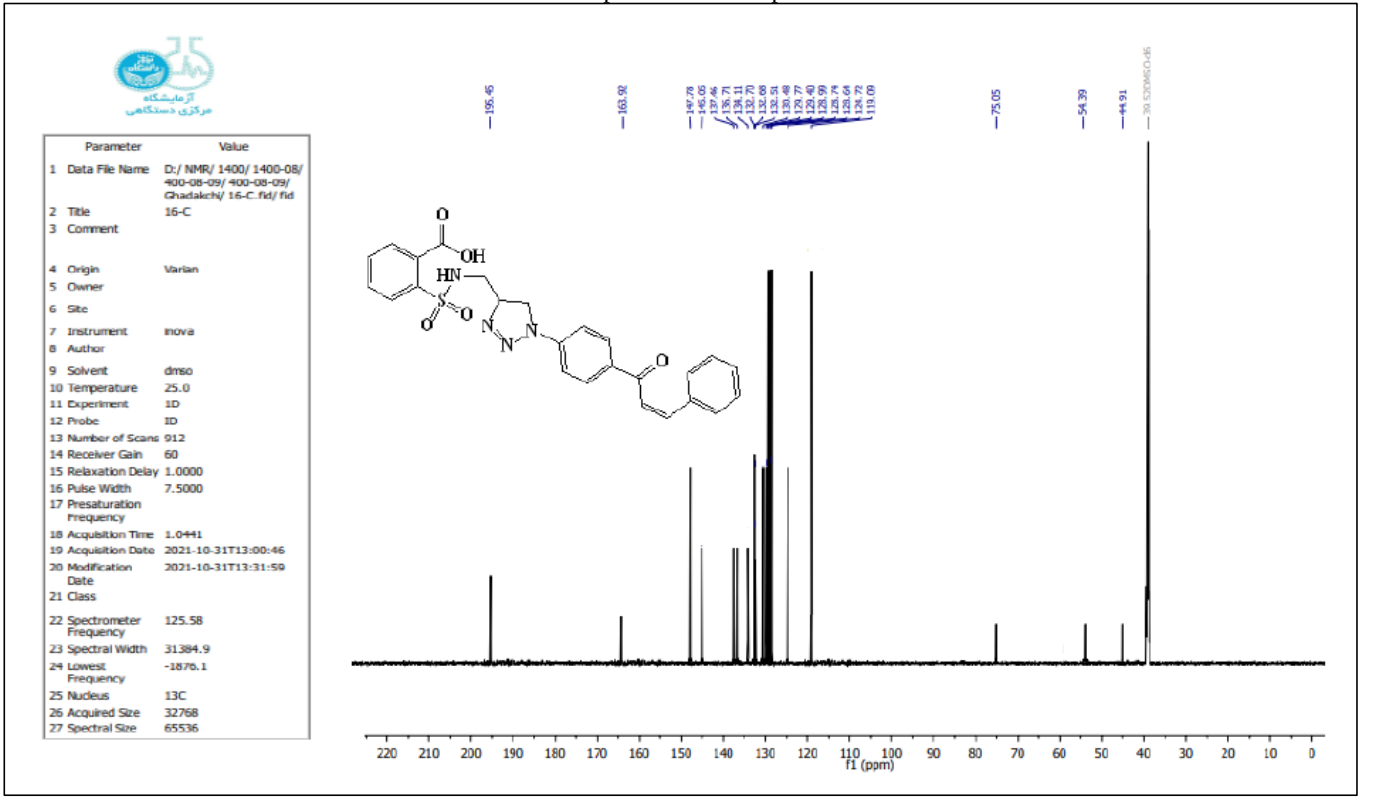
^{1}H NMR spectrum of compound **3e**



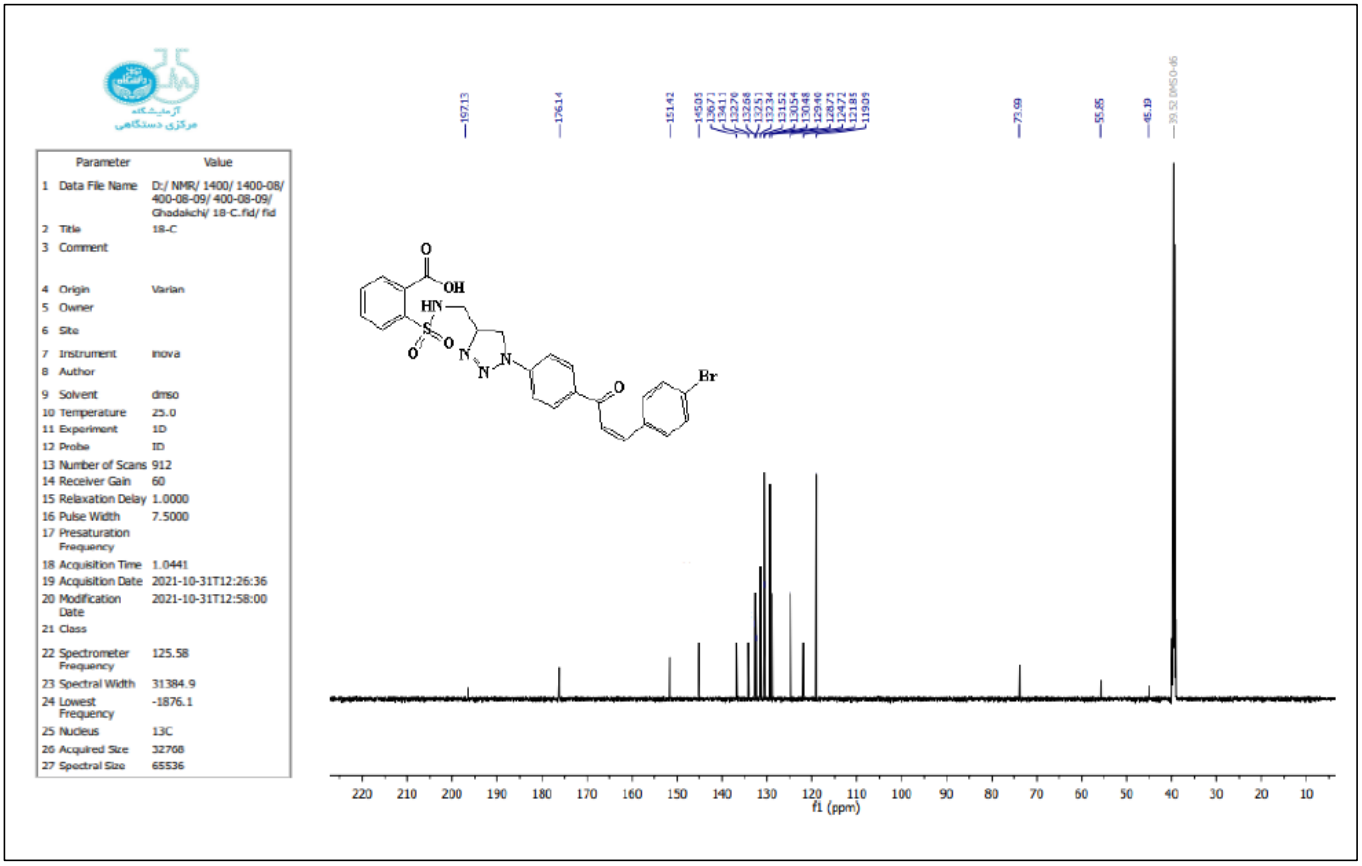
^{1}H NMR spectrum of compound **4b**



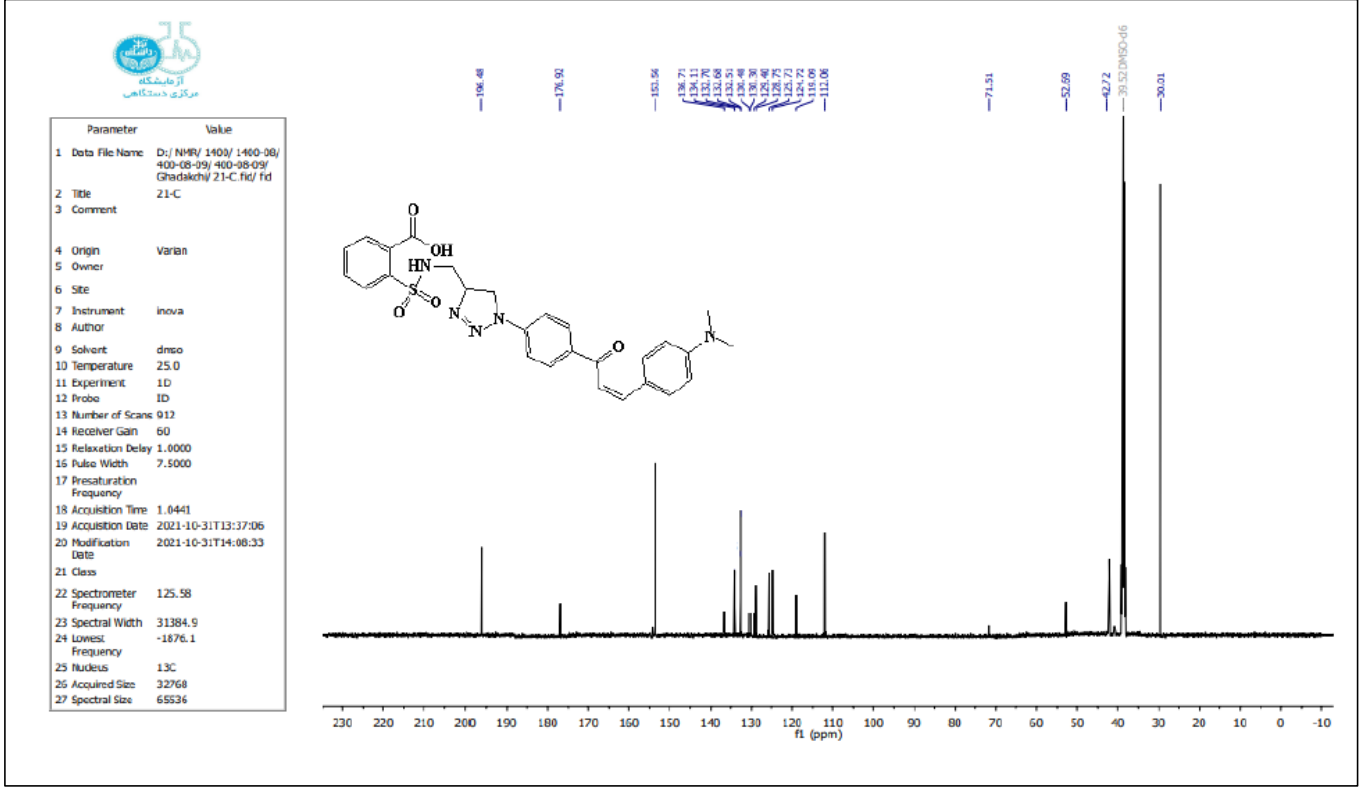
^{1}H NMR spectrum of compound **4d**



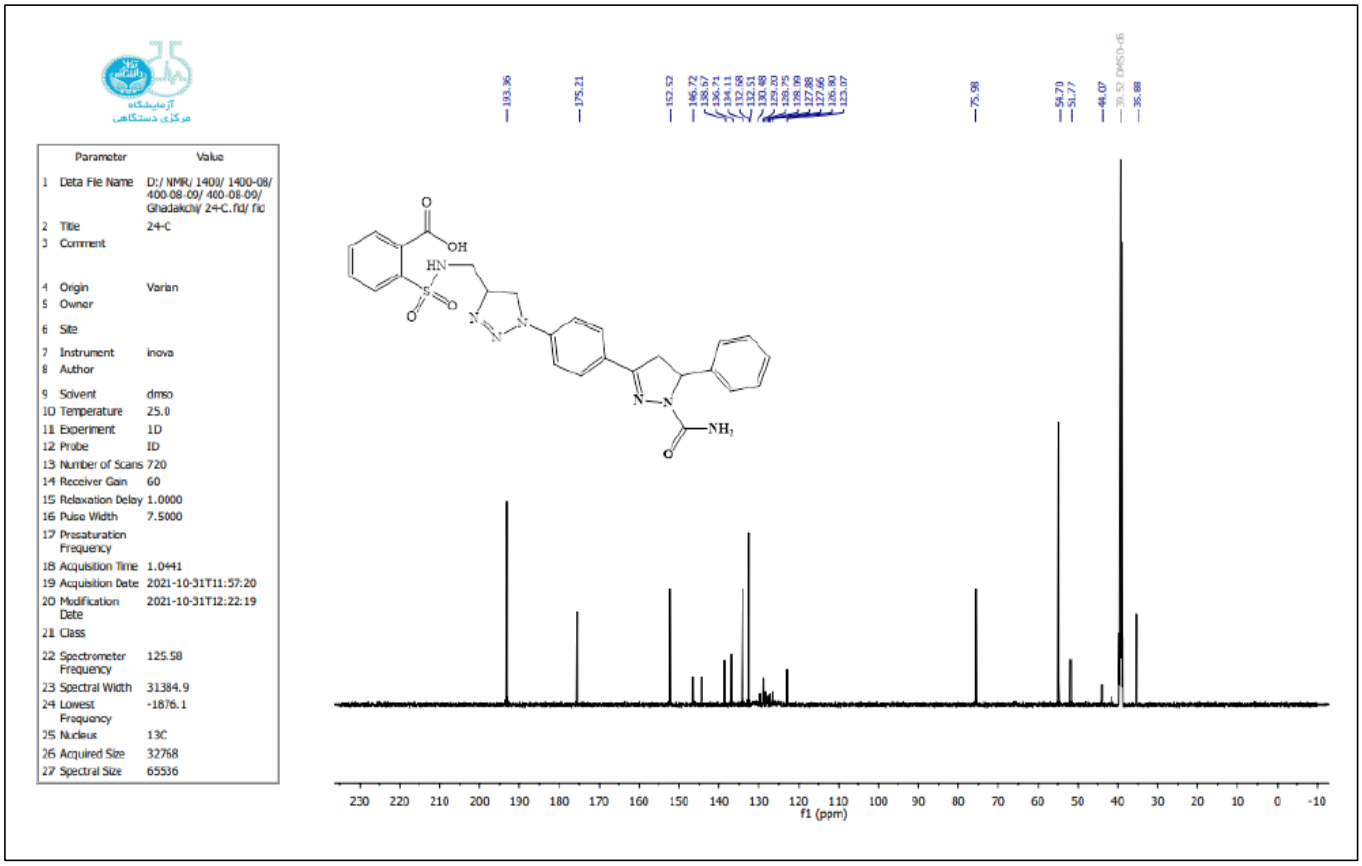
^{13}C NMR spectrum of compound **2a**



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



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



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7	Instrument	inova
8	Author	
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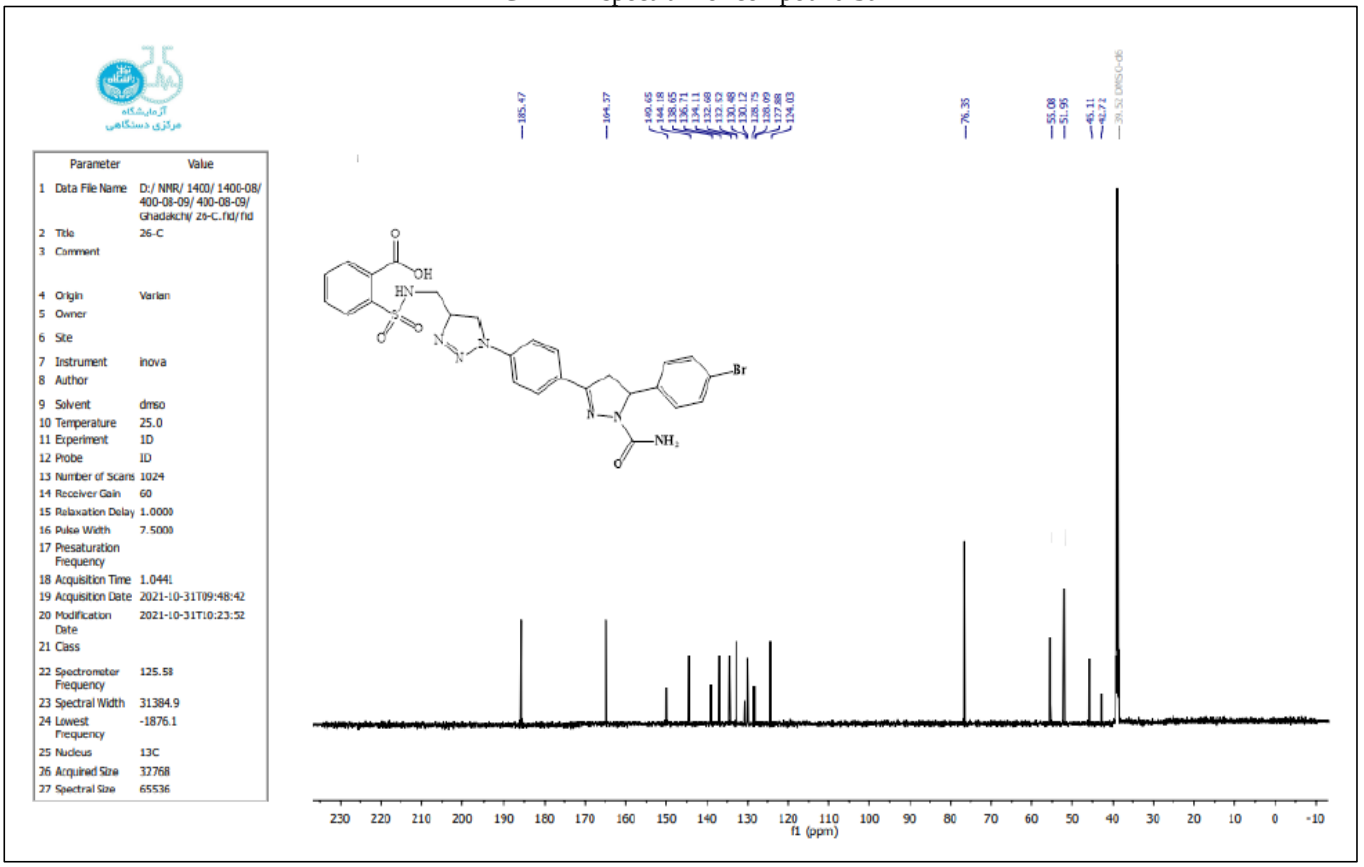
25	Nucleus	13C
26	Acquired Size	32768
27	Spectral Size	65536

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



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6	Site	
7	Instrument	inova
8	Author	
9	Solvent	dmso
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11	Experiment	1D
12	Probe	ID
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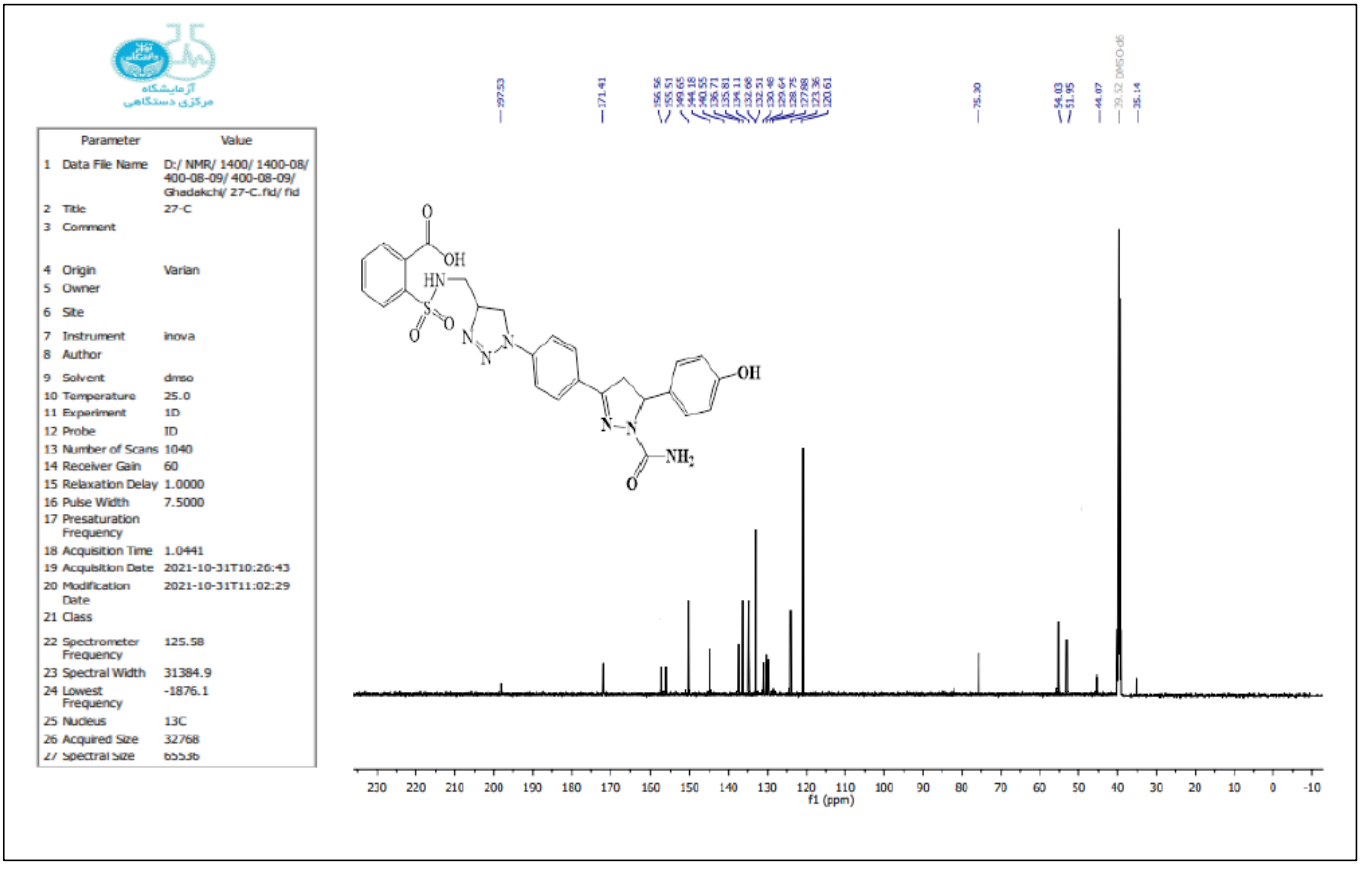
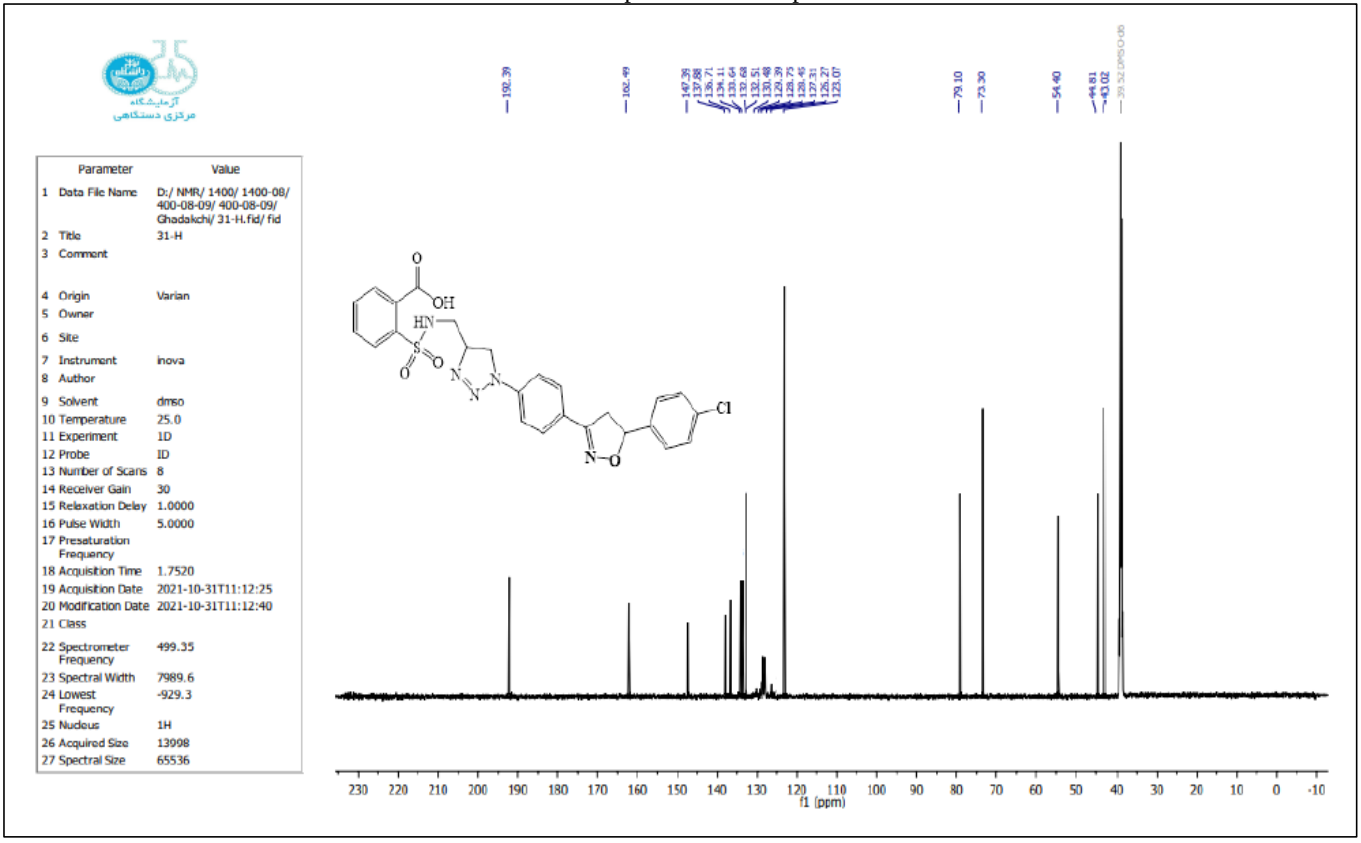
23	Spectral Width	31384.9
24	Lowest Frequency	-1876.1
25	Nucleus	13C
26	Acquired Size	32768
27	Spectral Size	65536

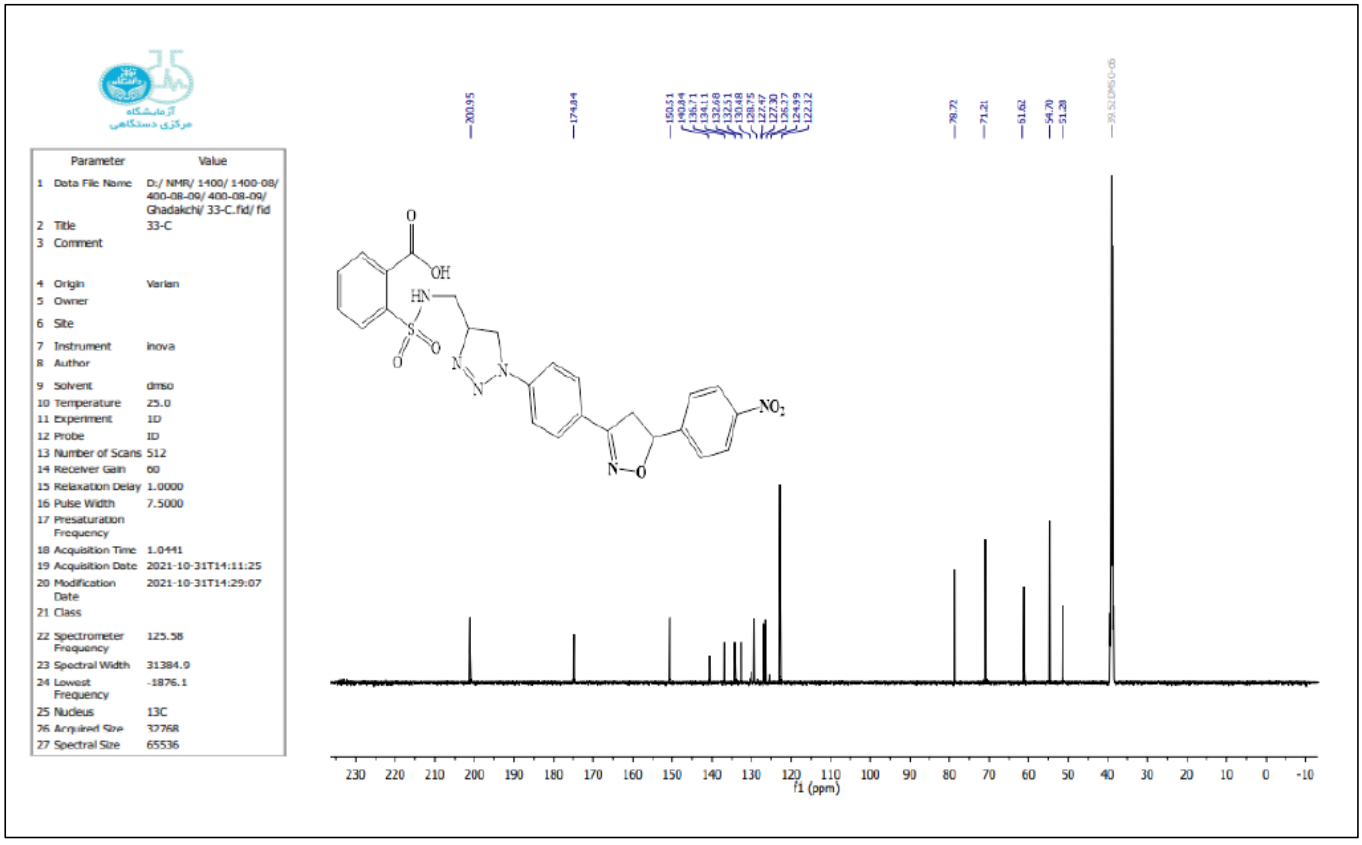
^{13}C- NMR spectrum of compound **3c**



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



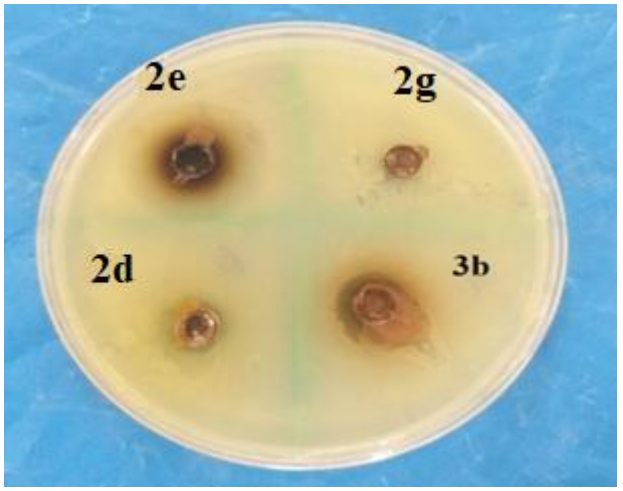
^{13}C- NMR spectrum of compound **4b**

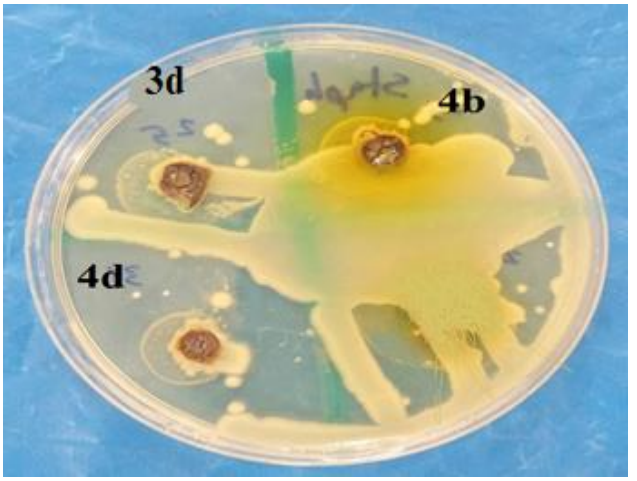


^{13}C- NMR spectrum of compound **4d**

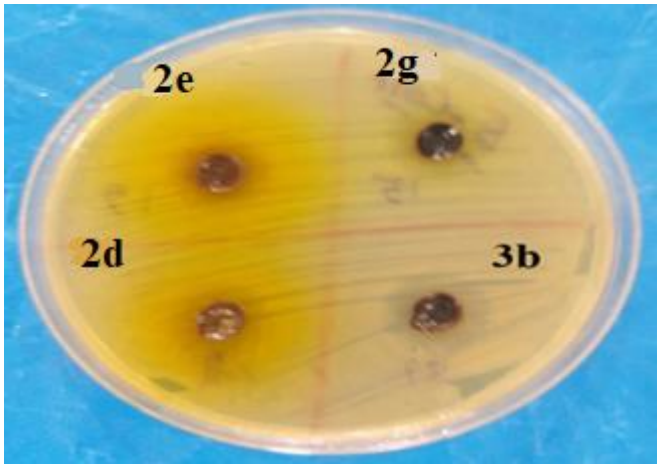
B. Antibacterial photographs

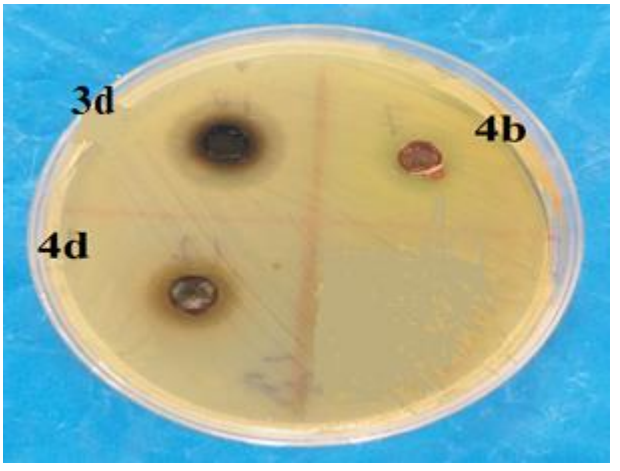
B.1. Antibacterial photographs of Chalcone, pyrazoline and isoxazoline derivatives 2e, 2g, 2d, 3b, 3d, 4b and 4d against Staphylococcus aurous



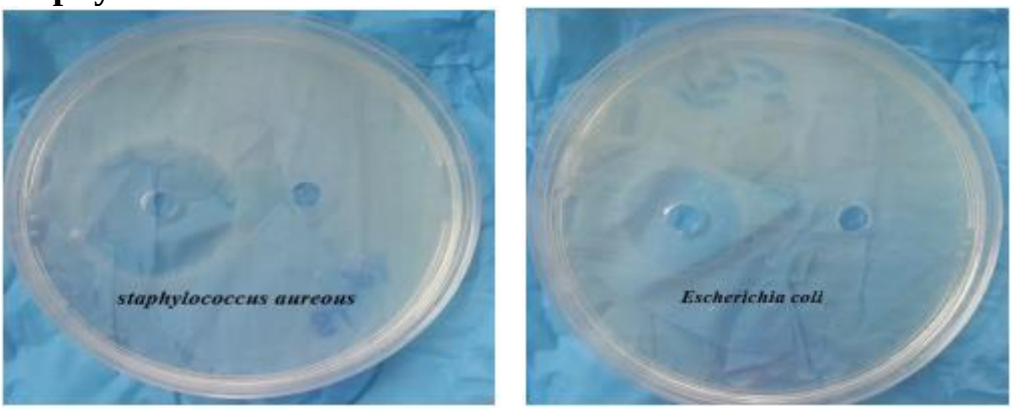


B.2. Antibacterial photographs of Chalcone, pyrazoline and isoxazoline derivatives 2e, 2g, 2d, 3b, 3d, 4b and 4d Escherichia Coli





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



Antibacterial activity of Ceftriaxone against Staphylococcus aureus and Escherichia coli.