DOI : https://doi.org/10.31351/vol32issSuppl.pp188-203

Molecular Docking, ADMET Study, Synthesis, Characterization, and Preliminary Antiproliferative Activity of Potential Histone Deacetylase Inhibitors with Isoxazole as New Zinc Binding Group [#]

Ali Mohammed Saeed[*,1] and Ayad Abed Ali Al-Hamashi[2]

[#] 2nd Scientific Conference for Postgraduate Students Researches.

[1] Ministry of Health and Environment, Babylon Healthcare Directorate, Babylon, Iraq

[2]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad,Iraq

Abstract

Histone acetylation is a highly interesting epigenetic target for drug therapy. Histone deacetylase enzymes (HDACs) are overexpressed in several diseases, including cancers. Most of the clinically used HDAC inhibitors involve hydroxamate group as a zinc-binding group (ZBG). Hydroxamates have a poor pharmacokinetic properties and high toxicity profile. Therefore, developing non-hydroximate HDAC inhibitors is a promising strategy for potency and selectivity enhancement. In this work, we designed new HDAC inhibitors with isoxazole moiety as ZBG using Ligand Designer from Glide (Schrodinger LLC). The cap group and the linker were optimized through trying various aliphatic and aromatic residues. The potential inhibition over HDAC8 for the optimally designed products was virtually evaluated using licensed Schrodinger modelling software. The results showed that the isoxazole has a potential bidentate interaction with HDAC8 active site zinc ion with acceptable fitness. ADMET study performed to predict the pharmacokinetic properties for the final compounds. The final compounds showed a decent estimated drug-like properties. The intermediates and final compounds were successfully synthesized and purified using column chromatography. The chemical structure for intermediates and final compounds were characterized by IR and NMR spectroscopy. Compounds Va and **Vb** revealed an encouraging antitumor activity in colon cancer cells (LS-174T) with IC_{50} of 0.8 µM and 0.88 µM, respectively, which is comparable to vorinostat inhibition activity IC_{50} of 0.6 µM.

Keywords: Histone Deacetylase, Molecular Docking, ADMET, Cancer, Vorinostat.

الرسو الجزئي ودراسة الحركية الدوائية وتخليق وتشخيص مثبطات هيستون ديسيتيلاز المحتملة ودراسة اولية للنشاط المثبط لنمو خلايا سرطانية مكونة الأيزوكسازول كمجموعة رابطة جديدة للزنك[#]

علي محمد علي سعيد عبد الامير[*،١] و اياد عبد علي جياد الهماشي[٢]

#المؤتمر العلمي الثاني لطلبة الدراسات العليا

[*،١] وزارة الصحة والبيئة , دائرة صحة بابل، بابل ، العراق

[٢]فرع الكيمياء الصيدلانية، كلية الصيدلة، جامعة بغداد، بغداد، العراق.

الخلاصة

أستله الهيستون هو هدف جيني مهم في مجال تصنيع وتصميم العلاجات. لوحظ زيادة في تكوين انزيمات هستون ديستيلاز بشكل مفرط في العديد من الأمراض، بما في ذلك السرطانات. تتضمن معظم مثبطات المستخدمة سريريا مجموعة الهيدروكسيمات كمجموعة رابطة للزنك (ZBG). الهيدروكسيمات لها خصائص دوائية ضعيفة وسمية عالية. لذلك، يعد تطوير مثبطات الهستون ديستيلاز تحتوي على مجموعات غير الهيدروكسيمات استراتيجية واعدة لتعزيز الفاعلية والانتقائية. في هذا العمل، قمنا بتصميم مثبطات هستون ديستيلاز جديدة مع مجموعة إيزوكسازول كمجموعة رابطة للزنك باستخدام برنامج التصميم الدوائي من شرودنكر. تم تحسين مجموعة الغطاء والرابط من خلال تجربة العديد من المجموعات الكيميائية الأليفاتية والاروماتية. تم تقييم التثبيط المحتمل على هستون ديستيلاز للمنتجات المصممة على النحو الأمثل تقريبا باستخدام برنامج نمذجة شرودنجر المرخص. أظهرت النتائج أن الأيزوكسازول لديه تفاعل محتمل مع أيون الزنك للهستون ديستيلاز الثامن. تم إجراء دراسة تنبؤية للحركية الدوائية المحتملة والسمية للمركبات النهائية المقترحة. أظهرت المركبات النهائية خصائص تقديرية لائقة شبيهة للأدوية. تم تصنيع المركبات الوسيطة والنهائية وتنقيتها بنجاح باستخدام الكروماتوغرافيا العمودية. تم تشخيص التركيب الكيميائي للمركبات الوسيطة والنهائية بواسطة التحليل الطيفي بالأشعة تحت الحمراء والتحليل الطيفي بالرنين المغناطيسي النووي. كشف المركبان **Va** و **Vb** عن نشاط مضاد للأورام مشجع لتثبيط نمو خلايا سرطان القولون (LS-174T) مع IC_{50} قيمته 0.8 ميكرومول و 0.88 ميكرومول ، على التوالي. وهو ما يمكن مقارنته بنشاط تثبيط الفورينوستات IC50من 0.6 ميكرومول.

الكلمات المفتاحية: هستون دي استيلاز، الرسو الجزيئي، الحركية الدوائية، أورام، دواء فورينوستات.

Introduction

Uncontrolled epigenetic modifications are associated with human diseases including cancer, Huntington's, Alzheimer's, and Parkinson's diseases, spinocerebellar ataxia, and amyotrophic lateral sclerosis [(1)]. Cancer is one of the major causes of death, and it gained the interest of many scientists to develop a new drug that can stop cancer development [(2)]. A strong correlation between cancer and abnormal epigenetic modifications was recorded [(1)]. Histone acetylation is a known epigenetic modification in the development of cancers and other diseases. Histone acetylation is a dynamic process that controlled by two enzymes of histone acetylase (HAT) and histone deacetylase

[1]Corresponding author E-mail: ali.mohammed1200e@copharm.uobaghdad.edu.iq

Received: 19/5 /2023

Accepted: 26/6 /2023

(HDAC) [2–4]. Inhibition of HDAC enzyme is promising anticancer therapy strategy [5].HDAC inhibitors of vorinostat, romidepsin, panobinostat, and belinostat are FDA approved for treating cancer as monotherapy or in combination with other anti-cancer medications. Three of the clinically used HDAC inhibitors are involving hydroxamate moiety as zinc binding group (ZBG). Hydroxamate claimed to have high toxicity and poor pharmacokinetics properties [6].

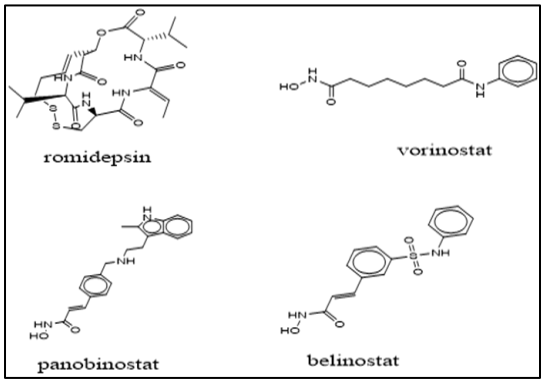


Figure 1. FDA approved HDAC inhibitors.

In continuous with the work [6-9], a docking study was performed using Glide software embedded with MAESTRO from licensed Schrödinger modelling software [10]. The reference compound used was vorinostat which is co-crystallized with HDAC8 protein (1T69). Virtual ligand generated using Ligand Designer to design a new zinc binding group focusing on heterocyclic ring as new ZBG. Virtual design showed isoxazole as new ZBG. Cap group and linker were added using general pharmacophoric properties of HDAC inhibitors. ADMET study was carried out to predict the pharmacokinetic properties of the designed molecules and to overcome poor pharmacokinetics and toxicity associated with hydroxamate ZBG. The designed compounds were docked against HDAC8 (1T69) [11] isoform that downloaded from PDB [12], prepared using protein preparation wizard within the software, Virtual compounds generated from ligand designer prepared, then docked against prepared HDAC8 isoform (1T69) using highly precise XP docking [12]. Compounds that showed accepted docking score and fitness to the receptor with bidentate zinc chelation were selected for synthesis.

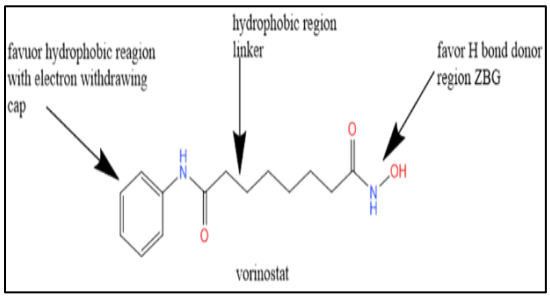


Figure 2. pharmacophoric characters of vorinostat as reference compound

Materials and Method

Molecular Docking

Docking study was performed using Glide application embedded with the maestro software from licensed Schrodinger's modelling suite version 13.0135. Virtual compounds were generated by Ligand Designer to design a new zinc binding group. The designed compounds were completed by adding cap and linker using the general pharmacophoric properties based on vorinostat [10,13]. The protein was chosen from homosapiens to simulate compound that may work on humans with vorinostat as co-crystallized ligands. Crystal structure of HDAC8 (1T69) is obtained from protein data bank [10]. The protein was prepared using protein preparation wizard including preprocessing of the protein to assign bond order, adding hydrogen, adding terminal oxygen to the chain, deleting water beyond 3 A^0, water beyond 3A° from the ligand forming less than 3 hydrogen bond with ligand or amino acid had less probability to affect the docking, from active site, and generating het stat with EPiK Optimizing H-bond assignments was performed using default setting and clean up the structure using OPLS (2005) force field built in with the Schrodinger software 13.1 (1-2023). Receptor grid was generated using the cocrystallized ligand that interacted with the protein using the default setting limiting the size of the grid to 15A*15A*15A [10,13]. Using ligprep, identify and prepare the set of ligands to be docked, ligprep goes beyond simple 2D and 3D structure conversion by including tautomeric, stereochemical and ionization state as well as energy minimization using OPLS (2005) force field [14,15]. The prepared ligands were docked against HDAC8 (1T69) using the default setting of XP docking limiting out to 10 poses. The generated poses were visualized by observing the fitness of the ligand into the active site.

ADMET studies

The prepared ligands undergo ligand base ADMET prediction, to assess drug likeness of designed compounds. QIKProp setting the software to identify the 5 most similar drug molecules, the output data checked for drugability of the designed compounds [16].

Chemical synthesis

Materials

Starting materials, reagents and catalysts were purchased from commercial suppliers (Sigma Aldrich, Glentham, Fluorochem, Macklin, Liyan, Thomas Beaker, Merck). All solvents used were dried using molecular sieves 3A°. Thin-layer chromatography (TLC) was performed using TLC silica gel 60 F254 sheet of 20*20 cm from Merck KGaA and detected under UV light of 254 nm. FT-IR spectroscopy carried out using Shimadzu IRAffinity-1 Spectrometer (Shimadzu, Japan) at the University of Baghdad-College of Pharmacy. ^{1}H-

NMR and ^{13}C-NMR analyses were performed at 400 MHz and 100 MHz respectively (*d6*-DMSO as the solvent) using Bruker Avance III, 400 MHz spectrometer at University of Basra

General procedure for the synthesis of 4-(((tert-butyldimethylsilyl)oxy)methyl)aniline (compound 1)

To a round bottom flask of 50 ml equipped magnetic stirrer, 1.5 g of TBDMSCl (10 mmol), 1.23 g of 4-aminobenzyl alcohol (10 mmol), 25 mmol imidazole added to 30 ml of acetonitrile and the reaction was stirred at room temperature overnight. The reaction was monitored with TLC (3 hexane: 1 ethyl acetoacetate). Solvent removed and residue was mixed with hexane to remove unreacted imidazole. The reaction was separated using column chromatography using silica eluted with ethyl acetate / hexane to yield 1 g (43%) of product as colourless liquid [17]. I.R: 3363, 2954, 2927, 2854, 1253 cm^{-1}.

General procedure for the synthesis compound II

To a round bottom flask equipped with magnetic stirrer was added 3 mmol of compound I (711 mg), 3 mmol of substituted benzoic acid, 3 mmol of EDC.HCl (573 mg), 0.3 mmol of HOBt (40 mg), 3 mmol of DMAP (366 mg) and 2 equivalent of DIPEA, and 12 ml DCM. The reaction was monitored by TLC (1 ethyl acetate: 3 hexane) and stirred at room temperature for 18 hours. The resultant residue was washed with 10% $NaHCO_3$, and 5% HCl. Further purification was performed using column chromatography on neutralized silica gel using (ethyl acetate: hexane) as eluent to afford the products as white glistering powder [18].

Compound **IIa** yield is (80%). I.R: 3363, 3055, 2951, 2931, 2897, 2854, 1658, 1597, 1523. ^{1}H NMR (400 MHz, DMSO) δ 10.18 (s, 1H), 7.91 – 7.84 (m, 2H), 7.70 – 7.63 (m, 2H), 7.56 – 7.41 (m, 3H), 7.21 (d, *J* = 8.2 Hz, 2H), 4.60 (s, 2H), 0.82 (d, *J* = 5.7 Hz, 6H), 0.82 (s, 9H).

Compound **IIb** yield is (50%) .IR: 3363, 3055, 2952, 2927, 2893, 2864, 1658, 1597, 1523. ^{1}H NMR (400 MHz, DMSO) δ 10.10 (s, 1H), 7.84 – 7.77 (m, 2H), 7.69 – 7.62 (m, 2H), 7.50 – 7.43 (m, 2H), 7.20 (d, *J* = 8.3 Hz, 2H), 4.60 (s, 2H), 1.24 (s, 9H), 0.82 (d, *J* = 5.4 Hz, 1H), 0.82 (s, 6H).

General procedure for synthesize of compound III

To a 20 ml beaker, 1 mmol of compound **II** and 3 ml of dry methanol were added. At 0 C^o, 0.25 mmol of acetyl chloride (20 mg) was added, the reaction mixture is monitored by TLC (4 ethyl acetate in 10 hexane) for consumption of the starting material after 15-60 minutes. The solvent was evaporated, and the residue was quenched and neutralized by adding saturated sodium bicarbonate, solvent was removed. Silica column chromatography was performed using gradient eluent (hexane: ethyl acetate). Yield is 70-80% as white powder [19].

N-(4-(hydroxymethyl)phenyl)-4-methyl benzamide compound (**IIIa**), I.R : 3336, 3055, 2916, 2862, 1651, 1597, 1523.^{1}H NMR (400 MHz, DMSO) δ 10.15 (s, 1H), 7.88 (d, *J* = 7.8 Hz, 2H), 7.73 (d, *J* = 8.0 Hz, 2H), 7.31 (dd, *J* = 15.7, 7.8 Hz, 4H), 5.15 (d, *J* = 5.7 Hz, 1H), 4.47 (d, *J* = 5.5 Hz, 2H), 2.38 (s, 3H).

4-chloro-N-(4-(hydroxymethyl) phenyl) benzamide compound (**IIIb**), I.R: 3344, 3035, 2924, 2865, 1651, 1600, 1523, 825. ^{1}H NMR (400 MHz, DMSO) δ 10.26 (s, 1H), 7.98 (d, *J* = 7.5 Hz, 2H), 7.76 (d, *J* = 8.1 Hz, 2H), 7.63 – 7.55 (m, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 5.19 (t, *J* = 5.7 Hz, 1H), 4.49 (d, *J* = 5.7 Hz, 2H).

General synthesis of 3-hydroxy 5-methyl isoxazole (compound IV)

To a round bottom flask of 25 ml equipped with magnetic stirred, 4 mmol of Meldrum reagent (576 mg) was added to 10 ml of dichloromethane, cooled to -20 C^o using dry ice. Pyridine 8.2 mmol was added drop wise at -20 C^0, stirred for 15 minutes, followed by acetyl chloride 17.6 mmol using insulin syringe. The resulting mixture was stirred at 0 C^0 for 2 hours, then at room temperature for 6 hours. The reaction mixture monitored by TLC (ethyl acetate: hexane) for the consumption of the starting material. The organic layer was washed with 5% HCl twice, dried over $MgSO_4$, solvent was removed to get acyl Meldrum as a dark red needle shape powder that undergo further reactions without purification. Yield is 643 mg (87%) of crude.

The resultant acyl Meldrum 2 mmol (374 mg) was added into 15 ml of toluene in 100 ml round bottom flask, N,O-diboc was added to the mixture and heated to 65 C^0 for 8 hours. Monitored by TLC (5 ethyl acetate: 10 hexane), solvent was removed to get an oily mass which further purified using column chromatography (ethyl acetate: hexane as eluent) to yield is 378 mg (65%) of tert-butyl ((tert-butoxycarbonyl)oxy)(3-oxobutanoyl)carbamate. In the next step 450 mg (1.42 mmol) of tert-butyl ((tert-butoxycarbonyl)oxy)(3-oxobutanoyl)carbamate in 5 ml methanol, 11 ml concentrated HCl was added and heated to reflux for 3 hours, sodium bicarbonate added to adjust the PH between 2-3, solvent was removed to produce a light brown powder, purified with column chromatography (5 ethyl acetate: 95 hexane: 1 acetic acid), yield is 40 % of compound (**IV**) [20]. IR: 3159, 3012, 2939,1631, 1527. ^{1}H NMR (400 MHz, DMSO) δ 11.03 (s, 1H), 5.74 (s, 1H), 2.26 (s, 3H). 13C NMR (101 MHz, DMSO) δ 170.93, 170.17, 93.82, 12.87.

General procedure for the synthesis of final compounds (compound V)

A solution of Di-p-chlorobenzyl Azodicarboxylate (DCAD) (1.1 mmol) in 3 ml dichloromethane in ice bath under argon atmosphere using Schlenk flask of 25 ml was added dropwise to a solution of triphenylphospine (1.1 mmol), 3-

hyroxyisoxazole (1.1 mmol) and benzyl alcohol derivative 1 mmol (compound **IIIa** and compound **IIIb**), the mixture is stirred at 0 Co for 3 hours, then at room temperature for 12 hours. The solvent was removed to leave a gummy mass that was purified by column chromatography (1.8 ethyl acetate: 8.2 hexane) to get a white powder. Yield is 30%. [21-23].

N-(4-(((5-methylisoxazol-3-yl)oxy) methyl) phenyl)benzamide (compound **Va**), I.R: 3336, 2924, 2854, 1708, 1651, 1597, 1523, 1261. ^{1}H NMR (400 MHz, DMSO) δ 10.17 (s, 1H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.76 – 7.69 (m, 2H), 7.39 – 7.32 (m, 2H), 7.26 (d, *J* = 7.9 Hz, 2H), 5.93 (d, *J* = 1.0 Hz, 1H), 5.08 (s, 2H), 2.31 (s, 3H), 2.23 (s, 3H), 2.18 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 171.86, 171.16, 170.86, 170.21, 165.86, 142.13, 139.83, 132.40, 131.39, 129.42, 129.40, 128.19, 120.59, 120.26, 93.79, 93.48, 71.11, 39.33, 21.49, 12.98, 12.92.

4-chloro-N-(4-(((5-methylisoxazol-3-yl)oxy) methyl)phenyl)benzamide (compound **Vb**) I.R: 338, 3055, 2927, 2854, 1647, 1597, 1523, 1253, 898 cm^{-1}. ^{1}H NMR (400 MHz, DMSO) δ 10.99 (s,1H), 8.14 – 8.06 (m, 2H), 7.72 – 7.64 (m, 2H), 7.48 – 7.35 (m, 2H), 6.54 – 6.46 (m, 2H), 5.99 (m, 1H), 5.14 (d, *J* = 18.0 Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 172.66, 166.32, 162.04, 140.35, 132.49, 130.59, 129.92, 128.99, 126.63, 97.14, 40.58, 40.37, 40.17, 39.96, 39.75, 39.54, 39.33, 25.98, 13.19, -2.77.

Antiproliferative Activity Study

The MTT test is used to assess the cell survival through identifying the mitochondrial activity of viable cells, [24]. In this work, vorinostat, compounds **Va**, and **Vb** were chosen for cell line MTT assay with colon cancer cells. The stock for all compounds was 0.100 mg/1ml, with serial dilution at 50% for each (0.05, 0.025, 0.0125, 0.00625, 0.00313 mg/ml). 2 ml of the above dilutions was added to 198 ml of cell line and incubate for 24 hours. After the drug exposure period was complete (24 hours), the medium was removed from the wells and the cells washed with phosphate buffer saline PBS. To assess formazan residue, a blank control was used. To attain a concentration of 0.5 mg/ml, 1.2 ml MTT solution (5mg/ml) was added to 10.8 ml medium. After that, each well was filled with 200µl of the resulting solution. Plate was incubated at 37°C for 3 hours until purple intracellular formazan crystals were visible under an inverted microscope. After the supernatant was removed, 100 µl of DMSO was added to each well to dissolve the resultant formazan crystals. The plate was incubated for 30 minutes at room temperature until the cells lysed and the purple crystals dissolved. The percentage of cell viability or proliferation was calculated by dividing the absorbance; readings of test samples by those of the control samples and multiplying by 100 [24-26].

Results and Discussion

Docking Study, the proposed compounds of **Va** and **Vb** revealed docking score of -3.75, -4.35 kcal/mole respectively, while vorinostat showed a docking score of -6.16 kcal/mol. After a carful visual inspection of 2D ligand-receptor interaction, compounds **Va** and **Vb** impart an accepted receptor fitness through the formation of bidentate zinc chelation, and interaction with several residues inside the active site. Similar to vorinostat, the cap amide carbonyl group for **Va** and **Vb** revealed a virtual hydrogen bond formation with PHE208. Additionally, the availability of linker phenyl residue forming a descent π-π stacking with the side chain ofHIE180This interaction is not available for vorinostat due the absence of aromatic moieties in its linker group. Most interestingly, the isoxazole moiety in **Va** and **Vb** is chelating the zinc ion in a bidentate manner and forming π-π stacking with HIS 143; while vorinostat hydroxamate group chelating zinc ion and forming two hydrogen bonds with TYR306 and HIS143 residues (Figure 4).

The designed compounds showed an accepted active site fitness supported by filling the active site and bidentate interaction with zinc binding group. The replacing of promiscuous and metabolically unstable hydroxamate moiety with a heterocyclic isoxazole moiety might slightly reduce the virtual binding affinity into the HDAC8 enzyme. However, the descent enzyme fitting, in addition to a promising antiproliferative activity and metabolic profile for novel ZBG of isoxazole might open the avenue for developing biologically active HDAC inhibitors.

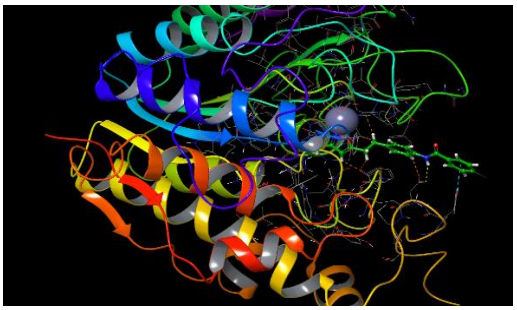
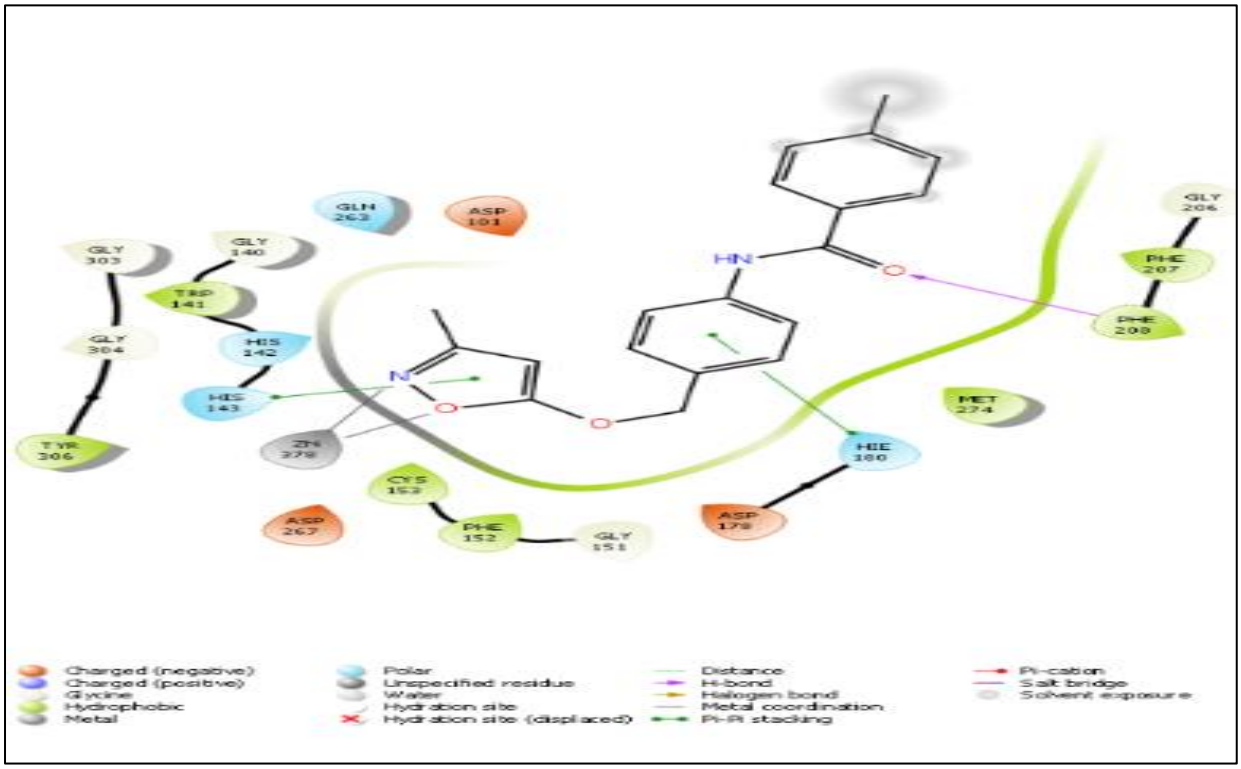
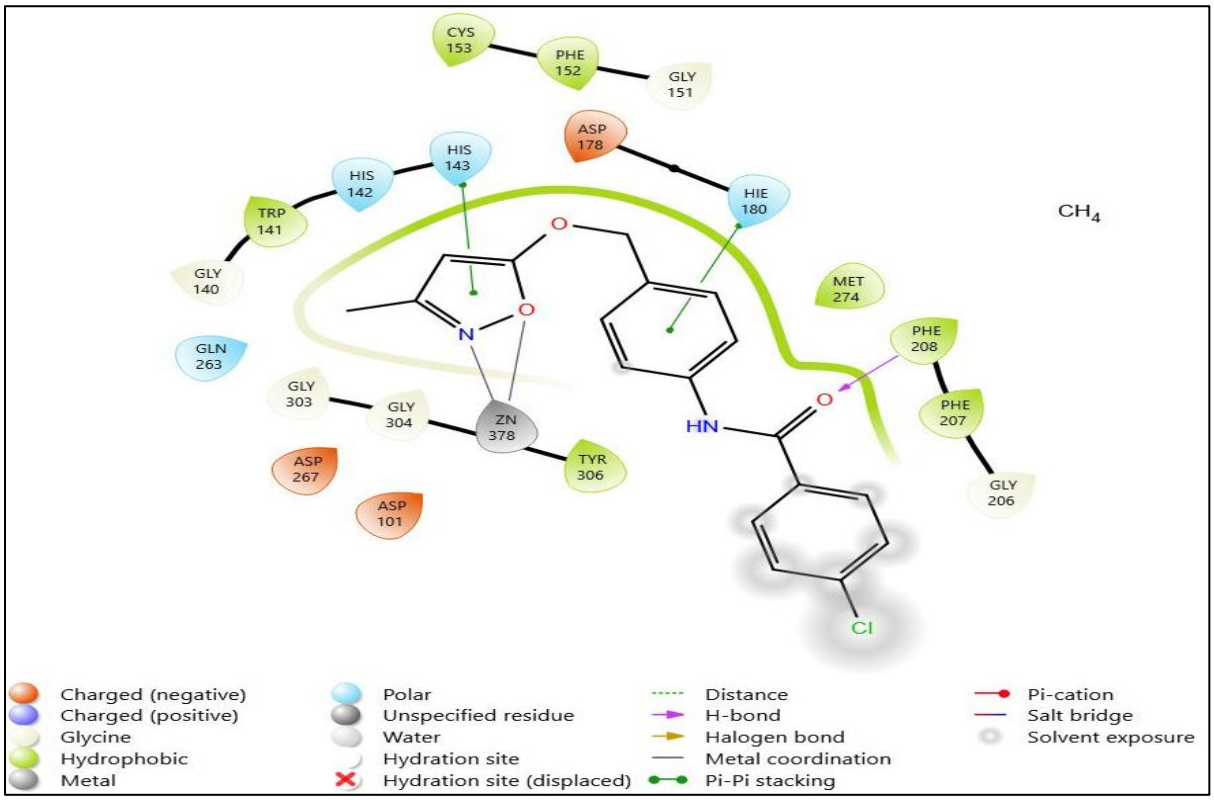


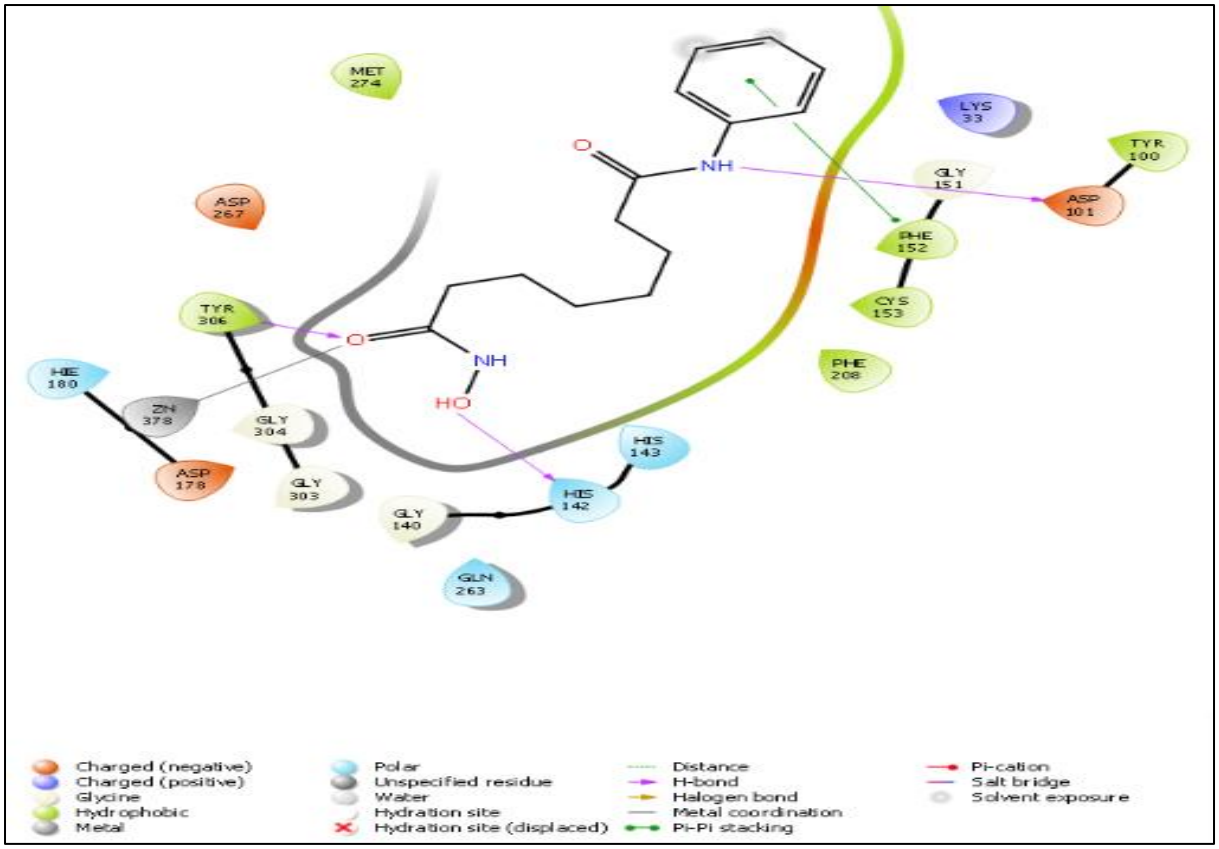
Figure 1. The 3D poses for the interaction of compound Vb with HDAC8


GLN 263
ASP 101
GLY 303
GLY 140
TRP 141
HIS 142
GLY 304
HIS 143
TYR 306
ZN 378
CYS 153
ASP 267
PHE 152
GLY 151
HIE 180
ASP 178
MET 274
PHE 208
PHE 207
GLY 206
HN
N
O
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

A


CYS 153
PHE 152
GLY 151
ASP 178
HIS 143
HIS 142
HIE 180
TRP 141
GLY 140
MET 274
PHE 208
GLN 263
GLY 303
GLY 304
ZN 378
PHE 207
HN
TYR 306
ASP 267
ASP 101
GLY 206
CH4
Cl
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

B



C

Figure 4. Two-Dimensional Interaction Diagram of (A) Va, (B) Vb, and (C) Vorinostat with HDAC8 isoform (1t69).

ADME-TOX Studies

Several structural features and properties should be considered for the designed molecules to considered as drug-like molecule, such as the rule of five and rule of three for orally administered drug also is a vital approach for avoiding expensive late preclinical trial and clinical trials frustration.

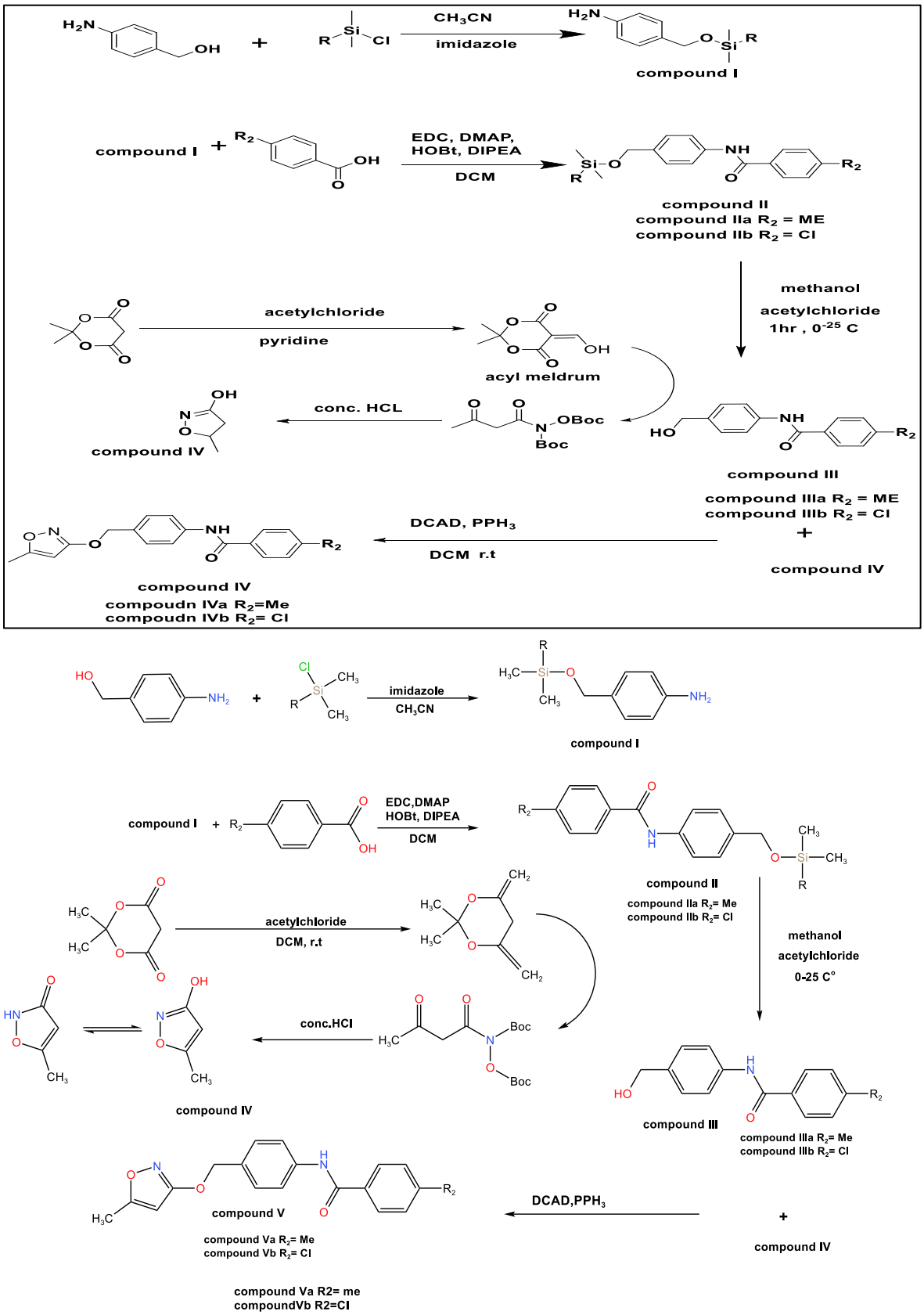
Compounds **Va** and **Vb** showed acceptable estimated pharmacokinetic properties. As the results indicated several hydrogen bonds tendency and metabolic stability. In addition, molecules having decent calculated oral absorptivity and no violation for drug-like molecules rules (Table 1). [27,28]

Table 1. The predicted ADMET data for the synthesized compounds

Compound	CNS	#metab	HumanOrallAbsorption	PercentHumanOralAbsorption	RuleOfFive	RuleOfThree
Va	0	3	3	100	0	0
Vorinostat	-2	3	3	67	0	0
Vb	0	4	3	90	0	0

Chemical Synthesis

Compound **I** was prepared by the reaction of 4-aminobenzylalcohol with equimolar tertiary butyl dimethyl silyl chloride (TBDMSCl) in presence of imidazole to selectively protect the hydroxyl group. Compound **I** underwent an amidation reaction with various acids in the presence of EDC.HCl, DMAP, HOBt, and DIPEA at room temperature followed by simple work-up and purification method to produce compound **II** in an excellent yield. Compound **III** was obtained by compound **II** silyl deprotection using 0.25 equivalent of acetyl chloride in dry methanol to produce benzylalcohol derivatives. The isoxazole moiety (compound **IV**) was prepared by the reaction of acyl Meldrum with N,O-diboc. The final compounds (compound **V**) were synthesized through the reaction of compound **IV** with isoxazole derivative of compound **IV** in presence of 1.2 equivalent of DCAD, 1.1 equivalent triphenylphosphine (pph_3) (Scheme 1).

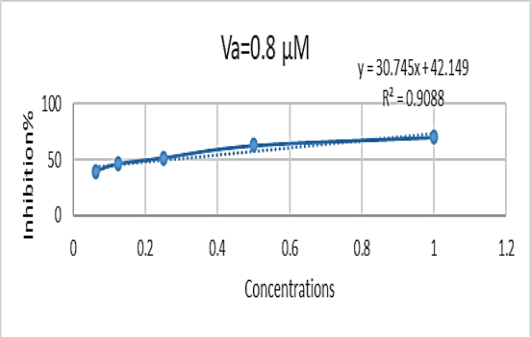


Scheme 1. Synthetic Pathway for Final Compounds

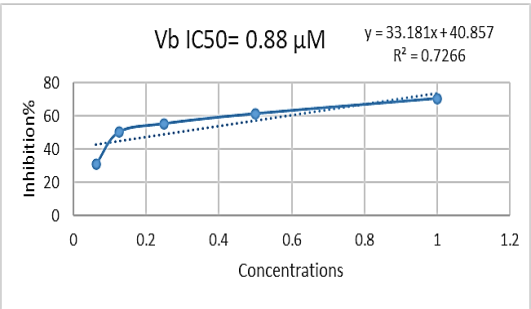
Antiproliferative Activity

The preliminary cancer cell growth inhibition assay (MTT assay) indicated that the synthesized compound **Va** and **Vb** showed a submicromolar inhibition activity with IC_{50} of 0.8 µM and 0.88 µM, respectively, that is comparable to vorinostat

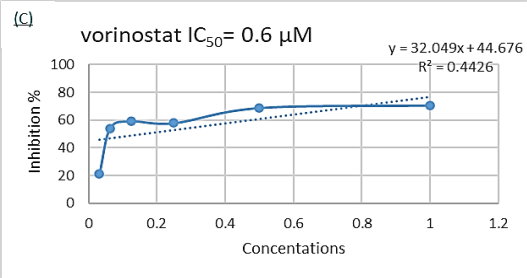
inhibition activity in IC_{50} of 0.6 µM in colon cancer cells (LS-174T) (Figure 5).



A



B



C

Figure 5. The IC_{50} for (A) Compound Va, (B) Compound Vb, (C) Vorinostat, in colon cancer cells (LS-174T).

Conclusion

Potential HDAC inhibitors were designed by the instillation of new zinc binding group of isoxazole. The zinc chelation tendency was virtually studied through the molecular docking studies using the licensed Glide software. The final compounds were virtually bound to HDAC8 at lower docking score; however, these molecules are chelating zinc ion in a bidentate manner through the isoxazole amine and oxygen moieties with accepted fitness. Final compounds showed accepted pharmacokinetic properties through the virtual ADMET studies. The designed compounds were successfully synthesized by applying the excellent mild organic synthesis methods with accepted yields. All the intermediates and final products were characterized by FTIR and NMR spectroscopy. The antiproliferative activity study indicated that the synthesized compounds exhibited a promising preliminary cancer cell inhibition which is comparable to the clinically used HDAC inhibitor of vorinostat.

Funding

Self-funding.

Ethics statement

We confirm as authors that our signing of this form is to guarantee that the submitted manuscript is in accordance with the ethical considerations and we have received the ethical approval from the related institution(s) and no animal or humans involved in this work.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Ayad Abed Ali. , Ali mohammed saeed; data collection: Ali mohammed saeed; analysis and interpretation of results: Ayad abed ali, Ali mohammed saeed. ; draft manuscript preparation: Ali mohammed saeed, draft editing and arrangemants: Ayad abed ali . All authors reviewed the results and approved the final version of the manuscript.

Conflict of Interest

The author declared no conflict of interest.

References

1. Jenke R, Reßing N, Hansen FK, Aigner A, Büch T. Anticancer Therapy with HDAC Inhibitors: Mechanism-Based Combination Strategies and Future Perspectives. MDPI [Internet]. 2020 Dec 30;13(4):634.
2. Cheng Y, He C, Wang M, Ma X, Mo F, Yang S, et al. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. Signal Transduct Target Ther. 2019 Oct 24;4(1):62.
3. Lu Y, Chan YT, Tan HY, Li S, Wang N, Feng Y. Epigenetic regulation in human cancer: the potential role of epi-drug in cancer therapy. Mol Cancer. 2020 Apr 27;19(1):79.
4. Alwash AH, Alani BG, Ibrahim IT. RECENT PROGRESS IN DEVELOPING SELECTIVE HISTONE. kerbala.jps. 2021 Apr 24;Pages 28-50.
5. Al-Amily D, Mohammed MH. Design, Synthesis and Cytotoxicity Study of Primary Amides as Histone Deacetylase Inhibitors. Iraqi J Pharm Sci. 2019;28(2) 2019:151–8.
6. Al-Hamashi AAAC. Design, Synthesis, and Biological Evaluation of Novel Histone Deacetylase Inhibitors as Anti-Cancer Agents. 2018.
7. Al-Hamashi AA, Abdulhadi SL, Ali RMH. Evaluation of Zinc Chelation Ability for Non-

Hydroxamic Organic Moieties. Egypt J Chem. 2023 May 1;66(5):215–21.
8. Al-Hamashi AA, Koranne R, Dlamini S, Alqahtani A, Karaj E, Rashid MS, et al. A new class of cytotoxic agents targets tubulin and disrupts microtubule dynamics. Bioorg Chem. 2021;116:105297.
9. Hasan Y, Al-Hamashi A. Identification of Selisistat Derivatives as SIRT1-3 Inhibitors by in Silico Virtual Screening. Turk Comput Theor Chem. 2023;8(2):1–11.
10. Friesner, Murphy RA;, Repasky RB;, Frye MP;, Greenwood LL;, Halgren JR;, et al. "Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes, J Med Chem,. 2006;49, 6177–6196(49, 6177–6196).
11. RCSB PDB - 1T69: Crystal Structure of human HDAC8 complexed with SAHA [Internet]. [cited 2022 May 13].
12. RCSB PDB: Homepage [Internet]. [cited 2022 Aug 10]. Available from: https://www.rcsb.org/
13. Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, et al. Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. J Med Chem. 2004;47(7):1750–9.
14. Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. J Comput Aid Mol Des. 2013;27(3):221–34.
15. Oleiwi MA, Zalzala MH. Synthesis, Molecular Docking Study and Cytotoxicity Evaluation of some Quinazolinone Derivatives as Nonclassical Antifolates and Potential Cytotoxic Agents. Iraqi J Pharm Sci. 2022;31(2):283–96.
16. Yadav R, Imran M, Dhamija P, Chaurasia DK, Handu S. Virtual screening, ADMET prediction and dynamics simulation of potential compounds targeting the main protease of SARS-CoV-2. J Biomol Struct Dyn. 2020;39(17):6617–32.
17. Bartoszewicz A, Kalek M, Nilsson J, Hiresova R, Stawinski J. A New Reagent System for Efficient Silylation of Alcohols: Silyl Chloride-N -Methylimidazole-Iodine. Synlett. 2008;2008(1):37–40.
18. Ghosh⇑ AK, Shahabi D. synthesis of amide derivative for electron deficient amines and functionalized carboxylic acid using EDC and DMAP and a catalyst amout of hobt as coupling reagent.pdf. Elsevier. 2019.
19. Khan AT, Mondal E. A Highly Efficient and Useful Synthetic Protocol for the Cleavage of tert-Butyldimethylsilyl (TBS) Ethers Using a Catalytic Amount of Acetyl Chloride in Dry Methanol. Cheminform. 2003;34(31).
20. Sørensen US, Falch E, Krogsgaard-Larsen P. A Novel Route to 5-Substituted 3-Isoxazolols. J org C. 1999;J. Org. Chem. 2000, 65, 1003-1007:1003–7.
21. Fletcher S. The Mitsunobu reaction in the 21 st century. Org Chem Front. 2015;2(6):739–52.
22. Lipshutz BH, Chung DW, Rich B, Corral R. Simplification of the Mitsunobu Reaction. Di-p-chlorobenzyl Azodicarboxylate: A New Azodicarboxylate. Org Lett. 2006;8(22):5069–72.
23. Chen L, Fletcher S. O-Alkylation of 3-hydroxyisoxazoles predominates under Mitsunobu conditions. Tetrahedron Lett. 2014;55(10):1693–6.
24. Hussien MS, Al-Hamashi AA. Phytosterol Profile in Iraqi Lactuca serriola after Purification and Isolation by Combiflash and HPLC (Conference Paper). Iraqi J Pharm Sci. 2023;31(Suppl.):54–61.
25. Mohammed ZY, Al-Jumaily EF, Yaseen NY. In Vitro Cytotoxic Study for Purified Resveratrol Extracted from Grape Skin Fruit Vitis vinifera. Iraqi J Pharm Sci. 2017;18(Suppl.):19–25.
26. Nadhum SA, Mohammed MH. Design, Synthesis, Characterization and Preliminary Anticancer Study for Methotrexate Silibinin Conjugates. Iraqi J Pharm Sci. 2017;24(1):74–84.
27. Jorgensen WL. The Many Roles of Computation in Drug Discovery. Science. 2004;303(5665):1813–8.
28. Kouassi1 KAR, Ganiyou2 A, Benié1 A, Koné1 MGR, Nobel1 N kouakou, Bohoussou1 KV, et al. Identification of Potential C-kit Protein Kinase Inhibitors Associated with Human Liver Cancer: Atom-based 3D-QSAR Modeling, Pharmacophores-based Virtual Screening and Molecular Docking Studies [Internet]. 2019 [cited 2022 Aug 22]. Available from: http://www.sciepub.com/reference/350880

Supplementary information

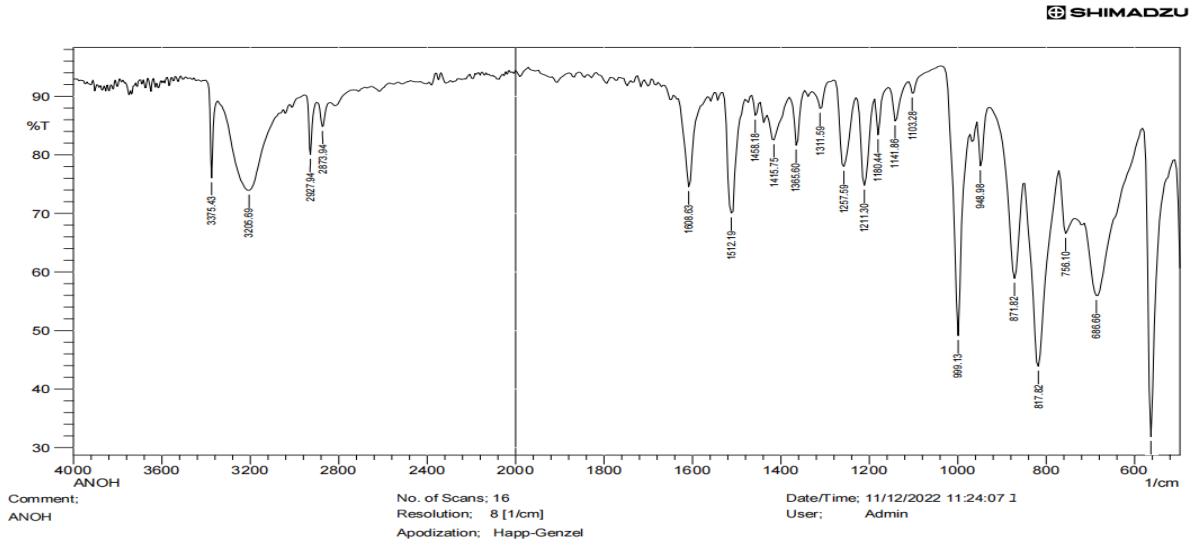


Figure 2. I.R spectrum of 4-aminobenzyl alcohol.

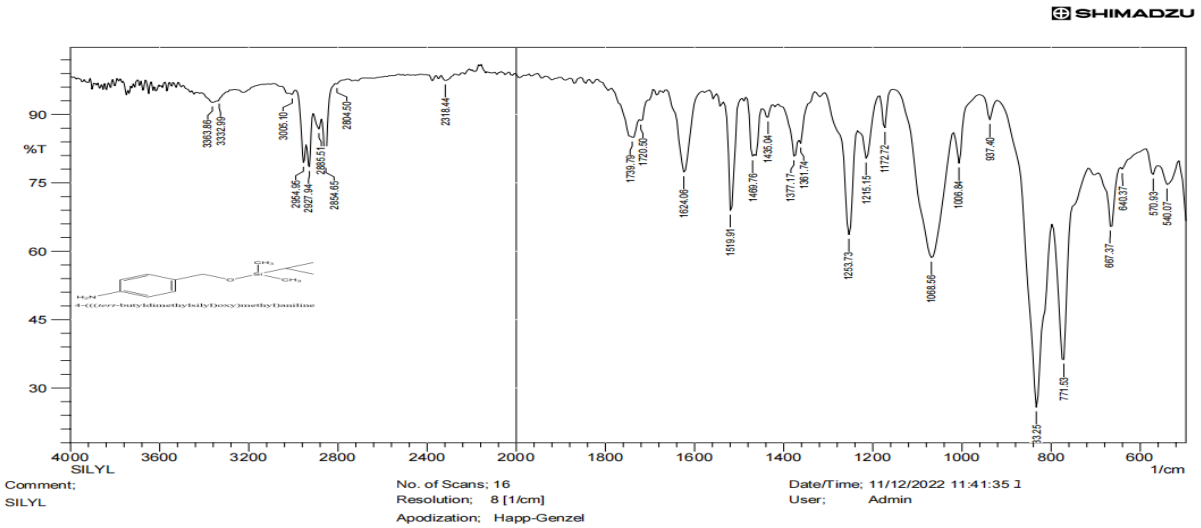


Figure 3. I.R spectra of compound I.

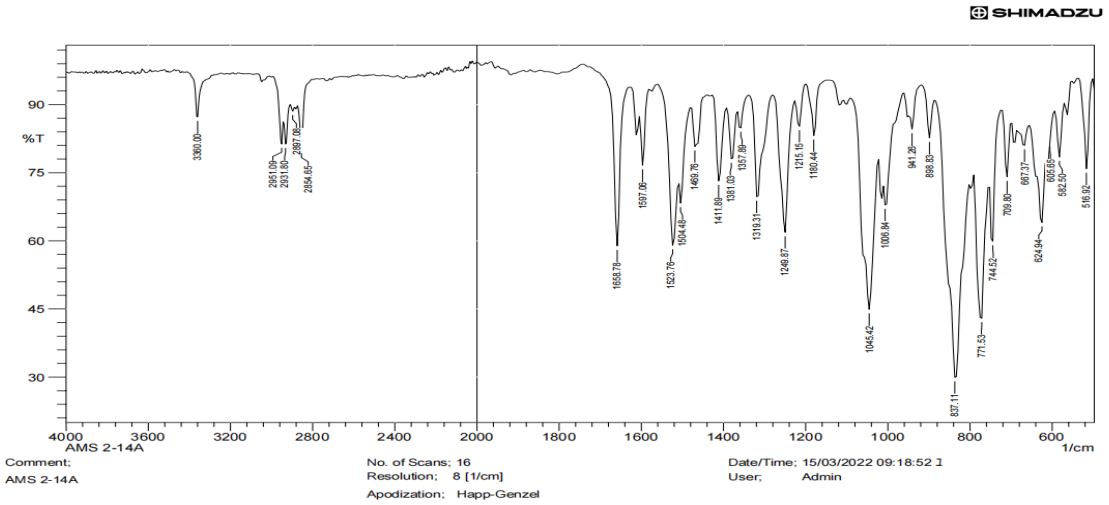


Figure 4. I.R spectrum of compoundIIa.

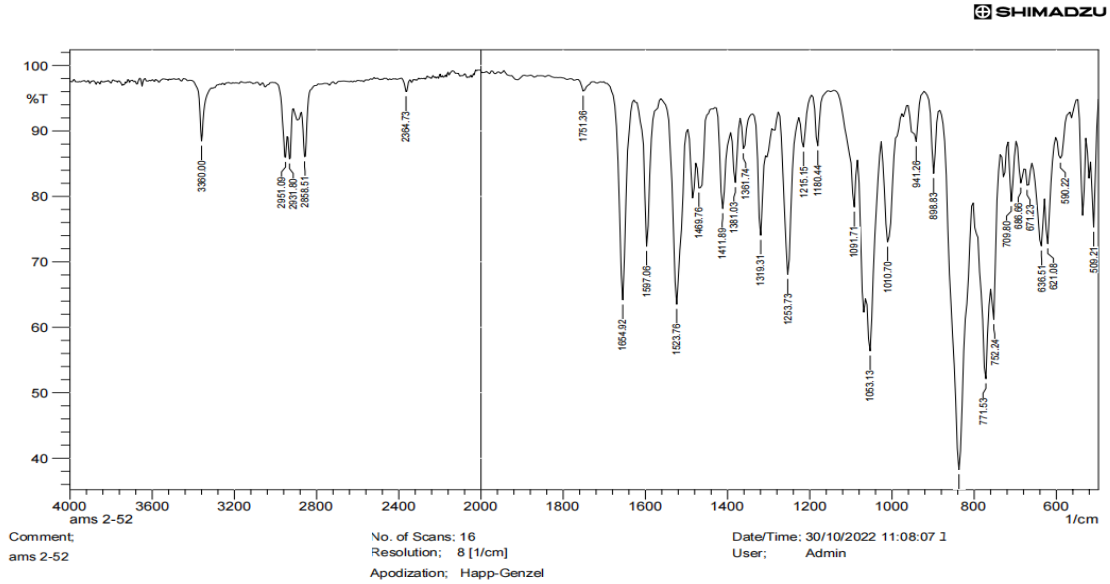


Figure 5. IR spectra of compound IIb.

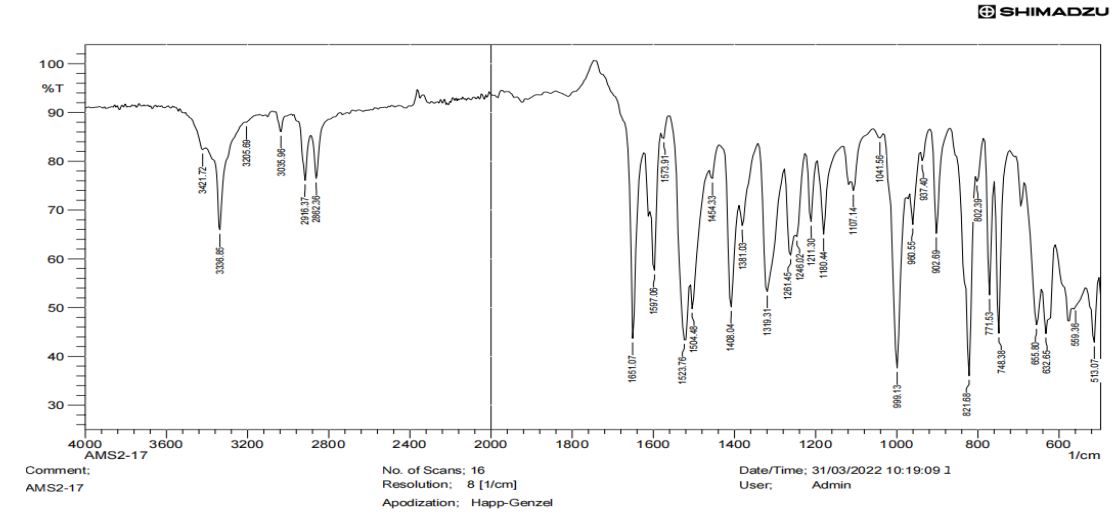


Figure 6. I.R spectrum of compound IIIa.

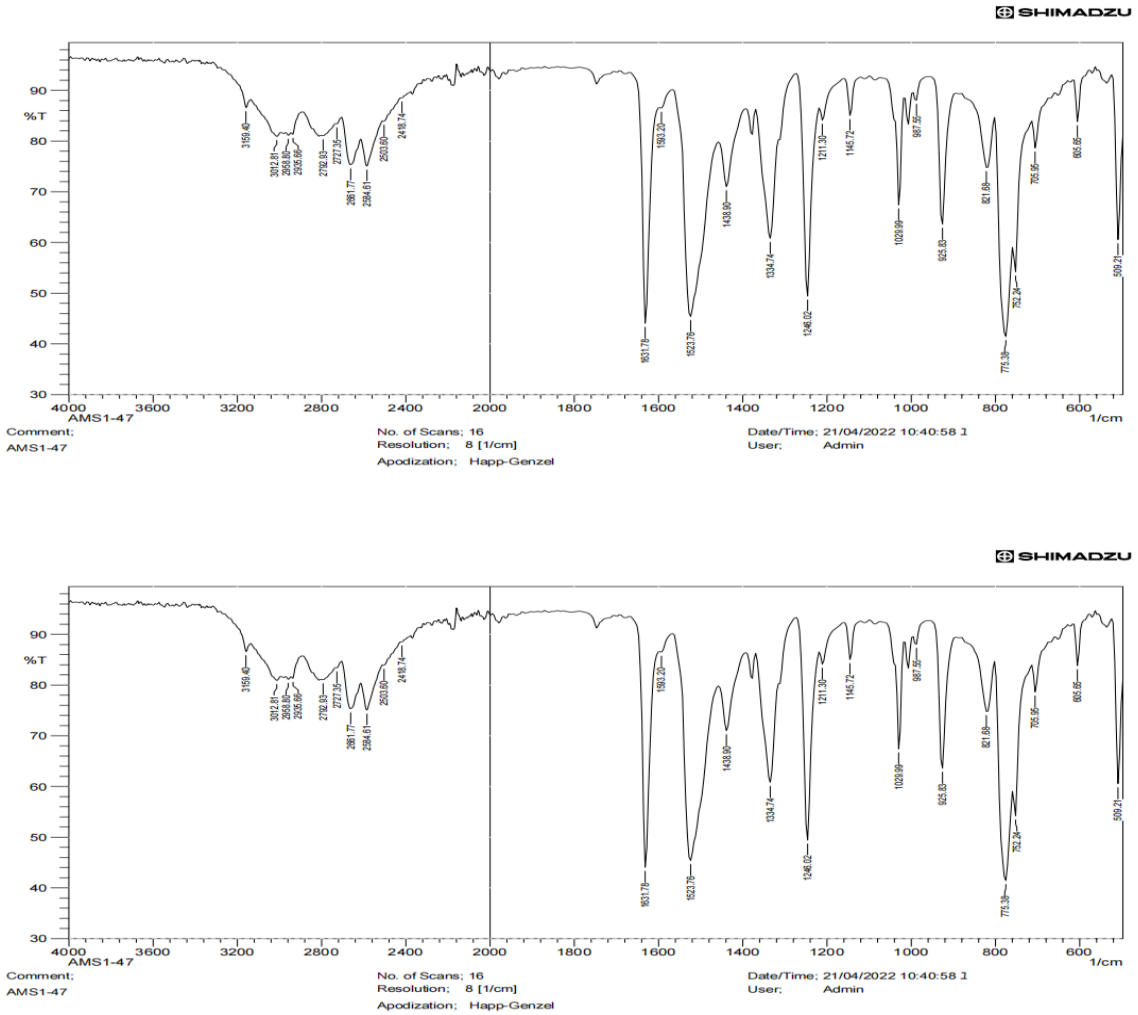


Figure 7. I.R Spectra of 5-methyl, 3-hyroxyisoxazole.

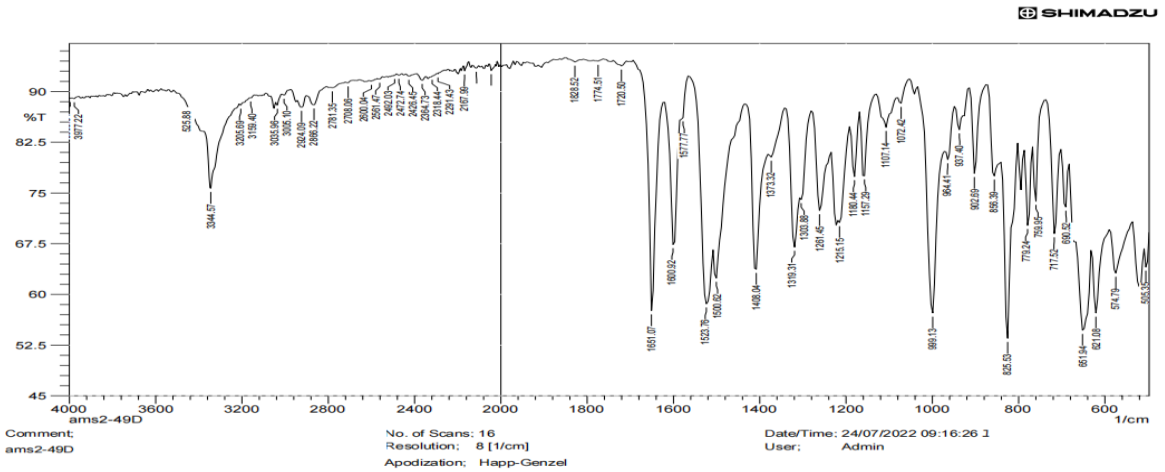


Figure 8. I.R spectrum of compound IIIb.

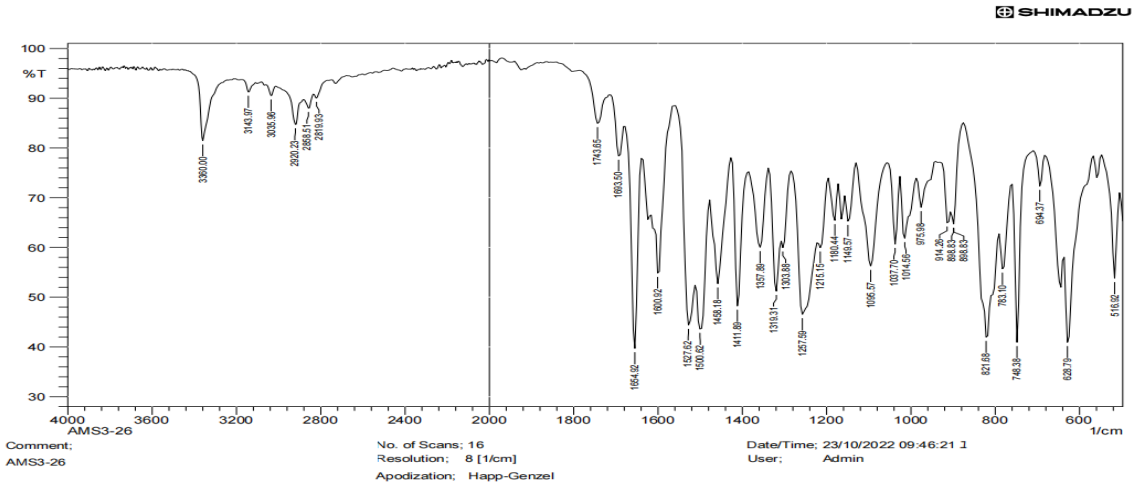


Figure 9. IR spectra of compound Va.

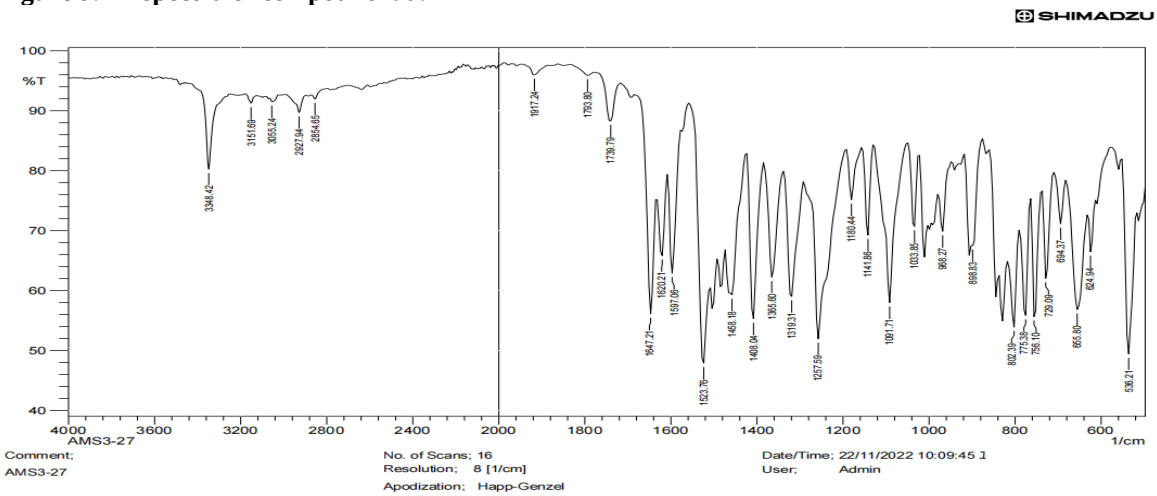


Figure 10. I.R spectrum of compound Vb.

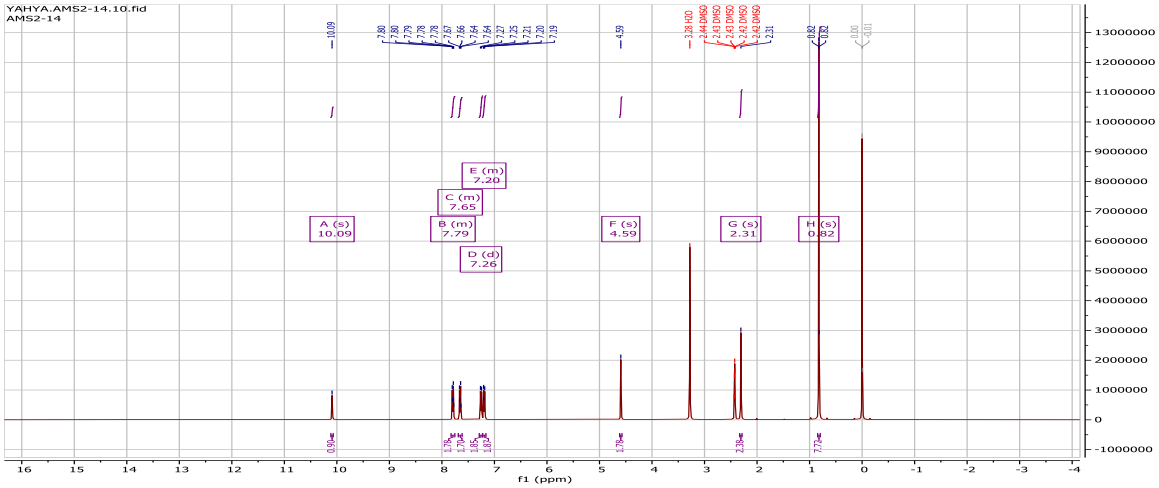


Figure 11. [1]HNMR spectra of compound IIa.

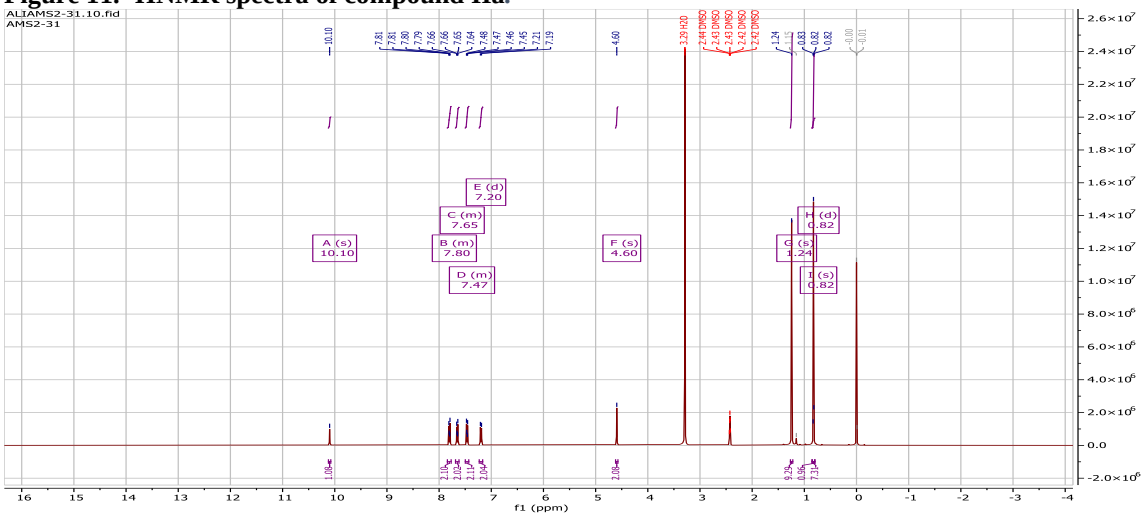


Figure 12.1HNMR spectrum of compound IIb

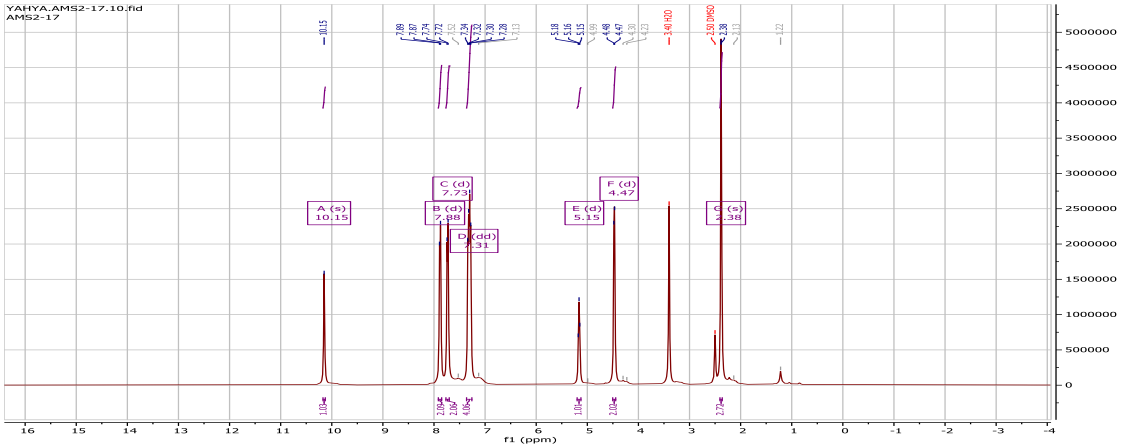


Figure 13. 1HNMR spectrum of compound IIIa.

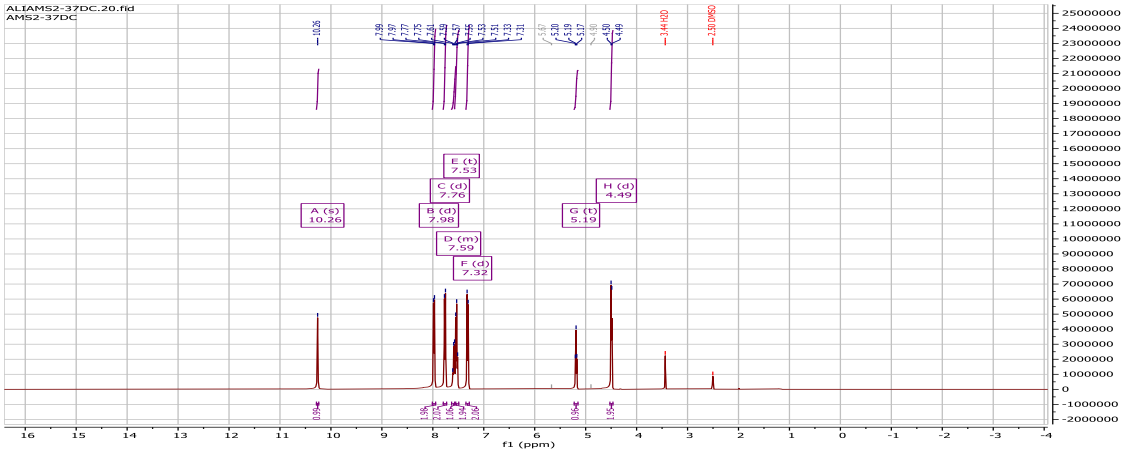


Figure 14.1HNMR spectrum of compound IIIb

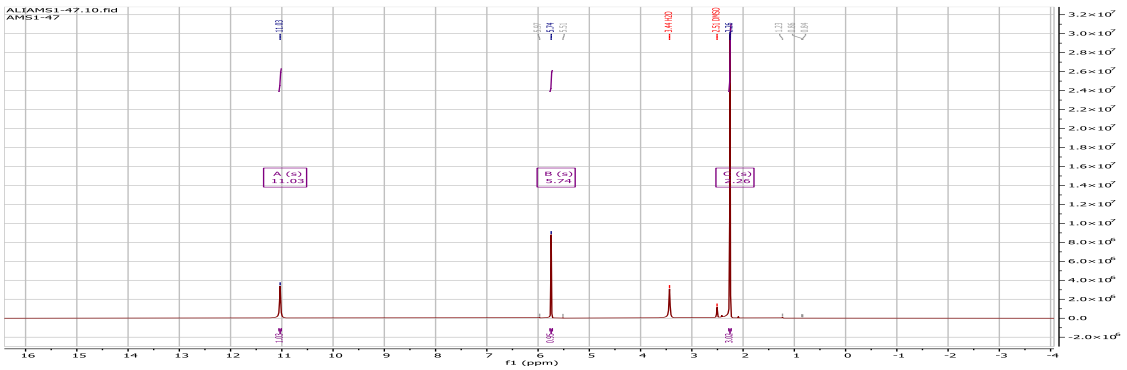


Figure 15. 1HNMR spectrum of compound IV

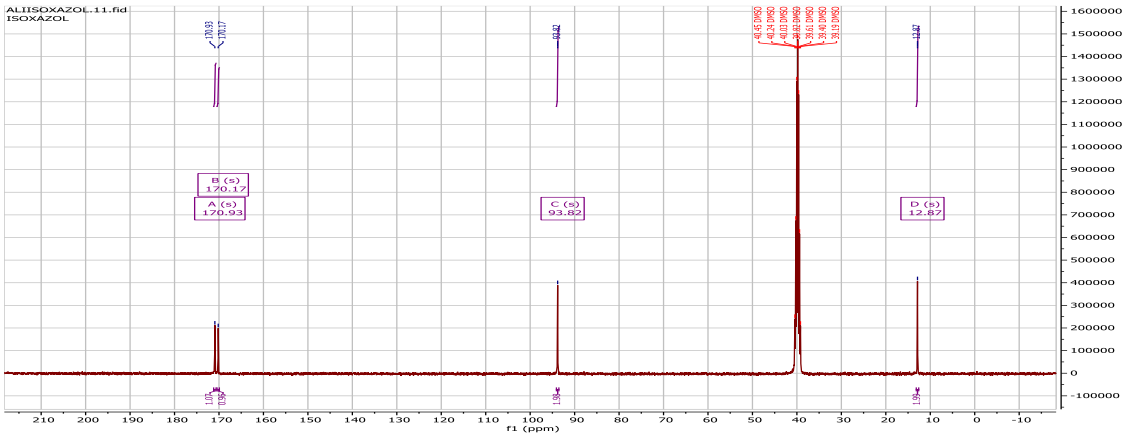


Figure 16 C^{13} NMR of compound IV

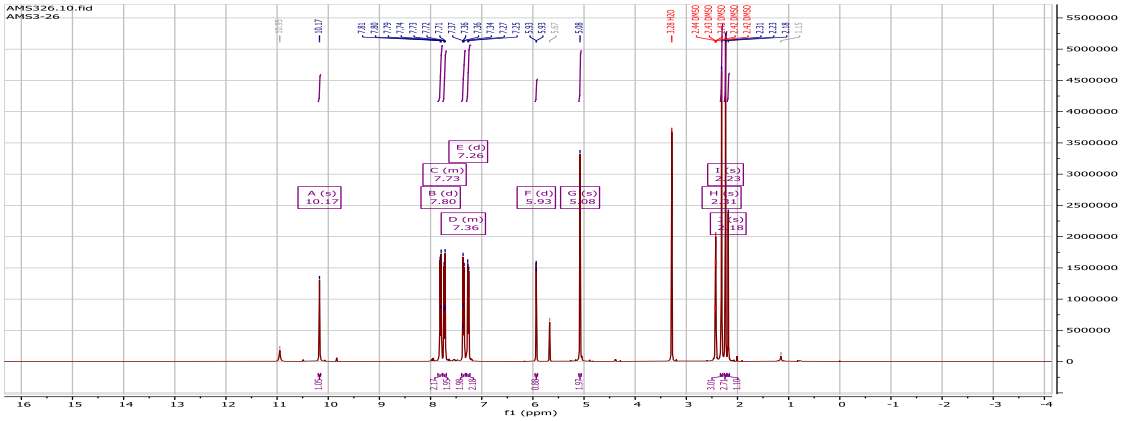


Figure 17. 1HNMR spectrum of compound Va

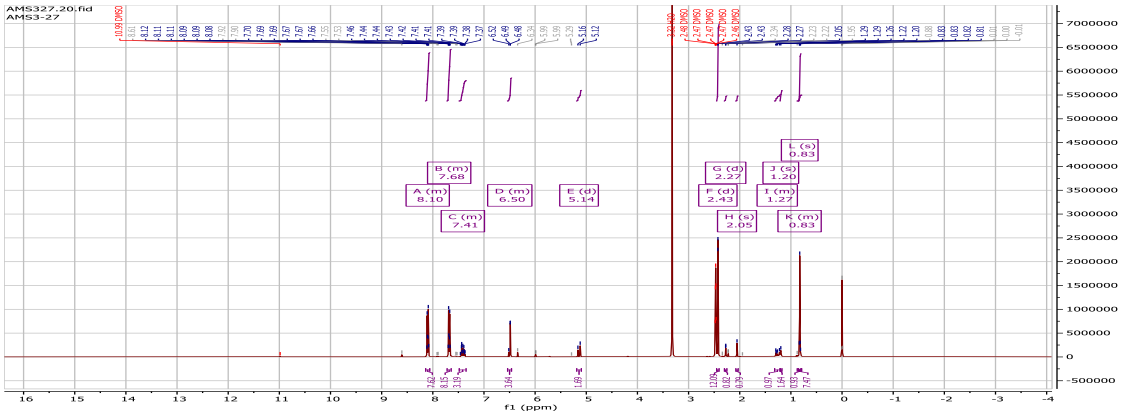


Figure 18.1HNMR spectrum of compound Vb

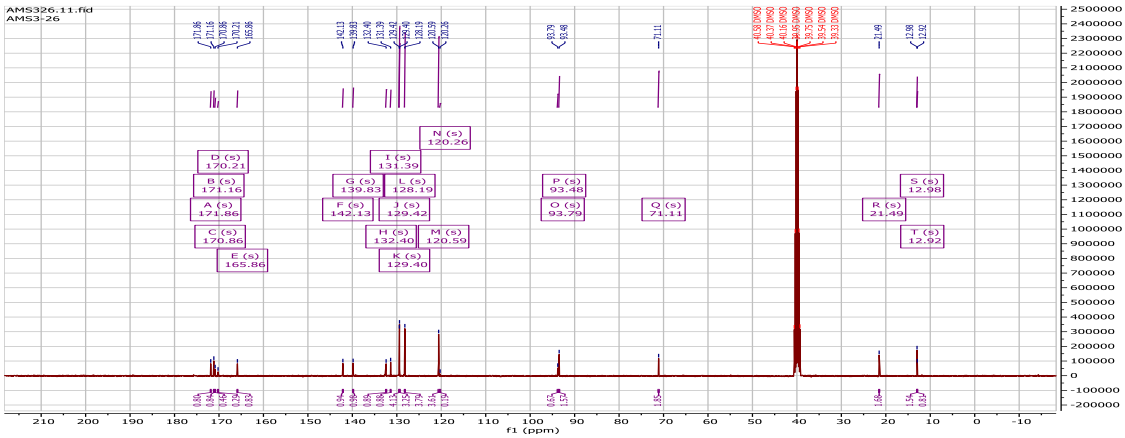


Figure 19. C[13]NMR spectra of compound Va