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Synthesis, Absorption, Distribution, Metabolism, Excretion, Toxicology (ADMET) and molecular docking studies of some pyridin-2(1H)-one derived from a Apocynin in Thi-Qar Governorate

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***Abstract*— The present study includes synthesized of some pyridin-2(1H)- one of 1-(4-hydroxy-3-methoxyphenyl) ethan-1-one. The [6-(4-hydroxy-3-methoxyphenyl) - 4 - (4-methoxyphenyl) - 2 - oxo-1,2-dihydropyridine - 3-carbonitrile, 4 - (4-bromophenyl) - 6 -(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2- dihydropyridine - 3- carbonitrile, 4 - (4-bromophenyl) – 6 - (4-hydroxy-3-methoxyphenyl)-2-oxo-1, 2 - dihydropyridine - 3-carbonitrile] derivatives have been synthesized by cyclization reaction of the 1-(4-hydroxy-3-methoxyphenyl)ethan-1-one with various aldehydes (4-methoxy benzaldehyde, 4-bromo benzaldehyde ,4-dimethyamino benzaldehyde) respectively and ethyl cyanoacetate in the presence of ammonium acetate. The structures of the prepared compounds were confirmed by the different available spectroscopic methods, such as FTIR, ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. The physical properties were assessed. The antioxidant activity of the synthesized compounds was evaluated by the use of 2,2-diphenyl-1-picrylhydrazyl. The compounds 4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile,and4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile, showed the highest activity as antioxidants (79.05, 67.28)%, which can be compared with ascorbic acid 82.71%, while the antioxidant activity of compound 6-(4-hydroxy-3-methoxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile, showed less effectiveness for the antioxidant 17.55% at a concentration of 12 ppm. Also, the prepared compounds were assessed for in vitro biological activity against the two types of bacteria [*staphylococcus aureus*, *Escherichia coli*] respectively which displayed moderate inhibition. In an attempt to understand the ligand–protein interactions in terms of the binding affinity, docking studies were performed using Py-Rx and BIOVIA\Discovery Studio 2021 for the compounds. The binding affinities calculated were in agreement with the minimum inhibitory concentration [MIC] values.**

***Keyword*—Pyridin-2-(1H)-one, Antioxidants activity, Molecular docking, Biological activity**

I. INTRODUCTION

The development of new routes to substituted pyridines with a wide variety of substituents continues to attract considerable attention for application in heterocyclic chemistry Substituted pyridines are a common type of N-heterocycle found in natural products, bioactive compounds, functional materials, and medicines [1]. Compounds have been associated with antitumor, antifungal, antibacterial, antiviral, antithrombotic, psychotherapeutic and anti-HIV properties [2, 5]. The 2-pyridone derivatives are heterocyclic compounds with vital substructures of many naturally compounds and has a wide spread applications. Some synthetic 2-pyridone intermediates and its metabolites demonstrate a broad spectrum in biological applications [6, 8] Naturally 2-pyridone derivatives like Rici nine [9] remarkable as CNS stimulant activities and the analogs albamycin [10, 12], Some authors consider that biological activity of 3-cyanopyridine-2(1 H)-ones depends on the substituent at a nitrogen atom of the pyridinone ring [12]. Some pyridinone derivatives containing aromatic substituents have valuable properties and display a variety of biological activities [13]. In our previous report, we described the synthesis of some substituted 3-cyanopyridine-2(1 *H*)-ones with various aliphatic substituents at positions 1 and 4 of the pyridinone ring [14]. Taking this into account, we report herein a new and simple path for synthesizing *N* -substituted 3-cyanopyridine-2(1 *H*)-ones with aromatic substituents. Recent studies showed that PDE3, PDE4, and PDE5 are overexpressed in cancerous cells compared with in normal cells. In addition, inhibition of tumor cell growth and

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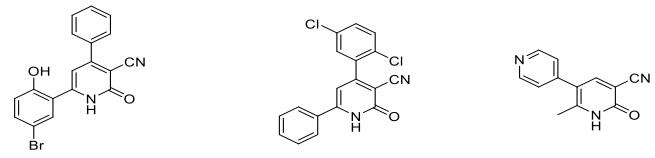
angiogenesis may be due to cross inhibition of PDE3 together with other PDEs [15]. Sulfonyl biscompounds carrying 2-pyridone moiety exhibited a good anticancer activity against human breast cell line (MCF7) [16]. The creation of novel 2-pyridone compounds with minimal toxicity can be used as antibacterial [17] and anti-inflammatory medicines [18]. As part of our ongoing efforts [19,20].



a- PIM-1 Kinase inhibition b- Survivin inhibition c- PDE3 inhibition

Fig1. Various 3-cyano-2-oxopyridine derivatives with potential growth inhibitory and/or antiangiogenic actions through PIM-1 kinase inhibition (a), survivin inhibition (b) or PDE3 inhibition (c).

II. MATERIALS AND METHODS

A. Material

The chemicals used are 4-methoxy 3-hydroxy acetophenone (%98), ethanol solvent (%99), 4-methoxy-3-hydroxyacetophenone, 4-methoxy benzaldehyde (%99), 4-bromo benzaldehyde (%98) and 4-dimethyl amino benzaldehyde (%98) from Sigma Aldrich, ethyl cyanoacetate (%99.8) and ammonium acetate(%98) was obtained from Alpha Chemika.

Characterization.

Melting points of the solid chemicals were determined in an open capillary tube by using SMP3 and were uncorrected. Reactions performed in the present study were followed by thin layer chromatography (TLC) using eluent (ethyl acetate: hexane) 1:1 and iodine vapor for the visualization of formed TLC spots. The synthesized compounds were characterized by the technology of FTIR spectra using a K-Br disc on Perkin Elmer, tenser 27 (Bruker). In the characterization,^{1}H-NMR and^{13}C-NMR spectra were used; they were listed on a Bruker– DRX system AL 400 MHz spectrometer with internal standard TMS in Basra.

SYNTHESIS OF DERIVATIVES T_1-T_3

Mixture of ethyl cyanoacetate (0.01 mol, 1.1gm), 1-(4-hydroxy-3-methoxyphenyl)ethan-1-one (0.0l mol ,1.66gm), and ammonium acetate (0.08 mol, 6.16gm) and 0.01 mol, 1.36, 1.85,1.49 gm) of the (4-methoxy benzaldehyde, 4-bromo benzaldehyde and 4-dimethyl amino benzaldehyde) respectively in 50ml ethanol ,then the mixture was heated under reflux for 48 hr. The progress of the reaction was monitored by TLC (hexane: ethyl acetate 1:1. After the completion of the reaction, the formed precipitated solid materials were filtered and then washed with water, recrystallized from ethanol solvent [23]. The physical properties of the synthesized compound are shown in Table 1.

6-(4-HYDROXY-3-METHOXYPHENYL)-4-(4-METHOXYPHENYL)-2-OXO-1,2-DIHYDROPYRIDINE-3- CARBONITRILE (T_1)

FTIR (vmax cm−1, KBr): 3369 (O-H Phenol), 3137 (N-H) , 3068,3051 (C-H aromatic), 2959 2933, 2830 (C-H aliphatic) , 2229 (C≡N), 1665(C=O) 1579, 1517, (C=C aromatic) 1292 ,1073 (C-O) 1466, 1453, 1430, 1399 (C-C aliphatic) 1218 (C-N bending out plane) 774 (N-H bending out plane) 983 ,964, 822 (C-H bending out plane) Fig2. 1H NMR (400 MHz, DMSO-d6) δ 12.5 ((s, 1H , N-H tautomer)), 9.8(s., 1H, O-H Phenol), 7.7 (d, J = 8.3 Hz, 2H , aromatic proton), 7.4(s, 1H, aromatic proton), 7.4 (d, J = 8.4 Hz, 1H, aromatic proton), 7.1 (d, J = 8.3 Hz, 2H ,aromatic proton), 6.8 (d, J = 8.3 Hz, 1H ,aromatic proton), 6.7 (s, 1H , CH=C-), 3.88 (s, 3H,-OCH3),3.84(s, 3H ,-OCH3). Fig3. 13C-NMR (101 MHz, DMSO-d6) δ 162.22 (C=O), 160.99 , 159.28 (C-O-CH3), 149.83, 147.73 (C-O-CH3,=C-OH), 129.97, 128.30, 122.75, 121.33(aromatic carbons)117.17(C≡N), 115.68, 114.15, 111.30(aromatic carbons) 95.40 (CH=C), 55.80(CH3-O aliphatic), 55.43 (CH3-O aliphatic) Fig4.M.WT, m/z: 348.2(100.0%),334,11 (21.6%).Fig5.

4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (T2):

FTIR (ν_{max} cm^{-1}, KBr): 3377 (O-H Phenol) 3140 (N-H) , 3010 (C-H aromatic), 2959,2939 (C-H aliphatic) , 2230 (C≡ *N*) , 1656 ((C=O), 1607(CH=C), 1580, 1515, (C=C aromatic) 1295 ,1070 (C-O) 1434,1392 (C-C aliphatic) 1212 (C-N bending out plane) 805 (N-H bending out plane) 982 ,960 (C-H bending out plane) Fig6. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.65 ((s, 1H , N-H tautomer)), 9.87(S, 1H, O-H Phenol), 7.77 (d, *J* = 8.1 Hz, 2H , aromatic proton), 7.66 (d, 2H, aromatic proton), 7.48 (s, *J* = 8.4 Hz, 1H, aromatic proton), 7.42 (d, *J* = 8.0 Hz, 1H ,aromatic proton), 6.88 (d, *J* = 8.3 Hz, 1H ,aromatic proton), 6.80 (s, 1H , CH=C-), 3.87 (s, 3H,-OCH_3) Fig7. ^{13}C-NMR (101 MHz, DMSO-d_6) δ 161.94 (C=O), 161.94 , 158.60 (C-O-CH3), 150.01, 147.75 (C-O-CH_3,=C-OH), 131.72, 130.34, 123.91, 121.48(aromatic carbons)116.66(C≡ *N*), 115.69, 114.15, 111.34(aromatic carbons), 104 (CH=C), 55.81(CH_3-O aliphatic) Fig8.M.wt, m/z: 396.01(100.0%), 398.01(97.3%) Fig9.

4-(4-(dimethylamino)phenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile(T_3):

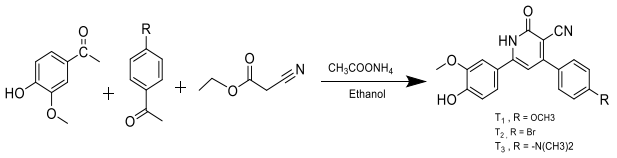
FTIR (ν_{max} cm^{-1}, KBr): 3365 (O-H Phenol) 3138 (N-H) , 3090-3067 (C-H aromatic), 2986-2932 (C-H aliphatic) , 2210 (C≡ *N*) , 1657 (N-H bending) 1573, 1529, (C=C aromatic) 1297 ,1088 (C-O) 1463,1384 (C-C aliphatic) 1227 (C-N bending out plane) 803 (N-H bending out plane) 997 ,966 (C-H bending out plane) Fig10. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 ((s, 1H , N-H tautomer)), 9.77(S, 1H, O-H Phenol), 7.95 (d, *J* = 90 Hz, 2H , aromatic proton), 7.65 (d, 2H, aromatic proton), 7.38 (s, *J* = 8.9 Hz, 1H, aromatic proton), 6.83 (d, *J* = 9.0 Hz, 2H ,aromatic proton), 6.70 (d, *J* = 8.3 Hz, 1H 159.76ppm (C-O-CH3),,aromatic proton), 3.88 (s, 3H,-OCH_3), 3.01 (s, 6H ,CH_3). Fig11. ^{13}C NMR (101 MHz, DMSO) δ 163.45ppm (HN-C=O, 154.15ppm (C-O-CH3), 153.70ppm ((CH=C), 151.70, 149.63, 147.68, 133.78, 121….111,12ppm(aromatic carbons, 115.65ppm(C ≡ *N*), 116.14, 114.61, 111.76 (aromatic carbons), 91.98 (CH=C aliphatic proton), 61.42ppm (CH_3-O aliphatic), 55.77ppm (CH_3-N aliphatic) Fig12. M.wt, m/z: 361.14(100.0%), 362.15(22.7%) Fig13.

Table1: physical properties of the synthesized compounds T_1-T_3

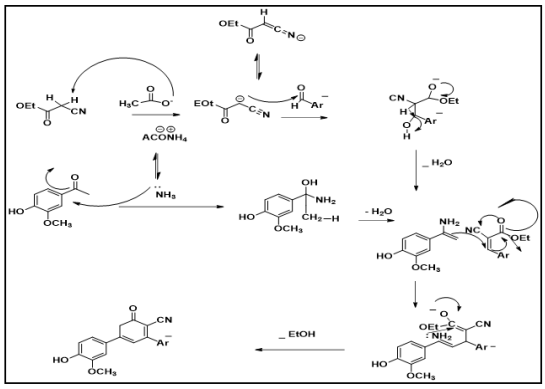
Compound no	Molecular formula	Color	M.wt g/mol	m.p °C
T_1	$C_{20}H_{16}N_2O_4$	Yellow	348.36	285-288
T_2	$C_{19}H_{13}BrN_2O_4$	Yellow	397.23	210-212
T_3	$C_{12}H_{19}N_3O_3$	Orange	361.40	273-275

III. RESULTS AND DISCUSSION

In the present study, three derivatives of 4-hydroxy-3-methoxy acetophenone T_1-T_3 were synthesized through the cyclization reaction of the 4-hydroxy-3-methoxy acetophenone,(4-methoxy benzaldehyde, 4-bromo benzaldehyde and 4-dimethyl amino benzaldehyde) respectively and ethyl cyanoacetate and ammonium acetate as catalyst in ethanol solvent. As shown in the following Scheme1.



Scheme1. Synthesis of 1-(4-hydroxy-3-methoxyphenyl) ethan-1-one derivatives $T_1 - T_3$



Mechanism of reaction

The synthesized compounds have been characterization by the determination physical properties as shown in table1 and the spectroscopy methods such as FTIR, ^{1}H-NMR, ^{13}C-NMR. The FTIR spectra of compounds T_1-T_3 showed absorption bands at 3369 , 3375, 3365 cm^{-1} respectively due to the stretching vibration of OH group and showed absorption bands 3137, 3140, 3138 cm^{-1} due to the stretching vibration of the N-H also a strong band at 1665,1665, 1657 cm^{-1} respectively, which is illustrated to stretching vibration of carbonyl group of the heterocyclic six member ring the appearance of bands at 2229 , 2230, 2210 cm^{-1}refer to the C≡N. The results of the FTIR spectra displayed that compounds have the anther bands at 3068,3010, 3067 cm^{-1}regions corresponding to stretch C-H aromatic, the appearance of bands at 2959 , 2939, 2986 cm^{-1}refer to the aliphatic C-H . Absorption bands at 1517 ,1515, 1529 respectively belonging to the C=C aromatic .On the other hand, [24]. the band at 1466, 1468, 1463 cm^{-1} mentioned to stretching vibration of the aliphatic C-H band, the stretching vibration of C-O band occurs at 1292, 1295, 1297 $cm^{-1}$of the compound T_1.T_3 .

The ^{1}H-NMR analysis show the important signal at of compound T_1 12.51 ppm due to N-H tautomer, and the appearance of signal at 9.82 ppm due to O-H phenol and two signal singlet at 3.88 , 3.84 ppm refer to two methoxy groups. The ^{13}C-NMR data gives signals at 162.68 ppm refer to carbon of the carbonyl group , the important signal at 95.86ppm exhibited to the -CH=C ,the signal at 56.26 ppm due to -O-CH_3 ,the signal at 55.89 ppm due to (-O-CH_3).

The ^{1}H-NMR analysis show the important signal of compound T_2 at 12.65 ppm due to N-H tautomer, and the appearance of signal at 9.87ppm due to O-H phenol and signal singlet at 3.87ppm methoxy group. The ^{13}C-NMR data gives signals at 161.94 ppm refer to carbon of the carbonyl group , the important signal at 104ppm exhibited to the -CH=C ,the signal at 55.81 ppm due to -O-CH_3.

The ^{1}H-NMR analysis show the important signal of compound T_3 at 12.30 ppm due to N-H tautomer, and the appearance of signal at 9.77 ppm due to O-H phenol and signal singlet at 3.88 ppm refer methoxy group and signal singlet at 3.01ppm dute to (N-CH_3). The ^{13}C-NMR data gives signals at 163.45 ppm refer to carbon of the carbonyl group , the important signal at 91.98ppm exhibited to the -CH=C ,the signal at 61.42 ppm due to -O-CH_3 ,the signal at 55.77 ppm due to (N-CH_3) [25].

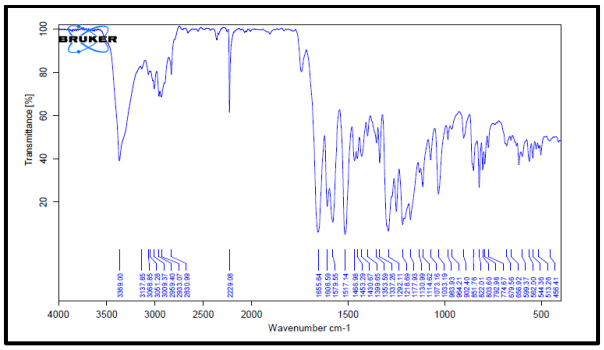


Fig 2: FT-IR spectrum of compound 6-(4-hydroxy-3-methoxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

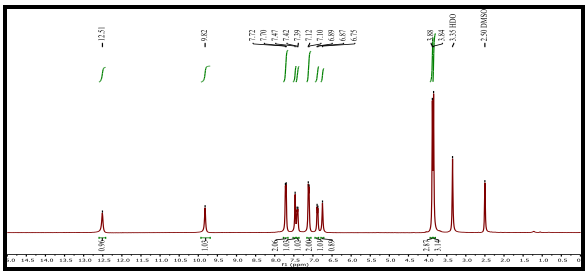


Fig 3: ^{1}H-NMR spectrum of compound 6-(4-hydroxy-3-methoxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile.

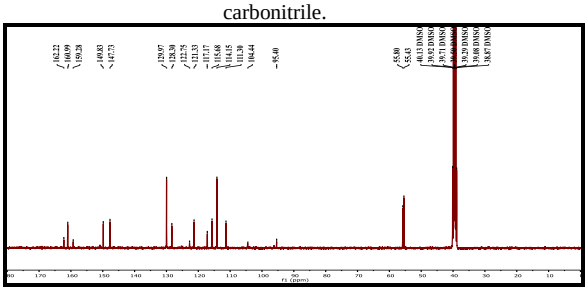


Fig 4: ^{13}C-NMR spectrum of compound 6-(4-hydroxy-3-methoxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

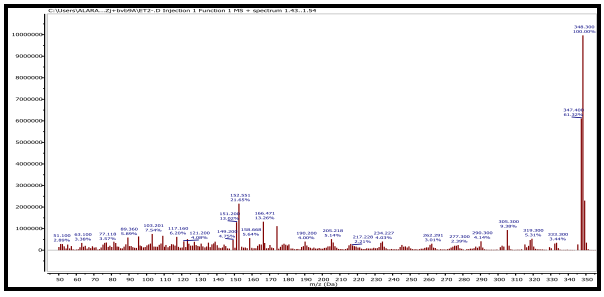


Fig 5: mass spectrum of6-(4-hydroxy-3-methoxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

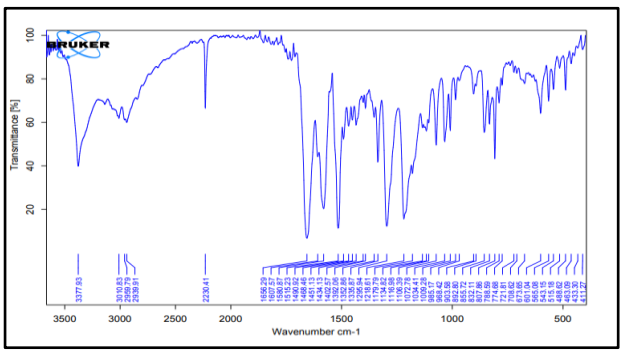


Fig6: FT-IR spectrum of compound 4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

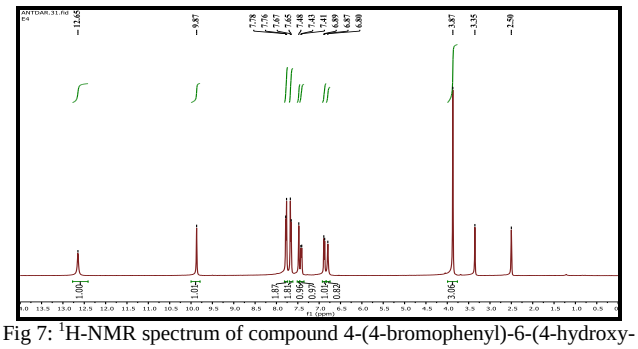


Fig 7: ^{1}H-NMR spectrum of compound 4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

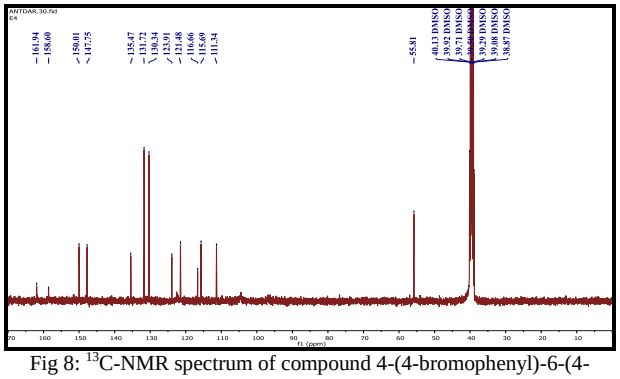


Fig 8: ^{13}C-NMR spectrum of compound 4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

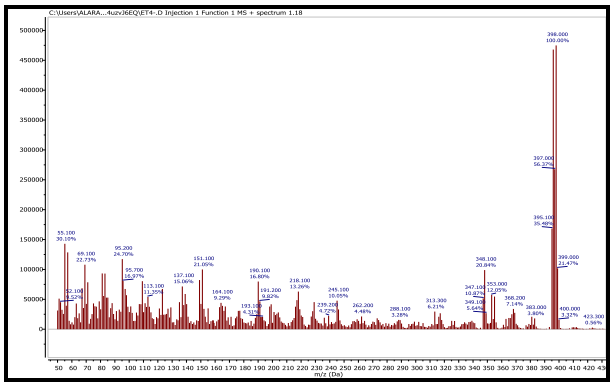


Fig 9: mass spectrum of 4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

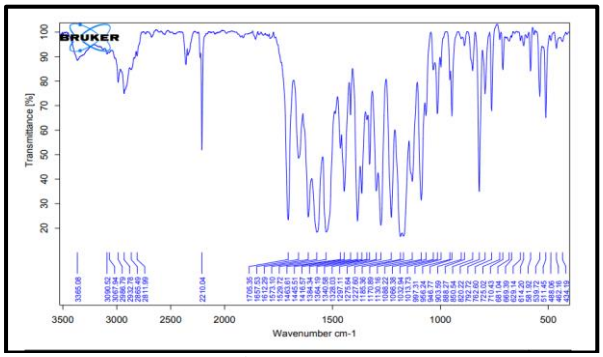


Fig 10: FT-IR spectrum of compound 4-(4-(dimethylamino)phenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

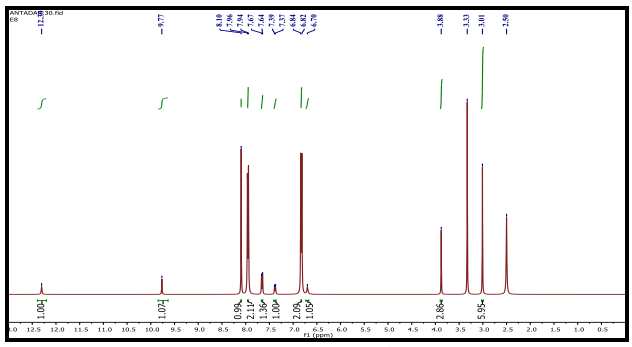


Fig 11: 1H-NMR spectrum of compound 4-(4-(dimethylamino)phenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

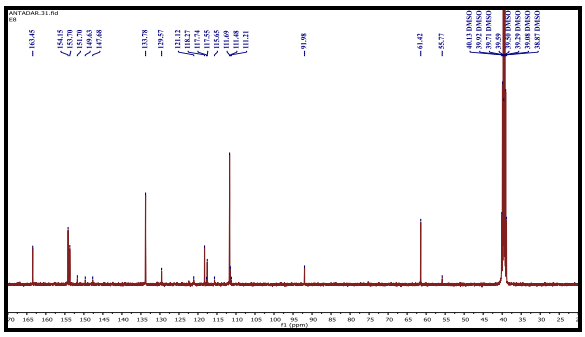


Fig 12: ^{13}C-NMR spectrum of compound 4-(4-(dimethylamino)phenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

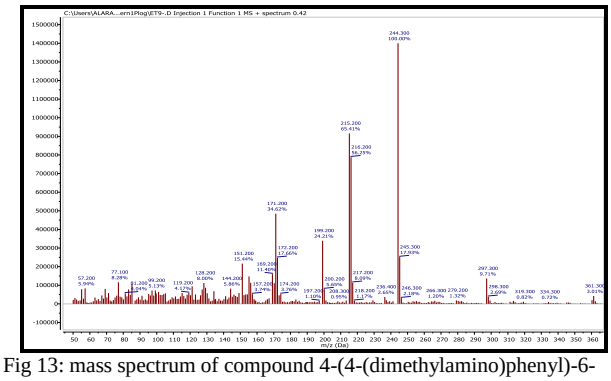


Fig 13: mass spectrum of compound 4-(4-(dimethylamino)phenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

A, Biological activity of synthesis compounds:

The biological activity of new synthesized compounds was assessed by the agar diffusion method using gentamicin as a reference. The compounds were examined, the plates were incubated for 24 hours at 37℃ and the inhibitory zone was recorded in (mm) The chemicals' biological effects on two series of bacteria [*Staphylococcus aureus, Escherichia coli*] respectively which displayed moderate inhibition. As shown in the following Table.2 [26]

Table.2: illustrates the antibacterial activity of compounds T_1-T_3

Compound symbol	Conc.(Mg/ml)	Staphylococcus aureus(Gram positive)	Escherichia coli(Gramnegative)
T_1	100	2	1
	50	1	-
T_2	100	2	1
	50	1	1
T_3	1000	2	-
	500	1	-

B.Antioxidant activity of synthesis compounds:

According to Table (3) all the synthesized compounds displayed good antioxidant activity by comparing the results with ascorbic acid (standard) [27]. was evaluated by the use of 2,2- diphenyl-1-picrylhydrazyl.The compounds 4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile,and4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile, showed the highest activity as antioxidants (79.05, 67.28)%, which can be compared with ascorbic acid 82.71%, while the antioxidant activity of compound 6-(4-hydroxy-3-methoxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile,showed less effectiveness for the antioxidant 17.55% at a concentration of 12 ppm.

Table.3: DPPH radical scavenging activity of compounds T_1–T_3.

	Ascorbic acid		T_1		T_2		T_3	
Conc. µ g/ml	Mean	SD	Mean	SD	mean	SD	mean	SD
200	82.716	2.7783	58.063	3.74925	79.051	1.66937	67.284	0.984491
100	74.80733	1.446881	45.52767	7.923938	68.827	4.343983	55.47833	0.333708
50	64.313	3.142829	33.14033	3.984654	54.47533	2.695338	45.64067	3.373077
25	52.73933	3.183176	26.929	2.645374	40.818	5.208505	34.41367	2.546881
12	39.275	1.351383	17.554	2.409608	28.974	4.207328	19.25167	3.558762

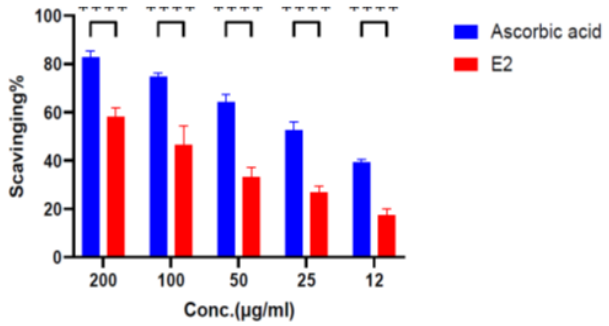


Fig14: Radical scavenging activity (RSA) for the synthesized compound 6-(4-hydroxy-3-methoxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

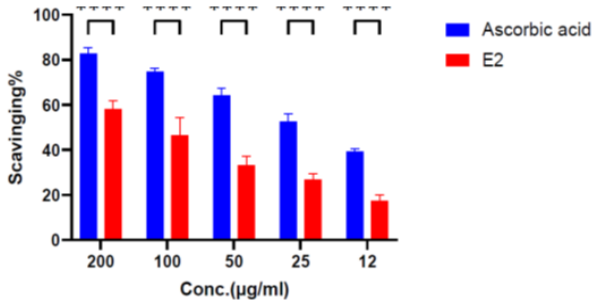


Fig15: Radical scavenging activity (RSA) for the synthesized compound 4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

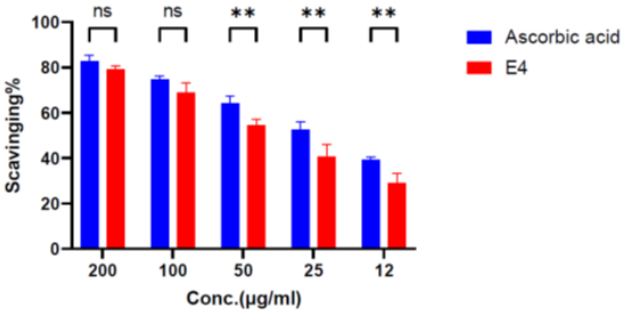


Fig16: Radical scavenging activity (RSA) for the synthesized compound 4-(4-(dimethylamino)phenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

C. Computational Studies:

Study docking molecular

The binding affinity (BA) and interaction of the synthesized compounds T1-T3 with active sites of the protein (4H2M) were analyzed using molecular docking studies via used PyRx and Pymol programs. The docking of receptors 4H2M (E.coli) for antibacterial activity. The 2D and 3D representations of compounds was shown in Fig7, with as the standard drug. The docking study of antibacterial activity results showed that the docking scores of compounds (T1-T3 with4H2M were found to be in the range of-7.6 to-7.9kcal/mol. The compound T2 shows the least binding energy of -7.9 kcal/mol and One hydrogen bonds. The most active compound was T1 from T2 and T3 interaction with the protein (PDB: 4H2M) exhibited conventional hydrogen bond interactions with HIS: 43and ASN: 144. Pi-Pi T-shaped, Pi-Alkyl PHE: 89 Alkyl [28]. Pi-Cation HIS: 43. Pi-Pi Stacked. The Gemifloxacin was used as the reference standard for comparison (-7.0 Kcal/mol). The standard interactions with the protein Conventional Hydrogen Bond TYR: A;145, TYR: B;211, ASN; A;203, and SER; A:71, Results were tabulated in Table 4.

Table 4. Molecular interactions for antibacterial activity of compounds T_1-T_3 and Gemifloxacin with 4M2H.

Comp	Binding affinity (kcal/mol)	RMSD lower bound	H- bond interaction	H- bond length in Å	Hydrophobic and other interactions
T1	-7.6	2.06	HIS: A;43 ASN: A;144	2.60 1.97	PHE:89, HIS.:43, TYR.:68, ALA:69, ALA:143, MET:86
T2	-7.9	1.13	ASN: A;28	2.65	PHE:89, HIS:43, TYR:68, ALA:69, LEU:85, MET:86.
T3	-7.8	15.311	TYR: A;145 HIS: A;43	2.15 2.55	ALA:69,LEU:85.
Gemifloxacin	-7.0	1.659	TYR: A;145 TYR: B;211 ASN; A;203 SER;A:71	2.43 2.65 2.63 2.21	PHE;A:70, MET;A:86.

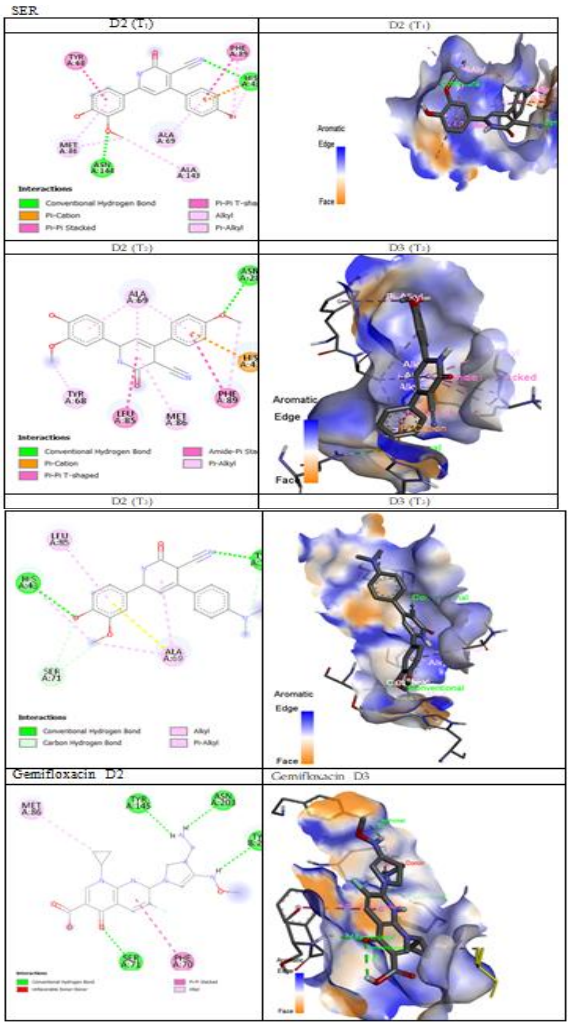


Figure 17. D_2 and D_3dimensional representations of molecular interactions between 4H2M and compounds T_1-T_3 and reference Gemifloxacin.

D.Analysis of ADMET properties

Study of physicochemical properties.

The physicochemical properties are necessary for the design of new compounds used in drugs. A drug-likeness the profile can be evaluated via these parameters of the molecule such as molecular weight (M.Wt), the number of heavy atoms (No heavy atoms), hydrogen bond acceptors(HBA), hydrogen bond donors(HBD), rotatable bonds, molar refractivity, and Topological polar surface areas(TPSA). These parameters were calculated for compounds T_1-T_3 and shown in Table 5.

Table5. Physicochemical properties of synthesized compounds (T_1-T_2)

Compd	Formula	Mwt g/mol	Heavy atoms	HBA	HBD	Rotatable Bond	Fraction Csp3	Molar Refractivity	TPSA (Å²)
T1	$C_{19}H_{13}BrN_2O_3$	397.22	25	3	2	3	0.05	98.86	88.11
T2	$C_{20}H_{16}N_2O_4$	348.35	26	5	2	4	0.01	97.66	95.34
T3	$C_{21}H_{19}N_3O_3$	361.39	27	4	2	4	0.14	105.37	89.35

The drug-likeness profiles were calculated based on Lipinski's (MW ≤ 500; HBA ≤ 10 and HBD ≤ 5), Ghose's (160 ≤ MWt ≤ 480; 40 ≤ MR ≤ 130 and 20 ≤ atoms ≤ 70), Veber's (rotatable bonds ≤ 10 and TPSA ≤ 140), Egan (TPSA ≤ 131.6) and Muegge (200 ≤ MW ≤ 600; the number of aromatic rings ≤ 7; several rotatable bonds ≤ 15; HBA ≤ 10 and HBD ≤ 5).28.29 The rule-based score defines the compounds into four probability score classes i.e. 11%, 17%, 55%, and 85%. The acceptable probability score is 55% which indicates that it passed the rule of five. All compounds T1-T3 have appeared with a score of 85% indicating compounds obeyed all the five rules without any violations with good bioavailability. Further, the synthetic accessibility of the compounds was assessed to quantify the complexity of the molecular structure. The results showed that the score was in the range of 2.79 - 2.99 revealing that the compounds do not have a complex synthetic route Table 6.

Table 6: Drug likeness, bioactivity and synthetic accessibility score.

Compd	Lipinski	Ghose	Veber	Egan	Muegge	bioactivity Score	synthetic accessibility
T_1	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55	2.78
T2	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55	2.90
T3	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55	2.99

The mean predicted lipophilicity values were evaluated to decide whether the compounds were soluble in aqueous or non-aqueous media and they were calculated by considering the consensus log Po/w.32 According to this, if a molecule is more soluble then its consensus log $P_{o/w}$ values will be more negative. The results of the compounds T1-T3 showed that the compounds were not soluble in a non-aqueous medium. Consensus log S (log S < -10: poorly soluble, < - 6: moderately soluble, < -4: soluble, < -2: very soluble, and <0: high soluble) values indicated that, the compounds were moderately soluble (except T_2) in an aqueous medium. The pharmacokinetic parameters like absorption, skin permeation, distribution, metabolism, and excretion were predicted. According to this predictive model, if a molecule falls in the white region indicates passive gastrointestinal absorption [29], whereas in the yellow region indicates passive brain permeation. Predicted distribution parameters of the compounds (T1-T3) suggested that all the synthesized compounds have high GI absorption. While all the compounds have no blood-brain permeate, hence there was no possibility of causing harmful toxicants in the brain and bloodstream. If the molecules have more negative log Kp value, it is said to be less skin permeate. The compounds (T_1-T_3) have more negative log Kp values, therefore these compounds are the least skin permeate Table 7.

Table 7. Predicted absorption parameters of compounds (T_1-T_3)

Compd.	Consensus Log Po/w	Consensus Log S (ESOL)	Solubility Class
T1	3.46	-4.69	Moderately soluble
T2	2.85	-3.84	Soluble
T3	2.87	-4.00	Moderately soluble

Table8. Predicted distribution parameters of the compounds

Comp	GI	BBB	Log Kp (cm/s)
T1	High	No	-6.42
T_2	High	No	-6.63
T_3	High	No	-6.59

Metabolism plays an important role in the bioavailability of drugs as well as drug-drug interactions. Metabolism parameters are important to understanding whether the compounds acted as an inhibitor or a non-inhibitor of certain proteins. then, the synthesized compounds T1-T3 were evaluated for their metabolism parameters and the results revealed that all compounds are found to be non-

substrates of permeability glycoprotein (P-gp). The P-gp is an important protein in assessing active efflux through biological membranes and cytochrome P450 (CYP) enzymes. All the compounds T_1-T_3 were also found to be non-substrates of CYP2C19 inhibitor and compound T_1 non substrates of CYP2D6. All Compounds were found to be substrates of CYP1A2 inhibitor, CYP2C9 inhibitor and CYP3A4 inhibitor Table 9.

Table 9: Predicted metabolism parameters of the compounds T_1-T_3

Comp	p-gp substrate	CYP1A2 inhabitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
T1	No	Yes	No	Yes	No	yes
T2	No	Yes	No	Yes	Yes	Yes
T3	No	Yes	No	Yes	Yes	Yes

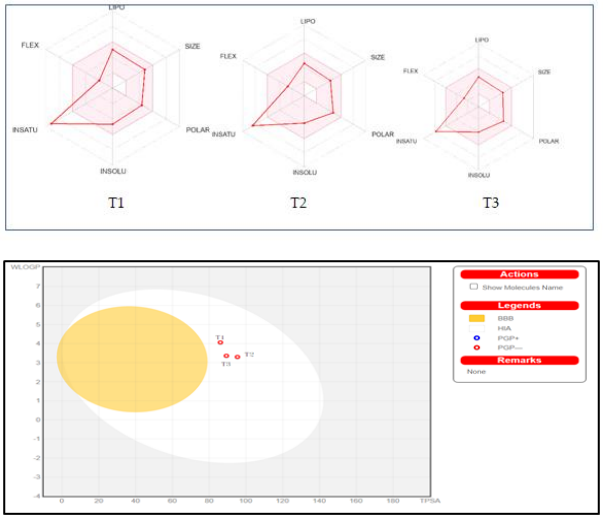


Figure 18: BOILED egg plot of synthesized compound T1-T3 from the Swiss ADME web tool.

*E.*Prediction results:

The synthesized compounds TI-T3 were calculated toxicity by using the online software ProTox-II. Toxicological endpoints results suggested that all the compounds were predicted to be hepatotoxicity, non-Mutagenicity, and compounds (T1 and T2) were predicted to be non-cytotoxicity Compounds T2 and T3 were non-carcinogenic but the compound T1 was carcinogenic. The toxicity class to all commpounds was 4 .[Class I: fatal if swallowed (LD50 ≤ 5) Class II: fatal swallowed (5 < LD50 ≤ 50) Class III: toxic if swallowed (50 < LD50 ≤ 300) Class IV: harmful if swallowed (300 < LD50 ≤ 2000) Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000), Class VI: non-toxic (LD50 > 5000). The predicted LD50. results suggested that the synthesized compounds were harmful if swallowed and belong to class IV (Table 10).

Table10 : The synthesized compounds T_1-T_3 were subjected to an insilico toxicity evaluation.

Comp	Organ Toxicity	Toxicity - endpoints				Predicted LD50(mg/kg)	Predicted Toxicity Class
	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity		
T1	Active	active	active	Inactive	Inactive	400mg/kg	4
T2	Active	Inactive	Inactive	Inactive	Inactive	400mg/kg	4
T3	Active	inactive	active	inactive	active	400mg/kg	4

Conclusion

In this study, three derivatives of 1-(4-hydroxy-3-methoxyphenyl) ethan-1-one T1-T3 were synthesized from cyclization reaction of 1-(4-hydroxy-3-methoxyphenyl) ethan-1-one , 4-methoxy benzaldehyde and 4-bromo benzaldehyde) respectively ,ethyl cyanoacetate in the presence ammonium acetate. The prepared compounds were confirmed by FTIR, 1H-NMR, 13C-NMR and mass spectroscopy. The physical properties were assessed. The antioxidant activity of the synthesized compounds was evaluated by the use of 2,2- diphenyl-1-picrylhydrazyl.The compounds 4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile,and4-(4-bromophenyl)-6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile, showed the highest activity as antioxidants (79.05, 67.28)%, which can be compared with ascorbic acid 82.71%, while the antioxidant activity of compound 6-(4-hydroxy-3-methoxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile, showed less effectiveness for the antioxidant 17.55% at a concentration of 12 ppm. Also the prepared compounds were assessed for in vitro biological activity against the two type of bacteria(staphylococcus aureus ,Escherichia coli). In an attempt to understand the ligand–protein interactions in terms of the binding affinity, docking studies were performed using Py-Rx and BIOVIA\Discovery Studio 2021 for the compounds. the binding affinities calculated were in agreement with the MIC values.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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