RESEARCH ARTICLE

Synthesis, Antimicrobial Evolution, Defibrillation Threshold Studies, Docking Studies, Silico Admet Analysis and PER-Metabolism Study of Some New Dihydropyrmidine Derivatives

Sahar B. Al-Juboori

College of Al-Rafidain University-Department of Pharmacy, Hay Al-Mustansiriya, 10001, Baghdad, Iraq

Received: 22th Dec, 19; Revised: 16th Jan, 20, Accepted: 13th Feb, 20; Available Online: 25th Mar, 2020

ABSTRACT

Dihydropyrimidinone and dihydropyrimidine derivatives are reported to possess broad biological activities. Many synthetic samples have been studied as antibacterial, antiviral, and anticancer agents. We decided to synthesize novel compounds of new pyrimidine derivatives**.**

The present work involves the synthesis of new dihydropyridine derivatives. The starting vanillin, compound (1) was used as the key intermediate to prepare the 5-acetyl-4-(4-hydroxy-3-methoxyphenyl)-6-methyl pyrimidine-2(1H)-one(2),Ethyl 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1,2-dihydropyrimidine-5-carboxylate (3), 4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydro quinazoline-2(1H)-one (4), respectively, through the reaction with urea and acetylacetone or ethyl acetoacetate or cyclohexanone but 4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydro quinazoline-2(1H)-thione (5) reacted with thiourea and cyclohexanone.

FTIR,^{1}H-NMRand^{13}C-NMR spectroscopy characterized all the synthesized compounds. The synthesized derivatives were screened for their in vitro, antibacterial activity against two gram-positive bacteria: *Bacillus subtilis, and Staphylococcus aureus* and two gram-negative bacteria: *Klebsiella pneumonia and Salmonella typhi* and the results showed that most of them have good antibacterial activity. While their antifungal activity against three fungi species: (*Aspergillus fumigates, Aspergillus niger, and Rhizopus*), revealed that compounds (1-5) displayed the most potent antifungal activity. Density functional theory (DFT) calculations for the synthesized compounds (1-5) were conducted, using a molecular structure with optimized geometry. Highest occupied molecular orbital/lowest unoccupied molecular orbital energies and structures are demonstrated. The antimicrobial activity indicates that compounds 3 and 4 are the most active than the compounds 2 and 5. Molecular docking revealed that compounds (4) and (5), with cyclohexyl groups are important to block the active centers of glucose -6-phosphate synthase in the bacteria and fungi.

Keywords: Antimicrobial, Cyclohexanone, Density functional theory (DFT), Vanillin, Urea.

International Journal of Drug Delivery Technology (2020); DOI: 10.25258/ijddt.10.1.5

How to cite this article: Al-Juboori, S.B. (2020). Synthesis, Antimicrobial Evolution, DFT Studies, Docking Studies, Silico Admet Analysis and PER-Metabolism Study of Some New Dihydropyrmidine Derivatives. International Journal of Drug Delivery Technology, 10(1): 21-32.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Multicomponent reactions (MCRs) are of increasing importance in organic and medicinal chemistry. In times where a premium is put on speed, diversity, and efficiency in the drug discovery process, MCR strategies offer significant advantages over conventional linear-type syntheses. In such reactions, three or more reactants come together in a single reaction vessel to form new products that contain portions of all the components.[1-5] In an ideal case, the individual building blocks are commercially available or are easily synthesized and cover a broad range of structural variations. One such MCR that belongs in the latter category is the venerable Biginelli dihydropyrimidine synthesis. In 1893, Italian chemist Pietro Biginelli reported on the acid-catalyzed cyclo condensation reaction of ethyl acetoacetate, benzaldehyde and urea.[6] Dihydropyrimidinone and dihydropyrimidine derivatives have broad biologically activities. Many synthetic samples have been studied as antibacterial, antiviral, antihypertensive, and anticancer agents. and the natural products containing these heterocyclic moieties have been studied as new leads for AIDS therapies.[7] One of the most famous pyrimidine derivatives is 5-florouracil (5- Fu), considered as one of the most important drugs for the treatment of colorectal, head and neck, pancreatic and breast carcinomas.[8] Reported in this paper is synthesis of

**Author for Correspondence: saharbalkat@gmail.com*

dihyropyrmidine by the reaction of a (β-keto ester or ketone), an aldehyde, and (urea or thiourea) is considered as one of the most efficient ways to synthesize dihydropyrimidinones. This acid-catalyzed reaction can be conducted under conventional.

MATERIAS AND METHODS

Chemicals used during the synthesis, supplied by Sigma-Aldrich. Solvents were dried and distilled before use. Completion of reactions and the purity of compounds were ascertained by thin-layer chromatography (TLC), using Silica gel GF_{254} (type 60) pre-coated Aluminium sheets, Merck (Germany).Melting points were measured using Kofler hot stage apparatus and are uncorrected. IR(KBr) spectra (υ,cm^{-1}) were recorded using Thermo Scientific™ Nicolet™ iS™10 FTIR Spectrometer in College of Education Ibn al-Haytham-Iraq, ^{1}H-NMR and ^{13}CNMR spectra were recorded on DMSO-$_{d6}$ with TMS as an internal standard on Bruker spectrophotometer at 300 MHz and 75 MHz respectively, (chemical shifts in δ ppm), the NMR work was done at University of Kashan - Iran). Computational study was performed for the prepared compounds using Chemical Bio Office 2016,version 16, DFT study with 3_21G* basis, While molecular docking study was run using Molecular Operation Environment,(MOE) program,2105., All physical data and biological effects are listed in Tables 1, 2 and 3 respectively

Preparation of 5-acetyl-4-(4-hydroxy-3-methoxyphenyl)-6-methylpyrimidin-2(1H)-one (2)

General procedure: A mixture of compound 1 (1.52 g, 0.01 mole), urea (0.6 g, 0.01 mole), acetylacetone (1 g, 0.01 mole) in absolute ethanol (40 mL) containing few drops of concentrated hydrochloric acid was refluxed for 8 hours. The formed precipitate was filtered, washed with ether several time, dried and recrystallized to give compounds 2 are shown in Scheme 1.

IR (KBr),(υ,cm^{-1}): compound 2 :- 3311 cm^{-1} (O-H), 3220, 3127 cm^{-1} (NH, NH) str.,2932 cm^{-1} Ar–(C-H), 2885,2730 cm^{-1} (C-H alphatic) 1719 cm^{-1} (C=O ketone), 1676 cm^{-1} (CONH), 1642 Ar(C=C), 1198(C-O). 1548 cm^{-1} (N-H) bend, 1286 cm^{-1} C-N) str, 1029 & 952 cm^{-1} in plane& out of plane Ar (C-H) bend.; 733 cm^{-1} out of plane Ar(C=C) bend, ^{1}HNMR(300MHz, DMSO-$_{d6}$,δ=ppm):12.7(1H,s,OH Ar), 9.23(1H,s,NHCO), 7.47(1H,s,NHCO); 7.83,8.42(2H,d, 2 Ar-H), 3.45 (s, 3H, O-CH_3), 7.77 (1H,s, Ar-H), 4.54 (s, 3H, C-H of ketone), 5.22 (s, 2H, C-H of dihydopyrimidine). ^{13}C-NMR (75 MHz, DMSO-$_{d6}$): δPPM 196.2, 166.7, 150.9, 143.3, 137.6, 121.5, 113.3, 109.0, 99.0, 57.1, 65.6, 45.6& 33.4.

Table 1: The physical and analytical data of all compounds.

Comp. no.	*M.p. (°C)*	*(Cryst. solvent)*	*Yield %*	*Mol. formula*	*(Mol. Wt.)*	*PK_a*
1	84	1-Propanol /water	-	C_8H_8O	152.3	7.781
2	165	Methanol	80	$C_{14}H_{14}N_2O_4$	274.28	7.998
3	185	Propanol	76	$C_{15}H_{16}N_2O_5$	304.3	7.898
4	198	Methanol	65	$C_{15}H_{16}N_2O_3$	272.3	8.178
5	219	Ethanol	70	$C_{15}H_{16}N_2O_2S$	288.37	8.117

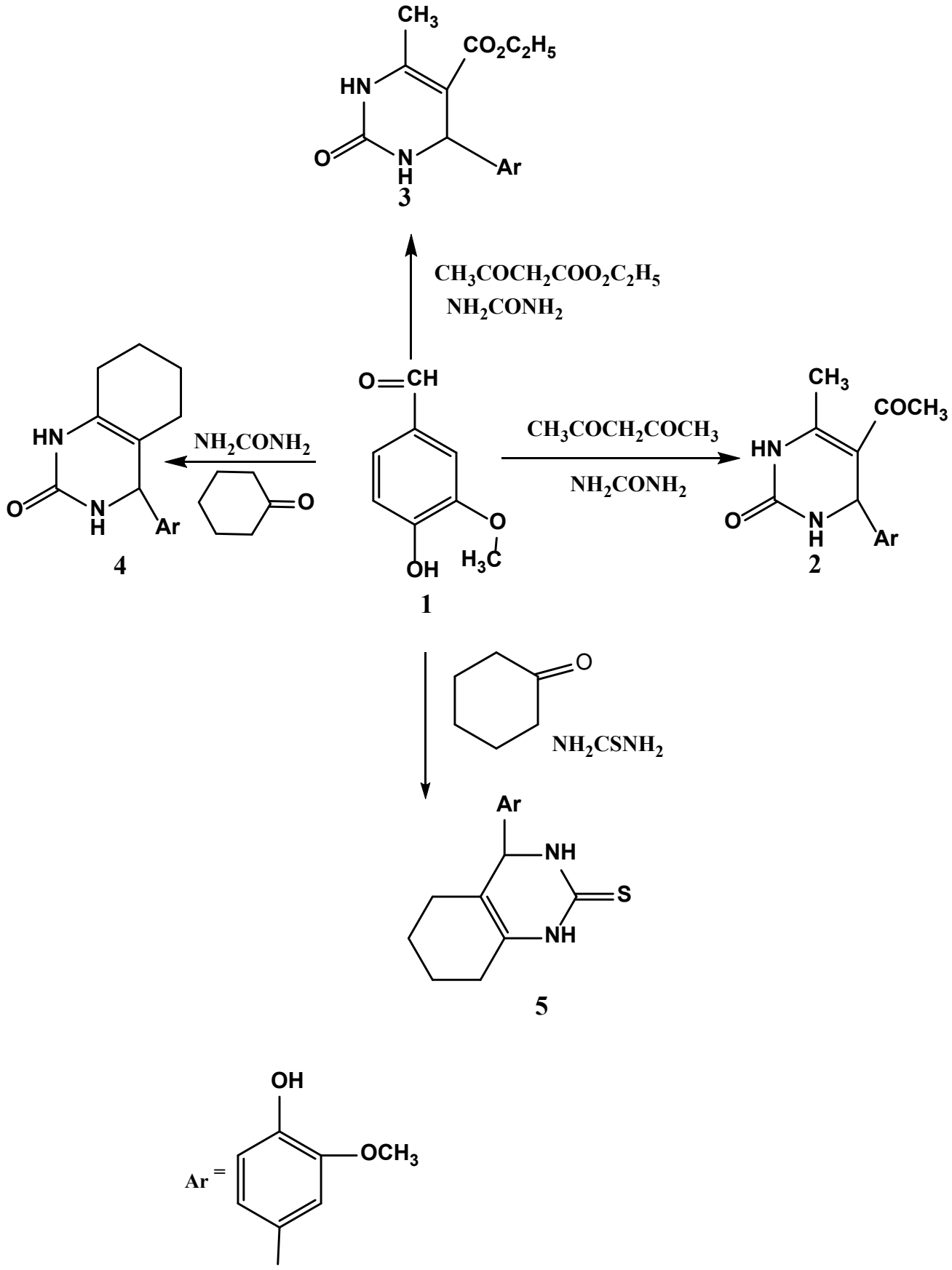
Table 2: Antifungal activity of the tested compounds

	Zone of inhibition(mm)					
Compound no.	*Aspergillus fumigates*		*Aspergillus Terrus*		*Rhizopus*	
Conc. µg/mL	100	50	100	50	100	50
1	11	7	15	10	15	9
2	25	16	28	16	30	16
3	30	20	38	26	45	27
4	28	18	33	22	34	20
5	23	13	26	11	30	12
DMSO	0	0	0	0	0	0

Table 3: Antibacterial activity of the tested compounds

	Zone of Inhibition (mm)							
Compound.	*Gram-positive*				*Gram-negative*			
No.	*Staphylococcus aureus*		*Bacillus subtilis*		*Klebsiella pneumoniae*		*Salmonella typhi*	
Conc. µg/mL	100	50	100	50	100	50	100	50
1	12	7	10	5	16	8	15	10
2	16	12	20	12	22	12	26	13
3	20	10	27	16	32	15	45	15
4	16	12	22	14	27	14	35	14
5	18	10	22	10	20	12	25	13
DMSO	0	0	0	0	0	0	0	0

Highly sensitive (>15 mm)=+++; moderately sensitive (10–15 mm)=++; slightly sensitive (5–10 mm)=+; and not sensitive (0 mm)=-, SD: Standard deviation, DMSO: Dimethyl sulfoxide



Scheme 1: Synthesis of some new dihydropyrimidine derivatives

Preparation of Ethyl4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1,2dihydropyrimidine -5-carboxylate (3)

General procedure: A mixture of compound 1 (1.52 g, 0.01 mole), urea (0.6 g, 0.01 mole), ethyl acetoacetate (1.46g, 0.01 mole) in absolute ethanol (45 mL) containing few drops of concentrated hydrochloric acid was refluxed for 64 hours. The formed precipitate was filtered, washed with ether several time, dried and recrystallized to give compounds 3 are shown in Scheme 1.

IR(KBr),(υ,cm^{-1}): compound 3 :- 3235 cm^{-1} (O-H), 3422, 3278 cm^{-1} (NH, NH) str, 1735 (C=O ester), 2912 cm^{-1} Ar(C=C)), 2835,2744 cm^{-1} (C-H alphatic), 1652cm^{-1} (CONH), 1615 cm^{-1} Ar(C=C), 1245 cm^{-1} (C-O), 1524 cm^{-1} (N-H) bend, 1252 cm^{-1} C-N) str, 1119 & 929 cm^{-1} in plane& out of plane Ar (C-H) bend.; 763 cm^{-1} out of plane Ar(C=C) bend,. ^{1}HNMR(300MHz, DMSO-$_{d6}$,δ=ppm):12.31(1H,s,OH Ar), 9.54(1H,s,NHCO), 7.83(1H,s,NHCO); 7.96,8.64(2H,d, 2 Ar-H), 3.76 (s, 3H, O-CH_3), 7.54 (1H,s, Ar-H), 3.06 (q, 3H, C-H of ester), 4.76 (t, 3H, C-H of ester), 5.69 (s, 2H, C-H of dihydopyrimidine).

^{13}C-NMR (75 MHz, DMSO-$_{d6}$): δPPM 215.2, 196.7, 175.3, 162.4, 157.9, 146.0, 136.7, 125.1, 103.3, 112.4, 69.5, 57.1, 42.6, and 35.3

Preparation of 4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydroquinazolin-2(1H)-one (4)

General procedure: A mixture of compound 11.52 g, 0.01 mole), urea (0.6g, 0.01 mole) cyclohexanone(0.98g, 0.01mole) in absolute ethanol (50 mL) containing few drops of concentrated hydrochloric acid was refluxed for 5 hours. After cooling the formed precipitate was filtered, washed with ether several time, dried and recrystallized to give compounds 4 are shown in scheme 1.

IR(KBr), (υ,cm^{-1}): compound 4:- 3315 cm^{-1} (O-H), 3389, 3292 cm^{-1} (NH, NH) str, 2929 (CH_2, cyclohexane), 2945 cm^{-1} Ar(C=C)), 2875,2704 cm^{-1} C-H alphatic), 1664(CONH), 1644 cm^{-1} Ar(C=C), 1265 cm^{-1} (C-O), 1555 cm^{-1} (N-H) bend, 1242 cm^{-1} C-N) str, 1135 and 946 cm^{-1} in plane and out of plane Ar (C-H) bend.; 737 cm^{-1} out of plane Ar(C=C) bend. ^{1}HNMR(300MHz, DMSO-$_{d6}$,δ=ppm):12.18(1H,s,OH Ar),9.18(1H,s,NHCO), 7.35(1H,s,NHCO); 7.37,8.44(2H,d, 2 Ar-H), 3.24 (s, 3H, O-CH_3), 7.29 (1H,s, Ar-H), 3.76- 5.29 (m, 8 H, C-H of cyclohexane) 5.67 (s, 2H, C-H of dihydopyrimidine).

^{13}C-NMR (75 MHz, DMSO-$_{d6}$): δPPM 163.6, 154.8, 144.3, 127.6, 116.3, 99.4, 75.1, 65.4, 55.6, 45.9, 35.2, 29.0, 26.6, 22.8 and 19.9

Preparation of 4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydroquinazoline-2(1H)-thione (5)

General procedure: A mixture of compound 1(1.52 g, 0.01 mole), (0.76 g, 0.01 mole) thiourea, (0.98 g, 0.01 mole) cyclohexanone in absolute ethanol (40 mL) containing few drops of concentrated hydrochloric acid was refluxed for 6 h. After cooling the formed precipitate was filtered, washed with ether several time, dried and recrystallized to give compounds 5 are shown in scheme 1.

IR(KBr),(υ,cm^{-1}): compound 5:- 3329 cm^{-1} (O-H), 3309, 3222 cm^{-1} (NH, NH) str, 2949 (CH2, cyclohexane), 2960 cm^{-1} (C-H aromatic), 2815,2734 cm^{-1} C-H alphatic), 1589 cm^{-1} Ar(C=C), 1229 cm^{-1} (C-O), 1163 cm^{-1} (C=S), 1575 cm^{-1} (N-H) bend, 1272 cm^{-1} (C-N) str, 1215 & 973 cm^{-1} in plane and out of plane Ar (C-H) bend.; 766 cm^{-1} out of plane Ar(C=C) bend. ^{1}HNMR(300MHz, DMSO-$_{d6}$,δ=ppm):12.84(1H,s,OH Ar),9.48(1H,s,NHCS), 8.85(^{1}H,s,NHCS); 7.85,8.74(2H,d, 2 Ar-H), 4.27 (s, 3H, O-CH_3), 7.06 (1H,s, Ar-H), 3.45, 5.62 (m, 8H, C-H of cyclohexane), 5.93 (s, 2H, C-H of dihydopyrimidine).

^{13}C-NMR (75 MHz, DMSO-$_{d6}$): δPPM 174.7, 166.9, 157.1, 141.4, 121.3, 109.4, 86.3, 75.3, 63.1, 56.7, 49.1, 33.8, 28.2, 24.0 and 20.8

Antifungal Screening

The titled compounds 1-5 were screened for their preliminary The antifungal activity by using the agar disc diffusion method against pathogenic strains of *Aspergillus fumigates, Aspergillus niger* and *Rhizopus*. The compounds (1-5) were stored dry at room temperature at concatenation (50 and 100) µg/ml. It is prepared in the quality control on feed department/ animals resources directorate. The Sabouraud's agar media (15 cm^3), kept at 45ºC, was poured in the petri-dishes, then allowed to solidify. Sterile, filter paper discs of 10mm diameter were impregnated with prepared derivative (50 µL) and placed on to the media, seeded with fungus. The plates were then incubated at 37ºC for one day. The test compounds were previously dissolved in (DMSO), which is used as a control, the zone of inhibition (ZI) was measured in (mm).

Antibacterial Screening

The antimicrobial activities of the synthesized derivatives were measured using well diffusion technique, and it was done in the quality control on feed department/animals resources directorate. The synthesized derivatives had been evaluated *in vitro* against Gram-positive *Bacillus subtilis*, *Staphylococcus aureus*, and Gram-negative *Klebsiella pneumonia* and *Salmonella typhi*. Dimethyl sulfoxide (DMSO) was used as a solvent and control.[9]

RESULTS AND DISCUSSION

The first compound (1) was used as the key intermediate, to prepare 5-acetyl-4-(4-hydroxy-3-methoxyphenyl)-6-methylpyrimidin-2(1H)-one (2), Ethyl 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1,2-dihydropyrimidine-5-carboxylate (3), 4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydroquinazolin-2(1H)-one (4), and 4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydroquinazoline-2(1H)-thione (5), respectively, through its reaction with vanillin with urea or thiourea. The analytical and physical data are listed in Table 1:-

Compound 2 prepared from vanillin l by its reaction with urea, and acetylacetone in ethanol containing hydrochloric acid. Thus, vanillin 1 reacted with urea and ethyl acetoacetate in ethanol containing hydrochloric acid to give the corresponding.[3] When the vanillin reacts with cyclohexanone (urea or thiurea) in ethanol containing hydrochloric acid to give the corresponding 4 and 5 respectively.

Antimicrobial Activity

The antimicrobial activity was evaluated by filter paper disc

agar diffusion method. For antibacterial studies, Hi-media bacteriological nutrient broth and bacteriological nutrient agar were used against two Gram-positive *Bacillus subtilis, Staphylococcus aureus*, and two Gram-negative *Klebsiella pneumonia and Salmonella typhi*. Antifungal studies were carried out using Sabouraud´s dextrose broth and dextrose agar against *Aspergillus fumigates, Aspergillus niger, and Rhizopus*. The concentrations of the compounds taken were 50 and 100 µg/ml. The sensitivity of microorganisms to the compounds is identified in Tables 3 and 4. Electron-rich nitrogen heterocyclic plays an essential role in diverse biological activities. Introducing pyrimidine derivative rings (compounds 1, 2, 3, 4 and 5) influences the antibacterial or pharmacokinetic properties. The antimicrobial activity of compounds 1-5 were tested, and the results are shown in Table 2 and 3.

The evaluation of the new compounds 3 and 4 at conc. 100 µg /mL have shown the highest inhibitory activity against Gram-positive bacteria, *Bacillus, subtilis and Staph. Aureus* and the gram-negative bacteria. The compounds 2 and 5 at conc 100 µg/mL have shown highest inhibitory activity against Gram-positive bacteria *Bacillus, subtilis, and Staph. aureus*, while compounds 2 and 5 have shown moderate inhibitory activity against the Gram-negative bacteria. The evaluation of the compounds (1) at conc. 100 µg/mL have shown the moderate inhibitory activity against Gram-positive bacteria, *Bacillus, subtilis, and Staph. aureus*, also, have displayed the same slight inhibitory activity against the Gram-negative bacteria but the compounds 1 at conc. 50 µg /mL have shown the moderate inhibitory activity against Gram-positive bacteria, *Bacillus, subtilis, and Staph. aureus*, also, have displayed the slight inhibitory activity against the Gram-negative bacteria. For the fungi used, *Aspergillus fumigates, Aspergillus terrus, and Rhizopus*. The compounds 2, 3, and 4 revealed highly inhibitory activity towards all of the fungi in all conc., while compounds 1 were moderate active at conc. 100 µg /mL and slight active at conc. 50 µg /mL against the tested fungi. the compound 5 and was highest active against the tested fungi at conc. 100 µg /mL and moderate active at conc. 50 µg /mL, against the tested fungi are shown in Figure 1 and 2.

DFT Study

DFT calculations were carried out for 2-mercapto pyrimidine compound. The compounds 1, 2, 3,4 and 5, respectively, with optimized geometries and 3D geometrical structures, are given in Figure 3.

The determined bond angle and twist angle, stretch, bend, stretch-bend, torsion, and total energy, and the 3D geometrical structure confirmed that compound(1) have no stereochemistry, while compound 2 and 3, the stereochemistry: C5-C6 is E, C 4 is S and compound 4 and 5 the stereochemistry: C5-C6 is Z,

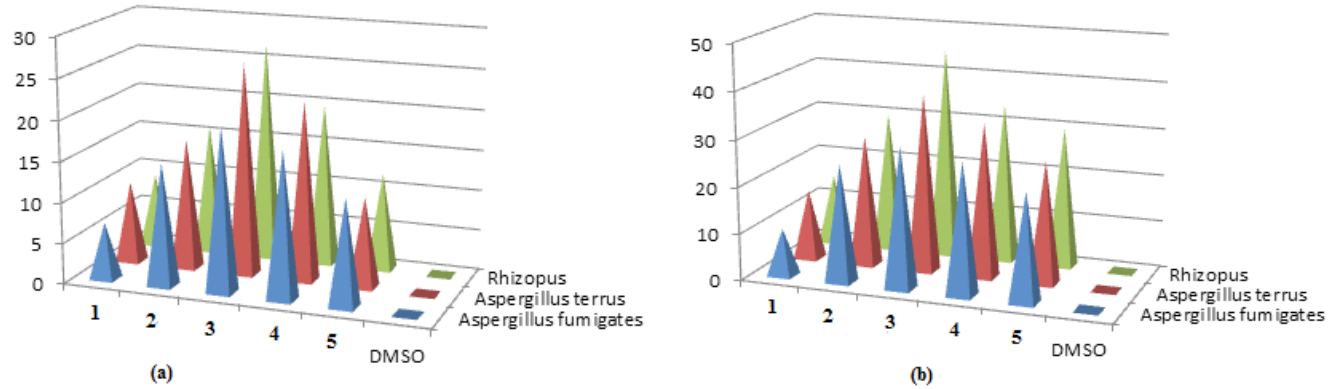


Figure 1: Antifungal activity of compounds 1–5: (a) Conc. =50 µg/ml; (b) conc. =100 µg/mL

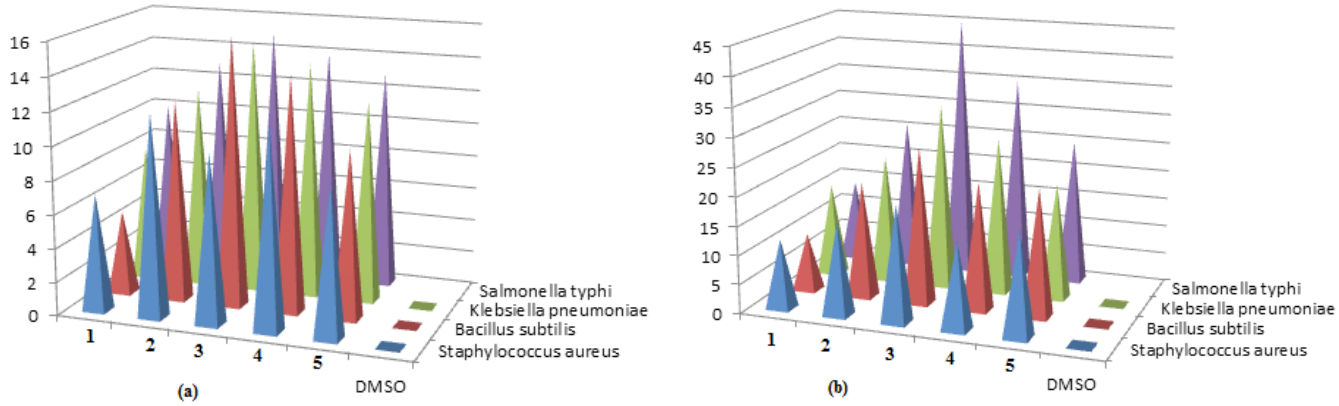


Figure 2: Antibacterial activity of compounds (1–5): (a) Conc. =50 µg/mL, (b) conc. = 100 µg/mL

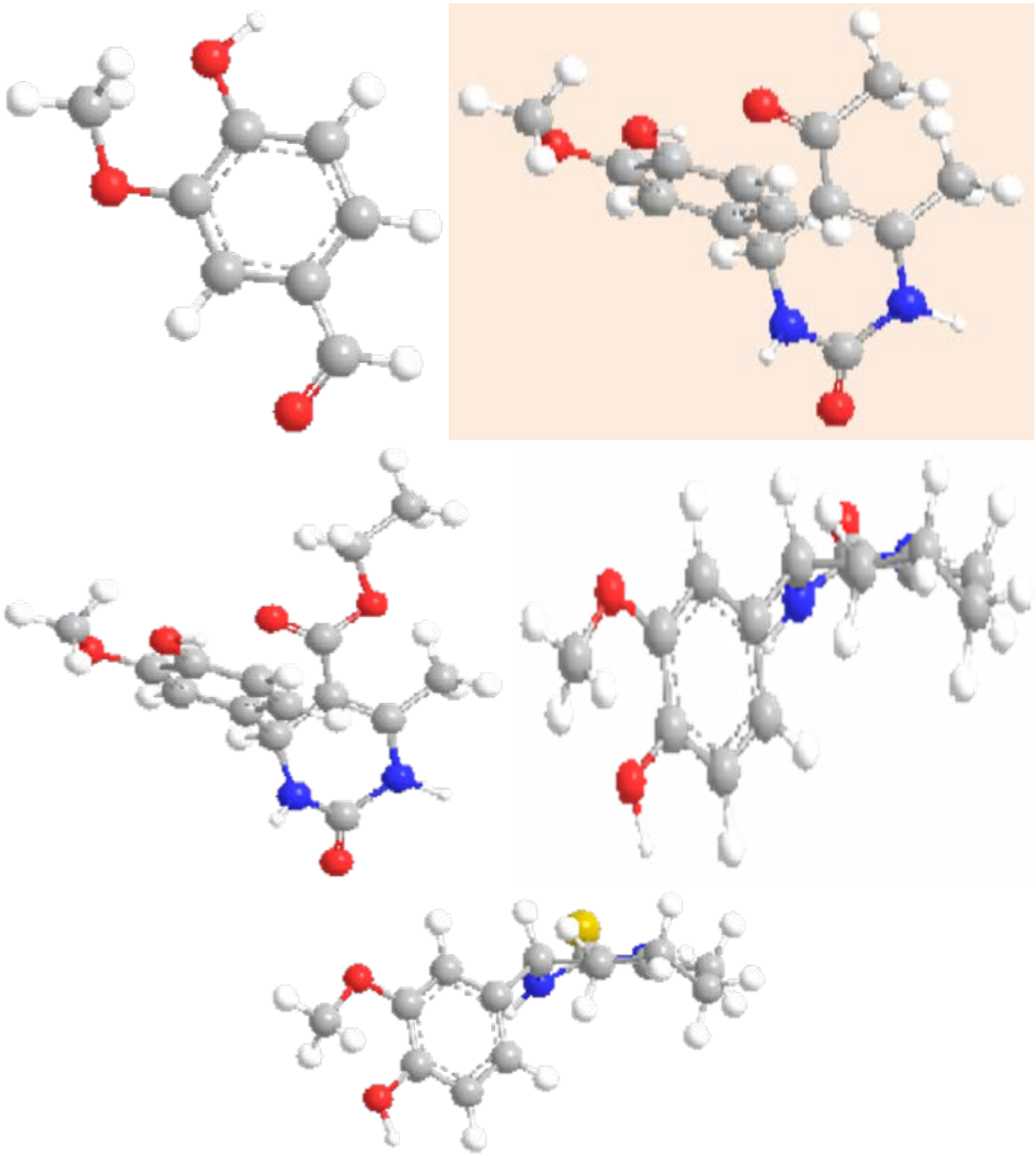


Figure 3: Optimized 3D geometrical structures for compounds 1, 2, 3,4, and 5.

Table 4: physical parameters for compounds 1,2,3,4 and 5 by using DFT with 3-21G* basis

Parameter	*Compound 1*	*Compound 2*	*Compound 3*	*Compound 4*	*Compound 5*
Stretch	0.8019	2.0712	2.3000	1.2411	1.1343
Bend	6.3669	12.0439	11.1864	7.4341	7.1711
Stretch-Bend	0.0724	0.1343	0.1629	0.0734	0.0405
Torsion	-5.9138	-6.2677	-7.7470	-4.2639	-5.5919
Log P	1.27	-0.04	0.71	1.44	2.43
Total energy: kcal/mol	11.0827	2.0108	3.9472	-5.6634	7.9170

C 4 is S. The physical parameters are calculated and displayed in Table 4.

Molecular Modeling Studies

The HOMO and the LUMO studies have been carried out, using DFT based quantum chemical descriptors. Figure 4 shows that charge transfer occurs within the molecules and the calculated HOMO and LUMO in electron volt value.The HOMO energies of compound (1-5) are : -11.317 eV, -9.722 eV, -9.493 eV, -8.525 and –8.120 eV, respectively. While the LUMO energies of synthetic compound (1-5) are calculated as-4.51eV, -2.382eV, -1.704eV, 1.044eV and 1.089eV, respectively are shown in Table 5.

Global Reactivity Descriptors and Electronic Properties

The energy gap (6.807 eV, 7.34 eV, 11.19 7eV, 9.569 eV and 9.209 eV) of synthesized compounds (1-5), Table 3. The charge density delocalization over the amide group in the compounds (1-5) detect measurable differences for HOMO and LUMO of compounds (1-5). For the band gaps, it was clear that the highest bandgap was for compound (1), which was -6.807eV, and the next value is for compound (2), -7.34eV are shown in (Table 5). The charge density, reactivity index, and bond properties of compounds (1-5) were calculated depending on the dipole moment. It can be calculated with the help of a dipole moment of bonds. The values of dipole moment for compounds (1-5) are shown in Table 5. The ionization potential (IP) and electron affinity (EA) can be expressed as the total of energy released when an electron absorbed by a neutral molecule,[10] which is approximated as (IP) = E_{HOMO} and EA= $-E_{LUMO}$, respectively, based on Koopman's theorem**.** The electrophilicity index has been used as a structural chemical potential (μ), chemical hardness (η), and electrophilicity index

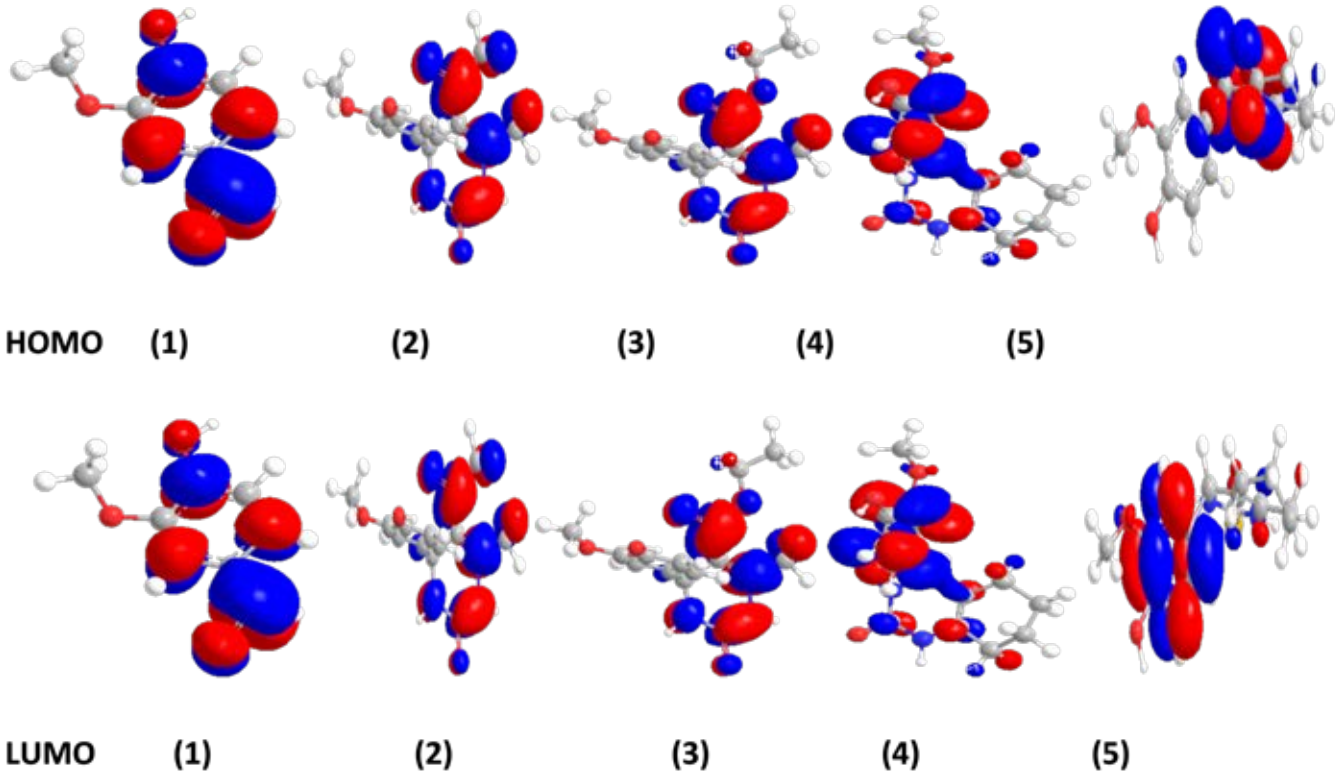


Figure 4: Highest occupied molecular orbital (HOMO), the lowest unoccupied molecular orbital (LUMO) for compounds (1-5)

Table 5: Electronic properties of synthesized compounds(1-5)by using DFT with3-21G* basis

Parameter	*Compound 1*	*Compound 2*	*Compound 3*	*Compound 4*	*Compound 5*
Dipole moment (Debye)	-0.0604	-13.2898	-11.1322	-14.5236	-3.3363
Ionization potential (IP) eV	11.317	9.722	9.493	8.525	8.12
Electron affinity (EA) eV	4.510	2.382	-1.704	1.044-	1.089-
Hardness (η)	3.4035	3.67	5.599	4.785	4.605
Electronegativity (μ)	7.9135	6.052	-3.895	-3.74	-3.16
Electrophilic index (ω)	9.199	4.99	1.35	1.46	1.08
E_{HOMO} eV	-11.317	-9.722	-9.493	-8.525	-8.12
E_{LUMO} eV	-4.510	-2.382	1.704	1.044	1.089
Band gap=E_{HOMO} - E_{LUMO} eV	-6.807	-7.34	-11.197	-9.569	-9.209
energy gap= E_{LUMO} - E_{HOMO} eV	6.807	7.34	11.197	9.569	9.209

(ω) of a molecule descriptor for the analysis of the chemical reactivity. A molecule with a lower value of μ and ω considered the more reactive nucleophile, while, in the opposite, a molecule with a high value of μ, and ω, recognized as a good electrophile. The electro-negativity and hardness are most commonly used to predict the chemical behavior to clarify aromaticity in organic compounds".[12] The molecule that has a large HOMO–LUMO gap is considered a hard molecule, while a molecule that has small HOMO–LUMO, considered a soft molecule. The HOMO as the electron-donating ability of a molecule and LUMO represents electron(s) accepting ability. The energy HOMO, LUMO, bandgap, dipole moment, softness, electrophilicity Index (ω), chemical potential, index are calculated and displayed in Table 5.[11-13]

Molecular Docking Studies

Molecular docking studies were done to give insights of molecular binding modes of the synthesized compounds inside the pocket of gyrase enzyme using MOE 2015 software. The binding sites were generated from the co-crystallized ligand, within gyrase enzyme crystal protein (PDB code: 4zvi).[14-16] At first, water molecules were removed from the complex. Then, the crystallographic disorders and unfilled valence atoms were corrected using protein report, and utility and clean protein options Protein-energy was minimized by applying CHARMM and MMFF94 force fields. The rigid of the binding site was a structure of the protein was obtained by applying fixed atom constraint. The protein essential amino acid defined and prepared for the docking process. 2D structures of the synthesized compounds were drawn using Chem-Bio Draw Ultra 14.0 and saved in MDL-SD file format From MOE 2015 software, the saved file was opened, 3D structures were protonated and energy was minimized by applying 0.05 RMSD kcal/mol. CHARMM force field. Then, the minimized structures were prepared for docking using prepare ligand protocol. The docking process was carried out using CDOCKER protocol. CDOCKER is a grid-based molecular docking method that employs CHARMM-based molecular dynamics (MD) scheme to dock ligands into a receptor binding site The receptor was held rigid while the ligands were allowed to be flexible during the refinement Each molecule was allowed to produce seven different interaction poses with the protein. Then docking scores (-CDOCKER interaction energy) of the best-fitted poses with the active site at Gyrase enzyme were recorded) (Table 6).[17]

We use all these processes to predict the proposed binding mode, affinity, preferred orientation of each docking pose, and binding free energy (ΔG) of the synthesized compounds and co-crystallized ligand with gyrase enzyme. The calculated interaction energies for the synthesized compounds were in complete agreement with the experimental result, which showed that 3, 4, 2 and 5 care potent inhibitors of gyrase enzyme as compared to the other members and reference standard. The critical binding site of Gyrase enzyme has been reported by the kinds of literature, consisting amino acid Asp73 and residues of Gly77. The proposed binding mode of crystal ligand showed affinity value of -4.531 kcal/ mol.N-H group in pyrrole ring binding by hydrogen bond interaction with Asp73 only. Figure 5.

The proposed binding mode of compound 2 showed an affinity value of -4.12 kcal/mol. It showed one hydrogen bonding between (OH) group and residues of Asp73. (Figure 6)

The proposed binding mode of compound 3 showed affinity value of -5.07 kcal/mol. It showed two hydrogen bonding between (N-H, C=O) group and residues of Asp73,Gly77 amino

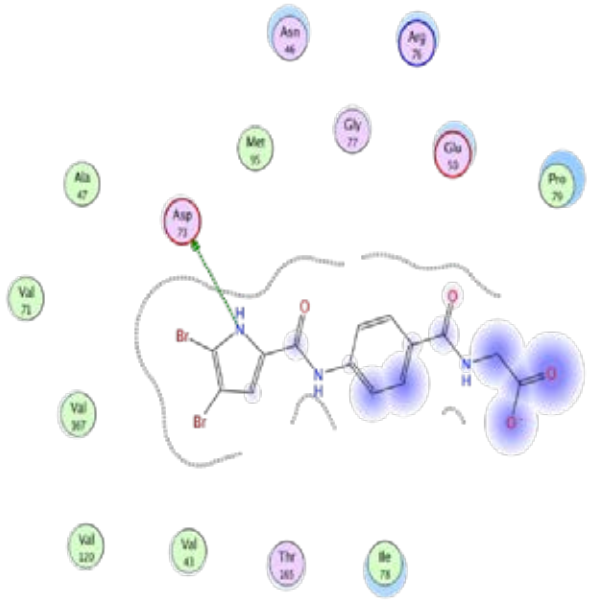


Figure 5: showing a binding mode of crystal ligand of gyrase enzyme as a potent inhibitor

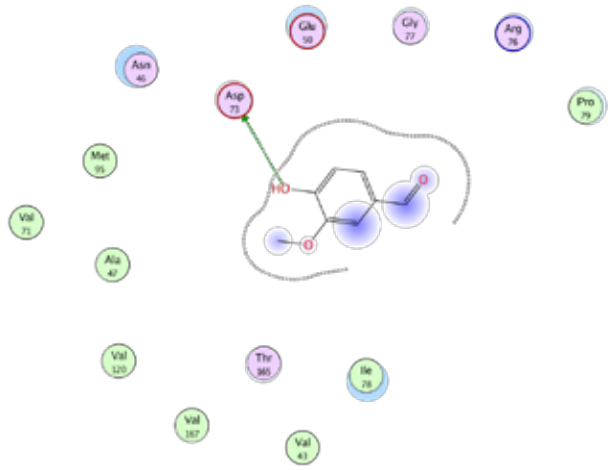


Figure 6: showing binding mode of comp. 2 gyrase enzyme as potent gyrase enzyme inhibitor

Table 6: Binding free energies and No. of bonds for synthesis compounds 1-5

Compounds No.	*Binding free energies (DG) kcal/mol*	*No. of bonds*	
		Pi	*H.b*
1	-0.41	1	0
2	-5.07	2	1
3	-7.09	2	1
4	-6.56	2	1
5	-4.74	1	1

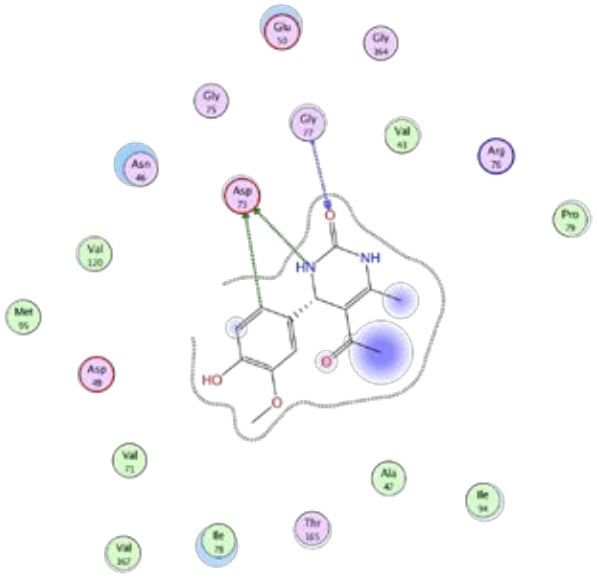


Figure 7: showing binding mode of comp. 3 gyrase enzyme as potent gyrase enzyme inhibitor

acid Respectively while aromatic moiety interacted with Asp73 by hydrophobic interaction. (Figure 7).

The proposed binding mode of compound 4 showed affinity value of -6.56 kcal/mol. It showed two hydrogen bonding between (N-H, C=O) group and residues of Asp73,Gly77 amino acids Respectively while aromatic moiety interacted with Asp73 by hydrophobic interaction (Figure 8).

While The proposed binding mode of compound 5 showed affinity value of -4.77kcal/mol. It showed one hydrogen bonding between (C=S) group and residues of Gly77 amino acid. In addition, the aromatics moieties demonstrated aromatic Stacking (bi-interaction) interaction with Asn46. (Figure 9)

Compound 3 showed binding mode with pocket better than to the general pattern observed by crystal ligand, The affinity value of -7.09 kcal/mol. It showed two hydrogen bonding between (N-H, C=O) group and residues of Asp73,Gly77 amino acid Respectively while aromatic moiety interacted with Asp73 by hydrophobic interaction. are shown in Figure 10.

C log P, Molecular Polar Surface Area and Molecular Volume Correlation

The relationship between the lipophilicity of the newly synthesized compounds and their biological activities was measured through the correlation of gyrase enzyme inhibitors with the C log P values for all of the compounds. The C log P value expresses the degree of lipophilicity of the chemical compound. An increase in this value indicates an increase in the lipophilic character of the tested compound. It is worthwhile to note that the C log P values for our synthesized compounds were ranging from 1.02 to 3.05.

These values may explain the variation in their biological activity compared with their lipophilicity. Interestingly, the C log P values for the most compounds lied in the ideal rang of lipophilicity Based on these results, we noted a correlation between the gyrase inhibition of the targeted compounds

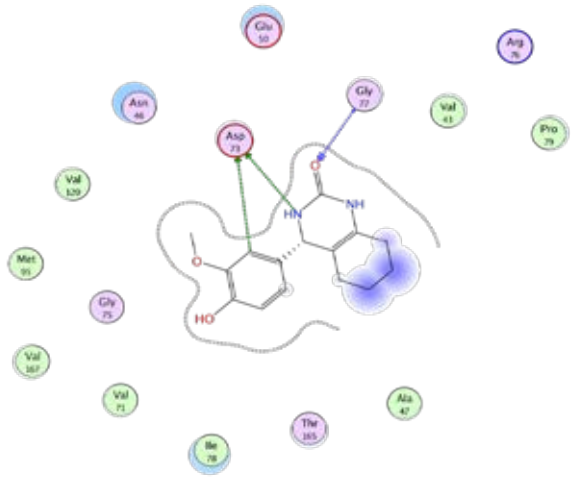


Figure 8: Showing binding mode of comp. 4 gyrase enzyme as potent gyrase enzyme inhibitor

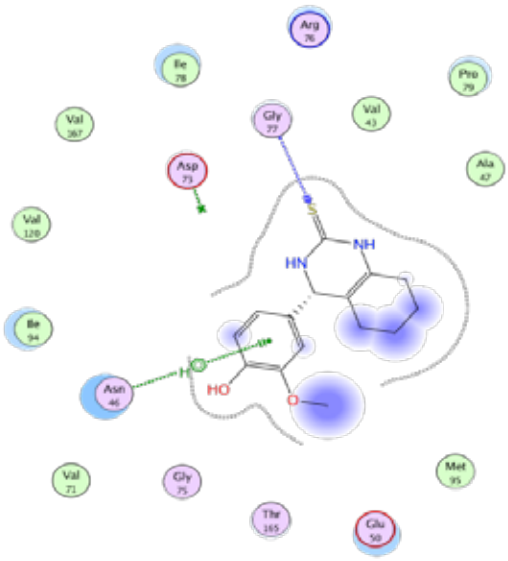


Figure 9: showing binding mode of comp. 5 gyrase enzyme as potent gyrase enzyme inhibitor

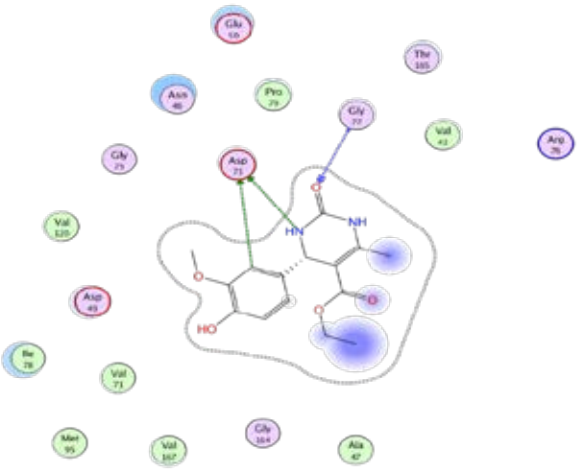


Figure 10: binding mode of comp. 3 gyrase enzyme as potent gyrase enzyme inhibitor

and their lipophilic characters. In addition, the total polar surface area (TPSA) is another key property linked to drug bioavailability; the passively absorbed molecules with TPSA >140 have low oral bioavailability so the synthesized compound showed acceptable values of TPSA. Moreover, molecular volume (M.V) descriptor determines transport characteristics of molecules, such as intestinal absorption It was observed that the synthesized compounds exhibited good molecular volume values, The C log P values, TPSA and M.V values were calculated using Chem-informatics on the Web (http://www.molinspiration.com) and summarized in (Table7).

In-silico ADMET Analysis

ADMET the prediction was carried out with the absorption, distribution, metabolism, excretion and toxicity by ADMET descriptor module of the small molecules protocol of pre-ADMET online software. These descriptors include human intestinal absorption, the solubility of each compound in the water at 25°C, blood-brain penetration (blood-brain barrier BBB) after oral administration, Cytochrome P450 2D6 (CYP2D6) enzyme inhibition and plasma protein binding (Table 8).

It was observed that ability of our compounds to penetrate BBB is very low. So that, all the synthesized compounds were expected to be safe toward central nervous system.

Moreover it was found that most of the synthesized compounds have good human intestinal absorption, It is well known that many drug candidates have failed during clinical tests because of problems related to their absorption properties if HIA value less than 40. The enhancement of absorption is expected to be due to the hydrophobic moiety, which increases the lipophilicity. By investigation of the our compounds it was found that their aqueous solubility logarithmic level equal 4,3 and 2 in most members indicating excellent to moderate aqueous solubility.

The plasma protein binding model predicts good binding ability of a compound to plasma Protein. The cytochrome P450 2D6 model predicts the inhibitory and non-inhibitory behavior of chemical structure. It was found that all the titled compounds are non-inhibitors of CYP2D6. So that, their liver dysfunction effect are not expected upon administration.

Finally, the closer the carcinogenicity scores to one, the more the probability of predict a cancer, while the closer the carcinogenicity scores to zero, the less the probability of predict a cancer so most of the designed compounds are predicted to be safe Table 9.

Pre-Metabolism study.

It is scientifically known that any chemical compounds within the human body must pass on the liver microsomal enzymes and a process of decomposition or metabolism take place in different sites of the compounds based on the type and method of enzyme in dealing with it. These are results of pre-metabolism study experiment show that 3A4 liver microsomal enzyme has lower effect on our compound than 1A2 and 2D6 enzymes, the most metabolic reaction can occur is de alkylation of O-CH3 then reduction occur to be OH group, that's easy to execrate it from human body Table 10.

CONCLUSION

In the present work a series of new dihydropyrimidine derivatives have been synthesized from vanillin by refluxing of urea and acetyl actone or ethylaceto acetate or cyclohenxane, in an acidic medium, then formation of different dihydro pyrmidine, but the reaction of compound (1) with or cyclohenxane and thiourea to give compounds (5). The titled dihydro pyrimidine derivatives were screened in vitro, for their preliminary antibacterial and antifungal activities using (100 and 50μg/ml) conc. New dihydropyrimidine derivatives, (3) and (2) showed good antibacterial activity against, Gram-positive, Gram-negative bacteria, and against fungi used in the evaluation. Docking results revealed that to design optimal compounds that act as Glutaminase domain inhibitor, we

Table 7: C log P, molecular polar surface area and molecular volume of the synthesized compounds 1-5

Comp.	*Clog P*	*MV*	*TPSA*
1	1.07	136.59	96.53
2	1.02	246.90	87.66
3	1.74	272.69	46.89
4	2.51	251.16	40.59
5	3.05	260.04	53.52

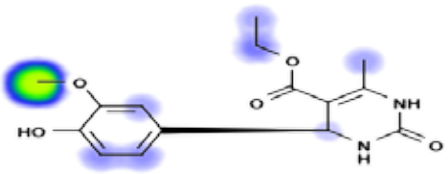
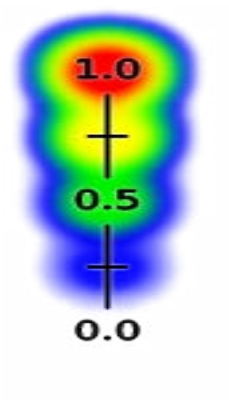
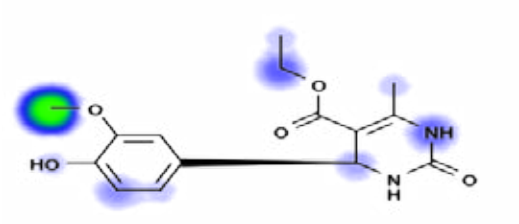
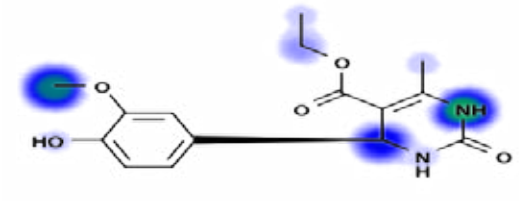
Table 8: Carcinogenicity prediction of the synthesized compounds

Comp.	*Ames test*	*Carcinogen on mouse*	*Carcinogen on rat*	*Carcinogenicity*	*TA100-NA*
1	Mutagen	Negative	Positive	1	Positive
2	Mutagen	Negative	Positive	0	Negative
3	Mutagen	Negative	Negative	0	Negative
4	Mutagen	Negative	Positive	0	Negative
5	Mutagen	Negative	Negative	0	Negative

Table 9: The designed compounds 1-5 by ADMET

Comp. no.	*BBB level*	*HIA*	*CYP2D6*	*PPB*	*Solubility level*
1	0.561	93.05	0	63.14	4
2	0.307	85.13	0	41.77	4
3	0.164	84.49	0	48.98	3
4	1.48	86.90	0	72.78	2
5	2.408	91.77	0	86.43	1

Table 10: Effect of liver microsomal enzymes on compound 3

ENZYME	*CYT. P450 enzymes effect on compound no. 3*	*Reference scale bar*
1A2		
2D6		
3A4		

must consider the following: The synthesized compounds should contain many hydrogen bond acceptor and donator centers to bind with specific amino acid in receptor active site, (Figure 10). A convenient choice of bulky hydrophobic moiety that give more blocking action on active site as compound 3 and 4, Figure 9 and 10 the closer the carcinogenicity scores to one, the more the probability of predicting a cancer. In contrast, the closer the carcinogenicity scores to zero, the less the probability of predict cancer so most of the designed compounds are predicted to be safe. we noted a correlation between the gyrase inhibition of the targeted compounds and their lipophilic characters. Besides, the total polar surface area (TPSA) is another key property linked to drug bioavailability. These are results of the pre-metabolism study experiment show that the 3A4 liver microsomal enzyme has a lower effect on our compound than 1A2 and 2D6 enzymes, the most metabolic reaction can occur is de alkylation of O-CH3 then reduction occur to be OH group, that's easy to execrate it from human body.

ACKNOWLEDGMENT

The authors are grateful to Dr. Abdulrahman M. Saleh, abdo.saleh240@gmail.com, Faculty of pharmacy for boys – Al-Azhar University, Cairo – Egypt. for running docking study and supporting us with the useful data.

REFERENCES

1. Ugi I, Dömling A, Hörl W. Multicomponent reactions in organic chemistry. Endeavour. 1994 Jan 1;18(3):115-122.
2. Armstrong RW, Combs AP, Tempest PA, Brown SD, Keating TA. Multiple-component condensation strategies for combinatorial library synthesis. Accounts of Chemical Research. 1996 Mar 13;29(3):123-131.
3. Tietze LF, Lieb ME. Domino reactions for library synthesis of small molecules in combinatorial chemistry. Current opinion in chemical biology. 1998 Jun 1;2(3):363-371.
4. Dax SL, McNally JJ, Youngman MA. Multi-Component Methodologies Synthesis. Current medicinal chemistry. 1999;6(3):255-270.
5. Dömling A. Isocyanide based multi component reactions in combinatorial chemistry. Combinatorial chemistry & high throughput screening. 1998 Apr;1(1):1-22.
6. Biginelli P. Aldehyde-Urea Derivatives of Aceto- and Oxaloacetic Acids. Gazz. Chim. Ital. 1893; 23: 360-413.
7. Piqani B, Zhang W. Synthesis of diverse dihydropyrimidine-related scaffolds by fluorous benzaldehyde-based Biginelli reaction and post-condensation modifications. Beilstein journal of organic chemistry. 2011 Sep 16;7(1):1294-1298.
8. Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. Nature reviews cancer. 2003 May;3(5):330-338.
9. Dabholkar VV, Gavande RP. Synthesis and antimicrobial activities of novel 1, 4-benzothiazine derivatives. Arabian J Chem., 2016; 9: 225-229.
10. Husseein MD, Mahmood AA, Aljuboori S. Synthesis, antibacterial and DFT study of derivatives derived from oxidation 7-hydroxy 4-methyl coumarin. Int. J. Pharm. Sci. Rev. Res. 2017; 46(2): 196-206.
11. Husseein MD, Ammar MA, Aljuboori S. Synthesis, antibacterial and DFT study of derivatives derived from oxidation 7-hydroxy

4-methyl coumarin. Int. J. Pharm. Sci. Rev. Res. 2017; 46, 196-206.
12. Husseein MD, Aljuboori S, Ammar MA. Synthesis, characterization and antibacterial evaluation with computational study of new schiff bases derived from 7-hydroxy-4-methyl coumarin. Orient. J. Chem. 2017; 33, 768-782.
13. Vektariene A, Vektaris G, Svoboda J. A theoretical approach to the nucleophilic behavior of benzofused thieno[3,2-b]furans using DFT and HF based reactivity descriptors, ARKIVOC. 2009; 7, 311-329.
14. Špirtović-Halilović S, Salihović M, Veljović E, Osmanović A, Trifunović S, Završnik D. Chemical reactivity and stability predictions of some coumarins by means of DFT calculations. Glas Hem Tehnol Bosne Herceg. 2014;43:57-60.
15. I. Laponogov X-S, Pan DA, Veselkov KE, McAuley LM, Fisher MR. Sanderson Structural basis of gate-DNA breakage and resealingby type II topoisomerases, PLoS ONE. 2010; 3:1-5.
16. El-Gamal KM, El-Morsy AM, Saad AM, Eissa IH, Alswah M. Synthesis, docking, QSAR, ADMET and antimicrobial evaluation of new quinoline-3-carbonitrile derivatives as potential DNA-gyrase inhibitors, Journal of Molecular Structure. 2018; 04:010.
17. Sahar B. Al-juboori, Ammara. R.M., Synthesis, antimicrobial evaluation and docking studies of some new 2-Mercapto Pyrimidine Schiff base, Asian J. Pharm. Clin. Res. 2019;12:496-502.