



In silico study of compounds from four tropical medicinal plants as allosteric gyrase inhibitor of multidrug-resistant *E. coli*

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ABSTRACT

The enormity and economic implications due to *Escherichia coli* infections and its resistance to valued antibiotics including fluoroquinolones in poultry production demand urgent alternative drugs from medicinal plants with a novel mechanism of action. Fifteen identified compounds from the four tropical medicinal plants, *Petiveria alliacea*, *Solanum erianthum*, *Alternanthera brasiliana*, and *Hoslundia opposita*, were subjected to molecular docking against the DNA gyrase of *E. coli* using Schrödinger software. The 3-dimensional (3D) x-ray chrystallographic structure of DNA gyrase A of *E. coli* with PDB ID-8QQI was retrieved in a co-crystallized form with isoquinoline-5-sulfonamide from protein data bank (PDB). The ligands were docked into its allosteric site occupied by the co-crystallized ligand. Post docking analyses were carried out through molecular mechanics/generalized born surface area (MM/GBSA), pharmacophore modelling and docking validation. Absorption, distribution, metabolism, excretion and toxicity (ADMET) of the ligands were predicted with appropriate online servers. The docking scores (kcal/mol) revealed gallic acid (-8.37), chlorogenic acid (-7.97), catechin (-7.20) and caffeic acid (-7.02) to be best among others, and also better than that of the reference drug isoquinoline-5-sulfonamide (-3.54). Nevertheless, the co-crystallized ligand recorded higher ΔG_{bind} value (-71.93 kcal/mol) than the hit compounds (-37.50, -36.47, -52.77 and -29.87 kcal/mol). Gallic acid, chlorogenic acid, catechin, and caffeic acid were deemed drug-like following their docking scores, favourable predicted pharmacokinetic, physicochemical and ADMET properties. They are thus suggested for further studies ranging from *in vivo* to hits optimization in order to validate the results from the present study.

Keywords: Medicinal plants, bioactive-compounds, drug-likeness, allosteric site, DNA gyrase

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<https://li01.tci-thaijo.org/index.php/JBAP>

1. Introduction

Avian pathogenic *E. coli* (APEC) has been implicated as the causative agent of many infectious diseases of poultry generally referred to as “colibacillosis”, which causes, for example, air sacculitis, coligranuloma, omphalitis, peritonitis, synovitis, arthritis, salpingitis, osteomyelitis and panophthalmitis.¹ These infections are usually controlled through the administration of antibiotic drugs like furazolidone, neomycin, flumquine, tetracycline, fluoroquinolones etc. Rajesh et al.² However, indiscriminate use of these drugs has led to the problem of antibiotic resistance.³ The detrimental effects of which have been reported in animals, human, plants, environment and soil by Bennani et al.⁴ According to the estimated values by Van Boeckel et al.⁵ 131,109 tonnes of antimicrobials were used in food animals, and this is projected to rise to about 200, 234 tonnes by the year 2030. In animals, multi-drug-resistant pathogens have emerged after the application of sub-therapeutic doses of tetracycline in feed for the purpose of growth promotion. It was observed that after a few days, chickens excreted tetracycline-resistant *E. coli* and at two weeks, the excreted *E. coli* was resistant to several antibiotics as reported by Nhung et al.⁶ *E. coli* from the gastrointestinal tract of animals and humans can be described as a reservoir for a wide variety of multi-drug-resistant genes. The first generation quinolones, such as nalidixic acid, were optimized with fluorine and thus gave rise to the second generation of antibiotics including ciprofloxacin, levofloxacin and norfloxacin. Further developments produced the third (sparfloxacin) and fourth (delafloxacin and moxifloxacin) generation drugs.⁷ The fluoroquinolone antibiotics are validated antibiotics in the market for clinical usage. They exhibit a dual-targeting mechanism of inhibiting DNA gyrase and topoisomerase IV. Inhibition of two enzymes simultaneously has made it difficult for bacteria to develop resistance rapidly, which makes it promising to develop novel antimicrobials of this type.⁸⁻⁹

DNA gyrase is a topoisomerase protein which consists of an A₂B₂ heterotetramer. This enzyme functions in catalyzing the stepwise negative supercoiling of DNA with the concomitant hydrolysis of an ATP to ADP + P_i. It has been reported that DNA gyrases are inhibited by a number of substances including novobiocin from the coumarin family of antibiotics and ciprofloxacin, a member of the quinolone family of antibiotics. These drugs are known as potential inhibitors of bacterial DNA replication and RNA transcription.¹⁰

Despite the success of the fluoroquinolones, resistance to these drugs has been frequently reported in clinics.¹¹⁻¹² Regrettably, for over forty years of administering fluoroquinolones in clinical use, target-mediated drug resistance has rapidly increased and thus reduced the medical potential of this important antibacterial class against many infections.

The production of new drugs is one of the approaches to reduce antibiotic resistance, especially those that can offer entirely new mechanisms of action that could effectively counteract the common resistance.¹³ Fortunately, gyrase and topoisomerase IV have again become central to the development of antibacterials. Triazaace-naphthylenes and spiropyrimidinetriones (two novel classes of compounds) were developed for the treatment of some infections via DNA gyrase and topoisomerase IV targets. These compounds interact with different amino acids at topoisomerase binding sites compared to fluoroquinolones. Positive outcomes in phase III clinical trials were reported on two members of both classes, i. e., gepotidacin and zoliflodacin.⁷

Further drug mechanisms targeting allosteric sites of these validated enzymes have been reported. This discovery offers a promising possibility for future structure-based molecular design strategies at these sites. The introduction of advanced computational modeling techniques has become crucial for designing and synthesizing dual-targeting inhibitory compounds.¹⁴ Chan et al.

have reported the inhibitory activity of thiophene carboxamide against *Staphylococcus* species.¹⁵ In a co-crystal structure, this test drug was observed to be bound within a pocket between the GyrA and GyrB subunits. This pocket represents a completely different site from the normal binding site of the fluoroquinolones and is referred to as an allosteric inhibition site. The drug thiophene carboxamide was discovered to be efficacious against *Escherichia coli* DNA gyrase by Chan et al¹⁵ and Thalji et al¹⁶, as the organism contains topoisomerase I and topoisomerase III (type I), both of which are dimeric and capable of reducing negative supercoiling in DNA.¹⁷ Biphenyl-based allosteric inhibitors and the antibiotic evyactin were also reported by Orritt et al to have the same activity as thiophenes against type II topoisomerase.¹⁸

In line with finding a solution to the global problem of antimicrobial resistance, medicinal plants have been reported to contain compounds of high prophylactic and therapeutic potentials, potentially preventing the establishment and spread of multi-drug resistant bacteria.¹⁹ This study therefore seeks to identify potential natural antibacterial compounds from *Petiveria alliacea*, *Solanum erianthum*, *Alternanthera brasiliana*, and *Hoslundia opposita* that could act as allosteric inhibitors of *E. coli* DNA gyrase by targeting the same binding site as that of the co-crystallized isoquinoline sulfonamide.

2. Materials and Methods

2.1 Preparation of the bioactive compounds

In our previous study (unpublished), 15 ligands including gallic acid, chlorogenic acid, catechin, caffeic acid, protocatechuic acid, o-hydroxybenzoic acid, sinapic acid, vanillic acid, t-ferulic acid, m-hydroxybenzoic acid, biochanin A, 1-anthrol, p-coumaric acid, syringic acid, and t-cinnamic acid were characterized from four tropical medicinal plants, namely, *Petiveria alliacea*, *Solanum erianthum*, *Alternanthera brasiliana*, and *Hoslundia opposita*. The preparation of the

compounds was done through the ligprep panel on Schrödinger Maestro software.²⁰ Force field was set to OPLS4 at a pH of 7.0 +/- 2.0 while the stereoisomer generation and output format were left at default according to Omoboyowa et al.²¹

2.2 Retrieval of target protein and preparation

The three-dimensional (3D) x-ray chrystallographic structure of DNA gyrase A of *E. coli* was retrieved in a co-crystallized form with Isoquinoline-5-sulfonamide from the protein data bank (PDB-ID 8QQI). Isoquinoline-5-sulfonamide thus serves as a reference compound in this study. The crystal structure of the protein was uploaded to the workspace of Schrödinger Maestro, pre-processed, optimized and minimized using the protein preparation wizard. During the preparation of the protein, zero bonds and disulfide bonds were created. Bond orders were assigned, water molecules were deleted and other chains were deleted, leaving only chain A. The glide grid was generated with X, Y, Z, coordinates of 204.23, 222.12, and 180.53, respectively.

2.3 Glide docking

Following the preparation of glide grid, the receptor grid was generated. The docking was performed in the allosteric binding site of the protein, as identified by the co-crystallized ligand¹² by employing the extra precision (XP) module. Rotational bonds were set free and the programme was allowed to run as described by Omoboyowa et al.²¹

2.4 Molecular mechanics/generalized born surface area (MM/GBSA)

MM/GBSA was calculated following docking in order to establish the stability of the binding complex through the prime MM/GBSA tool. OPLS4 field was selected while other parameters were left at default (Eleje et al).²²

2.5 Docking validation

The molecular docking was followed by validating the docking protocol on the workspace of Maestro. This involved re-docking the co-crystallized compound into

the active site of DNA gyrase. The compounds were aligned and the Root Mean Square Deviation (RMSD) was measured in angstrom, following the protocol of Olajubutu et al.²³

2.6 E-pharmacophore modelling

A pharmacophore as defined by International Union of Pure and Applied Chemistry (IUPAC) is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response (Wermuth et al).²⁴ Pharmacophores are an essential concept in computer-aided drug design (CADD). They are useful during the optimization of hit compounds. A four-point pharmacophore was developed in this study, based on the standard drug complex using partitioning features as reported by Saibu et al.²⁵

2.7 General properties, drug-likeness, pharmacokinetics and ADMET predictions of the characterized compounds

The general properties, physicochemical properties, drug-likeness, pharmacokinetics including absorption, distribution, metabolism, excretion and toxicity (ADMET) predictions were carried out using ADMETlab and SwissADME Web server tools as reported by Olugbogi et al.²⁶

3. Results

The results from this study are presented in Tables 1 to 6 and Figures 1 to 3. They show docking, MM/GBSA and fitness scores, hydrogen bonds and hydrophobic interactions of hit compounds with DNA gyrase.

Table 1 Docking, MM/GBSA and fitness scores.

S/N	Compound names	CID	Docking Score (kcal/mol)	MM/GBSA score ΔG_{bind} (kcal/mol)	Fitness score
1.	Gallic acid	370	-8.37	-37.50	0.29
2.	Chlorogenic acid	1794427	-7.97	-36.47	0.29
3.	Catechin	9064	-7.20	-52.77	0.29
4.	Caffeic acid	689043	-7.02	-29.87	0.29
5.	Protocatechuic acid	72	-6.91	-56.43	0.22
6.	o-Hydroxybenzoic acid	338	-6.54	--	0.29
7.	Sinapic acid	637775	-6.53	-34.82	0.29
8.	Vanillic acid	8468	-6.49	-29.15	0.29
9.	t-Ferulic acid	445858	-6.24	-42.77	0.29
10.	m-Hdroxybenzoic acid	7420	-5.74	-43.69	0.27
11.	Biochanin A	5280373	-5.68	-36.43	0.29
12.	1-Anthrol	123077	-5.52	-31.71	0.09
13.	p-Coumaric acid	637542	-5.34	-30.33	0.29
14.	Syringic acid	10742	-5.29	- 0.24	----
15.	t-Cinnamic acid	444539	-4.63	-40.78	0.09
16.	Isoquinoline-5-sulfonamide (LRL)	171662160	-3.54	-71.93	2.01

MM/GBSA= Molecular Mechanics/Generalized Born Surface Area

Table 2 Hydrogen bonds, hydrophobic and other interacting amino acids within the DNA gyrase allosteric pocket.

S/N	Compound names	Docking Score (kcal/mol)	Number of H-Bonds	H-bond interacting amino acids	Hydrophobic interacting amino acids	Other interacting amino acids	Pi-Pi Stacking
1.	Gallic acid	-8.37	5	ILE 112, VAL 268, GLN 267, PHE 96	ILE 112, PHE 96, VAL 268, TYR 266, PRO 218, PRO 265, ILE 264	SER 97, LYS 270, ASN 269, THR 219, ASP 113, GLY 114	1
2.	Chlorogenic acid	-7.97	7	GLN 267, VAL 268, PHE 96, SER 111, GLN 94, GLY 114, ASP 115	PRO 218, TYR 266, PRO 265, ILE 264, VAL 268, PHE 96, ILE 112, ALA 117	SER 97, GLN 94, ASN 269, GLN 267, THR 219, GLY 110, GLY 114, ASP 115, SER 116	1
3.	Catechin	-7.20	4	ASP 115, ILE 112, VAL 268, PHE 96	ILE 112, VAL 268, TYR 266, ILE 264, PHE 96	SER 97, SER 116, GLY 114, SER 111, GLY 110, LYS 270, ASN 269, THR 219, GLN 267, GLN 94	1
4.	Caffeic acid	-7.02	4	VAL 268, SER 97, GLN 94	ILE 112, VAL 268, TYR 266, PRO 265, ILE 264, PHE 96	ASN 269, GLN 267, ARG 91	1
5.	Protocatechuic acid	-6.91	3	VAL 268, GLN 267, PHE 96	ILE 112, PHE 96, VAL 268, TYR 266, PRO 265, ILE 264, PRO 218	GLY 114, ASN 269, THR 219, SER 97	1

Table 2 (Cont.)

S/N	Compound names	Docking Score (kcal/mol)	Number of H-Bonds	H-bond interacting amino acids	Hydrophobic interacting amino acids	Other interacting amino acids	Pi-Pi Stacking
6.	o-Hydroxybenzoic acid	-6.54	1	PHE 96	ILE 112, VAL 268, TYR 266, PRO 218, ILE 264, PHE 96	ASN 269, GLN 267, THR 219, SER 97	1
7.	Sinapic acid	-6.53	2	ILE 112, PHE 96	ILE 112, VAL 268, TYR 266, PRO 218, ILE 264, PHE 96, PRO 265	SER 111, ASP 113, GLY 114, ASN 269, GLN 267, SER 97, THR 219	1
8.	Vanillic acid	-6.49	2	GLN 267, PHE 96	ILE 112, VAL 268, TYR 266, PRO 218, ILE 264, PHE 96	GLY 114, SER 111, ASN 269, THR 219, SER 97	2
9.	t-Ferulic acid	-6.24	3	ILE 112, PHE 96, GLN 267	ILE 112, PHE 96, PRO 218, ILE 264, PRO 265, TYR 266, VAL 268	SER 111, ASP 113, GLY 114, SER 97, THR 219, GLN 267, ASN 269, ARG 272	1
10.	m-Hydroxybenzoic acid	-5.74	3	ILE 112, GLN 267, PHE 96	ILE 112, VAL 268, TYR 266, PHE 96	GLY 114, ASP 113, LYS 270, ASN 269, THR 219, SER 97	1
11.	Biochanin A	-5.68	4	VAL 268, PHE 96, SER 97, ARG 91	ILE 112, VAL 268, TYR 266, PRO 265, ILE 264, PHE 96, VAL 90	ASN 269, GLN 267, THR 219, GLN 94, ASP 87	2

Table 2 (Cont.)

S/N	Compound names	Docking Score (kcal/mol)	Number of H-Bonds	H-bond interacting amino acids	Hydrophobic interacting amino acids	Other interacting amino acids	Pi-Pi Stacking
12.	1-Anthrol	-5.52	2	GLN 267, PHE 96	ILE 112, VAL 268, TYR 26, ILE 264, PHE 96	ASN 269, THR 219, SER 97, ARG 91	2
13.	p-Coumaric acid	-5.34	2	ILE 112, PHE 96	ILE 112, VAL 268, TYR 266, PRO 265, ILE 264, PHE 96, PRO 218	SER 111, ASP 113, GLY 114, ASN 269, GLN 267, SER 97, THR 219	1
14.	Syringic acid	-5.29	4	ILE 112, GLN 267, PHE 96	ILE 112, VAL 268, TYR 266, PRO 265, ILE 264, PHE 96	GLY 114, SER 111, LYS 270, ASN 269, THR 219, GLN 94, SER 97	1
15	t-Cinnamic acid	-4.63	2	ASN 165, GLY 170	HET 92, LEU 98, LEU 166, LEU 41, LEU 102	ARG 91, THR 88, GLY 40, ASN 169, SER 171	1
16	Isoquinoline-5-sulfonamide (LRL)	-3.54	2	GLY 170, ASN 165	MET 92, LEU 41, LEU 102, LEU 166, ILE 112, VAL 268, TYR 266, ILE 264, PHE 96, LEU 98	SER 97, GLY 40, SER 172, LYS 42, THR 83, THR 219, GLN 267, ASN 269, SER 171, ASN 169, ASN 165, ARG 91, HIE 45	2

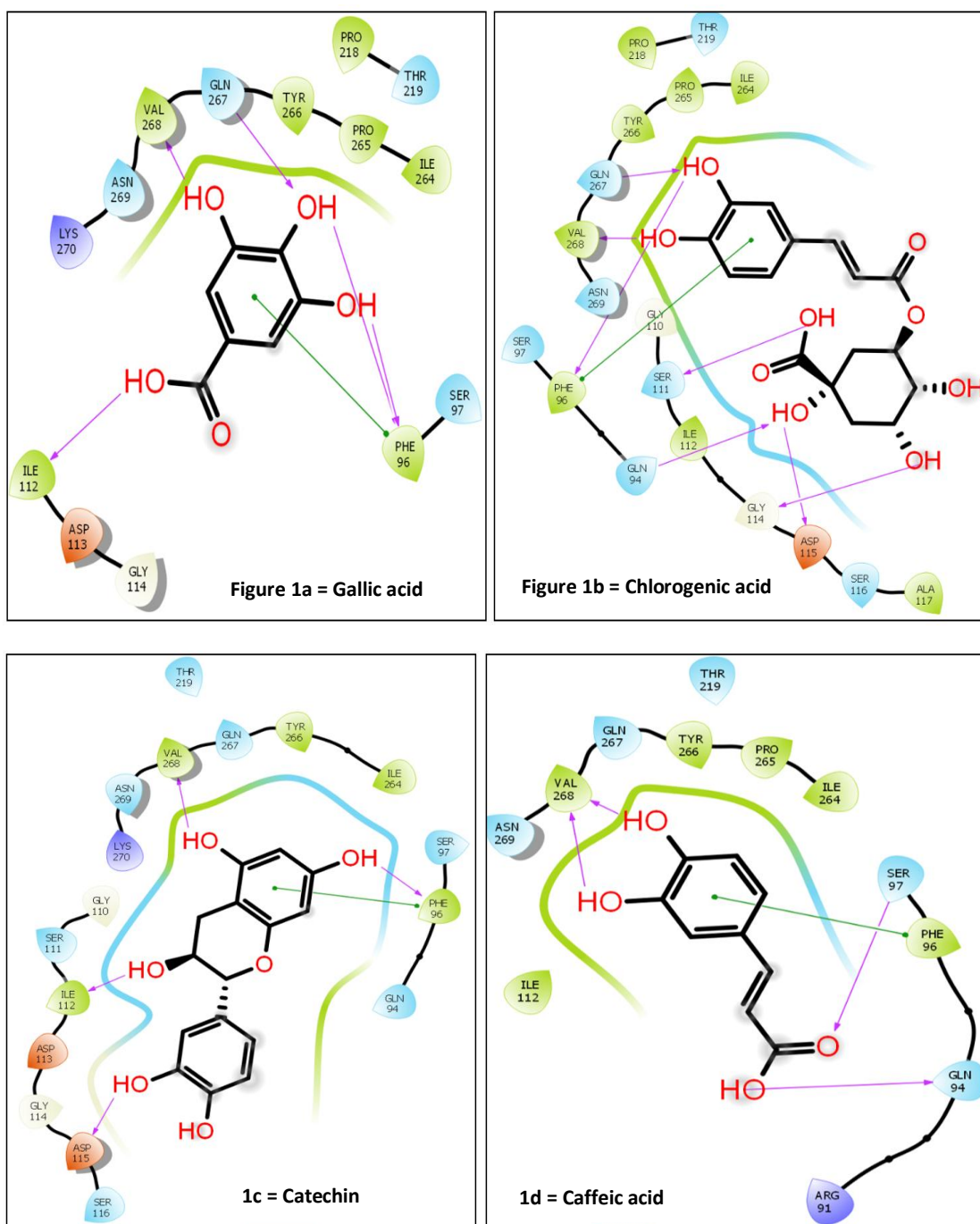
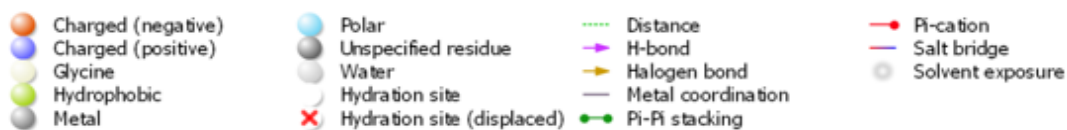


Fig. 1. (a, b, c & d) Compounds in the allosteric binding pocket of DNA gyrase of *E. coli* in 2D.



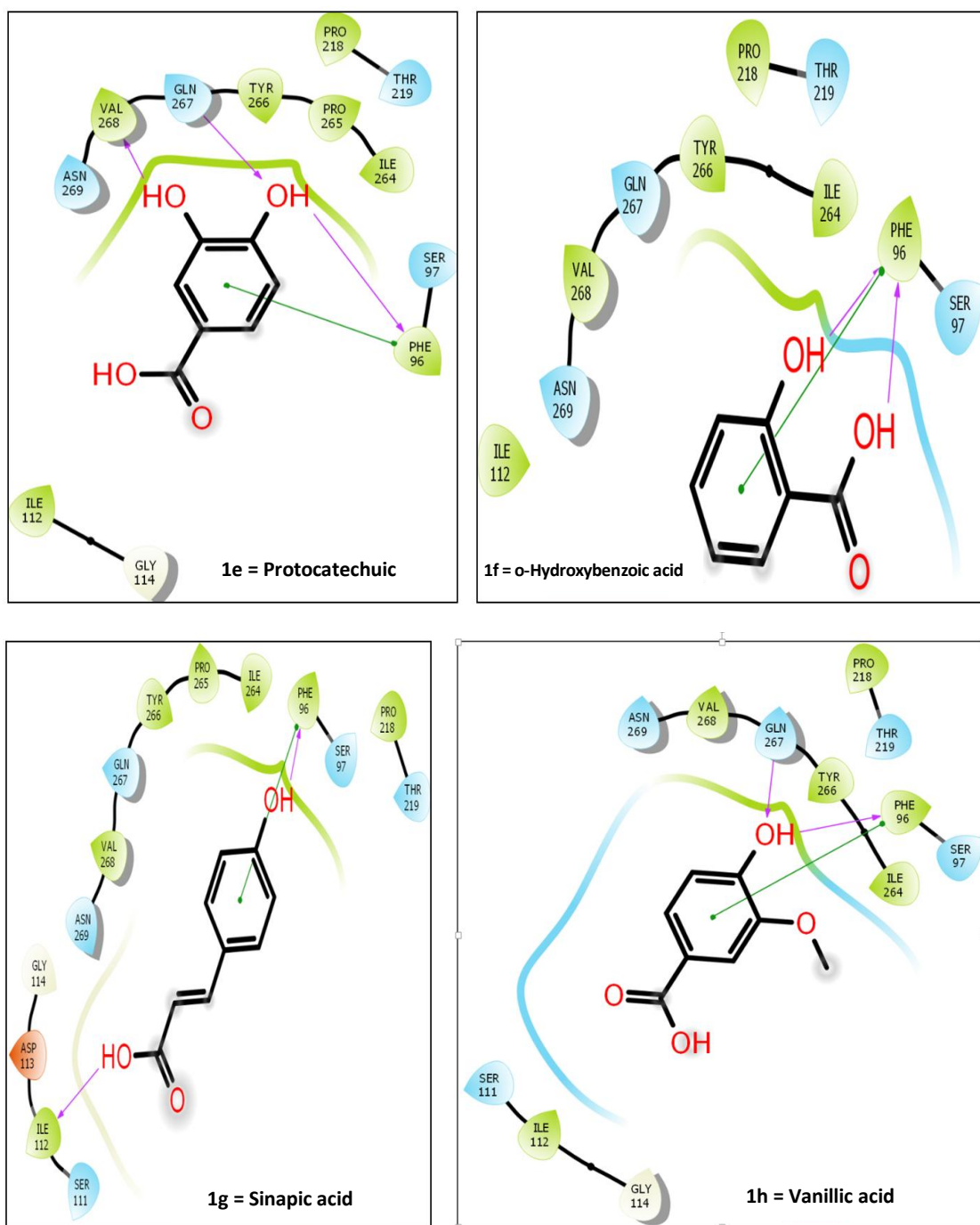
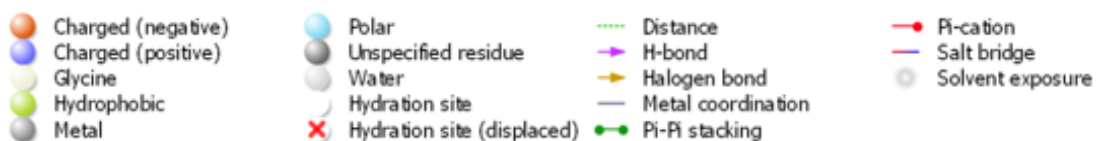


Fig. 1. (e, f, g & h) Compounds in the allosteric binding pocket of DNA gyrase of *E. coli* in 2D.



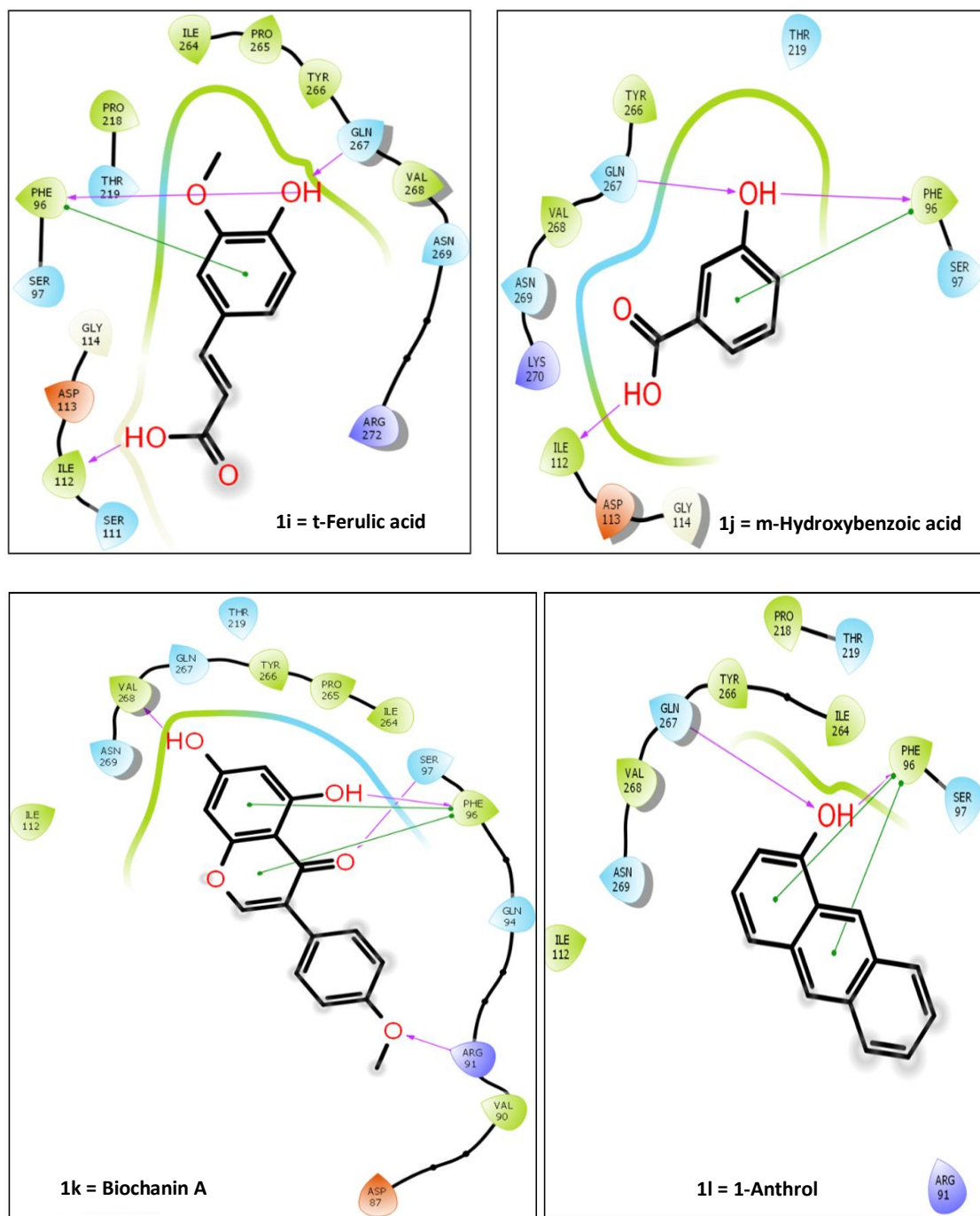
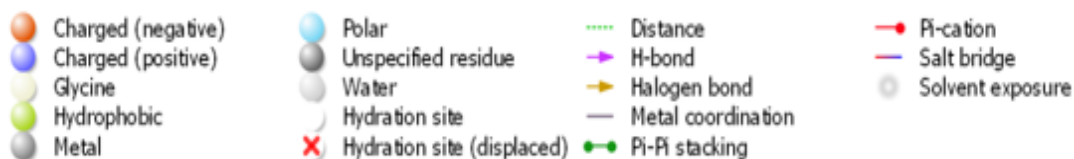


Fig.1. (i, j, k & l) Compounds in the allosteric binding pocket of DNA gyrase of *E. coli* in 2D.



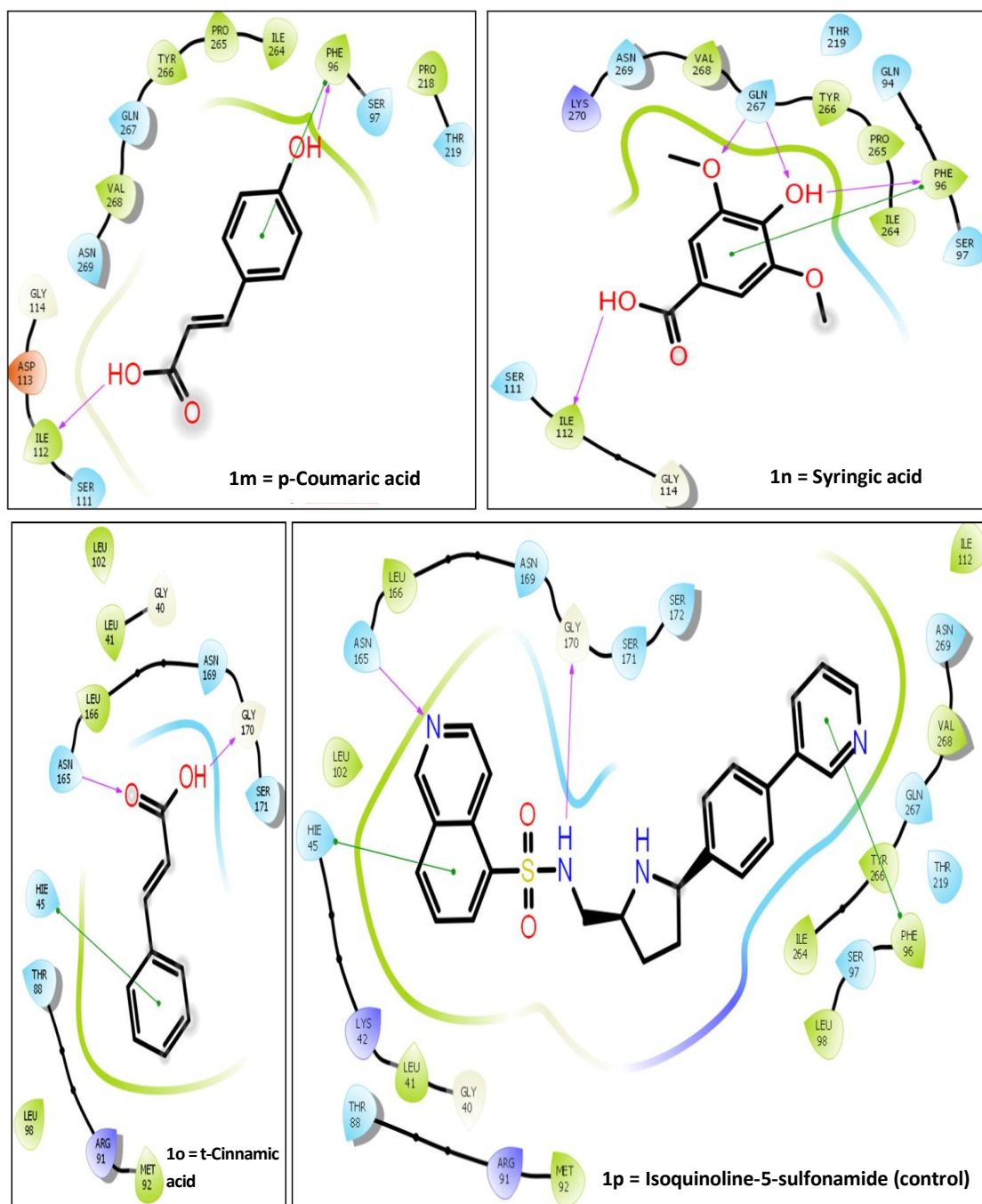


Fig. 1. (m, n, o & p) Compounds in the allosteric binding pocket of DNA gyrase of *E. coli* in 2D.

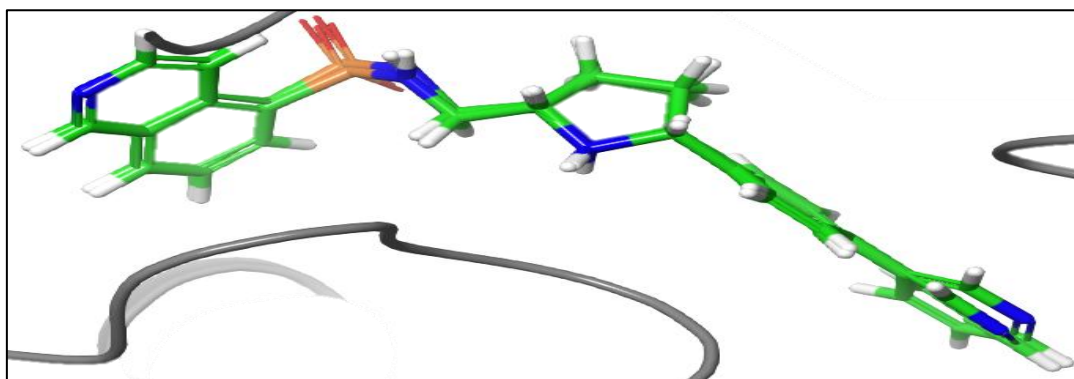


Fig. 2. Superposition of docked (under) and standard (upper) compounds, showing their alignment (RMSD = 0.28 Å).

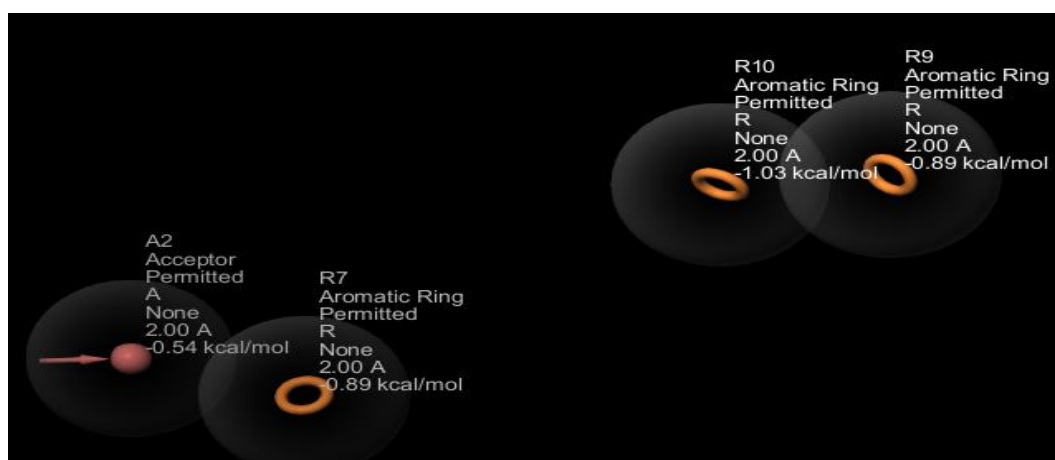


Fig. 3a. Generated pharmacophore hypothesis of isoquinoline-5-sulfonamide.

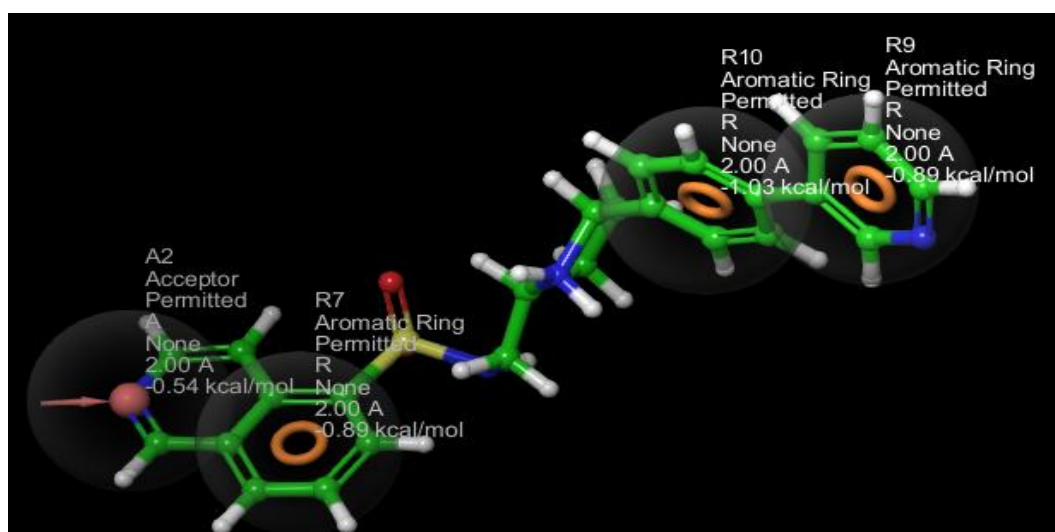


Fig. 3b. Pharmacophore hypothesis aligned with isoquinoline-5-sulfonamide.

Table 3 Characterized compounds and their general properties.

S/N	Compound	Solb	RB	BP (0°)	MP (0°)	HFE	VAP	pKa Acid	pKa Base
1	Gallic acid	Very soluble	1	376.47	231.74	-12.64	-6.13	5.69	3.71
2	Chlorogenic acid	Soluble	5	456.41	201.67	-9.41	-13.66	3.22	7.01
3	Catechin	Soluble	1	459.8	231.88	-12.51	-8.71	7.08	6.31
4	Caffeic acid	Soluble	2	356.62	288.17	-11.66	-6.19	5.49	4.68
5	Protocatechuic acid	Soluble	3	334.16	240.99	-11.84	-5.33	5.48	1.72
6	o-Hydroxybenzoic acid	Soluble	1	281.63	162.06	-8.94	-4.3	3.23	-0.93
7	Sinapic acid	Soluble	4	332.74	190.95	-11.35	-6.42	5.04	3.44
8	Vanillic acid	Soluble	2	301.37	209.06	-10.2	-5.03	4.16	2.51
9	t-Ferulic acid	Soluble	3	316.4	199.13	-9.95	-5.98	5.3	3.91
10	m-Hydroxybenzoic acid	Soluble	1	299.07	191.24	-9.63	-4.64	4.34	-1.19
11	Biochanin A	Moderately soluble	2	435.02	233.41	-12.4	-7.87	7.63	4.86
12	1-Anthrol	Moderately soluble	0	368.67	166.94	-7.83	-6.9	9.75	5.32
13	p-Coumaric acid	Soluble	2	325.12	253.12	-9.28	-5.36	4.49	2.29
14	Syringic acid	Soluble	3	317.02	195.17	-11.36	-5.39	4.17	2.63
15	t-Cinnamic acid	Soluble	2	287.63	172.9	-5.68	-3.19	4.04	-0.69
16	Isoquinoline-5-sulfonamide (LRL)	Moderately soluble	6	540.81	209.67	-3.42	-11.06	8.39	8.43

Solb = Solubility, RB = Number of rotatable bonds (≤ 10), BP = boiling point, MP = melting point, HFE = hydration free energy (kcal/mol), VAP = vapour pressure (LogVP), pK_a Acid = $-\log K_a$, pK_a Base = $-\log K_a$

Table 4 Physicochemical and drug-likeness predictions of hit compounds.

S/N	Compound	MW (g/mol) ≤500	HBA ≤10	HBD ≤5	MLOGP ≤4.5	TPSA (Å ²)	ROF	Remark
1	Gallic acid	170.12	5	4	-0.16	97.99	0	Yes
2	Chlorogenic acid	354.31	9	6	-1.05	164.75	1	Yes
3	Catechin	290.27	6	5	0.24	110.38	0	Yes
4	Caffeic acid	180.16	4	3	0.7	77.76	0	Yes
5	Protocatechuic acid	154.12	4	3	0.40	77.76	0	Yes
6	o-Hydroxybenzoic acid	138.12	3	2	0.99	57.53	0	Yes
7	Sinapic acid	224.21	5	2	0.73	75.99	0	Yes
8	Vanillic acid	168.15	4	2	0.74	66.76	0	Yes
9	t-Ferulic acid	194.18	4	2	1.00	66.76	0	Yes
10	m-Hydroxybenzoic acid	138.12	3	2	0.99	57.53	0	Yes
11	Biochanin A	284.26	4	2	0.77	79.90	0	Yes
12	1-Anthrol	194.23	1	1	3.45	20.23	0	Yes
13	p-Coumaric acid	164.16	3	2	1.28	57.53	0	Yes
14	Syringic acid	198.17	5	2	0.49	75.99	0	Yes
15	t-Cinnamic acid	148.16	2	1	1.9	37.30	0	Yes
16	Isoquinoline-5-sulfonamide	444.56	6	2	1.69	92.36	0	Yes

MW = molecular weight (≤ 500), HBA = hydrogen bond acceptors (≤ 10), HBD = hydrogen bond donors (≤ 5), MLOGP = Moriguchi octanol-water partition coefficient (≤ 4.15), $\text{LogP} > 0$ = lipophilic, $\text{LogP} < 0$ = hydrophilic, TPSA = topological polar surface area (≤ 140), and ROF= number of rule of five violations

Table 5 Compounds' absorption, distribution and metabolism prediction.

S/N	Compound names	IA	BBB	1A2	2C9	2D6	2C19
1.	Gallic acid	+	--	NI	NI	NI	NI
2.	Chlorogenic acid	+	+	NI	NI	NI	NI
3.	Catechin	+	--	NI	NI	NI	NI
4.	Caffeic acid	+	--	NI	NI	NI	NI
5.	Protocatechuic acid	+	--	NI	NI	NI	NI
6.	o-Hydroxybenzoic acid	+	+	NI	NI	NI	NI
7.	Sinapic acid	+	+	NI	NI	NI	NI
8.	Vanillic acid	+	--	NI	NI	NI	NI
9.	t-Ferulic acid	+	+	NI	NI	NI	NI
10.	m-Hydroxybenzoic acid	+	+	NI	NI	NI	NI
11.	Biochanin A	+	--	I	I	NI	I
12.	1-Anthrol	+	+	I	NI	NI	NI
13.	p-Coumaric acid	+	+	NI	NI	NI	NI
14.	Syringic acid	+	+	NI	NI	NI	NI
15.	t-Cinnamic acid	+	+	NI	NI	NI	NI
16.	Isoquinoline-5-sulfonamide	+	+	NI	NI	NI	I

IA = intestinal absorption, BBB = blood brain barrier, 1A2, 2C9, 2D6 and 2C19 are cytochrome P450 isozymes, I = inhibitor and NI = non-inhibitor

Table 6 Excretion and toxicity predictions of the compounds.

S/N	Compounds	Clearance rate	Half life (< 8 h)	AVT	MUT	CAR	TCL	BIOD
1.	Gallic acid	9.83	> 3 h	Safe	Safe	Safe	III	RBD
2.	Chlorogenic acid	6.32	> 3 h	Safe	Safe	Safe	III	NRBD
3.	Catechin	13.60	< 3 h	Safe	Safe	Safe	IV	NRBD
4.	Caffeic acid	9.38	> 3 h	Safe	Safe	Safe	IV	RBD
5.	Protocatechuic acid	9.06	> 3 h	Safe	Safe	Safe	III	RBD
6.	o-Hydroxybenzoic acid	5.22	< 3 h	Safe	Safe	Safe	III	RBD
7.	Sinapic acid	10.73	< 3 h	Safe	Safe	Safe	III	RBD
8.	Vanillic acid	13.28	< 3 h	Safe	Safe	Safe	III	RBD
9.	t-Ferulic acid	10.86	< 3 h	Safe	Safe	Safe	IV	RBD
10.	m-Hydroxybenzoic acid	6.10	< 3 h	Safe	Safe	Safe	III	RBD
11.	Biochanin A	3.04	< 3 h	Safe	Safe	Safe	III	NRBD
12.	1-Anthrol	6.74	< 3 h	Safe	Safe	Safe	III	NRBD
13.	p-Coumaric acid	5.48	< 3 h	Safe	Safe	Safe	III	RBD
14.	Syringic acid	12.13	< 3 h	Safe	Safe	Safe	II	RBD
15.	t-Cinnamic acid	4.84	< 3 h	Safe	Safe	Safe	III	RBD
16.	Isoquinoline-5-sulfonamide	6.64	< 3 h	Safe	Safe	Safe	III	NRBD

AVT = avian toxicity, Clearance Rate (< 15 mL/min/kg), MUT = mutagenicity, CAR = carcinogenicity, TCL = toxicity classification, BIOD = biodegradability, RBD = readily biodegradable, NRBD = non-readily biodegradable. Class I: $LD_{50} \leq 50$ mg/kg, Class II: $50 \text{ mg/kg} < LD_{50} < 500$ mg/kg, Class III: $500 \text{ mg/kg} < LD_{50} < 5000$ mg/kg and Class IV: $LD_{50} > 5000$ mg/kg.

4. Discussion

Molecular docking evaluates the inhibitory potential of plant phytochemicals against target proteins,²⁷ requiring precise selection of the protein and organism for maximum efficiency. In *E. coli*, DNA gyrase is essential for replicating its circular chromosomes,²⁸⁻²⁹ making it an ideal antibiotic target.³⁰ Fluoroquinolones (e.g., ciprofloxacin, levofloxacin, and nalidixic acid) act by binding to the topoisomerase-DNA interface, preventing DNA re-ligation and causing lethal chromosomal fragmentation³¹ that induces cell death. However, rising bacterial resistance to fluoroquinolones necessitates the development of new drugs with novel mechanisms of action. To address this, Bakker et al¹² discovered LEI-800, a compound effective against Gram-negative bacteria. Unlike fluoroquinolones, LEI-800 targets a unique allosteric binding pocket on DNA gyrase. This study explores that same allosteric site for targeted drug interactions.

Virtual screening revealed that gallic acid achieved the highest docking score (-8.37 kcal/mol), followed by chlorogenic acid (-7.97 kcal/mol), catechin (-7.20 kcal/mol), and caffeic acid (-7.02 kcal/mol), while the reference drug isoquinoline-5-sulfonamide recorded -3.54 kcal/mol. Both molecular docking and MM/GBSA calculations demonstrated interactions with numerous amino acid residues, likely contributing to the ligands' inhibitory activity within the DNA gyrase allosteric pocket. Hydrogen bonds play an essential role in catalysis, protein folding, and ligand-protein interactions.³² In this study, gallic acid, chlorogenic acid, catechin, and caffeic acid formed substantial hydrogen bond networks, establishing five, seven, four, and four hydrogen bonds, respectively. In contrast, the reference drug formed only two hydrogen bonds. This structural stability highlights the potential efficacy of these compounds as plant-derived antimicrobials.

Bakker et al.¹² highlighted the importance of key interacting residues within the allosteric pocket, including ASN269, ILE112, PHE96, LEU98, ASN169, LYS42, MET92, HIS45, and SER172, noting that LYS42, SER97, and SER172 formed hydrogen bonds with their reference compound. This aligns with the present study, where SER97 also established hydrogen bonds with caffeic acid while phenylalanine (PHE) primarily formed hydrogen bonds with our ligands, it engaged chiefly in hydrophobic interactions in the study by Olugbogi et al.²⁶ Furthermore, five critical residues ILE112, PHE96, VAL268, TYR266, and ILE264 consistently mediated hydrophobic interactions for both the four hits and the reference drug, while ASN269 consistently acted as a common polar interacting residue. Additionally, a higher frequency of Pi-Pi stacking interactions was observed across all ligands and isoquinoline-5-sulfonamide in this work. These Pi-Pi interactions are highly advantageous for drug delivery systems because they do not compromise the functional or structural properties of the cargo.³³ Ultimately, the interplay of hydrogen bonds, hydrophobic interactions, and electrostatic forces remains crucial for driving specific biological processes.³⁴⁻³⁵

MM/GBSA calculations provide deeper insights into binding affinities, offering critical information regarding the strength and stability of interactions between the *E. coli* target protein and each ligand.³⁶ In this study, the reference drug exhibited the strongest binding free energy ($\Delta G_{\text{bind}} = -71.93$ kcal/mol), followed by catechin (-52.77 kcal/mol), gallic acid (-37.50 kcal/mol), and chlorogenic acid (-36.47 kcal/mol), while caffeic acid showed the weakest affinity (-29.87 kcal/mol). Surprisingly, protocatechuic acid (one of the identified bioactive compounds); despite its modest docking score of -6.91 kcal/mol yielded a ΔG_{bind} value of -56.43 kcal/mol, outperforming all the plant-derived hits. This indicates that further

structural optimization of protocatechuic acid could enhance its potential as a promising drug candidate.

Root Mean Square Deviation (RMSD) is a critical post-docking analysis used to assess the accuracy of a docking protocol. Effective docking typically yields a low RMSD, indicating a close structural resemblance between the native and re-docked ligand conformations. Conversely, high RMSD points to significant conformational changes in the ligand upon binding. In this study, the RMSD obtained between the native and re-docked ligand was 0.28 Å. This close alignment demonstrates that the docking protocol produced highly accurate compound positioning, falling well within the scientifically accepted threshold of ≤ 2 Å for valid conformations.³⁷

Pharmacophore modeling is a powerful tool in computer-aided drug design used to identify compounds possessing the molecular features essential for macromolecular binding and biological activity.³⁸ These pharmacophoric features typically include hydrogen bond donors, hydrogen bond acceptors, hydrophobic regions, aromatic rings, and charged centers.³⁹ In this study, the generated pharmacophore model mapped four features: three aromatic rings and one hydrogen bond acceptor. The reference drug achieved the highest fitness score (2.01). In contrast, gallic acid, chlorogenic acid, catechin, caffeic acid, o-hydroxybenzoic acid, sinapic acid, vanillic acid, t-ferulic acid, biochanin A, and p-coumaric acid all shared a fitness score of 0.29. Lower scores were observed for m-hydroxybenzoic acid (0.27), protocatechuic acid (0.22), and both 1-anthrol and t-cinnamic acid (0.09), while syringic acid failed to yield a score. The failure of syringic acid to map suggests it lacks the essential structural features aligned with the reference drug's pharmacophore. These low fitness scores underscore the necessity for structural optimization and bioisosteric replacement, particularly on the top hits, to improve pharmacophoric alignment. Nevertheless, the positive correlation of the

remaining compounds' fitness score indicates reasonable alignment within the binding core of the target protein, corroborating the findings of Aribigbola et al.⁴⁰ regarding the significance of positive score correlations between reference and bioactive compounds from the medicinal plants in the present study.

Drug development and candidate selection prioritize the assessment of ADMET profiles to identify viable hits and eliminate unsuitable compounds.⁴¹ To reach systemic circulation, an oral drug must first cross the intestinal epithelial membrane.⁴² All compounds evaluated in this study exhibited high intestinal absorption, highlighting their potential for oral bioavailability. For central nervous system (CNS) targets, blood-brain barrier (BBB) permeability is a critical factor.⁴³ Gallic acid, catechin, caffeic acid, protocatechuic acid, vanillic acid, and biochanin A were predicted to be non-BBB permeant while the remaining compounds were BBB-positive. Regarding oral drug-likeness criteria, violating more than two rules in Lipinski's Rule of Five generally indicates poor bioavailability.⁴⁴⁻⁴⁵ Notably, none of the studied compounds violated more than one rule. Furthermore, the topological polar surface area (TPSA) a key determinant of cell membrane permeation for all compounds fell within the recommended range of 7 to 200 Å².^{21,46} Finally, optimal lipophilicity (LogP) is vital for efficacy, with values ideally remaining below 5 for oral drugs, centering around 2 for BBB permeation, and exceeding 5 for sublingual absorption.⁴⁵

Cytochrome P450 (CYP) enzymes play a critical role in drug metabolism, with six major isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 mediating nearly 90% of all CYP-mediated drug oxidation.⁴⁷ These enzymes dictate clearance rates and systemic drug residence times. CYP overactivation can diminish therapeutic efficacy, whereas enzyme inhibition can cause toxic drug accumulation. In this study, the majority of

the evaluated compounds did not inhibit CYP1A2, CYP2C9, CYP2C19, or CYP2D6, suggesting a low risk of adverse metabolic drug-drug interactions. Exceptions included biochanin A (which inhibited CYP1A2, CYP2C9, and CYP2C19), 1-anthrol (which inhibited CYP1A2), and the reference drug isoquinoline-5-sulfonamide (which inhibited CYP2C19).

Pharmacokinetic elimination refers to the irreversible removal of a drug from the body, quantified via clearance (CL), the ratio of the elimination rate to plasma concentration.⁴⁸ Excretion and toxicity profiling of the top hits (gallic acid, chlorogenic acid, catechin, and caffeic acid) revealed clearance rates within standard pharmacodynamic ranges. Furthermore, these compounds are not mutagenic, nephrotoxic, or carcinogenic to avians as predicted in this study, thus confirming their safety profiles for further development.⁴⁹ Ultimately, targeting allosteric binding sites remains a highly promising strategy for developing novel therapeutics against multidrug-resistant bacteria strains.⁵⁰

5. Conclusion

Gallic acid, chlorogenic acid, catechin and caffeic acid demonstrate favorable drug-like properties based on their binding affinity, pharmacokinetics, ADMET prediction, as well as the presence of several important interacting amino acids at the allosteric pocket of *E. coli* DNA gyrase. These identified bioactive compounds appear promising to act as allosteric inhibitors, offering a new avenue for developing novel antibiotics that could be effective against fluoroquinolone-resistant strains. The results from the present study warrant further in vitro and in vivo experimental validation to confirm their allosteric inhibitory activity.

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Conflicts of interest

None to declare

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