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Molecular Docking and ADME Profiles of β-Carboline Analogues as Potential Antibiotic Agents Targeting DNA Gyrase

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Antibiotic resistance remains a major threat to humans worldwide, owing to the ability of bacteria and fungi to mutate over time, as well as a dramatic decline in the antibiotic pipeline. Plants are widely recognised as sources for various bioactive secondary metabolites that can be developed as a hit compound for further antibiotic discoveries. β-Carboline has been recognised as one of the hit compounds exhibiting various biological activities including antibacterial properties. However, the optimisation and development of the hit compound always hampered by long and expensive procedures. The *in-silico* approaches involving molecular docking and ADME profiling can be expedite the process. Herein, an in-house library of β-carboline and its 19 analogues were virtually screened to evaluate their antibiotic activities and drug-likeness properties using molecular docking and ADME profiling respectively. Docking studies showed that all 19 β-carboline analogues strongly bound to the target protein (-6.8 to -9.4 kcal/mol) except **1o** (-6.7 kcal/mol), which exhibited binding energy comparable to the reference drug, novobiocin (-6.8 kcal/mol). Of these, derivatives **1l** bound the strongest (-9.4 kcal/mol) mainly due to the hydrogen bond interactions that occurred between the carboxylic acid moiety with Val71. ADME profiling showed that all β-carboline analogues demonstrated favourable drug-likeness properties and obey the Lipinski Rule of 5 (Ro5). The analogues **1l** showed only one inhibition on CYP2D6 suggesting less toxicity properties. Thus, through this work, the derivatives of β-carboline, especially **1l**, may serve as hit compound for future development of finding effective antibiotic agent.

Keywords: β-carboline; DNA Gyrase; antibiotic; molecular docking; ADME Profile

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Antibiotic resistance has become a major global public health threat that is contributing to the escalating morbidity and mortality. This is mainly due to the extensive use and decreasing number of new antibiotics approved by the Food and Drug Administration (FDA), which are causing bacteria to adapt to the antibiotic drugs currently on the market and be able to mutate over time [1]. Intrinsically, antibiotic resistance is more common in Gram-negative bacteria such as *Escherichia coli, Pseudomonas sp*, *Enterobacter sp.* and *Salmonella sp.* than Gram-positive bacteria, owing to the outer membrane that serves as their first line of defence [2]. These bacteria can be immune to designed antibiotic through three mechanisms. Firstly, they can synthesise enzymes to modify the active site of the antibiotics, making them unable to interact with their target [3]. Secondly, bacteria can also create a membrane barrier to prevent or reduce the bacteria's cell permeability, limiting the capacity for antibiotics to penetrate the membrane. Lastly, the active efflux of the drugs from cells is a common antibiotic resistance mechanism in bacteria and can make such drugs ineffective [4].

DNA gyrase is a prokaryotic type II topoisomerase enzymes that is often used as the active target when designing a novel antibiotic drug. It is a vital enzyme for all types of bacteria as it performs all the metabolic processes involving DNA during replication by introducing negative supercoiling [5]. Mechanism of DNA gyrase inhibition is known to occur in two ways: the inhibitors bind to DNA gyrase directly or bind to DNA as to change its conformation resulting in unrecognised by the bacterial enzyme [6]. Since this protein is absent in human and presents across all the microbes, it demonstrated a good potential target for designing newly antibiotic [7].

β-Carbolines is a type of heterocyclic natural and synthetic indole alkaloid that consists of tricyclic rings (A, B and C) with pyridine-fused indole scaffolds and contains two nitrogen atoms (Figure 1) [8]. β-Carboline alkaloids were first isolated from the seeds of *Peganum harmala*, a plant native to the Middle

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East, North Africa and Northwest China that was previously used to treat alimentary tract cancer and malaria [9]. Until now, these alkaloids have been isolated from various sources, including plants, marine creatures, microorganisms, insects, food products, as well as human tissues and physiological fluids [10, 11]. Synthesis of β-carbolines is also possible, mainly involving the Pictet-Spengler condensation reaction [12].

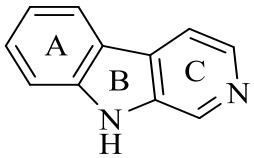

Figure 1. β-carboline scaffold.

Natural and synthetic β-carbolines have been linked to various biological activities such as anticancer [10], antimalarial [13], anticonvulsant [14], antiviral [15], antituberculosis [16] including anti-microbial [11]. There were substantial studies on β-carboline analogues to evaluate their *in vitro* anti-bacterial activity against Gram-positive and Gram-negative bacteria using minimum inhibitory concentration (MIC) method. Interestingly, β-carbolines exhibited stronger inhibition activity towards *S. aureus* (Gram-positive bacteria) than *E. coli* (Gram-negative bacteria), highlighting the scaffold's potential to combat antibiotic-resistant infections [17]. Unfortunately, most of the natural product compound shows lower efficacy *in vivo* despite displaying good activity *in vitro* animal models or cell culture resulting to poor absorption, distribution, metabolism, and excretion (ADME) profile [18]. A potential drug with a favourable pharmacokinetic profile must be absorbed into the bloodstream (absorption), transported to the action site (distribution), remain unaltered long enough to have a therapeutic effect, be converted to safe metabolites (metabolism) and eliminated appropriately (excretion) [19]. As such, *in-silico* approaches such as molecular docking and ADME prediction are frequently employed in all stages of drug design campaigns to reduce the cost and time required to optimise the hit and lead molecules so that drugs with high potency and safety are produced [20].

Due to the biological significance of β-carboline scaffold, particularly as antibiotic agents, the present study aimed to evaluate the ability of our previously synthesised β-carboline analogues [21] to bind to DNA gyrase by evaluating their binding affinities and interactions *via* molecular docking. In addition, ADME prediction was performed to provide details on the drug-likeness properties of the β-carboline analogues since the early estimation of ADME parameters can significantly reduce the pharmacokinetic failure rate in the clinical stages of the discovery process [22]. The β-carboline analogues with a good binding affinity as well as possessed a good pharmacokinetic profile can be a promising lead compounds that need further optimisation and development to be design as newly antibiotic agent.

EXPERIMENTAL

Ligands Preparation

β-carboline (**1o**) and its derivatives (**1a-1n** and **2a-3b**) were obtained from compounds synthesised by Ash'ari et al. [21] in which novobiocin (**1**) was used as the positive control as this drug co-crystallised in the target enzyme (1AJ6). The 3D structures of all the ligands are shown in Figure 2 were sketched and optimised using the AutoOptimize tool in Avogadro 2.0 [23]. The optimised ligands were saved separately in PDB format and then utilised in AutoDockTools (ADT) 1.5.6 [24]. The Gasteiger charges were computes and the files which were saved in PDBQT format for molecular docking.

Protein Preparation

DNA gyrase was selected as the target enzyme and its crystal structure (PDB ID: 1AJ6) with a resolution of 2.0 Å was downloaded from the Protein Data Bank (https://www.rcsb.org/). Water molecules and bound ligands were removed using PyMOL [25] before the addition of polar hydrogen atoms and Kollman charges were added using ADT 1.5.6. The optimised protein was then saved in PDBQT format for molecular docking.

Defining Grid Box for Docking

The grid box was created by selecting AutoGrid in ADT 1.5.6 to cover all the amino acid residues at the DNA gyrase active site, including the area of the co-crystallised ligand (novobiocin). Thus, the grid cavity sizes were selected to be 18 Å × 18 Å ×16 Å which were centred at 60.536 × -7.898 × 37.625 in the dimensions of the x, y, z axes, respectively, using a grid spacing of 1.0 Å.

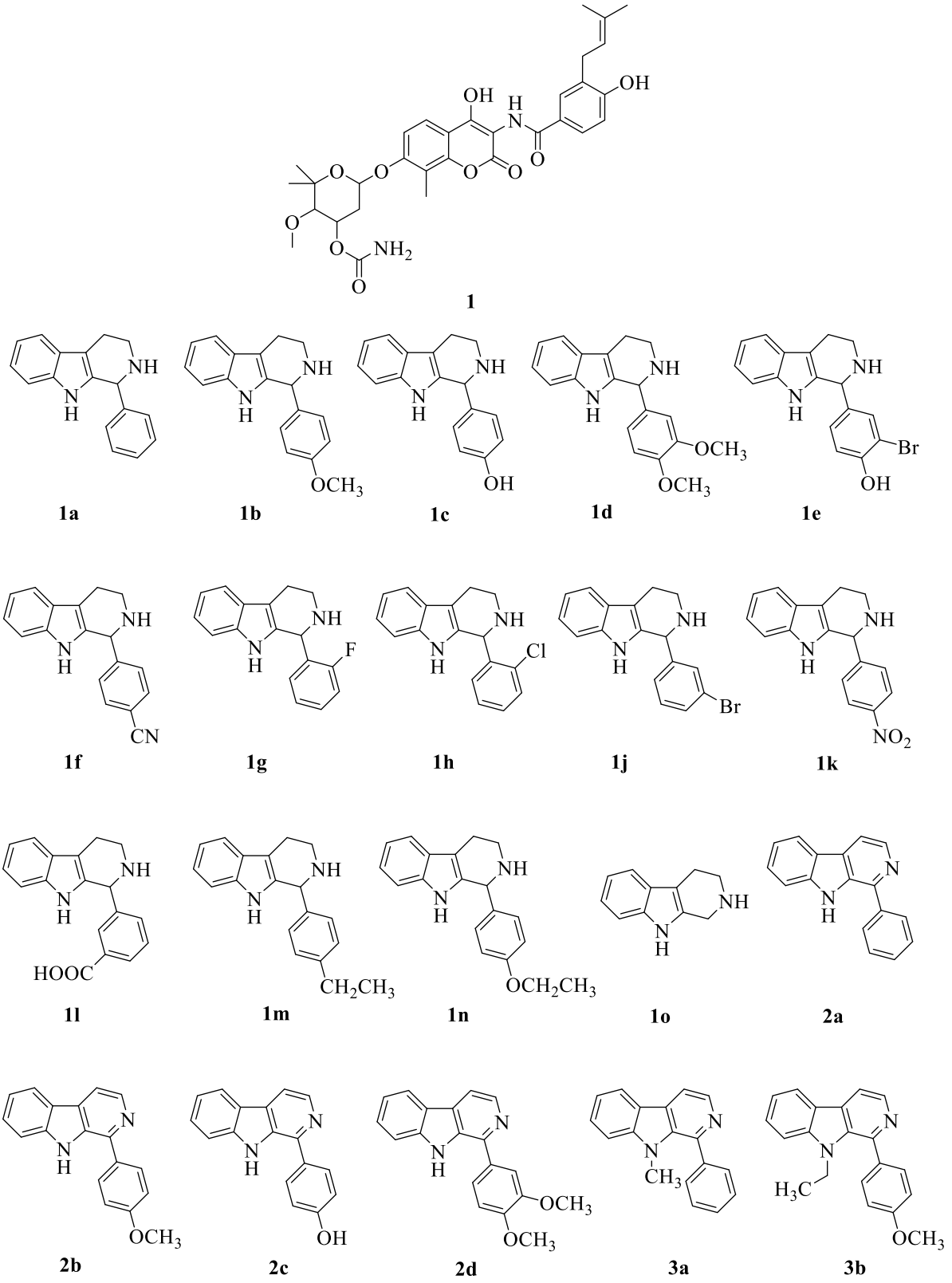

Figure 2. 2D structures of all compounds involved for *in-silico* studies.

Molecular Docking

The molecular docking was performed using AutoDock Vina 1.2.0, a flexible-rigid docking system that uses a genetic algorithm and semi-empirical calculation of free energy as its search algorithm and scoring function, respectively [26]. Before the docking procedure, the input configuration file was prepared to include all the

necessary information such as the name of the protein and ligand and the grid coordinates as previously defined. The number of modes was set at 10, while the energy range and exhaustiveness were fixed at 9 and 24 respectively. The docking process was then executed using command mode, and the results were ranked according to their binding affinity values (kcal/mol). The results were further visualised using PyMOL, Discovery Studio [27] and Maestro 13.3 [28] for 3D and 2D representations, to analyse chemical interactions such as the hydrogen bonds, hydrophobic interactions and bond distances.

In-silico ADME Profiling

The drug-likeness score for all the ligands were evaluated using the SwissADME server by submitting the SMILES notations generated during ligand preparation [29]. Drug-likeness scores help to describe the pharmacokinetic properties of the target compounds to predict important parameters such as absorption, distribution, metabolism and excretion (ADME). These parameters include molecular weight (g/mol), sp^3 fraction, number of rotatable bonds, topological polar surface area (TPSA), xlog *P* calculation, solubility estimation (ESOL) ESOL Log S calculation [30,31] and drug-likeness properties, mainly by considering the Lipinski Rule of 5. The predicted rates of the phase 1 metabolism of the top compounds and β-carboline involving the Cytochrome P450 (CYP) enzyme inhibitor and the P-glycoprotein (Pgp) substrate, were also evaluated.

RESULTS AND DISCUSSION

The molecular docking protocol was validated by redocking the co-crystallised novobiocin (which was also used as a reference drug) in the DNA gyrase cavity (PDB ID: 1AJ6). The default docking software parameters were used, except that the grid spacing was defined as 1.0 Å. Figure 3 shows the novobiocin was satisfactorily redocked with a root-mean-square-deviation (RMSD) value of 1.088 Å. As the RMSD value is below 2.0 Å, this indicates good docking accuracy, whereby the docked molecule had almost the same position as the bound ligand in the x-ray crystal structure of DNA gyrase [32]. All subsequent docking studies were performed according to the same protocol.

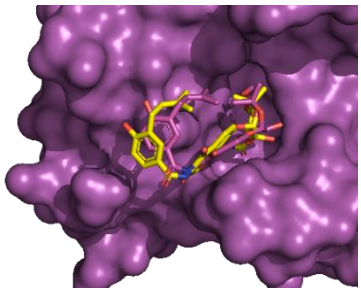

Figure 3. Superposition of novobiocin (purple) as bound and redocked ligand (yellow) in DNA gyrase.

Table 1. Docking results of β-carboline and analogues against DNA gyrase (PBD ID:1AJ6) using Maestro.

Compounds	Binding affinities (kcal/mol)	Amino acid residue having interaction within 4Å distance
1l	-9.4	Val71, Asp73, Ile78, Thr165, Pro79, Ile78, Gly77, Glu50, Ile94, Met95, Val120, Ala47, Asn46, Val43, Gln72, Met166, Val167
1g	-9.3	Val43, Asn46, Asp73, Ile78, Pro79, Gly77, Glu50, Ala47, Thr165, Val167, Met95, Ile94, Val120
1j	-9.1	Asn46, Asp73, Ile78, Thr165, Ile94, Met95, Val120, Val43, Ala47, Glu50, Gly77, Pro79, Val71, Val167
1h	-8.4	Asn46, Asp73, Ile78, Gly77, Arg76, Thr165, Val167, Glu50, Ala47, Val43, Ile94, Met95
2a	-8.2	Ile78, Thr165, Pro79, Gly77, Glu50, Ala47, Asn47, Vla43, Val167, Met95, Ile94, Val120
2b	-8.0	Asn46, Ile78, Pro79, Gly77, Glu50, Ala47, Val43, Thr165, Val167, Met95, Ile94
2c	-8.0	Ile78, Thr165, Pro79, Gly77, Glu50, Ala47, Asn46, Val43, Vla167, Met95, Ile94
1a	-7.8	Thr165, Val167, Met95, Val120, Asp73, Arg76, Gly77, Ile78, Val43, Asn46, Ala47, Asp49, Glu50

1f	-7.7	Ile78, Arg76, Asp73, Glu50, Ala47, Asn46, Val43, Val120, Met95, Ile94
3a	-7.7	Ile78, Pro79, Gly77, Arg76, Asp73, Thr165, Val167, Val43, Asn46, met95, Ile94
1b	-7.6	Asn46, Glu50, Ile94, Met95, Val120, Val167, Thr165, Val71, Asp73, Arg76, Gly77, Ile78, Pro79, Glu50, Ala47, Val43
2d	-7.6	Thr165, Pro79, Ile78, Gly77, Glu50, Ala47, Asn46, Val43, Val 167, Val120, Met95, Ile94
1m	-7.5	Ile78, Arg76, Asp73, Glu50, Ala47, Asn46, Val43, Met95, Ile94, Val167, Thr165
1k	-7.4	Asn46, Ile78, Arg76, Asp73, Glu50, Ala47, Val43, Thr165, Val167, Met95, Ile94.
1c	-7.2	Glu50, Gly77, Ile78, Arg76, Gly75, Thr165, Asp73, Asn46, Ala47, Asp49, Ala53
1e	-7.2	Asn46, Asp49, Glu50, Gly77, Ala53, Ala47, Ile78, Arg76, Gly75, Asp73, Thr165
1n	-7.2	Asn46, Ile78, Arg76, Asp73, Glu50, Ala47, Thr165, Val167, Val43, Met95, Ile94, Val120
3b	-7.2	Asn46, Ile78, Thr165, Pro79, Gly77, Arg76, Asp73, Val167, Val43, Met95, Ile94
1d	-6.8	Ile78, Gly119, Val120, Ile94, Pro79, Gly77, Arg76, Thr165, Glu50, Asp49, Asn46
1 Novobiocin	-6.8	Arg76, Ile78, Ala90, Thr165, Glu50, Ala90, Asn46, Asp73, Ile94, Gly77, Pro79
1o β-carboline	-6.7	Glu50, Asp73, Thr165, Glu50, Asn46, Ile74, Arg76, Gly77, Ile78, Pro79, Ala90

Table 1 lists the binding affinities by all the ligands, ranked from the lowest to the highest binding energy, as well as the amino acid residues involved in the binding interactions within 4 Å distance. Molecular docking studies have demonstrated that almost all β-carboline analogues bound strongly to the DNA gyrase protein, with binding energies ranging from -7.2 kcal/ mol to -9.4 kcal/mol, except analogues **1d**, **1o** and the reference drug, novobiocin, which displayed comparable binding values (~ -6.8 kcal/mol). Of these, β-carboline analogues, only two (**1l** and **1g**) exhibited the strongest binding interactions with the protein, which is worth further investigation.

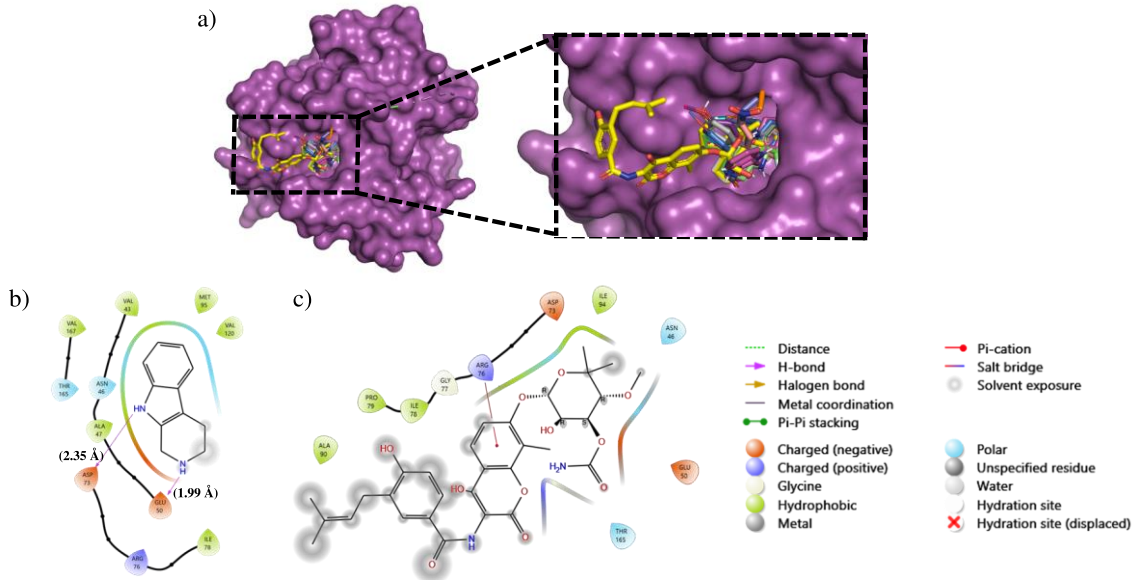

Figure 4. (a) 3D representation showing the docking poses of all the ligands docked at DNA gyrase active site and 2D representations of compound (b) β-carboline, **1o** and (c) novobiocin, **1**. The distance between amino acid residues and the ligand was set to less than 4 Å.

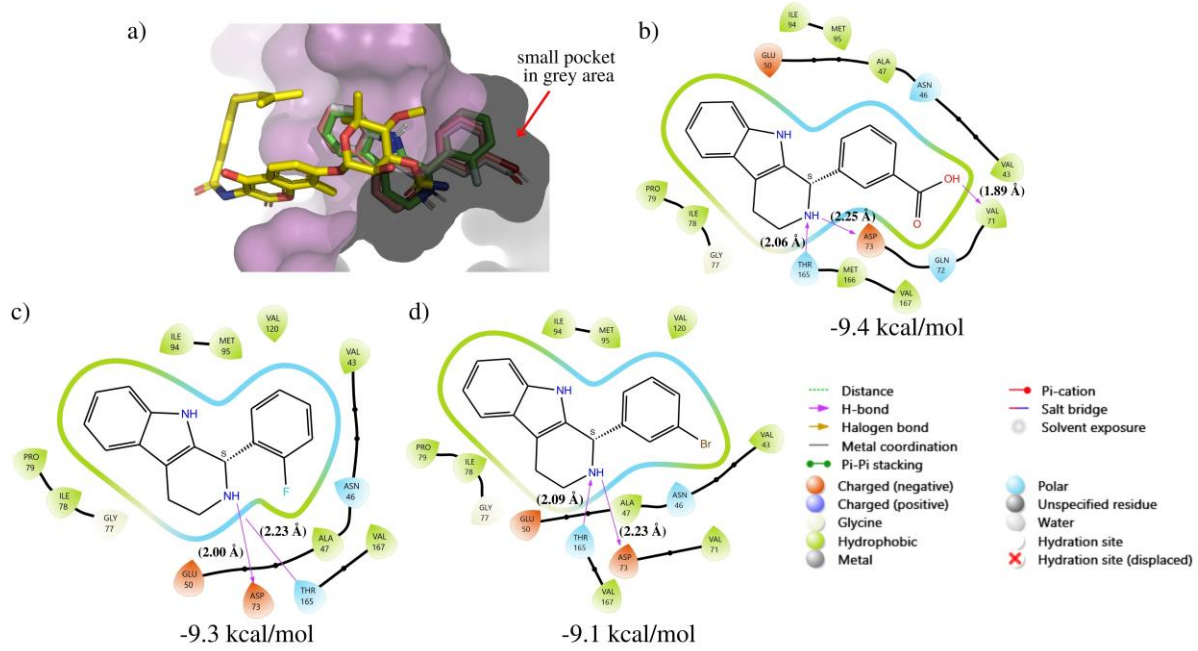

Figure 5. (a) 3D representation shows the docking poses of all the ligands docked at DNA gyrase active site and 2D representations of analogue (b) **1l**, (c) **1g** and d) **1j**. The distance between the amino acid residues and the ligand was set to less than 4 Å

The 3D representation of the complex DNA gyrase-ligands revealed that all the target compounds could dock at the active site of the protein (Figure 4a). β-Carboline scaffold, **1o** interacted with the Glu50 and Asp73 residues through the formation of hydrogen bond interactions between the amine moieties in the scaffold (Figure 4b). In contrast, novobiocin exhibited hydrophobic interactions, mainly with the formation of pi-cation interactions between the Arg76 residues and the benzene ring in the structure, where some functional groups of the molecule were exposed to the solvent (Figure 4c).

The active site of DNA gyrase has a deep pocket that can accommodate a small molecule, but it did not fit with its bound ligand, novobiocin. Interestingly, the pocket fit the β-carboline analogues well, particularly analogues **1l**, **1g** and **1j**, that demonstrated the comparable binding affinities observed (-9.1 to -9.4 kcal/mol) (Figure 5a). From the 2D representations in Figure 5, it is illustrated that all three derivatives showed the same interaction involving the amine moieties in the scaffold with Asp73 and Thr165 *via* hydrogen bonding. However, the presence of carboxylic acid moiety in **1l** (-9.4 kcal/mol) that fit into the deep pocket of the DNA gyrase interacted strongly with Val73 residue through the formation of additional hydrogen bonds (Figure 5b) which explained the slightly higher binding affinity compared to **1g** (-9.3 kcal/mol) and **1j** (-9.1 kcal/mol). In addition, the presence of halogens substituents (fluorine and bromine) at ortho and meta positions of benzene ring were found to be tolerated inside the deep pocket of the target protein implying the importance of halogens substituents at that position.

In-silico ADME Assessment

In-silico ADME is beneficial in the early drug discovery process to aid in the selection of potential hits for further optimisation and to gain a better understanding of structure activity relationship (SAR), which can help in designing a new molecule with better pharmacological activity [33]. SwissADME analyses each individual descriptor and provides information about whether the drug-likeness of the chemical satisfies the five distinct rule-based filters proposed by Lipinski, Ghose, Veber, Egan, and Muegge [29]. Herein, six important physicochemical properties were considered to predict the compound's ability to undergo absorption and distribution in *in-vivo*. Lipophilicity and solubility are important to ensure the absorption of drugs throughout the body. Drug molecules need to pass through both lipid and aqueous environments to reach the desired target [34]. The lipophilicity and aqueous solubility parameters were calculated using *X*logP (logarithm of the compound's partition coefficient between *n*-octanol and water partition) and Log S values, respectively. High XLOGP value indicate that a drug is too hydrophobic, easily trapped in the lipid phase and has difficulty in crossing membranes and re-entering the aqueous phase [19]. All the compounds are shown to have favourable lipophilicity and water solubility values, suggesting that they could be easily absorbed and transported (Table 2).

Table 2. Molecular weight, sp^3 fraction, number of rotatable bonds, TPSA value, XLOGP and ESOL value of novobiocin and β-carboline derivatives.

Derivatives	**Molecular weight** (150 g/mol ≤ MW ≤ 500 g/mol)	**sp^3 fraction** (≥0.25)	**Number of rotatable bonds** (0 < n < 10)	**TPSA** (20 Å2 ≤ TPSA ≤ 130 Å2)	**XLOGP** (-0.7 ≤ XLOGP3 ≤ +5.0)	**ESOL Log S** (Log S ≥ -6)	**LIPINSKI RULE OF FIVE**
1 Novobiocin	612.62	0.39	10	200.1	3.32	-5.34	Yes
1a	248.32	0.18	1	27.82	3.18	-3.9	Yes
1b	278.35	0.22	2	37.05	3.15	-3.95	Yes
1c	264.32	0.18	1	48.05	2.83	-3.75	Yes
1d	308.37	0.26	3	46.28	3.13	-4.01	Yes
1e	343.22	0.18	1	48.05	3.52	-4.65	Yes
1f	273.33	0.17	1	51.61	2.90	-3.82	Yes
1g	266.31	0.18	1	27.82	3.28	-4.05	Yes
1h	282.77	0.18	1	27.82	3.81	-4.48	Yes
1j	327.22	0.18	1	27.82	3.87	-4.80	Yes
1k	293.32	0.18	2	73.64	3.01	-3.93	Yes
1l	292.33	0.17	2	65.12	0.43	-2.30	Yes
1m	276.38	0.26	2	27.82	3.98	-4.46	Yes
1n	292.37	0.26	3	37.05	3.52	-4.18	Yes
1o β-carboline	172.23	0.27	0	27.82	1.49	-2.36	Yes
2a	244.29	0.00	1	28.68	3.98	-4.54	Yes
2b	274.32	0.06	2	37.91	3.95	-4.57	Yes
2c	260.29	0.00	1	48.91	3.62	-4.37	Yes
2d	304.34	0.11	3	47.14	3.92	-4.61	Yes
3a	258.32	0.06	1	17.82	3.93	-4.55	Yes
3b	302.37	0.15	3	27.05	4.20	-4.77	Yes

*(red=poor, green=good, yellow=intermediate)

The number of rotatable bonds and TPSA value reflect the flexibility of a drug and the presence of a polar surface area from the sum of the hydrogen donors and acceptors. These descriptors also relate to the ability of compounds to penetrate the membrane and blood-brain barrier (BBB). According to Veber's rule, good oral bioavailability should have 10 or fewer rotatable bonds and a TPSA value of less than 130 Å [35]. The predicted values demonstrated that all the compounds had acceptable flexibility, except for compound **3a**, which showed a low polar surface area. The sp^3 fraction descriptor, on the other hand, determines the complexity and chiral centres of a drug. Having both properties result in greater selectivity and fewer off-target effects as the spatial subtleties of the target proteins can be accessed [30]. For this series, most compounds displayed a low sp^3 fraction, which was related to the presence of several aromatic moieties on the β-carbolines. β-Carboline and all its analogues met all the requirements of Lipinski Rule of 5 (Ro5), suggesting their suitability for oral administration. This guideline recommends that an orally active medicine should not violate more than one of the following criteria: no more than five hydrogen bond donors (HBD), no more than 10 hydrogen bond acceptors (HBA), a molecular weight (MW) no greater than 500 Daltons and *c*Log*P* (lipophilicity) no greater than five [36].

The early prediction of the metabolites formed by the given molecules is necessary to minimise any potential safety liabilities due to the formation of toxic or reactive metabolites brought into early development [37]. Herein, β-carboline and the leading three analogues with strong binding affinity were further examined to determine whether these compounds could become P-glycoprotein (Pgp) substrates and inhibit CYP isoenzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4) during metabolism. Pgp substrates function as drug efflux transporters, excreting foreign compounds from the body [38] and preventing drug absorption from the systemic circulation diffusing into the brain [39]. Enzyme inhibition reduces metabolism, resulting in clinically important drug-drug interactions that might result in adverse side effects or treatment failures [36]. Table 3 depicts data showing that all the leading compounds became Pgp substrates, suggesting that these compounds can be eliminated from the body through the bile and urine. Meanwhile, for CYP interaction, only β-carboline, **1o** and **1l** inhibited a single type of enzyme CYP1A2 and CYP2D6 respectively.

Table 3. Predicted Pgp substrate and enzyme inhibition of β-carboline and its analogues, **1g**, **1j** and **1l**.

Compound	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
1o β-carboline	Yes	Yes	No	No	No	No
1g	Yes	Yes	No	No	Yes	Yes
1j	Yes	Yes	Yes	No	Yes	Yes
1l	Yes	No	No	No	Yes	No

Compound **1g** can potentially inhibit three iso-enzymes (CYP1A2, CY2D6 and CYP3A4), while compound **1j** displayed inhibition for all the iso-enzymes except CYP2C9. The ability of **1g** and **1j** to inhibit multiple CYP isoenzymes suggests that these analogues may have drug toxicity problems that require additional research, particularly in terms of dosage and enzyme-inhibition potency [40].

CONCLUSION

In conclusion, the molecular docking of β-carboline and its 19 analogues targeting DNA gyrase exhibited higher binding affinity (-6.8 kcal/mol to -9.4 kcal/mol) than novobiocin (-6.8 kcal/mol) and β-carboline, **1o** alone (-6.7 kcal/mol). Of these analogues, compound **1l** exhibited the highest binding affinity (-9.4 kcal/mol) due to the presence of a carboxylic moiety, which can occupy the deep pocket near the active site and form additional hydrogen interaction with Val71. Furthermore, *in-silico* ADME study revealed that β-carboline and all its analogues followed the Lipinski Rule of 5 (Ro5) guidelines, making them suitable for oral administration. In addition, the leading three analogues (**1g, 1j** and **1l**) were predicted able to be transported out through the Pgp protein, and compound **1l** was found to inhibit only one CYP isoform, compared to other derivatives. The findings suggest that analogue **1l** could be a promising candidate as an antibiotic agent for DNA gyrase due to its good ADME profile.

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