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Original article

Synthesis, biological evaluation and docking studies of 4-aryloxymethyl coumarins derived from substructures and degradation products of vancomycin

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ABSTRACT

Two series of 4-aryloxymethyl coumarins derived from the reaction of 4-bromomethyl coumarins with ethyl gallate and ethyl ester of N-Benzoyl tyrosine have been synthesized. Gallate ethers **3a**–**3g** and tyrosine derivatives **4e**–**4j** were most effective against *Entercoccus faecalis*. They were also found to be effective against *Aspergillus niger* and *Candida albicans*. Comparative docking studies with novobiocin have indicated better binding ability and higher '*C*' score values than novobiocin.

1. Introduction

Coumarins are a group of bioactive molecules, found extensively in nature with a wide range of structural modifications [1]. They exhibit antiviral [2], anti-cancer [3,4], anti-fungal [5], anti-inflammatory [6,7], anti-HIV [8] properties. They have been known to be particularly effective against Gram-positive species [9]. Coumarin based anti-biotics viz. novobiocin and clorobiocin affect the functioning of DNA gyrase, which is the basis for their broad spectrum antibacterial activity [10].

Hydroxy coumarins like scopoletin and gallic acid have been found to occur in *Pelargonium sidoides*, *Pelargonium reniforme* [14] and other plant species, exhibiting a range of biological activities [11–14]. Ester conjugates of 7-hydroxy coumarin with gallic acid have been found to be anti-proliferative against human cancer cell lines [15], methyl gallates with bis-aryl ether linkage and tyrosine moiety are common substructures to the anti-biotics of vancomycin family [16,17]. Naturally occurring bromotyrosine derivatives have been found to possess anti-microbial effect on the methicillin resistant *S. aureus* (MRSA) [18]. 4-aryloxymethyl coumarins with alkoxy and chloro substituents were found to be effective against *E. coli* [19].

Incorporation of bio-compatible fragments like vanillin and paracetamol has resulted in 4-aryloxymethyl coumarins exhibiting anti inflammatory activity (Fig. 1) [20].

In view of the importance associated with the above cited moieties in their potent anti-microbial activity, it was thought of considerable interest to employ derivatives of gallic acid and N-Benzoyl tyrosine for the generation of new 4-aryloxymethyl coumarins which are represented in the Scheme 1.

2. Chemistry

4-Bromoethylacetoacetate obtained from bromination of ethylacetoacetate, was treated with various substituted phenols under Pechmann cyclisation conditions using neat sulphuric acid as condensing agent. The reaction resulted in the formation of substituted 4-bromomethyl-coumarins **1** [21]. The allylic nucleophilic displacement was brought about by the reaction of **1** with ethyl gallate **2** (prepared from gallic acid and ethanol) to obtain compounds **3a**–**3j**.

4-Bromomethyl coumarins **1** were also condensed with *N*-benzoyl tyrosine ethyl ester, to obtain compounds **4a**–**4j**. In both

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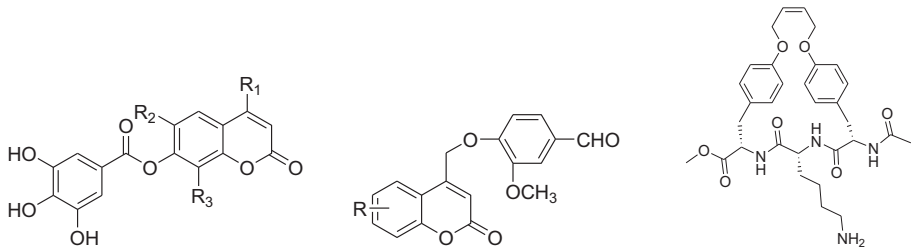
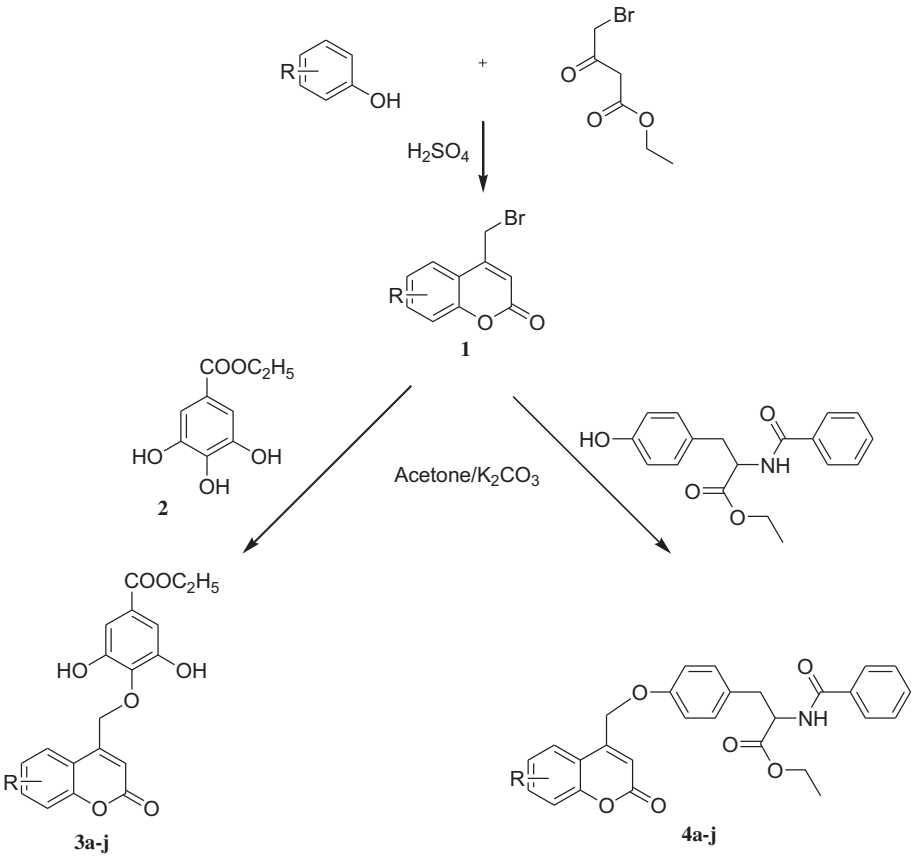


Fig. 1. Biologically active structurally related molecules.

the cases reactions were carried out in dry acetone in presence of anhydrous potassium carbonate at room temperature. Anhydrous potassium carbonate abstracts proton from phenolic –OH to give resonance stabilized phenoxide anion, which then reacts with 4-bromomethyl-coumarins to give desired compounds.

3. Results and discussion

Formation of products **3** and **4** is well supported by spectroscopic analysis. In case of compound **3a**, (R = 6-CH_3) IR spectrum exhibited two bands at 1711 cm^{-1} and 3444 cm^{-1} due to lactone and –OH stretching bands of coumarin and gallate moiety respectively. Formation of ethers **3a** was further confirmed by ^1H NMR, wherein the O–CH_2 protons appear as a singlet at 5.25 ppm which is characteristic of 4-aryloxymethyl coumarins [19]. The C_3–H of coumarin was observed at 6.73 ppm and the C_6–CH_3 protons appear as singlet at 2.34 ppm. Two aromatic protons resonating at 7.02 corresponded to the protons on gallate moiety [24], C5–H of coumarin resonated as singlet at 7.65, whereas C7–H and C8–H resonated as doublets with J = 8.0 Hz at 7.32 and 7.44 ppm respectively. Ethoxy protons appeared as triplet quartet pattern at 1.26 and 4.23 ppm with J = 7.0 Hz. The downfield D_2O exchangeable signal at 9.83 ppm corresponded to the phenolic –OH proton. ^{13}C NMR provides additional support for structure of the compounds. Lactone carbonyl resonates at 160 ppm and ester carbonyl of gallate resonates at 165 ppm, O–CH_2 resonates at 69 ppm, the methylene



R= 6-Me; 7-Me; 6-Cl; 7-Cl; 6-OMe; 7-OMe; 5,7-Me; 7,8-Me; 5,6 Benzo; 7,8 Benzo;

Scheme 1. Synthesis of gallate and tyrosinate ethers.

and methyl group of ester resonated at 60 and 14 ppm respectively, aromatic carbons were observed in accordance with expected values in the range of 112–151 ppm. The assignments are in agreement with the values reported for ethyl gallate (24). Molecular ion peak at 370 *m*/*z* (80%) in the EI-MS confirmed the proposed structure.

Compound **4a** (R = 6-CH_3) exhibited three prominent bands in the IR spectra at 3320 cm^{-1}, 1658 cm^{-1} and 1722 cm^{-1} due to the NH, amide carbonyl and lactone carbonyl frequencies respectively. In ^{1}H NMR spectrum, the signals observed at 5.25 (s); 4.09 (s) J = 7. 2 Hz and 3.7 ppm (d) are due to O–CH_2–Ar, O–CH_2 (ethoxy) and Ar–CH_2 protons respectively. The three proton signals at 1.14 (q) J = 7.2 Hz and 2.39 ppm (s) correspond to the CH_3 of ethoxy group and C_6–CH_3 respectively. Aromatic protons appear in the range of 7.02–7.83 ppm. The –NH proton appear as a doublet at 8.82 ppm with a coupling constant of J = 7.6 Hz, whereas the vicinal C–H proton appear at 4.63 ppm as multiplet. In the ^{13}C NMR lactone, ester and amide carbonyl resonates at 159, 166 and 171 ppm respectively, the methylene and methyl group of ester resonated at 60 and 13 ppm respectively, O–CH_2 was observed at 64 ppm, the –CH group flanked by Ester and amide functionalities resonates at 54, in addition the methyl group on coumarin resonates at 20 ppm.

The molecular ion peak in EI-MS at 485 was not observed, instead a peak at *m*/*z* 364 (15%) indicated the loss of C_6H_5–CONH moiety. Various compounds synthesized with their data are indicated in the Experimental section.

4. Biological screening

4.1. Anti-bacterial screening

All the newly synthesized compounds were evaluated for their in-vitro antibacterial activity against *Enteroccocus faecalis* (MTCC 3382), *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 1034), *Escherichia coli* (MTCC 1089) by Broth microdilution method [22]. Table 1 revealed that the compounds **3a**–**3j** were highly active against *S. faecalis* and *S. aureus*. In the activity against *S. aureus*, the efficacy of compounds decreased in the order of –Cl (**3i**), –OCH_3 (**3g**) and –CH_3 (**3a**) when located at C_6 positions. In case of groups at C_7 positions, the activity was least with 7-CH_3 (**3b**) and 7-Cl (**3j**) groups, the 7-OCH_3 group (**3h**) was ten times more active than the other two. All the gallate ethers **3a**–**3j** with exception of **3h** were most effective against *E. faecalis*, showing the least MIC value of 0.2 μg/mL. However they were inactive against *P. aeruginosa* and *E. coli*.

The anti-bacterial screening of compounds **4a**–**4j** revealed that the *N*-benzoyl tyrosine ethyl ester ethers were only active against *E. faecalis* with compounds **4e**, **4f**, **4h**–**4j** showing activity of 0.2 μg/mL which is significantly lower than standard drug used (Table 2). Even in these compounds the same trend was observed as in compounds **3**, with the efficacy decreasing in the order –Cl (**4i**), –OCH_3 (**4c**) and –CH_3 (**4a**) when located at C_6 positions. The activity were similar for –CH_3 (**4b**) and –OCH_3 (**4d**) at C_7 positions, however 7-Cl showed better activity than the other two. However, compounds of both the series, i.e **3** and **4** were inactive against Gram-negative bacteria.

4.2. Antifungal screening

The compounds of series **3** and **4** were screened for their antifungal activity against *Candida albicans* and *Aspergillus niger* by Broth dilution method [22]. All the gallate ethers **3a**–**3j** were inactive against both the fungal strains, with MIC as high as 100 μg/mL.

Compounds **4**, were highly active against *A. niger* with all the compounds showing activity 40 times better than the standard used. However in the case of *C. albicans*, –CH_3 and –OCH_3 attached at C_6 (**4a**, **4b**) and C_7 (**4c**, **4d**) respectively showed significantly higher growth inhibition than the standard used, almost 80 times better. Di substituted compounds **4e** and **4f**, along with benzo substituted compounds **4g** and **4h** showed appreciable activity while the –Cl substituted compounds at both C_6 and C_7 positions showed poor activity growth inhibition.

5. Computational studies

To understand the mechanism of anti-microbial activity of the compounds synthesized, molecular modelling and docking studies were performed on X-ray crystal structure of *E. coli* 24 kDa domain in complex with clorobiocin (PDB code: 1KZN; resolution 2.30 Å) using Surflex-Dock programme of Sybyl-X software. Clorobiocin was found to have hydrogen bonding interactions with Asp73 (1.911 Å), Thr165 (2.109 Å), Asn46 (2.034 Å) and Arg136 (2.071 Å). **3c** forms six hydrogen bonding interactions, phenol containing two OH group having four hydrogen bonding interactions second position OH; H interact with Asp49 (1.908 Å) and O interact with Asn46 (2.660 Å). In case of sixth position OH; H interact with Glu50 (1.812 Å) and O interact with Arg76 (2.524 Å) and two hydrogen bonding interaction with CO of coumarin moiety Gly77 (2.016 Å), Thr165 (2.549 Å). **4c** forms three hydrogen bonds, two hydrogen bonds with CO of coumarin moiety Arg136 (2.014 Å) and Arg136 (1.996 Å) and one hydrogen bond with ester CO and H of Thr165 (1.826 Å). All compounds have shown good *C*-score 8.56–6.02 kcal/mol (Tables 3 and 4). Compounds **3a**–**j** and **4a**–**j** were

Table 1
Antibacterial and antifungal activity of compounds **3**.

Compounds	R	Antibacterial				Antifungal	
		Gram positive		Gram negative			
		S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
3a	6-CH_3	1.6	0.2	100	100	100	100
3b	7-CH_3	12.5	0.2	100	100	100	100
3c	5,7-CH_3	0.8	0.2	100	100	100	100
3d	7,8-CH_3	0.2	0.2	100	100	100	100
3e	5,6 benzo	0.4	0.2	100	100	100	100
3f	7,8 benzo	1.6	0.2	100	100	100	100
3g	6-OCH_3	0.8	0.2	100	100	100	100
3h	7-OCH_3	1.6	0.8	100	100	100	100
3i	6-Cl	0.4	0.2	100	100	100	100
3j	7-Cl	12.5	3.125	100	100	100	100
Ciprofloxacin		2	2	2	2	–	–
Fluconazole		–	–	–	–	16	8

Table 2
Antibacterial and antifungal activity of compounds **4**.

Compounds	R	Antibacterial				Antifungal	
		Gram positive		Gram negative			
		S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
4a	6-CH_3	100	0.8	100	100	0.2	0.2
4b	7-CH_3	–	0.8	100	100	0.2	0.2
4c	6-OCH_3	100	0.4	100	100	0.2	0.2
4d	7-OCH_3	100	0.8	100	100	0.2	0.2
4e	5,7-CH_3	–	0.2	100	100	1.6	0.2
4f	7,8-CH_3	100	0.2	100	100	0.8	0.2
4g	5,6 benzo	–	0.4	100	–	1.6	0.2
4h	7,8 benzo	50	0.2	100	100	12.5	0.2
4i	6-Cl	100	0.2	–	100	50	0.2
4j	7-Cl	–	0.2	–	100	50	0.2
Ciprofloxacin		2	2	2	2	–	–
Fluconazole		–	–	–	–	16	8

having better hydrogen bonding, complex (ligand-protein), and internal (ligand–ligand) energies than novobiocin. Helmholtz free energies of interactions for protein-ligand atom pairs for all compounds showed less than chlorobiocin and novobiocin. Charge and van der Waals interactions between the protein and the ligand suggest that **3b**–**3d**, **4b**–**4d** and **4f**–**4i** are superior ligands than novobiocin to bind with DNA gyrase. Scoring of compounds with respect to the reward for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept terms revealed that compounds **3c**–**3e**, **4a**–**4b**, **4e** and **4g**–**4i** have more interactions with the protein than novobiocin and other compounds. The docking models of the compounds **3c** and **4c** along with clorobiocin are given below (Figs. 2 and 3).

6. Experimental

6.1. Chemistry

The melting points were determined by open capillary method and are uncorrected. IR spectra (KBr disc) were recorded on Nicolet –5700 FT-IR spectrometer. ^{1}H NMR spectra were recorded on Bruker 400 MHz Spectrometer using DMSO d_6 as solvents and TMS as internal standard. The Chemical shifts are expressed in δ ppm. Mass spectra were recorded using Shimadzu GCMS-QP2010S. The elemental analyses were carried out using Hereaus CHN rapid analyser. Purity of the compound was checked by TLC. All the chemicals purchased were of analytical grade and were used without further purification unless otherwise stated.

6.1.1. Synthesis of substituted 4-bromomethyl-7-methyl coumarin (*1*)

The required substituted 4-bromomethyl coumarin have been synthesized by the Pechmann cyclization of various substituted phenols with 4-bromoethylacetoacetate [23].

6.1.2. Synthesis of ethyl gallate (*2*)

It was synthesized using known procedure [24].

*6.1.2.1. Synthesis of ethyl 3,5-dihydroxy-4-((6-methyl-2-oxo-2H-chromen-4-yl)methoxy) benzoate (**3a**).* A mixture of 0.216 g of ethyl gallate (2) (0.001 M) and 0.138 g of anhydrous potassium carbonate (0.001 M) were stirred for 30 min in dry acetone (30 mL),

Table 3
Surflex-Dock score (kcal/mol) of compounds **3**.

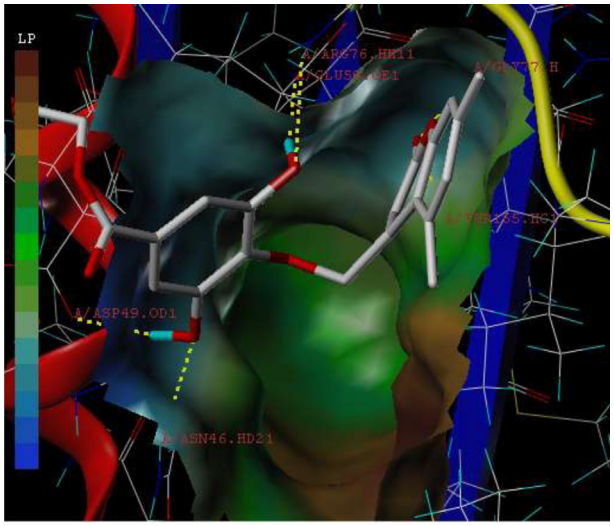
Compound	*C*-Score[a]	Crash score[b]	Polar score[c]	*G* score[d]	PMF score[e]	*D* score[f]	Chem score[g]
Clorobiocin	11.49	–1.50	3.77	–285.755	–81.590	–166.382	–32.321
Novobiocin	7.41	–1.72	3.84	–163.279	–77.294	–134.332	–23.450
3a	7.88	–1.42	1.61	–247.259	–28.464	–159.034	–23.846
3b	8.53	–1.10	1.00	–277.349	–30.339	–157.048	–22.652
3c	8.56	–1.04	3.55	–208.220	–45.019	–123.775	–22.064
3d	7.01	–1.46	1.49	–216.957	–36.187	–131.762	–21.255
3e	8.03	–1.14	1.29	–237.866	–39.442	–159.241	–27.880
3f	6.82	–1.48	2.57	–214.684	–31.019	–127.611	–22.393
3g	7.01	–0.94	1.75	–165.384	–37.433	–127.515	–18.688
3h	6.82	–1.48	2.57	–214.684	–31.019	–127.611	–22.393
3i	6.02	–1.27	3.41	–177.806	–35.186	–121.226	–22.467
3j	7.31	–1.27	3.29	–186.516	–24.328	–127.040	–23.724

[a] *C*-Score (Consensus-score) reports the output of total score.
[b] Crash-score revealing the inappropriate penetration into the binding site.
[c] Polar region of the ligand.
[d] G-score showing hydrogen bonding, complex (ligand–protein), and internal (ligand–ligand) energies.
[e] PMF-score indicating the Helmholtz free energies of interactions for protein–ligand atom pairs (Potential of Mean Force, PMF).
[f] *D*-Score for charge and van der Waals interactions between the protein and the ligand.
[g] Chem-score points for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term.

Table 4
Surflex-Dock score (kcal/mol) of compounds **4**.

Compound	*C*-Score[a]	Crash score[b]	Polar score[c]	G score[d]	PMF score[e]	D score[f]	Chem score[g]
Clorobiocin	11.49	–1.50	3.77	–285.755	–81.590	–166.382	–32.321
Novobiocin	7.41	–1.72	3.84	–163.279	–77.294	–134.332	–23.450
4a	7.98	–1.53	1.34	–245.947	–46.486	–139.192	–22.158
4b	7.87	–1.60	0.87	–258.828	–33.347	–151.901	–23.115
4c	8.35	–1.39	3.05	–222.002	–47.348	–121.572	–25.220
4d	6.75	–1.35	1.73	–183.994	–46.655	–122.156	–21.714
4e	7.04	–2.04	1.02	–220.515	–42.005	–136.690	–24.939
4f	7.03	–2.81	1.69	–245.009	–26.412	–145.345	–23.863
4g	7.08	–1.88	1.10	–286.362	–28.531	–153.314	–22.241
4h	7.13	–1.28	3.66	–195.059	–65.163	–114.734	–24.672
4i	8.21	–2.59	2.17	–345.424	1.079	–178.360	–27.819
4j	6.75	–1.35	1.73	–183.994	–46.655	–122.156	–21.714

[a] *C*-Score (Consensus-score) reports the output of total score.
[b] Crash-score revealing the inappropriate penetration into the binding site.
[c] Polar region of the ligand.
[d] G-score showing hydrogen bonding, complex (ligand–protein), and internal (ligand–ligand) energies.
[e] PMF-score indicating the Helmholtz free energies of interactions for protein–ligand atom pairs (Potential of Mean Force, PMF).
[f] D-score for charge and van der Waals interactions between the protein and the ligand.
[g] Chem-score points for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term.



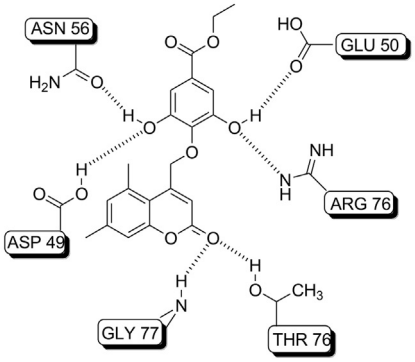


Fig. 2. Docking study of 24 kDa fragment DNA gyrase subunit A (1KZN) with compound **3c**,which shows the consensus score (*C*-score) of 8.56 and schematic representation of compound **3c** bound to the DNA subunit.

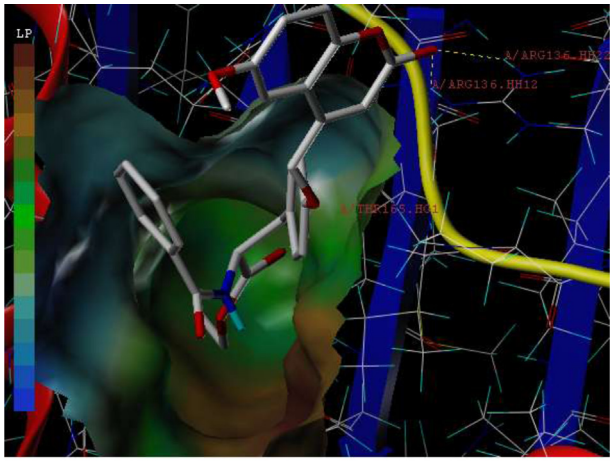
to this 0.253 g of 6-methyl 4-bromomethyl coumarin (1) was added and stirring was continued for 24 h, the reaction was monitored using TLC. After completion, the reaction mixture was quenched in crushed ice. The separated solid was filtered and washed with 1:1 HCl. The compound was dried and crystallized from methanol. Brownish white; yield 73%; m.p: 222–225 °C; IR (KBr) cm^{-1}1711 (C=O), 3444 (–OH); ^{1}H NMR (DMSO, 400 MHz, TMS): 1.26 (t, 3H, J = 7.2 Hz, O–CH_2–CH_3), δ ppm 2.34 (s, 3H, C6–CH_3), 4.21 (q, 2H, O–CH_2–CH_3, J = 7.2 Hz), 5.25 (s, 2H, O–CH_2), 6.73 (s, 1H, C3–H), 7.00 (s, 2H, Ar–H gallate), 7.32 (d, 1H, C7–H of coumarin); 7.44 (d, 1H, C8–H of coumarin), 7.65 (s, 1H, C5–H of coumarin), 9.83 (s, 2H, –OH, D_2O Exchangeable), ^{13}C NMR(400 MHz, DMSO d_6, δ ppm) 14.1,20.3,60.4,69.0,108.6, 112.5,115.3,116.5,124.5,125.2,132.7,133.1, 138.0,150.6,151.1,151.6,160.0,165.3 *m*/*z* 370 (25%); Anal Calcd. For $C_{20}H_{18}O_7$ (%) Calcd. C, 64.86; H, 4.90, found: C, 64.26; H, 4.40.

*6.1.2.2. Ethyl 3,5-dihydroxy-4-((7-methyl-2-oxo-2H-chromen-4-yl) methoxy) benzoate(**3b**).* Pale yellow; yield 70%; m.p: 235–240 °C; IR (KBr) cm^{-1} 1713 (C=O), 3404 (–OH); ^{1}H NMR (DMSO, 400 MHz, TMS): 1.26 (t, 3H, J = 7.2 Hz, O–CH_2–CH_3), δ ppm 2.34 (s, 3H, C7–CH_3), 4.21 (q, 2H, O–CH_2–CH_3, J = 7.2 Hz), 5.25 (s, 1H, O–CH_2), 6.73 (s, 1H, C3–H), 7.00 (s, 2H, Ar–H gallate), 7.30 (d, 1H, C7–H of coumarin), 7.40 (d, 1H, C8–H of coumarin), 7.60 (s, 1H, C5–H of coumarin), 9.84 (s, 2H, –OH, D_2O Exchangeable),; *m*/*z* 370; Anal Calcd. For $C_{20}H_{18}O_7$ (%) Calcd.: C, 64.86; H, 4.90, found: C, 64.46; H, 4.80.

*6.1.2.3. Ethyl 3,5-dihydroxy-4-((5,7-dimethyl-2-oxo-2H-chromen-4-yl)methoxy) benzoate (**3c**).* Pale yellow; yield 68%; m.p: 210–214 °C; IR (KBr) cm^{-1} 1710 (C=O), 3394 (–OH); ^{1}H NMR (DMSO, 400 MHz, TMS): δ ppm 1.26 (t, 3H, J = 7.2 Hz, O–CH_2–CH_3), 2.34 (s, 3H,C5–CH_3), 2.65 (s, 3H, C5–CH_3), 4.31 (q, 2H, O–CH_2–CH_3, J = 7.2 Hz), 5.25 (s, 1H, O–CH_2), 6.70 (s, 1H, C3–H), 7.00 (s, 2H, Ar–H gallate) 7.10 (s, 1H, C8–H of coumarin), 7.30 (s, 1H, C6–H of coumarin), 9.83 (s, 2H, –OH D_2O Exchangeable); *m*/*z* 384 (20%); Anal Calcd. For $C_{21}H_{20}O_7$ (%), Calcd: C, 65.62; H, 5.24, found: C, 65.43; H, 5.15.

*6.1.2.4. Ethyl 3,5-dihydroxy-4-((7,8-dimethyl-2-oxo-2H-chromen-4-yl)methoxy) benzoate (**3d**).* Off-white; yield 69%; m.p: 160–162 °C; IR (KBr) cm^{-1} 1710 (C=O), 3385 (–OH); ^{1}H NMR (DMSO, 400 MHz, TMS): δ ppm 1.26 (t, 3H, J = 7.2 Hz, O–CH_2–CH_3), 2.34 (s, 3H, C7–CH_3), 2.75 (s, 3H, C8–CH_3), 4.21 (q, 2H, O–CH_2–CH_3, J = 7.2 Hz), 5.25 (s, 1H, O–CH_2), 6.76 (s, 1H, C3–H), 7.00 (s, 2H, Ar–H gallate), 7.30 (d, 1H, C6–H of coumarin), 7.40 (d, 1H, C5–H of coumarin), 9.83 (s, 2H, –OH D_2O Exchangeable); *m*/*z* 384; Anal Calcd. For $C_{21}H_{20}O_7$ (%), Calcd: C, 65.62; H, 5.24, found: C, 65.13; H, 5.10.

*6.1.2.5. Ethyl 3,5-dihydroxy-4-((3-oxo-3H-benzo[f]chromen-1-yl) methoxy) benzoate(**3e**).* Yellow; yield 72%; m.p: 220–222 °C; IR (KBr) cm^{-1} 1700 (C=O), 3416 (–OH); ^{1}H NMR (DMSO, 400 MHz, TMS): δ ppm 1.26 (t, 3H, J = 7.2 Hz, O–CH_2–CH_3), 4.24 (q, 2H, O–



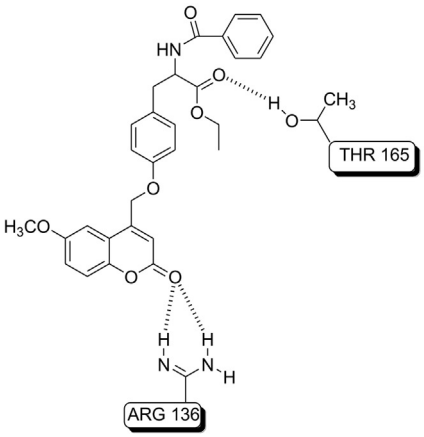


Fig. 3. Docking study of 24 kDa fragment DNA gyrase subunit A (1KZN) with compound **4c**,which shows the consensus score (*C*-score) of 8.35 and schematic representation of compound **4c** bound to the DNA subunit.

CH_2-CH_3, $J = 7.2$ Hz), 5.67 (s, 2H, O–CH_2), 7.00 (s, 2H, Ar–H gallate), 7.02 (s, 1H, C3–H), 7.04–8.35 (8H, Ar–H) 9.86 (s, 2H, –OH D_2O Exchangeable); *m/z* 406 (35%); Anal Calcd. For $C_{23}H_{18}O_7$ (%), Calcd: C, 67.98; H, 4.46, found: C, 67.75; H, 4.33.

*6.1.2.6. Ethyl 3,5-dihydroxy-4-((2-oxo-2H-benzo[h]chromen-4-yl) methoxy) benzoate(**3f**).* Buff coloured; yield 75%; m.p: 230–232 °C; IR (KBr) cm^{-1}1704 (C=O), 3401 (–OH); ^1H NMR (DMSO, 400 MHz, TMS): δ ppm 1.26 (t, 3H, $J = 7.2$ Hz, O–CH_2–CH_3), 4.24 (q, 2H, O–CH_2–CH_3, $J = 7.2$ Hz), 5.68 (s, 2H, O–CH_2), 7.00 (s, 2H, Ar–H gallate), 7.05 (s, 1H, C3–H), 7.04–8.35 (8H, Ar–H) 9.86 (s, 2H, –OH D_2O Exchangeable); *m/z* 406 (30%); Anal Calcd. For $C_{23}H_{18}O_7$ (%), Calcd: C, 67.98; H, 4.46, found: C, 67.11; H, 4.23.

*6.1.2.7. Ethyl 3,5-dihydroxy-4-((6-methoxy-2-oxo-2H-chromen-4-yl) methoxy) benzoate(**3g**).* Pale yellow; Yield 75%; m.p: 230–232 °C; IR (KBr)cm^{-1} 1704 (C=O),3401 (-OH); ^1H NMR (DMSO, 400 MHz, TMS): δ ppm 1.26 (t, 3H, $J = 6.8$ Hz, O–CH_2–CH_3), 3.79 (s, 3H, C6–OCH_3), 4.21 (q, 2H, O–CH_2–CH_3, $J = 6.8$ Hz), 5.23 (s, 1H, O–CH_2), 6.72 (s, 1H, C3–H), 7.00 (s, 2H, Ar–H gallate), 7.10 (d, 1H, C8–H of coumarin), 7.20 (d, 1H, C6–H of coumarin), 7.38 (s, 1H, C5–H of coumarin), 9.83 (s, 2H, –OH, D_2O Exchangeable); *m/z* 386 (20%); Anal Calcd. For $C_{20}H_{18}O_8$ (%), Calcd: C, 62.17; H, 4.70, found: C, 62.17; H, 4.51.

*6.1.2.8. Ethyl 3,5-dihydroxy-4-((7-methoxy-2-oxo-2H-chromen-4-yl) methoxy) benzoate(**3h**).* Off-white; Yield 72%; m.p: 210–212 °C; IR (KBr) cm^{-1} 1710 (C=O), 3355 (–OH); ^1H NMR (DMSO, 400 MHz, TMS) : δ ppm 1.26 (t, 3H, $J = 7.2$ Hz, O–CH_2–CH_3), 3.73 (s, 3H, C7–OCH_3), 4.21 (q, 2H, O–CH_2–CH_3, $J = 7.2$ Hz), 5.25 (s, 1H, O–CH_2), 6.75 (s, 1H, C3–H), 7.00 (s, 2H, Ar–H gallate) 7.3 (d, 1H, C8–H of coumarin) 7.4 (d, 1H, C8–H of coumarin), 7.6 (s, 1H, C5–H of coumarin), 9.83 (s, 2H, –OH, D_2O Exchangeable); *m/z* 386 (20%); Anal Calcd. For $C_{20}H_{18}O_8$ (%), Calcd: C, 62.17; H, 4.70, found: C, 62.0; H, 4.50.

*6.1.2.9. Ethyl 3,5-dihydroxy-4-((6-chloro-2-oxo-2H-chromen-4-yl) methoxy) benzoate (**3i**).* White coloured; yield 76%; m.p: 240–242 °C; IR (KBr) cm^{-1}1711 (C=O),3401 (-OH); ^1H NMR (DMSO, 400 MHz, TMS): δ ppm 1.26 (t, 3H, $J = 7.2$ Hz, O–CH_2–CH_3),4.23 (q, 2H, O–CH_2–CH_3, $J = 7.2$ Hz), 5.22 (s, 2H, O–CH_2), 6.78 (s, 1H, C3–H), 7.00 (s, 2H, Ar–H gallate) 8.06 (s, 1H, C5–H of coumarin), 7.6 (d, 1H, C7–H of coumarin), 7.4 (d, 1H, C8–H of coumarin), 9.86 (s, 2H, –OH, D_2O Exchangeable); *m/z* 390(M) (35%), 392(M + 2) (15%); Anal Calcd. For $C_{19}H_{15}ClO_7$ (%), Calcd: C, 58.40; H, 3.87, found: C, 58.10; H, 3.64.

*6.1.2.10. Ethyl 3,5-dihydroxy-4-((7-chloro-2-oxo-2H-chromen-4-yl) methoxy) benzoate (**3j**).* White coloured; yield 70%; m.p: 225–227 °C; IR (KBr) cm^{-1} 1712 (C=O), 3400 (–OH); ^1H NMR (DMSO, 400 MHz, TMS): δ ppm 1.27 (t, 3H, $J = 7.2$ Hz, O–CH_2–CH_3), 4.24 (q, 2H, O–CH_2–CH_3, $J = 7.05$ Hz), 5.27 (s, 2H, O–CH_2), 6.79 (s, 1H, C3–H), 7.00 (s, 2H, Ar–H gallate) 7.4 (s, 1H, C5–H of coumarin), 7.62 (s, 1H, C8–H of coumarin), 7.9 (d, 1H, C6–H of coumarin); 9.77 (s, 2H, –OH, D_2O Exchangeable), *m/z* 390$(M)^+$ (20%), 392$(M + 2)^+$ (8%); Anal Calcd. For $C_{19}H_{15}ClO_7$ (%), Calc: C, 58.40; H, 3.87, found: C, 58.35; H, 3.63.

*6.1.2.11. Synthesis of ethyl 2-benzamido-3-(4-((6-methyl-2-oxo-2H-chromen-4-yl)methoxy)phenyl)propionate (**4a**).* A mixture of 0.313 g of N-Benzoyl tyrosine ethyl ester (0.001 M) and 0.138 g of anhydrous potassium carbonate (0.001 M) were stirred for 30 min in dry acetone (30 ml), 0.253 g of 6-methyl 4-bromomethyl coumarin (1) was added and stirring was continued for 24 h, the reaction was monitored using TLC. After completion, the reaction mixture was quenched in crushed ice. The separated solid was filtered and washed with 1:1 HCl. The compound was dried and crystallized from ethanol. White coloured; yield 62%; m.p:176–180 °C; IR (KBr) cm^{-1} 1726 (C=O) 3318 (–NH) 1650 (amide C=O); ^1H NMR (DMSO, 400 MHz, TMS): 1.14 (t, 3H, O–CH_2–CH_3, $J = 7.2$ Hz), δ ppm 2.33 (s, 3H, Ar–CH_3), 3.09 (m, 2H, Ph-CH_2), 4.09 (q, 2H, O–CH_2–CH_3, $J = 7.2$ Hz), 4.61 (t, 1H, Ph–CH_2–CH, $J = 7.2$ Hz), 5.31 (s, 2H, O–CH_2), 6.51(s, 1H, C3–H), 7.04–7.88 (m, 12H, Ar–H), 8.84 (d, 1H, NH–CO, $J = 7.2$ Hz, D_2O Exchangeable); ^{13}C NMR(400 MHz,DMSO-d_6, δ ppm) 13.1,20.3,30.4,35.40,54.52,60.47,64.92,111.6,114.8,116.3,124.4,127.3, 128.2,130.2,130.4,131.4,132.9,133.6,151.1,151.2,156.2,159.7,166.4, 171.69; *m/z* 364 (10%); Anal Calcd. For $C_{29}H_{27}NO_6$ (%), Calcd: C, 71.74; H, 5.61; N, 2.88, found: C, 71.44; H, 5.45; N, 2.64.

*6.1.2.12. Ethyl 2-benzamido-3-(4-((7-methyl-2-oxo-2H-chromen-4-yl)methoxy)phenyl)propionate (**4b**).* Buff coloured; Yield 63%; m.p: 117–120 °C; IR (KBr) cm^{-1}1722 (C=O) 3320 (–NH) 1658 (amide C=O); ^1H NMR (DMSO, 400 MHz, TMS): δ ppm 1.19 (t, 3H, O–CH_2–CH_3, $J = 7.2$ Hz), 2.33 (s, 3H, Ar–CH_3), 3.09 (m, 2H, Ph-CH_2), 4.09 (q, 2H, O–CH_2–CH_3, $J = 7.2$ Hz), 4.61 (t, 1H, Ph–CH_2–CH, $J = 7.2$ Hz), 5.31 (s, 2H, O–CH_2), 6.51(s, 1H, C3–H), 7.06–7.88 (m, 12, Ar–H), 8.72 (d, 1H, NH–CO, $J = 7.2$ Hz, D_2O Exchangeable); *m/z* 364 (10%); Anal Calcd. For $C_{29}H_{27}NO_6$ (%), Calcd: C, 71.74; H, 5.61; N, 2.88, found: C, 71.23; H, 5.43; N, 2.74.

*6.1.2.13. Ethyl2-benzamido-3-(4-((6-methoxy-2-oxo-2H-chromen-4-yl)methoxy)phenyl)propionate (**4c**).* Pale yellow; Yield 61%; m.p: 140–144 °C; IR (KBr) cm^{-1} 1727 (C=O) 3289 (–NH) 1615 (amide C=O); ^1H NMR (DMSO, 400 MHz, TMS): δ ppm 1.14 (t, 3H, O–CH_2–CH_3, $J = 7.2$ Hz), 3.09 (m, 2H, Ph-CH_2), 3.81 (s, 3H, C6–OCH_3), 4.08 (q, 2H, O–CH_2–CH_3, $J = 7.2$ Hz), 4.60 (t, 1H, Ph–CH_2–CH, $J = 7.6$ Hz), 5.37 (s, 2H, O–CH_2), 6.53 (s, 1H, C3–H), 7.07–7.80 (m, 12, Ar–H), 8.78 (d, 1H, NH–CO, $J = 7.6$ Hz, D_2O Exchangeable); Anal Calcd. For $C_{29}H_{27}NO_7$ (%), Calcd: C, 69.45; H, 5.43; N, 2.79, found: C, 69.15; H, 5.23; N, 2.67.

*6.1.2.14. Ethyl2-benzamido-3-(4-((7-methoxy-2-oxo-2H-chromen-4-yl)methoxy)phenyl)propionate (**4c**).* Grey coloured; Yield 56%; m.p: 150–154 °C; IR (KBr) cm^{-1}1722 (C=O) 3278 (-NH) 1640 (amide C=O); ^1H NMR (DMSO, 400 MHz, TMS): δ ppm 1.19(t, 3H, O–CH_2–CH_3, $J = 7.2$ Hz), 3.09 (m, 2H, Ph-CH_2), 3.75 (s, 3H, C7–OCH_3), 4.09 (q, 2H, O–CH_2–CH_3, $J = 7.2$ Hz), 4.61 (t, 1H, Ph–CH_2–CH, $J = 7.2$ Hz),5.31 (s, 2H, O–CH_2), 6.51 (s, 1H, C3–H), 7.10–7.89 (m, 12H, Ar–H), 8.83 (d, 1H, NH–CO, $J = 7.2$ Hz, D_2O Exchangeable); Anal Calcd. For $C_{29}H_{27}NO_7$ (%), Calcd: C, 69.45; H, 5.43; N, 2.79, found: C, 69.35; H, 5.17; N, 2.62.

*6.1.2.15. Ethyl2-benzamido-3-(4-((5,7-dimethyl-2-oxo-2H-chromen-4yl)methoxy)phenyl)propionate (**4e**).* White coloured; yield 60%; m.p:167–170 °C; IR (KBr) cm^{-1} 1726 (C=O) 3286 (–NH) 1642 (amide C=O); ^1H NMR (DMSO, 400 MHz, TMS): δ ppm 1.14 (t, 3H, O–CH_2–CH_3, $J = 5.1$ Hz), 2.34 (s, 3H, C5–CH_3), 2.65(s, 3H, C7–CH_3), 3.07(m, 2H, Ph-CH_2),4.14 (q, 2H, O–CH_2–CH_3, $J = 5.1$ Hz), 4.61(t, 1H, Ph–CH_2–CH, $J = 7.2$ Hz),5.43 (s, 2H, O–CH_2), 6.49 (s, 1H, C3–H), 7.01–7.85 (m, 11H, Ar–H), 8.75 (d, 1H, NH–CO, $J = 5.7$ Hz, D_2O Exchangeable); Anal Calcd. For $C_{30}H_{29}NO_6$ (%), Calc: C, 72.13; H, 5.85; N, 2.80, found: C, 72.03; H, 5.63; N, 2.72.

*6.1.2.16. Ethyl 2-benzamido-3-(4-((7,8-dimethyl-2-oxo-2H-chromen-4yl)methoxy)phenyl) propionate (**4f**).* Buff coloured; yield 57%; m.p:90–94 °C; IR (KBr) cm^{-1}1725 (C=O) 3290 (-NH) 1648 (amide C=O); ^1H NMR (DMSO, 400 MHz, TMS): δ ppm 1.12 (t, 3H, O–CH_2–CH_3, $J = 7.2$ Hz), 2.24 (s, 3H, Ar–CH_3), 2.46 (s, 3H, Ar–CH_3), 3.07(m, 2H, Ph-CH_2, $J = 7.6$ Hz), 4.07 (q, 2H, O–CH_2–CH_3, $J = 7.2$ Hz), 4.58 (t,

1H, Ph–CH_2–CH, J = 7.6 Hz), 5.33 (s, 2H, O–CH_2), 6.46 (s, 1H, C3–H), 7.03–7.82 (m, 11, Ar–H), 8.78 (d,1H, NH–CO, J = 7.6 Hz D_2O Exchangeable); Anal. Calcd. for $C_{30}H_{29}NO_6$ (%), Calcd.: C, 72.13; H, 5.85; N, 2.80, found: C, 72.06; H, 5.71; N, 2.68.

*6.1.2.17. Ethyl2-benzamido-3-(4-((3-oxo-3H-benzo[f]chromen-1-yl) methoxy)phenyl)propionate (**4g**).* Light brown; Yield 56%; m.p: 155–157 °C; IR (KBr) cm^{-1}1728 (C=O) 3370 (–NH) 1647 (amide C=O); ^{1}H NMR (DMSO, 400 MHz, TMS): δ ppm 1.10 (t, 3H, O–CH_2–CH_3, J = 7.2 Hz), 3.12 (m, 2H, Ph-CH_2, J = 6.8 Hz), 4.05 (q, 2H, O–CH_2–CH_3, J = 7.2 Hz), 4.61(t, 1H, Ph–CH_2–CH, J = 7.6 Hz), 5.22 (s, 2H, O–CH_2), 6.81 (s, 1H, C3–H), 7.00–8.54 (m, 15H, Ar–H), 8.79 (d, 1H, NH–CO, J = 7.6 Hz, D_2O Exchangeable); Anal. Calcd. for $C_{32}H_{27}NO_6$ (%), Calcd: C, 73.69; H, 5.22; N, 2.69, found: C, 73.63; H, 5.19; N, 2.69.

*6.1.2.18. Ethyl2-benzamido-3-(4-((2-oxo-2H-benzo[h]chromen-4-yl) methoxy)phenyl)propionate (**4h**).* Light brown; Yield 61%; m.p:190–194 °C; IR (KBr) cm^{-1} 1727 (C=O) 3361 (–NH) 1647 (amide C=O); ^{1}H NMR (DMSO, 400 MHz, TMS): δ ppm 1.12 (t, 3H, O–CH_2–CH_3, J = 7.2 Hz), 3.02 (m, 2H, Ph-CH_2, 6.8 Hz),4.07 (q, 2H, O–CH_2–CH_3, J = 7.2 Hz), 4.61(t, 1H, Ph–CH_2–CH, J = 7.6 Hz), 5.24 (s, 2H, O–CH_2), 6.81 (s, 1H, C3–H), 7.02–8.62 (m, 15H, Ar–H), 8.73 (d, 1H, NH–CO, J = 7.6 Hz D_2O Exchangeable); Anal. Calcd. for $C_{32}H_{27}NO_6$ (%), Anal. Calcd. for $C_{28}H_{24}ClNO_6$ (%), Calcd: C, 73.69; H, 5.22; N, 2.69, found: C, 73.11; H, 5.16; N, 2.49.

*6.1.2.19. Ethyl2-benzamido-3-(4-((6-chloro-2-oxo-2H-chromen-4yl) methoxy)phenyl)propionate (**4i**).* Off white; Yield 57%; m.p:152–155 °C; IR (KBr) cm^{-1} 1726 (C=O) 3350 (-NH) 1648 (amide C=O); ^{1}H NMR (DMSO, 400 MHz, TMS): δ ppm 1.13 (t, 3H, O–CH_2–CH_3, J = 7.2 Hz), 3.09 (m, 2H, Ph-CH_2), 4.09 (q, 2H, O–CH_2–CH_3, J = 7.2 Hz), 4.60 (t, 1H, Ph–CH_2–CH, J = 7.6 Hz), 5.31 (s, 2H, O–CH_2), 6.59 (s, 1H, C3–H), 7.05–8.09 (m, 11H, Ar–H), 8.78 (d, 1H, NH–CO, J = 7.6 Hz, D_2O Exchangeable); Anal. Calcd. for $C_{28}H_{24}ClNO_6$(%), Calc: C, 66.47; H, 4.78; N, 2.77, found: C, 66.43; H, 4.65; N, 2.63.

*6.1.2.20. Ethyl 2-benzamido-3-(4-((7-chloro-2-oxo-2H-chromen-4yl) methoxy)phenyl)propionate (**4j**).* Light brown; Yield 62%; m.p:110–114 °C; IR (KBr) cm^{-1}1724 (C=O) 3340 (-NH) 1648 (amide C=O), ^{1}H NMR (DMSO, 400 MHz, TMS): δ ppm 1.14 (t, 3H, O–CH_2–CH_3, J = 7.2 Hz), 3.09 (m, 2H, Ph-CH_2), 4.08 (q, 2H, O–CH_2–CH_3, J = 7.2 Hz), 4.61(t, 1H, Ph–CH_2–CH, J = 7.2 Hz),5.35 (s, 2H, O–CH_2), 6.59 (s, 1H, C3–H), 7.01–8.19 (m, 11H, Ar–H), 8.86 (d,1H, NH–CO, J = 7.2 Hz, D_2O Exchangeable); Calc: C, 66.47; H, 4.78; N, 2.77, found: Calc: C, 66.44; H, 4.67; N, 2.65.

6.2. Anti-microbial screening

The antibacterial activity of the synthesized compounds were performed in-vitro against (i) Gram-positive bacteria: *E. faecalis* (ATCC no.35550), *S. aureus* (ATCC no. 12598) (ii) Gram-negative bacteria: *E. coli* (ATCC No. 25922), *P. aeruginosa* (ATCC No.25619) by broth dilution methods [22]. The MIC determination of the tested compounds was carried out in comparison with Ciprofloxacin.

The Anti-fungal activity were performed against these standard strains: *C. albicans* (ATCC no.2091) and *A.niger* (ATCC no. 9029). The MIC determination of the tested compounds was carried out in comparison with Fluconazole by broth dilution method [22].

Nine dilutions of each drug were prepared with BHI (brain heart infusion) for MIC. In the initial tube 20 μL (μL) of drug was added into the 380 μL of BHI broth. Then from the initial tube 200 μL was transferred to the first tube containing 200 μL of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200 μL was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution for each drug. From the maintained stock cultures of required organisms, 5 μL was taken and added into 2 mL of BHI broth. In each serially diluted tube 200 μL of above culture suspension was added. The tubes were incubated for 24 h at 37 °C in the incubator and observed for turbidity.

6.3. Computational methods

6.3.1. Preparation of the ligands

The three-dimensional structures of coumarin derivatives were constructed by using Sybyl X-2.0 version (Tripos Inc.) [25], running on Dual-core Intel (R) core (TM) i3-2130 CPU 3.40 GHz, RAM Memory 2 GB under the Windows 7 system.

6.3.2. Docking assay

For the docking of ligands to protein active sites and for estimating the binding affinities of docked compounds, Surflex-Dock module, a fully automatic docking tool available on Sybyl X-2.0 version (Tripos Inc.) was used in this study. Docking simulations: The X-ray Crystal Structure of *E. coli* 24 kDa Domain in Complex with clorobiocin (PDB code: 1KZN; resolution 2.30 Å; http://www.rcsb.org) [26] was obtained from protein data bank in PDB format as starting point. Protein structure with all water molecules deleted was used for docking simulations. Mislabelled atom types from the pdb file were corrected, subsequently, proline F angles were fixed at 70°, side chain amides were checked to maximize potential hydrogen bonding, side chains were checked for close van der Waals contacts, and essential hydrogens were added. The model was checked for conformational problems using the module ProTable from Sybyl. Ramachandran plot [27] of the backbone torsion angles phi and psi, local geometry and the location of buried polar residues/exposed non-polar residues were examined. The protein was subjected to energy minimization following the gradient termination of the Powell method for 3000 iterations using Kollman united force field with non-bonding cut-off set at 9.0 and the dielectric constant set at 4.0 [28,29] and energy minimization for synthesized compounds, including clorobiocin and novobiocin were carried out by Powell method for 3000 iterations using Tripos force field [30] and Gasteiger [31] charge with non-bonding cut-off set at 9.0 and the dielectric constant set at 4.0. The synthesized compounds and the standard compounds tested in this study were docked to DNA gyrase subunit A (PDB code: 1KZN) using Surflex-Dock programme in Sybyl software by incremental construction approach of building the structure in the active site so as to favour the binding affinity [32,33]. Finally, the docked ligands were ranked based on a variety of scoring functions that have been compiled into the single consensus score (*C*-score) [34].

7. Conclusion

The present investigation has shown that introduction of gallic acid and tyrosine at the allylic position in the coumarins leads to molecules with enhanced degree of specificity in their antimicrobial activity which is further supported by docking studies also.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.10.047.

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