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The Crystal Structure of 3-Amino-1-(4-Chlorophenyl)-9-Methoxy-1*H*-Benzo[*f*]Chromene-2-Carbonitrile: Antimicrobial Activity and Docking Studies

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Abstract: Compound 3-amino-1-(4-chlorophenyl)-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitrile (**4**), was synthesized via the reaction of 7-methoxynaphthalen-2-ol (**1**), 4-chlorobenzaldehyde (**2**), and malononitrile (**3**) in an ethanolic piperidine solution under microwave irradiation. The synthesized pyran derivative **4** was asserted through spectral data and X-ray diffraction. The molecular structure of compound **4** was established unambiguously through the single crystal X-ray measurements and crystallized in the Triclinic, P-1, a = 8.7171 (4) Å, b = 10.9509 (5) Å, c = 19.5853 (9) Å, α = 78.249 (2)°, β = 89.000 (2)°, γ = 70.054 (2)°, V = 1717.88 (14) Å^3, Z = 4. The target molecule has been screened for antibacterial and antifungal functionality. Compound **4** exhibited favorable antimicrobial activities that resembled the reference antimicrobial agents with an IZ range of 16–26 mm. In addition, MIC, MBC, and MFC were assessed and screened for molecule **4**, revealing bactericidal and fungicidal effects. Lastly, a molecular docking analysis was addressed and conducted for this desired molecule.

Keywords: 1*H*-benzo[*f*]chromene; antimicrobial activity; MIC; MBC; MFC; Docking; X-ray

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1. Introduction

The crystalline configurations of drug candidates have amassed substantial appreciation as a critical criterion for rational drug design with the manipulation of their functional moieties impacting the drug's structure–activity relationship. Generally, the attained crystallographic data offer explicit/precise structural identification and absolute configuration [1–6], which accordingly elucidates the performance of the novel drugs without triggering the adverse response of the biological system stimuli. Of the drug candidates with an elevated disposition to forge crystallographic structures, chromene compounds are among the most notorious and prosperous [7–9]. The comprehensive biomedical features of chromene molecules have motivated scientific figures within the drug discovery biosphere to cultivate new derivatives of this class of materials and explore their novel biological characteristics. Chromenes have been renowned for their incredible biological

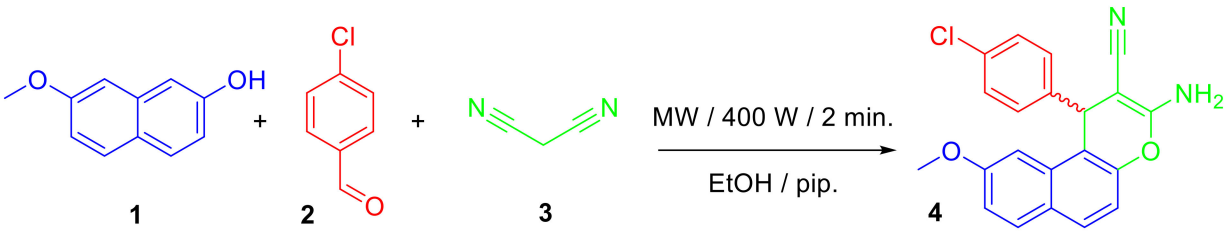
functions, which assisted their assimilation into various applications such as antimicrobial activities [10–14], hypolipidemic [15], antileishmanial, antiviral, anti-HIV, antianaphylactic activities [16–18], insecticidal [19], targeting of *c*-Src kinase enzyme [20,21], anticancer and cytotoxic activities [22–25], cell cycle analysis, apoptotic effects, caspase 3/7, and inhibition of the topoisomerase enzyme [26–33]. Among the synthetic strategies to acquire chromene molecules, microwave irradiation is one of the most efficacious and eco-friendly procedures, which facilitates the isolation of the desired compounds in a short period of time and results in good yields [34–37]. Furthermore, the dihydrofolate reductase (DHFR) enzyme used as a therapeutic target in the treatment of infections through NADPH is used in the reduction of DHFR and is involved in the synthesis of cell proliferation raw material [38]. DHFR inhibitors are widely used in the treatment of fungal, bacterial, and mycobacterial infections through block DNA replication as well as in fighting cancer [39]. In addition, the chromene derivatives are used as potential agents against DHFR [40].

In continuation of our efforts to discover oxygen-heterocyclic derivatives with promising antimicrobial and antitumor activities [41–57], we present the synthesis of 3-amino-1-(4-chlorophenyl)-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitrile and portray its crystallographic structure. Moreover, the antimicrobial behavior of the target molecule is evaluated and its minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentrations (MFC) are appraised. Additionally, a molecular docking assessment of the novel compound is addressed and granted.

2. Results and Discussion

2.1. Chemistry

The route adopted for the preparation of compound **4** is depicted in Scheme 1. The synthesis was initiated by reacting 7-methoxynaphthalen-2-ol (**1**) with 4-chlorobenzal- dehyde (**2**) and malononitrile (**3**) in an ethanolic–piperidine solution under microwave irradiation conditions to furnish 3-amino-1-(4-chlorophenyl)-9-methoxy-1*H*-benzo[*f*]-chromene-2-carbonitrile (**4**). By repeating the reaction at various watt powers (200, 300, 400 W) and time intervals (1, 1.5, 2 min.), the best results were obtained by employing 400 W with a 2 min. reaction period, which delivered the maximum yield for compound **4**. TLC was employed to monitor the reaction.



Scheme 1. Synthesis of 3-amino-1-(4-chlorophenyl)-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitrile (**4**).

2.2. Optical Activity

Compound **4** has a chiral feature; consequently, this specific rotation was gauged, utilizing a Carl Zeiss polarimeter to attribute the stereochemistry of the 1-position to the 1H-benzo[*f*]-chromene moiety. Results revealed that compound **4** has zero rotation (meaning the molecule is optically inactive) and is obtained in the form of a racemic (±) mixture [26–28], as illustrated in Scheme 1.

2.3. Spectroscopic Data

The structure and purity of compound **4** were substantiated through spectral analyses, including: IR, ^{1}H NMR, ^{13}C NMR, MS, and X-ray single crystal (see Supplementary Materials, Figures S1–S3).

2.4. Crystal Data

In the title compound **4**, $C_{21}H_{15}ClN_2O_2$, the crystallographic data and purification information are outlined in Table 1. The asymmetric unit of molecule **4** incorporates two independent compounds, which is witnessed in Figure 1. All the bond lengths and angles are in normal ranges [58]. As displayed in the crystal packing (Figure 2), the molecular components of compound **4** were linked through two intermolecular hydrogen bonds and two intramolecular hydrogen bonds, as shown in Table 2.

Table 1. X-ray experimental details for compound **4**.

Crystal Data	
Chemical formula	$C_{21}H_{15}ClN_2O_2$
Mr	362.80
Crystal system, space group	Triclinic, *P*-1
Temperature (K)	293
***a*, *b*, *c* (Å)**	8.7171 (4), 10.9509 (5), 19.5853 (9)
α, β, γ (°)	78.249 (2), 89.000 (2), 70.054 (2)
V (Å^3)	1717.88 (14)
Z	4
Radiation type	Cu *Kα*
μ (mm^{-1})	2.12
Crystal size (mm)	0.22 × 0.14 × 0.12
Data collection	
Diffractometer	Bruker APEX-II D8 venture diffractometer
Absorption correction	Multi-scan SADABS Bruker 2018
Tmin, Tmax	0.901, 0.937
No. of measured, independent and observed [I > 2σ(I)] reflections	15,845, 5040, 2471
R_{int}	0.044
Refinement	
R[F^2 > 2σ(F^2)], wR(F^2), S	0.107, 0.308, 1.26
No. of reflections	5040
No. of parameters	471
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.53, −0.33
CCDC No.	2,054,799

Table 2. Hydrogen-bond geometry (Å, °) for compound **4**.

D*—H···*A	***D*—H**	**H···*A***	***D*···*A***	***D*—H···*A***
N1A—H1AA●●●N2A [i]	0.860	2.3900	3.153 (14)	149.00
N1A—H1AB●●●O1B	0.860	2.5000	3.353 (12)	170.00
N1B—H1BA●●●N2B [ii]	0.860	2.3800	3.188 (15)	156.00
N1B—H1BB●●●O1A	0.860	2.4200	3.272 (12)	171.00

Symmetry codes: [i]: x, y + 1, z; [ii]: −x + 3, −y−2, −z + 2.

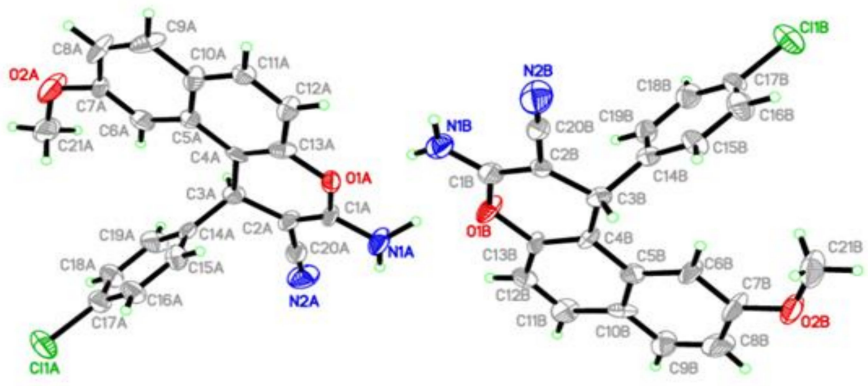


Figure 1. ORTEP diagrams of the titled compound **4**. Displacement ellipsoids are plotted at the 40% probability level for non-H atoms.

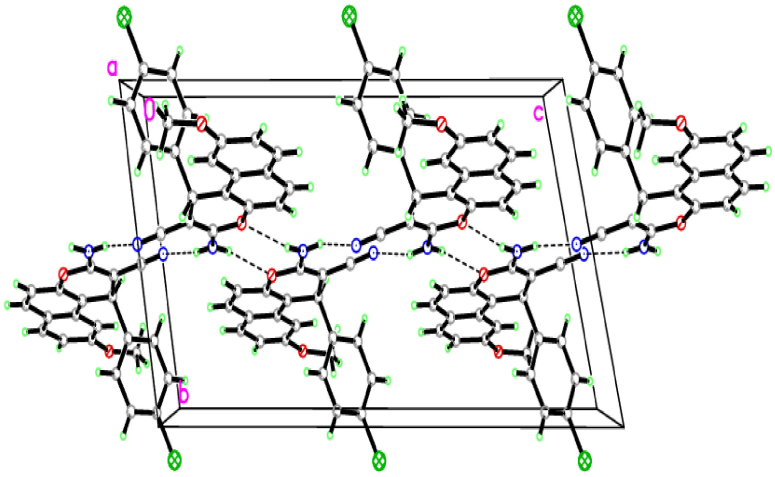


Figure 2. Molecular packing of compound **4**. Hydrogen bonds are drawn as dashed lines.

2.5. Biological Activity

2.5.1. Antimicrobial Activity Assay

Molecule **4** was estimated through a preliminary screening of its antibacterial activity via the agar diffusion methodology, employing a Mueller–Hinton agar medium for bacteria and a Sabouraud's agar medium for fungi [59]. The analyzed collections encompassed three Gram-positive species of pathogenic bacteria: *Staphylococcus aureus* (RCMB 000106), *Bacillus subtilis* (RCMB 000108), and *Staphylococcus epidermtitis* (RCMB 000107); three Gram-negative bacteria: *Enterococcus cloaca* (RCMB 000101), *Escherichia coli* (RCMB 000103), and *Salmonella typhimurium* (RCMB 000103), utilizing reference antibiotic drugs Ampicillin and Gentamycin (5 µg/mL). Compound **4** was also scrutinized against three fungi: *Aspergillus fumigatus* (RCMB 002003), *Aspergillus flavus* (002002), and *Candida albicans* (RCMB 005003), utilizing the reference antibiotic Ketoconazole (5 µg/mL). The minim zone of inhibition (IZ) in mm ± standard deviation beyond the well diameter (6 mm) was established, employing a 5 µg/mL concentration of compound **4**. Dimethyl sulfoxide (DMSO) was utilized as a blank and exhibited no antimicrobial activity. The inhibitory impacts of the synthetic compound in evaluation against these organisms are illustrated in Table 3. Compound **4** showed lower IZ than reference drugs against most of the tested microorganisms (*S. aureus, S. epid, E. cloaca, S. typhi and A. fumigates*). Furthermore, its compound displayed the same IZ with reference inhibitors against *B. subtili and A. flavus*.

Table 3. Antimicrobial screening for compound **4**.

	Diameter of Inhibition Zone (mm)								
	Gram + ve bacteria			*Gram − ve bacteria*			*Fungi*		
Compound	*S. aureus*	*B. subtilis*	*S. epid.*	*E. cloaca*	*E. coli*	*S. typhi.*	*A. fumigates*	*A. flavus*	*C. Albicans*
4	22 ± 0.7	26 ± 0.6	25 ± 0.4	26 ± 0.4	13 ± 0.1	16 ± 0.5	15 ± 1.1	16 ± 1.2	21 ± 0.7
Ampicillin	24 ± 1.1	26 ± 1.0	28 ± 1.4	-	-	-	-	-	-
Gentamycin	-	-	-	27 ± 0.6	30 ± 1.5	17 ± 0.4	-	-	-
Ketoconazole	-	-	-	-	-	-	17 ± 1.2	16 ± 1.1	20 ± 0.2

Diameter of the hole = 6 mm; Data are expressed in the form of mean ± SD. Not active (<8 mm), Weak activity (8–12 mm), Moderate activity (13–16 mm), Strong activity (≥17 mm). Solvent: DMSO (8 mm).

2.5.2. MIC, MBC/MFC Studies

MIC denotes the minimum inhibitory concentration (the lowest concentration required to inhibit bacterial growth), MBC to the mean bactericidal concentration (the lowest concentration of the synthesized drugs required to kill specific bacteria), and MFC to the minimum fungicidal concentration (the lowest concentration of the synthesized drugs required to kill specific fungi). MIC, MBC, and MFC were assayed for the active compound **4** in µg/mL. The examined antimicrobial data (MICs/MBCs and MICs/MFCs) of the desired molecule 4 and their standardized drugs are supplied in Table 4.

Table 4. The MIC (MBC/MIc) in µg/mL of compound **4**.

	Gram + ve bacteria				*Gram − ve bacteria*			*Fungi*	
Compound	*S. aureus*	*B. subtilis*	*S. epid.*	*E. cloaca*	*E. coli*	*S. typhi.*	*A. fumigates*	*A. flavus*	*C. Albicans*
4	6.25 (12.5)	12.5 (25)	25 (50)	25 (50)	25 (100)	50 (100)	6.25 (12.5)	12.5 (25)	25 (100)
Ampicillin	6.25	6.25	6.25	-	-	-	-	-	-
Gentamycin	-	-	-	12.5	6.25	12.5	-	-	-
Ketoconazole	-	-	-	-	-	-	6.25	12.5	6.25

Antimicrobials are regularly perceived as bactericidal/fungicidal if the MBC/MFC quantities do not exceed four times the MIC [60]. Molecule 4 has 2 × MIC = MBC value in the instance of *S. aureus*, *B. subtilis*, *E. cloaca*, and *S. typhimurium* bacteria. Furthermore, molecule 4 possesses 2 × MIC = MFC value in the instance of *A. fumigatus* and *A. flavus* fungi. Results in Tables 3 and 4 indicated that molecule 4 demonstrated much stronger antibacterial behavior against *S. aureus*, *B. subtilis*, *S. epidermtitis*, *E. cloaca*, *E. coli*, and *S. typhimurium* with an inhibition zone (Figure S4, Supplementary Material) ranging from 16–26 mm and MIC & MBC of 6.25–50 µg/mL compared to the quantities of the reference drug Ampicillin (IZ = 24–28 mm and MIC/MBC = 6.25 µg/mL) and Gentamycin (IZ = 17–30 mm and MIC/MBC = 6.25–50 µg/mL). Moreover, compound **4** (IZ = 15 and 16 mm) yielded a much stronger antifungal activity against *A. fumigatus* and *A. flavus* with MIC/MFC of 6.25 and 12.5 µg/mL in appraisal against Ketoconazole (IZ = 17 and 16 mm).

2.5.3. Structure-Activity Relationship (SAR) Study

The antimicrobial activity of molecule 4 is depicted in Table 3. The SAR study revealed that compound **4** with inhibitory effects ranging from 22–26 and 16–31 mm illustrated stronger vitality against the Gram-positive tested bacteria (*S. aureus, B. subtilis, S. epidermtitis*), Gram-negative tested bacteria (*E. cloaca*, *E. coli*, *S. typhimurium*), and inhibitory effects ranging from 15–21 mm against the tested fungi (*A. fumigates*, *A. flavus*, *C. Albicans*) in evaluation of the standard antibiotics Ampicillin (IZ = 24–28 mm), Gentamycin (IZ = 17–30 mm), and Ketoconazole (IZ = 16–20 mm), respectively. The presence of a hydrophobic electron-withdrawing substituent (the chlorine atom at the para-position on the phenyl group at the 1-position) alongside an electron-donating substituent (the methoxy group at the 9-position of the 1*H*-benzo[*f*]chromene moiety) has enhanced the antimicrobial behavior significantly.

2.6. Molecular Docking Analysis

According to the inhibitory functionalities of molecule 4, the molecular docking was performed against dihydrofolate reductase "DHFR", and its positioning of the active compound within the substrate binding pocket assists in the comprehension of its mode of interaction. We selected two crystal structures for the hDHFR protein ((PDB): 4DFR [61]) and (PDB ID: 3NTZ [62]). Compound **4**, the reference inhibitor Methotrexate, and reference drugs (Ampciline, Gentamicin, Ketocwere) were stationed in the binding pocket of enzyme s, and its binding interactions were illustrated in Figures 3 and 4.

The mGenTHERADER generated the 3D loop structure of DHFR and the applied docking framework. The biological behavior was represented as binding-interaction BI term for 3-amino-1-(4-chlorophenyl)-9-methoxy-1H-benzo[*f*]chromene-2-carbonitrile over DHFR, then compared with Methotrexate and reference drugs. Compound **4** re-docked and showed promising (root mean square deviation, RMSD = 0.93, 0.52 Å) compared to other compounds against both enzymes. The reference inhibitor reported interaction with vital binding site of 4DFR (ASP27, ILE5, ILE94, ARG52, ARG57). Compound **4** showed *BI* = −7.69 Kcal/mol. compared to Methotrexate BI = −8.86 Kcal/mol (Table 5). BI was arranged as 4 > **Gentamicin** > **Methotrexate** > **Ketoconazole** > **Ampicillin**. Compound **4** formed a strong H-bond between methoxy and vital ASP27 with a distance 1.2° and E = −1.69(kcal/mol), compared to Methotrexate, which showed an H-bond with ASP27 and formed a distance of 2.88° and ΔE = −5.4 (kcal/mol. The interaction mode for compound **4** and reference drugs in the binding pocket had the same manner as the reference inhibitor and might be responsible for the high inhibitory activity for compound **4**.

Table 5. The binding-affinity for tested compounds **4** with docking score (kcal/mol) against hDHFR.

	4DFR					3NTZ				
	ΔE	**rmsd**	**H.B**	**Int.**	**E_ele**	**ΔE**	**rmsd**	**H.B**	**Int.**	**E_ele**
4	−7.53	0.93	−36.95	−28.96	−24.33	−9.34	0.52	−203.47	−26.13	−11.01
Methotrexate	−8.82	1.90	−21.08	−20.66	−56.52	−6.79	2.28	−21.52	−31.78	−9.75
Ampicillin	−7.19	1.69	74.28	−21.28	−13.81	−7.44	1.73	72.46	−21.70	−11.83
Gentamicin	−8.27	2.93	313.10	−2.70	−19.63	−9.27	2.66	212.67	−21.96	−14.70
Ketoconazole	−7.53	2.32	23.93	−22.05	−10.80	−8.45	2.40	30.81	−14.48	−8.75

ΔE: Free binding energy of the ligand, Int.: Affinity binding energy of hydrogen bond interaction with receptor, H.B.: Hydrogen bonding energy between protein and ligand. E_ele: Electrostatic interaction with the receptor.

In case of 3NTZ; Compound **4** showed the highest ***BI*** = −9.43 Kcal/mol. compared to other inhibitors (Table 5). BI was arranged for other inhibitors as **Gentamicin** > **Ketoconazole** > **Ampciline** > **Methotrexate**. The active site of 3NTZ comprises the following amino acid residues: Val 6, Ala 9, Leu 22, Pro 25, Asp 27, Leu 28, Glu30, Gln35, Phe 31, Ser 49, Ile 50, Thr56, Leu62, and Thr 111. Compound **4** formed a strong H-bond between the amino group and Ala9 and formed a π-π bond between Leu22. Methotrexate showed an H-bond with Glu30 and Arg70 (Figure 4). Therefore, the interaction with vital amino acids of hDHFR plays an important role in the inhibitory effects of this compound. Furthermore, the activities of compound **4** were due to the presence of the amino and cyano groups.

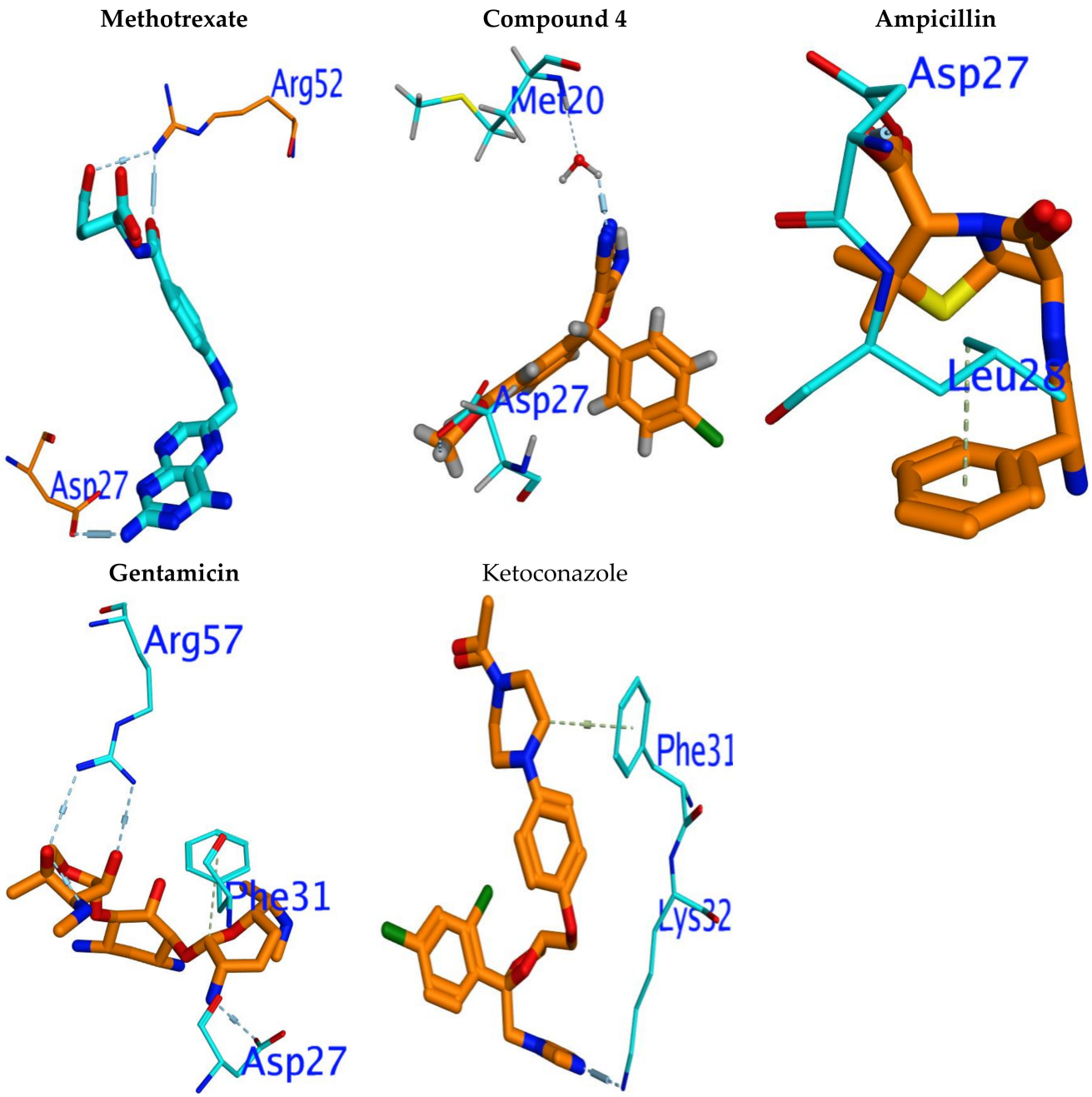


Figure 3. Predicted binding mode of compounds **4** and reference inhibitors into 4DFR H-bondings represented in blue lines, while π-π bond in green line.

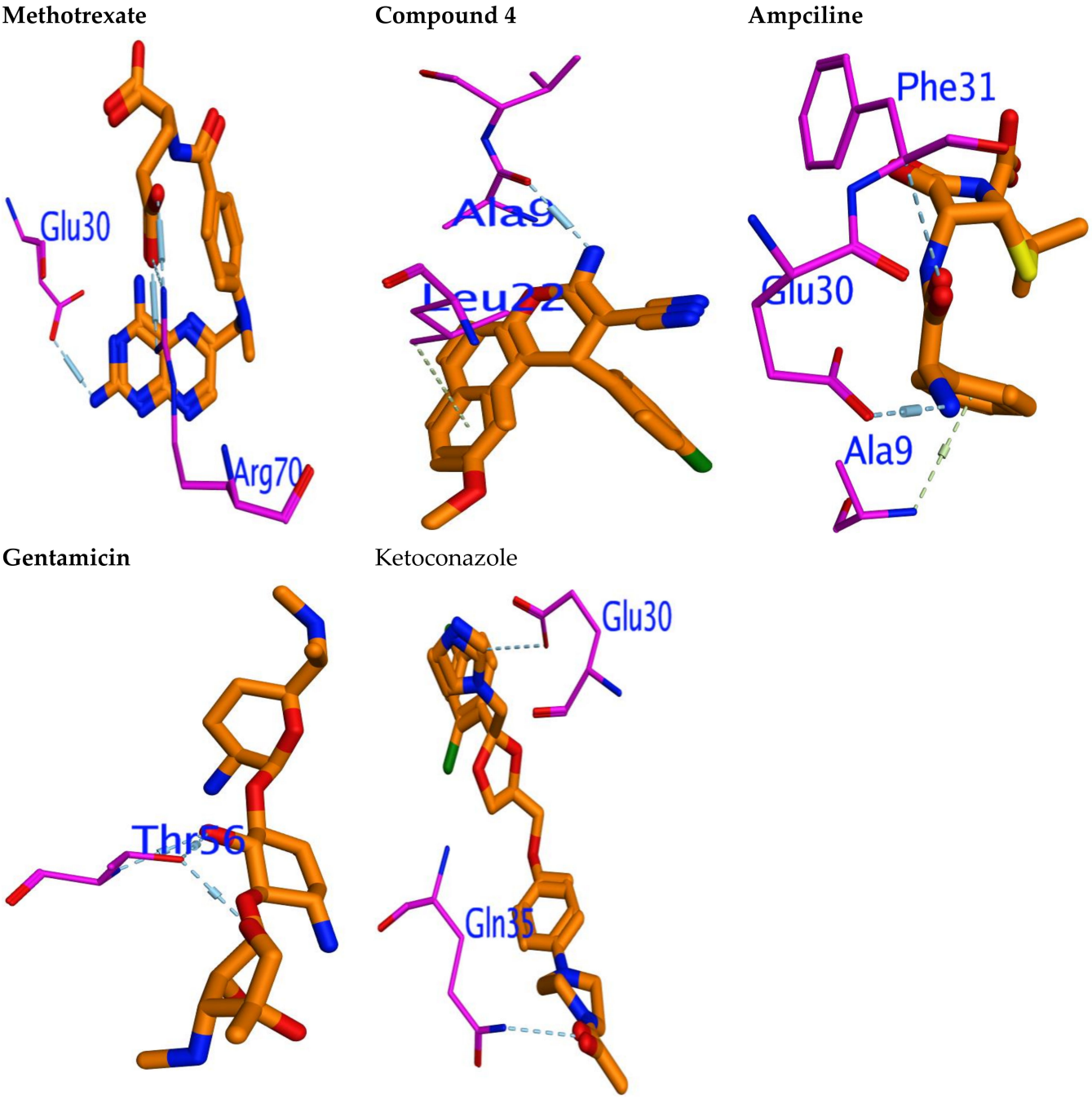


Figure 4. Predicted binding mode of compounds **4** and reference inhibitors into 3NTZ H-bondings represented in blue lines, while π-π bond in green line.

3. Experimental Section

3.1. Materials and Equipment's

All chemicals purchased and instruments used are mentioned in the Supplementary Material.

*3.2. Synthesis of 3-Amino-1-(4-Chlorophenyl)-9-Methoxy-1H-Benzo[f]Chromene-2-Carbonitrile (**4**)*

Yellow needles, yield 89%, m.p. 257–258 °C (Literature procedure: ionic liquids condition, yield 79%; m.p. 257–259 °C [63] microwave condition, yield 89%; m.p. 257–258 °C [64]).

3.3. Biological Screening

Compound **4** was screened for its in vitro antimicrobial activities against Gram-positive species of pathogenic bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, and *Staphylococcus epidermtitis*; three Gram-negative bacteria *Enterococcus cloaca*, *Escherichia coli*, and *Salmonella typhimurium* using the standard antibiotics Ampicillin and Gentamycin as reference drugs. The investigation also included three fungi: *Aspergillus fumigatus*, *Aspergillus flavus*, and *Candida albicans* using the standard antibiotic, Ketoconazole, as a reference drug [59]. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentrations (MFC) were determined as previously reported [60]. The antimicrobial activities were performed at the Regional Centre for Mycology & Biotechnology (RCMP), Al-Azhar University.

3.4. X-ray Crystallography Analysis

Compound **4** was obtained as single crystals by slow evaporation from an ethanol solution of the pure compound at room temperature with CCDC 2054799. Data were collected on a Bruker APEX-II D8 Venture area diffractometer, equipped with graphite monochromatic Mo Kα and Cu Kα radiations at 293 (2) K. Cell refinement and data reduction were carried out by Bruker SAINT. SHELXTL-2018/3 [65,66] was used to solve the structure.

3.5. Docking Assay

Small ligands (4 and methotrexate) were prepared using the DFT theory with the Becke3-Lee-Yang-parr (B3LYP) level using 6-311G ** basis as implemented in Gaussian 09W [67]. The optimization geometry for molecular structures was carried out and used in the docking experiment.

The 3D crystal-structure for the GHFR model was prepared using the glide-tool as described [68,69].

4. Conclusions

In an effort to develop potent antimicrobial agents, compound **4** was synthesized and characterized employing an X-ray diffraction technique. Subsequently, the antimicrobial behavior of molecule 4 was appraised against different pathogenic bacterial and fungal strains, which demonstrated promising antimicrobial activities in correspondence with the reference antimicrobial agents exhibiting an IZ range of 16–31 mm. Furthermore, the values of MIC, MBC, and MFC were ascertained for compound **4**, disclosing its bactericidal and fungicidal activites. The molecular docking analysis was performed to relate our biological findings with the chemical structure and to show their ability to bind with the DHFR active site similar to Methotrexate.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cryst12070982/s1, Figure S1: ^{1}H NMR of compound **4**; Figure S2: ^{1}H NMR 8–6 ppm of compound **4**; Figure S3: ^{13} C NMR of compound **4**, and Compound 4_checkCIF; Figure S4: The inhibition zone of compound **4**.

Author Contributions: A.M.E.-A., A.M.F., A.E.-G.E.A., A.M.N., R.M.O. and A.A.A. designed the proposed methods and analyzed the spectral data; A.M.E.-A. performed the experiment and implemented the biological study; A.A.E. performed DFT theoretical calculations; M.A.B. analyzed the biological data and reviewed and R.M.O. edited the draft. H.A.G. carried out and wrote the X-ray processes. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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