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Design, Synthesis, Molecular Docking and Biological Evaluation of New Carbazole Derivatives as Antimicrobial Agents

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KEYWORDS

Carbazole; Carbazole derivatives; Antimicrobial; Antifungal; Docking study

ABSTRACT:

AIM: Design, synthesis, molecular docking and biological evaluation of new carbazole derivatives as antimicrobial agents

Background: Around 1.2 million people die from microbial illness each year, making it the second greatest cause of mortality in the world. As a result, medicinal chemists are constantly looking for fresh or better ways to treat microbial diseases. Carbazole derivatives have demonstrated notable biological activities among a variety of nitrogen-containing heterocyclics, with their antibacterial and antifungal properties receiving the most attention.

Objectives: To perform synthesis and biological evaluation of carbazole derivatives

Methods: The present study started with a precursor named 9H-carbazole to prepare novel heterocyclic derivatives. Treatment of carbazole with bromoethane gave intermediate-1 (InM-1), also named 9-ethyl-9H-carbazole. InM-1 was transformed into 9-ethyl-9H-carbazole-3,6-dicarbaldehyde (InM-2) through treatment with phosphorous oxychloride. The reaction of InM-2 with sodium borohydride or MeOH afforded (9-ethyl-9H-carbazole-3.6-diyl)dimethanol(InM-3).4,4'-((9-ethyl-9H-carbazole-3,diyl)bis(methylene)bis(oxy)bis(4-oxobutanoic acid) (InM-4) was obtained from the reaction of InM-3 with succinic anhydride and pyridine. InM-5 (diacid) was also obtained from InM-3 with the reaction of glutaric anhydride and pyridine. InM-4 was used to synthesize the compounds 1, 2, 3, 7, 8, and 9, while InM-5 gave the compounds 4, 5, 6, 10, 11, and 12. Synthesized compounds were characterized using TLC, MASS, and NMR spectroscopy. The molecular docking of these compounds was studied for active compounds and showed lead like characteristics.

Results: A total of 15 carbazole derivatives were synthesized, with percentage yields of 72%, 75%, 68%, 71%, 75%, 74%, 76%, 80%, 71%, 76%, 80%, 71%, 70%, 70% and 72% respectively. TLC, Mass, and NMR spectroscopy were used to characterize newly synthesized compounds, as stated in the results section. All synthesized compounds were evaluated for antifungal and antimicrobial activity against B. subtilis ATCC 6633 and E. coli ATCC 35210. The gold scores of AR101 and DHFR were found to be 61.46, while the gold scores of synthesized compounds were found in the range of 31.31–55.71. The gold score of three compounds, BR2, BR8, and BR14, was found to be above 55. Synthesized compounds proved as potential antibacterial agents.

Conclusion: Compounds 8 and 11 were synthesized with the highest yield, i.e. 80%. Synthesized compounds 3, 9, and 14 at a concentration of 3.1± 0.4 showed the most potent activity against gram-negative bacteria, i.e., E. *coli*, while compounds 2, 6, and 8 at the same concentration were found to be most effective against gram-positive bacteria, i.e., B. *subtilis*. Furthermore, compounds 5, 6, and 15 showed the most potential as therapeutic antifungals.

Introduction:

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Microorganisms cause a variety of diseases in people and animals. The discovery of chemotherapeutic drugs was critical in controlling and treating such disorders. The discovery of chemotherapeutic drugs was critical in chemotherapeutic agents are either isolated from living organisms as antibiotics such as penicillin and tetracycline, or they are chemical compounds synthesized by chemists such as sulfa medicines [1]. The propensity of microorganisms to develop resistance to these chemotherapeutic drugs poses significant challenges for the treatment of microbial illnesses [2]. For this reason, attempts have been made to develop novel antibiotics or new chemical molecules with significant antimicrobial properties that may be acceptable for use as chemotherapeutic drugs [3]. The hunt for new antimicrobial agents is a constant process. Significant classes of nitrogen-containing aromatic heterocyclic compounds, carbazole and its derivatives, have a large p-conjugated system, attractive electrical and charge-transport properties, and the ability to incorporate a variety of functional groups into the architecturally rigid carbazolyl ring [4]. Due to these qualities, carbazole-based derivatives have a wide range of potential applications in the fields of photoelectrical substances, dyes, supramolecular detection, and pharmaceutical applications such as antioxidants, anti-inflammatory agents, antimicrobials, antihistaminics, psychotropic agents, and antimicrobials. Carbazole ring compounds were found in various natural sources, such as Murrayaeuchrestifolia Hayata [5]. But these natural compounds were found to be less effective. To achieve significant effectiveness, we have synthesized carbazole derivatives. The synthesized compounds were characterized by TLC, NMR, and MASS and evaluated for antimicrobial and antifungal activity an followed by molecular docking investigations, docking studies also gave favourable results for finding potent antimicrobial agents with a good gold score.

Material and methods:

All ingredients were obtained from commercial sources and utilized without additional purification.TLC on silica gel-protected aluminium sheets (type 60 F254, Merck) was used to determine the quality of all the newly synthesized compounds, and the spots were detected using iodine gasses and a UV lamp at 254 nm. The melting points were determined in untreated, open capillary tubes. The infrared (FT-IR) spectra were measured using the K-Br pressed pellet method on a 470-Shimadzu infrared spectrophotometer, and the results are expressed in cm1. The 13C-NMR spectra were measured using CDCl3 as a solvent on a Bruker DRX-300 instrument. The chemical shifts are measured in ppm downfield from a tetra-methylsilane internal standard (TMS). The splitting patterns are denoted by the letters s, d, and m, which stand for singlet, doublet, and multiplet, respectively. A Shimadzu 2010A LC-MS spectrometer was used to obtain the mass spectra as listed in table 1. Elemental analyses were carried out on an Elemental Vario EL III Carlo Erba 1108 instrument, with the results falling within 0.04% of the theoretical values [6, 7].

Scheme for synthesis compounds (1-15).

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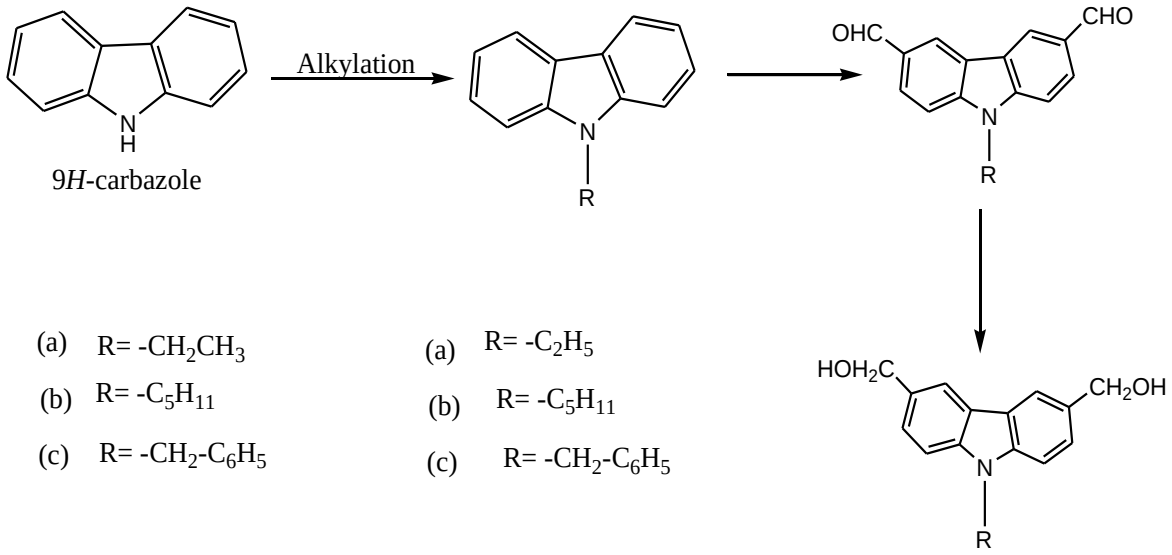
Scheme Step -1



(a) R= $-CH_2CH_3$
(b) R= $-C_5H_{11}$
(c) R= $-CH_2-C_6H_5$

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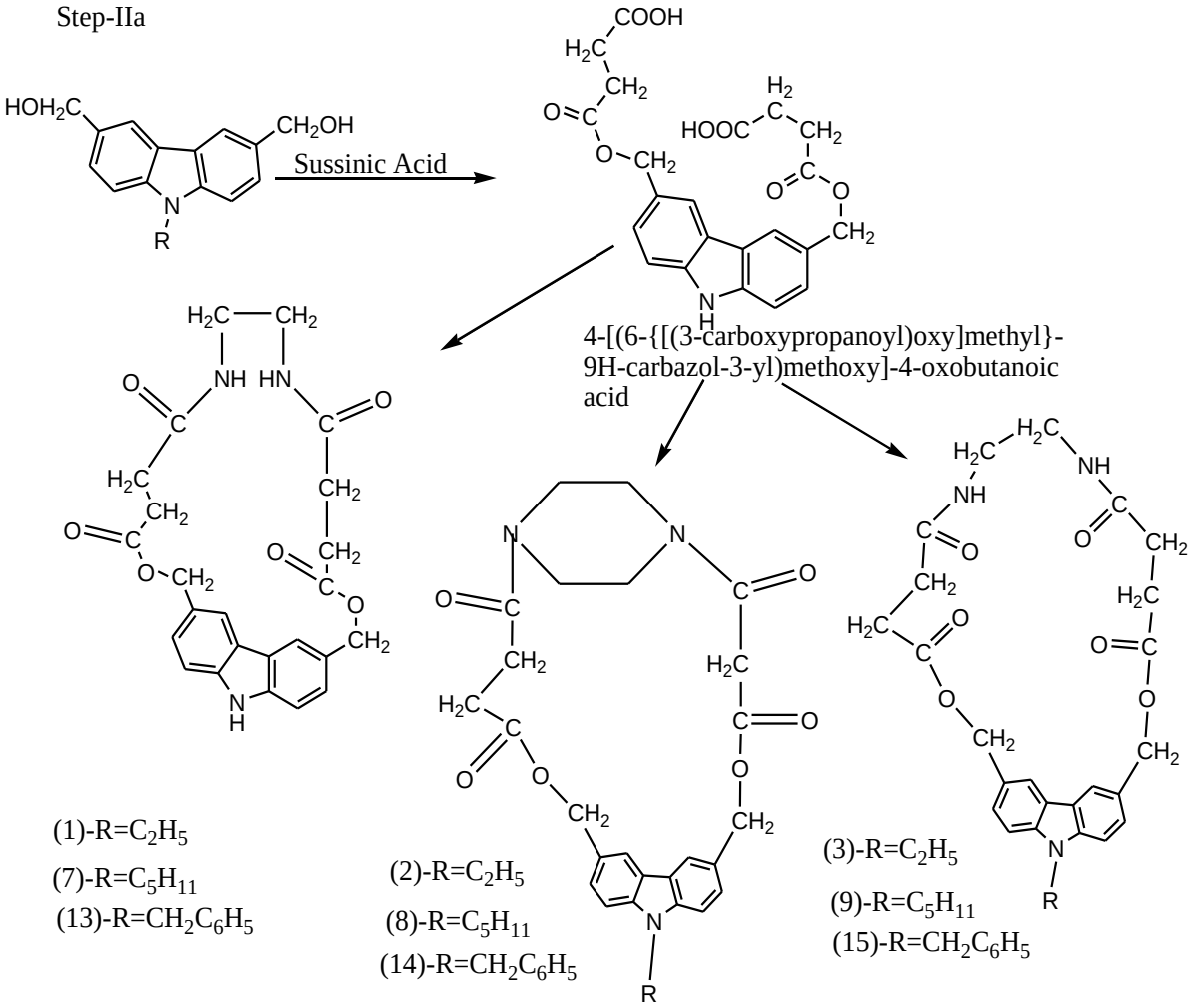
(b) R= $-C_5H_{11}$
(c) R= $-CH_2-C_6H_5$

Step-IIa



(1)-R=C_2H_5
(7)-R=C_5H_{11}
(13)-R=$CH_2C_6H_5$

(2)-R=C_2H_5
(8)-R=C_5H_{11}
(14)-R=$CH_2C_6H_5$

(3)-R=C_2H_5
(9)-R=C_5H_{11}
(15)-R=$CH_2C_6H_5$

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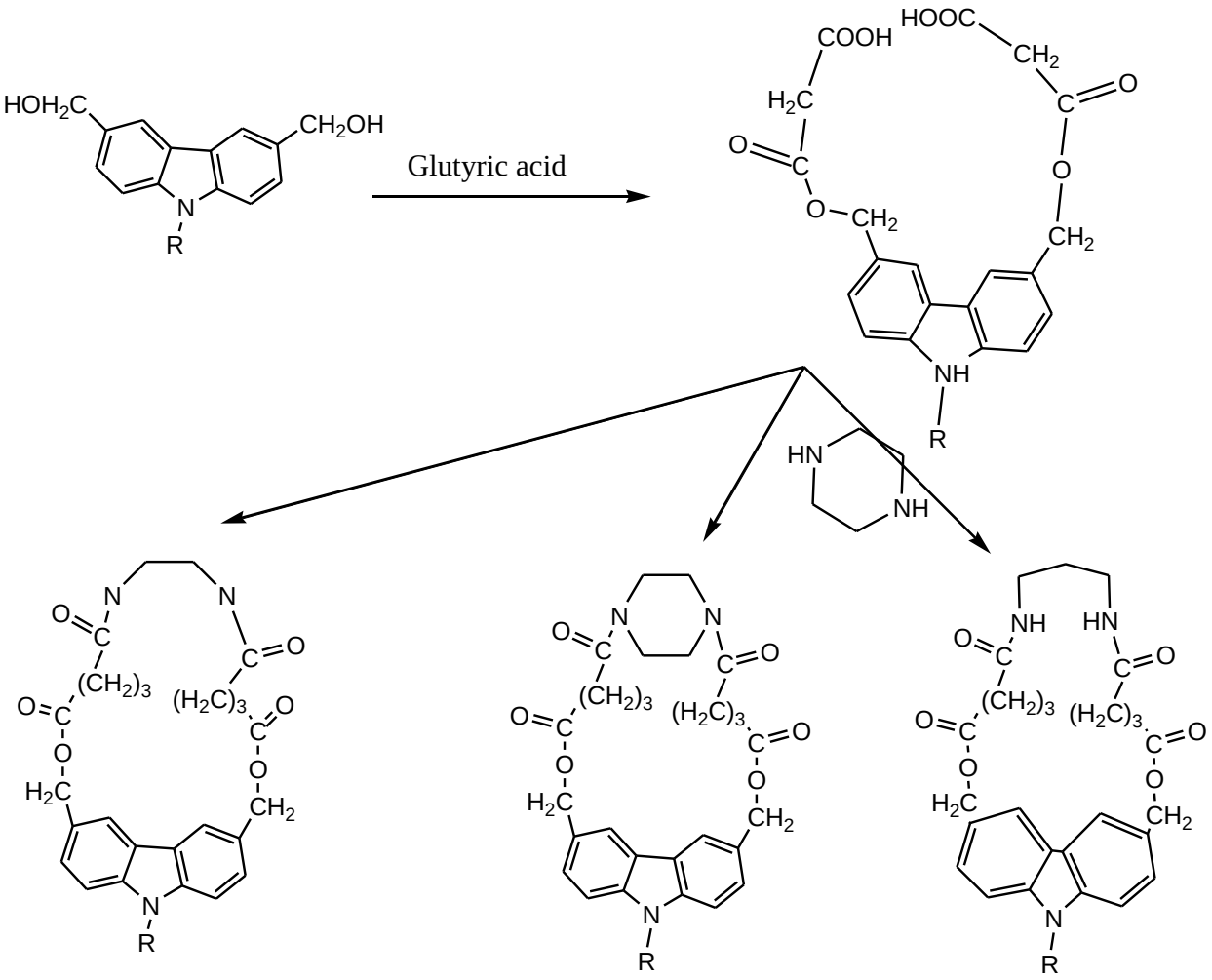
Step-IIb



(4)-R=C_2H_5

(10)-R=C_5H_{11}

(5)-R=C_2H_5

(11)-R=C_5H_{11}

(6)-R=C_2H_5

(12)-R=C_5H_{11}

Determination of antimicrobial activity:

The serial dilution method was used to test the antibacterial activities of the newly synthesized compounds against the bacterial strains *B. subtilis* ATCC 6633 (gram-positive) and *E. coli* ATCC 35210 (gram-negative). The serial dilution was impregnated with the test compounds dissolved in serial dilution at concentrations of 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 3.1 µg/mL in the disc diffusion method. Because of the free solubility of the test compounds, a test tube impregnated with serial dilution was used as a solvent control. The bacterial strains were inoculated on nutrient agar medium in petri dishes, and discs impregnated with solutions of various concentrations of the compounds were placed over them. The plates were incubated for 24 hours at 35ºC. Around the diameters, a zone of inhibition was observed, indicating that microorganism growth was inhibited. To compare the efficacy of the tested compounds, ciprofloxacin (50 µg mL^{-1}) was used as a standard (50 µg mL^{-1}) [8].

Determination of antifungal activity:

The newly synthesized compounds were tested for antifungal activity against the fungal strain *S. aureus*

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ATCC 27853 that was grown on agar medium using the paper serial dilution method. The activity testing procedure was similar to the antibacterial testing procedure described above, and the same concentrations of the tested compounds were used [7]. The results were recorded and reported after the fungal strains were incubated for 48 hours at 25°C with fluconazole as the standard (50µg mL-1). The diameters of the zones of inhibition were observed on and around discs, indicating that the prepared compounds inhibited microbial activity, which was observed and reported. Each experiment was carried out three times [9].

Docking Protocol

A computer-aided method called molecular docking is used to determine how ligands interact with target proteins' active sites.The X-ray 2D-structures of antibacterial targets were retrieved from the Protein Data Bank (www.rcsb.org) (Table 2). The coordinates of S. aureus dihydrofolate reductase (DHFR) complexes with a DHFR inhibitor called AR-101 (5-[[(2R)-2-cyclopropyl-7,8-dimethoxy-2H-chromen-5-yl]methyl]pyrimidine-2,4-diamine) were obtained using PDB entry: 3FYV (Resolution 2.20 Å) [9]. All chemical structures were created using Chem Draw, and in the MOPAC module, utilizing the AM1 technique for closed shell systems, implemented in the CS Chem3D Ultra, they were subjected to energy minimization. Using GOLD 5.3.0 (Gold CCDC), the ligands were docked into the active site of DHFR [10]. Gold offers four scoring functions: i. Goldscore; ii. Chem score; iii. ASP; and iv. Chem PLP. The docking procedure was validated using standard procedures. Every compound was docked ten times, and each position was rated according on how well it performed the Gold Score fitness test. The docking process's steps are listed below in brief:

1. **Target preparation:** 3FYV contains 158 amino acid residues and 91 water molecules. All water molecules were removed, and hydrogen atoms (1482 hydrogens) were added to the target protein.
2. **Binding site:** A co-crystallized ligand-binding site was selected as the binding site for the synthesized compounds.
3. The genetic algorithm parameters were set to automatic, which run between 10000 to 125000 operations.
4. **Number of conformations:** A maximum of 10 conformations were generated for compounds.

Results:

Total fifteen compounds are synthesized and evaluated with various parameters as listed in table 1.

Table 1: Synthesized compounds with their % yield, Rf value, NMR and Mass results

Compound	% Yield	Rf Value	NMR	Mass
Compound 1	72%.	0.6	**^{1}H NMR (400 MHz, DMSO-d6):** δ 8.14 (s, 2H), 7.89 (s, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 7.6 Hz, 2H), 5.21 (s, 4H), 4.40 (bs, 2H), 3.07 (bs, 4H), 2.55 (t, J = 6.4 Hz, 4H), 2.38 (t, J = 6.4 Hz, 4H), 1.25 (t, J = 6.8 Hz, 3H).	$C_{26}H_{29}N_3O_6$, 479.52 $[M]^+$; found, 480.2 $[M+H]^+$.
Compound 2	75%.	0.65	**^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.10 (s, 2H), 7.47 (d, J = 8.2 Hz, 2H), 7.37 (d, J = 8.2 Hz, 2H), 5.30 (s, 4H), 4.35 (bs, 2H), 3.59-3.41 (m, 8H), 2.71-2.59 (m, 8H), 1.41-1.40 (m, 3H).	$C_{28}H_{31}N_3O_6$, 505.56 $[M]^+$; found, 506.3 $[M+H]^+$.
Compound 3	68%.	0.6	**^{1}H NMR (400 MHz, DMSO-d6):** δ 8.15 (s, 2H), 7.84 (bs, 2H), 7.57-7.37 (m, 4H), 5.21 (s, 4H), 4.41 (bs, 2H), 3.03 (m, 4H), 2.36 (m, 8H), 1.48 (bs, 2H), 1.26 (m, 3H).	$C_{27}H_{31}N_3O_6$, 493.55 $[M]^+$; found, 494.1 $[M+H]^+$.
Compound 4	71%.	0.6	**^{1}H NMR (400 MHz, DMSO-d6):** δ 8.08 (bs, 2H), 7.45-7.26 (m, 6H), 5.28-5.25 (m, 4H), 4.33 (m, 2H), 3.21 (m, 4H), 2.42-1.92 (m, 12H), 1.39-1.38 (m, 3H).	$C_{28}H_{33}N_3O_6$, 507.58 $[M]^+$; found, 508.7 $[M+H]^+$.
Compound 5	75%.	0.6	**^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.10 (s, 2H), 7.47-7.37 (m, 4H), 5.29 (s, 4H), 4.33 (m, 2H), 3.53-3.09 (m, 8H), 2.43-1.95 (m, 8H), 1.75 (m, 4H), 1.47 (m, 3H).	$C_{30}H_{35}N_3O_6$, 533.62 $[M]^+$; found, 534.5 $[M+H]^+$.
Compound	74%.	0.65	**^{1}H NMR (400 MHz, DMSO-d6):** δ 8.07-8.01 (m, 2H), 7.42-	$C_{29}H_{35}N_3O_6$, 521.6

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6			7.36 (m, 6H), 5.25 (s, 4H), 4.31 (m, 2H), 3.10-3.00 (m, 4H), 2.36 (m, 4H), 2.17 (m, 4H), 1.92 (m, 4H), 1.36 (m, 3H).	$[M]^+$; found, 522.5 $[M+H]^+$.
Compound 7	76%.	0.6	**^{1} H NMR (400 MHz, $CDCl_3$):** δ 8.01 (m, 2H), 7.36-7.26 (m, 4H), 5.30-5.21 (m, 4H), 4.17 (m, 2H), 3.25-3.18 (m, 4H), 2.65 (m, 4H), 2.43 (m, 4H), 1.37-1.25 (m, 6H), 0.88-0.83 (m, 3H).	$C_{29}H_{35}N_3O_6$, 521.6 $[M]^+$; found, 522.5 $[M+H]^+$.
Compound 8	80%.	0.65	**^{1} H NMR (400 MHz, $CDCl_3$):** δ 8.10 (s, 2H), 7.47 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 5.30 (s, 4H), 4.27 (m, 2H), 3.73-3.33 (m, 8H), 2.72-2.61 (m, 8H), 1.84 (m, 2H), 1.34-1.26 (m, 4H), 0.86 (m, 3H).	$C_{31}H_{37}N_3O_6$, 547.64 $[M]^+$; found, 548.2 $[M+H]^+$.
Compound 9	71%.	0.6	**^{1} H NMR (400 MHz, $CDCl_3$):** δ 8.04 (s,2H), 7.40-7.32 (m, 4H), 5.24 (s, 4H), 4.21 (s, 2H), 3.18-3.16 (m, 4H), 2.67 (m, 4H), 2.46 (m, 4H), 2.20-1.70 (m, 6H), 1.29-1.26 (m, 6H), 0.84 (m, 3H).	$C_{30}H_{37}N_3O_6$, 535.63 $[M]^+$; found, 536.0 $[M+H]^+$.
Compound 10	76%	0.6	**^{1} H NMR (400 MHz, $CDCl_3$):** δ 8.09 (m, 2H), 7.50-7.29 (m, 4H), 5.32-5.28 (m, 4H), 4.32-4.25 (m, 2H), 3.28-3.17 (m, 4H), 2.48-2.32 (m, 4H), 2.05-1.85 (m, 4H), 1.46-1.25 (m, 12H), 0.94-0.86 (m, 3H).	$C_{31}H_{39}N_3O_6$, 549.66 $[M]^+$; found, 550.7 $[M+H]^+$.
Compound 11	80%.	0.6	**^{1} H NMR (400 MHz, $CDCl_3$):** δ 7.84 (m, 2H), 7.57-7.37 (m, 4H), 5.21 (m, 4H), 4.42 (m, 2H), 3.22-3.03 (m, 8H), 2.50-2.36 (m, 8H), 1.88-1.75 (m, 8H), 1.26 (m, 3H).	$C_{33}H_{41}N_3O_6$, 575.7 $[M]^+$; found, 576.7 $[M+H]^+$.
Compound 12	71%.	0.65	**^{1} H NMR (400 MHz, $CDCl_3$):** δ 8.23 (m, 2H), 7.42-7.33 (m, 4H), 5.24 (m, 4H), 4.18 (m, 2H), 3.33-3.16 (m, 4H), 2.67 (m, 4H), 2.46 (m, 4H), 1.82 (m, 4H), 1.54 (m, 4H), 1.27 (m, 6H), 0.84 (m, 3H).	$C_{32}H_{41}N_3O_6$, 563.68$[M]^+$; found, 564.7 $[M+H]^+$.
Compound 13	70%.	0.6	**^{1} H NMR (400 MHz, $CDCl_3$):** δ8.13-8.06 (m, 2H), 7.36-7.03 (m, 9H), 6.82-6.51 (bs, 2H) 5.47-5.20 (m, 6H), 3.22-3.00 (m, 4H), 2.65-2.39 (m, 8H).	$C_{31}H_{31}N_3O_6$, 541.59$[M]^+$; found, 542.8 $[M+H]^+$.
Compound 14	70%.	0.6	**^{1} H NMR (400 MHz, $CDCl_3$):** δ 8.19 (s, 2H), 7.44-7.10 (m, 9H), 5.48 (s, 2H), 5.30 (s, 4H), 3.69-3.40 (m, 8H), 2.75-2.59 (m, 8H).	$C_{33}H_{33}N_3O_6$, 567.63$[M]^+$; found, 568.8 $[M+H]^+$.
Compound 15	72%.	0.65	**^{1} H NMR (400 MHz, $CDCl_3$):** δ 8.07 (s, 2H), 7.36-7.05 (m, 9H), 6.69 (bs, 2H), 5.39 (s, 2H), 5.23 (s, 4H), 3.16 (m, 4H), 2.65 (m, 4H), 2.42 (m, 4H), 1.50 (m, 2H).	$C_{32}H_{33}N_3O_6$, 555.62$[M]^+$; found, 556.4 $[M+H]^+$.

Antimicrobial and antifungal activity:

Antimicrobial and antifungal activities of synthesized compounds were determined using E. *coli*, B. *subtilis*, and S. *aureus*. Many synthesized compounds were found to have lower MIC values compared to the standard drugs ciprofloxacin and fluconazole as listed in table 2.

Table 2: Antimicrobial and antifungal active compound with their MIC

S. No.	Compound	Bacteria (MIC)		Fungus (MIC)	
		E. Coli	B. Subtilis	S. Aureus	A Brasiliensis
1	1	12.5±0.1	25±0.5	12. 5±0.1	12.5±0.1
2	2	6.25±0.3	3.1±0.4	--	--
3	3	3.1±0.4	--	--	3.1±0.4
4	4	6.25±0.3	25±0.5	25±0.5	--
5	5	--	--	3.1±0.4	12.5±0.1

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6	6	--	3.1±0.4	3.1±0.4	6.25±0.3
7	7	6.25±0.3	--	--	12.5±0.1
8	8	--	3.1±0.4		--
9	9	3.1±0.4	--	--	--
10	10	6.25±0.3	6.25±0.3	12. 5±0.1	3.1±0.4
11	11	12.5±0.1	6.25±0.3	--	6.25±0.3
12	12	-	-	-	-
13	13	12.5±0.1	--	25±0.5	--
14	14	3.1±0.4	--	--	12.5±0.1
15	15	6.25±0.3	--	3.1±0.4	3.1±0.4
16	Ciprofloxacin	12.5±0.1	12. 5±0.1	--	--
17	Fluconazole	--	--	12. 5±0.1	3.1±0.4

Selection of the target receptor for docking study

Several possible antibacterial targets such as DNA gyrase, ligase, ribosome, and dihydrofolate reductase (DHFR) were explored for the selection of the most viable target for the synthesized compounds. The binding cavity of each target was visually analyzed. As the size of the synthesized molecules is large, the targets with large and hollow cavities were selected for further analysis. This resulted in the identification of three antibacterial targets. The final target was chosen by recreating the structure of its co-crystallized ligand in Gold 5.3.0 software utilizing four different docking scores listed in table 3 & 4. Between co-crystallized and docked conformations, the root mean square deviation (RMSD) was determined. The RMSD values showed that the DHFR is the most appropriate target for further study (Table 5). DHFR is a crucial enzyme in the metabolism of folate and a useful target for antibiotics [5].

Table 3: Details of various antibacterial drug targets

S. No.	PDB ID	Receptor	Resolution
1	1JIJ	TyrRS from S. aureus in its combination with SB-239629 in the crystal structure (LIGASE)	3.20
2	1KZN	E. coli 24k Da Domain in Complex with Clorobiocin: Crystal Structure (DNA GYRASE SUBUNIT B)	2.3
3	2MLM	Sortase A from Staphylococcus aureus in combination with a benzo[d]isothiazol-3-one-based inhibitor (sortase A transpeptidase.)	-
4	1XBP	Pleuromutilins inhibit the formation of peptide bonds; the 50S ribosomal subunit from Deinococcus radiodurans in association with Tiamulin (RIBOSOME)	3.50
5	2XCT	The S. *aureus* Gyrase complex with Ciprofloxacin and DNA in the twinned 3.35A structure (Gyrase)	3.35
6	3FRA	Iclaprim and Staphylococcus *aureus* F98Y DHFR complexed (DHFR)	2.35
7	3FYV	NADPH and AR-102 complexed with Staph. aureus DHFR	2.20
8	4URM	Staph GyraseB 24kDa in Complex with Kibdelomycin: Crystal Structure	2.94
9	4URO	Crystal structure of Novobiocin-complexed Staph GyraseB, 24 kDa	2.59
10	6F86	E. coli GyraseB 24kDa in Complex with 4: Crystal Structure-(4-bromo-1H-pyrazol-1-yl)-6-[(ethylcarbamoyl)amino]-N-(pyridin-3-yl)pyridine-3-carboxamide	1.90
11	5T8Z	Burkholderia multivorans' peptide deformylase's crystal structure in combination with actinonin	1.85
12	5IGY	Complex of macrolide 2'-phosphotransferase type II with erythromycin and GDP	1.45
13	4WFN	The 50S large ribosomal subunit of Deinococcus radiodurans, with a three residue insertion in L22, is crystallized in association with erythromycin.	3.54

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Table 4: PDB ID of antibacterial drug targets and their resolution

PDB ID	Enzyme category	Resolution
1JIJ	*S. aureus* tyrosyl-tRNA synthetase	3.20 Å
1KZN	E. coli DNA gyrase	2.30 Å
3FYV	*S. aureus* DHFR	2.20 Å

Table 5: **RMSD between co-crystalized and docked conformation**

PDB ID	RMSD[a]				Remarks
	Goldscore[b]	Chemscore[c]	ASP[d]	ChemPLP[e]	
1JIJ	3.008	1.552	1.909	0.825	-
1KZN	1.365	2.830	3.463	2.790	-
3FYV	0.169	0.638	0.307	1.493	*S. aureus* DHFR with Goldscore was selected for docking of synthesized compounds

a, RMSD: root mean square deviation

b, Goldscore takes into consideration elements including ligand torsion strain, metal contact, H-bonding energy, and van der Waals energy.

c, Chem Score takes account of hydrophobic-hydrophobic contact area, hydrogen bonding, ligand flexibility, and metal interaction.

d, the ASP An atom-atom distance potential known as the fitness function is constructed from a database of protein-ligand complexes.

e, Chem PLP van der Waals and repulsive forces are modeled using several linear potentials and the hydrogen bonding term from the Chem Score [6].

Validation of docking protocol and selection of fitness function:

By replicating the structure of the co-crystallized ligand (AR-101), the docking approach was proven to be effective. The RMSD between the co-crystallized and docked conformations was determined after the AR-101 was docked using four scoring (fitness) functions: i. goldscore; ii. chemscore; iii. ASP; and iv. chemPLP, as given by Gold program. The docking of the produced molecules used the scoring function with the lowest RMSD. Goldscore was chosen as the fitness function for the docking of the synthesized compounds because it provided the lowest RMSD (RMSD = 0.169; Table 5, Figure 1) of any of the available methods. Gold uses ligand docking with a genetic algorithm to enhance the ligand's conformation at the receptor binding site. It consists of four components: protein-ligand hydrogen bond energy, protein-ligand van der Waals (VDW) energy, ligand internal VDW energy, and ligand torsional strain energy, which are evaluated using GoldScore fitness functions. Each isomer received a 10 times docking, and each position was rated based on how well it met the Gold Score fitness criteria. For discussion, the conformations with the highest scores were chosen.

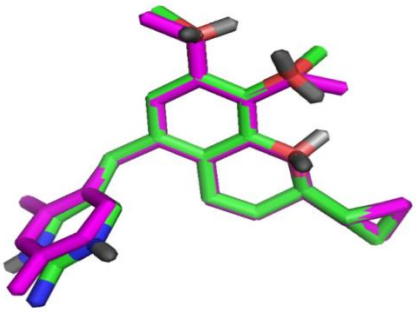

Fig. 1. Overlay of co-crystalized (magenta) and docked conformation (green) of AR-101 (RMSD = 0.169)

Docking study of the synthesized compounds Molecular docking experiments on S. *aureus dihydrofolate reductase* (DHFR) were carried out to obtain a thorough and precise understanding of the molecular mechanism behind the antibacterial activity of the most active chemical. Each of the fifteen

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substances was docked to DHFR's binding site. Table 6 displays each compound's Goldscore and key binding site residues. The co-crystallized ligand and non-classical DHFR inhibitor AR-101 [10] received a gold score of 61.46. The synthesized compounds had a gold score between 31.31 and 55.71. Three compounds, BR2, BR8, and Br14, had gold scores that were higher than 55 and comparable to those of AR-101. The compounds BR2, BR8, and Br14 stand out from the others because they have a piperazine ring structure sandwiched between two carbonyl functionalities. Only the terminal alkyl group distinguishes them from one another.

Major Drug-Receptor interactions: The binding site cavity of DHFR consists of several hydrophobic and polar residues such as Leu5, Val6, Ala7, Ile14, Gly15, Asn18, Gln19, Leu20, Asp27, Leu28, Val31, Thr46, Ser49, Phe92, Gly93, Gly94, Phe98, and Thr121. The binding site residues and overall binding modes of BR2, BR8, and Br14 suggest that these compounds fit well in the binding cavity and get stabilized by various electrostatic interactions. Here, BR2 is taken as a lead to discuss the binding interactions in detail. The major interactions between BR2 and DHFR include seven hydrogen bonds (H-bond donor: HBD and H-bond acceptor: HBA), dispersive interactions, and van der Waal's interactions. The carbonyl group of BR2 (a: HBA; d = 1.252Å; Figure 2) is involved in strong H-bond interactions with the NH group of Ala7. Several alkyl hydrogens were also involved in H-bond interactions with DHFR [11-14]. In this, the piperazine ring hydrogen is involved in H-bond interactions with the carbonyl group of Ile14 (b:HBD, d = 2.581Å). A hydrogen atom of the active methylene group showed H-bond interaction with the OH group of Thr46 (c: HBD, d = 2.661Å). The hydrogen of the methylene group adjacent to the carbazole ring is involved in H-bond interaction with the carbonyl group of Asn18 (d: HBD; d = 2.037Å). The terminal methyl function linked to carbozole nitrogen showed H-bond interactions with Ile50 (e: HBD; d = 2.359Å ;). The carbonyl group of the ester function (f and g) acts as HBA and shows two H-bonds with Val31 and Phe92, respectively (f: HBA; d = 2.137Å, d =2.214 Å) [15]. The other binding site residues, such as Leu6, Val6, Gln19, and Leu28, are involved in van der Waals interactions. The docking study clearly indicates that BR2 efficiently binds at the DHFR binding site via several H-bond and van der Waals interactions, and therefore, it may be suggested that the antibacterial activity of BR2 may be attributed to its ability to inhibit DHFR activity (Figure 2).

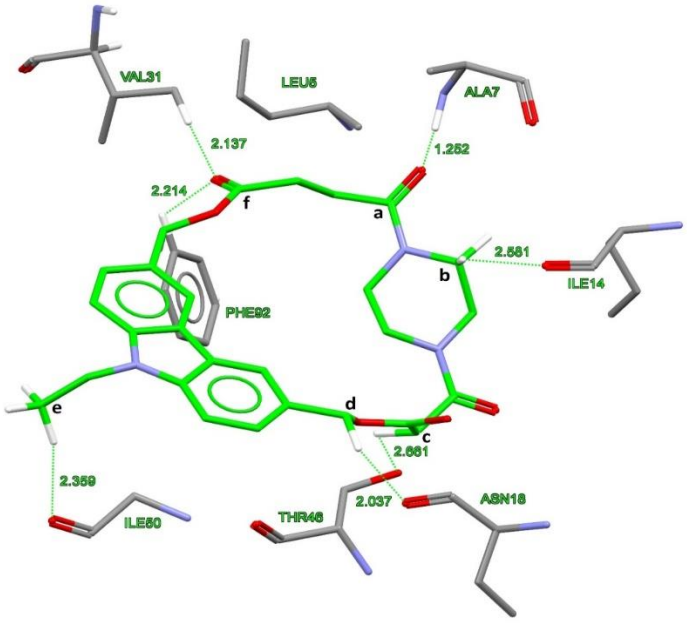

Fig. 2. Docked conformation of BR2 (green), only hydrogen atoms which are involved in H-bond interaction are shown; amino acid residues are shown in grey.

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Table 6. Goldscore and binding site residues of the docked compounds

S. No.	Comp. No.	Conf. No[a]	Goldscore	Amino acid residues
1	BR1	4	31.31	**Val6**, **Ala7**, Ile14, Asn18, Gln19, **Leu20**, **Leu28**, **Val31**, Thr46, **Ser49**, **Ile50**, **Leu45**, **Phe92**, Phe98
2	BR2	1	55.02	**Leu5**, **Val6**, **Ala7**, Ile14, Asn18, Gln19, **Leu20**, **Leu28**, **Val31**, Thr46, **Ser49**, **Ile50**, **Phe92**, Gly94
3	BR3	3	45.45	**Leu20**, Asn18, Gln19, **Leu28**, **Val31**, Thr46, **Ser49**, **Ile50**, **Leu45**, **Phe92**
4	BR4	1	45.97	**Leu5**, Gln19, **Leu20**, **Leu28**, **Val31**, , Thr46, **Ser49**, **Ile50**, **Phe92**,
5	BR5	2	40.75	**Val6**, **Ala7**, Ile14, Gly15, Asn18, Gln19, **Leu20**, **Asp27**, **Leu28**, **Val31**, Thr46, **Ser49**, **Ile50**, **Leu45**, Phe98, **Phe92**, Gly93, Gly94
6	BR6	2	19.69	-
7	BR7	2	54.72	**Leu5**, **Val6**, **Ala7**, Ile14, Asn18, Gln19, **Leu20**, Trp22, **Asp27**, **Val31**, **Thr46**, **Ser49**, **Ile50**, **Phe92**, Gly94
8	BR8	1	55.30	**Leu5**, **Val6, Ala7**, Ile14, Asn18, Gln19, **Leu20**, **Asp27**, **Leu28**, **Val31**, Lys45, Thr46, **Ser49**, **Ile50**, **Phe92**, Gly94, Phe98
9	BR9	3	38.66	**Leu5, Val6, Ala7**, Asn18, Gln19, **Leu20**, **Leu28**, **Val31**, Lys45, Thr46, **Ser49**, **Ile50**, **Leu45**, **Phe92**, Gly94, Thr96, Phe98
10	BR10	3	42.50	Gly15, Asn18, Gln19, **Leu20**, **Leu28**, **Val31**, Thr46, **Ser49**, **Ile50**, **Leu45**, **Phe92**
11	BR11	2	47.14	**Val6, Ala7**, Gln19, **Leu20, Asp27**, **Leu28**, **Val31**, Thr46, **Ser49**, **Ile50**, **Leu45**, **Phe92**, Thr111
12	BR12	1	49.25	Gln19, **Leu20**, **Leu28**, **Val31**, **Ser49**, **Ile50**, **Phe92**
13	BR13	4	51.10	Asn18, Gln19, **Leu20**,**Leu28**, Lys29, **Val31**, Lys32, Thr46, **Ser49**, **Ile50**, Lys52, **Leu45**, Pro55, Asn57, **Phe92**
14	BR14	3	55.71	**Leu5**, **Val6**, **Ala7**, Ile14, Asn18, Gln19, **Leu20**, **Leu28**, **Val31**, Thr46, **Ser49**, **Ile50**, **Leu45**, **Phe92**, Gly94
15	BR15	3	46.43	**Val6**, **Ala7**, Ile14, Gly15, Asn18, Gln19, **Leu20**, **Asp27**, **Leu28**, **Val31**, Thr46, **Ser49**, **Phe92**, Gly93, Phe98, Thr121
16	AR-101[b]	7	61.46	Leu5, Val6, Ala7, Leu20, Asp27, Leu28, Val31, Ser49, Ile150, Leu45, Phe92,

A: Gold conformation number; b: co-crystalized ligand; Residues common with AR-101 are shown in bold.

Discussion:

Before the present study, various researchers had worked to modify the carbazole moiety with improved antimicrobial activity. Some of them are discussed below. According to Saha *et al*., carbazole compounds have antibacterial effects on both gram-positive (B. *subtilis* and S. *aureus*) and gram-negative (E. coli and Pseudomonas) bacteria. The MIC of synthetic drugs against S. *aureus* is 50 g/mL [16]. Ochung *et al.* isolated three carbazole alkaloids from the stem bark of Alysicarpus ovalifolius: koenidine, koenimbine, and mohanimbine, using different solvents such as n-hexane, CH2Cl2, and MeOH, respectively [17]. Using the disc diffusion method, the three isolated compounds were tested for antibacterial activity against yeast-like and filamentous fungi, as well as various Gram-positive and Gram-negative bacteria. Dichloromethane extract had the highest activity against Candida *albicans* and Staphylococcus *aureus*, with zones of inhibition measuring 13.2 ±0.1 and 15.3 ±0.1 mm in diameter, respectively, when compared to the standard drugs fluconazole and amoxicillin, which had zones of inhibition measuring 17.3± 0.2 and mm, respectively. A chemical inhibited C. *albicans* and S. *aureus* the most, with zones of inhibition of 14.5± 0.1 and 13.8±

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0.1, respectively. Prasad *et al.* reported the one-pot production and assessment of carbazole derivatives as antibacterial agents. The chloro-substituted derivative had exceptional antibacterial action against E. *coli*, S. *aureus*, P. *aeruginosa*, and B. *subtilis*, but the two carbazole derivatives demonstrated significantly lesser activity against all bacterial species tested. In comparison to the regular medicine carbendazim, the carbazole derivative displayed excellent antifungal effectiveness against all fungi tested. Its increased activity could be attributed to the compound's inclusion of chloro- and N-oxide groups [18]. Rajakumar *et al.* investigated the antibacterial activity of a series of carbazole-based macrocyclic diamides with sulfur and oxygen connections. The MIC of carbazole amides against bacterial species was determined to be concentrations ranging between 5 and 80 µg/mL. When compared to the other macrocyclic diamides and the conventional antibiotic tetracycline at low concentrations ranging from 5 to 10 µg/mL, the macrocyclic diamide demonstrated considerable antibacterial activity against all of the screened bacterial species. In contrast, the MICs of macrocyclic diamides against fungal species were determined to be concentrations between 10 and 45 µg/mL. As compared to the other macrocyclic diamides and the conventional fungicide, carbendazim [19], macrocyclic diamide significantly inhibited all four fungal species with concentrations ranging from 10 to 20 µg/mL [19]. *Xue et al.* synthesized another large set of carbazoles with a common miscellaneous substituent at position 3 of the carbazole backbone and tested them for antimicrobial activity against three gram-positive bacteria (S. *aureus* (4220), S. *mutans* (3289), and S. *aureus* (MRSA CCARM 3167)), one gram-negative strain (E. coli (1924), and one fungus (Candida albicans (7535)) [20]. Nair *et al.* isolated carbazole alkaloids from Murraya *koenigii* leaves. Carbazole alkaloid was shown to have good activity against C. *kruseii*, C. *parapsilosis*, E. *coli*, S. *aureus*, and S. *pyogenes*, but other modified carbazole alkaloids were found to have limited activity against these test species. While some other carbazole alkaloids have a minimum inhibitory concentration (MIC) of 25 µg/mL against S. *aureus* and S. *pyogenes* [21]. Rahman et al modified the carbazole ring and confirmed its structure using multiple spectroscopic approaches. The antimicrobial potential of the modified carbazole compound was determined against E. *coli*, P. *vulgaris*, A. *niger*, and C. *albicans* species [22].Surendiran *et al.* synthesized carbazole derivatives and reported them as having good antibacterial potential due to the presence of chloro and methoxy groups in heterocyclic rings. Gu *et al.* synthesized a novel 1H-dibenzo carbazole using dehydroabietic acid and determined its antimicrobial activity against B. subtilis (1.9 µg/mL) [23]. Liu *et al.* formulated a set of 39 N-alkylated carbazoles with replacements on position 4 of the carbazole [24]. Farghaly et al. developed several thiazole-containing carbazoles using microwave-assisted [25]. Sharma et al looked into a number of novel 5-[(9H-carbazol-9-yl) methyl] compounds. -N-[(substituted phenyl) (piperazin-1-yl) methyl] -1,3,4-oxadiazol-2-amines compounds. The antimicrobial potential of the synthesized compound was determined using the disc diffusion method and found to be significant at 50 µg/mL [26]. Raju *et al.* synthesized sulfonamide and carbamate derivatives of 4-(oxiran-2-ylmethoxy)-9-Hcarbazole. Synthesized compounds were tested against S. *aureus*, B. *subtilis*, and E. *coli* for antimicrobial activity and against F. oxysporum, C. albicans, and A. niger for antifungal activity [27]. Bandgar et al looked into the antimicrobial properties of a new class of carbazole chalcones. Synthesized carbazole chalcones were tested for antibacterial and antimicrobial activity against P. *vulgaris*, E. *coli*, and S. *aureus* [28].

Conclusion:

A total of 15 carbazole derivatives were synthesised, and percentage yields were found to be 87.3%, 51%, 36%, 87%, 72%, 75%, 68%, 88%, 71%, 75%, 74%, 70%, 52.6%, 32%, 90%, 76%, 80%, 71%, 92%, 76%, 80%, 71%, 97.3%, 45%, 32%, 91%, and 70%, respectively. Further synthesised compounds were characterised by TLC, mass, and NMR spectroscopy, as discussed in the results section.

Finally, synthesized carbazole derivatives were evaluated for their antimicrobial and antifungal activity. Synthesized compounds 3, 9, and 14 at a concentration of 3.1± 0.4 showed the most potent activity against gram-negative bacteria, i.e., E. *coli*, while compounds 2, 6, and 8 at the same concentration were found to be most effective against gram-positive bacteria, i.e., B. *subtilis*. Furthermore, compounds 5, 6, and 15 showed the most potential as therapeutic antifungal. Synthesized compounds (BR1–15) were used in

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molecular docking to find out the accurate interaction of ligands with the active site of target proteins for antibacterial potential. Docking was started with several possible antibacterial targets such as dihydrofolate reductase (DHFR), ligase, ribosome, and DNA gyrase as per Table 3. These targets were shortlisted (table 4) based on visual analysis in reference to a large and hollow cavity.Additionally, target was selected by reproducing the conformation of its co-crystallized ligand (AR-101) using four different docking scores using Gold 5.3.0 software. Finally, AR-101 was found most suitable based on the scoring of Goldscore, Chescore, ASP, and ChemPLP using Gold software 5.3.O. All compounds (BR1–15) were docked at the binding site of DHFR. All docking results in the form of a Goldscore, conformal number, and amino acid residue were reported in Table 4. The gold scores of AR101 and DHFR were found to be 61.46, while the gold scores of synthesized compounds were found in the range of 31.31–55.71. The gold score of three compounds, BR2, BR8, and BR14, was found to be above 55. For possible use in future antitumor and antibacterial medicines, the therapeutic potential of carbazole derivatives could be further investigated.

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Conflict of interest: None

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