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Original Article

Unveiling the Bioactive Potential of Mannich Bases: Synthesis, Characterization, and Biological Perspectives

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ABSTRACT

Background: This study evaluates the antimicrobial and molecular docking profiles of 12 novel Mannich bases (MB1-MB12) to find potential candidates effective against *Escherichia coli, Staphylococcus aureus*, and *Candida albicans*. This study discusses the urgent need for new therapeutic agents with enhanced efficacy to combat the fast-growing problem of antimicrobial resistance. The compounds were synthesized using the Mannich reaction, which includes the reaction of aromatic amines, formaldehyde, and aromatic aldehydes under reflux conditions. **Materials and Methods:** Molecular docking on 2B35 and 4P8O protein targets determined binding affinity, interactions, and energy scores. Analytically, the compounds were characterized and appraised for synthetic feasibility and drug-likeness using ADME predictions and Lipinski's rule of five. MIC testing assessed antimicrobial effectiveness. To assess their therapeutic potential, the compounds' drug-likeness, GI absorption, and synthetic accessibility were examined. **Results:** The best docking compounds were MB7, MB3, and MB2, which had great binding affinities and significant interactions with key 2B35 residues. MB7 showed stronger antibacterial activity against *E. coli* (12.5 µg/mL), *S. aureus* (6.25 µg/mL), and *C. albicans*. Antifungal activity of MB3 and MB5 was substantial, with MIC values of 1.6 µg/mL against *C. albicans*. Antibacterial activity was higher in compounds with balanced physicochemical properties such moderate Log P values, hydrogen bonding, and high GI absorption. **Conclusion:** MB7 emerged as the most promising candidate, demonstrating robust docking and antibacterial activity against bacterial and fungal infections. MB3 and MB5 also showed promise as antifungal agents. These findings emphasize the compounds' potential as leads for further preclinical and mechanistic investigations to tackle drug-resistant illnesses.

Keywords: Mannich bases, Molecular docking studies, ADME prediction, Antitubercular activity, Anti-microbial activity.

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INTRODUCTION

The Mannich reaction has been a pivotal technique in synthetic organic chemistry since its discovery in 1917, enabling the efficient synthesis of β-amino carbonyl compounds, or Mannich bases, from aldehyde, amine, and ketone components.[1] These compounds have proven versatile in medicinal chemistry and natural product synthesis, with their structural modifications often enhancing biological activity.[2-5] Recent research has expanded the scope of the Mannich reaction to include the synthesis of compounds with potential antimalarial, antitubercular, and anti-inflammatory properties.[6-11] Despite its historical significance, the Mannich reaction has faced challenges such as lack of selectivity and competing side reactions. However, modern variations employing innovative catalysts and reaction conditions have addressed these limitations, making Mannich reactions valuable tools for organic chemists.[12-15] Metal complexes of Mannich bases, in particular, have attracted attention due to their sensitivity to different metal ions. Given the broad utility and potential of Mannich bases, this study aims to further explore their antitubercular and antibacterial properties.[16,17] By investigating novel derivatives and leveraging the versatile reactivity of Mannich reactions, the research seeks to contribute to ongoing efforts in combating tuberculosis and bacterial infections.[18-23] Ultimately, the study aims to advance our understanding of Mannich bases therapeutic potential and their role in addressing global health challenges.

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MATERIALS AND METHODS

Commercial-grade chemicals, including Benzocaine, Acetophenone, Acetanilide, Methanol, O-Phenylene diamine, O-Toluidine, P-Toluidine, O-Dianisidine, 4-Amino benzoic acid, 4-Dimethyl aminobenzaldehyde, Diphenylamine, and Ethanol, were sourced from reputable suppliers such as Himedia Laboratories Pvt. Ltd., ThermoFisher Scientific India Pvt. Ltd., Loba Chemie Pvt. Ltd., Moly Chemie Pvt. Ltd., Excelar, Merck, and Chagshu Hongsheng Fine Chemicals Ltd. Amines utilized in Mannich base synthesis included Benzocaine, Aniline, Acetanilide, Para amino benzoic acid, p-nitro aniline, and Indole-3-butyric acid. High-quality Borosil glassware, including beakers, conical flasks, glass rods, Petri dishes, standard flasks, and pipettes, were sourced from SL Scientific's. Molecular docking studies employed Schrodinger-Glide, with additional equipment including Swiss ADME, a parallel synthesizer, a magnetic stirrer, BRUKER IR Spectroscopy, ^{1}H NMR, and Mass Spectroscopy.

Molecular Docking studies

Molecular docking on the crystal structure of triclosan-inhibited Mycobacterium TB enoyl reductase (InhA) 2B35

Using triclosan as an inhibitor, molecular docking studies were used to find the best molecule to fit into the active site of Mycobacterium tuberculosis enoyl reductase (InhA), utilized Glide Schrodinger software (PDB code-2B35). This enzyme, belonging to the Oxidoreductase category, was obtained from *Escherichia coli* via X-ray diffraction analysis with a resolution of 2.30Aº. The 3D structure of Enoyl-[acyl-carrier-protein] reductase [NADH] comprises six chains (A-F), each with 269 amino acids and devoid of mutations. Ligands NAD and triclosan are present in every chain. Protein Data Bank (PDB) facilitated the import of the protein structure using code 2B35, followed by preprocessing involving hydrogen addition and bond ordering assignment.[24-26] The 3D structure of 2B35 downloaded from PDB shown in Figure 1.

In ligand preparation using Maestro Schrödinger software (Version 2020_1), the library's structures in SDF format were imported, followed by selection and placement of all ligands in the workspace. Ligprep was then employed with appropriate parameters to ensure chirality, ionization, and calculation accuracy. For protein preparation and grid generation of the 2B35 protein, Maestro's protein energy minimization wizard was utilized to address disulfide bonds and other energy-related tasks. Chain A (269 amino acid residues) was selected, while remaining chains (B-F) were excluded due to uniqueness. Water molecules were removed, retaining the co-crystal ligand TCL (Triclosan). The chosen protein underwent optimization and subsequent minimization in the refine tab.

Receptor grid creation involved selecting an atom from the ligand molecule in the workspace to generate the grid at the active binding site of the protein. This process resulted in a grid box positioned around the co-crystal in the 2B35 protein, with coordinates of 6.43, 9.76, and 26.06 in X, Y, and Z axes, respectively.

Molecular docking on antibacterial protein of 4P8O

Crystal structures of *Staphylococcus aureus* gyrase attached to an aminobenzimidazole urea inhibitor (PDB Code 4P8O) were obtained via X-Ray Diffraction with a resolution of 2.40Aº, featuring gyrase subunit B with two chains, A and B, totaling 187 amino acids.[25] Figure 1 depicts 4P8O's three-dimensional structure downloaded from PDB. These chains contain a single ligand, amino benzimidazole with code 883. Standard preprocessing, optimization, and minimization were performed on the proteins. Ramachandran graphs allowed one to assess the torsional angles (phi and psi) of amino acid residues. Using receptor grid construction, a grid box comprising coordinates of 49.9, -3.9, and 18.69 in the X, Y, and Z axes correspondingly was positioned around the co-crystal in the 4P8O protein. A Ramachandran two-dimensional graph was obtained after minimization of the proteins by Schrodinger 2020_1 version shows the allowed and disfavoured values of ψ and φ of the amino acid residues in proteins 2B35 and 4P8O.

The glide grid's zip files-along with ligand outmaegz-were loaded from the working directory during ligand docking. We choose the docking setting "Write SP descriptor information". Using binding energy, which was indexed from least to greatest, the amino acid residues of 2B35 and 4P8O helped sort the virtual screening findings.

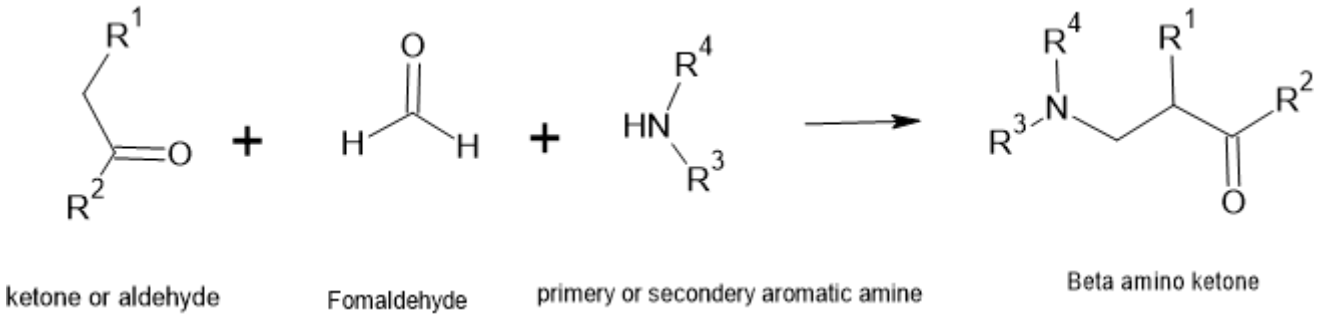
ADME Studies

The Swiss ADME web tool was employed to anticipate the ADME of the library. It offers data on various properties, including gastrointestinal absorption, lead likeness, molecular weight, rotational bonds, hydrogen bond donors, topological polar surface area, molar refractivity, log Kp (skin permeability), BBB permeation, cytochrome P450 inhibition, synthetic accessibility, and additional characteristics.

Synthetic Scheme

Mechanism

The amine undergoes a reaction with formaldehyde, which results in the formation of an iminium ion through the processes of protonation, deprotonation, and dehydration. After going through the processes of protonation and deprotonation, the ketone, which is composed of an alpha hydrogen, is transformed into an enol intermediate. Following depolarization, the production of the beta amino ketone arises as a consequence of the enol intermediate's attack on the iminium ion.[26]



Mannich bases MB1-MB12 synthesis general procedure

Mix 0.05 moles of aromatic amine with 0.05 moles of formaldehyde until they are completely combined. Heat 10 mL of ethanol and mix in 0.05 moles of aromatic aldehyde. The solutions should be mixed together and heated to 80°C for 8 hr. Rinse the product with methanol, let it to cool on ice, and then recrystallize it with hot ethanol. To monitor the completion of the reaction using thin-layer chromatography, use a mobile phase that consists of ethyl acetate and n-hexane at a ratio of 7:3.

This method is used to synthesize Mannich bases. The analysis of the compounds that were created was performed utilizing mass spectral data, ^{1}H NMR, infrared spectra, and melting points.[27,28]

Pharmacological Screening

Antitubercular activity screening

In order to find the MIC against Mycobacterium TB H37Rv within a concentration range of 25 to 1 µg/mL, the broth dilution method was employed. No changes were made to the composition of the 7H9 medium as it was diluted. After diluting the culture suspension to a 1:10 ratio, each well contained 1x10^5 cells that were corrected to a 1 McFarland standard. The medicine rifampicin (Sigma-Aldrich) was used as a reference, and its critical concentration was 1 µg/mL. One group served as a solvent control, while the other group served as a culture control (DMSO). In order to ascertain whether or not Mycobacterium tuberculosis growth suppression was noted following five days of incubation at 37°C, the presence of serpentine chords was examined. The Minimal Inhibitory Concentration (MIC) was determined by determining the dosage that inhibited Mtb growth, and the test doses varied between 100 and 0.19 µg/mL.[29,30]

Antimicrobial screening

In vitro antibacterial efficacy against *S. aureus* (G+ve bacterium), *Escherichia coli* (G-ve bacteria), and *Candida albicans* (fungal species) was assessed using the synthesized mannich bases at MIC µg/mL. Brain Heart Infusion (BHI) broth was used to control the growth of the microbes. This broth is a nutrient-rich, buffered culture medium that was autoclaved at 121°C for about fifteen minutes. Sodium chloride, dextrose, protease peptone, cow heart and calf brain infusions, and disodium phosphate are some of its constituents. After 5 µL were removed from the stock cultures, the previously listed microorganisms were mixed with 2 mL of BHI broth. The antibacterial activity of each compound was tested using the serial dilution method at various final concentrations. For every chemical, there were ten tubes. To dilute the chemical, 380 µL of BHI broth was combined with 20 µL of the compound in each tube. Each of the remaining nine tubes was given 200 µL to dilute the BHI broth separately. Since the second tube already contained 200 µL of BHI broth, 200 µL was transferred from the first tube to it, leading to a dilution of 10-1. 200 µL of the solution was transferred from the 10-1 tube to the third tube in order to reach a 10-2 dilution. To achieve a concentration of 10-9 µg/mL, the process is repeated until all compounds have been diluted. In the end, concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.6, 0.8, 0.4, and 0.2 µg/mL were produced. In the end, after adding 200 µL to tubes that had been diluted in a serial fashion, the culture suspension was incubated for 24 hr. 30, 31, 32; we recorded the compounds' MIC and checked the tubes for turbidity growth.

RESULTS

Molecular docking studies

Docking studies were conducted to evaluate the binding affinities of selected ligands with proteins 2B35 and 4P8O. Results indicated that ligands generally exhibited stronger interactions with 2B35. Among the ligands, MB2 demonstrated the highest docking score (-7.84) and significant interactions with ASP 148 and LYS 165. MB3 showed the lowest Glide energy (-41.81), reflecting strong binding stability. For 4P8O, the cocrystal 883 displayed the best docking score (-3.88) and robust interactions with ASP 81 and ARG 144. Residues ILE 194 (2B35) and ASP 81, SER 55 (4P8O) were keys for ligand binding represented in Table 1.

These findings highlight MB2 and MB3 as potential candidates for further optimization with 2B35, while cocrystal 883 serves as a reference for enhancing ligand affinity with 4P8O represented in Figure 1.

ADME Studies

Twelve compounds (MB1-MB12) were evaluated for their physicochemical properties, ADMET profiles, and drug-likeness. Molecular weights ranged from 225.29 (MB6) to 335.40 (MB3), with Log P values (2.01-3.44) and TPSA (29.10-74.92) falling

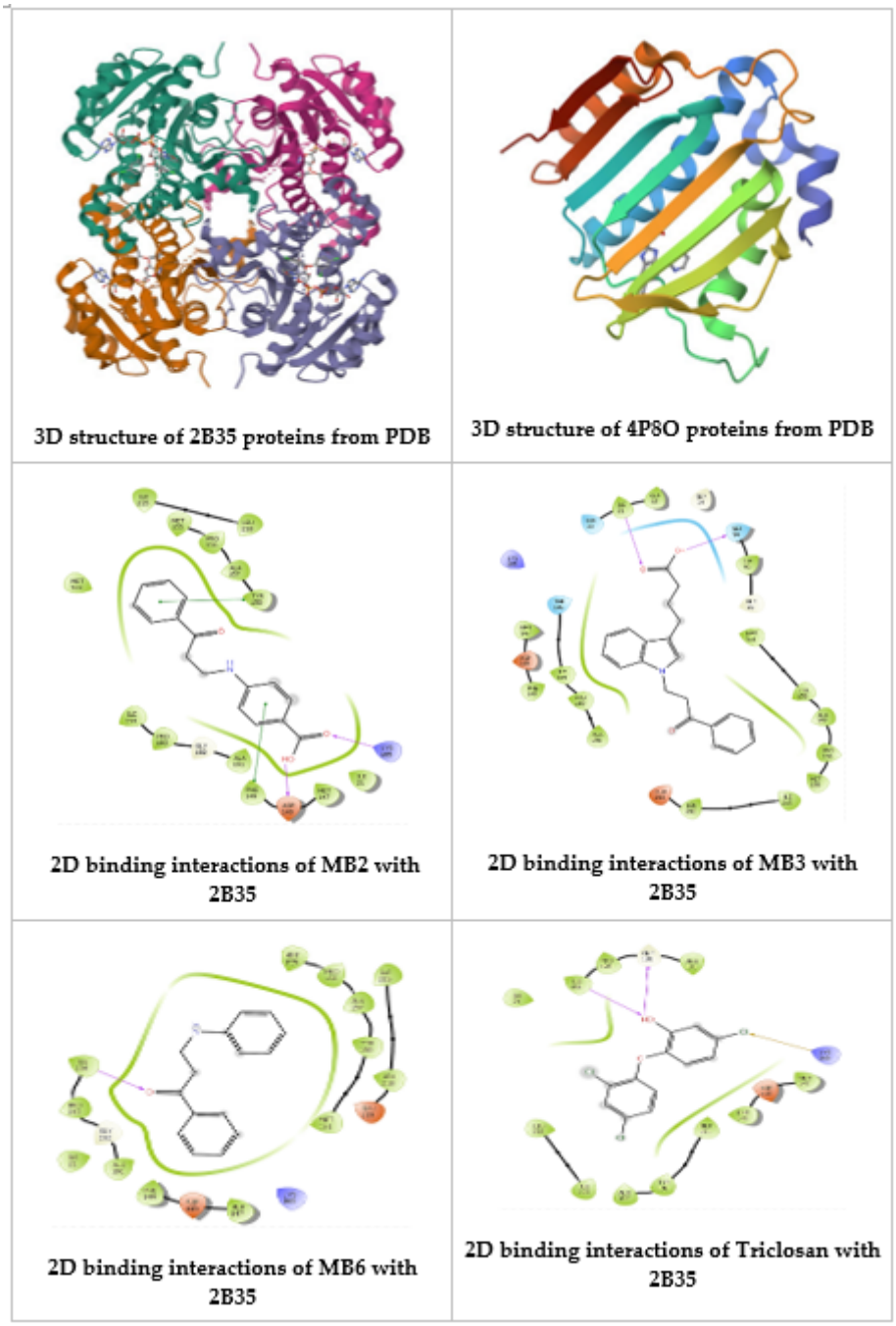
within acceptable ranges for drug-likeness. All compounds exhibited high gastrointestinal absorption and BBB permeability.

CYP450 enzyme inhibition varied, with MB7 and MB8 showing the broadest inhibition profiles across all tested isoforms (1A2, 2C19, 2C9, 2D6, and 3A4). Most compounds met lead-likeness criteria, except MB3 and MB12, while synthetic accessibility scores (1.31-2.42) indicated moderate ease of synthesis represented in Table 2. Compounds MB2, MB3, MB7 and MB8 emerged as promising leads, combining favourable ADMET profiles, drug-likeness, and synthetic feasibility, making them strong candidates for further optimization and development.

Synthesis and characterization

MB1(N-(3-oxo-3-phenylpropyl)-N-phenylacetamide)

Molecular formula $C_{17}H_{17}NO_2$; m.p: 110-115; yield 86%; IR (KBr, cm^{-1}): 1663.29 (C=O stretch, α, β-unsaturated, acyclic), 2926.24 (C-H stretch, -CH_2-), 1494.11 (C-H deformation, -CH_2-), 2977.28 (C-H stretch, -$COCH_3$), 1367.55 (C-H deformation, -$COCH_3$), 1319.74 (C-N stretch, 3º amine); ^{1}H NMR (DMSO-d6): 2.09, (s, 3H, -$COCH_3$), 4.32-4.36 (t, 2H, -CH_2-), 7.04-7.51 (t, q, n 10H, aromatic); MS m/z: 267.3 [M]+2; Anal. Found: C, 76.38; H, 6.41; N, 5.24; O, 11.97%.



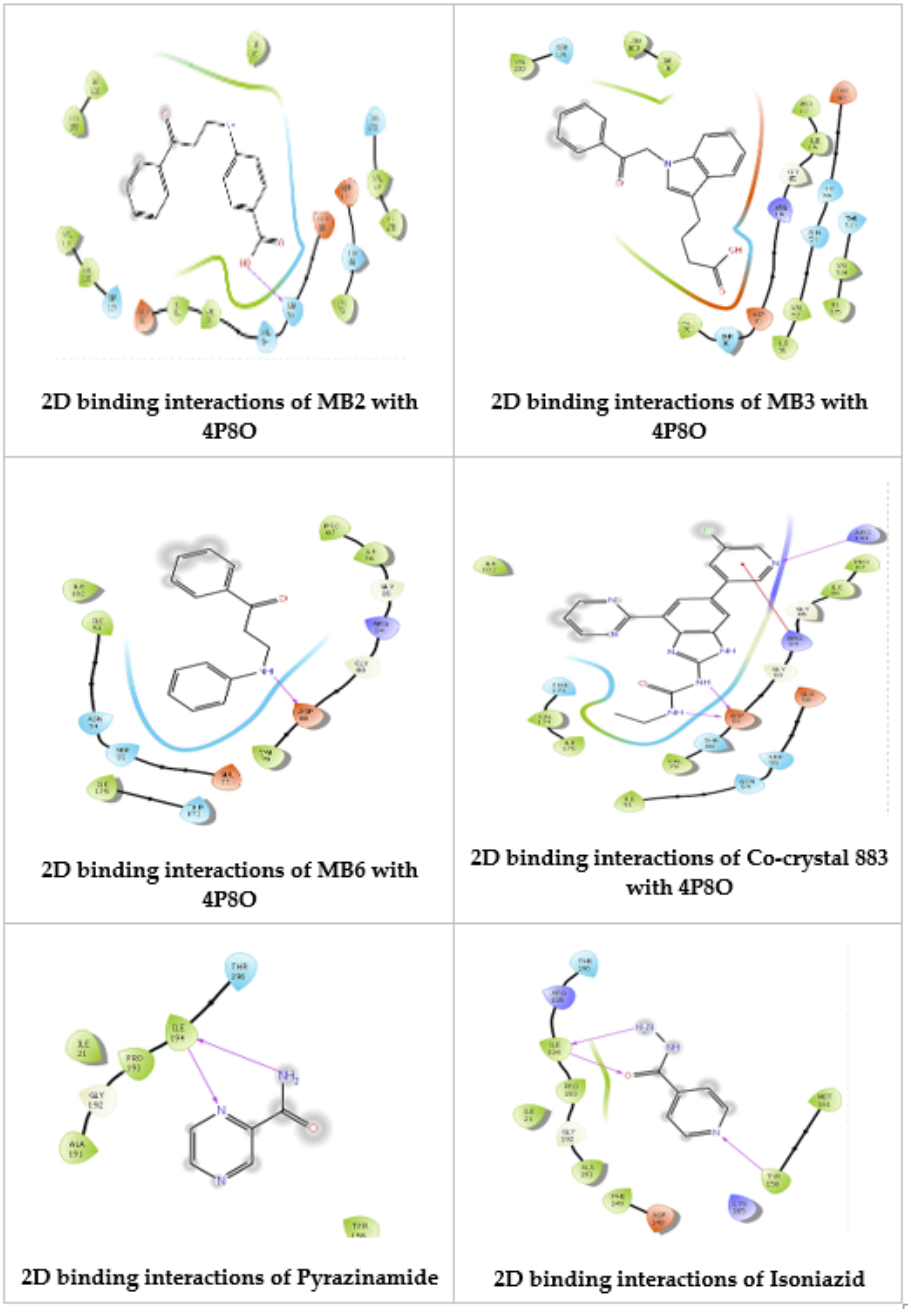


Figure 1: 3D structure of 2B35, 4P8O proteins from PDB database and 2D binding interactions of some synthesized compounds.

MB2 (4-[(3-oxo-3-phenylpropyl)amino]benzoic acid

Molecular formula $C_{16}H_{15}NO_3$; m.p: 138-141; yield 64%; IR (KBr, cm^{-1}): 1659.54 (C=O stretch, α, β-unsaturated, acyclic), 2928.99 (C-H stretch, -CH_2-), 1447.29 (C-H deformation, -CH_2-), 1279.12 (C-N stretch, 2° amine), 3454.96 (N-H stretch, 2° amine), 1601.46 (N-H deformation, 2° amine), 3547.12 (O-H stretch), 1731.12 (C=O aryl conjugated), 1385.26 (C-O stretch), 1206.54 (O-H deformation); ^{1}H NMR (DMSO-d6): 2.52-2.54 (t, 2H, -CH_2-), 3.478 (s, 1H, -NH), 4.57-4.59 (t, 2H, -CH_2-), 6.54 -7.97 (d, t, q, n, 9H, aromatic), 11.45 (s, 1H, -OH); MS (ESI) m/z: 269.2 [M]+1; Anal. Found: C, 71.56; H, 6.71; N, 4.91; O, 16.82%.

MB3 (4-[1-{3-oxo-3-phenylpropyl}-1H-indol-3-yl] butanoic acid)

Molecular formula $C_{21}H_{21}NO_3$; m.p: 114-118; yield 69%; IR (KBr, cm^{-1}): 1699.04 (C=O stretch, α, β-unsaturated, acyclic), 2928.59 (C-H stretch, -CH_2-), 1493.36 (C-H deformation, -CH_2-), 1323.53 (C-N stretch, 3° amine), 3392.63 (O-H stretch), 1429.50 (C-O stretch), 1276.81 (O-H deformation); ^{1}H NMR (DMSO-d6):

2.51-2.43 (t, 2H, -CH_2), 2.80-2.83 (t, 2H, -CH_2-), 4.34-4.38 (q, 2H, CH_2), 6.95-7.92 (d, t, q, n 10H, aromatic), 10.6 (s, 1H, OH); MS (ESI) m/z: 335.39 [M]-1; Anal. Found: C, 75.30; H, 6.31; N, 4.18; O, 14.31%.

MB4 (3-(4-nitroanilino)-1-phenylpropan-1-one)

Molecular formula $C_{16}H_{15}NO_4$; m.p: 177-178; yield 85%; IR (KBr, cm^{-1}): 1642.58 (C=O stretch, α, β-unsaturated, acyclic), 2922.52 (C-H stretch, -CH_2-), 1470.35 (C-H deformation, -CH_2-), 1291.39 (C-N stretch, 2° amine), 3434.56 (N-H stretch, 2° amine), 1592.00 (N-H deformation, 2° amine), 1522.73 (NO_2 stretch), 884.01 (C-N stretch, NO_2); ^{1}H NMR (DMSO-d6): 1.30-1.38 (q, 2H, CH_2), 3.95 (s, 1H, -NH), 4.34-4.39 (q, 2H, CH_2), 7.26-7.97 (t, q, n 9H, aromatic); MS (ESI) m/z: 285.29 [M]+1; Anal. Found: C, 67.36; H, 5.30; N, 4.91; O, 22.43%.

MB5 (methyl 4-[(3-oxo-3-phenylpropyl) amino] benzoate)

Molecular formula $C_{17}H_{17}NO_3$; m.p: 170-173; yield 76%; IR (KBr, cm^{-1}): 1601.08 (C=O stretch, α, β-unsaturated, acyclic), 2919.46 (C-H stretch, -CH_2-), 1472.97 (C-H deformation, -CH_2-), 1320.54 (C-N stretch, 2° amine), 3434.65 (N-H stretch, 2° amine), 1530.06 (N-H deformation, 2° amine), 1684.81 (C=O aryl conjugated), 2851.31 (C-H stretch, -OCH_3), 1396.11 (C-H deformation, -OCH_3); ^{1}H NMR (DMSO-d6): 1.25-1.29 (t, 3H, -CH_3), 3.36 (s, 1H, -NH), 2.51-2.52 (q, 2H, -CH_2-), 4.19-4.23 (q, 2H, -CH_2-), 6.74-7.96 (t, q, n 9H, aromatic); MS (ESI) m/z: 303.74 [M]-1; Anal. Found: C, 72.07; H, 6.05; N, 4.94; O, 16.94%.

MB6 (3-anilino-1-phenylpropan-1-one)

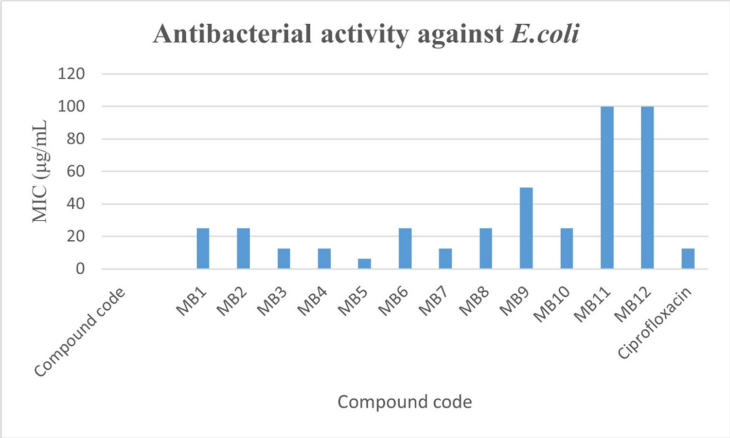
Molecular formula $C_{15}H_{15}NO$; m.p: 106-108; yield 72%; IR (KBr, cm^{-1}): 1675.72 (C=O stretch, α, β-unsaturated, acyclic), 2981.04 (C-H stretch, -CH_2-), 1454.93 (C-H deformation, -CH_2-), 1316.26 (C-N stretch, 2° amine), 3410.31 (N-H stretch, 2° amine), 1597.29 (N-H deformation, 2° amine); ^{1}H NMR (DMSO-d6): 3.22-3.24 (t, 1H, -NH), 3.57-3.59 (t, 2H, -CH_2-), 6.61-7.92 (d, t, q, n, 10H, aromatic); MS (ESI) m/z: 225.28 [M]+1; Anal. Found: C, 79.97; H, 6.71; N, 6.22; O, 7.10%.

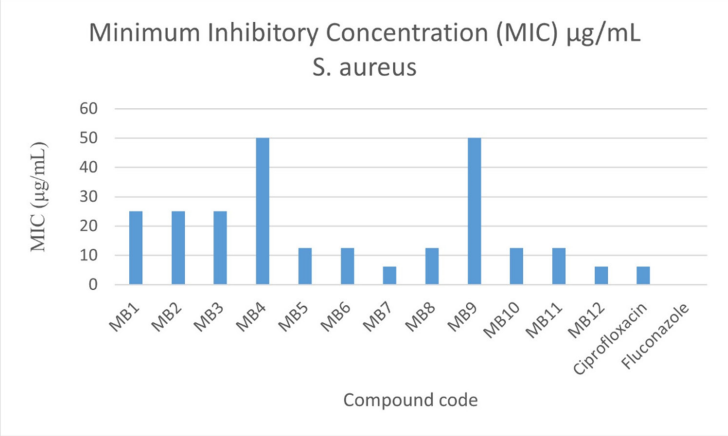
MB7 (3-(3-amino-4-methoxyphenylamino)-1-phenylpropan-1-one)

Molecular formula $C_{16}H_{18}N_2O_2$; m.p: 96-98; yield 61%; IR (KBr, cm^{-1}): 1651.12 (C=O stretch, α, β-unsaturated, acyclic), 2898.12 (C-H stretch, -CH_2-), 1441.93 (C-H deformation, -CH_2-), 1322.26 (C-N stretch, 2° amine), 3347.31 (N-H stretch, 2° amine), 1574.22 (N-H deformation, 2° amine); ^{1}H NMR (DMSO-d6): 7.34-7.89 (m, 5-CH, benzene), 2.78 (d, 1- CH_2, methylene), 3.25 (s, 1- CH_2, methylene), 4.0 (t, 1H, aromatic C-NH), 5.52-5.68 (m, 3-CH, benzene), 3.73 (t, 1 CH_3, methyl), 4.0 (d, 1-NH, aromatic C-NH).

Table 1: 2B35 and 4P8O protein docking scores and ligand interactions.

Protein Name	Ligand interactions and docking score with the 2B35 protein			Ligand interactions and docking score with the 4P8O protein		
Compound code	Docking Score	Glide Energy	H-Bond Interactions	Docking Score	Glide Energy	H-Bond Interactions
MB1	-5.56	-36.18	TYR 158, ILE 194	-2.70	-28.87	---
MB2	-7.84	-36.59	ASP 148, LYS 165	-4.66	-34.57	SER 55
MB3	-7.74	-41.81	ILE 21, SER 94	-5.10	-38.20	---
MB4	-4.89	-33.72	ILE 194	-3.48	-29.06	----
MB5	-6.34	-34.66	SER 94, ILE 194	-3.76	-30.37	---
MB6	-6.87	-28.48	ILE 194	-2.99	-26.92	ASP 81
MB7	-5.48	-30.25	TYR 158, ILE 194	-3.97	-23.42	--
MB8	-4.96	-32.74	ASP 148, LYS 165	-2.84	-25.58	ASP 81
MB9	-5.90	-29.86	ILE 194	-3.55	-21.23	--
MB10	-5.26	-31.53	SER94	-2.59	-18.75	SER 55
MB11	-4.98	-28.74	SER 94, ILE 194	-2.87	-17.69	SER 55
MB12	-5.53	-33.68	ILE 194	-2.64	-18.42	-
Co-crystal Triclosan	-7.36	-34.83	GLY 192, ILE 194	-	-	-
Co-crystal 883	-	-	-	-3.88	-51.53	ASP 81, ARG 144
Pyrazinamide	-5.08	-18.46	ILE 194	-4.98	-20.59	ASP 81, SER 55
Isoniazid	-3.69	-20.21	TYR 158, ILE 194	-4.50	-22.34	ASP 81, SER 55





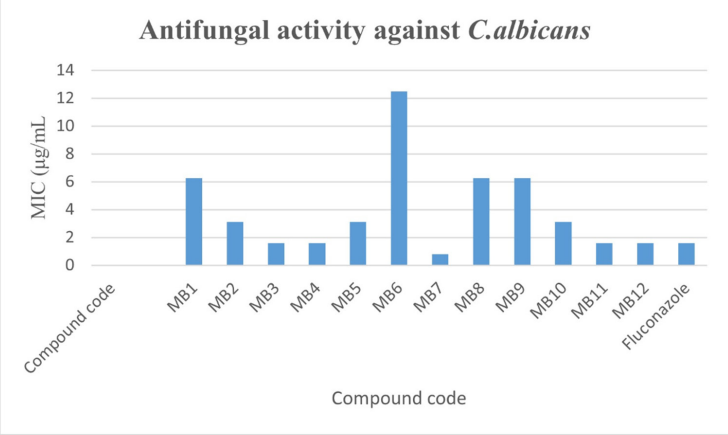


Figure 2: MIC values of compounds against *E. coli, S. aureus*, and *C. Albicans*.

Table 2: ADME properties of compounds by Swiss ADME web tool.

Compound Code	Mol. Wt	Log P	Molar refractivity	HBA	HBD	RB	TPSA	GI Absorption and BBB Permeant	Log Kp cm/s	Logs	Inhibition of Cytochrome P450 2E					Lead mimicry	Synthetic accessibility
											1A2	2C19	2C9	2D6	3A4		
MB1	267.32	2.73	80.18	2	0	6	37.38	High and yes	-6.09	-3.18	Yes	Yes	No	Yes	No	Yes	1.58
MB2	269.3	2.11	77.23	3	2	6	66.40	High and yes	-5.60	-3.64	Yes	No	Yes	No	No	Yes	1.55
MB3	335.40	3.44	98.88	3	1	8	59.30	High and yes	-5.96	-3.95	Yes	Yes	Yes	Yes	Yes	No	2.42
MB4	270.28	2.01	79.09	3	1	6	74.92	High and yes	-5.39	-4.86	Yes	Yes	Yes	Yes	No	No	1.82
MB5	283.32	2.66	81.55	3	1	7	55.40	High and yes	-5.46	-5.80	Yes	Yes	Yes	Yes	No	No	1.74
MB6	225.29	2.39	70.27	1	1	5	29.10	High and yes	-5.00	-3.80	Yes	Yes	Yes	Yes	No	No	1.31
MB7	270.33	2.40	81.17	2	2	6	64.35	High and yes	-5.78	-5.49	Yes	Yes	Yes	Yes	Yes	Yes	1.87
MB8	254.33	2.30	79.64	1	2	5	55.12	High and yes	-5.40	-5.76	Yes	Yes	Yes	Yes	Yes	Yes	1.77
MB9	240.30	2.01	74.67	1	2	5	55.12	High and yes	-5.57	-5.37	Yes	Yes	Yes	Yes	Yes	No	1.40
MB10	255.31	2.67	76.76	2	1	6	38.33	High and yes	-4.98	-5.86	Yes	Yes	Yes	Yes	No	No	1.64
MB11	239.31	2.46	75.24	1	1	5	29.10	High and yes	-4.82	-6.12	Yes	Yes	Yes	Yes	No	No	1.45
MB12	332.40	2.96	101.19	2	2	7	64.35	High and yes	-5.05	-7.59	Yes	Yes	Yes	Yes	Yes	No	2.04

MB8 (3-(3-amino-4-methylphenylamino)-1-phenylpropan-1-one)

Molecular formula $C_{16}H_{18}N_2O$; m.p: 95-97; yield 55%; IR (KBr, cm^{-1}): 1583.22 (C=O stretch, α, β-unsaturated, acyclic), 2875.26 (C-H stretch, -CH_2-), 1469.14 (C-H deformation, -CH_2-), 1385.37 (C-N stretch, 2º amine), 3358.42 (N-H stretch, 2º amine), 1432.27 (N-H deformation, 2º amine); ^{1}H NMR (DMSO-d6): 7.24-7.56 (m, 5-CH, benzene), 2.36 (d, 1- CH_2, methylene), 3.25 (s, 1- CH_2, methylene), 4.42 (t, 1H, aromatic C-NH), 6.59-5.67 (m, 3-CH, benzene), 2.35 (t, 1 CH_3, methyl), 4.2 (d, 1-NH, aromatic C-NH).

MB9 (3-(4-aminophenylamino)-1-phenylpropan-1-one)

Molecular formula $C_{15}H_{16}N_2O$; m.p: 107-109; yield 48%; IR (KBr, cm^{-1}): 1602.22 (C=O stretch, α, β-unsaturated, acyclic), 2784.36 (C-H stretch, -CH_2-), 1563.12 (C-H deformation, -CH_2-), 1334.34 (C-N stretch, 2º amine), 3327.54 (N-H stretch, 2º amine), 1458.37 (N-H deformation, 2º amine); ^{1}H NMR (DMSO-d6): 7.34-7.89 (m, 5-CH, benzene), 2.78 (d, 2-CH_2, methylene), 6.18-6.24 (d, 2H, benzene), 4.02 (t, 1H, aromatic C-NH), 6.18-6.24 (m, 3-CH, benzene), 4.0 (d, 1-NH, aromatic C-NH).

MB10 (3-(2-methoxyphenylamino)-1-phenylpropan-1-one)

Molecular formula $C_{16}H_{17}NO_2$; m.p: 112-114; yield 58%; IR (KBr, cm^{-1}): 1649.83 (C=O stretch, α, β-unsaturated, acyclic), 2775.24 (C-H stretch, -CH_2-), 1581.89 (C-H deformation, -CH_2-), 1340.35 (C-N stretch, 2º amine), 3327.44 (N-H stretch, 2º amine), 1387.48 (N-H deformation, 2º amine); ^{1}H NMR (DMSO-d6): 7.45-7.96 (m, 5-CH, benzene), 2.65 (d, 2-CH_2, methylene), 4.25 (t, 1H, aromatic C-NH), 6.47-6.55 (m, 2H, benzene), 6.60-6.47 (m, 4-CH, benzene), 3.73 (d, 1-CH, methyl).

MB11 (3-(p-toluidino)-1-phenylpropan-1-one)

Molecular formula $C_{16}H_{17}NO$; m.p: 75-78; yield 43%; IR (KBr, cm^{-1}): 1672.24 (C=O stretch, α, β-unsaturated, acyclic), 2781.73 (C-H stretch, -CH_2-), 1565.21 (C-H deformation, -CH_2-), 1345.27 (C-N stretch, 2º amine), 1372.56 (N-H deformation, 2º amine); ^{1}H NMR (DMSO-d6): 7.34-7.89 (m, 5-CH, benzene), 2.78-3.25 (m, 2-CH_2, methylene), 4.38 (t, 1H, aromatic C-NH), 6.84-6.96 (m, 4H, benzene), 2.35 (d, 1-CH, methyl).

MB12 (3-(4-(4-aminophenoxy)phenylamino)-1-phenylpropan-1-one)

Molecular formula $C_{21}H_{20}N_2O_2$; m.p: 95-97; yield 66%; IR (KBr, cm^{-1}): 1614.34 (C=O stretch, α, β-unsaturated, acyclic), 2757.82 (C-H stretch, -CH_2-), 1585.45 (C-H deformation, -CH_2-), 1338.67 (C-N stretch, 2° amine), 1323.42 (N-H deformation, 2° amine); ^{1}H NMR (DMSO-d6): 7.59-7.88 (m, 5-CH, benzene), 2.68-3.36 (m, 2-CH_2, methylene), 4.20 (d, 1-NH, aromatic), 6.42-6.70 (t, 7H, aromatic benzene), 4.35 (d, 2-NH, C-NH).

Pharmacological Screening

Antitubercular activity

The MIC evaluation of 12 compounds (MB1-MB12) demonstrated varying degrees of antimicrobial activity. Among the tested compounds, MB7 stood out as the most promising, displaying consistent and potent activity across all concentrations (100-0.19 µg/mL). Compounds MB2, MB3, MB8, and MB9 exhibited concentration-dependent activity, with efficacy observed at higher concentrations but diminishing at lower thresholds. Specifically, MB2 and MB3 became inactive below 3.125 µg/mL, while MB8 and MB9 lost activity below 12.5 µg/mL. In contrast, MB1, MB5, and MB6 showed activity only at the highest concentrations (100-50 µg/mL) and became inactive below 25 µg/mL, indicating weaker antimicrobial potential.

Notably, MB4, MB10, MB11, and MB12 were completely inactive across all tested concentrations, suggesting no observable antimicrobial effect. Based on these findings, MB7 is identified as the most potent candidate for further development, while MB2, MB3, MB8, and MB9 show moderate promise with concentration-dependent efficacy. The remaining compounds, particularly MB4, MB10, MB11, and MB12, require structural modifications to enhance their activity.

Antimicrobial activity

For this investigation, twelve compounds (MB1-MB12) were evaluated against *Candida albicans, Staphylococcus aureus*, and *Escherichia coli* to determine their Minimum Inhibitory Concentration (MIC). Figure 2 shows that MB7 had the strongest antibacterial activity, with MIC values of 12.5 µg/mL for *E. coli*, 6.25 µg/mL for *S. aureus*, and 0.8 µg/mL for *Candida albicans*. A MIC value of 6.25 µg/mL for *E. coli* and 12.5 µg/mL for *S. aureus* showed that MB5 was highly efficient, particularly against *C. albicans*. Compounded MB3 had doses of 12.5 µg/mL against *E. coli* and 1.6 µg/mL against *Candida albicans*; compounds MB1 and MB2 had modest efficiencies, while MB3 and MB4 had similar results. When testing MB6, a MIC value of 25 µg/mL was noted for *E. coli* and 12.5 µg/mL for the other pathogens. In Table 3, it is indicated that the Minimum Inhibitory Concentration (MIC) values for *E. coli* and *S. aureus* were 50 µg/mL for MB9 and MB10, while they were effective against *Candida albicans* at 6 µg/mL.[31,32]

DISCUSSION

The current work merged molecular docking and antimicrobial activity assessments to evaluate the potential of 12 new compounds (MB1-MB12) as antimicrobial agents against major diseases. The docking tests were done using two protein targets, 2B35 and 4P8O, and the results revealed variable levels of binding affinity and interactions. Notably, MB3 and MB2 had the highest docking scores of -7.74 and -7.84 against 2B35, respectively, indicating substantial binding affinity. Key hydrogen bond

Table 3: Antimicrobial screening of compounds.

	A concentration of µg/mL is required to inhibit its action		
	E. coli	*S. aureus*	*C. albicans*
MB1	25	25	6.25
MB2	25	25	3.12
MB3	12.5	25	1.6
MB4	12.5	50	1.6
MB5	6.25	12.5	3.12
MB6	25	12.5	12.5
MB7	12.5	6.25	0.8
MB8	25	12.5	6.25
MB9	50	50	6.25
MB10	25	12.5	3.12
MB11	100	12.5	1.6
MB12	100	6.25	1.6
Ciprofloxacin	12.5	6.25	----
Fluconazole	----	----	1.6

interactions, such as those with ASP148, LYS165 (MB2), and ILE21, SER94 (MB3), were found, confirming their potential as strong inhibitors. However, the binding scores against 4P8O were generally lower, with MB3 reaching a docking score of -5.10.

The pharmacokinetic characteristics of the compounds demonstrated strong gastrointestinal absorption and acceptable blood-brain barrier permeability for most, confirming their potential as orally accessible medicines. The synthetic accessibility scores further highlighted the viability of producing these molecules for large-scale applications.

The antibacterial activity of the compounds was further verified using MIC tests against *E. coli, S. aureu*s, and *C. albicans*. Among the examined compounds, MB7 consistently displayed higher activity, with MIC values of 12.5 µg/mL against *E. coli*, 6.25 µg/mL against *S. aureu*s, and 0.8 µg/mL against *C. albicans*. This is noteworthy since MB7 also showed positive docking contacts with hydrogen bonds involving TYR158 and ILE194 in 2B35, confirming its *in silico* promise and experimental efficacy. MB5 and MB3 had high antibacterial activity, especially against *C. albicans*, with MIC values of 3.12 µg/mL and 1.6 µg/mL, respectively

Antimicrobial standards Ciprofloxacin and Fluconazole have MIC values of 12.5 µg/mL against *S. aureu*s and 1.6 µg/mL against *C. albicans*. MB7, MB5, and MB3 exhibit equivalent or greater action against various diseases, highlighting their therapeutic potential. Compounds such as MB1 and MB2 demonstrated considerable antibacterial activity, although MB9, MB11, and MB12 had low efficacy, notably against *E. coli*.

The structure-activity relationship indicates that compounds with smaller molecular weights, moderate Log P values, and a balanced number of Hydrogen Bond Donors (HBD) and Acceptors (HBA) have better antibacterial characteristics. Furthermore, most drugs exhibited high gastrointestinal absorption and blood-brain barrier permeability, indicating excellent pharmacokinetic properties.

In summary, MB7 emerged as the most promising lead, with strong docking contacts and antibacterial efficacy against all tested pathogens. Compounds MB3 and MB5 also deserve additional exploration due to their dual in silico and *in vitro* effectiveness. These findings lay a solid framework for further improvement and development of these compounds as potential antibacterial agents. Future research will investigate the mechanism of action, cytotoxicity, and *in vivo* efficacy to aid clinical translation.

CONCLUSION

This work successfully combined computational docking analysis with experimental antimicrobial evaluations to find compounds MB1-MB12 that showed promise as antibacterial agents. Through docking tests, it was found that MB3 and MB2 had substantial binding affinities to the 2B35 protein. On the other hand, MB7 consistently showed strong contacts and robust antimicrobial activity. According to the computational predictions, the most promising candidates were MB7, MB3, and MB5, which showed significant activity against *E. coli, S. aureu*s, and *C. albicans* in experimental MIC evaluations.

Specifically, MB7 showed better antimicrobial effectiveness than Ciprofloxacin and Fluconazole in certain cases, with MIC values of 12.5 µg/mL, 6.25 µg/mL, and 0.8 µg/mL against *E. coli, S. aureu*s, and *Candida albicans*, respectively. More so, pharmacokinetic profiling showed that the majority of drugs had promising absorption and permeability characteristics, which bodes well for their future development.

These results demonstrate that MB7, MB3, and MB5 have promising antibacterial therapeutic potential as lead molecules. The therapeutic feasibility of these treatments will be determined in the future through mechanistic research, cytotoxicity assessments, and preclinical trials. In order to combat infections that are resistant to more than one treatment, our study adds to the increasing demand for new antimicrobial medicines.

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ABBREVIATIONS

ADME: Absorption, distribution, metabolism, and excretion; ***Mtb*:** *Mycobacterium tuberculosis*; ***E. coli*:** *Escherichia coli;* ***S. aureus*:** *Staphylococcus aureus;* ***C. albicans*:** *Candida albicans;* **MIC:** Minimum inhibitory concentration; **m.p:** melting point; **TPSA:** Topological Polar Surface Area; **HBA:** Hydrogen Bond Acceptor; **HBD:** Hydrogen Bond Donor; **RB:** Rotatable Bonds; **BBB:** Blood-Brain Barrier; **CYP Inhibition:** Inhibition of cytochrome P450 (CYP450) enzymes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The antibacterial potential of twelve new compounds (MB1-MB12) was examined in this work using molecular docking and MIC tests. Among the three MBs that docked with the 2B35 protein, MB7 was the most consistently effective, although MB3 and MB2 were also powerful binders. The MIC testing demonstrated that MB7 had better action against *E. coli, S. aureu*s, and *Candida albicans*, at concentrations of 12.5 µg/mL, 6.25 µg/mL and 0.8 µg/mL, respectively. MB3 and MB5

showed significant antifungal action as well. Their potential for development is enhanced by their favorable pharmacokinetics and synthetic accessibility. Potentially fruitful avenues for further investigation into drug-resistant infections include MB7, MB3, and MB5.

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