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Synthesis and characterization of amide derivatives of N-phenyl anthranilic acid

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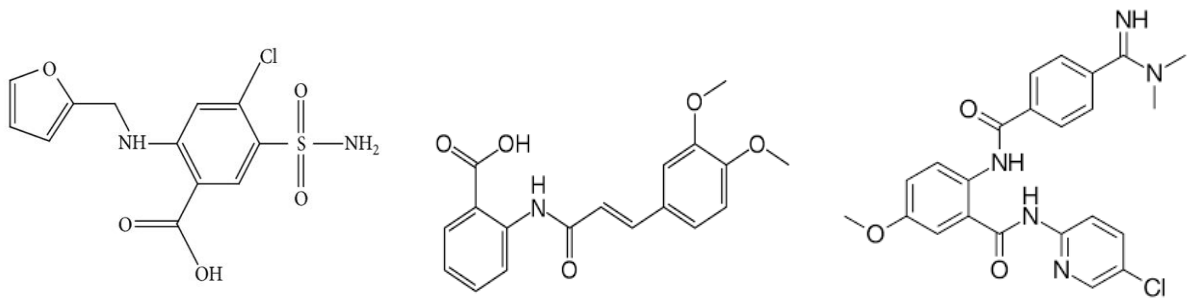
Abstract: N-Phenyl anthranilic acid (fenamic acid) is an amino-benzoic acid and the N-phenyl derivative of anthranilic acid. It serves as the fundamental structure for synthesizing several non-steroidal anti-inflammatory drugs, antibacterial drugs and also functions as a modulator of membrane transport. A series of amide derivatives of N-Phenyl Anthranilic acid that are 2-(Phenyl amino) benzamide, 2-(Phenyl amino) N, N- diphenylbenzamide, 2-(Phenyl amino) N-methyl N- phenyl benzamide and 2-(Phenyl amino) N-(2-hydroxy) phenyl benzamide were synthesized. Similar to the parent compound, these derivatives can exhibit antimicrobial and antibacterial activities. Current research aims to enhance the solubility and bioavailability of fenamic acid derivatives to reduce dose-related side effects of existing drugs. The chemical structures and functional groups of these derivatives were confirmed using elemental analysis, physical chemical characteristics, and information derived from spectral analysis. Moreover, the antimicrobial activity of the samples was assessed against Escherichia coli (a Gram-negative bacterium) and Staphylococcus aureus (a Gram-positive bacterium) using the agar-well diffusion technique. Additionally, molecular docking studies were conducted to predict the binding interactions of the synthesized compounds with DNA gyrase.

Keywords: N-Phenyl anthranilic acid, amide derivatives, antimicrobial, antibacterial, docking, agar-well diffusion.

Introduction

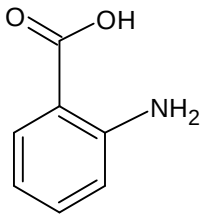
Anthranilic acid, or 2-aminobenzoic acid, is a valuable and economical precursor for the synthesis of benzo-fused heterocycles (Wiklund & Bergman, 2006). In the development of drugs aimed at treating various diseases, N-phenyl anthranilic acid and its derivatives offer distinctive pharmacophoric properties and serve as fundamental structural motifs. (Prasher & Sharma, 2021). The production of various commercially available drugs, such as furosemide (a diuretic), tranilast (an anti-allergic), betrixaban (an anticoagulant), and fenamates (analgesic and anti-inflammatory agents), is thought to be facilitated by these derivatives (Figure 1), which are also thought to be an inexpensive and effective starting precursor. In the last thirty years, a number of anthranilic acid analogs have shown diverse biological activities, including insecticidal, antiviral, anticancer, antibacterial, and anti-inflammatory properties (Nasr et al., 2022).



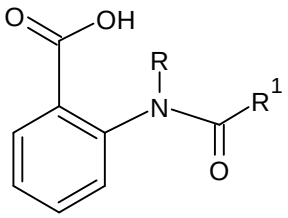
Furosemide **Tranilast** **Betrixaban**

Figure 1: Chemical structures of furosemide, tranilast and betrixaban.

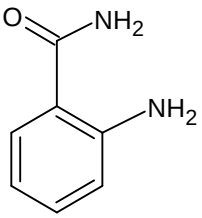
Historically, n-phenyl anthranilic acid was utilized in studies to induce and examine renal papillary necrosis in rats (Hardy, 1970), while research into fenamic acid derivatives revealed their potential as novel anti-atopic medications (Azuma et al., 1976). Building on these early insights, we have now progressed to a point where these precursors and compounds exhibit significant anti-viral, anti-cancer, and anti-diabetic properties (Figure 2). The functional groups in anthranilic acid and its analogues, such as carboxyl, amino, amide, carbomethoxy, and acetamide, enable their conjugation and derivatization. This flexibility allows for the development of specifically designed molecules aimed at effectively interacting with particular biological targets (Kwon et al., 2017; Schrey et al., 2019; Teponno et al., 2017).



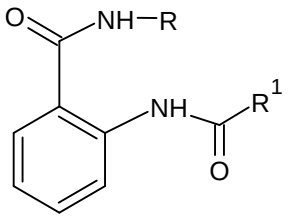
Anthranilic acid



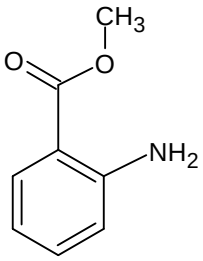
Anthranilic anilide



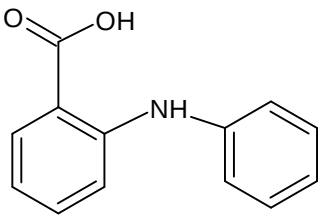
Anthranilamide



Anthranilic diamide



Methyl anthranilate



N-Phenyl Anthranilic acid (Fenamic acid)

Figure2: Chemical structures of anthranilic acid analogues.

N-Phenyl anthranilic acid, also known as fenamic acid, is a derivative of anthranilic acid and serves as the active metabolite of aspirin, paving the way for experimental research (Omar et al., 1996). Clinically, fenamates, which are N-substituted derivatives of anthranilic acid, are utilized as nonsteroidal anti-inflammatory drugs (NSAIDs) to alleviate fever, pain, and inflammation. These compounds work by inhibiting the cyclooxygenase (COX) enzyme, which decreases the production of prostaglandins. As non-steroidal anti-inflammatory drugs (NSAIDs), fenamates represent an interesting category of medications that could be used in combination with potent GABAa receptor allosteric agonists to alleviate pain, inflammation, as well as associated anxiety and depression. Headache, dizziness, blurred vision, and upset stomach are possible side effects (Buttgereit et al., 2001) peptic ulcer disease, gastrointestinal bleeding, and aspirin sensitivity are among conditions that make using NSAIDs contraindicated (Metwally et al., 2007). The novel synthesized derivatives could be used to overcome the unwanted side effects of already existing fenamates. The esters and amides being more soluble could be used to increase the solubility and bioavailability in the body thereby minimizing the dose related side effects (Bala et al., 2013; Husain et al., 2005; Lanza, 1998).

Experimental

Materials and Method

Grade solvents and reagents were purchased from commercial suppliers. The melting points of the synthesized compounds were measured using a capillary method with a melting point apparatus. The percentage yield was calculated and TLC method was used to determine the R_f values of the related derivatives. Infrared (IR) spectra were obtained using an FT-IR spectrophotometer (IR affinity-1, Shimadzu) using KBr pallets. Additionally, ^{1}H-NMR and ^{13}C-NMR spectra were obtained using an NMR Spectrometer (Avance III), 400 MHz: Bruker by using solvents $CDCl_3$ and DMSO. The Mass Spectrometer used is LCMS (Liquid Chromatography Mass Spectrometry) SCIEX Triple TOF 5600 and 5600+/SCIEX with mass range of 100 to 2000Da using solvents methanol and acetonitrile.

Drug-likeness

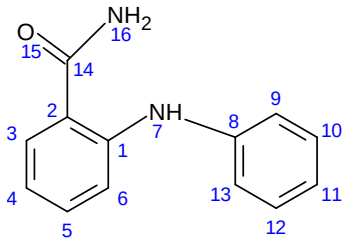
In silico techniques for assessing absorption, distribution, metabolism, and excretion (ADME) parameters utilize statistically developed models that establish connections between compound structural characteristics and biological behaviors. This method is gaining popularity for its efficient use of resources (Gleeson et al., 2011). As a result, the ADME characteristics of compound 73-KS5-51 were examined with the SwissADME online tool (Daina et al., 2017), and Lipinski's rule of five was utilized to assess its suitability as a drug candidate (Lipinski, 2004).

Synthesis of amide of N-Phenyl Anthranilic acid (2-Phenyl amino benzamide)-

An equal amount of N-Phenyl anthranilic acid and $SOCl_2$ was placed in a 250 mL round-bottom flask fitted with an air-cooled condenser. A vigorous reaction occurred, followed by the addition of liquid ammonia. After allowing the mixture to cool overnight, the crude product was filtered using a pump. The sea-green flakes obtained were subjected to recrystallization with hot acetonitrile. The resulting compound, named MB-6, was dried to determine its percentage yield.

MB-6 - 2-(Phenyl amino) benzamide- IR (KBr) values in cm^{-1}: 3232 (symmetric) and 3333 (asymmetric) for amide N-H stretching, 3036 for aromatic C-H stretching, 1662 (broad) for C=O stretching of the amide, 1585 and 1521 for C=C stretching in the aromatic ring, and

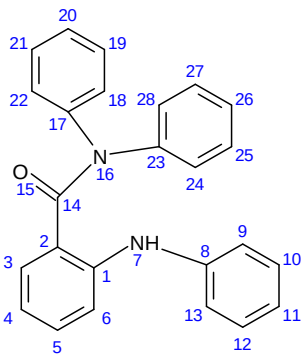
1253 for N-H stretching of a secondary amine.; ^{1}H-NMR (DMSO) values in δppm- 8.3-6.75 (multiplet, 9H, Aromatic), 11.9 (singlet, 2H, NH), 9.8 (broad singlet, 1H, NH); ^{13}C-NMR (DMSO) values in ppm- C1-116, C2-147, C3-132, C4-121.9, C5-134.9, C6-127, C8-122.5, C9-120, C10-118, C11-116, C12-114, C13-113, C14-170.09



Synthesis of diphenyl amide of N-Phenyl Anthranilic acid (2-Phenyl amino N, N-diphenylbenzamide)-

An equimolar mixture of N-Phenyl anthranilic acid, diphenylamine, and boric acid was heated in a round-bottom flask at 160°C to 180°C for 20 minutes without a solvent. This reaction led to the complete melting of the mixture, yielding the final product. After taking the flask off the heat, it was permitted to cool to normal temperature. A solution of liquid ammonia was then added to the crude product, and the mixture was heated with stirring to remove any unreacted acid. The remaining boric acid was filtered out and washed with distilled water. The off-white flakes obtained were recrystallized using hot acetonitrile. The resulting compound, designated as MB-7, was dried to calculate its percentage yield.

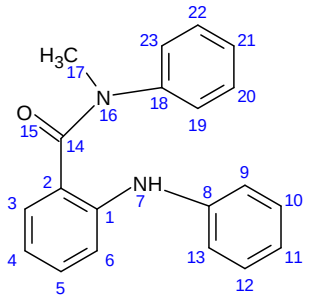
MB-7- 2-(Phenyl amino) N, N- diphenylbenzamide- IR (KBr) values in cm^{-1}: 3381 (asymmetric) for amide N-H stretching, 3039 for aromatic C-H stretching, 1600 (broad) for C=O stretching of the amide, 1514 and 1487 for C=C stretching in the aromatic ring, and 1253 for N-H stretching of a secondary amine; ^{1}H-NMR (DMSO) values in δppm-8.2 (singlet, 1H, NH), 7.26 - 6.8 (multiplet, 9H, Aromatic), 3.49 (multiplet, 5H, Aromatic), 2.5 (multiplet, 5H, Aromatic); ^{13}C-NMR (DMSO) values in ppm- C1-120, C2 to C6-117, C8-120, C9 to C13-117, C14-144, C17-120, C18 to C22-120.9, C23-120, C24 to C28-120.9



Synthesis of methyl phenyl amide of N-Phenyl Anthranilic acid (2-(Phenyl amino) N-methyl N- phenyl benzamide)-

An equimolar mixture of N-Phenyl anthranilic acid, N-methyl aniline, and boric acid was heated in a round-bottom flask at 160°C to 180°C for 20 minutes without any solvent. The material melted completely during the reaction. After heating, the mixture was allowed to cool to room temperature. To eliminate unreacted acid, the crude product was heated with stirring in a liquid ammonia solution. Remaining boric acid was removed by filtering and washing the product with distilled water. The resulting pale green precipitates were recrystallized using acetonitrile and then dried. The percentage yield and melting point of the final compound, designated as MB-8, were measured.

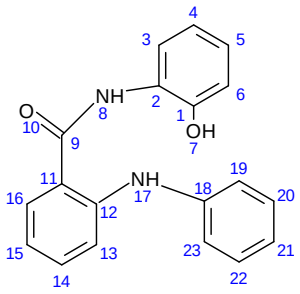
MB-8 - 2-(Phenyl amino) N-methyl N- phenyl benzamide- IR (KBr) values in cm^{-1}: 3335 (asymmetric) for amide N-H stretching, 3097 for aromatic C-H stretching, 1666 (broad) for C=O stretching of the amide, 1591 and 1521 for C=C stretching in the aromatic ring, 1323 for C-H bending of the methyl group, and 1255 for N-H stretching of a secondary amine; ^{1}H-NMR (DMSO) values in δppm-8.3-6.79 (multiplet, 9H, Ar), 9.7 (broad singlet, 1H, NH), 2.5 (singlet, 5H, Aromatic), 1.02 (singlet, 3H, CH); ^{13}C-NMR (DMSO) values in ppm- C1-116, C2-147, C3-132, C4-121.9, C5-134.9, C6-127, C8-122.5, C9-120, C10-118, C11-116, C12-114, C13-113, C14-170.09, C17-142, C18-147, C19-134, C20-125.9, C21-122, C22-121, C23-120



Synthesis of 2-amino phenol amide of N-Phenyl Anthranilic acid (2-Phenyl amino N-2-hydroxy phenyl benzamide)

An equimolar mixture of N-Phenyl anthranilic acid, 2-amino phenol, and boric acid was heated in a round-bottom flask at 160°C to 180°C for 20 minutes without any solvent. During heating, the reaction mixture completely melted, indicating the formation of the final product. After halting the heating, the mixture was allowed to cool to ambient temperature. A liquid ammonia solution was then added to the crude product while stirring and heating to eliminate any un-reacted acid. The product was subsequently filtered to eliminate any residual boric acid and washed with distilled water. The resulting green-black precipitates were recrystallized using acetonitrile and dried. The percentage yield and melting point of the final compound, labeled as MB-9, were determined.

MB-9 - 2-(Phenyl amino) N-(2-hydroxy) phenyl benzamide- IR (KBr) values in cm^{-1}: 3250 (symmetric) and 3333 (asymmetric) for amide N-H stretching, 3011 for aromatic C-H stretching, 1658 (broad) for C=O stretching of the amide, 1597 and 1581 for carbon double bonds stretching in the aromatic ring, and 1255 for NH bond stretching of a secondary amine; ^{1}H-NMR (DMSO) values in δppm-7.4-6.75 (multiplet, 9H, Aromatic), 9.72 (singlet, 1H, NH), 7.89 (singlet, 1H, NH), 2.5 (singlet, 1H, OH), 2.1 (singlet, 2H, Ar), 1.3 (singlet, 2H, Ar); ^{13}C-NMR (DMSO) values in ppm- C1-150, C2-141, C3-130, C4-127, C5-124, C6-120, C9-170.09, C11-147, C12-116, C13-127, C14-134.9, C15-121.9, C16-132, C18-122.5, C19-120, C20-118, C21-116, C22-114, C23-113



Antimicrobial activity

For the antimicrobial studies, ampicillin was used as the positive control and DMSO as the negative control. Both ampicillin and the test complex were dissolved in DMSO to achieve concentrations of 500 mM and 250 mM, respectively. Microorganism strains were inoculated into 15 mL of nutrient agar media, which were then transferred to Petri plates. Eight-

millimeter wells were created in the agar and filled with the test compounds. The plates were incubated at 37°C for eighteen hours. The diameter of the inhibitory zone (measured in millimeters) was used to assess the effectiveness of the test compounds. The average diameter of the inhibitory zone was calculated based on triplicate experiments.

Docking

Non-polar hydrogens were combined, and polar hydrogens were added using AutoDock Tools, with the structure saved in PDBQT format (Morris et al., 2009). For the ligands, 2D structures were drawn with Marvin Sketch and converted to 3D mol2 format. The ligands were saved in PDBQT format. Docking studies and binding affinity evaluations were conducted using AutoDock Vina (Trott and Olson, 2010).

Toxicity

The toxicity of the test compounds was evaluated in silico using the pkCSM online tool, which provides predictions based on computational models (Pires et al., 2015; Salgueiro et al., 2016; Pires et al., 2018).

Result and Discussion

ADME study

Test compounds exhibited favorable Pharmacokinetic features for oral bioavailability and pharmaceutical properties as summarized in Table 1

Table 1: ADME properties of the N-phenyl anthranilic acid derivatives predicted using the SwissADME web server.

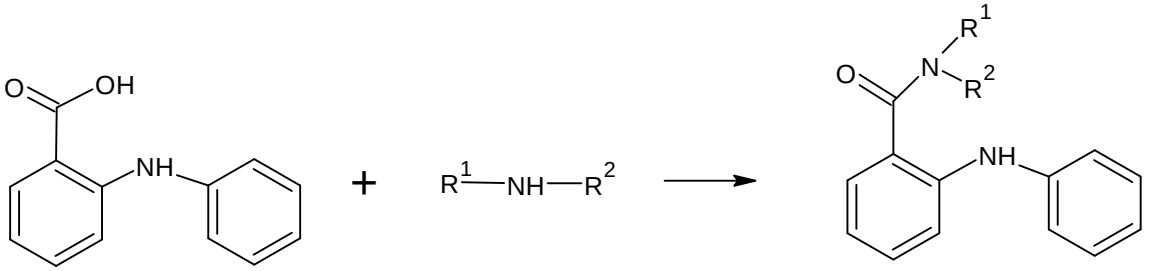
Cpd.	MW	HBAs	HBDs	TPSA	MR	Log P	Log S	GI absorption
MB-6	212.25	1	2	55.12	64.08	1.77	-3.85 (Soluble)	High
MB-7	364.44	1	1	32.34	115.34	3.56	-7.01 (Poor)	High
MB-8	302.37	1	1	32.34	95.10	3.12	-4.77 (Moderate)	High
MB-9	304.34	2	3	61.36	92.22	2.37	-5.29	High

							(Moderate)	

MW: Molecular weight (g/mol); HBAs: Number of hydrogen bond acceptors; HBDs: Number of hydrogen bond donors; TPSA: Topological polar surface area (Å²); MR: Molar refractivity; Log P: Partition coefficient; Log S: Estimated solubility (soluble, moderate, or poor); GI absorption: Gastrointestinal absorption.

Chemistry

A series of esters of N-phenyl anthranilic acidwere prepared as shown in Scheme 1. The purity of the prepared compounds was detected using silica gel-G TLC and was further verified by spectral data. Yield and physico-chemical characteristics of the prepared derivatives are presented in Table 2.



Scheme 1: Synthesis of amide derivatives of N-phenyl anthranilic acid.

Table 2: Physiochemical properties, R_f value, M.P., and % yield of the synthesized N-phenyl anthranilic acid derivatives.

Cpd.	**R1**	**R2**	**Mol. Formula**	**MW**	**Rf**	**M.P.(°C)**	**Yield (%)**
MB-6	H	H	$C_{13}H_{12}N_2O$	212	0.78	184-205	68
MB-7	C_6H_5	C_6H_5	$C_{25}H_{20}N_2O$	364	0.67	190-215	72
MB-8	CH_3	C_6H_5	$C_{20}H_{18}N_2O$	302	0.72	188-212	66
MB-9	H	C_6H_4OH	$C_{19}H_{16}O_2N_2$	304	0.70	182-195	70

Starting materials N-Phenyl Anthranilic acid and SOCl2, when treated with liquid ammonia, undergo amide formation. Compound MB-6 exhibits specific FTIR absorption peaks: a symmetric stretching at 3232 cm^{-1} and an asymmetric –N-H stretching at 3333 cm^{-1}

indicative of the amide group. A peak at 3036 cm^{-1} corresponds to =C-H stretching of the aromatic ring. The carbonyl stretching of the amide group appears as a broad peak at 1662 cm^{-1}. C=C stretching in the aromatic ring is shown by double peaks at 1585 cm^{-1} and 1521 cm^{-1}. Additionally, a peak at 1253 cm^{-1} indicates N-H stretching typical of a secondary amine. The ^{1}H-NMR spectrum of this compound display a multiplet signal between the region 8.3 to 6.75 δppm attributed to aromatic proton. The singlet at 11.9 δppm indicates the protons of amide group and 9.8 δppm for the NH proton of N-phenyl Anthranilic acid. The ^{13}C-NMR of compound shows two peaks at 147-116 ppm and 122.5-113 ppm indicating two benzene rings. The peak at 170.09 ppm is due to the presence of carbonyl group of amide. For MB-7, a single peak at 3381 cm^{-1} indicates N-H stretching in the amide group, additionally, a peak was observed at 3039 cm^{-1} for =C-H aromatic stretching. A broad peak at 1600 cm^{-1} indicates C=O stretching in the amide, while double peaks at 1514 cm^{-1} and 1487 cm^{-1} correspond to C=C stretching in the aromatic ring. N-H stretching of a secondary amine is observed at 1253 cm^{-1}. The ^{1}H-NMR spectrum of this compound exhibited a singlet at 8.2 δppm specific to the NH proton of N-phenyl Anthranilic acid, and a multiplet signal ranging from 7.26 δppm to 6.8 δppm for aromatic protons. There was also a multiplet signal ranging from 3.49 δppm to 2.5 δppm related to aromatic protons of the diphenyl group. The ^{13}C-NMR of compound shows four peaks at 120-117 ppm & 120-117 ppm and 120.5-113 ppm & 120.5-113 ppm respectively, indicating the presence of four aromatic rings. The peak at 144 ppm is due to the presence of carbonyl group of amide. Compound MB-8 displays a peak at 3335 cm^{-1} attributed to N-H stretching in the amide group, accompanied by a peak at 3097 cm^{-1} indicating =C-H aromatic ring stretching. A broad spectrum observed at 1666 cm^{-1} suggests C=O stretching in the amide, while double peaks at 1591 cm^{-1} and 1521 cm^{-1} correspond to C=C stretching in the aromatic ring. Additionally, N-H stretching of a secondary amine was observed at 1255 cm^{-1}. The ^{1}H-NMR spectrum of this compound exhibited a multiplet ranging from 8.3 to 6.79 δppm for aromatic protons, along with a broad singlet at 9.7 δppm specific to the NH proton of N-phenyl Anthranilic acid. A singlet was observed at 2.5 δppm for aromatic protons, and another singlet appeared at 1.02 δppm for methyl group protons. The ^{13}C-NMR of compound shows three peaks at 147-116 ppm & 122.5-113 ppm and 147-120 ppm respectively, indicating the presence of three aromatic rings. The peak at 170.09 ppm is due to the presence of carbonyl group of amide and at 142 ppm shows the presence of methyl group. Compound MB-9 exhibits symmetric stretching at 3250 cm^{-1} and asymmetric –N-H stretching at 3333 cm^{-1}, indicative of the amide group. A broad peak at 1658 cm^{-1} indicates C=O stretching in the amide, while double peaks at 1597

cm^{-1} and 1581 cm^{-1} correspond to C=C stretching in the aromatic ring. N-H stretching of a secondary amine is observed at 1255 cm^{-1}. The ^{1}H-NMR spectrum of this compound showed a multiplet from 7.4 to 6.75 δppm for aromatic protons, along with a singlet at 9.72 δppm specific to the NH proton of N-phenyl Anthranilic acid. Additionally, a singlet was observed at 7.89 δppm for the NH group of the amide, and another singlet appeared at 2.5 δppm for the –OH proton. There were two multiplets observed at 2.1 δppm and 1.3 δppm for aromatic protons. The ^{13}C-NMR of compound shows three peaks at 141-120 ppm, 147-116 ppm and 122.5-113 ppm respectively, indicating the presence of three aromatic rings. The peak at 170.09 ppm is due to the presence of carbonyl group of amide and at 150 ppm shows the presence of hydroxy group.

Anti microbial Activity

MB-7, identified as 2-(Phenyl amino) N, N-diphenylbenzamide, demonstrates significantly greater antibacterial activity against Escherichia coli and Staphylococcus aureus than the other compounds (Table-3). This enhanced activity is attributed to the presence of the diphenyl group and its strategic placement within the molecule. Establishing structure-activity relationships through further studies could elucidate and optimize these beneficial effects.

Table 3: Antimicrobial activity of N-phenyl anthranilic acid derivatives

Cpd.	**Zone of inhibition (mm)**	
	Escherichia coli	***Staphylococcus aureus***
MB-6	15±0.5773	14±0.5773
MB-7	24±1.0	27±1.154
MB-8	22±0.5773	20±1.0
MB-9	18±1.154	18±0.5773

Docking study

The docked ligand exhibited a pose similar to that of the co-crystallized ligand with DNA gyrase, confirming the reliability of the docking protocol used in this study. Subsequently, ester derivatives of N-phenyl anthranilic acid designed in the study were docked with DNA gyrase (PDB entry: 4CKL) to predict their binding interactions. All derivatives demonstrated significant binding in the active site, as evidenced by analysis of hydrogen bonds,

hydrophobic interactions, and binding free energies (docking scores, ΔG values in kcal/mol) of the top-ranked ligand-protein complexes (Table 4).

Table 4: Docking score and binding interactions of the N-phenyl anthranilic acid derivatives with DNA gyrase.

Cpd.	**ΔG**	**Hydrogen bond interactions (bond distance)**	**Hydrophobic interactions (residues involved)**
MB-6	-6.7	Asn169 (2.69 Å), Gly170 (2.05 Å), and Tyr266 (2.42 & 3.23 Å)	Pi-Sulfur (Met92), and Pi-Alkyl (Leu98)
MB-7	-6.6	Gln94 (3.06 Å), and Ser97 (2.40 Å)	Pi-Pi T-Shaped (Val90 & Arg91), and Pi-Alkyl (Phe96)
MB-8	-6.4	Ser97 (3.02Å)	Pi-Sigma (Val90 & Arg91), and Pi-Alkyl (Gln267)
MB-9	-7.0	Ser171 (2.88 Å), and Ser172 (3.18 Å)	Pi-Cation (Lys42 & Arg91), Pi-Pi Stacked (Tyr266), and Pi-Alkyl (Arg91 & Leu98)

The best docked pose for compound MB-9 was further studied in minutiae employing Discovery Studio for exploring the orientation, pattern and binding interactions of this derivative in the active site of DNA gyrase (Figure 3). Compound MB-9 showed hydrogen bond interactions with Ser171 and Ser172 residues (with bond distance of 2.88 and 3.18 Å, respectively) and hydrophobic interactions with Lys42 & Arg91 residues (Pi-Cation), Tyr266 residue (Pi-Pi Stacked), and Arg91 & Leu98 residue (Pi-Alkyl). Docking of the newly designed N-phenyl anthranilic acid derivatives with the DNA gyrase protein revealed that these compounds bind potently with DNA gyrase and could serve as potential antimicrobial agents inhibiting DNA gyrase.

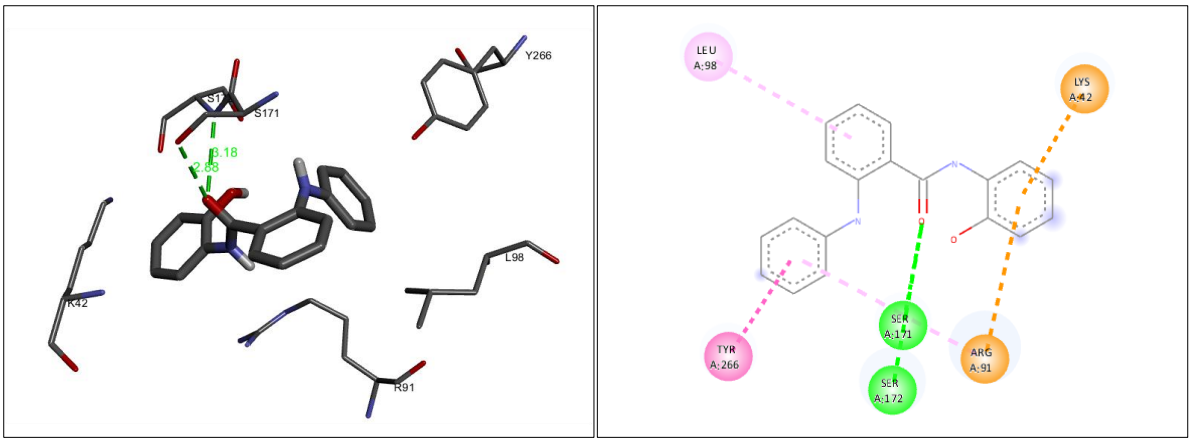


Figure 3: Docking interaction analysis of MB-9 with DNA gyrase.

In the docking interaction analysis of MB-9 with DNA gyrase, both 3D and 2D representations highlight crucial aspects of their binding. The 3D docked pose reveals specific hydrogen bond interactions between MB-9 and amino acid residues within the active site of DNA gyrase. These hydrogen bonds, illustrated as dashed lines, signify strong interactions essential for stabilizing the ligand-protein complex. On the other hand, the 2D docked pose provides a simplified view emphasizing not only hydrogen bonds but also crucial hydrophobic interactions between MB-9 and the protein. These hydrophobic contacts, depicted as lines or arcs, involve non-polar residues that contribute significantly to the binding affinity and overall stability of the complex. Together, this detailed analysis elucidates the molecular interactions guiding the binding of MB-9 with DNA gyrase, offering valuable insights into its potential as a therapeutic agent or for further drug development.

Toxicity

The potential toxicity of the designed compounds, including mutagenicity, carcinogenicity, cardiotoxicity, immunotoxicity, skin irritation, and reproductive toxicity, was evaluated using the pkCSM online tool (Table 5).

Table 5: The probable toxicity prediction for the designed compounds obtained using the pkCSM program.

Cpd.	**AMES toxicity**	**Max. tolerated dose (hum**	**hERG I inhibitor**	**hERG II inhibitor**	**Oral Rat Acute Toxic**	**Oral Rat Chronic Toxici**	**Hepato-toxicity**	**Skin Sensitization**	***Tetrahymena pyriformis***	**Minnow toxicity**

		an)			**ity (LD50)**	**ty (LOAEL)**			**toxicity**	
MB-6	Yes	0.386	No	No	2.069	1.488	No	No	1.26	1.03
MB-7	No	0.594	No	Yes	3.510	0.109	Yes	No	0.29	0.29
MB-8	Yes	0.576	No	Yes	2.392	0.384	Yes	No	0.60	0.01
MB-9	Yes	0.738	No	Yes	2.568	1.607	No	No	0.33	1.05

Conclusion

The successful synthesis and characterization of ester derivatives of N-phenyl anthranilic acid with promising antibacterial activity demonstrate the potential for developing new antimicrobial agents. Derived compounds were tested for antibacterial activity where MB-7 [2-(Phenyl amino) N, N- diphenylbenzamide] exhibited highly antibacterial activities than other compounds. The *in vitro* antimicrobial and *in silico* docking results showed a potential of these molecules to act as DNA gyrase inhibitors and these derivatives could serve as preliminary molecules for development of effective antimicrobial agents.

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Interest conflict

All authors affirm that they have no conflicts of interest.

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