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SYNTHESIS, CHARACTERIZATION AND MOLECULAR DOCKING OF NAPHTHO[2,1-b]FURAN DERIVATIVES FOR ANTIBACTERIAL SCREENING

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ABSTRACT

A novel synthesis of N-formyl-N-(formyloxy)-5-nitronaphtho[2,1-b]furan-2-carboxamides derivatives 4(a-f) from the staring precursors of ethyl naphtho[2,1-b]furan-2-carboxylate 1 is mixed with acetic acid, HNO_3 and conc. H_2SO_4 to get the compound ethyl 5-nitronaphtho[2,1-b]furan-2-carboxylate 2. Compound 2 reacts with hydroxyl amine hydrochloride, sodium hydroxide, ethanol, and HCl to get a compound N-hydroxy-5-nitronaphtho[2,1-b]furan-2-carboxamide 3 followed by the reaction of compound 3 with acetyl chloride, H_2SO_4 to get an desired product. The structural confirmation of the prepared compounds was done by several analytical technique methods such as FTIR, ^{1}H-NMR, Mass, and Elemental analysis. The confirmed compounds were used for several G+ve and G-ve antibacterial activity against *E.Faecalis, S.auerus*, *E. Coli*, and *S. Pneumoniae* with Standards for use in antibacterial was chloramphenicol, the zone of inhibition of the compounds were compared to the reference medication. It shows excellent results of two G+ve (*E. faecalis and S.auerus)* and two G-ve (*E. coli* and *S. Pneumoniae)* antibacterial activity. The structural morphology of the compound was done by the molecular docking studies which showed an excellent result.

Keywords: Naphtho[2,1-*b*]furan, Triazole, Molecular Docking, Antibacterial Activity, Antifungal Activity.
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INTRODUCTION

Naphthofurans are key components of significant natural products and exhibit a wide spectrum of biological functions.[1-3] Because of their wide range of pharmacological properties, such as antibacterial[4], anticancer[5], anthelmintic[6], antifertility[7], mutagenic, growth inhibitory, and oestrogenic activities[8], many compounds containing a naphthofuran ring have received a lot of interest. The spectrum of biological activities that are constituents of important natural products. These bioactive moieties have a wide range of pharmacological potential[9], which has drawn the attention of many organic and medicinal chemists, who have created effective syntheses. The encouraging findings of earlier research encouraged us to broaden the scope of our investigation to include the synthesis of annulated heterocyclic systems with potential biological applications. We are describing here the synthesis of further analogs of the naphthofuran moiety as a basis unit and their antibacterial properties as a follow-up to our earlier work.[10-11] This study discusses the structural activity relationships to connect the substituent effects and the activities that help in medication creation. Traditional medicines are made from these plant extracts, and some of them, including mansonone D, Dunnione, and others, are also essential biologically active substances.[12] In nature, naphthofurans are neither fused nor linked with nitrogen heterocycles. Except for a few reports of such compounds from our lab, the synthesis of naphthofurans fused or coupled with nitrogen heterocycles has not been documented. Since the discovery of penicillin, azetidinone-based

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heterocycles have been a significant class of medications with a broad range of therapeutic activity.[13] Additionally, heterocycles containing quionolines moieties continue to have a wide range of biological duties. In light of these findings, we investigate the viability and effectiveness of a method for synthesizing naphthofuran in combination with quinolines and an azetidinone nucleus[14], which turns out to have strong antibacterial properties. The hydroxamic acids contain an ester with a hydroxyl amine group is significant in a number of industries. According to a literature review, several hydroxamic acids and their derivatives have a wide range of uses in the biological and pharmaceutical fields.[15] Our present study describes how to synthesize various derivatives of naphthofurans and evaluate their antibacterial properties in accordance with the approach provided below, taking into account the various biological activities of heterocyclic compounds that comprise the naphthofuran entity. By using elemental analysis, UV-visible, Fourier transform infrared radiation (FT-IR), nuclear magnetic resonance (NMR), and mass spectral examinations, the derivatives of the title compounds were confirmed. The confirmed compounds were used for several G+ve and G-ve antibacterial activity against *E.Faecalis, S.auerus, E. Coli,* and *S. Pneumoniae* with Standards for use in antibacterial was *chloramphenicol*, the zone of inhibition of the compounds were compared to the reference medication. It shows excellent results of two G+ve (*E. Faecalis,* and *S. auerus*) and two G-ve (*E. coli* and *S. Pneumoniae*) antibacterial activity. The structural morphology of the compound was done by the molecular docking studies which showed an excellent result.

EXPERIMENTAL

Chemicals and Instruments

Sigma Aldrich is where all of the chemicals and reagents were bought. Every one of the necessary components is prepared using double distilled water. Using an FTIR Frontier Perkin Elmer instrument, FTIR spectra in the 4000-300 cm-1 region were used to examine the functional groups of the synthesized compounds. The Agilent VNMRS-400 NMR instruments were utilized to detect the 1H-NMR spectrum. Using Water's SYNAPT G2 QTOF LCMS instruments, the molecular weight of the molecule was determined.

Synthesis of ethyl 5-nitronaphtho[2,1-b]furan-2-carboxylate 2

Compound 2 was synthesized by the reaction of ethyl naphtho[2,1-b]furan-2-carboxylate 1 (1.75 g, 0.02 M) is mixed with glacial acetic acid (30 mL) at 0-6 ^{0}C, then to this added the cooled mixture of conc. HNO_3 and conc. H_2SO_4 (1:2, 40 mL) dropwise with constant stirring for about 4-5 h for about 40 min. Then obtained product was transferred into ice-cold water, and the solid yellow-colored compound was obtained. It was filtered and recrystallized from ethanol to get the required pure compound 2.

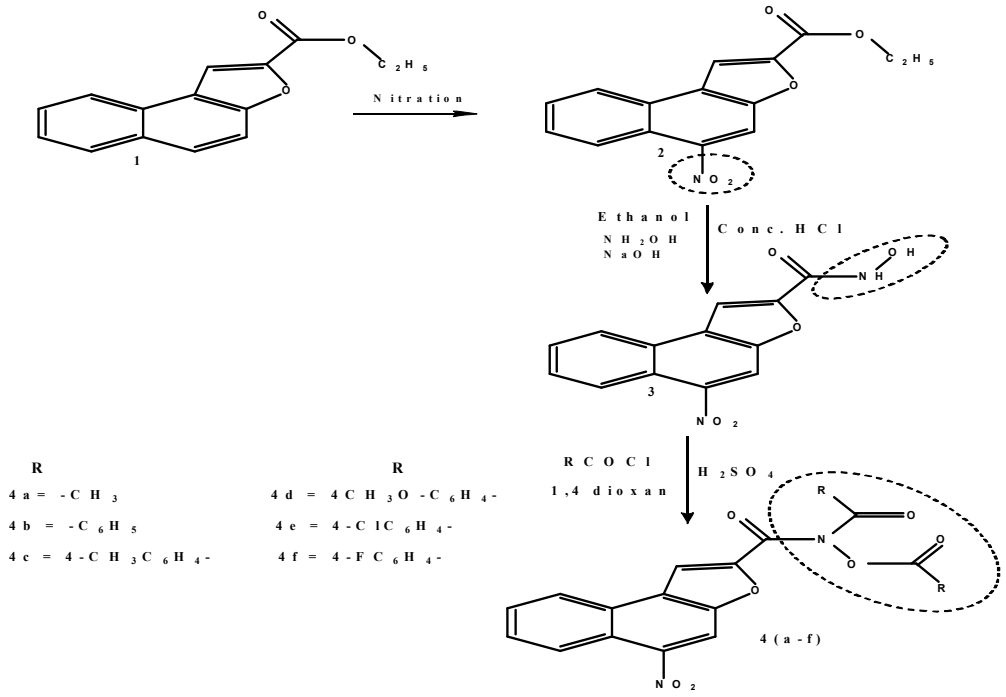
Synthesis of *N*-hydroxy-5-nitronaphtho[2,1-b]furan-2-carboxamide 3

The compound ethyl 5-nitronaphtho[2,1-b]furan-2-carboxylate 2 (2 g) was dissolved in 85 mL of ethanol. To this added hydroxyl amine hydrochloride (1.2 g) and sodium hydroxide (1.8 g). Then this reaction mixture was refluxed in a water bath for about 4 hours and then poured into cold water. The solution was filtered and followed by acidification with conc. HCl to get a white precipitate of N-hydroxy-5-nitronaphtho[2,1-b]furan-2-carboxamide 3. This product was filtered, washed with distilled water, and dried and it was purified using ethanol. Yield: 90.2%; mp: 198 °C. FT-IR (KBr, cm^{-1}) 1697 (-C=O), 3054 (-OH), 2729 (-NH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.37 (s, 1H, –NH), δ 13.55 (s, 1H, –OH), δ 7.5-8.43 (aromatic proton). MS (m/z): 272.69.

Synthesis of N-formyl-N-(formyloxy)-5-nitronaphtho[2,1-b]furan-2-carboxamides derivatives 4(a-f)

N-hydroxy-5-nitronaphtho[2,1-b]furan-2-carboxamide 3 (0.227 g; 0.01 M) was dissolved in dioxane (5 mL). To this acetyl chloride (0.05 M) and a catalytic amount of conc. H_2SO_4 was added. This reaction mixture was stirred at room temperature till the reaction was completed. Then the reaction mixture was poured into ice water to get N-formyl-N-(formyloxy)-5-nitronaphtho[2,1-b]furan-2-carboxamides 4a. The completion of the reaction was confirmed by TLC. The pure product was extracted with hexane. The remaining compounds in this series 4(b-f) were synthesized by following the same procedure as in the synthesis of compound 4a. Yield recorded 51.4%; mp: 76 °C. FT-IR (KBr, cm^{-1}) 1803, 1729 and 1679 (-C=O). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.4 and at δ 2.7 (s, 3H, -CH_3), δ 7.8-8.3 (aromatic proton). MS

(m/z): 357.12. 4b Yield: 53.7%; mp: 158 °C. FT-IR (KBr, cm^{-1}) 1802, 1728 and 1677 (-C=O)[16-17]. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.81 (t, 1H), δ 8.35 (s, 1H), δ 7.54-8.97 (aromatic proton). MS (m/z): 480.09. 4c and 4d Yield: 76-77%; mp: 162 and 170 °C. FT-IR (KBr, cm^{-1}) 1800, 1719 and 1669 (-C=O). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.42 (s, 3H, $-CH_3$), δ 8.35 (s, 1H), δ 7.31-8.97 (aromatic proton). MS (m/z): 508.12 & 540.11. 4e and 4f Yield: 81-90%; mp: 180 and 175 °C. FT-IR (KBr, cm^{-1}) 1800, 1720 and 1670 (-C=O). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H,), δ 7.56-8.97 (aromatic proton). MS (m/z): 548.01 & 516.07.



Scheme-1: Synthetic Route of Naphthofuran Derivatives

RESULTS AND DISCUSSION

Spectral Characterization

The structure of N-hydroxy-5-nitronaphtho[2,1-b]furan-2-carboxamide 3 was confirmed by following IR, NMR, and Mass spectral studies. The IR spectrum of 3 showed an absorption band due to a -OH bond at a stretching frequency of 3054 cm^{-1} and an absorption band due to a -NH bond at a stretching frequency of 2729 cm^{-1} (Fig.-1). The ^{1}H-NMR ($CDCl_3$) spectrum showed a singlet with very weak intensity at δ 3.37 integrated for –NH proton, another singlet with very weak intensity at δ 13.55 integrated for -OH proton, and a multiplet at δ 7.5-8.43 integrated for six aromatic protons (Fig.-2). The mass spectrum of 3 showed a molecular ion peak at m/z = 272.69 which further confirmed the structure of 3 (Fig.-3). The structure of 4a was confirmed by IR, NMR, and Mass spectral studies. The IR spectrum of 4a exhibited three sharp absorption bands at 1803, 1729, and 1679 cm^{-1} due to three C=O groups (Fig.-4). The ^{1}H-NMR ($CDCl_3$) spectrum exhibited two singlets at δ 2.4 and at δ 2.7 integrated for two $-CH_3$ protons of two acetyl groups, and a multiplet at δ 7.8-8.3 integrated for six aromatic protons (Fig.-5). The mass spectrum of 4a showed an (M+1) peak at m/z =357.12 which further confirmed the structure of 4a (Fig.-6). 4b Yield: 53.7%; mp: 158 °C. FT-IR (KBr, cm^{-1}) 1802, 1728 and 1677 (-C=O). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.81 (t, 1H), δ 8.35 (s, 1H), δ 7.54-8.97 (aromatic proton). MS (m/z): 480.09. 4c and 4d Yield: 76-77%; mp: 162 and 170 °C. FT-IR (KBr, cm^{-1}) 1800, 1719 and 1669 (-C=O). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.42 (s, 3H, $-CH_3$), δ 8.35 (s, 1H), δ 7.31-8.97 (aromatic proton). MS (m/z): 508.12 & 540.11. 4e and 4f Yield: 81-90%; mp: 180 and 175 °C. FT-IR (KBr, cm^{-1}) 1800, 1720 and 1670 (-C=O). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H,), δ 7.56-8.97 (aromatic proton). MS (m/z): 548.01 & 516.07. The Elemental analysis of the synthesized compounds shows a formation of compound Table-1.

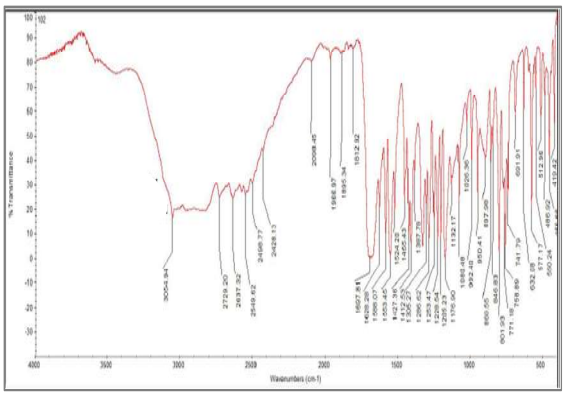


Fig.-1: FTIR Spectra of Compound 3

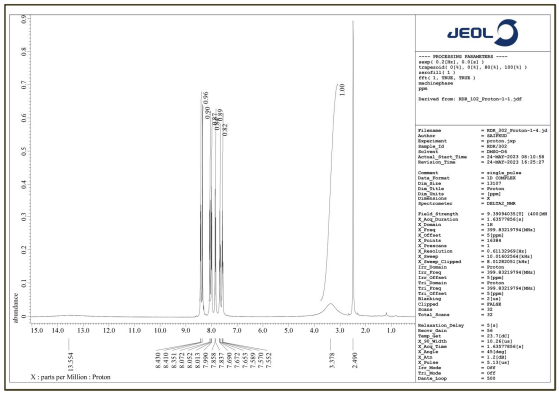


Fig.-2: NMR Spectra of Compound 3

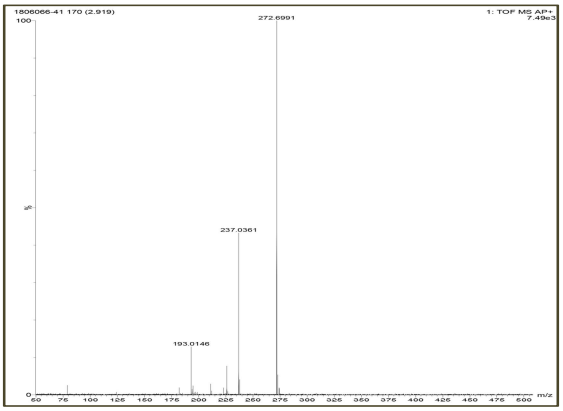


Fig.-3: Mass Spectra of Compound 3

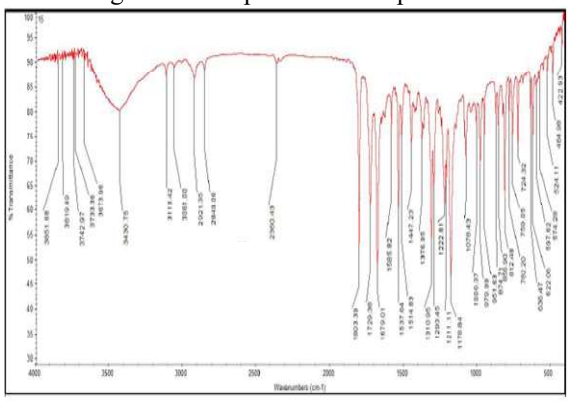


Fig.-4: FTIR Spectra of Compound 4a

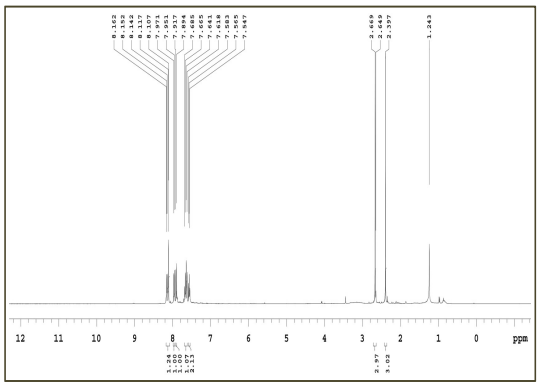


Fig.-5: NMR Spectra of Compound 4a

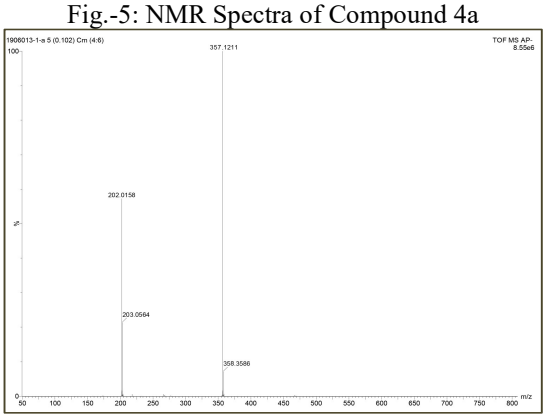


Fig.-6: Mass Spectra of Compound 4a

Table-1: Elemental Data of Newly Synthesized Compounds

Comp.	R	M.P. ^{0}C	Yield (%)	Mol. formula	Clad (Found)%				
					C	H	N	O	Halogen
2				$C_{15}H_{11}NO_5$	63.16 (63.14)	3.89 (3.85)	4.89 (4.85)	28.04 (28.00)	-
3		198	90.2	$C_{13}H_8N_2O_5$	57.36 (57.33)	2.29 (2.25)	10.29 (10.25)	29.39 (29.38)	-
4a	-CH_3	76	51.4	$C_{17}H_{12}N_2O_7$	57.31 (57.30)	3.39 (3.37)	7.86 (7.84)	31.43 (31.40)	-
4b	C_6H_5-	158	53.7	$C_{27}H_{16}N_2O_7$	67.50 (67.47)	3.36 (3.34)	5.83 (5.80)	23.31 (23.30)	-
4c	4-CH_3-C_6H_5	162	77.5	$C_{29}H_{22}N_2O_7$	68.23 (68.20)	4.34 (4.30)	5.49 (5.45)	21.94 (21.90)	-
4d	4-CH_3O-C_6H_5	170	76.2	$C_{29}H_{20}N_2O_9$	64.45 (64.40)	3.73 (3.70)	5.18 (5.16)	26.64 (26.61)	-
4e	4-Cl-C_6H_5	180	90.2	$C_{27}H_{14}C_{12}N_2O_7$	59.04 (59.01)	2.57 (2.55)	5.10 (5.08)	20.39 (20.36)	Cl-12.91 (12.90)
4f	4-F-C_6H_5	178	81.8	$C_{27}H_{14}F_2N_2O_7$	62.80 (62.78)	2.73 (2.70)	5.42 (5.40)	21.69 (21.67)	F-7.36 (7.35)

Biological Activity of Synthesized Compounds

Antibacterial Activity

Using cultures of six bacteria and two fungi that had been cultured for twenty-four hours, the in vitro antibacterial activity was tested using the cup-plate method.[18] The compounds 4a-f have been determined for their antibacterial activity against *S. aureus, E.Faecalis S. Pneumoniae, and E.coli*. The standard for use in antibacterial was chloramphenicol. The compounds were evaluated at varying concentrations of 25, 50, 75, and 100 µg/ml versus all species in DMF. The zone of inhibition was compared to the reference

medicine during a 24-hour incubation period at 25 ^{0} C for antibacterial activity. The results are displayed in Table-2. The antibacterial activity was evaluated by measuring the zone of inhibition versus the test organisms while comparing it to that of the employed standard reference. Table-2 illustrates the inhibitory zones that were identified. As seen in Fig.-7, this resulted in a good zone of inhibition in the primary screening process against the bacterial strains. It was discovered that the presence of electron-withdrawing groups attached to the naphthofuran ring displayed strong effects on the antibacterial activity. In this regard, among all the compounds in this series that were examined, compounds 4c and 4e showed the best antibacterial activity due to the inclusion of nitro and chloro substituents in their structures.

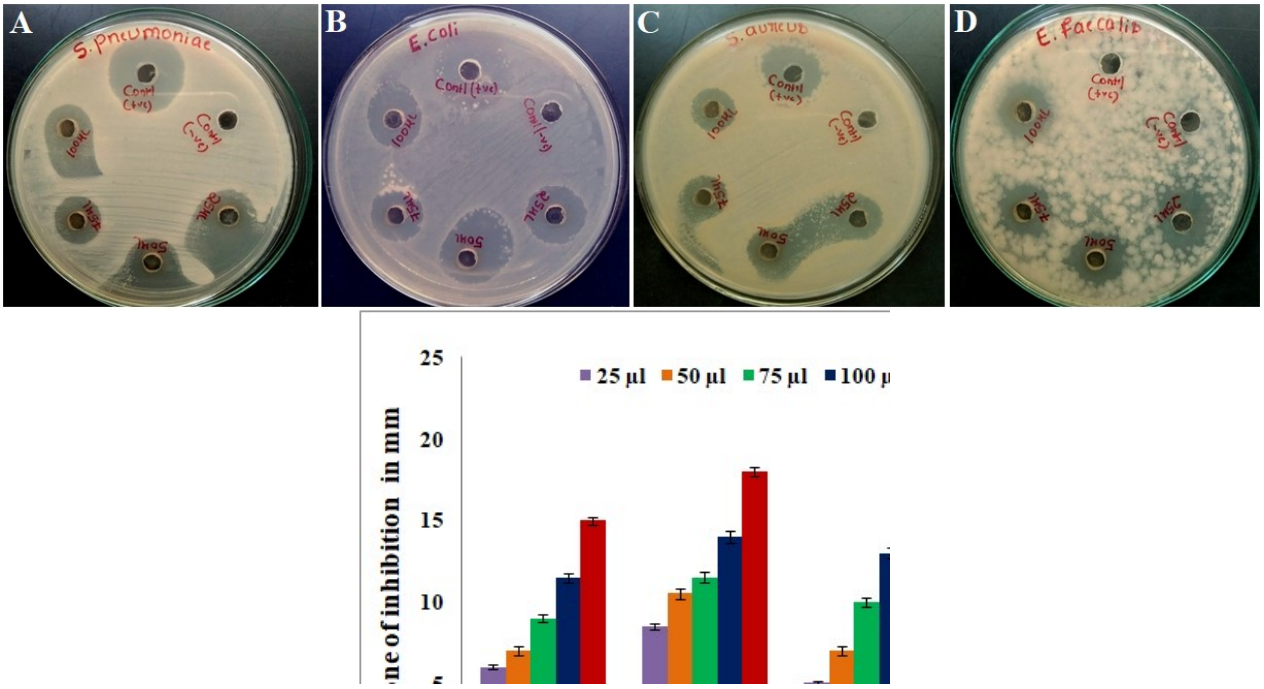


Fig.-7: Antibacterial Activity of Different Concentrations of Compound 4a. S. aureus, E. Faecalis S. Pneumoniae, and E. coli Representation of the Antibacterial Activity of Inhibition Zones of Compound 4a Against Tested Pathogens

Table-2: Antibacterial Activity of Compounds 4a-f

Compound	Concentration in *µg/ml*	Growth inhibition against *bacteria in mm*			
		E.Faecalis	*S.auerus*	*E. Coli*	*S. Pneumoniae*
4a	25	06.26±0.23	05.68±0.26	08.48±0.21	06.26±0.14
	50	09.21±0.01	07.29±0.27	10.57±0.06	07.26±0.13
	75	12.85±0.14	13.85±0.03	14.78±0.02	12.32±0.15
	100	17.23±0.12	18.26±0.21	17.66±0.01	15.24±0.13
4b	25	07.84±0.25	08.38±0.46	09.28±0.10	06.22±0.33
	50	16.92±0.08	17.26±0.27	14.88±0.05	15.23±0.13
	75	14.44±0.25	15.18±0.26	15.48±0.20	15.12±0.13
	100	17.59±0.13	21.13±0.23	20.56±0.00	21.22±0.13
4c	25	06.23±0.15	07.13±0.26	07.18±0.23	05.38±0.12
	50	20.46±0.08	19.35±0.30	20.48±0.05	20.26±0.22
	75	16.43±0.05	17.83±0.16	17.99±0.16	15.58±0.22
	100	24.46±0.32	23.48±0.35	28.45±0.02	24.15±0.22
4d	25	06.22±0.14	09.17±0.15	09.83±0.35	08.16±0.21
	50	16.25±0.16	14.48±0.15	15.84±0.18	15.27±0.15

	75	11.26±0.13	12.87±0.35	12.86±0.25	12.26±0.26
	100	21.15±0.58	22.77±0.26	22.43±0.14	19.45±0.11
4e	25	06.85±0.24	06.45±0.13	08.18±0.13	08.39±0.23
	50	18.55±0.55	17.24±0.01	19.54±0.07	19.21±0.16
	75	16.85±0.14	16.85±0.03	15.78±0.02	15.32±0.15
	100	25.25±0.13	24.12±0.06	25.21±0.14	23.22±0.07
4f	25	08.18±0.16	06.50±0.34	09.23±0.12	07.33±0.32
	50	18.45±0.23	17.51±0.09	17.24±0.20	19.22±0.13
	75	18.78±0.26	16.90±0.12	12.25±0.02	15.23±0.22
	100	22.53±0.19	21.52±0.05	21.82±0.03	20.25±0.15
Control	100	-	-	-	-
Std	100	28.15±0.02	26.04±0.32	26.06±0.44	24.01±0.02

Molecular Docking Studies

The described methodology was ultimately used to finish the docking of molecules examinations.[19-20] The Protein Data Bank (PDB: http://www.rcsb.org/pdb) crystal structure of the antitubercular receptor (PDB code: 2MBR) was used to perform in-silico molecular docking. Prior to screening, heteroatoms and water molecules had been ignored. Prior to being employed in the docking analysis, the receptor structure was constructed using the HEX modeling program 8.0's protein preparations module. All hetero and water molecules from the crystal structure were removed during protein production, with the exception of the water molecules that were 5Å away from the ligand. The three-dimensional configurations of each ligand coupled with the receptor binding interactions were visualized to maximize quality using Discovery Studio 3.2. At the active binding areas of the receptor, every molecule was docked. The results of the in silico molecular docking procedure provide important information about the newly created medicines' possible affinity for the receptor's active areas. We employed the derived docking values to direct our dry study of the antimicrobial activity. Through Vander walls, hydrogen bonds, pi-alkyl alkyl, and other interactions with a variety of receptor amino acids, all derivatives demonstrated better binding interactions with receptors close by, potentially inhibiting the receptor's ability to cause bacterial infections. By using the key of amino acid residues, all of the complexes' binding energies demonstrated strong binding interactions with the *E. coli* murbenzyme receptor. LEU104, LEU178, ARG327, TRP89, VAL132, PHE328, VAL52, LEU53, VAL326, ASN51, GLN120, GLU325, GLU334, LEU107, ILE122, VAL326, and ILE110 are some key of amino acid residues. The hydrophobic and hydrophilic spheres are utilized to identify the interaction locations that will be predicted ligand binding sites in each conceivable location. In the end, the molecular docking investigations for the chosen compounds demonstrated that the compounds that were generated are antimicrobial competitive inhibitors in contrast to antimicrobial murbenzyme receptor binding receptors. Following Fig.-8 to Fig.-13 demonstrate the bonding interactions.

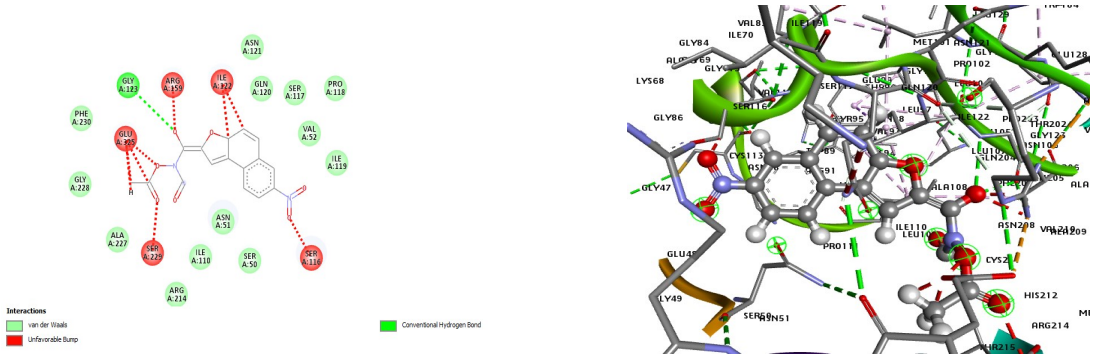


Fig.-8: 2D and 3D Bonding Interactions of 2MBR Receptor with Compound 4a

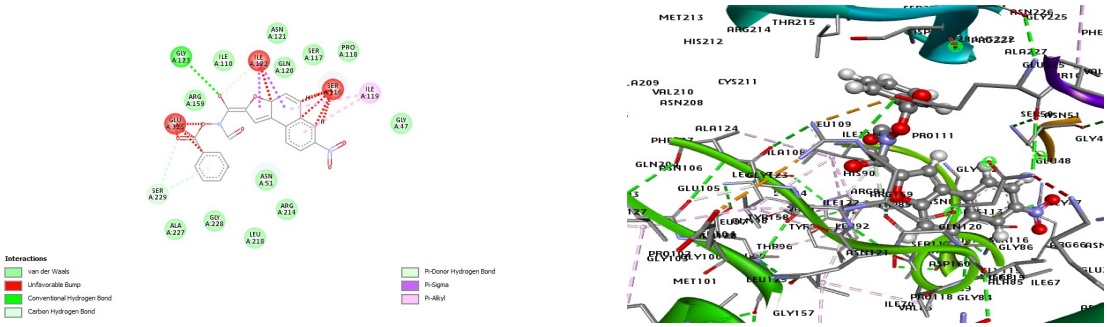


Fig.-9: 2D and 3D Bonding Interactions of 2MBR Receptor with Compound 4b

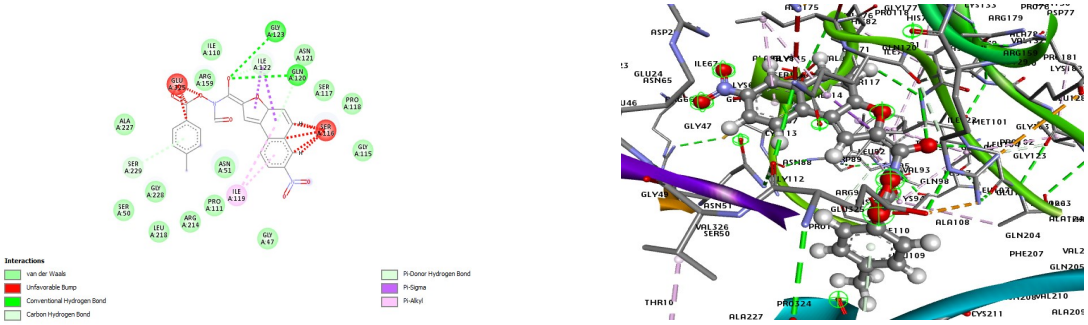


Fig.-10: 2D and 3D Bonding Interactions of 2MBR Receptor with Compound 4c

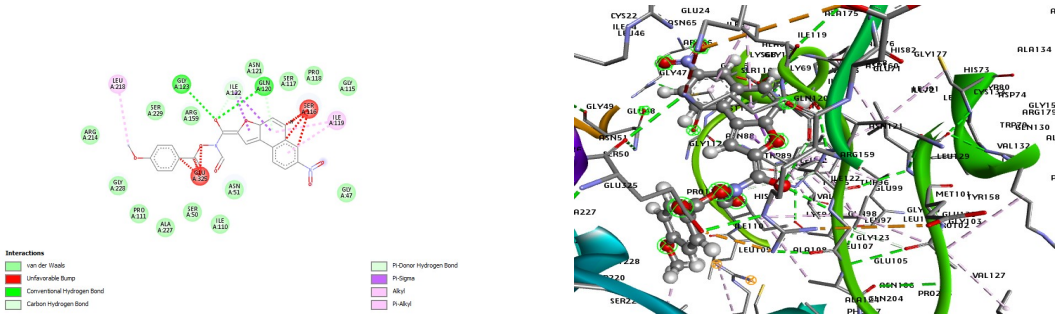


Fig.-11: 2D and 3D Bonding Interactions of 2MBR Receptor with Compound 4d

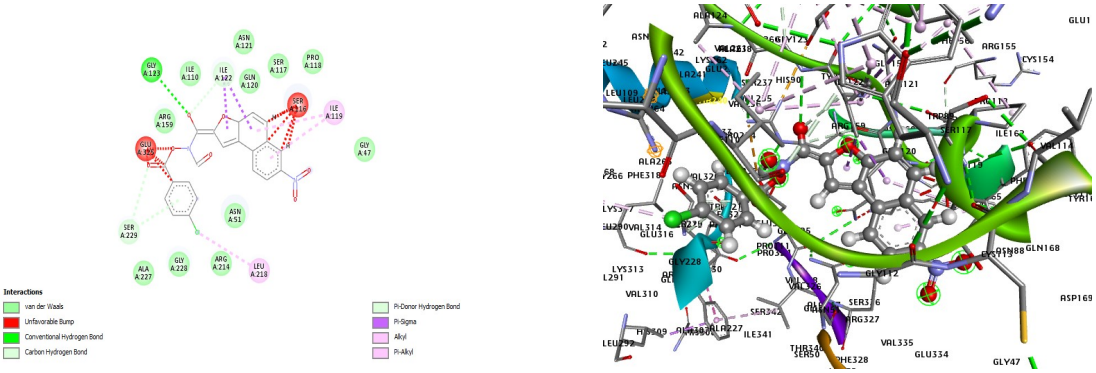


Fig.-12: 2D and 3D Bonding Interactions of 2MBR Receptor with Compound 4e

In order to identify possible biological targets for the antibacterial receptor, the synthetic molecules 4(a-f) performed molecular docking (2MBR: PDB ID). The binding energies of the synthesized compounds 4(a-f) with respective receptors were identified using computational docking studies with the HEX 8.0 engine. These docking energy levels and probable binding interaction types are listed in Table-3. Antibacterial docking results indicated that 4b, 4e, and 4f could interact. The 4c has a higher affinity (-330.29 kcal mol^{-1}) than the other drugs for the 2MBR receptor. The amino acids 4a, 4d, and 4e interact with other amino

acids in the protein that surrounds the receptor molecule in hydrophobic (Pi-alkyl), alkyl-alkyl, and hydrogen bonding ways. The optimal docking positions of receptors 4(a–f) and 2MBR are listed in Fig.-8 to Fig.-13, as reported in Table-3.

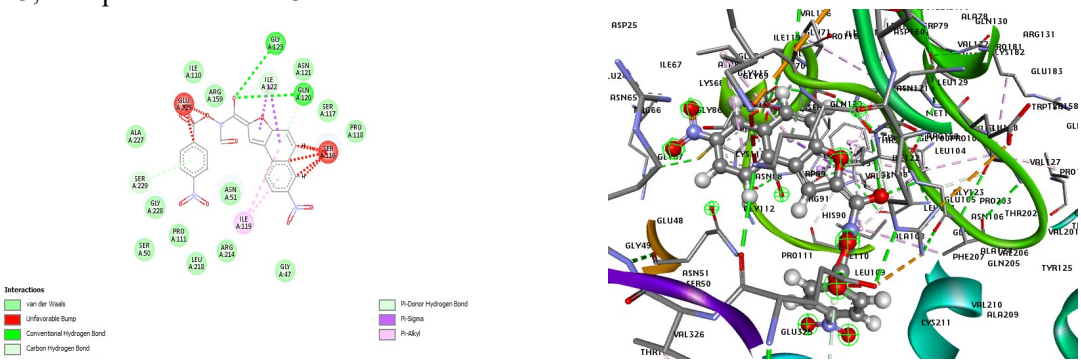


Fig.-13: 2D and 3D Bonding Interactions of 2MBR Receptor with Compound 4f

Table-3: Binding Energies of Compound 4(a–f) with 2MBR Receptor

Entry	Receptor PDB code	ΔG (Kcal/mol) With MurB
4a	2MBR	-300.39
4b	2MBR	-312.46
4c	2MBR	-330.29
4d	2MBR	-307.01
4e	2MBR	-325.39
4f	2MBR	-319.06
Ciprofloxacin (STD)	2MBR	-237.66

CONCLUSION

In our work we have successfully confirmed the Synthesized compounds of N-formyl-N-(formyloxy)-5-nitronaphtho[2,1-b]furan-2-carboxamides derivatives 4(a-f), ethyl naphtho[2,1-b]furan-2-carboxylate 1, ethyl 5-nitronaphtho[2,1-b]furan-2-carboxamide 2, N-hydroxy-5-nitronaphtho[2,1-b]furan-2-carboxamide 3. The structural confirmation of the synthesized compounds was done by several analytical technique methods such as FTIR, 1H-NMR, Mass, and Elemental analysis. The confirmed compounds were used for several G+ve and G-ve antibacterial activity against *E.Faecalis, S.auerus*, *E. Coli*, and *S. Pneumoniae* with Standards for use in antibacterial was *chloramphenicol,* the zone of inhibition of the compounds were compared to the reference medication. It shows excellent results of two G+ve (*E. Faecalis, and S. auerus*) and two G-ve (*E. coli and S. Pneumoniae*) antibacterial activity. The structural morphology of the compound was done by the molecular docking studies which showed an excellent result.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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