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Original Article

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One pot synthesis and docking study of some tetrahydrobenzo[b]pyran derivatives as extended spectrum class lactamase inhibitors for urinary tract infection

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ABSTRAC

Objective: The objective of the present investigation was to develop extended spectrum class lactamase inhibitors for urinary tract infection. **Methods:** With this aim a series of tetrahydrobenzo[b] pyran derivatives were synthesized by one pot strategy and screened for the antimicrobial activity against Proteus vulgaris. The docking and pharmacophore identification were carried out using Vlife MDS 4.3. **Results:** Structures of newly synthesized compounds were confirmed by physicochemical, spectral, and elemental analysis. The 4 chloro and 4 hydroxy derivatives are more active than other derivatives. The docking analysis showed that the all the inhibitors are showing the activity by blocking extended spectrum class lactamase. The pharmacophore identification showed the presence of negative ionizable, hydrogen bond donor, hydrogen bond acceptor, and aliphatic are important features required for extended spectrum class lactamase inhibition. **Conclusions:** Synthesized tetrahydrobenzo[b]pyran molecules were found to having moderate to good antimicrobial activity. Docking analysis shows that the possible mode of action of these synthesised derivatives is through lactamase inhibition. Molecules having chloro group 2-amino-3-carbonitrile-4- (2-chlorophenyl)-5-oxo-5,6,7,8 tetrahydro-4H-pyran were found to be most active.

INTRODUCTION

Proteus vulgaris is a rod-shaped, gram-negative bacterium major causative organism in humans, especially in urinary tract infection and wound infection. The pathogenicity of this organism is associated with the presence of enzyme extended-spectrum class lactamase which is responsible for the inactivation of β-lactum antibiotics such as penicillin and cephalosporin [1-5]. Nowadays, increase in antibiotic resistance is a major tricky worldwide and therefore theire is need to develop potent, selective inhibitor with fewer side effects [6]. Benzopyran derivatives are known for various pharmacological activities such as anticoagulant, antibacterial, antifungal, and vascular smooth muscle relaxants [7-9]. Emergence of computational chemistry rapidly increased specificity and speed of drug discovery [10]. Virtual screening is utilized to study the ligand-receptor binding study, which is useful in establishing the mechanism of action of designed drugs. Pharmacophore is nothing but the three dimensional description of molecular features such as hydrogen bond donor, hydrogen bond acceptor, hydrophobic, aliphatic, negative ionizable, positive ionizable which are responsible for the activity. Here, we report one pot synthesis, docking, and pharmacophore identification of series of tetrahydrobenzo[b]pyran derivatives as antimicrobials.

MATERIALS AND METHODS

All aryl aldehydes, malononitrile, and dimedone, were purchased from S. D. FINE, SPECTROCHEM Co. and were used without further purification.

NMR analysis was performed on Brucker–Avance 300 MHz, NMR spectrophotometer. For ^{1}H NMR analysis, DMSO was used as solvent and tetramethylsilane as an internal standard; Infrared spectra were recorded on Perkin Elmer 1310 FT-IR spectrometer with KBr pellets. Liquid chromatography–mass spectrometry analysis was performed on mass spectrometer – API 5500Qtrap (Applied biosystems, Canada). The column used for analysis were, Atlantis dC18 (100 mm × 2 mm × 5 um) Waters India Pvt Ltd., Bengaluru. The mobile phase used for sample is: 5 mM ammonium formate in methanol 5 mM ammonium formate in water and flow rate was 0.4 mL/min.

Synthesis of Tetrahydrobenzo[b]pyran

A solution of aromatic aldehyde (1 mmol), malononitrile (1 mmol) and dimedone (1 mmol), and CuI (37 mg, 20 mol%) in 5 mL ethanol + water (30:70 v/v) was stirred at room temperature. The product tetrahydrobenzo[b]pyran was separated and purified by recrystallization in ethanol (Figure 1).

Computational Analysis

Ligand preparation [10,11]

The structure of 3-amino-6,6-dimethyl-8-oxo-1-phenyl-5,6,7,8-tetrahydronaphthalene-2-carbonitrile was used as the template to built the molecules in the dataset in builder module of Vlife MDS 4.2. The ligands were optimized by energy minimization using Merck molecular force field till a gradient of 0.001 kcal/mol/Å was reached, maintaining the template structure rigid during the minimization.

Docking studies

Docking simulations were carried out using biopredicta module of Vlife MDS 4.2 using crystal structure of extended spectrum class lactamase (PDB ID 1HZO) downloaded from rcsb.org.

Antimicrobial screening

15-20 mL of molten agar was poured into each sterile plate. The plates are inoculated with the help of sterile loop using spread plate technique. The incubated plates were allowed to stand for 3 to 15 min before applying paper disc. Using sterile forceps, the paper absorbent disc of 6 mm diameter coated with respective chemical was placed on the surface of plate. The plates were incubated at temperature of 37°C. For comparison, the DMF solvent as control was run under similar condition to know activity of blank. The zone of inhibition if any developed, was measured for *P. vulgaris* for all compounds under study.

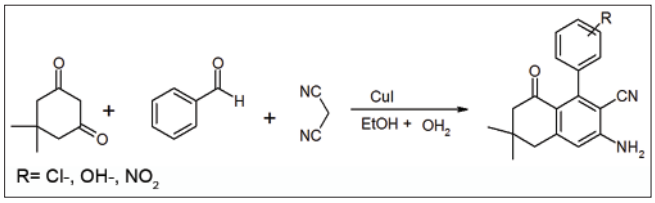


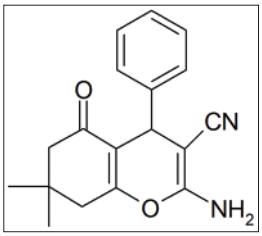
Figure 1: Scheme of synthesis of tetrahydrobenzo[b]pyran

Pharmacophore Modeling

Pharmacophore modeling was also carried out in Vlife MDS 4.2 using Mol sign module. The minimum number of pharmacophore features generated for an alignment is taken 4 and tolerance is kept to 10 Å [10,11]. Synthesis of tetrahydrobenzo[b]pyran.

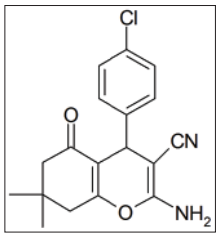
Characterization of Synthesized Compounds

The IR spectrum of the compound exhibited stretching frequencies (spectrum 2.1) at 3412, 3334, 2199, 1693/cm indicating the presence of -NH_2, -CN and C=O functionalities, respectively. The ^{1}H NMR spectrum of same compound (Spectrum 2.2) displayed a singlet at δ 4.1 due to C4-H and a broad singlet at δ 7.1 for NH_2 protons which confirmed the structure of targeted compound. The spectral and physical data of the compound was found to be in agreement with the literature data. Encouraged by this result, we planned to diversify the methodology by varying the substitution at C-4 position of benzopyran. The above methodology was extended to the use of aromatic aldehydes carrying either electron donating or withdrawing substituents gives high yield of the product with good purity.



2-amino-3-cyano-7,7-dimethyl-5-oxo-5,6,7,8 tetrahydro 4H-benzopyran

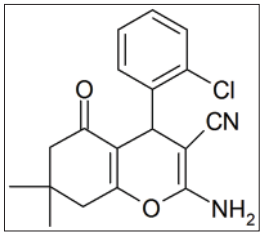
Yield of 88% with melting point 227-230°C, IR (KBr disc) 3396, 3324, 12199, 1686/cm. The ^{1}H NMR (300 MHz $CDCl_3$) δ (ppm): 0.938 (s, 3H), 1.02 (s, 3H), 2.1 (d, J=15.9 2H), 2.49 (s, 2H), 3.84 (s, 1H) pyran, 7.00 (s, 2H), 7.106-7.29 (m, 5H) (spectrum 2.2). The ^{13}C NMR (300 MHz $CDCl_3$) δ (ppm): 27.24, 28.83, 32.25, 36.01, 46.98, 50.42, 58.77, 62.07, 113.17, 120.16, 127.02, 127.58, 128.78, 145.18, 158.93, 162.96, 196.14, and MS: M+1 = 295.



2-amino-3-carbonitrile-4(4-chlorophenyl)-5-oxo-5,6,7,8 tetrahydro-4H-pyran

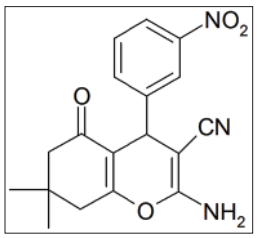
Yield of 92% with melting point 205-207°C, IR (KBr disc) 3373, 3311, 2193, 1649/cm, ^{1}H NMR (300 MHz) δ: 0.928 (s, 3H), 1.016 (s, 3H), 2.109 (s, 2H), 2.263 (s, 2H) for methylene, 4.175 (s, 1H) pyran, 7.075 (s, 2H) primary amine, 7.166 (d, 2H), and 7.349 (d, 2H) J=8.4Hz. ^{13}C NMR (300 MHz) δ: 27.29, 28.75, 32.25, 35.54, 46.99, 50.37, 58.20, 112.75,

120.01, 128.74, 129.56, 131.55, 144.19, 158.93, 163.09, 196.17, MS: M+1 = 329.



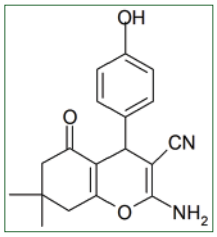
2-amino-3-carbonitrile-4-(2-chlorophenyl)-5-oxo-5,6,7,8 tetrahydro- 4H-pyran

Yield of 85% with melting point 222-224°C, IR (KBr disc) 3380, 3327, 2197, 1681/cm, ^{1}H NMR (300 MHz) δ: 0.968 (s, 3H), 1.030 (s, 3H), 2.070 (s, 2H), 2.264 (s, 2H), 7.014 (s, 2H) amino group, 4.015 (s, 1H), 7.13-7.36 (m, 4H), ^{13}C NMR (300 MHz) δ: 27.31, 28.85, 32.21, 33.28, 45.77, 46.99, 47.34, 50.37, 57.25, 62.07, 112.21, 119.70, 127.89, 128.67, 129.90, 130.40, 132.52, 142, 159.12, 163.62, 196.04, MS: M+1 = 328.



2-amino-3-carbonitrile-4-(3-nitrophenyl)-5-oxo-5,6,7,8 tetrahydro-4H-pyran

Yield of 85% with melting point 200-201°C, IR (KBr disc) 3430, 3334, 2186, 1530/cm, ^{1}H NMR (300 MHz) δ: 0.943 (s, 3H), 1.030 (s, 3H), 2.125 (s, 2H), 2.285 (s, 2H), 4.399 (s, 1H) pyran, 7.1 (s, 2H) primary amine, 7.579-8.084 (m, 4H), ^{13}C NMR (300 MHz) δ: 27.19, 28.77, 32.28, 35.86, 50.32, 57.66, 62.08, 112.23, 119.80, 122.09, 122.23, 130.48, 134.63, 147.44, 148.23, 159.09, 163.63, 196.34, MS: M+1=340.



2-amino-3-carbonitrile-4-(4-hydroxyphenyl-5-oxo-5,6,7,8 tetrahydro-4H-pyran

Yield of 85% with melting point 174-175207°C, IR (KBr disc) 3465, 3365, 3201, 2198, 1661 cm^{-1} (Spectrum 2.17), ^{1}H NMR (300 MHz) δ: 0.925 (s, 3H), 1.00 (s, 3H), 2.126 (s, 2H), 2.190 (s, 2H) methylene, 4.150 (s, 1H) pyran, 6.6 (s, 2H) (J=6.9 Hz), 6.9 (s, 2H) (J=7.2 Hz). Unfortunately, the exchangeable protons of amino group and phenolic OH group have not detected in the spectrum, ^{13}C NMR (300 MHz) δ: 27.52, 28.10, 28.84, 32.07, 34.78, 50.69, 114, 115, 128.45, 129.45, 156.10, 195.95, MS: M+1 = 309.

Antimicrobial Activity

All the compounds synthesized are screened for the antimicrobial activity against *P. vulgaris*. All the compounds showed significant activity. The 4 chloro and 4 hydroxy derivatives are more active than other derivatives, while 2-Amino-3-carbonitrile-4-(4-hydroxyphenyl-5-oxo-5,6,7,8 tetrahydro-4H-pyran and 2-Amino-3-carbonitrile-4-(4-chlorophenyl-5-oxo-5,6,7,8 tetrahydro-4H-pyran showed similar activity. 2-amino-3-cyano-7,7-dimethyl-5-oxo-5,6,7,8 tetrahydro 4H-benzopyran and 2-amino-3-carbonitrile-4-(3-nitrophenyl)-5- oxo-5,6,7,8 tetrahydro-4H-pyran these two derivatives are inactive as shown in Table 1.

Docking Study

To explore the mechanism of action of molecules under study, the docking simulations are carried out in Biopredicta

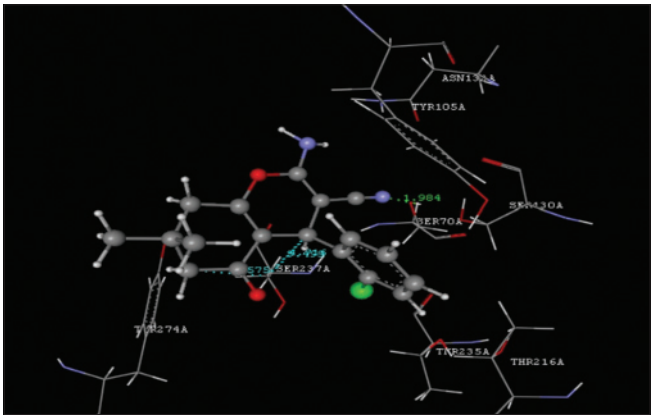


Figure 1: The binding interaction of synthesized derivative with extended spectrum class lactamase

Table 1: The antimicrobial activity of synthesized derivatives

Compound	Zone of inhibition (mm)
2-amino-3-cyano-7,7-dimethyl-5-oxo-5,6,7,8 terahydro 4H-benzopyran	-
2-amino-3-carbonitrile-4 (4-chlorophenyl)-5-oxo-5,6,7,8 tetrahydro-4H-pyran	15
2-amino-3-carbonitrile-4-(2-chlorophenyl)-5-oxo-5,6,7,8 tetrahydro-4H-pyran	14
2-amino-3-carbonitrile-4-(3-nitrophenyl)-5-oxo-5,6,7,8 tetrahydro-4H-pyran	-
2-amino-3-carbonitrile-4-(4-hydroxyphenyl-5-oxo-5,6,7,8 tetrahydro-4H-pyran	17
Penicillin	10
Ampicillin	16

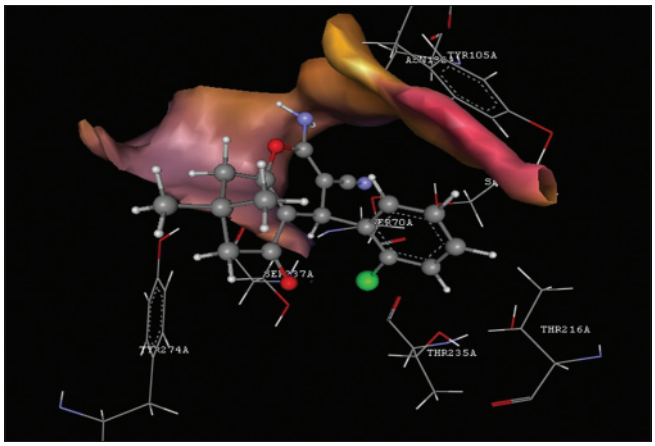


Figure 2: The binding conformation of synthesized derivative with extended spectrum class lactamase

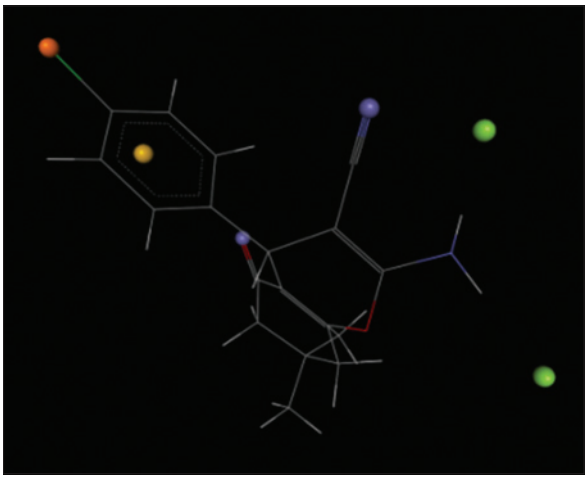


Figure 3: The pharmacophoric feature of synthesized derivatives

module of the v life MDS 4.2 using crystal structure of extended spectrum class lactamase (PDB ID 1HZO). All the compounds showed nice fit in the active site of extended spectrum class lactamase enzyme. The derivatives are founds to showing Hydrophobic interactions with ASP 163, ARG161, ARG 65, GLN 41, GLN 267, ARG 153, and hydrogen bond interactions with ALA 146, GLU 269. The vander wall interactions with ASP 163, ARG161, ARG 65, GLN 41, GLN 267, ARG 153, ALA 146, and GLU 269 as shown in Figures 2 and 3.

Pharmacophore Modeling Studies

To identify key structural features which are responsible for antibacterial activity of the tetrahydrobenzo[b]pyran derivatives the pharmacophore modeling is carried out in the mol sign module of v life MDS 4.2. The each of hypothesis showed features such as hydrogen bond donor, hydrogen bond acceptor, hydrophobic, aliphatic, positive ionizable are important for antibacterial activity of tetrahydrobenzo[b] pyran derivatives as shown is Figure 4.

CONCLUSION

Series of synthesized tetrahydrobenzo[b]pyran molecules were found to be having moderate to good antimicrobial activity. Docking analysis resulted their possible mode of action of these derivatives is through lactamase inhibition. Molecules substituted with chloro group 2-amino-3-carbonitrile-4-(2-chlorophenyl)-5-oxo-5,6,7,8 tetrahydro-4H-pyran were found to be most active. Docking analysis shows that all the molecules designed were best fited in active site of extended spectrum class lactamase (PDB ID 1HZO) insights gained from the study could be employed to design antimicrobial leads with enhanced potency, selectivity, and reduced toxicity. Development of potent antimicrobials for urinary tract infection are developed using multicomponent chemical reactions.

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