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RESEARCH ARTICLE

The QSAR study of pyridothienopyrimidine derivatives as antimicrobial activities against *pseudomonas aeruginosa*

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Abstract

Background: Antimicrobial resistance (AMR) cases have been widespread in the last decade. The discovery and development of new drugs need to be done to overcome this. **Objective:** This research aims to develop antimicrobial candidates; some research has found that some compounds with pyridothienopyrimidine derivatives can inhibit the growth of *pseudomonas aeruginosa.* **Methods:** The *in silico* approach method, along with the quantitative structure-activity relationship (QSAR) technique, plays an important role in discovering and developing new drugs. This study focused on developing pyridothienopyrimidine derivatives that are much more potent by making the best QSAR equation of 12 pyridothienopyrimidine derivatives tested *in vitro* for its antimicrobial activity against *pseudomonas aeruginosa.* **Results:** The best QSAR equation was obtained from pyridothienopyrimidine derivatives as antimicrobial activity *pseudomonas aeruginosa*, with pMIC=-0.102 (±1.418) Log S-1.017 (±0.370) E_{LUMO} -0.017 (±0.012) MR-3.544 (±1.418) (n=12; Sig = 0.001; R = 0.943; R^2 = 0.890; *F*= 21.558; Q^2=0.62). **Conclusions:** Increasing the antimicrobial activity of pyridothienopyrimidine derivatives against *pseudomonas aeruginosa* can be achieved by decreasing Log S, E_{LUMO}, and molar refractivity. The best QSAR equation can be a tool to obtain a more potential new chemical structure model and reduce trials and errors.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a gram-negative bacterium known as an opportunistic pathogen in humans (Qin *et al.*, 2022). *P. aeruginosa* ranks fourth among the causes of nosocomial infections, following *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus*, according to data from the China Antimicrobial Surveillance Network (Qin *et al.*, 2022). Several infections caused by *P. aeruginosa* include respiratory tract infections (COPD), urinary tract infections (UTI), gastrointestinal tract infections (GIT Infection), skin infections, burns, osteomyelitis, and sepsis (Tuon *et al.*, 2022). Infections attributed to *P. aeruginosa* have seen numerous cases of multi-drug resistance (MDR) (Qin *et al.*, 2022). Epidemiological data indicates that approximately 700,000 individuals succumb annually to MDR cases involving *P. aeruginosa* (Qin *et al.*, 2022).

P. aeruginosa can form biofilms as a defense mechanism against antibiotics employed by clinicians (Wahyudi *et al.*, 2019; Wahyunita *et al.*, 2021; Tuon *et al.*, 2022). Apart from biofilm formation, other mechanisms, such as the presence of flagella, pili, lipopolysaccharides, and quorum sensing, contribute to *P. aeruginosa's* development of MDR (Thi *et al.*, 2020;

Tuon *et al.,* 2022). The high prevalence of MDR cases in *P. aeruginosa* has prompted researchers to seek novel compounds that are more potent than existing therapeutic guidelines (Daikos *et al.*, 2021).

One potential compound that could be developed as an antibiotic for *P. aeruginosa* is the pyridothienopyrimidine derivatives (Ge Zayda *et al.*, 2020). In previous studies, 12 pyridothienopyrimidine derivative compounds were successfully synthesised, and their *in vitro* antimicrobial activity against *P. aeruginosa* showed promising results for further development (Ge Zayda *et al.*, 2020). The objective of this research is to explore pyridothienopyrimidine derivative compounds to discover derivatives with significantly greater potential using quantitative structure-activity relationship (QSAR) techniques.

Methods

QSAR procedure

The process of QSAR conducted in this study utilised ligand-based drug design (LBDD). The study involved three main steps to derive the most optimal QSAR equation. The first step involved the collection of molecular descriptor data from various software, such as Chembiodraw and SwissADME. The second step encompassed performing a multivariate analysis using the multiple linear regression (MLR) method, including the utilisation of the Pearson correlation. The identification of the best QSAR equation was accomplished through an internal validation method, taking into account parameters like r, R^2, F, sigma, and Q^2. The correlation coefficient (r) quantifies the degree of association between biological activity data from experimental observations and calculated results derived from regression analysis equations. A higher r value compared to the critical value in the r table indicates a stronger correlation between the experimental biological activity data and the calculated results. The coefficient of determination (R^2) represents the proportion of the variance in biological activity that can be explained by the physical-chemical properties or descriptors used in the analysis. The Fisher criterion (F) measures the significance of this relationship when compared to the critical *F* value. A higher F value suggests that the relationship defined by the regression equation is statistically significant and not due to random chance. Finally, the cross-validation coefficient (Q^2) assesses the predictive power of the QSAR equation in forecasting the bioactivity of compounds (Adeniji *et al.,* 2018; Putra *et al.,* 2023; Kesuma *et al.,* 2024). Subsequently, armed with the best QSAR equation, the study proceeded to formulate a new compound model with enhanced potential.

Collection of molecular descriptors

The 12 compounds were derived from pyridothienopyrimidine that have been synthesised and tested for their antibacterial activities against *P. aeruginosa* (ATCC-27853) (Ge Zayda *et al.*, 2020). These were drawn in 2D and 3D using the Chembiodraw application ver. 16.0, see Figure 1 below. The QSAR equation obtained from the minus logarithm of minimum inhibitory concentration (pMIC) that has been obtained from the antibacterial against *P. aeruginosa* (ATCC-27853) is equated with various descriptors obtained from the Chembiodraw and pkCSM applications. Descriptors used represent several Hansch QSAR parameters, which are grouped based on three parameters these are hydrophobic, electronic and steric (Hansch & Fujita, 1964; Kubinyi, 1993).

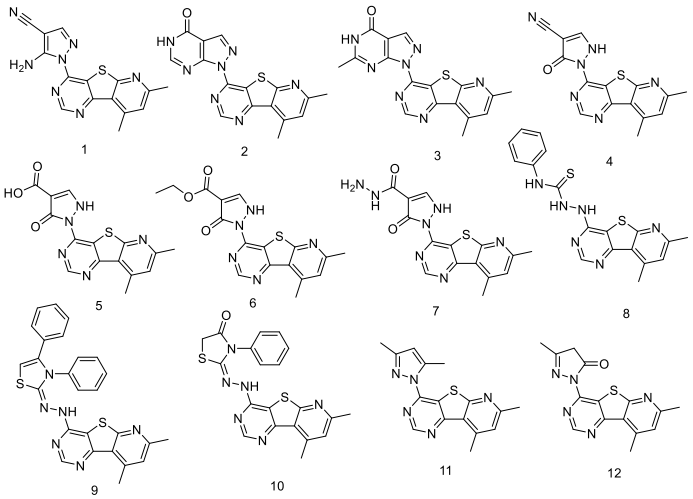


Figure 1: Pyridonthienopyrimidine derivatives

Multivariate analysis and validation of the QSAR equation

Pursuing the optimal equation model involved variables contingent upon the growth inhibition activity. All variables underwent analysis via the MLR method, commencing with an initial screening through the application of the Pearson correlation or matrix correlation method using SPSS software ver. 24.0. The results yielded QSAR equations and statistical parameters, including r, R^2, and Fischer criteria (F) values (Hardjono *et al.*, 2018).

The best QSAR equation is validated using several parameters, encompassing Fischer criteria (F), the difference between R^2 and Q^2 (R^2 - Q^2), a coefficient of determination (R^2) > *0.6*, a cross-validation coefficient or leave one out (Q^2) > *0.5*, and a difference between R^2 and Q^2 not exceeding 0.3 (Chirico & Gramatica, 2011; Adeniji *et al.*, 2018). These parameters are computed following the specified formulas below.

$$R^2 = 1 - \left[\frac{\sum(Y_{exp} - Y_{Pred})^2}{\sum(Y_{exp} - \bar{Y}_{training})^2}\right]$$

$$Q^2 = 1 - \left[\frac{\sum(Y_{pred} - Y_{exp})^2}{\sum(Y_{exp} - \bar{Y}_{training})^2}\right]$$

New models of chemical structure

The best QSAR equation obtained is used to find a better compound candidate by considering the pMICpred value. Synthesis was conducted, and an *in vitro* test was also redone on some compound candidates.

Results

Descriptor identification results of 12 pyridothienopyrimidine derivative compounds. The descriptor represents several Hansch QSAR parameters: hydrophobic, electronic and steric (Kubinyi, 1993; Todeschini & Consonni, 2009). Hydrophobic parameters are Log P, ClogP, Log S; electronic parameters are TPSA, E_{Homo}, E_{Lumo}; while steric parameters are molar refractivity (MR), molecular weight (Mr), and molecular volume (MV). The descriptor results of hydrophobic, electronic and steric parameters are shown in Table I.

Table I: Descriptors of hydrophobic, electronic and steric parameters of 12 pyridothienopyrimidine derivatives

Compound	Y-axis	X-axis								
	pMIC	Hydrophobic			Electronic			Steric		
		Log P	ClogP	Log S	TPSA	E_{HOMO}	E_{LUMO}	MR	Mr	MV
1	-2.097	3.29	1.53	-3.90	102.49	-8.64	-2.46	91.41	321.36	134.59
2	-2.000	2.88	0.78	-3.65	94.14	-8.48	-2.66	95.24	349.37	143.77
3	-2.398	3.00	1.28	-3.86	94.14	-8.53	-2.62	99.75	363.40	150.14
4	-1.875	2.82	1.15	-3.62	93.21	-8.78	-2.59	88.34	322.34	133.41
5	-2.097	2.34	1.55	-3.91	106.72	-8.79	-2.59	89.06	341.34	137.97
6	-2.000	2.94	2.22	-4.16	95.72	-8.79	-2.58	99.29	369.40	151.02
7	-2.000	1.44	-0.03	-3.66	124.54	-8.80	-2.56	95.87	355.37	144.04
8	-2.699	4.95	3.06	-5.85	73.17	-6.31	-2.04	110.93	380.49	159.22
9	-3.000	8.16	7.60	-9.29	64.71	-5.28	-1.85	140.87	480.61	203.48
10	-3.000	5.09	3.14	-6.05	81.78	-6.73	-1.97	116.62	420.51	174.43
11	-2.097	4.91	3.56	-4.21	52.68	-8.87	-2.64	91.11	309.39	131.22
12	-2.097	2.99	1.31	-3.44	69.75	-8.27	-2.51	86.37	311.36	130.07

Results

To obtain the best correlation between pMIC (Y-axis) and several descriptors (X-axis), screening was used applying the Pearson correlation or matrix correlation method, which was then carried out by multiple linear regression (MLR) (Hardjono *et al*., 2016). The Pearson correlation or matrix correlation results can be seen in Table II.

Table II: Pearson correlation or matrix correlation of PMIC with its various descriptors

Descriptor	pMIC	Log P	ClogP	LogS	TPSA	E_{HOMO}	E_{LUMO}	MR	Mr	MV
pMIC	1									
Log P	-0.805	1								
ClogP	-0.736	0.968	1							
LogS	0.864*	-0.923	-0.929	1						
TPSA	0.444	-0.747	-0.683	0.475	1					
E_{HOMO}	-0.917	0.858	0.801	-0.934	-0.488	1				
E_{LUMO}	-0.926*	0.807	0.749	-0.899	-0.402	0.963	1			
MR	-0.886*	0.848	0.840	-0.967	-0.356	0.920	0.880	1		
Mr	-0.848	0.725	0.740	-0.908	-0.188	0.856	0.827	0.972	1	
MV	-0.872	0.789	0.794	-0.941	-0.273	0.889	0.854	0.990	-0.075	1

Descriptors with a meaningful relationship with pMIC can be seen from results close to 1 or -1. Only three descriptors were included in the QSAR equation, represented by each parameter, because the number of compounds made from this equation is only 12, n = 12. One descriptor parameter can enter the QSAR equation with n = 5 (Topliss & Costello, 1972). From the results of the matrix correlation in Table II, which still meets the requirements, the hydrophobic parameter is represented by Log S with a value of 0.864; the electronic parameter is represented by an E_{LUMO} with a value of -0.926, while the steric parameter is represented by MR with a value of -0.886.

The QSAR equation of screening matrix correlation is created and compared with several other QSAR equations for the MLR process. MLR process was carried out by analysing the Fischer criteria (F), coefficient of determination (R^2), and cross-validation coefficient by leaving one out (Q^2) with the results in Table III.

Table III: Multiple linear regression of QSAR equations

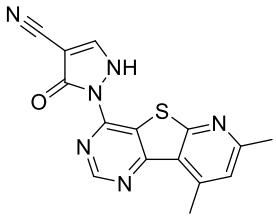
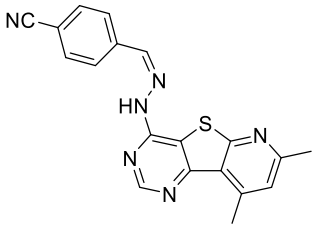
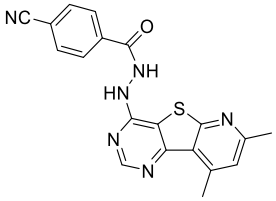
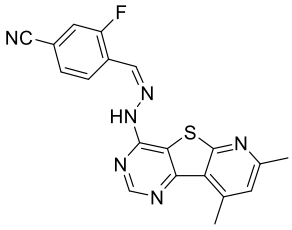
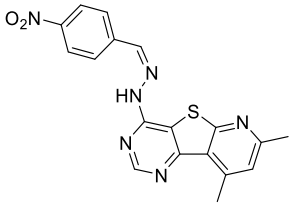
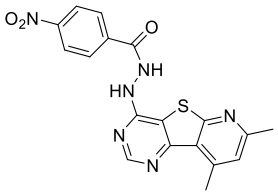
Number of equations	QSAR equations					
1	pMIC=-0.102 (±1.418) Log S-1.017 (±0.370) E_{LUMO} -0.017 (±0.012) MR-3.544 (±1.418)					
Log S; E_{LUMO}; MR	N = 12	Sig = 0.001	*r* = 0.943	R^2=0.890	*F* = 21.558	Q^2= 0.62; R^2-Q^2=0.27
2	pMIC=-0.013(±0.053)log P-0.0872(±0.365)E_{LUMO}-0.007(±0.008)-3.635(±1.472)					
Log P; E_{LUMO}; MR	N = 12	Sig = 0.001	*r* = 0.938	R^2=0.881	*F* = 19.683	Q^2= 0.51; R^2-Q^2=0.37
3	pMIC=0.013(±0.047)Clog P-0.233(±0.102)E_{HOMO}-0.006(±0.006)-3.358(±1.547)					
ClogP ; E_{HOMO}; MV	N = 12		*r* = 0.926	R^2=0.858	*F* = 16.053	Q^2= 0.46; R^2-Q^2=0.40
4	pMIC= 0.013 (±0.47)ClogP-0.233 (±0.102)E_{HOMO}-0.006(±0.006)MV-3.358 (±1.547)					
LogS, E_{HOMO}; MR	N = 12	Sig = 0.001	*r* = 0.926	R^2=0.858	*F* = 16.063	Q^2= 0.41; R^2-Q^2=0.45
5	pMIC=0.013 (±0.132)Log S+0.006 (±0.005) TPSA-0.006(±0.004) Mr-0.539(±0.747)					
LogS, TPSA; Mr	N = 12	Sig = 0.001	*r* = 0.896	R^2=0.729	*F* = 10.863	Q^2=0.20; R^2-Q^2=0.53

Determination of new compound design

Based on the QSAR equation, developing a more potent compound with a lower MIC or a higher pMIC value involves formulating a compound meeting three criteria. First, the compound should possess a lower Log S value, indicating the presence of a nonpolar group. Second, the E_{LUMO} of the molecular structure must be decreased. Third, the MR should exhibit a more negative value. MR quantifies the total polarization of a mole of a substance and is influenced by factors such as temperature, refractive index, and pressure.

The descriptor and pMIC prediction of the new chemical structure of the model can be seen in Table IV.

Table IV: Descriptor and pMIC prediction of new model chemicals structure

Compounds	Log S	E_{LUMO}	MR	pMIC Prediction
	-3.62	-2.59	88.34	-2.0416
Compound four				
	-5.702	-3.18	106.16	-1.5331
New one				
	-5.389	-3.524	105.92	-1.2111
New two				
	-5.935	-3.064	106.57	-1.6342
New three				
	-6.016	-5.14	108.95	0.4449
New four				
	-5.716	-5.493	108.49	0.7811
New five				

Discussion

Based on the MLR analysis of the five QSAR equations, the coefficient of determination (R^2) with acceptance criteria > 0.6, all five of the QSAR equations meet the criteria (Chirico & Gramatica, 2011; Adeniji *et al.*, 2018; Sumartha *et al.*, 2022; Putra *et al*., 2023; Kesuma *et al*., 2024). Based on the Fischer criteria (F) value, with acceptance criteria F count > F table (4.066) or F count/F table > 1, all five of the QSAR equations meet

the Fischer criteria (F) value requirements. Based on internal validation by cross-validation coefficient or leaving one out (Q2) with acceptance criteria Q2 > 0.5, the QSAR equations that meet the requirements are equations one and two (Gramatica, 2007; Adeniji *et al.*, 2018; Chirico & Gramatica, 2011). Based on Internal validation using the difference between R^2 and Q^2 with acceptance criteria ≤ 0.3, the QSAR equation that met the conditions is equation one with a value of 0.27. Based on the overall analysis of the MLR, the equation that m*et a*l criteria requirements was equation one. Equation one was chosen to be the best QSAR equation with consideration of the statistical data

The best QSAR equation is equation one, namely pMIC=-0.102 (±1.418) Log S-1.017 (±0.370) ELumo -0.017 (±0.012) MR-3.544 (±1.418) with sig, r, R^2, F and Q^2 values that have m*et a*l the requirements if the criteria (Hardjono *et al.*, 2016; Luo *et al.*; 2012; Adeniji *et al.*, 2018; Chirico & Gramatica, 2011).

Compound four, which has both experimental pMIC values and the highest predicted pMIC values, is employed as reference standards to identify prospective compounds with enhanced potency. Analysing the three criteria —Log S value, E_{LUMO}, and MR— yields a novel compound design. This analysis identifies five potential candidate compounds for resynthesis, labelled as 'new one – five'. Descriptor outcomes for compound four and new one - five are provided in Table IV. The pMIC predictions are as follows: new five > new four > new two > new one > new three > compound four. This indicates that the new five is particularly promising as it boasts the highest pMIC value among the candidates, making it a compelling choice for further consideration.

Conclusion

To increase the antimicrobial activity of pyridothienopyrimidine derivatives against *P. aeruginosa*, Log S, E_{LUMO}, and MR must be decreased. This can be achieved by selecting the best QSAR equation, which can be a tool to obtain a potential new chemical structure model and reduce trial and error.

Source of funding

This project did not receive funding.

Conflict of interest

The authors declare no conflict of interest.

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