Research Article

Lipophilicity-Driven QSAR Models For Antimicrobial Azaphenanthrene Scaffolds

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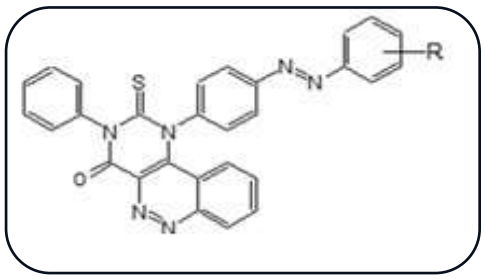
Abstract



Nitrogen-fused polycyclic aromatic heterocycles, particularly azaphenanthrenes, represent versatile scaffolds in medicinal chemistry owing to their enhanced electron distribution, hydrogen-bonding capacity, and metabolic stability. This study reports the synthesis and antimicrobial evaluation of azaphenanthrene derivatives bearing diverse substituents, alongside quantitative structure-activity relationship (QSAR) modeling to elucidate structure-activity trends. Compounds exhibited potent activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas diminuta, often surpassing ampicillin, with lipophilicity (log P) emerging as the primary correlate of potency (r = 0.86–0.96)

Key Words: Polycyclic Aromatic Heterocycles, QSAR, Lipophilicity, Ampicillin, Antimicrobial, Physicochemical properties.

INTRODUCTION:

Fused nitrogen heterocycles have long been recognized as essential scaffolds in medicinal chemistry due to their structural flexibility and broad pharmacological potential.[i] Azaphenanthrenespolycyclic aromatic heterocycles formed by nitrogen substitution in the phenanthrene frameworkhave garnered significant attention, as nitrogen incorporation enhances electron distribution, hydrogen bonding, and metabolic stability, thereby improving drug-likeness and receptor affinity.[ii,III]

Over the past decade, azaphenanthrene derivatives have exhibited diverse bioactivities, including anticancer effects via DNA intercalation, kinase inhibition, and apoptosis modulation, potent antimicrobial/antifungal activity against multidrug-resistant pathogens and antioxidant/enzyme-inhibitory properties. Their synthetic versatility sustains research into efficient strategies, structural diversification, and bioevaluations to optimize therapeutic profiles.[iv-vi]

As nitrogenous polycyclic aromatic hydrocarbons (PAHs), azaphenanthrenes serve as natural product synthons with antimalarial, anticancer, and emetic traits, accessed via ring annulation, cyclizations, or controlled pyrolysis/combustion. They metabolize to bay-region diol epoxides linked to mutagenicity/carcinogenicity, yet hold pharmacological promise in anti-infectives.Recent dehydrocyclizations of naphthyridines yield complex fused systems resembling bioactive motifs.[vii-xi]

This study focuses on evaluating antimicrobial activity and QSAR modelssynthesized azaphenthrene heterocycles to elucidate their therapeutic applications. [xii,xiii]

EXPERIMENTAL SECTION:

All the chemical used are of pure grade. Minimum inhibitory concentrations (MICs) and minimum bactericidal/fungicidal concentrations (MBC/MFC) were determined via broth microdilution per CLSI M07-A10 and M27-A3 guidelines.

Inhibition zone data were expressed as mean ± standard deviation (SD) from triplicates. MIC/MBC values were determined from three independent experiments. Statistical significance was analyzed using one-way ANOVA followed by Tukey's post-hoc test (GraphPad Prism v9.0; $p < 0.05$).

RESULT AND DISCUSSION:

A. Biological activities:

The antimicrobial evaluation of the synthesized substituted azaphenanthrene derivatives was carried out against a panel of representative pathogenic bacteria. Nutrient agar medium was utilized for bacterial cultivation, and inocula containing approximately 10^7 CFU/mL were prepared from log-phase broth cultures. The inoculated plates were incubated at 37 °C for 24 hours under standard aerobic conditions. Four microbial strains Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas diminuta were selected for the antibacterial assay based on their clinical relevance and occurrence in recent antimicrobial studies.[xix-xx] Ampicillin trihydrate served as the reference antibacterial agent and was assessed under identical conditions. Notably, all synthesized diazaphenanthrene derivatives exhibited significant antimicrobial activity, outperforming the standard drug in several cases, with minimum inhibitory concentration (MIC) values ranging between 4–48 µg/mL. These results are consistent with recent reports highlighting the strong bioactivity of nitrogen-enriched heterocyclic scaffolds.

The screening results depicted in Table-I, reveal that reported compounds showed a remarkable effect on the bacteriocidal/ bacterostatic potency. Substituent R on the azaphenanthrene ring affects antibacterial potency; lower MIC means higher activity.Relative activity of substituents for all four bacteria, activity order is:

3-OC_2H_5> 4-OC_2H_5> 3-C_2H_5> 4-C_2H_5> 3-OCH_3> 3-NO_2> 4-NO_2> 3-COOH > 3-Cl > 4-Cl > 3-CH_3> 4-CH_3> 3-OH > 4-OH > H.

Ethoxy groups give the strongest activity: 3-OC_2H_5 has MIC 6, 8, 5, 4 µg/ml against B.S,S.A, E.C, and P.D respectively; 4-OC_2H_5 is slightly less active but still much better than H.The unsubstituted compound (H) and phenolic OH derivatives show the weakest activity (MICs around 45–54 µg/ml).All substituents follow the same trend across organisms: P. diminuta is the most sensitive (lowest MICs), then E. coli, B. subtilis, and finally S. aureus (highest MICs).

P.diminuta > E. coli > B. Subtilis > S.aureus

Increasing lipophilicity and bulk (ethyl and ethoxy groups, especially at the 3-position) markedly enhances antibacterial activity, consistent with the QSAR result that higher log P correlates with higher −log MIC.More polar or less hydrophobic groups (OH, COOH) and the unsubstituted ring reduce activity, suggesting that adequate hydrophobic character is crucial for interaction with bacterial targets or penetration of cell membranes.

B. QSAR Activity:

- **Calculation of physicochemical properties**

  In order to deduce the relationship of observed activity, in terms of MIC (µg/mL) of synthesized compounds with different structural parameters, systematic QSAR studies have been performed by using the model proposed by Hansch and coworkers.

  The Minimum Inhibitory Concentration represents the concentration of synthesized drug inhibited visible growth of microbs. The same are further expressed as –log MIC on molar basis and used as dependent variables to get linear relationship in QSAR model. The calculated parameters used in present studies include VDW, CAA, CMA, CSEV, DDENE and log P. These parameters were calculated by using Chem 3D6.0 software. Further, HOMO and LUMO energies were calculated by semiempiricalPM3. studies using MOPAC 6.0 package.

- **Multiple linear regression analysis**

  Multiple linear regression (MLR) analysis was used to investigate the significant relationship between biological activity and physicochemical properties. The MLR was performed by using the VALSTATby the stepwise method. The highest correlation of independent variables with dependent variable was chosen for deriving the QSAR model. The statistical values, multiple correlation coefficient (r), standard errors (s), cross validation r2 (q2) and standard error of prediction (SPRESS) were used to evaluate the obtained QSAR models. Several combinations of independent variables were firstly attempted using three variables (one representative from each property) for individual models, and then, more variables were added in order to

optimize the statistical values but not more than five independent variables were used. The best model derived from the MLR analysis was used to predict the inhibitory activity of the synthesized compounds.

Furthermore, in order to deduce effective QSAR models, a correlation matrix, which is required to have a better selection for calculated parameters, is shown in Tables VII and VIII.

The resulting mono parametric models are depicted in Eqs. 1-5, along with statistical parameters of the regression. No outliers have been determined and the equations were derived using the entire data set (n=16).

QSAR model for S. aureus

-log MIC = [2.946(± 0.236)] +log P [0.571(± 0.101)] (1)

n=16, r=0.86 , s=0.090, F=147.51

The comparison of above correlation coefficient (Table-II) shows that the partition coefficient (log P) plays a dominating role in the theoretical modeling of the activity and also shows the direct relationship with the activity. Comparison also demonstrates the significance of other parameters in deciding the activity quantitatively. It also emphasizes the domination of steric properties over the volumetric properties for modeling the activity against specific type of bacteria.

Bi parametric QSAR model for S. aureus

In order to have further improvement in statistics CSEV has been included in eq. (9) and following bi-parametric correlation was resulted:

-log MIC = [2.264(±0.683)]+log P[0.501(±0.112)] + CSEV [0.005 (± 0.004)] (2)

n=16, r=0.86, s=0.079, F=98.63

Results from the bi-parametric combination [10] are not encouraging and model obtained is not statistically significant. This also confirms the results of Eq. (1)

QSAR model for B. subtilis

-log MIC = [2.945(± 0.389)] +log P [0.501(± 0.167)] (3)

n=16, r=0.88, s=0.148, F=41.91

QSAR model for E. coli

-log MIC = [2.921(± 0.326)] +log P [0.466(± 0.140)] (4)

n=16, r=0.88, s=0.124, F=51.85

QSAR model for P.diminuta

-log MIC = [2.952(± 0.315)] +log P [0.440(± 0.135)] (5)

n =16, r =0.96, s =0.120, F =49.60

The F-values obtained in Eqs. 1and 2-5 are found statistically significant at 99% level. Similarly, cross validation of obtained equations were checked by employing the leave one out (LOO) method ($r^2_{cv} > 0.83$). The calculated and predicted activities of the synthesized compounds were in accordance with the observed activities as shown in Figs. 1.

CONCLUSION

Conclusively, a series of azaphenthrene scaffolds has been fused as effective antimicrobial agents. Furthermore, QSAR analysis executed on these derivatives have revealed that the positive results of the hydrophobicity of the molecule, proposedthat there is a significant correlation between lipophilicitybiological activities of molecule.

Table I: The in vitro antimicrobial activity of Azaphenanthrene Derivatives

S.No.	R	Bacteria							
		B.S		S.A		E.C		P.D	
		MIC (μg/ml)	-logMIC	MIC (μg/ml)	-logMIC	MIC (μg/ml)	-logMIC	MIC (μg/ml)	-logMIC
1	H	51	3.586	54	3.562	49	3.661	47	3.622
2	3-Cl	33	3.841	35	3.815	32	3.915	31	3.868
3	4-Cl	37	3.791	39	3.768	35	3.854	34	3.828
4	3-CH_3	40	3.718	43	3.686	38	3.815	37	3.751
5	4-CH_3	43	3.690	45	3.666	41	3.740	39	3.729

6	3-C_2H_5	12	4.269	14	4.202	11	4.307	9	4.394
7	4-C_2H_5	15	4.172	17	4.117	14	4.202	12	4.269
8	3-OH	46	3.661	48	3.643	44	3.718	42	3.701
9	4-OH	49	3.629	51	3.616	47	3.690	45	3.671
10	3-OC_2H_5	6	4.600	8	4.475	5	4.670	4	4.776
11	4-OC_2H_5	9	4.424	11	4.337	8	4.475	7	4.533
12	3-OCH_3	18	4.096	19	4.073	17	4.121	15	4.176
13	4-OCH_3	21	4.029	23	3.990	21	5.029	18	4.096
14	3-NO_2	24	4.000	26	3.965	25	3.982	22	4.037
15	4-NO_2	26	3.965	29	3.917	27	3.948	25	3.982
16	3-COOH	33	3.901	32	3.873	32	3.873	31	3.931
17	A	-	3.90	-	4.60	-	4.43	-	4.60

A = Ampicillin;BS = B. subtilis; SA = S. aureus; EC=E.coli; P.D = P. diminuta.

Table II:Values of selected descriptors calculated for Azaphenanthrene Derivatives

R	**$HOMO_{ene}$**	**$LUMO_{ene}$**	**DDENE**	**VDW**	**NVDW**	**Log P**	**CSEV**	**CAA**	**CMA**
H	-8.879	-0.72	0.824	8.969	0.649	1.525	154.957	372.622	188.529
3-Cl	-9.014	-0.936	1.302	8.787	0.607	2.646	165.713	396.206	203.133
4-Cl	-8.915	-0.891	1.432	8.984	5.645	2.646	159.326	382.523	194.503
3-CH_3	-8.878	-0.721	0.824	8.968	0.652	2.321	161.898	389.889	198.594
4-CH_3	-8.842	-0.712	0.801	9.061	7.606	2.320	160.999	379.482	194.232
3-C_2H_5	-5.306	-0.858	0.823	9.686	0.517	2.851	175.577	175.577	214.678
4-C_2H_5	-8.616	-0.663	0.815	9.896	3.107	2.849	179.391	409.081	212.356
3-OH	-8.829	-0.724	0.893	7.045	0.443	2.227	149.536	367.044	184.884
4-OH	-8.748	-0.75	0.904	8.409	-1.316	1.821	145.238	359.245	179.996
3-OC_2H_5	-8.749	-0.6601	0.906	11.583	1.248	2.843	185.519	185.519	226.678
4-OC_2H_5	-8.696	-0.6793	0.739	11.426	16.547	2.843	186.216	186.216	224.425
3-OCH_3	-8.778	-0.682	0.906	10.922	1.341	2.314	168.689	403.945	206.428
4-OCH_3	-8.713	-0.692	0.736	10.719	12.089	2.314	168.579	398.019	204.833
3-NO_2	-11.36	-5.565	1.646	9.06	1.490	1.470	164.745	394.089	201.94
4-NO_2	-11.243	-5.431	1.978	8.235	-1.849	1.470	167.695	392.422	201.305

3-COOH	-9.139	-1.242	2.563	7.826	0.926	2.047	165.713	396.206	203.133
A	-9.337	-0.827	0.675	7.455	2.439	-1.204	280.439	555.251	304.619

A = Ampicillin

Table III: Correlation matrix of used molecular descriptors

	$HOMO_{ene}$	$LUMO_{ene}$	DDENE	VDW	NVDW	LogP	CSEV	CAA	CMA
$HOMO_{ene}$	1.000								
$LUMO_{ene}$	0.720	1.000							
DDENE	0.503	0.574	1.000						
VDW	0.242	0.255	0.480	1.000					
NVDW	0.106	0.275	0.336	0.548	1.000				
Log P	0.650	0.643	0.423	0.521	0.407	1.000			
CSEV	0.182	0.0149	0.086	0.766	0.412	0.595	1.000		
CAA	0.539	0.219	0.320	0.515	0.258	0.509	0.579	1.000	
CMA	0.217	0.035	0.077	0.774	0.379	0.609	0.988	0.634	1.000

Table IV: Cross-validation parameters

Eq.	PRESS	SSY	PRESS/SSY	S_{PRESS}	SDEP	r^2_{CV}	r^2_{bsp}
1	0.156	1.009	0.155	0.105	0.098	0.913	0.892
2	0.414	0.926	0.447	0.172	0.160	0.749	0.718
3	0.294	0.807	0.364	0.144	0.135	0.787	0.757
4	0.273	0.719	0.380	0.140	0.131	0.779	0.759

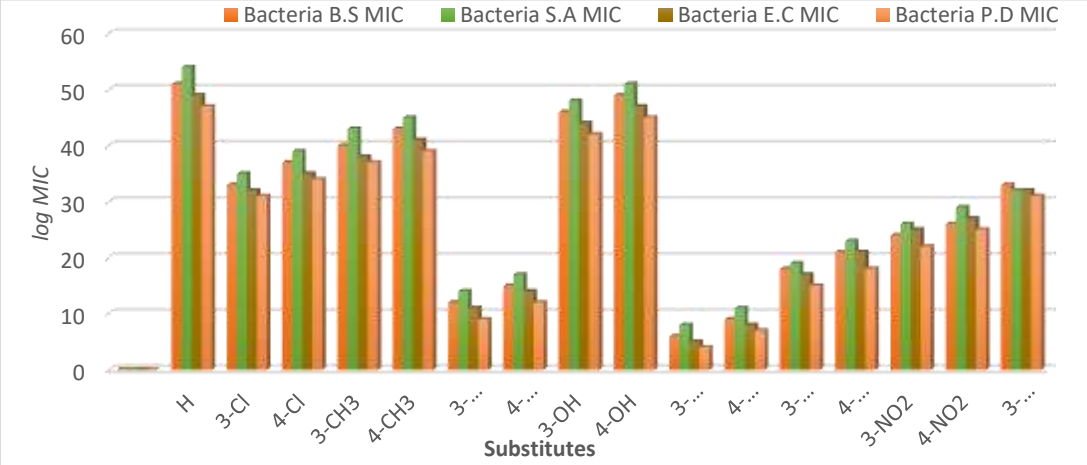


Fig. 1. Plots of observed vs predicted activity of azaphenthrene against different bacteria.

References

1. Ahmed S, Rao K. Bioactive Nitrogen-Containing PAHs: Antimalarial and Anticancer Potential. Bioorg Chem. 2020;95:103546. .
2. Sharma D, Rai S, Arora S, Gupta P, Sharma R, Chopra AK. Study of the trace elements in Aloe vera L. (Aloe barbadensis Miller) (Liliaceae) and its biological and environmental importance. J Chem Pharm Res. 2011;3:64–68.

3. Chen L, Wu X, Zhou H. Advances in Annulation Strategies for Polycyclic Heterocycles. Org Chem Front. 2022;9:2110-2125.].
4. Gupta R, Singh T. Inter- and Intramolecular Cyclization in Heterocyclic Synthesis. J Org Chem. 2019;84:7012-7024.
5. Hernández P, López R, Martín A. Formation of Nitrogen-Containing PAHs under Combustion Conditions. Environ Sci Technol. 2023;57:3457-3466.
6. Heo J, Lim C, Park S. Nitrogen-Fused Aromatic Systems as Privileged Scaffolds in Medicinal Chemistry. Eur J Med Chem. 2022;237:114398.
7. Kumar M, Patel D, Suresh R. Recent Trends in the Pharmacology of Azaphenanthrene Derivatives. Med Chem Res. 2023;32:1204-1218.
8. Li Y, Chen H, Zhao Q. Natural Product-Inspired Azaphenanthrenes: Chemistry and Biological Relevance. Nat Prod Rep. 2021;38:2234-2251.
9. Morgan D, Ellis J. Bay-Region Epoxide Pathways in Nitrogen-Containing PAHs. Chem Res Toxicol. 2022;35:678-689.
10. Park J, Kim S, Lee H. Pharmacological Significance of Polycyclic Nitrogen Heterocycles. Pharmacol Ther. 2019;203:107395.
11. Sato K, Fujii T, Nakahara Y. Nitrogen Incorporation in PAHs during Pyrolysis. J Anal Appl Pyrolysis. 2018;134:328-336.
12. Singh R, Verma P. Catalytic Dehydrocyclization Strategies for Azaphenanthrene Synthesis. Synthesis. 2021;53:4481-4490.
13. Sharma M, Maheshwari A, Bindal N. A Novel Procedure to Synthesize 3-Chloro-1-(4a,10b-Diazaphenanthrene-2-yl)-4-Phenyl Azetidin-2-ones and Exploration of Their Anti-Inflammatory Activity. J Heterocycl Chem. 2013;50:E116.
14. Sharma P, Kumar A, Sharma M, Upadhyay S. Synthesis, Characterization and Electrochemical Behaviour of Some Substituted 2-Phenyl-4-(4′-Arylazo)phenyl-3-thioxo-3,4,4a,10a-tetrahydro-2H-2,4,9,10-tetraazaphenanthrene-1-ones. Indian J Chem Sect B. 2004;43:2653-2660.
15. Wang L, Zhang J, Huang Y. Metabolic Activation of Aza-PAHs: Diol Epoxide Pathways and Toxicological Impact. Toxicol Lett. 2020;323:15-25.
16. Zhang K, Liu C. Structural Importance of Azaphenanthrenes in Natural Product Chemistry. Chem Eur J. 2021;27:11672-11684.
17. Zhao Y, Xiang H, Du S. Cyclization Methodologies for Constructing Phenanthrene-Based Heterocycles. Org Biomol Chem. 2020;18:6374-6385.
18. Khan MA, Rahman M, Lee J. Advances in Heterocyclic Antimicrobial Agents: Design, Activity, and Mechanisms. J Mol Struct. 2021;1240:130555.
19. Oliveira DS, Lima MN, Torres RA. Evaluation of Antimicrobial Activity Using Updated MIC-Based Methodologies. Microb Pathog. 2023;179:106123.
20. Singh R, Patel V. Nitrogen-Containing Fused Heterocycles as Emerging Antibacterial Leads. Eur J Med Chem. 2022;236:114352.
21. Zhang X, Hu Y, Chen L. Recent Developments in QSAR-Guided Drug Discovery of Polycyclic Heterocycles. Bioorg Chem. 2024;144:106868.