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SYNTHESIS, ANTIMICROBIAL EVALUATION AND DOCKING STUDY OF SCHIFF BASE OF 2-CHLOROACRIDONE

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ABSTRACT

In this study six schiff bases of 2-Choloroacridone were prepared. The structures of compounds were established by their physicochemical and spectral means (IR and ^{1}HNMR). The synthesized compounds were further screened for their antimicrobial activity against *S. aureus*, *B. subtilis* (Gram positive), and *P. aeruginosa*, *E. coli* (Gram negative) bacteria and *C. albican* and *A. niger* fungal strain. Among these synthesized compounds, some of the compounds showed significant antimicrobial activity. Besides, antimicrobial activity the docking study of synthesized compounds was done on target protein Thymidylate kinase (TMPK) (PDB ID: 4QGG) using Autodock Vina. Compound 6 showed maximum docking score.

KEYWORDS: Acridone, Schiff bases, Antimicrobial, Antibacterial, Antifungal, Docking.

INTRODUCTION

Acridone, also known as acridin-9(10H)-one in its chemical nomenclature, is a captivating heterocyclic organic compound that has garnered substantial attention across various scientific fields, especially in the realms of chemistry and pharmacology. This fascination stems from its diverse array of applications, notably its exceptional antimicrobial properties. At its core, acridone boasts a tricyclic structure, characterized by the fusion of two benzene rings with a central pyridine ring. This unique molecular arrangement confers several remarkable chemical properties upon acridone, such as its aromaticity, planarity, and the presence of conjugated double bonds within its structure. These features render acridone an

exceptionally versatile compound with immense potential for a wide range of chemical interactions.

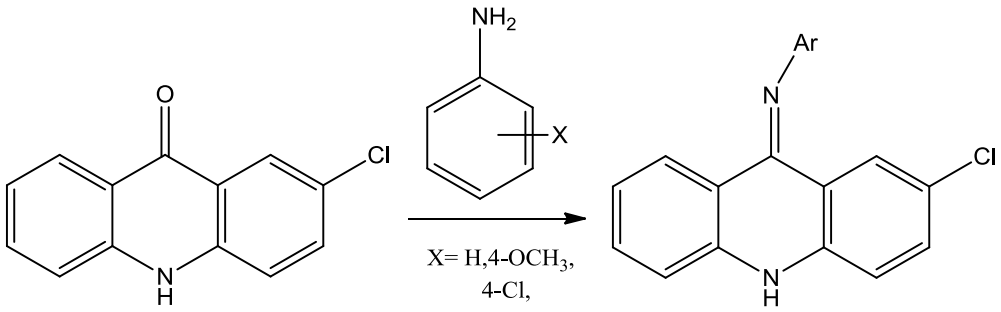
The chemical characteristics of acridone, rooted in its distinctive structure, make it an intriguing subject of study in various scientific disciplines. Beyond its antimicrobial prowess, acridone exhibits a multitude of other bioactive properties. The literature abounds with evidence showcasing acridone's capabilities, including its role as an anticancer agent[1], its effectiveness against malaria[2],[3], its anti-inflammatory properties[4], its potential as an antitubercular agent[5], and its antiviral activity.[6] Moreover, acridone has also demonstrated antibacterial qualities[7],[8], underscoring its broad spectrum of applications in combating microbial threats.

MATERIAL AND METHOD

All chemicals and solvents employed in this study were of laboratory-grade quality. The purity of the compounds was assessed *via* preparatory silicagel-G plate Thin Layer Chromatography (TLC). To further characterize these compounds, Infrared (IR) and Proton Nuclear Magnetic Resonance (H^1 NMR) spectroscopy were employed. IR spectra were obtained by recording measurements on a FT IR spectrometer, using KBr pellets as the medium. H^1NMR spectra, on the other hand, were acquired using a spectrometer in DMSO, with tetramethylsilane serving as the internal standard. The chemical shifts of the compounds were reported in parts per million (ppm). Melting points were determined using the capillary method and are uncorrected. Spot visualization was facilitated by the use of an iodine chamber and UV lamp.

Chemistry

Synthesis of Schiff base of 2-Chloroacridone



2-Chloroacridone

Schiff's base of 2-Choloro acridone

Compounds	-Ar	Compounds	-Ar
1	$-C_6H_5$	4	$2\text{-}OH\text{-}C_6H_4$
2	$4\text{-}Cl\text{-}C_6H_4$	5	$3\text{-}OH\text{-}C_6H_4$
3	$4\text{-}OCH_3\text{-}C_6H_4$	6	4- OH-C6H4

Synthesis of schiff's bases of 2-Chloroacridinone (1-6)

In a small evaporating basin, 0.1 moles of a 2-Chloroacridinone, along with 0.1 moles of a substituted aromatic amine derivative (as detailed in Table 1), were combined with zinc chloride. This mixture was then gently stirred using a glass rod and placed on a boiling water bath. Shortly thereafter, water droplets began to form on the oily layer. After approximately 45 minutes, the basin was shifted to an ice-water bath, and the contents were thoroughly stirred, leading to rapid solidification. The solid material in the basin was subsequently broken down, transferred into a conical flask, and subjected to recrystallization using rectified spirit as the solvent.[9],[10],[11],[12]

Table 1: Quantity of aromatic amines.

S. No	Aromatic amines	Quantity (moles)	S. No	Aromatic amines	Quantity (moles)
1	Aniline	9.3 ml (0.1)	4	*o*-aminophenol	10.9 g (0.1)
2	4-chloroaniline	12.8 g (0.1)	5	*m*-aminophenol	10.9 g (0.1)
3	4-methoxyaniline	12.3 g (0.1)	6	*p*-aminophenol	10.9 g (0.1)

Spectral analysis

Compound 1: (2-Chloro-10H-acridin-9-ylidene)-phenyl-amine

IR (KBr) cm^{-1}: 3368.0-3365.1 (N–H *str*), 2987.5-2982.3 (C–H *str* aromatic), 1767-1758.6 (C–N *str*), 1663.6-1641.4 (C=N), 1609-1601.7 (C=C *str* aromatic)

^{1}H-NMR, (DMSO) δ ppm: 4.06 (s, 1H, NH), 6.58- 7.71 (m, 12H, Ar-H).

Compound 2: (2-Chloro-10H-acridin-9-ylidene)-(4-chloro-phenyl)-amine

IR (KBr) cm^{-1}: 3368.9-3366.3 (N–H *str*), 2988.3-2981.9 (C–H *str* aromatic), 1766.3-1757.5 (C–N *str*), 1663.8-1640.9 (C=N), 1610.1-1602.3 (C=C *str* aromatic)

^{1}H-NMR, (DMSO) δ ppm: 4.11 (s, 1H, NH), 6.62- 7.74 (m, 11H, Ar-H).

Compound 3: (2-Chloro-10H-acridin-9-ylidene)-(4-methoxy-phenyl)-amine

IR (KBr) cm^{-1}: 3368.5-3366.1 (N–H *str*), 2988.7-2986.7 (C–H *str* aromatic), 1765.6-1757.1 (C–N *str*), 1667.6-1636.9 (C=N), 1661.4-1643.9 (C=C *str* aromatic).

^{1}H-NMR, (DMSO) δ ppm: 3.87 (s, 1H,-OCH_3), 4.21 (s, 1H, NH), 6.75- 7.76 (m, 11H, Ar-H).

Compound 4: 2-(2-Chloro-10H-acridin-9-ylideneamino)-phenol

IR (KBr) cm^{-1}: 3367.5-3366.8 (N–H *str*), 2989.3-2983.0 (C–H *str* aromatic), 1765.3-1756.4(C–N *str*), 1667.8-1637.4 (C=N), 1661.0-1642.9 (C=C *str* aromatic)

^{1}H-NMR, (DMSO): δ ppm: 3.73 (s, 1H,-OH), 4.22 (s, 1H, NH), 6.70- 7.81 (m, 11H, Ar-H).

Compound 5: 3-(2-Chloro-10H-acridin-9-ylideneamino)-phenol

IR (KBr) cm^{-1}: 3367.1-3366.3 (N–H *str*), 2989.4-2983.6 (C–H *str* aromatic), 1765.8-1756.9(C–N *str*), 1667.0-1637.7 (C=N), 1661.1-1642.7(C=C *str* aromatic)

^{1}H-NMR, (DMSO): δ ppm: 3.74(s, 1H,-OH), 4.25 (s, 1H, NH), 6.73- 7.82 (m, 11H, Ar-H).

Compound 6: 4-(2-Chloro-10H-acridin-9-ylideneamino)-phenol

IR (KBr) cm^{-1}: 3367.4-3366.7 (N–H *str*), 2989.5-2983.4 (C–H *str* aromatic), 1765.1-1756.6(C–N *str*), 1667.2-1637.6 (C=N), 1661.3-1642.9 (C=C *str* aromatic)

^{1}H-NMR, (DMSO): δ ppm: 3.74(s, 1H,-OH), 4.26 (s, 1H, NH), 6.74- 7.81 (m, 11H, Ar-H).

Antimicrobial Activity

Antimicrobial activity testing was conducted on a series of synthesized compounds using a panel of six different microbial strains. Among the bacterial strains, two were Gram-positive bacteria, namely *Bacillus subtilis* and *Staphylococcus aureus*, while the Gram-negative bacterial strains included *Escherichia coli* and *Pseudomonas aeruginosa*. In addition to the bacterial strains, two fungal strains, *Candida albicans* and *Aspergillus niger*, were also employed in the antimicrobial evaluation.

For the present investigation, cup or well method was used. The nutrient agar media was sterilized by autoclaving at 15 psi and 121^{o}C for 20 minutes. Muller Hinton agar medium was used to inoculate bacterial cultures and Sabourand's dextrose agar medium was used for fungal cultures. Two fold dilutions of each test compound and standard drugs were made to provide final concentration of 100, 50, 25, and 12.5 µg/ml. Wells were made in the media with sterile borer and filled with solution of synthesized compounds in DMSO. These petri-dishes were incubated at 30- 35^{o}C in incubator. The petri dishes were examined for the zone of inhibitions after 24 hrs. Antifungal activity was also carried out employing cup or well method but the plates were incubated for 48 hrs at 22- 25 ^{o}C. The activity was compared on the basis of zone of inhibition with reference to standard drug ciprofloxacin (antibacterial) and fluconazole (antifungal). The observed activity index is presented in table 2. [13, 14, 15]

OBSERVATIONS

Following the required incubation period, the diameter of the inhibition zone was determined using a calibrated measuring device, specifically a vernier caliper. The Activity Index was calculated by averaging the inhibition zone sizes of both test and standard compounds, as per the following formula.

CALCULATIONS

$$\text{Activity Index} = \frac{\text{Zone of inhibition of test compounds } (T_{avg})}{\text{Zone of inhibition of standard compounds } (S_{avg})}$$

Table 2: Activity Index for antibacterial and antifungal activity.

S. No	Comp.	Activity Index					
		B.subtilis	***S.aureus***	***P.aeruginosa***	***E.coli***	***C.albicans***	***A.niger***
1	C-1	0.74	0.59	0.63	0.47	0.78	0.74
2	C-2	0.76	0.62	0.64	0.52	0.82	0.78
3	C-3	0.77	0.60	0.62	0.49	0.80	0.80
4	C-4	0.80	0.64	0.66	0.54	0.82	0.82
5	C-5	0.78	0.62	0.64	0.50	0.81	0.78
6	C-6	0.82	0.65	0.68	0.54	0.84	0.78
7	Cipro.	1.0	1.0	1.0	1.0	NA	NA
8	Fluco.	NA	NA	NA	NA	1.0	1.0

Docking Studies

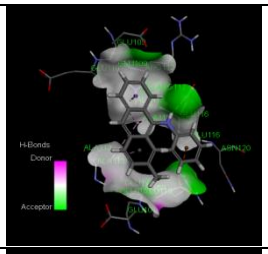
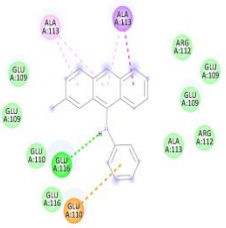
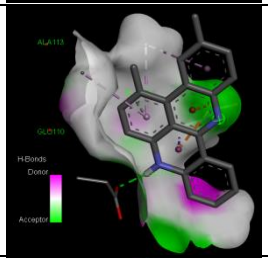
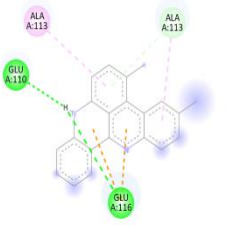
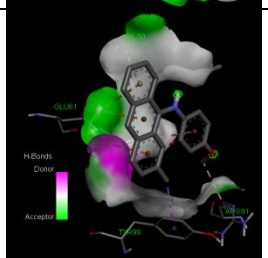
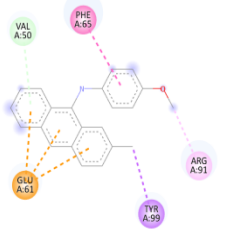
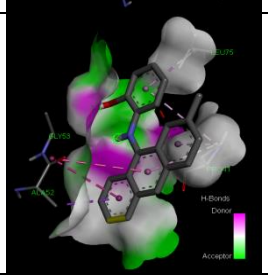
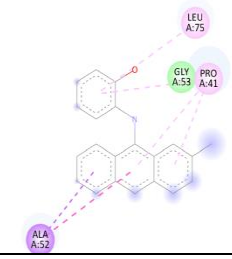
Anti-bacterial drugs typically operate through various mechanisms, such as inhibiting cell wall synthesis, blocking protein synthesis, disrupting nucleic acid (DNA) synthesis, and interfering with metabolic processes. These antibiotics generally target specific cellular proteins responsible for these vital functions. For instance, Thymidylate kinase (TMPK), an enzyme involved in the biosynthesis of dTTP, plays a crucial role in bacterial DNA synthesis. In the case of ciprofloxacin, it acts by inhibiting DNA gyrase, which is essential for unwinding bacterial DNA during cell division. Consequently, our focus in this study is on TMPK as the target protein, aiming to investigate the molecular interactions between newly synthesized ligands and this protein.[16] In the docking study, Autodock vina was used for molecular interaction. The crystal structures of Thymidylate kinase (TMPK) (PDB ID: 4QGG) was downloaded from Protein Data Bank (PDB) (www.rcsb.org). As TMPK is an essential enzyme for the biosynthesis of bacterial DNA. Therefore it can serve an attractive therapeutic target for the development of novel antibacterial agents. The docking score (lowest energy, Kcal/mol) of the synthesized compounds is given in table 3. The highest docking score (lowest binding energy) comes out for compound number 6. The docking pose

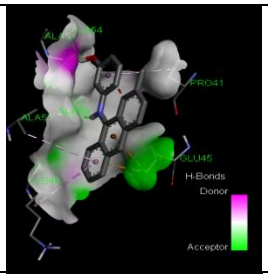
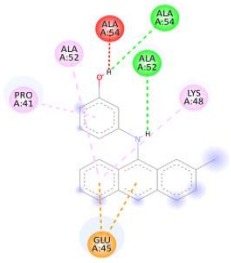
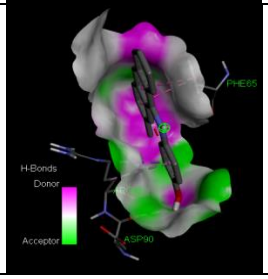
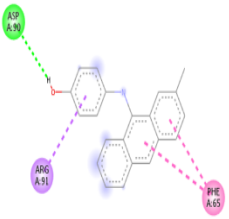
of all the compounds, with the amino acid of target protein (TMPK) involved in interaction are shown in table 4.

Table 3: Docking score of synthesised compounds.

Comp.	Grid Center	Docking score (Lowest Binding Energy) Kcal/mol	Comp.	Grid Center	Docking score (Lowest Binding Energy) Kcal/mol
1	X 20.16; Y -0.12 ; Z -10.46	-5.670	4	X 21 Y -0.12 Z -10.46	-6.781
2	X 22; Y 4; Z -10.46	-5.716	5	X 20.16 Y -0.12 Z -10.46	-6.019
3	X 20.16 Y -0.12 Z -10.46	-6.095	6	X 20.16 Y 0 Z -8	-8.444

Table 4: Lowest binding energy docking pose (3-D and 2-D) of synthsised compounds.

Compounds	3-D intraction	2-D intraction	Amino acid involved in Interaction
1			ALA(A:113), GLU(A:116), GLU(A:110)
2			ALA(A:113),GLU(A:116), GLU(A:110)
3			GLU(A:61),TYR(A:99), ARG(A:91), PHE(A:65), VAL(A:50)
4			ALA(A:52), LEU(A:75), GLY(A:53), PRO(A:41)

5			GLU(A:45), LYS(A:48), ALA(A52), ALA(A54), PRO(A:41)
6			ARG(A:91), ASP(A:90), PHE(A:65)

RESULT AND DISCUSSION

In this study six Schiff bases were synthesized as per the procedure reported in literature. Evaluation of the compounds for antibacterial activity showed variable result against all bacterial strains activity index ranging from 0.47 to 0.82. Compounds C-6 showed maximum activity among all the compounds. All the compounds were having maximum activity against *B. subtilis* while minimum activity was observed against *E. coli* (0.47 to 0.54). Compound 4 and compound 5 have comparable activity. Result of antifungal activity was also variable. Maximum activity was shown by compound 6 against *C. albicans* while compound 4 showed maximum activity against *A.niger*. Docking study of the compounds was carried out by autodock vina on target protein Thymidylate kinase (TMPK) (PDB ID: 4QGG).[17],[18] Lowest binding energy (Higest docking score) was found with compound 6 with docking score of -8.444 Kcal/mol. The amino acid involved in interaction were ARG (A: 91), ASP (A: 90), PHE (A: 65).

CONCLUSION

In the present study schiff base of 2-Cholroacridone were prepared in good yield. These compounds were further evaluated for antimicrobial activity. Among the synthesized compounds variable antimicrobial activity was observed. The synthesized compounds showed good activity index against *B. subtilis* while least activity was observed against *E. coli*. Moreover, in the synthesized compounds, compound bearing 4-OH hydroxyl group showed more activity as compared to other groups. Docking study of compounds also showed that compound 6 have lowest binding energy.

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