EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 11
165-169
Year: 2023

SYNTHESIS, *IN-VITRO* ANTIMICROBIAL EVALUATION AND DOCKING STUDY OF 9-(SUBSTITUTED-BENZYLIDENE)-2-METHOXY-9, 10-DIHYDRO-ACRIDINE ANALOGUES

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Article Received on 04/09/2023 **Article Revised on 25/09/2023** **Article Accepted on 16/10/2023**

ABSTRACT
In this study six 9-(Substituted-Benzylidene)-2-methoxy-9, 10-dihydro-acridine analogues were prepared by the procedure as reported in literature. The characterization of compounds was done by their physicochemical and spectral means (IR and ^{1}HNMR). The synthesized compounds were further screened for their antimicrobial activity against different microbial strains *viz. S. aureus, B. subtilis* (Gram positive), and *P. aeruginosa, E. coli* (Gram negative) bacteria and *C. albican, A. niger* fungal strain. Among these synthesized compounds, some of the compounds showed significant antibacterial and antifungal activity. Docking study of the two compounds showing maximum activity, were carried out by Autodock vina on protein Thymidylate kinase (TMPK).

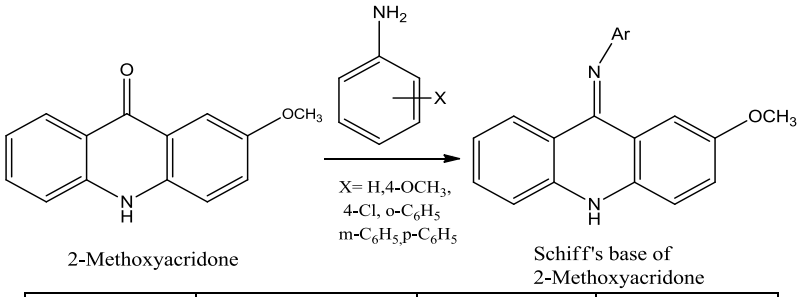
KEY WORDS: Acridine, antimicrobial, antibacterial, antifungal.

INTRODUCTION
Acridine is a unique and versatile chemical compound that has attracted significant attention in the fields of chemistry and pharmacology due to its intriguing molecular structure and diverse pharmacological activities. This heterocyclic aromatic molecule, composed of a tricyclic arrangement of two benzene rings fused to a central pyridine ring, exhibits a wide range of chemical reactivity and biological effects. Its rich chemistry and pharmacological significance have made it a subject of intense research and exploration. In the literature acridine is reported to have various pharmacological properties *viz.* antimicrobial[1], anticancer[2], Alzheimer's Disease[3] etc. A number of marketed preparations based on the acridine nucleus are available. These preparations represent various pharmacological activities. Bucricaine, [butyl-(1, 2, 3, 4-tetrahydroacridin-9-yl)-amine] is used topically for surface anesthesia of eye and given by injection for infilteration anesthesia, peripheral nerve block and spinal anesthesia. Quinacrine, [2-methoxy-6-chloro-9-(1-diethylamino-3-methylpropanamine)-acridine] is also known as mepacrine. It acts as gametocytocide. It destroys the sexual erythrocytic forms of plasmodia and act as antimalarial agent. 9-Aminoacridine acts as disinfectant. Proflavin, (3, 6-diaminoacridine) is found to be active as bacteriostatic against many Gram positive bacteria.[4]

MATERIALS AND METHOD
Chemicals and solvents used in the study were of laboratory-grade quality. The purity of the compounds was checked by Thin Layer Chromatography (TLC). The compounds were prepared by the methods reported in literature. Compounds were further characterized by Infrared (IR) and Proton Nuclear Magnetic Resonance (H1 NMR) spectroscopy. IR spectra were obtained using FT IR spectrometer, using KBr pellets as the medium. H^{1}NMR spectra were ontained by spectrometer in DMSO, using 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 9-(Substituted-Benzylidene)-2-methoxy-9, 10-dihydro-acridine analogues



2-Methoxyacridone

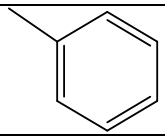
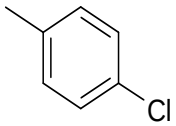
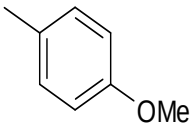
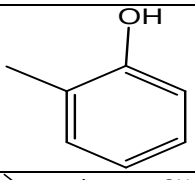
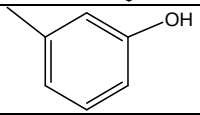
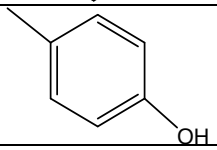
Schiff's base of 2-Methoxyacridone

Compounds	-Ar	Compounds	-Ar
1	-C6H5	4	-2-OH-C_6H_4
2	-4-Cl-C6H4	5	-3-OH-C_6H_4
3	-4-OMe-C6H4	6	-4-OH-C_6H_4

General method of Synthesis of compounds

In a small evaporating basin, 0.1 mol each of 2-Methoxyacridone and a substituted aromatic amine (Aniline, 4-chloroaniline, 4-Methoxyaniline, 2-Hydroxyaniline, 3-Hydroxyaniline, 4-Hydroxyaniline), in equimolar quantities, were combined along with zinc chloride. This mixture was gently agitated using a glass rod and then placed onto a boiling water bath. As the reaction proceeded, small water droplets started to form on the oily layer. Approximately 45 minutes later, the basin was carefully transferred to an ice-water bath, and the contents were vigorously stirred, resulting in rapid solidification. The resulting solid material in the basin was subsequently fragmented, collected, and transferred into a conical flask. To purify the solid material, a recrystallization process was employed, using rectified spirit as the solvent.[5][6][7][8] The physiochemical data of the compounds are reported in table 1.

Table 1: Physical parameters of synthesized compounds of acridone derivatives.

Compounds	-Ar	-R	% Yield	Melting point (^OC)	R_f Value
1		-OCH_3	63	205-207	0.49
2		-OCH_3	55	218-220	0.71
3		-OCH_3	63	221-223	0.61
4		-OCH_3	55	209-211	0.23
5		-OCH_3	58	214-217	0.66
6		-OCH_3	62	215-218	0.51

Spectral analysis

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

IR (KBr) cm^{-1}: 3364.0-3369.2 (N–H *str*), 2960.0-2967.4 (C–H *str* aromatic), 1751.4-1756.1 (C–N *str*), 1645.6-1661.4 (C=N), 1603.0-1608.6 (C=C *str* aromatic)
^{1}H-NMR, (DMSO) δ ppm: 3.70 (3H, -OCH_3), 3.97 (s, 1H, NH), 6.56- 7.65 (m, 11H, Ar-H).

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

IR (KBr) cm^{-1}: 3364.4-3368.3 (N–H *str*), 2961.6-2966.1 (C–H *str* aromatic), 1751.3-1755.6 (C–N *str*), 1646.1-1660.9 (C=N), 1603.1-1608.3 (C=C *str* aromatic)
^{1}H-NMR, (DMSO) δ ppm: 3.69 (3H, -OCH_3), 3.98 (s, 1H, NH), 6.58- 7.66 (m, 11H, Ar-H).

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

IR (KBr) cm^{-1}: 3364.9-3368.8 (N–H *str*), 2960.3-2966.5 (C–H *str* aromatic), 1751.6-1754.8 (C–N *str*), 1647.5-1660.1 (C=N), 1604.2-1608.6 (C=C *str* aromatic)
^{1}H-NMR, (DMSO) δ ppm: 3.76 (s, 6H,-OCH_3), 4.02 (s, 1H, NH), 6.62- 7.74 (m, 11H, Ar-H).

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

IR (KBr) cm^{-1}: 3364.1-3368.0 (N–H *str*), 2961.1-2965.9 (C–H *str* aromatic), 1751.3-1755.1 (C–N *str*), 1648.6-1659.0 (C=N), 1604.6-1607.7 (C=C *str* aromatic)
^{1}H-NMR, (DMSO) δ ppm: 3.77 (s, 3H,-OCH_3), 4.04 (s, 1H, NH), 6.65- 7.71 (m, 11H, Ar-H).

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

IR (KBr) cm^{-1}: 3364.5-3368.2 (N–H *str*), 2961.6-2965.1 (C–H *str* aromatic), 1751.4-1755.8 (C–N *str*), 1648.0-1659.5 (C=N), 1603.9-1607.1 (C=C *str* aromatic)
^{1}H-NMR, (DMSO) δ ppm: 3.77 (s, 3H,-OCH_3), 4.01 (s, 1H, NH), 6.66- 7.70 (m, 11H, Ar-H).

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

IR (KBr) cm^{-1}: 3364.5-3368.7 (N–H *str*), 2961.4-2965.9 (C–H *str* aromatic), 1751.0-1755.6 (C–N *str*), 1648.3-1659.8 (C=N), 1604.2-1606.6 (C=C *str* aromatic)
^{1}H-NMR, (DMSO) δ ppm: 3.78 (s, 3H,-OCH_3), 4.02 (s, 1H, NH), 6.68- 7.77 (m, 11H, Ar-H).

Antimicrobial Activity

Antimicrobial activity testing was carried out on a series of synthesized compounds by cup or well method. A set of six different microbial strains, comprising two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and two fungal strains (*Candida albicans* and *Aspergillus niger*) were used in study. 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 culture the bacterial strains, while Sabourand's dextrose agar medium was employed for the fungal strains. Two-fold dilutions of each test compound and standard drugs were prepared to achieve final concentrations of 100, 50, 25, and 12.5 µg/ml. Wells were created in the media using a sterile borer and then filled with solutions of the synthesized compounds dissolved in DMSO. Following this, the petri dishes were incubated at temperatures between 30 to 35°C for bacterial and 22 to 25°C for fungal cultures. After incubation for a period of 24 hours for bacteria and 48 hours for fungi, the petri dishes were examined to determine the zones of inhibition. The diameter of the inhibition zone was determined using a calibrated measuring device, specifically a vernier caliper. The antimicrobial activity was evaluated by comparing the observed zones of inhibition with those of the reference standard drugs, ciprofloxacin (for antibacterial activity) and fluconazole (for antifungal activity). The results of this analysis are presented in Table 2. The Activity Index was calculated by dividing the average of zone of inhibition test and standard compounds.[9][10][11]

Table: 2 Activity Index for antibacterial and antifungal activity.

S.N.	Comp.	Activity Index					
		B.subtilis	***S.aureus***	***P.aeruginosa***	***E.coli***	***C.albicans***	***A.niger***
1	C-1	0.73	0.60	0.62	0.50	0.74	0.74
2	C-2	0.74	0.60	0.63	0.60	0.75	0.74
3	C-3	0.80	0.68	0.66	0.54	0.78	0.78
4	C-4	0.76	0.65	0.64	0.58	0.76	0.74
5	C-5	0.78	0.64	0.65	0.58	0.76	0.74
6	C-6	0.81	0.66	0.66	0.56	0.82	0.80
7	Cipro.	1.0	1.0	1.0	1.0	NA	NA
8	Fluco.	NA	NA	NA	NA	1.0	1.0

Docking study

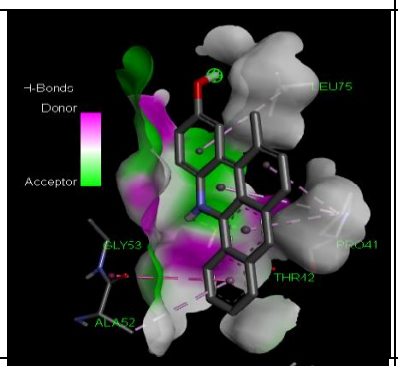
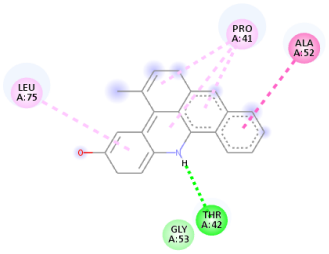
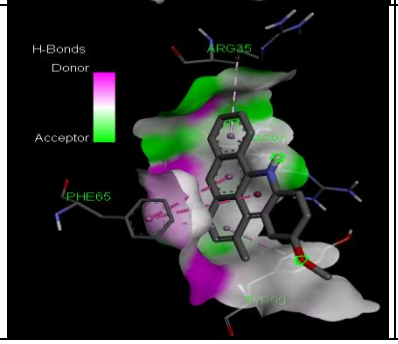
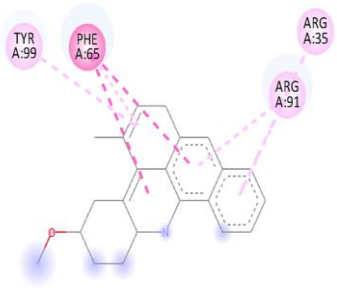
Anti-bacterial medications exert their effects through a variety of mechanisms. These antibiotics are generally designed to target specific cellular proteins responsible for these crucial functions. For instance, consider Thymidylate kinase (TMPK), an enzyme critical in the creation of bacterial DNA's dTTP component**.** In the case of ciprofloxacin, it achieves its effects by inhibiting DNA gyrase, which is essential in unwinding bacterial DNA during cell division. As a result, our research focus in this study involves the molecular interaction of target protein (TMPK) with two most active compounds (Compounds 3 and compound 6). The study was conducted on software Autodock Vina.[12][13] The crystal

structures of Thymidylate kinase (TMPK) (PDB ID: 4QGG) were obtained from the Protein Data Bank (PDB) (www.rcsb.org**).** The resulting docking scores, representing the lowest binding energy (affinity) in Kcal/mol, are listed in Table 3, where compound 3 exhibits the highest docking score of -7.882 Kcal/mol and compound 6 showed docking score of -6.338. The 3D and 2D interactions of compounds and protein was done through Biovia Discovery studio 2021.

Table 3: Docking score of synthesized compounds.

Compound	Grid Center	Grid Size	Docking Score (Lowest Binding Energy) Kcal/mol
3	X 18; Y 0; Z -8	X 29; Y 30; Z 29	-7.882
6	X 20.16; Y -0.12; Z -10.46	X 31; Y 32; Z 32	-6.338

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

Compound	3-D Intraction	2-D intraction	Amino acid involved in interactions
3			LEU (A:75), PRO (A:41), ALA (A:52), THR (A:42)
6			TYR (A:99), PHE (A:65), ARG (A:91) ; ARG (A:35)

RESULT AND DISCUSSION

In this study six analogues of 9-(Substituted-Benzylidene)-2-methoxy-9, 10-dihydro-acridine were synthesized as per the procedure reported in literature. The structures of the compounds were established by their IR and ^{1}HNMR data. The values of spectral data are in good agreement with the literature values. Physical parameters of synthesized analogue are reported in table 1. Screening of the compounds for in vitro antibacterial activity was carried out by cup or well method. Antimicrobial activity showed variable result, activity index ranging from 0.50 to 0.82. Compounds C-6, C3 and C5 showed maximum activity among all the compounds. Maximum activities of the compounds were reported against *B. subtilis* while minimum activity was observed against *E. coli*. Result of antifungal activity was also variable. Maximum activity was shown by compound 6 against *C. albicans* while compound 3 showed maximum activity against *A.niger*. The docking study of two most active compound was studied to see the molecular interaction (table 4). The amino acid involved in interaction of compounds 3 are LEU (A:75), PRO (A:41), ALA (A:52), THR (A:42) and for compound 6 are TYR (A:99), PHE (A:65), ARG (A:91) ; ARG (A:35).

CONCLUSION

In the present study compounds 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, study revealed that in the synthesised compounds, compound bearing 4-OH group and 4-OCH3 group showed more activity as compared to other groups. The docking score of the compounds showed that compound 3 have more binding affinity than compound 6.

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