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STRUCTURE-ACTIVITY INVESTIGATION OF ATYPICAL ACRIDINE DERIVATIVES AS ANTIMICROBIAL AGENTS

Mohamed I. Al-Ashmawi, Mohamed A. El-Sadek, Mohamed A. El-Bermawy, Ashraf K. Mohamed* and Osama I. Al-Sabbagh

*Department of Medicinal Chemistry, *Department of Microbiology, Faculty of Pharmacy, University of Zagazig, Egypt.*

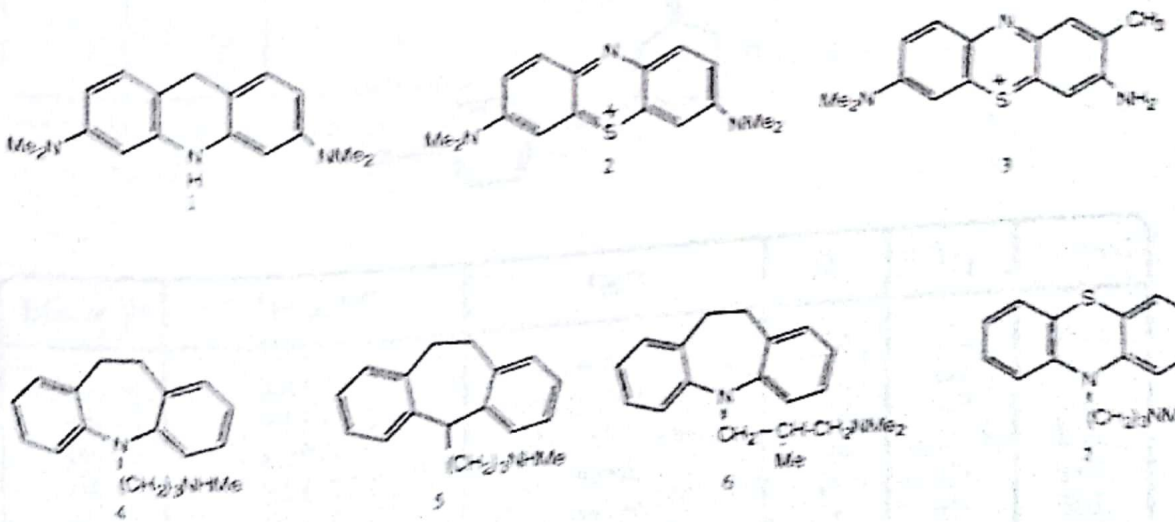
ABSTRACT

Although acridines and many tricylic compounds are known to possese potent antimicrobial activity, yet most of these compounds are either toxic (cannot be used systemically) or have other pharmacological activities. To develop safe and effective agents, we report the design and synthesis of nonclassical acridine derivatives. Biological evaluation of these acridine analogues showed a consistent structure-activity relationship. Some of these acridines showed potent antimicrobial activity, compound **22** exhibited MIC of 7 ug / mL.

INTRODUCTION

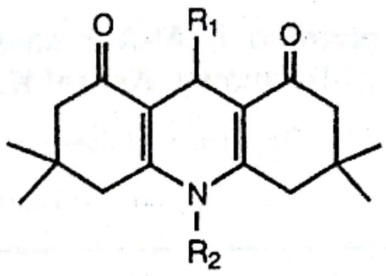
Acridine analogues such as acridine orange **1** and other tricyclic agents, e.g., methylene blue **2** and toluidine blue **3**, have been used externally as antimicrobial agents[1]. In addition, tricylic psychotropic compounds such as desipramine **4**, protriptyline **5**, trimeprazine **6**, promazine **7** and several others possess bacteriosatic activity[2].

These tricyclic compounds, as antimicrobial agents, have some disadvantages. Many of them are toxic if used internally as in the case of methylene blue **2** and acridine orange **1**. Other tricyclic agents produce CNS effects, e.g., promazine **7** and desipramine **4**. To develop useful antimicrobial drugs, the mechanism of action should be fully or even partially known.



Also, consistent structure-activity relationship is essential in order to understand the optimum structure activity requirements needed to develop agents with high selectivity for the target microbial cells[3].

The exact mechanism of antimicrobial action of the known acridines and tricyclic related analogues is not fully understood. One of the proposed mechanisms is the intercalation of these compounds into bacterial DNA[1,3,4]. There are no consistent and reliable structure-activity relationship studies reported to date that can help in understanding the exact requirements for optimal antimicrobial activity.



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Scheme 1:

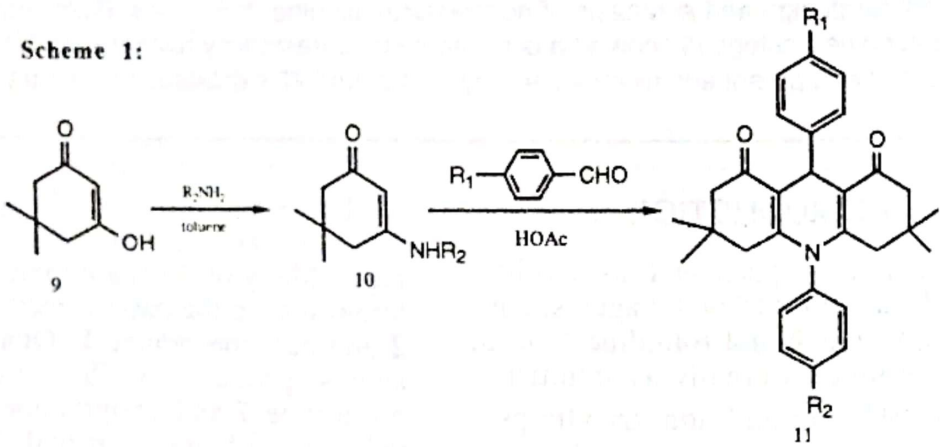
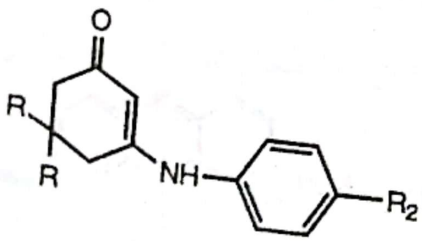


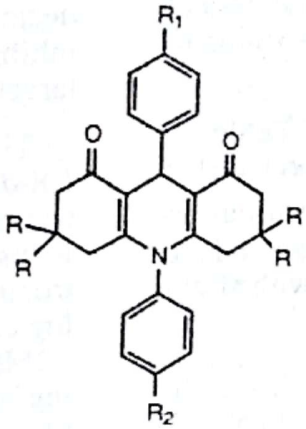
Table (1): Physicochemical data for enaminone derivatives.



Compd.	R^2	R	RS[a]	Mp, °C	% yield
10a	H	Me	Toluene	182-184	82
10b	Cl	Me	aq.[b] EtOH	211-213	87
10c	Br	Me	aq. EtOH	221-223	95
10d	OMe	Me	Toluene	177-179	85
10e	Cl	H	Toluene	193-195	95
10f	Br	H	aq. EtOH	188-190	93

[a] RS: Recrystallization solvent. [b] aq. : aqueous

Table (2): Physicochemical data for hexahydroacridinedione derivatives.



Comp.	R_1	R_2	R	mp, °C	RS*	% yield
11	H	H	Me	242-244[a]	aq. HOAc	80
12	Cl	H	Me	241-243[b]	aq. dioxane	84
13	NO_2	H	Me	262-264[c]	aq. EtOH	86
14	OMe	H	Me	226-228[d]	aq. EtOH	75
15	H	Cl	Me	293-295	aq. HOAc	81
16	H	Br	Me	300-302	aq. HOAc	88
17	H	OMe	Me	216-218	aq. HOAc	74
18	Cl	Cl	Me	285-287	gl. HOAc	81
19	OMe	OMe	Me	214-216	aq. EtOH	77
20	NO_2	Cl	Me	303-305	aq. dioxane	88
21	H	Br	H	256-257	aq. HOAc	78
22	NO_2	Cl	H	299-301	aq. dioxane	82

* RS: Recrystallization solvent. a: Reported in Ref. 7: 236 °C.
b : 233-234 °C[(8)], c : 260- 261°C[(8)], d : 185-187°C.[(8)]

We here report the rational design and synthesis of tricyclic compounds. Biological evaluation of these agents showed that some of the designed hexahydroacridinediones possess excellent antimicrobial effect.

Two positions (9 and 10) of hexahydroacridine-1,8-dione derivatives were subjected to investigation as shown in model **8.** In many tricyclic antimicrobial agents such as acridines and the tricyclic psychotropic agents changes in these positions showed sensitivity towards the bacteriostatic activity[(1-2)]. The proposed model **8** has some advantages, for example it is: 1) tricyclic, like the agents mentioned above 2) rigid 3) close to planar 4) easily manipulated and handled 5) easily synthesized, therefore less costly, and it also gives us the opportunity to conduct conclusive structure-activity studies.

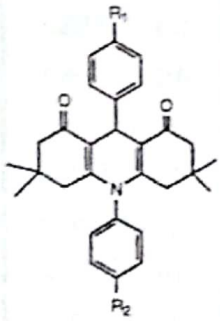
RESULTS AND DISCUSSION

The synthesis of the nonclassical acridine derivatives (**11-22**) is summarized in Scheme 1.

Enaminones **10a - 10f** (Table 1) were synthesized in fairly good yields by reacting dimedone[9] with various aromatic amines using similar methods to the ones previously reported with slight modifications[4,5].

The final products; hexahydroacridinedione analogues **11-22** (Table 2) were prepared from the enaminones **10** by reacting them with bimolecular ratios of substituted aromatic aldehydes. This provides the final products **11-22** in better yields than previously reported[7-9].

Table (3): Microbiological data for hexahydroacridinedione derivatives.



Comp.	R_1	R_2	MIC*
11	H	H	3.25
12	Cl	H	0.055
13	NO_2	H	0.031
14	OMe	H	0.063
15	H	Cl	3.75
16	H	Br	2.20
17	H	OMe	1.50
18	Cl	Cl	0.125
19	OMe	OMe	1.500
20	NO_2	Cl	0.062

* MIC : Minimum Inhibitory Concentration, mg/ mL

The antimicrobial evaluation of hexahydroacridinedione derivatives was conducted on Escherishia coli. The minimum inhibitory concentrations (MIC) for the target compounds are shown in Table 3.

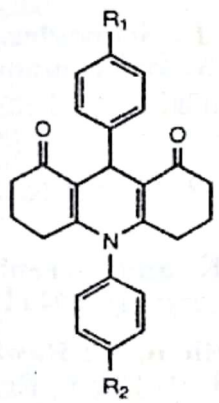
The 9,10-diphenyl-hexahydroacridine-1,8-dione **11** showed a reasonable antibacterial effect (MIC = 3.25 mg/ml). We focused our study on examining the electronic effects in correlation with the biological activity. Electron activation group (OMe), compound **14** has MIC = 0.063 mg/ml. It has fifty two times the activity of compound **11**. The 4-chloro derivative **12** is more potent than the methoxy analgoue **14**. The antimicrobial activity of **12** is fifty nine folds the activity of the parent compound. The most potent agent in this series is the 4-nitrophenyl derivative **13** (MIC = 0.031 mg/ml). The electron withdrawing substituent enhances the bacteriostatic activity one hundred and four times more than the unsubstituted analogue **11.** Substitution at the 4-position of the phenyl ring (**12-14**) enhances bacteriostatic potency. Best results were obtained when the electron deactivating group was present as in compound **13.**

Substituents at 9-phenyl-hexahydroacridine analogues **15-17** were biologically examined. The electron releasing group OMe, as in compound **17,** has a higher activity (MIC = 1.5 mg/ml) than the corresponding unsubstituted derivative **11.** Slightly deactivating substituent, Cl, as in **15** produces a small decrease in antimicrobial activity. The bromine atom at the 4 position, compound **16,** showed slight increase in activity. The bromine atom has higher activation effect on the phenyl group than the chlorine atom.

Combination of substituents at both positions 9 and 10 was also examined. Placing Cl at both p-position of the two phenyl groups **18** enhanced the activity of twenty six times more than the unsubstituted analogue **11.** Replacing the two chlorines with methoxy groups (compound **19**), resulted in enhancement of activity by more than two and half folds. When the nitro group replaced the 4-Cl at the 10-phenyl group

(compound **20**), it produced a high antimicrobial effect. There was a fifty two-fold increase in activity more than the parent molecule **11.**

The geminal methyls at positions 3 and 6 affect the planarity of the acridine nucleus. They also increase the lipophilicity of the system. Therefore, we synthesized an analogue of compound **16** which has MIC of 2.2 mg/ml. Surprizingly, the non-methylated derivative **21** showed 17 fold-increase (MIC = 0.127 mg/ml) in activity more than the corresponding methylated analogue **16**. Compound **20,** which has MIC = 0.062, is one of the best hexahydroacridine derivative in the series mentioned above. Based on the biological difference between the methylated **19** and the non-methylated analogues, we designed the corresponding non-methylated derivative **22**. We assumed that compound **22** should possess an activity of at least seventeen times higher than the methylated-analogue **20;** which has high antimicrobial potency. Luckily, the actual MIC of compound **22** was found to be 0.007 mg/ml, i.e., an increase in potency of 464 times, more than compound **11** and nine times more than compound **20.**



21: R_1 = H, R_2 = Br

22: R_1 = NO_2, R_2 = Cl

Current research is ongoing to explain the effect of the geminal methyl groups at positions 3 and 6 of the hexahydroacridine nucleus. The decrease in the antimicrobial potency of the methylated derivative might be due to: a) altering the planarity of the tricylic structure, b) changing the lipophilicity of the acridine derivatives, c) steric effect of the geminal methyls or d) combination of any or all of the factors mentioned above.

EXPERIMENTAL

Melting points were determined using Electrothermal Digital Melting point apparatus and are uncorrected. Proton magnetic resonance (^{1}HNMR) spectra were recorded on a Varian EM, 90 MHz spectrometer using TMS as internal standard. Infrared (IR) spectra (KBr) were recorded on a Pye Unicam Sp-1100 spectrometer. All spectral data are in agreement with the assigned structures.

Enaminodimedones 10a- 10d[6,7].

Equivalent amounts of dimedone **9** and aromatic amines were heated under reflux in toluene for 4 h. The solution was slightly concentrated then allowed to cool at room temperature. The formed precipitant was filtered, dried and recrystallized from the proper solvent (Table 1). The enamines of cyclohexane-1,3-dione were prepared in a similar fashion (Table 1).

Hexahydroacridine-1,8-diones (11-22):

Acridines **11-22** were synthesized using methods reported before[6-9] with slight modifications as follows. Enamines **(10a-10f)** dissolved in the least amount of glacial acetic acid was mixed with double equivalent amount of various substituted aromatic aldehydes. The mixture was heated under reflux for 4 h, diluted with H_2O (half the amount of acetic acid used), then allowed to cool at room temperature. The resulted solid was isolated by filtration and recrystallized from the appropriate solvent (Table 2).

Microbiological Evaluation:

Agar dilution susceptibility tests:

The determination of MIC for one or more bacterial isolates by different drugs under test was performed in Mueller-Hinton medium which is recommended

for most susceptibility tests. The drug is incorporated into a liquified agar medium (45-50 °C), which is then mixed, poured into petri plates and allowed to solidify[10]. A series of petri plates are prepared with different concentrations of the drug. Five different E. coli isolates were inoculated into each plate. After overnight incubation at 35 °C, the MIC end point is read as the lowest concentration which completely inhibits growth agar dilution test has been found to be comparably reproducible[11,12].

Preparation of antimicrobial plates:

Dilution of the antimicrobial agents were prepared in dimethylformamide (DMF) as they were insoluble in distilled water. The agar medium is then prepared in flasks, or tubes and allowed to cool in a 50 °C water bath. Sufficient volumes are prepared to fill each 9-cm petri plate with 20 ml of agar. The diluted antimicrobial solutions are added to the melted medium in a ratio of 0.1 part antimicrobial agent to 9.9 parts medium (i.e., 0.2 ml drug to 19.8 ml of agar for each petri plate). The medium is then mixed gently by inverting the tube or flask several times and the content is poured into petri plates[10,13]. The plates are then set aside on flat, horizontal surface and allowed to harden. At least one control plate containing the test medium, without drugs, and another control plate containing the DMF to rule out the antibacterial activity of the solvent are prepared for each series of dilution.

Inoculation of test plates:

The density of viable cells in the inoculum is one of the most important variables that influences the results of susceptibility tests. In order to obtain reproducible results, the inoculum density must be carefully standardized. In performing agar dilution susceptibility tests, in general, the inoculum should be applied as a spot that covers a circle about 5-8 mm in diameter and each spot should contain about 1×10^4 viable cells[11-14] of about 1×10^8 colony forming units (CFU)/ml of original culture suspension of microorganism. The broth cultures in the stationary phase of growth were used in adjusting inoculum density. In that growth phase a maximum cell concentration of about 1×10^9 CFU/ml will reach after 12-16 hours of incubation. A 1:10 dilution of such stationary phase broth cultures should yield inocula containing about 1×10^8 CFU/ml.

Incubation of test plates[11]:

The inoculated plates are allowed to stand undisturbed until the spots of inoculum have absorbed completely. The plates are then inverted and allowed to incubate at 35 °C overnight.

REFERENCES

(1) **a- Yamabe, S.:** Arch. Biochem., 154: 19 (1973).

b- Molnar, J., Mandi, Y.; Kiraly; J.: Acta Microbiol. Acad. Sci. Hung. 23: 45 (1976).

(2) **Farkas, S. and Molnar, J.:** Acta Microbiol. Acad. Sci. Hung., 26: 351 (1979).

(3) **Wilson, W.D.; Jones, R.L.;** Adv. Pharmacol and Chemoth., 18: 177 (1981).

(4) **Molnar, J.; Schneider, B.; Mandi, Y.; Farkos, S. and Holland, I.B.;** Acta Microbiol Acad. Sci. Hung., 27: 309 (1950).

(5) **Lerman, L.S.;** J. Mol. Biol., 3: 18 (1961).

(6) **Dixon, K. and Greenhill, J.V.;** J. Chem. Soc. Perkin II: 164 (1974).

(7) **Viswanathan, N.; Rawle, N.N. and Gawad, D.H.;** J. Chem. Res. (M): 2760 (1985).

(8) **Al-Ashmawi, M.I.; Sakr, S.M.; Abdel-Aal, E. and Abou-Sier, A.H.;** Bull. Fac. Phar. Cairo Univ., 29 (3): 9 (1991).

(9) **Al-Ashmawi, M.I.; Abou-Sier, A.H. and Abdel-Aal, E.H.;** Alex. J. Pharm. Sci., 5 (2): 197 (1991).

(10) **Snyder, R.J.; Kohner, P.C.; Ilstrup, D.M. and Washington, J.A.;** Antimicrob. Agents Chemother., 2: 74 (1976).

(11) **Barry, A.L.;** In "Antibiotics in Laboratory Medicine". V. Lorian (ed). Waverly Press, Inc. Williams & Wilkins, Baltimore, pp. 1 -26 (1986).

(12) **Barry, A.L.; Schoenknecht, F.D.; Shadomy, S.; Sherris, J.C.; Thornsberry, C.; Washington, J.A. and Kramer, R.B.;** Curr. Microbiol., 1: 277 (1978).

(13) **Barry, A.L. and Lasner, R.A.;** Antimicrob. Agents Chemother., 2: 549 (1976).

(14) **Ericson, H.M. and Sherris, J.C.;** Acta Pathol. Microbiol. Scand. B., Suppl., 217 (1971).

تشييد مركبات أكريدينية غير تقليدية كمضادات للميكروبات من خلال دراسة العلاقة بين التركيب البنائى لهذه المركبات وفاعليتها البيولوجية

محمد إبراهيم العشماوى - محمد الحسينى الصادق - محمد عبد الشافى البرماوى

أشرف قدرى محمد* - أسامة إبراهيم الصباغ

قسم الكيمياء الطبية - كلية الصيدلة - جامعة الزقازيق

* *قسم الميكروبيولوجى - كلية الصيدلة - جامعة الزقازيق*

من المعروف أن كثير من مشتقات الأكريدين وكذلك المركبات ثلاثية الحلقات لها فاعلية كبيرة كمضادات للميكروبات. ولكن وجد أن معظمها له آثار جانبية ضارة أو غير مستحبه وذلك بسبب عدم توافر العلاقة بين التركيب البنائى. والخواص البيولوجية لهذه المواد. وعلي العكس هذه الدراسة تقدم دراسة كامله عن تصميم وتشييد بعض مشتقات الأكريدين الغير تقليدية والتي أمكن من خلالها إرساء العلاقة بين التركيب الكيميائى والخواص البولوجية لهذه المركبات. بناءاً علي هذه الدراسة تم الوصول إلي مشتقات أكريدينية ذات فاعلية عالية كمضادات للميكروبات.