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Research Communication

ANTIMICROBIAL ACTIVITY OF SYNTHESISED NOVEL N^{10}-ALKYL SUBSTITUTED ACRIDINE-9-ONE DERIVATIVES

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Abstract: *N-Phenyl anthranilic acid was prepared by Ullmann condensation of o-chlorobenzoic acid and aniline. The N-phenyl anthranilic acid was cyclized with polyphosphoric acid over a water bath at 100°C to form acridin-9(10H)-one. N^{10}-Alkylated acridin-9-ones were synthesized in two series, in first series, N^{10}-alkylation with 1-bromo-3-chloro propane and in second series with 1, 2-dichloroethane were done by using tetrabutyl ammonium bromide as phase transfer catalyst. Later both were derivatized by refluxing with different secondary amines in presence of potassium carbonate using anhydrous acetonitrile as solvent. The newly synthesized compounds were purified by column chromatography. The formation of title compounds confirmed by physical and spectral data. The synthesized compounds were subjected to microbiological screening.*

Keywords: *Ullmann condensation, acridin-9(10H)-one, N^{10}-alkyl substituted derivatives, antimicrobial activity.*

Introduction

Acridine and its derivatives constitute an important series of chemotherapeutic drugs and dyestuffs, they are bactericidal and bacteriostatic against both Gram positive and Gram negative organisms[1-2]. Acridin-9-(10H)-one, a derivative of acridine, is an interesting compound of tricyclic nitrogen containing heterocyclic and was first used in 19th century against malaria[3]. Acridone containing alkaloids have been isolated from the bark and leaves of several trees, for instance *Melicopoe fareana* & *Evodia xanthoxyloids*. These trees are found in forests of Queensland (Australia) and the important alkaloids are melicopicine, melicopidine and eroxanthine[4]. Acridone are reported to possess a wide spectrum of biological activities such as antileishmanial, antitumor, anti-HIV, antimicrobial etc. In addition, drug resistance and poor bioavailability are also associated with acridone[5]. We synthesized some newer N^{10}-alkyl substituted acridin-9-ones. In the first series, N^{10}-alkylation with 1-bromo-3-chloropropane and in second series, N^{10}-alkylation with 1,2-dichloroethane were done by using tetrabutyl ammonium bromide as phase transfer catalyst. Later both were derivatized with various secondary amines by refluxing for different time intervals in presence of potassium carbonate using anhydrous acetonitrile as solvent as per scheme (5a-5e, 6a-6b). The synthesized compounds have shown satisfactory spectral data which are in conformity of the proposed structures. All the synthesized compounds were screened for antimicrobial activity[6-9].

Materials and Methods

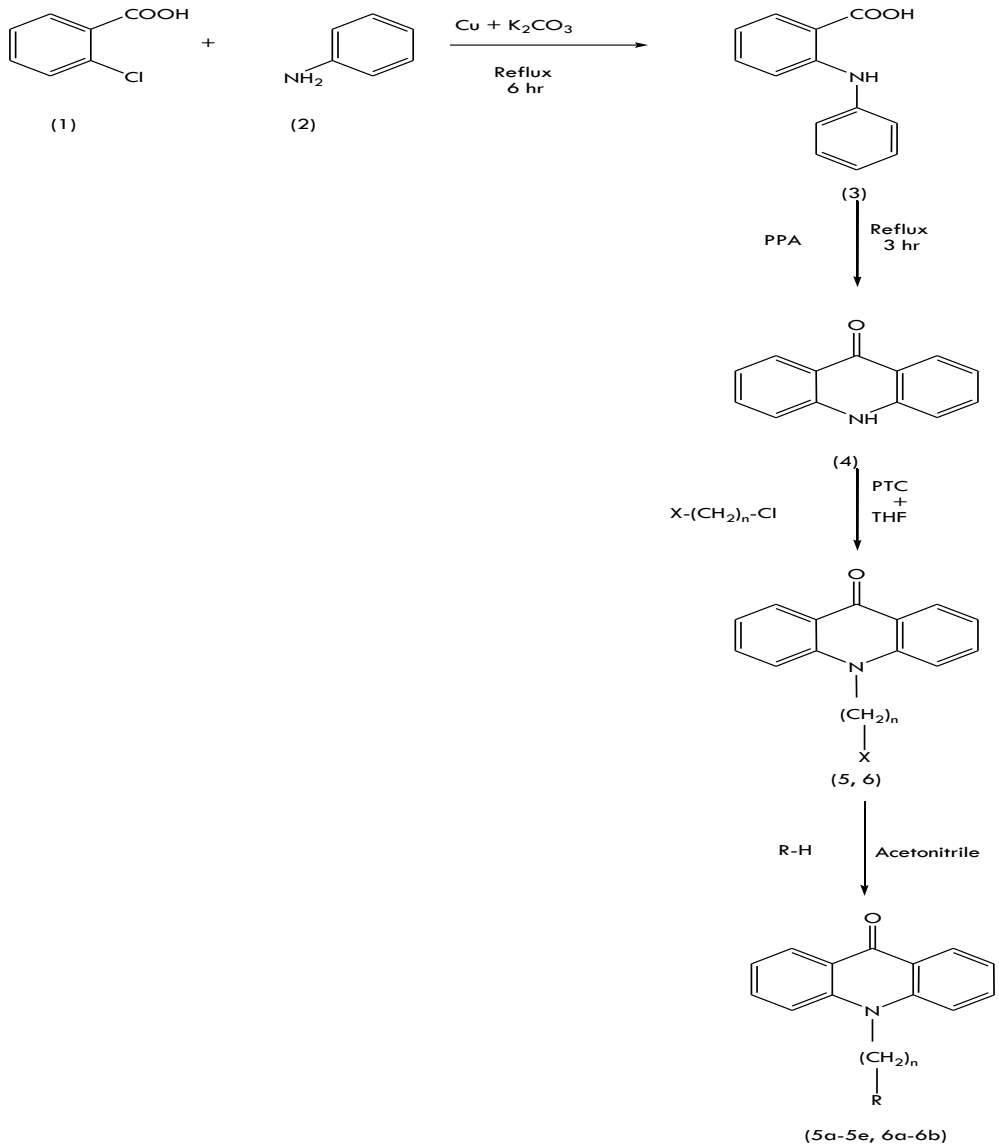
The chemicals and reagents used in the present project were of AR and LR grade, procured from Aldrich, Hi-Media, Merck, Sigma and Ranbaxy. Melting points of the synthesized compounds were determined by open capillary method and were uncorrected. IR spectral analysis was carried out using FTIR-8400S, SHIMADZU, ^{1}HNMR spectral analysis were carried out using instrument amx-400 and the solvent used was deuterated chloroform and dimethyl sulfoxide. The mass spectral data were recorded from LCMS 2010A, SHIMADZU.

N[10]-Alkylated acridine-9-ones were prepared as per procedure discussed in literature[10-11].

Method of preparation of N-phenyl anthranilic acid (3):

Ullmann condensation:

To a mixture of o-chlorobenzoic acid (20g, 0.128mol), aniline (11.8ml, 0.128mol) and copper metal (0.5g) were taken in a 500ml round bottom flask, to which isoamyl alcohol (100ml) was added with stirring. To this mixture dry potassium carbonate (20g) was slowly added with stirring and the reaction mixture was allowed to refluxed for 6 hr in a light liquid paraffin oil bath at 135-140°C.



The isoamyl alcohol was removed by steam distillation and mixture poured into 2L of hot water and acidified with concentrated hydrochloric acid. The bluish black precipitate formed was filtered, washed with hot water & collected. The crude acid was dissolved in aqueous 10% sodium hydroxide solution, boiled in presence of activated charcoal & filtered. On acidification of the filtrates with concentrated hydrochloric acid, light yellowish precipitate was obtained,

which was washed with hot water. The crude acid was recrystallized from aqueous methanol to give a light yellow solid.

Scheme of Synthesis

Compound	X	n	R
5	Cl	3	--
5a	Cl	3	N,N-diethylamine
5b	Cl	3	N,N dimethylamine
5c	Cl	3	N,N diisopropylamine
5d	Cl	3	N,N diphenylamine
5e	Cl	3	pyrrolidin-2-one
6	Cl	2	--
6a	Cl	2	N,N-diethylamine
6b	Cl	2	N,N dimethylamine

Method of preparation of acridin-9-one (4):

N-Phenyl anthranilic acid (18g, 0.084mol) was taken in a 500ml of round bottom flask to which polyphosphoric acid (180g, 0.5327mol) was added, shaken well and refluxed on a water bath at 100°C for 3 hr. Appearance of yellow colour indicated the completion of reaction. Then, it was poured into 2 L of hot water and made alkaline by 25% ammonia solution. The yellow precipitate formed was filtered, washed with hot water and collected. The crude acridin-9(10H)-one was recrystallized from acetic acid.

Method of preparation of 10-(3'-chloropropyl) acridin-9-one (5):

Potassium hydroxide (67.5g) and tetrabutyl ammonium bromide (5g) as a catalyst were taken in a 500ml iodine flask at room temperature. To this mixture acridin-9(10H)-one (10g, 0.051mol) was added with stirring, then tetrahydrofuran (200ml) was added slowly, the reaction mixture was stirred for 30min. 1-Bromo-3-chloro propane (20ml, 0.127mol) was slowly added into reaction mixture and stirred for 40 hr at room temperature. Tetrahydrofuran was evaporated and aqueous layer was extracted with chloroform. The chloroform layer was washed thrice with water, dried over anhydrous sodium sulfate and rotavaporated. The crude 10-(3'-chloropropyl) acridin-9-one was purified by column chromatography by using solvent system chloroform: acetone (8:1) to give a yellow solid.

Method of preparation of 10-[3'-(N, N-diethyl amino) propyl] acridin-9-one (5a):

10-(3'-Chloropropyl) acridin-9-one (5g, 0.0184mol) was taken in 500ml round bottom flask, to which acetonitrile (200ml) was added with stirring. To this mixture potassium iodide (7.5g) and potassium carbonate (12.5g) were added and refluxed for 30min at 60°C. Then N, N-diethylamide (10ml, 0.14mol) was added slowly and refluxed for 18 hr. The contents were cooled, diluted with water and extracted with chloroform. The chloroform layer was washed thrice with water, dried over anhydrous sodium sulfate and rotavaporated. The crude product was purified by column chromatography using the solvent system chloroform: acetone (8:1) to give a light yellow solid of 10-[3'-(N, N-diethylamino) propyl] acridin-9-one.

Method of preparation of 10-[3'-(N, N-dimethylamino) propyl] acridin-9-one (5b):

The procedure used for 5a was repeated with 5g (0.0184mol) of 5, 7.5g of KI, 12.5g of K_2CO_3 and 10g (0.222mol) of N, N dimethylamine by refluxing 16 hr. The product was purified by column chromatography to give a light yellow solid.

Method of preparation of 10-[3'-(N, N-diisopropylamino) propyl] acridin-9-one (5c):

The procedure used for 5a was repeated with 5g (0.0184mol) of 5, 7.5g of KI, 12.5g of K_2CO_3 and 10ml (0.099mol) of N, N diisopropylamine by refluxing 20 hr. The product was purified by column chromatography to give a yellow solid.

Method of preparation of 10-[3'-(N, N-diphenylamine) propyl] acridin-9-one (5d):

The procedure used for 5a was repeated with 5g (0.0184mol) of 5, 7.5g of KI, 12.5g of K_2CO_3 and 10g (0.059mol) of N, N diphenylamine by refluxing 19 hr. The product was purified by column chromatography to give a light yellow solid.

Method of preparation of 10-[3'-(N-pyrrolidin-2''-one) propyl] acridin-9-one (5e):

The procedure used for 5a was repeated with 5g (0.0184mol) of 5, 7.5g of KI, 12.5g of K_2CO_3 and 10ml (0.1175mol) of pyrrolidin-2-one by refluxing 21 hr. The product was purified by column chromatography to give a light yellow solid.

Method of preparation of 10-(2'-chloroethyl) acridin-9-one (6):

Compound 6 in the pure form was synthesized following the procedure used for 5 with 10g (0.051mol) of acridin-9-one, 5g of tetrabutyl ammonium bromide and 1,2-dichloroethane (20ml, 0.2021mol). The crude product was purified by column chromatography to give an yellowish grey solid.

Method of preparation of 10-[2'-(N, N-diethylamino) ethyl] acridin-9-one (6a):

The experimental step used for 5a were repeated by taking 5g (0.0194mol) of 6, 7.5g KI, 12.5 g K_2CO_3 and 10ml (0.14mol) of N,N diethylamine. The product was puriefied by column chromatography to give a light yellow solid.

Method of preparation of 10-[2'-(N, N-dimethylamino) ethyl] acridin-9-one (6b):

The experimental step used for 5b were repeated by taking 5g (0.0194mol) of 6, 7.5g KI, 12.5 g K_2CO_3 and 10g (0.222mol) of N,N dimethylamine. The product was puriefied by column chromatography to give yellowish grey crystal.

Antibacterial activity:

Antibacterial activity of the synthesized compounds was determined by the cup-plate method against the gram-positive organisms *Staphylococcus aureus, Bacillus subtilis* and gram-negative organisms *Escherichia coli, Pseudomonas aeuroginosa, Shigella* at 100µg/ml concentration. The bacteria were subcultured on Nutrient Agar medium. The petridishes were incubated at 37°C for 24hr. Ampicillin (10 mcg/disc) (Std.1) and Ciprofloxacin (30mcg/disc) (Std.2) were used as standards. The results are presented in Table 2.

Antifungal activity:

The antifungal activity of the synthesized compounds was carried out against the fungi *Candida albicans* and *Aspergillus niger* at 100µg/ml concentration. The fungi were subcultured in Sabouraud Dextrose Agar medium. The fungal susceptibility testing was done by cup-plate method using Fluconazole (10 mcg/disc) (Std.1), Amphotericin B (100 units/disc) (Std.2) and Clotrimazole (100 mcg/disc) as std.3. The petridishes were incubated for 48hr at 25°C. The results are presented in Table 2.

Results and Discussion

N-phenyl anthranilic acid (3): (m.p. 181°C), **IR (KBr), CM^{-1}**: 3041.53 (C-H str. aromatic); 1514.02, 1452.30 (C=C str. aromatic); 1658.67 (C=O str.); 3330.84 (N-H str.); 2500-3300 (C=O bend.).

Acridin-9-one (4): (m.p. 340°C); **IR(KBr), CM^{-1}**: 3352.05 (N-H str.); 3097.47, 3062.75, 3031.89 (C-H str. aromatic); 1641.31 (C=O str.); 1535.23, 1473.51 (C=C str. aromatic).

10-(3'-chloropropyl) acridin-9-one (5): (m.p. 180°C); **IR(KBr), CM^{-1}**: 3070.46, 3018.39 (C-H str. aromatic); 2943.17, 2864.09 (C-H str. alkanes); 1610.45 (C=O str.);1556.45, 1492.80 (C=C str. aromatic); 567.03, 543.89 (C-Cl str.); 754.12 (C-H rock methyl); **1H NMR ($CDCl_3$),**: δ 7.1 - 8.5 {m, Ar-H (8H)}; δ 4-6 - 4-8 {t,Hc (2H)}; δ 4.4 - 4.6 {s, Ha (2H)}; δ 1.4 - 1.8 {t, Hb (2H)}; **MS: (m/z)** 272 (M+1), M+2 and others peaks are observed at 273, 254, 236, 197, 196.

10-[3'-(N,N-diethylamino) propyl] acridin-9-one (5a): (m.p. 152°C); **IR(KBr), CM^{-1}**: 3097.47, 3033.82 (C-H str. aromatic); 2993.32, 2948.96 (C-H str. alkanes); 1596.95, 1492.80 (C=C str. aromatic); 1631.67 (C=O str.); 752.19 (C-H rock, methyl); **1H NMR ($CDCl_3$)**: δ 7.1 - 8.5 {m, Ar-H (8H)}; δ 3.6 - 4.0 {t,Ha (2H)}; δ 3.1 - 3.4 {m, Hd (4H)}; δ 2.3 - 2.0 {t, Hc (2H)}; δ 1.7-1.9 {m, Hb (2H)}; δ 0.8 -1.0 {s, He (6H)}; **MS: (m/z)** 308 (M+), M+1, M+2 and other peaks are observed at 309, 310, 249, 200, 181.

10-[3'-(N,N-dimethylamino) propyl] acridin-9-one (5b): (m.p. 220°C); **IR(KBr), CM^{-1}**: 3097.47, 3031.89 (C-H str. aromatic); 2993.32, 2948.96 (C-H str. alkanes); 1598.88, 1531.37 (C=C str. aromatic); 1635.52 (C=O str.); 752.19 (C-H rock, methyl).

10-[3'-(N,N-diisopropylamino) propyl] acridin-9-one (5c): (m.p.170°C); **IR(KBr), CM^{-1}**: 3095.54, 3033.82 (C-H str. aromatic); 2956.67, 2867.95 (C-H str. alkanes); 1598.88, 1494.73 (C=C str. aromatic); 1633.59 (C=O str.); 752.19 (C-H rock, methyl).

10-[3'-(N, N-diphenylamino) propyl] acridin-9-one (5d): (m.p. 240°C); **IR (KBr), CM^{-1}**: 3099.39, 3031.89 (C-H str. aromatic); 2993.32, 2896.88 (C-H str. alkanes); 1598.88, 1488.94 (C=C str. aromatic); 1635.52 (C=O str.); 752.19 (C-H rock, methyl).

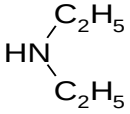
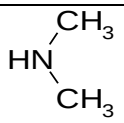
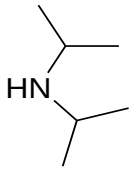
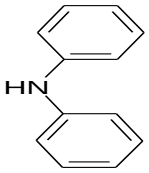
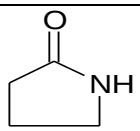
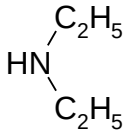
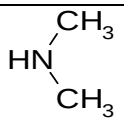
10-[3'-(N-pyrrolidin-2''-one) propyl] acridin-9-one (5e): (m.p. 124°C); **IR (KBr), CM^{-1}**: 3095.54, 3033.82 (C-H str. aromatic); 2995.25, 2869.88 (C-H str. alkanes);1596.95, 1494.73 (C=C str. aromatic); 1631.67 (C=O str.); 750.26 (C-H rock, methyl).

10-(2'-chloroethyl) acridin-9-one (6): (m.p. 160°C); **IR(KBr), CM^{-1}**:3045.39, 3010.67 (C-H str. aromatic); 2960.53, 2873.74 (C-H str. alkanes); 750.26 (C-H rock, methyl); 1573.81, 1487.01 (C=C str. aromatic); 673.11, 551.60 (C-Cl str.); 1625.88 (C=O str.); **1H NMR ($CDCl_3$)**: δ 7.2-8.4 {m, Ar-H (8H)}; δ 3.5--3.9 {m,Hb (2H)}; δ 3.0--3.2 {m, Ha (2H); **MS: (m/z)** 257 (M+), M+1, M+2 and other peaks are observed at 258, 259, 221, 63.

10-[2'-(N, N-diethylamino) ethyl] acridin-9-one (6a): (m.p. 190°C); **IR(KBr), CM^{-1}**: 3095.54, 3031.89 (C-H str. aromatic); 2991.39, 2898.81 (C-H str. alkanes); 1600.81, 1531.37 (C=C str. aromatic); 1635.52 (C=O str.); 750.26 (C-H rock, methyl).

10-[2'-(N,N-dimethylamino) ethyl] acridin-9-one (6b): (m.p. 240°C); **IR(KBr), CM^{-1}**: 3099.39, 3031.89 (C-H str. aromatic); 2993.32, 2896.88 (C-H str. alkanes);1598.88, 1531.37 (C=C str. aromatic); 1635.52 (C=O str.); 752.19 (C-H rock, methyl); **1H NMR ($CDCl_3$)**: δ 7.2-8.5 {m, Ar-H (8H)}; δ 3.2--3.4 {m,Ha (2H)}; δ 2.5-2.7 {m, Hb (2H)}; δ2.1-2.4{s,Hc(6H)}; **MS: (m/z)** 266 (M+), M+1 and other peaks are observed at 267, 199, 159, 71.

Table 1:
Physical Data of the Synthesized Compounds

Compound Code	n	R	Mol. Formula	% Yield	R_f*	M.P.
5	3	--	$C_{16}H_{14}NOCl$	46.69%	0.88	180°C
5a	3		$C_{20}H_{24}N_2O$	44.09%	0.75	152°C
5b	3		$C_{18}H_{20}N_2O$	48.45%	0.72	220°C
5c	3		$C_{22}H_{28}N_2O$	42.00%	0.68	170°C
5d	3		$C_{28}H_{24}N_2O$	48.39%	0.65	240°C
5e	3		$C_{20}H_{20}N_2O_2$	45.84%	0.70	124°C
6	2	--	$C_{15}H_{12}NOCl$	47.04%	0.93	160°C
6a	2		$C_{19}H_{22}N_2O$	49.04%	0.79	190°C
6b	2		$C_{17}H_{18}N_2O$	52.22%	0.74	240°C

*Stationary Phase : Silica Gel G
Mobile Phase : Chloroform: Acetone: 8:1

Antibacterial activity:

Most of the compounds exhibited mild to moderate antibacterial activity against all the microbes (*S.aureus, B.subtilis, E.coli, P.aeuroginosa, Shigella*) tested. All the compounds have shown antibacterial activity as indicated by the diameter of zone of inhibition (Table-2). Among them compound 5C was found to possess highest activity against both Gram positive and Gram

negative organism compared to other derivatives however 5d was found to possess least activity against Gram positive, Gram negative and fungal organisms. The antibacterial activity of 5c (100 µg/ml) was comparable with that of ciprofloxacin (30 mcg/disc) against *E.coli* and *B.subtilis.*

Antifungal activity:

The antifungal activity of the compounds was determined against two fungal species. Most of the compounds showed reasonable antifungal activity against both the strains (C.*albicans, A.niger*) tested. The antifungal activity of 5a (100 µg/ml) against *C.albicans* was on par with that of fluconazole (10 mcg/disc), amphotericin B (10 units/disc) whereas higher than that of clotrimazole (100 mcg/disc).

Table 2:
Biological Activity Data of the Synthesized Compounds

Compound Code	Zone of Inhibition (in mm)						
	B.subtilis	**S.aureus**	**E.coli**	**P.aeuroginosa**	**Shigella**	**C.albicans**	**A.niger**
5a	17	14	19	18	17	22	13
5b	19	15	22	22	19	18	4
5c	28	20	28	21	20	4	14
5d	13	12	2	5	7	3	3
5e	21	12	7	7	8	1	19
6	NI	NI	3	6	NI	4	4
6a	NI	NI	8	10	12	12	12
6b	NI	NI	21	18	10	7	11
Std 1	3	4	NI	9	6	23	17
Std 2	33	37	30	27	40	31	8
Std 3	--	--	--	--	--	18	23
Control	NI	NI	NI	NI	NI	NI	NI

Note : Average zone diameter in mm of triplicates
NI : No inhibition
Control : DMSO

Conclusion

The derivatives of N^{10}-(3'-chloropropyl) acridin-9-one with various secondary amines, show potent antimicrobial activity when compared to the derivatives of N^{10}-(2'-chloroethyl) acridin-9-one. With these encouraging result, all the synthesized compounds can be further explored for detailed microbiological and pharmacological investigation to arrive at possible newer potent drugs.

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