



Research Article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL N¹⁰-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. N10-Alkylated acridin-9-ones were synthesized in two series, in first series, N10-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, N10-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 organisms¹⁻². 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 malaria³. 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 eroxanthine⁴. 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

acridone⁵. We synthesized some newer N¹⁰-alkyl substituted acridin-9-ones. In the first series, N¹⁰-alkylation with 1-bromo-3-chloropropane and in second series, N¹⁰-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 activity⁶⁻⁹.

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, ^1H NMR 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 literature¹⁰⁻¹¹.

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 reflux for 6 hr in a light liquid paraffin oil bath at 135-140°C.

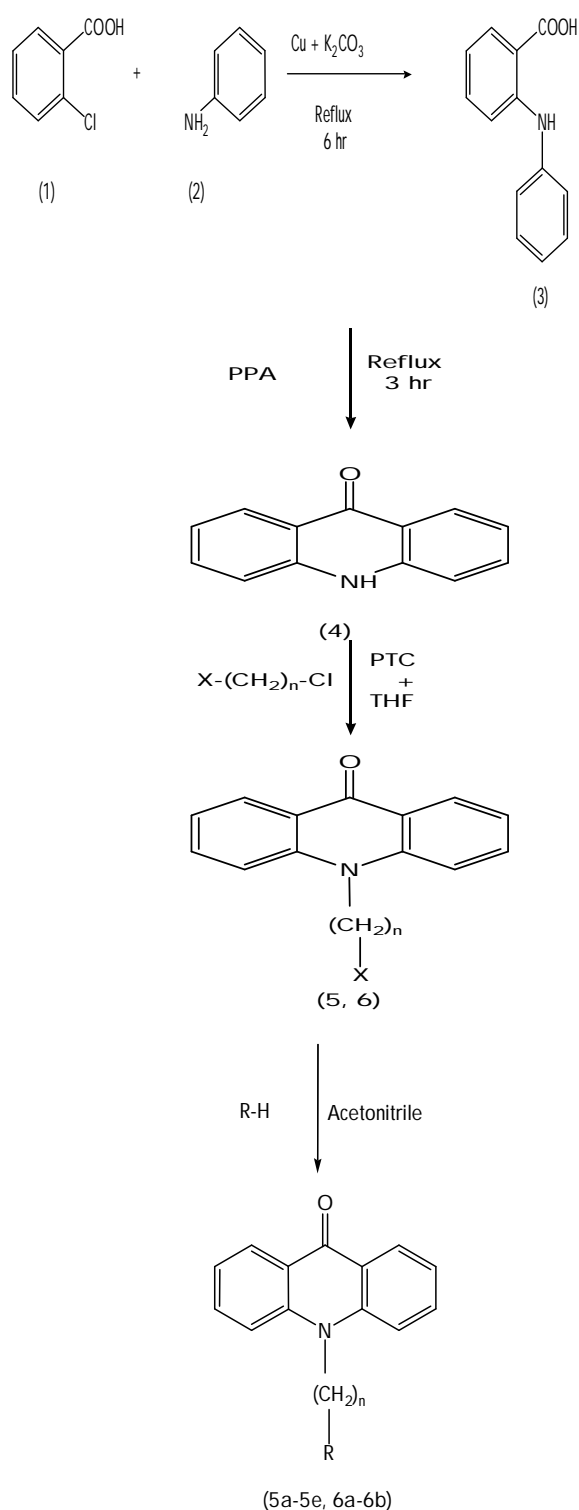
The isoamyl alcohol was removed by steam distillation and mixture poured into 2L of hot water and acidified with concentrated hydrochloric acid. The bluish black precipitate formed was filtered, washed with hot water & collected. The crude acid was dissolved in aqueous 10% sodium hydroxide solution, 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.

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 500 ml iodine



Scheme of Synthesis

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-diethylamino) 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-diethylamine (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 K₂CO₃ 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 K₂CO₃ and 10ml (0.099mol) of N,N diisopropylamine by refluxing 20 hr. The product was purified by column chromatography to give an yellow solid.

Table 1: Derivatives synthesized

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

Method of preparation of 10-[3'-(N,N-diphenylamino) 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 K₂CO₃ 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 K₂CO₃ 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.

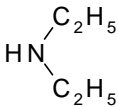
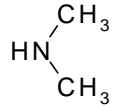
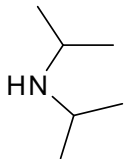
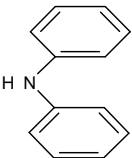
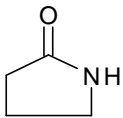
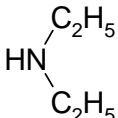
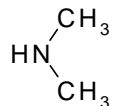
Method of preparation of 10-[2'-(N,N-diethylamino) ethyl] acridin-9-one (6a):

The experimental step used for 5a were repeated by taking 5g (0.0194mol) of 6, 7.5g KI, 12.5 g K₂CO₃ and 10ml (0.14mol) of N,N diethylamine. The product was purified 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 K₂CO₃ and 10g (0.222mol) of N,N dimethylamine. The product was purified by column chromatography to give yellowish grey crystal.

Table 2: 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 ₂ O	44.09%	0.75	152°C
5b	3		C ₁₈ H ₂₀ N ₂ O	48.45%	0.72	220°C
5c	3		C ₂₂ H ₂₈ N ₂ O	42.00%	0.68	170°C
5d	3		C ₂₈ H ₂₄ N ₂ O	48.39%	0.65	240°C
5e	3		C ₂₀ H ₂₀ N ₂ O 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 ₂ O	49.04%	0.79	190°C
6b	2		C ₁₇ H ₁₈ N ₂ O	52.22%	0.74	240°C

*Stationary Phase : Silica Gel G

Mobile Phase : Chloroform : Acetone :: 8:1

Table 3: 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

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 3.

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 3.

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 (CDCl₃):** 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+), 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 (CDCl₃):** 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 (CDCl₃):** 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 (CDCl₃):** 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)}; δ 2.1-2.4 {s, Hc (6H)}; **MS: (m/z)** 266 (M+), M+1 and other peaks are observed at 267, 199, 159, 71.

Antibacterial activity:

Most of the compounds exhibited mild to moderate antibacterial activity against all the microbes (*S.aureus*, *B.subtilis*, *E.coli*, *P.aeruginosa*, *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).

CONCLUSION:

The derivatives of N¹⁰-(3'-chloropropyl) acridin-9-one with various secondary amines, show potent antimicrobial activity when compared to the derivatives of N¹⁰-(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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REFERENCES:

1. John HB, John MB. Wilson and Gisvold's Text book of Organic Medicinal and Pharmaceutical Chemistry. 11th ed. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 1.
2. Bansal RK. Heterocyclic Chemistry. 3rd ed. New Delhi: New Age International (P) Ltd; 1999. p. 360-5.
3. Peter GS, John BT. Comprehensive Medicinal Chemistry: The Rational Design, Mechanistic Study & Therapeutic Applications of Chemical Compounds. Oxford: Pergamon Press; 2005. vol 4 p. 4-5.
4. Bentley PH, O'Hanlon PT. Anti-infectives: Recent Advances in Chemistry and Structure Activity Relationships. Cambridge: The Royal Society of Chemistry; 1996. p. 15.
5. Valerie S, Stephane M, Valerie P, Galy JP. Synthesis of new bis and tetra acridines. Molecules 2001;6:673-82.

6. Pelczer MJ Jr., Chan ECS, Krieg NR. Microbiology. 5th ed. New Delhi: Tata Mc Graw-Hill Publishing Company Ltd; 2004. p. 505, 535-7.
7. Dalby DK. Prescott & Dunn's Industrial Microbiology. 4th ed. New Delhi: CBS Publishers & Distributors; 1999. p. 48-50.
8. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1996;45:493-6.
9. Priya V., Mary A. R., Vajjeyanthi P., Mathiyalagan N., "Pharmacologically active n-chloroisonicotinamide in the kinetic study of oxidation of p-methylbenzaldehyde di-n-butyl acetal", Int. J. Res. Dev. Pharm. L. Sci., 2014, 3(6), pp. 1304-1309.
10. Richard FDA, Lisa H. Evaluation of a rapid inoculum preparation method for agar disk diffusion susceptibility testing. J Clin Micro 1982;15(2):282-5.
11. Hegde R, Thimmaiah P, Yerigeri MC, Krishnegowda G, Thimmaiah KN, Peter JH. Anti-calmodulin acridone derivatives modulate vinblastin resistance in multidrug resistance (MDR) cancer cell. Eur J Med Chem 2004;39:161-77.
12. Krishnegowda G, Thimmaiah P, Hegde R, Dass C, Peter JH, Thimmaiah KN. Synthesis and chemical characterization of 2-methoxy-N10-substituted acridones needed to reverse vinblastin resistance in multidrug resistant (MDR) cancer cells. Bioorg Med Chem 2002; 10: 2367-80.

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