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Bernthsen synthesis, antimicrobial activities and cytotoxicity of acridine derivatives

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ABSTRACT

The condensation reaction of diphenylamine with 2-oxo-2*H*-(substituted chromen)-4-yl acetic acid in presence of anhydrous zinc chloride afford 4-(acridine-9-ylmethyl)-2*H*-(substituted chromen)-2-one. The synthesized compounds were characterized by spectral studies and elemental analysis and screened for their in vitro antibacterial activity against *Staphylococcus aureus*, *Staphylococcus pyogenes* (gram +ve), *Escherichia coli*, *Pseudomonas aeruginosa* (gram −ve) and antifungal activity against *Aspergillus niger* and anticancer activity (HL-60, Hep-2 & HEK293T) by MTT assay. Chloro substituted compounds showed antimicrobial and anticancer activity with IC_{50} values in the low micromolar range.

Acridine-based pharmacophores have wide applications in antimicrobial and anticancer therapy. The discovery of acridines as antimalarial and antitumor agents have attracted the attention of organic chemists and thus led to intensive interest in the synthesis of several drugs based on acridine.[1,2] Acridines are known to be biologically versatile compounds possessing several biological activities.[3,4] Some of the acridine derivatives bearing a heterocyclic/aromatic ring system as one of the substituents have been found to be associated with biological activities. Nitrogen-containing heterocycles are indispensable structural units for medicinal chemists. Many compounds containing the acridine chromophore were synthesized and tested, and the aminoacridines found wide use,[5,6] both as antibacterial agents and as antimalarial. Acridines show a wide spectrum of biological activities such as DNA intercalating agents, anticancer,[7] antitumor,[8] analgesic,[9] anticonvulsant,[10] hypertensive and anti-inflammatory.[11]

The Benzopyrones are a group of compounds whose members include coumarins and flavonoids. Dietary exposure to benzopyrones is quite significant, as these compounds are found in vegetables, fruits, seeds, nuts, coffee, tea and wine. Coumarins have multiple biological activities including disease prevention, growth modulation and anti-oxidant properties.[12] Coumarin is a natural substance that has shown anti-tumor activity in vivo, with the effect believed to be due to its metabolites (e.g., 7-hydroxycoumarin). Based on the survey of recent studies on acridines and coumarins, the focus of our work is synthesis of relevant compounds and their therapeutic importance.

The synthesis of the compounds was carried as outlined in Scheme 1. The compound, (2-oxo-2*H*-(substituted chromen)-4-yl)acetic acid **2**(**a**–**h**) was synthesized by pechmann condensation using various derivative of phenol **1**(**a**–**h**) and obtained as white needle crystals with good 68% yield.[13,14] We report a simple and an efficient one pot synthesis of a few acridine derivatives through the condensation reaction of diphenylamine (**3**) with various (2-oxo-2*H* (substituted chromen)-4-yl)acetic acid **2**(**a**–**h**). Synthesis of 4-(acridin-9-ylmethyl)-2*H*-(substituted chromen)-2-one **4**(**a**–**h**) as a first approach, the reactants (diphenylamine, zinc chloride and (2-oxo-2*H*-(substituted chromen)-4-yl)acetic acid) were mixed in the stoichiometry ratio (1:5:3) used in literature for the conventional heating, by acid-catalyzed Bernthsen synthesis.[15] Molecular formula and structures of the compounds were in accordance with by spectral data and elements analytical data.[16]

All the synthesized compounds were evaluated for their in vitro antibacterial activity against *Staphylococcus aureus*, *Staphylococcus pyogenes* (gram +ve), *Escherichia coli*, *Pseudomonas aeruginosa* (gram −ve) and for their antifungal activity against *Aspergillus niger* by agar plate disk diffusion method and compared with standard drugs. The results are given in Table 1. The synthesized compounds are tested against bacterial species with MIC values ranging from 25 to 1000 μg/mL.[17] While considering all the newly synthesized compounds of this series together, we may conclude that: ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and greseofulvin were use as standard drugs. From the screening results, molecule **4g** (R = H, R^1 = Cl) showed outstanding activity against

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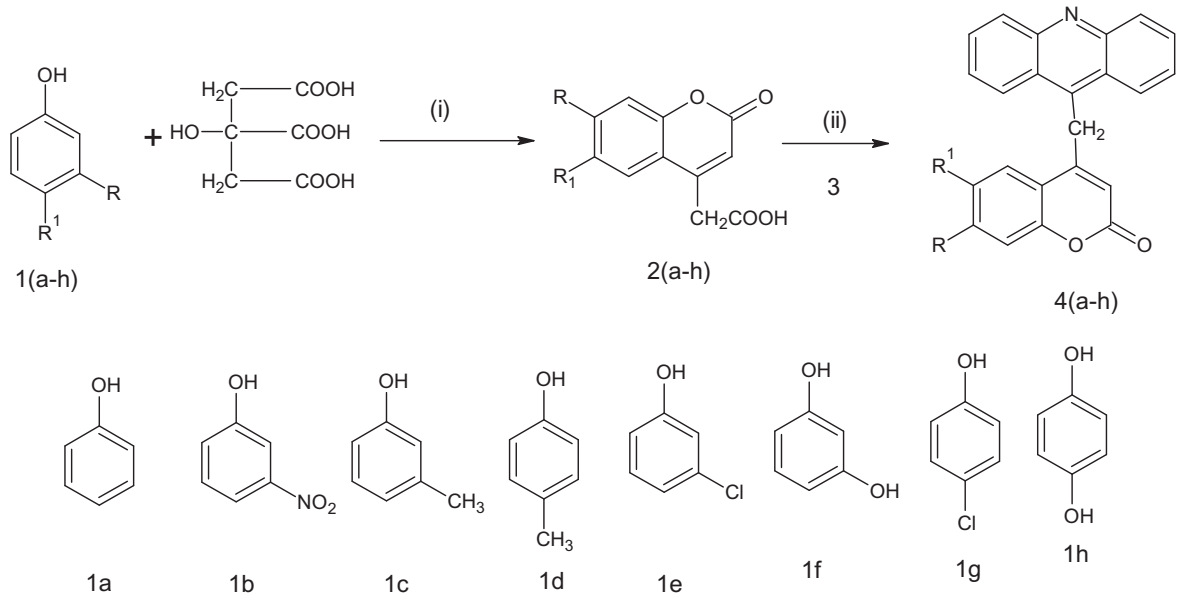

Scheme 1. Reagents and conditions: (i) concd H_2SO_4; (ii) $ZnCl_2$, 230 °C, 20 h.

Table 1
Antimicrobial activity of synthesized compounds (**4a–h**)

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungal
	S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*A. niger*
4a	250	250	250	250	500
4b	250	500	100	200	500
4c	500	50	150	62.5	1000
4d	125	100	125	125	500
4e	50	250	50	500	1000
4f	500	500	200	250	>1000
4g	250	75	75	25	1000
4h	500	250	50	100	250
Amicillin	250	100	100	100	–
Chloramphenicol	50	50	50	50	–
Ciprofloxacin	50	50	25	25	–
Norfloxacin	10	10	10	10	–
Nystatin	–	–	–	–	100
Greseofulvin	–	–	–	–	100

Gram-negative *P. Aeruginosa*, as compared with chloramphenicol and ciprofloxacin (Table 1) whereas compounds **4e** (R = Cl, R^1 = H) and **4h** (R = H, R^1 = OH) displayed brilliant activity against Gram-negative bacteria *E. coli*. Compound **4e** (R = Cl, R^1 = H) showed excellent antimicrobial activity against the gram-positive bacteria *S. Aureus* as compared with ampicillin. Compound **4c** (R = CH_3, R^1 = H) exhibits exceptional activity against gram-positive bacteria *S. Pyogenes* whereas other compounds showed moderate to poor activity against all bacterial strains compared with the standard drugs. All the synthesized compounds showed only moderate anti fungal activity.

In vitro cytotoxicity evaluation of substituted 4-(acridine-9-yl-methyl)-2*H*-(substituted chromen)-2-one (**4a–g**) derivatives were carried out against HL-60 (Human leukemia) and Hep-2 (Human larynx carcinoma) and one normal cell line HEK293T (Human embryonic kidney). The cytotoxicity assay was performed by MTT in Table 2. The MTT Assay is a well-established colorimetric assay, which can be used to detect the effects of agents on cellular metabolism. Derivatives of acridine, compounds **4e** and **4g** showed good cytotoxicity against HL-60 cells. None of the derivatives showed cytotoxicity against Hep-2 cells and HEK293T cells in the compound concentration range of 0.005–100 μM. Cyclophosphamide (CP), a widely used anticancer and immunosuppressive agent, is itself a prodrug, and IC_{50} value of standard anticancer drug (Cyclophosphamide) was evaluated against same cell lines. Cyclophosphamide was more potent against HL-60 (Human leukemia) and compared to Hep-2 (Human larynx carcinoma) and HEK293T (Human embryonic kidney) in vitro.

Table 2
IC_{50} values (μM) of Acridine derivatives on three different cell lines

Sample code	IC_{50} values (μM)		
	Hep-2	HL-60	HEK293T
4a	>100	>100	>100
4b	>100	>100	>100
4c	>100	>100	>100
4d	>100	>100	>100
4e	>100	**27.53**	>100
4f	>100	>100	>100
4g	>100	**28.72**	>100
4h	>100	>100	>100
Cyclophosphamide	>100	**8.79**	>100

* IC_{50} is the concentration of compound required to inhibit the cell growth by 50%.

In conclusion, acridine and coumarin related compounds have proved for many years to have significant therapeutic potential. They come from a wide variety of natural sources and new acridine derivatives are being discovered or synthesised on a regular basis. However, their vital role in plant and animal biology has not been fully exploited. Series of 4-(acridine-9-ylmethyl)-2*H*-(substituted chromen)-2-one have been prepared in one pot synthesis. It is evident from the research described that synthesized compounds are a plentiful source of potential anti-cancer drugs deserving further study.

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16. *Spectral data of compounds*: 4-(acridin-9-ylmethyl)-2*H*-chromen-2-one (**4a**): Yield 68%; mp 250–252 °C; IR (KBr) 2828, 1765, 1455, 1025 cm^{-1}; ^1H NMR(400 MHz, $CDCl_3$) δ 2.57 (s, 2H, $-CH_2-$), 7.00–7.69 (m, 15H, Ar-H); $C_{23}H_{15}NO_2$ requires C, 81.88; H, 4.48; N, 4.15; founds C, 81.85; H, 4.45; N, 4.11. 4-(Acridin-9-ylmethyl)-7-nitro-2*H*-chromen-2-one (**4b**): Yield 72%; mp 280–284 °C; IR (KBr) 2850, 1770, 1459, 1028 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.60 (s, 2H, CH_2-), 7.10–7.89 (m, 14H, Ar-H); $C_{23}H_{14}N_2O_4$ requires C, 2.00; H, 7.59; N, 16.68; founds C, 72.25; H, 7.69; N, 16.74. 4-(Acridin-9-ylmethyl)-7-methyl-2*H*-chromen-2-one (**4c**): Yield 72%; mp 267–270 °C; IR (KBr) 2885, 1768, 1458, 1027 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.30 (s, 3H, CH_3-), 2.65 (s, 2H, CH_2-), 7.15–7.90 (m, 17H, Ar-H); $C_{24}H_{17}NO_2$ requires C, 81.96; H, 4.78; N, 3.90; founds C, 82.03; H, 4.88; N, 3.99. 4-(Acridin-9-ylmethyl)-6-methyl-2*H*-chromen-2-one (**4d**): Yield 72%; mp 250–254 °C; IR (KBr) 2860, 1758, 1460, 1022 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.22 (s, 3H, CH_3-), 2.58 (s, 2H, CH_2-), 7.13–7.80 (m, 17H, Ar-H); $C_{24}H_{17}NO_2$ requires C, 82.00; H, 4.80; N, 3.91; founds C, 82.03; H, 4.88; N, 3.99. 4-(Acridin-9-ylmethyl)-7-chloro-2*H*-chromen-2-one (**4e**): Yield 69%; mp 255–259 °C; IR (KBr) 2838, 1740, 1462, 1015, 810 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.608 (s, 2H, CH_2-), 7.00–7.75 (m, 14H, Ar-H); $C_{23}H_{14}ClNO_2$ requires C,74.17; H, 3.79; N, 3.67; founds C,74.30; H, 3.80; N, 3.77. 4-(Acridin-9-ylmethyl)-7-hydroxy-2*H*-chromen-2-one (**4f**): Yield 70%; mp 250–252 °C; IR (KBr) 3378, 2839, 1770, 1462, 1024 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.89 (s,1H, –OH), 2.39 (s, 2H, CH_2-), 7.03–7.81 (m, 15H, Ar-H); $C_{23}H_{15}NO_3$ requires C, 78.12; H, 4.22; N, 3.90; founds C, 78.17; H, 4.28; N, 3.96. 4-(Acridin-9-ylmethyl)-7-chloro-2*H*-chromen-2-one (**4g**): Yield 69%; mp 277–280 °C; IR (KBr) 2852, 1742, 1454, 1350, 1005 cm^{-1}; ^1H NMR (400 MHz,$CDCl_3$) δ 2.71 (s, 2H, $-CH_2-$), 7.13–7.75 (m, 14H, Ar-H); $C_{23}H_{14}ClNO_2$ requires C, 74.22; H, 3.75; N, 3.64; founds C, 74.30; H, 3.80; N, 3.77. 4-(Acridin-9-ylmethyl)-6-hydroxy-2*H*-chromen-2-one (**4h**): Yield 70%; mp 250–252 °C; IR (KBr) 2927, 1765, 1460, 1030 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1H, –OH), 2.41 (s, 2H, CH_2-), 7.10–8.00 (m, 15H, Ar-H); $C_{23}H_{15}NO_3$ requires C, 78.10; H, 4.23; N, 3.92; founds C, 78.17; H, 4.28; N, 3.96.
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