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Original article

Synthesis, structure and DNA cleavage studies of coumarin analogues of tetrahydroisoquinoline and protoberberine alkaloids

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Dedicated to Professor V.D. Patil on his 75th birthday.

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ABSTRACT

Novel molecular matrices have been derived from coumarin-4-acetic acids and β-phenylethylamines using the Bischler–Napieralski protocol which has led to the synthesis of analogues of tetrahydropapaverine in which the dimethoxybenzene moiety has been replaced by substituted coumarins. One carbon homologation has led to cyclization at the C3 position of coumarin generating the protoberberine skeleton. Structures have been confirmed by diffraction studies. The results showed that compounds **6e**, **6f**, **7e** and **7f** were found to be very effective against DNA samples of Gram positive bacterium *Staphylococcus aureus* and fungus *Aspergillus niger*.

1. Introduction

Coumarin analogues of isoquinoline alkaloids represent an unknown class of heterocyclic templates that originate from the basic framework of biologically active compounds, mimicking the core groups of natural products.

Isoquinolines and protoberberines have been a part of one of the largest groups of alkaloids and have attracted a great deal of interest over the decades due to their potent biological activities [1–3]. Several plants and mammalian species contain simple 1,2,3,4-tetrahydroisoquinolines (THIQs). THIQs are the constituent of several drugs as they exhibit anti-tumor [4], cardiovascular to β-adrenergic receptor antagonistic [5], antispasmodial [6], antitubercular [7], antimicrobial [8–10], antifungal [11], and non-competitive AMPA receptor antagonism [12]. Clinically, papavarine is employed as a vasodilator because of its relaxatory effect on vascular smooth muscle [13]. The protoberberines, tetracyclic isoquinoline alkaloids also display a broad spectrum of biological activities [14,15] and feature predominantly as active components in many folklore medicines especially in China and other Asian countries. A wide range of synthetic methods have been reported for protoberberines and these have been the subject of several reviews [16,17]. Coumarins with diverse structural features and versatile biological properties such as antimicrobial, anticancer, anti-inflammatory and anti-HIV activities have been recently reviewed [18]. DNA gyrase is mainly inhibited by quinolines and coumarins, some of which are widely used for the treatment of bacterial infectious diseases (e.g., ciprofloxacin) [19]. Unfortunately, of late multi-drug resistant Gram positive bacteria have started posing serious issues in medical science to deal with. To overcome the limitations of the known drugs, it has become imperative to identify a new class of compounds.

However, to date, no reports have been dedicated to the synthesis and biological evaluation of coumarin analogues of isoquinoline alkaloids. Herein, in continuation to extend our research on polycyclic compounds of coumarin [20] (Fig. 1) we designed a series of novel coumarin analougues of 1,2,3,4-tetrahydroisoquinolines and protoberberine alkaloids. The synthesized compounds are tested for DNA cleavage against Gram negative *Escherischia coli*, Gram positive *Staphylococcus aureus* and fungus *Aspergillus niger*.

2. Chemistry

As shown in Scheme 1, coumarin-4-acetic acids **1**, were prepared according to literature methods [21]. A coupling component, ethyl

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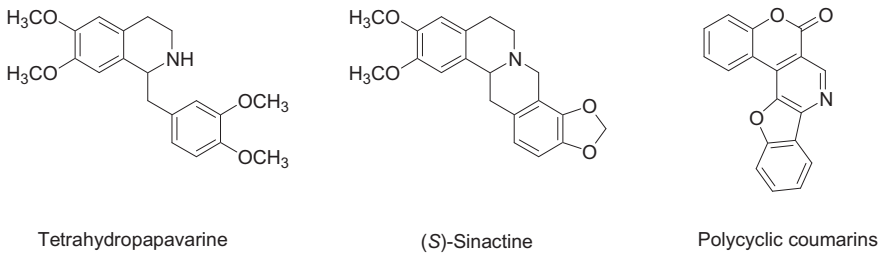


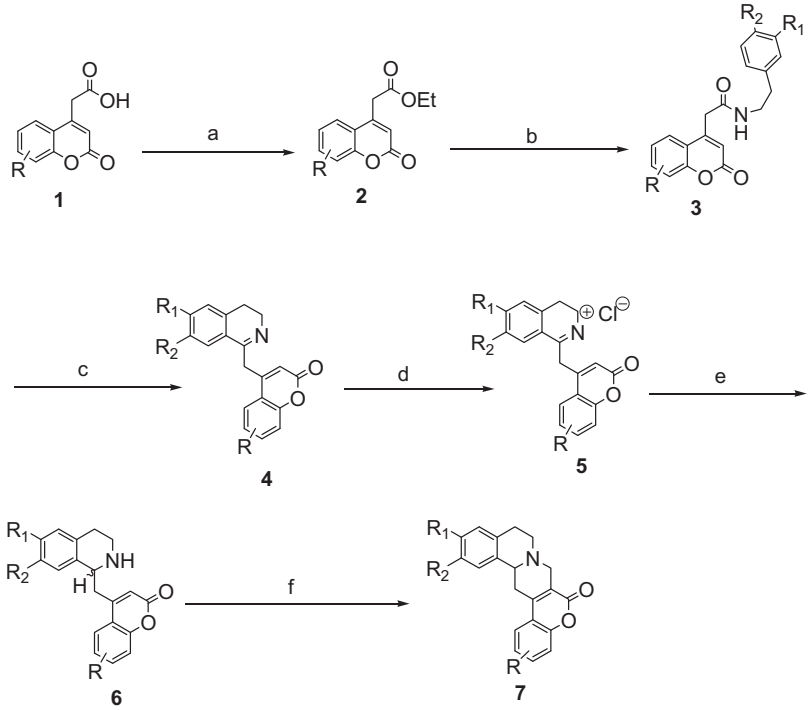
Fig. 1. Some biologically important coumarin and isoquinoline derivatives.

ester of coumarin-4-acetic acids **2**, was prepared from **1** through esterification. The other coupling component 2-phenylethanamine was procured commercially. The formation of the amide bond was accomplished by refluxing the ethyl ester **2** and 2-phenylethanamine in the presence of toluene to furnish **3**. Alternatively, microwave chemistry, a non-conventional popular technique, has been successfully employed in the preparation of **3**, in which ethyl ester **2** reacted with 2-phenylethylamines in solvent free condition in a household microwave oven to form amides **3**. The amide **3** subjected to Bischler–Napieralski reaction in the presence of P_2O_5 as a dehydrating agent afforded 3,4-dihydroisoquinoline **4** as a bright greenish yellow solid. Because the direct reduction of 3,4-dihydroisoquinoline **4** with sodium borohydride was deemed too sluggish, 3,4-dihydroisoquinoline **4** was converted to their corresponding hydrochloride salts **5** to accomplish smooth reduction to yield 1,2,3,4-tetrahydroisoquinoline **6**. Further, the Mannich reaction upon compound **6** led to one carbon homologation through nitrogen–carbon–carbon bond formation to yield **7**, which possesses the carbon skeleton of coumarin analogues of protoberberine alkaloids. The numbering of the skeleton **7** is shown in Fig. 2.

In summary, the synthesis of the coumarin analogues of isoquinoline and protoberberine alkaloids was successfully carried out with two key steps, viz., Bischler–Napieralski reaction to get 3,4-dihydroisoquinolines **4** and at a later stage Mannich reaction to furnish the desired final product **7**. All the synthesized compounds (Table 1) were characterized by spectral analysis like ^1H, ^{13}C, 2D-HETCOR NMR and X-ray crystal diffraction studies wherever necessary.

2.1. X-ray diffraction studies

Single crystals of two molecules [Scheme 1, **6a** and **7a**] were obtained by slow evaporation from methanol solution. Crystals suitable for X-ray studies were selected under a polarizing microscope and then mounted on a BRUKER AXS SMART APEX CCD diffractometer [22] with a crystal to detector distance of 6.06 cm. The data were reduced using SAINT PLUS [22] and the structures were solved by direct methods or SIR92 [23a] and refined using SHELXL [23b] to *R* values of 0.042 and 0.049 respectively. The packing diagrams were generated using CAMERON [24]. Hydrogen



R = 6-Me, 7-Me, 7,8-benzo, 5,6-methylenedioxy; $R_1 = R_2 = H$ and $R_1 = R_2 = OCH_3$

Scheme 1. Synthetic route for the coumarin analogues of 1,2,3,4-tetrahydroisoquinoline **6** and protoberberine alkaloids **7**. Reagents and conditions: (a) ethanol, H_2SO_4, reflux, 24 h; (b) 2-phenethylamines, toluene, reflux, 6 h; (c) P_2O_5, dry xylene, reflux, 12 h; (d) 20% HCl, heat; (e) $NaBH_4$, methanol, RT, 1 h; (f) HCHO/AcOH, reflux, 12 h.

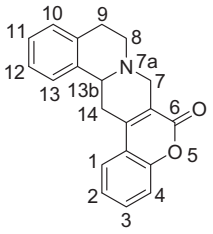


Fig. 2. Numbering of the skeleton.

atoms were located by using difference Fourier map and refined isotropically. However, in cases where location was not possible, the H-atoms were fixed in geometrically calculated positions. The crystallographic information of two structures is available in CCDC [25].

Among the two crystals, compound **6a** crystallizes in an orthorhombic system, space group $P\ 2_12_12_1$, whereas the rest of the molecules crystallize in the centrosymmetric monoclinic system, space group $P\ 2_1/n$. ORTEP [26] diagrams of both the systems are shown in Figs. 3 and 4. All the crystal structures are stabilized via a network of both weak and strong hydrogen bonds with weak intermolecular C–H···π and π···π interactions (see supplementary information; Tables S1–S2 and Figures S1–S2).

3. Pharmacology

The DNA cleavage studies of all the reported coumarin analogues of 1,2,3,4-tetrahydroisoquinolines (**6a**–**6f**) and the corresponding protoberberines (**7a**–**7f**) have been carried out against *S. aureus* 6538, *E. coli* ATCC 35218 and fungus *A. niger* by agarose gel electrophoresis method.

Gel electrophoresis technique works on the migration of DNA under the influence of electric potential. Gel electrophoresis

Table 1
Coumarin analogues of 1,2,3,4-tetrahydroisoquinolines and protoberberine skeletons with two points of structural diversity.

Entry	R	R_1	R_2	Isolated yields (%)
3a	6-CH_3	H	H	83
3b	6-CH_3	OCH_3	OCH_3	80
3c	7-CH_3	H	H	82
3d	7-CH_3	OCH_3	OCH_3	82
3e	7,8-benzo	H	H	76
3f	7,8-benzo	OCH_3	OCH_3	77
3g	6,7-OCH_2O	OCH_3	OCH_3	75
4a	6-CH_3	H	H	75
4b	6-CH_3	OCH_3	OCH_3	50
4c	7-CH_3	H	H	70
4d	7-CH_3	OCH_3	OCH_3	77
4e	7,8-benzo	H	H	67
4f	7,8-benzo	OCH_3	OCH_3	71
4g	6,7-OCH_2O	OCH_3	OCH_3	65
6a	6-CH_3	H	H	74
6b	6-CH_3	OCH_3	OCH_3	78
6c	7-CH_3	H	H	70
6d	7-CH_3	OCH_3	OCH_3	72
6e	7,8-benzo	H	H	74
6f	7,8-benzo	OCH_3	OCH_3	77
7a	2-CH_3	H	H	77
7b	2-CH_3	OCH_3	OCH_3	76
7c	3-CH_3	H	H	70
7d	3-CH_3	OCH_3	OCH_3	74
7e	3,4-benzo	H	H	72
7f	3,4-benzo	OCH_3	OCH_3	76

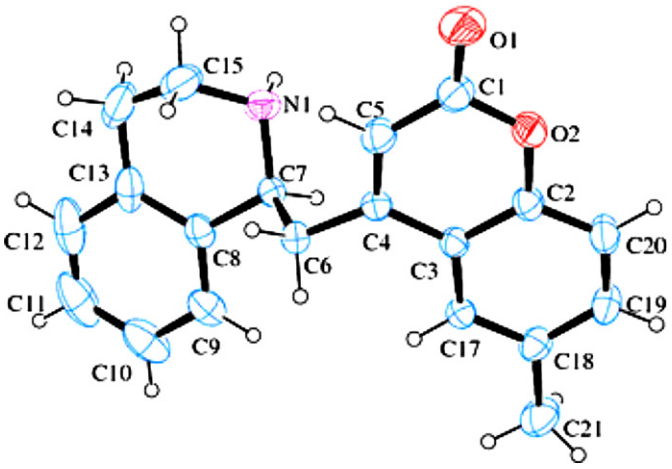


Fig. 3. ORTEP diagram with the displacement ellipsoids at 30% probability level (**6a**).

pictures are shown in Figs. 5–7. The photograph (Fig. 5) shows the molecular weight differences compared to control and is the differentiating criterion for the DNA cleaving ability of the tested compounds with *S. aureus*. Control experiments using DNA alone does not indicate any significant cleavage of DNA even after long exposure time. After marker M and control C, the first six lanes correspond to tetrahydroisoquinolines and in this series the compounds **6a**–**6d** with methyl groups in the coumarin moiety and corresponding to unsubstituted and 6,7-dimethoxy substituted isoquinoline moiety do not show any changes when compared with the control. It is interesting to note that the 7,8-benzo substituted derivates **6e** and **6f** show difference in molecular weight and tailing to a great extent which could be attributed to the DNA cleavage. Even the corresponding pentacyclic protoberberines **7a**–**7d** have yielded the same result. The benzo substituted derivates **7e** and **7f** have clearly showed the absence of the marker band thus providing a proof for their DNA cleaving ability of *S. aureus*.

It is of considerable interest to see that all the twelve compounds **6a**–**6f** and **7a**–**7f** did not show any interaction with the DNA of *E. coli* which shows that these compounds are inactive against Gram negative bacterial species (Fig. 6).

Against fungal DNA of *A. niger*, the lanes corresponding to tetrahydroisoquinolines **6a**–**6d** with methyl groups on the coumarin

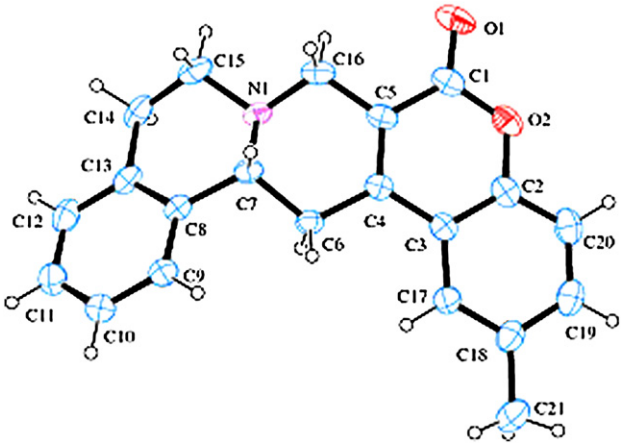


Fig. 4. ORTEP diagram with the displacement ellipsoids at 30% probability level (**7a**).

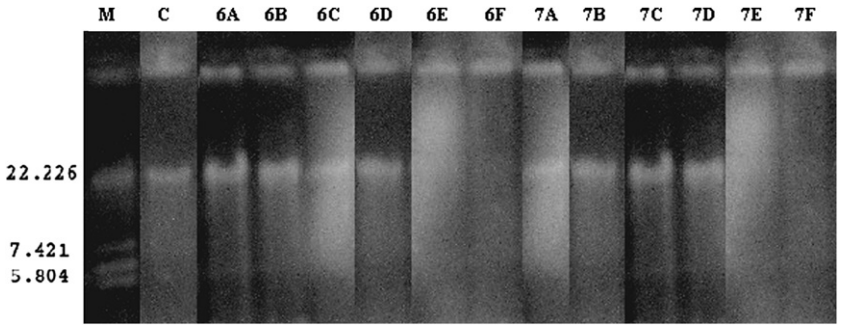


Fig. 5. Gel electrophoresis picture of coumarin analogues of isoquinoline alkaloids. Photograph showing the effect of representative compounds on DNA of *S. aureus*. Lane M: DNA marker, Lane C: untreated DNA, Lanes 6A, 6B, 6C, 6D, 6E, 6F, 7A, 7B, 7C, 7D, 7E and 7F correspond to compounds **6a**, **6b**, **6c**, **6d**, **6e**, **6f**, **7a**, **7b**, **7c**, **7d**, **7e** and **7f** respectively.

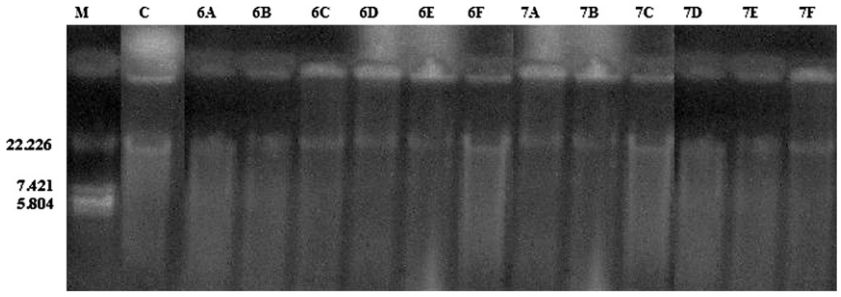


Fig. 6. Gel electrophoresis picture of coumarin analogues of isoquinoline alkaloids. Photograph showing the effect of representative compounds on DNA of *E. coli*. Lane M: DNA marker, Lane C: untreated DNA, Lanes 6A, 6B, 6C, 6D, 6E, 6F, 7A, 7B, 7C, 7D, 7E and 7F correspond to compounds **6a**, **6b**, **6c**, **6d**, **6e**, **6f**, **7a**, **7b**, **7c**, **7d**, **7e** and **7f** respectively.

ring corresponding to unsubstituted and dimethoxy substituted isoquinolines and the corresponding protoberberines **7a**–**7d** show no conspicuous differences in bandwidth or molecular weight indicating their inertness towards the fungal DNA (Fig. 7). The corresponding benzo analogues **6e**, **6f**, **7e** and **7f** were found to exhibit considerable molecular weight differences and tailing and absence of marker which showed that these compounds have the ability to cleave the fungal DNA.

In conclusion it can be seen that compounds exhibit specific affinity for the Gram positive bacterial and fungal species. These structure activity relationship studies also indicate that among the tetrahydroisoquinolines **6a**–**6f** and the polycyclic protoberberines **7a**–**7f**, the 7,8-benzo substitution which is also naturally occurring is associated with significant DNA cleaving activity which is probably due to the angularly fused cyclic skeleton. However, the nature of the reactive intermediates involved in the DNA cleavage by the compounds has not been clear. As these compounds observed to cleave the DNA, it can be concluded that the compounds **6e**, **6f**, **7e** and **7f** inhibit the growth of the pathogenic organisms by cleaving the genome.

4. Conclusion

A series of novel coumarin analogues of 1,2,3,4-tetrahydoisoquinolines and protoberberine alkaloids **6** and **7** were synthesized via intermediates **3**, **4** and **5** were well characterized and evaluated for DNA cleavage studies by agarose gel electrophoresis method against Gram positive bacteria *S. aureus* ATCC 6538, Gram negative bacteria *E. coli* ATCC 35218 and fungus *A. niger*. The results show that compounds **6e**, 6**f**, **7e** and **7f** show selectivity towards the Gram positive bacteria *S. aureus* and *A. niger*. These compounds can act as potent antibacterial agents by genomic cleavage.

5. Experimental

5.1. Analysis and instruments

The melting points were determined by open capillaries on a Buchi-apparatus and are uncorrected. The IR spectra were recorded on a Nicolet-Impact-410 FT-IR spectrometer, using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded respectively,

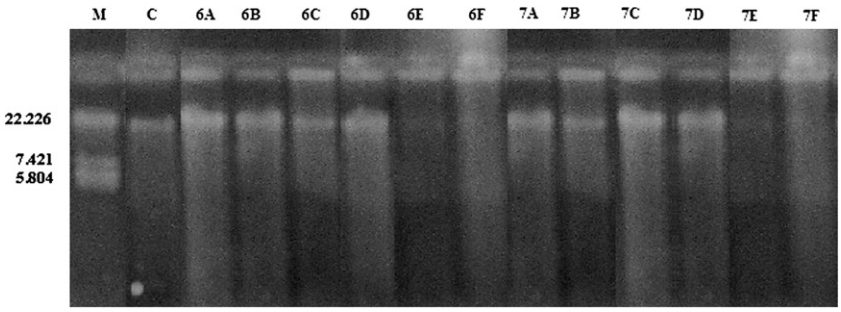


Fig. 7. Gel electrophoresis picture of coumarin analogues of isoquinoline alkaloids. Photograph showing the effect of representative compounds on DNA of *A. niger*. Lane M: DNA marker, Lane C: untreated DNA. Lanes 6A, 6B, 6C, 6D, 6E, 6F, 7A, 7B, 7C, 7D, 7E and 7F correspond to compounds **6a**, **6b**, **6c**, **6d**, **6e**, **6f**, **7a**, **7b**, **7c**, **7d**, **7e** and **7f** respectively.

using Bruker 300 MHz and 75 MHz spectrometer. All the chemicals purchased from Sigma–Aldrich were used without further purification. The mass spectra were recorded using Agilent Single quartz LC MS. The elemental analysis was carried out using Heraus CHN rapid analyzer.

5.2. X-ray studies

X-ray diffraction study was performed on a BRUKER AXS SMART APEX CCD diffractometer.

5.3. Syntheses

5.3.1. General procedure for the preparation of compound (**3**)

Ethyl coumarin-4-acetate (0.002 mol, 1 equiv) was taken in 10 mL of dry toulene. Added 2-phenylethanamine (0.006 mol, 3 equiv) and refluxed for 6 h. Solvent removed under reduced pressure. The residue obtained was taken in a small quantity of alcohol and on cooling a colorless solid separated out. Filtered and the solid was washed several times with cold ethanol. Further the pure product was obtained by recrystallization in hot ethanol.

*5.3.1.1. 2-(6-Methyl-2-oxo-2H-coumarin-4-yl)-N-phenethyl-acetamide (**3a**).* Mp. 178–180 °C, IR (KBr) ν_{max}/cm^{-1}: 3284 (NH), 3082, 2918, 1732 ($\nu_{C=O}$ lactone), 1640 ($\nu_{C=O}$ amide), 1574, 1498, 908, 826 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.42 (s, 3H, C6-CH_3), 2.75 (t, 2H, $J = 6.5$ Hz, Ar-CH_2), 3.53 (t, 2H, $J = 6.5$ Hz, N-CH_2), 3.64 (s, 2H, coumarin-C4-CH_2), 5.81 (s (br), 1H, NH, D_2O exchangeable), 6.28 (s, 1H, C3-H), 7.43 (s, 1H, C5-H), 6.98–7.39 (m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.4, 35.7, 40.9, 41.2, 117.1, 117.4, 118.8, 125.0, 127.0, 128.9, 128.9, 129.0, 129.0, 133.7, 134.8, 138.7, 149.6, 152.2, 160.9, 167.6; LC MS: 322 $[M + 1]^+$; Anal. cald. for $C_{20}H_{19}NO_3$: C, 74.75; H, 5.96; N, 4.36. Found: C, 74.84; H, 5.78; N, 4.48.

5.3.2. General procedure for the synthesis of (**4**)

2-(2-Oxo-2*H*-coumarin-4-yl)-*N*-phenethyl-acetamide (2 g) was taken in 50 mL of dry xylene and 10 g of P_2O_5 as dehydrating agent. This mixture was refluxed for 10 h on an oil bath at 150 °C. Reaction mixture was cooled and carefully poured over 1000 mL ice-cold water. Separated solid was filtered and strongly basified with ammonia. In some cases product was soluble in water then aqueous layer was washed with benzene (20 mL × 2) and strongly basified with ammonia to afford the bright yellow colored solid which was filtered, dried and purified over column chromatography.

*5.3.2.1. 4-(3,4-Dihydro-isoquinoline-1-ylmethyl)-6-methyl-coumarin-2-one (**4a**).* Mp. 148–150 °C, IR (KBr) ν_{max}/cm^{-1}: 1711 cm^{-1}(C=O for lactone carbonyl), 1661 cm^{-1} (C=N); ^1H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H, C6′-CH_3), 2.77 (t, 2H, $J = 7.2$ Hz, C3-CH_2,), 3.78 (t, 2H, $J = 7.2$ Hz, C4-CH_2), 4.21 (s, 2H, α-CH_2), 6.22 (s, 1H, C3′-H), 7.26–7.86 (m, 7H, Ar-H); LC MS: 304 $[M + 1]^+$; Anal. cald. for $C_{20}H_{17}NO_7$ (%): C, 79.19; H, 5.65; N, 4.62. Found: C, 79.87; H, 5.78; N, 4.73.

5.3.3. General procedure for the synthesis of (**6**)

Compound **4** (5 g) was treated with 20% HCl (100 mL) and on heating clear solution was resulted which was filtered and allowed to cool. Colorless to brown crystals separated out in the form of their corresponding hydrochlorides **5**. These were used further without purification.

To the hydrochloride of **5a** (2 g, 0.0058 mol) in methanol (30 mL) was added sodium borohydride (1 g) gradually during 10 min interval. The reaction mixture was stirred at room temperature for 1 h (TLC monitored). The colorless solid separated was filtered, washed with cold water, dried and recrystallized from ethanol.

*5.3.3.1. 6-Methyl-4-(1,2,3,4-tetrahydro-isoquinolin-1-ylmethyl)-coumarin-2-one (**6a**).* Mp. 140–142 °C, IR (KBr) ν_{max}/cm^{-1}: 3349, 3022, 2950, 1703 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.7 (s (br), NH), 2.46 (s, 3H, C6′-CH_3), 2.89 (m, 2H, C4-CH_2), 3.05 (dd, 1H, $J = 10.2$, 4.2 Hz, C3-CH_2), 3.02 (dd, 1H, $J = 8.1$, 3.9 Hz, Cα-CH_2), 3.24 (dd, 1H, $J = 12.0$, 6.3 Hz, Cα-CH_2), 3.40 (dd, 1H, $J = 12.0$, 2.4 Hz, C3-CH_2), 4.41 (dd, 1H, $J = 12.0$, 7.8 Hz, C1-H), 6.41 (s, 1H, C3′-H), 7.18–7.39 (m, 5H, Ar-H), 7.56 (s, 1H, C5′-H), 7.38 (d, 1H, C8′-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.5, 30.0, 39.1, 40.5, 54.9, 116.2, 117.6, 119.4, 124.6, 126.4, 126.5, 127.1, 130.1, 133.2, 134.3, 135.7, 138.1, 152.4, 153.5, 161.2; LC MS: 306 $[M + 1]^+$, 131 [100%]; Anal. cald. for $C_{20}H_{19}NO_2$ (%): C, 78.66; H, 6.27; N, 4.59. Found: C, 78.84; H, 6.37; N, 4.73.

5.3.4. General procedure for the synthesis of (**7**)

6-Methyl-4-(1,2,3,4-tetrahydro-isoquinolin-1-ylmethyl)-coumarin-2-one (1 g, 0.003 mol) was refluxed with 20 mL of formalin and 1 mL of acetic acid for 12 h. Reaction mixture was cooled and basified with liquor ammonia carefully. Violet colored solid separated was column purified using hexane/ethyl acetate (8/2) to afford the colorless crystalline solid 2-methyl-8,9,13b,14-tetrahydro-7*H*-5-oxa-7a-aza-dibenzo[*a*,*j*] anthracene-6-one (0.8 g, 77%).

*5.3.4.1. 2-Methyl-8,9,13b,14-tetrahydro-7H-5-oxa-7a-aza-dibenzo[a,j]anthracene-6-one (**7a**).* Mp. 173–176 °C; IR (KBr) ν_{max}/cm^{-1}: 3056, 2927, 1710; ^1H NMR (300 MHz, $CDCl_3$): δ 2.44 (s, 3H, C2-CH_3), 2.73–2.90 (m, 3H, C9-H, C14-H, C8-H), 3.18–3.26 (m, 2H, C9-H, C8-H), 3.42–3.51 (m, 2H, C14-H, C7-H), 3.76–3.79 (m, 1H, C13b-H), 4.06–4.11 (m, 1H, C7-H), 7.19–7.38 (m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.4, 29.7, 33.4, 51.0, 53.8, 58.4, 117.0, 119.5, 121.8, 123.4, 125.7, 126.6, 126.9, 129.5, 132.2, 134.3, 135.0, 137.1, 146.0, 150.9, 160.6; LC MS: 317 $[M^+]$, 132 [100%]; Anal. cald. for $C_{21}H_{19}NO_2$ (%): C, 79.47; H, 6.03; N, 4.41. Found: C, 79.62; H, 6.18; N, 4.53.

5.4. Bioassay conditions

5.4.1. DNA cleavage experiment

5.4.1.1. Preparation of culture media. DNA cleavage experiments were done according to the literature [27]. Nutrient broth [peptone, 10; NaCl, 10 and Yeast extract, 5 (g/L)] was used for culturing of *E. coli* (Gram negative) and *S. aureus* (Gram positive) and potato dextrose broth [potato, 250; dextrose, 20; in g/L] was used for the culture of *A. niger*. The 50 mL media was prepared, autoclaved for 15 min at 121 °C, 15 lb pressure. The autoclaved media were inoculated with the seed culture. *E. coli* and *S. aureus* were incubated for 24 h and *A. niger* for 48 h at 37 °C.

5.4.1.2. Isolation of DNA. The fresh bacterial culture (1.5 mL) is centrifuged to obtain the pellet which is then dissolved in 0.5 mL of lysine buffer (100 mM Tris pH 8.0, 50 mM EDTA, 10% SDS). To this 0.5 mL of saturated phenol was added and incubated at 55 °C for 10 min. Then centrifuged at 10,000 rpm for 10 min and to the supernatant, equal volume of chloroform:isoamyl alcohol (24:1) and 1/20th volume of 3 M sodium acetate (pH 4.8) were added. Then centrifuged at 10,000 rpm for 10 min and to the supernatant, 3 volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in TAE buffer (10 mM Tris pH 8.0, 1 mM EDTA) and stored in cold condition.

5.4.1.3. Agarose gel electrophoresis. Cleavage products were analysed by agarose gel electrophoresis method [27]. Test samples (1 mg/mL) were prepared in DMF. The samples (50 μg) were added to isolated DNA of *S. aureus*, *E. coli* and *A. niger*. The samples were incubated for 2 h at 37 °C and then 20 μL of the DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into

the electrophoresis chamber wells along standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L) and finally loaded on agarose gel and passed the constant 50 V of electricity for around 30 min. Removed the gel and stained with 10 μg/mL ethidium bromide for 10–15 min and the bands observed under Vilberlourmate Gel documentation system and photographed to determine the extent of DNA cleavage. Then the results are compared with standard DNA marker.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2010.04.041.

References and notes

[1] K.W. Bentley, The Isoquinoline Alkaloids. Harwood Academic Publishers, Amsterdam, 1998, pp. 219–264.
[2] (a) A. Capasso, S. Piacente, N. De Tommasi, L. Rastrelli, C. Pizza, Curr. Med. Chem. 13 (2006) 807–812;
(b) H. Guinudeau, M. Lebouef, A. Cave, J. Nat. Prod. 57 (1990) 1033–1035.
[3] K.W. Bentley, Nat. Prod. Rep. 18 (2001) 148–170.
[4] J.D. Scott, R.M. Williams, Chem. Rev. 102 (2002) 1669–1730.
[5] H. Dong, C.M. Lee, W.L. Huang, S.X. Peng, Br. J. Pharmacol. 107 (1992) 262–268.
[6] V.S. Bernan, D.A. Montenegro, J.D, Korshalla, W.M. Maiese, M. Steinberg, GreensteinD.A, J. Antibiot. 47 (1994) 1417–1424.
[7] P. Kumar, K.N. Dhawan, K. Kishore, K.P. Bhargava, J. Heterocycl. Chem. 19 (1982) 677–679.
[8] H.S. Gunes, B. Ozturk, J. Fac. Pharm. Gazi 18 (2001) 81–88.
[9] K. Iwasa, M. Moriyasu, Y. Tachibana, H. Kim, Y. Wataya, W. Wiegrebe, K. F. Bastow, L.M. Cosentio, M. Kozuka, K. Lee, Bioorg. Med. Chem. 9 (2001) 2871–2884.
[10] A.S. Capilla, M. Romero, M.D. Pujol, D.H. Caignard, P. Renard, Tetrahedron 57 (2001) 8297–8303.
[11] L.T. Lia, Y.-C. Lin, C.J. Wang, M.S. Lin, S. Yen, H. Chen, Bioorg. Med. Chem. Lett. 6 (1996) 1335–1338.
[12] R. Gitto, M.L. Barreca, L.D. Luca, G.D. Sarro, G. Ferreri, S. Quartarone, E. Russo, A. Constanti, A. Chimirri, J. Med. Chem. 46 (2003) 197–200.
[13] T. Uruno, I. Takayanagi, M. Tokunaga, K. Kubota, K. Takagi, Jpn. J. Pharmacol. 24 (1974) 681–686.
[14] D.S. Bhakuni, S. Jain, Protoberberine Alkaloids, , In: The Alkaloids, vol. 28. Academic Press, 1986, pp. 95–99.
[15] K. Iwasa, M. Moriyasu, T. Yamori, T. Turuo, D.-U. Lee, W. Wiegrebe, J. Nat. Prod. 64 (2001) 896–898.
[16] K.W. Bentley, Nat. Prod. Rep. 20 (2003) 342–365.
[17] K.W. Bentley, Nat. Prod. Rep. 19 (2002) 332–356.
[18] M.V. Kulkarni, G.M. Kulkarni, C.-H. Lin, C.M. Sun, Curr. Med. Chem. 13 (2006) 2795–2818.
[19] T.P. Tran, E.L. Ellsworth, J.P. Sanchez, B.M. Watson, M.A. Stier, H.D. Hollis Showalter, J.M. Domagala, M.A. Shapiro, E.T. Joannides, S.J. Grachek, D. Q. Nguyen, P. Bird, J. Sharadendu, A. Yip, C. Ha, S. Ramazeni, X. Wuc, R. Singh, Bioorg. Med. Chem. Lett. 17 (2007) 1312–1320.
[20] I.A. Khan, M.V. Kulkarni, M. Gopal, M.S. Shahabuddin, C.-M. Sun, Bioorg. Med. Chem. Lett. 15 (2005) 3584–3587.
[21] B.B. Dey, K.K. Row, J. Indian Chem. Soc. (1924) 107–124.
[22] Bruker SMART and SAINT. Bruker AXS Inc., Madison, Wisconsin, USA, 1998.
[23] (a) G.M. Sheldrick, SHELXS97 and SHELXL97. University of Göttingen, Germany, 1997;
(b) A. Altomare, G. Cascarano, C. Giacovazzo, A. Gualardi, J. Appl. Crystallogr. 26 (1993) 343–350.
[24] D.J. Watkin, L. Pearce, C.K. Prout, CAMERON. Chemical Crystallography Laboratory, University of Oxford, England, 1993.
[25] Crystal data **6a**: $C_{20}H_{19}N_1O_2$, $M = 305.4$, orthorhombic, space group $P\,2_12_12_1$, $a = 8.357(1)$ Å, $b = 12.208(2)$ Å, $c = 16.021(3)$ Å, $V = 1634.6(1)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.24$ g cm^{-3}, $F(0\,0\,0) = 647.9$, $\mu = 0.080$ mm^{-1}, $R = 0.042$, $wR = 0.101$, GOF = 1.087 for 1375 reflections with $I > 2\sigma(I)$, CCDC 671669. **7a**: $C_{21}H_{19}N_1O_2$, $M = 317.4$, monoclinic, space group $P\,2_1/c$, $a = 10.428(1)$ Å, $b = 20.159(2)$ Å, $c = 7.704(1)$ Å, $\beta = 94.731(2)^\circ$, $V = 1614.1(1)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.31$ g cm^{-3}, $F(0\,0\,0) = 671.9$, $\mu = 0.084$ mm^{-1}, $R = 0.049$, $wR = 0.117$, GOF = 1.072 for 2260 reflections with $I > 2\sigma(I)$, CCDC 671672. The CIF files have been submitted to the Cambridge Crystallographic Data Centre. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).
[26] L.J. Farrugia, J. Appl. Crystallogr. 30 (1997) 565–565.
[27] T.A. Brown, Molecular Biology—A Practical Approach, vol. 1 (1990) pp. 51–52.