Monatshefte für Chemie 137, 1477–1486 (2006)
DOI 10.1007/s00706-006-0537-6

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

New 3,4-Annelated Coumarin Derivatives: Synthesis, Antimicrobial Activity, Antioxidant Capacity, and Molecular Modeling

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Received March 16, 2006; accepted April 6, 2006
Published online October 5, 2006

Summary. The one pot reaction preparation, spectral analysis, and molecular modeling experiments on the new 3,4-annelated coumarin systems with bioactivity associated structural features are described. These provided the insight into the equilibrium of the respective tautomeric forms making possible the reconciliation of previously published spectral data with the structure assignments as well as the correction of erroneously established structures. The synthesized compounds were tested in a standard disk diffusion assay and displayed strong to moderate antimicrobial activity, with a promising new lead prototype compound (7-amino-9-hydroxy-5-oxa-7a,8,11-triazacyclopenta[*b*]phenanthren-6-one (**5**)) possessing greater activity that the antibiotics Ampicillin and Nystatin. Antioxidant capacities of the compounds, determined spectrophotometrically using a phosphomolybdenum method, were greater, and in the case of compound **5** four times the activity of α-tocopherol acetate.

Keywords. 3,4-Annelated coumarin systems; Enamino and imino tautomeric forms; Antimicrobial activity; Antioxidant capacity; One pot synthesis.

Introduction

There has been a dramatic increase in pathogen resistance to both pharmaceutical and agrochemical antimicrobial agents. New prototypes (lead compounds) are needed to address this situation. Coumarins have a variety of bioactivities including anticoagulant, estrogenic, dermal, photosensitizing, antimicrobial, vasodilator, molluscacidal, antihelmintic, sedative and hypnotic, analgesic and hypothermic activity

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[1, 2]. The synthesis of coumarin derivatives has attracted considerable attention of organic and medicinal chemists as these are widely used as fragrances, pharmaceuticals, and agrochemicals [2]. There are reports on efficacies of pure coumarins against *Gram*-positive and *Gram*-negative bacteria as well as fungi [3]. Fungitoxicity of 1,3,4-thiadiazolo[3,2-*a*]-*s*-triazine-5*H*-thiones was previously reported and the structure-activity relationship discussed [4]. Phenols are the most abundant and widely used natural and synthetic antioxidants. Their mechanism of action as antioxidants relies on their ability to transfer their phenolic H-atom to a chain-carrying peroxyl radical at a rate much faster than that at which the chain-propagation step of lipid peroxidation proceeds. *Pratt et al.* [5] introduced 5-pyrimidols and related compounds as novel chain-breaking antioxidants that are more effective than phenols. In continuation of our previous work [6] in this paper we report the one pot synthesis of 3,4-annelated coumarin derivatives containing mentioned activity associated structural features, their elucidation by spectral methods (1D and 2D NMR, IR, UV, HRMS, chemical analysis) and simple chemical transformations, their *in vitro* antimicrobial screening, as well as antioxidant capacity. The position of the equilibrium between tautomeric forms of the synthesized compounds was assessed by spectral means and molecular modeling experiments. Structural misassignments of related compounds previously reported in the literature are brought to attention and corrected.

Results and Discussion

Several new coumarin containing systems (**1**–**7**, Fig. 1) that are expected to possess antimicrobial activity and high antioxidant capacity, having in mind the possible enamino and/or imino tautomeric forms, were prepared in the following one pot reaction manner [7]: an acetonitrile solution of 3-cyano-4-chlorocoumarin and a heteroarylamine (6-amino-3-pyridinol, 1*H*-imidazol-2-amine, 3-amino-5-hydroxypyrazole, 2-amino-5-(ethylthio)-1,3,4-thiadiazole, 2-quinolinamine, respectively, 1:1 mol ratio of starting materials) was reacted at reflux temperature in the presence

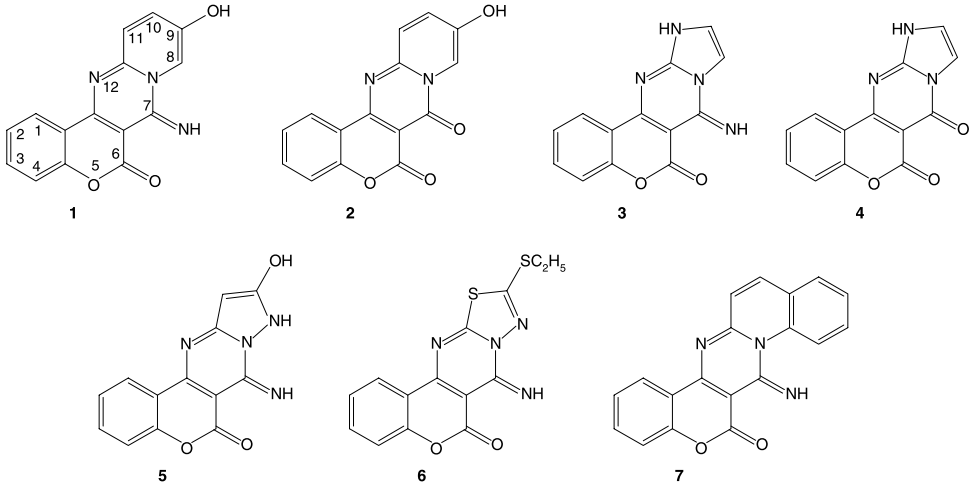

Fig. 1. Structures of the new synthesized 3,4-annelated coumarin derivatives **1**–**7**

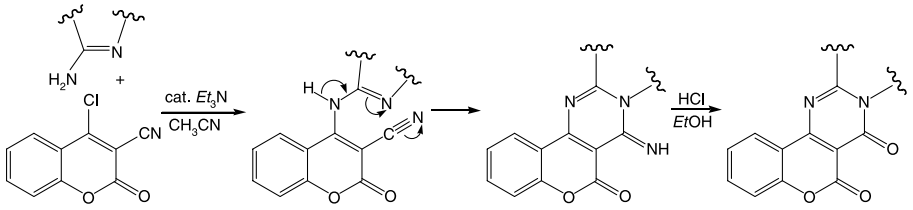

Fig. 2. General reaction sequence leading to the new polyheterocyclic 3,4-annelated coumarin derivatives

of triethylamine as a base catalyst, leading to the formation of an intermediate product (Fig. 2). No efforts were made to isolate that intermediate but instead it was cyclized *in situ* into the desired heteroaromatic products (**1**, **3**, **5**–**7**), presumably by means of a mechanism involving a proton transfer from amino to cyano group with the synchronous building of a pyrimidine ring. In the case of compounds **1** and **3** the obtained polycyclic imine (**1**) and enamine (**3**, see below) were also subjected to acidic hydrolysis giving the corresponding oxoderivatives (Fig. 2).

The structure of compound **1** (Fig. 1), giving a molecular ion at $m/z = 279$ (HRMS (EI): $m/z = 279.0639$ corresponding to the formula $C_{15}H_9N_3O_3$) in MS (EI), was established based on spectral data, especially 1D and 2D NMR. The ^{13}C NMR showed 14 resonances, one conjugated lactone carbonyl ($\delta = 163.0$ ppm), and additional five (C-11a, C-4a, C-12a, C-7, and C-9) signals corresponding to quaternary heteroatom bonded C-sp^2, six aromatic methyne shifts (C-1, C-2, C-3, C-4, C-8, C-10, and C-11, the signals of C-1 and C-4 were accidentally isochronous) (DEPT), and a quaternary C-sp^2 (C-12b) with a high field shift at $\delta = 95.6$ ppm. The ^1H-^1H COSY and HSQC spectra permitted the assignment of the proton shifts of the coumarin moiety and the cross peaks in the HMBC spectrum between H-1 and C-12a and C-12b, as well as H-11 and C-11a corroborated the connectivity of the pyrimidine ring. Protons H-8, H-10, and H-11 formed a spin system typical for a 1,2,4-trisubstituted aromatic ring. An additional proof of **1** was obtained by chemical transformation of the imino function of the pyrimidine ring (giving a band of $\bar{\nu} = 1648\,cm^{-1}$ in the IR) into a carbonyl group. As expected this conversion was smoothly (reaction time was under 0.5 h) achieved with boiling 15% ethanolic solution of HCl leading to compound **2**.

Compound **3** was synthesized starting from 1*H*-imidazol-2-amine and obtained as a bluish crystalline solid. The molecular formula ($C_{13}H_8N_4O_2$) of **3** was deduced from the HRMS (EI) of the M^+ ion at $m/z = 252.0609$ ($\Delta = -3.8$ mmu of the calcd. value). All thirteen carbons were found in the ^{13}C NMR which resembled that of compound **1** except in signals originating from the forth heterocyclic ring. The protons H-8 and H-9 formed an AX spin system with a coupling constant of 3.1 Hz, while the HMBC spectrum showed correlations of H-8 and H-9 with C-10a. The spectral data were in good agreement with those previously published for related systems. However, the ^1H NMR spectrum also showed a broad singlet at $\delta = 9.47$ ppm corresponding to two protons of an $-NH_2$ group indicating the sole existence of the enamino form in the tauromeric equilibrium. This was not completely unexpected since related compounds had the ratio of enamino to imino in favor of the enamino form [8]. In order to address this matter molecular modeling

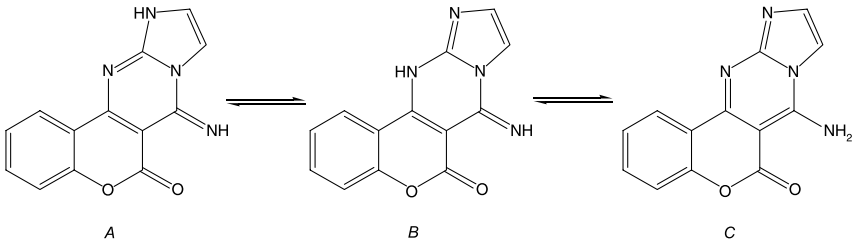

Fig. 3. The structures of tautomeric forms *A*–*C* of compound **3** in equilibrium

experiments on the tautomers of compound **3** were undertaken. The structures of tautomeric forms *A*–*C* (Fig. 3) were optimized to correspond to the global energetic minimum by the use of MM^+ and AM1 force fields (at semiempirical level), with the *Polak-Ribiere* (conjugate gradient) minimization method with an energy convergence criterion of 0.04 kJ/mol, incorporated in the HyperChem 7 software package. The obtained results showed clearly that the enamino form *C* is more thermodynamically stable than the imino form *B* by 43.14 kJ/mol and 154.31 kJ/mol more stable than form *A*, resulting in only trace levels of forms *A* and *B* (the order of magnitude 10^{-6}–10^{-18}%) in the equilibrium mixture. This argument was also reflected in the easiness of hydrolysis to the corresponding, now, phenol derivative compound **4** (in Fig. 1 the keto form is presented). Compared to the hydrolysis of compound **1** to **2** which was accomplished in under 30 min with the yield of 76%, the reaction with the refluxing ethanolic HCl of compound **3** converting it to compound **4** took 24 h and proceeded with lower yield (68%, no starting material could be reisolated from the reaction mixture). The structure of compound **4** was elucidated by spectral means and confirmed the phenolic nature of the tautomeric form (see experimental section) analogous to form *C* of the starting compound **3**. These results seem to contradict the ones published by *Stefanovic-Kaljaj et al.* [9]. They state that the 7-imino-7,12-dihydro-5-oxa-7a,12,13-triazaindeno[1,2-*b*]phenanthren-6-one takes the form of the readily (reaction time unreported) hydrolysable imine, giving no clear assignment to the second (if present at all) N–H proton in the molecule existing in one of the imino tautomeric forms. In light of the before mentioned arguments and previously published results [8] it seems reasonable to expect the enamine tautomeric (*C*-type) form to be a major contributor to the equilibrium if not the predominant one.

Compounds **5**–**7** were characterized and their structures confirmed by the analysis of the 2D NMR spectra (^{1}H-^{1}H COSY, HSQC, and HMBC) as well as DEPT and 1D ^{1}H and ^{13}C NMR which permitted the resolution of some overlapping proton signals. Their structures were also corroborated by comparison of their spectral data (IR, UV, NMR, and MS) to those published in the literature [6, 8]. All of the compounds exhibited M^+ ion peaks in the HRMS (EI) corresponding to their molecular formula. It is obvious that compounds **6** and **7** exist only in the presented imino forms, while compound **5** according to spectral data and molecular modeling is in fact 7-amino-9-hydroxy-5-oxa-7a,8,11-triazacyclopenta[*b*]phenanthren-6-one. Compound **7** was allegedly previously synthesized [8], however, by consideration of the starting material (1-aminoisoquinoline) used by the authors in the same reaction sequence as the current, one concludes that *Govori*

Table 1. The antimicrobial activity (growth inhibition zone, mm, including disk diameter, 6 mm) of the synthesized compounds **1**–**7**

Compound/ Microorganism	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*	*S. enteritidis*	*A. niger*	*C. albicans*
1	16[a]	+26[b]	+21	+20	+20	19	+18
2	17	+23	+17	+18	+19	17	+20
3	16	na	+15	+17	na	+18	na
4	na	na	+13	na	na	+16	na
5	17	18	19	18	16	19	16
6	na	na	na	na	na	+15	na
7	na	na	+16	na	na	+25	na
Ampicillin	15	16	16	16	17	nt	nt
Nystastin	nt	nt	nt	nt	nt	17	19

na Not active; *nt* not tested; [a] bactericidal or fungicidal activity; [b] bacteriostatic or fungistatic activity

Table 2. The total antioxidant capacity of the synthesized compounds **1**–**7**, at the concentration of 100 μg/cm^3, expressed as equivalents of α-tocopherol acetate (μmol/cm^3) and as ratio between the activity of α-tocopherol acetate and that of the synthesized compounds at the same mol/cm^3 concentration

Compound	Equivalents of α-tocopherol acetate (μmol/cm^3)	Antioxidant capacity ratio of the compounds **1**–**7** and α-tocopherol acetate
1	0.5721	1.60
2	0.6161	1.73
3	0.4987	1.26
4	0.9975	2.52
5	1.4668	3.93
6	1.0561	3.49
7	0.9094	2.85

et al. [8] prepared 7-imino-7*H*-5-oxa-7a,14-diazadibenzo[*a*,*j*]anthracen-6-one and not 7-imino-7*H*-5-oxa-7a,14-diazadibenzo[*a*,*h*]anthracen-6-one. Hence, the published structural assignment needs revision.

The results of the antimicrobial and antioxidant assays are presented in Tables 1 and 2.

The compounds showed strong to moderate activity in reducing the microbial growth compared to the standard antibiotics Ampicillin and Nystatin. *E. coli* was the most susceptible microorganism of all to the tested compounds, and *A. niger* among the filamentous fungal organisms. Significant reductions of bacterial growth were obtained in the case of medically important pathogen *P. aeruginosa*. Most of the compounds showed in general considerable bacteriostatic activity, although compounds **6** and **7** (a rather unexpected result for compound **6** [4]) were almost completely inactive towards the tested bacteria, however some marked fungistatic activity against *A. niger*. Compound **5** was the most active and unselective in its action, it superseded the activity of the positive controls in several instances. It

seems that the *Gram*-positive *S. aureus* was equally resistant to the compounds as the *Gram*-negative bacteria, although it is recognized that presence of certain cell wall lipopolysaccharides is to be considered responsible for the greater resistance of *Gram*-negative bacteria [10].

In regard to their antioxidant capacity the synthesized compounds can be arranged in the following decreasing order of activity: **5**>**6**>**7**>**4**>**2**>**1**>**3**. All of the compounds possess greater activity compared to α-tocopherol acetate reaching approximately 4 times increase in the activity in the case of compound **5**. It is evident that the hydrolysis products **2** and **4**, relative to their parent compounds have greater antioxidant activity. That is especially clear in the relationship between compounds **3** and **4** where the hydrolysis reaction generates an additional phenolic group (the major tautomeric form), instead of a carbonyl formed in the conversion of **1** to **2**, although still an extra oxygen atom in the molecule seems to benevolently influence the stability of the formed radical. Similarly, in general, the antioxidant capacity of these highly conjugated systems **1**–**7** follow the expected order of their ability to transfer their acidic H-atom. The obvious exceptions being the sulfur containing compound **6** and pentacyclic compound **7**.

Conclusions

In summary, the preparation, spectral analysis, and molecular modeling experiments on the new 3,4-annelated coumarin systems provided insight into the equilibrium of the respective tautomeric forms making possible the reconciliation of previously published spectral data with the structure assignments as well as the correction of erroneously established structures. The synthesized compounds displayed strong to moderate antimicrobial activity, with compound **5** possessing greater activity than the standard antibiotics Ampicillin and Nystatin making it a promising new lead prototype compound for future studies. Antioxidant capacities of the compounds were greater, and in the case of compound **5** four times the activity of α-tocopherol acetate. Therefore, one can conclude that this one pot reaction can successfully be employed in the preparation of new polyheterocyclic coumarin derivatives having interesting physiological properties.

Experimental

General Methods

Melting points were determined on a *Kofler* hot-plate apparatus and are uncorrected. Microanalysis of carbon, hydrogen, nitrogen, and sulfur were carried out with a Carlo Erba 1106 microanalyser; their results agreed favorably with this calculated values. MS (EI, 70 eV), including HRMS (EI), spectra were recorded on a Finnigan-MAT 8230 BE mass spectrometer. The IR measurements were carried out with a Perkin-Elmer 457 grating FT instrument in KBr tablets. UV spectra (in methanol) were measured on a Perkin-Elmer Lambda 15 UV-VIS spectrophotometer. The NMR spectra were recorded on a Varian Gemini 200 (^{1}H at 200 MHz, ^{13}C at 50 MHz) spectrometer, using *DMSO*-d_6 or D_2O with a few drops of *TFA-d* as the solvents. Chemical shifts are expressed in δ (ppm) using *TMS* (Me_4Si) as an internal standard. 2D experiments (^{1}H-^{1}H COSY, HSQC, and HMBC) and DEPT were run on a Varian Gemini 300 instrument with the usual pulse sequences. For TLC, silica gel plates (Kiesel 60 F_{254}, Merck) were used.

Starting Materials

4-Chlorocoumarin-3-carbonitrile was obtained from 3-hydroxy-4-chlorocoumarin by reaction with $POCl_3$ in dimethylformamide (*DMF*) as the solvent [11]. All other chemicals were commercially available (1*H*-imidazol-2-amine, 2-quinolinamine, 2-amino-5-(ethylthio)-1,3,4-thiadiazole, 3-amino-5-hydroxypyrazole were purchased from Sigma-Aldrich, while 6-amino-3-pyridinol was obtained from Merck) and used as received, except that the solvents were purified by distillation.

General Procedure for the Preparation of 3,4-Annelated Coumarins

The solution of 4-chlorocoumarin-3-carbonitrile (1.0 g, 48 mmol) and the appropriate heteroarylamine (49 mmol) in acetonitrile (30 cm^3) in the presence of catalytic amounts of triethylamine (2 cm^3) was refluxed for 0.5–15 h. After cooling, the precipitate solid was filtered off, washed with acetonitrile, and recrystallized from *DMF*. The purity of the synthesized compounds was checked by TLC.

In the case of compounds **1** and **3**, 15 mmol thereof were heated under reflux (2–24 h) with 15% hydrochloric acid in ethanol (25 cm^3). After cooling, a precipitate was formed which was filtered off and washed with a saturated (5%) sodium bicarbonate solution, and then with water. Recrystalization from *DMF* yielded compounds **2** and **4**.

9-Hydroxy-7-imino-7H-5-oxa-7a,12-diazabenzo[a]anthracen-6-one (**1**, $C_{15}H_9N_3O_3$)

Yield 62%, mp 275–277°C (*DMF*); IR (KBr): $\bar{\nu} = 3302–3210$ (N–H, O–H), 3164 (Ar–H), 1681 (C=O), 1648 (C=N), 1615 (C=C), 1499, 1319, 1154, 1091, 857, 747 cm^{-1}; UV (*Me*OH): $\lambda(\log \varepsilon) =$ 425.6 (4.78), 312.8 (4.93), 255.2 (4.84), 227.2 (4.91) nm. ^{1}H NMR (*DMSO*-d_6): $\delta = 10.24$ (brs, 1H, O–H), 9.89 (brs, 1H, =N–H), 9.00 (d, 1H, H-1), 8.90 (d, 1H, H-10), 7.71 (t, 1H, H-3), 7.45 (m, 2H, H-8, H-11), 7.40 (d, 1H, H-4), 7.25 (t, 1H, H-2) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 163.0$ (s, C-6), 155.0 (s, C-11a), 154.7 (s, C-4a), 153.4 (s, C-12a), 151.7 (s, C-7), 147.4 (s, C-9), 135.4 (d, C-10), 128.8 (d, C-3), 126.1 (d, C-2), 121.6 (d, C-1, C-4), 120.0 (s, C-6a), 118.5 (d, C-11), 118.1 (d, C-8), 95.6 (s, C-12b) ppm; MS (EI): m/z (%) = 279 $[M]^+$ (100), 251 $[M–CO]^+$ (30), 212 (75), 184 (5), 140 (7), 93 (11), 66 (6), 39 (5); HRMS (EI): M^+ ($C_{15}H_9N_3O_3$) $m/z =$ found 279.0639, calcd. 279.0644.

9-Hydroxy-5-oxa-7a,12-diazabenzo[a]anthracene-6,7-dione (**2**, $C_{15}H_8N_2O_4$)

Yield 76%, mp 289–292°C (*DMF*); IR (KBr): $\bar{\nu} = 3350–3320$ (O–H), 1681 (C=O), 1610 (C=C), 1510, 1318, 1154, 1090, 856, 744 cm^{-1}; UV (*Me*OH): $\lambda(\log \varepsilon) = 424.8$ (4.72), 312.8 (4.86), 255.2 (4.76), 216.8 (4.89) nm; ^{1}H NMR (*DMSO*-d_6): $\delta = 10.57$ (brs, 1H, O–H), 8.97 (d, 1H, H-1), 8.85 (d, 1H, H-8), 7.73 (t, 1H, H-3), 7.50 (d, 1H, H-10), 7.42 (d, 1H, H-11), 7.33 (d, 1H, H-4), 7.30 (t, 1H, H-2) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 161.8$ (s, C-6), 155.7 (s, C-12a), 153.3 (s, C-11a), 153.1 (s, C-7), 153.0 (s, C-4a), 146.5 (s, C-9), 134.4 (d, C-10), 134.2 (d, C-3), 133.7 (d, C-8), 127.2 (d, C-1), 124.5 (d, C-2), 119.3 (s, C-6a), 118.0 (d, C-11), 116.4 (d, C-4), 97.3 (s, C-12b) ppm; MS (EI): m/z (%) = 280 $[M]^+$ (87), 279 $[M–H]^+$ (70), 251 $[M–H–CO]^+$ (93), 212 (75), 164 (90), 150 (100), 134 (90), 63 (1); HRMS (EI): M^+ ($C_{15}H_8N_2O_4$) $m/z =$ found 280.0502, calcd. 280.0484.

7-Imino-7,10-dihydro-5-oxa-7a,10,11-triazacyclopenta[b]phenanthren-6-one
(**3**, $C_{13}H_8N_4O_2$) *(7-amino-5-oxa-7a,10,11-triazacyclopenta[b]phenanthren-6-one)*

Yield 55%, mp 244–246°C (*DMF*); IR (KBr): $\bar{\nu} = 3352$ (N–H), 3219 (Ar–H), 1705 (C=O), 1659 (C=N), 1609 (C=C), 1471, 1297, 1189, 1060, 912, 755 cm^{-1}; UV (*Me*OH): $\lambda(\log \varepsilon) = 324.0$ (4.58), 257.6 (4.90), 230.4 (4.74), 214.4 (4.76) nm; ^{1}H NMR (*DMSO*-d_6): $\delta = 9.47$ (brs, 2H, NH_2), 8.43 (d, 1H, H-1), 8.32 (d, 1H, H-8), 7.65 (t, 1H, H-3), 7.42 (t, 1H, H-2), 7.38 (d, 1H, H-4), 6.64 (d, 1H, H-9) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 161.8$ (s, C-6), 152.5 (s, C-4a), 149.7 (s, C-7), 149.1 (s, C-10a), 148.3 (s, C-11a), 133.1 (d, C-9), 130.6 (d, C-3), 124.9 (d, C-2), 122.8 (d, C-1), 118.8 (s, C-6a), 117.1 (d, C-4), 97.2 (d, C-8), 85.4 (s, C-11b) ppm; MS (EI): m/z (%) = 252 $[M]^+$ (10), 250 $[M–2H]^+$ (100), 185 (15), 155 (11), 128 (8), 86 (5), 52 (7); HRMS (EI): M^+ ($C_{13}H_8N_4O_2$) $m/z =$ found 252.0609, calcd. 252.0647.

10H-5-Oxa-7a,10,11-triazacyclopenta[b]phenanthrene-6,7-dione (**4**, $C_{13}H_7N_3O_3$)
(7-hydroxy-5-oxa-7a,10,11-triazacyclopenta[b]phenanthren-6-one)
Yield 68%, mp 238–239°C (*DMF*); IR (KBr): $\bar{\nu} = 3351$ (O–H), 3218 (Ar–H), 1706 (C=O), 1654 (C=N), 1637, 1597 (C=C), 1472, 1213, 1158, 919, 755 cm^{-1}; UV (*Me*OH): $\lambda(\log \varepsilon) = 334.6$ (4.63), 257.5 (4.88), 230.4 (4.64), 210.3 (4.51) nm; ^{1}H NMR (*DMSO*-d_6): $\delta = 9.72$ (brs, 1H, O–H), 8.94 (d, 1H, H-8), 8.44 (d, 1H, H-1), 7.82 (t, 1H, H-3), 7.48 (t, 1H, H-2), 7.39 (d, 1H, H-4), 6.05 (d, 1H, H-9) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 162.0$ (s, C-6), 153.6 (s, C-4a), 152.1 (s, C-7), 149.7 (s, C-11a), 147.3 (s, C-10a), 130.9 (d, C-3), 129.0 (d, C-9), 125.2 (d, C-2), 123.1 (d, C-1), 117.3 (s, C-6a), 117.2 (d, C-4), 103.1 (d, C-8), 85.2 (s, C-11b) ppm; MS (EI): m/z (%) = 253 $[M]^+$ (20), 252 $[M–H]^+$ (100), 224 (5), 186 (10), 157 (11), 115 (3), 76 (5), 36 (23); HRMS (EI): M^+ ($C_{13}H_7N_3O_3$) m/z = found 253.0493, calcd. 253.0487.

9-Hydroxy-7-imino-7,8-dihydro-5-oxa-7a,8,11-triazacyclopenta[b]phenanthren-6-one
(**5**, $C_{13}H_8N_4O_3$) *(7-amino-9-hydroxy-5-oxa-7a,8,11-triaza-cyclopenta[b]phenanthren-6-one)*
Yield 62%, mp 275–278°C (*DMF*); IR (KBr): $\bar{\nu} = 3397–3320$ (N–H, O–H), 3170 (Ar–H), 1715 (C=O), 1630 (C=N), 1604 (C=C), 1534, 1108, 972, 763 cm^{-1}; ^{1}H NMR (*DMSO*-d_6): $\delta = 11.23$ (brs, 1H, O–H), 9.83 (brs, 2H, NH_2), 8.29 (d, 1H, H-1), 7.60 (t, 1H, H-3), 7.37 (m, 2H, H-2, H-4), 5.46 (s, 1H, H-10) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 171.8$ (s, C-9), 161.2 (s, C-6), 152.2 (s, C-4a), 149.9 (s, C-10a), 149.4 (s, C-7), 148.8 (s, C-11a), 132.7 (d, C-3), 124.6 (d, C-1), 124.5 (d, C-2), 118.5 (s, C-6a), 116.8 (d, C-4), 85.2 (s, C-11b), 74.4 (d, C-10) ppm; MS (EI): m/z (%) = 268 $[M]^+$ (22), 239 (52), 212 (100), 182 (32), 113 (71), 76 (73), 43 (78); HRMS (EI): M^+ ($C_{13}H_8N_4O_3$) m/z = found 268.0604, calcd. 268.0596.

9-Ethylsulfanyl-7-imino-7H-5-oxa-10-thia-7a,8,11-triazacyclopenta[b]phenanthren-6-one
(**6**, $C_{14}H_{10}N_4O_2S_2$)
Yield 41%, mp 305–310°C (*DMF*); IR (KBr): $\bar{\nu} = 3368$ (N–H), 3148 (Ar–H), 2980 (C–H), 1710 (C=O), 1653 (C=N), 1617 (C=C), 1554, 1367, 1059, 771, 617 (C–S) cm^{-1}; UV (*Me*OH): $\lambda(\log \varepsilon) =$ 323.2 (4.84), 255.6 (4.28), 232.4 (4.66), 213.1 (4.23) nm; ^{1}H NMR (*DMSO*-d_6): $\delta = 9.74$ (brs, 1H, =N–H), 8.35 (d, 1H, H-1), 7.74 (t, 1H, H-3), 7.45 (t, 1H, H-2), 7.40 (d, 1H, H-4), 3.21 (q, 2H, CH_2), 1.43 (t, 3H, CH_3) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 162.0$ (s, C-10a), 161.3 (s, C-6), 153.8 (s, C-9), 151.0 (s, C-4a), 147.8 (s, C-7), 147.3 (s, C-11a), 133.9 (d, C-3), 125.2 (d, C-2), 124.9 (d, C-1), 117.8 (s, C-6a), 117.3 (d, C-4), 84.1 (s, C-11b), 18.4 (t, CH_2), 15.4 (q, CH_3) ppm; MS (EI): m/z (%) = 330 $[M]^+$ (4), 329 (5), 255 (93), 240 (100), 226 (62), 212 (57), 185 (20), 157 (26), 114 (25), 91 (27), 68 (24), 43 (51); HRMS (EI): M^+ ($C_{14}H_{10}N_4O_2S_2$) m/z = 330.0254, calcd. 330.0245.

7-Imino-7H-5-oxa-7a,14-diazadibenzo[a,h]anthracen-6-one (**7**, $C_{19}H_{11}N_3O_2$)
Yield 58%, mp 310–312°C (*DMF*); IR (KBr): $\bar{\nu} = 3302$ (N–H), 3078 (Ar–H), 1701 (C=O), 1641 (C=N), 1614 (C=C), 1510, 1260, 1075, 869, 762 cm^{-1}; UV (*Me*OH): $\lambda(\log \varepsilon) = 324.0$ (4.26), 259.2 (4.69), 207.2 (4.55) nm; ^{1}H NMR (D_2O, *TFA*): $\delta = 8.21$ (d, 1H, H-1), 7.69 (t, 1H, H-3), 7.44 (m, 2H, H-2, H-4), 7.05 (m, 1H, H-11), 6.90 (m, 1H, H-12), 6.89 (m, 1H, H-9), 6.57 (m, 1H, H-10), 6.41 (brd, 1H, H-8), 5.70 (brd, 1H, H-13) ppm; ^{13}C NMR (D_2O, *TFA*): $\delta = 165.0$ (s, C-6), 163.4 (s, C-7), 158.7 (s, C-4a), 141.5 (s, C-13a), 141.4 (s, C-14a), 138.2 (d, C-12), 135.5 (s, C-7b), 133.2 (d, C-3), 131.6 (d, C-1), 130.7 (m, C-6a, C-11), 129.7 (d, C-2), 127.5 (d, C-9), 123.8 (d, C-4), 121.8 (s, C-11a), 121.4 (d, C-10), 116.2 (d, C-8), 110.5 (d, C-13), 96.2 (s, C-14b) ppm; MS (EI): m/z (%) = 313 $[M]^+$ (100), 285 $[M–CO]^+$ (30), 243 (15), 177 (3), 128 (20), 101 (12), 76 (11), 41 (15); HRMS (EI): M^+ ($C_{19}H_{11}N_3O_2$) m/z = 313.0842, calcd. 313.0851.

Test Microorganisms
The *in vitro* antimicrobial activities of the compounds **1**–**7** were tested against a panel of laboratory control strains belonging to the American Type Culture Collection Maryland, USA: *Gram*-positive: *Staphylococcus aureus* (ATCC 6538), *Gram*-negative: *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enteritidis* (ATCC 13076), and fungal organisms *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 10231), except for the *Gram*-negative

bacteria *Escherichia coli* 95, which was obtained from the Institute of Immunology and Virology "Torlak", Belgrade, Serbia and Montenegro.

Antimicrobial Assay

A disk diffusion method, according to the NCCLS [12], was employed for determination of the antimicrobial activity of compounds **1–7**. The following nutritive media were used: Antibiotic Medium 1 (Difco Laboratories, Detroit Michigan USA) for growing *Gram*-positive and *Gram*-negative bacteria and Tripton soy agar (TSA- Torlak, Belgrade) for *Candida albicans* and *Aspergillus niger*. Nutritive media were prepared according to the instructions of the manufacturer. All agar plates were prepared in 90 mm *Petri* dishes with 22 cm^3 of agar, giving a final depth of 4 mm. 0.1 cm^3 of a suspension of the tested microorganisms (10^8 cells per cm^3) were spread on the solid media plates. Sterile filter paper disks ("Antibiotica Test Blattchen", Schleicher and Schuell, Dassel, Germany, 6 mm in diameter) were impregnated with 50 mm^3 of the solutions of compounds **1–7** in methanol, 1 mg/cm^3, giving 50 μg per disk (all solutions were filter-sterilized using a 0.45 μm membrane filter), and placed on inoculated plates. These plates, after standing at 4°C for 2 h, were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the fungi. Standard disks of Ampicillin and Nystatin (origin – Institute of Immunology and Virology "Torlak", 30 μg of the active component, diameter 6 mm) were used individually as positive controls, while the disks imbued with 50 mm^3 of pure methanol were used as a negative control. The diameters of the inhibition zones were measured in millimeters using a "Fisher-Lilly Antibiotic Zone Reader" (Fisher Scientific Co. USA). Each test was performed in quintuplicate and repeated three times; the results were then analyzed for statistical significance and mean values were selected.

Determination of the Total Antioxidant Capacity

The total antioxidant capacity of the synthesized compounds **1–7** was evaluated by the method of *Prieto et al.* [13]. The antioxidant capacity of their methanol solutions (100 $\mu g/cm^3$) was measured spectrophotometrically using a phosphomolybdenum method, based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate/Mo(V) compounds with a maximum absorption at $\lambda = 695$ nm. A 0.1 cm^3 aliquot of sample solution (100 $\mu g/cm^3$) was combined in an Eppendorf tube with 1 cm^3 of the reagent solution (0.6 *M* sulfuric acid, 28 m*M* sodium phosphate, and 4 m*M* ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each sample was measured at 695 nm against a blank solution, using a Perkin-Elmer Lambda 15 UV-VIS spectrophotometer. A typical blank solution contained 1 cm^3 of reagent solution and the appropriate volume of methanol used for the dissolution of the samples and it was incubated under the same conditions as the other samples. Stock solutions of α-tocopherol acetate were prepared in methanol just prior to use. Exact concentrations were determined spectrophotometrically on the basis of the absorption coefficients from literature. The total antioxidant capacity was expressed as equivalents of α-tocopherol acetate ($\mu mol/cm^3$), as well as the ratio between the activity of α-tocopherol acetate and that of the synthesized compounds at the same $mmol/cm^3$ concentration.

Acknowledgements

The authors acknowledge the Ministry of Science and Environmental Protection of Serbia for financial support.

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Verleger: Springer-Verlag GmbH, Sachsenplatz 4–6, 1201 Wien, Austria. – Herausgeber: Österreichische Akademie der Wissenschaften, Dr.-Ignaz-Seipel-Platz 2, 1010 Wien, und Gesellschaft Österreichischer Chemiker, Eschenbachgasse 9, 1010 Wien, Austria. – Redaktion: Getreidemarkt 9/163-OC, 1060 Wien, Austria. – Satz und Umbruch: Thomson Press Ltd., Chennai, India. – Offsetdruck: Manz Crossmedia, 1051 Wien, Austria. – Verlagsort: Wien. – Herstellungsort: Wien. – Printed in Austria.