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Structure–activity relations of azafluorenone and azaanthraquinone as antimicrobial compounds

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Abstract—Antimicrobial activities of two azafluorenones, four 1-azaanthraquinones, five 2-azaanthraquinones, and one 2-azaquinone were tested. Several azaanthraquinones possessed broad, potent activity, while the azafluorenones demonstrated weak activity. The following structure–activity relationship was postulated: (1) activity decreased in the order 2-azaanthraquinones > 1-azaanthraquinones > azafluorenones; and (2) a hydroxyl group at the peri-carbonyl group enhanced activity. In addition, correlations among reduction potential, hydrophobic parameter, and antimicrobial activity were discussed.
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Quinones (anthraquinones, naphthoquinones, and heteronaphthoquinones) are important, naturally occurring pigments widely distributed in nature, which demonstrate antimicrobial and anticancer activities. An azafluorenone alkaloid, onychine (**1**), occurring in *Cleistopholis patens* (Annonaceae), was active against *Candida*, with a minimum inhibitory concentration (MIC) of 3.12 μg/mL against *C. albicans* B311 in yeast–nitrogen broth.[1] An azaanthraquinone alkaloid, cleistopholine (**3**), was isolated from the same plant.[2] Onychine and cleistopholine are related to diazafluoranthene eupolauridine through a common hypothetical biosynthetic precursor derived from oxoaporphine liriodenine.[3]

Benzo[g]isoquinoline-5,10-dione (**7**) showed potent teratogenic and embryotoxic activity in *Acheta domesticus*[4] and possesses antifungal activity.[5,6]

In studies on the structure–activity relations of drugs, standard redox potential is one of the most important parameters for estimating physiological activity. Cyclic voltammetry was employed to determine the standard redox potentials of 1-and 2-azaanthraquinones at physiological pH 7.2. A definite correlation between standard redox potential and inhibition ($\log IC_{50}$) of EBV-EA activation was reported previously.[7,8] In addition, introduction of the electronic property[7,8] as an additional parameter supports the correlation.

This paper presents preliminary results of the structure–activity relations of several azafluorenones and azaanthraquinones.

Onychine (**1**),[9] 4-methyl-1-azafluorenone (**2**),[9] and cleistopholine (**3**)[10] were synthesized by constructing cycloalkenopyridines using the oxidative thermal rearrangement as previously reported. 9-Hydroxy-4-methyl-1-azaanthraquinone (**4**), 6,9-dihydroxy-4-meth- yl-1-azaanthraquinone (**5**), and 9-hydroxy-3-methyl-1-azaanthraquinone (**6**) were prepared by the Diels–Alder reaction followed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. The Diels–Alder reaction of isoquinoline-5,8-dione (**13**) and cyclohexa-1,3-diene followed by oxidation with Ag_2O and thermal elimination of ethylene gave 2-azaanthraquinone (**7**) in 30% yield from **13**. 6-Methoxy-(**8**) and 9-methoxy-2-azaanthraquinone (**9**) were synthesized from **13** and 1-methoxy-1,3-cyclohexadiene, respectively, in the same manner as above. Demethylation of **8** and **9** with BBr_3 afforded 6-hydroxy-(**10**) and 9-hydroxy-2-azaanthraquinone (**11**) in 80% and 78% yields, respectively.

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Thieno[2,3-*g*]isoquinoline-4,9-dione (**12**) was prepared by tandem-directed metalation reaction[11] of *N*,*N*-diethylisonicotinamide and 3-thiophenecarboxaldehyde (Fig. 1).

Viabilities of compounds (**1**, **3**–**9**, **11**: 32 nmol) for Raji cells were 70%, 70%, 40%, 60%, 30%, 60%, 60%, 60%, and 60%, respectively.[8,12,13]

Compounds **1**–**12** were tested against the representative strains of Gram-positive bacteria (*Staphylococcus aureus* NCTC 8530, *Bacillus subtilis* IFO 3007), Gram-negative bacteria (*Escherichia coli* IFO 3545, *Proteus vulgaris* IFO 3851, *Pseudomonas aeruginosa* IFO 3080), yeasts (*Saccharomyces cerevisiae* IFO 0203, *Schizosaccharomyces pombe* IFO 0342, *Candida utilis* OUT 6020, *Rhodotorula rubra* IFO 0001), and filamentous fungi (*Aspergillus niger* ATCC 6275, *Penicillium chrysogenum* IFO 4626, *Rhizopus chinensis* IFO 4768, *Mucor mucedo* IFO 7684). The MIC was determined by the 2-fold broth dilution method. All compounds were tested at an initial concentration of 100 μg/mL. Each bacterium was cultured in a test tube with 5 mL of peptone medium containing the test compound at 37 °C for 2 days. Yeast and filamentous fungi were cultured on wort at 25 °C for 2 days. The growth of bacteria and yeast was measured by optical density at 600 nm, and that of filamentous fungi was estimated visually.

Cyclic voltammetry was performed by a conventional three-electrode system using a laboratory-constructed microcomputer-controlled system, in which a potentiostat (Hokuto Denko, HA-301) was used to control the working electrode potential. A plastic-formed-carbon (PFC) electrode with surface area = 0.071 cm^2 (BAS, PFCE-3), an Ag/AgCl (saturated NaCl) electrode, and a platinum coil electrode functioned as the working, reference, and counter electrodes, respectively. Before recording each voltammogram, pretreatment of the working electrode was conducted as previously described.[6] The test solution was 2:1 (v/v) phosphate buffer (pH 7.2)-ethanol containing 0.1 mM test compound, and was degassed with prepurified N_2 gas prior to the voltammetric measurements. The electrolytic cell was water-jacketed to maintain the temperature at 25 ± 0.1 °C.

The antimicrobial activities of compounds **1**–**12** are summarized in Table 1. Onychine (**1**) did not possess a strong anticandidal activity as reported by Clark and co-workers.[1] All compounds tested were inactive against *E. coli*. In general, the azafluorenones (**1**, **2**) possessed weak activity. The 2-azaanthraquinones (**7**–**11**) exhibited stronger activity than did the 1-azaanthraquinones and were active against all microorganisms except for *E. coli* and *P. vulgaris*. Hydroxyl derivatives of the 1-azaanthraquinones **4**–**6** were more active than was **3**. Compound **5**, with two hydroxyl groups at the peri-carbonyl position, was the most active compound among the 1-azaanthraquinones. The 2-azaanthraquinones showed the same activity trend as did the 1-azaanthraquinones. 6-Hydroxy (**10**) and 9-hydroxyquinone (**11**) were more active than were **7**–**9**. Among the compounds prepared, **12** was the most potent (0.39–6.25 μg/mL) against the microorganisms tested, except for *E. coli* and *P. vulgaris*.

Cytotoxicity against human A549 cells was also tested for two compounds **7** and **12**.[14] LC_{50} values of **7** and **12** were 1.3 and 0.16 μg/mL, respectively. Compound **7** had less toxicity to A549 cells and antimicrobial activity than **12**. It was found that there was no relationship between viability for Raji cells and antimicrobial activity.

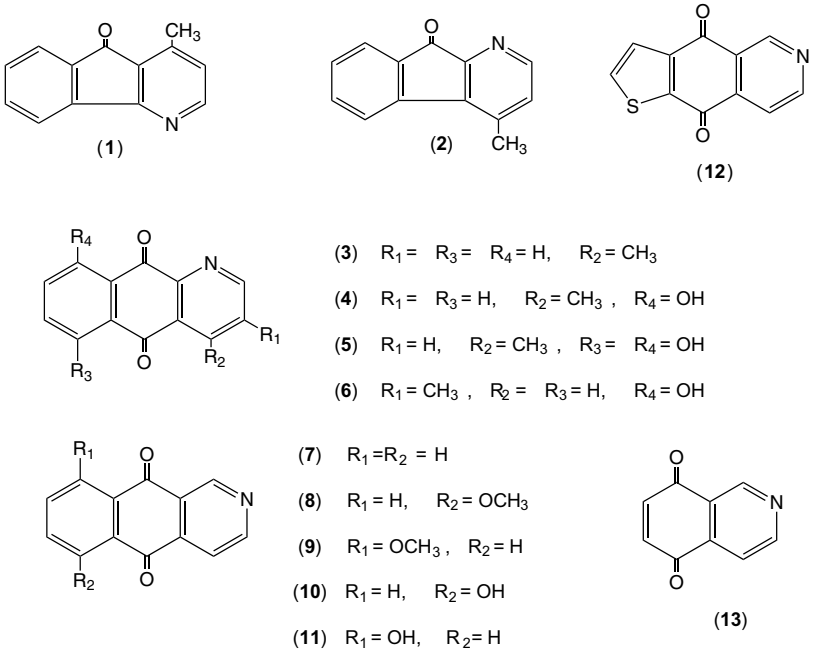

Figure 1. Structures of compounds.

Table 1. Minimum inhibitory concentrations[a] of azafluorenones, 1-azaanthraquinones, and 2-azaanthraquinones

	1	2	3	4	5	6	7	8	9	10	11	12
S. aureus	>100	100	25	12.5	12.5	12.5	6.25	12.5	12.5	1.56	1.56	1.56
B. subtilis	100	50	25	12.5	6.25	6.25	3.13	6.25	3.13	3.13	3.13	6.25
E. coli	>100	>100	>100	>100	>100	>100	100	>100	>100	>100	>100	100
P. vulgaris	100	100	50	50	100	100	50	50	50	25	25	50
P. aeruginosa	100	50	50	25	12.5	25	6.25	6.25	6.25	3.13	3.13	1.56
S. cerevisiae	100	>100	100	100	12.5	100	12.5	25	25	3.13	3.13	1.56
S. pombe	50	100	25	12.5	6.25	12.5	3.13	1.56	3.13	0.20	0.78	0.39
C. utilis	100	100	50	50	12.5	25	12.5	25	25	6.25	12.5	3.13
R. rubra	100	100	50	50	12.5	50	6.25	12.5	6.25	3.13	3.13	1.56
A. niger	100	100	100	>100	50	>100	12.5	12.5	12.5	3.13	3.13	0.78
P. chrysogenum	50	50	50	25	6.25	25	3.13	12.5	12.5	1.56	1.56	0.78
R. chinensis	>100	>100	>100	>100	12.5	>100	12.5	25	25	6.25	12.5	6.25
M. mucedo	>100	100	50	50	12.5	25	6.25	12.5	25	6.25	3.13	3.13

[a] MICs are expressed as μg/mL.

Cyclic voltammograms of **2**, **7**, and **12**, which were obtained at 20 mV s^{-1}, are shown in Figure 2. As represented by the voltammogram for compound **7**, many compounds, except for **2**, **11**, and **12**, had two reduction peaks (the cathodic and anodic peak potentials are summarized in Table 2). However, when voltage scan rate was increased (e.g., up to 200 mV s^{-1}), the second reduction peak became larger than the first reduction peak, and therefore, should be considered an adsorption wave due to the reduction of a pyridine derivative adsorbed at the electrode surface. In contrast, the first reduction peak usually is proportional to the square root of the scan rate, indicating that the peak is limited by diffusion of the pyridine derivative. Unfortunately, the corresponding anodic peak was not detected for some compounds (**1**, **2**, **11**), probably because of the instability of the reduction products. Accordingly, we chose the first reduction peaks at 20 mV s^{-1}, which reflect the ''thermodynamic'' redox potentials, to examine their connections with microorganism MICs.

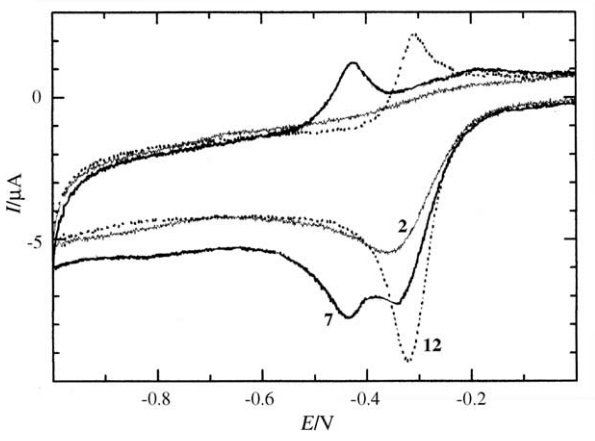

Figure 2. Cyclic voltammograms of azafluorenone and 2-azaanthraquinones.

Furthermore, we calculated some electronic properties of these compounds by the PM3 method using the CAChe MOPAC program[15] and the logarithm of the octanol–water partition coefficient of the molecule (log *P*).[16] Table 3 contains the electronic properties (LUMO energy, HOMO energy, steric energy, and total energy) and the hydrophobic parameter (log *P*). Table 4 shows the results of regression analysis between MIC and first reduction potential ($E_{pc\text{-}1}$). Good to moderate correlations between MIC and $E_{pc\text{-}1}$ were obtained with the exception of activity against *E. coli*, *P. vulgaris*,

Table 2. The first and second cathodic peak potentials ($E_{pc\text{-}1}$ and $E_{pc\text{-}2}$) and the anodic peak potentials (E_{pa}) versus Ag/AgCl (saturated NaCl) obtained at 20 mV s^{-1} for azafluorenones, 1-azaanthraquinones, and 2-azaanthraquinones

Compound	E_{pc-1} (V)	E_{pc-2} (V)	E_{pa} (V)
1	−0.388	−0.788	ND[a]
2	−0.356	ND	ND
3	−0.339	−0.460	−0.447
4	−0.338	−0.488	−0.479
5	−0.330	−0.536	−0.524
6	−0.328	−0.495	−0.485
7	−0.345	−0.432	−0.422
8	−0.320 (sh[b])	−0.373	−0.355
9	−0.324 (sh)	−0.363	−0.353
10	−0.320	−0.456	−0.444
11	−0.350	ND	ND
12	−0.319	ND	−0.303

[a] ND: not detected.
[b] The first reduction peak was obtained as a shoulder at 5 mV s^{-1}.

Table 3. Electronic properties of azafluorenones, 1-azaanthraquinones, and 2-azaanthraquinones

Compound	LUMO (eV)	HOMO (eV)	Steric energy (kcal/mole)	Total energy (hartree)	log *P*
1	−1.199	−9.434	−1.006	−99.191	2.460
2	−1.206	−9.366	3.830	−99.195	2.460
3	−1.477	−10.209	−13.581	−116.660	2.014
4	−1.534	−9.522	−16.951	−121.723	1.262
5	−1.771	−9.009	−12.087	−141.146	1.445
6	−1.659	−9.498	−18.283	−128.913	1.729
7	−1.634	−10.339	−19.074	−109.484	1.147
8	−1.528	−9.641	−15.259	−128.872	0.895
9	−1.533	−9.643	−15.293	−128.859	0.895
10	−1.795	−9.622	−18.591	−121.732	0.863
11	−1.790	−9.609	−18.595	−121.729	0.863
12	−1.914	−10.157	−10.419	−106.178	−0.026

Table 4. Correlation coefficients for MIC of azafluorenones, 1-azaanthraquinones, and 2-azaanthraquinones

	E_{pc-1}	LUMO	HOMO	Steric energy	Total energy	log *P*	Multiple regression with E_{pc-1} and log *P*
S. aureus	0.863	0.799	0.292	0.808	0.624	0.726	0.878
B. subtilis	0.866	0.794	0.229	0.805	0.656	0.737	0.884
E. coli	0.142	0.391	0.703	0.115	0.389	0.494	0.575
P. vulgaris	0.411	0.500	0.536	0.636	0.111	0.687	0.696
P. aeruginosa	0.828	0.814	0.193	0.707	0.554	0.845	0.906
S. cerevisiae	0.495	0.796	0.243	0.665	0.452	0.818	0.827
S. pombe	0.628	0.805	0.280	0.887	0.626	0.785	0.792
C. utilis	0.772	0.914	0.280	0.818	0.598	0.844	0.879
R. rubra	0.730	0.862	0.306	0.767	0.554	0.886	0.898
A. niger	0.251	0.406	0.288	0.136	0.013	0.581	0.623
P. chrysogenum	0.622	0.868	0.128	0.674	0.471	0.880	0.880
R. chinensis	0.514	0.703	0.159	0.450	0.355	0.792	0.795
M. mucedo	0.855	0.838	0.275	0.765	0.601	0.764	0.884

S. cerevisiae, *A. niger*, and *R. chinensis*. The first reduction potential at pH 7.2 may be set a parameter determining the antimicrobial activity of the pyridine derivatives. The more positive E_{pc-1}, the stronger the antimicrobial activity. Among these electronic properties, LUMO energy and steric energy demonstrated definite correlations. Good correlation of log *P* and MIC was also obtained. The low log *P* value of the compounds indicates potent antimicrobial activity. Thus, we introduced log *p* as an additional parameter, and performed multiple regression analyses, which resulted in slight improvement in correlation, as shown in Table 4.

In conclusion, some azaanthraquinones possess potent antimicrobial activity. Reduction potentials measured at physiological pH 7.2, log *P*, and LUMO energy were useful parameters for estimating the antimicrobial activity of azafluorenones, 1-azaanthraquinones, and 2-azaanthraquinones.

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