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Synthesis and bioactivity evaluation of 2-aza-anthraquinones

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Abstract

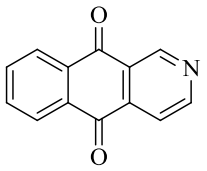
2-Aza-anthraquinones can be found in nature. Many of these azaanthraquinones derivatives have indeed been discovered to possess diverse and pronounced biological activities, including antimicrobial, antiparasitic, antiviral and anticancer properties. These compounds have been shown to be an intercalating agent to DNA. In continuation of our synthetic interest in physiologically active quinone derivatives, the present report describes the synthesis and bioactivity evaluation of 2-aza-anthraquinones. The biological assessment of all these compounds demonstrated a significant cytotoxic effect of 2-azaanthraquinones against different cancer cell lines.

Keywords. Synthesis, 2-aza-anthraquinones, bioactivity, benz[*g*]isoquinoline.

1. INTRODUCTION

Naturally occurring 2-aza-anthraquinone, benz[*g*]isoquinoline-5,10-dione 1, was isolated from *Psychotricha camponutans* and *Mitracarpus scaber*, and exhibited growth inhibition against multi-drug resistant pathogens, e.g. *Staphylococcus aureus* and *Plasmodium falciparum* [1,2] Bostrycoidin **2a** and 9-*O*-methylbostrycoidin **2b**, two metabolites of numerous *Fusarium* species, showed antibiotic activity against the tubercle bacil and G^+ bacteria, respectively[3,4]. Tolypocladin **2c**, which was isolated from the mycelium of *Tolypocladium inflatum*, displayed metal-chelating properties [5]. In addition, 2-azaanthraquinone derivatives interfered with the activity of DNA topoisomerases and attracted considerable attention in cancer chemotherapy as intercalating DNA binding agents [6].

In continuation of our synthetic interest in physiologically active quinone derivatives [7-16], we now report synthesis and bioactivity evaluation of 2-aza-anthraquinones. Furthermore, the cytotoxicity with regard to different cancer cell lines and the antimicrobial potential of these compounds will be discussed as well.



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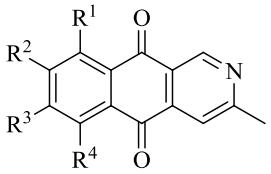

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2a R^1=R^4=OH, R^2=H, R^3=OMe (bostrycoidin)
2b R^1=R^3=OMe, R^4=OH
2c R^1=R^3=R^4=OH, R^2=H (tolypocladin)
2d R^1=R^3=H, R^2=R^4=OMe (scorpinone)

Figure 1

2. EXPERIMENTAL

2.1. General information

All reactions were performed in the appropriate oven-dried glass aparatus and under nitrogen atmosphere. Unless otherwise stated, solvents and chemicals were obtained from commercial sources and used without further purification. Column chromatography was performed using silica gel (60Å, particle size 40-60 µm). NMR spectra were recorded on a Bruker Avance (500 MHz). Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) in hertz (Hz). High resolution mass spectrometry analysis (HRMS) were recorded on a Q-exactive or a Q-TOF2 instrument. IR analysis were recorded on Perkin Elmer Spectrum Two.

2.2. General procedure for the synthesis of compounds 6a-e [14]

To a solution of 2-alkyl-3-phenoxymethyl-l,4-naphthoquinones **5a-e** (1.5 mmol) in ethanol (30 ml) was added dropwise a solution of aqueous ammonia (25% NH_3 in H_2O) (15 mmol) in ethanol (5 ml). The solution was protected from light by aluminum foil and stirred for 2 days in an open vessel, allowing contact with the air. Then, most of the solvent was evaporated at reduced pressure and the resulting residue was dissolved in dichloromethane, washed with 1N hydrochloric acid and then with 2N sodium hydroxide. After drying ($MgSO_4$) and evaporation of the solvent at reduced pressure, compounds **6a-e** were obtained after recrystallization from methanol.

Compound 6a: Yielded 73 % as white powder, mp 172-174 °C. ^{1}H-NMR ($CDCl_3$): 2.77 (3H, s, CH_3), 7.81-7.88 (2H, m, H-7 and H-8), 7.89 (1H, s, H-4), 8.26-8.34 (2H, m, H-6 and H-9), 9.44 (1H, s, H-l). ^{13}C-NMR ($CDCl_3$): 25.1 (CH_3), 118.5 (C-4), 124.2 (=C_{quat}), 127.2 and 127.3 (C-6 and C-9), 133.2 (=C_{quat}), 133.3 (=C_{quat}), 134.5 and 135.0 (C-7 and C-8), 138.7 (C), 149.3 (C-l), 165.5 (=C_{quat}), 182.3 (C=O), 182.7 (C=O). IR (KBr) cm^{-1}: 2950; 1681 (C=O), 1666 (C=O), 1585 (C=C); 1475; 1355; 1258; 1170; 766. ESI-MS: 224 $[M+H]^+$.

Compound 6b: Yielded 75 % as yellow needles, mp 98 °C. ^{1}H-NMR ($CDCl_3$): 1.47 (9H, s, C($C\underline{H}_3$)), 7.82-7.87 (2H, m, H-7 and H-8), 8.11 (1H, d, J = 1.0 Hz, H-4), 8.29-8.34 (2H, m, H-6 and H-9), 9.49 (1H, d, J = 1.0 Hz, H-1). ^{13}C-NMR ($CDCl_3$): 29.8 (C($\underline{C}H_3)_3$), 38.5 ($\underline{C}(CH_3)_3$), 114.1 (C-4), 123.6 (C), 127.0 and 127.1 (C-6 and C-9), 133.0 (2 x =C_{quat}), 134.1 and 134.6 (C-7 and C-8), 138.5 (C), 148.7 (C-1), 176.5 (C-3), 182.2 (C=O), 182.9 (C=O). IR (KBr) cm^{-1}: 2970; 1676 (C=O), 1587 (C=C); 1455; 1376; 1254; 1130; 1070; 788. ESI-MS: 265 $[M+H]^+$.

Compound 6c: Yielded 70 % as yellow needles, mp 200-201 °C. ^{1}H-NMR ($CDCl_3$): 7.51-7.52 (3H, m, H-3'; H-4' and H-5'), 7.85-7.89 (2H, m, H-7 and H-8), 8.20-8.23 (2H, m, H-2' and H-6'), 8.33-8.38 (2H, m, H-6 and H-9), 8.51 (1H, d, J = 1.0 Hz, H-4), 9.62 (1H, d, J = 1.0 Hz, H-1). ^{13}C-NMR ($CDCl_3$): 115.3 (C-4), 124.6 (=C_{quat}), 127.3 and 127.4 (C-6 and C-9), 127.6 (C-2' and C-6'), 129.1 (C-3' and C-5'), 130.7 (C-4'), 133.2 (=C_{quat}), 133.3 (=C_{quat}), 134.4 and 135.0 (C-2 and C-8), 137.6 (C_{quat}), 139.3 (C_{quat}), 150.0 (C-1), 162.8 (C-3), 182.3 (C=O), 182.8 (C=O). IR (KBr) cm^{-1}: 1676, 1634, 1612, 1580, 1320, 1298, 1283, 711. ESI-MS: 286 $[M+H]^+$.

Compound 6d: Yielded 82 % as an orange powder, mp 212-214 °C. ^{1}H-NMR ($CDCl_3$): 7.51 (2H, d, J = 8.5 Hz, H-3' and H-5'), 7.85-7.92 (2H, m, H-7 and H-8), 8.16 (2H, d, J = 8.5 Hz, H-2' and H-6'), 8.33-8.38 (2H, m, H-6 and H-9), 8.47 (1H, d, J = 1.0 Hz, H-4), 9.56 (1H, d, J = 1.0 Hz, H-l). ^{13}C-NMR ($CDCl_3$): 115.1 (C-4), 124.8 (=C_{quat}), 127.3 and 127.4 (C-6 and C-9), 128.8 (C-2' and C-6'), 129.4 (C-3' and C-5'), 133.1 (C_{quat}), 133.2 (=C_{quat}), 134.4 and 135.1 (C-7 and C-8), 136.0 (C_{quat}), 137.1 (C_{quat}), 139.36 (=C_{quat}), 150.0 (C-l), 161.4 (C-3), 182.1 (C=O), 182.6 (C=O). IR (KBr) cm^{-1}: 1668, 1584, 1550, 1584, 1480, 1408, 1372, 1265, 1130, 924, 843, 746, 702. ESI-MS: 220 $[M+H]^+$.

Compound 6e: Yielded 76 % as an orange powder, mp 198-201 °C. ^{1}H-NMR ($CDCl_3$): 7.22 (2H, t, J = 8.5 Hz, H-3' and 8-5'), 7.81-7.91 (2H, m, H-7 and H-8), 8.23 (2H, d, J = 8.5 Hz, H-2' and H-6'), 8.34-8.36 (2H, m, H-6 and H-9), 8.41 (1H, d, J = 1.0 Hz, H-4), 9.56 (1H, d, J = 1.0 Hz, H-l). ^{13}C-NMR ($CDCl_3$): 114.9 (C-4), 116.0 and 116.3 (C-2' and C-6'), 124.5 (=C_{quat}), 127.3 and 127.4 (C-6 and C-9), 129.5 and 129.7 (C-3' and C-5'), 133.2 (=C_{quat}), 133.3 (=C_{quat}), 133.9 (=C_{quat}), 134.4 and 135.1 (C-7 and C-8), 139.3 (=C_{quat}), 150.1 (C-1), 161.8 (C-3), 164.5 (d, J_{C-F} = 245 Hz, =CF), 182.2 (C=O), 182.8 (C=O). IR (KBr): cm^{-1} 1683, 1664, 1648, 1598, 1584, 1551, 1296, 1153, 925, 844. ESI-MS: 304 $[M+H]^+$.

2.3. Cell culture and cell viability assay

Four human cancer cell lines KB, $HepG_2$, MCF 7 and Lu obtained from the American Type Culture Collection (USA) ATCC, were used for cytotoxic evaluation. The cells were grown in RPMI 1640

medium supplemented with 10 % fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere (95 % air and 5 % CO_2). The exponentially growing cells were used throughout the experiments. The inhibitory effects of the compounds on the growth of the human cancer cell lines were determined by measuring the metabolic activity using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, human cancer cell lines (1×105 cells/mL) were treated for 3 days with a series of concentrations of the compounds (in DMSO): 0.125, 0.5, 2.0, 8.0, 32.0, and 128.0 µg/mL. After incubation, 0.1 mg MTT solution (50 µL of a 2 mg/mL solution) was added to each well, and the cells were then incubated at 37 °C for 4 h. The plates were centrifuged at 1000 rpm for 10 min at room temperature, and the media were then carefully aspirated. Dimethylsulfoxide (150 µL) was added to each well to dissolve the formazan crystals. The plates were read immediately at 540 nm on a microplate reader (TECAN GENIOUS). All the experiments were performed three times, and the mean absorbance values were calculated. The results are expressed as the percentage of inhibition that produced a reduction in the absorbance by the treatment of the compounds compared to the untreated controls. A dose-response curve was generated, and the inhibitory concentration of 50 % (IC_{50}) was determined for each compound as well as each cell line.

2.4. Antibacterial and antifungal assay

Seven microorganism strains from American Type Culture Collection (*Staphylococcus aureus* ATCC 13709, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442, *Lactobacillus fermentum* ATCC 9338, *Salmonella enterica* ATCC 13076, and *Candida albicans* ATCC 10231) were used to evaluate antimicrobial activity. Assay was performed in 96-well microtiter plates. Each well containing 10 µL of compounds dissolved in DMSO was diluted together with 190 µL of microorganism suspension (5×105 CFU/mL). After 18-20 h incubation at 37^o C, microbial growth/viability was assessed fluorimetrically after addition of 10 µL resazurin per well using Tecan Genious microplate reader at 600 nm. The results were expressed as % reduction of microorganism growth/viability compared with control well, and IC50 values were determined from dose-response curve.

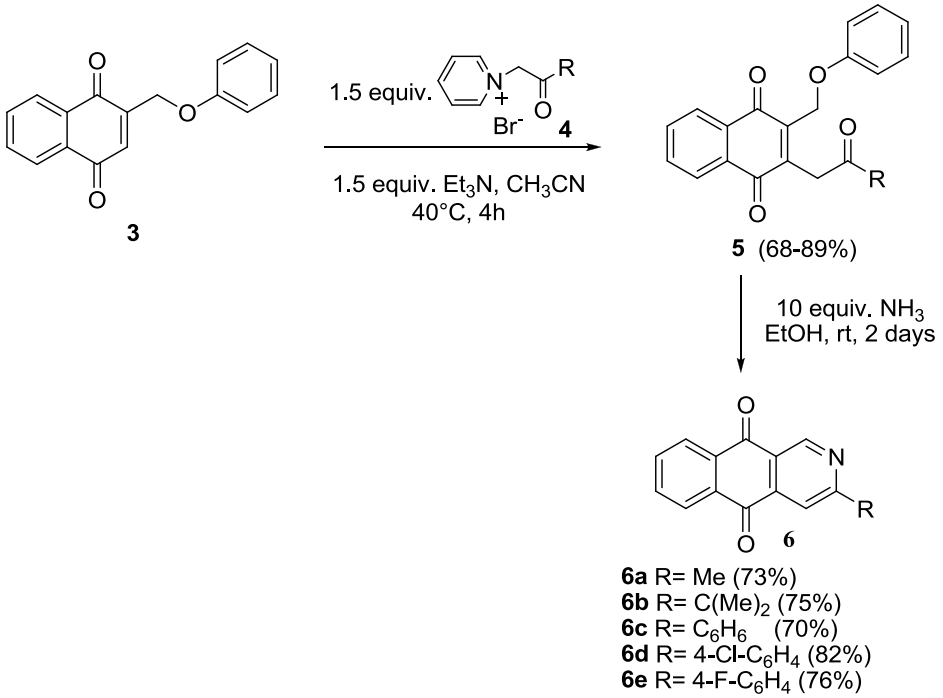
Statistical Analysis. All data represent the mean ± SD of at least three independent experiments performed in triplicates. Statistical significance is indicated as determined by one-way ANOVA followed by Dunnett's multiple comparison test using GraphPad Prism 6 program (GraphPad Software Inc., USA), $p < 0.05$.

3. RESULTS AND DISCUSSION

Recently, we published a straightforward synthesis of 2-azaanthraquinones via an ammonia-induced cyclization of 2-acyl-3-phenoxymethyl-1,4-naphthoquinones [7-16]. However, the bioactivity of these compounds has not been investigated. In this report, the synthesis of these derivatives was carried out and outlined in Scheme 1 [14]. In this way, 2-acyl-3-phenoxymethyl-1,4-naphtho quinone **4** reacted with *N*-(acylmethyl)pyridinium ylide, which was formed *in situ* by reaction of *N*-acylpyridinium chloride and triethylamine (Scheme 1) [14, 15]. The Michael adduct **5** was treated with 10 equivalents of ammonia, resulting in a nucleophilic addition across the carbonyl of the acyl group and intramolecular substitution of the phenoxylated carbon atom. The cyclized product oxidized spontaneously to afford 2-azaanthraquinones **6a-h** in 70-82 % yield. These compounds were synthesized for cytotoxicity and antimicrobial testing and to compare the biological activities with other pyranonaphthoquinone derivatives.

With this set of compounds in hand, the biological activity spectrum of these molecules was investigated in the next part. In particular, 2-azaanthraquinones **6a-e** were tested for their cytotoxicity against four cancer cell lines (KB, Hep-G2, MCF7 and Lu) and for their antimicrobial properties against two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and one yeast strain (*Candida albicans*).

The cytotoxicity test data clearly show that 2-azaanthraquinones **6a**, **6b** and **6d** also exhibit reasonable anticancer effects (table 1). On the other hand, 2-azaanthraquinones **6c** and **6d** display weak cytotoxic activity. Within the series of 2-azaanthraquinones, methyl derivatives **6a** and isopropyl derivatives **6b** seem to display a considerable cytotoxic effect against all four cell lines, and compound **6a** exhibits a particularly strong anticancer effect against Lu cells with an IC_{50}-value (1.24 (μg/ml) similar to that of ellipticine (1.3l (μg/ml).



Scheme 1

Table 1: Cytotoxic activity

Entry	Compounds	IC_{50} (μg/ml)			
		KB	HepG2	MCF 7	Lu
1	**6a**	**4.91**	**5.05**	**1.59**	**1.24**
2	**6b**	**5.83**	**7.20**	**5.15**	**3.77**
3	**6c**	>10	>10	>10	>10
4	**6d**	**7.8**	**6.5**	>10	>10
5	**6e**	>10	>10	>10	>10
6	**Ellipticine**	**0.31**	**0.35**	**0.45**	**1.31**

Table 2: Antibacterial activity

Entry	Compounds	IC_{50} (μg/ml)				
		Gram (+)		Gram (-)		Fungi
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albican*
1	**6a**	**3.5**	>10	>10	>10	>10
2	**6b**	>10	**6.5**	>10	>10	>10
3	**6c**	>10	>10	>10	>10	>10
4	**6d**	>10	8.5	>10	>10	>10
5	**6e**	>10	>10	>10	>10	>10
6	Ampicillin	0.08	0.004	0.8	-	-
	Cefotaxime	-	-	-	8.5	-
	Amphotericin B	-	-	-	-	0.43

The antimicrobial assessment of the same compounds, however, revealed a poor biological activity profile, and only **6a** and **6b** showed moderate *anti-B. subtilis* and *anti-S. aureus* activity.

In conclusion, a series of 2-azaanthraquinones was conveniently and selectively synthesized in

excellent yields via introduction of acylmethyl groups onto 2-hydroxymethyl-1,4-naphthoquinone by using acylmethylpyridinium ylides and a final intermolecular cyclization. The biological assessment of all these compounds demonstrated a significant cytotoxic effect of 2-azaanthraquinones against different cancer cell lines, pointing to the biological relevance of these scaffolds.

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REFERENCES

1. Krapcho A. P., Waterhouse D. *General sythetic pathway to oxygenated 3-methylbenz[g]isoquinoline-5,10*, J. Heterocycles **51**, 737-749 (1999).
2. Okunade A. L., Clark A. M., Hufford C. D., Oguntimein B. O. *Azaanthraquinone: An antimicrobial alkaloid from Mitracarpus scaber*, Planta Med., **65**, 447-448 (1999).
3. Arsenault, G. P. *The structure of bostrycoidin, a β-aza-anthraquinone from Fusariumsolani D_2 purple*, Tetrahedron Lett., **45**, 4033-4037 (1965).
4. Steyn P. S., Wessels P. L., Marasas W. O. F. *Pigment from fusariummoniliforme sheldon: Structure and ^{13}C nuclear magnetic resonance assignments of an azaanthraquinone and three naphthoquinones*, Tetrahedron, **35**, 1551-1555 (1979).
5. Gräfe U., Ihn W., Tresselt D., Miosga N., Kaden U., Schlegel B., Bormann E. -J., Sedmera P., Novak. *Tolypocladin - a new metal-chelating 2-aza-anthraquinone from Tolypocladium inflatum*, J. Biol. Metals, **3**, 39-44 (1990).
6. Khanapure S. P., Biehl E. R. *A convenient synthesis of azaanthraquinones via Polar Addition to hetaryne intermediates, use of carbanions devrived from 3-cyano-1(3H)-isobenzofuranoes*, Heterocycles, **27**, 2643-2650 (1988).
7. N. V. Tuyen, and N. De Kimpe. *Synthesis of pyranonaphthoquinone antibiotics involving the ring closing metathesis of a vinyl ether*, Tetrahedron Lett., **45**, 3443 (2004).
8. S. Claessens, G. Verniest, E. H. Samir, N. V. Tuyen, B. Kesteleyn, L. Van Puyvelde and N. De Kimpe. *The behaviour of the natural pyranonaphthoquinone pentalongin in alcoholic solvents*, Tetrahedron, **62**, 5152 (2006).
9. N. V. Tuyen and N. De Kimpe. *Synthesis of 6H-naphtho[2,3-c]chromene-7,12-diones via palladium-catalyzed intramolecular cyclization of 2-bromo-3-aryloxymethyl-1,4-naphthoquinones*, Tetrahedron, **59**, 5941 (2003).
10. N. V. Tuyen, B. Kesteleyn, N. De Kimpe. *Synthesis of 1,3-disubstituted naphtho[2,3-c]pyran-5,10-diones*, Tetrahedron, **57**, 4213 (2001).
11. N. V. Tuyen, S. Claessens, P. Habonimana, K. A. Tehrani, L. Van Puyvelde and N. De Kimpe. *Synthesis of Harounoside, A Naturally Occurring Pentalongin Hydroquinone Bisglucoside*, Synlett, 2469 (2006).
12. S. Claessens, G. Verniest, J. Jacobs, E. Van Hende, P. Habonimana, N. V. Tuyen, L. Van Puyvelde and N. De Kimpe. *A Survey of Synthetic Routes towards the Pyranonaphthoquinone Antibiotic Pentalongin and Syntheses of the Corresponding Nitrogen Derivatives*, Synlett, 829 (2007).
13. S. Claessens, G. Verniest, J. Jacobs, E. Van Hende, P. Habonimana, N. V. Tuyen, L. Van Puyvelde and N. De Kimpe. *A Survey of Synthetic Routes towards the Pyranonaphthoquinone Antibiotic Pentalongin and Syntheses of the Corresponding Nitrogen Derivatives*, Synlett, 1176 (2007).
14. B. Kesteleyn, N. V. Tuyen, N. De Kimpe. *Synthesis of 3-alkyl- and 3-aryl-2-aza-anthraquinones*, Tetrahedron, **55**, 2091 (1999).
15. Aldersley M. F., Christi S. H., Dean F. M., Douglas M. E., Ennis D. S. *Pyridinium ylides in syntheses of naphthopyrandiones and in regioselective syntheses of acylated anthraquinones related to fungal and bacterial metabolites*, J. Chem. Soc. Perkin Trans. I, 2163-2174 (1990).
16. Tuyen Nguyen Van, et al. *Synthesis and anticancer properties of new (dihydro)pyranonaphthoquinones and their epoxy analogs*, Bioorganic & Medicinal Chemistry Letters, **25**, 3355-3358 (2015).

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