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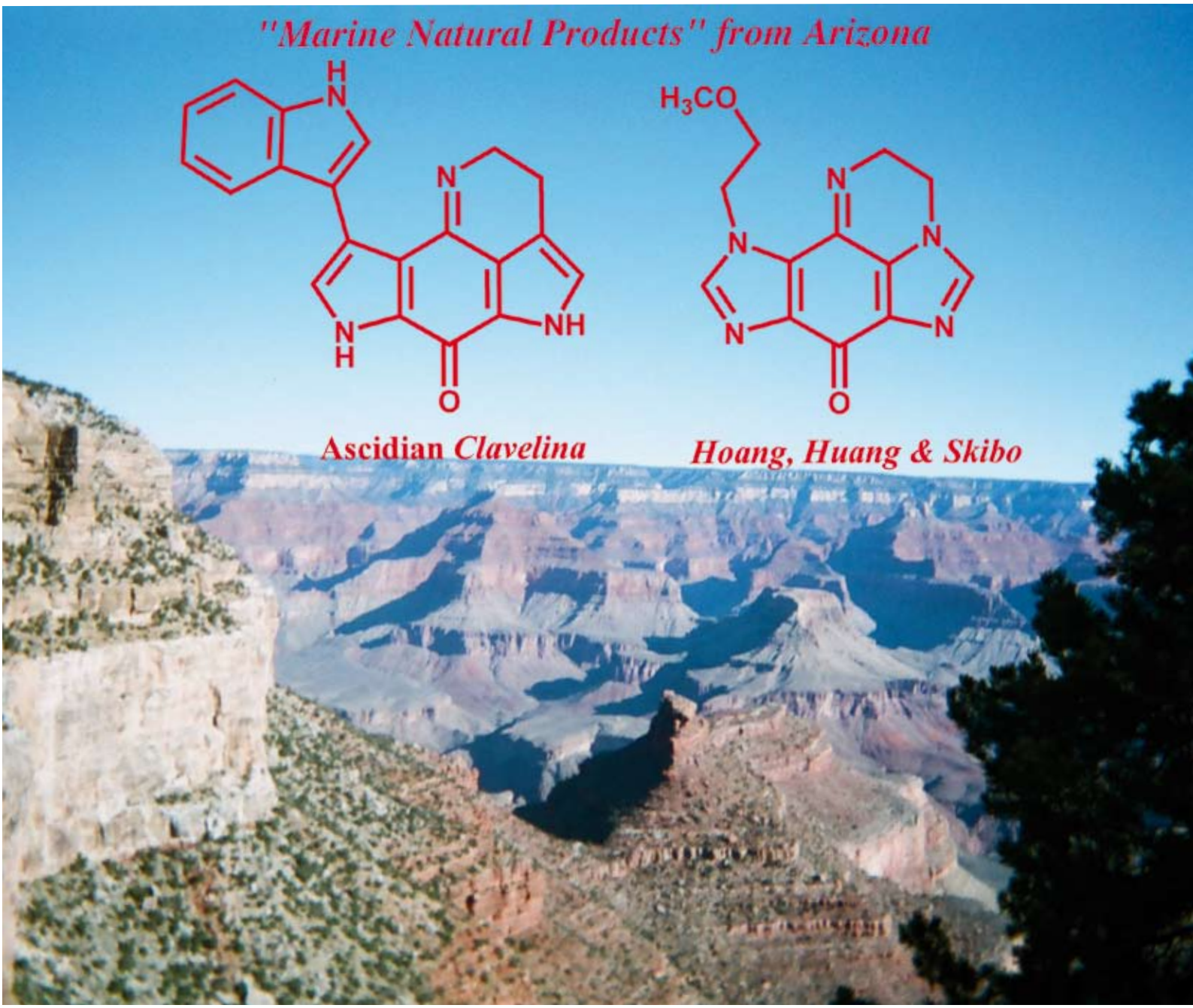

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FULL PAPER

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Synthesis and *in vitro* evaluation of imidazole-based wakayin analogues

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Analogues of wakayin based on diimidazo[1,5,4-*de*, 1,5,4-*h*]-quinoxaline have been prepared and evaluated with respect to cytostatic and cytotoxic activity. Assays in a 60-cell-line human cancer panel revealed selective activity against cells with the VEGF (Flt-1) receptor.

Introduction

Wakayin, shown in Fig. 1, was isolated from ascidian *Clavelina* species by Ireland and coworkers in 1991.[1] The inhibitory activity of wakayin against topoisomerase I,[2] and no doubt its interesting structure, has prompted analogue development over the past decade.[3–7] The most recent studies utilized pyrazolo rather than the pyrrolo rings of the natural product.[6,7] These analogues possess modest cytostatic activity and some inhibited topoisomerase I and II mediated relaxation of supercoiled DNA.

Recently, we reported the preparation and biological evaluation of imidazo analogues of the marine pyrroloquinoline alkaloids called imidazoquinoxalinones, Fig. 1.[8] The change from the pyrrolo ring of the natural products to the imidazo ring was characterized by a change in the cellular target for some analogues as well as their cytostatic/cytotoxic profile.

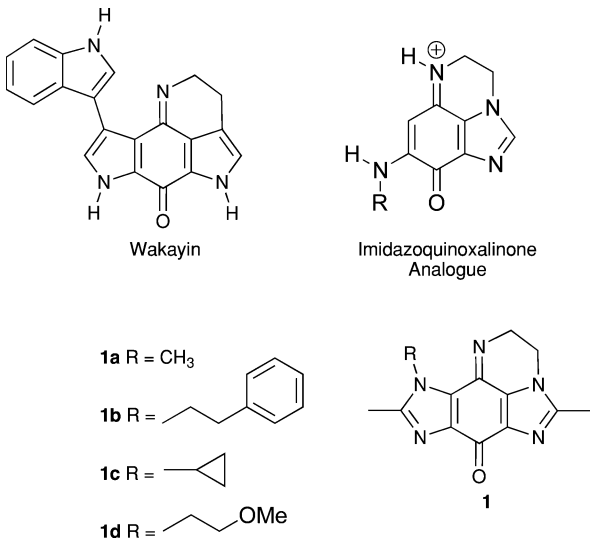

Fig. 1 Structures of wakayin and its imidazo analogues.

This switch affords a more electron deficient heterocycle (see graphical abstract) that bears a resemblance to the purine ring. As a result, the imidazo analogue may be a better intercalating agent or may interact with a purine-utilizing cellular target. In light of our results with the imidazoquinoxalinones,[8] we prepared imidazo analogues of wakayin, **1** in Fig. 1. Our goals were to assess the cytostatic/cytotoxic properties of **1** in a 60-cell-line human cancer panel to determine what types of analogues are worthy of further study and what cellular targets are responsible for biological activity.

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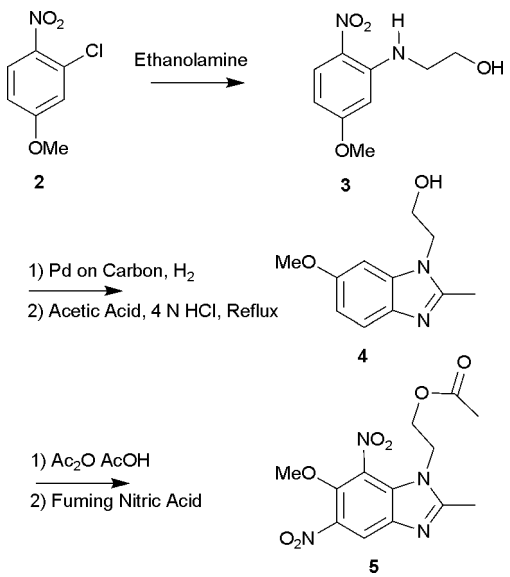
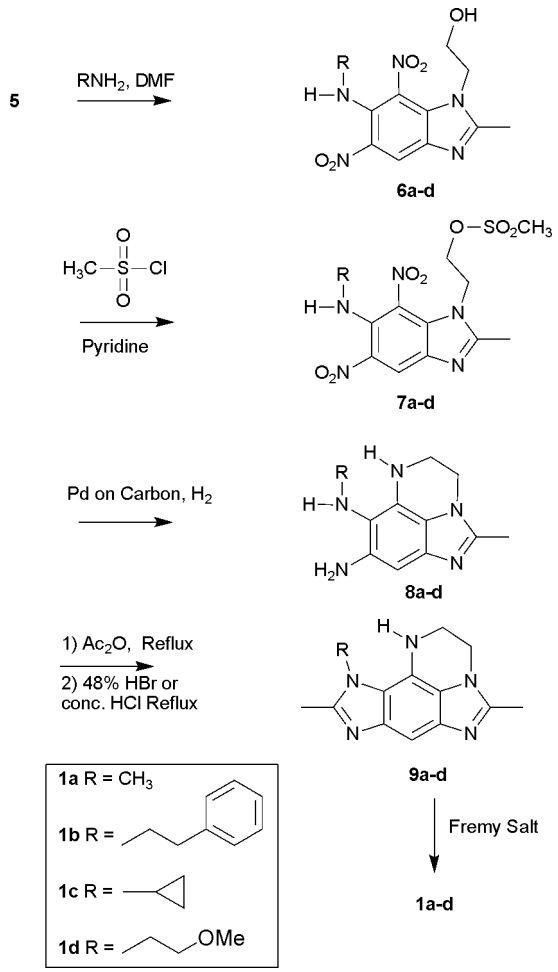
Results and discussion

Synthesis of analogues 1

The preparation of **1a–d** is described below in conjunction with Schemes 1 and 2. Described in Scheme 1 is the preparation of the key intermediate **5**, which was the starting point for the incorporation of the various R groups. The preparation of **5** started with the nitration of *m*-chlorophenol followed by methylation of the phenol to yield 3-chloro-4-nitroanisole **2**.[9] Treatment of **2** with ethanolamine resulted in nucleophilic aromatic substitution to afford **3**. The hydroxyethyl group of **3** eventually became the ethylene tether in **1**. Catalytic reduction of **3** resulted in an *ortho* diamine that was immediately treated with acetic acid to afford the Phillips[10] cyclization product **4**. *O*-Acetylation of **4** was carried out to protect the hydroxyl group from nitration during the fuming nitric acid treatment that afforded **5**.



Scheme 1 Synthesis of key intermediate **5**.



Scheme 2 Elaboration of **1** from key intermediate **5**.

We treated **5** with various primary alkylamines in DMF solvent resulting in nucleophilic aromatic substitution of the methoxy group along with *O*-deacetylation to afford **6a–d**. Aromatic amines such as aniline did not undergo this nucleophilic aromatic substitution reaction. *O*-Methanesulfonation of **6a–d** afforded **7a–d** that was converted to the tetrahydroquinoxoline intermediate **8a–d** by catalytic nitro group reduction followed by internal nucleophilic displacement of methanesulfonate. These cyclization products are overly electron rich and therefore susceptible to aerobic oxidation.

To prevent oxidation, **8a–c** was immediately cyclized to **9a–c** in the presence of acetic anhydride and hydrobromic acid. Compound **8d** was cyclized to **9b** with hydrochloric acid to preserve the methoxy functional group. Fremy salt[11] oxidation of **9a–d** afforded the final products **1a–d** as yellow-orange solids. We have used Fremy salt for the preparation of iminoquinones[12] and extended amidines[8] from aromatic amines.

Cytostatic and cytotoxic assays of 1

The mean cytostatic and cytotoxic parameters of **1** were measured in a 60 human cancer cell line panel consisting of the major histological tissue types. The cytostatic parameters include GI_{50} and TGI, which are the concentrations of drug required for 50% growth inhibition and total growth inhibition, respectively. The cytotoxic parameter is the LC_{50}, which is the concentration required for 50% cell kill.

These data were obtained under the *In Vitro* Cell Line Screening Project at the National Cancer Institute.[13–15] For compounds **1a–d**, the three parameters discussed above were determined for each of the 60 human cancer cell lines. The resulting 180 parameters provided a thorough assessment of the activity of these new wakayin analogues.

The results of these screens are shown with the bar graphs in Fig. 2. The cyclopropyl derivative **1c** was inactive against all cell lines; inactivity is defined as those possessing parameters with log values > -4. However, the methyl and phenethyl derivatives, **1a** and **1b**, displayed a degree of cytostatic activity against some histological cancer types; **1a** and **1b** were most active against the ovarian and melanoma cancer panels, respectively. However, **1d** (red bars) displayed the highest activity against the leukemia and renal histological cancer types.

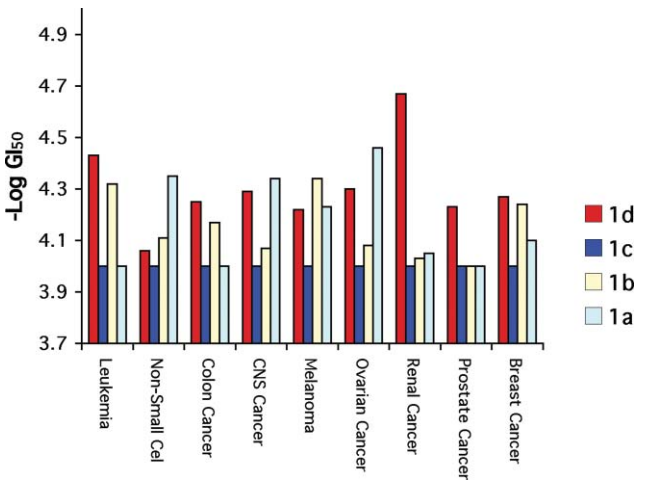


Fig. 2 Bar graphs of the average $-\log GI_{50}$ values for **1a–d** against histological cancer types.

The high average activity of **1d** against the renal panel is due to its cytostatic activity against the renal cancer cell line A498 (< -8.0). The ~10 000-fold increase in cytostatic activity accompanying the change from methyl to methoxyethyl indicates that such oxygen-containing side chains should be the subject of further development, Fig. 3.

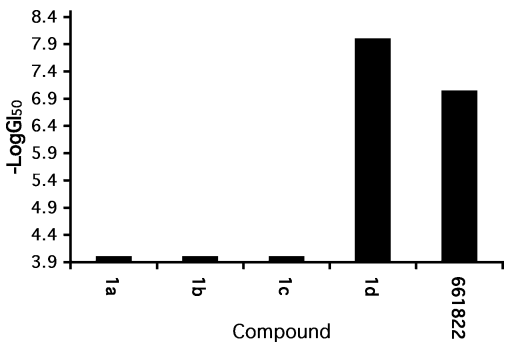


Fig. 3 Bar graph comparing the activities of analogues **1** against renal cancer cell line A498. The structure of 661822 is shown in Fig. 4.

COMPARE analysis

COMPARE analysis[13,16] provided insights into what might be the molecular target of the active analogue **1d**. This analogue exhibits high histologic selectivity (renal cancer) and would be amenable to a meaningful COMPARE analysis. In contrast, mean graphs with lower histological specificity (**1a**) are essentially flat and will correlate with other flat mean graphs providing little meaningful target information. The COMPARE analysis correlates mean graph data with known molecular target levels in cell lines and thereby generates hypotheses concerning the agent's mechanism of action. A molecular target is a protein or enzyme that has been measured in the National Cancer Institute's panel of 60 human tumor cell lines. The levels of over one thousand biologically relevant molecular targets have been determined in these tumor cell lines from measurements of mRNA and enzyme activity levels.[17]

COMPARE correlations indicated that the cytostatic activity of **1d** correlates (0.74 correlation coefficient) with the molecular target Flt-1, which is a receptor for vascular endothelial growth factor (VEGF). The renal cancer A498 has the highest level of Flt-1 receptor in the 60-cell-line panel, thus the sensitivity of this cell line to **1d**. VEGF induces vasculogenesis and angiogenesis, both of which are important for tumor development.[18] Consequently, both VEGF[19] and its receptor (Flt-1)[20] have been the subject of antitumor agent design.

We also did a COMPARE correlation of the cytostatic profile of **1d** with over 38 000 compounds in the National Cancer Institute's compound database. Visit http://dtp.nci.nih.gov/for access to NCI databases and the COMPARE algorithm. The goal was to discover other compounds that possess the cytostatic profile of **1d**. Shown in Fig. 4 is the structure of NSC 661822.[21] Although not a perfect structural match, this compound does have obvious structural similarities (electron deficient heterocycle with a side chain) to **1d**. Nevertheless, this compound is an order of magnitude less potent than **1d**, see Fig. 3. The COMPARE studies indicate that future analogues of **1d** should be screened for binding to the Flt-1 receptor.

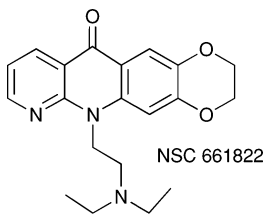


Fig. 4 Structure of NSC 661822.

Conclusions

We succeeded in preparing the previously unreported diimidazo[1,5,4-*de*, 1,5,4-*h*]-quinoxaline ring system **1** as a structural analogue of the marine natural product wakayin. Our synthesis permits the convenient incorporation of various R groups for analogue development.

In order to assess the cytostatic and cytotoxic activity of **1a–d**, we measured the GI_{50}, TGI, and LC_{50} parameters for each analogue in a 60 human cancer cell line panel consisting of the major histological tissue types. Analogue **1d** was the most active and libraries of **1** will include similar R groups: straight chains bearing heteroatoms (oxygen or nitrogen, based on the structure of NSC 661822 in Fig. 4).

COMPARE analysis suggests that **1d** targets the Flt-1 receptor. Libraries based on **1d** will therefore be screened for binding to this receptor. Work in this laboratory and elsewhere has shown COMPARE to be useful in drug discovery.[13,22,23] Recently, we reported the discovery of a quinolinedione-based antiangiogenesis agent using COMPARE.[23]

Finally, we conclude that the analogues of **1** are not true mimics of wakayin. The potential density maps shown in the graphical abstract reveals that the ring system of **1** is more electron-deficient than that of wakayin. This feature no doubt contributes to the different biological properties of **1** relative to wakayin.

Experimental

General

All analytically pure compounds were dried under high vacuum in a drying pistol over refluxing methanol. Many of the compounds were crystallized from reaction mixtures or elution solvents upon concentration and therefore no recrystallization solvent is specified. Elemental analyses of final products **1** that were assayed were run at Arizona State University. Melting points and decomposition points were determined with a Mel-Temp apparatus. All TLCs were performed on silica gel plates using a variety of solvent systems and a fluorescent indicator for visualization. IR spectra were taken as KBr pellets and only the strongest absorbances were reported. ^{1}H NMR spectra were obtained with a 300 or 500 MHz spectrometer. All chemical shifts are reported relative to TMS.

Synthesis of compounds

1-(2-Hydroxyethyl)-5-methoxy-2-nitroaniline (3). A solution of 1.0 g (5.3 mmol) of **2** in 5 ml of ethanolamine was heated to 50 °C under constant stirring for 4 h. The reaction was allowed to cool to room temperature and then diluted with 20 ml of water. After dilution with water, an orange precipitate resulted. This precipitate was collected and washed with cold water. The orange precipitate was then crystallized from 95% ethanol resulting in orange needles: 830 mg (73%) yield; Mp 106–109 °C; TLC (chloroform–methanol [99 : 1]) $R_f = 0.23$; FTIR 3504, 3315, 2974, 2941, 2891, 2837, 1631, 1577, 1431, 1334, 1232, 1207, 1031, 819, 752, 663 cm^{-}1; ^{1}H NMR ($CDCl_3$) δ 8.5 (broad s, 1H, amide NH), 8.15 (d, $J = 10$ Hz, 1H, C(3)), 6.26 (dd, $J = 2.7$ Hz and 10 Hz, 1H, C(4)), 6.22 (d, $J = 2.7$ Hz, 1H, C(6)), 3.95 and 3.49 (2t, $J = 5.4$ Hz, 4H, ethylene), 3.85 (s, 3H, methoxy); APCI+: calcd for $C_9H_{13}N_2O_4$ 213.0875, found 213.0845.

2-(2-Hydroxyethyl)-6-methoxy-2-methyl-1*H*-benzimidazole (4). A solution of 1.0 g (4.70 mmol) of **3** in 100 mL of methanol was purged with N_2 followed by addition of 100 mg of 5% Pd on carbon. This mixture was then reduced under 50 psi of H_2 for 4 h and the resulting reduced solution was filtered through Celite and concentrated to an oil under reduced pressure. To this oil were added 5 mL of acetic acid and 20 mL of 4 N hydrochloric acid and the resulting solution refluxed for 6 h. After reflux was

complete, the acidic reaction mixture was made neutral with 10% aq. $NaHCO_3$ and then extracted 3 times with 25 ml portions of chloroform. The organic layer was dried with Na_2SO_4, filtered and then concentrated under reduced pressure. The resulting oil was then crystallized using chloroform–hexane resulting in tan colored crystals: 680 mg (70%) yield; Mp 149 °C; TLC (chloroform–methanol [95 : 5]) $R_f = 0.31$; FTIR 3146, 2947, 2835, 1622, 1489, 1410, 1211, 1078, 1026, 806, 632 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.23 (d, $J = 9$ Hz, 1H, C(4)), 6.69 (d, $J = 2.4$ Hz, 1H, C(7)), 6.66 (dd, $J = 2.4$ Hz and 9 Hz, 1H, C(5)), 4.17 and 4.03 (2t, $J = 4.2$ Hz, 4H, ethylene), 3.82 (s, 3H, methoxy), 2.49 (s, 3H, 2-methyl); APCI+: calcd for $C_{11}H_{15}N_2O_2$ 207.1134, found 207.1122.

1-[2-Acetyloxyethyl]-6-methoxy-2-methyl-5,7-dinitro-1*H*-benzimidazole (5). A solution of 2.6 g (12.60 mmol) of **4** in 40 mL of acetic anhydride–acetic acid (1 : 1) was refluxed for 3 h. The solvent was then removed under reduced pressure to afford an oil. This oil was then chilled in an isopropanol–dry ice bath (−20 °C) and 30 ml of ice cold fuming nitric acid (≥ 90%) were slowly added to the cooled oil. The acidic mixture was allowed to slowly warm up to 50 °C and was stirred for 5 h. The resulting product was poured over crushed ice, neutralized with 10% aq. $NaHCO_3$ and extracted with ethyl acetate. The organic extract was dried over Na_2SO_4, concentrated to an oil under reduced pressure, and was purified by column chromatography using silica gel and chloroform–methanol (98 : 2) as the eluent. The eluents were concentrated to afford yellow crystals: 2.65 g (61%) yield; Mp 142–146 °C; TLC (chloroform–methanol [95 : 5]) $R_f = 0.49$; FTIR 3462, 3327, 3026, 2958, 1745, 1626, 1535, 1336, 1232, 1051, 736 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.44 (2, 1H, C(4)), 4.34 and 4.26 (2t, $J = 4.8$ Hz, 4H, ethylene), 4.06 (s, 3H, methoxy), 2.66 (s, 3H, acetyl), 2.00 (s, 3H, 2-methyl); APCI+: calcd for $C_{13}H_{15}N_4O_7$ 339.0941, found 339.0931.

1-(2-Hydroxyethyl)-2-methyl-6-(methylamino)-5,7-dinitro-1*H* benzimidazole (6a). A mixture of 270 mg (0.80 mmol) of **5** was dissolved in 3 mL of 24% methylamine in dry DMF and stirred at 40 °C for 4 h. The reaction mixture was then cooled to room temperature and concentrated to an oil under reduced pressure. The dark oil was purified by column chromatography using silica gel and dichloromethane/methanol (98:2) as the eluent. The eluents were concentrated to afford a red solid:120 mg (52%) yield; Mp 174–177 °C; TLC (dichloromethane, methanol [94:6]) $R_f = 0.33$; FTIR 3438, 3338, 3294, 3113, 3049, 1641, 1529, 1429, 1280, 1028, 896 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.42 (1 s, 1H, C(4)), 7.75 (q, $J = 5.1$ Hz, 1H, N-H), 4.98 (t, $J = 5.4$ Hz, 1H, alcohol), 4.07 (t, $J = 5.4$ Hz, N(1) methylene), 3.48 (q, $J = 5.4$ Hz, 2H, N(1) methylene), 2.77 (d, $J = 5.7$ Hz, 3H, methylamine), 2.56 (s, 3H, 2-methyl); APCI+: calcd for $C_{11}H_{14}N_5O_5$ 296.0995, found 296.0984.

General procedure for the synthesis of 6b–d

A solution of 100 mg (0.30 mmol) of **5** in 3 mL of the appropriate amine was stirred at 40–50 °C for 4 h. The reaction mixture was then cooled to room temperature and combined with 50 mL of chloroform. The organic solution was then washed with 25 mL of 1.0 N HCl and then 2 × 25 mL portions of water. The organic extract was dried over Na_2SO_4, concentrated to an oil under reduced pressure, and then purified by column chromatography using silica gel and dichloromethane–methanol (98 : 2). The eluents were concentrated to afford **6b–d** as red solids.

1-(2-Hydroxyethyl)-2-methyl-6(2-phenethylamino)-5,7-dinitro-1*H*-benzimidazole (6b). 62 mg (51% yield); Mp 132–134 °C; TLC (dichloromethane–methanol [90 : 10]) $R_f = 0.28$; FTIR 3302, 3026, 2933, 2881, 1635, 1583, 1525, 1440, 1257, 1246 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.21 (1 s, 1H, C(4)), 7.72 (broad s, 1H, N-H), 7.16 (multiplet, 5H, phenyl), 4.03 and 3.79 (2t, $J = 5.1$ Hz, 4H, N(1) ethylene), 3.26 (q, $J = 6.6$ Hz, 2H, C(6)), 2.86 (t, $J = 7.2$ Hz, 2H, C(6)), 2.5 (s, 3H, 2-methyl); APCI+: calcd for $C_{18}H_{20}N_5O_5$ 386.1464, found 386.1467.

1-(2-Hydroxyethyl)-2-methyl-5,7-dinitro-6-(cyclopropylamino)-1*H*-benzimidazole (6c). 53 mg (54% yield); Mp 182–184 °C; TLC (dichloromethane–methanol [95 : 5]) $R_f = 0.17$; FTIR 3365, 3246, 3095, 3014, 2972, 2885, 1631, 1581, 1520, 1431, 1286, 1125 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.31 (1 s, 1H, C(4)), 8.18 (broad s, 1H, N-H), 4.07 (t, $J = 4.5$, 2H, ethylene), 3.74 (broad t, N(1) ethylene), 3.03 (broad t, 1H, OH), 2.59 (multiplet, 1H, methylene), 2.45 (s, 3H, 2-methyl), 0.69 and 0.52 (2 doublet of triplet, $J = 5.4$ Hz and 6.6 Hz, 4H, ethylene); APCI+: calcd for $C_{13}H_{16}N_5O_5$ 322.1151, found 322.1152.

1-(2-Hydroxyethyl)-2-methyl-6-(2-methoxyethylamino)-5,7-dinitro-1*H*-benzimidazole (6d). 50 mg (50% yield); Mp 126–128 °C; TLC (dichloromethane–methanol [97 : 3]) $R_f = 0.23$; FTIR 3288, 3095, 2951, 2870, 2839, 1633, 1579, 1525, 1437, 1363, 1246, 1122, 1047 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.33 (1 s, 1H, C(4)), 8.0 (broad s, 1H, N-H), 4.07 and 3.79 (2t, $J = 4.8$ Hz, 4H, N(1) ethylene), 3.49 and 3.15 (2t, $J = 5.1$ Hz, 4H, C(6) ethylene), 3.30 (s, 3H, methoxy), 2.59 (s, 3H, 2-methyl); APCI+: calcd for $C_{13}H_{18}N_5O_6$ 340.1257, found 340.1257.

General procedure for the synthesis of 7a–d

To a solution of 300 mg of **6a–d** in 4 mL of dry prydine were added 400 μL of methane-sulfonylchloride. The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was combined with 50 ml of chloroform and the solution washed with water (1 × 25 mL) and with 1.0 N HCl (2 × 25 mL). The organic extract was then dried with Na_2SO_4, concentrated to an oil under reduced pressure, and then crystallized using dichloromethane–hexane.

1-(2-Methanesulfonoxyethyl)-2-methyl-6-(methylamino)-5,7-dinitro-1*H* benzimidazole (7a). 308 mg (81% yield); Mp 198–200 °C; TLC (dichloromethane–methanol [94 : 6]) $R_f = 0.47$; FTIR 3338, 3294, 3113, 3049, 1641, 1529, 1429, 1280, 1028, 896 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.45 (1 s, 1H, C(4)), 7.84 (q, $J = 5.1$ Hz, 1H, N-H), 4.35 (multiplet, 4H, N(1) ethylene), 3.06 (s, 3H, methanesulfonyl), 2.77 (d, $J = 5.4$ Hz, 3H, C(6) methylamine), 2.57 (s, 3H, 2-methyl); APCI+: calcd for $C_{12}H_{16}N_5O_7S$ 374.0770, found 374.0767.

1-(2-Methanesulfonoxyethyl)-2-methyl-6(2-phenethyl-amino)-5,7-dinitro-1*H*-benzimidazole (7b). 330 mg (92% yield); Mp 137 °C; TLC (dichloromethane–methanol [90 : 10]) $R_f = 0.70$; FTIR 3321, 3043, 2933, 2881, 1631, 1577, 1518, 1427, 1344, 1248, 1174 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.51 (1 s, 1H, C(4)), 7.82 (broad s, 1H, N-H), 7.24 (multiplet, 5H, phenyl), 4.27 (multiplet, 4H, N(1) ethylene), 3.28 and 2.87 (2t, $J = 6.9$ Hz, 4H, C(6)), 2.82 (s,

3H, methanesulfonyl) 2.56 (s, 3H, 2-methyl); APCI+: calcd for $C_{19}H_{22}N_5O_7S$ 464.1240, found 464.1218.

1-(2-Methanesulfonoxyethyl)-2-methyl-5,7-dinitro-6-(cyclopropylamino)-1*H*-benzimidazole (7c). 320 mg (86% yield); Mp 185–188 °C; TLC (dichloromethane–methanol [95 : 5]) $R_f = 0.46$; FTIR 3398, 3029, 3028, 2933, 1629, 1579, 1512, 1460, 1356, 1269 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.64 (1 s, 1H, C(4)), 8.39 (broad s, 1H, N-H), 4.40 (multiplet, 4H, ethylene), 2.98 (s, 3H, methanesulfonyl), 2.59 (multiplet, 1H, methylene), 2.66 (s, 3H, 2-methyl), 0.79 and 0.62 (2 doublet of triplets, $J = 5.4$ and 2.1, 4H, ethylene); APCI+: calcd for $C_{14}H_{18}N_5O_7S$ 400.0927, found 400.0920.

1-(2-Methanesulfonoxyethyl)-2-methyl-6-(2-methoxyethylamino)-5,7-dinitro-1*H*-benzimidazole(7d). 324 mg 88% yield); Mp 160–164 °C; TLC (dichloromethane–methanol [97 : 3]) $R_f = 0.36$; FTIR 3304, 3078, 2980, 2933, 2891, 2814, 1633, 1579, 1520, 1437, 1361, 1248, 1180, 1047 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.55 (1 s, 1H, C(4)), 4.29 (multiplet, 4H, N(1) ethylene), 3.57 and 3.15 (2t, $J = 4.8$ Hz, 4H, C(6) ethylene), 3.30 (s, 3H, methanesulfonyl), 2.84 (s, 3H, methoxy), 2.59 (s, 3H, 2-methyl); APCI+: calcd for $C_{14}H_{20}N_5O_8S$ 418.1032.

General procedure for the synthesis of 9a–d

A solution of 100 mg of **7a–d** in 60 mL of methanol was purged with N_2 and then combined with 100 mg of 5% Pd on carbon. This mixture was then shaken in a hydrogenation apparatus under 50 psi of H_2 for 24 h. After the reduction, the catalyst was filtered through a bed of Celite and the solution concentrated to an oil (**8a–d**) under reduced pressure. To this oil were added 5 mL of acetic anhydride and the mixture refluxed for 1 h. After completion of the reflux, the mixture was allowed to cool to room temperature and then concentrated under reduced pressure to an oil. For the synthesis of **9a–c**, 2 mL of 48% HBr were added to facilitate closure of the imidazo ring. For the synthesis of **9d**, 37% HCl was used to protect the side chain methoxy group. For **9a–d**, the respective acid mixtures were refluxed for 1 h. Afterwards, the mixture was allowed to cool to room temperature and concentrated under reduced pressure. The resulting dark oil was then crystallized using ethanol–ethyl acetate, yielding a hydrohalide salt.

5,6-Dihydro-2,7,8-trimethyl-7-diimidazo[1,5,4-*de*, 1,5,4-*h*]-quinoxaline (9a). 45 mg (46% yield); Mp ≥ 280 °C dec; TLC (*n*-butanol–acetic acid–water [5 : 2 : 3]) $R_f = 0.29$; FTIR 3431, 3346, 2920, 2750, 1655, 1529, 1462, 1423, 1317, 1195, 1072, 798, 563 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.30 (broad s, 1H, amine), 7.25 (1 s, 1H, C(10)), 4.42 and 3.71 (2t, $J = 5.4$ Hz, 4H, ethylene bridge), 2.79 and 2.78 (2 s, 6H, methyls); APCI+: calcd for $C_{13}H_{16}N_5$ 242.1406, found 242.1386.

5,6-Dihydro-2,8-dimethyl-7-(2-phenethyl)-diimidazo[1,5,4-*de*, 1,5,4-*h*]-quinoxaline (9b). 80 mg (75% yield); Mp ≥ 255 °C; TLC (*n*-butanol–acetic acid–water [5 : 2 : 3]) $R_f = 0.51$; FTIR 3188, 2920, 2741, 1653, 1512, 1421, 1340 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.17 (1 s, 1H, C(10)), 7.13 and 6.85 (2 multiplet, 5H, phenyl), 4.72 and 4.36 (2t, $J = 6$ Hz, 4H, N(7) ethylene), 3.63 and 3.04 (2t, $J = 6.6$ Hz, 4H, ethylene bridgehead), 2.70 and 2.15 (2 s, 6H, methyls); APCI+: calcd for $C_{20}H_{22}N_5$ 332.1875, found 332.1885.

7-Cyclopropyl-5,6-dihydro-2,8-dimethyl-diimidazo[1,5,4-*de*, 1,5,4-*h*]-quinoxaline (9c). 85 mg (80% yield); Mp dec ≥ 235 °C; TLC (*n*-butanol–acetic acid–water [5 : 2 : 3]) $R_f = 0.41$; FTIR 3310, 2982, 2918, 2758, 1655, 1516, 1334, 1205, 1047, 829, 557 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.11 (1 s, 1H, C(10)), 6.98 (broad s, 1H, N-H), 4.38 and 3.63 (2t, $J = 5.1$ Hz, 4H, ethylene bridgehead), 3.73 (multiplet, 1H, methylene), 2.71 and 2.69 (2 s, 6H, methyls), 0.79 and 0.62 (2 multiplet, 4H, ethylene); APCI+: calcd for $C_{15}H_{18}N_5$ 268.1562, found 268.1576.

5,6-Dihydro-2,8-dimethyl-7-(2-methoxyethyl)-diimidazo[1,5,4-*de*, 1,5,4-*h*]-quinoxaline (9d). 65mg (71% yield); Mp dec > 295 °C; TLC (*n*-butanol–acetic acid–water [5 : 2 : 3]) $R_f = 0.36$; FTIR 3396, 3306, 2931, 2814, 1655, 1577, 1633, 1516, 1431, 1342, 1194, 1039, 785, 559 cm^{-1}; too unstable for an ^1H-NMR; APCI+: calcd for $C_{15}H_{20}N_5O$ 286.1668, found 286.1645.

General procedure for the synthesis of 1a–d

A solution of 20–100 mg of **9a–d** in 20 ml of 0.2 M pH 7.0 phosphate buffer was combined with (excess) 250 mg of Fremy salt. This mixture was stirred for 5 min at room temperature and then the aqueous solution was extracted with 3 × 15 mL portions of chloroform. The organic layer was dried over Na_2SO_4 and then concentrated to a solid under reduced pressure. The final product was crystallized from ethyl acetate–hexane.

4,5-Dihydro-2,7,8-trimethyldiimidazo[1,5,4-*de*, 1,5,4-*h*]quinoxalin-10-one (1a). 5.0 mg (50% yield); Mp dec ≥ 280 °C; TLC (dichloromethane–methanol [90 : 10]) $R_f = 0.27$; FTIR 3493, 3041, 2982, 2947, 2850, 1685, 1655, 1606, 1541, 1249, 1103, 1028, 885 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.35 and 4.08 (2t, $J = 7.2$ Hz, 4H, ethylene bridge), 4.11 (s, 3H, N(7) methyl), 2.79 (s, 3H, C(2) methyl), 2.78 (s, 3H, 2-methyl); MALDI: calcd for $C_{13}H_{14}N_5O$ (M + 1) 256.120, found 256.115.

4,5-Dihydro-2,8-dimethyl-7-(2-phenethyl)-diimidazo[1,5,4-*de*, 1,5,4-*h*]-quinoxalin-10-one (1b). 6.5 mg (17% yield); Mp dec ≥ 264 °C; TLC (dichloromethane–methanol [96 : 4]) $R_f = 0.29$; FTIR 3431, 3149, 3028, 2930, 1655, 1560, 1438, 1174, 1111, 619 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.14 (t, 3H, phenyl (C3′, C4′ and C5′)), 6.94 (dd, $J = 1.8$ Hz and 6.0 Hz, phenyl (C2′ and C6′)), 4.40 and 3.96 (2t, $J = 7.2$ Hz, ethylene bridgehead), 4.25 and 2.96 (2t, $J = 7.2$ Hz, 4H, ethylene sidechain), 2.70 (s, 8-methyl), 2.15 (s, 3H, 2-methyl); MALDI: calcd for $C_{20}H_{20}N_5O$ (M + 1) 346.167, found 346.166.

4,5-Dihydro-2,8-dimethyl-7-cyclopropyl-diimidazo[1,5,4-*de*, 1,5,4-*h*]-quinoxalin-10-one (1c). 23 mg (33% yield); Mp dec ≥ 255 °C; TLC (dichloromethane–methanol [90 : 10]) $R_f = 0.27$; FTIR 3427, 3082, 3009, 2960, 2926, 1658, 1496, 1437, 1357, 1265, 1072 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.38 and 4.05 (2t, $J = 6.9$ Hz, 4H, ethylene bridge), 3.25 (sept, $J = 3.3$ Hz, 1H, methylene), 2.54 (s, 3H, 8-methyl), 2.41 (s, 3H, 2-methyl), 1.32 and 1.85 (2 multiplet, 4H, ethylene); MALDI: calcd for $C_{15}H_{16}N_5O$ (M + 1) 282.135, found 282.134.

4,5-Dihydro-7-(2-methoxyethyl)-2,8-dimethyl-diimidazo[1,5,4-*de*, 1,5,4-*h*]-quinoxalin-10-one (1d). 10.5 mg (28% yield); Mp dec ≥ 180 °C; TLC (dichloromethane–methanol [95 : 5]) $R_f = 0.32$; FTIR 3365, 3261, 3163, 2924, 2852, 1656, 1514, 1460, 1377, 1118, 1070, 659 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.89 and 3.79 (2t, $J = 5.4$ Hz, 4H, ethylene bridgehead), 4.34 and 4.07 (2t, $J = 6.6$ Hz, 4H, ethylene sidechain), 3.28 (s, 3H, methoxy), 2.50 (s, 3H,

8-methyl), 2.42 (s, 3H, 2-methyl); MALDI: Calcd for $C_{15}H_{18}N_5O$ (M + 1) 300.146, found 300.144.

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References

1 B. R. Copp, C. M. Ireland and L. R. Barrows, *J. Org. Chem.*, 1991, **56**, 4596.
2 J. M. Kokoshka, T. L. Capson, J. A. Holden, C. M. Ireland and L. R. Barrows, *Anti-Cancer Drugs*, 1996, **7**, 758.
3 L. M. Zhang, M. P. Cava, R. D. Rogers and L. M. Rogers, *Tetrahedron Lett.*, 1998, **39**, 7677.
4 R. Barret and N. Roue, *Tetrahedron Lett.*, 1999, **40**, 3889.
5 V. Beneteau, A. Pierre, B. Pfeiffer, P. Renard and T. Besson, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2231.
6 L. Legentil, L. Benel, V. Bertrand, B. Lesur and E. Delfourne, *J. Med. Chem.*, 2006, **49**, 2979.
7 L. Legentil, B. Lesur and E. Delfourne, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 427.
8 H. Hoang, D. V. LaBarbera, K. A. Mohammed, C. M. Ireland and E. B. Skibo, *J. Med. Chem.*, 2007, **50**, 4561.
9 H. H. Hodgson and R. Smith, *J. Chem. Soc.*, 1939, 263.
10 M. A. Phillips, *J. Chem. Soc.*, 1928, 2393.
11 H. Zimmer, D. C. Lankin and S. W. Horgan, *Chem. Rev.*, 1971, **71**, 229.
12 I. Islam and E. B. Skibo, *J. Org. Chem.*, 1990, **55**, 3195.
13 D. K. Paull, R. H. Shoemaker, L. Hodes, A. Monks, D. A. Scudiero, L. Rubinstein, J. Plowman and M. R. Boyd, *J. Natl. Cancer Inst.*, 1989, **81**, 1088.
14 R. H. Shoemaker, *Nat. Rev.*, 2006, **6**, 813–823.
15 M. R. Boyd and K. D. Paull, *Drug Dev. Res.*, 1995, **34**, 91.
16 E. A. Sausville, D. Zaharevitz, R. Gussio, L. Meijer, M. Louarn-Leost, C. Kunick, R. Schultz, T. Lahusen, D. Headlee, S. Stinson, S. G. Arbuck and A. Senderowicz, *Pharmacol. Ther.*, 1999, **82**, 285.
17 D. T. Ross, U. Scherf, M. B. Eisen, C. M. Perou, C. Rees, P. Spellman, V. Iyer, S. S. Jeffrey, M. Van De Rijn, M. Waltham, A. Pergamenschikov, J. C. E. Lee, D. Lashkari, D. Shalon, T. G. Myers, J. N. Weinstein, D. Botstein and P. O. Brown, *Nat. Genet.*, 2000, **24**, 227.
18 M. Shibuya and L. Claesson-Welsh, *Exp. Cell Res.*, 2006, **312**, 549.
19 G. Ranieri, R. Patruno, E. Ruggieri, S. Montemurro, P. Valerio and D. Ribatti, *Curr. Med. Chem.*, 2006, **13**, 1845.
20 H. Zhong and J. P. Bowen, *Curr. Top. Med. Chem.*, 2007, **7**, 1379.
21 F. Chatel, S. Morel, G. Boyer and J. P. Galy, *Heterocycles*, 2000, **53**, 2535.
22 W. S. Chen, H. J. Kung, W. K. Yang and W. C. Lin, *Int. J. Cancer*, 1999, **83**, 579.
23 R. H. J. Hargreaves, C. L. David, L. J. Whitesell, D. V. LaBarbera, A. Jamil, J. C. Chapuis and E. B. Skibo, *J. Med. Chem.*, 2008, **51**, 2492.

