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ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 4601–4608
Bioorganic & Medicinal Chemistry

Substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones: Synthesis, structure–activity relationships, and cytotoxic activity on selected human cancer cell lines

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Received 3 March 2007; revised 29 March 2007; accepted 4 April 2007
Available online 19 April 2007

Abstract—An efficient synthesis and the cytotoxic activity of a series of substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** is described. The synthesis was accomplished in an expedient manner (seven-steps) from commercially available starting materials. Several of the derivatives tested demonstrated significant in vitro cytotoxic activity against the human cancer cell lines H460 ($\geqslant$7 nM) and LCC6 ($\geqslant$28 nM). Following SAR and pharmacokinetic studies a derivative was further evaluated for its in vivo anti-tumor activity against a highly angiogenic human melanoma xenograft where it demonstrated significant efficacy as a mono-therapy and in combination with Taxol and Cisplatin.

1. Introduction

Xenorhabdus sp. are bacterial symbionts of soil-living, entomopathogenic nematodes and have been a rich source of biologically active compounds, most notably antibiotics.[1] It has been theorized that the antibiotics produced by the bacteria act to minimize competition from secondary microbial contaminants within their host and thus provide an optimal environment for growth.[2] Recently, while evaluating the bioactivity of small molecule metabolites isolated from *Xenorhabdus* sp. we demonstrated that the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** (Fig. 1) have significant anticancer activity.[3] There are several known natural products with related molecular structures such as the antibiotics holomycin **2** (R^1 = H and R^2 = Me) and thiolutin **3** (R^1 and R^2 = Me), both of which were isolated from the bacteria *Streptomyces* sp.[4] As well as displaying antimycobacterial activity, thiolutin **3** (R^1 and R^2 = Me) has been shown to suppress tumor-induced angiogenesis via the inhibition of focal adhesion kinase (FAK).[5] Thiomarinol A **4** is another structurally related and potent antibiotic which was isolated from marine bacteria *Alteromonas rava*.[6] Hall and co-workers have recently reported the asymmetric total synthesis of a thiomarinol antibiotic.[7] The total syntheses of holomycin **2** (R^1 = H and R^2 = Me) and thiolutin **3** (R^1 and R^2 = Me) have been reported in the literature.[8] However, the reported synthetic routes were not amendable to the preparation of derivatives with aromatic substituents on the pyrrolone nitrogen atom (R^1 = Ar), which were required for the present study. In this paper, we report the synthesis, structure–activity relationships, and cytotoxic activity on selected human cancer cell lines of a series of substituted 6-amino-4*H*-[1,2]dithiolo-[4,3-*b*]pyrrol-5-ones **1a**–**q**.

2. Results and discussion

2.1. Chemistry

The synthesis of the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** was accomplished in six steps from 1,3-bis(*tert*-butylsulfanyl)propan-2-one **6** (Scheme 1). The 1,3-bis(*tert*-butylsulfanyl)propan-2-

Keywords: Synthesis; 6-Amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones; Cytotoxicity; Anti-tumor.
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doi:10.1016/j.bmc.2007.04.017

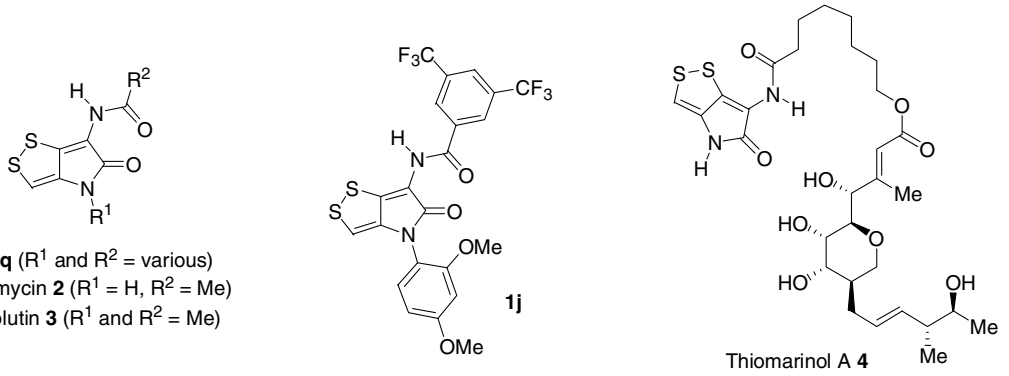
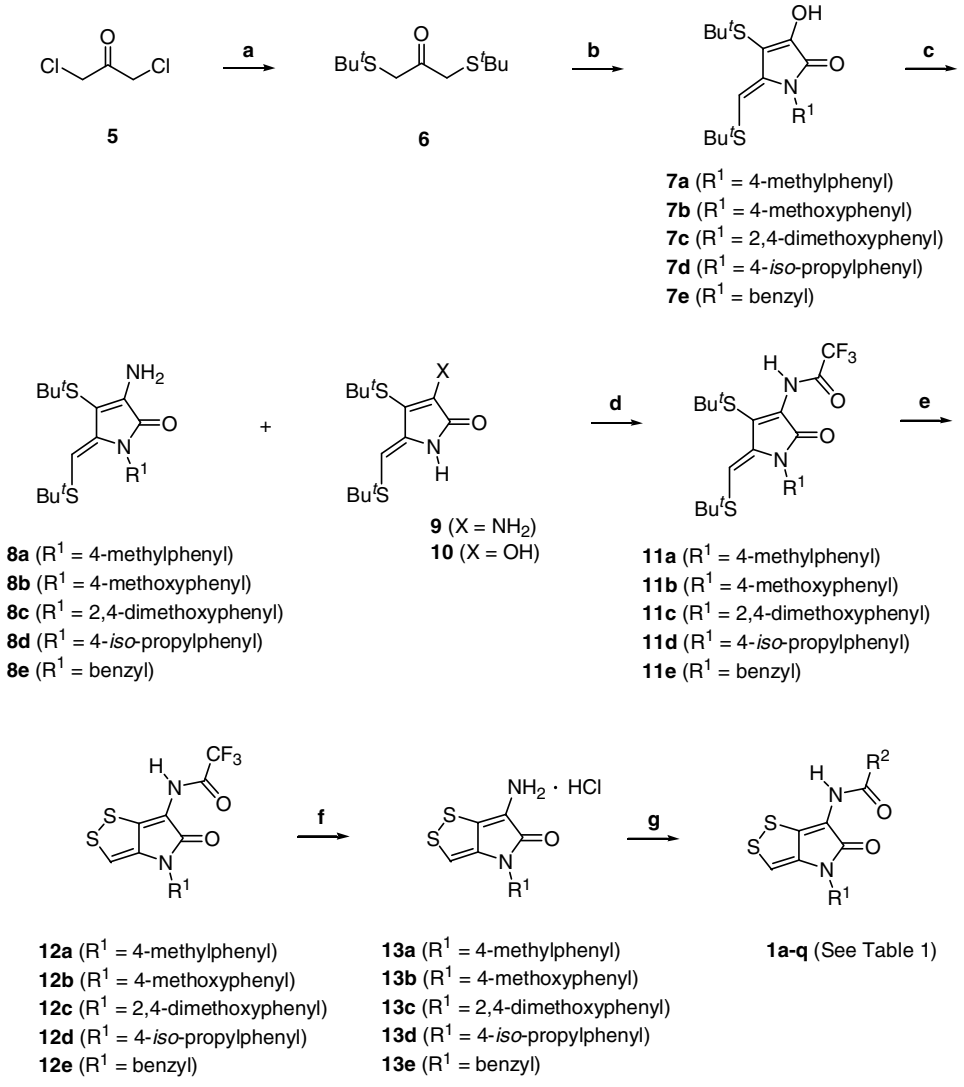


Figure 1. Substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q**, compound **1j**, and the structurally related natural products holomycin **2** (R^1 = H, R^2 = Me), thiolutin **3** (R^1 and R^2 = Me), and thiomarinol A **4**.



Scheme 1. Synthesis of the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q**. Reagents and conditions: (a) *tert*-butylmercaptan, NaOH (aq), DMF, rt; (b) R^1NH_2, $TiCl_4$, THF, 0 °C then oxalyl chloride, NEt_3, 0 °C–rt (60–70% yield, over two steps); (c) ammonium butanoate, 100 °C (80–96% yield); (d) trifluoroacetic anhydride, THF, rt (90–95% yield); (e) $Hg(OAc)_2$, TFA, rt then I_2, CH_2Cl_2, rt (50–75% yield); (f) HCl (concd), MeOH, reflux (65–80% yield); (g) R^2COCl, NEt_3, THF, rt (90–95% yield).

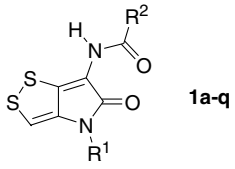
one **6** was prepared on a kilogram scale from commercially available 1,3-dichloroacetone **5** upon reaction with two equivalents of *tert*-butylmercaptan in the presence of sodium hydroxide.[9] The ketone **6** was reacted with the appropriate primary amine in tetrahydrofuran in the presence of titanium tetrachloride and triethylamine,

and the corresponding imines were subsequently reacted (without isolation) with oxalyl chloride to afford the cyclic enols **7a**–**e** in good yield (60–70% yield, over two steps). In all instances the cyclic enols **7a**–**e** were obtained as a single geometrical isomer. The cyclic enols **7a**–**e** were then converted to the cyclic enamines **8a**–**e** upon heating in molten ammonium acetate at 150 °C. However, under these reaction conditions significant amounts of the reaction byproducts **9** and **10** (up to 90% combined yield) were isolated along with the desired reaction products **8a**–**e**. The quantity of the byproducts isolated was substrate dependent and purification of the reaction mixtures required tedious chromatography. It was subsequently determined that using ammonium butanoate at 100 °C led to significantly improved yields of the desired reaction products **8a**–**e** (up to 95% yield).[10] Reaction of the enamines **8a**–**e** with trifluoroacetic anhydride in tetrahydrofuran afforded the corresponding trifluoroacetates **11a**–**e** in high yields. The *tert*-butyl protecting groups were then removed upon treatment with mercuric acetate in trifluoroacetic acid and the resultant dithiols were directly cyclized to the dithiopyrrolones **12a**–**e** under oxidative conditions upon treatment with iodine. Hydrolysis of the trifluoroacetate moieties with concentrated hydrochloric acid and methanol afforded the bicyclic enamines **13a**–**e** in good yield. It was essential to isolate the bicyclic enamines **13a**–**e** as their hydrochloride salts as they were unstable in their neutral form. In the final step of the synthesis, the bicyclic enamines **13a**–**e** were elaborated to the desired substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** upon treatment with the appropriate acyl chloride in the presence of triethylamine. Compounds **1a**–**q** were isolated as highly stable and brightly colored crystalline solids.

2.2. Biological results I (in vitro cytotoxicity)

For evaluation of their antiproliferative activity, the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** were tested in vitro against the actively growing human cancer cell lines H460 (lung) and LCC6 (breast). The effects are expressed as IC_{50} values below (Table 1). A number of the derivatives tested displayed notable cytotoxic activity against H460 ($\geqslant$7 nM) and LCC6 ($\geqslant$28 nM). The natural product **1a** (R^1 = H, R^2 = 1-methylpentyl, entry 1), which was isolated from *Xenorhabdus* sp., exhibited IC_{50} values of 200 nM for both H460 and LCC6. Significant enhancements of cytotoxic activity, particularly against H460, were observed with aromatic substituents on the pyrrolone nitrogen atom. Compound **1f** (R^1 = 2,4-dimethoxyphenyl, R^2 = methyl, entry 6) exhibited IC_{50} values of 30 and 190 nM for H460 and LCC6, respectively. Incorporation of a second aromatic substituent on the exocyclic amide moiety led to a further increase in potency. Compound **1h** (R^1 = 2,4-dimethoxyphenyl, R^2 = 4-trifluoromethylphenyl, entry 8) exhibited IC_{50} values of 7 and 85 nM for H460 and LCC6, respectively. Decreases in cytotoxicity were observed when benzyl substituents were incorporated on the pyrrolone nitrogen atom. Compound **1q** (R^1 = benzyl, R^2 = 3,5-bistrifluoromethylphenyl, entry 17) exhibited IC_{50} values of 706 and 1180 nM for H460 and LCC6, respectively. In general it was observed that aromatic subsituents on the pyrrolone nitrogen atom as well as the exocyclic amide moiety

Table 1. IC_{50} values of the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q**



1a-q

Entry	Compound	R^1	R^2	Cytotoxicity IC_{50}[a] (nM)	
				H460	LCC6
1	**1a**	H	1-Methylpentyl	200 ± 29	200 ± 25
2	**1b**	4-Methoxyphenyl	Methyl	53 ± 6	220 ± 31
3	**1c**	4-Methoxyphenyl	Trifluoromethyl	190 ± 24	220 ± 35
4	**1d**	4-Methylphenyl	Methyl	44 ± 7	220 ± 19
5	**1e**	2,4-Dimethoxyphenyl	Trifluoromethyl	170 ± 29	60 ± 9
6	**1f**	2,4-Dimethoxyphenyl	Methyl	30 ± 6	190 ± 43
7	**1g**	2,4-Dimethoxyphenyl	2,4-Dimethoxyphenyl	11 ± 1	251 ± 31
8	**1h**	2,4-Dimethoxyphenyl	4-Trifluoromethylphenyl	7 ± 1	85 ± 11
9	**1i**	2,4-Dimethoxyphenyl	3,5-Difluorophenyl	11 ± 1	349 ± 36
10	**1j**	2,4-Dimethoxyphenyl	3,5-Bis-trifluoromethylphenyl	130 ± 19	160 ± 22
11	**1k**	2,4-Dimethoxyphenyl	2-Furan	50 ± 5	28 ± 2
12	**1l**	2,4-Dimethoxyphenyl	2-Thiophene	8 ± 1	210 ± 34
13	**1m**	4-*iso*-Propylphenyl	3,5-Dihydroxy-4-*iso*-propylphenyl	150 ± 27	40 ± 7
14	**1n**	2,4-Dimethoxyphenyl	3,5-Dihydroxy-4-*iso*-propylphenyl	72 ± 11	70 ± 13
15	**1o**	Benzyl	Methyl	900 ± 137	—
16	**1p**	Benzyl	3,5-Dihydroxy-4-*iso*-propylphenyl	170 ± 32	250 ± 48
17	**1q**	Benzyl	3,5-Bis-trifluoromethylphenyl	706 ± 143	1180 ± 168

[a] IC_{50} values are reported as means ± SE of three independent determinations.

led to compounds with significantly increased potency with respect to the natural product **1a** (R^1 = H, R^2 = 1-methylpentyl, entry 1).

Following the preliminary investigations in our laboratory against the H460 and LCC6 cancer cell lines, the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** were tested on the National Cancer Institute's (NCI) panel of 60 cancer cell lines. It was demonstrated that the cytotoxicity of the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** was selective for solid cancer cells over hematological cancer cells since the LC_{50} values were significantly lower (~100-fold) in all of the solid cancer cell lines with respect to leukemia and lymphoma cell lines. Similar selectivity was not observed in the IC_{50} values which measure growth inhibition and thus, as anticancer agents, the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** may be most effective against solid tumors. During the NCI testing, compounds **1j** and **1n** emerged as the derivatives which demonstrated the most promising overall in vitro cytotoxicity (NCI data not shown).

The NCI's COMPARE algorithm did not yield any significant activity correlations with any other compounds in the database which indicates that the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** may exert their cytotoxic effect via a unique mode of action. Of note, we have demonstrated that the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** induce apoptosis in both human endothelial cells and human caner cells using the Annexin V apoptosis assay. Moreover, the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** do not affect cell-cycle progression and thus the demonstrated cytotoxic effect is not simply due to the inhibition of cell proliferation.

2.3. Biological results (pharmacokinetics study)

To assess the pharmacokinetic (PK) properties of this class of substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q**, compounds **1j** and **1n** were administered by intraperitoneal injection (IP) to female mice. These two compounds were chosen due to their superior in vitro cytotoxicity profiles that were observed in the NCI's extensive cell line screening. The mean plasma concentrations were plotted versus time following an IP dose of 20 mg/kg (Fig. 2).

Each of the compounds **1j** and **1n** was absorbed rapidly into the blood stream and their C_{max} values (57 and 106 μM, respectively) were reached within 30 min of administration. Over the next 8 h, the plasma concentrations of **1j** and **1n** decreased significantly. The plasma concentrations of compounds **1j** and **1n** exceeded their corresponding IC_{50} values for both the H460 and LCC6 cell lines for the entire duration of the PK experiments (8 h). The overall IP pharmacokinetic profiles of compounds **1j** and **1n** are summarized below (Table 2). The systemic plasma clearances (CL) for compounds

Table 2. The IP pharmacokinetic profile of compounds **1j** and **1n** in female mice[a]

Parameter	Unit	Compound **1j**	Compound **1n**
Dose	μmol/kg	36.8	38.2
C_{max}	μM	57	106
T_{max}	min	30	3
$T_{1/2}$	min	160	94
$AUC_{(0-480)}$	μM min	12,629	6097
CL	mL/min/kg	2.9	6.3

[a] Reported values are means of two mice/time/concentration data points.

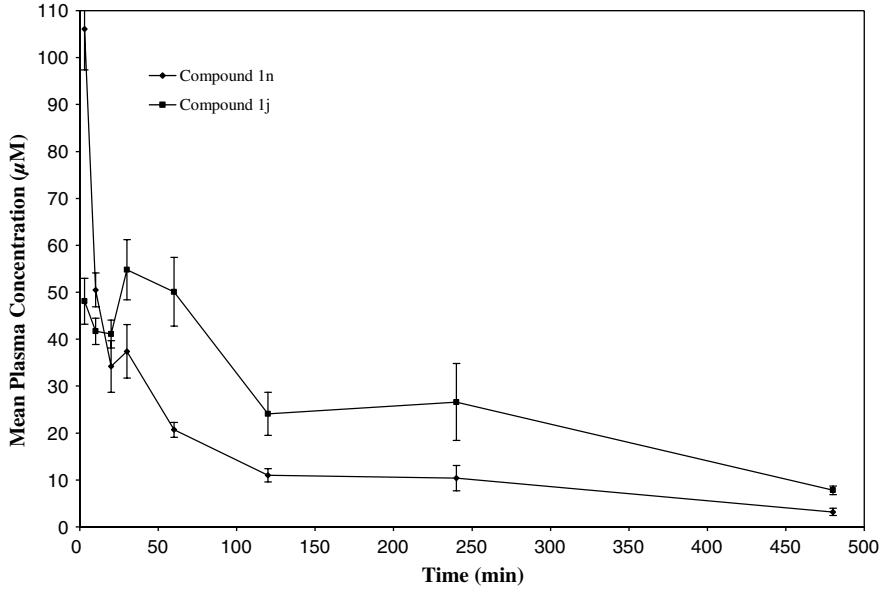


Figure 2. A plot of the mean plasma concentration versus time for compounds **1j** and **1n** following intraperitoneal injection (20 mg/kg body weight). The error bars represent the overall data distribution ($\pm SD/n^{1/2}$).

1j and **1n** were 2.9 mL/min/kg and 6.3 mL/min/kg, respectively. The calculated half-life ($T_{1/2}$) for compounds **1j** and **1n** was 160 min and 94 min, respectively.

Similar pharmacokinetic profiles were observed when compounds **1j** and **1n** were orally administered. High calculated oral bioavailabilities (F_{po}) of greater than 70% were noted. Following an analysis of the pharmacokinetic and in vitro cytotoxicity data, compound **1j** was selected for advancement into in vivo anti-tumor efficacy studies.

2.4. Biological results (in vivo anti-tumor activity)

The in vivo anti-tumor activity of compound **1j** was evaluated as a mono-therapy and as a combination-therapy with the commercial chemotherapy drugs Taxol or Cisplatin in male mice (*Mus musculus*) implanted subcutaneously with a human SKMEL-28 V+ tumor xenograft. SKMEL-28 V+ tumor cells are a variant of SKMEL-28 cells that have been engineered to overexpress vascular endothelial growth factor (VEGF) and thus display high angiogenic activity. This particular tumor was chosen due to the hypothesis that compound **1j** may inhibit tumor-induced angiogenesis.[4] In the mono-therapy experiments, compound **1j** was administered by intraperitoneal injection daily for 24 days (5 and 10 mg/kg body weight) or every second day (20 mg/kg body weight). Upon completion of the experiment, compound **1j** had demonstrated significant in vivo anti-tumor activity in the absence of gross (e.g., loss of body weight) or histological indications of toxicity. Dose–response efficacy was observed and, at the highest dose of 20 mg/kg given every second day, tumor growth was inhibited by approximately 58% as compared to vehicle mice (Fig. 3).

In the combination-therapy experiments, compound **1j** was administered by intraperitoneal injection daily for 24 days (5 and 10 mg/kg body weight) and in addition either Taxol (10 mg/kg body weight) or Cisplatin (3 mg/kg body weight) was administered by intraperitoneal injection every second day for 24 days (total of 12 doses). In combination with Taxol, compound **1j** at 10 mg/kg every day inhibited SKMEL-28 V+ tumor growth by approximately 87% compared to vehicle mice (Taxol alone induced approximately 37% inhibition, Fig. 3). In combination with Cisplatin, compound **1j** at 10 mg/kg every day inhibited SKMEL-28 V+ growth by approximately 76% (Cisplatin alone induced approximately 26% inhibition, Fig. 3).

2.5. Conclusion

We have described the efficient synthesis and preliminary biological evaluation of a novel class of substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** as potential anticancer agents. SAR studies demonstrated that aromatic substituents on the pyrrolone nitrogen atom as well as the exocyclic amide moiety led to compounds with significant in vitro antiproliferative activity against H460 ($\geqslant$7 nM) and LCC6 ($\geqslant$28 nM) cancer cell lines while displaying favorable pharmacokinetic profiles. Ultimately, compound **1j** was selected for in vivo anti-tumor activity studies upon human SKMEL-28 V+ xenografts as both a mono-therapy and in combination with commercial Cisplatin or Taxol where strong efficacy was demonstrated. Work to determine the molecular target(s) responsible for the anti-cancer activity of the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1a**–**q** is currently underway and will be reported in due course.

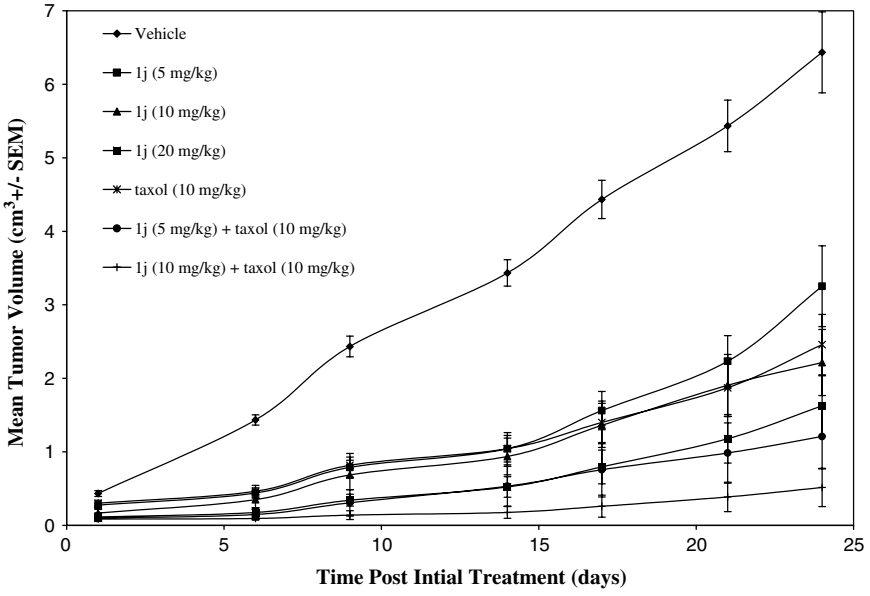


Figure 3. The in vivo efficacy of compound **1j** (5, 10, and 20 mg/kg) as a mono-therapy and in combination with Taxol (10 mg/kg) or Cisplatin (3 mg/kg) on SKMEL-28 V+ human tumor xenograft growth compared to vehicle. The error bars represent the overall data distribution ($\pm SD/n^{1/2}$).

3. Experimental

3.1. General experimental

3.1.1. Chemistry. All non-aqueous reactions were performed under an atmosphere of dry nitrogen and in flame- or oven-dried glassware unless stated otherwise. The reaction temperatures stated were those of the external bath. All solvents and reagents were purified according to standard procedures or used as supplied.[11] Silica gel chromatography ('flash chromatography') was carried out using Merck silica gel 60 (230–400 mesh).[12] All proton and carbon nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR, respectively) were recorded on a Varian 400 FT spectrometer at ambient temperature. The chemical shifts for all compounds are listed in parts per million downfield from tetramethylsilane using the NMR solvent as an internal reference. The reference values used for deuterated chloroform ($CDCl_3$) were 7.26 and 77.16 ppm for ^{1}H and ^{13}C NMR spectra, respectively. Low resolution mass spectra (MS) were recorded on a Hewlett–Packard 5985 GC-mass spectrometer. The mode of ionization used was chemical ionization (CI) with isobutane. Microanalyses were performed on a Carlo Erba Model 1106 CHN analyzer.

3.1.2. Determination of IC_{50} values. The human cancer cell lines, H460 and LCC6, were purchased from the American Type Culture Collection (ATCC) and were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS) which was purchased from Invitrogen. The drug solutions were prepared in DMSO and the cells were treated with the appropriate concentrations for 48 h. The cells were then incubated with MTT (0.5 mg/mL) for 4 h, the resultant MTT color precipitates were dissolved in DMSO, and the absorbance was determined at 470 nm. The percentage of cell growth versus log concentration was plotted to generate a linear equation. The IC_{50} value of the compound is the concentration that gives a 50% growth.[13]

3.1.3. Pharmacokinetic studies. The pharmacokinetic studies with the substituted 6-amino-4*H*-[1,2]dithiolo-[4,3-*b*]pyrrol-5-ones **1n** and **1j** were conducted in conscious male mice ($n = 2$ per study). The compounds **1n** and **1j** were administered by either intraperitoneal injection in a vehicle of 5% chremophore in saline at a dose of 20 mg/kg or orally via gavage in a vehicle of soybean oil at a dose of 20 mg/kg. Blood samples of 300 μL were drawn from the tail vein for determination of the plasma concentrations of the substituted 6-amino-4*H*-[1,2]dithiolo[4,3-*b*]pyrrol-5-ones **1n** and **1j** at multiple time points up to 8 h after administration. Calibration standards were prepared via serial dilution from a 5 μg/mL acetonitrile standard. Blood samples of 100 μL were centrifuged and the resultant plasma was extracted with 300 μL of cold acetonitrile. HPLC analysis for quantification was performed on a Waters 2695 instrument with a Waters μ Boundpack C_{18} column. The mobile phase was comprised of 30% water (containing 0.2% triethylamine and 0.5% phosphoric acid) and 70% acetonitrile at a flow rate of 1.0 mL/min. The retention times for compounds **1n** and **1j** were 8.13 min and 5.51 min, respectively.

3.1.4. Tumor animal models and treatment. The animal experiments were approved by McGill University Ethics Committee and were conducted at the Lady Davis Institute Animal Facility in accordance with the Canadian Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act. Clinical grade Cisplatin and Taxol were purchased from the Oncology Pharmacy at the Jewish General Hospital (McGill University) and stored at room temperature or at 4 °C, respectively. To induce tumor formation in mice, SKMEL-28 cells (originally from the ATCC) were engineered to overexpress mouse VEGF and were grown to 60% confluence in DMEM that was purchased from Invitrogen and supplemented with 10% FBS and then harvested using trypsin–EDTA solution. The cells were centrifuged and washed twice with phosphate-buffered saline solution and then re-suspended at a dilution of $1–2 \times 10^6$ cells/0.1 mL. The cell viability was confirmed by Trypan blue staining. Only those cells with >95% viability and 'normal' morphology were used for the in vivo experiments. Approximately 2 million cells were used to induce primary tumors in five mice. Once the tumor size reached approximately 1 cm^3, the tumor was isolated, dissected to remove non-tumor tissue, washed three times with phosphate-buffered saline (PBS), and then sliced into small pieces of approximately 1 mm^3. Tissue explants were then inoculated subcutaneously into individual mice. Once the tumors became palpable, the mice were randomized into experimental groups and the treatment was initiated. All drugs were administered by intraperitoneal injection daily or every second day. Primary tumor growth was monitored every second to fifth day using a caliper. Relative tumor volume (cm^3) was estimated using the formula $[(\text{length(cm)} \times \text{width(cm)}^2)/2]$. At the end of the experiment, the mice were sacrificed by cervical dislocation and a full autopsy was conducted.

3.2. Chemical experimental

3.2.1. 4-*tert*-Butylsulfanyl-5-*tert*-butylsulfanylmethylene-1-(2,4-dimethoxyphenyl)-3-hydroxy-1,5-dihydro-pyrrol-2-one (7c). To a stirred solution of 1,3-bis(*tert*-butylsulfanyl)propan-2-one **6** (2.34 g, 10.0 mmol) in tetrahydrofuran (100 mL) were added 2,4-dimethoxyaniline (1.53 g, 10.0 mmol) and triethylamine (2.8 mL, 20 mmol) and the resultant solution was cooled to 0 °C. A solution of titanium tetrachloride (1.04 g, 5.50 mmol) in hexanes (15 mL) was added dropwise over the course of 30 min. After the addition was complete, the reaction mixture was allowed to warm to room temperature and was then heated at reflux temperature for 2 h. The reaction mixture was cooled to −10 °C and oxalyl chloride (0.84 mL, 10 mmol) was added followed by the dropwise addition of a solution of triethylamine (2.8 mL, 20 mmol) in tetrahydrofuran (100 mL) over the course of 30 min. After the addition was complete, the reaction mixture was allowed to warm to room temperature and was stirred for 15 h. The solution was then filtered and the precipitate was washed with ether (250 mL). The

combined filtrate was washed with water (3× 50 mL), dried over anhydrous sodium sulfate, and concentrated in vacuo to afford the crude product. The crude product was recrystallized from ethyl acetate/hexanes to afford the *title compound* **7c** (2.97 g, 70%) as a light yellow crystalline solid. Mp >190 °C, ethyl acetate/hexanes; ^{1}H NMR ($CDCl_3$) δ 1.30 (9H, s, C(CH_3)$_3$), 1.41 (9H, s, C(CH_3)$_3$), 3.76 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 6.45 (1H, s, vinyl C*H*), 6.53–6.60 (2H, m, Ar*H*), 7.09–7.17 (1H, m, Ar*H*); **MS** (CI) *m*/*z* (rel. intensity) 424 (M+H, 100); Anal. Calcd for $C_{21}H_{29}NO_4S_2$: C, 59.54; H, 6.90; N, 3.31. Found: C, 59.34; H, 6.97; N, 3.19.

3.2.2. 4-*tert*-Butylsulfanyl-5-*tert*-butylsulfanylmethylene-1-(2,4-dimethoxyphenyl)-3-amino-1,5-dihydro-pyrrol-2-one (8c). A mixture of the enol **7c** (2.12 g, 5.00 mmol) and ammonium butanoate (50 g, 478 mmol) was heated to 100 °C and held at that temperature for 15 min. The reaction mixture was allowed to cool to approximately 50 °C and was poured into water (100 mL) and the resultant mixture was extracted with ether (3× 100 mL). The combined organic extracts were washed with an aqueous solution of sodium hydroxide (10% w/w, 25 mL) and then with water (2× 25 mL). The organic solution was then dried over anhydrous sodium sulfate and concentrated in vacuo to afford the crude product. Flash chromatography using hexanes/ethyl acetate (1:1) as the eluant afforded the *title compound* **8c** (2.01 g, 95%) as a yellow crystalline solid. Mp 123–124 °C, ethyl acetate/hexanes; ^{1}H NMR ($CDCl_3$) δ 1.28 (9H, s, C(CH_3)$_3$), 1.40 (9H, s, C(CH_3)$_3$), 3.77 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.43 (2H, broad s, NH_2), 6.14 (1H, s, vinyl C*H*), 6.52–6.58 (2H, m, Ar*H*), 7.13–7.16 (1H, m, Ar*H*); **MS** (CI) *m*/*z* (rel. intensity) 423 (M+H, 100); Anal. Calcd for $C_{21}H_{30}N_2O_3S_2$: C, 59.68; H, 7.16; N, 6.63. Found: C, 59.30; H, 7.27; N, 6.52.

3.2.3. *N*-[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl]-2,2,2-trifluoroacetamide (12c). To a stirred solution of the enamine **8c** (2.11 g, 5.00 mmol) in tetrahydrofuran (20 mL) was added trifluoroacetic anhydride (0.76 mL, 5.5 mmol) and the resultant solution was stirred for 30 min. The reaction mixture was concentrated in vacuo to afford the trifluoroacetamide **11c** (2.60 g, 100%) as a yellow crystalline solid. This material was taken up in trifluoroacetic acid (50 mL) and was treated with mercuric acetate (1.59 g, 5.00 mmol) and the resultant solution was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo and the residue was taken up in acetonitrile (100 mL). Hydrogen sulfide was then bubbled through the reaction mixture for 1 h. The reaction mixture was then degassed of excess hydrogen sulfide via bubbling nitrogen through the reaction mixture for 30 min. A solution of iodine (1.27 g, 5.00 mmol) in dichloromethane (50 mL) was then added dropwise over the course of 10 min and the reaction mixture was then stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo to afford the crude product. Flash chromatography using ethyl acetate/hexanes/dichloromethane (1:1:1) as the eluant afforded the *title compound* **12c** (1.36 g, 67%) as a yellow crystalline solid. Mp 151–152 °C, ethyl acetate/hexanes/dichloromethane; ^{1}H NMR ($CDCl_3$) δ 3.73 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 6.52 (1H, s, vinyl C*H*), 6.53–6.56 (2H, m, Ar*H*), 7.14–7.16 (1H, m, Ar*H*), 8.43 (1H, s, N*H*COCF_3); MS (CI) *m*/*z* (rel. intensity) 405 (M+H, 100); Anal. Calcd for $C_{15}H_{11}F_3N_2O_4S_2$: C, 44.55; H, 2.74; N, 6.93. Found (V_2O_5 added): C, 44.35; H, 2.96; N, 6.69.

3.2.4. *N*-[4-(2,4-Dimethoxyphenyl)-5-oxo-4,5-dihydro-[1,2]-dithiolo[4,3-*b*]pyrrol-6-yl]-3,5-bis-trifluoromethyl benzamide (1j). To a stirred solution of the trifluoroacetamide **12c** (1.00 g, 2.47 mmol) in methanol (150 mL) was added concentrated hydrochloric acid (5 mL) and the resultant solution was heated at reflux temperature for 2 h. The reaction mixture was allowed to cool to room temperature and was then concentrated in vacuo to afford the hydrochloride salt **13c** (760 mg, 89%) as a green crystalline solid. This material was taken up in tetrahydrofuran (30 mL) and was treated with 3,5-bis-trifluoromethylbenzoyl chloride (622 mg, 2.25 mmol). Triethylamine (0.55 mL, 4.2 mmol) was then added dropwise over the course of 2 min and the resultant solution was stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo to afford the crude product. Flash chromatography using ethyl acetate/hexanes/dichloromethane (1:1:1) afforded the *title compound* **1j** (977 mg, 81%) as a bright orange crystalline solid. Mp 135–138 °C, ethyl acetate/hexanes/dichloromethane; ^{1}H NMR ($CDCl_3$) δ 3.78 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 6.53–6.57 (3H, m, vinyl C*H* and Ar*H*), 7.21 (1H, d, J = 7 Hz, Ar*H*), 8.06 (1H, s, ArH), 8.48 (2H, s, Ar*H*), 8.89 (1H, s, N*H*CO); **MS** (CI) *m*/*z* (rel. intensity) 549 (M+H, 100); **Anal.** Calcd for $C_{22}H_{14}F_6N_2O_4S_2$: C, 48.18; H, 2.57; N, 5.11. Found: C, 47.90; H, 2.90; N, 4.73.

Acknowledgments

We are grateful to the National Research Council of Canada (NRC) for financial support (NRC-IRAP #611050). MPAL thanks the National Sciences and Engineering Research Council of Canada (NSERC) for an industrial postdoctoral research fellowship. We also thank the National Cancer Institute (NCI) for in vitro screening and Simon Fraser University for NMR services.

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