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Natural product based inhibitors of the thioredoxin–thioredoxin reductase system †

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Spiroketal naphthodecalins are readily assembled by Barton's base mediated Ullmann binaphthyl ether coupling, Dakin reactions and hypervalent iodine spirocyclization. The core structures can be further diversified by enone addition and Stille coupling reactions. Nanomolar inhibitors for the Trx/TrxR redox control system were prepared by this approach and compared to series of natural product isolates. Cytotoxicity in MCF-7 cell assays ranged from an IC_{50} of 1.6 to >100 μM.

Introduction

Natural products continue to provide structurally complex but highly original lead structures for drug discovery programs.[1] We have recently discovered that the naphthoquinone spiroketal pharmacophore of the palmarumycin family of fungal metabolites produces potent inhibitors of the thioredoxin–thioredoxin reductase cellular redox system.[2,3] Thioredoxin (Trx) is the major cellular protein disulfide reductase in a broad range of organisms, including humans. Trx itself is reduced by electrons originating from NADPH *via* the enzymatic assistance of thioredoxin reductase (TrxR). Trx protein levels are significantly elevated in several human primary cancers with highly significant correlations between increased Trx levels, tumor proliferation and inhibited apoptosis.[4,5] Recent reports indicate that increased Trx expression is an independent prognostic factor for decreased patient survival in patients with colon cancer and non small cell lung cancer.[6,7] Redox activity is essential for the biological effects of Trx.[8] Mechanisms by which Trx-1 produces its effects (Fig. 1) include increased levels of the hypoxia inducible factor-1α transcription factor subunit that mediates the growing tumor's response to hypoxia and its constant need for increased oxygen and nutrient supply through increased angiogenesis;[9] decreased binding of the NF-E2-related factor 2 and polyamine modulated factor-1 (Nrf-2/PMF-1) transcription factor leading to decreased expression of spermidine/spermine N^1-acetyltransferase (SSAT), a regulator of polyamine induced apoptosis;[10] and inhibition of the tumor suppressor protein PTEN, a phosphatidylinositol (PtdIns)-3-phosphatase that attenuates the activity of the PtdIns-3-kinase/

† Electronic supplementary information (ESI) available: experimental procedures for **S1**–**S10**. Copies of ^{1}H and ^{13}C NMR spectra for all new compounds. See http://www.rsc.org/suppdata/ob/b4/b402431a/

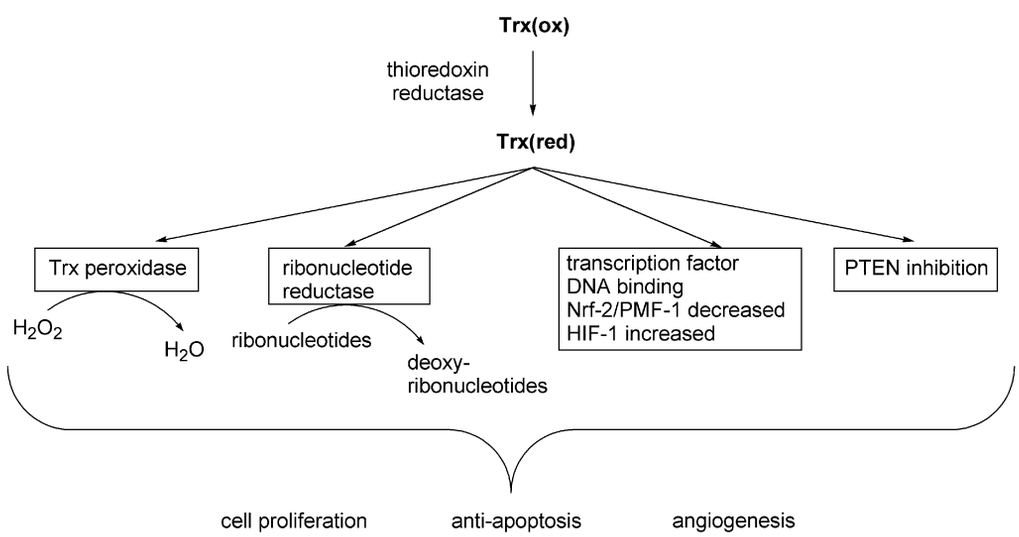

Fig. 1 Some mechanisms by which Trx stimulates tumor growth. Trx is reduced by thioredoxin reductase (TrxR). Reduced Trx is a cofactor for thioredoxin peroxidases and ribonucleotide reductase; it increases the HIF-1 transcription factor, thus increasing tumor angiogenesis. It also decreases the DNA binding of the Nrf-2/PMF-1 transcription factor leading to decreased levels of apoptosis and inducing acetylated polyamines; and it binds and inhibits PTEN, thus de-repressing the Akt cell survival signaling pathway.

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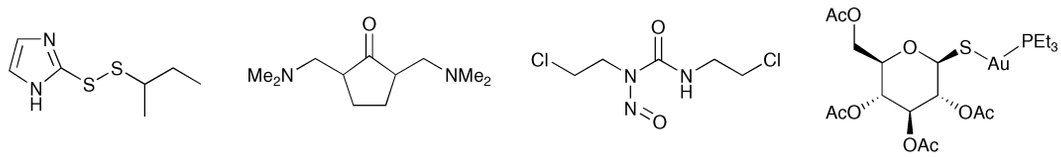


Fig. 2 Chemotherapeutic inhibitors of the Trx/TrxR system.

Akt (protein kinase B) cell survival signaling pathway.[11] Trx is also implicated in defence against damage caused by H_2O_2-based oxidative stress acting through thioredoxin peroxidases, members of the peroxiredoxin family that use Trx as a source of reducing equivalents to scavenge H_2O_2.[12]

The Trx/TrxR system is of medicinal interest due to its inherent role as a broad based indicator of diseases such as AIDS, rheumatoid arthritis, and certain forms of cancer and represents an attractive target for further pharmacological examination. Over the past decade, biological screening has identified a number of diverse small organic and organometallic molecules as chemotherapeutic inhibitors of Trx and TrxR (Fig. 2).[13] Nitrosoureas such as BCNU were found early on to be effective irreversible inhibitors of TrxR.[14] However, these compounds are quite toxic, relatively non-selective, and known to alkylate DNA. Alkyl 2-imidazolyl disulfides such as PX-12 and the symmetrical cyclopentanone NSC 131233 were revealed as inhibitors through screening by a COMPARE analysis from over 50 000 compounds tested by the National Cancer Institute. PX-12 is currently in Phase I clinical trials and demonstrated antitumor activity in immunodeficient mice xenograft models.[15] Auranofin, commonly used for the treatment of rheumatoid arthritis, was also discovered to be a selective tight-binding inhibitor of Trx/TrxR.[16]

Our biological assays revealed that palmarumycin CP_1 inhibited the Trx/TrxR system with an IC_{50} of 350 nM, a level of activity that compared well with that reported for the structurally more complex *para*-quinone pleurotin (Fig. 3).[2,17]

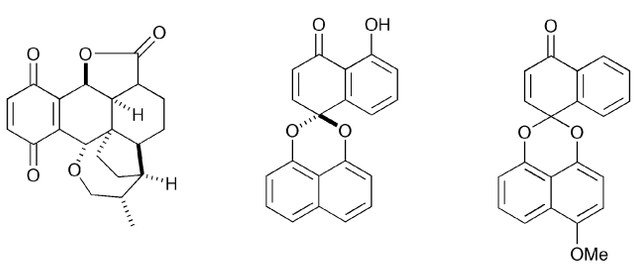


Fig. 3 Small molecule inhibitors of the Trx/TrxR system.

Moreover, palmarumycin CP_1 was found to affect growth inhibition at 4–20 times lower concentrations than pleurotin, and several members of a small library of analogs possessing a naphthoquinone spiroketal moiety were found to be potent and selective inhibitors of Trx-1/TrxR.[2] Both the phenol group and the enone functionality present in palmarumycin CP_1 appeared to be important for maximizing enzymatic activity. Additionally, it was determined that the presence of the naphthalenediol ketal enhances Trx-1 over TrxR selectivity compared to aliphatic ketals. It should be noted that many of these derivatives also demonstrated potent cytotoxicity as evidenced through low micromolar activities for growth inhibition against two breast cancer cell lines.[2] We now report a new SAR study of naphthoquinone spiroketal analogs of the palmarumycin family of fungal metabolites; many of these derivatives were again found to be potent inhibitors of the thioredoxin–thioredoxin reductase cellular redox system.

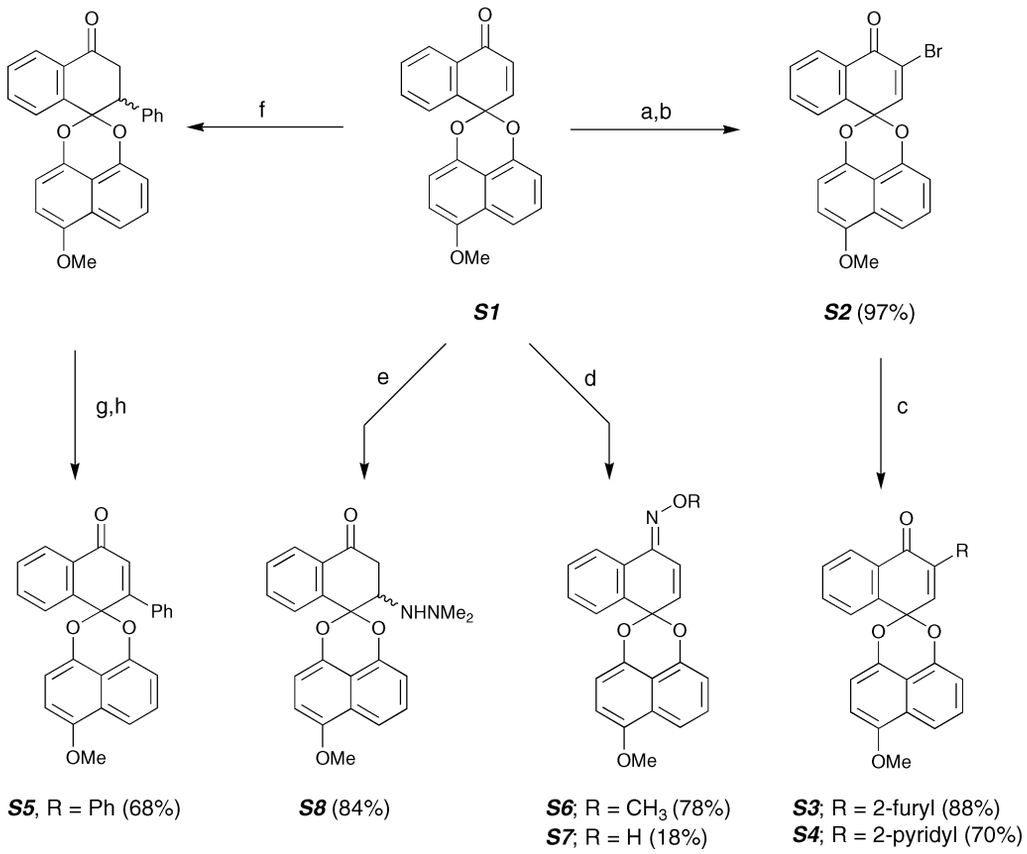
Results and discussion

Synthesis

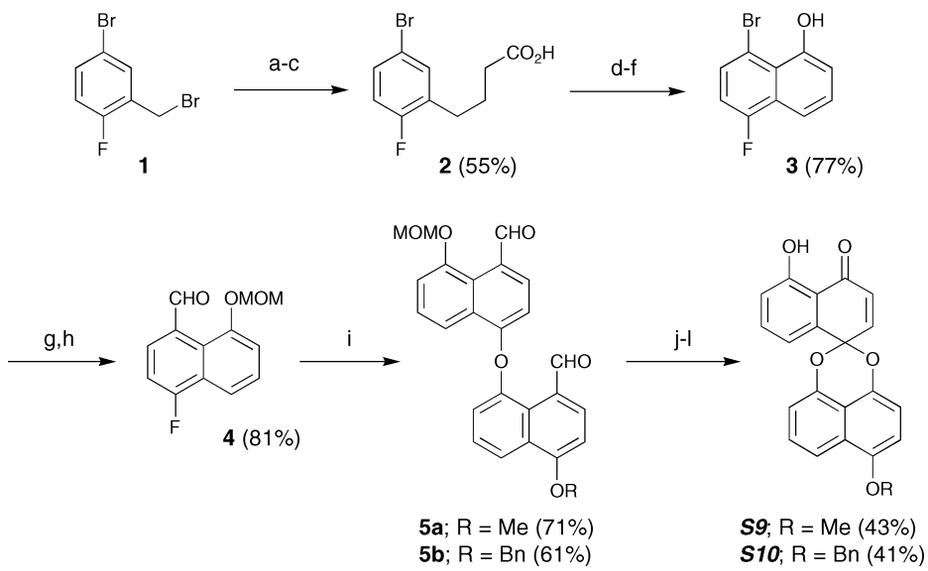
Previous work in our laboratories had identified a rapid synthetic access toward palmarumycin analogs such as **S1**.[18] Interestingly, although this compound lacks the hydroxyl substituent on one of the arenes, it was found to be a moderately potent inhibitor of Trx-1/TrxR. In order to further investigate the pharmacology of this new structural motif, we envisioned introducing structural diversity into the **S1** scaffold at several key sites in close proximity to the naphthoquinone spiroketal. α-Substitution at the ketone was achieved in 97% yield by a bromination/elimination sequence using $PhMe_3NBr_3$ and Et_3N (Scheme 1). The bromide **S2** was further functionalized through a palladium-mediated coupling with organostannanes to afford the heteroaryl derivatives **S3** and **S4** in good yield. In order to introduce enone β-functionalization, a 3 step protocol was devised. Methylaluminium bis(2,6-di-*tert*-butyl-4-methylphenoxide) (MAD) promoted Michael addition of phenyllithium into the naphthoquinone monoketal was conducted according to the procedure of Swenton.[19] The resulting diastereomeric mixture of products was reoxidized in a bromination/dehydrohalogenation sequence using $PhMe_3NBr_3$ and Li_2CO_3 and resulted in the formation of **S5** in good yield. The carbonyl group of **S1** was also converted to either the oxime (**S7**) or the oxime ether (**S6**) by treatment with hydroxylamine or methoxylamine, respectively, in pyridine at ambient temperature. Repeated attempts to obtain the hydrazone from **S1** under a variety of conditions resulted exclusively in conjugate addition to afford the hydrazine **S8** as the sole product.

The more highly oxygenated **S9** was synthesized analogous to **S1** as outlined in Scheme 2. The known benzyl bromide **1**[20] was substituted by allylmagnesium Grignard reagent to afford an intermediate butene which was subjected to hydroboration/oxidation, followed by Jones oxidation to give the acid **2** in 55% yield for the 3 step sequence. The acid underwent cationic ring closure in the presence of PPA to afford the substituted tetralone which was oxidized in good yield to the naphthol **3** through a bromination/dehydrohalogenation protocol. The bromonaphthol was protected as its MOM ether and then converted to the fluoronaphthaldehyde **4** by treatment with *t*-BuLi and quenching with DMF. This key intermediate **4** underwent nucleophilic aromatic substitution (S_NAr) by 5-hydroxy-1-methoxynaphthalenecarboxaldehyde[21] in the presence of Barton's base[18] to afford the dinaphthyl ether **5a** in 71% yield. Exposure of the bis-formylated **5a** to excess *m*-CPBA followed by $NaBH_4$-mediated reduction of the resulting bisformate ester gave an electron-rich bisnaphthol which smoothly participated in the intramolecular oxidative spirocyclization[22] initiated by $PhI(OCOCF_3)_2$ to provide the palmarumycin analogue **S9** in 74% yield. Apparently, the slightly acidic environment in the spirocyclization step resulted in the concomitant loss of the MOM ether. An additional derivative (**S10**) bearing a benzyl ether in place of the methyl ether was prepared from fluoronaphthaldehyde **4** *via* dinaphthyl ether **5b** using analogous methodology.

O-Demethylation of **S1** and **S9** under a variety of conditions led to extensive decomposition, and therefore we had to devise an alternative route to access the desired free phenols (Scheme 3). MOM-protection of **6**[23] was followed by



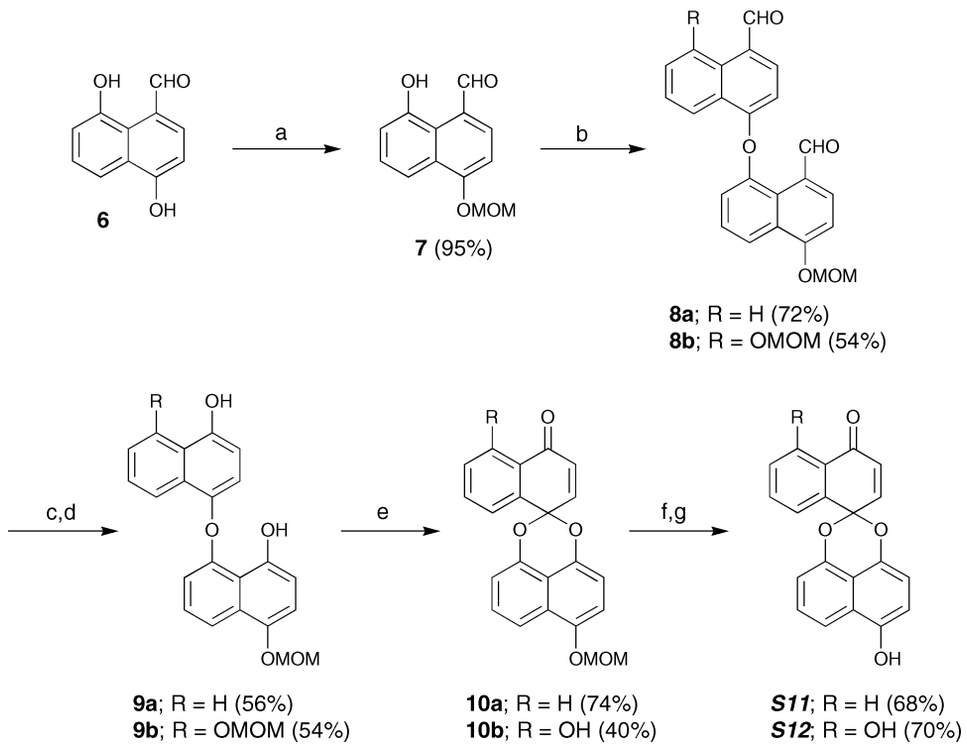
Scheme 1 *Reagents and conditions*: (a) $PhMe_3NBr_3$, THF, rt, 3 h. (b) Et_3N, CH_2Cl_2, 0 °C, 1 h. (c) Bu_3SnR, $Pd(PPh_3)_4$ (5 mol%), CuI (40 mol%), DMF, 80 °C, 2 h. (d) $RONH_2$·HCl, pyridine, rt, 24 h. (e) Me_2NNH_2, EtOH, 80 °C, 1 h. (f) Methylaluminium bis(2,6-di-*tert*-butyl-4-methylphenoxide), toluene/CH_2Cl_2, −78 °C; PhLi, −78 °C to rt, 1 h. (g) $PhMe_3NBr_3$, THF, rt, 30 min. (h) Li_2CO_3, LiBr, DMF, 130 °C, 1 h.



Scheme 2 *Reagents and conditions*: (a) Allyl-MgBr, THF. (b) BH_3·DMS, THF; NaOH, H_2O_2. (c) Jones reagent, acetone. (d) PPA, 120 °C. (e) $PhMe_3NBr_3$, THF. (f) Li_2CO_3, LiBr, DMF, 130 °C. (g) NaH, MOMCl, DMF. (h) *t*-BuLi, Et_2O, −78 °C; DMF. (i) Barton's base, 5-hydroxy-1-methoxy-4-naphthalenecarboxaldehyde or 5-hydroxy-1-benzyloxy-4-naphthalenecarboxaldehyde, CH_3CN, 70 °C. (j) *m*-CPBA, CH_2Cl_2. (k) $NaBH_4$, MeOH/THF (1 : 1), 0 °C. (l) $PhI(OCOCF_3)_2$, CH_3CN, 0 °C.

substitution with 4-fluoro-1-naphthalenecarboxaldehyde[24] or the fluoride **4** in the presence of Barton's base[18] to give the diaryl ethers **8a** and **8b**, respectively. Dakin reactions, reductive deformylations, and oxidative spirocyclizations provided the MOM ethers **10a** and **10b**, which were subsequently deprotected with TMS-Br[25] in good yields to give the desired **S11** and **S12**. The crude reaction mixture contained significant amounts of silyl enol ether derived from conjugate addition of bromide ion to the enone, and therefore a treatment with TBAF was used as part of the workup protocol.

We were able to augment this synthetic library of palmarumycin and **S1** analogs by several natural product naphthalenediol ketals.[26] As part of INBio's current initiatives for identifying trypanothione reductase (TR) inhibitors, natural products derived from fermentation broths of microfungi have been evaluated. The anamorph of an unidentified Ascomycete fungi[27] produced several naphthalenediol ketals in culture. The original teleomorph was collected in 1996 in the Guanacaste Conservation Area, Costa Rica, from decaying trunk material and identified originally to belong to the *Rhytidhysteron* genus.[28]



Scheme 3 *Reagents and conditions*: (a) K_2CO_3, MOMCl, acetone, rt. (b) 4-Fluoro-1-naphthalenecarboxaldehyde or **4**, Barton's base, CH_3CN, 80 °C. (c) *m*-CPBA, CH_2Cl_2. (d) $NaBH_4$, MeOH/THF (1 : 1), 0 °C. (e) $PhI(OCOCF_3)_2$, CH_3CN, 0 °C. (f) TMSBr, 4 Å molecular sieves, CH_2Cl_2. (g) TBAF, THF.

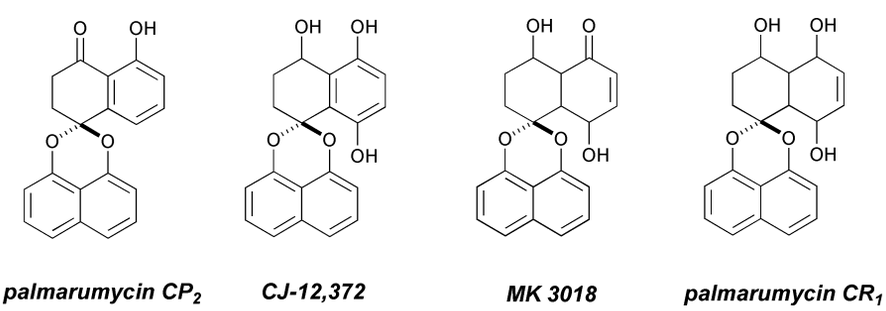


Fig. 4 Natural products screened for Trx/TrxR inhibition.

Three agar plugs containing the preserved material were activated for 15 days, and the resulting inocula were further cultured for an additional 15 days at 25 °C in 100 mL of Czapek Dox broth media (100 mL × 60 flasks) in an orbital shaker at 150 rpm for a final volume of 6 L. Approximately 100 mL of 95% ethanol were added to each culture media and left overnight. Cell disruption was further assisted with a biomixer on the following day. Filtration to separate mycelia and evaporation to 10% of its original volume was achieved under vacuum. The resulting hydroalcoholic solution was added to water and extracted with three portions of methylene chloride and three portions of ethyl acetate, and all organic extractions were combined and evaporated. The resulting 1.97 g of extract were separated on MPLC using RP-18 to yield five main fractions. The fraction with the correct NMR profile was collected, yielding 565 mg. The final step of separation was achieved using a BioXplore© HPLC system whereby 98 mg of MK-3018,[29] 87 mg of the new compound palmarumycin CR_1,[30] 54 mg of CJ-12,372[31] and 5 mg of palmarumycin CP_2[32] were obtained (Fig. 4). All structures were elucidated by means of ^{1}H-/^{13}C-NMR, COSY, HSQC, DEPT and HMBC experiments and the molecular weight was corroborated with HR-MS. These natural products are currently under evaluation for their TR and hGR inhibitory activity at INBio's laboratories, and we were also interested in determining their specificity by assays in the mammalian Trx/TrxR system.

Trx-1/TrxR inhibition and cell growth inhibition assays

For all compounds, Trx/TrxR activities were measured in microtiter plate colorimetric assays using purified human placenta TrxR and recombinant human Trx.[33] TrxR activity was measured as the increase in absorbance at 405 nm which occurs as dithionitrobenzoic acid (DTNB) is reduced by the enzyme-mediated transfer of reducing equivalents from NADPH. Trx activity was measured as the Trx dependent reduction of insulin in the presence of TrxR and NADPH, detected by the reduction of DTNB at the end of the incubation. Antiproliferative activity was measured using an estrogen receptor positive, p53 positive MCF-7 human breast cancer cell line. The cells were seeded at 4000 cells/well in 96-well microtiter plates and allowed to attach overnight. After exposure to the compounds for 72 h, viable cells were stained with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide. Plates were incubated for 3 h in the dark and measured spectrophotometrically at 540 nm.[34] Results are summarized in Table 1.

In contrast to palmarumycin CP_1 and MK 3018, the naturally occurring naphthalenediol spiroketals palmarumycin CP_2, CJ-12,372, and palmarumycin CR_1 showed no cytotoxicity and only insignificant inhibition of the Trx-1/TrxR redox system. This demonstrates that the presence of an enone function is necessary for biological activity in this class of fungal

Table 1 IC_{50} values [μM] for Trx-1/TrxR inhibition and cell growth inhibition[35]

Entry	Compound	Trx-1/TrxR	MCF-7
1	Pleurotin	0.17	4.1
2	Palmarumycin CP_1	0.35	1.0
3	Palmarumycin CP_2	>100	>50
4	CJ-12,372	14.4	>50
5	MK 3018	8.4	10.0
6	Palmarumycin CR_1	>50	>100
7	**S9**	1.0	14.0
8	**S10**	5.2	14.2
9	**S1**	3.2	9.2
10	**S6**	>50	80.0
11	**S7**	23.6	10.2
12	**S2**	3.1	2.6
13	**S3**	6.4	6.0
14	**S4**	5.0	1.6
15	**S5**	>50	>100
16	**S8**	16.6	10.4
17	**S11**	0.34	2.8
18	**S12**	0.20	2.6

metabolites. The introduction of an oxime or oxime ether in place of the carbonyl group present in **S1** also led to a significant reduction of the Trx-1/TrxR inhibition, although cytotoxicity was conserved in the oxime **S7**. We were particularly interested in reducing the inherent electrophilicity of the α,β-unsaturated carbonyl moiety by chemical modifications at the α- and β-sites in order to reduce the possibility for unselective alkylations of other cellular thiols. Substitution at the enone β-position was not tolerated for cytotoxicity. An increase in biological activity in both the cellular and the enzymatic assays over the parent **S1** was only accomplished with the brominated derivative **S2**. However, the cytotoxicity increased several-fold in **S4**, the 2-pyridyl derivative. Since the 2-furyl derivative **S3** also showed a low-micromolar cytotoxicity, it is possible to generalize that substitution at the α-position of the enone is well tolerated by the Trx-1/TrxR complex and can be used to increase cellular activity. Interestingly, while we expected a significant boost in the Trx-1/TrxR activity due to the presence of the phenolic hydroxy group in **S9** *versus* the lead structure **S1**, the increase in enzymatic inhibitory activity was only 3-fold, and the cellular activity dropped off to 14 μM. Conversely, the *O*-demethylated analog, bis-phenolic **S12**, as well as the monophenol **S11** demonstrated excellent Trx-1/TrxR activities of 0.20 and 0.34 μM, respectively, while maintaining a high level of cytotoxicity in the MCF-7 cell line. **S12** represents therefore the first thioredoxin inhibitor equipotent to pleurotin, in spite of the considerably simpler structure of the newer compound. **S11** is also of considerable interest for this structure–activity analysis because it demonstrates that the substituents of the naphthalene moiety of **S1** are indeed strongly influencing biological activity.

Conclusion

We have been able to expand the structural diversity and the SAR of naphthalenediol spiroketal-based low-micromolar Trx-1/TrxR inhibitors by the preparation of a focused library of **S1** derivatives that takes advantage of selective and high-yielding chemical modifications of the pharmacophore moiety. The enone function is critical for biological activity, but a β-hydrazine adduct (**S8**) could likely function as a precursor of this group. Carbon-substitution at the β-position tends to reduce the activity, in particular in the MCF-7 assay. Cytotoxicity can be increased significantly by the presence of α-substituents at the enone, and several derivatives that lack the phenolic hydroxy group found in palmarumycin CP_1 but maintain a good overall activity profile could be identified. Two synthetic derivatives, **S11** and **S12**, were found that match or exceed the Trx-1/TrxR activities of the potent natural products palmarumycin CP_1 and pleurotin. Further biological studies, in particular *in vivo* assays of suitable derivatives, will be reported in due course.

Experimental

General

All reactions were performed in flame-dried or oven-dried glassware under a dry nitrogen atmosphere. THF and ether were distilled over Na/benzophenone, and CH_2Cl_2 was purified by passage through a column of activated alumina. All other reagents and solvents were used as received unless otherwise noted. NMR spectra were recorded in $CDCl_3$ (unless otherwise noted) at either 300 MHz (^{1}H NMR) or 75 MHz (^{13}C NMR) using a Bruker Avance 300 with XWIN-NMR software. Melting points were determined on a Mel-Temp II and are uncorrected.

8-Hydroxy-4-methoxymethoxynaphthalene-1-carbaldehyde (7)

To a solution of 4,8-dihydroxynaphthalene-1-carbaldehyde (**6**, 1.00 g, 5.31 mmol) in acetone (25 mL) was added K_2CO_3 (1.47 g, 10.6 mmol) followed by chloromethyl methyl ether (80%; 0.46 mL, 4.8 mmol). The reaction mixture was stirred at room temperature for 1.5 h, quenched with H_2O and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and passed through a 1″ pad of SiO_2 (CH_2Cl_2) to provide 1.06 g (95%) of **7** as a yellow solid: mp 64–65 °C (CH_2Cl_2); IR (neat) 2904, 2806, 2704, 1649, 1519, 1273, 1222 cm^{-1}; ^{1}H NMR δ 12.11 (d, 1H, J = 0.9 Hz), 9.61 (d, 1H, J = 0.9 Hz), 7.91 (d, 1H, J = 8.2 Hz), 7.87 (dd, 1H, J = 8.3, 1.2 Hz), 7.50 (t, 1H, J = 8.0 Hz), 7.18 (dd, 1H, J = 7.8, 1.2 Hz), 7.12 (d, 1H, J = 8.2 Hz), 5.49 (s, 2H), 3.56 (s, 3H); ^{13}C NMR δ 196.1, 160.9, 156.0, 145.4, 128.9, 128.6, 126.4, 122.9, 116.9, 113.8, 106.2, 95.0, 57.1; MS (EI) *m*/*z* (rel intensity) 232 (M^+, 100), 202 (22), 170 (28), 131 (22), 115 (25), 77 (20); HRMS (EI) calcd for $C_{13}H_{12}O_4$ 232.0736, found 232.0735.

4-(5-Methoxymethoxy-8-formylnaphthalen-1-yloxy)-naphthalene-1-carbaldehyde (8a)

To a solution of naphthol **7** (510 mg, 2.20 mmol) and 4-fluoro-1-naphthalenecarbaldehyde[36] (348 mg, 2.00 mmol) in CH_3CN (8 mL) at room temperature was added 2-*tert*-butyl-1,1,3,3-tetramethylguanidine (0.48 mL, 2.4 mmol). The reaction mixture was heated at 70 °C for 1 h, additional Barton's base (0.10 mL, 0.50 mmol) was added, and heating was continued for 2 h. The reaction mixture was then cooled to room temperature, poured into 1.0 M HCl (30 mL), and extracted with CH_2Cl_2 (2 × 40 mL). The combined organic layers were washed with H_2O (30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography of the residue on SiO_2 (hexanes/EtOAc, 7 : 3) gave 552 mg (72%) of **8a** as a pale yellow solid: mp 100–101 °C (hexanes/EtOAc); IR (neat) 2904, 2735, 1682, 1576, 1507, 1419, 1321, 1219, 1161 cm^{-1}; ^{1}H NMR δ 10.90 (d, 1H, J = 0.5 Hz), 10.22 (s, 1H), 9.35 (d, 1H, J = 8.5 Hz), 8.47 (dd, 1H, J = 8.4, 0.7 Hz), 8.35 (dd, 1H, J = 8.5, 1.1 Hz), 8.15 (d, 1H, J = 8.3 Hz), 7.79–7.74 (m, 1H), 7.78 (d, 1H, J = 8.0 Hz), 7.69–7.64 (m, 1H), 7.53 (dd, 1H, J = 8.4, 7.6 Hz), 7.30 (dd, 1H, J = 7.6, 1.1 Hz), 7.24 (d, 1H, J = 8.3 Hz), 6.76 (d, 1H, J = 8.0 Hz), 5.50 (s, 2H), 3.59 (s, 3H); ^{13}C NMR δ 192.2, 192.1, 158.8, 157.7, 152.2, 138.0, 132.6, 131.0, 130.2, 128.3, 127.6, 127.5, 127.4, 126.7, 126.3, 126.2, 125.3, 122.3, 120.5, 119.9, 110.1, 108.0, 95.0, 56.9; MS (EI) *m*/*z* (rel intensity) 386 (M^+, 57), 359 (54), 331 (26), 159 (87), 130 (27), 105 (100); HRMS (EI) calcd for $C_{24}H_{18}O_5$ 386.1154, found 386.1147.

4-(5-Methoxymethoxy-8-hydroxynaphthalen-1-yloxy)-naphth-1-ol (9a)

To a solution of dialdehyde **8a** (464 mg, 1.20 mmol) in CH_2Cl_2 (25 mL) was added 70% *m*-CPBA (890 mg, 3.60 mmol). The reaction mixture was stirred at room temperature for 15 h. A solution of 10% $Na_2S_2O_3$ (30 mL) was added and stirring was continued for 1 h. The organic layer was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography of the residue on SiO_2 (hexanes/EtOAc, 3 : 2) afforded 340 mg (68%) of 4-(5-methoxymethoxy-8-formyloxynaphthalen-1-yloxy)-1-formyloxynaphthalene as a pale yellow foam: IR (neat) 2945, 1739, 1601, 1508, 1461, 1369, 1254, 1113 cm^{-1}; ^1H NMR δ 8.50 (s, 1H), 8.33–8.29 (m, 1H), 8.18 (dd, 1H, J = 8.5, 1.0 Hz), 8.08 (s, 1H), 7.99–7.96 (m, 1H), 7.66–7.56 (m, 2H), 7.42 (dd, 1H, J = 8.4, 7.8 Hz), 7.17 (d, 1H, J = 8.3 Hz), 7.14 (d, 1H, J = 8.1 Hz), 7.08 (d, 1H, J = 8.4 Hz), 7.01 (dd, 1H, J = 7.6, 1.0 Hz), 6.70 (d, 1H, J = 8.3 Hz), 5.42 (s, 2H), 3.58 (s, 3H); ^{13}C NMR δ 160.5, 159.9, 152.3, 151.7, 141.4, 138.7, 129.3, 127.9, 127.8, 127.3, 127.1, 126.5, 122.6, 121.6, 121.1, 120.1, 119.0, 117.7, 117.5, 111.5, 108.2, 95.2, 56.6; MS (EI) *m*/*z* (rel intensity) 418 (M^+, 74), 390 (72), 345 (100), 317 (41), 299 (37), 159 (42), 144 (86), 115 (67); HRMS (EI) calcd for $C_{24}H_{18}O_7$ 418.1053, found 418.1065.

To a solution of this bisformate ester (248 mg, 0.593 mmol) in 1 : 1 MeOH/THF (10 mL) at 0 °C was slowly added $NaBH_4$ (50.0 mg, 1.30 mmol). The reaction mixture was stirred at 0 °C for 1 h, diluted with EtOAc (25 mL) and washed with H_2O. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was *immediately* subjected to chromatography on SiO_2 (hexanes/EtOAc, 3 : 2) to afford 177 mg (83%) of **9a** as a white foam: IR (neat) 3433 (br), 1608, 1460, 1395, 1262, 1228, 1152 cm^{-1}; ^1H NMR δ 9.20 (s, 1H), 8.29–8.26 (m, 1H), 7.96–7.91 (m, 2H), 7.55–7.43 (m, 2H), 7.17 (d, 1H, J = 8.4 Hz), 7.16 (dd, 1H, J = 8.4, 7.8 Hz), 7.09 (d, 1H, J = 8.1 Hz), 6.97 (d, 1H, J = 8.4 Hz), 6.76 (d, 1H, J = 8.1 Hz), 6.50 (dd, 1H, J = 7.7, 0.9 Hz), 6.46 (s, 1H), 5.36 (s, 2H), 3.61 (s, 3H); ^{13}C NMR δ 156.6, 150.2, 148.8, 146.0, 143.0, 129.1, 128.2, 127.6, 126.3, 125.9, 125.7, 122.7, 121.8, 118.3, 117.1, 115.8, 111.7, 110.0, 109.6, 108.1, 95.9, 56.5; MS (EI) *m*/*z* (rel intensity) 362 (M^+, 67), 317 (57), 299 (14), 143 (100), 115 (45); HRMS (EI) calcd for $C_{22}H_{18}O_5$ 362.1154, found 362.1153.

1-Oxo-1,4-dihydronaphthalene-4-spiro-2′-naphtho[4″-methoxymethoxy-1″,8″-*de*][1′,3′]dioxin (10a)

To a solution of bisnaphthol **9a** (285 mg, 0.786 mmol) in dry CH_3CN (15 mL) at 0 °C was added $PhI(OCOCF_3)_2$ (370 mg, 0.860 mmol) in one portion. The reaction mixture was stirred at 0 °C for 30 min and concentrated under reduced pressure. The residue was dissolved in EtOAc (40 mL), washed with H_2O (20 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography on SiO_2 (hexanes/EtOAc, 4 : 1) gave 207 mg (74%) of **10a** as a yellow foam: IR (neat) 2955, 1675, 1611, 1425, 1385, 1299, 1266, 1157, 1047 cm^{-1}; ^1H NMR δ 8.16 (dd, 1H, J = 7.8, 1.1 Hz), 7.97 (dd, 1H, J = 7.8, 0.8 Hz), 7.90 (dd, 1H, J = 8.5, 0.8 Hz), 7.75 (td, 1H, J = 7.6, 1.4 Hz), 7.62 (td, 1H, 7.6, 1.2 Hz), 7.47 (dd, 1H, J = 8.5 and 7.6 Hz), 7.10 (d, 1H, J = 8.3 Hz), 7.01 (dd, 1H, J = 7.6, 0.7 Hz), 7.00 (d, 1H, J = 10.6 Hz), 6.88 (d, 1H, J = 8.3 Hz), 6.37 (d, 1H, J = 10.6 Hz), 5.37, 5.35 (AB-system, 2H, J = 6.7 Hz), 3.57 (s, 3H); ^{13}C NMR δ 183.5, 148.4, 147.7, 141.9, 138.9, 138.7, 134.0, 130.4, 130.3, 130.2, 128.1, 127.3, 126.7, 126.5, 116.4, 113.9, 110.8, 110.3, 109.8, 95.6, 93.2, 56.4; MS (EI) *m*/*z* (rel intensity) 360 (M^+, 71), 315 (100), 264 (7), 202 (7), 174 (62), 158 (33); HRMS (EI) calcd for $C_{22}H_{16}O_4$ 360.0998, found 360.0993.

1-Oxo-1,4-dihydronaphthalene-4-spiro-2′-naphtho[4″-hydroxy-1″,8″-*de*][1′,3′] dioxin (S11)

To a solution of spirocycle **10a** (80 mg, 0.22 mmol) in CH_2Cl_2 (3 mL) containing powdered 4 Å molecular sieves (200 mg) at 0 °C was added bromotrimethylsilane (0.12 mL, 0.88 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature, stirred for 12 h and filtered over Celite (CH_2Cl_2). The filtrate was concentrated under reduced pressure. The residue was dissolved in THF (3 mL), and TBAF (1.0 M in THF; 0.22 mL, 0.22 mmol) was slowly added. The reaction mixture was stirred at room temperature for 15 min, quenched with H_2O and extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was passed through a short pad of SiO_2 (CH_2Cl_2) to afford 48 mg (68%) of **S11** as an orange solid: mp 195 °C (acetone, dec); IR (neat) 3344 (br), 1666, 1597, 1415, 1385, 1264, 1044 cm^{-1}; ^1H NMR (acetone-d_6) δ 8.99 (s, 1H), 8.12 (ddd, 1H, J = 7.8, 1.4, 0.5 Hz), 8.05 (ddd, 1H, J = 7.8, 1.3, 0.5), 7.90 (dd, 1H, J = 8.5, 0.8), 7.88 (td, 1H, J = 7.6, 1.4 Hz), 7.74 (td, 1H, J 7.6, 1.3 Hz), 7.51 (dd, 1H, J = 8.5, 7.6 Hz), 7.14 (d, 1H, J = 10.6 Hz), 7.03 (dd, 1H, J = 7.6, 0.8 Hz), 6.94 (d, 1H, J = 8.3 Hz), 6.86 (d, 1H, J = 8.3 Hz), 6.42 (d, 1H, J = 10.6 Hz); ^{13}C NMR (acetone-d_6) δ 183.0, 148.6, 147.8, 140.2, 139.3, 139.2, 134.0, 130.6, 130.2, 130.0, 128.4, 126.6, 125.9, 125.5, 116.6, 114.0, 110.4, 110.1, 109.5, 93.1; MS (EI) *m*/*z* (rel intensity) 316 (M^+, 100), 219 (6), 174 (24), 159 (29), 144 (28), 131 (17), 115 (15), 102 (22); HRMS (EI) calcd for $C_{20}H_{12}O_4$ 316.0736, found 316.0734.

1-(5-Methoxymethoxy-8-formylnaphthalen-1-yloxy)-5-methoxymethoxy-naphthalene-4-carbaldehyde (8b)

To a solution of naphthol **7** (592 mg, 2.55 mmol) and fluoride **4** (400 mg, 1.70 mmol) in CH_3CN (6 mL) at room temperature was added 2-*tert*-butyl-1,1,3,3-tetramethylguanidine (0.52 mL, 2.6 mmol). The reaction mixture was heated at 80 °C for 5 h, cooled to room temperature, poured into 1.0 M HCl (20 mL), and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were washed with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on SiO_2 (hexanes/EtOAc, 7 : 3) afforded 100 mg (25%) of fluoride **4** and 410 mg (54%) of **8b** as a pale yellow solid: mp 123–124 °C (hexanes/EtOAc); IR (neat) 1675, 1505, 1417, 1321, 1260, 1220, 1159, 1000 cm^{-1}; ^1H NMR δ 11.09 (s, 1H), 10.94 (s, 1H), 8.30 (dd, 1H, J = 8.4, 1.0 Hz), 8.16 (d, 1H, J = 8.3 Hz), 8.10 (dd, 1H, J = 8.3, 0.8 Hz), 7.88 (d, 1H, J = 8.1 Hz), 7.53 (t, 1H, J = 8.4 Hz), 7.50 (t, 1H, J = 8.4 Hz), 7.40 (d, 1H, J = 7.8 Hz), 7.25 (d, 1H, J = 8.3 Hz), 7.21 (dd, 1H, J = 7.7, 1.0 Hz), 6.80 (d, 1H, J = 8.1 Hz), 5.50 (s, 2H), 5.43 (s, 2H), 3.59 (s, 3H), 3.55 (s, 3H); ^{13}C NMR δ 194.2, 192.5, 157.7, 157.2, 154.2, 152.8, 130.9, 130.8, 128.8, 128.2, 127.6, 127.5, 126.6, 126.2, 125.7, 120.0, 119.1, 116.4, 112.1, 111.9, 108.0, 95.4, 95.0, 56.9, 56.8; MS (EI) *m*/*z* (rel intensity) 446 (M^+, 63), 401 (100), 373 (7), 341 (10), 215 (13), 171 (45), 114 (17); HRMS (EI) calcd for $C_{26}H_{22}O_7$ 446.1366, found 446.1370.

1-(5-Methoxymethoxy-8-hydroxynaphthalen-1-yloxy)-5-methoxymethoxynaphth-4-ol (9b)

To a solution of dialdehyde **8b** (320 mg, 0.717 mmol) in CH_2Cl_2 (15 mL) was added 70% *m*-CPBA (525 mg, 2.13 mmol). The reaction mixture was stirred at room temperature for 15 h, treated with a solution of 10% $Na_2S_2O_3$ (15 mL) and stirred for another 1 h. The organic layer was separated and diluted with CH_2Cl_2 then washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography of the residue on SiO_2 (hexanes/EtOAc, 3 : 2) afforded 206 mg (61%) of 1-(5-methoxymethoxy-8-formyloxynaphthalen-1-yloxy)-5-methoxymethoxy-4-formyloxynaphthalene as a yellow foam: IR (neat) 2950, 1739, 1601, 1506, 1416, 1369, 1254, 1117 cm^{-1}; ^1H NMR δ 8.38 (s, 1H), 8.17 (dd, 1H, J = 8.5, 1.0 Hz), 8.08 (s, 1H), 7.98 (dd, 1H, J = 8.5, 0.9 Hz), 7.46 (t, 1H, J = 8.1 Hz), 7.42 (dd, 1H, J = 8.4, 7.7 Hz), 7.25 (dd, 1H, J = 7.8, 0.9 Hz), 7.16 (d, 1H, J = 8.4 Hz), 7.07 (d, 1H, J = 8.4 Hz), 7.00 (d, 1H, J = 8.3 Hz), 6.99 (dd, 1H, J = 7.6, 1.0 Hz), 6.68

(d, 1H, J = 8.3 Hz), 5.42 (s, 2H), 5.32 (s, 2H), 3.57 (s, 3H), 3.55 (s, 3H); ^{13}C NMR δ 160.7, 160.5, 152.7, 152.3, 151.7, 151.6, 141.0, 138.7, 129.3, 127.5, 126.5, 121.1, 120.3, 120.1, 119.5, 119.0, 117.5, 116.2, 112.3, 111.6, 108.2, 95.3, 95.2, 56.7, 56.6; MS (EI) *m*/*z* (rel intensity) 478 (M^+, 85), 450 (100), 422 (44), 373 (54), 345 (54), 315 (51), 187 (63), 174 (70); HRMS (EI) calcd for $C_{26}H_{22}O_9$ 478.1264, found 478.1241.

To a solution of this bisformate ester (200 mg, 0.418 mmol) in 1 : 1 MeOH/THF (8 mL) at 0 °C was slowly added $NaBH_4$ (35 mg, 0.92 mmol). The reaction mixture was stirred at 0 °C for 1 h, diluted with EtOAc (20 mL) and washed with H_2O. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was *immediately* subjected to chromatography on SiO_2 (hexanes/EtOAc, 3 : 2) to afford 157 mg (89%) of **9b** as a white foam: IR (neat) 3432 (br), 1632, 1608, 1461, 1395, 1264, 1226, 1158, 1010 cm^{-1}; ^{1}H NMR δ 9.36 (s, 1H), 8.99 (s, 1H), 7.89 (dd, 1H, J = 8.5, 0.9 Hz), 7.63 (dd, 1H, J = 8.5, 0.8 Hz), 7.30 (t, 1H, J = 8.2 Hz), 7.26 (d, 1H, J = 8.3 Hz), 7.17–7.12 (m, 3H), 6.91 (d, 1H, J = 8.3 Hz), 6.90 (d, 1H, J = 8.4 Hz), 6.46 (dd, 1H, J = 7.7, 0.9 Hz), 5.48 (s, 2H), 5.32 (s, 2H), 3.62 (s, 3H), 3.56 (s, 3H); ^{13}C NMR δ 156.5, 154.1, 152.6, 148.9, 145.9, 141.6, 130.0, 129.0, 127.2, 125.5, 120.5, 117.0, 116.6, 116.3, 115.7, 111.5, 110.0, 109.9, 109.2, 108.9, 95.9, 95.8, 57.1, 56.3; MS (EI) *m*/*z* (rel intensity) 422 (M^+, 32), 345 (22), 264 (38), 205 (31), 189 (35), 173 (100); HRMS (EI) calcd for $C_{24}H_{22}O_7$ 422.1366, found 422.1361.

4′-Methoxymethoxypalmarumycin CP$_1$ (10b)

To a solution of bisnaphthol **9b** (230 mg, 0.544 mmol) in dry CH_3CN (8 mL) at 0 °C was added $PhI(OCOCF_3)_2$ (253 mg, 0.588 mmol) in one portion. The reaction mixture was stirred at 0 °C for 30 min and concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL), washed with H_2O and saturated aqueous $NaHCO_3$, dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography on SiO_2 (hexanes/EtOAc, 7 : 3) gave 80 mg (40%) of **10b** as a yellow foam: IR (neat) 2955, 1662, 1610, 1456, 1425, 1266, 1240, 1010 cm^{-1}; ^{1}H NMR δ 12.17 (s, 1H), 7.91 (dd, 1H, J = 8.5, 0.7 Hz), 7.65 (t, 1H, J = 8.0 Hz), 7.50–7.44 (m, 2H), 7.13 (dd, 1H, J = 8.4, 1,0 Hz), 7.11 (d, 1H, J = 8.3 Hz), 7.02 (dd, 1H, J = 7.5, 0.6 Hz), 7.01 (d, 1H, J = 10.5 Hz), 6.89 (d, 1H, J = 8.3 Hz), 6.35 (d, 1H, J = 10.5 Hz), 5.38, 5.35 (AB-System, 2H, J = 6.7 Hz), 3.57 (s, 3H); ^{13}C NMR δ 189.0, 162.1, 148.5, 147.5, 141.8, 140.0, 139.2, 136.7, 129.9, 127.3, 126.7, 119.8, 119.5, 116.5, 114.1, 113.8, 110.9, 110.3, 109.9, 95.7, 93.1, 56.5; MS (EI) *m*/*z* (rel intensity) 376 (M^+, 14), 331 (15), 174 (100), 149 (21), 118 (41); HRMS (EI) calcd for $C_{22}H_{16}O_6$ 376.0947, found 376.0948.

4′-Hydroxypalmarumycin CP$_1$ (S12)

To a solution of spirocycle **10b** (45 mg, 0.12 mmol) in CH_2Cl_2 (2 mL) containing powdered 4 Å molecular sieves (120 mg) at 0 °C was added bromotrimethylsilane (65 µL, 0.50 mmol). The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for 12 h. The reaction mixture was filtered over Celite (CH_2Cl_2) and the filtrate was concentrated under reduced pressure. The residue was dissolved in THF (3 mL), and TBAF (1.0 M in THF; 0.12 mL, 0.12 mmol) was slowly added. The reaction mixture was stirred at room temperature for 15 min, quenched with H_2O and extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was passed through a short pad of SiO_2 (CH_2Cl_2) to afford 28 mg (70%) of **S12** as a yellow–brown solid: mp 280 °C (acetone, dec); IR (neat) 3400 (br), 1661, 1609, 1456, 1417, 1347, 1265, 956 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 12.18 (s, 1H), 8.99 (s, 1H), 7.90 (dd, 1H, J = 8.5, 0.8 Hz), 7.77 (dd, 1H, J = 8.2, 7.8 Hz), 7.50 (dd, 1H, J = 8.5, 7.6 Hz), 7.49 (dd, 1H, J = 7.6, 1.0 Hz), 7.17 (d, 1H, J = 10.5 Hz), 7.15 (dd, 1H, J = 8.4, 1.0 Hz), 7.03 (dd, 1H, J = 7.5, 0.8 Hz), 6.94 (d, 1H, J = 8.1 Hz), 6.86 (d, 1H, J = 8.1 Hz), 6.44 (d, 1H, J = 10.5 Hz); ^{13}C NMR (acetone-d_6) δ 190.0, 162.6, 149.3, 148.3, 141.6, 140.7, 140.4, 137.6, 130.2, 127.3, 126.2, 120.4, 120.1, 117.3, 114.6, 114.5, 111.1, 110.9, 110.2, 93.6; MS (EI) *m*/*z* (rel intensity) 332 (M^+, 100), 303 (6), 275 (6), 174 (28), 118 (34), 102 (35), 92 (26), 63 (41); HRMS (EI) calcd for $C_{20}H_{12}O_5$ 332.0685, found 332.0694.

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27 Current molecular and morphological studies are being carried out to assess the taxonomy of both, the anamorph and teleomorph of the original isolate and will be published shortly. Both anamorph and teleomorph specimens are preserved at INBio's laboratories for future reference, under accession number 3352.
28 Collection permit number 005-96-VUI.
29 Identified by $^{1}H/^{13}C$-NMR, HSQC, DEPT, COSY and HMBC experiments and MS data. MK3018 was originally reported in H. Ogishi, N. Chiba, T. Mikawa, T. Sasaki, S. Miyagi and M. Sezaki, *JP 01,294,686,1989; Chem. Abstr.*, 1990, **113**, 38906q.
30 Palmarumycin CR_1 spectroscopic data: ^{1}H-NMR (400 MHz, CD_3OD, δ/ppm): 7.52 (d br, 2H, H-2′ and 7′ overlapped), 7.46 (dd br, 2H, H-3′ and 6′ overlapped), 6.98 (dd br, H, H-4′ or 5′), 6.95 (dd br, 1H, H-5′ or 4′), 5.92 (m, 2H, H-6 and 7), 4.76 (m, 1H, H-8), 4.54 (m, 1H, H-5), 4.31 (s br, 1H, H-4), 2.38 (m, 2H, H-4a and 8a), 1.90 (m, 3H, H-2 and 3_2 overlapped), 1.76 (m, 1H, H-3_1); ^{13}C NMR (100 MHz, CD_3OD, δ ppm): 149.0 s (C-1′ or C-8′), 147.8 s, (C-8′ or C-1′), 135.7 s (C-4′a), 134.8 d (C-6 or C-7), 129.3 d (C-7 or C-6), 128.6 d (C-3′ or C-6′), 128.4 d (C-6′ or C-3′), 121.7 d (C-2′ or C-7′), 121.3 d (C-7′ or C-2′), 115.2 s (C-8′a), 110.6 d (C-4′ or C-5′), 110.2 d (C-5′ or C-4′), 105.1 s (C-1), 67.7 d (C-5), 64.2 d (C-4), 63.2 d (C-8), 43.3 d (C4a or C8a), 42.3 d (C8a or C4a), 29.6 t (C-3), 26.7 t (C-2); HR-MS: obs. 340.1301, calc. 340.1311 for $C_{20}H_{20}O_5$. Full assignments and further data will be published shortly.
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32 Identified by ^{1}H-/^{13}C-NMR, HSQC and HMBC experiments and MS data, and compared with published spectroscopic information in Barrett *et al.*, ref. 31. Originally reported in K. Krohn, A. Michel, U. Floerke, H.-J. Aust, S. Draeger and B. Schulz, *Annalen*, 1994, 1093–1097 and 1099–1108.
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