Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2272–2276
Bioorganic & Medicinal Chemistry Letters

Synthesis of a small library of 2-phenoxy-1,4-naphthoquinone and 2-phenoxy-1,4-anthraquinone derivatives bearing anti-trypanosomal and anti-leishmanial activity

Maria Laura Bolognesi,[a,*] Federica Lizzi,[a] Remo Perozzo,[b] Reto Brun[c] and Andrea Cavalli[a,*]

[a]*Department of Pharmaceutical Sciences, Alma Mater Studiorum, Bologna University, Via Belmeloro 6, I-40126 Bologna, Italy*
[b]*Pharmaceutical Biochemistry Group, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland*
[c]*Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland*

Received 7 February 2008; revised 3 March 2008; accepted 3 March 2008
Available online 7 March 2008

Abstract—Taking advantage of the structural features of natural products showing anti-trypanosomatid activity, we designed and synthesized a small library of 2-phenoxy-1,4-naphthoquinone and 2-phenoxy-1,4-anthraquinone derivatives. The library was obtained following a parallel approach and using readily available synthons. All the derivatives showed inhibitory activity toward either *Trypanosoma* or *Leishmania* species, with **8**, **10**, and **16** being the most active compounds against *Trypanosoma brucei rhodesiense*, *Leishmania donovani*, and *Trypanosoma cruzi* cells (IC_{50} = 50 nM, IC_{50} = 0.28 μM, and IC_{50} = 1.26 μM, respectively).

Trypanosomatids are the causative agents of various lethal parasitic diseases such as Chagas' disease (*Trypanosoma cruzi*), African sleeping sickness (*Trypanosoma brucei* with the two sub-species *T. b. gambiense* and *T. b. rhodesiense*), and leishmaniases (*Leishmania donovani*, *L. major*, and *L. tropica*).[1] Such diseases mainly affect the least developed countries and almost half a billion people are presently at risk. Despite its epidemiological importance, the therapy of trypanosomatid infections remains an unmet challenge.[2,3] No vaccines are currently available to prevent these diseases, and the recommended drugs have high toxicity and limited efficacy.[4] In addition, rapidly-developing drug resistance has become a major problem. Although new anti-protozoan drugs are in development for these diseases,[5] the lead identification still represents a real bottleneck,[6] and the drug development pipeline is currently almost empty.[7]

Combinatorial chemistry is one potential strategy for drug discovery.[8] Drug discovery for these diseases can be tailored to academic settings where cost-effectiveness in synthesizing new anti-parasitic agents is a major consideration. Attention should therefore focus on a strategy that can yield molecules with high hit rates and a concomitant reduced library size. In this respect, the concept of natural-product-derived compound collections is particularly attractive. This strategy recognizes that natural product fragments have been evolutionarily selected and biologically pre-validated. They are therefore appropriate starting points for the development of compound collections.[9–11] This approach may be particularly fruitful if a link already exists between a given compound class and the desired biological activity.[11]

Here, we present a small library of compounds endowed with anti-trypanosomatid activity, obtained through a parallel approach. In the library design, we selected the quinone unit as the core structure for combinatorial derivatization. Naphthoquinones and other related quinone compounds are one of the major natural product classes with significant activity against *Leishmania* and *Trypanosoma*.[12] For instance, lapachol (**1**, Fig. 1) was reported to exhibit marked anti-trypanosomal and leish-

Keywords: Anti-parasitic; Anti-protozoan; Neglected diseases; Tropical diseases; Compound library; Parallel synthesis; Whole-cell assays.
* Corresponding authors. Tel.: +39 051 2099700; e-mail addresses: marialaura.bolognesi@unibo.it; andrea.cavalli@unibo.it

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.03.009

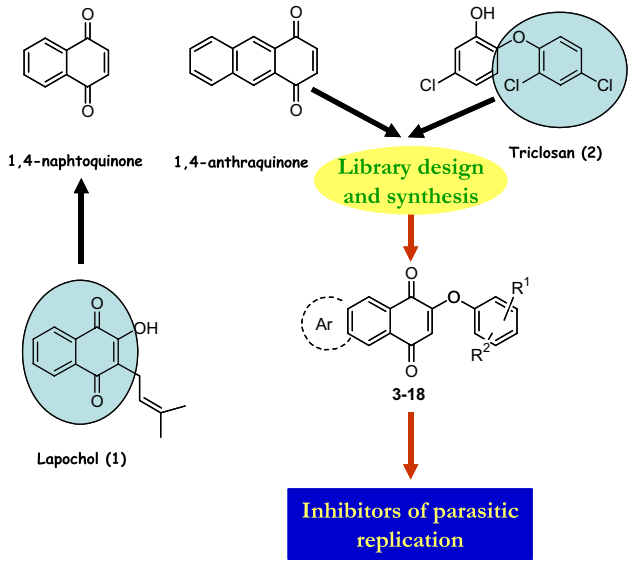


Figure 1. Design strategy for compounds **3**–**18**.

manicidal activity, while displaying no serious toxic effects in humans.[13] Therefore, based on the 1,4-naphthoquinone and 1,4-anthraquinone natural scaffolds, we synthesized 16 compounds (**3**–**18**, Table 1), which incorporated, at position 2, a selection of aromatic groups that would mimic a structural element of triclosan (**2**, Fig. 1). Triclosan is a general biocide which was recently demonstrated to kill both procyclic forms and bloodstream forms of *T. brucei*[14] (see Fig. 1 for the design strategy).

For the development of an efficient and cheap parallel synthesis approach, we focused our attention on the displacement reaction of 2-bromoquinones (**19a** and **19b**) with phenoxides (**20a**–**h**) (Table 1). We were able to carry out a one-pot reaction at room temperature that, in most cases, could achieve the quantitative conversion of the starting reactant within 3 h (Scheme 1). Moreover, we developed an operationally simple and versatile workup protocol, which involved the recovery of high-purity final products by filtration upon addition of water to the reaction mixture.[15] As **19a** is the only reagent that is not commercially available, we carried out an efficient bromination procedure starting from the readily available 1,4-anthraquinone (Scheme 2).[16]

Table 2 reports the activity of compounds **3**–**18** against *T. b. rhodesiense*, *T. cruzi*, and *L. donovani* as well as their cytotoxicity against L6 cells (rat skeletal myoblasts). The anti-parasitic potential of all compounds was analyzed with regard to the WHO/TDR screening activity criteria.[6] In the following paragraphs, we discuss the results of compounds **3**–**18** against (i) *T. b. rhodesiense*, (ii) *T. cruzi*, and (iii) *L. donovani*.

(i) All the derivatives showed good activity against the blood trypomastigote form of *T. b. rhodesiense*. The unsubstituted 2-phenoxy-1,4-anthraquinone derivative (**8**) was the most potent of the series, showing an IC_{50} value of 50 nM. Compound **8** was just fivefold less active than the arsenic derivative melarsoprol (**21**, 10 nM), which was used as a reference compound (Fig. 2). Unfortunately, **8** was also quite cytotoxic against L6 cells (IC_{50} = 1000 nM) with a selectivity index (SI = IC_{50}L6/IC_{50}parasite) of 20. Adding substituents to **8** did not improve its activity against the parasitic cells. Furthermore, L6 cytotoxicity was usually increased by the introduction of halogens on the phenyl ring to mimic triclosan structure. However, a fairly good increase of SI was observed with compound **3** (i.e., 2-(2,4-dichlorophenoxy)-1,4-anthraquinone), which retained nanomolar activity (IC_{50} = 65 nM) and showed SI > 30. Concerning the 2-phenoxy-1,4-naphthoquinone derivatives, **11**–**18** showed quite interesting profiles against *T. b. rhodesiense*, with **16** being active in the nanomolar range, and **11**–**15** and **17**–**18** being active in the micromolar range. Compound **16** was the most interesting compound we discovered. It showed an IC_{50} value of 80 nM and an SI of 74, which is very close to the value considered a hit by WHO/TDR (SI more than 100).[6] All other 2-phenoxy-1,4-naphthoquinone derivatives (**11**–**15** and **17**–**18**) showed both lower inhibitory activities and lower SIs when compared to **16**.

(ii) Concerning the amastigote form of *T. cruzi*, most of our derivatives showed activity in the micromolar range (see Table 2). The most interesting result was again obtained with **16**, which exhibited an IC_{50} value of 1.26 μM. Compound **16** was slightly more potent than the reference compound benznidazole (**22** in Fig. 2), which has an IC_{50} of 1.70 μM. Here too, however, the low SI (<5) was the main drawback for **16**, pointing to the need for further investigation of the present compound series to decrease cell cytotoxicity. The profiles of **13** (IC_{50} = 2.47 μM and SI = 2.1) and **15** (IC_{50} = 2.88 μM and SI = 1.7) were very similar to that of the parent compound. Among the 2-phenoxy-1,4-anthraquinone derivatives, the most active inhibitor was **6**, showing IC_{50} and SI values of 2.87 μM and 0.39, respectively. Remarkably, in contrast to their activity against *T. b. rhodesiense*, the 2-phenoxy-1,4-anthraquinone derivatives were generally less potent against *T. cruzi* cells than the 2-phenoxy-1,4-naphthoquinones.

(iii) When tested against the axenic amastigote form of *L. donovani*, the derivatives were all less potent than they were against *T. b. rhodesiense*. All the compounds were active in the micromolar range. The most potent of the present series was **10** (i.e., 2-(2,4-difluoro-phenoxy)-1,4-anthraquinone) with an IC_{50} value of 0.28 μM. Remarkably, this value was very similar to that of the reference compound miltefosine (**23** in Fig. 2), which has an IC_{50} of 0.31 μM. This indicates **10** as a possible hit candidate for the development of new anti-leishmanial derivatives. However, **10** also showed low selectivity with an SI of 7. Among the 2-phenoxy-1,4-naphthoquinone derivatives, **13** was the most interesting compound, endowed with an IC_{50} value of

Table 1. The compound library obtained by a one-pot parallel synthesis approach

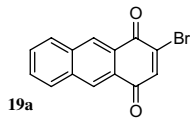
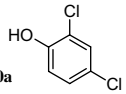
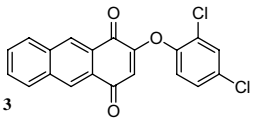
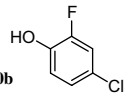
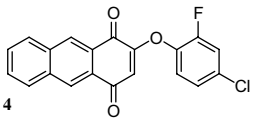
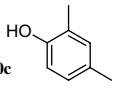
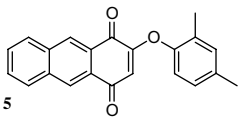
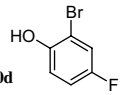
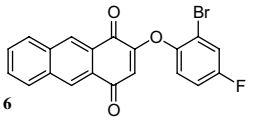
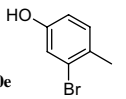
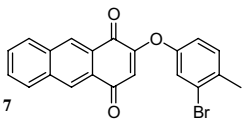
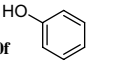
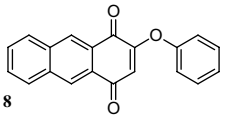
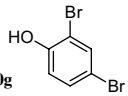
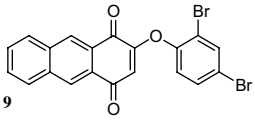
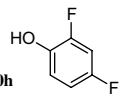
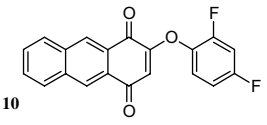
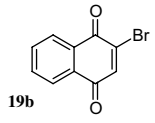
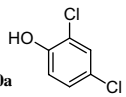
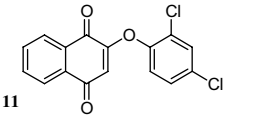
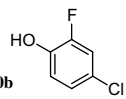
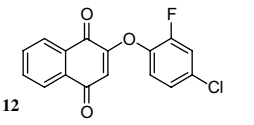
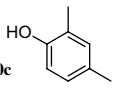
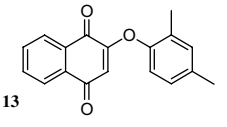
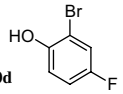
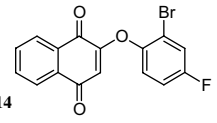
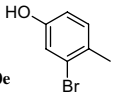
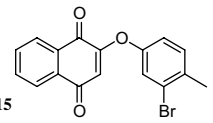
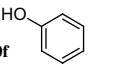
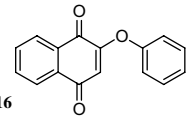
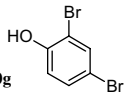
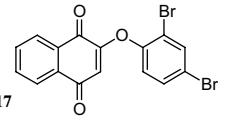
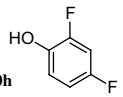
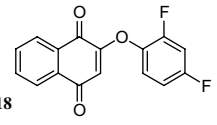
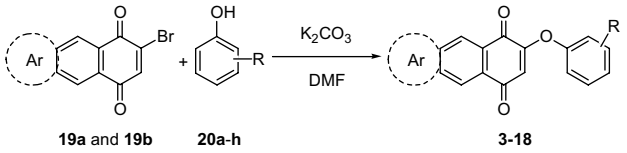
Quinones (**19**)	Phenols (**20**)	Entries (**3**–**18**)
 **19a**	 **20a**	 **3**
	 **20b**	 **4**
	 **20c**	 **5**
	 **20d**	 **6**
	 **20e**	 **7**
	 **20f**	 **8**
	 **20g**	 **9**
	 **20h**	 **10**
 **19b**	 **20a**	 **11**
	 **20b**	 **12**
	 **20c**	 **13**

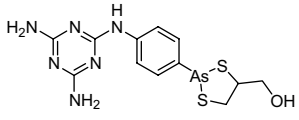
Table 1 (*continued*)

Quinones (**19**)	Phenols (**20**)	Entries (**3**–**18**)
	 **20d**	 **14**
	 **20e**	 **15**
	 **20f**	 **16**
	 **20g**	 **17**
	 **20h**	 **18**

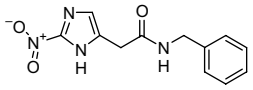


19a and **19b** **20a-h** **3-18**

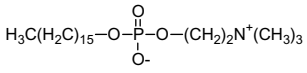
Scheme 1. Synthesis of the compound library. Reagents and conditions: phenol, K_2CO_3 in DMF, 30 min, then bromoquinone, room temperature, 3 h.



21 (melarsoprol)



22 (benznidazole)



23 (miltefosine)

Figure 2. Chemical structure of some currently marketed drugs used as reference compounds (**21**–**23**).

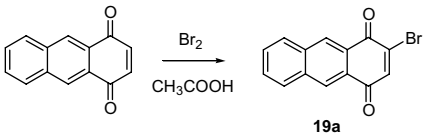
0.51 μM and an SI of 10. Here too, the potency was fairly good, while the selectivity needs to be increased to reduce possible adverse effects.

As a general observation, cytotoxicity is a common problem for many natural products[2] as they are usually

Table 2. Anti-trypanosomatid activity of the compound library expressed as IC_{50}[a] (μM)

Compound	*T. b. r.*	*T. c.*	*L. d.*	L6	SI *T. b. r.*	SI *T. c.*	SI *L. d.*
3	0.065	13.7	0.49	2.01	31.0	0.15	4.15
4	0.21	21.5	0.53	2.64	12.4	0.12	5.01
5	0.31	22.5	0.55	4.13	13.2	0.18	7.53
6	0.08	2.87	0.35	1.13	14.1	0.39	3.24
7	0.15	6.36	0.36	2.56	17.4	0.40	7.10
8	0.05	4.66	0.34	1.00	20.0	0.21	2.94
9	0.14	16.4	0.47	2.19	15.4	0.13	4.71
10	0.24	7.14	0.28	1.98	8.43	0.28	7.01
11	0.29	5.95	1.32	5.18	17.8	0.87	3.94
12	0.37	4.43	0.70	4.58	12.5	1.03	6.54
13	0.22	2.47	0.51	5.26	24.4	2.13	10.3
14	0.36	3.74	1.81	4.66	13.1	1.25	2.58
15	0.30	2.88	0.92	4.92	16.4	1.71	5.34
16	0.08	1.26	1.26	5.92	74.0	4.70	4.69
17	0.51	34.3	2.37	28.5	55.7	0.83	12.1
18	0.39	5.00	2.29	5.51	14.2	1.10	2.41
21	0.01						
22		1.70					
23			0.31				

[a] IC_{50} values for the blood trypomastigote form of *Trypanosoma brucei rhodesiense* (*T. b. r.*), the amastigote form of *Trypanosoma cruzi* (*T. c.*) in L6 cells, and the axenic amastigote form of *Leishmania donovani* (*L. d.*) are reported. The L6 cytotoxicity as well as the selectivity index (SI = IC_{50} for L6/IC_{50} for parasite) for all parasites is also shown. Compounds **21**, **22**, and **23** are the standard drugs melarsoprol, benznidazole, and miltefosine, respectively. A detailed description of the assays can be found in Refs. 17 and 18.



19a

Scheme 2. Synthesis of 2-bromo-anthracene-1,4-dione. Reagents and condition: Br_2 dropwise, cooling, 2.5 h.

produced to work as biological defense mechanisms. With respect to safety, however, a greater tolerance may be acceptable for anti-trypanosomatid candidates, given the short course of therapy and the scarce safety profile of the currently available drugs.[7]

In conclusion, we report on the design, synthesis, and anti-parasitic screening of a small library of natural-product-derived naphtho- and anthra-quinones against trypanosomatid parasites. Notwithstanding the small number of synthesized compounds, we discovered some very potent inhibitors against *T. b. rhodesiense*, and some compounds that were active against *T. cruzi* and *L. donovani*. Although the present derivatives were overall quite cytotoxic toward L6 cells, we identified a promising hit compound. Indeed, **16** showed an IC_{50} value of 80 nM against *T. b. rhodesiense* cells and an SI of 74, which is very close to the specifications required by WHO/TDR for **16** to be considered an anti-trypanosomatid hit. This confirms the rationale of our design strategy (Fig. 1). In addition, the low-cost criterion was met by using the devised straight synthetic route, which also made use of readily available synthons. In the light of these results, we plan to complement the present study with two strategies: (1) further exploitation of the versatile synthetic route to enlarge the present series of compounds, with the aim of tuning down human cell toxicity; (2) application of biochemical and molecular biology tools[19] in order to identify the target protein(s) of these compounds. Here, a chemical proteomics approach will allow us to elucidate the SARs in more detail and, thus, to more effectively address the design of further less toxic derivatives. It is worth noting that, in addition to a possible target-related mechanism, the general free-radical-generation mechanism of quinones (probably at the basis of their general cytotoxicity) may be exploited to prevent resistance development.[20]

Acknowledgments

This research was supported by the University of Bologna. We thank C. Melchiorre and M. Recanatini for the critical reading of the manuscript. Ms. S. Vitillo is gratefully acknowledged for her technical assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.009.

References and notes

1. Available from: http://www.who.int/tdr.
2. Pink, R.; Hudson, A.; Mouries, M. A.; Bendig, M. *Nat. Rev. Drug Disc.* **2005**, *4*, 727.

3. Gelb, M. H.; Hol, W. G. *Science* **2002**, *297*, 343.
4. Linares, G. E.; Ravaschino, E. L.; Rodriguez, J. B. *Curr. Med. Chem.* **2006**, *13*, 335.
5. Hotez, P. J.; Molyneux, D. H.; Fenwick, A.; Kumaresan, J.; Sachs, S. E.; Sachs, J. D.; Savioli, L. *N. Engl. J. Med.* **2007**, *357*, 1018.
6. Nwaka, S.; Hudson, A. *Nat. Rev. Drug Disc.* **2006**, *5*, 941.
7. Renslo, A. R.; McKerrow, J. H. *Nat. Chem. Biol.* **2006**, *2*, 701.
8. Gutteridge, W. E. *Parasitology* **1997**, *114*, S145.
9. Koch, M. A.; Wittenberg, L. O.; Basu, S.; Jeyaraj, D. A.; Gourzoulidou, E.; Reinecke, K.; Odermatt, A.; Waldmann, H. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 16721.
10. Breinbauer, R.; Vetter, I. R.; Waldmann, H. *Angew. Chem. Int., Ed. Engl.* **2002**, *41*, 2879.
11. Lessmann, T.; Leuenberger, M. G.; Menninger, S.; Lopez-Canet, M.; Muller, O.; Hummer, S.; Bormann, J.; Korn, K.; Fava, E.; Zerial, M.; Mayer, T. U.; Waldmann, H. *Chem. Biol.* **2007**, *14*, 443.
12. Kayser, O.; Kiderlen, A. F.; Croft, S. L. *Parasitol. Res.* **2003**, *90*, S55.
13. Krohn, K.; Hussain, H.; Viqar, U. A.; Ghulam, A. M.; Green, I. R. *Arkivoc* **2007**, *ii*, 145.
14. Paul, K. S.; Bacchi, C. J.; Englund, P. T. *Eukaryot. Cell* **2004**, *3*, 855.
15. General procedure for the synthesis of the library. A carousel reaction tube was charged with the selected phenol (0.35 mmol) and DMF (9 mL). After 1 min, K_2CO_3 (1.05 mmol) was added and the reaction mixture was stirred for 1 h at room temperature. 2-Bromo-anthracene-1,4-dione (0.35 mmol) or 2-bromo-naphthalene-1,4-dione (0.35 mmol) was added. After standing for 3 h, the mixture was treated with cold water and filtered. The obtained solid was then washed and dried under vacuum at 40 °C for 6 h. The above synthetic protocol was repeated using a total of eight different phenols to produce eight 2-phenoxy-[1,4]-naphthoquinone and eight 2-phenoxy-anthracene-1,4-dione derivatives as yellow solids. If needed, **3**–**18** can be crystallized by petroleum ether (for details see Supplementary Material).
16. A solution of Br_2 (0.25 mL, 4.80 mmol) in glacial acetic acid (10 mL) was added dropwise to a suspension of 1,4-anthraquinone (1 g, 4.80 mmol) in glacial acetic acid (20 mL) in an ice-water bath. The reaction mixture was stirred for 2.5 h with cooling, then treated with cold water and filtered. The formed solid was collected by filtration and crystallized from ethanol to give **19a** as an orange solid: 81% yield; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.65 (s, 1H), δ 7.73–7.78 (m, 2H, J = 10 Hz), δ 8.08–8.13 (m, 2H, J = 10 Hz), δ 8.66 (s, 1H), δ 8.75 (s, 1H). ESI-MS: found 309 (M^++Na^+, ^{79}Br); 311 (M^++Na^+, ^{81}Br).
17. Orhan, I.; Sener, B.; Atici, T.; Brun, R.; Perozzo, R.; Tasdemir, D. *Phytomedicine* **2006**, *13*, 388.
18. Kuettel, S.; Zambon, A.; Kaiser, M.; Brun, R.; Scapozza, L.; Perozzo, R. *J. Med. Chem.* **2007**, *50*, 5833.
19. Lokey, R. S. *Curr. Opin. Chem. Biol.* **2003**, *7*, 91.
20. Efferth, T. *Drug Resist. Updat.* **2005**, *8*, 85.