Journal of
Medicinal Chemistry

Article

Structure-Activity Relationship of Antischistosomal Ozonide Carboxylic Acids

Jianbo Wu, Xiaofang Wang, Francis C. K. Chiu, Cecile Haeberli, David M. Shackleford, Eileen Ryan, Sriraghavan Kamaraj, Vivek J. Bulbule, Alexander Wallick, Yuxiang Dong, Karen White, Paul H Davis, Susan A. Charman, Jennifer Keiser, and Jonathan L. Vennerstrom

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.0c00069 • Publication Date (Web): 05 Mar 2020

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Structure-Activity Relationship of Antischistosomal Ozonide Carboxylic Acids

Jianbo Wu[†], Xiaofang Wang[†], Francis C. K. Chiu[§], Cécile Häberli[‡,£], David M. Shackleford[§], Eileen Ryan[§], Sriraghavan Kamaraj[†], Vivek J. Bulbule[†], Alexander I. Wallick[∞], Yuxiang Dong[†], Karen L. White[§], Paul H. Davis[∞], Susan A. Charman[§], Jennifer Keiser[‡,£], and Jonathan L. Vennerstrom[†]*

[†]College of Pharmacy, University of Nebraska Medical Center, 986125 Nebraska Medical Center, Omaha, NE, United States

[§]Centre for Drug Candidate Optimisation, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052, Australia

[‡]Swiss Tropical and Public Health Institute, Socinstrasse 57, CH-4002 Basel, Switzerland

[£]University of Basel, CH-4003 Basel, Switzerland

[∞]Department of Biology, University of Nebraska at Omaha, Omaha, NE, United States

*J.L.V.: Phone: 402 559 5362. E-mail: jvenners@unmc.edu

Abstract

The semisynthetic artemisinins and other bioactive peroxides are best known for their powerful antimalarial activities, but also have substantial activity against schistosomes - another hemoglobin-degrading pathogen. Building on this discovery, we now describe the initial structure-activity relationship (SAR) of antischistosomal ozonide carboxylic acids OZ418 (**2**) and OZ165 (**3**). Irrespective of lipophilicity, these ozonide weak acids have relatively low aqueous solubilities and high protein binding values. Ozonides with *para*-substituted carboxymethoxy and *N*-benzylglycine substituents had high antischistosomal efficacies. It was possible to increase solubility, decrease protein binding and maintain high antischistosomal activity in mice infected with juvenile and adult *Schistosoma mansoni* by incorporating a weak base functional group in these compounds. In some cases, adding polar functional groups and heteroatoms to the spiroadamantane substructure increased solubility and metabolic stability, but in all cases, decreased antischistosomal activity.

Keywords: 1,2,4-trioxolane, ozonide, antischistosomal, artemisinin, OZ418

Introduction

Artemisinin and its semisynthetic derivatives dihydroartemisin, artemether and artesunate (Figure 1) are best known for their powerful antimalarial activities,[1,2] but also have substantial activity against schistosomes - another hemoglobin-degrading pathogen.[3-7] With their high activity against juvenile stage schistosomes, the semisynthetic artemisinins have promise for the chemoprophylaxis and prevention of schistosomiasis.[4,6,8] In contrast, praziquantel, the only drug available for treatment of this disease, has little activity against the young developmental stages of the parasite and is rarely curative.[4,7,8] Abundant data indicates that the pharmacophoric peroxide bond[9] of semisynthetic artemisinins and other bioactive peroxides undergoes one-electron reduction by heme released during parasite hemoglobin digestion[10,11] to produce carbon-centered radicals that alkylate heme and parasite proteins.[10,12-21]

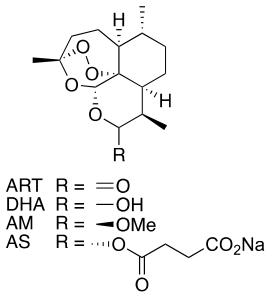


Figure 1. Artemisinin (ART) and its semisynthetic derivatives dihydroartemisinin (DHA), artemether (AM), and artesunate (AS).

We have shown that one class of synthetic peroxides - ozonides (1,2,4-trioxolanes) - have promising antischistosomal activity.[5] The most active of these were OZ418 (**2**) and OZ165 (**3**),[22-24] carboxylic acid analogs of next-generation antimalarial ozonide OZ439 (**1**) (artefenomel).[25] For example, single 400 mg/kg oral doses of **2** administered to *Schistosoma mansoni*-, *S. japonicum*- or *S. haematobium*-infected mice reduced adult worm burden by 80, 69

and 86%, respectively.[23,24] Like the semisynthetic artemisinins, ozonides **2** and **3** are even more effective against the juvenile form of the parasite; single 200 mg/kg oral doses of **2** and **3** administered to *S. mansoni*-infected mice reduced juvenile worm burden by 84 and 100%, respectively.[24] However, with IC_{50} values of 44 and 39 μM against ex vivo *S. mansoni* and *S. japonicum*, **2** has very weak in vitro activity.[23,26] The structure-activity relationship (SAR) of antischistosomal ozonides has demonstrated that: 1) the spiroadamantane ring system and peroxide bond are essential for activity; 2) the core 1,2,4-trioxolane is superior to the corresponding 1,2,4-trioxane or 1,2,4,5-tetraoxane isosteres; and 3) ozonides with carboxylic acid, but not neutral or basic, functional groups have the highest antischistosomal activity. For example, **1** has no antischistosomal activity.[24] In this paper, we profile ozonides **4**-**25** to expand the structure-activity relationship (SAR) of **2** and **3**.

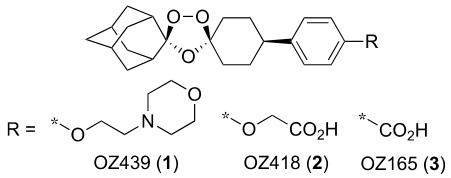
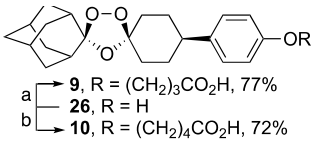


Figure 2. Ozonides OZ439 (**1**), OZ418 (**2**), and OZ165 (**3**).

Chemistry

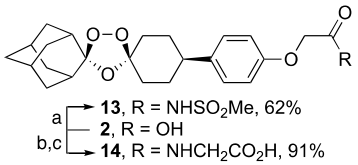
Ozonides **4**-**25** were prepared as described in Schemes 1-12. Ozonide carboxylic acids **9** and **10** were prepared in 77 and 72% yield by a one-pot alkylation of ozonide phenol **26**[27] with the corresponding bromoalkyl esters followed by hydrolysis (Scheme 1). Ozonide acylsulfonamide **13** was prepared in 62% yield by a DCC-mediated coupling of **2** with methanesulfonamide (Scheme 2). HOBt/EDC-mediated condensation of **2** with the ethyl ester of glycine followed by ester hydrolysis afforded ozonide carboxyamide **14** in 91% yield (Scheme 2).

Scheme 1[a]



[a]Reagents and conditions: 4-bromobutanoate (a) or 5-bromopentanoate (b), powdered NaOH, tetrabutylammonium hydrogensulfate, dimethoxyethane, rt to 60 °C, 12 h, then aq. AcOH.

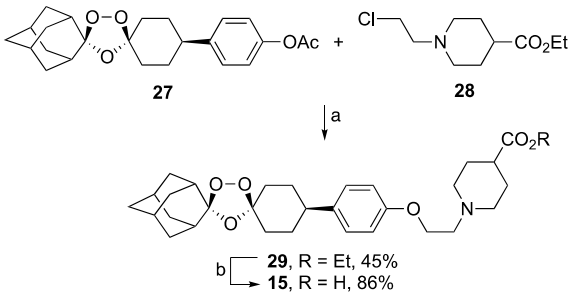
Scheme 2[a]



[a]Reagents and conditions: (a) methanesulfonamide, DCC, DMAP, CH_2Cl_2, rt, 24 h; (b) glycine ethyl ester hydrochloride, HOBt, EDC, DIPEA, DMF, rt, 12 h; (c) 2% aq. NaOH, EtOH, rt, 12 h, then aq. AcOH.

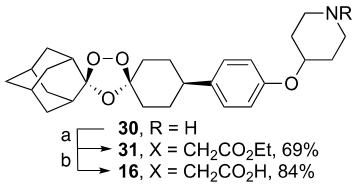
Reaction of ozonide acetate **27**[27] with *N*-chloroethylpiperidine **28** according to the method of Charman et al.[25] furnished ozonide ester **29** in 45% yield; hydrolysis of **29** gave ozonide piperidine carboxylic acid **15** as its sodium salt in 86% yield (Scheme 3). Alkylation of ozonide piperidine **30**[25] with ethyl bromoacetate produced ozonide ester **31** in 69% yield (Scheme 4). Hydrolysis of **31** afforded ozonide piperidine carboxylic acid **16** in 84% yield.

Scheme 3[a]



[a]Reagents and conditions: (a) powdered NaOH, tetrabutylammonium hydrogensulfate, CH_3CN, rt to 60 °C, 12 h; (b) powdered NaOH, EtOH, rt, 24 h.

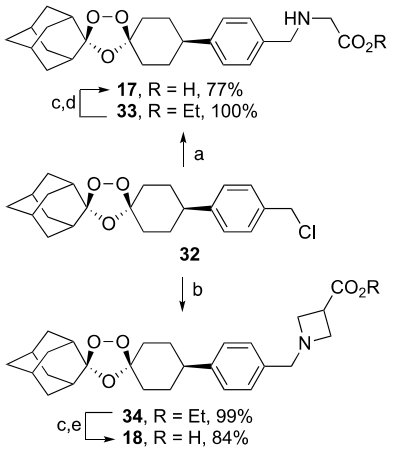
Scheme 4[a]



[a]Reagents and conditions: (a) ethyl bromoacetate, K_2CO_3, 12:1 THF:H_2O, rt, 12 h; (b) 1 M aq. NaOH, EtOH, 50 °C, 20 h, then aq. AcOH.

Ozonide esters **33** and **34** were formed in 99-100% yield by alkylation of ozonide benzyl chloride **32**[28] with the ethyl esters of glycine and azetidine-3-carboxylic acid, respectively (Scheme 5). Ester hydrolysis of **33** and **34** followed by treatment with methanesulfonic acid afforded the aza ozonide carboxylic acids **17** and **18** as their mesylate salts in 77 and 84% yield, respectively.

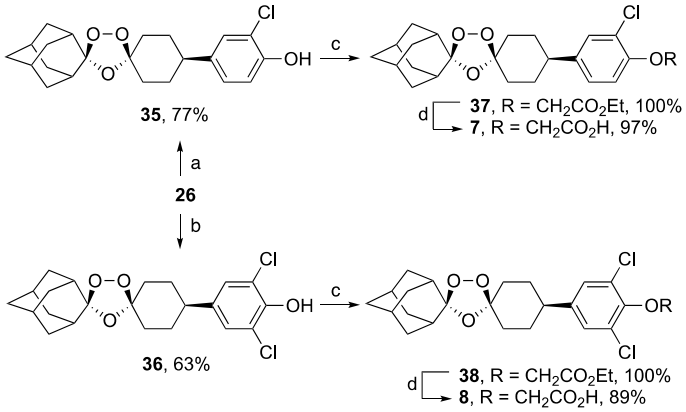
Scheme 5[a]



[a]Reagents and conditions: glycine ethyl ester hydrochloride (a) or ethyl azetidine-3-carboxylate hydrochloride (b), DIPEA, DMA, 50 °C, 5 h; (c) 1 M NaOH, aq. THF, rt, 12 h, then aq. AcOH; (d) methanesulfonic acid, Et_2O, rt, 1 h; (e) methanesulfonic acid, EA, rt, 1 h.

Chlorination of ozonide phenol **26**[27] with one and nine molar equivalents of *N*-chlorosuccinimide (NCS) furnished the chlorinated phenol intermediates **35** (77%) and **36** (63%), respectively (Scheme 6). Alkylation of **35** and **36** with ethyl bromoacetate afforded a quantitative yield of ozonide esters **37** and **38**; ester hydrolysis of the latter produced the target ozonide carboxylic acids **7** and **8** in 97 and 89% yield, respectively.

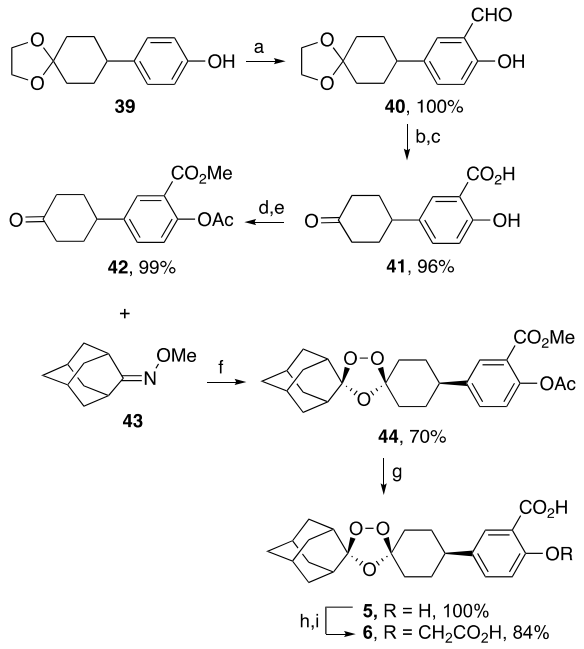
Scheme 6[a]



[a]Reagents and conditions: (a) 1 eq. NCS, DMF rt, 24 h; (b) 9 eq. NCS, DMF rt, 24 h; (c) ethyl bromoacetate, K_2CO_3, acetone, 60 °C, 12 h; (d) 1 M aq. NaOH, THF, rt, 12 h, then aq. AcOH.

Ozonide salicylic acid **5** was synthesized in a multistep sequence starting from phenol ketal **39**[29] (Scheme 7). Formylation of **39** led to a quantitative yield of salicylaldehyde **40** which then underwent successive silver oxide oxidation and ketal deprotection to afford keto salicylate **41** in 96% yield. Successive Fisher esterification and acetylation of **41** furnished the keto diester **42** in 99% yield. Griesbaum coozonolysis[30] of **42** and oxime ether **43**[31] formed ozonide diester **44** in 70% yield. Ester hydrolysis of **44** afforded the desired ozonide salicylate **5** in quantitative yield. Ozonide dicarboxylic acid **6** was obtained in 84% yield by alkylation of **5** with ethyl bromoacetate followed by hydrolysis.

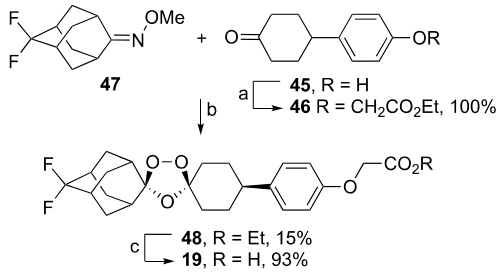
Scheme 7[a]



[a]Reagents and conditions: (a) paraformaldehyde, $MgCl_2$, Et_3N, THF, rt to reflux, 5 h; (b) Ag_2O, H_2O, 100 °C, 12 h; (c) 6 N HCl, THF, rt, 4 h; (d) acetyl chloride, MeOH, 0 °C to reflux, 4 h; (e) Ac_2O, pyridine, DCM, 0 °C to rt, 5 h; (f) O_3, 4:1 cyclohexane:DCM, 0 °C, 0.25 h; (g) KOH, 4:4:1 H_2O:MeOH:THF, rt to 50 °C, 4 h; (h) ethyl bromoacetate, K_2CO_3, acetone, 60 °C, 12 h; (i) 1 M aq. NaOH, 5:1 THF:H_2O, rt, 12 h, then aq. AcOH.

Coozonolysis[30] of oxime ether **47**[28] and keto ester **46**, formed by alkylation of keto phenol **45** with ethyl bromoacetate, furnished ozonide ester **48** in 15% yield (Scheme 8). Ester hydrolysis of **48** afforded the desired ozonide carboxylic acid **19** in 93% yield.

Scheme 8[a]

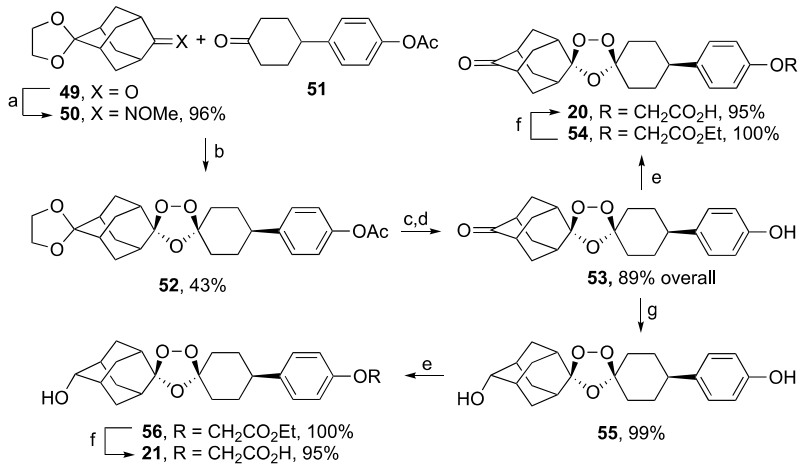


[a]Reagents and conditions: (a) ethyl bromoacetate, K_2CO_3, acetone, 60 °C, 12 h; (b) O_3, 5:2 cyclohexane:DCM, 0 °C, 0.25 h; (c) 15% aq. KOH, THF, rt, 12 h, then aq. AcOH.

As shown in Scheme 9, ozonide carboxylic acids **20** and **21** were synthesized from **53**, a common ozonide keto phenol precursor. The synthesis of **53** began with conversion of the *mono*-ketal of adamantane-2,6-dione (**49**)[32] to its oxime ether **50** in high yield. This was followed by coozonolysis[30] of **50** and keto ester **51**[27] to form ozonide ester ketal **52** in 43% yield. Successive ester and ketal deprotection of **52** furnished **53** in an overall yield of 89%. Alkylation

of **53** with ethyl bromoacetate to form ozonide ester **54** followed by hydrolysis afforded ozonide carboxylic acid **20** in 95% overall yield. Reduction of **53** with sodium borohydride afforded the corresponding secondary alcohol **55** in 99% yield. Alkylation of **55** with ethyl bromoacetate to form ozonide ester **56** followed by hydrolysis afforded carboxylic acid **21** in 95% overall yield.

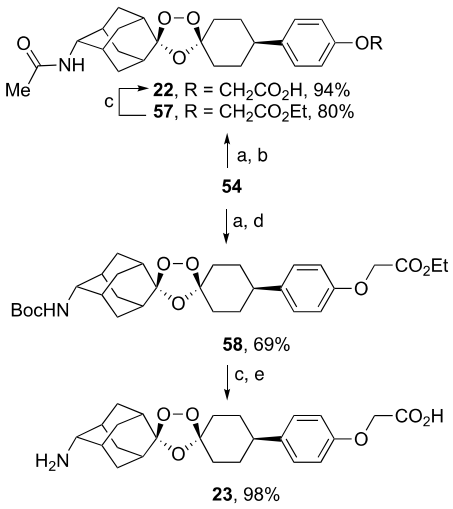
Scheme 9[a]



[a]Reagents and conditions: (a) methoxyamine HCl, pyridine, EtOH, rt, 12 h; (b) O_3, 3:1 cyclohexane:DCM, 0 °C, 0.25 h; (c) 1 M aq. NaOH, THF, rt, 12 h; (d) 1 M MsOH, 5:1 acetone:H_2O, rt, 12 h; (e) ethyl bromoacetate, K_2CO_3, acetone, 55 °C, 12 h; (f) 5:1 1 M aq. NaOH:THF, rt, 12 h, then aq. AcOH; (g) $NaBH_4$, MeOH, 0 °C, 2 h.

Reductive amination of ozonide ester **54** followed by acetylation afforded ozonide ester **57** in 80% yield (Scheme 10). Ester hydrolysis of **57** furnished ozonide acetamide carboxylic acid **22** in 94% yield. Similarly, Boc protection of the crude amino ester ozonide formed by reductive amination of **54** afforded **58** in 69% yield. Ester hydrolysis of **58** followed by treatment with methanesulfonic acid afforded ozonide amino acid **23** as its mesylate salt in 98% yield.

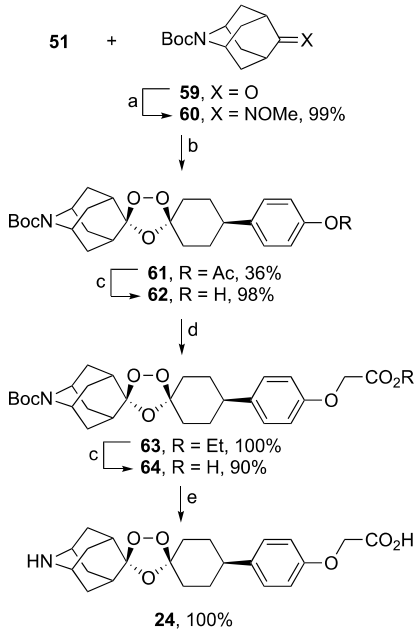
Scheme 10[a]



[a]Reagents and conditions: (a) $NaBH_3CN$, NH_4OAc, AcOH, MeOH, rt, 12 h, then 1 M aq. NaOH; (b) acetyl chloride, pyridine, DCM, rt, 6 h; (c) 1 M aq. KOH, THF, rt, 12 h, then aq. AcOH; (d) $(Boc)_2O$, DIPEA, DCM, 0 °C to rt, 12 h; (e) 1 M methanesulfonic acid in EtOAc, rt, 12 h.

The synthesis of ozonide **24** began with formation of oxime ether **60** from the Boc-protected azaadamantanone **59**[33] which then underwent coozonolysis[30] with keto ester **51** to form ozonide ester **61** in 36% yield (Scheme 11). Ester hydrolysis of **61** furnished ozonide phenol **62** in 98% yield which was then alkylated with ethyl bromoacetate to form ozonide ester **63** in quantitative yield. Ester hydrolysis of **63** produced ozonide carboxylic acid **64** in 90% yield. Boc deprotection of **64** with methanesulfonic acid afforded the mesylate salt of ozonide carboxylic acid **24** in quantitative yield.

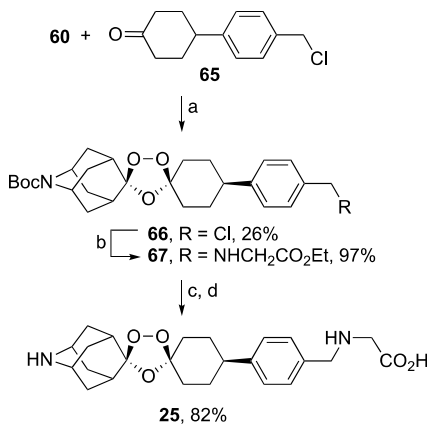
Scheme 11[a]



[a]Reagents and conditions: (a) methoxyamine hydrochloride, pyridine, EtOH, rt, 12 h; (b) O_3, 10:1 cyclohexane:DCM, 0 °C, 0.25 h; (c) 15 % aq. KOH, THF, rt, 12 h, then aq. AcOH; (d) ethyl bromoacetate, K_2CO_3, acetone, 60 °C, 12 h; (e) methanesulfonic acid, THF, rt, 12 h.

The synthesis of target ozonide **25** began with coozonolysis[30] of oxime ether **60** and ketone **65** to form ozonide **66** in 26% yield (Scheme 12). Alkylation of glycine ethyl ester with **66** afforded ozonide ester **67** in 97% yield. Intermediate **67** then underwent successive ester hydrolysis and Boc deprotection with methanesulfonic acid to afford the dimesylate salt of ozonide carboxylic acid **25** in 82% yield. Ozonides **2**-**4**, **11**, and **12** were obtained as previously described.[34-36]

Scheme 12[a]



[a]Reagents and conditions: (a) O_3, 10:1 cyclohexane:DCM, 0 °C, 0.25 h; (b) glycine ethyl ester hydrochloride, DIPEA, DMA, 50 °C, 5 h; (c) 1 M aq. NaOH, THF, rt, 12 h, then aq. AcOH; (d) methanesulfonic acid, THF, rt, 12 h.

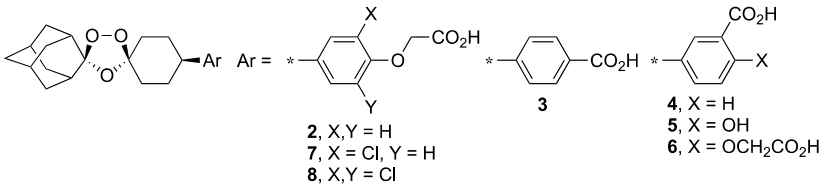
Physicochemical, in vitro ADME, and Antischistosomal Properties

When considering the physicochemical and *in vitro* ADME properties of these ozonides (Tables 1-3), we note several overarching trends. First, the calculated polar surface area (PSA) values of between 68 and 117 Å^2 indicate that the polarity of these compounds is unlikely to be a rate-limiting factor for membrane permeability and oral bioavailability.[37] Second, most of the compounds have low aqueous solubilities even those with $LogD_{7.4}$ values ≤ 3. Third, these compounds had high estimated protein binding values ranging from 94.2 to >99.5%, an unsurprising result for these ozonide weak acids.

The data in Table 1 show the SAR of the phenyl substructure of **2** and **3**. The only compounds with high aq. solubilities at pH 6.5 were salicylate **5** and dicarboxylic acid **6**; the latter was also metabolically stable. Ozonide **7**, the more lipophilic chloro analog of **2**, was also metabolically

stable. Ozonide **4**, the meta isomer of **3**, was somewhat less stable to metabolism; interestingly, addition of a phenol functional group in **5** improved metabolic stability in mouse microsomes, but substantially decreased metabolic stability in human microsomes. Of these analogs (Table 1), only **7** had *in vivo* activity against chronic *S. mansoni* infections equal or superior to that of the two prototype ozonides **2** and **3**. For comparison, at this same 400 mg/kg oral dose, dihydroartemisinin[5] and praziquantel[41] reduce worm burden by 66 and 96%, respectively.

Table 1. Physicochemical, in vitro ADME, and antischistosomal data for ozonides **2-8**.



Compd	gLogD$_{7.4}$[a]	PSA (Å^2)[b]	Sol$_{2.0}$/Sol$_{6.5}$ (μg/mL)[c]	h/m CL$_{int}$ (μL/min/mg protein)[d]	cPPB (%)[e]	*S. mansoni* WBR (%) 1 x 400 mg/kg po[f]
2	2.9	77.1	<1.6/6.3-12.5	ND[g]	ND[h]	80[i]*
3	3.0	67.8	<3.1/<1.6	ND[g]	98.8	74[i]*
4	3.0	67.8	<1.6/<1.6	12/30	98.9	63
5	3.0	88.1	<1.6/>100	108/20	99.0	36
6	1.9	117.2	<3.1/>100	<7/8	99.1	0
7	3.2	77.1	<3.1/3.1-6.3	<7/8	>99.5	84
8	3.3	77.1	<3.1/3.1-6.3	ND[g]	ND	32

[a]Log D$_{7.4}$ values were estimated by chromatography[38]

[b]Calculated PSA values were generated using JChem for Excel.

[c]Kinetic solubilities at pH 6.5 phosphate buffer and 0.01 M HCl (approx. pH 2.0)[39]

[d]In vitro intrinsic clearance (CL_{int}) values in human and mouse liver microsomes.

[e]Protein binding values were estimated by chromatography.[40]

[f]Groups of five *S. mansoni*-infected NMRI mice were treated on day 49 post-infection with ozonides dissolved or suspended in 7% v/v Tween 80, 3% v/v ethanol. At 28 d post-treatment, animals were sacrificed and dissected to assess total worm burden reduction (WBR).

[g]Analytical difficulties precluded measurement.

[h]Poor chromatography precluded determination.

[i]data from Keiser et al.[24]

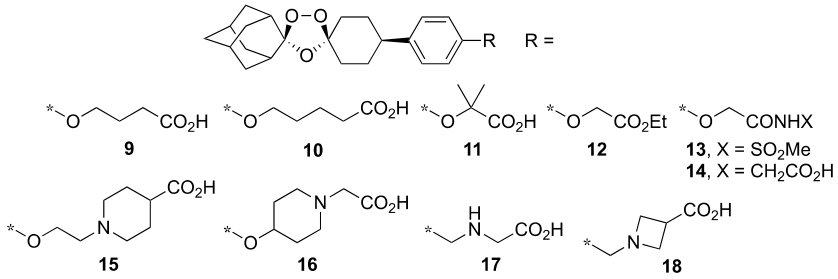
*p <0.02 from the Kruskal–Wallis test comparing the medians of the responses between the treatment and control groups.

ND = not determined

The data in Table 2 show the SAR of the carboxymethoxy substructure of **2**. Extending the length of the side chain to carboxypropoxy (**9**) or carboxybutoxy (**10**) decreased aq. solubility, increased protein binding, and decreased antischistosomal activity compared to **2**. Compound **11**, the *gem*-dimethyl analog of **2**, also had very high protein binding and was less active than **2**. With the exception of ethyl ester **12** and glycine conjugate **14**, the other compounds were metabolically stable; **12** was rapidly converted to **2** in the both human and mouse liver microsomes, whereas **14** was converted to **2** in human but not mouse liver microsomes. Of these two compounds, only **14** had significant antischistosomal activity. Interestingly, **14** was more and less soluble than **2** at pH 2.0 and 6.5, respectively. Acyl sulfonamide **13** had the same solubility profile as **2**, but had only modest antischistosomal activity. The amphoteric analogs **15-18** show the effects of adding a weak base functional group to the overall profiles of these

ozonides. Compound **17**, an aza isostere of **9** had a much superior profile than the latter. For example, **17** was less lipophilic, had lower protein binding and was much more active than **9** against *S. mansoni in vivo*. However, for the two piperidine carboxylic acids **15** and **16**, only the former had significant antischistosomal activity. Like **15**, azetidine carboxylic acid **18** was less lipophilic than **2**, but had only weak antischistosomal activity.

Table 2. Physicochemical, in vitro ADME, and antischistosomal data for ozonides **9-18**.



Compd	gLogD$_{7.4}$	PSA (Å^2)	Sol$_{2.0}$/Sol$_{6.5}$ (μg/mL)	h/m CL$_{int}$ (μL/min/mg protein)	cPPB (%)	*S. mansoni* WBR (%) 1 x 400 mg/kg po
9	3.6	77.1	<3.1/<1.6	<7/8	>99.5	55
10	4.0	77.1	<1.6/<1.6	<7/13	>99.5	51
11	3.1	77.1	<1.6/3.1-6.3	<7/7	>99.5	48
12	>5.3	63.2	<1.6/<0.78	ND[a]	98.9	60
13	3.0	97.4	<1.6/6.3-12.5	<7/12	99.0	53
14	3.0	106.2	12.5-25/<3.1	<7/CND[a,b]	98.5	76*
15	3.8	81.5	3.1-6.3/<1.6	<7/9	98.0	0
16	ND[c]	81.5	ND[d]	<7/<7	ND	75*
17	3.3	84.4	<12.5/<6.3	<7/10	97.6	90*

18	ND[c]	72.3	6.3-12.5/<1.6	7/10	ND	43

[a]apparent non-NADPH-mediated degradation evident

[b]Putative amide hydrolysis product **2** was detected in controls and NADPH samples.

[c]Poor chromatography precluded determination

[d]Poor solubility in DMSO (<1 mg/mL) precluded determination of solubility

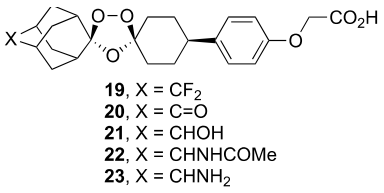
*p <0.02 from the Kruskal–Wallis test comparing the medians of the responses between the treatment and control groups.

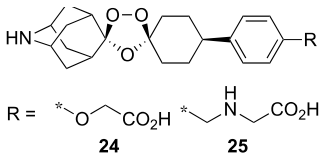
CND = could not determine

ND = not determined

The data in Table 3 summarize the SAR of the spiroadamantane substructure of **2**. Replacing the distal methylene carbon of **2** with a difluoromethylene (**19**) or a ketone (**20**) increased aq. solubility, decreased protein binding, and decreased both metabolic stability and antischistosomal activity. Decreasing lipophilicity by adding a secondary alcohol (**21**), acetamide (**22**), or primary amine (**23**) to the distal methylene carbon of **2** increased aq. solubility at pH 6.5 for **21** and **22**, but abolished antischistosomal activity for all three ozonides. Interestingly, the amphoteric **23** had the lowest protein binding (94.2%) for this series of ozonides. Compounds **24** and **25**, azaadamantane analogs of **2** and **17**, show the consequences of replacing the methylene carbon of **2** with a nitrogen atom. Unfortunately, **24** had low aq. solubility, intermediate metabolic stability, and low antischistosomal activity. Even though **25** was metabolically stable and was the sole ozonide in this series with high aq. solubility at both pH 2.0 and 6.5, it also had weak antischistosomal activity.

Table 3. Physicochemical, in vitro ADME, and antischistosomal data for ozonides **19**-**25**.





Compd	gLogD$_{7.4}$	PSA (Å^2)	Sol$_{2.0}$/Sol$_{6.5}$ (μg/mL)	h/m CL$_{int}$ (μL/min/mg protein)	cPPB (%)	*S. mansoni* WBR (%) 1 x 400 mg/kg po
19	2.9	77.1	<1.6/12.5-25	23/130	99.4	57
20	1.9	94.1	<1.6/50-100	ND[a]	97.8	24
21	1.7	97.3	<1.6/25-50	<7/<7	98.5	14
22	1.8	106.2	3.1-6.3/12.5-25	10/<7	98.2	0
23	1.4	104.7	6.3-12.5/<1.6	13/8	94.2	30
24	1.3	93.7	3.1-6.3/<1.6	35/27	ND[b]	29
25	1.6	101.0	>100/50-100	<7/<7	ND[b]	41

[a]apparent non-NADPH-mediated degradation

[b]poor chromatography precluded determination

As the data in Tables 1-3 reveal, we identified **7**, **14**, **16**, and **17** – four new ozonides with high activity against adult *S. mansoni* in a mouse model. As a measure of their antischistosomal selectivity, these ozonides were tested for cytotoxicity against four human cell lines: foreskin fibroblast (HFF), osteosarcoma (U-2 OS), kidney (HEK 293T), and hepatocyte (HC-04). None of the compounds inhibited these cell lines at concentrations up to 50 μM (data not shown). Although our primary objective was to identify ozonides with high activity against the adult stage of *S. mansoni*, we wanted to find compounds with high activities against both adult and juvenile stages. Therefore, the most active ozonides were also tested for activity

against juvenile *S. mansoni* in infected mice (Table 4). At a single 200 mg/kg dose, all of the compounds reduced worm burden by more than 80% in mice with juvenile stage *S. mansoni* infections (Table 4); of these, **2**, **14**, and **17** were the most effective with WBR values of 97-100%.

Table 4. In vivo activity of selected ozonides against the juvenile stage of *S. mansoni*.

Compd	*S. mansoni* WBR (%) 1 x 200 mg/kg po
2	100[a]
3	84[a]
7	92*
14	97*
16	74*
17	97*

[a]data from Keiser et al.[24]

*p <0.05 from the Kruskal–Wallis test comparing the medians of the responses between the treatment and control groups.

Summary

In summary, these ozonide weak acids had relatively low aqueous solubilities and high protein binding values that were independent of lipophilicity. It was possible to increase solubility, decrease protein binding and maintain high antischistosomal activity in mice infected with juvenile and adult *Schistosoma mansoni* by incorporating a weak base functional group in these compounds. Ozonides with *para*-substituted carboxymethoxy and *N*-benzylglycine substituents had high antischistosomal activities. These are exemplified by **7**, **14**, **16**, and **17** – four new ozonides with $LogD_{7.4}$ values ranging from 3.0 to 3.3, similar to that of prototype ozonide **2** ($LogD_{7.4}$ of 2.9). Adding polar functional groups and heteroatoms to the spiroadamantane

substructure could increase solubility and metabolic stability, but in all cases, decreased antischistosomal activity.

Experimental Section

General. Melting points are uncorrected. Unless otherwise noted, 1D ^{1}H and ^{13}C NMR spectra were recorded on a 500 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvents. All chemical shifts are reported in parts per million (ppm) and are relative to internal $(CH_3)_4Si$ (0 ppm) for ^{1}H and $CDCl_3$ (77.2 ppm) or DMSO-d_6 (39.5 ppm) for ^{13}C NMR. Silica gel (sg) particle size 32−63 μm was used for all flash column chromatography. Reported reaction temperatures are those of the oil bath. GCMS data was generated with an Agilent 6890-5973N GC-MS system using a DB-5 column and helium flow rate of 1 mL/min. Combustion analysis confirmed that all target compounds have a purity of at least 95%. X-ray crystallographic data for all ozonides with 8'-aryl substituents (like **2**-**25**) demonstrate that they have cis configurations.[27,42]

5-(Dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)-2-hydroxybenzoic acid (5). Step 1. To a mixture of anhydrous magnesium chloride (12.21 g, 128.2 mmol), paraformaldehyde (5.78 g, 192.3 mmol) and triethylamine (17.9 ml, 128.2 mmol) in anhydrous THF (150 mL) at rt was added dropwise a solution of 4-(1,4-dioxaspiro[4.5]decan-8-yl)phenol (**39**)[29] (15.0 g, 64.1 mmol) in THF (100 mL) under Ar. The reaction mixture was then refluxed for 5 h. After completion of the reaction, an insoluble solid was filtered and washed with THF (2 x 25 mL). After solvent removal in vacuo, the residue was dissolved in DCM (150 mL), washed with 10% HCl (3 x 25 mL), water (3 x 25 mL) and dried over $MgSO_4$. After filtration, the solvent was removed in vacuo to afford 2-hydroxy-5-(1,4-dioxaspiro[4.5]decan-8-yl)benzaldehyde (**40**) as viscous oil (16.8 g, 100%). GCMS: m/z: 262 [M^+], ^{1}H NMR ($CDCl_3$) δ

1.66–1.90 (m, 8H), 2.50–2.60 (m, 1H), 3.98 (s, 4H), 6.90–6.92 (m, 1H), 7.40–7.42 (m, 2H), 9.85 (s, 1H), 10.86 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 31.4, 34.8, 41.8, 64.1, 108.1, 117.3, 120.2, 131.1, 135.9, 137.99, 159.79, 196.5. **Step 2**. To a stirred solution of silver nitrate (7.13 g, 41.98 mmol) in water (25 mL) was added dropwise a solution of NaOH (3.35 g, 83.96 mmol) in water (25 mL) at 0 ºC. After stirring for 5 min, silver oxide was obtained as a brown semisolid. To this mixture, a solution of **40** (5.5 g, 20.99 mmol) in water (25 mL) was added and the reaction mixture was stirred at 100 ºC for 12 h. After filtration of the black silver suspension, the aq. solution was neutralized (pH = ~7) with 10% HCl at 0 ºC to obtain a solid that was filtered, washed with water and dried to afford 2-hydroxy-5-(1,4-dioxaspiro[4.5]decan-8-yl)benzoic acid as a colorless solid (5.6 g, 96%). To a stirred solution of hydroxy-5-(1,4-dioxaspiro[4.5]decan-8-yl)benzoic acid (5.6 g, 20.1 mmol) in THF (75 mL) was added 6 N HCl (20 mL) dropwise at rt. After completion of the reaction, the solvent was removed in vacuo and the residue was dissolved in DCM (100 mL), washed with water (3 x 25 mL) and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed in vacuo to afford 2-hydroxy-5-(4-oxocyclohexyl)benzoic acid (**41**) as a colorless solid (4.69 g, 100%). ^{1}H NMR (DMSO-d_6) δ 1.78–1.90 (m, 2H), 2.02–2.08 (m, 2H), 2.22–2.30 (m, 2H), 2.52–2.62 (m, 2H), 3.01–3.08 (m, 1H), 6.92 (d, J = 8.42 Hz, 1H), 7.47 (dd, J = 1.47, 8.42 Hz, 1H), 7.71 (d, J = 1.47 Hz, 1H), 11.02 (bs, 1H), 14.0 (bs, 1H). ^{13}C NMR (DMSO-d_6) δ 33.6, 40.8, 40.9, 112.8, 117.4, 128.1, 134.5, 136.2, 159.9, 172.2, 210.4. **Step 3**. To a solution of **41** (6.54 g, 27.9 mmol) in MeOH (200 mL) was added acetyl chloride (2 mL) dropwise at 0 ºC. Then the reaction mixture was heated to reflux for 4 h. Solvents were then removed in vacuo and the residue was dissolved in DCM (100 mL), washed with water (3 x 25 mL) and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed in vacuo to afford methyl 2-hydroxy-5-(4-oxocyclohexyl)benzoate as a

viscous oil (6.98 g, 100%). GCMS: m/z: 248 [M^+]. Successive dropwise additions of pyridine (11.28 mL, 139.6 mmol) and acetic anhydride (13.2 ml, 139.6 mmol) were made to a solution of methyl 2-hydroxy-5-(4-oxocyclohexyl)benzoate (6.98 g, 28.2 mmol) in DCM (50 mL) at 0 ºC. The reaction mixture was stirred at rt for 5 h before solvent removal in vacuo. The residue was cooled to 0 °C to obtain a solid that was filtered, washed with water (3 x 25 mL) and dried to afford methyl 2-acetoxy-5-(4-oxocyclohexyl)benzoate (**42**) as a colorless solid (8.0 g, 99%). GCMS: m/z: 290 [M^+], ^{1}H NMR ($CDCl_3$) δ 1.80–1.90 (m, 2H), 2.02–2.08 (m, 2H), 2.26 (s, 3H), 2.38–2.46 (m, 4H), 2.96–3.04 (m, 1H), 3.79 (s, 3H), 6.98 (d, J = 8.30 Hz, 1H), 7.35 (dd, J = 1.95, 8.30 Hz, 1H), 7.81 (d, J = 1.95 Hz, 1H). **Step 4.** A solution of **42** (5.89 g, 20.29 mmol) and *O*-methyl-2-adamantanone oxime (**43**)[31] (5.45 g, 30.44 mmol) in cyclohexane (320 mL) and DCM (80 mL) at 0 °C was treated with ozone according to the method of Dong et al.[42] Solvents were removed in vacuo and the residue was triturated with 95% aq. EtOH to obtain a solid that was filtered, washed with 95% aq. EtOH and dried to afford methyl 2-acetoxy-5-(dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)benzoate (**44**) as a colorless solid (6.5 g, 70%). mp 132–134 °C. ^{1}H NMR ($CDCl_3$) δ 1.68–2.08 (m, 22H), 2.34 (s, 3H), 2.56–2.62 (m, 1H), 3.86 (s, 3H), 7.01 (d, J = 8.30 Hz, 1H), 7.38 (dd, J = 2.44 & 8.30 Hz, 1H), 7.85 (d, J = 1.95 Hz, 1H). **Step 5.** To a solution of **44** (2.0 g, 4.39 mmol) in 4:4:1 H_2O:MeOH:THF (45 mL) was added solid KOH (2.46 g, 43.9 mmol) at rt. After the addition, the reaction mixture was stirred at 50 ºC for 4 h. After cooling to rt, the reaction mixture was concentrated to 10 mL in vacuo, diluted with water (50 mL) and neutralized (pH = ~ 7) with 10% aq. HCl at 0 °C to obtain a solid that was filtered, washed with water and dried to afford **5** (1.8 g, 100%) as a colorless solid. mp 159–161 ºC. ^{1}H NMR (DMSO-d_6) δ 1.46–1.56 (m, 2H), 1.64–1.96 (m, 20H), 2.43–2.52 (m, 1H), 6.55 (d, J = 8.30 Hz, 1H), 6.99 (dd, J = 2.44, 8.30 Hz, 1H), 7.52 (d, J = 1.95 Hz,

1H). ^{13}C NMR (DMSO-d_6) δ 26.0, 26.5, 31.7, 34.4, 34.5, 36.0, 36.3, 41.1, 108.5, 110.7, 115.8, 120.1, 127.8, 129.2, 133.4, 161.2, 171.8. Anal. calcd for $C_{23}H_{27}NaO_6$ H_2CO_3: C, 59.50; H, 6.03. Found: C, 60.09; H, 5.77.

***cis*-Adamantane-2-spiro-3'-8'-[3-carboxy-4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (6). Step 1.** A mixture of **5** (200 mg, 0.50 mmol), ethyl bromoacetate (167 mg, 1.00 mmol) and potassium carbonate (208 mg, 1.50 mmol) in acetone (20 mL) was stirred at 60 °C for 12 h. A solid was filtered and washed with acetone, and the filtrate was concentrated in vacuo to afford *cis*-adamantane-2-spiro-3'-8'-[3-chloro-4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane as colorless oil (250 mg, 87%). ^{1}H NMR ($CDCl_3$) δ 1.28 (m, 6H), 1.77–1.65 (m, 8H), 1.87–1.77 (m, 6H), 1.93 (d, J = 12.9 Hz, 2H), 2.07–1.97 (m, 6H), 2.54 (t, J = 12.3, 1H), 4.25 (m, 4H), 4.69 (s, 2H), 4.81 (s, 2H), 6.85 (d, J = 8.6 Hz, 1H), 7.30 (d, J = 8.4, 1H), 7.78 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 14.1, 26.5, 26.9, 31.4, 34.6, 34.8, 36.4, 36.8, 41.88, 61.0, 61.2, 61.3, 67.0, 108.2, 111.4, 114.9, 119.9, 130.6, 131.9, 139.5, 156.4, 164.8, 167.8, 168.6. **Step 2.** A mixture of *cis*-adamantane-2-spiro-3'-8'-[3-chloro-4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (250 mg, 0.44 mmol) and 1 M aq. NaOH (2 mL) in THF (10 mL) was stirred at rt for 12 h. The solvent was removed in vacuo and the residue was diluted with H_2O (10 mL) and acidified with AcOH (0.2 mL). The resultant precipitate was collected by filtration and washed with cold water, dried in vacuo at 50 °C to afford **6** as a white solid (190 mg, 95%). mp 164–165 °C. ^{1}H NMR (DMSO-d_6) δ 1.51 (q, J = 13.0, 2H), 1.98–1.62 (m, 20H), 2.59 (t, J = 12.3, 1H), 4.61 (s, 2H), 7.04 (d, J = 8.6 Hz, 1H), 7.28 (d, J = 8.2 Hz, 1H), 7.39 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 26.3, 26.7, 31.6, 34.5, 34.7, 36.2, 36.6, 40.9, 68.4, 108.5, 111.1, 116.2, 124.2, 128.9, 131.0, 139.4, 155.5, 168.3, 171.7. Anal. calcd for

$C_{25}H_{30}O_8$ H_2O: C, 63.01; H, 6.77. Found: C, 63.38; H, 6.58. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{25}H_{30}O_8Na$ 481.1838; Found 481.1838.

***cis*-Adamantane-2-spiro-3'-8'-[3-chloro-4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decan (7). Step 1.** A solution of *N*-chlorosuccinimide (1.25 g, 9.36 mmol) in dry DMF (10 mL) was added to a solution of **26**[27] (3.32 g, 9.31 mmol) in dry DMF (30 mL) and stirred under nitrogen at rt for 24 h protected from light. The mixture was poured on ice water and then extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with water (100 mL) and brine (25 mL), dried with $MgSO_4$ and concentrated in vacuo to give a semisolid that was purified by chromatography (sg, 9:1 hexane:ethyl acetate) to afford 2-chloro-4-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenol (**35**) (2.80 g, 77%) as a white solid. mp 119–121 °C. ^{1}H NMR ($CDCl_3$) δ 1.62–2.01 (m, 22H), 2.44–2.50 (m, 1H), 5.41 (s, 1H), 6.92 (d, J = 8.0 Hz, 1H), 7.01 (dd, J = 8.0, 2.0 Hz, 1H), 7.15 (d, J = 2.0 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 26.7, 27.1, 31.5, 31.7, 34.7, 34.8, 34.99, 35.00, 36.6, 37.0, 42.1, 108.4, 111.7, 116.2, 119.8, 126.8, 126.9, 127.3, 139.8, 149.7. **Step 2**. A mixture of **35** (210 mg, 0.54 mmol), ethyl bromoacetate (180 mg, 1.08 mmol) and potassium carbonate (224 mg, 1.62 mmol) in acetone (20 mL) was stirred at 60 °C for 12 h. The solid was filtered and washed with acetone and the filtrate was concentrated *in vacuo* to afford *cis*-adamantane-2-spiro-3'-8'-[3-chloro-4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**37**) as a colorless oil (0.257 g, 100%). ^{1}H NMR ($CDCl_3$) δ 7.22 (d, J = 2.1 Hz, 1H), 7.01 (dd, J = 8.5, 2.1 Hz, 1H), 6.77 (d, J = 8.5 Hz, 1H), 4.66 (s, 2H), 4.25 (q, J = 7.1 Hz, 2H), 2.48 (t, J = 12.1 Hz, 2H), 2.07–1.97 (m, 6H), 1.93 (d, J = 12.8 Hz, 2H), 1.87–1.59 (m, 15H), 1.28 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 14.1, 26.5, 26.9, 31.4, 34.6, 34.8, 36.4, 36.8, 41.8, 61.4, 66.5, 108.2, 111.4, 114.2, 123.1, 125.5, 129.0, 140.8, 151.9, 168.5. **Step 3.** A mixture of **37** (110 mg, 0.23 mmol) and 1 M aq. NaOH (1

mL) in THF (10 mL) was stirred at rt for 12 h. The solvent was removed *in vacuo* and the residue was diluted with H_2O (10 mL) and acidified with AcOH (0.2 mL). The precipitate was filtered, washed with water and dried in vacuo at 50 °C to afford **7** as a white solid (100 mg, 97%). mp 145–146 °C. ^{1}H NMR ($CDCl_3$) δ 2.12–1.54 (m, 22H), 2.48 (t, J = 12.4 Hz, 1H), 4.67 (s, 2H), 6.79 (d, J = 8.4 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 7.23 (s, 1H), 8.07 (brs, 1H). ^{13}C NMR ($CDCl_3$) δ 26.5, 26.9, 31.4, 34.6, 34.8, 36.4, 36.8, 41.9, 66.2, 108.2, 111.5, 114.4, 123.2, 125.9, 129.1, 141.3, 151.4, 173.2. Anal. calcd for $C_{24}H_{29}ClO_6 H_2O$: C, 61.73; H, 6.69. Found: C, 62.00; H, 6.40.

***cis*-Adamantane-2-spiro-3'-8'-[3,5-dichloro-4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decan (8). Step 1**. To a solution of **26**[27] (410 mg, 1.0 mmol) in dry DMF (5 mL) was added dropwise a solution of *N*-chlorosuccinimide (1.25 g, 9.36 mmol) in dry DMF (3 mL). The reaction mixture was stirred under nitrogen at rt for 24 h. The mixture was poured on ice and then extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with water (30 mL) and brine (20 mL), dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude product was purified by chromatography (sg, hexane:EA, 25:1) to afford *cis*-adamantane-2-spiro-3'-8'-(3,5-dichloro-4-hydroxyphenyl)-1',2',4'-trioxaspiro[4.5]decane (**36**) (270 mg, 63%) as a white solid. mp 143–145 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61–1.87 (m, 14H), 1.90–2.08 (m, 8H), 2.45 (tt, J = 12.5, 3.2 Hz, 1H), 5.70 (s, 1H), 7.10 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.5, 26.9, 31.4, 34.5, 34.8, 36.4, 36.8, 41.8, 108.0, 111.6, 120.8, 126.6, 139.6, 146.0. **Step 2**. A mixture of **36** (270 mg, 0.63 mmol), ethyl bromoacetate (159 mg, 0.95 mmol) and potassium carbonate (175 mg, 1.26 mmol) in acetone (20 mL) was stirred at 60 °C for 12 h. The solid was removed by filtration and washed with acetone and the filtrate was concentrated *in vacuo* to afford *cis*-adamantane-2-spiro-3'-8'-[3,5-dichloro-4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-

trioxaspiro[4.5]decane (**38**) (322 mg, 100%) as a white solid. mp 99–100 °C. ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7.1 Hz, 3H), 1.58–1.69 (m, 6H), 1.69–1.84 (m, 8H), 1.87–1.93 (m, 2H), 1.94–2.05 (m, 6H), 2.46 (tt, J = 12.3, 3.3 Hz, 1H), 4.28 (q, J = 7.1 Hz, 2H), 4.56 (s, 2H), 7.11 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 14.1, 26.4, 26.8, 31.1, 34.4, 34.8, 36.4, 36.7, 41.9, 61.3, 69.3, 107.8, 111.5, 127.3, 128.8, 144.3, 148.4, 167.9. **Step 3.** A mixture of **38** (322 mg, 0.63 mmol) and 1 N aq. NaOH (2 mL) in THF (20 mL) was stirred at rt for 12 h. Removal of the solvents *in vacuo* gave a white residue which was suspended in H_2O (10 mL) and acidified with AcOH to pH 3. The precipitate was filtered, washed with water and dried in vacuo at 50 °C to afford **8** (270 mg, 89%) as a white solid. mp 142–143 °C. ^{1}H NMR ($CDCl_3$) δ 1.61–1.87 (m, 14H), 1.90–2.09 (m, 8H), 2.49 (tt, J = 12.3, 3.4 Hz, 1H), 4.64 (s, 2H), 7.15 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 26.5, 26.9, 31.2, 34.4, 34.8, 36.4, 36.8, 42.0, 68.9, 107.9, 111.6, 127.4, 128.7, 144.8, 148.0, 171.2. Anal. calcd for $C_{24}H_{28}Cl_2O6$: C, 59.63; H, 5.84. Found: C, 59.89; H, 6.00.

***cis*-Adamantane-2-spiro-3'-8'-[4-(carboxypropoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (9).** To a solution of **26**[27] (250 mg, 0.70 mmol) in dry dimethoxyethane (25 mL) were added powdered NaOH (168 mg, 4.20 mmol) and Bu_4NHSO_4 (48 mg, 0.15 mmol) and the resulting mixture was stirred at rt for 30 min before addition of ethyl 4-bromobutanoate (205 mg, 1.05 mmol). The reaction mixture was stirred at 60 °C for 12 h, then concentrated in vacuo. The residue was suspended in H_2O (20 mL) and acidified with AcOH to pH 3. The precipitate was filtered, washed with H_2O and dried in vacuo at 50 °C to afford **9** (240 mg, 77%) as a white solid. mp 148–149 °C. ^{1}H NMR ($CDCl_3$) δ 1.87–1.57 (m, 14H), 1.94 (d, J = 12.8 Hz, 2H), 2.06 – 1.97 (m, 6H),

2.09 (p, *J* = 6.7 Hz, 2H), 2.49 (tt, *J* = 12.1, 3.3 Hz, 1H), 2.57 (t, *J* = 7.3 Hz, 2H), 3.98 (t, *J* = 6.1 Hz, 2H), 6.81 (d, *J* = 8.3 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 24.5, 26.5, 26.9, 30.6, 31.7, 34.77, 34.83, 36.4, 36.8, 42.1, 66.5, 108.5, 111.4, 114.4, 127.6, 138.5, 157.1, 179.2. Anal. calcd for $C_{26}H_{34}O_6$: C, 70.56; H, 7.74. Found: C, 70.70; H, 7.75.

***cis*-Adamantane-2-spiro-3'-8'-[4-(carboxybutoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decan (10).** To a solution of **26**[27] (250 mg, 0.70 mmol) in dry dimethyoxyethane (25 mL) were added powdered NaOH (168 mg, 4.20 mmol) and Bu_4NHSO_4 (48 mg, 0.15 mmol) and the resulting mixture was stirred at rt for 30 min before addition of methyl 5-bromopentanoate (220 mg, 1.05 mmol). The reaction mixture was stirred at 60 °C for 12 h, then concentrated in vacuo. The residue was suspended in H_2O (20 mL) and acidified with AcOH to pH 3. The precipitate was filtered, washed with H_2O and dried in vacuo at 50 °C to afford **10** (230 mg, 72%) as a white solid. mp 144–145 °C. ^{1}H NMR ($CDCl_3$) δ 1.87–1.61 (m, 18H), 1.94 (d, *J* = 12.8 Hz, 2H), 2.06–1.97 (m, 6H), 2.43 (d, *J* = 6.9 Hz, 2H), 2.48 (tt, *J* = 15.7, 5.0 Hz, 1H), 3.93 (t, *J* = 5.3 Hz, 2H), 6.80 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.5, 26.5, 26.9, 28.7, 31.7, 33.8, 34.77, 34.83, 36.4, 36.8, 42.1, 67.3, 108.5, 111.4, 114.4, 127.6, 138.3, 157.3, 179.3. Anal. calcd for $C_{27}H_{36}O_6$: C, 71.03; H, 7.95. Found: C, 70.84; H, 7.78.

***cis*-Adamantane-2-spiro-3'-8'-[4-[2-[methylsulfonamino]-2-oxoethoxy]phenyl]-1',2',4'-trioxaspiro[4.5]decane (13).** To a mixture of **2** (150 mg, 0.36 mmol), methanesulfonamide (47

mg, 0.40 mmol) and DMAP (10 mg, 0.07 mmol) in DCM (20 mL) was added DCC (82 mg, 0.40 mmol). The resulting mixture was stirred at rt for 24 h. The solid was filtered and the filtrate was concentrated *in vacuo* to produce a residue that was purified by chromatography (sg, DCM) to afford **13** (110 mg, 62%) as a white solid. mp 148–149 °C; ^{1}H NMR ($CDCl_3$) δ 2.08–1.68 (m, 22H), 2.55 (td, J_1 = 12 Hz, J_2 = 3 Hz), 3.38 (s, 3H), 4.85 (s, 2H), 6.88 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 2H), 8.84 (br, 1H); ^{13}C NMR ($CDCl_3$) δ 26.5, 26.9, 31.6, 34.7, 41.7, 42.1, 67.4, 108.3, 111.5, 114.7, 128.2, 140.9, 154.8, 167.7. Anal. calcd for $C_{25}H_{33}NO_7S$ 0.25 H_2O: C, 60.53; H, 6.81; N, 2.82. Found: C, 60.87; H, 6.86; N, 2.84.

***cis*-Adamantane-2-spiro-3'-8'-[4-[2-[(carboxymethyl)amino]-2-oxoethoxy]phenyl]-1',2',4'-trioxaspiro[4.5]decane (14). Step 1.** To a mixture of **2** (415 mg, 1.0 mmol) and HOBt (162 mg, 1.2 mmol) in DMF (10 mL) was added EDC (230 mg, 1.2 mmol) followed by addition of glycine ethyl ester hydrochloride (154 mg, 1.1 mmol) and DIPEA (155 mg, 1.2 mmol). The resulting mixture was stirred at rt for 12 h and then diluted with EA (50 mL), washed with brine (2 x 50 mL), 1 N HCl (20 mL), 1 N NaOH (20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to give a colorless residue that was dissolved in a mixture of ethanol (40 mL) and 2% aq. NaOH (4 mL). The resulting mixture was stirred at rt for 12 h and then concentrated in vacuo to afford a white residue that was suspended in H_2O (50 mL), acidified with AcOH to pH 3, filtered, washed with H_2O, and dried in vacuo to afford **14** (430 mg, 91%) as a white solid. mp 147–148 °C; ^{1}H NMR (DMSO-d_6) δ 1.50–1.58 (m, 2H), 1.66–1.93 (m, 19H), 2.59 (t, J = 15 Hz, 1H), 3.81 (d, J = 7.2 Hz, 2H), 4.49 (s, 2H), 6.91

(d, *J* = 11.2 Hz, 2H), 7.15 (d, *J* = 11.2 Hz, 2H), 8.35 (t, *J* = 7.2 Hz, 1H), 12.63 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 26.3, 26.7, 31.7, 34.6, 34.7, 36.3, 36.6, 40.9, 41.2, 67.4, 108.6, 111.0, 115.2, 127.9, 139.2, 156.5, 168.7, 171.4. Anal. calcd for $C_{26}H_{33}NO_7$: C, 66.23; H, 7.05; N, 2.97. Found: C, 66.40; H, 6.96; N, 2.60.

Sodium 1-(2-(4-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)ethyl)piperidine-4-carboxylate (15). Step 1. To a solution of *cis*-adamantane-2-spiro-3'-8'-(4'-acetoxyphenyl)-1',2',4'-trioxaspiro[4.5]decane (**27**)[27] (430 mg, 1.1 mmol) in dry acetonitrile (20 mL) were added powdered NaOH (259 mg, 6.5 mmol) and tetrabutylammonium hydrogensulfate (110 mg, 0.3 mmol) according to the method of Charman et al.[25] The mixture was stirred at rt for 0.5 h. Then, ethyl 1-(2-chloroethyl)piperidine-4-carboxylate (**28**)[43] (475 mg, 2.2 mmol) was added and the reaction stirred at 60 ºC for 12 h. The inorganic solid was filtered off and washed with CH_2Cl_2. After removal of the solvents in vacuo, the residue was dissolved in EtOAc (50 mL). The organic layer was washed with water, brine, and dried over $MgSO_4$. Removal of the solvent in vacuo afforded ethyl 1-(2-(4-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)ethyl)piperidine-4-carboxylate (**29**) (260 mg, 45%) as a white solid. mp 70–72 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.3 Hz, 3H), 1.69–2.04 (m, 26H), 2.15–2.19 (m, 2H), 2.26–2.30 (m, 1H), 2.46–2.51 (m, 1H), 2.77 (t, J = 5.9 Hz, 3H), 2.95 (brs, 2H), 4.07 (t, J = 5.9 Hz, 2H), 4.13 (q, J = 7.3 Hz, J = 14.2 Hz, 2H), 6.83 (d, J = 8.3 Hz, 2H), 7.10 (d, J = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 26.5, 26.9, 28.2, 31.6, 34.7, 34.8, 36.4, 36.8, 41.0, 42.0, 53.4, 57.4, 60.3, 66.0. 108.4, 111.3, 114.4, 127.6, 138.5, 157.1, 175.0. **Step 2.** To a solution of **29** (223 mg, 0.41 mmol) in EtOH (5 mL) was added powdered NaOH (50 mg, 1.2 mmol) and the reaction mixture was stirred for 24 h at rt. Filtration afforded **15** (190 mg, 86%)

as a white solid. mp 165–167 °C; ^{1}H NMR (CD_3OD) δ 1.66–2.23 (m, 29H), 2.50–2.55 (m, 1H), 2.77 (t, J = 5.5 Hz, 3H), 3.01 (brs, 2H), 4.09 (t, J = 5.5 Hz, 2H), 6.84 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 2H); ^{13}C NMR (CD_3OD) δ 28.2, 28.6, 30.4, 33.0, 36.0, 38.0, 38.1, 43.4, 45.6, 55.3, 58.7, 66.7, 109.8, 112.4, 115.7, 128.8, 140.0, 158.7, 183.9. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{30}H_{42}NO_6$ 512.3012; Found 512.3026.

2-(4-(4-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)piperidin-1-yl)acetic acid (16). Step 1. To a solution of *cis*-adamantane-2-spiro-3-8-[4-(4-piperidinyloxy)phenyl]-1,2,4-trioxaspiro[4.5]decane (**30**)[25] (0.30 g, 0.68 mmol) and ethyl 2-bromoacetate (0.14 g, 0.84 mmol) in THF (12 mL) was added a solution of potassium carbonate (0.09 g, 0.65 mmol) in water (1 mL). The reaction mixture was stirred at rt for 12 h. After solvent removal in vacuo, the crude product was purified by crystallization from 4:1 EtOH:H_2O to afford ethyl 2-(4-(4-(dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)piperidin-1-yl)acetate (**31**) as a colorless solid (0.25 g, 69%). mp 106–107°C; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J = 7.0 Hz, 3H), 1.64–2.08 (m, 26H), 2.44–2.56 (m, 3H), 2.76–2.85 (m, 2H), 3.24 (s, 2H), 4.19 (q, J = 7.0 Hz, 2H), 4.26–4.34 (m, 1H), 6.82 (d, J = 8.5 Hz, 2H), 7.10 (d, J = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 26.5, 26.9, 30.6, 31.6, 34.7, 34.8, 36.4, 36.8, 42.0, 50.3, 59.6, 60.6, 71.9, 108.4, 111.4, 116.0, 127.7, 138.5, 155.7, 170.5. **Step 2.** To a solution of **31** (0.53 g, 1.0 mmol) in EtOH (20 mL) was added 1 N aq. NaOH (2 mL). The resulting mixture was stirred at 50 °C for 20 h. The reaction mixture was then diluted with water (5 mL) and AcOH (5 mL). The precipitate was filtered, washed with water and dried in vacuo at 40 °C to afford **16** (0.42 g, 84%) as colorless solid. mp 171–172°C. ^{1}H NMR (DMSO-d_6) δ 1.42–1.60 (m, 2H), 1.61–2.10 (m, 24H), 2.44–2.56 (m, 1H), 2.68–2.82 (m, 2H), 2.90–3.08 (m, 2H), 3.22 (s, 2H), 4.32–4.46 (m, 1H), 6.88 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H); ^{13}C NMR (DMSO-d_6)

δ 26.3, 26.7, 30.7, 31.7, 34.6, 34.7, 36.3, 36.6, 41.2, 48.3, 55.4, 71.0, 108.6, 111.0, 116.4, 128.1, 139.2, 155.2, 167.7. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{29}H_{40}NO_6$ 498.2856; Found 498.2854.

***cis*-Adamantane-2-spiro-3'-8'-[4-[[(carboxymethyl)amino]methyl]phenyl]-1',2',4'-trioxaspiro[4.5]decane mesylate (17). Step 1.** A mixture of *cis*-adamantane-2-spiro-3'-8'-[4-(chloromethyl)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**32**)[28] (0.30 g, 0.77 mmol), glycine ethyl ester hydrochloride (1.30 g, 9.32 mmol) and DIPEA (1.50 g, 11.65 mmol) in DMA (20 mL) was stirred at 50 °C for 5 h. The reaction mixture was then cooled to rt and diluted with EA (100 mL), washed successively with H_2O (50 mL), 1 M HCl (15 mL) and brine (2 x 50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated and dried in vacuo at 50 °C to afford *cis*-adamantane-2-spiro-3'-8'-[4-[[(2-ethoxy-2-oxoethyl)amino]methyl]phenyl]-1',2',4'-trioxaspiro[4.5]decane (**33**) (340 mg, 97%) as a light yellow solid. mp 77–78 °C. ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J = 7.0 Hz, 3H), 1.69–2.05 (m, 22H), 2.53 (t, J = 12.0 Hz, 1H), 1.89 (s, 2H), 3.40 (s, 2H), 4.18 (q, J = 7.0 Hz, 2H), 7.16 (d, J = 8.0 Hz, 2H), 7.24 (d, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 14.3, 26.5, 26.9, 31.5, 34.7, 42.6, 50.2, 53.0, 60.7, 108.4, 111.4, 126.9, 128.4, 137.4, 145.1, 172.5. **Step 2.** A mixture of **33** (340 mg, 0.75 mmol) and 1 M aq. NaOH (1 mL) in THF (20 mL) was stirred at rt for 12 h. After removal of the solvents in vacuo, the residue was suspended in H_2O (20 mL) and acidified with AcOH (0.2 mL). The resultant precipitate was filtered, washed with water and dried in vacuo at 50 °C to afford *cis*-adamantane-2-spiro-3'-8'-[4-[[(carboxymethyl)amino]methyl]phenyl]-1',2',4'-trioxaspiro[4.5]decane (290 mg, 90%) as a white solid. To a suspension of *cis*-adamantane-2-spiro-3'-8'-[4-[[(carboxymethyl)amino]methyl]phenyl]-1',2',4'-trioxaspiro[4.5]decane (230 mg, 0.54 mmol) in Et_2O (10 mL) was added methanesulfonic acid (150 mg, 1.56 mmol) in Et_2O (2 mL) and the

resulting mixture was stirred at rt for 1 h. The precipitate was then filtered, washed with Et_2O and dried in vacuo at 50 °C to afford **17** as a white solid (240 mg, 85%). mp 142–143 °C. ^{1}H NMR (DMSO-d_6) δ 1.55–1.93 (m, 22H), 2.30 (s, 3H), 2.65 (t, J = 12.0 Hz, 1H), 3.85 (s, 2H), 4.12 (s, 2H), 7.29 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 9.24 (br, 2H), 13.79 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 26.3, 26.7, 31.4, 34.5, 34.7, 36.3, 36.6, 41.7, 46.9, 50.2, 108.5, 111.1, 127.4, 129.7, 130.7, 147.5, 168.4. Anal. calcd for $C_{26}H_{37}NO_8S$: C, 59.64; H, 7.12; N, 2.67. Found: C, 59.26; H, 6.90; N, 2.41.

***cis*-Adamantane-2-spiro-3'-8'-[4-((3-(carbonyl)azetidin-1-yl)methyl)phenyl]-1',2',4'-trioxaspiro[4.5]decane mesylate (18). Step 1.** A mixture of **32** (0.22 g, 0.57 mmol), ethyl azetidine-3-carboxylate hydrochloride (1.02 g, 6.17 mmol) and DIPEA (0.99 g, 7.65 mmol) in DMA (15 mL) was stirred at 50 °C for 5 h. Then reaction mixture was cooled to rt and diluted with EA (100 mL), washed successively with H_2O (50 mL), 1 M HCl (15 mL) and brine (2 x 50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The obtained residue was dried in vacuo at 50 °C to afford *cis*-adamantane-2-spiro-3'-8'-[4-((3-(ethoxycarbonyl)azetidin-1-yl)methyl)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**34**) (270 mg, 99%) as a white solid. mp 144–145 °C. ^{1}H NMR ($CDCl_3$) δ 1.64–1.76 (m, 8H), 1.76–1.86 (m, 6H), 1.92 (d, J = 12.9 Hz, 2H), 1.96–2.05 (m, 6H), 2.51 (tt, J = 12.0, 3.2 Hz, 1H), 3.34 (d, J = 3.8 Hz, 2H), 3.60 (d, J = 11.2 Hz, 4H), 4.14 (qd, J = 7.1, 1.8 Hz, 2H), 7.14 (t, J = 4.7 Hz, 2H), 7.19 (d, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 26.5, 26.9, 31.4, 33.9, 34.7, 34.8, 36.4, 36.8, 42.6, 56.6, 60.9, 62.7, 108.4, 111.4, 126.9, 128.7, 134.6, 145.4, 172.9. **Step 2.** A mixture of **34** (270 mg, 0.56 mmol) and 1 M aq. NaOH (2 mL) in THF (15 mL) was stirred at rt for 12 h, and then concentrated in vacuo. The residue was diluted with H_2O (20 mL) and acidified with AcOH to pH 5. The precipitate was filtered, washed with water and dried in vacuo at 50 °C to afford *cis*-

adamantane-2-spiro-3'-8'-[4-((3-(carbonyl)azetidin-1-yl)methyl)phenyl]-1',2',4'-trioxaspiro[4.5]decane (250 mg, 98%) as a white solid. To a stirred solution of *cis*-adamantane-2-spiro-3'-8'-[4-((3-(carbonyl)azetidin-1-yl)methyl)phenyl]-1',2',4'-trioxaspiro[4.5]decane (250 mg, 0.55 mmol) in EA (10 mL) was added dropwise a solution of methanesulfonic acid (106 mg, 1.10 mmol) in EA (2 mL). The resulting mixture was stirred at rt for 1 h. The precipitate was filtered, washed with Et_2O and dried in vacuo at 50 °C to afford **18** as a white solid (262 mg, 86%). mp 151–152 °C. ^{1}H NMR (DMSO-d_6) δ 1.57 (qd, J = 13.0, 4.1 Hz, 2H), 1.62–1.96 (m, 20H), 2.34 (s, 3H), 2.64 (tt, J = 12.2, 3.5 Hz, 1H), 3.62 (q, J = 9.1 Hz, 1H), 4.08–4.28 (m, 4H), 4.34 (d, J = 17.8 Hz, 2H), 7.29 (d, J = 7.8 Hz, 2H), 7.39 (d, J = 7.9 Hz, 2H), 10.16 (s, 1H), 13.12 (brs, 1H); ^{13}C NMR (DMSO-d_6) δ 26.3, 26.7, 31.4, 32.5, 34.5, 34.7, 36.3, 36.6, 41.7, 55.2, 55.5, 57.3, 108.5, 111.1, 127.7, 128.6, 130.6, 147.7, 171.8. Anal. calcd for $C_{28}H_{39}NO_8S$: C, 61.18; H, 7.15; N, 2.55. Found: C, 61.00; H, 7.30; N, 2.37.

***cis*-6,6-Difluoroadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (19). Step 1.** A mixture of 4-(4-hydroxyphenyl)cyclohexanone (**45**) (5.71 g, 30 mmol), ethyl bromoacetate (6.01 g, 36 mmol) and K_2CO_3 (8.29 g, 60 mmol) in acetone (150 mL) was stirred at reflux for 12 h. The solid was removed by filtration and the filtrate was concentrated *in vacuo* to afford ethyl 2-(4-(4-oxocyclehexyl)phenoxyl)acetate (**46**) as a light yellow oil (8.29 g, 100%). ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7.0 Hz, 3H), 1.90 (dt, J_1 = 12.5 Hz, J_2 = 6.0 Hz, 2H), 2.19–2.22 (m, 2H), 2.48–2.53 (m, 4H), 2.98 (tt, J_1 = 9.0 Hz, J_2 = 3.0 Hz, 1H), 4.27 (q, J = 7.0 Hz, 2H), 4.60 (s, 2H), 6.87 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 34.1, 41.4, 41.9,

61.4, 65.6, 114.8, 127.7, 138.0, 156.5, 169.0, 211.1. **Step 2. 46** (0.51 g, 1.86 mmol) and 6,6-difluoroadamantan-2-one *O*-methyl oxime (**47**)[28] (0.40 g, 1.86 mmol) in cyclohexane (100 mL) and DCM (40 mL) was treated with ozone according to the method of Dong et al.[41] After removal of the solvents in vacuo, the residue was recrystallized from MeOH to afford *cis*-6,6-difluoroadamantane-2-spiro-3'-8'-[4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**48**) (0.13 g, 15%) as a white solid. mp 112–113 °C. ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J = 7.0 Hz, 3H), 1.68 (m, 2H), 1.79–2.06 (m, 18H), 2.50 (t, J = 12.5 Hz, 1H), 4.27 (q, J = 7.0 Hz, 3H), 4.59 (s, 2H), 6.83 (d, J = 8.5 Hz, 2H), 7.13 (d, J = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 30.7 (t, ^3J = 3.5 Hz), 30.9 (t, ^3J = 3.5 Hz), 34.2 (t, ^2J = 34.3 Hz), 34.4, 34.7, 42.2, 61.3, 65.6, 108.9, 109.5, 114.6, 124.0 (^1J = 121.7 Hz), 127.8, 139.2, 156.3, 169.0. **Step 3.** A mixture of **48** (120 mg, 0.25 mmol) and 15% aq. KOH (0.5 mL) in THF (10 mL) was stirred at rt for 12 h. Removal of the solvents *in vacuo* gave a white residue which was suspended in H_2O (10 mL) and acidified with AcOH (0.5 mL). The precipitate was filtered, washed with water and dried in vacuo at 50 °C to afford **19** (105 mg, 93%) as a white solid. mp 155–156 °C. ^{1}H NMR (CD_3OD) δ 1.65 (qd, J_1 = 13.0 Hz, J_2 = 3.0 Hz, 2H), 1.81–2.10 (m, 18H), 2.56 (t, J = 12.0 Hz, 1H), 4.48 (s, 2H), 6.86 (d, J = 8.5 Hz, 2H), 7.13 (d, J = 8.5 Hz, 2H); ^{13}C NMR (CD_3OD) δ 30.36 (t, ^3J = 3.9 Hz), 30.42 (t, ^3J = 3.9 Hz), 31.4, 34.2, 34.3 (t, ^2J = 21.9 Hz), 34.7 (t, ^3J = 21.9 Hz), 34.8, 41.8, 53.4, 66.0, 108.77, 108.80, 114.2, 123.7 (t, ^1J = 150.8 Hz), 127.1, 138.6, 156.9, 173.6. Anal. calcd for $C_{24}H_{28}F_2O_6$: C, 63.99; H, 6.27. Found: C, 63.81; H, 6.07.

***cis*-6-Oxoadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (20). Step 1.** To a solution of adamantane-2,6-dione mono ethylene ketal (**49**)[32] (3.90 g, 18.72 mmol) in dry EtOH (100 mL) was added pyridine (2.45 g, 30.90 mmol) followed by methoxyamine hydrochloride (2.40 g, 28.09 mmol). The reaction mixture was stirred at rt for 12 h, concentrated *in vacuo*, and partitioned between DCM (100 mL) and H_2O (50 mL). The organic phase was separated, and the aq. layer was extracted with DCM (3 x 30 mL). The organic layers were combined and washed with 1 M HCl (30 mL), sat. aq. $NaHCO_3$ (30 mL) and brine (30 mL), dried over $MgSO_4$ and concentrated to afford adamantane-2,6-dione mono ethylene ketal *O*-methyl oxime (**50**) (4.27 g, 96%) as a white solid. mp 90–91 °C. ^{1}H NMR ($CDCl_3$) δ 1.74 (d, J = 12.5 Hz, 2H), 1.80 (d, J = 12.5 Hz, 2H), 1.91 (s, 2H), 2.47 (s, 2H), 3.41 (s, 2H), 3.82 (s, 3H), 3.99 (s, 4H); ^{13}C NMR ($CDCl_3$): δ 27.9, 34.5, 34.7, 35.8, 36.3, 61.0, 64.4, 109.8, 165.2. **Step 2.** A mixture of **50** (4.24 g, 17.91 mmol) and 4-(4-oxocyclohexyl)phenyl acetate (**51**) (4.16 g, 17.91 mmol) in cyclohexane (450 mL) and DCM (150 mL) was treated with ozone according to the method of Dong et al.[42] After solvent removal in vacuo, the residue was recrystallized from EtOH to afford *cis*-6-oxoadamantane-2-spiro-3'-8'-(4-acetoxyphenyl)-1',2',4'-trioxaspiro[4.5]decane ethylene ketal (**52**) (3.35 g, 43%) as a white solid. mp 156–157 °C. ^{1}H NMR ($CDCl_3$) δ 1.66 – 2.05 (m, 20H), 2.28 (s, 3H), 2.54 (t, J = 12.0 Hz, 1H), 3.94 (s, 4H), 6.99 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 21.1, 31.5, 31.6, 34.6, 34.8, 35.1, 35.3, 42.4, 64.3, 108.5, 109.9, 110.3, 121.4, 127.7, 143.6, 148.9, 169.7. **Step 3.** A mixture of **52** (3.35 g, 7.34 mmol), 1 M NaOH (10 mL) and THF (100 mL) was stirred at rt overnight, then concentrated *in vacuo*. The residue was suspended in H_2O (30 mL) and acidified with AcOH (2 mL). The resulting precipitate was filtered, washed with water, and dried in vacuo to afford *cis*-6-oxoadamantane-2-spiro-3'-8'-(4-hydroxyphenyl)-1',2',4'-trioxaspiro[4.5]decane

ethylene ketal (2.97 g, 98%) as a white solid. mp 164–165 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.64 – 2.04 (m, 20H), 2.48 (t, J = 12.0 Hz, 1H), 3.95 (s, 4H), 4.98 (brs, 1H), 6.79 (d, J = 8.5 Hz, 2H), 7.10 (d, J = 8.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 31.6, 34.7, 34.8, 35.1, 35.3, 42.0, 64.3, 108.6, 110.0, 110.2, 115.2, 127.8, 138.4, 153.9. **Step 4.** A suspension of *cis*-6-oxoadamantane-2-spiro-3'-8'-(4-hydroxyphenyl)-1',2',4'-trioxaspiro[4.5]decane ethylene ketal (3.10 g, 7.48 mmol) in 1 M methanesulfonic acid in 5:1 acetone:H_2O (42 mL) was stirred at rt for 12 h. The resulting solid was filtered, washed with water, and dried in vacuo to afford *cis*-6-oxoadamantane-2-spiro-3'-8'-(4-hydroxyphenyl)-1',2',4'-trioxaspiro[4.5]decane (**53**) (2.52 g, 91%) as a white solid. mp 187–188 °C. ^{1}H NMR (DMSO-d_6) δ 1.52 (q, J = 12.0 Hz, 2H), 1.75 (d, J = 11.5 Hz, 2H), 1.86 (d, J = 12.0 Hz, 2H), 1.95 (s, 4H), 2.07 (s, 4H), 2.18 (d, J = 12.0 Hz, 2H), 2.33 (d, J = 16.0 Hz, 2H), 2.51 (s, 1H), 6.66 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 8.5 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 31.8, 34.5, 35.50, 35.53, 35.7, 41.2, 44.5, 45.0, 109.2, 109.4, 115.5, 127.8, 136.5, 156.0, 214.6. **Step 5.** A mixture **53** (600 mg, 1.62 mmol), ethyl bromoacetate (325 mg, 1.94 mmol) and potassium carbonate (447 mg, 3.24 mmol) in acetone (30 mL) was stirred at 55 °C for 12 h. After filtering and washing with acetone, the filtrate was concentrated *in vacuo* to afford *cis*-6-oxoadamantane-2-spiro-3'-8'-[4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**54**) as a white solid (740 mg, 100%). mp 138–139 °C. ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7.0 Hz, 3H), 1.68–1.75 (m, 2H), 1.84–1.90 (m, 4H), 1.92 (d, J = 13.0 Hz, 2H), 1.99 (d, J = 13.0 Hz, 2H), 2.07 (d, J = 13.0 Hz, 2H), 2.15 (s, 2H), 2.27 (d, J = 13.0 Hz, 2H), 2.35 (d, J = 12.5 Hz, 2H), 2.48 (d, J = 16.0 Hz, 2H), 2.52 (t, J = 12.0 Hz, 2H), 4.27 (q, J = 7.0 Hz, 2H), 4.60 (s, 2H), 6.84 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 31.5, 34.6, 35.68, 35.74, 35.9, 42.0, 44.7, 45.2, 61.3, 65.6, 109.0, 109.3, 114.7, 127.7, 139.2, 156.3, 169.1, 215.9. **Step 6.** A mixture of **54** (230 mg, 0.50 mmol) and 1 M aq. NaOH (2 mL) in THF

(15 mL) was stirred at rt for 12 h. Removal of the solvents *in vacuo* gave a white residue which was suspended in H_2O (10 mL) and acidified with AcOH (0.5 mL). The precipitate was filtered, washed with water and dried in vacuo at 50 °C to afford **20** (205 mg, 95%) as a white solid. mp 171–172 °C. ^{1}H NMR (CD_3OD) δ 1.67–1.75 (m, 2H), 1.85–1.90 (m, 4H), 1.94 (d, J= 13.0 Hz, 2H), 1.99 (d, J= 13.0 Hz, 2H), 2.07 (d, J= 12.5 Hz, 2H), 2.15 (s, 2H), 2.27 (d, J= 13.0 Hz, 2H), 2.35 (d, J= 12.0 Hz, 2H), 2.49 (d, J= 16.5 Hz, 2H), 2.53 (t, J= 12.5 Hz, 1H), 4.66 (s, 2H), 6.86 (d, J= 8.5 Hz, 2H), 7.15 (d, J= 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 31.5, 34.6, 35.7, 35.8, 35.9, 42.0, 45.1, 65.0, 109.1, 109.2, 114.7, 127.9, 139.7, 155.8, 172.8, 216.3. Anal. calcd for $C_{24}H_{28}O_7$: C, 67.28; H, 6.59. Found: C, 67.54; H, 6.36.

***cis*-6-Hydroxyadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (21). Step 1.** To a solution of **53** (100 mg, 0.27 mmol) in methanol (10 mL) at 0 °C was added $NaBH_4$ (17 mg, 0.68 mmol). The resulting mixture was stirred at 0 °C for 2 h and then quenched with H_2O (1 mL). After solvent removal in vacuo, the residue was extracted in EA (20 mL), washed with saturated $NaHCO_3$ (10 mL), water (10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to afford *cis*-6-hydroxyadamantane-2-spiro-3'-8'-(4-hydroxyphenyl)-1',2',4'-trioxaspiro[4.5]decane (**55**) (99 mg, 99%) as a white solid. mp 159–160 °C. ^{1}H NMR (DMSO-d_6) δ 1.46–2.10 (m, 20H), 2.48 (t, J= 12.0 Hz, 1H), 5.59 (d, J= 2.0 Hz, 1H), 4.67 (d, J= 3.0 Hz, 1H), 6.66 (d, J= 8.5 Hz, 2H), 6.98 (d, J= 8.5 Hz, 2H), 9.13 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 28.5, 31.8, 32.9, 33.3, 34.61, 34.63, 35.2, 35.9, 41.2, 71.5, 108.6, 111.0, 115.5, 127.8, 136.6, 156.0. **Step 2.** A mixture of **55** (90 mg, 0.24 mmol), ethyl bromoacetate (48 mg, 0.29 mmol) and potassium carbonate (67 mg, 0.48 mmol) in acetone (10 mL) was stirred at 55 °C for 12 h. The solid was filtered and washed with acetone and the filtrate was concentrated *in vacuo* to afford *cis*-6-hydroxyadamantane-2-spiro-3'-8'-[4-(2-

ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**56**) as a white solid (110 mg, 100%). mp 138–139 °C. ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J = 7.0 Hz, 3H), 1.62–2.14 (m, 20H), 2.49 (t, J = 12.0 Hz, 2H), 3.81 (s, 1H), 4.26 (q, J = 7.0 Hz, 2H), 4.58 (s, 2H), 6.82 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 28.2, 31.6, 32.9, 33.29, 33.32, 34.7, 35.1, 35.8, 42.0, 61.3, 65.6, 73.2, 108.6, 110.8, 114.6, 127.7, 139.4, 156.3, 169.1. **Step 3.** A mixture of **56** (190 mg, 0.41 mmol) and 1 M aq. NaOH (2 mL) in THF (15 mL) was stirred at rt for 12 h. Removal of the solvents *in vacuo* gave a white residue which was suspended in H_2O (10 mL) and acidified with AcOH (0.5 mL). The precipitate was filtered, washed with water and dried in vacuo at 50 °C to afford **21** (170 mg, 95%) as a white solid. mp 153–154 °C. ^{1}H NMR (DMSO-d_6) δ 1.46–1.92 (m, 18H), 2.02 (d, J = 11.5 Hz, 1H), 2.08 (d, J = 11.0 Hz, 1H), 2.56 (t, J = 12.0 Hz, 1H), 2.58 (s, 1H), 4.60 (s, 2H), 4.66 (br, 1H), 6.79 (d, J = 8.5 Hz, 2H), 7.10 (d, J = 8.5 Hz, 2H), 12.96 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 28.5, 31.8, 32.9, 33.3, 34.6, 35.2, 35.9, 41.2, 65.0, 71.5, 108.6, 111.0, 114.8, 127.89, 138.9, 156.5, 170.8. Anal. calcd for $C_{24}H_{30}O_7$: C, 66.96; H, 7.02. Found: C, 66.96; H, 6.89.

***cis*-6-Acetamidoadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (22). Step 1.** To a solution of **54** (1.02 g, 2.23 mmol) in MeOH (50 mL) were added ammonium acetate (2.27 g, 26.81 mmol) and acetic acid (0.5 mL). The resulting mixture was stirred at rt for 0.5 h before addition of sodium cyanoborohydride (0.58 g, 8.94 mmol). The reaction mixture was stirred at rt for 12 h and then quenched with 1 M aq. NaOH (10 mL). After solvent removal in vacuo, the residue was extracted with EA (100 mL), and washed with water (30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to afford *cis*-6-aminoadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane as a grey residue (1.02 g, 100%), which was used as starting material for

next step without purification. **Step 2.** *Cis*-6-aminoadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (220 mg, 0.48 mmol) was treated with acetyl chloride (57 mg, 0.72 mmol) and pyridine (76 mg, 0.96 mmol) in DCM (10 mL) at 0 °C. After stirring at rt for 6 h, the reaction mixture was quenched with H_2O (1 mL), diluted with DCM (10 mL), washed successively with 1 M HCl (5 mL), H_2O (5 mL) and brine (5 min) and then dried over $MgSO_4$. After filtration and solvent removal in vacuo, the residue was purified by chromatography (sg, 10:1 DCM:MeOH) to afford *cis*-6-acetamidoadamantane-2-spiro-3'-8'-[4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**57**) (200 mg, 83%) as a white solid. mp 89–90 °C. ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J = 7.0 Hz, 2H), 1.69–2.10 (m, 23H), 2.49 (t, J = 12.0 Hz, 1H), 3.97 (d, J = 7.0 Hz, 1H), 4.26 (q, J = 7.0 Hz, 2H), 4.58 (s, 2H), 5.77 (d, J = 7.0 Hz, 1H), 6.83 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 23.7, 29.1, 29.2, 30.2, 30.5, 31.5, 31.6, 34.0, 34.6, 34.7, 35.3, 35.5, 42.0, 52.4, 61.3, 65.6, 108.8, 110.5, 114.6, 127.7, 139.3, 156.3, 169.1, 169.5. **Step 3.** A mixture of **57** (170 mg, 0.34 mmol) and 1 M aq. NaOH (1 mL) in THF (10 mL) was stirred at rt for 12 h. After solvent removal in vacuo, the resulting residue was suspended in H_2O (10 mL) and acidified with AcOH (0.5 mL). The precipitate was filtered, washed with water and dried in vacuo at 50 °C to afford **22** (150 mg, 94%) as a white solid. mp 163–164 °C. ^{1}H NMR (DMSO-d_6) δ 1.51–2.02 (m, 26H), 2.55 (t, J = 12.0 Hz, 1H), 3.75 (d, J = 6.0 Hz, 1H), 4.56 (s, 2H), 6.79 (d, J = 8.5 Hz, 2H), 7.10 (d, J = 8.5 Hz, 2H), 7.81 (d, J = 7.0 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 23.2, 28.8, 28.9, 30.3, 30.7, 31.7, 34.2, 34.5, 34.6, 35.4, 35.5, 41.2, 52.4, 65.4, 108.7, 110.7, 114.7, 127.8, 138.7, 156.7, 169.2, 170.9. Anal. calcd for $C_{26}H_{33}NO_7$: C, 66.23; H, 7.05; N, 2.97. Found: C, 66.44; H, 6.90; N, 2.73.

***cis*-6-Aminoadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane mesylate (23). Step 1.** *cis*-6-Aminoadamantane-2-spiro-3'-8'-[4-

(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (700 mg, 1.53 mmol) was treated with $(Boc)_2O$ (667 mg, 3.07 mmol) and DIPEA (793 mg, 6.13 mmol) in DCM (20 mL) at 0 °C. The reaction mixture was then stirred at rt overnight, diluted with DCM (30 mL), washed successively with 1 M HCl (10 mL), saturated $NaHCO_3$ (10 mL) and brine (20 min), dried over $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (sg, hexane:EA 5:1) to afford *cis*-6-((tert-butoxycarbonyl)amino)adamantane-2-spiro-3'-8'-[4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**58**) (590 mg, 69%) as a white solid. mp 89–90 °C. ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J = 7.0 Hz, 3H), 1.45 (s, 9H), 1.63–2.09 (m, 20H), 2.49 (t, J = 12.0 Hz, 1H), 3.67 (s, 1H), 4.26 (q, J = 7.0 Hz, 2H), 4.56 (s, 2H), 4.86 (s, 1H), 6.82 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.1, 28.4, 28.9, 29.0, 30.6, 30.9, 31.49, 31.53, 34.0, 34.1, 34.6, 35.3, 35.5, 42.0, 53.5, 61.3, 65.6, 79.2, 108.6, 110.6, 114.6, 127.7, 139.3, 155.2, 156.2, 169.0. **Step 2.** A mixture of **58** (250 mg, 0.75 mmol) and 1 M aq. NaOH (1 mL) in THF (10 mL) was stirred at rt for 12 h. After solvent removal in vacuo, the residue was suspended in H_2O (20 mL) and acidified with AcOH (0.2 mL). The precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford *cis*-6-((tert-butoxycarbonyl)amino) adamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane as a white solid, which was then treated with 1 M methanesulfonic acid in EtOAc (6 mL) at rt for 12 h. The precipitate was filtered, washed with EA and dried in vacuo at 50 °C to afford **23** as a white solid (232 mg, 98% over two steps). mp 182–183 °C. ^{1}H NMR (DMSO-d_6) δ 1.52 (q, J = 12.5 Hz, 2H), 1.65 (d, J = 13.5 Hz, 1H), 1.75–2.04 (m, 17H), 2.34 (s, 3H), 2.55 (t, J = 12.0 Hz, 1H), 3.30 (s, 1H), 4.61 (s, 2H), 6.81 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 7.95 (s, 3H), 12.92 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 27.28, 27.33, 28.8, 29.2, 31.7, 33.57, 33.61, 34.5, 34.9, 35.1, 40.2,

41.1, 53.9, 64.9, 109.0, 110.0, 114.8, 127.9, 138.8, 156.5, 170.8. Anal. calcd for $C_{25}H_{35}NO_9S$: C, 57.13; H, 6.71; N, 2.66. Found: C, 56.95; H, 6.59; N, 2.57.

***cis*-6-Azaadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane mesylate (24). Step 1.** To a solution of 2-Boc-2-azaadamantan-6-one (**59**)[33] (4.00 g, 15.91 mmol) in dry EtOH (100 mL) was added pyridine (2.08 g, 26.25 mmol) followed by methoxyamine hydrochloride (1.99 g, 23.87 mmol). The resulting mixture was stirred at rt for 12 h and then concentrated in vacuo. The residue was partitioned between DCM (100 mL) and H_2O (50 mL). The organic phase was separated, and the aqueous layer was extracted with DCM (3 x 30 mL). The combined organic layers were successively washed with 1 M HCl (30 mL), saturated $NaHCO_3$ (30 mL) and brine (30 mL). After drying over $MgSO_4$, the solvent was removed *in vacuo* to afford 2-Boc-2-azaadamantan-6-one *O*-methyl oxime (**60**) (4.41 g, 99%) as a white solid. mp 90–91 °C. ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 10H), 1.78 (t, J = 10.2 Hz, 1H), 1.86 (t, J = 9.6 Hz, 1H), 1.96 (t, J = 13.5 Hz, 1H), 2.05 (t, J = 13.5 Hz, 1H), 2.69 (s, 1H), 3.61 (s, 1H), 3.83 (s, 3H), 4.30 (s, 1H), 4.42 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 27.7, 28.5, 34.6, 35.7, 36.0, 37.0, 37.4, 45.6, 47.1, 61.1, 79.5, 154.2, 163.8. **Step 2. 60** (841 mg, 3.0 mmol) and ethyl 4-(4-oxocyclohexyl)phenyl acetate (**51**) (697 mg, 3.0 mmol) in cyclohexane (120 mL) and DCM (20 mL) was treated with ozone according to the method of Dong et al.[42] After solvent removal in vacuo, the residue was recrystallized from MeOH to afford *cis*-6-Boc-6-azaadamantane-2-spiro-3'-8'-(4-acetoxyphenyl)-1',2',4'-trioxaspiro[4.5]decane (**61**) as

a white solid (541 mg, 36%). mp 162–163 °C. ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9H), 2.09–1.66 (m, 16H), 2.15 (s, 2H), 2.28 (s, 3H), 2.56 (t, *J* = 12.3 Hz, 1H), 4.14 (d, *J* = 11.2 Hz, 1H), 4.27 (d, *J* = 21.8 Hz, 1H), 7.00 (d, *J* = 8.5 Hz, 2H), 7.20 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.1, 28.5, 31.4, 33.2, 33.5, 34.6, 35.0, 42.3, 44.4, 44.8, 45.8, 46.2, 79.3, 108.9, 110.0, 121.4, 127.7, 143.5, 148.9, 154.2, 169.6. **Step 3.** A mixture of **61** (250 mg, 0.50 mmol) and 15% aq. KOH (1 mL) in THF (10 mL) was stirred at rt for 12 h, and then concentrated *in vacuo*. The residue was suspended in H_2O (20 mL) and acidified with AcOH to pH 4. The precipitate was filtered, washed with water and dried in vacuo to afford *cis*-6-Boc-6-azaadamantane-2-spiro-3'-8'-(4-hydroxyphenyl)-1',2',4'-trioxaspiro[4.5]decane (**62**) (225 mg, 98%) as a white solid. mp 176–177 °C. ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9H), 1.73–1.66 (m, 2H), 1.75–2.08 (m, 14H), 2.15 (s, 2H), 2.49 (t, *J* = 11.4 Hz, 1H), 4.15 (d, *J* = 11.5 Hz, 1H), 4.27 (d, *J* = 21.7 Hz, 1H), 5.56 (brs, 1H), 6.77 (d, *J* = 8.3 Hz, 2H), 7.06 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 28.5, 31.6, 33.2, 33.5, 34.7, 34.9, 42.0, 44.5, 44.9, 45.9, 46.3, 79.6, 109.1, 109.9, 115.2, 127.7, 138.0, 154.2, 154.3. **Step 4.** A mixture of **62** (225 mg, 0.49 mmol), ethyl bromoacetate (100 mg, 0.60 mmol) and potassium carbonate (104 mg, 0.74 mmol) in acetone (20 mL) was stirred at 60 °C for 12 h. A solid was filtered and washed with acetone before concentrating the filtrate *in vacuo* to afford *cis*-6-Boc-6-azaadamantane-2-spiro-3'-8'-[4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**63**) (266 mg, 100%) as a white solid. mp 139–140 °C. ^{1}H NMR ($CDCl_3$) δ 1.30 (t, *J* = 7.1 Hz, 2H), 1.47 (s, 9H), 1.61–2.08 (m, 16H), 2.15 (s, 2H), 2.51 (t, *J* = 11.8 Hz, 1H), 4.14 (d, *J* = 10.9 Hz,

1H), 4.25–4.30 (m, 3H), 4.59 (s, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 28.5, 31.5, 33.2, 33.5, 34.6, 35.0, 42.0, 44.4, 44.8, 45.8, 46.2, 61.3, 65.6, 79.3, 109.0, 110.0, 114.6, 127.7, 139.3, 154.2, 156.3, 169.1. **Step 5.** A mixture of **63** (265 mg, 0.49 mmol) and 15% aq. KOH (1 mL) in THF (10 mL) was stirred at rt for 12 h. Removal of the solvent *in vacuo* gave a white residue which was suspended in H_2O (10 mL) and acidified with AcOH to pH 3. The precipitate was filtered, washed with water and dried in vacuo at 50 °C to afford *cis*-6-Boc-6-azadamantane-2-spiro-3'-8'-carboxymethyl-1',2',4'-trioxaspiro[4.5]decane (**64**) (232 mg, 90%) as a white solid. mp 158–160 °C. ^{1}H NMR ($CDCl_3$) δ 1.46 (d, *J* = 1.7 Hz, 9H), 1.65 (q, *J* = 12.4 Hz, 2H), 1.73–2.06 (m, 14H), 2.13 (s, 2H), 2.47 (t, *J* = 12.2 Hz, 1H), 4.14 (d, *J* = 11.7 Hz, 1H), 4.26 (d, *J* = 28.8 Hz, 1H), 4.48 (s, 2H), 6.78 (d, *J* = 8.2 Hz, 2H), 7.06 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 28.5, 31.5, 33.2, 33.5, 34.6, 35.0, 41.9, 44.4, 44.8, 45.8, 46.2, 65.4, 79.5, 108.9, 109.9, 114.6, 127.8, 154.3, 155.9. **Step 6.** A mixture of **64** (232 mg, 0.45 mmol) and methanesulfonic acid (480 mg, 5.00 mmol) in THF (5 mL) was stirred at rt for 12 h. The precipitate was filtered and washed with Et_2O to afford **24** (230 mg, 100%) as a white solid. mp 183–184 °C. ^{1}H NMR (DMSO-d_6) δ 1.53 (q, *J* = 13.3, 2H), 1.71–2.18 (m, 16H), 2.37 (s, 3H), 2.57 (t, *J* = 12.1 Hz, 1H), 3.55 (s, 1H), 3.59 (s, 1H), 4.62 (s, 2H), 6.82 (d, *J* = 8.6 Hz, 2H), 7.12 (d, *J* = 8.7 Hz, 2H), 8.73 (brs, 2H), 12.95 (brs, 1H); ^{13}C NMR (DMSO-d_6) δ 30.8, 31.7, 33.2, 34.3, 41.0, 45.5, 45.9, 65.0, 108.7, 109.7, 114.8, 127.9, 138.7, 156.6, 170.8. Anal. calcd for $C_{24}H_{33}NO_9S$: C, 56.35; H, 6.50; N, 2.74. Found: C, 56.01; H, 6.70; N, 2.59.

***cis*-6-Azaadamantane-2-spiro-3'-8'-[4-[[(carboxymethyl)amino]methyl]phenyl]-1',2',4'-trioxaspiro[4.5]decane mesylate (25) Step 1. 60** (400 mg, 1.43 mmol) and 4-(4-(chloromethyl)phenyl)cyclohexan-1-one (**65**) (318 mg, 1.43 mmol) in cyclohexane (120 mL) and DCM (20 mL) was treated with ozone according to the method of Dong et al.[42] After solvent removal in vacuo, the residue was recrystallized from MeOH to afford *cis*-6-Boc-6-azaadamantane-2-spiro-3'-8'-[4-(chloromethyl)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**66**) as a white solid (182 mg, 26%). mp 157–158 °C. ^{1}H NMR ($CDCl_3$) δ 1.47 (d, J = 2.1 Hz, 9H); 1.66– 2.09 (m, 20H), 2.15 (s, 2H), 2.56 (t, J = 11.5 Hz, 1H), 4.15 (d, J = 10.8 Hz, 1H), 4.27 (d, J = 21.9 Hz, 1H), 4.56 (s, 2H), 7.20 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 7.9 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 28.5, 31.3, 33.2, 33.5, 34.9, 34.5, 42.5, 42.6, 44.3, 44.7, 45.8, 46.1, 46.2, 79.3, 108.8, 110.0, 127.1, 128.7, 135.4, 146.3, 154.2. **Step 2.** A mixture of **66** (182 mg, 0.37 mmol), glycine ethyl ester hydrochloride (622 mg, 9.32 mmol) and DIPEA (1.50, 11.65 mmol) in DMA (20 mL) was stirred at 50 °C for 5 h. Then reaction mixture was cooled to rt, diluted with EA (100 mL), and washed successively with H_2O (3 x 50 mL) and brine (50 mL). The organic layer was separated and dried over $MgSO_4$, filtered and concentrated and dried in vacuo at 50 °C to afford *cis*-6-Boc-6-azaadamantane-2-spiro-3'-8'-[4-[[(2-ethoxy-2-oxoethyl)amino]methyl]phenyl]-1',2',4'-trioxaspiro[4.5]decane (**67**) (200 mg, 97%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J = 7.0 Hz, 3H); 1.47 (d, J = 2.2 Hz, 9H), 1.66–2.07 (m, 16H), 2.16 (s, 2H), 2.55 (t, J = 11.8 Hz, 1H), 3.41 (s, 2H), 3.77 (s, 2H), 4.15 (d, J = 10.8 Hz, 1H), 4.19 (q, J = 7.1 Hz, 2H), 4.28 (d, J =

21.2 Hz, 1H), 7.17 (d, *J* = 8.2 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 28.5, 31.4, 33.2, 33.5, 34.6, 35.0, 42.5, 44.4, 44.8, 45.8, 46.2, 50.1, 53.0, 60.8, 79.3, 109.0, 110.0, 126.8, 128.4, 137.3, 144.9, 154.2, 172.3. **Step 3.** A mixture of **67** (200 mg, 0.36 mmol) and 1 M aq. NaOH (1 mL) in THF (10 mL) was stirred at rt for 12 h and then concentrated in vacuo. The residue was suspended in H_2O (10 mL) and acidified with AcOH to pH 3. The precipitate was filtered, washed with water and dried in vacuo at 50 °C to afford *cis*-6-Boc-6-azaadamantane-2-spiro-3'-8'-[4-[[(carboxymethyl)amino]methyl]phenyl]-1',2',4'-trioxaspiro[4.5]decane, which was treated with methanesulfonic acid (480 mg, 5 mmol) in THF (5 mL). The resulting mixture was stirred at rt for 12 h before addition of Et_2O (10 mL). The supernatant was removed and to the oily residue was added Et_2O (20 mL). The resulting mixture was stirred at rt for 12 h. The precipitate was filtered and washed with Et_2O to afford **25** (183 mg, 82%) as a white solid. mp 174–175 °C. ^{1}H NMR (DMSO-d_6) δ 1.58 (qd, *J* = 13.1, 3.9 Hz, 2H); 1.76–2.18 (m, 16H), 2.34 (s, 6H), 2.67 (tt, *J* = 12.4, 3.2 Hz, 1H), 3.55 (s, 1H), 3.59 (s, 1H), 3.85 (s, 2H), 4.13 (s, 2H), 7.30 (d, *J* = 7.9 Hz, 2H), 7.41 (d, *J* = 7.9 Hz, 2H), 8.73 (s, 2H), 9.26 (s, 2H), 13.77 (brs, 1H); ^{13}C NMR (DMSO-d_6) δ 30.77, 30.84, 31.4, 33.2, 34.2, 41.6, 45.5, 45.9, 46.9, 50.2, 108.7, 109.6, 127.4, 129.8, 130.7, 147.3, 168.4. Anal. calcd for $C_{26}H_{40}N_2O_{11}S$: C, 50.31; H, 6.50; N, 4.51. Found: C, 50.66; H, 6.58; N, 4.34. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{24}H_{33}N_2O_5$ 429.2389; Found 429.2397.

Polar Surface Area (PSA). PSA values were calculated using ChemAxon Instant JChem (ver 16.4).

Partition Coefficient. Partition coefficient values (Log D) of the test compounds were estimated by correlation of their chromatographic retention properties against the characteristics of a series of standard compounds with known partition coefficient values using gradient HPLC (modification of a method reported by Lombardo et al.[38]

Protein Binding. Protein binding values of the test compounds were estimated by correlation of their chromatographic retention properties on a human albumin column against the characteristics of a series of standard compounds with known protein binding values. The method employed is a gradient HPLC based derivation of the method developed by Valko et al.[40]

Kinetic Solubility. Compounds in DMSO (10 mg/mL) were diluted into either pH 6.5 phosphate buffer or 0.01 M HCl (approx. pH 2.0) with the final DMSO concentration being 1%. After 30 minutes, samples were then analysed via nephelometry to determine a solubility range.[39]

In Vitro Metabolic Stability. As fully described in Coteron et al.,[44] metabolic stability assays were performed by incubating test compounds in liver microsomes at 37 °C and 0.4 mg/mL protein concentration. The metabolic reaction was initiated by the addition of an NADPH-regenerating system and quenched at various time points over a 60 min incubation period by the addition of acetonitrile containing diazepam as internal standard. Control samples (containing no

NADPH) were included (and quenched at 2, 30 and 60 min) to monitor for potential degradation in the absence of cofactor.

Antischistosomal Screen. All animal experiments were performed in line with national guidelines (permit 2070). All animals were kept in polycarbonate cages under environmentally-controlled conditions (temperature: 25 °C, humidity: 70%, 12:12 h light/dark photocycle), had free access to tap water and rodent food and acclimatized for one week prior to infection. Cercariae of *S. mansoni* were obtained from infected *Biomphalaria glabrata*. As described by Lombardo et al.,[45] four-week-old female NMRI mice (Charles Rivers, Sulzfeld, Germany) were infected subcutaneously with approximately 100 *S. mansoni* cercariae. At 21 d or 49 d after infection, groups of four mice were treated with single oral doses of compounds in a 7% (v/v) Tween 80% and 3% (v/v) ethanol vehicle (10 mL/kg). Untreated mice (n = 8) served as controls. At 21 d post-treatment, animals were killed by the CO_2 method and dissected. Worms were removed by picking, then sexed and counted.

Cytotoxicity Screen. As fully described in Sanford et al.,[45] cell lines were maintained in D10 media. Once confluent, increasing concentrations of test compound (0 to 100 μM) were added and incubated for 24 h. Alamar blue (10 mM) was then added to each well and incubated for 4 h. A BioTek Synergy HT plate reader was then used to determine fluorescence.

Corresponding Author:

J.L.V.: Phone: 402.559.5362. E-mail: jvenners@unmc.edu

Present Addresses:

†For V.J.B.: Allexcell, Inc., 135 Wood Street, West Haven, CT 06516, United States.

†For S.K.: Organic Chemistry Division, School of Advanced Sciences, Vellore Institute of Technology, Vellore-632 014, India.

Notes: The authors declare no competing interests.

Acknowledgment

We acknowledge the U.S. National Institutes of Health (AI116723-01) and the European Research Council (ERC-2013-CoG 614739-A HERO) for financial support.

Supporting Information: The Supporting Information is available free of charge on the ACS Publications website at

^{1}H and $^{13}C\{^{1}H\}$ NMR spectra (500 MHz) of **5**-**10** and **13**-**25** and SMILES string computer readable identifiers for **2**-**25.**

Abbreviations Used: CL_{int}, in vitro intrinsic clearance; DCC, dicyclohexylcarbodiimide; EDC, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, Hydroxybenzotriazole; WBR, worm burden reduction.

References

(1) Eastman, R. T.; Fidock, D. A. Artemisinin-based combination therapies: A vital tool in efforts to eliminate malaria. *Nat. Rev. Microbiol.* **2009**, *7,* 864–874.

(2) Tu, Y. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. *Nat. Med.* **2011**, *17,* 1217–1220.

(3) Klayman, D. L. Qinghaosu (artemisinin): an antimalarial drug from China. *Science* **1985**, *228,* 1049–1055.

(4) Keiser, J.; Utzinger, J. Antimalarials in the treatment of schistosomiasis. *Curr. Pharm. Design* **2012**, *18,* 3531–3538.

(5) Zhang, X.-G.; Z., Li, G.-X.; Zhao, S.-S.; Xu, F.-L.; Wang, Y.-H.; Wang, W. A review of dihydroartemisinin as another gift from traditional Chinese medicine not only for malaria control but also for schistosomiasis control. *Parasitol. Res.* **2014**, *113,* 1769–1773.

(6) Saeed, M. E. M.; Krishna, S.; Greten, H. J.; Kremsner, P. G.; Efferth, T. Antischistosomal activity of artemisinin derivatives in vivo and in patients. *Pharmacol. Res.* **2016**, *110,* 216–226.

(7) Caffrey, C. R.; El-Sakkary, N.; Mäder, P.; Krieg, R.; Becker, K.; Schlitzer, M.; Drewry, D. H.; Vennerstrom, J. L.; Grevelding, C. G. Drug discovery and development for schistosomiasis. In Neglected Tropical Diseases: Drug Discovery and Development; Swinney, D.; Pollastri, M., Eds.; Wiley-VCH: Weinheim, Germany, **2019**, pp. 187–225.

(8) Bergquist, R.; Utzinger, J.; Keiser, J. Controlling schistosomiasis with praziquantel: How much longer without a viable alternative? *Infect. Dis. Poverty* **2017**, *6,* 74.

(9) Kaiser, M.; Wittlin, S.; Nehrbass-Stuedli, A.; Dong, Y.; Wang, X.; Hemphill, A.; Matile, H.; Brun, R.; Vennerstrom, J. L. Peroxide bond-dependent antiplasmodial specificity of artemisinin and OZ277 (RBx11160). *Antimicrob. Agents Chemother.* **2007**, *51,* 2991–2993.

(10) Meshnick, S. R. Artemisinin: mechanisms of action, resistance and toxicity. *Int. J. Parasitol*. **2002**, *32,* 1655−1660.

(11) Klonis, N.; Creek, D. J.; Tilley L. Iron and heme metabolism in *Plasmodium falciparum* and the mechanism of action of artemisinins. *Curr. Opin. Microbiol.* **2013**, *16,* 722–727.

(12) Creek, D. J.; Charman, W. N.; Chiu, F. C. K.; Prankerd, R. J.; Dong, Y.; Vennerstrom, J. L.; Charman, S. A. Relationship between antimalarial activity and haem alkylation for spiro- and dispiro-1,2,4-trioxolane antimalarials. *Antimicrob. Agents Chemother.* **2008**, *52,* 1291-1296.

(13) Meunier, B.; Robert, A. Heme as trigger and target for trioxane-containing antimalarial drugs. *Acc. Chem. Res.* **2010**, *43,* 1444–1451.

(14) Tilley, L.; Charman, S. A.; Vennerstrom, J. L. Semisynthetic artemisinin and synthetic peroxide antimalarials. In Neglected Diseases and Drug Discovery; Palmer, M. J., Wells, T. N. C., Eds.; Royal Society of Chemistry: Cambridge, U.K., **2012**; pp 33−64.

(15) Portela, J.; Boissier, J.; Gourbal, B.; Pradines, V.; Colliere V.; Coslédan, F.; Meunier, B.; Robert, A. Antischistosomal activity of trioxaquines: In vivo efficacy and mechanism of action on *Schistosoma mansoni*. *PLoS Negl. Trop. Dis.* **2012**, *6,* e1474.

(16) Thétiot-Laurent, S. A.-L.; Boissier, J.; Robert, A.; Meunier, B. Schistosomiasis chemotherapy. *Angew. Chem. Int. Ed.* **2013**, *52,* 7936¬–7956.

(17) Wang, J.; Zhang, C. J.; Chia, W. N.; Loh, C. C.; Li, Z.; Lee, Y. M.; He, Y.; Yuan, L. X.; Lim, T. K.; Liu, M.; Liew, C. X.; Lee, Y. Q.; Zhang, J.; Lu, N.; Lim, C. T.; Hua, Z.-C.; Liu, B.; Shen, H. M.; Tan, K. S.; Lin, Q. Haem-activated promiscuous targeting of artemisinin in *Plasmodium falciparum*. *Nat. Commun.* **2015**, *6,* 10111.

(18) Ismail, H. M.; Barton, V. E.; Panchana, M.; Charoensutthivarakul, S.; Biagini, G. A.; Ward, S. A.; O'Neill, P. M. A click chemistry-based proteomic approach reveals that 1,2,4-trioxolane and artemisinin antimalarials share a common protein alkylation profile. *Angew. Chem. Int. Ed. Engl.* **2016**, *55,* 6401–6405.

(19) Jourdan, J.; Matile, H.; Reift, E.; Biehlmaier, O.; Dong, Y.; Wang, X.; Mäser, P.; Vennerstrom, J. L.; Wittlin, S. Monoclonal antibodies that recognize the alkylation signature of

antimalarial ozonides OZ277 (arterolane) and OZ439 (artefenomel). *ACS Infect. Dis.* **2016**, *2,* 54–61.

(20) Jourdan, J.; Walz, A.; Matile, H.; Schmidt, A.; Wu, J.; Wang, X.; Dong, Y.; Vennerstrom, J. L.; Schmidt, R. S.; Wittlin, S.; Mäser, P. Stochastic protein alkylation by antimalarial peroxides. *ACS Infect. Dis.* **2019**, *5,* 2067–2075.

(21) Giannangelo, C.; Anderson, D.; Wang, X.; Vennerstrom, J. L.; Charman, S. A.; Creek, D. J. Ozonide antimalarials alkylate heme in the malaria parasite *Plasmodium falciparum*. *ACS Infect. Dis.* **2019**, *5,* 2076–2086.

(22) Xiao, S.-H.; Keiser, J.; Chollet, J.; Utzinger, J.; Dong, Y.; Vennerstrom. J. L.; Endriss, Y.; Tanner, M. In vitro and in vivo activities of synthetic trioxolanes against major human schistosome species. *Antimicrob. Agents Chemother.* **2007**, *51,* 1440–1445.

(23) Xue J.; Wang, X.; Dong, Y.; Vennerstrom, J. L.; Xiao, S.-H. Effect of ozonide OZ418 against *Schistosoma japonicum* harbored in mice. *Parasitol. Res.* **2014**, *113,* 3259–3266.

(24) Keiser, J.; Ingram, K.; Vargas, M.; Chollet, J.; Wang, X.; Dong, Y., Vennerstrom, J. L. In vivo activity of aryl ozonides against Schistosoma species. *Antimicrob. Agents Chemother.* **2012**, *56,* 1090–1092.

(25) Charman, S. A.; Arbe-Barnes, S.; Bathurst, I. C.; Brun, R.; Campbell, M.; Charman, W. N.; Chiu, F. C. K.; Chollet, J.; Craft, J. C.; Creek, D. J.; Dong, Y.; Matile, H.; Maurer, M.; Morizzi, J.; Nguyen, T.; Papastogiannidis, P.; Scheurer, C.; Shackleford, D. M.; Sriraghavan, K.; Stingelin, L.; Tang, Y.; Urwyler, H.; Wang, X.; White, K. L.; Wittlin, S.; Zhou, L.; Vennerstrom, J. L. Synthetic ozonide drug candidate OZ439 offers new hope for a single-dose cure of uncomplicated malaria. *Proc. Natl. Acad. Sci. U. S. A.* **2011,** *108*, 4400-4405.

(26) Leonidova, A.; Vargas, M.; Huwyler, J.; Keiser, J. Pharmacokinetics of the

antischistosomal lead ozonide OZ418 in uninfected mice determined by liquid chromatography-tandem mass spectrometry. *Antimicrob. Agents Chemother.* **2016**, *60,* 7364–7371.

(27) Tang, Y.; Dong, Y.; Karle, J. M.; DiTusa, C. A.; Vennerstrom, J. L. Synthesis of tetrasubstituted ozonides by the Griesbaum coozonolysis reaction: Diastereoselectivity and functional group transformations by post-ozonolysis reactions. *J. Org. Chem.* **2004**, *69,* 6470-6473.

(28) Dong, Y.; Wang, X.; Kamaraj, S.; Bulbule, V. J.; Chiu, F. C. K.; Chollet, J.; Dhanasekaran, M.; Hein, C. D.; Papastogiannidis, P.; Morizzi, J.; Shackleford, D. M.; Barker, H.; Ryan, E.; Scheurer, C.; Tang, Y.; Zhao, Q.; Zhou, L.; White, K. L.; Urwyler, H.; Charman, W. N.; Matile, H.; Wittlin, S.; Charman, S. A.; Vennerstrom, J. L. SAR of the antimalarial ozonide artefenomel (OZ439). *J. Med. Chem.* **2017**, *60,* 2654–2668.

(29) Chu, X.-J.; Bartkovitz, D.; Danho, W.; Swistok, J.; Cheung, A. W.-H.; Kurylko, G.; Rowan, K.; Yeon, M.; Franco, L.; Qi, L.; Chen, L.; Yagaloff, K. Discovery of 1-amino-4-phenylcyclohexane-1-carboxylic acid and its influence on agonist selectivity between human melanocortin-4 and -1 receptors in linear pentapeptides. *Bioorg. Med. Chem. Lett.* **2005**, *15,* 4910–4914.

(30) Griesbaum, K.; Liu, X.; Kassiaris, A.; Scherer, M. Ozonolyses of *O*-alkylated ketoximes in the presence of carbonyl groups: A facile access to ozonides. *Liebigs Ann./Recueil* **1997**, 1381–1390.

(31) Dong, Y.; Vennerstrom, J.L. Dispiro-1,2,4,5-tetraoxanes via ozonolysis of cycloalkanone *O*-methyl oximes: A comparison with the peroxidation of cycloalkanones in acetonitrile sulfuric acid media. *J. Org. Chem.* **1998**, *63,* 8582–8585.

(32) Ayres, F. D.; Khan, S. I.; Chapman, O. L.; Kaganove, S. N. Improvements in the synthesis of adamantane-2,6-dione and preparation of the novel adamantane-2,6-dione *mono*-ketal. *Tetrahedron Lett.* **1994**, *35,* 7151–7154.

(33) Wu, J.; Leas, D. A.; Dong, Y.; Wang, X.; Ezell, E. L.; Stack, D. E.; Vennerstrom, J. L. Synthesis of 2-azaadamantan-6-one: A missing isomer. *ACS Omega* **2018**, *3,* 11362–11367.

(34) Dong, Y.; Tang, Y.; Chollet, J.; Matile, H.; Wittlin, S.; Charman, S. A.; Charman, W. N.; Santo Tomas, J.; Scheurer, C.; Snyder, C.; Scorneaux, B.; Bajpai, S.; Alexander, S. A.; Wang, X.; Padmanilayam, M.; Cheruku, S. R.; Brun, R.; Vennerstrom, J. L. Effect of functional group polarity on the antimalarial activity of spiro and dispiro-1,2,4-trioxolanes. *Bioorg. Med. Chem.* **2006**, *14,* 6368–6382.

(35) Vennerstrom, J. L.; Dong, Y.; Chollet, J.; Matile, H.; Padmanilayam, M.; Tang, Y.; Charman, W. N. Spiro and dispiro 1,2,4–trioxolane antimalarials. U. S. Patent 6,825,230, Nov 30, 2004.

(36) Vennerstrom, J. L.; Dong, Y.; Charman, S. A.; Wittlin, S.; Chollet, J.; Wang, X.; Sriraghavan, K.; Zhou, L.; Matile, H.; Charman, W. N. Spiro and dispiro 1,2,4-trioxolane antimalarials. PCT WO 2009/091433 A2, Jul 23, 2009.

(37) Palm, K.; Stenberg, P.; Luthman, K.; Artursson, P. Polar molecular surface properties predict the intestinal absorption of drugs in humans. *Pharm. Res.* **1997**, *14,* 568–571.

(38) Lombardo, F.; Shalaeva, M. Y.; Tupper, K. A.; Gao, F. ElogD$_{oct}$: A tool for lipophilicity determination in drug discovery. 2. basic and neutral compounds. *J. Med. Chem.* **2001**, *44,* 2490–2497.

(39) Bevan, C. D.; Lloyd, R. S. A high-throughput screening method for the determination of aqueous drug solubility using laser nephelometry in microtiter plates. *Anal. Chem.* **2000**, *72,* 1781–1787.

(40) Valko, K.; Nunhuck, S.; Bevan, C.; Abraham, M. H.; Reynolds, D. P. Fast gradient HPLC method to determine compounds binding to human serum albumin. Relationships with octanol/water and immobilized artificial membrane lipophilicity. *J. Pharm. Sci.* **2003**, *92,* 2236–2248.

(41) Dong, Y.; Chollet, J.; Vargas, M.; Mansour, N. R.; Bickle, Q.; Alnouti, Y.; Huang, J.; Keiser, J.; Vennerstrom, J. L. Praziquantel analogs with activity against juvenile *Schistosoma mansoni*. *Bioorg. Med. Chem. Lett.* **2010**, *20,* 2481–2484.

(42) Dong, Y.; Chollet, J.; Matile, H.; Charman, S. A.; Chiu, F. C. K.; Charman, W. N.; Scorneaux, B.; Urwyler, H.; Santo Tomas, J.; Scheurer, C.; Snyder, C.; Dorn, A.; Wang, X.; Karle, J. M.; Tang, Y.; Wittlin, S.; Brun, R.; Vennerstrom, J. L. Spiro and dispiro-1,2,4-trioxolanes as antimalarial peroxides: Charting a workable SAR using simple prototypes. *J. Med. Chem.* **2005**, *48,* 4953−4961.

(43) Dumele, O.; Schreib, B.; Warzok, U.; Trapp, N.; Schalley, C. A.; Diederich, F. Halogen-bonded supramolecular capsules in the solid state, in solution, and in the gas phase. *Angew. Chem. Int. Ed.* **2017**, *56,* 1152–1157.

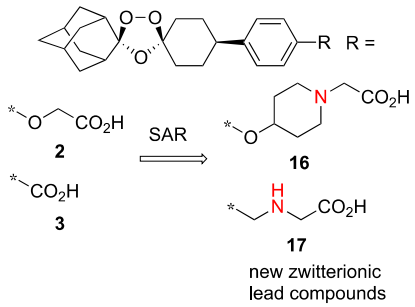
(44) Coteron, J. M.; Marco, M.; Esquivias, J.; Deng, X.; White, K. L.; White, J.; Koltun, M.; El Mazouni, F.; Kokkonda, S.; Katneni, K.; Bhamidipati, R.; Shackleford, D. M.; Angulo-Barturen, I.; Ferrer, S. B.; Jimenez-Diaz, M. B.; Gamo, F. J.; Goldsmith, E. J.; Charman, W. N.; Bathurst, I.; Floyd, D.; Matthews, D.; Burrows, J. N.; Rathod, P. K.; Charman, S. A.; Phillips, M. A., Structure-guided lead optimization of triazolopyrimidine-ring substituents identifies potent

Plasmodium falciparum dihydroorotate dehydrogenase inhibitors with clinical candidate potential. *J. Med. Chem.* **2011**, *54*, 5540–5561.

(45) Lombardo, F. C.; Pasche, V.; Panic, G.; Endriss, Y.; Keiser, J. Life cycle maintenance and drug-sensitivity assays for early drug discovery in *Schistosoma mansoni*. *Nat. Protoc.* **2019**, 14, 461–481.

(46) Sanford, A. G.; Schulze, T. T.; Potluri, L. P.; Hemsley, R. M.; Larson, J. J.; Judge, A. K.; Zach, S. J.; Wang, X.; Charman, S. A.; Vennerstrom, J. L.; and Davis, P. H. Novel *Toxoplasma gondii* inhibitor chemotypes. *Parasitol. Int.* **2018**, *67,* 107–111.

Table of Contents Graphic


R R =
CO2H
2
SAR
16
CO2H
3
H
N
CO2H
17
new zwitterionic
lead compounds