Novel Angular Benzophenazines: Dual Topoisomerase I and Topoisomerase II Inhibitors as Potential Anticancer Agents

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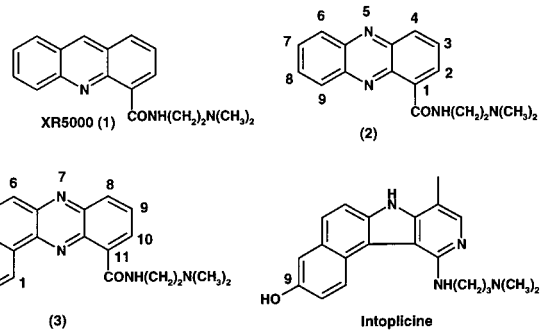
A series of substituted angular benzophenazines were prepared using a new synthetic route via a novel regiocontrolled condensation of 1,2-naphthoquinones and 2,3-diaminobenzoic acids. The synthesis and biological activity of this new series of substituted 8,9-benzo[*a*]phenazine carboxamide systems are described. The analogues were evaluated against the H69 parental human small cell lung carcinoma cell line and H69/LX4 resistant cell line which overexpresses P-glycoprotein. Selected analogues were evaluated against the COR-L23 parental human non small cell lung carcinoma cell line and the COR-L23/R resistant cell line which overexpresses multidrug resistance protein. This series of novel angular benzophenazines were potent cytotoxic agents in these cell lines and may be able to circumvent multidrug resistance mechanisms which result in the lack of efficacy of many drugs in cancer chemotherapy. These compounds show dual inhibition of topoisomerase I and topoisomerase II and thus target two key enzymes responsible for the topology of DNA that are active at different points in the cell cycle. The introduction of chirality into the carboxamide side chain of these novel benzophenazine carboxamides has resulted in the discovery of a potent enantiospecific series of cytotoxic agents, exemplified by 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-1-(*R*)-methyl-ethyl)-amide, XR11576 (**(R)-4j″**). In vivo activity has been demonstrated for 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-1-(*R*)-methyl-ethyl)-amide, XR11576, after intravenous administration to female mice, and this compound has been selected as a development candidate for further evaluation.

Introduction

The topoisomerases are essential enzymes in the regulation of DNA topology, which is required if cells are to divide and proliferate,[1] and are important cellular targets for a number of successful chemotherapeutic agents.[1] Drugs that target topoisomerase II, for example doxorubicin and etoposide, have been widely used in cancer chemotherapy,[2] while those that specifically target topoisomerase I, principally the camptothecin analogues, have made an important impact more recently for the treatment of colon cancer.[3] A shortfall of many of these specific inhibitors of either topoisomerase I or topoisomerase II is their inability to overcome multidrug resistance (MDR).[4,5] Several dual inhibitors of topoisomerase I and II have been identified; these include intoplicine,[6] XR5000 (DACA),[7] and TAS-103,[8] all of which are in clinical evaluation. Recently it has been suggested that TAS-103 predominantly acts as a topoisomerase IIα inhibitor.[9] An advantage of both XR5000 and TAS-103 is their ability to circumvent MDR and to target two key enzymes that affect the topology of DNA which are active at different points in the cell cycle.

XR5000 (**1**) is an acridine derivative which acts as a DNA intercalating agent, and the steric and electronic effects that determine its cytotoxicity have recently been discussed in detail.[10] To find a novel structural class of second generation dual topoisomerase inhibitors, a program of work was initiated to discover an orally active dual inhibitor of topoisomerase I and II that avoided MDR.



The phenazines are different chemically and structurally from the acridines but have similar shape and have been shown to fulfill the fundamental physicochemical requirements for DNA intercalation.[11] A strategy of modifying the phenazine template to give structurally novel dual topoisomerase inhibitors was adopted. The

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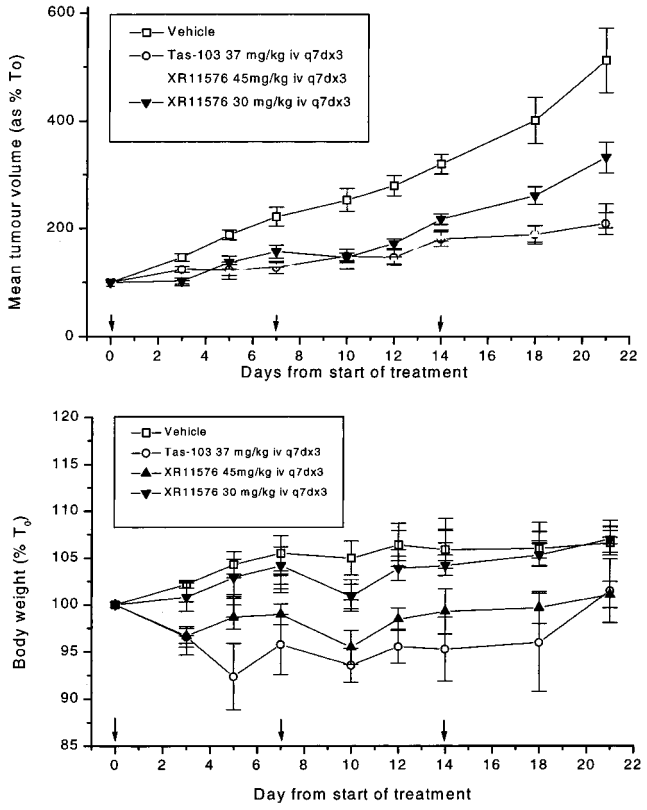


Figure 1.

synthesis and antitumor activity of substituted phenazine-1-carboxamides have previously been reported.[11] The substituent effects on phenazine-1-carboxamides (**2**) have previously indicated that substitution at the 9-position gave improved cytotoxic potency in vitro when evaluated against the L1210 and the P388 leukemia cell lines.[11] In addition, benzofusion at the 3,4-, 6,7-, and 8,9-positions, to give angular tetracyclic benzophenazines, indicated that the 8,9-benzo[*a*]phenazine-1-carboxamide (**3**) was the most active compound in this series when tested on these cell lines.[11]

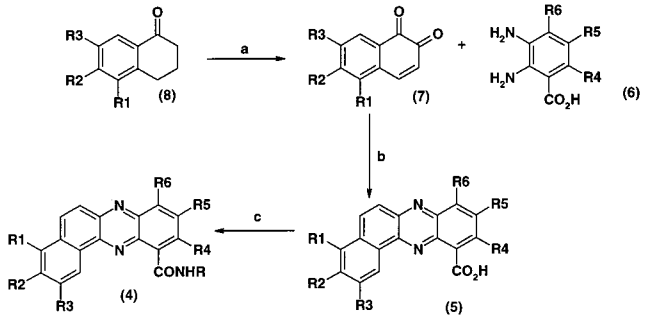
The dual topoisomerase I and II inhibitor Intoplicine has a benzo-fused ring in a similar position to the benzofusion in (**3**), and it has been shown in the Intoplicine series that substitution in the 9-position with a hydroxyl moiety gave improved biological activity in vitro and in vivo when compared to the unsubstituted analogue and was also shown to be essential for dual inhibition of topoisomerase I and topoisomerase II.[6] It was our postulate that novel substituted benzo[*a*]-phenazine carboxamides would show similar trends in biological activity and lead to the discovery of novel dual inhibitors of the topoisomerases I and II.

In this paper we extend these studies to novel substituted angular tetracyclic phenazines with solid tumor activity by reporting the synthesis, biological activity, and structure−activity relationships (SAR) for substituted derivatives of (**3**) and the effects of modifying the carboxamide side chain of (**3**) on these parameters.

Chemistry

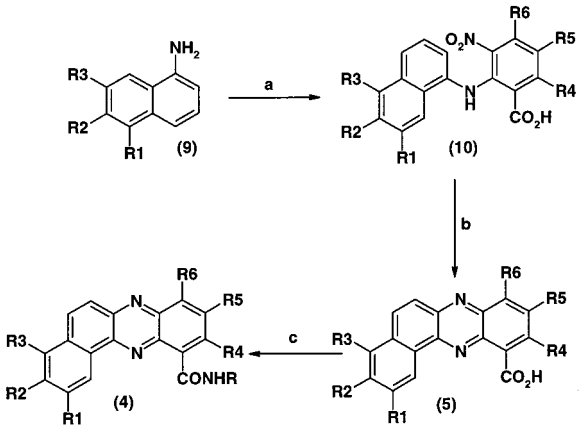
Substituted benzo[*a*]-11-carboxamides (**4**) were obtained from amide bond formation between an activated form of the corresponding acid (**5**) and the appropriate

Scheme 1[a]



[a] Reagents: (a) SeO_2, AcOH; (b) EtOH, concentrated HCl; (c) CDI, DMF, Et_3N, RNH_2 or $SOCl_2$, DCM, Et_3N, RNH_2.

Scheme 2[a]



[a] Reagents: (a) CuCl, Cu powder, *N*-ethylmorpholine, ethylene glycol; (b) 2 M NaOEt, $NaBH_4$, EtOH; (c) CDI, DMF, Et_3N, RNH_2 or $SOCl_2$, DCM, Et_3N, RNH_2.

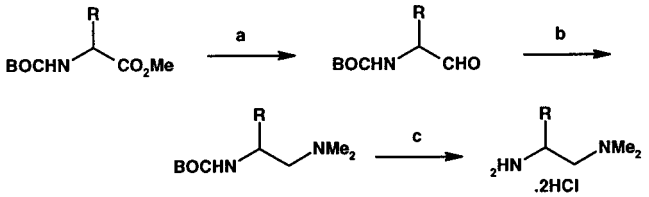
amines. The novel acids (**5**) were obtained from a regiocontrolled condensation under acidic conditions between 1,2-naphthoquinones (**7**) and 2,3-diaminobenzoic acids (**6**). The naphthoquinones (**7**) were in turn prepared by oxidation of the corresponding tetralones (**8**) using selenium dioxide as indicated in Scheme 1 below.

Substituted benzo[*a*]-11-carboxamides (**4**) could also be prepared according to the sequence shown in Scheme 2. An Ullman coupling of 2-bromo-3-nitrobenzoic acid with naphthylamines (**9**) and subsequent reductive ring closure of **10** to give **5**, according to the method of Denny et al.,[11] followed by amide bond formation then gave the desired compounds (**4**).

In some cases the methyl ester of **5**, formed from **5** under standard conditions, could be treated directly with the relevant amine to give **4**. A general synthesis from protected amino acid derivatives of some of the amines used to form **4** is outlined in Scheme 3 and is applicable to racemic and enantiomerically pure compounds.

The starting protected amino acid derivatives were available after standard functional group manipulation. Protection of the amino group as its butyloxycarbonyl derivative was achieved using di-*tert*-butyl dicarbonate and triethylamine in acetonitrile, and esterification of the carboxylic acid group to give the methyl ester was achieved using methanol HCl. These steps were high yielding and typically >95%. Methyl ester reduction mediated with diisobutylaluminum hydride (DIBAL) at

Scheme 3[a]



[a] Reagents: (a) DIBAL, toluene, − 78 °C; (b) Me_2NH·HCl, $NaCNBH_3$, NaOAc, MeOH; (c) 4 M HCl dioxan.

−78 °C gave the corresponding aldehyde without any racemization of enantiopure compounds. Reductive amination of the aldehyde using dimethylamine in the presence of sodium cyanoborohydride gave the required *N,N*-dimethylamines. Removal of the *tert*-butyloxycarbonyl group was achieved in >95% yield using 4 M HCl in dioxan. Where the R group in the side chain contained a hydroxyl group, this was protected as the *tert*-butyldimethylsilyl ether by reaction with *tert*-butyldimethylsilyl chloride in dichloromethane in the presence of imidazole in quantitative yield. Removal of the *tert*-butyldimethylsilyl group was simultaneous with the removal of the butyloxycarbonyl group in step c using 4 M HCl in dioxan. The amines either synthesized or commercial were coupled to an activated form of **5** to form **4**. Some functionality (R1−R6) in compounds **4** or **5** could be further modified to give compounds of the same general structure.

Results and Discussion

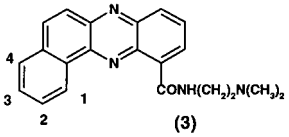
The cytotoxicity of compounds of general structure **4** was measured using the H69 parental (H69/P) human small cell lung carcinoma cell line and the drug resistant human small cell lung carcinoma cell line H69/LX4 which overexpresses P-glycoprotein (Pgp). The cytotoxicity of compounds was also measured using the COR-L23 parental (COR-L23/P) human non small cell lung carcinoma cell line and also the drug resistant human non small cell lung carcinoma cell line COR-L23/R which overexpresses multidrug resistance associated protein (MRP). The activities of novel compounds designed to examine the effects of substitutions in the benzo[*a*]fused ring in these cell lines are indicated in Table 1.

In the design of novel substituted benzo[*a*]phenazines, the effects of substitution at the 2−4-positions were examined in order to investigate any overlap with the 9-hydroxyl of Intoplicine. It has been previously indicated by the 1-aza compound that substitution in the 1-position was least tolerant to substitution probably due to the steric and electronic effects of this position on the side chain and the phenazine nitrogen at the 12-position.[11] The activity of a series of fused heteroaromatic[*a*]phenazines which confirms this observation is published elsewhere.[12] Substitution in the 2−4-positions is tolerated with the 4-position in general being most favored, providing the most potent compounds in this series—the 4-OH **4f** and 4-OMe **4c** analogues. There does not appear to be any major electronic effect of the substituents on activity, with less than 3-fold difference in activity with strongly electron withdrawing substituents (e.g., NO_2, **4i**) and strongly electron donating substituents (e.g., OMe, **4c**). This new finding that activity is retained or improved by substitution at the 4-position gives scope for further structural modification and SAR development.

As substitution at the 4-position had given the most potent compounds, the tolerances to substitution at this position were examined in detail and the cytotoxicity of compounds in this series is listed in Tables 2 and 3. SAR throughout are discussed in terms of activity on the parental cell line compared with compound **4c**.

Substitution with a wide range of functionality groups such as thiomethyl **4j**, halogen **4m**−**o**, methyl **4p**, nitrile **4q**, ester **4r**, hydroxymethyl **4t**, and amino **4u** was tolerated with only marginal loss in activity (2−3-fold). These results in general indicate that a variety of small groups are tolerated in this region. In contrast the corresponding methyl sulfone **4k**, sulfonylamino **4l**, carboxylic acid **4s**, dimethylamino **4w**, acetylamino **4x**, and sulfonamide moieties **4y**,**z** gave a dramatic loss in

Table 1. Substitution in the Benzo[*a*]fused Ring of **3** and **4a**−**i**

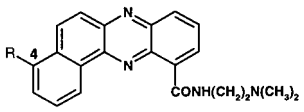


(3)

compound	substituent	H69/P IC_{50} (nM)[a]	H69/LX4 IC_{50} (nM)[a]	Rf[b]	L23/P IC_{50} (nM)[a]	L23/R IC_{50} (nM)[a]	Rf[b]
TAS-103		21.0 ± 7.7[c] (n = 45)	22.6 ± 8.3[c] (n = 45)	1.1	17.3 ± 4.2[c] (n = 18)	42.9 ± 22[c] (n = 17)	2.5
doxorubicin		27.3 ± 30.6[c] (n = 22)	3874 ± 2791[c] (n = 22)	142	20.1 ± 7.0[c] (n = 19)	319.3 ± 145[c] (n = 16)	16
topotecan		15.9 ± 5.3[c] (n = 20)	62.9 ± 30.0[c] (n = 20)	3.9	13.2 ± 3.6[c] (n = 20)	20.0 ± 4.1[c] (n = 20)	1.5
3		67	130	1.9	102	140	1.4
4a	2-OMe	110	112	1.0	NT	NT	-
4b	3-OMe	141	185	1.3	68	79	1.2
4c	4-OMe	35	49	1.4	14	20	1.4
4d	2-OH	86	282	3.3	NT	NT	-
4e	3-OH	109	193	1.8	67	96	1.4
4f	4-OH	35	48	1.4	15	17	1.1
4g	2-NO_2	94	102	1.1	NT	NT	-
4h	3-NO_2	49	69	1.4	NT	NT	-
4i	4-NO_2	96	120	1.3	20	24	1.2
XR11576		23.0 ± 5.9[c] (n = 8)	29.0 ± 14.9[c] (n = 8)	1.2	10.1 ± 4.2[c] (n = 7)	12.1 ± 5.6[c] (n = 7)	1.2

[a] Each assay was performed in quadruplicate with the number of determinations $N \geq 2$, and the results correspond to a mean value where the IC_{50} = concentration of drug (nM) to reduce the cell number to 50%. [b] The Rf is the resistance factor and is the ratio of the IC_{50} on the resistant cell line over the IC_{50} on the parental cell line in the cell lines shown. NT (not tested). [c] Mean ± SD.

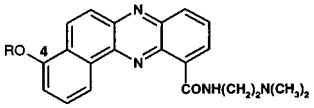
Table 2. Substitution at the 4-Position of the Benzo[*a*]phenazine Carboxamides, **4j**−**4z**



compound	substituent R	H69/P IC_{50} (nM)[a]	H69/LX4 IC_{50} (nM)[a]	Rf[b]
TAS-103		21	22	1.1
doxorubicin		27	3874	142
topotecan		16	62	3.9
4c	4-OMe	35	49	1.4
4j	4-SMe	121	94	0.8
4k	4-SO_2Me	1364	3813	2.8
4l	4-SO_2NHMe	638	>5000	>7.8
4m	4-F	111	78	0.7
4n	4-Cl	117	159	1.4
4o	4-Br	77	171	2.2
4p	4-Me	117	98	0.8
4q	4-CN	101	147	1.5
4r	4-CO_2Me	103	91	0.9
4s	4-CO_2H	>5000	>5000	-
4t	4-CH_2OH	117	423	3.6
4u	4-NH_2	111	193	1.7
4v	4-NHCH_2CN	103	155	1.5
4w	4-NMe_2	778	925	1.2
4x	4-NHCOMe	468	>5000	>10.7
4y	4-NHSO_2Me	2133	4987	2.3
4z	4-N(SO_2Me)$_2$	2137	>5000	>2.3

[a,b] See footnotes of Table 1.

Table 3. Oxygen Linked 4-Substituted Benzo[*a*]phenazine Carboxamides, **4a′**−**4p′**



compound	substituent R	H69/P IC_{50} (nM)[a]	H69/LX4 IC_{50} (nM)[a]	Rf[b]
TAS-103		21	22	1.1
doxorubicin		27	3874	142
topotecan		16	62	3.9
4f	4-OH	35	48	1.4
4c	4-OMe	35	49	1.4
4a′	4-OEt	49	182	3.7
4b′	4-O^{i}Pr	420	874	2.1
4c′	4-OBn	556	882	1.6
4d′	4-OCH_2CCH	40	61	1.5
4e′	4-OCH_2CN	28	24	0.9
4f′	4-$OCH_2CH_2CH_2$CN	39	58	1.5
4g′	4-OCOMe	24	67	2.8
4h′	4-OCONHEt	64	103	1.6
4i′	4-OCH_2CH_2OMe	90	124	1.4
4j′	4-OCH_2CH_2OH	303	235	0.8
4k′	4-OCH_2CO_2Et	587	506	0.9
4l′	4-OCH_2COMe	45	138	3.1
4m′	4-OCH_2CONH_2	89	328	3.7
4n′	4-OCH_2CO_2H	3844	>5000	>1.3
4o′	4-$OCH_2CH_2CH_2NMe_2$	116	91	0.8
4p′	4-OCH_2CH_2morpholino	99	236	2.4

[a,b] See footnotes of Table 1.

cytotoxicity (>10-fold). The extent to which functionality is tolerated is dependent on the size and direction of the electronic effects of the functionality, with strongly acidic groups being disfavored. The cyanomethylamino compound **4v** only lost ∼3-fold activity, indicating that the electronic effects of small linear functionality is allowed in this region. All of the compounds in Table 2 are less cytotoxic than the 4-methoxy analogue **4c**. In compound **4c**, attachment to the phenazine nucleus is by an oxygen linkage, and this enables rapid synthetic access from a common intermediate **4f** to a diverse set of O-linked substituents at the 4-position to further broaden SAR and examine substituent effects. The SAR of analogues that are attached at the 4-position by oxygen are listed in Table 3.

Replacement of methyl **4c** by ethyl **4a′** does not result in any loss of potency. However, the isopropyl **4b′** derivative loses ∼12-fold activity, indicating a steric constraint in this region, and this is supported by the loss of cytotoxicity for benzyl analogue **4c′**. In addition, the propargyl **4d′** and the cyanomethoxy **4e′** compounds are equipotent with the **4c** analogue, confirming that small groups with directional functionality are well tolerated. The 3-cyanopropoxy moiety is also tolerated (**4f′**), and this coupled with the result above in Table 2 for the cyanomethylamino compound **4v** supports this hypothesis.

The ethylamide **4h′** is ∼2-fold less potent, and the acetyloxy compound **4g′** retains activity but may be metabolized to the potent phenolic analogue **4f** within the cell.

These data also show that the methoxyacetyl analogue **4l′** is equipotent and the methoxycarboxamide **4m′** is 2.5-fold less active. A dramatic loss of activity is observed for the methoxyethylcarboxylate **4k′**, and the methoxycarboxylic acid **4n′** loses 100-fold activity again showing acidic functionality is disfavored.

The ethoxymethoxy **4i′** and ethoxymorpholino **4p′** analogues lose 2−3-fold activity, and the ethoxyhydroxy analogue **4j′** loses 8−9-fold activity. The propyloxydimethylamino compound **4o′** loses ∼3-fold activity, and this together with the ethoxymorpholino result **4p′** indicates that basic groups can be tolerated in this region.

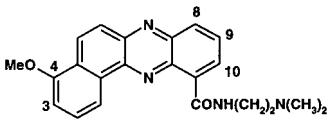
The results in Tables 2 and 3 show that the most potent compounds tend to have small substituents with a directional effect in a limited region, and although larger groups are tolerated, the positioning of functionality on them and the directional effects are more limited.

To investigate the effect of disubstitution in the benzo[*a*]fused ring, the 3,4-dimethoxy analogue **4q′** was prepared. This was found to be ∼12-fold less potent than the parent **4c** (Table 4). Further disubstitution in the benzo[*a*]fused ring was not examined. Previously Denny had shown in the phenazine series that substitution in the 6- and 7-positions (5,6-positions in this series) was disfavored, so work concentrated on the substituent effects at the 8-, 9-, and 10-positions.

A methyl substituent at the 8-position **4r′** occupies a position similar to that of the 8-methyl group in Intoplicine, and it was found that this compound was only ∼2-fold less active than the parent. The introduction of Cl **4s′** and OMe **4u′** at the 9-position gave analogues approximately equipotent with the parent **4c**, showing substitution at this position was tolerated. The 9-bromo analogue **4t′** lost ∼4.5-fold activity, indicating there may be some size constraint in this region.

Methoxy substitution at the 10-position **4v′** resulted in >75-fold loss of cytotoxicity, indicating the impact of substitution at this position on the geometry of the side chain at the 11-position. This effect is emphasized

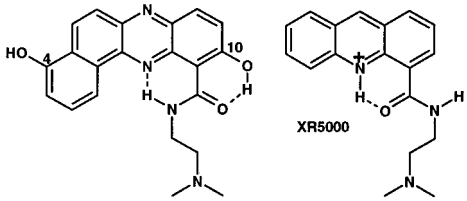
Table 4. Substituent Effects at the 3-, 8-, 9-, and 10-Positions of the 4-Methoxy-benzo[*a*]phenazine Carboxamides **4q′**−**4z′**



compound	substituent	H69/P IC_{50} (nM)[a]	H69/LX4 IC_{50} (nM)[a]	Rf[b]
TAS-103		21	22	1.1
doxorubicin		27	3874	142
topotecan		16	62	3.9
4c	4-OMe	35	49	1.4
4q′	3,4-di-OMe	436	804	1.8
4r′	4-OMe, 8-Me	87	168	1.9
4s′	4-OMe, 9-Cl	20	21	1.0
4t′	4-OMe, 9-Br	159	100	0.6
4u′	4,9-di-OMe	19	25	1.3
4v′	4,10-di-OMe	2640	3619	1.4
4w′	4,10-di-OH	19	25	1.3
4x′	4-OMe, 10-OH	103	107	1.0
4y′	4-OMe, 10-NH_2	34	29	0.85
4z′	4-OMe, 10-NHMe	127	143	1.1

[a,b] See footnotes of Table 1.

further in the 4,10-dihydroxy analogue **4w′**, which is equipotent with the parent. In XR5000 the postulated active conformation has an internal hydrogen bond between the amide carbonyl and the protonated acridine nitrogen. In the phenazine series, however, with a much



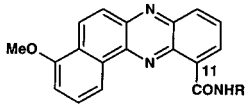
lower pK_a, a hydrogen bond exists between the amide N−H and the phenazine nitrogen, the amide proton in the NMR spectrum having a chemical shift of ∼10 ppm. In the 10-methoxy substituted analogue, the chemical shift of the amide proton in the NMR spectrum is ∼6 ppm, indicating that it is not hydrogen bonded in the active conformation as evidenced from the dramatic loss of potency.

This theory is reinforced by the introduction of hydroxyl **4x′**, amino **4y′**, and methylamino **4z′** groups at the 10-position. These changes would enable an internal hydrogen bond to form with the side chain carbonyl, and these analogues are within a 1−3-fold activity range of the parent **4c**.

Having studied the effects of substitution at the 10-position on activity with the dimethylaminoethylamide side chain, the SAR of side chain variation was explored in more detail. This work was conducted using the template from analogue **4c** shown below, and the effects of side chain variation are discussed in comparison to the dimethylaminoethylamide side chain. The results are summarized in Table 5.

Extending the chain length by one carbon unit (**4a″**) resulted in ∼13-fold loss of activity, and this clearly indicates the importance of chain length and the positioning of the basic side chain for activity. Replacement of the dimethylamino moiety with diethylamino (**4c″**),

Table 5. Varation of the Amide Side Chain at the 11-Position of the 4-Methoxy-benzo[*a*]phenazine Carboxamides **4a″**−**4s″**



compound	substituent R	H69/P IC_{50} (nM)[a]	H69/LX4 IC_{50} (nM)[a]	Rf[b]
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doxorubicin		27	3874	142
topotecan		16	62	3.9
4c	$-(CH_2)_2NMe_2$	35	49	1.4
4a″	$-(CH_2)_3NMe_2$	470	597	1.3
4b″	$-CH_2CH(NMe_2)CH_2NMe_2$	126	480	3.8
4c″	$-(CH_2)_2NEt_2$	412	1001	2.4
4d″	$-(CH_2)_2$piperidino	156	330	2.1
4e″	$-CH_2CH(OH)CH_2NH_2$	515	>5000	>9.7
4f″	$-(CH_2)_2$morpholino	597	1004	1.7
4g″	$-(CH_2)_2N(CH_2CH_2OH)_2$	614	3378	5.5
4h″	$-(CH_2)_2$pyrrolidino	40	77	1.9
4i″	$-(CH_2)_2NHMe$	99	234	2.4
4j″	$-CH(Me)CH_2NMe_2$	30	44	1.4
4k″	$-CH_2CH(Me)NMe_2$	101	126	1.2
4l″	$-C(Me)_2CH_2NMe_2$	435	642	1.5
4m″	$-CH(Et)CH_2NMe_2$	107	118	1.1
4n″	$-CH(^iPr)CH_2NMe_2$	35	25	0.7
4o″	$-CH(Bn)CH_2NMe_2$	478	513	1.1
4p″	$-CH(CH_2OH)CH_2NMe_2$	23	28	1.2
4q″	$-CH(CH_2CH_2OH)CH_2NMe_2$	26	28	1.1
4r″	$-CH(CO_2Me)CH_2NMe_2$	468	>5000	>10
4s″	$-CH(CO_2H)CH_2NMe_2$	211	>5000	>23

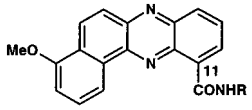
[a,b] See footnotes of Table 1.

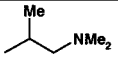
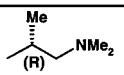
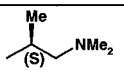
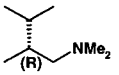
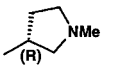
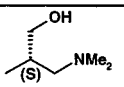
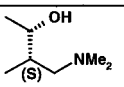
piperidino (**4d″**), morpholino (**4f″**), and $N(CH_2CH_2OH)_2$ (**4g″**) all resulted in reduced activity. An extended side chain analogue (**4b″**) with a dimethylamino group β to the amide lost significant activity, and a primary amino group at the terminus with an hydroxyl group β to the amide (**4e″**) also gave a dramatic loss of potency. The pyrrolidino (**4h″**) and methylamino (**4i″**) compounds were equiactive and ∼3-fold less active than the parent **4c**. These results show that there is a steric constraint at the side chain terminus with small hydrophobic groups being favored and hydrophilic substituents not being well tolerated. The dimethylamino moiety at the side chain terminus linked by a two-carbon unit to the amide was optimal in this series. This important observation led to the investigation of substituent effects in the ethylamide side chain.

Introduction of a methyl group α **4j″** or α **4k″** to the amide resulted in compounds that were equipotent and ∼3-fold less active, respectively. However, the *gem*-dimethyl analogue **4l″** lost significant activity (∼12-fold), showing this amount of steric bulk was not tolerated α to the amide group. The above observations led to substitution α to the amide being extended to a range of substituents with diverse stereoelectronic effects, with compounds being made in racemic form in the first instance.

The steric tolerance α to the amide was examined by replacement of the methyl by ethyl (**4m″**), isopropyl (**4n″**), and benzyl (**4o″**). The ethyl compound **4m″** was ∼3-fold less active, the isopropyl **4n″** was equiactive, and the benzyl **4o″** was ∼14-fold less active. It appears there is reasonable tolerance in this region as the isopropyl result **4n″** indicates, but larger groups such as benzyl **4o″** and the result from the *gem* dimethyl

Table 6. Enantiospecific Side Chain Variation at the 11-Position of 4-Methoxy-benzo[*a*]phenazine Carboxamides



Compound	Substituent (R)	H69/P IC_{50} (nM)[a]	H69/LX4 IC_{50} (nM)[a]	Rf[b]
TAS-103		21	22	1.1
Doxorubicin		27	3874	142
Topotecan		16	62	3.9
4j″		30	44	1.4
(R) – 4j″ XR11576		23	29	1.2
(S) – 4j″		96	121	1.3
(R) – 4n″		21	20	1.0
(R) – 4t″		79	128	1.6
(S) – 4p″		20	25	1.2
(S) – 4u″		19	19	1

[a,b] See footnotes of Table 1.

compound **4l″** above indicate that if the side chain adopts an unfavorable conformation, then potency is reduced. The effect of introducing hydrophilic groups in this region was examined, and both the hydroxymethyl **4p″** and hydroxyethyl **4q″** moieties gave compounds at least equipotent with the parent **4c**. Introduction of ester **4r″** and acid **4s″** groups lead to significant loss of activity and were inactive on the resistant cell line. The results from these substitutions have led to the important observation that substitution with both hydrophobic and hydrophilic moieties is tolerated, enabling the physicochemical properties of the molecule to be varied.

Having examined the tolerances in the racemic series, the enantiomerically pure compounds were synthesized for some of the more potent analogues in the racemic series. These analogues are listed in Table 6.

The (*R*)-enantiomer of the methyl analogue **4j″** was more potent than its racemate, and the (*S*)-enantiomer which was only ~80% optically pure was ~4-fold less active than the pure (*R*)-enantiomer. This indicated the importance of the chirality at this center and the directional positioning of the substituent on activity and is to our knowledge the first report of side chain chirality in DNA intercalating agents having an important influence on biological activity. The (*R*)-enantiomer of the isopropyl compound **4n″** was also more active than its racemate and the cyclic (*R*)-*N*-methylpyrrolidine compound **4t″** 3−4-fold less active compared to **4j″**. Knowing these chirality and directionality effects, the hydroxymethyl **4p″** and the 2-hydroxyethyl analogues **4u″** were synthesized as the (*S*)-enantiomers, and as predicted, the potent activity was retained.

Table 7. Pharmacokinetics in XR11576 in Female Balb/c Mice

	Balb/c mice		
admistration	20 mg/kg iv	50 mg/kg iv	50 mg/kg po
C_{max} (μg/mL)	0.8 ± 0.3	2.9 ± 0.8	0.35 ± 0.29
AUC (μg·min/mL)	64 ± 6	156 ± 9	114 ± 30
Cl (mL/min)	6.3 ± 0.6	6.4 ± 0.4	N/A
K_{el} (min^{-1})	0.0044 ± 0.0002	0.0030 ± 0.00034	0.002 ± 0.001
V_{ss} (L)	1.3 ± 0.4	1.9 ± 0.6	N/A
F (%)			72 ± 25

Selected compounds were studied for their ability to stabilize cleavable complexes in the presence of either topoisomerase I or II as described previously.[7] The presence of cleavable complexes was indicated by an increase in the number and intensity of bands observed on the gel after electrophoresis and autoradiography. From these data it can be concluded that a representive set of potent cytotoxic novel angular phenazines are dual inhibitors of topoisomerase I and II.[7] As an example, compound **(R)-4j″** inhibited both topoisomerase I and II in a dose-dependent manner between 0.03 and 1 μM.[7]

The mechanism of inhibition of topoisomerase I and II is unknown for these compounds. However, it has recently been shown that an acridine-4-carboxamide intercalates into duplex DNA with a specific hydrogen bonded interaction to the N7 of guanine in the major groove.[13] This study showed that a single hydrogen bond is formed by the protonated *N*,*N*-dimethylamino group of the *N*-(2-(dimethylamino)ethyl)-4-carboxamide side chain to N7 of one of the guanine residues at the intercalation site. The change in electrostatic potential in the major groove resulting from the neutralization of the partial negative charge on guanine by the protonated *N*,*N*-dimethylamino group, together with the steric blocking of the groove by the side chain, is likely to be important in the inhibition of the topoisomerases. As many of the phenazines described here have the same or a similar 11-carboxamide side chain to the analogue described as well as a flat tetracyclic moiety, intercalation in the minor groove and additional binding to the major groove in guanine rich regions is a suggested mechanism for the inhibition of the topoisomerases by these compounds.

From the SAR in this novel series of angular phenazines, the enantiomerically pure compound **(R)-4j″** (XR11576) was a dual inhibitor of topoisomerase I and II and a potent cytotoxic agent in all the cell lines in which it was tested. The results in the H69 and L23 parental and resistant cell lines compared to known topoisomerase inhibitors are summarized in Table 1.

Due to XR11576 having a favorable profile in vitro to other compounds in this series and known topoisomerase inhibitors it was chosen for pharmacokinetic and xenograft studies in vivo. The plasma pharmacokinetics following intravenous and oral administration of XR11576 in female mice is summarized in Table 7.

These results indicate that XR11576 has an oral bioavailability of ~72% in female mice and demonstrates linear pharmacokinetics after iv dosing. In addition, XR11576 exhibits a relatively large volume of distribution and low clearance.

Using a H69/P Xenograft, the effect of XR11576 on tumor growth and body weight of female nude tumor bearing mice was examined. The results of these studies are shown in Figure 1.

Data in Figure 1 is expressed as mean ± SEM. The effect of treatment on tumor volume is plotted as a percentage of that seen on day 0 (To) which is the start of treatment. The results indicate that XR11576 given q7d×3 at 30 and 45 mg/kg iv evoked a dose related tumor growth delay in this model.

Summary

The discovery and SAR of a novel series of substituted benzo[*a*]phenazine carboxamides that are potent cytotoxic agents on a number of human cancer cell lines has been described. These compounds have been designed and shown to act as dual inhibitors of topoisomerase I and II and thus target two key enzymes that affect the topology of DNA at different points of the cell cycle. The ability of these compounds to overcome potential multidrug resistance mechanisms has been demonstrated by their cyctotoxicity on Pgp and MRP overexpressing cell lines. One of the most potent compounds on all cell lines examined is XR11576, and this compound has been shown to be orally bioavailable and has demonstrated tumor growth delay in female mice by both the oral and iv routes. The novel substituted benzo[*a*]phenazine carboxamide XR11576 has been selected as a candidate for further evaluation.

Experimental Section

Methods and Materials. Reagents, starting materials, and solvents were purchased from common commercial suppliers and used as received or distilled from the appropriate drying agent. Reactions requiring anhydrous conditions were performed under an atmosphere of nitrogen or argon. Precoated aluminum backed silica gel 60 F_{254} plates with a layer thickness of 0.25 mm were used for thin-layer chromatography, and the stationary phase for preparative column chromatography using medium pressure was silica gel 60, mesh size 40−60 μm from E. Merck, Darmstadt, Germany.

NMR spectra were obtained using a Bruker ACF 400 operating at 400 MHz, and the (^{1}H) ppm were calibrated to residual $CHCl_3$ in $CDCl_3$ at 7.26 ppm. Mass spectra were obtained in the indicated mode using a Finnigan SSQ 710L machine. Melting points were determined using an electrothermal 9100 series apparatus.

The compound purity on all compounds tested in biological systems was assessed as being >95% using HPLC using a water/acetonitrile gradient at 30 °C on a Waters symmetry C_{18} 150 mm × 4.6 mm column and by NMR spectroscopy. Microanalyses were performed on a representation of compounds by MEDAC Ltd. U.K. Where analyses are indicated only by the symbols of the elements, results obtained were within 0.4% of the theoretical values.

Chemistry. General Method for the Preparation of 1,2-Naphthoquinones. 1,2-Naphthoquinones were prepared from the corresponding substituted α-tetralones by oxidation with selenium dioxide in acetic acid using the method of Bekaert.[14] In a typical example, commercially available 5-methoxy-1-tetralone (15.35 g, 87.1 mmol) and ground selenium dioxide (24.2 g, 217.75 mmol) in glacial acetic acid (160 mL) were warmed at 65 °C for 7 h. The mixture was then cooled to room temperature and the acetic acid removed in vacuo. The residue was treated with ethyl acetate (100 mL) and filtered through Celite and a plug of silica eluting with ethyl acetate, the organics were concentrated, and the product precipitated on standing at 4 °C overnight to yield the product as a red solid (11.1 g, 68%). If the required tetralones were not commercially available, they were prepared using standard literature methods. The following tetralones were prepared: 5-methyl-1-tetralone;[15] 5-fluoro-1-tetralone;[15] 5,6-dimethoxy-1-tetralone;[14] 5-bromo-1-tetralone;[16] 5-cyano-1-tetralone was prepared from 5-bromo-1-tetralone by treatment with copper(I) cyanide in DMF under standard conditions;[17] 5-chloro-1-tetralone was prepared from 5-amino-1-tetralone[18] by diazotization under standard conditions, followed by treatment with copper(I) chloride;[19] 5-ethoxy-1-tetralone was prepared from 5-hydroxy-1-tetralone by alkylation with ethyl iodide under standard conditions; 5-methylsulfanyl-1-tetralone was also prepared from 5-hydroxy-1-tetralone as described in the literature;[20] 5-methylsulfonyl-1-tetralone was prepared from 5-methylsulfanyl-1-tetralone using MCPBA under standard conditions;[21] and 7-nitro-1-tetralone.[22] These tetralones were oxidized to the corresponding 1,2-naphthoquinones using the procedure described above.

General Method for the Synthesis of Substituted Benzo[*a*]phenazine-11-carboxylic Acids. 4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid. A mixture of 5-methoxy-[1,2]naphthoquinone[14] (1.98 g, 9.3 mmol), 2,3-diamino-benzoic acid, diacetate salt,[23] (4.03 g, 14.8 mmol), and concentrated hydrochloric acid (2.2 mL) was heated to reflux in ethanol (20 mL) for 4 h. The reaction mixture was cooled and the precipitate collected by filtration and washed with ethanol and ether to yield the title compound as a beige solid (2.74 g, 97%). ^{1}H NMR (DMSO-d_6) δ 4.05 (s, 3H), 7.50 (d, 1H), 7.84−7.87 (m, 1H), 7.99 (d, 1H), 8.08−8.10 (m, 1H), 8.41−8.49 (m, 3H), 8.63 (d, 1H). MS (DCI/NH_3) *m/e* 305 $(M + H)^+$.

The following substituted benzo[*a*]phenazine-11-carboxylic acids were prepared by the general method above.

4-Methyl-benzo[*a*]phenazine-11-carboxylic Acid (32%). ^{1}H NMR (DMSO-d_6) δ 2.79 (s, 3H), 7.85−7.78 (m, 2H), 8.11−8.05(m, 2H), 8.47−8.44 (m, 2H), 8.51 (dd, 1H), 9.02 (d, 1H).

4-Fluoro-benzo[*a*]phenazine-11-carboxylic Acid (31%). ^{1}H NMR (DMSO-d_6) δ 7.80 (m, 1H), 7.95 (m, 1H), 8.11−8.06 (m, 2H), 8.38 (d, 1H), 8.43 (dd, 1H), 8.51 (dd, 1H), 8.97 (d, 1H).

3,4-Dimethoxy-benzo[*a*]phenazine-11-carboxylic Acid (74%). ^{1}H NMR ($CDCl_3$) δ 3.98 (s, 3H), 4.04 (s, 3H), 7.76 (d, 1H), 7.95 (d, 1H), 8.05 (t, 1H), 8.37 (d, 1H), 8.48 (d, 2H), 8.79 (d, 1H).

4-Bromo-benzo[*a*]phenazine-11-carboxylic Acid (54%). ^{1}H NMR (DMSO-d_6) δ 7.87 (t, 1H), 8.08 (t, 1H), 8.20 (d, 1H), 8.26 (m, 1H), 8.33 (m, 1H), 8.48 (m, 2H), 9.24 (d, 1H).

4-Cyano-benzo[*a*]phenazine-11-carboxylic Acid (63%). ^{1}H NMR (DMSO-d_6) δ 8.10−8.18 (m, 2H), 8.33 (d, 1H), 8.43 (m, 2H), 8.48−8.54 (m, 2H), 9.48 (d, 1H). MS (DCI/NH_3) *m/e* 300 $(M + H)^+$.

4-Chloro-benzo[*a*]phenazine-11-carboxylic Acid (36%). ^{1}H NMR (DMSO-d_6) δ 7.93 (t, 1H), 8.08 (dd, 1H), 8.11 (d, 1H), 8.19 (d, 1H), 8.43, (dd, 1H), 8.52, (t, 2H), 9.17, (d, 1H).

4-Methanesulfonyl-benzo[*a*]phenazine-11-carboxylic Acid (30%). ^{1}H NMR (DMSO-d_6) δ 8.16 (m, 2H), 8.32 (m, 1H), 8.43 (d, 1H), 8.56 (m, 2H), 9.03 (d, 1H), 9.61 (d, 1H). MS (DCI/NH_3) *m/e* 353 $(M + H)^+$.

Benzo[*a*]phenazine-4,11-dicarboxylic Acid 4-methyl Ester (28%). ^{1}H NMR (DMSO-d_6) δ 4.03 (s, 3H), 8.10 (m, 2H), 8.20 (d, 1H), 8.44 (m, 2H), 8.53 (d, 1H), 9.47 (d, 1H).

4-Ethoxy-benzo[*a*]phenazine-11-carboxylic Acid (76%). ^{1}H NMR (DMSO-d_6) δ 1.52 (q, 3H), 4.33 (t, 2H), 7.50 (d, 1H), 7.85−7.90 (m, 1H), 8.00 (d, 1H), 8.08−8.11 (m, 1H), 8.45−8.55 (m, 3H), 8.68 (d, 1H).

2-Nitro-benzo[*a*]phenazine-11-carboxylic acid was prepared from 7-nitro[1,2]naphthoquinone and 2,3-diamino-benzoic acid, diacetate salt using the previously described methods[23] for the synthesis of benzo[*a*]phenazine-11-carboxylic acids. The 7-nitro[1,2]naphthoquinone was prepared from 7-nitrotetralone.[22] The acid was used without further purification.

4-Hydroxy-benzo[*a*]phenazine-11-carboxylic Acid. To a solution of 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (441 mg, 1.45 mmol) in dichloromethane (30 mL) cooled to 0 °C was added a 1.0 M solution of boron tribromide in dichloromethane (7.25 mL, 7.25 mmol). The reaction mixture

was allowed to warm to room temperature and then stirred for 16 h. The mixture was then poured onto ice/water (20 mL) yielding 4-hydroxy-benzo[*a*]phenazine-11-carboxylic acid as a red/brown solid which was collected by filtration and air-dried (230 mg, 55%). ^{1}H NMR (DMSO-d_6) δ 7.35 (d, 1H), 7.75 (m, 1H), 7.92 (d, 1H), 8.08 (m, 1H), 8.47−8.55 (m, 4H), 10.72 (br, s, 1H).

4-Benzyloxy-benzo[*a*]phenazine-11-carboxylic Acid. A mixture of 4-hydroxy-benzo[*a*]phenazine-11-carboxylic acid (80 mg, 0.28 mmol), sodium hydroxide (34 mg, 0.85 mmol), and benzyl bromide (100 μL) in ethanol (2 mL) was heated to reflux for 4 h. The reaction mixture was then cooled, diluted with ethyl acetate (15 mL), washed with 1M HCl, and dried ($MgSO_4$) ,and the solvent was removed in vacuo to yield the crude title compound as a brown solid (50 mg, 47%). ^{1}H NMR (DMSO-d_6) includes δ 5.35 (s, 2H). This compound was used directly in the next step without further purification.

The following compounds were prepared in an analogous manner from 4-hydroxy-benzo[*a*]phenazine-11-carboxylic acid using the appropriate alkylating reagent.

4-Prop-2-ynyloxy-benzo[*a*]phenazine-11-carboxylic acid was prepared using propargyl bromide (64%).

4-Isobutoxy-benzo[*a*]phenazine-11-carboxylic acid was prepared using isobutyl bromide (13%). The above materials were used without further purification.

4-(2-Methoxy-ethoxy)-benzo[*a*]phenazine-11-carboxylic acid was prepared using 2-bromoethyl methyl ether (40%). ^{1}H NMR (DMSO-d_6) δ 3.40 (s, 3H), 3.85 (t, 2H), 4.40 (t, 2H), 7.51 (d, 1H), 7.87 (t, 1H), 8.01 (d, 1H), 8.10 (t, 1H), 8.50 (m, 3H), 8.67 (d, 1H).

4-Ethoxycarbonylmethoxy-benzo[*a*]phenazine-11-carboxylic acid was prepared using ethyl bromoacetate (78%). Sodium ethoxide in dry ethanol was used for this reaction. ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H), 4.25 (q, 2H), 4.81 (s, 2H), 7.07 (d, 1H), 7.64 (t, 1H), 7.84 (dd, 1H), 7.90 (d, 1H), 8.38 (m, 2H), 8.59 (d, 1H), 9.04 (d, 1H).

4-[2-(*tert*-Butyl-dimethyl-silanyloxy)-ethoxy]-benzo[*a*]-phenazine-11-carboxylic acid was prepared using *tert*-butyl-(2-iodo-ethoxy)-dimethylsilane (6%). ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 6H), 0.80 (s, 9H), 4.00 (t, 2H), 4.19 (t, 2H), 7.21 (d, 1H), 7.65 (t, 1H), 7.83 (d, 1H), 7.90 (t, 1H), 8.41 (dd, 1H), 8.52 (d, 2H), 8.82 (dd, 1H), 15.75 (br. s, 1H). The *tert*-Butyl-(2-iodo-ethoxy)-dimethyl-silane was prepared using standard procedures from 2-iodo-ethanol (99%). ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 6H), 0.82 (s, 9H), 3.12 (t, 2H), 3.74 (t, 2H).

Benzo[*a*]phenazine-11-carboxylic Acid Methyl Ester. A mixture of 1,2-naphthoquinone (2.0 g, 12.6 mmol) and 2,3-diamino-benzoic acid, diacetate salt (3.79 g, 13.9 mmol) was heated to reflux in acetic acid (30 mL) for 2 h. The reaction mixture was cooled and the solvent removed in vacuo to yield a gum. This was purified using flash chromatography to yield a 2:1 mixture of the desired title compound to the undesired benzo[*a*]phenazine-8-carboxylic acid (885 mg). The mixture of benzo[*a*]phenazine-11-carboxylic acid and benzo[*a*]phenazine-8-carboxylic acid (885 mg) was heated to reflux in a mixture of methanol (40 mL) and acetyl chloride (920 μL) for 90 min. The reaction mixture was then cooled slowly to yield the title compound as a single isomer which was collected by filtration (377 mg, 11%). ^{1}H NMR (DMSO-d_6) δ 4.21 (s, 3H), 7.95−8.01 (m, 2H), 8.02−8.09 (m, 1H), 8.10−8.11 (m, 1H), 8.35 (d, 1H), 8.47(d, 1H), 8.57 (d, 1H), 9.06 (d, 1H), 9.53−9.55 (m, 1H). This compound was identical spectroscopically to the title compound prepared by a different route.[11]

4-Methylsulfamoyl-benzo[*a*]phenazine-11-carboxylic Acid and 3-Methylsulfamoyl-benzo[*a*]phenazine-11-carboxylic Acid. Benzo[*a*]phenazine-11-carboxylic acid methyl ester (220 mg, 0.72) was heated under nitrogen to 180 °C in chlorosulfonic acid (2 mL) for 6 h. The reaction was then cooled and poured onto ice/water and the pale yellow solid collected by filtration to give approximately a 1:1 mixture of 4-chlorosulfonyl-benzo[*a*]phenazine-11-carboxylic acid and 3-chlorosulfonyl-benzo[*a*]phenazine-11-carboxylic acid (182 mg, 0.48 mmol). ^{1}H NMR (DMSO-d_6) includes δ 9.30 (s, 1H), 9.41 (d, 1H). This mixture was used directly in the next step without purification without further purifcation. The mixture of 4-chlorosulfonyl-benzo[*a*]phenazine-11-carboxylic acid and 3-chlorosulfonyl-benzo[*a*]phenazine-11-carboxylic acid (182 mg, 0.48 mmol) was dissolved in dichloromethane (5 mL). To this was added a 40% solution of methylamine in water (5 mL), and the reaction mixture was stirred vigorously for 4 h. The reaction mixture was then poured onto dichloromethane, acidified (2 N HCl), extracted into dichloromethane, and dried ($MgSO_4$) and the solvent removed in vacuo to give a ∼1:1 mixture of the title compounds (160 mg, 0.44 mmol, 91%). The two isomers were separated after further chemical modification. ^{1}H NMR ($CDCl_3$/MeOH-d_4) includes δ 2.55 (s, 3H), 2.61 (s, 3H), 9.22 (d, 1H), 9.38 (s, 1H).

4-Dimethylsulfamoyl-benzo[*a*]phenazine-11-carboxylic acid and 3-dimethylsulfamoyl-benzo[*a*]phenazine-11-carboxylic acid (168 mg, 91%) were prepared in an analogous manner by reaction of dimethylamine with the mixture of 4-chlorosulfonyl-benzo[*a*]phenazine-11-carboxylic acid and 3-chlorosulfonyl-benzo[*a*]phenazine-11-carboxylic acid (180 mg, 0.47 mmol). The two isomers were separated after further chemical modification. NMR (DMSO-d_6) of mixture includes δ 9.06 (d, 1H), 9.42 (s, 1H), 2.93 (s, 6H).

4-Nitro-benzo[*a*]phenazine-11-carboxylic acid and 3-nitro-benzo[*a*]phenazine-11-carboxylic acid. Concentrated sulfuric acid (5 mL) and concentrated nitric acid (5 mL) were mixed together at 0 °C. To this mixture was added benzo[*a*]-phenazine-11-carboxylic acid (100 mg, 0.36 mmol), and the reaction mixture was allowed to warm slowly to room temperature. After 24 h the reaction mixture was poured onto water yielding a yellow precipitate, this was collected by filtration to yield a 4:1 mixture of 4-nitro-benzo[*a*]phenazine-11-carboxylic acid and 3-nitro-benzo[*a*]phenazine-11-carboxylic acid (84%). The two isomers were separated after further chemical modification. ^{1}H NMR (DMSO-d_6) includes δ 9.82 (d, 1H), 9.46 (d, 1H), 1:4 ratio. MS (DCI/NH_3) *m/e* 320 $(M + H)^+$.

9-Bromo-4-methoxy-benzo[*a*]phenazine-11-carboxylic Acid. To a solution of 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (100 mg, 0.33 mmol) in chloroform (7 mL) was added dropwise bromine (5 mL). Stirring was continued for 48 h. The chloroform and bromine were removed in vacuo, and the residue was purified by flash chromatography to give a yellow gum (25 mg, 20%). ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 3H), 5.76 (d, 1H), 6.11 (d, 1H), 6.99 (d, 1H), 7.79 (d, 1H), 7.95 (t, 1H), 8.33 (dd, 1H), 8.85 (dd, 1H), 14.46 (br. s, 1H).

4-Amino-benzo[*a*]phenazine-11-carboxylic Acid and 3-Amino-benzo[*a*]phenazine-11-carboxylic Acid. To the 4:1 mixture of 4-nitro-benzo[*a*]phenazine-11-carboxylic acid and 3-nitro-benzo[*a*]phenazine-11-carboxylic acid (52 mg, 0.16 mmol) in ethanol (5 mL) was added ammonium chloride solution (3 mL) and indium (cat.). The reaction mixture was heated to reflux, then cooled, and filtered through a bed of Celite. The filtrate was diluted with water, extracted into dichloromethane, and dried ($MgSO_4$) and the solvent removed in vacuo to yield the title compounds as a mixture (48 mg, 0.15 mmol, 94%). ^{1}H NMR (DMSO-d_6) δ 6.95 (d, 1H), 7.55 (t, 1H), 7.75 (m, 2H), 8.05 (m, 2H), 8.20 (d, 1H), 8.65 (d, 1H). The mixture was used directly in the next step and the constituents separated after further modification.

2-Methoxy-benzo[*a*]phenazine-11-carboxylic Acid. To a solution of 8-amino-naphthalen-2-ol (8.00 g, 50 mmol) in dry DMF (80 mL) was carefully added sodium hydride (60% dispersion in mineral oil, 3.2 g, 222 mmol). After being stirred for 4 h, the reaction mixture was cooled in an ice bath and methyl iodide (3.13 mL) was added dropwise. The reaction mixture was then stirred at room temperature for 3 days. Water (10 mL) was then added, and the volatiles were removed in vacuo. The residue was dissolved in chloroform, washed with water, and dried ($MgSO_4$) and the solvent removed in vacuo to yield a dark oil which was purified using flash chromatography eluting with chloroform to yield 7-methoxy-naphthalen-1-ylamine as a dark brown liquid (2.92 g, 16.9 mmol). Treatment of 7-methoxy-naphthalen-1-ylamine (1.19 g, 6.88 mmol) with 2-bromo-3-nitro-benzoic acid (1.8 g, 6.88 mmol)

according published methods,[11] to yield 2-(7-methoxy-naphthalen-1-ylamino)-3-nitro-benzoic acid (0.47 6, 21%), and reductive cyclization of 7-methoxy-naphthalen-1-ylamine using sodium borohydride[11] yielded the desired title compound (43%). ^{1}H NMR (DMSO-d_6) δ 4.02 (s, 3H), 7.56 (dd, 1H), 7.86 (d, 1H), 8.10−8.04 (m, 2H), 8.23 (d, 1H), 8.42 (dd, 1H), 8.48 (dd, 1H), 8.55 (d, 1H), 14.44 (s, 1H). MS (DCI/NH_3) *m/e* 305 $(M + H)^+$.

3-Methoxy-benzo[*a*]phenazine-11-carboxylic acid was prepared in an analogous manner starting from 5-amino-2 naphthol to give the title compound. ^{1}H NMR (DMSO-d_6) δ 3.99 (s, 3H), 7.55 (dd, 1H), 7.67 (d, 1H), 7.96−8.10 (m, 2H), 8.23 (d, 1H), 8.51 (d, 2H), 8.94 (d, 1H), 14.60 (br. s, 1H). MS (DCI/NH_3) *m/e* 305 $(M + H)^+$.

4,10-Dimethoxy-benzo[*a*]phenazine-11-carboxylic Acid. 2-Amino-6-methoxy-3-nitro-benzoic acid methyl ester was prepared using a procedure analogous to that described in the literature.[24] Hydrolysis of 2-amino-6-methoxy-3-nitro-benzoic acid methyl ester (1.00 g, 4.42 mmol) was achieved using potassium hydroxide in refluxing ethanol for 2 h to yield 2-amino-6-methoxy-3-nitro-benzoic acid (773 mg, 82%). Hydrogenation of the 2-amino-6-methoxy-3-nitro-benzoic acid (1.0 g, 4.72 mmol) group was performed in acetic acid/water over 10% palladium on carbon catalyst on a Parr apparatus at 50 psi using hydrogen to yield 2,3-diamino-6-methoxy-benzoic acid (730 mg, 52%). 2,3-Diamino-6-methoxy-benzoic acid (729 mg, 2.41 mmol) was reacted with 5-methoxy-[1,2]naphthoquinone as described in the general method to yield the desired title compound (628 mg,78%). ^{1}H NMR (DMSO-d_6) δ 4.05 (s, 3H), 4.10 (s, 3H), 7.45 (d, 1H), 7.78−7.82 (m, 1H), 7.92 (d, 1H), 8.03 (d, 1H), 8.36−8.40 (m, 2H), 8.78 (d, 1H).

4-Methoxy-8-methyl-benzo[*a*]phenazine-11-carboxylic Acid. 2,3-Diamino-4-methylbenzoic acid was prepared from 4-methyl anthranilic acid according to the literature.[11] This was reacted with 5-methoxy[1,2]naphthoquinone as described in the general method[14] to yield the desired title compound. ^{1}H NMR (DMSO-d_6) δ 2.97 (s, 3H), 4.06 (s, 3H), 7.50 (d, 1H), 7.85−7.90 (m, 1H), 7.97 (d, 1H), 8.02 (d, 1H), 8.45−8.50 (m, 2H), 8.57 (d, 1H).

9-Chloro-4-methoxy-benzo[*a*]phenazine-11-carboxylic Acid. 5-Chloro-3-nitroanthranilic acid was prepared according to the procedure described by Flippin.[24] Hydrogenation of this material in ethyl acetate using 10% palladium on carbon at 50 psi using hydrogen for 2 h yielded 2,3-diamino-5-chlorobenzoic acid. This was reacted with 5-methoxy[1,2]naphthoquinone as described in the general method to yield the desired title compound. ^{1}H NMR (DMSO-d_6) δ 4.05 (s, 3H), 7.48 (d, 1H), 7.82−7.86 (m, 1H), 7.92 (d, 1H), 8.30 (d, 1H), 8.48 (d, 1H), 8.54 (d, 1H), 8.68 (d, 1H), 14.1 (br. s, 1H).

4-Methoxy-10-methylamino-benzo[*a*]phenazine-11-carboxylic Acid Methyl Ester. 2,3-Diamino-6-fluoro-benzoic acid methyl ester (1.0 g, 5.43 mmol) was coupled with 5-methoxy[1,2]naphthoquinone (0.73 g, 3.88 mmol) as previously described in the general methods to give 10-fluoro-4-methoxybenzo[*a*]phenazine-11-carboxylic acid methyl ester (530 mg, 77%). The 10-fluoro-4-methoxybenzo[*a*]phenazine-11-carboxylic acid methyl ester (0.1 g, 0.29 mmol) was dissolved in a 2 M solution of methylamine in tetrahydrofuran (5 mL). The reaction was stirred for 15 h at room temperature. The solvent and methylamine were removed in vacuo to give the crude product obtained (0.10 g, 97%). ^{1}H NMR ($CDCl_3$) δ 4.05 (s, 3H), 4.18 (s, 3H), 7.18 (d, 1H), 7.50 (d, 1H), 7.70 (t, 1H), 7.87 (d, 1H), 8.18 (d, 1H), 8.36 (br. s, 1H), 8.40 (d, 1H), 8.95 (d, 1H).

10-Amino-4-methoxy-benzo[*a*]phenazine-11-carboxylic Acid Methyl Ester. 10-Fluoro-4-methoxybenzo[*a*]phenazine-11-carboxylic acid methyl ester (0.47 g, 1.40 mmol) was dissolved in DMF (10 mL), sodium azide (0.90 g, 13.9 mmol) was added, and the reaction was heated at 90 °C for 15 h. Water (50 mL) and 2 M sodium hydroxide (25 mL) were then added, and the resulting brown precipitate was filtered and washed with water and diethyl ether to give the title product (0.24 g, 51%). ^{1}H NMR ($CDCl_3$) δ 4.20 (s, 3H), 7.24 (d, 1H), 7.70 (t, 1H), 7.72 (d, 1H), 7.90 (d, 1H), 8.46 (d, 1H), 8.51 (d, 1H), 8.85 (d, 1H).

The amines used to couple to a derivative of the substituted benzo[*a*]phenazine-11-carboxylic acids above were either commercially available or synthesized by known methods as outlined below.

4-Aza-DL-leucine Methyl Ester Dihydrochloride. Methanol (150 mL) was saturated with anhydrous hydrogen chloride gas, and to this solution was added 4-aza-DL-leucine (4.86 g, 23.7 mmol). The reaction mixture was stirred overnight at room temperature. The solvent was removed in vacuo to yield the title compound (5.2 g, 100%). ^{1}H NMR (d_3-MeOD) δ 3.16 (s, 6H), 3.71 (m, 1H), 3.96 (td, 1H), 4.04 (s, 3H), 4.78 (t, 1H).

***N*^{1},*N*^{1}-Dimethyl-butane-1,2-diamine Dihydrochloride.** Methyl *N*-(*tert*-butoxycarbonyl)-2-aminobutyrate was prepared as described in the literature.[26] Dropwise treatment over 45 min of methyl *N*-(*tert*-butoxycarbonyl)-2-aminobutyrate (4.34, 20 mmol) in toluene (50 mL) with 1 M diisobutyl aluminum hydride in toluene (50 mL, 50 mmol) at −78 °C followed by stirring for a further 45 min yielded *N*-(*tert*-butoxycarbonyl)-2-aminobutanal (2.58 g, 76%) as previously described.[27] A mixture of *N*-(*tert*-butoxycarbonyl)-2-aminobutanal (1.85 g, 13.1 mmol), dimethylamine hydrochloride (1.61 g, 19.7 mmol), sodium acetate (1.21 g, 14.8 mmol), and sodium cyanoborohydride (0.83 g, 13.2 mmol) in methanol was stirred at room temperature for 24 h. The pH was adjusted to 6−7 using acetic acid and monitored during the reaction. The reaction mixture was then concentrated in vacuo, and the residue was dissolved in ethyl acetate and washed with water. The organic layer was dried ($MgSO_4$) and the solvent removed in vacuo to yield a colorless oil, which was purified on silica eluting with ethyl acetate 70% and hexane 30% to yield the desired dimethylamine derivative as a pale oil (0.56 g, 3.3 mmol). To this compound (260 mg, 1.53 mmol) was added a 4.0 M solution of HCl in dioxane (2 mL) carefully, and the reaction mixture was stirred for 90 min. The reaction mixture was then concentrated in vacuo to yield the desired title compound as an off-white solid in quantitative yield. ^{1}H NMR (d_3-MeOD) δ 1.20 (t, 3H), 1.90 (m, 2H), 3.09 (s, 6H), 3.53 (m, 2H), 3.82 (m, 1H).

3*N*^{1},*N*^{1}-Trimethyl-butane-1,2-diamine Dihydrochloride. 2-Methyl-3-[*N*-(*tert*-butoxycarbonyl)amino]butanal was prepared from DL-valine methyl ester hydrochloride as previously described.[26] A mixture of 2-methyl-3-[*N*-(*tert*-butoxycarbonyl)amino]butanal (3.76 g, 18.7 mmol), dimethylamine hydrochloride (3.04 g, 37.3 mmol), sodium acetate (2.45 g, 29.9 mmol), and sodium cyanoborohydride (1.76 g, 28.0 mmol) in methanol (80 mL) was stirred at room temperature for 18 h. The solvent was removed in vacuo, the residue was dissolved in ethyl acetate (300 mL), washed with water (300 mL), and dried ($MgSO_4$), the solvent was removed in vacuo, and the product was purified on silica eluting with 90% dichlorometane and 10% methanol to yield *N*^{1},*N*^{1}-Dimethyl-2-(*N*-*tert*-butoxycarbonyl)-3-methyl-butane-1,2-diamine as a white solid (2.2 g, 51%). To *N*^{1},*N*^{1}-Dimethyl-2-(*N*-*tert*-butoxycarbonyl)-3-methyl-butane-1,2-diamine (2.2 g, 9.5 mmol) was added a 4.0 M solution of hydrochloric acid in dioxane (10 mL) at room temperature. After the mixture was stirred for 30 min, the volatile components were removed in vacuo to yield the desired title compound as a white solid (1.8 g, 93%). MH^+ 131. ^{1}H NMR (d_3-MeOD) δ 1.20 (d, 6H), 2.20 (m, 1H), 3.10 (s, 6H), 3.50 (dd, 1H), 3.60 (dd, 1H), 3.75 (m, 1H).

(*R*)-3*N*^{1},*N*^{1}-Trimethyl-butane-1, 2-diamine diydrochloride salt was prepared as described above by using D-valine as the starting material. ^{1}H NMR (d_3-MeOD) δ 1.20 (d, 6H), 2.20 (m, 1H), 3.10 (s, 6H), 3.50 (dd, 1H), 3.60 (dd, 1H), 3.75 (m, 1H).

***N*^{1},*N*^{1}-Dimethyl-3-phenyl-propane-1,2-diamine Dihydrochloride.** 1-Phenyl-2-[*N*-(*tert*-butoxycarbonyl)amino]propanal was prepared from DL-phenylalanine methyl ester hydrochloride as previously described.[26] A mixture of 1-phenyl-2-[*N*-(*tert*-butoxycarbonyl)amino]propanal (1.39 g, 5.58 mmol), dimethylamine hydrochloride (0.91 g, 11.2 mmol), sodium acetate (731 mg, 8.9 mmol), and sodium cyanoborohydride (526 mg, 8.36 mmol) in methanol (30 mL) was stirred at room temperature for 18 h. The solvent was removed in vacuo, and

the residue was dissolved in ethyl acetate (200 mL), washed with water (200 mL), and dried ($MgSO_4$), the solvent was removed in vacuo, and the product was purified on silica eluting with 90% dichlorometane and 10% methanol to yield N^1,N^1-dimethyl-2-(*N*-*tert*-butoxycarbonyl-3-phenyl-propane-1,2-diamine as a white solid (912 mg, 59%). To N^1,N^1-dimethyl-2-(*N*-*tert*-butoxycarbonyl-3-phenyl-propane-1,2-diamine (912 mg, 3.2 mmol) was added a 4.0 M solution of hydrochloric acid in dioxane (10 mL) at room temperature. After the mixture was stirred for 30 min, the volatile components were removed in vacuo to yield the desired title compound as a white solid (826 mg, 100%). ^{1}H NMR (d_3-MeOD) δ 3.00 (br s, 1H), 3.15 (m, 2H), 3.50 (dd, 1H), 4.15 (m, 1H), 7.40−7.50 (m, 5H).

(*S*)-N^1,N^1-Dimethyl-propane-1,2-diamine Diydrochloride. 2-(*S*)-[*N*-(*tert*-Butoxycarbonyl)amino]propanal was prepared from L-alanine methyl ester hydrochloride according to the literature procedure.[26] A mixture of the 2-(*S*)-[*N*-(*tert*-butoxycarbonyl)amino]propanal (1.58 g, 9.1 mmol), dimethylamine hydrochloride (1.49 g, 18.3 mmol), sodium acetate (1.20 g, 14.6 mmol), and sodium cyanoborohydride (0.86 g, 13.7 mmol) in methanol (10 mL) was stirred at room temperature for 18 h. The reaction mixture was dissolved in ethyl acetate (40 mL), washed with water (30 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to yield a viscous oil. This was dissolved in dichloromethane (30 mL), extracted with citric acid (10 mL), basified with 1 M sodium hydroxide, and re-extracted with ethyl acetate (2 × 20 mL). The organic layer was reduced in vacuo, and the product was purified on silica eluting with 90% dichlorometane and 10% methanol to yield (*S*)-N^1,N^1-dimethyl-propane-1,2-diamine as a white solid (537 mg 15%). To (*S*)-N^1,N^1-dimethyl-propane-1,2-diamine (502 mg, 2.5 mmol) was added a 4.0 M solution of hydrochloric acid in dioxane (5.5 mL) at room temperature. After the mixture was stirred for 30 min, the volatile components were removed in vacuo to yield the desired title compound as a viscous oil (450 mg, 100%). ^{1}H NMR (d_3-MeOD) δ 1.20 (d, 3H), 3.27 (m, 1H), 3.44 (m, 1H), 3.57 (s, 6H), 3.64 (dd, 1H).

(*R*)-N^1,N^1-Dimethyl-propane-1,2-diamine Dihydrochloride. 2-(*R*)-[*N*-(*tert*-Butoxycarbonyl)amino]propanal was prepared from D-alanine methyl ester hydrochloride according to the procedure described in the literature.[26]

A mixture of 2-(*R*)-[*N*-(*tert*-butoxycarbonyl)amino]propanal (16.21 g, 94 mmol), dimethylamine hydrochloride (15.28 g, 187.6 mmol), sodium acetate (11.53 g, 140 mmol), and sodium cyanoborohydride (8.24 g, 131 mmol) in methanol (250 mL) was stirred at room temperature for 18 h maintaining pH at 6−7 by the dropwise addition of acetic acid. The reaction mixture was dissolved in ethyl acetate (400 mL), washed with water (200 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to yield a viscous oil which was purified on silica eluting with 90% dichlorometane and 10% methanol to yield (*R*)-N^1,N^1-dimethyl-propane-1,2-diamine as a white solid (10.81 g, 57%). To this compound (3.17 g, 15.7 mmol) was added a 4.0 M solution of hydrochloric acid in dioxane (20 mL) at room temperature. After the mixture was stirred for 1 h, the volatile components were removed in vacuo to yield the desired title compound as a viscous oil (4.28 g, 100%). ^{1}H NMR (d_3-MeOD) δ 1.57 (d, 3H), 3.08 (s, 6H), 3.50 (dd, 1H), 3.62, (dd, 1H), 4.03, (q, 1H).

(*S*)-2-Amino-3-(dimethylamino)-propan-1-ol Dihydrochloride. *N*-[(*tert*-Butoxy)carbonyl]-*O*-(*tert*-butyldimethylsilyl)-*R*-serine methyl ester was prepared according to the literature from D-serine methyl ester hydrochloride.[27] Treatment of *N*-[(*tert*-butoxy)carbonyl]-*O*-(*tert*-butyldimethylsilyl)-*R*-serine methyl ester with diisobutyl aluminum hydride in toluene at −70 °C for 2 h yielded the correponding aldehyde.[27] A mixture of the crude aldehyde (4.43 g, 22 mmol), dimethylamine hydrochloride (2.26 g. 27.7 mmol), sodium cyanoborohydride (1.31 g, 20.8 mmol), and sodium acetate (1.83 g, 22.2 mmol) was stirred in methanol (55 mL) for 24 h at room temperature. Aqueous workup yielded the dimethylamino derivative which was dissolved in dioxane, and to this was added a 4.0 M solution of hydrochloric acid in dioxane and the mixture stirred for 20 min. Concentration of the mixture in vacuo and purification of the product on silica eluting with 90% dichlorometane and 10% methanol yielded the desired title compound as a white solid (1.94 g, 27%). ^{1}H NMR (d_3-MeOD) δ 3.10 (s, 6H), 3.54 (m, 1H), 3.66 (dd, 1H), 3.93 (m, 3H).

3(*S*)-Amino-4-(dimethylamino)-butan-2(*S*)-ol Dihydrochloride. Hydrogen chloride gas was bubbled through a solution of D-threonine (20 g, 168 mmol) in methanol (50 mL). The resulting solution was stirred for 5 h at room temperature and then reduced in vacuo to yield D-threonine methyl ester hydrochloride as a white solid in quantitative yield. Treatment of D-threonine methyl ester (28.45 g, 168 mmol, with di-*tert*-butyl dicarbonate (36.64, 168 mmol) in acetonitrile (275 mL) and triethylamine (16.9 g, 168 mmol) yielded *N*-(*tert*-butoxycarbonyl)-D-threonine methyl ester (32.7 g, 140 mmol). Treatment of *N*-(*tert*-butoxycarbonyl)-D-threonine methyl ester (32.7 g, 140 mmol) with *tert*-butyldimethylsilyl chloride (26.2 g, 174 mmol) in dichloromethane (170 mL) with imidazole (19,1 g, 281 mmol) yielded the corresponding TBDMS protected alcohol (35.5 g, 102 mmol). Treatment of this compound (15 g, 43 mmol) in toluene (130 mL) with 1 M diisobutyl aluminum hydride in toluene (52 mL, 52 mmol) at −78 °C for 4 h yielded the corresponding aldehyde (12.1 g, 38 mmol).[27] Reductive amination was carried out as described previously to give the dimethylamino derivative which was treated with 4.0 M HCl in dioxane as described previously to yield a golden oil. Trituration with ether gave the title compound as a white solid (3.98 g, 30%). ^{1}H NMR (d_3-MeOD) δ 1.42 (d, 3H), 3.10 (s, 6H), 3.53 (dd, 1H), 3.68 (dd, 1H), 3.80 (m, 1H), 4.17 (dd, 1H).

3-Amino-4-(dimethylamino)-butan-1-ol Dihydrochloride. DL-Homoserine (6.0 g, 50.4 mmol) was treated with methanol saturated with HCl gas (100 mL) and stirred at room temperature for 18 h. The solvent was removed in vacuo to yield a white solid which was treated with with di-*tert*-butyl dicarbonate (11.5 g, 52.9 mmol) in acetonitrile (90 mL) and triethylamine (7.7 mL, 55.4 mmol) to yield the *N*-*tert*-butoxycarbonyl protected derivative (4.52 g, 22.5 mmol). Treatment of this compound (2.55 g, 12.7 mmol) in toluene (40 mL) with 1 M diisobutyl aluminum hydride in toluene (22.8 mL, 22.8 mmol) at −78 °C for 4 h yielded the corresponding lactol (2.0 g, 9.8 mmol).[27] Reductive amination on the lactol (502 mg, 2.47 mmol) was carried out as described previously to yield 3-[*N*-(*tert*-butoxycarbonyl)amino]-4-(dimethylamino)-butan-1-ol (140 mg, 0.6 mmol) which was treated with 4.0 M HCl in dioxane as described above to yield the desired title compound as a white solid (138 mg, 100%). ^{1}H NMR (d_3-MeOD) δ 2.10 (m, 2H), 3.10 (s, 6H), 3.59 (ddd, 2H), 3.92 (m, 2H), 4.05 (m, 1H).

1-Methyl-3-(*R*)-aminopyrrolidine Dihydrochloride. A solution of 3-*R*-(−)-1-benzyl-3-aminopyrrolidine (847 mg, 4.8 mmol) in *tert*-butyl alcohol (10 mL) and 1.0 N sodium hydroxide solution (4.8 mL) was treated dropwise with a solution of di-*tert*-butyl dicarbonate (1.06 g, 4.8 mmol) in *tert*-butyl alcohol (5 mL). After 1.5 h, the *tert*-butyl alcohol was removed in vacuo, the residue was dissolved in ethyl acetate (50 mL), washed with water (50 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to yield the desired 3-*N*-*tert*-butoxycarbonyl protected derivative as a colorless gum (1.3 g, 4.7 mmol). A solution of the 3-*N*-*tert*-butoxycarbonyl protected derivative (800 mg, 2.9 mmol) in tetrahydrofuran (5 mL) and ethanol (5 mL) was stirred over palladium hydroxide catalyst under an atmosphere of hydrogen for 24 h. The reaction mixture was then filtered through Celite and the solvent removed in vacuo to yield the desired 3-*N*-*tert*-butoxycarbonyl-3-(*R*)-aminopyrrolidine in quantitative yield. To a solution of *N*-*tert*-butoxycarbonyl-3-(*R*)-aminopyrrolidine (437 mg, 2.35 mmol) in methanol (10 mL) was added 37% formaldehyde solution in H_2O (0.52 mL) and sodium borohydride (271 mg, 7.0 mmol). The reaction mixture was stirred for 24 h at room temperature and then reduced in vacuo. The residue was dissolved in chloroform (10 mL), washed with brine (10 mL) and $NaHCO_3$ solution (10 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to yield the desired 3-*N*-*tert*-

butoxycarbonyl-3-(*R*)-amino-1-methylpyrrolidine as a gum (390 mg, 1.95 mmol), deprotection with 4.0 M HCl solution in dioxane as described above yielded the desired title compound (337 mg, 100%) which was used without further purification.

***N*¹,*N*¹,*N*²,*N*²-Tetramethyl-propane-1,2,3-triamine Trihydrochloride.** To ice cooled thionyl chloride (35 mL) was added 1,3-bis(dimethylamino)-propan-2-ol (4.91 g, 33.58 mmol) dropwise over 45 min with stirring. After the addition was complete, the mixture was stirred for a further 4 h. Excess thionyl chloride was removed under reduced pressure to give the product as the hydrochloride salt as a cream solid (8.9 g). The free base was obtained by treating a suspension of the hydrochloride salt in toluene (18 mL) with sodium hydroxide (2.4 equiv) in water (14 mL) for 30 min. The organic layer was removed, dried over $MgSO_4$, and filtered, and the solvent was removed in vacuo to yield 2-chloro-*N*,*N*,*N*¹,*N*¹-tetramethylpropane-1,3-diamine as a yellow liquid. 2-Chloro-*N*,*N*,*N*¹,*N*¹-tetramethylpropane-1,3-diamine (1.6 g, 9.71 mmol) as a solution in toluene (14 mL) was treated with potassium phthalimide (1.98 g, 10.68 mmol). The stirred mixture was heated at reflux for 18 h and cooled to ambient temperature, the solvent was removed under reduced pressure to yield a beige solid, and recrystallization from diethyl ether afforded a fawn solid (1.85 g, 6.7 mmol). This solid (0.953, 3.46 mmol) as a solution in ethanol (10 mL) was treated with hydrazine hydrate (0.22 mL, 6.93 mmol), and the mixture was stirred at ambient temperature for 18 h. The suspension was removed by filtration, the filtrate acidified with 2 mL of 2 M hydrochloric acid, and the solvent removed in vacuo to give the product as a cream solid (795 mg, 90%). MH^+ 172. ^{1}H NMR (d_3-MeOD) δ 2.40 (s, 6H), 2.45 (s, 6H), 2.54 (m, 1H), 2.65 (m, 1H), 2.97 (m, 1H), 3.01 (t, 1H), 3.12 (m, 1H).

General Methods for the Preparation of Substituted Benzo[*a*]-11-carboxamides. Method A. Activation of 5 with 1,1′-Carbonyl Diimidazole (CDI). 4-Methoxy-benzo-[*a*]phenazine-11-carboxylic Acid (2-(Dimethylamino)-ethyl)-amide (4c). A mixture of 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (129 mg, 0.42 mmol) and CDI (138 mg, 0.85 mmol) was stirred in dry DMF (8 mL) at room temperature for 4 h. To this mixture was added *N*,*N*-dimethylethylenediamine (0.5 mL, 4.5 mmol), and the reaction mixture was stirred at room temperature for a further 30 min. The volatile components were then removed in vacuo, the residue was dissolved in dichloromethane (20 mL), washed with water (2 × 20 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to provide crude product. This was purified using flash chromatography eluting with 5% methanol in dichloromethane to yield the title compound as a bright yellow solid (120 mg, 76%), mp 166−169 °C. ^{1}H NMR (DMSO-d_6) δ 2.36 (s, 6H), 2.61 (t, 2H, J = 5.5 Hz), 3.72 (q, 2H, J = 5.5 Hz), 4.02 (s, 3H), 7.44 (dd, 1H, J = 2.65, 8.9 Hz), 7.64 (d, 1H, J = 2.6 Hz), 7.95−8.03 (m, 2H), 8.24 (d, 1H, J = 9.3 Hz), 8.40 (dd, 1H, J = 1.49, 8.2 Hz), 8.74 (dd, 1H, J = 1.4, 7.6 Hz), 9.32 (d, 1H, J = 8.9 Hz), 10.39 (t, 1H). MS (DCI/NH_3) *m*/*e* 375 $(M + H)^+$. Anal. ($C_{22}H_{22}N_4O_2$) C, H, N.

Method B: Activation of 5 with Thionyl Chloride. 4-Methoxy-benzo[*a*]phenazine-11-carboxylic Acid (2-(Dimethylamino)-1,1-dimethyl-ethyl)-amide (4l″). A mixture of 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (1.0 g, 3.2 mmol) and thionyl chloride (10 mL) was heated to reflux for 6 min. The thionyl chloride was then removed in vacuo. The residue was dissolved in dry dichloromethane (10 mL), the solution cooled to 0 °C, and 1-(dimethylamino)-2-methyl-2-aminopropane (5 mL) was added. After being stirred for 2 h, the reaction mixture was diluted with dichloromethane (50 mL), washed with sodium bicarbonate solution (50 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to provide crude product which was purified on silica eluting with 95% dicloromethane and 5% methanol to give the title compound as a yellow solid (945 mg, 74%), mp 158−162 °C. ^{1}H NMR (DMSO-d_6) δ 1.56 (s, 6H), 2.30 (s, 6H), 2.78 (s, 2H), 4.07 (s, 3H), 7.48 (d, 1H), 7.86 (m, 1H), 7.98 (d, 1H), 8.07 (m, 1H), 8.43 (d, 1H), 8.50 (d, 1H), 8.56 (d, 1H), 8.95 (d, 1H), 9.67 (br, 1H). MS (DCI/NH_3) *m*/*e* 403 $(M + H)^+$.

Method C: Reaction of Amines Directly with the Substituted Benzo[*a*]-11-carboxylic Acid Methyl Esters. 4-Methoxy-benzo[*a*]phenazine-11-carboxylic Acid (2-(Dimethylamino)-1-methyl-ethyl)-amide (4j″). A mixture of 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid methyl ester (350 mg, 1.1 mmol) and 1-(dimethylamino)-2-propylamine (2 mL, 15.5 mmol) was heated to 110 °C for 4 h. The reaction mixture was then cooled, and the excess amine was removed in vacuo. The residue was then purified using flash chromatography on silica (ethyl acetate and then 25% methanol in ethyl acetate) to yield the title compound (164 mg, 38%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.53 (d, 3H, J = 6.5 Hz), 2.37 (s, 6H), 2.55 (dd, 1H, J = 12.2, 6.2 Hz), 2.84 (dd, 1H J = 12.2, 7.8 Hz), 4.10 (s, 3H), 4.55−4.65 (m, 1H), 7.28−7.30 (m, 1H), 7.75−7.80 (m, 1H), 7.95−8.02 (m, 2H), 8.42 (dd, 1H J = 8.4, 1.5 Hz), 8.57 (d, 1H, J = 9.5 Hz), 8.81 (d, 1H, J = 8.2 Hz), 9.02 (dd, 1H, J = 7.3, 1.6 Hz), 10.9 (d, 1H, J = 7.2 Hz). MS (DCI/NH_3) *m*/*e* 389 $(M + H)^+$.

The following compounds were prepared in an analogous manner from the appropriate substituted benzo[*a*]-11-carboxylic acid activated as the acyl imidazole and reacted with the appropriate amine using general method A.

3-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4b) was prepared from 3-methoxy-benzo[*a*]phenazine-11-carboxylic acid (129 mg, 0.90 mmol) and *N*,*N*-dimethylethylenediamine (0.5 mL, 4.5 mmol) to yield the title compound as a yellow solid (281 mg, 84%), mp 172−173 °C. ^{1}H NMR (DMSO-d_6) δ 2.36 (s, 6H), 2.61 (t, 2H, J = 5.5 Hz), 3.72 (q, 2H, J = 5.5 Hz), 4.02 (s, 3H), 7.44 (dd, 1H, J = 2.65, 8.9 Hz), 7.64 (d, 1H, J = 2.6 Hz), 8.03−7.95 (m, 2H), 8.24 (d, 1H, J = 9.3 Hz), 8.40 (dd, 1H, J = 1.49, 8.2 Hz), 8.74 (dd, 1H, J = 1.4, 7.6 Hz), 9.32 (d, 1H, J = 8.9 Hz), 10.39 (t, 1H). MS (DCI/NH_3) *m*/*e* 375 $(M + H)^+$. Anal. ($C_{22}H_{22}N_4O_2$) C, H, N.

2-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4a) was prepared from 2-methoxy-benzo[*a*]phenazine-11-carboxylic acid (184 mg, 0.61 mmol) and *N*,*N* dimethylethylenediamine (0.5 mL, 4.5 mmol) to give the title compound as a yellow solid 191 mg, 84%), mp 181−184 °C. ^{1}H NMR (DMSO-d_6) δ 2.47 (s, 6H), 2.90 (t, 2H, J = 2.91 Hz), 3.79 (q, 2H, J = 6.8 Hz, 4.06 (s, 3H), 7.56 (m, 1H), 7.81 (d, 1H J = 9.20 Hz), 8.04 (m, 2H), 8.18 (d, 1H J = 9.24 Hz), 8.41(d, 1H J = 7.48 Hz), 8.55 (m, 2H), 9.97 (br.t, 1H). MS (DCI/NH_3) *m*/*e* 375 $(M + H)^+$.

4-Nitro-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4i) was prepared from the mixture of 4-nitro-benzo[*a*]phenazine-11-carboxylic acid, 3-nitro-benzo[*a*]phenazine-11-carboxylic acid (69 mg, 0.21 mmol), and *N*,*N*-dimethylethylenediamine (0.25 mL, 2.25 mmol) and then purified using flash chromatography to give the title compound as a yellow gum (26 mg, 36%). ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 6H), 2.75 (t, 2H), 3.95 (q, 2H), 7.95 (t, 1H), 8.10 (t, 1H), 8.30 (d, 1H), 8.45 (d, 1H), 8.50 (d, 1H), 8.70 (d, 1H), 9.10 (dd, 1H), 9.90 (d, 1H), 10.50 (br, 1H). MS (DCI/NH_3) *m*/*e* 390 $(M + H)^+$.

3-Nitro-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4h) was prepared from the mixture of 4-nitro-benzo[*a*]phenazine-11-carboxylic acid, 3-nitro-benzo[*a*]phenazine-11-carboxylic acid (69 mg, 0.21 mmol), and *N*,*N*-dimethylethylenediamine (0.25 mL, 2.25 mmol) and then purified using flash chromatography to give the title compound as a yellow solid (7 mg, 9%), mp 245.5−246.5 °C. ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 6H), 3.10 (t, 2H), 4.05 (q, 2H), 8.10 (dd, 1H), 8.15 (dd, 2H), 8.20 (d, 1H), 8.45 (d, 1H), 8.60 (dd, 1H), 9.0 (dd, 1H), 9.90 (d, 1H), 10.40 (br. s, 1H). MS (DCI/NH_3) *m*/*e* 390 $(M + H)^+$.

4-Benzyloxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4c′) was prepared from 4-benzyloxy-benzo[*a*]phenazine-11-carboxylic acid (46 mg, 0.12 mmol) and *N*,*N*-dimethylethylenediamine (70 μL, 0.60 mmol) to give the title compound as a yellow solid (20 mg, 37%), mp 172−173 °C. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 6H), 2.75 (t, 2H J = 6.0 Hz), 3.89−3.93 (m, 2H), 5.35 (s, 2H), 7.33−7.49 (m, 4H),

7.55 (d, 2H, J = 7.2 Hz), 7.73−7.77 (m, 1H), 7.95−8.0 (m, 2H), 8.40 (dd, 1H, J = 8.6, 1.5 Hz), 8.63 (d, 1H, J = 9.4 Hz), 9.00−9.07 (m, 2H), 10.90 (br. s, 1H). MS (DCI/NH_3) *m/e* 451 $(M + H)^+$.

4-Prop-2-ynyloxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4d′) was prepared from 4-prop-2-ynyloxy-benzo[*a*]phenazine-11-carboxylic acid (55 mg, 0.17 mmol) and *N*,*N*-dimethylethylenediamine (100 μL, 0.85 mmol) to give the title compound as a yellow solid (19 mg, 28%), mp 173−174 °C. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 6H), 2.55 (t, 1H, J = 2.4 Hz), 2.70 (t, 2H, J = 6.0 Hz), 3.82−3.87 (m, 2H), 4.95 (d, 2H J = 2.4 Hz), 7.32 (d, 1H, J = 8.3 Hz), 7.70−7.74 (m, 1H), 7.90−7.95 (m, 2H), 8.35 (dd, 1H, J = 8.50, 1.6 Hz), 8.50 (d, 1H, J = 9.8 Hz), 8.95 (dd, 1H, J = 7.30, 1.5 Hz), 9.01 (d, 1H, J = 8.2 Hz), 10.80 (br. s, 1H). MS (DCI/NH3) *m/e* 399 $(M + H)^+$.

3,4-Dimethoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4q′) was prepared from 3,4-dimethoxy-benzo[*a*]phenazine-11-carboxylic acid (45 mg, 0.13 mmol) and *N*,*N*-dimethylethylenediamine (44 μL, 0.40 mmol) to give the title compound as a yellow solid (21 mg, 39%), mp 155−159 °C. ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 6H), 2.70 (t, 2H, J = 6.0), 3.84 (q, 2H, J = 6.0 Hz), 4.01 (s, 3H), 4.04 (s, 3H), 7.40 (d, 1H, J = 9.0 Hz), 7.90−7.81 (m, 2H), 8.40−8.30 (m, 2H), 8.91 (d, 1H J = 7.3 Hz), 9.11 (d, 1H, J = 8.9 Hz), 10.82 (br. s, 1H). MS (DCI/NH3) *m/e* 405 $(M + H)^+$.

4-Ethoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4a′) was prepared from 4-ethoxy-benzo[*a*]phenazine-11-carboxylic acid (240 mg, 0.75 mmol) and *N*,*N*-dimethylethylenediamine (411 μL, 3.75 mmol) to give the title compound as a yellow solid (56 mg, 19%), mp 151−152 °C. ^{1}H NMR (DMSO-d_6) δ 1.52 (t, 3H, J = 6.9 Hz), 2.35 (s, 6H), 2.65 (t, 2H, J = 5.9 Hz), 3.72−3.75 (m, 2H), 4.30 (q, 2H, J = 6.9 Hz), 7.43 (d, 1H, J = 7.9 Hz), 7.80−7.84 (m, 1H), 7.92 (d, 1H, J = 9.5 Hz), 8.05−8.10 (m, 1H), 8.42 (dd, 1H, J = 8.6, 1.6 Hz), 8.45 (d, 1H, J = 9.5 Hz), 8.72 (dd, 1H, J = 7.1, 1,5 Hz), 8.94 (d, 1H, J = 8.2 Hz), 10.25 (t, 1H, J = 5.0 Hz). MS (DCI/NH_3) *m/e* 389 $(M + H)^+$. Anal. $C_{23}H_{24}N_4O_2$ C, H, N.

4-Isobutoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4b′) was prepared from 4-isobutoxy-benzo[*a*]phenazine-11-carboxylic acid (10.8 mg, 0.031 mmol) and *N*,*N*-dimethylethylenediamine (17 μL, 0.16 mmol) to give the title compound as a yellow solid (9.6 mg, 74%), mp 152−154 °C. ^{1}H NMR ($CDCl_3$) δ 10.87 (br, 1H), 8.93 (m, 2H), 8.54 (d, 1H, J = 9.5 Hz), 8.35 (dd, 1H, J = 8.4, 1.5 Hz), 7.89 (m, 2H), 7.69 (t, 1H, J = 8.0 Hz), 7.19 (m, 1H), 3.93 (d, 2H, J = 6.4 Hz), 3.87 (m, 2H), 2.75 (m, 2H), 2.40 (s, 6H), 2.24 (m, 1H), 1.11 (d, 6H, J = 6.8 Hz). MS (EI) *m/e* 416 (M^+).

4-(2-Methoxy-ethoxy)-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4i′) was prepared from 4-(2-methoxy-ethoxy)-benzo[*a*]phenazine-11-carboxylic acid (45 mg, 0.13 mmol) and *N*,*N*-dimethylethylenediamine (70 μL, 0.64 mmol) to give the title compound as a yellow solid (5 mg, 9%). ^{1}H NMR ($CDCl_3$) δ 2.69 (s, 6H), 2.92−3.10 (m, 2H), 3.49 (s, 3H), 3.83−3.93 (t, 2H, J = 4.4 Hz), 3.94−4.10 (m, 2H), 4.28−4.40 (t, 2H, J = 4.4 Hz), 7.22 (d, 1H, J = 8.0 Hz), 7.80 (t, 1H), 7.84−7.96 (m, 2H, J = 4.7, 8.3 Hz), 8.37 (d, 1H, J = 8.2 Hz), 8.54 (d, 1H, J = 9.4 Hz), 8.82 (d, 1H, J = 7.4 Hz), 8.90 (d, 1H, J = 6.9 Hz), 11.10 (br. s, 1H). MS (DCI/NH_3) *m/e* 419 (M^+).

[11-(2-Dimethylamino-ethylcarbamoyl)-benzo[*a*]phenazin-4-yloxy]-acetic acid ethyl ester (4k′) was prepared from 4-ethoxycarbonylmethoxy-benzo[*a*]phenazine-11-carboxylic acid (144 mg, 0.39 mmol) and *N*,*N*-dimethylethylenediamine (210 μL, 1.95 mmol) to give the title compound as a yellow solid (34 mg, 22%), mp 167−168 °C. ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, J = 7.1 Hz), 2.36 (s, 6H), 8.94 (dd, 1H, J = 7.3, 1.5 Hz); 8.58 (d, 1H, J = 9.5 Hz) 8.34 (dd, 1H, J = 8.4, 1.5 Hz), 7.91 (m, 2H), 7.66 (t, 1H, J = 8.1 Hz), 7.08 (d, 1H, J = 8.0 Hz), 4.83 (2H, s), 4.27 (q, 2H, J = 7.1 Hz), 3.83 (m, 2H), 2.69 (m, 2H), 9.01 (d, 1H, J = 8.2 Hz), 10.76 (br. s, 1H). MS (DCI/NH_3) *m/e* 447 $(M + H)^+$. Anal.($C_{25}H_{26}N_4O_4$) C, H, N.

4-Methyl-benzo[*a*]phenazine-11-carboxylic acid(2-(dimethylamino)-ethyl)-amide (4p) was prepared from 4-methyl-benzo[*a*]phenazine-11-carboxylic acid (100 mg, 0.35 mmol) and *N*,*N*-dimethylethylenediamine (0.2 mL, 1.73 mmol) to give the title compound as a yellow solid (26 mg, 26%), mp 166−171 °C. ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 6H), 2.60 (t, 2H, J = 5.7 Hz), 2.75 (s, 3H), 3.91 (q, 2H, J = 5.7 Hz), 7.69 (t, 1H, J = 6.9 Hz), 7.73 (t, 1H, J = 7.6 Hz), 7.98 (d, 1H, J = 7.3 Hz), 8.02 (d, 1H, J = 9.6 Hz), 8.31 (d, 1H, J = 9.4 Hz), 8.42 (dd, 1H, J = 8.4, 1.5 Hz), 9.02 (dd, 1H, J = 7.3, 1.5 Hz), 9.34 (d, 1H, J = 7.9 Hz), 10.88 (br. s, 1H). MS (DCI/NH_3) *m/e* 359 $(M + H)^+$.

4-Fluoro-benzo[*a*]phenazine-11-carboxylic acid(2-(dimethylamino)-ethyl)-amide (4m) was prepared from 4-fluoro-benzo[*a*]phenazine-11-carboxylic acid (100 mg, 0.34 mmol) and *N*,*N*-dimethylethylenediamine (0.2 mL, 1.71 mmol) to give the title compound as a yellow solid (29 mg, 29%), mp 172−175 °C. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 6H), 2.68 (t, 2H, J = 5.8 Hz), 3.83 (q, 2H, J = 5.5 Hz), 7.47 (m, 1H), 7.71 (m, 1H), 7.93 (dd, 1H, J = 8.5, 7.2 Hz), 7.97 (d, 1H, J = 9.5), 8.29 (d, 1H, J = 9.5 Hz), 8.36 (dd, 1H, J = 8.4, 1.5 Hz), 8.97 (dd, 1H, J = 7.3, 1.5 Hz), 9.22 (d, 1H, J = 8.1 Hz), 10.67 (br. s, 1H). MS (DCI) *m/e* 363 $(M + H)^+$.

4-Methylsulfanyl-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4j) was prepared from 4-methylsulfanyl-benzo[*a*]phenazine-11-carboxylic acid which was prepared from 4-Fluoro-benzo[*a*]phenazine-11-carboxylic acid (58 mg, 0.2 mmol) by treatment with sodium thiomethoxide (55 mg, 1 mmol) in DMSO (3 mL). The mixture was heated at 130 °C for 2 h, quenched with acetic acid (2 mL) and water (5 mL), and extracted with ethyl acetate (2 × 10 mL). The organics were dried ($MgSO_4$) and reduced in vacuo to give the product which was used crude in the next step. ^{1}H NMR (DMSO-d_6) δ 2.70 (s, 3H), 7.91 (m, 2H), 8.11 (m, 2H), 8.45 (d, 1H), 8.52 (m, 2H), 9.00 (d, 1H). MS (DCI/NH3) *m/e* 321 $(M + H)^+$. 4-Methylsulfanyl-benzo[*a*]phenazine-11-carboxylic acid (223 mg, 0.17 mmol) and *N*,*N*-dimethylethylenediamine (100 μL, 0.85 mmol) gave the title compound as a yellow glass (4.7 mg, 9%). ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 6H), 2.66 (s, 3H), 2.75 (m, 2H), 3.90 (m, 2H), 7.76 (m, 2H), 7.99 (m, 1H), 8.05 (d, 1H, J = 9.7 Hz), 8.42 (dd, 1H, J = 8.4, 1.5 Hz), 8.63 (d, 1H, J = 9.7 Hz), 9.02 (dd, 1H, J = 7.2, 1.5 Hz), 9.32 (m, 1H), 10.79 (br. s, 1H). MS (EI) *m/e* 390 (M^+).

4-Bromo-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4o) was prepared from 4-bromo-benzo[*a*]phenazine-11-carboxylic acid (52 mg, 0.15 mmol) and *N*,*N*-dimethylethylenediamine (81 μL, 0.75 mmol) to give the title compound as a yellow solid (13 mg, 21%), mp 171−172 °C. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 6H), 2.69 (br. t, 2H, J = 5.9 Hz), 3.84 (m, 2H), 7.61 (t, 1H, J = 7.9 Hz), 7.94 (m, 1H), 8.02 (m, 2H), 8.36 (dd, 1H, J = 8.4, 1.5 Hz), 8.47 (d, 1H, J = 9.8 Hz), 8.97 (dd, 1H, J = 7.3, 1.6 Hz), 9.41 (d, 1H, J = 8.0 Hz), 10.61 (br. s, 1H). MS (DCI/NH_3) *m/e* 423:425, 1:1 $(M + H)^+$. Anal. ($C_{21}H_{19}BrN_4O$) C, H, N.

4-Cyano-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4q) was prepared from 4-cyano-benzo[*a*]phenazine-11-carboxylic acid (1.0 g, 3.34 mmol) and *N*,*N*-dimethylethylenediamine (5 mL, 45 mmol) to give the title compound as a yellow solid (200 mg, 16%), mp 172−176 °C. ^{1}H NMR (DMSO-d_6) δ 2.35(s, 6H), 2.62 (t, 2H), 3.72 (q, 2H), 8.00 (m, 2H), 8.10−8.74 (dd, 1H), 8.24 (d, 1H), 8.35 (d, 1H), 8.41 (m, 2H), 9.62 (d, 1H), 9.99 (t, 1H). MS (DCI) *m/e* 370 $(M + H)^+$.

4-Methanesulfonyl-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4k) was prepared from 4-methanesulfonyl-benzo[*a*]phenazine-11-carboxylic acid (120 mg, 0.34 mmol) and *N*,*N*-dimethylethylenediamine (186 μL, 1.7 mmol) to give the title compound as a yellow solid (85 mg, 59%), mp 204 °C dec. ^{1}H NMR (DMSO-d_6) δ 2.99 (s, 6H), 3.53 (br. t, 2H, J = 6.3 Hz), 3.57 (s, 3H), 4.04 (br. m, 2H), 8.24 (m, 2H), 8.40 (d, 1H, J = 9.8 Hz), 8.59 (m, 2H), 8.65 (m, 1H), 9.10 (d, 1H, J = 9.8 Hz), 9.64 (d, 1H, J = 8.0 Hz), 9.79 (br. m, 1H). MS (DCI/NH_3) *m/e* 423 $(M + H)^+$.

4-Chloro-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4n) was prepared from 4-chloro-benzo[*a*]phenazine-11-carboxylic acid (75 mg, 0.24 mmol) and *N*,*N*-dimethylethylenediamine (100 μL, 0.85 mmol) to give the title compound as a yellow solid (19 mg, 21%). ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 6H), 2.62−2.70 (t, 2H, J = 8.0 Hz), 3.78−3.86 (m, 2H, J = 5.4, 3.0 Hz), 7.69 (t, 1H, J = 8.0 Hz), 7.72 (d, 1H, J = 0.9, 6.8 Hz), 7.94 (t, 1H, J = 1.3, 7.2 Hz), 8.04 (d, 1H, J = 9.5 Hz), 8.37 (d, 1H, J = 1.5, 6.9 Hz), 8.51 (d, 1H, J = 9.4 Hz), 8.98(d, 1H, J = 1.5, 5.6 Hz), 9.99 (d, 1H, J = 8.0 Hz), 10.60 (br. s, 1H). MS (DCI/NH_3) *m/e* 379 (M + H)$^+$.

4-Acetyloxy-11-(2-dimethylamino-ethylcarbamoyl)-benzo[*a*]phenazine (4r) was prepared from 4-acetyloxy-benzo-[*a*]phenazine-11-carboxylic acid (46 mg, 0.13 mmol) and *N*,*N*-dimethylethylenediamine to give the title compound as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 6H), 2.70 (t, 2H, J = 5.8 Hz), 3.87 (q, 2H, J = 5.8 Hz), 4.03 (s, 3H), 7.81 (t, 1H, J = 7.9 Hz), 7.96 (t, 1H, J = 7.8), 8.07 (d, 1H, J = 9.8 Hz), 8.35−8.40 (m, 2H), 9.00 (d, 1H, J = 7.2 Hz), 9.13 (d, 1H, J = 9.8 Hz), 9.68 (d, 1H, J = 8.1 Hz), 10.65 (br. s, 1H). MS (DCI/NH_3) *m/e* 403 (M + H)$^+$.

4-Methylsulfamoyl-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide; trifluoro-acetate (4l) was prepared from the mixture of 4-methylsulfamoyl-benzo[*a*]phenazine-11-carboxylic acid, 3-methylsulfamoyl-benzo[*a*]phenazine-11-carboxylic acid, and *N*,*N*-dimethylethylene-diamine. The two isomers were separated using preparative HPLC to give the title compound as a yellow solid, mp 224−226 °C. ^{1}H NMR ($CDCl_3$/d_4-MeOD) δ 2.67 (s, 3H), 3.05 (s, 6H), 3.62 (t, 2H), 4.25 (t, 2H), 8.00−8.10 (m, 3H), 8.45−8.50 (m, 2H), 8.88 (d, 1H), 8.99 (d, 1H), 9.35 (d, 1H). MS (DCI/NH_3) *m/e* 438 (M + H)$^+$.

2-Nitro-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4g) was prepared from 2-nitro-benzo[*a*]phenazine-11-carboxylic acid and *N*,*N*-dimethyleth-ylenediamine, to give the title compound as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 2.92 (d, 6H, J = 5.0 Hz), 3.59 (q, 2H, 5.6 Hz), 4.26 (q, 2H, 6.1 Hz), 7.98 (dd, 1H, J = 7.3, 8.5 Hz), 8.10−8.03 (m, 2H), 8.13 (d, 1H, J = 9.3 Hz), 8.41 (dd, 1H, J = 8.0, 1.4 Hz), 8.56 (dd, 1H, J = 8.5, 2.3 Hz), 8.80 (dd, 1H, J = 6.6, 1.4 Hz), 9.98 (d, 1H, J = 2.2 Hz), 10.72 (br. t, 1H). MS (DCI/NH_3) *m/e* 390 (M + H)$^+$.

9-Bromo-4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4t′) was prepared from 9-bromo-4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (24 mg, 0.064 mmol) and *N*,*N*-dimethylethylenediamine (0.5 mL, 4.25 mmol) to give the title compound as a yellow solid (24 mg, 86%), mp 161−163 °C. ^{1}H NMR δ 2.33 (s, 6H), 2.74 (t, 2H), 3.85 (q, 2H), 4.09 (s, 3H), 7.10 (d, 1H), 7.90−8.04 (m, 3H), 8.38 (dd, 1H), 8.55 (t, 1H), 8.92 (dd, 1H), 10.73 (br.t, 1H). MS (DCI/NH_3) *m/e* 453/455 (M + H)$^+$, 1:1.

The following compounds were prepared in an analogous manner from the appropriate substituted benzo[*a*]-11-carboxylic acid activated as the acid chloride and reacted with the appropriate amine using general method B.

3-Dimethylamino-2-[(4-methoxy-benzo[*a*]phenazine-11-carbonyl)-amino]-propionic acid methyl ester (4r″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (1.9 g, 6.25 mmol) and 4-aza-DL-leucine methyl ester hydrochloride (1 g, 6.9 mmol) in the presence of pyridine (30 mL). The product was purified using preparative HPLC (isocratic 60% water/40% MeCN) to yield the trifluoroacetate salt of the desired compound as a yellow solid (165 mg, 5%), mp 200 °C dec. ^{1}H NMR (DMSO-d_6) δ 3.04(s, 6H), 3.90−3.70 (m, 2H), 3.96 (s, 3H), 4.10 (s, 3H), 5.61 (m, 1H), 7.34 (d, 1H, J = 7.95 Hz), 7.88 (t, 1H, J = 8.15 Hz), 7.97 (m, 2H), 8.52 (dd, 1H, J = 8.6, 1.5 Hz), 8.60 (d, 1H, J = 9.52 Hz), 8.94 (m, 2H), 11.93 (br. d, 1H). MS (DCI/NH_3) *m/e* 433 (M + H)$^+$.

4,10-Dimethoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4v′) was prepared from 4,10-dimethoxy-benzo[*a*]phenazine-11-carboxylic acid (628 mg, 1.88 mmol) and *N*,*N*-dimethylethylenediamine (1.03 mL, 9.4 mmol) to give the title compound as a yellow solid (116 mg, 15%), mp 119 °C dec. ^{1}H NMR (DMSO-d_6) 2.25 (s, 6H), 2.80 (t, 2H, J = 6.1 Hz), 3.82−3.87 (m, 2H), 4.06 (s, 3H), 4.10 (s, 3H), 6.78 (br s 1H), 7.19 (d, 1H, J = 7.7 Hz), 7.63−7.70 (m, 2H), 7.85 (d, 1H, J = 9.4 Hz), 8.28 (d, 1H, J = 9.4 Hz), 8.45 (d, 1H, J = 9.4 Hz), 8.95 (d, 1H, J = 8.1 Hz). MS (DCI/NH_3) *m/e* 405 (M + H)$^+$.

4-Methoxy-8-methyl-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4r′) was prepared from 4-methoxy-8-methyl-benzo[*a*]phenazine-11-carboxylic acid (120 mg, 0.38 mmol) and *N*,*N*-dimethylethylenediamine (0.21 mL, 1.9 mmol) to give the title compound as a yellow solid (38 mg, 26%), mp 170−172 °C. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 6H), 2.76 (t, 2H, J = 6.0 Hz), 3.00 (s, 3H), 3.88−3.92 (m, 2H), 4.10 (s, 3H), 7.30 (d, 1H), 7.75−7.79 (m, 1H), 7.82 (d, 1H, J = 9.4 Hz), 8.03 (d, 1H, J = 9.7 Hz), 8.55 (d, 1H, J = 9.7 Hz), 8.90 (d, 1H, J = 7.4 Hz), 9.03 (d, 1H, J = 8.0 Hz), 10.95 (br. s, 1H). MS (DCI/NH_3) *m/e* 389 (M + H)$^+$.

9-Chloro-4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4s′) was prepared from 9-chloro-4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (245 mg, 0.72 mmol) and *N*,*N*-dimethylethylenediamine (0.4 mL, 3.6 mmol) to give the title compound as a yellow solid (67 mg, 23%), mp 188−190. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 6H), 2.75 (t, 2H, J = 5.9 Hz), 3.86−3.90 (m, 2H), 4.08 (s, 3H), 7.25 (d, 1H, J = 7.8 Hz), 7.72−7.77 (m, 1H), 7.89 (d, 1H, J = 9.3 Hz), 8.36 (d, 1H, J = 2.5 Hz), 8.56 (d, 1H, J = 9.6 Hz), 8.92 (d, 1H, J = 2.5 Hz), 8.95 (d, 1H, J = 8.1 Hz), 10.75 (br. s, 1H). MS (DCI/NH_3) *m/e* 411/409 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (1-dimethylaminomethyl-propyl)-amide (4m″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (48 mg, 0.16 mmol) and N^1,N^1-dimethyl-butane-1,2-diamine dihydrochloride (61 mg, 0.32 mmol) in the presence of triethylamine (67 μL, 0.96 mmol) to give the title compound as a yellow solid (19 mg, 30%), mp 110−114 °C. ^{1}H NMR ($CDCl_3$) δ 1.11 (t, 3H, J = 7.5 Hz), 1.78−1.89 (m, 1H), 1.98−2.08 (m, 1H), 2.36 (s, 6H), 2.59 (dd, 1H, J = 12.5, 6.1 Hz), 2.82 (dd, 1H, J = 12.5, 7.6), 4.10 (s, 3H, OMe), 4.46−4.54 (m, 1H), 7.27 (d, 1H, J = 9.0 Hz), 7.76 (t, 1H, J = 8.1 Hz), 7.94−8.01 (m, 2H), 8.41 (d, 1H, J = 8.5 Hz), 8.57 (d, 1H, J = 9.4 Hz), 8.79 (d, 1H, J = 8.2 Hz), 9.01 (d, 1H, J = 7.1 Hz), 10.92 (d, 1H, J = 8.3). MS (DCI/NH_3) *m/e* 403 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (1-dimethylaminomethyl-2-methyl-propyl)-amide (4n″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (87 mg, 0.29 mmol) and 3N^1,N^1-trimethyl-butane-1,2-diamine dihydrochloride salt (290 mg, 1.43 mmol) in the presence of triethylamine (0.48 mL, 3.43 mmol) to give the title compound as a yellow solid (77 mg, 64%). ^{1}H NMR ($CDCl_3$) δ 1.03 (d, 3H, J = 2.6 Hz), 1.04 (d, 3H, J = 2.6 Hz), 2.16 (m, 1H), 2.28 (s, 6H), 2.59 (dd, 1H, J = 12.6, 4.8 Hz), 2.83 (dd, 1H, J = 12.6, 9.1 Hz), 4.01 (s, 3H), 4.49 (m, 1H), 7.18 (d, 1H, J = 7.8 Hz), 7.68 (t, 1H, J = 8.1), 7.90 (m, 2H), 8.33 (dd, 1H, J = 8.6, 1.5 Hz), 8.48 (d, 1H, J = 9.6 Hz), 8.71 (d, 1H, J = 8.2 Hz), 8.95 (dd, 1H, J = 7.3, 1.4 Hz), 10.90 (br. d, 1H). MS (DCI/NH_3) *m/e* 417 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (1-dimethylaminomethyl-2-phenyl-ethyl)-amide (4o″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (98 mg, 0.32 mmol) and N^1,N^1-dimethyl-3-phenyl-propane-1,2-diamine dihydrochloride salt (81 mg, 0.32 mmol) in the presence of triethylamine (100 μL, 7.14 mmol) to give the title compound as a yellow solid (70 mg, 47%), mp 154−158 °C. ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 6H), 2.60 (dd, 1H, J = 12.5, 6.1 Hz), 2.76 (dd, 1H, J = 12.4, 8.3 Hz), 3.21 (dd, 1H, J = 13.7, 5.5 Hz), 3.27 (dd, 1H, J = 13.7, 5.9 Hz), 4.09 (s, 3H), 4.83 (m, 1H), 7.04 (t, 1H, J = 7.3 Hz), 7.16 (t, 2H, J = 7.5), 7.22 (d, 1H, J = 8.0 Hz), 7.32 (d, 2H, J = 7.1), 7.55 (t, 1H, J = 8.1 Hz), 7.96 (t, 1H, J = 9.2 Hz), 7.99 (d, 1H, J = 8.3 Hz), 8.24 (d, 1H, J = 8.2 Hz), 8.42 (dd, 1H, J = 8.5, 1.6 Hz), 8.55 (d, 1H, J = 9.4 Hz), 9.02 (dd, 1H, J = 7.2, 1.5 Hz), 11.0 (br. d, 1H). MS (DCI/NH3) *m/e* 465 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-1-(*S*)-methyl-ethyl)-amide ((S)-4j″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid

(362 mg, 1.19 mmol) and (*S*)-N^1,N^1-dimethyl-propane-1,2-diamine dihydrochloride salt (209 mg, 1.19 mmol) in the presence of triethylamine (0.35 mL, 2.5 mmol) to give the title compound as a yellow glass (9 mg, 2%). ^{1}H NMR ($CDCl_3$) δ 1.53 (d, 3H, J = 6.5 Hz), 2.37 (s, 6H), 2.55 (dd, 1H, J = 12.2, 6.2 Hz), 2.84 (dd, 1H, J = 12.2, 7.8 Hz), 4.10 (s, 3H), 4.55−4.65 (m, 1H), 7.28−7.30 (m, 1H), 7.75−7.80 (m, 1H), 7.95−8.02 (m, 2H), 8.42 (dd, 1H, J = 8.4, 1.5 Hz), 8.57 (d, 1H, J = 9.5 Hz), 8.81 (d, 1h, J = 8.2 Hz), 9.02 (dd, 1H, J = 7.3, 1.6 Hz), 10.9 (d, 1H, J = 7.2 Hz). MS (DCI/NH_3) *m/e* 389 (M + H)$^+$. This compound was 80% optically pure as determined by NMR using the chiral shift reagent 2,2,2-trifluoro-1-(9-anthranyl) ethanol and comparing to the racemate (**4j″**) and the >95% optically pure enantiomer (**(R)-4j″**).

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-1(*R*)-methyl-ethyl)-amide ((R)-4j″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (207 mg, 0;68 mmol) and (*R*)-N^1,N^1-dimethyl-propane-1,2-diamine diydrochloride salt (238 mg, 1.36 mmol) in the presence of triethylamine (0.3 mL, 21 mmol) to give the title compound as a yellow solid (73 mg, 28%), mp 116−118 °C; $[\alpha]^{23}_D$ −120.0° (c = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.53 (d, 3H, J = 6.5 Hz), 2.38 (s, 6H,), 2.51−2.60 (m, 1H), 2.82−2.90 (m, 1H), 4.11 (s, 3H), 4.56−4.65 (m, 1H), 7.29 (d, 1H, J = 8.2 Hz), 7.78 (t, 1H, J = 8.1 Hz), 7.96−8.02 (m, 2H), 8.43 (d, 1H, J = 8.4 Hz), 8.58 (d, 1H, J = 9.5), 8.82 (d, 1H, J = 8.4 Hz), 9.03 (d, 1H, J = 7.3 Hz). MS (DCI/NH_3) *m/e* 389 (M + H)$^+$. This compound was >95%% optically pure as determined by NMR using the chiral shift reagent 2,2,2-trifluoro-1-(9-anthranyl)ethanol and comparing to the racemate (**4j″**). Treatment of the above compound with HCl in ether and recrystallization from ether and aqueous ethanol gave the hydrated monohydrochloride salt as a yellow solid, mp 235−236 °C; $[\alpha]^{23}_D$ −127.4° (c = 0.833, H_2O); MS (DCI/NH_3) *m/e* 389 (M + H)$^+$. Anal. ($C_{23}H_{25}ClN_4O_2 \cdot 3(^1/_2)H_2O$) C, H; calcd 6.5; found 5.8; Cl; N.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-1(*S*)-hydroxymethyl-ethyl)-amide ((S)-4v″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (709 mg, 2.33 mmol) and (*S*)-2-amino-3-(dimethylamino)-propan-1-ol dihydrochloride salt (738 mg, 3.86 mmol) in the presence of triethylamine (10.8 mL, 77.4 mmol) to give the title compound as a yellow solid (165 mg, 18%), mp 88 °C dec. ^{1}H NMR ($CDCl_3$) δ 2.39 (6H, s), 2.88 (dd, 1H, J = 12.4, 7.4 Hz), 2.96 (dd, 1H, J = 12.4, 6.0 Hz), 4.06 (s, 3H), 4.17 (m, 2H), 4.63 (m, 1H), 5.32 (br. s, 1H), 7.21 (d, 1H, J = 8.0 Hz), 7.72 (t, 1H, J = 8.0 Hz), 7.86 (d, 1H, J = 9.5 Hz), 7.90 (m, 1H), 8.35 (dd, 1H, J = 8.6, 1.5 Hz), 8.41 (d, 1H, J = 9.5 Hz), 8.76 (d, 1H, J = 8.2 Hz), 8.91 (dd, 1H, J = 7.2, 1.5 Hz), 10.97 (br. s, 1H). MS (DCI/NH_3) *m/e* 405 (M + H)$^+$. This compound was >95%% optically pure as determined by NMR using the chiral shift reagent 2,2,2-trifluoro-1-(9-anthranyl) ethanol and comparing to the racemate (**4p″**).

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (1(*S*)-dimethylaminomethyl-2(*S*)-hydroxy-propyl)-amide ((S)-4u″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (500 mg, 1.65 mmol) and 3(*S*)-amino-4-(dimethylamino)-butan-2(*S*)-ol dihydrochloride salt (1.1 g, 5.25 mmol) in the presence of triethylamine (4.57 mL, 32.9 mmol) to give the title compound as an orange solid (161 mg, 23%), mp 189−190 °C. ^{1}H NMR ($CDCl_3$) δ 1.37 (d, 3H, J = 6.4 Hz), 2.36 (s, 6H), 2.88 (dd, 1H, J = 13.1, 4.8 Hz), 3.06 (dd, 1H, J = 13.1, 5.2 Hz), 3.48 (s, 1H), 4.06 (s, 3H), 4.43 (m, 2H), 7.35 (d, 1H, J = 8.0 Hz), 7.75 (t, 1H, J = 8.2 Hz), 7.99−7.91 (m, 2H), 8.42 (dd, 1H, J = 8.6, 1.5 Hz), 8.53 (d, 1H, J = 9.6 Hz), 9.02 (dd, 1H, J = 7.3, 1.5 Hz), 9.27 (d, 1H, J = 8.2 Hz), 11.21 (br. s, 1H). MS (DCI/NH_3) *m/e* 419 (M + H)$^+$. Anal. ($C_{24}H_{26}N_4O_3$) C, H, N. This compound was >95% optically pure as determined by NMR using the chiral shift reagent 2,2,2-trifluoro-1-(9-anthranyl) ethanol.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid [1-(dimethylamino)-1-(2-hydroxyethyl)]-ethylamide (4q″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (150 mg, 0.49 mmol) and 3-amino-4-(dimethylamino)-butan-1-ol diydrochloride salt (122 mg, 0.58 mmol) in the presence of triethylamine (1.5 mL, 10.8 mmol) to give the title compound as a yellow solid (112 mg, 54%), mp 143−146 °C. ^{1}H NMR ($CDCl_3$) δ 1.99 (m, 1H), 2.10 (m, 1H), 2.35 (s, 6H), 2.63 (dd, 1H, J = 12.2, 2.1 Hz), 3.44 (dd, 1H, J = 12.2, 6.0 Hz), 3.80−3.67 (m, 2H), 4.02 (s, 3H), 4.59 (q, 1H, J = 7.1 Hz), 7.19 (d, 1H, J = 7.7 Hz), 7.69 (t, 1H, J = 8.1 Hz), 7.94−7.86 (m, 2H), 8.35 (dd, 1H, J = 8.5, 1.5 Hz), 8.48 (d, 1H, J = 9.5 Hz), 8.53 (d, 1H, J = 8.2 Hz), 8.92 (dd, 1H, J = 7.2, 1.5 Hz), 11.05 (d, 1H). MS (DCI/NH_3) *m/e* 419 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-piperidin-1-yl-ethyl)-amide (4d″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (250 mg, 0.82 mmol) and 1-(2-aminoethyl) piperidine (0.59 mL, 4.1 mmol) to give the title compound as a yellow solid (95 mg, 28%), mp 141−143 °C. ^{1}H NMR ($CDCl_3$) δ 1.35 (m, 2H), 1.45 (m, 4H), 2.47 (m, 4H), 2.7 (t, 2H), 3.7 (m, 2H), 4.07 (s, 3H), 7.5 (d, 1H) 7.83 (m, 1H), 7.95 (d, 1H), 8.07 (m, 1H), 8.43 (m, 1H), 8.5 (m, 1H), 8.62 (m, 1H), 8.73 (m, 1H), 10.05 (br, 1H). MS (DCI/NH_3) *m/e* 414 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-morpholin-4-yl-ethyl)-amide (4f″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (250 mg, 0.82 mmol) and 4-(2-aminoethyl) morpholine (0.54 mL, 4.11 mmol) to give the title compound as a yellow solid (187 mg, 54%), mp 201−202 °C. ^{1}H NMR ($CDCl_3$) δ 2.62 (t, 4H), 2.90 (t, 2H), 3.65 (t, 4H), 3.93 (m, 2H), 4.20 (s, 3H), 7.25 (m, 1H), 7.70 (t, 1H), 8.00 (m, 2H), 8.45 (m, 1H), 8.60 (d, 1H), 8,75 (d, 1H), 9.0 (d, 1H), 10.9 (br, 1H). MS (DCI/NH_3) *m/e* 417 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide (4h″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (220 mg, 0.72 mmol) and 1-(aminoethyl)pyrrolidine (0.46 mL, 3.6 mmol) to yield the title compound as a yellow solid (222 mg, 77%), mp 148−150 °C. ^{1}H NMR ($CDCl_3$) δ 1.82 (t, 4H), 2.75 (t, 4H), 3.20 (t, 2H), 3.90−3.99 (m, 2H), 4.11 (s, 3H), 7.26−7.31 (m, 1H), 7.78 (t, 1H), 7.95−7.98 (m, 1H), 7.98−8.03 (m, 1H) 8.4−8.46 (m, 1H), 8.55−8.61 (m, 1H), 8.78−8.83 (m, 1H) 8.98−9.30 (m, 1H). MS (DCI/NH_3) *m/e* 401 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-diethylamino-ethyl)-amide (4c″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (255 mg, 0.84 mmol) and *N*,*N*-diethylethylenediamine (0.59 mL, 4.2 mmol) to give the title compound as an orange solid (70 mg, 34%), mp 74 °C dec. ^{1}H NMR (DMSO-d_6) δ 0.99 (t, 6H), 2.65 (q, 4H), 2.79 (t, 2H), 3.65−3.72 (m, 2H), 4.07 (s, 3H), 7.49−7.53 (m, 1H), 7.8−7.87 (m, 1H), 7.98 (d, 1H), 8.04−8.11 (m, 1H), 8.45 (m, 1H), 8.51 (m, 1H), 8.67 (m, 1H), 8.89 (m, 1H),10.15−10.25 (br, 1H). MS (DCI/NH_3) *m/e* 403 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid {2-[bis-(2-hydroxy-ethyl)-amino]-ethyl}-amide (4g″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (250 mg, 0.82 mmol) and *N*,*N*-bis(2-hydroxyethyl)ethylenediamine (0.57 mL, 4.11 mmol) to give the title compound as a yellow gum (131 mg, 37%). ^{1}H NMR ($CDCl_3$) δ 2.75 (t, 4H), 3.00 (t, 2H), 3.56 (t, 4H), 3.80−3.89 (m, 2H), 4.40 (s, 3H), 7.16−7.22 (m, 1H), 7.69−7.75 (m, 1H), 7.85−7.89 (m, 1H), 7.89−7.93 (m, 1H), 8.33−8.39 (m, 1H), 8.47−8.51 (m, 1H), 8.51−8.55 (m, 1H), 8.89−8.95 (m, 1H), 11.29 (br, 1H). MS (DCI/NH_3) *m/e* 435 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (1-methyl-pyrrolidin-3-(*R*)-yl)-amide (4t″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (586 mg, 1.93 mmol) and 1-methyl-3-(*R*)-aminopyrrolidine dihydrochloride salt (383 mg, 2.21 mmol) in the presence of triethylamine (0.92 mL, 6.5 mmol) to yield the title compound as a yellow solid (138 mg, 19%), mp 164−167 °C. ^{1}H NMR ($CDCl_3$) δ 2.02−2.10 (m, 1H), 2.40−2.46 (m, 1H), 2.52−2.61 (m, 1H), 2.58 (s, 3H), 2.75−2.79 (m, 1H), 2.99−3.02 (m, 1H), 3.13−3.18 (m, 1H), 4.10 (s, 3H), 4.90−4.98 (m, 1H), 7.26 (d, 1H), 7.75−7.80 (m, 1H), 7.95−8.00 (m, 2H), 8.42(dd, 1H, J = 8.4, 1.5 Hz), 8.58 (d, 1H, J = 9.6 Hz), 8.99−9.03 (m, 2H), 11.32 (d, 1H). MS (DCI/NH_3) *m/e* 387 (M + H)$^+$. Anal. ($C_{23}H_{22}N_4O_2$) C, H, N.

(*R*)-4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (1-dimethylaminomethyl-2-methyl-propyl)-amide ((R)-4n″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (504 mg, 1.66 mmol) and (*R*)-3N^1,N^1-trimethyl-butane-1, 2-diamine dihydrochloride (504 mg, 2.48 mmol) in the presence of triethylamine (3.0 mL, 21.3 mmol) to give the title compound as a yellow solid (350 mg, 51%). ^{1}H NMR ($CDCl_3$) δ 1.10 (d, 3H, J = 2.4 Hz), 1.12 (d, 3H, J = 2.5 Hz), 2.22 (m, 1H), 2.34 (s, 6H), 2.64 (dd, 1H, J = 12.6, 5.1 Hz), 2.89 (dd, 1H, J = 12.6, 9.0 Hz), 4.09 (s, 3H,), 4.56 (m, 1H), 7.26 (d, 1H, J = 7.7 Hz), 7.75 (t, 1H, J = 8.1 Hz), 7.97 (m, 2H), 8.41 (dd, 1H, J = 8.5, 1.5 Hz), 8.56 (d, 1H, J = 9.5 Hz), 8.80 (d, 1H, J = 8.3 Hz), 9.03 (dd, 1H, J = 7.3, 1.6 Hz), 11.0 (br d, 1H). MS (DCI/NH_3) *m/e* 417 (M + H)$^+$. This compound was >95%% optically pure as determined by NMR using the chiral shift reagent 2,2,2-trifluoro-1-(9-anthranyl) ethanol.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2,3-bis)-(dimethylamino)-propyl)amide (4b″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (210 mg, 0.69 mmol) and N^1,N^1,N^2,N^2-tetramethyl-propane-1,2,3-triamine trihydrochloride (273 mg, 1.1 mmol) to yield the title compound as a yellow solid (90 mg, 30%), mp 126.5−128 °C. ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 6H), 2.32−2.38 (m, 1H), 2.52 (s, 6H), 2.56−2.62 (m, 1H), 3.00−3.09 (m, 1H), 3.65−3.74 (m, 1H), 4.01−4.09 (m, 1H), 4.11 (s, 3H), 7.28 (d, 1H, J = 7.7 Hz), 7.78 (t, 1H, J = 8.0 Hz), 7.96−8.01 (m, 2H), 8.42 (d, 1H, J = 8.5 Hz), 8.58 (d, 1H, J = 9.6 Hz), 8.98−9.03 (m, 2H). MS (DCI/NH_3) *m/e* 432 (M + H)$^+$.

The following compounds were prepared in an analogous manner from the appropriate substituted benzo[*a*]-11-carboxylic acid methyl ester and the appropriate amine using general method C.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (3-(dimethylamino)-propyl)-amide (4a″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid methyl ester (50 mg, 0.16 mmol) and 3-(dimethylamino) propylamine (1.0 mL, 8.1 mmol) to give the title compound as a yellow solid (37 mg, 64%), mp 97−99 °C. ^{1}H NMR (DMSO-d_6) 1.92 (t, 2H, J = 7.0 Hz), 2.2 (s, 6H), 3.62 (q, 2H, J = 6.7 Hz), 4.07 (s, 3H), 7.3 (d, 1H, J = 8.0 Hz), 7.85 (t, 1H, J = 8.1 Hz), 7.98 (d, 1H, J = 9.5 Hz), 8.05 (t, 1H, J = 7.3 Hz), 8.42 (d, 1H, J = 8.6 Hz), 8.49 (d, 1H, J = 9.6 Hz), 8.52 (d, 1H, J = 6.9 Hz), 8.72 (d, 1H, J = 8.1 Hz), 9.95 (br, 1H). MS (DCI/NH_3) *m/e* 389 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (3-amino-2-hydroxy-propyl)-amide (4e″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid methyl ester (50 mg, 0.16 mmol) and 1,3-diamino-2-hydroxypropane (500 mg, 5.6 mmol) to yield the title compound as a beige solid (24 mg, 40%), mp 199 °C dec. ^{1}H NMR ($CDCl_3$) δ 2.81 (dd, 1H, J = 12.6, 7.5 Hz), 2.97 (dd, 1H, J = 12.6, 3.7 Hz), 3.70−3.75 (m, 1H), 3.90−4.00 (m, 2H), 4.01 (s, 3H), 7.15 (d, 1H, J = 8.0 Hz), 7.65−7.71 (m, 1H), 7.81 (d, 1H, J = 9.5 Hz), 7.85−7.90 (m, 1H), 8.31 (d, 1H, J = 7.5 Hz), 8.42 (d, 1H, J = 9.6 Hz), 8.80 (d, 1H, J = 8.2 Hz), 8.88 (d, 1H, J = 6.1 Hz), 11.2 (br, 1H). MS (DCI/NH_3) *m/e* 377 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-propyl)-amide (4k″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid methyl ester (100 mg, 0.31 mmol) and N^2,N^2-dimethyl-propane-1,2-diamine (0.5 mL, 3.7 mmol) to give the title compound as a yellow solid (30 mg, 25%). ^{1}H NMR ($CDCl_3$) δ 1.18 (d, 3H, J = 6.5 Hz), 2.45 (s, 6H), 3.08 (m, 1H), 3.70 (m, 1H), 3.93 (m, 1H), 4.05 (s, 3H), 7.23 (m, 1H), 7.75 (m, 1H), 7.95 (m, 2H), 8.40 (m, 1H), 8.52 (m, 1H), 8.97 (m, 2H), 10.76 (br s, 1H). MS (DCI/NH_3) *m/e* 389 (M + H)$^+$.

4-Dimethylamino-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4w) was prepared from the mixture of 4-(dimethylamino)-benzo[*a*]phenazine-11-carboxylic acid methyl ester and 3-(dimethylamino)-benzo[*a*]-phenazine-11-carboxylic acid methyl ester (289 mg, 0.87 mmol) and *N*,*N*-dimethylethylenediamine (3 mL, 27 mmol), followed by purification on silica eluting with 10% methanol in dichloromethane to remove the minor isomer, to give the title compound as a yellow solid (75 mg, 22%), mp 114−115 °C. ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 6H), 2.70 (t, 2H), 2.90 (s, 6H), 3.85 (q, 2H), 7.40 (d, 1H), 7.70 (t, 1H), 7.90 (dd, 2H), 8.40 (dd, 1H), 8.53 (d, 1H), 8.95 (dd, 1H), 9.10 (d, 1H), 10.85 (br, 1H). MS (DCI/NH_3) *m/e* 388 (M + H)$^+$.

4-Methoxy-10-methylamino-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4z′) was prepared from 4-methoxy-10-methylamino-benzo[*a*]phenazine-11-carboxylic acid methyl ester (104 mg, 0.30 mmol) and *N*,*N*-dimethylethylenediamine (5 mL, 45 mmol) to yield the title compound as an orange solid (32 mg, 27%), mp 310−312 °C. ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 6H), 2.77 (t, 2H, J = 6.5 Hz), 3.18 (d, 3H, J = 5.1 Hz), 3.83 (q, 2H, J = 6.2 Hz), 4.10 (s, 3H), 7.20 (d, 1H, J = 7.8 Hz), 7.56 (d, 1H, J = 9.6 Hz), 7.70 (t, 1H, J = 8.1 Hz), 7.90 (d, 1H, J = 9.5 Hz), 8.12 (d, 1H, J = 9.6 Hz), 8.40 (d, 1H, J = 9.5 Hz), 8.80 (d, 1H, J = 8.3 Hz), 11.12 (m, 1H), 11.92 (m, 1H). MS (DCI/NH_3) *m/e* 404 (M + H)$^+$.

10-Amino-4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4y′) was prepared from 10-amino-4-methoxy-benzo[*a*]phenazine-11-carboxylic acid methyl ester (100 mg, 0.30 mmol) and *N*,*N*-dimethylethylenediamine (3 mL, 27 mmol) to give the title compound as a red solid (16 mg, 14%), mp 170−172 °C. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 6H), 2.70 (t, 2H J = 6.4 Hz), 3.87 (q, 2H, J = 6.1 Hz), 4.09 (s, 3H), 7.20 (d, 1H, J = 7.7 Hz), 7.23 (d, 1H, J = 9.3 Hz), 7.72 (t, 1H, J = 8.1 Hz), 7.91 (d, 1H, J = 9.5 Hz), 8.01 (d, 1H, J = 9.3 Hz), 8.43 (d, 1H, J = 9.3 Hz), 8.80 (d, 1H, J = 8.2 Hz), 11.75 (m, 1H). MS (DCI/NH_3) *m/e* 390 (M + H)$^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-methylamino-ethyl)-amide (4i″) was prepared from 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid methyl ester (95.5 mg, 0.3 mmol) and *N*-methylethylenediamine (1 mL, 11 mmol) to give the title compound as a yellow solid (34 mg, 32%), mp 104−106 °C. ^{1}H NMR ($CDCl_3$) δ 2.64 (s, 3H), 3.12 (t, 2H), 3.90−3.96 (m, 2H), 4.11 (s, 3H), 7.29 (s, 1H), 7.63−7.71 (m, 1H), 7.92−7.95 (m, 1H), 7.95−8.01 (m, 1H), 8.38−8.45 (m, 1H), 8.55−8.61 (m, 1H), 8.89−8.95 (m, 1H), 8.95−9.02 (m, 1H), 11.08 (br, 1H). MS (DCI/NH_3) *m/e* 361 (M + H)$^+$.

Interconversion of Substituted Benzo[*a*]-11-carboxamides. 4-Hydroxy-benzo[*a*]phenazine-11-carboxylic Acid (2-(Dimethylamino)-ethyl)-amide Hydrobromide Salt (4f). To a solution of 4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (727 mg, 1.94 mmol) in dry dichloromethane (15 mL) cooled to −5 °C was added a 1.0 M solution of boron tribromide in dichloromethane (13.6 mL, 13.6 mmol). After being stirred for 4 h, the reaction mixture was poured onto ice/water (50 mL), yielding a precipitate which was collected by filtration. This was triturated from a hot methanol/ethyl acetate mixture to yield the title compound as a beige solid (505 mg, 72%), mp 220 °C dec. ^{1}H NMR (DMSO-d_6) 2.91 (s, 6H), 3.45 (t, 2H), 3.97−4.02 (m, 2H), 7.37 (d, 1H), 7.72−7.78 (m, 1H), 7.92(d, 1H), 8.08−8.12(m, 1H), 8.45−8.51 (m, 2H), 8.54−8.61 (m, 2H), 9.42 (br, 1H), 10.13 (t, 1H), 10.67 (s, 1H). MS (EI) *m/e* 360 (M^+). Anal. ($C_{21}H_{20}N_4O_2$) C, H, N.

3-Hydroxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4e). To a solution of 3-methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (170 mg, 0.45 mmol) in dry DMF (3 mL) was added sodium thioethoxide (380 mg, 4.5 mmol). The reaction mixture was then heated to reflux under argon for 3 h, cooled to room temperature, and acidified with 1 M HCl. The volatile components were removed in vacuo. The residue was purified using column chromatography (20% methanol in dichloromethane) to yield the title compound as a red solid (142 mg, 88%), mp 230 °C dec. ^{1}H NMR (DMSO-d_6) δ 2.50 (s, 6H), 2.58 (br, 2H), 3.88 (br, 2H), 7.41 (m, 2H), 7.89 (d, 1H, J = 9.4 Hz), 8.03 (m, 1H), 8.13 (d, 1H, J = 9.4 Hz), 8.42 (dd, 1H, J = 1.51, 8.4 Hz), 8.66 (m, 1H), 9.14 (br, 1H), 10.40 (br, 1H), 10.58 (s, 1H). MS (DCI/NH_3) *m/e* 361 (M + H)$^+$. Anal. ($C_{21}H_{20}N_4O_2$) C, H, N.

2-Hydroxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4d) was prepared in an analogous manner from 2-methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (200 mg, 0.54

mmol) to give the title compound as a yellow solid (151 mg, 78%), mp 172−174 °C. ^{1}H NMR (DMSO-d_6) δ 2.89 (s, 6H), 3.46 (t, 2H, J = 6.0 Hz), 4.07 (q, 2H, J = 6.0 Hz), 7.40 (m, 1H), 7.81 (d, 1H, J = 9.2 Hz), 8.02 (d, 1H, J = 8.5 Hz), 8.10 (m, 1H), 8.18 (d, 1H, J = 9.2 Hz), 8.49 (m, 2H), 8.56 (m, 1H), 10.13 (br.t, 1H), 10.56 (s, 1H). MS (DCI/NH_3) *m/e* 361 $(M + H)^+$.

4-Cyanomethoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4e′). To a suspension of 4-hydroxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide hydrobromide salt (230 mg, 0.52 mmol) in dry DMF (3 mL) were added potassium *tert*-butoxide (175 mg, 1.56 mmol) and then bromoacetonitrile (47 μL, 0.68 mmol). The reaction mixture was heated to 100 °C for 1 h and then cooled to room temperature, diluted with ethyl acetate (30 mL), washed with sodium carbonate solution (30 mL) and brine (30 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to the crude product. This was purified using flash chromatography on silica eluting with 25% MeOH in ethyl acetate to yield the title compound as a yellow solid (74 mg, 36%), mp 188−191 °C. ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 6H), 2.68 (t, 2H, J = 5.9 Hz), 3.81−3.84 (m, 2H), 5.02 (s, 2H), 7.30 (d, 1H, J = 8.1 Hz), 7.71−7.75 (m, 1H), 7.92−7.98 (m, 2H), 8.36−8.41 (m, 2H), 8.96 (dd, 1H, J = 7.2, 1.5 Hz), 9.15 (d, 1H, J = 8.3 Hz), 10.70 (br, 1H). MS (DCI/NH_3) *m/e* 400 $(M + H)^+$. Anal. ($C_{23}H_{21}N_5O_2$) C, H, N.

The following compounds were prepared in an analogous manner using 4-hydroxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide hydrobromide salt and the appropriate alkylating reagent.

4-(2-Morpholin-4-yl-ethoxy)-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4p′) was prepared using *N*-(2-chloroethyl)morpholine hydrochloride to yield the title compound as a yellow solid (51%), mp 135−137 °C. ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 6H), 2.68−2.71 (m, 4H), 2.75 (t, 2H J = 6.0 Hz), 3.01 (t, 2H, J = 5.6 Hz), 3.75−3.79 (m, 4H), 3.88−3.92 (m, 2H), 4.36 (t, 2H, J = 5.6 Hz), 7.25−7.27 (m, 1H), 7.71−7.75 (m, 1H), 7.95−8.01 (m, 2H), 8.41 (dd, 1H, J = 8.5, 1.5 Hz), 8.55 (d, 1H, J = 9.5 Hz), 9.01−9.05 (m, 2H), 10.8 (br, 1H). MS (DCI/NH_3) *m/e* 474 $(M + H)^+$.

4-(3-Cyano-propoxy)-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4f′) was prepared using 4-bromobutyronitrile to give the title compound as a yellow solid (37%), mp 168−170 °C. ^{1}H NMR ($CDCl_3$) δ 2.35−2.38 (m, 2H), 2.45 (s, 6H), 2.73−2.75 (m, 4H), 3.89−3.93 (m, 2H), 4.37 (d, 2H, J = 5.7 Hz), 7.28−7.30 (m, 1H), 7.78−7.80 (m, 1H), 7.98−8.01 (m, 2H), 8.45 (dd, 1H, J = 8.4, 1.5 Hz), 8.56 (d, 1H, J = 9.7 Hz), 9.05 (dd, 1H, J = 7.2, 1.5 Hz), 9.08 (d, 1H, J = 8.2 Hz), 10.8 (br, 1H). MS (DCI/NH_3) *m/e* 428 $(M + H)^+$.

4-(3-Dimethylamino-propoxy)-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4o′) was prepared using 3-dimethylaminopropyl chloride hydrochloride to give the title compound as a yellow solid (44%), mp 145−147 °C. ^{1}H NMR ($CDCl_3$) δ 2.15−2.21 (m, 2H), 2.34 (s, 6H), 2.44 (s, 6H), 2.63 (t, 2H, J = 7.2 Hz), 2.79 (t, 2H, J = 6.0 Hz), 3.90−3.95 (m, 2H), 4.30 (t, 2H, J = 6.3 Hz), 7.25−7.27 (m, 1H), 7.71−7.74 (m, 1H), 7.95−8.01 (m, 2H), 8.40 (dd, 1H, J = 8.40, 1.5 Hz), 8.55 (d, 1H, J = 9.5 Hz), 8.99−9.03 (m, 2H), 10.89 (br, 1H). MS (DCI/NH_3) *m/e* 446 $(M + H)^+$.

4-Carbamoylmethoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4m′) was prepared using 2-bromoacetamide to give the title compound as a yellow solid (5%), mp 228 °C dec. ^{1}H NMR ($CDCl_3$ and two drops of MeOH-d_4) δ 2.32 (s, 6H), 2.68 (t, 2H, J = 6.2 Hz), 3.78 (t, 2H, J = 6.2 Hz), 4.68 (s, 2H), 7.20 (d, 1H, J = 7.8 Hz), 7.70−7.72 (m, 1H), 7.90−7.95 (m,2H), 8.35 (dd, 1H, J = 1.5, 8.5 Hz), 8.53 (d, 1H, J = 9.8 Hz), 8.86 (dd, 1H, J = 7.2, 1.5 Hz), 8.95 (d, 1H, J = 8.3 Hz). MS (DCI/NH_3) *m/e* 418 $(M + H)^+$.

4-(2-Oxo-propoxy)-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4l′) was prepared using chloroacetone to give the title compound as orange crystals (25%), mp 182−183 °C. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 6H), 2.45 (s, 3H), 2.75 (t, 2H, J = 5.9 Hz), 3.88−3.91 (m, 2H), 4.83 (s, 2H), 7.12 (d, 1H, J = 7.8 Hz), 7.71−7.76 (m, 1H), 7.95−8.02 (m, 2H), 8.45 (d, 1H, J = 8.4, 1.4 Hz), 8.63 (d, 1H, J = 9.6 Hz), 9.03 (dd, 1H, J = 7.2, 1.6 Hz), 9.15 (d, 1H, J = 8.1 Hz), 10.80 (br, 1H). MS (DCI/NH_3) *m/e* 417 $(M + H)^+$.

Ethyl-carbamic acid 11-(2-(dimethylamino)-ethylcarbamoyl)-benzo[*a*]phenazin-4-yl ester (4h′). A mixture of 4-hydroxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide hydrobromide salt (540 mg, 1.2 mmol), triethylamine (0.51 mL, 3.6 mmol), and ethyl isocyanate (0.29 mL, 3.6 mmol) was stirred in dry DMF (3 mL). The product slowly precipitated from the reaction mixture and was collected by filtration and washed with ether to yield the title compound as a yellow solid (210 mg, 41%), mp 302 °C dec. ^{1}H NMR (DMSO-d_6) δ 0.9 (t, 3H, J = 7.4 Hz), 2.4 (s, 6H), 2.75 (br, 2H), 3.37 (t, 2H, J = 6.4 Hz), 5.3 (br, 2H), 7.57 (d, 1H, J = 7.2 Hz), 7.75 (t, 1H, J = 7.4 Hz), 7.91 (t, 2H, J = 7.1 Hz), 8.13 (d, 1H, J = 9.6 Hz), 8.3 (d, 1H, J = 8.4 Hz), 8.95 (d, 1H, J = 6.7 Hz), 9.21 (d, 1H, J = 7.6 Hz), 10.76 (br, 1H). MS (DCI/NH_3) *m/e* 432 $(M + H)^+$.

Acetic acid 11-(2-(dimethylamino)-ethylcarbamoyl)-benzo[*a*]phenazin-4-yl ester (4g′). A mixture of 4-hydroxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (46 mg, 0.13 mmol), triethylamine (71 μL, 0.52 mmol), and acetyl chloride (20 μL, o.29 mmol) in dichloromethane (2 mL) was stirred at room temperature for 2 h. The volatile components were removed in vacuo, and the residue was purified using column chromatography on silica eluting with 10% methanol in dichloromethane to yield the title compound as a yellow gum (27 mg, 52%). ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 6H), 2.52 (s, 3H), 2.65 (t, 2H, J = 6.0 Hz), 3.72 (q, 2H, J = 6.0 Hz), 7.74 (d, 1H, J = 7.0 Hz), 7.99 (t, 1H, J = 8.0 Hz), 8.08 (d, 1H, J = 9.6 Hz), 8.12 (t, 1H, J = 7.8 Hz), 8.31 (d, 1H, J = 9.6 Hz), 8.47 (d, 1H, J = 8.0 Hz), 8.75 (d, 1H, J = 7.0 Hz), 9.38 (d, 1H, J = 8.0 Hz). MS (DCI/NH_3) *m/e* 403 $(M + H)^+$.

[11-(2-Dimethylamino-ethylcarbamoyl)-benzo[*a*]phenazin-4-yloxy]-acetic acid trifluoroacetate salt (4n′). [11-(2-Dimethylamino-ethylcarbamoyl)-benzo[*a*]phenazin-4-yloxy]-acetic acid *tert*-butyl ester was prepared using *tert*-butyl bromoacetate (15%). ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 9H), 2.75 (t, 2H), 3.90 (q, 2H), 4.80 (s, 2H) 7.13 (d, 1H) 7.72 (t, 1H), 7.95 (d, 1H), 7. 98 (d, 1H), 8.41 (dd, 1H), 8.65 (d, 1H), 9.00 (dd, 1H), 9.07 (d, 1H). To a solution of [11-(2-(dimethylamino)-ethylcarbamoyl)-benzo[*a*]phenazin-4-yloxy]-acetic acid *tert*-butyl ester (18 mg, 0.04 mmol) in dry dichloromethane (1 mL) was added trifluoroacetic acid (1 mL). After the mixture was stirred for 4 h, the solvent was removed in vacuo to yield crude product. Trituration with ether yielded the title compound as a yellow solid (10 mg, 47%), mp 192−194 °C. ^{1}H NMR (DMSO-d_6) δ 2.70 (s, 6H), 3.18 (t, 2H), 3.89−3.95 (m, 2H), 5.00 (s, 2H), 7.42 (d, 1H, J = 8.0 Hz), 7.81−7.86 (m, 1H), 8.00 (d, 1H, J = 8.0 Hz), 8.08−8.12 (m, 1H), 8.46 (d, 1H, J = 7.8 Hz), 8.55−8.62 (m, 2H), 8.85 (d, 1H, J = 8.1 Hz), 10.1 (br, 1H). MS (DCI/NH_3) *m/e* 419 $(M + H)^+$.

11-(2-Dimethylamino-ethylcarbamoyl)-benzo[*a*]phenazine-4-carboxylic acid trifluoroacetate salt (4s). 11-(2-Dimethylamino-ethylcarbamoyl)-benzo[*a*]phenazine-4-carboxylic acid methyl ester (200 mg, 0.5 mmol) was sonicated in a mixture of methanol (4 mL) and ammonium hydroxide (20 mL). The suspension was then heated to 50 °C for 92 h. All volatiles were then removed in vacuo to yield crude product, which was purified using preparative HPLC on reverse phase (C_{18}) eluting with water 80% acetonitrile 20% to yield the title compound as a yellow solid (20 mg, 8%), mp 227−229 °C. ^{1}H NMR (DMSO-d_6) δ 2.94 (d, 6H, J = 3.7 Hz), 3.49 (m, 2H), 4.00 (q, 2H, J = 6.2 Hz), 8.05 (t, 1H, J = 7.9 Hz), 8.12−8.22 (m, 2H), 8.48−8.58 (m, 3H), 9.17 (d, 1H, J = 9.8 Hz), 9.41 (d, 1H, J = 8.2 Hz), 9.82 (t, 1H). MS (DCI/NH_3) *m/e* 389 $(M + H)^+$.

4-Hydroxymethyl-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4t). To a solution of 11-(2-(dimethylamino)-ethylcarbamoyl)-benzo[*a*]phenazine-4-carboxylic acid methyl ester (317 mg, 0.78 mmol) in tetrahydrofuran (18 mL) and 2-propanol (10 mL) at 0 °C was added lithium borohydride (2.0 M solution in tetrahydrofuran, 1.97

mL, 1.97 mmol). The reaction mixture was stirred at room temperature overnight and then quenched with ammonium chloride solution (10 mL). The reaction mixture was extracted with ethyl acetate (20 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified using flash chromatography on silica eluting with 10% MeOH in dichloromethane to yield the title compound as a yellow solid (98 mg, 31%), mp 175−176 °C. ^{1}H NMR ($CDCl_3$) δ 2.50 (br s, 6H), 2.85 (br m, 2H), 3.83 (m, 2H), 5.09 (s, 2H), 7.69 (m, 3H), 7.86 (m, 1H), 8.24 (m, 2H), 8.80 (dd, 1H, J = 7.1, 1.5 Hz), 8.92 (dd, 1H, dd, J = 7.1, 1.5 Hz) 10.64 (1H, br). MS (CI/NH_3) *m/e* 375 $(M + H)^+$. Anal. ($C_{22}H_{22}N_4O_2 \cdot 3(^1/_2)H_2O$) C, H, N.

4-(2-Hydroxy-ethoxy)-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4j′). 4-[2-(*tert*-Butyl-dimethyl-silanyloxy)-ethoxy]-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide was prepared from 4-[2-(*tert*-butyl-dimethyl-silanyloxy)-ethoxy]-benzo[*a*]-phenazine-11-carboxylic acid (358 mg, 0.8 mmol) and *N*,*N*-dimethylethylenediamine (439 μL, 4.0 mmol). The intermediate was purified on silica eluting with 5% methanol in dichloromethane to give (125 mg, 30%).

To a solution of 4-[2-(*tert*-butyl-dimethyl-silanyloxy)-ethoxy]-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (125 mg, 0.24 mmol) in tetrahydrofuran (5 mL) was added a 1.0 M solution of tetrabutylammonium fluoride (1.2 mL, 1.2 mmol). After being stirred for 1.5 h, the reaction mixture was diluted with ethyl acetate (20 mL), washed with water (20 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to yield crude product which was purified using flash chromatography eluting with 5% methanol in dichloromethane to yield the title compound as an orange solid (24 mg, 25%). ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 6H). 2.68 (t, 2H, J = 6.0 Hz), 3.83 (q, 2H J = 5.7 Hz), 4.27 (m, 2H), 4.11 (m, 2H), 7.11 (d, 1H, J = 7.9 Hz), 7.57 (t, 1H, J = 8.1 Hz), 7.85 (d, 1H, J = 9.6 Hz), 7.91 (m, 1H), 8.32 (dd, 1H, J = 8.3, 1.5 Hz), 8.47 (d, 1H, J = 9.6 Hz), 8.80 (d, 1H, J = 8.3 Hz), 8.93 (dd, 1H, J = 7.3, 1.5 Hz), 10.69 (br, 1H). MS (CI/NH_3) *m/e* 405 $(M + H)^+$.

4-Amino-benzo[*a*]phenazine-11-carboxylic Acid (2-(Dimethylamino)-ethyl)-amide (4u). A mixture of 4-nitro-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (176 mg, 0.45 mmol), indium (154 mg, 0.75 mmol), and saturated ammonium chloride solution (5 mL) in ethanol (20 mL) was heated to reflux for 3 h. The reaction mixture was cooled to room temperature, quenched with water (10 mL), and then filtered through Celite. The filtrate was concentrated in vacuo, the residue was treated with saturated sodium bicarbonate solution (10 mL), extracted into chloroform (2 × 10 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to yield the title compound as a red solid (163 mg, 100%), mp 243−244 °C. ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 6H), 2.90 (t, 2H), 3.95 (m, 2H), 7.10 (d, 1H) 7.65 (t, 1H), 7.90 (m, 2H), 8.05 (d, 1H), 8.35 (d, 1H), 8.70 (d, 1H), 8.95 (d, 1H), 11.0 (br, 1H). MS (DCI/NH_3) *m/e* 360 $(M + H)^+$.

4-Acetylamino-benzo[*a*]phenazine-11-carboxylic Acid (2-(Dimethylamino)-ethyl)-amide (4x). To a solution of 4-amino-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (20 mg, 0.056 mmol) in tetrahydrofuran (5 mL) was added pyridine (0.1 mL) and acetyl chloride (20 μL). After being stirred for 1 h, the reaction mixture was quenched with sodium bicarbonate solution (10 mL), extracted with ethyl acetate (20 mL), separated, and dried ($MgSO_4$). The solvent was removed in vacuo, and the residue was triturated with ether to yield the title compound as a yellow solid (10 mg, 45%), mp 233 234 °C. ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 6H), 2.40 (s, 3H), 2.65 (t, 2H), 3.80 (q, 2H), 7.45 (t, 1H), 7.70 (m, 2H), 7.90 (m, 2H), 8.20 (s, 1H), 8.25 (d, 1H), 8.70 (d, 1H), 8.80 (d, 1H), 10.10 (br, 1H). MS (DCI/NH_3) *m/e* 402 $(M + H)^+$.

4-Methanesulfonylamino-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4y) was prepared in an analogous manner from 4-amino-benzo[*a*]-phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (52 mg, 0.14 mmol) and methanesulfonyl chloride (10 μL) to give the title compound as a yellow solid (10 mg, 16%), mp 204 °C dec. ^{1}H NMR (MeOH-d_4) δ 3.18 (s, 6H), 3.24 (s, 3H), 3.70 (t, 2H, J = 6.0 Hz), 4.22 (t, 2H, J = 6.0 Hz), 8.02−7.92 (m, 3H), 8.09 (dd, 1H, J = 7.5, 8.2 Hz), 8.42 (d, 1H, J = 8.5 Hz), 8.65 (d, 1H, J = 9.7 Hz), 8.79 (d, 1H, J = 6.2 Hz), 8.98 (d, 1H, J = 7.9 Hz). MS (DCI/NH_3) *m/e* 438 $(M + H)^+$.

4-Bis-(Methanesulfonylamino)-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (4z) was prepared in a similar manner from 4-amino-benzo[*a*]-phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (19.4 mg, 0.054 mmol) using excess methanesulfonyl chloride (40 μL) and triethylamine (0.1 mL, 0.71 mmol) to give the title compound as a yellow solid (13 mg, 47%), mp 226−227 °C. ^{1}H NMR $CDCl_3$ δ 2.45 (s, 6H), 2.75 (t, 2H), 3.60 (s, 6H), 3.90 (q, 2H), 7.85 (d, 1H), 7.95 (t, 1H), 8.05 (t, 1H), 8.20 (d, 1H), 8.35 (d, 1H), 8.45 (d, 1H), 9.08 (d, 1H), 9.75 (d, 1H), 10.65 (br, 1H). MS (DCI/NH_3) *m/e* 516 $(M + H)^+$.

4-(Cyanomethyl-amino)-benzo[*a*]phenazine-11-carboxylic Acid (2-(dimethylamino)-ethyl)-amide (4v). To a solution of 4-amino-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (69 mg, 0.19 mmol) in methanol (10 mL) was added formaldehyde (37% solution, 1.0 mL), potassium cyanide (102 mg, 1.47 mmol), and 2 N HCl (1.0 mL). The reaction mixture was heated to 50 °C for 3 h. The reaction mixture was then cooled to room temperature, diluted with water (15 mL) and sodium bicarbonate solution (15 mL), extracted into dichloromethane (2 × 15 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to yield crude product. This was purified using flash chromatography eluting with 10% methanol in dichloromethane to yield the title compound as a violet solid (13 mg, 17%), mp 224−225 °C. ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 6H), 2.80 (t, 2H), 3.10 (s, 2H), 3.90 (q, 2H, 7.03 (d, 1H,), 7.75 (t, 1H), 7.95 (m, 2H), 8.12 (d, 1H), 8.40 (d, 1H), 8.80 (d, 1H), 9.05 (d, 1H), 10.95 (br, 1H). MS (DCI/NH_3) *m/e* 416 $(M\ NH_4)^+$.

3-Dimethylamino-2-[(4-methoxy-benzo[*a*]phenazine-11-carbonyl)-amino]-propionic Acid Hydrochloride (4s′). A mixture of 3-(dimethylamino)-2-[(4-methoxy-benzo[*a*]phenazine-11-carbonyl)-amino]-propionic acid methyl ester (150 mg, 0.35 mmol) and 1 M HCl (50 mL) was heated to reflux for 1 h. After cooling to room temperature, the volatile components were removed in vacuo to yield the title compound as a red solid (158 mg, 100%). ^{1}H NMR (DMSO-d_6) δ 2.95 (br s, 6H), 3.80−3.60 (m, 2H), 4.10 (s, 3H), 5.39 (m, 1H), 7.54 (d, 1H, J = 8.00 Hz), 7.86 (t, 1H, J = 8.19 Hz), 8.03 (d, 1H, J = 9.49 Hz), 8.14 (t, 1H, J = 7.25 Hz), 8.55 (m, 2H), 8.69 (d, 1H, J = 7.06 Hz), 9.01 (d, 1H, J = 8.19 Hz), 10.74 (br d, 1H). MS (DCI/NH_3) *m/e* 419 $(M + H)^+$.

4,10-Dihydroxy-benzo[*a*]phenazine-11-carboxylic Acid (2-(Dimethylamino)-ethyl)-amide (4w′). To a cold solution of 4,10-dimethoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (96 mg, 0.24 mmol) in dichloromethane (2 mL) was added a 1.0 M solution of boron tribromide in dichloromethane (2.14 mL, 2.14 mmol). The reaction mixture was stirred at room temperature for 16 h and then ice (5 g) was added with sodium carbonate (5 mL) and sodium chloride (5 mL). The organics were extracted into dichloromethane (2 × 10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo to yield an orange compound which was recrystallized from dichloromethane, methanol, and hexane to yield the title compound as an orange solid (6 mg, 8%), mp 257 °C (dec). ^{1}H NMR (DMSO-d_6) δ 2.33 (s, 6H), 2.67 (t, 2H, J = 5.7 Hz), 3.75−3.80 (m, 2H), 7.32 (d, 1H, J = 7.3 Hz), 7.64 (d, 1H, J = 9.4 Hz), 7.70−7.74 (m, 1H), 7.85 (d, 1H, J = 9.4 Hz), 8.3 (d, 1H, J = 9.4 Hz), 8.40 (d, 1H, J = 9.4 Hz), 8.78 (d, 1H, J = 8.1 Hz), 10.55 (br, 1H), 11.4 (br, 1H). MS (DCI/NH_3) *m/e* 377 $(M + H)^+$. Anal. ($C_{21}H_{20}N_4O_3$) C, H, N.

10-Hydroxy-4-methoxy-benzo[*a*]phenazine-11-carboxylic Acid (2-(Dimethylamino)-ethyl)-amide (4x′). To a cold solution of 4,10-dimethoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (300 mg, 0.74 mmol) in dichloromethane (25 mL) was added a 1.0 M solution of boron tribromide in dichloromethane (1.63 mL, 1.63 mmol). The

reaction mixture was stirred at room temperature for 6 h, and then ice (5 g) was added with sodium carbonate (5 mL) and sodium chloride (5 mL). The organics were extracted into dichloromethane (2 × 10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo to yield a yellow solid which was purified using flash chromatography on silica eluting with ethyl acetate and 15% methanol to yield the title compound as a yellow solid (61 mg, 21%), mp 181−182 °C. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 6H), 2.78 (t, 2H J = 6.0 Hz), 3.86−3.90 (m, 2H), 4.10 (s, 3H), 7.25 (d, 1H J = 7.8 Hz), 7.57 (d, 1H J = 9.4 Hz), 7.70−7.75 (m, 1H), 7.95 (d, 1H J = 9.4 Hz), 8.22 (d, 1H J = 9.4 Hz), 8.51 (d, 1H J = 9.6 Hz), 8.81 (d, 1H J = 8.2 Hz), 11.65 (br, 1H), 16.17 (s, 1H). MS (DCI/NH_3) *m/e* 391 $(M + H)^+$.

4,9-Dimethoxy-benzo[*a*]phenazine-11-carboxylic Acid (2-(Dimethylamino)-ethyl)-amide (4u′). A mixture of 9-chloro-4-methoxy-benzo[*a*]phenazine-11-carboxylic acid (2-(dimethylamino)-ethyl)-amide (85 mg, 0.21 mmol) and a 25% solution of sodium methoxide in methanol (4 mL) was heated to reflux for 6 h. The reaction mixture was then cooled to room temperature, diluted with ethyl acetate (15 mL), washed with water (15 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to yield a yellow solid which was purified using flash chromatography on silica eluting with ethyl acetate and 15% methanol to yield the desired title compound as a yellow solid (42 mg, 50%), mp 184−185 °C. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 6H), 2.75 (t, 2H, J = 6.0 Hz), 3.85−3.90 (m, 2H), 4.07 (s, 3H), 4.10 (s, 3H),7.22 (d, 1H J = 8.0 Hz), 7.64 (d, 1H J = 3.0 Hz), 7.72−7.77 (m, 1H), 7.91 (d, 1H J = 9.4 Hz), 8.55 (d, 1H J = 9.6 Hz), 8.70 (d, 1H J = 3.0 Hz), 8.99 (d, 1H J = 8.0 Hz), 10.92 (br, 1H). MS (ESI +ve) *m/e* 405 $(M + H)^+$.

4-Methoxy-benzo[*a*]phenazine-11-carboxylic Acid (2-(Dimethylamino)-1-hydroxymethyl-ethyl)-amide (4p″). A mixture of 3-(dimethylamino)-2-[(4-methoxy-benzo[*a*]phenazine-11-carbonyl)-amino]-propionic acid methyl ester (335 mg, 0.66 mmol) and lithium borohydride (72 mg, 3.3 mmol) in tetrahydrofuran (10 mL) and 2-propanol (10 mL) was stirred at room temperature for 18 h. A further 5 equiv of lithium borohydride were added portionwise, and the mixture was stirred for 18 h. The reaction was quenched with ammonium chloride solution (5 mL), extracted with ethyl acetate (2 × 20 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo to yield a brown gum. Purification using flash chromatography on silica eluting with ethyl acetate and 15% methanol and trituration with ether yielded the desired title compound as an orange powder (50 mg, 19%), mp 168−170 °C. ^{1}H NMR ($CDCl_3$) δ 2.35, (s, 6H), 2.87 (m, 2H), 4.05 (m, 5H), 4.55 (m, 1H), 7.20 (s, 1H), 7.71 (t, 1H, J = 8.14), 7.88 (m, 2H), 8.35 (dd, 1H, J = 1.48, 8.47 Hz), 8.50 (1H, d, J = 9.55 Hz), 8.85 (1H, d, J = 8.17 Hz), 8.91 (1H, dd, J = 1.47 Hz, 7.29 Hz), 11.05, (1H, d). MS (DCI/NH_3) *m/e* 405 $(M + H)^+$.

Biology. Cytotoxicity Assays. The cytotoxicity of compounds was measured using the H69 parental (H69/P) human small cell lung carcinoma cell line and the drug resistant human small cell lung carcinoma cell line H69/LX4 which overexpresses P-glycoprotein (Pgp). The cytotoxicity of selected compounds was also measured using the COR-L23 parental (COR-L23/P) human non small cell lung carcinoma cell line and also the drug resistant human non small cell lung carcinoma cell line COR-L23/R which overexpresses multidrug resistance associated protein (MRP). The cytotoxicity, as measured by the IC_{50} (concentration required to give 50% cell kill) in the resistant cell line, divided by the cytotoxicity in the parental cell line gives an indication of the degree to which a compound is affected by Pgp or MRP-dependent MDR and is termed the resistance factor (Rf) of the compound.

These cytotoxicity assays were performed as described previously.[28, 29] After addition of the cytotoxics, the cells were incubated for 5−6 days before adding Alamar Blue (H69/P, H69/LX4) or Sulfurhodamine B (COR-L23, COR0L23/R) to measure cell proloferation.

Phamacokinetic Studies. Female Balb/c (20−30 g) were used throughout the studies. Animals (n = 2−4 per time point) were administered XR11576 either iv via a lateral tail vein at 20 and 50 mg/kg or by oral gavage at 50 mg/kg. At various times (2 min−24 h) after dosing, blood samples were obtained from anaesthetized animals by cardiac puncture. The blood samples were collected using heparinized syringes and centrifuged to prepare plasma which was analyzed using HPLC.[30]

Xenograft Studies. Xenograft studies were performed using H69 SCLC (7 × 10^6) cells harvested from in vitro incubations in PBS, with inoculation performed subcutaneously into the right flank of female CD1 nude mice (20−28 g). Tumor volumes were estimated as described previously.[31] When tumors had reached an average size of between 0.3 and 0.7 cm, the animals were randomized into groups of 7−8 by tumor volume. The efficacy of XR11576 was evaluated following iv administration and compared with the clinical candidate TAS-103 (given iv).

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