Potential Chemopreventive Agents Based on the Structure of the Lead Compound 2-Bromo-1-hydroxyphenazine, Isolated from *Streptomyces* Species, Strain CNS284

Martin Conda-Sheridan,[†] Laura Marler,[‡] Eun-Jung Park,[‡] Tamara P. Kondratyuk,[‡] Katherine Jermihov,[§] Andrew D. Mesecar,[§] John M. Pezzuto,[‡] Ratnakar N. Asolkar,[‖] William Fenical,[‖] and Mark Cushman*,[†]

[†]*Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, and The Purdue University Center for Cancer Research, Purdue University, West Lafayette, Indiana 47907, United States,* [‡]*College of Pharmacy, University of Hawaii at Hilo, Hilo, Hawaii 96720, United States,* [§]*Center for Pharmaceutical Biotechnology and Department of Medicinal Chemistry and Pharmacognosy, Department of Biological Sciences, and The Purdue University Center for Cancer Research, Purdue University, West Lafayette, Indiana 47907, United States and* [‖]*Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92037, United States*

The isolation of 2-bromo-1-hydroxyphenazine from a marine *Streptomyces* species, strain CNS284, and its activity against NF-κB, suggested that a short and flexible route for the synthesis of this metabolite and a variety of phenazine analogues should be developed. Numerous phenazines were subsequently prepared and evaluated as inducers of quinone reductase 1 (QR1) and inhibitors of quinone reductase 2 (QR2), NF-κB, and inducible nitric oxide synthase (iNOS). Several of the active phenazine derivatives displayed IC$_{50}$ values vs QR1 induction and QR2 inhibition in the nanomolar range, suggesting that they may find utility as cancer chemopreventive agents.

Introduction

In 2008, cancer accounted for almost 8 millions deaths worldwide,[1] and it is the second leading cause of death in the United States.[2,3] Moreover, the number of cases is expected to increase by over 45% in the next 20 years.[3] Even with the recent advances in chemotherapy, the best strategy for dealing with the cancer problem is simply to prevent it from developing. Chemoprevention aims to prevent, delay, and ultimately reverse cancer development.[4] A strategy to avoid carcinogenesis is to avert the metabolic formation of carcinogens.[5] This may be achieved by inhibition of phase I enzymes,[6] such as aromatase.[7] These enzymes modify potential carcinogens, rendering them more active.[7,8] In some cases, quinone reductase 2 (QR2)[a] has behaved like a phase I enzyme by converting quinone substrates into species capable of causing carcinogenesis.[9–11] The same end could be achieved by induction of phase II enzymes, such as quinone reductase 1 (QR1)[12–15] or glutathione S-transferase (GST),[16] which detoxify carcinogens.[17] Finally, the transcription factor nuclear factor κ-light chain enhancer of activated B cells (NF-κB)[18,19] and inducible nitric oxide synthase (iNOS)[20] are also regarded as potential chemopreventive targets.

Small molecules that can affect more than one pathway or interact with multiple targets are especially attractive candidates for chemoprevention.[21–27] Molecules of the phenazine class have been observed as secondary metabolites from
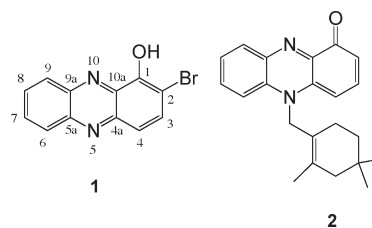

Figure 1. Phenazine natural products.

Streptomyces, *Pseudomonas*, and other marine microorganisms.[28] Natural or synthetic phenazines have shown antibiotic, anticancer, antifungal, and neuroprotective properties.[29–31] For example, 2-bromo-1-hydroxyphenazine (**1**, Figure 1)[32] and lavanducyanin (**2**, Figure 1)[33,34] present interesting biological activities, including cytotoxicity against cancer cells and antibiotic activity.

As described in the present report, the natural product **1**, which was isolated by activity-directed fractionation of a *Streptomyces* species, strain CNS284, was highly cancer cell cytotoxic (IC$_{50}$ = 0.1 μM against HCT-116) and showed weak to moderate activity in the NF-κB-luciferase assay (IC$_{50}$ = 73 μM). This result suggested further exploration of the chemopreventive activities of analogues of phenazine **1**. The phenazines **8**, **9**, and **10** were obtained during the synthesis of the natural product **1**. Examination of these compounds in a panel of chemopreventive targets revealed surprising activities vs a number of them. Phenazine **8** inhibited iNOS activity (IC$_{50}$ 4.2 μM), while compound **9** doubled the concentration of QR1 at a activity of 29.4 μM, and the phenazine **10** inhibited QR2 (IC$_{50}$ = 18.5 μM). These results suggested the further optimization of these activities through structure manipulation. However, in view of the disparate targets and inadequate knowledge of the mechanisms of action involved

*To whom correspondence should be addressed. Tel: 765-494-1465. Fax: 765-494-6790. E-mail: cushman@purdue.edu.

[a]Abbreviations: BBO, multinuclear broadband observe; CD, concentration to double QR1 induction; CI, chemopreventive index, IC$_{50}$/CD; DMEM, Dulbecco's modified Eagle's medium; IC$_{50}$, half maximal inhibitory concentration; iNOS, inducible nitric oxide synthase; IR, QR1 induction ratio; LPS, lipopolysaccharide; NF-κB, nuclear factor κ-light chain enhancer of activated B cells; QNP, quattro nucleus probe; QR1, quinone reductase 1; QR2, quinone reductase 2.

Scheme 1[a]
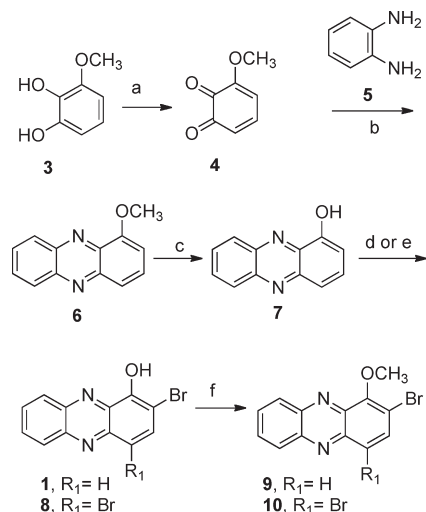
[a] Reagents and conditions: (a) *o*-Chloranil, ether. (b) PhH, HOAc. (c) HBr, HOAc, reflux. (d) One equivalent of NBS, PhCH$_3$. (e) Two equivalents of NBS, PhCH$_3$. (f) MeI, K$_2$CO$_3$, acetone.
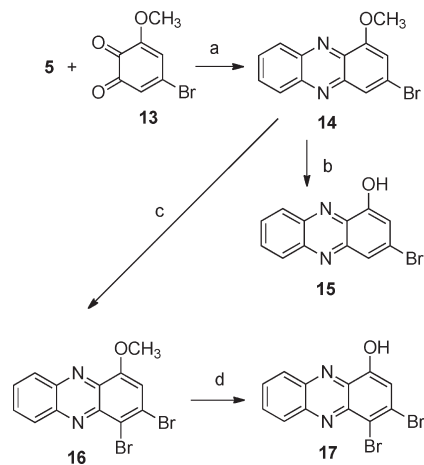
Scheme 2[a]
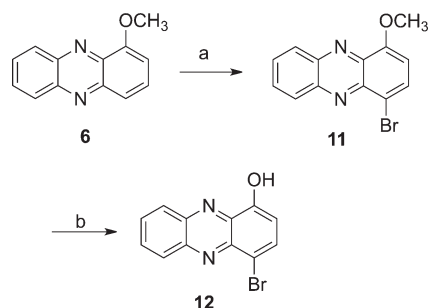
[a] Reagents and conditions: (a) One equivalent of NBS, PhCH$_3$, CH$_3$CN. (b) BBr$_3$, CH$_2$Cl$_2$.

Scheme 3[a]

[a] Reagents and conditions: (a) PhH, HOAc. (b) HBr, HOAc. (c) NH$_4$OAc, Br$_2$, HOAc. (d) BBr$_3$, CH$_2$Cl$_2$.

Scheme 4[a]

20 R$_1$= R$_4$= H, R$_2$= R$_3$= CH$_3$
21 R$_1$= CH$_3$, R$_3$= Br, R$_2$= R$_3$= H
22 R$_1$= R$_3$= Br, R$_2$= R$_4$= H
23 R$_1$= R$_3$= R$_4$= H, R$_2$= CH$_3$
24 R$_1$= R$_3$= R$_4$= H, R$_2$= OCH$_3$
25 R$_1$= R$_4$= H, R$_2$= R$_3$= OCH$_3$
26 R$_1$= Br, R$_3$= NO$_2$, R$_2$= R$_4$= H
27 R$_1$=R$_4$= OCH$_3$, R$_2$= R$_3$= H

28 R$_1$= R$_4$= H, R$_2$= R$_3$= CH$_3$
29 R$_1$= CH$_3$, R$_3$= Br, R$_2$= R$_4$= H
30 R$_1$= R$_3$= Br, R$_2$= R$_4$= H
31 R$_1$= R$_3$= R$_4$= H, R$_2$= CH$_3$
32 R$_1$= R$_3$= R$_4$= H, R$_2$= OCH$_3$
33 R$_1$= R$_4$= H, R$_2$= R$_3$= OCH$_3$
34 R$_1$= Br, R$_3$= NO$_2$, R$_2$= R$_4$= H
35 R$_1$=R$_4$= OCH$_3$, R$_2$= R$_3$= H

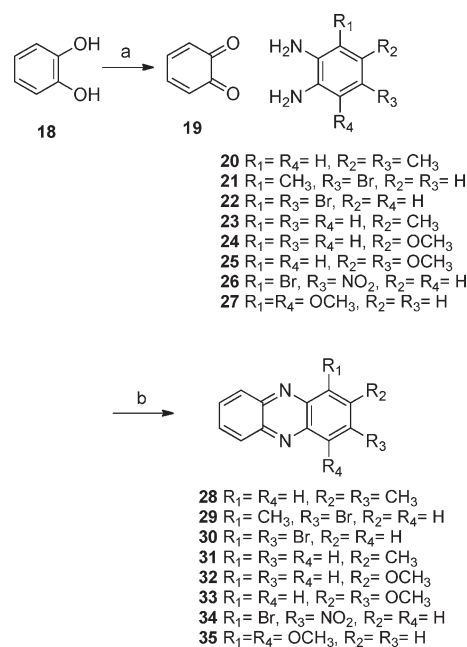[a] Reagents and conditions: (a) Ag$_2$O, acetone. (b) PhH, HOAc.

with a number of them, and the complete lack of any information about the chemopreventive activities of any phenazines, the initial approach has necessarily been empirical, consisting mainly of the synthesis of a random array of substituted phenazines that were brominated, chlorinated, methylated, methoxylated, and nitrated in various locations. The activities of the resulting compounds were determined vs QR1 induction, QR2 inhibition, iNOS inhibition, and NF-κB inhibition. Although several reports have evaluated the biological activities of phenazines,[28−34] to our knowledge, this is the first report on their chemopreventive properties, including QR1 induction and QR2 inhibition.

Results and Discussion

Chemistry. The core structure, 1-methoxyphenazine (**6**), was prepared following published procedures (Scheme 1).[35,36] Briefly, 1-methoxycatechol (**3**) was oxidized with *o*-chloranil and then condensed with *o*-phenylenediamine (**5**). Demethylation under standard conditions[35] yielded **7**. Subsequent bromination with 1 equiv of *N*-bromosuccinimide gave the natural product 2-bromo-1-hydroxyphenazine (**1**). The spectral properties of the synthetic compound were identical with those of the natural product isolated from the marine *Streptomyces* species, strain CNS284. Treatment of compound **7** with 2 equiv of *N*-bromosuccinimide yielded com-
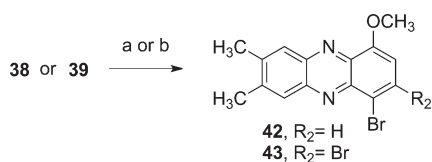
pound **8**. This compound showed a good QR1 induction ratio (IR) of 2.8; consequently, the position of the bromine substituent was altered to study the effect on activity.

The para-substituted phenazines **11** and **12** were synthesized by bromination and demethylation of compound **6** (Scheme 2). The synthesis of the *m*-bromo derivative **14** (Scheme 3) was achieved by condensation of the appropriate quinone **13** with *o*-phenylenediamine (**5**). Subsequent deprotection, bromination, and demethylation gave the analogues **15−17**. In addition, a series of simple mono- and disubstituted phenazines, **28−35**, were prepared to study the influence that different substitution patterns have on the biological activity (Scheme 4).

The next step consisted of the addition of methyl groups (**38** and **39**) or chloride (**40** and **41**) to the phenazine system (Scheme 5). This series also included some bromide substituents (**39** and **41−43**, Schemes 5 and 6). Compound **40** was
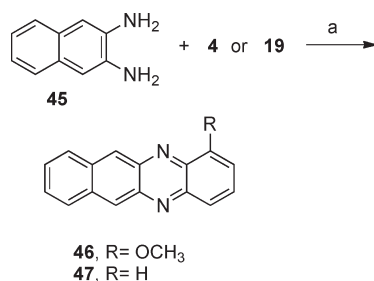
Scheme 5[a]

36, R$_1$= CH$_3$
37, R$_1$= Cl

4, R$_2$= H
13, R$_2$= Br

38, R$_1$= CH$_3$, R$_2$= H
39, R$_1$= CH$_3$, R$_2$= Br
40, R$_1$= Cl, R$_2$= H
41, R$_1$= Cl, R$_2$= Br
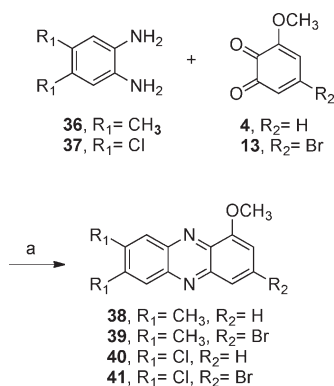
[a] Reagents and conditions: (a) PhH, HOAc.

Scheme 6[a]

38 or **39**

42, R$_2$= H
43, R$_2$= Br

[a] Reagents and conditions: (a) One equivalent of NBS, PhCH$_3$, CH$_3$CN. (b) Br$_2$, HOAc, NaOAc.

Scheme 7[a]

40

44
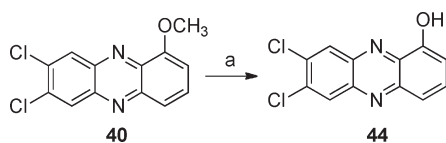
[a] Reagents and conditions: (a) HBr, HOAc.

Scheme 8[a]

45 + **4** or **19**

46, R= OCH$_3$
47, R= H

[a] Reagents and conditions: (a) PhH, HOAc.

Scheme 9[a]

45 + **48**

49

[a] Reagents and conditions: (a) PhH, HOAc.

Scheme 10[a]

50 **48** **51**

52 **53**

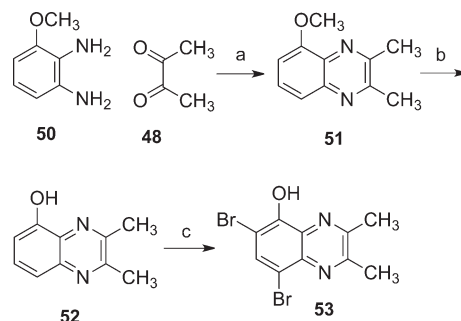[a] Reagents and conditions: (a) PhH, HOAc. (b) BBr$_3$, CH$_2$Cl$_2$. (c) Two equivalents of NBS, PhCH$_3$, CH$_3$CN.

demethylated yielding **44** (Scheme 7). In addition, the benzo-[*b*]phenazine analogues **46** and **47** were synthesized (Schemes 8). In compound **49** (Scheme 9), the positions of the nitrogens were changed. Finally, quinoxaline analogues of the phenazines were synthesized using the same condensation reaction (Scheme 10) to study the behavior of smaller fragments.

QR1 Inductive Activity. It is believed that chemoprevention can be achieved by induction of QR1, which deactivates potential carcinogens.[37] The effect of phenazines on QR1 induction was tested as described in the Experimental Section.[38] Analogues of the phenazine natural product **1** and compound **6** were prepared to establish structure–activity relationships for QR1 induction. Both the induction ratio (IR) and the concentration to double (CD) values were determined for each compound, where the IR is the QR1 activity in mouse hepatoma cells in the presence of a 50 μM concentration of the phenazine divided by the activity in the absence of the phenazine, and the CD is the concentration of the drug that doubles the QR1 activity. The experimental results (Table 1) indicate that a bromine substituent para to the oxygen atom at C-1 increases QR1 induction when substituents are absent on the second benzene ring. This effect is apparent in compounds **8** and **11**, which present induction ratios (IRs) of 2.8 and 2.7 and concentration to double QR1 activity (CD) values of 27.0 and 4.3 μM, respectively. On the other hand, a bromine substituent in the meta position has a negative effect on enzyme induction, that is, **14** and **15**. Methylation of the hydroxyl group has, in general, a positive effect on the activity. For example, compound **11** has an IR of 2.7, which is larger than its hydroxyl analogue **12**, which has an IR of 1.5. Furthermore, the methylated compound **16** has an IR of 1.6 versus a 1.0 value for the phenolic analogue **17**. This result documents the detrimental effect of a bromine substituent in the meta position, which is also seen with other compounds.

The next aim was to analyze how various substituents on the unsubstituted benzene ring affect QR1 induction. The parent compound **38** (IR = 4.7, and CD = 11.3 μM) was more active than its brominated analogues **39** and **43** (IR values of 1.2 and 1.4). The positive effect of a bromine substituent para to the methoxy group did hold for the analogue **42**, which shows an IR of 6.6 (CD = 1.83 μM). Given that the IR of **38** was also higher than the parent compound **6**, it was hypothesized that the better IR values of these compounds were also a consequence of the substituents at C-7 and C-8. This hypothesis was confirmed when other substituents were added to the ring. For example, the chlorinated compounds **40**, **41**, and **44** have IR values of 4.2, 3.1, and 2.2 (CD values = 9.42, 0.012, and 1.85 μM). The positive effect of the methoxy group on QR1 activity is also observed with these compounds. In addition, the data suggest a positive effect of oxygen substitution on the IR values, that is, compound **38** has an IR of 4.7, which is higher than the deoxygenated compound **28** with an IR of 2.4. The best inducer of QR1 is compound **46**, with an IR value of 7.9 at 305 nM (the IR at the testing concentration of 50 μM was 2.2) and a CD value of only 4.7 nM. This compound

Table 1. Induction of QR1 and Inhibition of NF-κB, iNOS, and QR2

compd	QR1 IR[a,b]	QR1 CD[c,d] (μM)	NF-κB-luciferase Luciferase activity % inhibition[b,e]	NF-κB-luciferase IC50[f] (μM)	Nitrite assay % max inhibition[b,g]	Nitrite assay IC50[f] (μM)	QR2 % max inhibition	QR2 IC50[f,h] (μM)
1	0.7		50.0 ± 0.7	73.0 ± 0.0	31.5 ± 2.5		N/A[h]	N/A
6	1.4		0		2.4 ± 1.1		88.6 ± 1.7	31.1 ± 1.7
8	2.8	27.0	17.5 ± 4.9		70.9 ± 1.1	4.2 ± 0.5	N/A	N/A
9	2.5	29.4	0		44.7 ± 2.2		94.9 ± 1.1	0.186 ± 0.03
10	1.8		0		24.7 ± 4.0		88.7 ± 7.5	18.5 ± 4.4
11	2.7	4.3	54.0 ± 8.5		47.5 ± 7.7		90.4 ± 2.9	36.8 ± 2.7
12	1.5		75.0 ± 5.2	5.19 ± 2.4	84.7 ± 0.0	31.5 ± 0.0	50.3 ± 2.7	26.9 ± 3.6
14	1.0		0		68.6 ± 5.1	26.5 ± 1.5	88.8 ± 2.24	0.69 ± 0.11
15	0.7		99.8 ± 0.1	5.42 ± 0.4	86.5 ± 0.4	28.4 ± 0.4	N/A	N/A
16	1.6		84.2 ± 2.0[j]		70.9 ± 0.4	33.7 ± 0.4	93.3 ± 0.64	0.16 ± 0.02
17	1.0		91.5 ± 4.8	4.74 ± 2.35	79.2 ± 3.3	17.4 ± 1.2	61.5 ± 2.3	1.4 ± 0.27
28	2.4	23.0	82.0 ± 5.7	9.15 ± 3.18	49.1 ± 1.8		90.2 ± 2.03	13.5 ± 0.93
29	1.2		0		5.5 ± 1.8		89.9 ± 0.5	0.45 ± 0.02
30	1.9		0		16.4 ± 0.4		42.1 ± 0.78	3.3 ± 0.27
31	0.8		10.5 ± 7.8		38.7 ± 1.8		109.1 ± 9.1	60.8 ± 9.86
32	0.8		16.0 ± 5.7		12.5 ± 4.4		90.0 ± 1.51	15.3 ± 0.76
33	0.8		0		29.4 ± 3.3		80.0 ± 2.47	18.2 ± 1.59
34	1.6		72.5 ± 15.2	13.1 ± 0.7	79.7 ± 3.3	7.2 ± 1.1	85.1 ± 0.4	0.32 ± 0.02
35	1.0		18.5 ± 9.2		5.0 ± 2.6		93.6 ± 9.3	35.5 ± 8.2
38	4.7	11.3	48.5 ± 9.2		53.8 ± 9.9	47.0 ± 1.4	100.5 ± 8.6	11.7 ± 3.17
39	1.2		79.2 ± 5.9	6.64 ± 0.76	8.8 ± 5.9		31.2 ± 2.5	10.5 ± 2.7
40	4.2	9.42	93.4 ± 0.2	16.17 ± 3.5	14.6 ± 9.5		74.4 ± 3.81	4.8 ± 0.95
41	3.1	0.012	77.4 ± 2.0	2.46 ± 0.5	0.0 ± 3.3		N/A	N/A
42	6.6	1.83	67.5 ± 3.5	17 ± 0.85	51.9 ± 2.9	48.9 ± 1.6	42.7 ± 3.6	11.3 ± 1.0
43	1.4		92.4 ± 2.3	1.39 ± 1.7	77.7 ± 5.5	34.5 ± 2.0	82.6 ± 1.12	4.1 ± 0.22
44	2.2	1.85	50.5 ± 3.3		51.2 ± 1.8	47.2 ± 1.2	76.7 ± 1.95	0.48 ± 0.09
46	7.9[i,j]	0.0047	99.5 ± 9.9	2.1 ± 1.3	98.9 ± 0.4	7.2 ± 1.0	64.4 ± 1.21	3.5 ± 0.28
47	0.6[j]	4.39	84.5 ± 6.4	2.48 ± 1.3	98.7 ± 3.7	5.9 ± 0.5	56.1 ± 1.9	11.2 ± 1.2
49	3.2	16.6	77.3 ± 7.9[j]		3.4 ± 11.4		N/A	N/A
51	1.4		52.0 ± 4.24		0.8 ± 13.6		N/A	N/A
52	1.7		52.5 ± 4.95[j]		0.0 ± 7.3		N/A	N/A
53	1.8		91.9 ± 2.4	1.93 ± 0.3	81.3 ± 0.4	45.6 ± 2.6	6.2 ± 0.76	2.9 ± 1.54
54		0.23						
55		0.26						

[a] IR, induction ratio. [b] Testing concentration, 50 μM (except for **1**, which was tested at 100 μM in the NF-κB assay). [c] CD is the concentration that doubles the activity. [d] CD values were determined for phenazines with induction ratios > 2. [e] NF-κB IC50 calculated when inhibition > 70% (except for **1**). [f] IC50, median inhibitory concentration. [g] Nitrite IC50 calculated when inhibition > 50%. [h] N/A, not applicable, no inhibition; compounds **1** and **8** react directly with the NRH substrate, so no inhibition data could be obtained. [i] Testing concentration, 305 nM. [j] Cytotoxic at testing concentration.

was moderately cytotoxic in Hepa 1c1c7 cells, with an IC50 of 9.58 μM, hence, the lower IR at the testing concentration. This cytotoxicity is not a concern, since the GI50 value is 3 orders of magnitude above the dose needed to significantly induce QR1. The chemopreventive index (CI) for this compound (GI50/CD) is 2038, where the GI50 is the concentration required to inhibit growth 50% relative to control (no drug) after a 48 h incubation period.

QR2 Inhibitory Activity. As stated earlier, in some cases, QR2 may produce toxic metabolites; therefore, its inhibition could prevent carcinogenesis.[10] Inhibition assays were performed on the synthetic compounds as described in the Experimental Section. The addition of chlorine atoms increases inhibitory activity, resulting in IC50 values of 4.8 ± 0.95 (compounds **40**) and 0.48 ± 0.09 μM (**44**) versus the unsubstituted compound **6**, with an IC50 of 31.1 ± 1.7 μM. Compounds with a bromine atom in the meta position, with respect to the oxygen, also showed good inhibition of QR2 with IC50 values of 0.69 ± 0.11 (compound **14**), 0.16 ± 0.02 (**16**), 1.4 ± 0.27 (**17**), and 4.1 ± 0.22 μM (**43**). Three other compounds that showed very good inhibitory activity were **9**, **29**, and **34**, with IC50 values of 0.18 ± 0.03, 0.45 ± 0.02, and 0.32 ± 0.02 μM.

NF-κB Inhibitory Activity. NF-κB is an inducible transcription factor that, when activated, promotes cell survival and differentiation. On the other hand, its down regulation renders cells more sensitive to apoptosis.[19] This fact makes NF-κB inhibitors good candidates for chemoprevention. Most of the compounds showed modest to good activity against NF-κB. In general, a bromine meta to the oxygen increased activity, that is, compounds **15−17**, **39**, **41**, and **43**. Also, molecules with two bromine atoms, such as **43**, were even more active than molecules **39** or **42**, with only one bromine substituent. Finally, bromine-substituted hydroxyphenazines showed a higher activity than their methylated analogues, that is, **11** vs **12** or **16** vs **17**.

Nitrite Assay. Inhibitors of iNOS have been considered as potential anti-inflammatory and cancer chemopreventive agents.[20] Therefore, the inhibitory activities of phenazines on the production of nitrite, a stable product of nitric oxide (NO), have been evaluated. Various phenazines showed moderate inhibitory iNOS activities. Most of the compounds that contain bromine atoms in the meta and/or para positions, with respect to the oxygen, were active. The hydroxyl compounds are more active than their methylated analogues, that is, **8** vs **10** or **15** and **17** vs **14** and **16**. Also, phenazines with methyl substituents on the nonoxygenated benzene ring were active. Overall, the most active compounds were **8**, **34**, **46**, and **47**.

Multiple Activities. Arguably, the most attractive feature of these phenazines is their ability to interact with multiple

Table 2. Rank Order of Compounds in the Nitrite, NF-κB, QR1, and QR2 Assays[a]

| nitrite		NF-κB		QR1		QR2	
compd[b]	IC$_{50}$ (μM)	compd[b]	IC$_{50}$ (μM)	compd[b]	CD (μM)	compd[b]	IC$_{50}$ (μM)
8	4.2	*43*	1.4	46	0.0047	**16**	0.16
47	5.9	*53*	1.9	41	0.012	**9**	0.19
46	7.2	*46*	2.1	42	1.8	**34**	0.32
34	7.2	**41**	2.5	44	1.8	**29**	0.45
17	17.4	*47*	2.5	11	4.3	**44**	0.48
14	26.5	*17*	4.7	47	4.4	**14**	0.69
15	28.4	*12*	5.2	40	9.4	**17**	1.4
12	31.5	*15*	5.4	38	11.3	**53**	2.9
16	33.7	**39**	6.6	49	16.6	**30**	3.3
43	34.5	*28*	9.2	28	23	**46**	3.5
53	45.6	*34*	13.1	8	27	**43**	4.1
38	47.0	**42**	17.0	9	29.4	**40**	4.8

[a] The compounds listed are the 12 most active compounds in each of the four assays out of 32 compounds tested. [b] The compound numbers that are italic appear in both the nitrite and the NF-κB columns.

targets (Table 1). The ability to block multiple pathways may create a synergistic effect that could lead to greater chemopreventive potential.[24−27,39−41] This is critical in chemoprevention because "cancer is a multifactorial disease that requires modulation of multiple pathways and multiple targets".[42] The most notable compounds are **9**, **16**, **28**, **34**, **38**, and **46**. These compounds present QR1 inductive or QR2, iNOS or NF-κB inhibitory activities on multiple targets at low micromolar concentrations. In some cases, the biological results were obtained in the nanomolar concentration range. 1-Methoxybenzo[b]phenazine (**46**) exhibited the best all-around activity vs all four targets: QR1 IR 7.9 and CD 0.0047 μM, NF-κB IC$_{50}$ 2.1 μM, nitrite IC$_{50}$ 7.2 μM, and QR2 IC$_{50}$ 3.5 μM. Out of the 32 compounds, it ranked first vs QR1 induction, third vs NF-κB inhibition, tied for third vs iNOS inhibition, and tenth vs QR2 inhibition. This clearly demonstrates that good activity vs all four targets can be achieved with a single compound.

Table 2 contains rank-order lists of the 12 most active compounds in each of the four assays. The numbers of the compounds that appear in both the nitrite and the NF-κB columns are italicized. Eight of the compounds appear in both the iNOS and the NF-κB lists, from which it may be concluded that some of the structural features that confer inhibitory activity vs iNOS (nitrite assay) also confer inhibitory activity in the NF-κB assay. Five of the eight compounds are brominated phenazines or phenazine analogues having either a phenol or a methyl ether in the same position or an equivalent position. The activity vs QR1 and QR2 seems to result from independent pharmacophores, with less overlap in their rank order lists.

Conclusion

Simple syntheses of the natural product **1** and a series of its analogues have been carried out. The activities of the natural product **1** were significantly optimized vs QR1 induction, inhibition of NF-κB-luciferase activity, inhibition of iNOS activity, and inhibition of QR2, through the synthesis of congeners and analysis of structure−activity relationships. Several of the compounds were active in the nanomolar range, including **41** and **46** vs QR1 and **9**, **14**, **16**, **29**, **34**, and **44** vs QR2. These results provide new phenazine leads compounds for the design and synthesis of chemopreventive agents targeting QR1 induction, QR2 inhibition, iNOS inhibition, and NF-κB inhibition. The CD value of 4.7 nM and CI value of 2038 for compound **46** vs QR1 are particularly noteworthy.
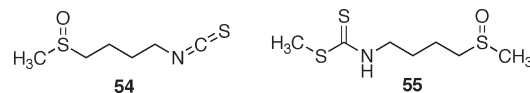
Figure 2. Structures of sulforaphane (**54**) and sulforamate (**55**).

The CD value of 4.7 nM is the lowest CD value ever recorded by our group for any QR1 inducer. For comparison, the standard compound sulforaphane (**54**, Figure 2), which is a natural product found in broccoli, has a CD value of 0.23 μM and a CI value of 42, while the synthetic compound sulforamate (**55**) has a CD of 0.26 μM and a CI of 137.[43] Sulforaphane has been proven to induce QR1 in rat mammary gland in vivo in a manner that is consistent with its chemopreventive activity.[44]

Various compounds synthesized in the present study inhibit or induce two or more distinct targets, which provides a foundation for the development of effective chemopreventive agents. Multiple targeting offers the likelihood of improved efficacy if the two protein targets operate in a synergistic way.

Experimental Section

NMR spectra were obtained at 300 (^{1}H) and 75 or 125 (^{13}C) MHz in CDCl$_3$ or DMSO-d_6 using Bruker ARX300 or Bruker DX-2 500 [QNP probe or multinuclear broadband observe (BBO) probe, respectively] spectrometers unless otherwise indicated. Flash chromatography was performed with 230−400 mesh silica gel. TLC was carried out using commercially available precoated glass silica gel plates of 2.5 mm thickness, and compounds were visualized with short-wavelength UV light. Melting points were determined using capillary tubes with a Mel-Temp apparatus and are uncorrected. IR spectra were obtained as films on salt plates with CH$_2$Cl$_2$ as the solvent, using a Perkin-Elmer 1600 series FTIR spectrometer. Microanalyses were performed at the Purdue Microanalysis Laboratory or Galbraith Laboratories, Inc. Natural product isolation was performed with Prep Nova-Pak HR C18, 6 μm, 60 Å, 300 mm × 40 mm (1st purification) or Phenomenex Luna C8 (2), 10 mm × 250 mm, 5 μm (2nd purification). HPLC analyses were performed on a Waters 1525 binary HPLC pump/Waters 2487 dual λ absorbance detector system using a 5 μM C-18 reverse phase column. All yields refer to isolated compounds. Unless otherwise stated, chemicals and solvents were of reagent grade and used as obtained from commercial sources without further purification. The purities of all of the tested compounds were >95% as determined by HPLC or elemental analysis.

Isolation of 2-Bromo-1-hydroxyphenazine (1) from *Streptomyces* Species, Strain CNS284. The producing strain was cultured in 2.8 L Fernbach flasks (25 × 1 L) containing A1BFe media for 7 days at 25−27 °C on a shaker at 230 rpm. The fermentation broth was then extracted by addition of 20 g/L of XAD-7 resin, which was subsequently collected by filtration and extracted with acetone. The organic residue (20 g) from the resin was subjected to silica normal-phase flash chromatography generating eight fractions (gradient solvent system: 100% isooctane, 20% ethyl acetate−isooctane, 40% ethyl acetate−isooctane, 60% ethyl acetate−isooctane, 80% ethyl acetate−octane, 100% ethyl acetate, 10% methanol−ethyl acetate, and 20% methanol−ethyl acetate). The active fractions 6 and 7 were purified by RP-HPLC (detection at 254 nm; 0−10 min, 40% aqueous CH$_3$CN; 10−25 min, linear gradient up to 65% aqueous CH$_3$CN) to afford three subfractions. These subfractions were then separately purified by RP HPLC (detection of 254 nm; 0−40 min, 30−65% aqueous CH$_3$CN; 40−50 min, 65−100% aqueous CH$_3$CN) giving 3.2 mg of the natural product, 2-bromo-1-hydroxyphenazine (**1**), as a light yellow amorphous solid. UV (CH$_3$OH) λ_{max} (log ε): 270 (4.43), 378 (2.19) nm. IR (neat): ν_{max} 3653, 2907, 1513, 1470, 1385, 1267, 1174, 1103, 882, 751 cm^{-1}. ^{1}H NMR (500 MHz, CDCl$_3$) and ^{13}C NMR (125 MHz, CDCl$_3$): see Table 3. ESIMS m/z 275 (MH$^+$),

Table 3. NMR Spectroscopic Data for 2-Bromo-1-hydroxyphenazine (**1**) in CDCl$_3$ at 500 MHz (Assignments Made by Analysis of 2D NMR Data)

C/H no.	δ_H (*J* Hz)	δ_C		COSY	HMBC
1		149.3	C		
2		103.9	C		
3	7.83 d (8.6)	135.5	CH	H-4	C-1, C-2, C-4a
4	7.63 d (8.6)	121.3	CH	H-3	C-2, C-3, C-10a
4a		100.0	C		
5					
5a		141.7	C		
6	8.18 d (8.4)	129.1	CH	H-7. H-8	C-8, C-9a
7	7.82 m	131.6	CH	H-6, H-8, H-9	C-7, C-
8	7.82 m	131.6	CH	H-6, H-7, H-9	C-7, C-9a
9	8.20 d (8.4)	129.5	CH	H-7, H-8	C-8, C-5a
9a		141.7	C		
10					
10a		134.2	C		
1-OH	8.52 br s				

297 (MNa$^+$), 751 (2MNa$^+$). HRESIMS *m/z*: calcd, 275.9814; found, 275.9814.

General Procedure for the Preparation of Phenazines 6, 38, 40, and 46. 3-Methoxycatechol (**3**, 1.03−2.10 g, 7.5−15.0 mmol, 1 equiv) was dissolved in ethyl ether (20−40 mL), and the mixture was cooled to −78 °C. *o*-Chloranil (1.84−3.67 g, 7.5−15.0 mmol, 1 equiv) was added to the reaction mixture. The solution was stirred for 4 h, keeping the temperature constant. The reaction mixture was filtered, giving the desired benzoquinone **4**, which was used without further purification. Compound **4** (1.03−2.10 g, 7.5−15.0 mmol, 1 equiv) was immediately added to a flask containing the appropriate *o*-phenylenediamine (1.02−2.67 g, 7.5−15.0 mmol, 1 equiv) dissolved in benzene (25−54 mL) and acetic acid (25−45 mL). The mixture was stirred for 24 h at room temperature. The solvent was removed in vacuo.

General Procedure for the Preparation of Phenazines 14, 39, and 41. 5-Bromo-3-methoxycatechol (**13**, 0.7−1.41 g, 3.05−6.10 mmol, 1 equiv) was dissolved in ether (15−30 mL), and the solution was cooled down to −78 °C. *o*-Chloranil (0.75−1.50 g, 3.06−6.11 mmol) was added slowly, and the reaction mixture was stirred at −78 °C for 6 h. The purple solid, benzoquinone **13**, was separated by filtration and immediately added to a solution of the appropriate *o*-phenylenediamine (0.33−0.83 g, 3.01−6.10 mmol, 0.99−1 equiv) in benzene (40−80 mL) and acetic acid (35−70 mL). The reaction mixture was stirred at room temperature for 12 h.

General Procedure for the Preparation of Phenazines 28−35, and 47. Catechol (**18**, 0.112−0.284 g, 1.01−2.64 mmol) and silver(I) oxide (0.564−1.44 g, 2.43−6.12 mmol, 2.31−2.40 equiv) were dissolved in acetone (20−50 mL), and the reaction mixture was stirred for 10 min. The obtained solid was filtered and added immediately, without purification, to a solution of the appropriate *o*-phenylenediamine (0.215−0.485 g, 0.81−2.89 mmol, 1.0−1.2 equiv) dissolved in benzene (20−30 mL) and acetic acid (17−25 mL). The flask was purged with argon and kept at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The solvent was removed in vacuo.

2-Bromo-1-hydroxyphenazine (1). 1-Hydroxyphenazine[35] (**7**, 0.51 g, 2.60 mmol) and *N*-bromosuccinimide (0.51 g, 2.85 mmol) were dissolved in toluene (50 mL), and the reaction mixture was heated at 50 °C for 8 h. The reaction mixture was cooled to room temperature, and the toluene was removed in vacuo. The sample was dissolved in dichloromethane (25 mL), silica gel (5 g) was added to the flask, and the solvent was removed. The product was purified by silica gel column chromatography, using dichloromethane to dichloromethane-ethyl acetate, 100:5. The product was obtained as a yellow solid (0.24 g, 34%): mp 233−235 °C. IR (film): 3359, 2907, 1575, 1551, 1530, 1419, 1385, 1103, 764, 751 cm^{-1}.

^{1}H NMR (CDCl$_3$): δ 8.53 (bs, 1 H), 8.27−8.24 (m, 2 H), 7.91−7.88 (m, 3 H), 7.72 (d, *J* = 9.5 Hz, 1 H). ESIMS *m/z* (rel intensity): 275 (MH$^+$, 66), 277 (MH$^+$, 61). Anal. calcd for C$_{12}$H$_6$BrN$_2$O: C, 52.39; H, 2.56; N, 10.18; Br, 29.05. Found: C, 52.05; H, 2.32; N, 10.06; Br, 29.15. Compound **8** (0.18 g, 22%) was also isolated as a by-product.

1-Methoxyphenazine (6). The general procedure provided a solid that was dissolved in ethyl acetate (25 mL), and water was added (10 mL). Sodium bicarbonate was added until the generation of CO$_2$ ceased. The aqueous layer was decanted, and the organic solvent was removed in vacuo. The compound was purified by silica gel column chromatography with the column containing 2 cm of neutral alumina on top of the silica gel. The compound was eluted using a gradient of hexane to ethyl acetate−hexane, 1:3, and ethyl acetate−dichloromethane, 1:3. The product was obtained as a yellow solid (0.63 g, 42%): mp 166−168 °C (lit.[35] mp 168−169 °C). ^{1}H NMR (CDCl$_3$): δ 8.38 (m, *J* = 10.0 Hz, 1 H), 8.20 (m, *J* = 10.0 Hz, 1 H), 7.84−7.79 (m, 3 H), 7.72 (t, *J*$_1$ = 7.0 Hz, *J*$_2$ = 12.0 Hz, 1 H), 7.04 (d, *J* = 7 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 154.7, 143.8, 143.1, 141.8, 136.4, 130.4, 130.1, 129.8, 128.9, 121.0, 106.0, 56.1. ESIMS *m/z* (rel intensity): 211 (MH$^+$, 100). Anal. calcd for C$_{13}$H$_{10}$N$_2$O: C, 74.27; H, 4.79; N, 13.33. Found: C, 74.04; H, 4.62; N, 13.29.

1-Hydroxyphenazine (7). 1-Methoxyphenazine (**6**, 2.13 g, 10.1 mmol) was dissolved in 48% hydrobromic acid (60 mL) and glacial acetic acid (60 mL), and the reaction mixture was heated at reflux for 8 h. Hydrobromic acid (48%, 30 mL) was added, and the reaction mixture was stirred for another 8 h. The aqueous solution was extracted with ethyl acetate (5 × 35 mL). The organic extracts were combined and washed with saturated sodium bicarbonate (2 × 30 mL), brine (1 × 30 mL), and water (2 × 30 mL). The solvent was removed in vacuo, and the compound was purified by silica gel column chromatography, using hexane−ethyl acetate, 1:1−1:10. The product was obtained as a yellow solid (1.35 g, 68%): mp 148−150 °C (lit.[16] mp 159−160 °C). ^{1}H NMR (CDCl$_3$): δ 8.23 (m, 2 H), 7.86 (t, *J* = 4.0 Hz, 1 H), 7.78 (t, *J* = 3.0 Hz, 1 H), 7.25 (t, *J* = 3.0 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 151.6, 143.9, 143.7, 141.1, 134.5, 131.7, 130.9, 130.6, 130.3, 129.5, 129.0, 119.8, 108.8. ESIMS *m/z* (rel intensity): 197 (MH$^+$, 100).

2,4-Dibromo-1-hydroxyphenazine (8). 1-Hydroxyphenazine (**7**, 0.55 g, 2.80 mmol) and *N*-bromosuccinimide (1.09 g, 6.16 mmol) were dissolved in toluene (50 mL), and the reaction mixture was heated at 50 °C for 8 h. The reaction mixture was cooled to room temperature, and the toluene was removed in vacuo. The product was purified by silica gel column chromatography, using dichloromethane to dichloromethane−ethyl acetate, 100:5. The product was obtained as a yellow solid (0.925 g, 93%): mp 196−198 °C. IR (film): 3320, 3068, 1620, 1592, 1514, 1466, 1404, 1371, 948, 758 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 11.55 (bs, 1 H), 8.39 (s, 1 H), 8.35−8.27 (m, 2 H), 8.06−8.01 (m, 2 H). ^{13}C NMR (DMSO-d_6): δ 150.9, 142.7, 141.3, 139.4, 136.8, 135.5, 132.2 (2 C), 129.4, 128.9, 111.4, 104.4. ESIMS *m/z* (rel intensity): 353 (MH$^+$, 100), 355 (MH$^+$, 41). Anal. calcd for C$_{12}$H$_6$Br$_2$N$_2$O: C, 40.71; H, 1.71, N, 7.91; Br, 45.14. Found: C, 40.34; H, 1.59; N, 7.77; Br, 45.22.

2-Bromo-1-methoxyphenazine (9). 2-Bromo-1-hydroxyphenazine (**1**, 30 mg, 0.11 mmol) was dissolved in acetone (5 mL). Potassium carbonate (28 mg, 0.2 mmol) was added to the reaction mixture, and the mixture was stirred for 1 min. Methyl iodide (0.1 mL) was added, and the reaction mixture was stirred at reflux for 3 h. The solvent was removed, and the compound was dissolved in chloroform (10 mL). The organic phase was washed with water (3 × 10 mL). The product was purified by preparative TLC, using chloroform as solvent, and obtained as a yellow solid (31 mg, 98%): mp 145−147 °C. IR (film): 1728, 1623, 1588, 1552, 1514, 1474, 1415, 1360, 1302, 929, 753 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.34 (m, 1 H), 8.21 (m, 1 H), 7.92 (m, 2 H), 7.86 (m, 2 H), 4.31 (s, 3 H). ^{13}C NMR (CDCl$_3$): δ 152.9, 143.5, 143.0, 142.6, 138.9, 134.3, 131.0, 130.9, 130.0, 129.4, 125.9, 116.4. EIMS *m/z* 288 (M$^+$). CIMS *m/z* (rel intensity) 289 (MH$^+$, 100). HREIMS

m/z: calcd, 287.9898; found, 287.9896. HPLC purity: 95.02% (MeOH, 100), 96.03% (MeOH−H$_2$O, 90:10).

2,4-Dibromo-1-methoxyphenazine (10). 2,4-Dibromo-1-hydroxyphenazine (**8**, 0.155 g, 0.438 mmol) contained in a high-pressure vessel was dissolved in tetrahydrofuran (10 mL) and dimethylformamide (1 mL), and the reaction mixture was cooled to −78 °C. Sodium hydride (0.100 g, 4.17 mmol) was added. The reaction vessel was purged with argon, and the reaction mixture was stirred for 30 min, keeping the atmosphere inert. The reaction mixture was warmed to 0 °C, and methyl iodide (0.5 mL) was added. The flask was closed and heated for 8 h at 100 °C. The reaction mixture was allowed to cool to room temperature and diluted with water (10 mL). The aqueous solution was extracted with ethyl acetate (3 × 15 mL). The organic extracts were combined and washed with aqueous ammonium chloride (20 mL), water (3 × 20 mL), and brine (20 mL). The solvent was removed in vacuo, and the product was purified by silica gel column chromatography, eluting with hexane−ethyl acetate, 100:1. The product was obtained as a yellow solid (0.149 g, 92%): mp 171−173 °C. IR (film): 3061, 2933, 1617, 1576, 1513, 1467, 1401, 968, 945, 797, 747 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.32−8.27 (m, 2 H), 8.26 (s, 1 H), 7.90−7.88 (m, 2 H), 4.30 (s, 3 H). ^{13}C NMR (CDCl$_3$): δ 152.8, 142.9, 142.6, 140.5, 138.8, 136.3, 131.7, 131.4, 129.7, 129.6, 118.9, 115.7, 62.6. EIMS *m/z* (rel intensity): 369 (MH$^+$, 100). Anal. calcd for C$_{13}$H$_8$Br$_2$N$_2$O: C, 42.22; H, 2.26; N, 7.36. Found: C, 42.43; H, 2.19; N, 7.61.

4-Bromo-1-methoxyphenazine (11).[45,46] 1-Methoxyphenazine (**6**, 240 mg, 1.14 mmol) was dissolved in toluene (20 mL) and acetonitrile (20 mL). *N*-Bromosuccinimide (213 mg, 1.19 mmol) was added with stirring, and the reaction mixture was heated to 50 °C for 8 h. Toluene was removed in vacuo, and the compound was purified by silica gel column chromatography, eluting with chloroform−hexane, 1:1, to chloroform−ethyl acetate, 1:1. The product was obtained as a yellow solid (249 mg, 75%): mp 148−150 °C (lit.[45,46] mp 155 °C). IR (film): 3060, 3004, 2961, 1621, 1600, 1551, 1521, 1476, 1461, 1281, 1095, 917, 754 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.38 (m, 2 H), 8.63 (d, *J* = 8.2 Hz, 1 H), 7.89 (m, 2 H), 6.94 (d, *J* = 8.1 Hz, 1 H), 4.15 (s, 3 H). ^{13}C NMR (CDCl$_3$): δ 154.9, 143.4, 142.1, 140.8, 136.9, 133.0, 131.2, 130.9, 129.7, 129.6, 114.0, 106.7, 56.5. EIMS *m/z* (rel intensity): 290 (M$^+$, 57), 288 (M$^+$, 57), 179 (M$^+$-OCH$_4$Br, 100). Anal. calcd for C$_{13}$H$_9$BrN$_2$O: C, 54.00; H, 3.14; N, 9.69; Br, 27.64. Found: C, 53.79; H, 3.11; N, 9.41; Br, 27.51.

4-Bromo-1-hydroxyphenazine (12).[32] 4-Bromo-1-methoxyphenazine (**11**, 400 mg, 1.38 mmol) was dissolved in dry dichloromethane (25 mL). The reaction mixture was purged with argon and cooled to −78 °C. Boron tribromide (1 M solution in dichloromethane, 4.0 mL) was added dropwise to the reaction mixture with stirring. The temperature was maintained at −78 °C, and the reaction mixture was stirred for 1 h. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. The reaction mixture was heated at reflux for 1 h. The solution was allowed to cool to room temperature, and saturated aqueous sodium bicarbonate (30 mL) at ~4 °C was slowly added. The solution was extracted with dichloromethane (3 × 40 mL). The reaction mixture was filtered through a column packed with Celite 545 and basic alumina on top (1 in.), and the solvent was removed in vacuo. The compound was purified by silica gel column chromatography (ethyl acetate−hexane, 1:1). The product was obtained as a yellow powder (322 mg, 85%): mp 199−201 °C. IR (film): 3359, 1731, 1628, 1518, 1472, 1421, 1372, 1343, 1319, 1300, 924, 766, 750 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.41−8.38 (m, 1 H), 8.25−8.21 (m, 1 H), 8.07 (d, *J* = 8.1 Hz, 1 H), 7.94−7.85 (m, 2 H), 7.13 (d, *J* = 8.1 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 151.5, 144.2, 141.1, 140.7, 134.8, 134.6, 131.3, 130.0, 128.7, 112.0, 109.3. CIMS *m/z* (rel intensity): 277 (MH$^+$, 100), 275 (MH$^+$, 98). HREIMS *m/z* calcd, 273.9742; found, 273.9740. HPLC purity: 100.00% (MeOH−H$_2$O, 95:5).

3-Bromo-1-methoxyphenazine (14). The general procedure was followed. The solvent was removed in vacuo, and the compound was purified by silica gel column chromatography

(1 in. of basic alumina was added to the top of the silica gel column), dichloromethane−ethyl acetate, 3:1. The product was obtained as a dark-yellow solid (0.41 g, 47%): mp 148−150 °C. IR (film): 3058, 2957, 2932, 1622, 1591, 1555, 1518, 1475, 1398, 1363, 1351, 1311, 1218, 1145, 1111, 864, 826, 755, 736 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.40−8.35 (m, 1 H), 8.25−8.17 (m, 2 H), 8.05 (d, *J* = 1.9 Hz, 1 H), 7.89−7.83 (m, 2 H), 7.14 (d, *J* = 1.9 Hz, 1 H), 4.18 (s, 3 H). ^{13}C NMR (CDCl$_3$): δ 155.2, 144.0, 143.7, 141.9, 135.5, 131.3, 130.4, 130.1, 129.2, 124.9, 123.5, 110.9, 56.7. CIMS *m/z* (rel intensity): 289 (MH$^+$, 100). Anal. calcd for C$_{13}$H$_9$N$_2$OBr: C, 54.00; H, 3.14; N, 9.69. Found: C, 53.94; H, 3.11; N, 9.49.

3-Bromo-1-hydroxyphenazine (15). 3-Bromo-1-methoxyphenazine (**14**, 203 mg, 0.71 mmol) was dissolved in 48% hydrobromic acid (20 mL) and glacial acetic acid (15 mL), and the reaction mixture was heated in an oil bath at 110 °C for 16 h. An extra amount of 48% hydrobromic acid (10 mL) was added, and the reaction mixture was heated in an oil bath at 110 °C for 8 h. The reaction mixture was allowed to cool to room temperature and extracted with ethyl acetate (5 × 20 mL). The combined organic layers were washed with aqueous sodium bicarbonate (2 × 25 mL) and water (2 × 30 mL). The solvent was removed, and the compound was purified by silica gel column chromatography, eluting with ethyl acetate−hexane, 2:1. The product was obtained as a light brown solid (135 mg, 69.9%): mp 200−202 °C. IR (film): 3360, 3070, 2917, 2849, 1624, 1604, 1557, 1517, 1476, 1417, 1380, 1353, 1318, 1162, 1121, 751 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.25−8.18 (m, 3 H), 7.99 (d, *J* = 1.7 Hz, 1 H), 7.88−7.86 (m, 2H), 7.34 (d, *J* = 1.7 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 151.9, 149.5, 143.7, 140.9, 133.4, 131.3, 130.7, 129.6, 129.1, 122.0, 113.1. CIMS *m/z* (rel intensity): 275 (MH$^+$, 100). HREIMS *m/z* calcd, 273.9742; found, 273.9748. Anal. calcd for C$_{12}$H$_7$BrN$_2$O·0.65EtOAc: C, 54.00; H, 3.14; N, 9.69. Found: C, 53.94; H, 3.11; N, 9.49. HPLC purity: 100.00% (MeOH−H$_2$O, 90:10), 97.85 (MeOH−H$_2$O, 95:5).

3,4-Dibromo-1-methoxyphenazine (16). 3-Bromo-1-methoxyphenazine (**14**, 270 mg, 0.93 mmol) was dissolved in toluene (20 mL). Bromine (148 mg, 0.93 mmol), ammonium acetate (200 mg, 2.43 mmol), and acetic acid (20 mL) were added. The reaction mixture was heated at reflux for 1 h. The solvent was removed in vacuo, and the orange solid was dissolved in ethyl acetate (40 mL). The organic phase was washed with brine (1 × 30 mL) and water (2 × 30 mL). The organic phase was removed in vacuo, and the compound was purified by silica gel column chromatography, eluting with hexane−ethyl acetate, 5:1 to 3:1. The product was obtained as a yellow solid (331 mg, 96%): mp 208−210 °C. IR (film): 3060, 2917, 1619, 1586, 1547, 1515, 1473, 1260, 1145, 750 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.32−8.27 (m, 2 H), 7.88−7.83 (m, 2 H), 7.20 (s, 1 H), 4.13 (s, 3 H). ^{13}C NMR (CDCl$_3$): δ 154.2, 143.7, 141.7, 135.6, 131.7, 131.1, 129.6, 127.9, 117.2, 111.6, 56.8. CIMS *m/z* (rel intensity): 369 (MH$^+$, 100). HREIMS *m/z* calcd, 365.9003; found, 365.9006. Anal. calcd for C$_{13}$H$_8$Br$_2$N$_2$O: C, 42.50; H, 2.18; N, 7.46. Found: C, 42.43; H, 2.19; N, 7.61.

3,4-Dibromo-1-hydroxyphenazine (17). 3,4-Dibromo-1-methoxyphenazine (**16**, 170 mg, 0.46 mmol) was dissolved in freshly distilled anhydrous dichloromethane (30 mL) under argon atmosphere. The reaction mixture was cooled to −78 °C. Boron tribromide (200 μL, 2.07 mmol) was added, keeping the temperature constant. The reaction mixture was stirred for 3 h at −78 °C and then for 21 h at room temperature. The reaction mixture was heated at reflux for 1 h. A saturated aqueous solution of sodium bicarbonate (40 mL) at 4 °C was slowly added. The reaction mixture was stirred for 10 min. The aqueous phase was removed, and the organic layer was washed with water (2 × 30 mL). The solvent was removed in vacuo, and the compound was purified by silica gel column chromatography, eluting with dichloromethane−ethyl acetate, 3:1. The product was obtained as a yellow solid (140 mg, 86%): mp 208−210 °C. IR (film): 3347, 3067, 2957, 2922, 2851, 1626, 1606, 1585, 1548, 1512, 1471, 1413, 1349, 1305, 922, 834 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.41 (m, 1 H), 8.24 (m, 2 H), 7.94−7.91 (m, 2 H), 7.50 (s, 1 H). ^{13}C NMR (CDCl$_3$): δ 151.0, 144.8, 141.0, 140.8, 133.9, 131.7, 131.5, 130.0,

129.3, 128.7, 115.1, 114.0. EIMS *m/z* (rel intensity): 354 (M$^+$, 100). HRCIMS *m/z* calcd, 351.8847; found, 351.8851. HPLC purity: 99.44 (MeOH−H$_2$O, 95:5).

2,3-Dimethylphenazine (28). The general procedure was followed. The compound was purified by silica gel column chromatography (1 in. of basic alumina was added to the top of the silica gel column), eluting with hexane−chloroform, 1:1. The product was obtained as a brown solid (62.2 mg, 20.3%): mp 170−172 °C (lit[47] mp 174−175 °C). ^{1}H NMR (CDCl$_3$): δ 8.22−8.19 (m, 2 H), 7.98 (s, 2 H), 7.81−7.77 (m, 2 H), 2.21 (s, 6 H). ^{13}C NMR (CDCl$_3$): δ 142.9, 142.8, 141.8, 129.6, 129.4, 127.8, 20.6. EIMS *m/z* (rel intensity): 208 (M$^+$, 100). HPLC purity: 95.46% (MeOH, 100).

3-Bromo-1-methylphenazine (29). The general procedure was followed. The compound was purified by silica gel column chromatography (1 in. of basic alumina was added to the top of the silica gel column), eluting with hexane−dichloromethane, 2:1. The product was obtained as a yellow powder (59 mg, 19%): mp 132−134 °C. IR (film): 1622, 1556, 1519, 1276, 764, 751 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.20−8.13 (m, 3 H), 7.82−7.78 (m, 2 H), 7.65 (s, 1 H), 2.85 (s, 3 H). ^{13}C NMR (CDCl$_3$): δ 143.6, 143.2, 142.5, 141.5, 139.6, 132.8, 130.8, 130.1, 129.9, 124.7, 17.3. EIMS *m/z* (rel intensity): 274 (M$^+$, 54), 272 (M$^+$, 54), 179 (M$^+$-Br, 100). Anal. calcd for C$_{13}$H$_9$BrN$_2$: C, 57.17; H, 3.32; N, 10.26. Found: C, 57.01; H, 3.27; N, 10.14.

1,3-Dibromophenazine (30). The general procedure was followed. The compound was purified by silica gel column chromatography (1 in. of basic alumina was added to the top of the silica gel column), eluting with hexane−dichloromethane, 2:1. The product was obtained as a yellow powder (124 mg, 36%): mp 179−181 °C. IR (film): 3051, 1621, 1582, 1502, 1342, 1129, 956, 862, 757, 748 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.38 (d, *J* = 2.0 Hz, 1 H), 8.32 (m, 1 H), 8.24 (m, 2 H), 7.89 (m, 2 H). ^{13}C NMR (CDCl$_3$): δ 143.8, 143.5, 143.4, 139.4, 136.4, 131.8, 131.4, 131.3, 130.0, 129.2, 125.2, 123.8. EIMS *m/z* (rel intensity): 338 (M$^+$, 100). Anal. calcd for C$_{12}$H$_6$Br$_2$N$_2$: C, 42.64; H, 1.79; N, 8.29. Found: C, 42.46; H, 1.74; N, 8.20.

2-Methylphenazine (31).[48] The general procedure was followed. The compound was purified by silica gel column chromatography, eluting with ethyl acetate−hexane, 1:1. The compound was obtained a light brown solid (49 mg, 9.7%): mp 107−108 °C (lit[49] mp 117 °C). IR (film): 3026, 2976, 2920, 1633, 1604, 1511, 1482, 1466, 1437 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.24 (m, 2 H), 8.14 (d, *J* = 8.9 Hz, 1 H), 8.00 (s, 1 H), 7.81 (m, 2 H), 7.28 (d, *J* = 8.9 Hz, 1 H), 2.66 (s, 3 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 143.5, 143.1, 142.8, 142.2, 141.1, 133.4, 130.1, 129.8, 129.5, 129.4, 128.9, 127.5, 22.1. EIMS *m/z* (rel intensity): 194 (M$^+$, 100). HPLC purity: 98.48% (MeOH−H$_2$O, 80−20).

2-Methoxyphenazine (32). The general procedure was followed. The compound was purified by silica gel column chromatography, eluting with ethyl acetate−dichloromethane, 1:1. The product was obtained as light brown solid (26 mg, 6.5%): mp 116−118 °C (lit[50] mp 126 °C). ^{1}H NMR (CDCl$_3$): δ 8.14 (m, 2 H), 8.04 (d, *J* = 9.5 Hz, 1 H), 7.75 (m, 2 H), 7.47 (d, *J* = 2.7 Hz, 1 H), 7.34 (s, 1 H), 3.97 (s, 1 H). ^{13}C NMR (CDCl$_3$): δ 149.7, 143.3, 142.8, 141.6, 137.4, 135.2, 130.7, 130.4, 129.5, 128.9, 121.9, 111.7, 55.8. EIMS *m/z* (rel intensity): 210 (M$^+$, 100). HPLC purity: 99.40% (MeOH−H$_2$O, 80:20).

2,3-Dimethoxyphenazine (33). The general procedure was followed. The compound was purified by silica gel column chromatography, eluting with ethyl acetate−dichloromethane, 1.5:1. The product was obtained as a yellow solid (113 mg, 25%): mp 215−227 °C (lit[51] mp 230−231 °C). ^{1}H NMR (CDCl$_3$): δ 8.13−8.09 (m, 2 H), 7.74−7.70 (m, 2 H), 7.34 (s, 2 H), 4.07 (s, 3 H). ^{13}C NMR (CDCl$_3$): δ 154.3, 141.8, 141.7, 128.9, 128.7, 105.1, 56.3. EIMS *m/z* (rel intensity): 240 (M$^+$, 100). HPLC purity: 96.82% (MeOH−H$_2$O, 80−20).

1-Bromo-3-nitrophenazine (34). The general procedure was followed. The compound was purified by silica gel column chromatography, eluting with ethyl acetate−hexane, 1.5:1. The

product was obtained as a dark yellow solid (39 mg, 6.7%): mp 214−216 °C. IR (film): 3089, 1624, 1599, 1558, 1535, 1509, 1461, 1411, 1346, 862, 758 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 9.16 (d, *J* = 2.3 Hz, 1 H), 8.90 (d, *J* = 2.3 Hz, 1 H), 8.41 (dd, *J* = 7.8 Hz, 2.1 Hz, 1 H), 8.32 (dd, *J* = 7.8 Hz, 2.0 Hz, 1 H), 8.03−7.98 (m, 2 H). ^{13}C NMR (CDCl$_3$): δ 147.4, 144.8, 142.0, 141.4, 133.2, 132.6, 130.1, 129.6, 126.4, 126.1. ESIMS *m/z* (rel intensity): 304 (MH$^+$, 100). HRESIMS *m/z* calcd, 302.9643; found, 302.9651. HPLC purity: 95.46% (MeOH−H$_2$O, 70:30), 98.05% (MeOH−H$_2$O, 90:10).

1,4-Dimethoxyphenazine (35). The general procedure was followed. The compound was purified by silica gel column chromatography (with one-half inch of basic alumina on top) eluting with dichloromethane. The product was obtained as a yellow solid (68 mg, 17%): mp 179−181 °C (lit[52] mp 185 °C). IR (film): 3056, 2994, 2935, 2835, 1626, 1606, 1485, 1463, 1397, 1383, 1247, 1231, 1092, 764, 752 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.34 (m, 2 H), 7.79 (m, 2 H), 6.88 (m, 2 H), 4.05 (s, 3 H). ^{13}C NMR (CDCl$_3$): δ 148.7, 142.1, 136.9, 130.6, 129.8, 105.7, 56.2. EIMS *m/z* (rel intensity): 240 (M$^+$, 86), 225 (M$^+$-CH$_3$, 100). CIMS *m/z* (rel intensity): 241 (MH$^+$, 100). HREIMS *m/z* calcd, 240.0899; found, 240.0900. HPLC purity: 95.00% (MeOH, 100), 98.05% (MeOH−H$_2$O, 90:10).

7,8-Dimethyl-1-methoxyphenazine (38). The general procedure provided a solid that was dissolved in hot ethyl acetate (70 mL). The solution was filtered through Celite. The Celite was washed with ethyl acetate (∼200 mL) until the filtered solution was light brown. Water (40 mL) was added, and the organic phase was collected. The organic layer was washed with a saturated aqueous solution of sodium bicarbonate (30 mL) and water (2 × 30 mL). The compound was purified by silica gel column chromatography (1 in. of basic alumina on top), using ethyl acetate−hexane, 1:1. The product was obtained as a yellow powder (713 mg, 39.9%): mp 187−189 °C (dec). IR (film): 3043, 2916, 2834, 1636, 1606, 1560, 1515, 1477, 1469, 1442, 1400, 1109, 871, 775, 739 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.12 (s, 1 H), 7.96 (s, 1 H), 7.78 (d, *J* = 8.8 Hz, 1 H), 7.7 (t, *J* = 7.6 Hz, 1 H), 7.01 (d, *J* = 7.3 Hz, 1 H), 4.15 (s, 3 H), 2.53 (s, 6 H). ^{13}C NMR (CDCl$_3$): δ 154.8, 143.5, 142.6, 141.8, 141.3, 141.1, 136.0, 129.4, 128.2, 127.3, 121.1, 105.6, 56.1, 20.4. CIMS *m/z* (rel intensity): 239 (M$^+$, 100). HREIMS *m/z* calcd, 238.1106; found, 238.1108. Anal. calcd for C$_{15}$H$_{14}$N$_2$O·0.2H$_2$O: C, 74.48; H, 6.00; N, 11.58. Found: C, 74.14; H, 6.00; N, 11.76.

7,8-Dimethyl-3-bromo-1-methoxyphenazine (39). The general procedure was followed. The solvent was removed in vacuo. The compound was purified by silica gel column chromatography (1 in. of basic alumina on top), eluting with dichloromethane−ethyl acetate, 95:5. The product was obtained as a yellow powder (919 mg, 47.5%); mp 229−231 °C. IR (film): 3054, 2986, 2918, 2849, 1691, 1632, 1593, 1557, 1516, 1465, 1442, 1421, 1265, 748, 705 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.09 (s, 1 H), 7.99 (d, *J* = 1.6 Hz, 1 H), 7.92 (s, 1 H), 4.16 (s, 3 H), 2.55 (s, 3 H), 2.54 (s, 3 H). ^{13}C NMR (CDCl$_3$): δ 155.2, 144.0, 143.8, 143.5, 142.1, 141.4, 128.4, 127.5, 123.7, 123.4, 110.4, 56.6, 20.6. EIMS *m/z* (rel intensity): 319 (M$^+$, 87), 317 (M$^+$, 85) 237 (M$^+$-HBr, 100). HREIMS *m/z* calcd, 317.0289; found, 317.0291. HPLC purity: 97.8% (MeOH−H$_2$O, 95:5), 96.11% (MeOH, 100).

7,8-Dichloro-1-methoxyphenazine (40). The general procedure provided a solid that was dissolved in hot ethyl acetate (70 mL). The solution was filtered through a column containing Celite 545 (∼1 in.) and basic alumina on top (∼1 in.). The Celite was washed with ethyl acetate until the filtered solution was light in color. The organic solutions were combined and washed with saturated sodium bicarbonate (50 mL) and water (50 mL). The compound was purified by silica gel column chromatography, eluting with ethyl acetate−hexane, 1:3. The product was obtained as a dark yellow powder (539 mg, 19.4%): mp 245- 247 °C. IR (film): 3041, 2964, 1619, 1555, 1518, 1503, 1466, 1413, 1396, 1284, 1254, 1111, 885, 763 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.53 (s, 1 H), 8.35 (s, 1 H), 7.79 (d, 2 H), 7.11 (t, *J* = 4.33 Hz, 1 H). ^{13}C NMR (CDCl$_3$): δ 155.0, 144.5, 141.8, 140.5, 137.1, 135.7, 135.0, 131.5, 130.2, 129.5, 121.3,

107.1, 56.5. EIMS *m/z* (rel intensity): 278 (M⁺, 100). HREIMS *m/z* calcd, 278.0014; found, 278.0011. HPLC purity: 96.74% (MeOH−H₂O, 95:5).

3-Bromo-7,8-dichloro-1-methoxyphenazine (41). The general procedure was followed. The solvent was removed under vacuum, and the solid was purified by silica gel column chromatography (with one-half inch of basic alumina on top) using hexane−ethyl acetate, 1:3. The product was obtained as a yellow solid (331 mg, 18%): mp: 249−251 °C. IR (film): 2962, 2922, 1609, 1578, 1554, 1505, 1452, 1431, 1260, 1099, 801 cm⁻¹. ¹H NMR (CDCl₃): δ 8.49 (s, 1 H), 8.33 (s, 1 H), 8.00 (s, 1 H), 7.15 (s, 1 H), 4.18 (s, 1 H). ¹³C NMR (CDCl₃): δ 155.1, 144.3, 142.1, 140.3, 136.4, 135.8, 135.5, 130.4, 129.4, 126.2, 123.4, 111.7, 56.9. EIMS *m/z* (rel intensity): 357 (M⁺, 100). HRCIMS *m/z* calcd, 356.9197; found, 356.9199. HPLC purity: 98.45% (MeOH−H₂O, 95:5).

4-Bromo-7,8-dimethyl-1-methoxyphenazine (42). 7,8-Dimethyl-1-methoxyphenazine (**38**, 330 mg, 1.38 mmol) was dissolved in toluene (13 mL) and acetonitrile (1.3 mL). *N*-Bromosuccinimide (246 mg, 1.38 mmol) was added to the flask, and the reaction mixture was heated at reflux for 12 h. The solvent was removed under vacuum, and the solid residue was purified by silica gel column chromatography, eluting with ethyl acetate−hexane, 2:1. The product was obtained as a yellow powder (223 mg, 51%): mp 194−196 °C. IR (film): 3048, 2931, 2835, 1630, 1600, 1549, 1515, 1472, 1415, 1385, 1343, 1287, 1235, 1123, 1097, 924, 727 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ 8.11 (s, 1 H), 8.09 (s, 1 H), 7.99 (d, *J* = 8.2 Hz, 1 H), 6.89 (d, *J* = 8.2 Hz, 1 H), 4.13 (s, 3 H), 2.53 (s, 6 H). ¹³C NMR (125 MHz, CDCl₃): δ 155.0, 142.8, 142.4, 141.6, 140.4, 136.4, 132.2, 128.0, 127.9, 113.9, 106.9, 106.2, 56.4, 20.6, 20.5. CIMS *m/z* (rel intensity): 397 (MH⁺, 100), 395 (MH⁺, 69). HREIMS *m/z* calcd, 393.9316; found, 393.9327. HPLC purity: 96.50% (MeOH−H₂O, 90:10).

7,8-Dimethyl-1-methoxy-3,4-dibromophenazine (43). 7,8-Dimethyl-3-bromo-1-methoxyphenazine (**39**, 270 mg, 0.93 mmol) was dissolved in toluene (20 mL). Bromine (148 mg, 0.93 mmol), sodium acetate (200 mg, 2.43 mmol), and acetic acid (20 mL) were added. The reaction mixture was heated at reflux for 1 h. The solvent was removed in vacuo, and the orange solid was dissolved in ethyl acetate (40 mL). The organic phase was washed with brine (1 × 30 mL) and water (2 × 30 mL). The organic phase was removed in vacuo, and the compound was purified by silica gel column, eluting with hexane−ethyl acetate, 5:1 to 3:1. The product was obtained as a yellow powder (276 mg, 74%): mp 225−227 °C. IR (film): 3047, 2973, 2931, 1633, 1584, 1459, 1315, 1131, 1000, 863, 763, 750 cm⁻¹. ¹H NMR (CDCl₃): δ 7.98 (s, 1 H), 7.95 (s, 1 H), 7.12 (s, 1 H), 4.08 (s, 3 H), 2.48 (s, 6 H). ¹³C NMR (CDCl₃): δ 154.2, 143.2, 142.9, 142.5, 141.0, 140.9, 134.9, 127.9, 127.8, 126.8, 117.0, 111.0, 56.6, 20.6, 20.5. CIMS *m/z* (rel intensity): 395 (MH⁺, 68). HREIMS *m/z* calcd, 393.9316; found, 393.9316. Anal. calcd for C₁₅H₁₂Br₂N₂O: C, 45.23; H, 2.96; N, 6.91. Found: C, 45.49; H, 3.05; N, 7.07

7,8-Dichloro-1-hydroxyphenazine (44).[53] 7,8-Dichloro-1-methoxyphenazine (**40**, 270 mg, 0.96 mmol) was dissolved in dry, freshly distilled dichloromethane (30 mL). The reaction mixture was purged with argon and cooled to −78 °C. Boron tribromide (1 M solution in dichloromethane, 2.5 mL) was added dropwise to the mixture with stirring. After 5 min, more boron tribromide (1 mL, 1 M solution in dichloromethane) was added. The temperature was maintained at −78 °C, and the reaction mixture was stirred for 2 h. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was heated at reflux for 1 h. The solution was allowed to cool to room temperature, and saturated aqueous sodium bicarbonate (40 mL) at 0 °C was slowly added. The solution was extracted with ethyl acetate (4 × 30 mL). The organic extracts were combined and washed with brine (30 mL) and water (30 mL). The solvent was removed, and the compound was purified by silica gel column chromatography, eluting with ethyl acetate−acetic acid, 100:1. The product was obtained as an orange very sticky solid (191 mg, 75%): mp 270−271 °C (dec). IR (film): 3398, 1630, 1505, 1462, 1450, 1419, 1275, 1260, 764, 750 cm⁻¹.

¹H NMR (DMSO-*d₆*): δ 10.80 (OH, br s, 1 H), 8.56 (s, 1 H), 8.55 (s, 1 H), 7.83 (t, *J* = 8.4 Hz, 1 H), 7.67 (d, *J* = 8.7 Hz, 1 H), 7.22 (d, *J* = 7.4 Hz, 1 H). ESIMS *m/z* (rel intensity): 265 (MH⁺, 100). HRESIMS *m/z* calcd; found, 264.9935. Anal. calcd for C₁₂H₆Cl₂N₂O·0.5CHCl₃: C, 46.23; H, 2.02; N, 8.63. Found; C, 46.50; H, 2.07; N, 8.85. HPLC purity: 97.93% (MeOH−H₂O, 95:5).

1-Methoxybenzo[*b*]phenazine (46). The general procedure provided a solid that was dissolved in hot ethyl acetate (100 mL). The solution was filtered through a column containing Celite 545 (∼1 in.) with basic alumina (∼1 in.) on top. The Celite was washed with ethyl acetate until the filtered solution was light in color. The organic solutions were combined and washed with saturated sodium bicarbonate (50 mL) and water (50 mL). The compound was purified by silica gel column chromatography, eluting with ethyl acetate−hexane, 1:3.5. The product was obtained as a dark red powder (577 mg, 17%): mp 245−247 °C. IR (film): 2932, 1732, 1582, 1548, 1536, 1463, 1406, 1284, 1254, 1111, 885, 763, 736 cm⁻¹. ¹H NMR (CDCl₃): δ 9.04 (s, 1 H), 8.83 (s, 1 H), 8.10−8.08 (m, 2 H), 7.78 (d, *J* = 9 Hz, 1 H), 7.68 (t, *J* = 7.5 Hz, 1 H), 7.51−7.47 (m, 2 H), 6.96 (d, *J* = 7.3 Hz, 1 H), 4.17 (s, 3 H). ¹³C NMR (CDCl₃): δ 154.9, 145.0, 139.8, 138.6, 138.4, 134.6, 134.2, 130.7, 128.6, 128.4, 127.1, 126.9, 126.6, 121.6, 105.6, 56.4. EIMS *m/z* (rel intensity): 260 (M⁺, 66), 231 (M⁺-CHO, 100). HREIMS *m/z* calcd, 260.0950; found, 260.0949. HPLC purity: 96.25% (MeOH−H₂O, 95:5).

Benzo[*b*]phenazine (47). The general procedure was followed. The compound was purified by silica gel column chromatography (with one-half inch of basic alumina on top) eluting with dichloromethane. The product was obtained as an orange solid (93 mg, 32%): mp 219−221 °C (lit[54] mp 233 °C). IR (film): 1686, 1581, 1528, 1456, 1381, 1365, 1326 cm⁻¹. ¹H NMR (CDCl₃): δ 8.84 (s, 2 H), 8.18 (dd, *J* = 6.8, *J* = 3.3 Hz, 2 H), 8.07 (dd, *J* = 6.6, *J* = 3.2 Hz, 2 H), 7.77 (dd, *J* = 6.9, *J* = 3.1 Hz, 2 H), 7.49 (dd, *J* = 6.7, *J* = 3.1 Hz, 2 H). ¹³C NMR (CDCl₃): δ 144.4 (2 C), 139.9 (2 C), 134.4 (2 C), 130.5 (2 C), 129.8 (2 C), 128.4 (2 C), 127.5 (2 C), 126.7 (2 C). EIMS *m/z* (rel intensity): 230 (M⁺, 100). HREIMS *m/z* calcd, 230.0844; found, 230.0843. HPLC purity: 95.18% (MeOH−H₂O, 90:10), 97.11% (MeOH−H₂O, 95:5).

2,3-Dimethylbenzo[*g*]quinoxaline (49).[55] 2,3-Butanedione (**48**, 0.43 g, 4.95 mmol) in benzene (6 mL) was added dropwise to a solution of 2,3-diaminonaphthalene (**45**, 0.53 g, 3.35 mmol) in benzene (10 mL) and glacial acetic acid (6 mL). The reaction mixture was stirred for 12 h. The solvent was removed in vacuo, and the solid was dissolved in ethyl acetate (50 mL). The solution was washed with saturated aqueous sodium bicarbonate (2 × 40 mL), brine (40 mL), and water (40 mL). The product was purified by silica gel column chromatography, eluting with ethyl acetate−hexane, 1:3. The product was obtained as a brown solid (0.31 g, 45%): mp 209−211 °C. IR (film): 2990, 2917, 1630, 1580, 1522, 1438, 1411, 1373, 1158, 899, 877, 787 cm⁻¹. ¹H NMR (CDCl₃): δ 8.46 (s, 2 H), 8.03−7.99 (m, 2 H), 7.50−7.46 (m, 2 H), 2.69 (s, 6 H). ¹³C NMR (CDCl₃): δ 154.3, 137.9, 133.1, 128.2, 126.3, 126.1, 23.5. CIMS *m/z* (rel intensity): 209 (MH⁺, 100). HREIMS *m/z* calcd, 208.1000; found, 208.0998. HPLC purity: 95.41% (MeOH−H₂O, 95:5).

5-Methoxy-2,3-dimethylquinoxaline (51). A solution of 2,3-butanedione (**48**, 0.26 g, 3.0 mmol) was added dropwise to a solution of 3-methoxy-*o*-phenylenediamine (**50**, 0.41 g, 3.0 mmol) in benzene (30 mL) and glacial acetic acid (25 mL). The reaction mixture was stirred for 12 h at room temperature. The solvent was removed in vacuo, and the solid was dissolved in ethyl acetate (50 mL). The solution was washed with saturated aqueous sodium bicarbonate (2 × 30 mL), brine (30 mL), and water (2 × 20 mL). The solvent was removed, and the compound was purified by silica gel column chromatography, using dichloromethane as the solvent. The product was obtained as a beige solid (322 mg, 57%): mp 118−119 °C (lit. mp[56] 118 °C). IR (film): 3088, 3054, 2993, 2948, 1633, 1609, 1571, 1480, 1467, 1446, 1402, 1376, 1196, 1094, 996, 823, 769 cm⁻¹. ¹H NMR

(CDCl$_3$): δ 7.56 (m, 2 H), 7.00 (t, J = 4.8 Hz, 1 H), 4.06 (s, 3 H), 2.76 (s, 3 H), 2.71 (s, 3 H). ^{13}C NMR (CDCl$_3$): δ 154.5, 153.7, 152.0, 141.8, 132.8, 128.6, 120.1, 107.0, 56.1, 23.2, 23.0. CIMS m/z (rel intensity): 189 (MH$^+$, 100). HRCIMS m/z calcd, 188.0950; found, 188.0952. HPLC purity: 95.95% (MeOH$-$H$_2$O, 95:5).

5-Hydroxy-2,3-dimethylquinoxaline (52).[57] 5-Methoxy-2,3-dimethylquinoxaline (**51**, 278 mg, 1.47 mmol) was dissolved in dry, freshly distilled dichloromethane (25 mL). The reaction mixture was purged with argon and cooled to -78 °C. Boron tribromide (1 M solution in dichloromethane, 3.0 mL) was added dropwise to the mixture with stirring. The temperature was maintained at -78 °C, and the reaction mixture was stirred for 1 h. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. The reaction mixture was heated at reflux for 1 h. The solution was allowed to cool to room temperature, and saturated aqueous sodium bicarbonate (30 mL) at $\sim$4 °C was slowly added. The solution was extracted with dichloromethane (3×35 mL). The solvent was removed, and the compound was purified by silica gel column chromatography, eluting with ethyl acetate$-$hexane, 1:1. The product was obtained as a light brown powder (203 mg, 79%): mp 139$-$140 °C. IR (film): 3358, 3022, 2997, 2954, 2919, 1619, 1576, 1488, 1477, 1442, 1427, 1408, 1383, 1217, 1193, 823, 764, 751 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.01 (s, 1 H), 7.52$-$7.43 (m, 2 H), 7.07 (dd, J = 7.2, J = 1.3 Hz, 1 H), 2.64 (s, 1 H), 2.59 (s, 1 H). ^{13}C NMR (CDCl$_3$): δ 154.1, 151.3, 151.0, 141.2, 130.7, 129.6, 118.5, 109.9, 22.9, 22.6. EIMS m/z (rel intensity): 174 (M$^+$, 100). HREIMS m/z calcd, 174.0793; found, 174.0790. HPLC purity: 96.94% (MeOH$-$H$_2$O, 95:5).

6,8-Dibromo-2,3-dimethylquinoxalin-5-ol (53). *N*-Bromosuccinimide (654 mg, 3.68 mmol) and 5-hydroxy-2,3-dimethyl quinoxaline (**52**, 320 mg, 1.84 mmol) were mixed together and dissolved in toluene (10 mL) and acetonitrile (5 mL). The reaction mixture was heated at reflux for 8 h. The solvent was removed in vacuo, and the compound was purified by silica gel column chromatography (ethyl acetate$-$hexane, 1:3). The product was obtained as a brown solid (301 mg, 49.3%): mp 108$-$109 °C. IR (film): 3408, 3057, 2995, 2956, 2922, 2852, 1713, 1687, 1610, 1560, 1459, 1424, 1401, 1381, 1371, 1203, 937, 734 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 8.05 (OH br s, 1 H), 7.93 (s, 1 H), 2.74 (s, 1 H), 2.70 (s, 1 H). ^{13}C NMR (CDCl$_3$): δ 155.2, 152.9, 148.5, 137.6, 135.0, 130.9, 111.7, 103.4, 23.2, 22.6. EIMS m/z (rel intensity): 330 (M$^+$, 88). HREIMS m/z calcd, 329.9792; found, 329.9005. HPLC purity: 98.31% (MeOH$-$H$_2$O, 90:10).

QR1 Assay. Hepa 1c1c7 (mouse hepatoma) cells were used in the assay. Cells were incubated in a 96-well plate with test compounds at a maximum concentration of 50 μM for 48 h, digitonin was used to permeabilize cell membranes, and enzyme activity was measured by the reduction of 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a blue formazan. Production was measured by absorption at 595 nm. A total protein assay using crystal violet staining was run in parallel. The total protein levels were determined using crystal violet staining in parallel to determine the cytotoxicity of test compounds.[58] Briefly, the media were discarded after 48 h of incubation with test compounds, and cells were stained with 100 μL of 0.2% crystal violet in 2% ethanol for 10 min. After cells were washed with tap water to remove excessive crystal violet, 200 μL of 0.5% SDS in 50% ethanol was added to dissolve the stained cells. The optical density was determined at 595 nm. 4'-Bromo-flavone (CD = 0.01 μM) was used as a positive control.

Expression and Purification of Human QR2. Human QR2 was expressed and purified from 3 L of *Escherichia coli* BL21(DE3) grown in Luria$-$Bertani medium supplemented with 100 μg/mL ampicillin following our previously reported procedures.[9] The concentration of purified QR2 was determined using the Bio-RAD Protein Assay. Frozen QR2, previously stored at -80 °C, was thawed on ice and then adjusted to the desired concentration, typically as a 4 mg/mL stock for enzyme kinetics measurements, by diluting in storage buffer without glycerol.

Steady-State Kinetic Assays and QR2 IC$_{50}$ Value Determination. The catalytic activity of QR2 was determined using MTT [3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide] and NMeH as substrates. Assays were run in 96-well plates with a final assay volume of 200 μL. Each assay mixture contained approximately 12 nM QR2, 17.5 μM NMeH, and 200 μM MTT in a reaction buffer containing 100 mM NaCl, 50 mM Tris, and 0.1% Triton-X100 (Fisher Biotech 93004). The assays were performed at 23 °C using a SpectraMax Plus 384 UV/vis microplate reader by monitoring the increase in absorbance at 612 nm, which is due to formation of the product formazan, the reduced form of the substrate MTT. All reactions were initiated by the addition of QR2. The initial slopes of the reaction (ΔAbs/Δtime) were measured and were used to calculate the initial rates of the reaction using a value of 11300 mM^{-1} cm^{-1} for the molar extinction coefficient.

IC$_{50}$ values were also determined in 96-well plates at 23 °C using the same assay conditions described above except that inhibitor concentrations were varied from 0.1 to 100 μM. Assays at each inhibitor concentration were performed in triplicate, and the average and standard deviations in the rate values were used to determine the final IC$_{50}$ values by calculating the percent inhibition (% I) at each inhibitor concentration versus the negative control with zero inhibitor. These data were then plotted as the percent inhibition versus inhibitor concentration, [I]. All data were fit to the equation: % I = % I$_{max}$/[(1 + [I]/IC$_{50}$)] using nonlinear regression via the Enzyme Kinetics Module of the program SigmaPlot from SPSS Scientific. IC$_{50}$ and % I$_{max}$ values are reported along with their standard error in the fitted parameters.

NF-κB Luciferase Assay. Studies were performed with NF-κB reporter stably transfected human embryonic kidney cells 293 from Panomics (Fremont, CA). This cell line contains chromosomal integration of a luciferase reporter construct regulated by NF-κB response element. The gene product, luciferase enzyme, reacts with luciferase substrate, emitting light, which is detected with a luminometer. Data were expressed as % inhibition at 50 μM or IC$_{50}$ values (i.e., concentration of test sample required to inhibit TNF-α activated NF-κB activity by 50%). After treated cells were incubated, they were lysed in Reporter Lysis buffer. The luciferase assay was performed using the Luc assay system from Promega, following the manufacturer's instructions. In this assay, *N*-α-tosyl-L-phenylalanine chloromethyl ketone (TPCK) was used as a positive control; IC$_{50}$ = 5.09 μM.

Nitrite Assay. RAW 264.7 cells were incubated in a 96-well culture plate for 24 h. The cells were treated with various concentrations of compounds dissolved in phenol red-free Dulbecco's modified Eagle's medium (DMEM) for 30 min followed by 1 μg/mL of lipopolysaccharide (LPS) treatment for 24 h. NO is an unstable molecule, easily oxidized to the stable end product, nitrite. Therefore, the nitrite level in cultured media was measured using Griess reagent [1:1 mixture (v/v) of 1% sulfanilamide in 5% H$_3$PO$_4$ and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride solution]. A standard curve was created by using known concentrations of sodium nitrite. The positive control in this assay was Na-L monomethyl arginine (L-NMMA); IC$_{50}$ = 19.7 μM.

Sulforhodamine B (SRB) Assay. To evaluate the cytotoxicity of test compounds toward NF-κB reporter stably transfected human embryonic kidney cells 293 and RAW 264.7 cells in the assay condition, SRB assay was performed as describe previously.[59] Briefly, after the fixation with 10% trichloroacetic acid, cells were stained with 0.4% SRB solution followed by dissolving bound SRB to cells in 10 mM Tris-buffer. The optical density was determined at 515 nm.

References

(1) Seffrin, J. R.; Hill, D.; Burkart, W.; Magrath, I.; Badwe, R. A.; Ngoma, T.; Mohar, A.; Grey, N. It Is Time to Include Cancer and Other Noncommunicable Diseases in the Millennium Development Goals. *CA Cancer J. Clin.* **2009**, *59*, 282–284.

(2) Jemal, A.; Siegel, R.; Ward, E.; Hao, Y. P.; Xu, J. Q.; Thun, M. J. Cancer Statistics, 2009. *CA Cancer J. Clin.* **2009**, *59*, 225–249.

(3) Smith, B. D.; Smith, G. L.; Hurria, A.; Hortobagyi, G. N.; Buchholz, T. A. Future of Cancer Incidence in the United States: Burdens Upon an Aging, Changing Nation. *J. Clin. Oncol.* **2009**, *27*, 2758–2765.

(4) Hong, W. K.; Sporn, M. B. Recent Advances in Chemoprevention of Cancer. *Science* **1997**, *278*, 1073–1077.

(5) Cuendet, M.; Oteham, C. P.; Moon, R. C.; Pezzuto, J. M. Quinone Reductase Induction as a Biomarker for Cancer Chemoprevention. *J. Nat. Prod.* **2006**, *69*, 460–463.

(6) Georgiadis, P.; Demopoulos, N. A.; Topinka, J.; Stephanou, G.; Stoikidou, M.; Bekyrou, M.; Katsouyianni, K.; Sram, R.; Autrup, H.; Kyrtopoulos, S. A. Impact of Phase I or Phase II Enzyme Polymorphisms on Lymphocyte DNA Adducts in Subjects Exposed to Urban Air Pollution and Environmental Tobacco Smoke. *Toxicol. Lett.* **2004**, *149*, 269–280.

(7) Dietz, B. M.; Kang, Y. H.; Liu, G. W.; Eggler, A. L.; Yao, P.; Chadwick, L. R.; Pauli, G. F.; Farnsworth, N. R.; Mesecar, A. D.; van Breemen, R. B.; Bolton, J. L. Xanthohumol Isolated from *Humulus lupulus* Inhibits Menadione-Induced DNA Damage through Induction of Quinone Reductase. *Chem. Res. Toxicol.* **2005**, *18*, 1296–1305.

(8) Barillari, J.; Iori, R.; Broccoli, M.; Pozzetti, L.; Canistro, D.; Sapone, A.; Bonamassa, B.; Biagi, G. L.; Paolini, M. Glucoraphasatin and Glucoraphenin, a Redox Pair of Glucosinolates of Brassicaceae, Differently Affect Metabolizing Enzymes in Rats. *J. Agric. Food Chem.* **2007**, *55*, 5505–5511.

(9) Calamini, B.; Santarsiero, B. D.; Boutin, J. A.; Mesecar, A. D. Kinetic, Thermodynamic, and X-Ray Structural Insights into the Interaction of Melatonin and Analogues with Quinone Reductase 2. *Biochem. J.* **2008**, *413*, 81–91.

(10) Buryanovskyy, L.; Fu, Y.; Boyd, M.; Ma, Y. L.; Hsieh, T. C.; Wu, J. M.; Zhang, Z. T. Crystal Structure of Quinone Reductase 2 in Complex with Resveratrol. *Biochemistry* **2004**, *43*, 11417–11426.

(11) Lisztwan, J.; Pornon, A.; Chen, B.; Chen, S. A.; Evans, D. B. The Aromatase Inhibitor Letrozole and Inhibitors of Insulin-like Growth Factor I Receptor Synergistically Induce Apoptosis in In Vitro Models of Estrogen-Dependent Breast Cancer. *Breast Cancer Res.* **2008**, *10*, R56.

(12) Tang, L.; Zhang, Y. S. Dietary Isothiocyanates Inhibit the Growth of Human Bladder Carcinoma Cells. *J. Nutr.* **2004**, *134*, 2004–2010.

(13) Eggler, A. L.; Gay, K. A.; Mesecar, A. D. Molecular Mechanisms of Natural Products in Chemoprevention: Induction of Cytoprotective Enzymes by Nrf2. *Mol. Nutr. Food Res.* **2008**, *52*, S84–S94.

(14) Misico, R. I.; Song, L. L.; Veleiro, A. S.; Cirigliano, A. M.; Tettamanzi, M. C.; Burton, G.; Bonetto, G. M.; Nicotra, V. E.; Silva, G. L.; Gil, R. R.; Oberti, J. C.; Kinghorn, A. D.; Pezzuto, J. M. Induction of Quinone Reductase by Withanolides. *J. Nat. Prod.* **2002**, *65*, 677–680.

(15) Fu, Y.; Buryanovskyy, L.; Zhang, Z. T. Quinone Reductase 2 Is a Catechol Quinone Reductase. *J. Biol. Chem.* **2008**, *283*, 23829–23835.

(16) Talalay, P.; Fahey, J. W.; Holtzclaw, W. D.; Prestera, T.; Zhang, Y. S. Chemoprotection Against Cancer by Phase 2 Enzyme Induction. *Toxicol. Lett.* **1995**, *82–3*, 173–179.

(17) Angeloni, C.; Leoncini, E.; Malaguti, M.; Angelini, S.; Hrelia, P.; Hrelia, S. Modulation of Phase II Enzymes by Sulforaphane: Implications for Its Cardioprotective Potential. *J. Agric. Food Chem.* **2009**, *57*, 5615–5622.

(18) Surh, Y. J. NF-kappa B and Nrf2 as Potential Chemopreventive Targets of Some Anti-inflammatory and Antioxidative Phytonutrients with Anti-inflammatory and Antioxidative Activities. *Asia Pac. J. Clin. Nutr.* **2008**, *17*, 269–272.

(19) Libermann, T. A.; Zerbini, L. F. Targeting Transcription Factors for Cancer Gene Therapy. *Curr. Gene Ther.* **2006**, *6*, 17–33.

(20) Aggarwal, B. B.; Shishodia, S. Molecular Targets of Dietary Agents for Prevention and Therapy of Cancer. *Biochem. Pharmacol.* **2006**, *71*, 1397–1421.

(21) Spector, N.; Xia, W. L.; El-Hariry, I.; Yarden, Y.; Bacus, S. Small Molecule HER-2 Tyrosine Kinase Inhibitors. *Breast Cancer Res.* **2007**, *9*, 205–212.

(22) Fabian, M. A.; Biggs, W. H.; Treiber, D. K.; Atteridge, C. E.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lelias, J. M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A. M.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J A Small Molecule-Kinase Interaction Map for Clinical Kinase Inhibitors. *Nat. Biotechnol.* **2005**, *23*, 329–336.

(23) Jin, X.; Gossett, D. R.; Wang, S.; Yang, D.; Cao, Y.; Chen, J.; Guo, R.; Reynolds, R. K.; Lin, J. Inhibition of AKT Aurvival Pathway by a Small Molecule Inhibitor in Human Endometrial Cancer Cells. *Br. J. Cancer* **2004**, *91*, 1808–1812.

(24) Maheshwari, R. K.; Singh, A. K.; Gaddipati, J.; Srimal, R. C. Multiple Biological Activities of Curcumin: A Short Review. *Life Sci.* **2006**, *78*, 2081–2087.

(25) Thangapazham, R. L.; Sharma, A.; Maheshwari, R. K. Multiple Molecular Targets in Cancer Chemoprevention by Curcumin. *AAPS J.* **2006**, *8*, E443–E449.

(26) Manson, M. M.; Holloway, K. A.; Howells, L. M.; Hudson, E. A.; Plummer, S. M.; Squires, M. S.; Prigent, S. A. Modulation of Signal-Transduction Pathways by Chemopreventive Agents. *Biochem. Soc. Trans.* **2000**, *28*, 7–12.

(27) Cross, J. V.; Deak, J. C.; Rich, E. A.; Qian, Y. Y.; Lewis, M.; Parrott, L. A.; Mochida, K.; Gustafson, D.; Vande Pol, S.; Templeton, D. J. Quinone Reductase Inhibitors Block SAPK/JNK and NF Kappa B Pathways and Potentiate Apoptosis. *J. Biol. Chem.* **1999**, *274*, 31150–31154.

(28) Laursen, J. B.; Nielsen, J. Phenazine Natural Products: Biosynthesis, Synthetic Analogues, and Biological Activity. *Chem. Rev.* **2004**, *104*, 1663–1685.

(29) Kerr, J. R.; Taylor, G. W.; Rutman, A.; Hoiby, N.; Cole, P. J.; Wilson, R. *Pseudomonas aeruginosa* Pyocyanin and 1-Hydroxyphenazine Inhibit Fungal Growth. *J. Clin. Pathol.* **1999**, *52*, 385–387.

(30) Shinya, K.; Shimizu, S.; Kunigami, T.; Furihata, K.; Hayakawa, Y.; Seto, H. Novel Neuronal Cell Protecting Substances, Aestivophoenins A and B, Produced by Streptomyces-Purpeofuscus. *J. Antibiot.* **1995**, *48*, 1378–1381.

(31) Shinya, K.; Shimizu, S.; Kunigami, T.; Furihata, K.; Seto, H. A New Neuronal Cell Protecting Substance, Lavanduquinocin, Produced by *Streptomyces* Viridochromogenes. *J. Antibiot.* **1995**, *48*, 574–578.

(32) Birkofer, L.; Birkofer, A. Bacteriostatic Effect of a Phenazine Derivative on *Mycobacterium tuberculosis* Type Gallinaceus. *Naturwissenschaften* **1949**, *36*, 92.

(33) Nakayama, O.; Arakawa, H.; Yagi, M.; Tanaka, M.; Kiyoto, S.; Okuhara, M.; Kohsaka, M. WS-9659 A and B, Novel Testosterone 5-Alpha-Reductase Inhibitors Isolated from a *Streptomyces* III. Biological Characteristics and Pharmacological Characteristics. *J. Antibiot.* **1989**, *42*, 1235–1240.

(34) Matsumoto, M.; Seto, H. Stimulation of Mammalian-Cell Proliferation by Lavanducyanin. *J. Antibiot.* **1991**, *44*, 1471–1473.

(35) Surrey, A. Pyocyanine. *Org. Synth.* **1955**, *3*, 753.

(36) Wrede, F.; Strack, E. Synthesis of Pyocyanine and Some of Its Homologues. *Ber. Desch. Chem. Ges.* **1929**, *62*, 2051–2057.

(37) Dinkova-Kostova, A. T.; Talalay, P. Persuasive Evidence that Quinone Reductase Type 1 (DT diaphorase) Protects Cells Against the Toxicity of Electrophiles and Reactive Forms of Oxygen. *Free Radical Biol. Med.* **2000**, *29*, 231–240.

(38) Schupp, P. J.; Kohlert-Schupp, C.; Whitefield, S.; Engemann, A.; Rohde, S.; Hemscheidt, T.; Pezzuto, J. M.; Kondratyuk, T. P.; Park, E. J.; Marler, L.; Rostama, B.; Wright, A. D. Cancer Chemopreventive and Anticancer Evaluation of Extracts and Fractions from Marine Macro- and Micro-organisms Collected from Twilight Zone Waters Around Guam. *Nat. Prod. Commun.* **2009**, *4*, 1717–1728.

(39) Lohar, M. V.; Mundada, R.; Bhonde, M.; Padgaonkar, A.; Deore, V.; Yewalkar, N.; Bhatia, D.; Rathos, M.; Joshi, K.; Vishwakarma, R. A.; Kumar, S. Design and Synthesis of Novel Furoquinoline Based Inhibitors of Multiple Targets in the PI3K/Akt-mTOR Pathway. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3603–3606.

(40) Xu, Q.; Briggs, J.; Park, S.; Niu, G. L.; Kortylewski, M.; Zhang, S. M.; Gritsko, T.; Turkson, J.; Kay, H.; Semenza, G. L.; Cheng, J. Q.; Jove, R.; Yu, H. Targeting Stat3 Blocks Both HIF-1 and VEGF Expression Induced by Multiple Oncogenic Growth Signaling Pathways. *Oncogene* **2005**, *24*, 5552–5560.

(41) Fan, Q. W.; Knight, Z. A.; Goldenberg, D. D.; Yu, W.; Mostov, K. E.; Stokoe, D.; Shokat, K. M.; Weiss, W. A. A Dual PI3 Kinase/mTOR Inhibitor Reveals Emergent Efficacy in Glioma. *Cancer Cell* **2006**, *9*, 341–349.

(42) Aggarwal, B. B.; Takada, Y.; Oommen, O. V. From Chemoprevention to Chemotherapy: Common Targets and Common Goals. *Expert Opin. Invest. Drugs* **2004**, *13*, 1327–1338.

(43) Gerhauser, C.; You, M.; Liu, J. F.; Moriarty, R. M.; Hawthorne, M.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer Chemopreventive Potential of Sulforamate, a Novel Analogue of Sulforaphane

that Induces Phase 2 Drug-metabolizing Enzymes. *Cancer Res.* **1997**, *57*, 272–278.

(44) Cornblatt, B. S.; Ye, L. X.; Dinkova-Kostova, A. T.; Erb, M.; Fahey, J. W.; Singh, N. K.; Chen, M. S. A.; Stierer, T.; Garrett-Mayer, E.; Argani, P.; Davidson, N. E.; Talalay, P.; Kensler, T. W.; Visvanathan, K. Preclinical and Clinical Evaluation of Sulforaphane for Chemoprevention in the Breast. *Carcinogenesis* **2007**, *28*, 1485–1490.

(45) Yosioka, I. Phenazines. XXI. Bromination of Phenazine Derivatives. *Chem. Pharm. Bull.* **1959**, *8*, 581–584.

(46) Ueda, K.; Yosioka, I. Studies on Phenazines. XXX. Bromination of Phenazine Derivatives by N-Bromosuccinimide (2). *Chem. Pharm Bull.* **1968**, *16*, 1521–1526.

(47) Teuber, H. J.; Staiger, G. Reactions with Nitrosodisulfonate. 7. Ortho-Benzoquinone and Phenazine. *Chem. Ber./Recl.* **1955**, *88*, 802–827.

(48) Shah, J. R.; Shah, C.; Velingkar, S. V. Synthesis and Antimicrobial Activity of Some Phenazine Analogues. *Indian J. Heterocycl. Chem.* **2003**, *13*, 175–176.

(49) Vivian, D. L.; Hartwell, J. L.; Waterman, H. C. Phenazine Syntheses. 3. Miscellaneous Phenazines. *J. Org. Chem.* **1954**, *19*, 1641–1645.

(50) Tietze, M.; Iglesias, A.; Merisor, E.; Conrad, J.; Klaiber, I.; Beifuss, U. Efficient Methods for the Synthesis of 2-Hydroxyphenazine Based on the Pd-Catalyzed N-Arylation of Aryl Bromides. *Org. Lett.* **2005**, *7*, 1549–1552.

(51) Vivian, D. L. Phenazine Syntheses. 7. Certain Disubstituted Phenazines. *J. Org. Chem.* **1956**, *21*, 824–825.

(52) King, F. E.; Clark, N. G.; Davis, P. M. H. Hydroxy-Quinoxalines and Hydroxy-Phenazines, and Experiments on the Preparation of Hydroxyquinoxaline Di-N-Oxides. *J. Chem. Soc.* **1949**, 3012–3016.

(53) Issidorides, C. H.; Atfah, M. A.; Sabounji, J. J.; Sidani, A. R.; Haddadin, M. J. Application of 1,2-Diketones in the Synthesis of Phenazine Oxides. *Tetrahedron* **1978**, *34*, 217–221.

(54) Vanallan, J. A.; Reynolds, G. A.; Adel, R. E. Polynuclear Heterocycles. 2. Addition Reactions of Benzophenazines. *J. Org. Chem.* **1962**, *27*, 2873–2878.

(55) Mariaud, M.; Levillain, P. New Quantitative Determination of 2,3-Dioxobutane (Diacetyl) in Alcoholic Beverages by an Indirect Spectrofluorometric Procedure with Use of 2,3-Diaminonaphthalene as Reagent of Derivatization. *Talanta* **1994**, *41*, 75–79.

(56) Lane, E. S.; Williams, C. Organic Complex-Forming Agents for Metals. 2. Preparation of 5-Hydroxy-Quinoxalines and 5–8-Dihydroxy-Quinoxalines and Related Compounds. *J. Chem. Soc.* **1956**, 2983–2986.

(57) Barret, R.; Daudon, M. Oxidation of Phenols to Quinones by Bis(trifluoroacetoxy)iodobenzene. *Tetrahedron Lett.* **1990**, *31*, 4871–4872.

(58) Su, B. N.; Gu, J. Q.; Kang, Y. H.; Park, E. J.; Pezzuto, J. M.; Kinghorn, A. D. Induction of the Phase II Enzyme, Quinone Reductase, by Withanolides and Norwithanolides from Solanaceous Species. *Mini-Rev. Org. Chem.* **2004**, *1*, 115–123.

(59) You, M.; Wickramaratne, D. B. M.; Silva, G. L.; Chai, H. Y.; Chagwedera, T. E.; Farnsworth, N. R.; Cordell, G. A.; Kinghorn, A. D.; Pezzuto, J. M. (−)-Roemerine, an Aporphine Alkaloid from Annona-Senegalensis That Reverses the Multidrug-Resistance Phenotype with Cultured-Cells. *J. Nat. Prod.* **1995**, *58*, 598–604.