Structure−Activity Studies of Substituted Quinoxalinones as Multiple-Drug-Resistance Antagonists

David S. Lawrence, Jean E. Copper, and Charles D. Smith*

Department of Pharmacology, Pennsylvania State University, Hershey, Pennsylvania 17033

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A significant problem in the clinical treatment of cancer relates to the development of tumor resistance to many chemotherapeutic agents. Acquired drug resistance is often mediated through overexpression of membrane transport proteins that effectively efflux anticancer agents. Two of the best-studied transporters, P-glycoprotein (Pgp) and MRP1, have pharmacological properties that only partially overlap. In our search for improved drug-resistance antagonists, we have identified a family of substituted quinoxalines that selectively antagonizes Pgp over MRP1. Consequently, a focused library of congeners was designed and synthesized starting with a parent bromomethylquinoxalinone. This parent quinoxalinone was then condensed with a series of phenols to yield a family of substituted phenoxymethylquinoxalinones. These compounds were evaluated for their toxicity toward drug-sensitive MCF-7 breast carcinoma cells and for their abilities to antagonize Pgp and MRP1 in drug-resistant cell lines (NCI/ADR and MCF-7/VP, respectively). The results of this structure−activity study indicate that compounds with carbonyl substitutions of the phenoxy group (ester, amide, or ketone moieties) demonstrate excellent antagonism of Pgp while having relatively low toxicity toward drug-sensitive cells. Importantly, none of these compounds antagonized MRP1. Because of their transporter selectivity, we predict that substituted quinoxalinones may be more effective MDR modulators in vivo than are nonselective transporter antagonists.

Introduction

A significant problem in the clinical treatment of cancer arises from the development of tumor resistance to a wide variety of chemotherapeutic agents. The overexpression of drug transport proteins is a major mechanism for this multiple drug resistance (MDR).[1,2] The drug transporter, P-glycoprotein (Pgp), is an ATP-dependent drug efflux pump whose biochemistry and pharmacology have been intensely studied. A series of homologous proteins termed multidrug-resistance-related proteins (MRPs) have been discovered more recently, and these proteins share many pharmacological characteristics with Pgp.[3−5] Because of the importance of MDR in clinical oncology, an intensive search for antagonists of these transporters has developed since the demonstration that verapamil has this property.[6] Consequently, a variety of compounds have since been shown to reverse Pgp-mediated MDR in vitro,[7] and several of these agents have been evaluated in clinical trials.[8,9] Unfortunately, most of these agents suffer clinically from their intrinsic toxicity or from undesired effects on the pharmacokinetics of the accompanying anticancer drugs.

It seems likely that these clinical results may stem from incomplete attention to the pharmacologies of the different transport proteins, making it worthwhile to compare the properties of Pgp and MRP1. One significant difference between Pgp and MRP1 relates to their tissue distributions. Studies have shown that Pgp is expressed by certain types of secretory cells, including capillary endothelial cells in the brain and testis, and at sites within the pancreas, kidney, and liver.[1,10] By comparison, MRP1 mRNA has been observed in virtually every type of tissue[11] and is prominently expressed in peripheral blood mononuclear cells.[3,12−15] An additional difference between Pgp and MRP1 relates to their expression in human tumors. Extensive expression analyses have demonstrated that Pgp is often increased in tumor cells from patients undergoing chemotherapy, indicating that this mechanism of MDR is clinically important.[16−19] In contrast, although MRP1 is expressed in a high percentage of solid tumors and leukemias,[20] its overexpression is not consistently found in tumors. For example, MRP1 levels detected in normal and malignant hematopoietic cells were equivalent,[12,13] and the MRP1 levels in lung tumors were found to be lower than those in normal lung tissue.[21] The MRP1 mRNA levels in malignant melanoma,[22] acute lymphocytic leukemia,[14] or chronic lymphocytic leukemia[10] were not altered by chemotherapy but did increase moderately in acute myelogenous leukemia.[13,14] Therefore, it seems that overexpression of Pgp activity is clinically more significant than elevation of MRP1 levels. A final difference between Pgp and MRP1 relates to their drug-resistance profiles which are only partially overlapping.[23,24] For example, the resistance factors for vincristine, etoposide, and doxorubicin in MRP1-transfected cells are greater than those for vinblastine and paclitaxel. Conversely, Pgp-overexpressing cells are extremely resistant to the latter two drugs. The capacity of certain compounds to antagonize either Pgp or MRP1 has also been suggested.[25−27]

* Address for correspondence: Dept. of Pharmacology, H078, Penn State College of Medicine, 500 University Dr., Hershey, PA 17033-2390. Tel: (717) 531-1672. Fax: (717) 531-5013. E-mail: cdsmith@psu.edu.

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Scheme 1. Synthesis of Substituted Quinoxalinones

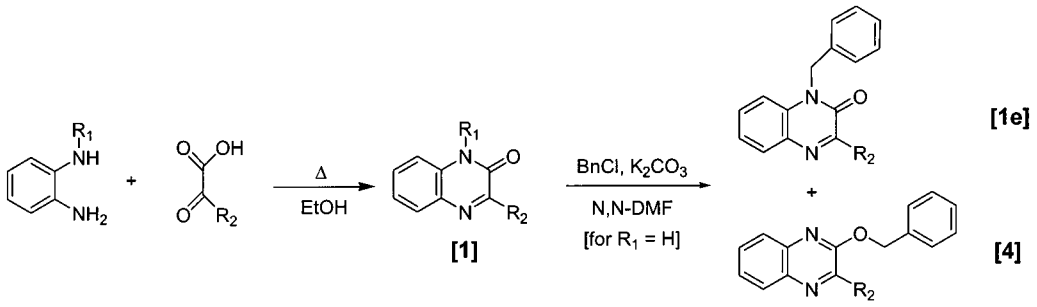
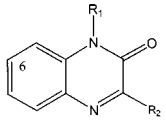


Table 1. Reversal of Pgp- and MRP1-Mediated Resistance by 2-Oxoquinoxalines



compd	R_1	R_2	benzo substituent	toxicity[a]	Pgp[b]	MRP1[c]	Pgp/MRP1
9	H	2-aminophenyl	none	22	1.7	1.1	1.5
10	H	2-acetamidophenyl	none	0	1.0	1.0	1.0
11	H	2-aminophenyl	benz[*g*]	37	4.6	1.3	3.5
12	H	2-amino-5-bromophenyl	benz[*g*]	49	9.2	1.3	7.1
13	H	2-methylamino-5-methoxyphenyl	benz[*g*]	25	1.1	1.0	1.1
14	CH_3	2-methylamino-5-methoxyphenyl	none	7	1.1	3.5	0.3
15	CH_3	2-methylaminophenyl	6-nitro	7	1.0	1.1	0.9
16	CH_3	2-methylamino-5-methoxyphenyl	benz[*g*]	3	1.1	2.1	0.5

[a] Toxicity is calculated as the percentage of MCF-7 cells killed by 10 μg/mL of the indicated compounds. [b] The Pgp antagonism score = percentage of NCI/ADR cells surviving in the absence of vinblastine/percentage of NCI/ADR cells surviving in the presence of vinblastine. [c] The MRP1 antagonism score = percentage of MCF-7/VP cells surviving in the absence of vincristine/percentage of MCF-7/VP cells surviving in the presence of vincristine.

Because of these issues, we hypothesize that the selectivity of individual MDR modulators toward Pgp or MRP1 will be critical in determining their therapeutic utility.[28] Antagonists of these transport proteins enhance the intracellular accumulation of many anticancer drugs, thereby increasing their toxicities. The MDR modulators most extensively evaulated in clinical trials, verapamil[23−26] and the cyclosporin analogues,[24,29,30] antagonize both Pgp and MRP1. Thus, the clinical failure of these nonselective modulators may result from increased systemic toxicity of the anticancer drug due to inhibition of MRP1 in normal tissues. Superior results may be obtained with modulators that are specifically directed toward Pgp, and some newer agents including XR9576,[31] LY335979,[32] and R101933[33] have demonstrated improved Pgp selectivity and pharmacological properties. To extend the portfolio of transporter-selective antagonists, we now describe structure−activity relationships (SARs) of substituted quinoxalinones that reverse MDR mediated by Pgp but not MRP1.

Results and Discussion

Screening 11 000 compounds from commercially available libraries identified several structural platforms with good potential as MDR antagonists. One of these, the 2-oxoquinoxaline or quinoxalinone heterocycle, showed promise as a versatile scaffold with which to study SARs. The initial screening was conducted at a single final dose of 10 μg/mL for the test compounds, equating to concentrations of approximately 10−50 μM. Table 1 shows that compounds **12** and **14** selectively potentiated the cytotoxicity of vinblastine toward NCI/ADR cells or vincristine toward MCF-7/VP cells, respectively, indicating selective antagonism of Pgp or MRP1. Compound **12** was moderately cytotoxic toward MCF-7 cells, a property undesired for an MDR modulator. We therefore set out to expand the diversity of compounds of this type to explore the quinoxalinone scaffold for its potential to provide selective Pgp and MRP1 antagonists without concomitant toxicity toward nonresistant cells.

A series of substituted quinoxalinones, shown in Table 2, were synthesized by standard literature techniques centered around the condensation of an *o*-phenylenediamine with an α-keto acid (Scheme 1). These simple structures were designed to assess some of the basic requirements for selective MDR antagonists as well as to determine parameters for their synthesis. Compound **1e** was formed by N-benzylation of compound **1b**, which also produced an enol ether side product **4**. While the quinoxalinones in Table 2 were essentially nontoxic to MCF-7 cells, they demonstrated only modest MDR antagonism scores. For comparison purposes, the well-established MDR antagonist verapmil demonstrated an $IC_{50} > 51$ μM toward MCF-7 cells, and Pgp and MRP1 scores of 5.9 and 1.9, respectively. Importantly, antagonism by the substituted quinoxalinones was selective for Pgp, whereas verapmil caused significant reversal of both Pgp- and MRP1-mediated MDR. Benzyl substitution in either position 1 or 3 resulted in enhanced Pgp antagonism over methyl substitution or no substitution (in position 1) and low toxicity to MCF-7 cells. Therefore, substitution in the 3-position was further explored to provide compounds with improved antagonism of Pgp.

In work reported by Corona et al.,[34] phenoxy- and phenoxymethylquinoxalines (without the 2-oxo moiety) were synthesized and tested for inhibitory activity

Scheme 2. Synthesis of Substituted Phenoxymethylquinoxalinones

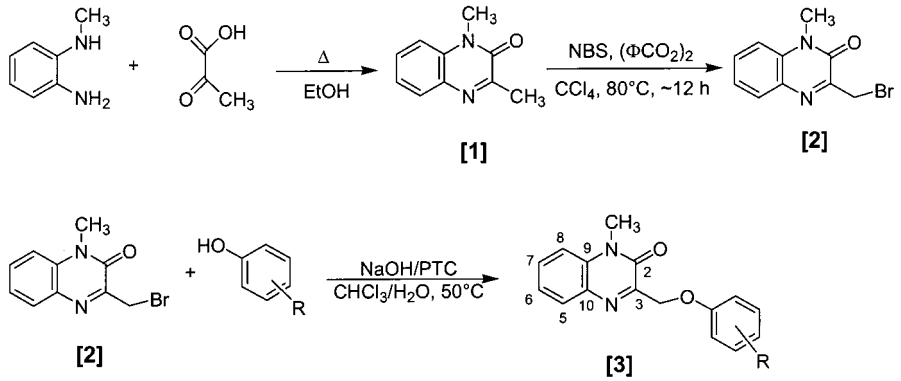
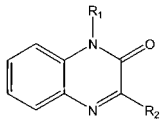


Table 2. Cytotoxicity and MDR Antagonism by Substituted Quinoxalinones

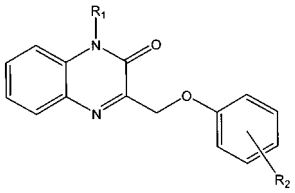


compd	R_1	R_2	IC_{50} (μM)[a]	Pgp	MRP1	Pgp/MRP1
1a	H	benzyl	>106	2.8	1.0	2.8
1b	H	CH_3	>156	1.0	1.1	0.9
1c	CH_3	CH_3	>144	1.1	0.7	1.7
1d	CH_3	benzyl	>100	2.9	1.1	2.7
1e	benzyl	CH_3	>100	3.1	0.8	3.7
verapamil			>51	5.9	1.9	3.1

[a] IC_{50} = concentration required to kill 50% of MCF-7 cells.

against dihydrofolate reductase and thymidylate synthase. This type of substitution is amenable to a parallel synthesis strategy and allows the production of many analogues in a facile manner and was therefore adapted to a quinoxalinone scaffold. As indicated in Scheme 2, the synthesis of substituted phenoxymethylquinoxalinones began with selective bromination of the 3-methyl group of compound **1c** (or **1e**) to give the parent bromomethylquinoxalinone **2** (or **2a**). The various phenoxymethylquinoxalinones were then formed in parallel by condensation of the bromomethylquinoxalinone with the appropriate phenolate anion. Because preliminary experiments demonstrated that the quinoxalinone structure is sensitive to some basic conditions (e.g. cesium carbonate in DMF at 70 °C), the reactions were performed under phase-transfer-catalyzed conditions to minimize exposure of the quinoxalinone heterocycle to base. Furthermore, because N-alkylation would occur with quinoxalinones unsubstituted in the 1-position, all of the parent bromomethylquinoxalinones were substituted with either methyl or benzyl groups. The selections of solvent and temperature were done to ensure convenience in a parallel synthesis approach. While the unoptimized reaction yields were generally low, this synthetic strategy allowed for sufficient quantities (>5 mg) of many different compounds to be made with relative ease. Workups and purifications were done with only slight variation among samples to maximize the number of compounds that could be synthesized even though reaction yield was thereby reduced. These reaction conditions were found to be unsuitable for several substituent groups, including $-NH_2$, $-CO_2H$, and indole, which did not form condensation products due to competing reactions. Additionally, methoxy-substituted

Table 3. Cytotoxicity and MDR Antagonism by Phenoxymethylquinoxalinones



compd	R_1	R_2	IC_{50} (μM)	Pgp	MRP1	Pgp/MRP1
3a	CH_3	H	>94	3.7	1.0	3.7
3b	CH_3	4-fluoro	26	1.2	0.8	1.4
3c	CH_3	4-cyano	>43[a]	3.2	0.8	3.9
3d	CH_3	2,3,4,5,6-pentafluoro	>70	2.1	0.8	2.6
3e	CH_3	4-benzyloxy	>40	1.9	0.9	2.2
3f	CH_3	3-*tert*-butyl	19	4.1	0.9	4.5
3g	CH_3	3,4-dichloro	>37[a]	1.1	0.9	1.3
3h	CH_3	4-benzoyl	>67	4.7	0.9	4.9
3i	CH_3	3-methyl-4-nitro	>77	1.6	1.1	1.4
3j	CH_3	4-CO_2CH_3-2-OCH_3	71	5.6	1.1	5.2
3k	CH_3	2-C(O)NH-phenyl	>32[a]	3.6	1.0	3.6
3l	CH_3	3-dimethylamino	57	1.1	0.9	1.1
3m	CH_3	ethyl 4-carboxylate	>74	3.8	0.9	4.2
3n	CH_3	3-(*N*-phenylamine)	25	1.3	0.9	1.5
3o	CH_3	2-benzyl	8.1	1.0	1.1	0.9
3p	CH_3	3-hydroxypyridine	>56	1.0	1.1	0.9
3q	CH_3	4-phenyl	30	2.5	0.7	3.7
3r	benzyl	2-C(O)NH-phenyl	>54	12.4	0.9	13.4
3s	CH_3	2-benzo	20	1.0	0.9	1.2
3t	CH_3	3-benzo	40	1.2	0.9	1.3

[a] Accurate IC_{50} values could not be obtained because of low solubilities of the compounds. The values indicate the maximum doses tested.

phenols subjected to these reaction conditions formed products that showed extra carbonyl stretches in the infrared spectra, suggesting that a base-catalyzed rearrangement of the methoxyphenol had taken place. Twenty substituted phenoxymethylquinoxalinones were synthesized and evaluated. Lack of substitution of the phenoxy moiety resulted in a nontoxic compound (**3a**) with a moderate Pgp score (Table 3). Activity was substantially improved in compounds with carbonyl substitutions of the phenoxy group. Thus, compounds **3h**, **3j**, **3k**, **3m**, and **3r** are substituted with ester, amide, or ketone moieties and demonstrate good antagonism of Pgp while having relatively low toxicity toward drug-sensitive cells. Additional active compounds include compounds **3c** and **3f**. While a cyano group (**3c**) can be viewed as a bioisostere to the carbonyl group, the *tert*-butyl group **(3f)** merely adds steric bulk and resulted in a compound more toxic than the parental phenoxymethylquinoxalinone. Notably, the replacement of the

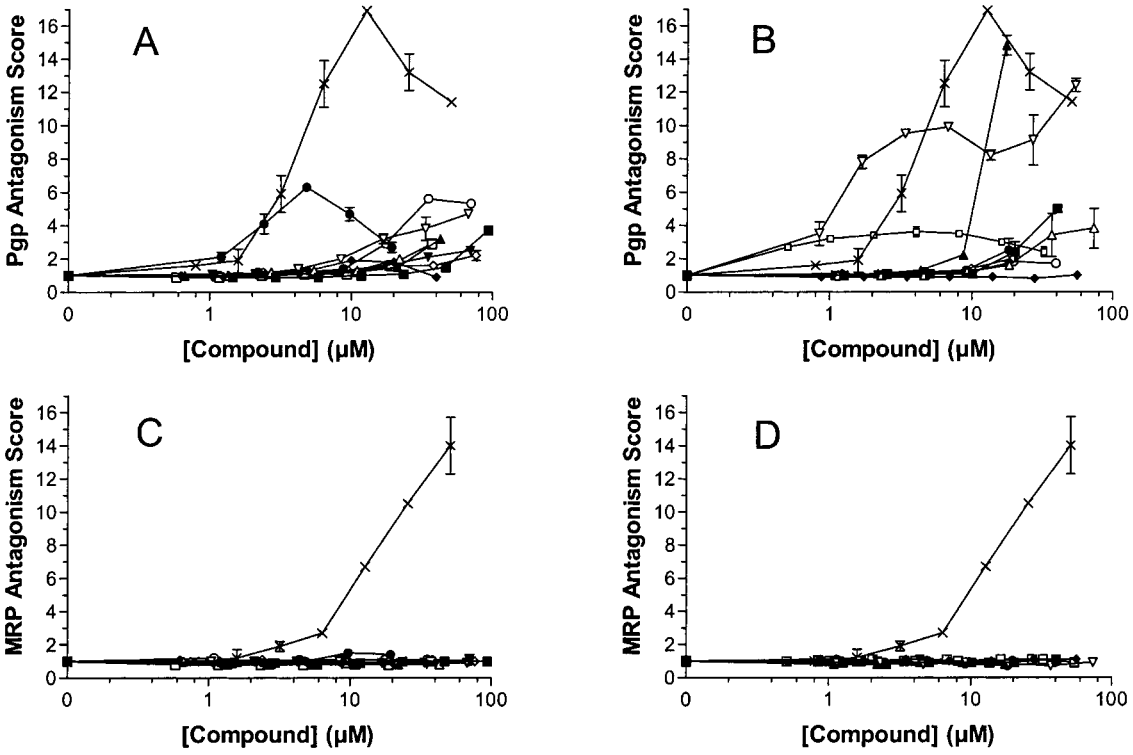


Figure 1. Dose−response curves for antagonism of Pgp and MRP1 by substituted phenoxymethylquinoxalinones. Pgp (A and B) or MRP1 (C and D) antagonism scores were calculated at the indicated concentration of test compound as described in the Experimental Section. No values beyond the IC_{50} of the compound toward MCF-7 cells are indicated. Compounds plotted in A and C include **3a** (■), **3b** (△), **3c** (▲), **3d** (▼), **3e** (◆), **3f** (●), **3g** (□), **3h** (▽), **3i** (◇), **3j** (○), verapamil (×). Compounds plotted in B and D include **3k** (■), **3l** (△), **3m** (▲), **3n** (▼), **3o** (◆), **3p** (●), **3q** (□), **3r** (▽), **3s** (◇), **3t** (○), verapamil (×).

N-methyl group of **3k** with an *N*-benzyl group of **3r** not only decreased cytotoxicity but also improved Pgp antagonism by more than 3-fold. Consequently, replacement of *N*-methyl with larger substituents throughout the entire sequence of substituted phenoxymethylquinoxalinones might yield larger antagonism scores. Of central importance to the goal of developing transporter-selective antagonists, none of the phenoxymethylquinoxalinones showed any antagonism toward MRP1, indicating clear pharmacophore differences between Pgp and MRP1.

The antagonism scores listed in Tables 2 and 3 were calculated at concentrations of the test compound that killed no more than a 25% of MCF-7 cells (in parallel experiments). While this cutoff is somewhat arbitrary, it simplifies the data significantly and makes a direct, compound-to-compound comparison possible. A more complete representation of the data relating to compounds **3a**−**3t** is given by Figure 1, where antagonism scores up to the IC_{50} (against MCF-7 cells) are plotted against the concentrations of test compounds. Results obtained with verapamil, the current "benchmark" MDR antagonist, demonstrate that this compound has little selectivity for Pgp versus MRP1. The plots also show that, while none of the substituted phenoxymethylquinoxalinones have higher peak Pgp scores than verapamil, compound **3r** is both more potent and more selective.

Conclusions

We began this project with the hypothesis that selective antagonism of Pgp versus MRP1 will be important to effectively clinically potentiate the toxicity of chemotherapeutic agents toward resistant tumors. Screening libraries of structurally diverse compounds resulted in the identification of several scaffolds on which to build potential MDR antagonists. The phenoxymethylquinoxalinone heterocycles were chosen for their amenability to synthesis and possession of several substitution sites. A qualitative SAR analysis of 20 substituted phenoxymethylquinoxalinones showed that the best combination of Pgp-selective antagonism and low intrinsic toxicity was demonstrated by compounds containing carbonyl substitutions of the phenoxy group, with maximal activity demonstrated by compound **3r**. This quinoxalinone possesses an *N*-phenylamide on the phenoxy moiety and a benzyl substitution on position 1. Further exploration of this platform to refine the structural requirements for selective Pgp and MRP1 antagonism is currently underway. Cell lines are widely used as model systems for the development of anticancer drugs and are highly reasonable systems for developing SARs but do not always adequately mimic clinical tumor responses. Therefore, the ultimate therapeutic utility of these agents will require evaluation through the established clinical trial process.

Experimental Section

Reagents and starting materials were generally obtained from Aldrich Chemical (Milwaukee, MN) and used as received unless otherwise noted. Solvents were either purchased as anhydrous or ACS grade and stored over 4 Å molecular sieves. Flash chromatography refers to the method of Still et al.[35] and generally used Selecto Scientific silica gel (32−63 μm). Thin-layer chromatography was performed on either BakerFlex IB-F or EM Science silica gel 60 F-254 plates (250-μm thick). Radial chromatography was performed on a Chromatotron model 7924T (Harrison Research, Palo Alto, CA) using 1-, 2-, or 4-mm

thick silica rotors. Melting points were determined in an open capillary on a MelTemp II melting point apparatus and are uncorrected. Infrared spectra were measured on a Nicolet Avatar 360 FT-IR; values are expressed in wavenumbers (cm^{-1}). ^1H and ^{13}C NMR spectra were obtained on a Bruker 200AM spectrometer. Chemical shifts are reported in ppm (δ) using tetramethylsilane (TMS) as reference and coupling constants (J) are reported in hertz. Mass spectra were obtained from Mass Consortium (San Diego, CA). Elemental analysis was performed by Midwest Microlab (Indianapolis, IN). All chemical yields are unoptimized and generally represent the result of a single experiment.

MCF-7 breast carcinoma cells and NCI/ADR cells, an MDR line that expresses Pgp,[36] were obtained from the Division of Cancer Treatment of the National Cancer Institute. Drs. Schneider and Cowan provided MCF-7/VP cells, which express MRP1 but not Pgp.[37] Libraries of compounds for screening were purchased from MicroSource Discovery Systems, Inc. (Gaylordsville, CT) and ChemBridge Corp. (San Diego, CA).

3-Benzyl-1*H*-quinoxalin-2-one (1a). 1,2-Phenylenediamine (1.00 g, 9.25 mmol) and phenylpyruvic acid (1.52 g, 9.25 mmol) were dissolved in EtOH (35 mL) and heated to reflux. After ~1.5 h, heat was removed and product spontaneously precipitated. Crude product was collected and recrystallized from EtOH to give 989 mg of off-white fibrous crystals (mp 178−179 °C, 4.19 mmol, 45.3%). FT-IR (KBr): 3425−3190 cm^{-1} (NH stretches), 3060−2975 (aromatic CH), 2900−2825 (aliphatic CH), 1660 (C═O), 1600 (C═N). ^1H NMR ($CDCl_3$): δ 12.39 (bs, 1H, NH), 7.835 (d, 1H, J = 8.1 Hz, H_8), 7.50−7.45 (m, 3H, $H_{5/6/7}$), 7.35−7.17 (m, 5H, phenyl), 4.30 (s, 2H, benzyl $-CH_2$). Anal. ($C_{15}H_{12}N_2O\cdot H_2O$) C, H; N: calcd, 11.02; found, 13.28.

3-Methyl-1*H*-quinoxalin-2-one (1b). 1,2-Phenylenediamine (1.00 g, 9.25 mmol) and pyruvic acid (0.643 mL, 9.25 mmol) were dissolved in EtOH (35 mL) and heated to reflux. After ~1.5 h, heat was removed and product spontaneously precipitated after cooling to 0 °C. Crude product was collected and recrystallized from EtOH to give 1.001 g of yellow needles (mp 241−243 °C dec, 6.25 mmol, 67.6%). FT-IR (KBr): 3450−3100 cm^{-1} (NH stretches), 3000−2950 (aromatic CH), 2900−2825 (aliphatic CH), 1660 (C═O), 1600 (C═N). ^1H NMR ($CDCl_3$): δ 7.82−7.79 (m, 1H, H_8), 7.51−7.47 (m, 1H, H_5), 7.36−7.31 (m, 2H, $H_{6/7}$), 2.63 (s, 3H, CH_3). Anal. ($C_9H_8N_2O$) C, H, N.

1,3-Dimethyl-1*H*-quinoxalin-2-one (1c). *N*-Methyl-1,2-phenylenediamine (6.68 g, 54.7 mmol) and pyruvic acid (3.8 mL, 54.7 mmol) were dissolved in EtOH (230 mL) and warmed to ~50 °C. At this point, the color changed dramatically from a dark, cloudy brown to clear orange. Ethanol was evaporated and the crude product was filtered through a silica bed with CH_2Cl_2/EtOAc (1:1) to give 7.36 g of product as a yellow solid (mp 75−77 °C, 42.3 mmol, 77.2%). FT-IR (KBr): 3075−2975 cm^{-1} (aromatic CH), 2950−2850 (aliphatic CH), 1650 (C═O), 1600 (C═N). ^1H NMR ($CDCl_3$): δ 7.80 (d, 1H, J = 7.9 Hz, H_8), 7.52 (t, 1H, J = 7.8 Hz, H_5), 7.33 (m, 2H, $H_{6/7}$), 3.70 (s, 3H, NCH_3), 2.59 (s, 3H, CH_3). Anal. ($C_{10}H_{10}N_2O$) C, H, N.

3-Benzyl-1-methyl-1*H*-quinoxalin-2-one (1d). *N*-Methyl-1,2-phenylenediamine (1.00 g, 8.18 mmol) and phenylpyruvic acid (1.34 g, 8.18 mmol) were dissolved in EtOH (31 mL) and the reaction was refluxed for 1.5 h. Ethanol was evaporated and the crude product was chromatographed through a Biotage flash chromatography silica cartridge with 40% EtOAc/Hex (at 10 mL/min) to give 400 mg of product as a light yellow solid (mp 72−74 °C, 1.60 mmol, 19.5%). FT-IR (KBr): 3075−3025 cm^{-1} (aromatic CH), 2950−2900 (aliphatic CH), 1650 (C═O), 1600 (C═N). ^1H NMR ($CDCl_3$): δ 7.83 (dd, 1H, J = 1.2, 8.0 Hz, H_8), 7.46 (d, 3H, J = 7.3 Hz, $H_{5/6/7}$), 7.30−7.19 (m, 5H, phenyl), 4.25 (s, 2H, benzyl CH_2), 3.55 (s, 3H, NCH_3). Anal. ($C_{16}H_{14}N_2O$) H, N; C: calcd, 76.78; found, 76.10.

1-Benzyl-3-methyl-1*H*-quinoxalin-2-one (1e). 3-Methylquinoxalinone (**1b**; 511 mg, 3.19 mmol) was stirred with benzyl chloride (0.367 mL, 3.19 mmol) and potassium carbonate (904 mg, 6.54 mmol) in DMF (10 mL) overnight. Reaction mixture was poured into 30 mL of water and extracted with EtOAc (3 × 30 mL). Combined EtOAc extracts were washed with brine (30 mL) and dried over $MgSO_4$. Recrystallization of the crude product from EtOH (0 °C) gave 384 mg of product as a light yellow solid. Purification of the concentrated mother liquor by radial chromatography (silica, 15% EtOAc/Hex) gave another 144 mg of product along with 110 mg (0.436 mmol, 13.7%) of a by-product (**2-benzyloxy-3-methyl-1,2-dihydroquinoxaline, 4**). Total yield of product was 528 mg (mp 90−91 °C, 2.11 mmol, 66.1%). FT-IR (KBr): 3033 cm^{-1} (aromatic CH), 2918 (aliphatic CH), 1649 (C═O), 1601 (C═N). ^1H NMR ($CDCl_3$): δ 7.82 (dd, 1H, J = 1.3, 7.7 Hz, H_8), 7.44−7.22 (m, 8H, $H_{5/6/7}$ + phenyl), 5.50 (s, 2H, NCH_2), 2.67 (s, 3H, CH_3). Anal. ($C_{16}H_{14}N_2O$) C, H, N. **4:** FT-IR (KBr): 3115−3029 cm^{-1} (aromatic CH), 2955−2889 (aliphatic CH), 1609−1581 (C═C, C═N). ^1H NMR ($CDCl_3$): δ 7.95 (d, 1H, J = 6.5 Hz, H_8), 7.93 (m, 1H, H_5), 7.62−7.38 (m, 7H, $H_{6/7}$, phenyl), 5.57 (s, 2H, CH_2O), 2.68 (s, 3H, CH_3). Anal. ($C_{16}H_{14}N_2O$) C, H, N.

3-Bromomethyl-1*H*-1-methylquinoxalin-2-one (2). Dimethylquinoxalinone (**1c**; 133 mg, 0.763 mmol) was dissolved in CCl_4 (10 mL); *N*-bromosuccinimide (144 mg, 0.809 mmol) and benzoyl peroxide (0.4 mg, 0.002 mmol) were added and the mixture was heated to reflux. After 10.5 h, TLC (silica, 40% EtOAc/Hex) showed the reaction to be complete. Reaction was cooled to room temperature and filtered through a plug of silica with 40% EtOAc/Hex. Purification of the crude product by radial chromatography (silica, 40% EtOAc/Hex) gave 71 mg of pure product (mp 180−182 °C dec, 0.28 mmol, 49%). FT-IR (KBr): 3045 cm^{-1} (aromatic CH), 2975 (aliphatic CH), 1645 (C═O), 1598 (C═N). ^1H NMR ($CDCl_3$): δ 7.87 (d, 1H, J = 8.0 Hz, H_8), 7.61 (t, 1H, J = 7.3 Hz, H_5), 7.41−7.33 (m, 2H, $H_{6/7}$), 4.66 (s, 2H, CH_2Br), 3.74 (s, 3H, NCH_3). ^{13}C NMR ($CDCl_3$): δ 155.2 (2), 153.9 (3), 134.0 (10), 132.7 (9), 131.4 (6), 130.7 (7), 124.2 (5), 114.0 (8), 29.5 (CH_3).

1-Benzyl-3-bromomethyl-1*H*-quinoxalin-2-one (2a). 1-Benzyl-3-methyl-1*H*-quinoxalin-2-one (**1e**; 3.20 g, 12.8 mmol) was dissolved in CCl_4 (200 mL); *N*-bromosuccinimide (2.41 g, 13.5 mmol) and benzoyl peroxide (6.2 mg, 0.026 mmol) were added and the mixture was heated to reflux. After 16.5 h, TLC (silica, 25% EtOAc/Hex) showed the reaction to be complete. Reaction was cooled to room temperature, CCl_4 was removed by evaporation, and the crude mixture was purified by flash chromatography (silica, 25% EtOAc/Hex) to give 1.41 g of pure product as a pale yellow solid (mp 131−133 °C dec, 4.28 mmol, 33.5%) along with 1.68 g (6.71 mmol, 52.4%) of unreacted starting material. FT-IR (CH_2Cl_2): 3061 cm^{-1} (aromatic CH), 2979 (aliphatic CH), 1660 (C═O), 1603 (C═N). ^1H NMR ($CDCl_3$): δ 7.88 (d, 1H, J = 7.8 Hz, H_8), 7.49−7.31 (m, 8H, phenyl), 5.54 (s, 2H, benzyl CH_2), 4.73 (s, 2H, CH_2Br).

General Condensation Procedure for Substituted 1-Methyl-3-phenoxymethyl-1*H*-quinoxalin-2-ones 3. Bromomethylquinoxalinone (**2**; 100 mg, 0.395 mmol) and a specific phenol (0.395 mmol) were dissolved in $CHCl_3$ (10 mL). A solution of NaOH (23.7 mg, 0.593 mmol) and benzyltriethylammonium chloride (9 mg, 0.040 mmol) in H_2O (10 mL) was added and the mixture stirred rapidly while heating to 50 °C. TLC (silica, 40% EtOAc/Hex or 10% EtOAc/CH_2Cl_2) was used to monitor the reaction progress. Workup consisted of cooling to room temperature, separating layers, extracting the aqueous layer with fresh $CHCl_3$ (2 × 10 mL), washing the combined chloroform extracts with brine (10 mL), and drying over Na_2SO_4. Evaporation of the solvent, followed by filtration through a plug of silica with 40% EtOAc/Hex, gave the crude product; purification by radial chromatography (silica, 40% EtOAc/Hex) gave the pure product.

1-Methyl-3-phenoxymethyl-1*H*-quinoxalin-2-one (3a): pale yellow film (41%). FT-IR (KBr): 3059 cm^{-1} (aromatic CH), 2933 (aliphatic CH), 1647 (C═O), 1603 (C═N). ^1H NMR ($CDCl_3$): δ 7.95 (d, 1H, J = 7.9 Hz, H_8), 7.59 (m, 1H, H_5), 7.39−7.26 (m, 4H, $H_{6/7}$ + phenolic H_b), 7.08 (d, 2H, J = 7.8 Hz, phenolic H_a), 6.97 (m, 1H, phenolic H_c), 5.38 (s, 2H, CH_2O), 3.74 (s, 3H, NCH_3). Anal. ($C_{16}H_{14}N_2O_2\cdot 0.5H_2O$) C, H, N.

3-(4-Fluorophenoxymethyl)-1-methyl-1*H*-quinoxalin-2-one (3b): clear film (14%). FT-IR (CCl_4): 3063 cm^{-1} (aromatic CH), 2922−2852 (aliphatic CH), 1651 (C═O), 1601 (C═N). ^1H

NMR ($CDCl_3$): δ 7.97−7.93 (m, 1H, H_8), 7.68−7.52 (m, 3H, $H_5 + H_\beta$), 7.38−7.34 (m, 2H, $H_{6/7}$), 7.01−6.97 (m, 2H, H_α), 5.33 (s, 2H, CH_2O), 3.75 (s, 3H, NCH_3). Anal. ($C_{16}H_{13}N_2O_2F \cdot 2.5H_2O$) C; H: calcd, 5.51; found, 4.03; N: calcd, 8.51; found, 9.71.

3-(4-Cyanophenoxymethyl)-1-methyl-1*H*-quinoxalin-2-one (3c): pale yellow solid (mp 133 °C dec, 17.4%). FT-IR (KBr): 3068 cm^{-1} (aromatic CH), 2923 (aliphatic CH), 2222 (C≡N), 1654 (C=O), 1606 (C=N). ^1H NMR ($CDCl_3$): δ 7.90 (d, 1H, J = 7.1 Hz, H_8), 7.59 (d, 3H, H_5 + phenolic H_b), 7.39 (t, 2H, J = 6.8 Hz, $H_{6/7}$), 7.12 (d, 2H, J = 8.8 Hz, phenolic H_a), 5.42 (s, 2H, CH_2O), 3.76 (s, 3H, NCH_3). Anal. ($C_{17}H_{13}N_3O_2 \cdot H_2O$) C, H; N: calcd, 13.58; found, 12.99.

1-Methyl-3-(2,3,4,5,6-pentafluorophenoxymethyl)-1*H*-quinoxalin-2-one (3d): pale yellow solid (mp 128−130 °C, 27%). FT-IR (KBr): 3059 cm^{-1} (aromatic CH), 2933 (aliphatic CH), 1647 (C=O), 1603 (C=N). ^1H NMR ($CDCl_3$): δ 7.88 (d, 1H, J = 7.9 Hz, H_8), 7.60 (m, 1H, H_5), 7.38 (m, 2H, $H_{6/7}$), 5.47 (s, 2H, CH_2O), 3.73 (s, 3H, NCH_3). Anal. ($C_{16}H_9N_2O_2F_5$) C, H, N; F: calcd, 26.66; found, 23.20.

3-(4-Benzyloxyphenoxymethyl)-1-methyl-1*H*-quinoxalin-2-one (3e): yellow film (9%). FT-IR (KBr): 3033 cm^{-1} (aromatic CH), 2926 (aliphatic CH), 1656 (C=O), 1603 (C=N). ^1H NMR ($CDCl_3$): δ 7.95 (d, 1H, J = 3.9 Hz, H_8), 7.63−7.55 (m, 1H, H_5), 7.42−7.22 (m, 7H, $H_{6/7}$ + benzyl), 7.07−6.83 (m, 4H, $H_{2'/3'/5'/6'}$), 5.32 (s, 2H, quinoxalines CH_2O), 5.01 (s, 2H, benzyl CH_2O), 3.74 (s, 3H, NCH_3). Anal. ($C_{23}H_{20}N_2O_3 \cdot 2H_2O$) C, N; H: calcd, 5.92; found, 5.18.

3-(3-*tert*-Butylphenoxymethyl)-1-methyl-1*H*-quinoxalin-2-one (3f): yellow film (6%). FT-IR (KBr): 3077 cm^{-1} (aromatic CH), 2963−2868 (aliphatic CH), 1658 (C=O), 1601 (C=N). ^1H NMR ($CDCl_3$): δ 8.32 (d, 1H, J = 6.3 Hz, NH), 7.88−6.98 (m, 8H, aromatic protons), 6.88 (d, 1H, J = 8.1 Hz, C=CH−O), 5.59 (s, 1H, CH_2O), 3.77 (s, 3H, NCH_3), 1.26 (s, 9H, $[CH_3]_3$).

3-(3,4-Dichlorophenoxymethyl)-1-methyl-1*H*-quinoxalin-2-one (3g): orange solid (mp 105−107 °C, 66%). FT-IR (KBr): 3084 cm^{-1} (aromatic CH), 2921 (aliphatic CH), 1658 (C=O), 1608 (C=N). ^1H NMR ($CDCl_3$): δ 7.95 (d, 1H, J = 7.9 Hz, H_8), 7.63−7.59 (m, 1H, H_5), 7.44−7.17 (m, 2H, $H_{6/7}$), 6.98−6.95 (m, 2H, phenolic $H_{5/6}$), 6.72 (d, 1H, J = 7.7 Hz, phenolic H_2), 5.34 (s, 2H, CH_2O), 3.76 (s, 3H, NCH_3). Anal. ($C_{16}H_{12}N_2O_2Cl_2 \cdot H_2O$) N; C: calcd, 54.41; found, 54.87; H: calcd, 4.00; found, 3.48; Cl: calcd, 20.07; found, 23.44.

3-(4-Benzoylphenoxymethyl)-1-methyl-1*H*-quinoxalin-2-one (3h): cream-colored solid (mp 145−147 °C, 45%). FT-IR (KBr): 3053 cm^{-1} (aromatic CH), 2903 (aliphatic CH), 1655 (C=O), 1600 (C=N). ^1H NMR ($CDCl_3$): δ 7.93 (dd, 1H, J = 1.1, 8.0 Hz, H_8), 7.85−7.73 (m, 4H, $H_{3''/5''/2''/6''}$), 7.62−7.42 (m, 4H, $H_{5/3''/4''/5''}$), 7.37 (d, 2H, J = 8.0 Hz, $H_{6/7}$), 7.13 (d, 2H, J = 8.9 Hz, $H_{2'/6'}$) 5.46 (s, 2H, CH_2O), 3.76 (s, 3H, NCH_3). Anal. ($C_{23}H_{18}N_2O_3 \cdot 2H_2O$) C, N; H: calcd, 5.46; found, 4.73.

1-Methyl-3-(3-methyl-4-nitrophenoxymethyl)-1*H*-quinoxalin-2-one (3i): off-white solid (mp 140−143 °C dec, 13%). FT-IR (KBr): 3088 cm^{-1} (aromatic CH), 2930 (aliphatic CH), 1661 (C=O), 1607 (C=N), 1509 (assym. NO_2), 1341 (symm. NO_2). ^1H NMR ($CDCl_3$): δ 8.08 (d, 1H, J = 9.8 Hz, $H_{5'}$), 7.89 (m, 1H, H_8), 7.60 (m, 1H, H_5), 7.43−7.35 (m, 2H, $H_{6/7}$), 6.94 (bs, 2H, $H_{2'/6'}$), 5.43 (s, 2H, CH_2O), 3.76 (s, 3H, NCH_3), 2.63 (s, 3H, CH_3). Anal. ($C_{17}H_{15}N_3O_4 \cdot H_2O$) C, N; H: calcd, 4.99; found, 4.46.

3-Methoxy-4-(4-methyl-3-oxo-3,4-dihydroquinoxalin-2-ylmethoxy)benzoic acid methyl ester (3j): yellow film (7.1%). FT-IR (KBr): 3087 cm^{-1} (aromatic CH), 2921−2850 (aliphatic CH), 1715 (ester C=O), 1653 (C=O), 1602 (C=N). ^1H NMR ($CDCl_3$): δ 7.92 (d, 1H, J = 7.7 Hz, H_8), 7.64−7.33 (m, 5H, $H_{5/6/7}$ + $H_{2'/6'}$), 6.99 (d, 1H, J = 8.4 Hz, $H_{5'}$), 5.49 (s, 2H, CH_2O), 3.97 (s, 3H, CO_2CH_3), 3.89 (s, 3H, OCH_3), 3.75 (s, 3H, NCH_3). Anal. ($C_{19}H_{18}N_2O_5 \cdot 2.5H_2O$) C, N; H: calcd, 5.80; found, 5.08.

2-(4-Methyl-3-oxo-3,4-dihydroquinoxalin-2-ylmethoxy)-*N*-phenylbenzamide (3k): yellow solid (mp 148 °C dec, 43%). FT-IR (KBr): 3315 cm^{-1} (amide NH), 3040 (aromatic CH), 2916 (aliphatic CH), 1656 (amide C=O), 1643 (C=O), 1598 (C=N). ^1H NMR ($CDCl_3$): δ 8.31 (dd, 1H, J = 1.8, 7.8 Hz, NH), 7.80−7.05 (m, 13H, aromatic protons), 5.59 (s, 2H, CH_2O), 3.76 (s, 3H, NCH_3). Anal. ($C_{23}H_{19}N_3O_3 \cdot 1.5H_2O$) C, N; H: calcd, 5.38; found, 4.68.

3-(3-Dimethylaminophenoxymethyl)-1-methyl-1*H*-quinoxalin-2-one (3l): orange-brown film (11%). FT-IR ($CDCl_3$): 3079 cm^{-1} (aromatic CH), 2924−2802 (aliphatic CH), 1657 (C=O), 1604 (C=N). ^1H NMR ($CDCl_3$): δ 7.95 (d, 1H, J = 7.7 Hz, H_8), 7.57 (d, 1H, J = 7.2 Hz, H_5), 7.40−7.33 (m, 2H, $H_{6/7}$), 7.14 (m, 1H, $H_{2'}$), 6.53 (m, 3H, $H_{4'/5'/6'}$), 5.37 (s, 2H, CH_2O), 3.74 (s, 3H, NCH_3), 2.94 (s, 6H, $N(CH_3)_2$). Anal. ($C_{18}H_{19}N_3O_2 \cdot 1.5H_2O$) H; C: calcd, 64.27; found, 64.96; N: calcd, 12.49; found, 10.08.

4-(4-Methyl-3-oxo-3,4-dihydroquinoxalin-2-ylmethoxy)-benzoic acid ethyl ester (3m): light yellow solid (mp 152 °C dec, 39%). FT-IR (KBr): 3071 cm^{-1} (aromatic CH), 2983−2910 (aliphatic CH), 1710 (ester C=O), 1656 (C=O), 1602 (C=N). ^1H NMR ($CDCl_3$): δ 8.02−7.90 (m, 2H, $H_{3'/5'}$), 7.61−7.56 (m, 2H, $H_{5/8}$), 7.41−7.33 (m, 2H, $H_{6/7}$), 7.11−7.00 (m, 2H, $H_{2'/6'}$), 5.46 (d, 2H, J = 16 Hz, CH_2O), 4.34 (q, 2H, J = 7,1 Hz, ester CH_2), 3.75 (s, 3H, NCH_3), 1.37 (t, 3H, J = 7.1 Hz, ester CH_3). Anal. ($C_{19}H_{18}N_2O_4 \cdot 1.75H_2O$) C, N; H: calcd, 5.86; found, 4.90.

1-Methyl-3-(3-phenylaminophenoxymethyl)-1*H*-quinoxalin-2-one (3n): yellow film (17%). FT-IR ($CDCl_3$): 3345 cm^{-1} (NH), 3050 (aromatic CH), 2923 (aliphatic CH), 1653 (C=O), 1595 (C=N). ^1H NMR ($CDCl_3$): δ 7.95 (d, 1H, J = 7.7 Hz, H_8), 7.58 (t, 1H, J = 7.7 Hz, H_5), 7.41−7.33 (m, 2H, $H_{6/7}$), 7.22−6.62 (m, 9H, phenyl), 5.34 (s, 2H, CH_2O), 3.73 (s, 3H, NCH_3). Anal. ($C_{22}H_{19}N_3O_2 \cdot 3H_2O$) C; H: calcd, 6.12; found, 5.35; N: calcd, 10.21; found, 9.24.

3-(2-Benzylphenoxymethyl)-1-methyl-1*H*-quinoxalin-2-one (3o): yellow film (6%). FT-IR ($CDCl_3$): 3026 cm^{-1} (aromatic CH), 2925 (aliphatic CH), 1655 (C=O), 1603 (C=N). ^1H NMR ($CDCl_3$): δ 8.07 (d, 1H, J = 8.6 Hz, H_8), 7.66−7.32 (m, 5H, $H_{5/6/7/3'/5'}$), 7.16 (bs, 5H, phenyl), 7.08−6.95 (m, 2H, $H_{4'/6'}$), 5.22 (s, 2H, CH_2O), 3.80 (s, 2H, benzyl CH_2), 3.75 (s, 3H, NCH_3).

1-Methyl-3-(pyridin-3-yloxymethyl)-1*H*-quinoxalin-2-one (3p): off-white film (3%). FT-IR ($CDCl_3$): 3070 cm^{-1} (aromatic CH), 2919−2850 (aliphatic CH), 1651 (C=O), 1602 (C=N). ^1H NMR ($CDCl_3$): δ 7.88 (d, 1H, J = 7.9 Hz, H_8), 7.60 (m, 1H, H_5), 7.42−7.19 (m, 6H, H_6 + pyridyl), 5.45 (s, 2H, CH_2O), 3.75 (s, 3H, NCH_3). Anal. ($C_{15}H_{13}N_3O_2$) C: calcd, 67.40; found, 45.68; H: calcd, 4.90; found, 3.95; N: calcd, 15.72; found, 7.48.

3-(Biphenyl-4-yloxymethyl)-1-methyl-1*H*-quinoxalin-2-one (3q): pale yellow film (27%). FT-IR ($CDCl_3$): 3031 cm^{-1} (aromatic CH), 2934 (aliphatic CH), 1654 (C=O), 1604 (C=N). ^1H NMR ($CDCl_3$): δ 7.96 (d, 1H, J = 6.0 Hz, H_8), 7.81−7.09 (m, 12H, phenyl), 5.44 (d, 2H, J = 11.5 Hz, CH_2O), 3.74 (s, 3H, NCH_3). Anal. ($C_{22}H_{18}N_2O_2 \cdot 3H_2O$) C, N; H: calcd, 6.10; found, 4.65.

2-(4-Benzyl-3-oxo-3,4-dihydroquinoxalin-2-ylmethoxy)-*N*-phenylbenzamide (3r): condensed as in **3k**, but using **1-benzyl-3-bromomethyl-1*H*-quinoxalin-2-one (2a)** as starting material to give product as a yellow solid (mp 167−169 °C, 70%). FT-IR (KBr): 3446−3318 cm^{-1} (amide NH), 3064−3060 (aromatic CH), 2924−2835 (aliphatic CH), 1655 (amide C=O), 1599 (C=N). ^1H NMR ($CDCl_3$): δ 10.79 (bs, 1H, NH), 8.34 (dd, 1H, J = 1.8, 7.9 Hz, $H_{3'}$), 7.79 (d, 2H, J = 7.6 Hz, $H_{8/5'}$), 7.67 (d, 1H, J = 7.0 Hz, phenyl), 7.52−7.14 (m, 14H, phenyl), 5.66 (s, 2H, benzyl CH_2), 5.54 (s, 2H, CH_2O). Anal. ($C_{29}H_{23}N_3O_3$) C, H, N.

1-Methyl-3-(naphthalen-1-yloxymethyl)-1*H*-quinoxalin-2-one (3s): light yellow film (12%). FT-IR ($CDCl_3$): 3053 cm^{-1} (aromatic CH), 2936 (aliphatic CH), 1653 (amide C=O), 1603 (C=N). ^1H NMR ($CDCl_3$): δ 8.49−8.44 (m, 1H, $H_{8'}$), 7.94 (d, 1H, J = 7.7 Hz, H_8), 7.82−7.78 (m, 1H, H_5), 7.64−7.32 (m, 7H, phenyl), 6.98 (d, 1H, J = 7.3 Hz, $H_{2'}$), 5.53 (s, 2H, CH_2O), 3.79 (s, 3H, NCH_3). Anal. ($C_{20}H_{16}N_2O_2 \cdot 1.25H_2O$) C, N; H: calcd, 5.50; found, 4.89.

1-Methyl-3-(naphthalen-2-yloxymethyl)-1*H*-quinoxalin-2-one (3t): light yellow film (17%). FT-IR ($CDCl_3$): 3057 cm^{-1} (aromatic CH), 2936 (aliphatic CH), 1653 (amide C=O), 1602 (C=N). ^1H NMR ($CDCl_3$): δ 7.97−7.89 (m, 1H, H_8), 7.79−7.33

(m, 10H, phenyl), 5.49 (s, 2H, CH_2O), 3.75 (s, 3H, NCH_3). Anal. ($C_{20}H_{16}N_2O_2 \cdot 1.5H_2O$) C, N; H: calcd, 5.58; found, 4.83.

Assay of Cytotoxicity and Reversal of MDR. To test for reversal of Pgp-mediated MDR, NCI/ADR cells were placed into 96-well tissue culture plates at approximately 15% confluency and were allowed to attach and recover for 24 h. The cells were then treated with varying concentrations (as allowed by solubility) of the test compound in the presence of 0 or 50 nM vinblastine for 48 h according to previously described procedures.[38,39] After 48 h, cell survival was assayed using the sulforhodamine B (SRB) binding assay.[40] The percentage of cells killed was calculated as the percentage decrease in SRB binding as compared with control cultures and was taken from the mean of the absorbance measurements of three equally treated wells. Reversal of MDR is indicated if the compound enhanced the toxicity of vinblastine toward the NCI/ADR cells. The reversal index (Pgp Antagonism Score) was calculated as the percentage of surviving NCI/ADR cells in the absence of vinblastine/the percentage of surviving NCI/ADR cells in the presence of vinblastine. Control cultures included equivalent amounts of ethanol (as the solvent control), which does not modulate the growth or drug sensitivity of these cells at the doses used in these studies. Inhibition of Pgp was manifested as the ability of the compound to potentiate the cytotoxicity of vinblastine toward the NCI/ADR cells. To assess the toxicity of the compounds toward drug-sensitive cells, the effects of the test modulators on the growth of drug-sensitive MCF-7 cells were determined by the same methods. To test for reversal of MRP1-mediated MDR, MCF-7/VP cells were placed into 96-well tissue culture plates at approximately 15% confluency and were allowed to attach and recover for 24 h. The cells were then treated with varying concentrations (as allowed by solubility) of the test compound in the presence of 0 or 1 nM vincristine for 48 h as above. After 48 h, cell survival was assayed using the SRB binding assay. Reversal of MDR is indicated if the compound enhanced the toxicity of vincristine toward the MCF-7/VP cells. The reversal index (MRP1 antagonism score) was calculated as the percentage of surviving MCF-7/VP cells in the absence of vincristine/the percentage of surviving MCF-7/VP cells in the presence of vincristine. Control cultures included equivalent amounts of ethanol (as the solvent control), which does not modulate the growth or drug sensitivity of these cells at the doses used in these studies. Inhibition of MRP1 was manifested as the ability of the compound to potentiate the cytotoxicity of vincristine toward the MCF-7/VP cells.

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