# II. Synthesis and Biological Evaluation of Some Bioisosteres and Congeners of the Antitumor Agent, 2-{4-[(7-Chloro-2-quinoxalinyl)oxy]phenoxy}propionic Acid (XK469)

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XK469 (**1**) is among the most highly and broadly active antitumor agents to have been evaluated in our laboratories. Subsequent developmental studies led to the entry of (*R*)-(+) **1** (NSC 698215) into phase 1 clinical trials (NIH UO1-CA62487). The antitumor mechanism of action of **1** remains to be elucidated, which has prompted a sustained effort to elaborate a pharmacophoric pattern of **1**. The present study focused on a strategy of synthesis and biological evaluation of topologically based, bioisosteric replacements of the quinoxaline moiety in the lead compound (**1**) by quinazoline (**4a−d**), 1,2,4-benzotriazine (**12a−18b**), and quinoline (**21a−g**) ring systems. The synthetic approach to each of the bioisosteres of **1** utilized the methodology developed in previous work (see Hazeldine, S. T.; Polin, L.; Kushner, J.; Paluch, J.; White, K.; Edelstein, M.; Palomino, E.; Corbett, T. H.; Horwitz, J. P. Design, Synthesis, and Biological Evaluation of Analogues of the Antitumor Agent 2-{4-[(7-Chloro-2-quinoxalinyl)oxy]phenoxy}propionic acid (XK469). *J. Med. Chem.* **2001**, *44*, 1758−1776.), which is extended to the procurement of the benzoxazole (**23a**,**b**), benzthiazole (**23c**,**d**), pyridine (**25a**,**b**), and pyrazine (**27**) congeners of **1**. Only quinoline analogues, bearing a 7-halo (**21a**,**b**,**d**,**e**) or a 7-methoxy substituent (**21g**), showed antitumor activities (Br > Cl > CH$_3$O > F ≈ I), at levels comparable to or greater than the range of activities manifested by **1** and corresponding analogues. At high individual dosages, the (*S*)-(−) enantiomers of **1** and **21b**,**d** all produce a reversible slowing of nerve−conduction velocity in the mice, the onset of which is characterized by a distinctive dysfunction of the hind legs, causing uncoordinated movements. The condition resolves within 5−10 min. However, at higher dosages, which approach a lethal level, the behavior extended to the front legs, lasting from 20 min to 1 h. By contrast, the (*R*)-(+) forms of these same agents did not induce the phenomenon of slowing of nerve−conduction velocity.

## Introduction

Compound **1** (XK469) (Figure 1) is among the most highly and broadly active antitumor agents to have been evaluated in our laboratories.[1−4] Subsequent developmental studies involved the National Cancer Institute (NCI), the DuPont Pharmaceuticals Company (DPC), and our laboratories. The collaborative effort led only recently to the entry of (*R*)-(+) **1** (NSC 698215) into phase 1 clinical trials (NIH UO1-CA62487). The mechanism of action of **1** remains unknown, though several common mechanisms of anticancer drug action have been excluded.[1a] In the absence of a validated molecular target, efforts were initiated to develop a pharmacophoric pattern,[1a,b] to delineate the supramolecular interactions of **1** with a putative biological target. It was found[1] that changes in the nature and location of substituents in ring A of **1** induced significant differences in both the in vitro and the in vivo activities of the 2-{4-[(2-quinoxalinyl)oxy]phenoxy}propionic acids. Thus, the 7-halogen derivatives proved to be the most active compounds exhibiting an order of relative antitumor activity of F ≈ Cl ≈ Br > I, whereas the 3-, 5-,
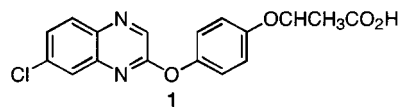


**Figure 1.**

6-, and 8-regioisomers of **1** were essentially all inactive. All alterations of the hydroquinone (1,4) connector linkage in **1** resulted in inactive compounds. By contrast, simple alkyl ester and amide derivatives of **1** showed only minor reductions in activity relative to that of the free acid. The second phase of this effort to elaborate a pharmacophore of **1** focused on a classical strategy of systematic generation and biological evaluation of topologically based, bioisosteric replacement of the quinoxaline moiety in the lead compound by quinazoline, 1,2,4-benzotriazine, and quinoline ring systems, which was extended to the congeneric benzoxazole, benzthiazole, pyrazine, and pyridine derivatives.
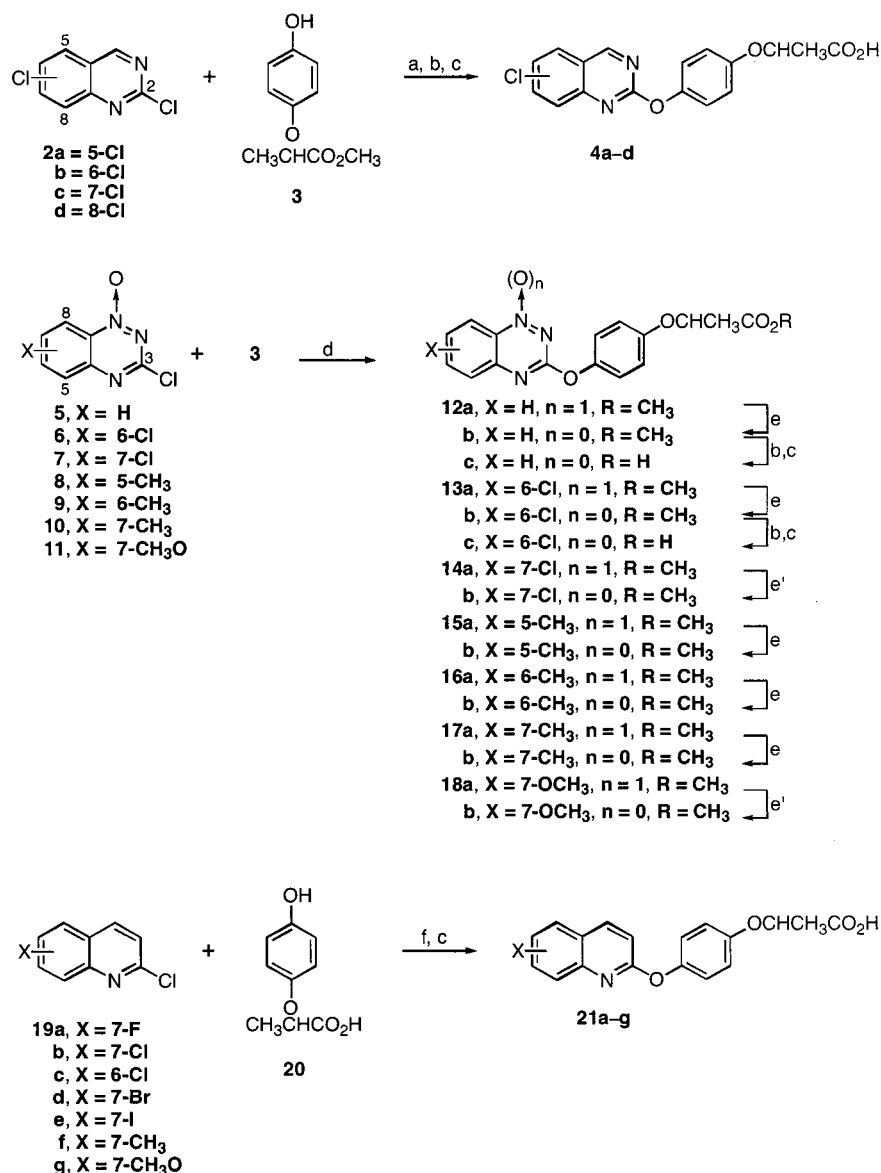
## Chemistry

**Quinazolines.** There is considerable patent literature that describes the preparation of a wide spectrum of heterocyclic, ether type, phenoxy fatty acids, including the bioisosteric structures listed above, which comprise an important group of herbicides for the selective control of weed grasses. The synthetic methodology leading to these agents, which was utilized extensively in our prior

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**Scheme 1**[a]



**2a** = 5-Cl
**b** = 6-Cl
**c** = 7-Cl
**d** = 8-Cl

**4a–d**

**5**, X = H
**6**, X = 6-Cl
**7**, X = 7-Cl
**8**, X = 5-CH$_3$
**9**, X = 6-CH$_3$
**10**, X = 7-CH$_3$
**11**, X = 7-CH$_3$O

**12a**, X = H, n = 1, R = CH$_3$
**b**, X = H, n = 0, R = CH$_3$
**c**, X = H, n = 0, R = H
**13a**, X = 6-Cl, n = 1, R = CH$_3$
**b**, X = 6-Cl, n = 0, R = CH$_3$
**c**, X = 6-Cl, n = 0, R = H
**14a**, X = 7-Cl, n = 1, R = CH$_3$
**b**, X = 7-Cl, n = 0, R = CH$_3$
**15a**, X = 5-CH$_3$, n = 1, R = CH$_3$
**b**, X = 5-CH$_3$, n = 0, R = CH$_3$
**16a**, X = 6-CH$_3$, n = 1, R = CH$_3$
**b**, X = 6-CH$_3$, n = 0, R = CH$_3$
**17a**, X = 7-CH$_3$, n = 1, R = CH$_3$
**b**, X = 7-CH$_3$, n = 0, R = CH$_3$
**18a**, X = 7-OCH$_3$, n = 1, R = CH$_3$
**b**, X = 7-OCH$_3$, n = 0, R = CH$_3$

**19a**, X = 7-F
**b**, X = 7-Cl
**c**, X = 6-Cl
**d**, X = 7-Br
**e**, X = 7-I
**f**, X = 7-CH$_3$
**g**, X = 7-CH$_3$O

**20**

**21a–g**

[a] Reagents: (a) K$_2$CO$_3$/CH$_3$CN; (b) aqueous NaOH/THF; (c) aqueous HCl; (d) K$_2$CO$_3$/butanone; (e) HCO$_2$NH$_4$/Pd-C/CH$_3$OH; (e) H$_2$/Pd-C/CH$_3$OH; (f) K$_2$CO$_3$/DMF.

work[1a] and is quite straightforward, is highlighted in Schemes 1 and 2. Thus, the etherification of methyl 2-(4-hydroxyphenoxy)propionate (**3**) with each of the 2,5-, 2,6-, 2,7-, and 2,8-dichloroquinazolines (Scheme 1, **2a–d**) was achieved with K$_2$CO$_3$ in acetonitrile[2] to provide the corresponding methyl 2-{4-[(5-, 6-, 7-, and 8-chloro-2-quinazolinyl)oxy]phenoxy}propionates in good yields. Hydrolysis of the latter with dilute NaOH, followed by acidification, gave the propionic acid derivatives (**4a–d**). The precursory dichloroquinazolines (**2a–d**) were prepared via the following known sequence of reactions: (i) selective photolytic (side chain) dichloronation of the corresponding (commercially available) chloro-*o*-tolylisocyanates to the benzal chloride derivatives;[3] (ii) conversion of the latter with anhydrous NH$_3$ to N-substituted ureas; and (iii) cyclization of each urea with NH$_4$OH to a chloro-2-quinazolone.[4] The latter, on treatment with POCl$_3$, gave the dichloroquinazolines.

**1,2,4-Benzotriazines.** Synthesis of the precursory 3-chloro-1,2,4-benzotriazines-1-oxide (Scheme 1, **5–11**) proceeded from correspondingly substituted 2-nitro-

anilines, according to the methods of Wolf and Pfister.[5a,b] Processes for the preparation of substituted 2-{4-[(1-oxide-1,2,4-benzotriazin-3-yl)oxy]phenoxy}propionate esters have also been described.[6] For example, the reaction of 3-chloro-1,2,4-benzotriazine-1-oxide (**5**) and **3**, under the conditions outlined in Scheme 1, provided methyl 2-{4-[(1-oxide-1,2,4-benzotriazin-3-yl)oxy]phenoxy}propionate (**12a**).[6] Reduction (HCO$_2$NH$_4$/Pd–C/MeOH) of the latter yielded methyl 2-{4-[(1,2,4-benzotriazin-3-yl)oxy]phenoxy}propionate (**12b**),[6] which, on saponification and acidification, afforded the unsubstituted 1,2,4-triazine derivative, **12c**. As noted above, simple alkyl (e.g., methyl and ethyl) esters differ only marginally in activity ratings from the free carboxylic acid. Moreover, if an ester failed to show activity, the acid also proved to be inactive. In accord with these findings, neither **12a–c** nor **13a–c** manifested significant antitumor activities (Table 1), which negated the need to saponify the methyl propionate esters **14b–18b**.

**Quinolines.** The synthesis of a series of 2-{4-[(6- and 7-monosubstituted-2-quinolinyl)oxy]phenoxy}-

**Scheme 2**[a]
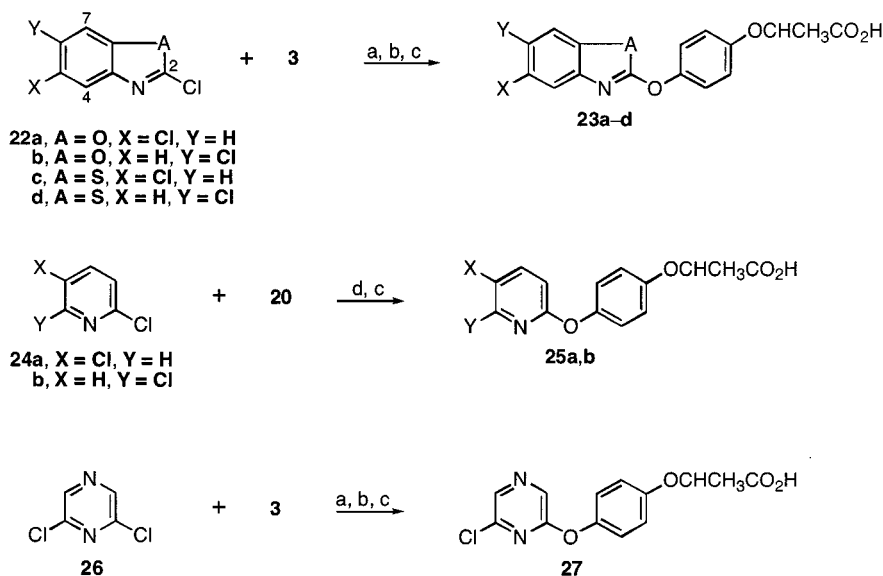


[a] Reagents: (a) $K_2CO_3/CH_3CN$; (b) aqueous NaOH/THF; for **22a**, aqueous $K_2CO_3/THF$; (c) aqueous HCl; (d) $K_2CO_3/DMF$.

**Table 1.** Evaluation of Selected Analogues of **1** Against Solid Tumors of Mice

| compd no. | enantiomeric form | SC tumor | no. of injections IV | total dose (mg/kg) | drug deaths | % body wt. loss at nadir | T/C (%) | regressions complete (partial) | log tumor cell kill[a] | cures[a] | activity rating[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** (Pos Cont) | racemic | Mam17/Adr* | 13 | 364 | 0/5 | 0 | 10.3 | | 2.82 | 0/5 | ++++ |
| **1** (Pos Cont) | R-(+) | Panc 03** | 7 | 560 | 0/7 | +2.5 | 24.5 | 0/7 | 2.0 | 0/7 | +++ |
| **21a** | racemic | Mam17/Adr* | 7 | 324 | 0/5 | −1.6 | 14 | | 1.5 | 0/5 | ++ |
| **21b** | R-(+) | Panc 03** | 7 | 560 | 0/7 | +3.7 | 14.1 | 0/7 (2/7) | 2.3 | 0/7 | +++ |
| **21b** | racemic | Panc 03* | 7 | 336 | 0/5 | −8.9 | 0 | | 2.6 | 0/5 | +++ |
| **21b** | racemic | Mam17/Adr* | 6 | 300 | 0/5 | −10.6 | 0 | | 3.8 | 0/5 | ++++ |
| **21c** | racemic | insufficient cytotoxcity in culture, not tested in mice | | | | | | | | | |
| **21d** | R-(+) | Panc 03** | 6 | 480 | 0/4 | +5.9 | 2.8 | 3/4 | 3.1 | 0/4 | ++++ |
| **21d** | racemic | Mam17/Adr* | 6 | 352 | 0/5 | −5.5 | 0 | | 4.2 | 0/5 | ++++ |
| **21e** | racemic | Mam17/Adr* | 7 | 370 | 0/5 | −4.0 | 2 | | 2.1 | 0/5 | ++ |
| **21f** | racemic | insufficient cytotoxcity in culture, not tested in mice | | | | | | | | | |
| **21g** | racemic | Mam17/Adr* | 6 | 450 | 0/5 | −5.0 | 0 | | 3.0 | 0/5 | ++++ |
| **21g** | racemic | Panc 03** | 5 | 450 | 0/5 | −2.0 | 45 | 0/5 | 0.8 | 0/5 | + |
| **23a** | racemic | Mam17/Adr* | 7 | 462 | 0/4 | −2.3 | >100 | | none | 0/4 | inactive |
| **23b** | racemic | Mam17/Adr* | 7 | 455 | 0/3 | 0 | >100 | | none | 0/3 | inactive |

[a] See Experimental Section; *, early; **, advanced.

propionic acids (**21a−g**) is highlighted in Scheme 1. Several examples of these have been previously described in the patent literature in context with the design of novel herbicides.[7] Preparations of intermediates 7-methoxy-, 7-methyl-, 6-, and 7-chloro-2-quinolinol have been described by Effenberger and Hartmann,[8] whereas syntheses of 7-bromo-2-quinolinol were reported by Campbell et al.[9a,b] The methodology, employed by the latter group, was applied successfully to the preparation of both the 7-fluoro- and 7-iodo-2-chloro-quinolines. Wherein preparations of (R)-(+) **21b** and **21d** were required (Table 1), racemic **20** was replaced with the commercially available (R)-(+) enantiomer to provide the corresponding product in >99% enantiomeric excess.

**Six Five-Membered Heteroaryl Derivatives.** Preparations of structures **23a−d** (Scheme 2), in which A is O or S, have been described in herbicidal patent literature for the selective control of weed grasses, e.g., **23b**, Fenoxaprop.[10] Processes for the preparation of the intermediates 2,5-(**22a**) and 2,6-dichlorobenzoxazole (**22b**) are readily accessible[11] as is 2,5-dichlorobenzthiazole[12] (**22c**). 2,6-Dichlorobenzthiaziole (**22d**) is commercially available.

**Six-Membered Heteroaryl Derivatives.** Preparations of selected pyrazine and pyridine analogues of **1** were carried out to ascertain the relative importance of a fused benzene ring to the biological activities of **1** and correspondingly substituted quinoline derivatives. Preparations of both 2-{4-[(5- and 6-chloro-substituted-2-pyridyl)oxy]phenoxy}propionic acids (Scheme 2, **25a,b**, respectively) are the subjects of patents in the herbicide literature[13,14] and are described as well as antiinflammatory and analgesic agents.[15] Interaction of the commercially available 2,5- and 2,6-dichloropyridines (**24a,b**, respectively) with **20** (Scheme 2) with $K_2CO_3$ in dimethyl formamide (DMF), followed by acidification, provided the corresponding 2-propionic acid derivatives, **25a,b**, respectively. The more reactive electrophilic 2-position of 2,6-dichloropyrazine (**26**) permitted the same displacement reaction with **3** to be carried out with $K_2CO_3$ in refluxing acetonitrile. Saponification of the intermediate ester, followed by acidification, gave the 2-pyrazinyloxy derivative (**27**).

**Biological Results and Discussion**

All newly synthesized analogues of **1** were initially evaluated, as previously described,[1a] in our in vitro, disk

diffusion soft agar colony formation assay, to determine cytotoxicity against leukemias, solid tumors, and normal cells (see the Experimental Section). None of the quinazoline, 1,2,4-benzotriazine, pyrazine, or pyridine congeners of **1** manifested sufficient activity in culture to warrant testing in mice. In sharp contrast, the 7-haloquinolines (**21a,b,d,e**) all exhibited impressive antitumor activities (Table 1). Interestingly, the 7-bromo derivative (**21d**) proved to be the most active, eliciting three out of four complete regressions (CRs) and a 3.1 log kill against advanced stage pancreatic ductal adenocarcinoma 03. On the other hand, the 7-chloro compound (**21b**) produced no CRs but a 2.3 log kill against the same tumor. Similarly, **1**, in the same trial, effected no CRs and a 2.0 log kill. In an evaluation against resistant mammary adenocarcinoma-17/adr (M17/adr, a *p*-glycoprotein positive, multiresistant tumor), **21d** gave rise to a 4.2 log kill. Bioisostere **21a**, though active (1.5 log kill) against M17/adr, proved to be inferior to both **21b** and **21d**. The relative antitumor activities of the 2-{4-[(7-halo-2-quinoxalinyl)oxy]phenoxy}propionic acids[1a] were found to be F ≈ Cl ≈ Br > I, whereas the corresponding quinoline derivatives showed an order of activity of Br > Cl > F ≈ I. The 7-methoxy quinoline analogue (**21g**) was also highly active (3.0 log kill) against M17/adr. However, activity fell off rapidly with dose reduction (62%), producing only a 1.4 log kill. Despite the limited testing performed to date, the effect of 7-methoxy substitution is noteworthy in that the antitumor effects parallel those of the 7-methoxy analogue of **1**. Both the 6-chloro (**21c**) and the 7-methyl (**21f**) derivatives showed insufficient cytotoxicity in culture to warrant testing in mice. The congeneric benzoxazole derivatives (**23a,b**) indicated modest in vitro activity but failed to exhibit meaningful cytotoxic results (Table 1) in the treatment of mice bearing M17/Adr. The pyridines (**25a,b**) and pyrazine (**27**) congeners failed to show activity in culture.

**Toxicity Differences Among Enantiomers and Racemic Mixtures.** The cumulative long term dose-limiting toxicity for all of the active agents (**21b,d** and **1**) was the same: gastrointestinal (GI) epithelial damage, with only minor marrow toxicity.[16] However, substantial differences were observed in the immediate (~15 s to 1 h), post-IV injection effects of the enantiomers of **21b,d** and **1**. At high individual dosages, the (*S*)-(−) enantiomers produce a reversible slowing of nerve conduction velocity in the mice. This precipitated a distinctive dysfunction of the hind legs, which folded backward, causing uncoordinated movements, precluding, thereby, support of the mouse. The condition resolved within 5−10 min; however, at higher dosages (approaching a lethal level), the behavior extended to the front legs, lasting from 20 min to 1 h. During recovery, the problem in the movement of the front legs vanished before the hind legs, indicating that the longer nerves were more affected than the shorter nerves. By contrast, the (*R*)-(+) forms of these same agents did not induce the observed aberrant behavior. It is important to point out that interconversion of the (*R*)-(+) and (*S*)-(−) enantiomers of **1** has been reported to occur in plasma of mice and higher animals with >90% existing in the (*R*)-(+) form at equilibrium.[17] These findings

prompted the selection of the (*R*)-(+) form of **1** for phase 1 trials.

## Summary

The second phase of an effort[1] to elaborate a pharmacophore of **1** focused on a strategy of synthesis and biological evaluation of topologically based, bioisosteric replacements of the quinoxaline moiety in the lead compound **1** by quinazoline, 1,2,4-benzotriazine, and quinoline ring systems. The study was extended to include the congeneric benzoxazole, benzthiazole, pyrazine, and pyridine derivatives. Only the quinoline analogues bearing a 7-halo or a 7-methoxy substituent showed levels of antitumor activities (Br > Cl > CH$_3$O > F ≈ I), comparable to or greater than those manifested by **1** and corresponding analogues.

It is apparent, then, that the interchange of the N$^4$ and C$^3$ atoms of the quinoxaline moiety in **1** to generate a 7-chloroquinazoline derivative (**4c**) or the replacement of the C$^3$ in the quinoxaline ring of **1** by N$^3$ to form a 1,2,4-triazine ring analogue (**13c**) produce, in both cases, inactive compounds. By contrast, the exchange of N$^4$ in **1** by C$^4$ generated a group of quinoline antitumor agents (**21a,b,d,e,g**) of at least equal activity.

The failure of either the pyridine (**25a,b**) or the pyrazine analogues (**27**) to manifest cytotoxicity points to the importance, relative to antitumor activity, of a 7-halo- or 7-methoxybenzene moiety of the quinoxaline and quinoline ring systems of **1** and **21a,b,d,e,g**, respectively. On the other hand, there is at present no ready explanation of the modest, in vitro activity of the benzoxazoles (**23a,b**) and lack of activity noted for the benzthiazole derivatives (**23c,d**).

At high individual dosages, the *S*-(−) enantiomers of **1** and **21b,d** each produce a reversible slowing of nerve−conduction velocity in mice, which resolves within 5−10 min. This phenomenon is not observed with corresponding *R*-(+) enantiomers and is also absent in *R*-(+) **1**.

## Experimental Section

**Chemistry.** All commercially available solvents and reagents were used without further purification. Fenoxaprop was obtained from the Fluka Chemical Corp. (U.S.A.). Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were measured on a Perkin-Elmer 1330 spectrometer in KBr pellets or as a thin film. Optical rotations were measured on a Jasco DIP-370 polarimeter at room temperature. Nuclear magnetic resonance ($^1$H and $^{13}$C NMR) spectra were recorded at room temperature and referenced to a residual solvent signal on either a Varian Unity 300, Varian Mercury 400, or GE Q300 instruments in the Department of Chemistry, Wayne State University, Detroit, MI. Chemical shifts are reported in parts per million downfield from tetramethylsilane (TMS). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; b, broad; m, unresolved multiplet. Mass spectra were recorded on a MS80RFA instrument and other instruments at Wayne State University. Flash column chromatography was carried out with silica gel 200−400 mesh, 60 Å (Aldrich), and the crude product was introduced on to the column as a CHCl$_3$ solution. Thin-layer chromatography was performed on Whatman PE SIL G/UV (250 $\mu$m) plates. Compounds were visualized by use of 254 or 366 nm light and I$_2$ vapor. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

**General Method of Preparation of Esters of Quinazolines, 1,2,4-Benzotriazines-1-oxides, Benzoxazoles, Benzthiazoles, and Pyrazines.** A mixture of the 2- or 3-chlorohetrocycle, **3**, anhydrous $K_2CO_3$, and an aprotic solvent ($CH_3$-CN or butanone) was refluxed until the reaction was complete. The hot mixture was filtered, the residue was washed with warm acetone, and the filtrate was evaporated to dryness. The residue was dissolved in $CH_2Cl_2$, filtered through silica gel, and washed with ether. The filtrate was shaken with saturated NaCl, containing a small amount of 1 N NaOH, followed by saturated NaCl. The dried solution ($MgSO_4$) was evaporated, and the crude residue was purified by flash column chromatography where required, followed by crystallization from a mixture of AcOEt−hexanes (heptane).

**General Method Preparation of the Reduced of 1,2,4-Benzotriazines-1-oxide Esters. Method A.** A mixture of the 1-oxide derivative, $HCO_2NH_4$, Pd−C, and $CH_3OH$ was refluxed until the reaction was complete. The solvent was evaporated, and the residue was mixed with hot AcOEt. The mixture was filtered through silica gel, the filtrate was evaporated, and the residue was purified by flash column chromatography. The product was crystallized from a mixture of AcOEt−hexanes (heptane).

**Method B.** A mixture of the 1-oxide, 2% Pd−C, and $CH_3$-OH was shaken with $H_2$ in a Parr apparatus until the reaction was complete. The catalyst was removed, and the filtrate was then concentrated and purified by flash column chromatography. The product crystallized from a mixture of AcOEt−hexanes (heptane).

**General Procedure for Hydrolysis of Carboxylic Acid Esters.** To a solution of the ester dissolved in tetrahydrofuran (THF) was added, in portions, 0.1 N base, and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated, filtered to remove insoluble material, and then cooled and adjusted to pH 3−4 with 0.25 N HCl. The solid that deposited on further cooling was collected, washed with ice−water, dried, and crystallized from aqueous ethanol. The benzoxazoles and benzthiazoles were crystallized from a mixture of AcOEt−hexanes. Attempts to crystallize the 1,2,4-benzotriazine derivatives resulted in significant decomposition.

**Preparation of Quinoline and Pyridine Carboxylic Acids.** A mixture of the 2-chlorohetrocycle, **20**, anhydrous base, and DMF was gently refluxed until the reaction was complete. After it was cooled, the reaction mixture was concentrated, water was then added, and the solution was filtered through Celite to remove insoluble material. After it was cooled, the filtrate was acidified with 1 N HCl to pH 3−4, and the solid was collected, washed with ice-water, and dried or extracted with AcOEt. The impure product was dissolved in AcOEt and filtered through silica gel to remove the dark, very polar contaminant. The filtrate was then concentrated, purified by flash column chromatography (if required), and crystallized from a mixture of AcOEt−hexanes (heptane).

**Preparation of the 2-Chloro-6- and 7-Substituted Quinolines.** Cyclization of the precursory E-(N)-(3-substituted phenyl)-3-ethoxypropenamides with an electron-withdrawing substituent (F, Cl, Br, and I) was effected in concentrated $H_2$-$SO_4$,[12,13] whereas cyclization of those bearing an electron-donating substituent ($CH_3$ and $CH_3O$) was effected in concentrated HCl[12] to give a mixture of isomeric 2-quinolinols (see ratios below). Treatment of the crude mixture with $POCl_3$ provided corresponding mixtures of the 2-chloroquinolines. The 2-chloro-7-substituted quinolines (**19a,b,d−g**) were obtained after removal of dark polar contaminants by filtering the latter, dissolved in $CHCl_3$ through silica gel. Fractional crystallization of the mixture of solids from 2-propanaol removed the 5-substituted isomers, providing thereby **19a,b,d−g**.

Cyclization of E-(N)-(4-chlorophenyl)-3-ethoxypropenamide in concentrated $H_2SO_4$ provided 6-chloro-2-quinolinol, which was then converted and isolated as described above to 2,6-dichloroquinoline (**19c**).

**2-Chloro-7-fluoroquinoline (19a).** Cyclization of E-(N)-(3-fluorophenyl)-3-ethoxy-propenamide gave a mixture of 7- and 5-fluoro-2-quinolinols in a ratio of 92:8. The product **19a** was obtained, as described above, in the form of white crystals; mp 102−103 °C. ^{1}H NMR (400 MHz, CDCl$_3$): $\delta$ 8.08 (d, J = 8.0 Hz, 1H), 7.81 (dd, J = 9.2, 6.4 Hz, 1H), 7.65 (dd, J = 9.6, 2.4 Hz, 1H), 7.38−7.31 (m, 2H).

**2,7-Dichloroquinoline (19b).** Cyclization of E-(N)-(3-chlorophenyl)-3-ethoxypropenamide gave a mixture of 7- and 5-chloro-2-quinolinols in a ratio of 70:30. The product **19b** was obtained, as described above, in the form of white crystals; mp 121−123 °C. ^{1}H NMR (300 MHz, CDCl$_3$): $\delta$ 8.09 (d, J = 8.7 Hz, 1H), 8.02 (d, J = 1.8 Hz, 1H), 7.76 (d, J = 8.7 Hz, 1H), 7.52 (dd, J = 8.7, 2.1 Hz, 1H), 7.39 (d, J = 8.7 Hz, 1H).

**2,6-Dichloroquinoline (19c).** Cyclization of E-(N)-(4-chlorophenyl)-3-ethoxypropenamide gave 6-chloro-2-quinolinol, which in turn provided **19c** as white crystals; mp 162−163 °C. ^{1}H NMR (300 MHz, CDCl$_3$): $\delta$ 8.03 (d, J = 8.7 Hz, 1H), 7.96 (d, J = 9.0 Hz, 1H), 7.80 (d, J = 1.8 Hz, 1H), 7.68 (dd, J = 8.7, 2.1 Hz, 1H), 7.42 (d, J = 8.7 Hz, 1H).

**7-Bromo-2-chloroquinoline (19d).** Cyclization of E-(N)-(3-bromophenyl)-3-ethoxypropenamide gave a mixture of 7- and 5-bromo-2-quinolinol in a ratio of 63:37. The desired product **19d** was obtained as white crystals; mp 120−121 °C. ^{1}H NMR (400 MHz, CDCl$_3$): $\delta$ 8.20 (d, J = 1.6 Hz, 1H), 8.07 (d, J = 8.0 Hz, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.65 (dd, J = 8.8, 1.6 Hz, 1H), 7.40 (d, J = 8.8 Hz, 1H).

**2-Chloro-7-iodoquinoline (19e).** Cyclization of E-(N)-(3-iodophenyl)-3-ethoxypropenamide gave a mixture of 7- and 5-iodo-2-quinolinols in a ratio of 57:43. The product **19e** was obtained as light yellow crystals; mp 122−123 °C. ^{1}H NMR (400 MHz, CDCl$_3$): $\delta$ 8.43 (d, J = 1.6 Hz, 1H), 8.05 (d, J = 9.2 Hz, 1H), 7.81 (dd, J = 8.4, 1.6 Hz, 1H), 7.51 (d, J = 9.2 Hz, 1H), 7.39 (d, J = 8.0 Hz, 1H).

**2-Chloro-7-methylquinoline (19f).** Cyclization of E-(N)-(3-methylphenyl)-3-ethoxypropen-amide gave a mixture of 7- and 5-methyl-2-quinolinols in a ratio of 55:45. The product **19f** was obtained as white crystals; mp 83−84 °C. ^{1}H NMR (400 MHz, CDCl$_3$): $\delta$ 8.05 (d, J = 8.0 Hz, 1H), 7.80 (s, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.39 (dd, J = 8.0, 1.6 Hz, 1H), 7.32 (d, J = 8.8 Hz, 1H), 2.56 (s, 3H).

**2-Chloro-7-methoxyquinoline (19g).** Cyclization of E-(N)-(3-methoxyphenyl)-3-ethoxypropenamide gave a mixture of 7- and 5-methoxy-2-quinolinols in a ratio of 82:18. The desired product, **19g**, was obtained as off-white crystals; mp 99−100 °C. ^{1}H NMR (400 MHz, CDCl$_3$): $\delta$ 8.01 (d, J = 8.0 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.35 (d, J = 2.8 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.20 (dd, J = 9.2, 2.4 Hz, 1H), 3.93 (s, 3H).

**2-{4-[(7-Fluoro-2-quinolinyl)oxy]phenoxy}propionic Acid (21a).** A mixture of **19a** (0.18 g, 1.0 mmol), **20** (0.19 g, 1.0 mmol), anhydrous $K_2CO_3$ (0.35 g, 2.5 mmol), and DMF (5 mL) were refluxed overnight. The pure product (0.20 g, 61% yield) was obtained after crystallization from AcOEt−heptane as white crystals; mp 135−137 °C. ^{1}H NMR (400 MHz, DMSO-$d_6$): $\delta$ 13.08 (bs, 1H), 8.38 (d, J = 8.8 Hz, 1H), 7.99 (dd, J = 8.8, 6.4 Hz, 1H), 7.40−7.32 (m, 2H), 7.18 (d, J = 8.8 Hz, 1H), 7.17−7.12 (m, 2H), 6.94−6.89 (m, 2H), 4.82 (q, J = 6.8 Hz, 1H), 1.51 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-$d_6$): $\delta$ 173.9, 163.6 (d, J = 245.5 Hz), 163.2, 155.2, 147.6 (d, J = 12.7 Hz), 147.3, 141.0, 130.8 (d, J = 10.4 Hz), 123.4, 123.2, 116.1, 115.1 (d, J = 24.5 Hz), 112.7, 111.7 (d, J = 20.8 Hz), 72.5, 19.0. ^{19}F NMR (376 MHz, CDCl$_3$): $\delta$ 76.33 (m). IR (KBr): 3415 (OH), 1710 (C=O) cm$^{-1}$. MS (EI): m/z (%) 327 (M$^+$, 59), 282 (15), 268 (15), 254 (67), 238 (8), 226 (4), 209 (4), 198 (3), 151 (5), 146 (100), 126 (12), 119 (7), 91 (7). HRMS (EI): m/z 327.0910 (M$^+$, calcd for $C_{18}H_{14}NFO_4$, 327.0907). Anal. ($C_{18}H_{14}NFO_4$) C, H, N.

**2-{4-[(7-Chloro-2-quinolinyl)oxy]phenoxy}propionic Acid (21b).** Quinoline **19b** (1.15 g, 5.81 mmol), **20** (1.06 g, 5.81 mmol), anhydrous $K_2CO_3$ (2.00 g, 14.5 mmol), and DMF (25 mL) were refluxed overnight. Pure material (1.68 g, 84% yield) was obtained after crystallization from AcOEt−heptane as white crystals; mp 149−150 °C. ^{1}H NMR (400 MHz, DMSO-$d_6$): $\delta$ 13.05 (bs, 1H), 8.40 (d, J = 8.8 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.66 (d, J = 2.8 Hz, 1H), 7.48 (dd, J = 8.8, 2.4 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 7.18−7.13 (m, 2H), 6.94−6.89

(m, 2H), 4.82 (q, $J$ = 6.4 Hz, 1H), 1.51 (d, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 173.6, 162.8, 155.0, 147.1, 146.6, 140.6, 135.0, 129.9, 126.1, 125.6, 124.3, 123.0, 116.0, 113.5, 72.4, 18.7. IR (KBr): 3440 (OH), 1705 (C=O) cm$^{-1}$. MS (EI): $m/z$ (%) 343 (M$^+$, 46), 298 (15), 284 (16), 270 (71), 254 (8), 236 (6), 167 (19), 162 (100), 155 (8), 127 (22), 114 (10), 97 (11), 91 (24), 83 (16), 81 (12), 73 (19), 71 (14), 69 (23), 67 (12), 63 (12), 60 (17), 57 (27), 55 (35), 45 (18). HRMS (EI): $m/z$ 343.0609 (M$^+$, calcd for C$_{18}$H$_{14}$NClO$_4$, 343.0611). Anal. (C$_{18}$H$_{14}$NClO$_4$) C, H, N. (*R*)-(+) enantiomer: mp 160–161 °C; [$\alpha$]$_D$ = +19.5° (*c* = 0.50, 0.1 N NaOH). Chiral HPLC separation ((*S*) enantiomer, 5.8 min, (*R*) enantiomer, 8.1 min) using Astec Chirobiotic T 250 mm × 4.6 mm, 65% H$_2$O, 35% CH$_3$OH, 20 mM NH$_4$NO$_3$ at 1 mL/min with detection at 250 nm.

**2-{4-[(6-Chloro-2-quinolinyl)oxy]phenoxy}propionic Acid (21c).** Quinoline **19c** (0.20 g, 1.0 mmol), **20** (0.18 g, 1.0 mmol), anhydrous K$_2$CO$_3$ (0.35 g, 2.5 mmol), and DMF (5 mL) were refluxed overnight. Pure material (0.15 g, 44% yield) was obtained after crystallization from AcOEt–heptane as white crystals; mp 173–174 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 13.03 (bs, 1H), 8.32 (d, $J$ = 9.0 Hz, 1H), 8.02 (s, 1H), 7.60 (s, 2H), 7.25 (d, $J$ = 8.7 Hz, 1H), 7.14 (d, $J$ = 8.4 Hz, 2H), 6.90 (d, $J$ = 8.4 Hz, 2H), 4.80 (q, $J$ = 6.6 Hz, 1H), 1.50 (d, $J$ = 6.6 Hz, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 173.5, 162.4, 155.0, 147.2, 144.6, 140.0, 130.6, 129.4, 126.9, 126.6, 123.0, 116.0, 114.3, 72.4, 18.8. IR (KBr): 3425 (OH), 1735 (C=O) cm$^{-1}$. MS (EI): $m/z$ (%) 343 (M$^+$, 8), 270 (9), 208 (27), 182 (54), 180 (6), 169 (26), 162 (21), 149 (7), 137 (6), 125 (10), 111 (19), 109 (14), 104 (15), 97 (42), 95 (33), 91 (18), 83 (58), 77 (32), 69 (91), 67 (57), 65 (12), 57 (64), 55 (100), 51 (16). HRMS (EI): $m/z$ 343.0608 (M$^+$, calcd for C$_{18}$H$_{14}$NClO$_4$, 343.0611). Anal. (C$_{18}$H$_{14}$NClO$_4$) C, H, N.

**2-{4-[(7-Bromo-2-quinolinyl)oxy]phenoxy}propionic Acid (21d).** Quinoline **19d** (0.79 g, 3.3 mmol), **20** (0.61 g, 3.3 mmol), anhydrous K$_2$CO$_3$ (1.13 g, 8.18 mmol), and DMF (20 mL) were refluxed overnight. Pure material (1.03 g, 82% yield) was obtained after crystallization from AcOEt–heptane as white crystals; mp 160–161 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 13.09 (bs, 1H), 8.39 (d, $J$ = 8.8 Hz, 1H), 7.88 (d, $J$ = 9.2 Hz, 1H), 7.80 (d, $J$ = 1.6 Hz, 1H), 7.60 (dd, $J$ = 9.2, 1.6 Hz, 1H), 7.25 (d, $J$ = 8.8 Hz, 1H), 7.18–7.13 (m, 2H), 6.94–6.89 (m, 2H), 4.82 (q, $J$ = 6.8 Hz, 1H), 1.51 (d, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 173.9, 163.0, 155.3, 147.2, 147.1, 141.0, 130.3, 129.6, 128.5, 124.9, 124.0, 123.3, 116.1, 114.0, 72.5, 19.1. IR (KBr): 3415 (OH), 1705 (C=O) cm$^{-1}$. MS (EI): $m/z$ (%) 387 (M$^+$, 42), 342 (10), 328 (10), 314 (31), 300 (6), 285 (7), 256 (22), 236 (13), 206 (53), 199 (18), 185 (10), 171 (8), 157 (8), 127 (44), 115 (15), 111 (13), 97 (27), 91 (28), 83 (33), 73 (57), 69 (45), 60 (58), 57 (56), 55 (69), 43 (100), 41 (66). HRMS (EI): $m/z$ 387.0107 (M$^+$, calcd for C$_{18}$H$_{14}$NBrO$_4$, 387.0106). Anal. (C$_{18}$H$_{14}$NBrO$_4$) C, H, N. (*R*)-(+) enantiomer: mp 166–167 °C; [$\alpha$]$_D$ = +22.0° (*c* = 0.50, 0.1 N NaOH). Chiral HPLC separation ((*S*) enantiomer, 5.9 min; (*R*) enantiomer, 8.5 min) using Astec Chirobiotic T 250 mm × 4.6 mm, 65% H$_2$O, 35% CH$_3$OH, 20 mM NH$_4$NO$_3$ at 1 mL/min with detection at 250 nm.

**2-{4-[(7-Iodo-2-quinolinyl)oxy]phenoxy}propionic Acid (21e).** Quinoline **19e** (0.29 g, 1.0 mmol), **20** (0.18 g, 1.0 mmol), anhydrous K$_2$CO$_3$ (0.35 g, 2.5 mmol), and DMF (5 mL) were refluxed overnight. Pure material (0.34 g, 78% yield) was obtained after crystallization from AcOEt as white crystals; mp 138–140 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 13.04 (bs, 1H), 8.34 (d, $J$ = 9.2 Hz, 1H), 7.99 (s, 1H), 7.73 (dd, $J$ = 8.4, 1.6 Hz, 1H), 7.68 (d, $J$ = 8.8 Hz, 1H), 7.23 (d, $J$ = 8.8 Hz, 1H), 7.18–7.12 (m, 2H), 6.95–6.89 (m, 2H), 4.82 (q, $J$ = 6.4 Hz, 1H), 1.52 (d, $J$ = 6.4 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 173.9, 162.7, 155.2, 147.3, 147.2, 141.0, 136.0, 133.8, 130.0, 125.1, 123.3, 116.2, 114.1, 97.5, 72.6, 19.1. IR (KBr): 3440 (OH), 1735 (C=O) cm$^{-1}$. MS (EI): $m/z$ (%) 435 (M$^+$, 100), 390 (14), 376 (20), 362 (49), 346 (4), 309 (10), 284 (41), 271 (53), 254 (84), 236 (23), 219 (6), 207 (6), 181 (5), 144 (5), 127 (44), 116 (14), 100 (7), 89 (7), 43 (6). HRMS (EI): $m/z$ 434.9961 (M$^+$, calcd for C$_{18}$H$_{14}$NIO$_4$, 434.9966). Anal. (C$_{18}$H$_{14}$NIO$_4$) C, H, N.

**2-{4-[(7-Methyl-2-quinolinyl)oxy]phenoxy}propionic Acid (21f).** To quinoline **19f** (0.34 g, 1.9 mmol), **20** (0.35 g, 1.9 mmol), and DMF (10 mL) was added, in portions, 60% NaH (0.23 g, 5.8 mmol), and the mixture was refluxed for 2 h. Pure material (0.20 g, 32% yield) was obtained, after crystallization from AcOEt–heptane, as light yellow crystals; mp 183–185 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 13.03 (bs, 1H), 8.29 (d, $J$ = 8.8 Hz, 1H), 7.78 (d, $J$ = 8.0 Hz, 1H), 7.43 (s, 1H), 7.28 (d, $J$ = 8.4 Hz, 1H), 7.16–7.10 (m, 3H), 6.93–6.89 (m, 2H), 4.82 (q, $J$ = 6.4 Hz, 1H), 2.41 (s, 3H), 1.51 (d, $J$ = 6.4 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 173.9, 162.4, 155.1, 147.6, 146.6, 140.6 (2C), 128.0, 127.4, 127.0, 124.0, 123.4, 116.1, 112.3, 72.5, 21.9, 19.1. IR (KBr): 3460 (OH), 1725 (C=O) cm$^{-1}$. MS (EI): $m/z$ (%) 323 (M$^+$, 57), 305 (6), 278 (9), 276 (7), 264 (13), 250 (60), 236 (10), 234 (6), 222 (5), 142 (100), 115 (17), 105 (6), 77 (6). HRMS (EI): $m/z$ 323.1164 (M$^+$, calcd for C$_{19}$H$_{17}$NO$_4$, 323.1158). Anal. (C$_{19}$H$_{17}$NO$_4$) C, H, N.

**2-{4-[(7-Methoxy-2-quinolinyl)oxy]phenoxy}propionic Acid (21g).** Quinoline **19g** (0.39 g, 2.0 mmol), **20** (0.36 g, 2.0 mmol), anhydrous K$_2$CO$_3$ (0.69 g, 5.0 mmol), and DMF (10 mL) were refluxed overnight. Pure material (0.45 g, 66% yield) was obtained after crystallization from AcOEt–heptane as light yellow crystals; mp 164–166 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 13.06 (bs, 1H), 8.25 (d, $J$ = 8.8 Hz, 1H), 7.78 (d, $J$ = 8.8 Hz, 1H), 7.16–7.10 (m, 2H), 7.06 (dd, $J$ = 8.8, 2.4 Hz, 1H), 7.02 (d, $J$ = 8.0 Hz, 1H), 6.99 (d, $J$ = 2.4 Hz, 1H), 6.94–6.88 (m, 2H), 4.82 (q, $J$ = 6.4 Hz, 1H), 3.81 (s, 3H), 1.51 (d, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 174.0, 162.9, 161.5, 155.1, 148.3, 147.7, 140.5, 129.5, 123.4, 120.9, 117.5, 116.1, 110.5, 107.0, 72.5, 56.1, 19.1. IR (KBr): 3415 (OH), 1735 (C=O) cm$^{-1}$. MS (EI): $m/z$ (%) 339 (M$^+$, 62), 323 (10), 294 (8), 280 (13), 266 (35), 250 (13), 175 (7), 158 (100), 142 (18), 115 (10), 77 (6). HRMS (EI): $m/z$ 339.1105 (M$^+$, calcd for C$_{19}$H$_{17}$NO$_5$, 339.1107). Anal. (C$_{19}$H$_{17}$NO$_5$) C, H, N.

**2-{4-[(5-Chloro-2-benzoxazolyl)oxy]phenoxy}propionic Acid (23a).** The methyl ester of **23a** was prepared by refluxing for 4 h a mixture of **22a** (0.47 g, 2.5 mmol), **3** (0.49 g, 2.5 mmol), anhydrous K$_2$CO$_3$ (0.41 g, 3.0 mmol), and CH$_3$CN (15 mL). Pure material (0.76 g, 87% yield), in the form of a light yellow oil, was obtained after chromatography (4:1 hexanes:AcOEt). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.46 (d, $J$ = 1.6 Hz, 1H), 7.31 (d, $J$ = 8.8 Hz, 1H), 7.32–7.28 (m, 2H), 7.19 (dd, $J$ = 8.4, 2.0 Hz, 1H), 6.97–6.91 (m, 2H), 4.75 (q, $J$ = 6.8 Hz, 1H), 3.77 (s, 3H), 1.63 (d, $J$ = 6.4 Hz, 3H). $^{13}$C NMR (400 MHz, CDCl$_3$): $\delta$ 172.6, 163.5, 156.0, 147.2, 147.0, 142.1, 130.2, 123.8, 121.5, 119.1, 116.5, 110.8, 73.4, 52.7, 18.8. IR (film): 1750 (C=O) cm$^{-1}$. MS (EI): $m/z$ (%) 347 (M$^+$, 100), 312 (2), 288 (84), 261 (34), 244 (6), 233 (4), 216 (6), 205 (5), 188 (15), 182 (18), 170 (11), 162 (9), 153 (13), 140 (16), 119 (23), 91 (24), 76 (41), 63 (33), 59 (50), 55 (19), 50 (21), 43 (14), 39 (15). HRMS (EI): $m/z$ 347.0563 (M$^+$, calcd for C$_{17}$H$_{14}$NClO$_5$, 347.0561).

Saponification of the methyl ester of **23a** (0.61 g, 1.8 mmol) was effected with 0.1 N K$_2$CO$_3$ (35 mL, 3.5 mmol) in THF (40 mL). The mixture was extracted with AcOEt, followed by filtration of the dried (MgSO$_4$) extract through silica gel and evaporation of the clear solution to dryness. Crystallization of the residue from AcOEt–hexanes gave **23a** (0.21 g, 36% yield) as an off-white solid; mp 167–168 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.65 (d, $J$ = 8.8 Hz, 1H), 7.61 (d, $J$ = 2.4 Hz, 1H), 7.43–7.38 (m, 2H), 7.30 (dd, $J$ = 8.4, 2.0 Hz, 1H), 6.99–6.93 (m, 2H), 4.87 (q, $J$ = 6.8 Hz, 1H), 1.51 (d, $J$ = 6.8 Hz, 3H). $^{13}$C NMR (400 MHz, DMSO-$d_6$): $\delta$ 173.7, 163.8, 156.4, 147.4, 146.8, 142.4, 129.5, 124.0, 122.3, 116.4, 112.1, 72.6, 18.9. IR (KBr): 2880 (OH), 1725 (C=O) cm$^{-1}$. MS (EI): $m/z$ (%) 333 (M$^+$, 100), 304 (4), 288 (40), 274 (77), 261 (92), 248 (23), 232 (12), 220 (30), 216 (16), 205 (10), 188 (48), 182 (42), 168 (33), 162 (23), 153 (35), 144 (12), 140 (41), 124 (15), 119 (24), 109 (25), 92 (45), 81 (26), 76 (88), 63 (90), 55 (32), 50 (47), 45 (56), 39 (45). HRMS (EI): $m/z$ 333.0401 (M$^+$, calcd for C$_{16}$H$_{12}$NClO$_5$, 333.0404). Anal. (C$_{16}$H$_{12}$NClO$_5$) C, H, N.

**Biologic Testing Methods: In Vitro.**[1a] A brief description of the methods follows. All materials were initially tested in a disk diffusion soft agar colony formation assay (disk assay). The disk assay is designed to compare the relative cytotoxicity

of an agent against leukemia cells, solid tumor cells (including multidrug resistant solid tumors), and normal cells. The inhibition is expressed in zone units, with 200 units = 6.5 mm. On average, a 10-fold dilution of a cytotoxic agent produces a 330 zone unit change. Activity against a drug sensitive leukemia (L1210 or P388) provides the reference point. The leukemic cell can represent antiproliferative leukemic active agents of past discoveries. The agent needs greater activity against the drug insensitive solid tumors than against the leukemia cells. Normal fibroblasts were used in current studies. For the operation of the assay, the tumor cells are isolated from live tissue, i.e., a tumor growing in a mouse. The cells are then seeded in the soft agar. The drug is placed on a filter paper disk (standard hole punch of Whatman no. 1), which is then placed on top of the soft agar (60 mm plate). The drug diffuses off the disk as the tumor cells are replicating, creating a zone of inhibition of colony formation. Those materials with sufficient cytotoxicity (>400 units) and tumor selectivity progressed to in vivo evaluation in tumor-bearing mice as described below.

**In Vivo.** Treatment was carried out against advanced/early stage pancreatic ductal adenocarcinoma-03 and/or early mammary adenocarcinoma-17/Adr. All are sensitive to **1**. The Mam-17/Adr is a *p*-glycoprotein positive multidrug resistant tumor.

**a. Tumor and Animal Maintenance.** Mouse tumors were maintained in the mouse strain of origin and were transplanted into the appropriate $F_1$ hybrid (or the inbred mouse of origin) for therapy trials. Individual mouse body weights for each experiment were within 5 g, and all mice were over 17 g at the start of therapy. The mice were supplied food and water ad libitum.

**b. Chemotherapy of Solid Tumors.** The animals were pooled, implanted subcutaneously with 30−60 mg tumor fragments by a 12 gauge trocar, and again pooled before unselective distribution to the various treatment and control groups (five or six mice per group). For early stage treatment, chemotherapy was started 1−3 days after tumor implantation while the number of cells is relatively small ($10^7$ to $5 \times 10^7$ cells). For advanced stage tumors, treatment was delayed until the tumors were in the 150−350 mg size ($1.5 \times 10^8$ to $3.5 \times 10^8$ cells). Tumors were measured with a caliper twice weekly or three times weekly for the more rapidly growing tumors. Mice were sacrificed when their tumors reached 1500 mg (i.e., before they could cause the animal discomfort). Tumor weights were estimated from two-dimensional measurements. Dose schedules were adjusted for toxicity; accordingly, doses reported in Table 1 are not the same, because of the toxicity encountered at the top dose(s), at which time treatment was terminated for all doses. Therefore, the highest achieved dose is reported.

**c. Tumor Weight.** The tumor weight (in mg) = $(a \times b^2)/2$, where $a$ and $b$ are the tumor length and width in (mm), respectively.

**d. Quantified End Points for Assessing Antitumor Activity for Solid Tumors.** The following quantified end points are used to assess antitumor activity.

**1. Tumor Growth Delay ($T−C$ Value).** $T$ is the median time (in days) required for the treatment group tumors to reach a predetermined size (e.g., 1000 mg), and $C$ is the median time (in days) for the control group tumors to reach the same size. Tumor-free survivors are excluded from these calculations (cures are tabulated separately). In our judgment, this value is the single most important criterion of antitumor effectiveness because it allows the quantification of tumor cell kill.

**2. Calculation of Tumor Cell Kill.** For subcutaneously (SC) growing tumors, the log cell kill is calculated from the following formula: log cell kill total (gross) = $T−C$ value in days (3.32) ($T_d$), where $T−C$ is the tumor growth delay as described above and $T_d$ is the tumor volume doubling time in days, estimated from the best fit straight line from a log−linear growth plot of the control group tumors in exponential growth (100−800 mg range). The conversion of the $T−C$ values to log cell kill is possible because the $T_d$ of tumors regrowing

posttreatment ($R_x$) approximates the $T_d$ values of the tumors in untreated control mice.

duration of treatment of solid tumor, 5−20 days

| antitumor activity | | gross log tumor cell kill |
|---|---|---|
| highly active | ++++ | >2.8 |
| | +++ | 2.0−2.8 |
| | ++ | 1.3−1.9 |
| | + | 0.7−1.2 |
| inactive | − | <0.7 |

**3. Regressions for Advanced Staged Tumors.** (i) Partial regression: regression of the tumor to less than 50% of the pretreatment size; (ii) complete regression: regression of the tumor to below limit of palpation (<50 mg); and (iii) cures: there are no cures in the experiments reported.

**4. Nonquantitative Determination of Antitumor Activity by Tumor Growth Inhibition ($T/C$ Value).** The treatment and control groups are measured when the control group tumors reach approximately 700−1200 mg in size (median of group). The median tumor weight of each group is determined, including zeros. The $T/C$ value in percent is an indication of antitumor effectiveness. A $T/C$ equal to or less than 42% is considered significant antitumor activity by the Drug Evaluation Branch of the Division of Cancer Treatment (NCI). A $T/C$ value <10% is considered to indicate highly significant antitumor activity and is the level used by NCI to justify a clinical trial if toxicity, formulation, and certain other requirements are met (termed DN-2 level activity). A body weight loss nadir (mean of group) of greater than 20% or greater than 20% drug deaths is considered to indicate an excessively toxic dosage in a single course trial.

**Supporting Information Available:** Experimental procedures for the series **4a−d**, **12a−c**, **13a−c**, **14a,b**, **15a,b**, **16a,b**, **17a,b**, **18a,b**, **23c,d**, **25a,b**, and **27** not tested in vivo and chiral HPLC separation of **21b**. This material is available free of charge via the Internet at http://pubs.acs.org/.

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