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Nitroarylhydroxymethylphosphonic Acids as Inhibitors of CD45

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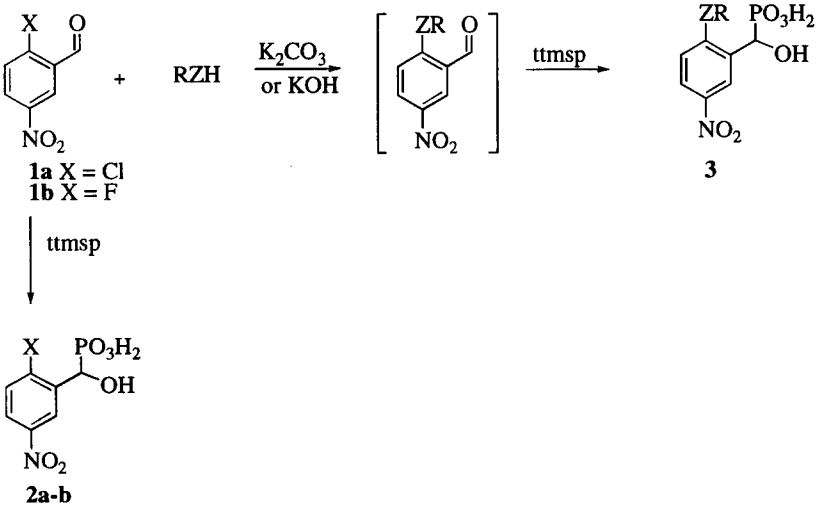
Abstract—A series of nitroarylhydroxymethylphosphonic acids was synthesized and evaluated as inhibitors of CD45. It was discovered that both the alpha hydroxy and nitro groups are essential for activity. Potency is enhanced by the addition of a large lipophilic group on the aryl ring adjacent to the phosphonic acid moiety. Kinetics studies have shown that these compounds are competitive inhibitors and thus bind at the active site of this enzyme © 1997 Elsevier Science Ltd.

Introduction

Tyrosine phosphorylation provides the molecular switch that regulates a wide range of cellular processes through transduction of extracellular signals. Examples of these essential processes include the mitogenic actions of insulin and growth factors[1–5] as well as lymphocyte growth and differentiation.[6] CD45 is expressed on all hematopoietic cells except those of erythrocyte lineage. It was shown to be a protein tyrosine phosphatase in 1988.[7] Its indispensable roles in the coupling of the T-cell antigen receptor with the phosphatidylinositol second messenger pathway as well as antigen-mediated proliferation of T lymphocytes[8] make CD45 a very attractive target for the treatment of tissue transplants and/or autoimmune diseases. In 1995 Miski et al.[9] reported on the ability of several aporphine alkaloids to inhibit CD45. This inhibition was shown to be dose dependent with IC_{50} values in the 5–200 μM range although it was not determined whether these compounds were binding in the active site (competitive inhibition). The design of our compounds was based on the rationale that a hydrolytically stable bioisostere of tyrosine phosphate (benzyl phosphonic acid) would potentially mimic the natural substrate of CD45. We wish to report on the synthesis and structure–activity relationship of a versatile new class of nitroarylhydroxymethylphosphonic acids that inhibit CD45 with IC_{50} values ranging from 2 to 12 μM.

Chemistry

The precursor aldehydes that were not commercially available were prepared in a variety of ways. Scheme 1 shows the displacement of the chlorine atom of **1a** by such nucleophiles as phenols, mercaptans, azide, and methylbenzylamine. The resulting 2-substituted benz-



Scheme 1.

aldehydes were then reacted with tris(trimethylsilyl)-phosphite (ttmsp) as described by Sekine[10] to give the phosphonic trimethylsilyl esters, which upon alcoholic/aqueous work up gave the target acid **3**.

Scheme 2 shows the use of compound **4** as a nucleophile, to react with benzyl bromide, to give 2-benzyloxy-5-nitrobenzaldehyde **7a**. The 2-cyclohexyloxy derivative **7b** was synthesized according to literature methodology.[11] Both **7a** and **7b** were reacted with ttmsp to give acids **8a** and **8b**. Aldehyde **4** failed to give the corresponding acid upon treatment with ttmsp so it was reacted with diethylphosphite, adsorbed onto basic alumina using a procedure described by Texier–Boullet[12], to give the diester **5**. Treatment of **5** with trimethylsilyl bromide (TMSBr) gave the acid **6**.

Scheme 3 shows an adaptation of Fry's procedure[13] for the synthesis of aldehyde **10** from commercially available **9**. The nitrile is alkylated with isopropyl chloride to give the nitrilium ion which is reduced using triethyl silane to the imine. This imine is readily hydrolysed to the aldehyde.

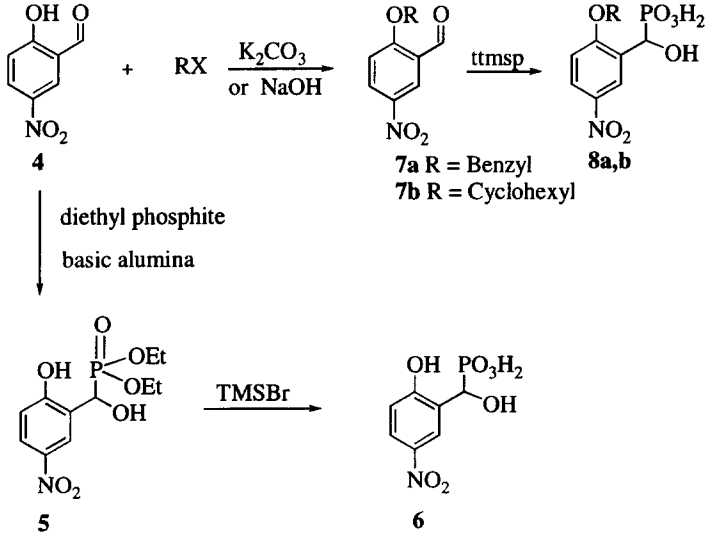
The synthesis of the benzylmethylene substituted derivatives **15**, **19**, and **21** are shown in Scheme 4. The commercially available aldehyde **12** was reduced by sodium borohydride in ethanol to give the alcohol **13**. The alcohol was brominated with phosphorus tribromide and subsequently heated in triethylphosphite in Arbuzov fashion to give the diester **14**. Treatment of the diester with TMSBr afforded the acid **15**. Aldehyde **12** was directly converted to the hydroxydiester **16** by reaction with diethyl phosphite on basic alumina. The Mitsunobu reaction with phthalimide gave **17**, albeit in low yield. Methanolic hydrazine removed the phthalimide giving **18**. Dealkylation with TMSBr gave **19**. Compound **16** was also reacted with diethylamino sulfur trifluoride (DAST) to give the fluorinated diester **20**. Dealkylation with TMSBr gave the acid **21**.

Scheme 5 shows the synthesis of thiophene and furan derivatives. Aldehyde **22d**, 3-bromo-5-nitrothiophene-2-carboxaldehyde (Table 2) was synthesized according to Gronowitz.[14] Aldehyde **22e**, 4-nitrothiophene-2-carboxaldehyde was synthesized using the method of Foye.[15] The rest of the aldehydes are commercially available and all were reacted with ttmsp to give the target acid **23**.

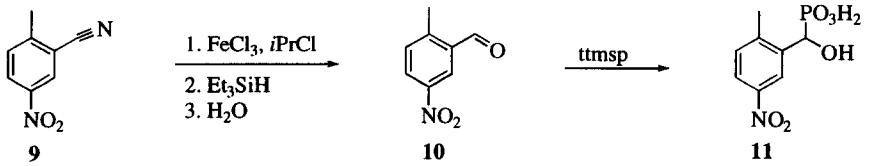
Scheme 6 shows the synthesis of compounds **25a** and **25b**. The aldehyde **24b** was synthesized according to the methods of Snieckus.[16] Scheme 7 shows the facile use of ttmsp on ketone **26** to give the alpha methyl hydroxymethyl phosphonic acid **27**.

Results and Discussion

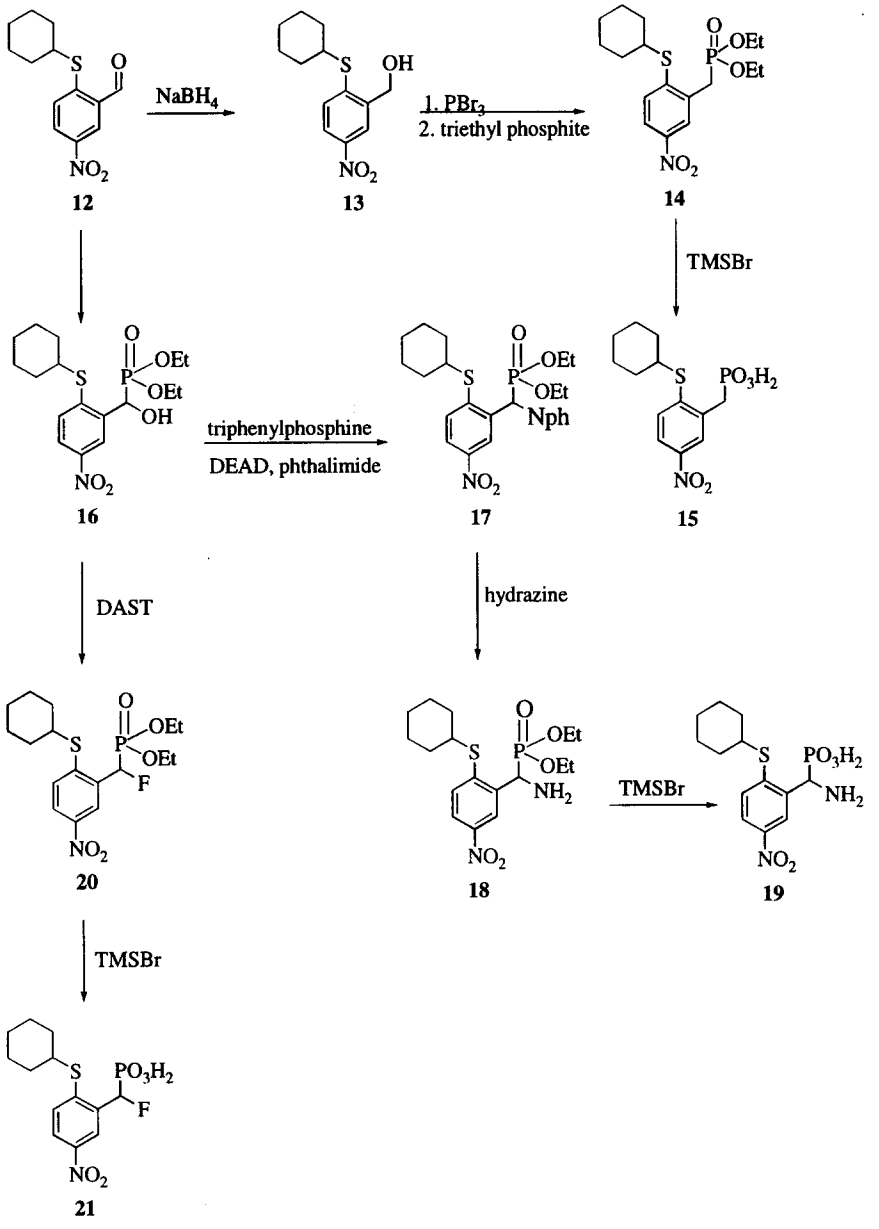
Table 1 shows the inhibition at 50 μM and/or IC_{50} values for all of the phenyl analogues. The negligible inhibition exhibited by compounds **6** and **11** at 50 μM is very interesting when compared to compounds **2a** and **2b**. The methyl group in **11** lacks the pi electron density of



Scheme 2.

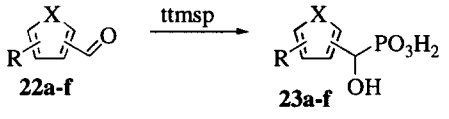


Scheme 3.

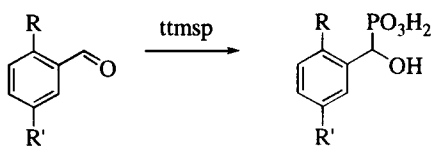


Scheme 4.

the halogens. Compound **6** was also all but devoid of inhibitory activity at 50 μM suggesting that a pi electron rich but nonpolar moiety is optimal for potency. The potency of **3a** seemed to confirm this hypothesis. The phenoxy derivatives in every instance were found to be more potent than their phenylthio counterparts. The potency of the 4-bromophenoxy analogue **3c**, as well as the active but less potent analogue **3m** are both indicative of how large the lipophilic pocket is which accommodates the 2-substituent. It is interesting to note that although the phenoxy derivatives were more potent than the phenylthio compounds the benzyloxy and cyclohexyloxy compounds **8a** and **8b** were both far less potent than their benzylthio (**3x**) and cyclohexylthio (**3mm**) counterparts. Indeed while the cyclohexyloxy



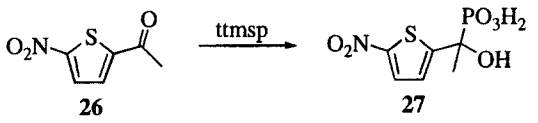
Scheme 5.



24a R = 4-Cl-phenylthio
R' = H
24b R = phenyl
R' = nitro

25a,b

Scheme 6.



26 27

Scheme 7.

compound showed 50% inhibition of CD45 and 50 μM compound **3mm** showed an IC_{50} of 4μM. The 2-thiosubstituted compounds show superior potency when there is at least one methylene group separating the sulfur atom from the phenyl ring. There is no significant difference in potency between the benzylthio compound **3x** and the homologues **3hh** (phenylethylthio), **3jj** (phenylpropylthio), and **3kk** (phenylbutylthio). The alkylthio compounds **3ll** and **3oo**, and cycloalkylthio analogues **3mm** and **3nn**, are also equipotent to each other as well as the phenylalkylthio derivatives. The lack of potency observed for compound **3n** is possibly due to its zwitterionic nature, which would place a positive charge on the nitrogen atom thereby eliminating its chance of binding at what is obviously a large but lipophilic region of the active site.

The complete lack of inhibition of **25a** at 50 μM compared to **3t** shows the importance of the nitro group. The greatly diminished potency of **25b** indicates that the perpendicular biphenyl conformation at the 2-position is definitely not optimal.

As Table 2 shows, 5-nitrothiophene-2-hydroxymethyl phosphonic acid (**23b**) is by far the best isomer of the thiophene series with an IC_{50} of 4 μM. The furanyl derivative **23a** was much less potent showing only 30–35% inhibition at 50 μM. Replacement of the nitro group with a methane sulfonyl as in **23f** abolished all inhibitory activity. Placement of a lipophilic group (bromine atom) at the 3-position gave **23d**, which showed a slight increase in potency (IC_{50} = 2 μM) analogous to the SAR of the phenyl series. This bromine atom can be quite instrumental for the introduction of many other moieties at the 3-position using well established coupling procedures (Stille, Castro–Stevens, Suzuki and so on) will be the subject of future investigations.

Table 3 shows the dramatic effects of altering the benzylhydroxyl moiety. Compound **15** illustrates the importance of the hydroxyl group (0% inhibition at 50 mM vs IC_{50} of 4 mM for compound **3mm**). The inability of either the amino or fluoro groups to replace the hydroxyl group is shown in compounds **19** and **21**. Finally the addition of a methyl group on the alpha carbon as in **27** also resulted in an inactive compound.

As Figures 1 and 2 show, the phenyl analogue **3nn** and the thiophene derivative **23b** are both competitive inhibitors of CD45. This represents the first reported series of compounds which are known to bind at the active site of CD-45.

Experimental

All melting points were taken on a Mel-Temp II open capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were taken on a General Electric QE-300 spectrometer. Signals are reported in parts per million using tetramethysilane as an internal standard. For column chromatography, silica gel 60 (Merck) was used. The basic alumina (activated) was purchased from ICN Biomedicals. Mass spectra (DCI) were obtained with a Finnigan MAT Incos 50 single quadrupole mass spectrometer while NEG FABMS were obtained using a Finnigan TSQ700 triple stage quadrupole. The matrix used was an equal volume each of glycerol, 2-thioglycerol and *m*-nitrobenzyl alcohol.

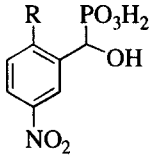
Expression of CD45

Recombinant CD45 cytoplasmic domain was produced in *Spodoptera frugiperda* Sf9 cells. DNA encoding Lys575 to Ser1281 as obtained from mRNA from human thymus (Clontech) by reverse transcription and the polymerase chain reaction. The cDNA was engineered into plasmid pAcUw43 (Pharmingen) for expression from the *Autographa californica* nuclear polyhedrosis virus p 10 promoter, and transferred into the virus genome by homologous recombination in Sf9 cells. Protein was harvested from Sf9 cells 2–3 days after infection with recombinant virus. The recombinant CD45 cytoplasmic domain was partially purified by sequential precipitation with ammonium sulfate.[17] Solid ammonium sulfate to 40% saturation was stirred into clarified lysates on ice, then centrifuged to recover the supernatant and precipitated again to 80% saturation. Precipitated material was pelleted by centrifugation, solubilized in 25 mM Hepes 50 mM NaCl 1mM DTT and dialysed against multiple changes of the same buffer prior to freezing in 10% glycerol with protease inhibitors.

CD45 Inhibitory assay

The assay used was a slight modification of the Malachite Green colorimetric assay described by Fisher.[18] Semi-purified CD45 (20 μL) in a pH 6.8 imidazole buffer was added to wells of a Corning Easy-Wash plate. Next, 10 μL of a compound diluted in 12.5% DMSO was added. Finally 20 μL of the fyn phosphorylated peptide, FTATEPQ(PHOSPHO)YQPGENL, was added to start the reaction which was allowed to proceed for 30 min at ambient temperature. The Malachite Green reagent (100 μL of 2.6 mM Malachite Green in 3.6 M sulfuric acid) was then added and the plate was reincubated for 30 min at ambient temperature. The optical density (OD) was then read on a Molecular Devices V_{max} spectrophotometer at 650 nm. Under these conditions color formation was linear between 0.5 and 10 nmol phosphate per well.

Table 1.



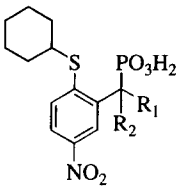
Compd	R	Percentage inhibition at 50 μM	IC_{50} (μM)	Mp (°C)	^1H NMR (DMSO) δ
2a	Cl	92,38[a]		220–220.5	4.98 (d, 1H)
2b	F	65		226–231	4.74 (d, 1H)
3a	N_3	92	8	190–190.5	4.82 (d, 1H)
3b	Phenoxy	90	6	211–213	5.05 (d, 1H)
3c	4-Br-Phenoxy	92	2	217–219	5.01 (d, 1H)
3d	4-Cl-Phenoxy	93	4	199.5–201	5.01 (d, 1H)
3e	2, 3-Di-Cl-phenoxy[b]		8	98–107	5.27 (d, 1H)
3f	2, 4-Di-Cl-phenoxy		7	163–168	5.27 (d, 1H)
3g	2-F-phenoxy[b]		3	105–110	5.29 (d, 1H)
3h	2-CH_3-Phenoxy	78	8	211.5–213	5.12 (d, 1H), 2.18 (s, 3H)
3i	3-CH_3-Phenoxy	86	8	197.5–199	5.05 (d, 1H), 2.30 (s, 3H)
3j	4-CH_3-phenoxy	92	11	175–176	5.05 (d, 1H), 2.31 (s, 3H)
3k	4-*t*-Bu-phenoxy	81	8	144–146	5.11 (d, 1H)
3l	3-Cyanophenoxy	20		148–150	4.93 (d, 1H)
3m	2-Naphthoxy[b]		12	113–118	5.32 (d, 1H)
3n	*N*-Methyl-*N*-benzyl	0		91–95	5.26 (d, 1H), 2.67 (s, 3H)
3o	Phenylthio	81, 43[a]		229–231	5.15 (d, 1H)
3p	2-Pyridylthio	94	10	219–206	5.19 (d, 1H)
3s	3-CH_3-Phenylthio	55		180–190	5.15 (d, 1H), 2.29 (s, 3H)
3t	4-Cl-Phenylthio	79, 37[a]		229–232	5.15 (d, 1H)
3u	4-*t*-Bu-phenylthio	63		168–175	5.13 (d, 1H)
3v	4-CH_3-Phenylthio	54		210–214	5.20 (d, 1H), 2.35 (s, 3H)
3w	2-Naphthylthio	50		201–210	5.22 (d, 1H)
3x	Benzylthio	93	5	219–221	5.00 (d, 1H), 4.35 (dd, 2H)
3y	2-Phenylbenzylthio		8	207–215	4.97 (d, 1H)
3z	(4-Phenyl)benzylthio	90	6	195–04	5.04 (d, 1H)
3z	3-Cyanobenzylthio[b]		4	150–155	5.19 (d, 1H), 4.46 (m, 2H)
3aa	4-Br-Benzylthio[b]		10	176–180	5.16 (d, 1H), 4.39 (dd, 2H)
3bb	4-Cl-Benzylthio		8	223–225	4.96 (d, 1H), 4.39 (s, 2H)
3cc	4-Ethylbenzylthio	90	8	218–219	4.92 (d, 1H), 4.29 (dd, 2H)
3dd	4-F-Benzylthio	92	7	211–218	5.00 (d, 1H), 4.33 (dd, 2H)
3ee	4-*t*-Bu-benzylthio	88	9	208–210	4.94 (d, 1H), 4.30 (dd, 2H)
3ff	3-CH_3-Benzylthio	87	8	195–196	4.98 (d, 1H), 4.29 (dd, 2H)
3gg	4-CH_3-Benzylthio	90	4	207–209	4.97 (d, 1H), 4.28 (dd, 2H)
3hh	Phenethylthio	90	6	225–226	4.95 (d, 1H), 3.30, 2.90 (t, 2H)
3ii	4-CH_3-Phenethylthio		15	223–225	4.95(d, 1H), 3.25, (t, 2H)
3jj	Phenylpropylthio	92	4	196–202	5.59 (d, 1H) 3.06 (t, 2H)
3kk	Phenylbutylthio	80	4	210–220	5.56 (d, 1H)
3ll	*n*-Hexylthio	94	6	208–212	4.99 (d, 1H), 3.03 (t, 2H)
3mm	Cyclohexylthio	97	4	217–220	5.05 (d, 1H)
3nn	Cycloheptylthio	95	4	190–200	5.04 (d, 1H)
3oo	*n*-Decylthio		6	215–220	4.97 (d, 1H), 3.02 (t, 2H)
6	OH	9		267 (dec)	4.65 (d, 1H)
8a	Benzyloxy[b]	50		178–182	5.29 (d, 2H), 5.21 (d, 1H)
8b	Cyclohexyloxy[b]	50		148–152	5.14 (d, 1H), 2.50 (s, 3H)
11	Methyl	10		202–204	4.75 (d, 1H), 2.50 (s, 3H)
25a	4-Cl-Phenylthio	0		173–178	5.16 (d, 1H, PCH)
25b	Phenyl	35		216–217	4.63 (d, 1H)

[a]Inhibition at 10 μM.
[b]Isolated as the anilinium salt.

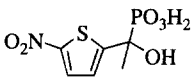
Derivation of Lineweaver–Burk

The CD45 enzymatic assay was carried out with four different concentrations of the fyn peptide substrate (125, 250, 500 and 1000 μM) and four different concentrations of compounds **3nn** and **23b** (see Figs 1 and 2). The assay was carried out as described above except that the fyn peptide and the inhibitor were

Table 3.



15, 19, 21



27

Compound	R_1	R_2	Percentage inhibition at 50 μM
15	H	H	0
19	NH_2	H	0
21	F	H	0[a]
27	OH	CH_3	0

[a]Tested at 20 μM.

premixed before being added to the assay wells already containing CD45 in order to expose the enzyme to both substrate and inhibitor at the same time. The data are plotted as the inverse of the initial rate of reaction (nmol PO_4 released per hour) vs. the inverse of the substrate concentration.

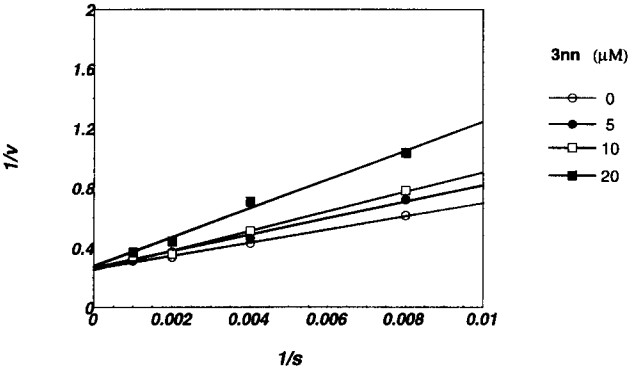


Figure 1.

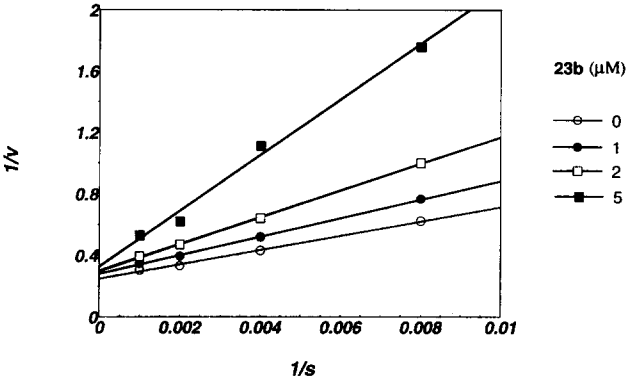


Figure 2.

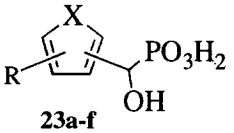
Derivation and analysis of data

The net increase in optical density at 650 nm (net OD) was calculated as the OD in the presence of enzyme and substrate (with and without test compound present) minus the OD obtained when only the substrate was present (background OD). The percent inhibition of enzyme activity was calculated as follows:

$$\% \text{ inhibition} = \left[1 - \left(\frac{\text{net OD with inhibitor}}{\text{net OD without inhibitor}}\right)\right] \times 100$$

General method for synthesis of 2-phenoxy compounds (3b–3m). Synthesis of compound **3b** is typical of the procedure. Into a 200 mL flask was placed DMF (50 mL), **1a** (2.56 g, 14.0 mmol), phenol (1.56 g, 17.0 mmol), and potassium hydroxide (1 g). The reaction was stirred at 100°C for 1.5 h before being poured into water. The product was extracted with ethyl acetate. The layers were separated and the organic layer was washed with water, dried over magnesium sulfate and evaporated to an oil (1.62 g, 48%). This oil was not further purified but was dissolved in THF and ttmsp (2.35 mL, 7.00 mmol) was added and the reaction was stirred for 3 h before being evaporated to an oil which was dissolved in ethanol:ether (1:1). Cyclohexylamine (1.00 mL, 8.40

Table 2.



23a-f

Compd	X	R	Acid[a]	IC_{50} (μM)	Mp °C	^{1}H NMR (DMSO-d_6) δ
23a	O	5-NO_2	2	>50	184 (dec)	4.55 (d, 1H)
23b	S	5-NO_2	2	4	185 (dec)	7.84 (d, 1H), 5.27 (d, 1H)[b]
23c	S	5-NO_2	3	48	221–224	8.15, 7.68 (s, 1H), 4.77 (d, 1H)[b]
23d	S	3-Br-5-NO_2	2	2	178–180	8.18 (s, 1H), 4.96 (d, 1H)
23e	S	4-NO_2	2	>50	222–225	8.61, 7.50 (s, 1H), 4.70 (d, 1H)
23f	S	5-SO_2CH_3	2	50	198–200	5.00 (d, 1H), 3.28 (s, 3H)

[a]Position of acid.
[b]Acetic acid-d_4 was used as the solvent.

mmol) was added and the resulting solid was filtered, washed with ether, and dried under vacuum to give 2.14 (36%). An analytical sample was prepared by recrystallization from ethanol. Calcd for $C_{19}H_{25}N_2O_7P \cdot 0.25\ H_2O$: C, 53.21 H, 5.99 N, 6.53. Found: C, 53.25 H, 6.03 N, 6.49. Melting points and ^1H NMR data for these compounds is shown in Table 1.

2-Benzylmethylamino-5-nitrobenzaldehyde. Aldehyde **1a** (1.00 g, 5.22 mmol) was dissolved in DMF (30 mL). Methylbenzylamine (0.70 mL, 5 .22 mmol) and potassium carbonate (1 g) were added. The reaction was stirred at 80°C for 1.5 h. The mix was then poured into water and the product was extracted with ethyl acetate. The organic layer was washed with water, dried over magnesium sulfate, and concentrated to an oil that was chromatographed on a silica gel column eluted with hexane:ethyl acetate (4:1) giving 0.89 g (62%) of the title compound; mp 71–72°C; ^1H NMR ($CDCl_3$) δ 10.08 (s, 1H), 4.63 (s, 2H), 3.07 (s, 3H).

General method for the synthesis of 2-thio compounds (3o–3oo). The synthesis of compound **3dd** is typical of the procedure used. Compound **1a** (2.22 g, 12.00 mmol) was dissolved in dimethylformamide (30 mL). To this was added 4-fluorobenzylmercaptan (1.70 g, 1 2.00 mmol) and potassium hydroxide (1 g). This was heated to 100 °C for 15 min before being poured into water. The product was extracted with ethyl acetate and the organic layer was washed with water, dried over magnesium sulfate and concentrated in vacuo to an oil that was dissolved in tetrahydrofuran (25 mL). To this solution was added ttmsp (3.97 mL, 12.0 mmol). This was stirred under nitrogen at ambient temperature for 3 h before being evaporated in vacuo. The residue was dissolved in ethanol (20 mL). Cyclohexylamine (1.37 mL, 12.0 mmol) was added and the solid was filtered and washed with ether to give 1.85 g (32.5% from **1a**). An analytical sample of the cyclohexylammonium salt was prepared by recrystallization from ethanol–ether. FABMS 372 (M-H); Calcd for $C_{20}H_{26}FN_2O_6PS$: C, 50.84 H, 5.55 N, 5.93. Found: C, 50.52 H, 5.42 N, 5.93. Melting points and ^1H NMR data for these compounds is shown in Table 1.

***O,O*-Diethyl-2-hydroxy-5-nitrophenylhydroxymethyl phosphonate (5)**. Compound **4** (2.96 g, 18.0 mmol) was mixed with diethyl phosphite (2.30 mL, 18.0 mmol) and dichloromethane (2 mL). The resulting clear solution was adsorbed onto basic alumina and this stood for 16 h before being extracted with dichloromethane: methanol (19:1). The extract was concentrated on a rotovap and chromatographed on a silica gel column eluted with ethyl acetate: dichloromethane (1:1) giving **5** (3.66 g, 67%) as an oil. ^1H NMR (DMSO-d_6) δ 5.26 (d, 1H, PCH).

2-Hydroxy-5-nitrophenylhydroxymethylphosphonic acid (6). The ester **5** (3.66 g, 12.0 mmol) was dissolved in dichloromethane. Bromotrimethylsilane (10.0 mL, 73.5 mmol) was added and the reaction was stirred under nitrogen for 16 h after which time the mixture was concentrated to an oil. The crude product was dissolved in ethanol and propylene oxide (2 mL) and cyclohexylamine (1.4 mL, 12.0 mmol) were each added. The product was filtered and washed with ether to give 2.12 g (48%) of **6**; mp 267–268 °C (dec); ^1H NMR (DMSO-d_6) δ 8.28 (s, 1H), 7.93 (d, 1H), 6.76 (d, 1H), 4.65 (d, 1H, PCH). Calcd for $C_{13}H_{21}N_2O_7P \cdot H_2O$: C, 42.63 H, 6.33 N, 7.65. Found: C, 42.90 H, 6.47 N, 7.52.

2-Benzyloxy-5-nitrobenzaldehyde (7a). 2-Hydroxy-5-nitrobenzaldehyde (1.00 g, 5.98 mmol) was dissolved in dimethylformamide (15 mL). To this was added potassium carbonate (1.65 g, 11.97 mmol) and benzyl bromide (0.71 mL, 5.98 mmol). This mixture was stirred at 65 °C for 4 h before being poured into ice. The resulting solid (1.35 g, 88%) was filtered, washed with water, and dried under vacuum. It was used without further purification. Mp 119–121 °C ^1H NMR ($CDCl_3$) δ 10.51 (s, 1H), 8.75 (d, 1H), 8.41 (dd, 1H), 7.43 (s, 5H), 7.20 (d, 1H), 5.31 (s, 2H).

2-Methyl-5-nitrobenzaldehyde (10). Compound **9** (2.00 g, 12.3 mmol) was dissolved in isopropyl chloride (80 mL). The solution was cooled in an ice–water bath before addition of ferric chloride (2.00 g, 12.3 mmol). The reaction was then refluxed for 5 h after which time the mixture was concentrated in vacuo. To the residue was added dichloro methane (100 mL) and triethylsilane (2.60 mL, 16.0 mmol). This was again refluxed for 1 h. The mixture was cooled and water (25 mL) was added. After additional stirring for 15 min the layers were separated. The organic layer was dried over magnesium sulfate and concentrated to an oil which was chromatographed on a silica gel column eluted with ethyl acetate: hexane (1:6) giving 1.20 g (60%) of **10**. Spectral data agree with that in the literature.[19]

2-Cyclohexylthio-5-nitrobenzyl alcohol (13). The aldehyde **12** (10.0 g, 38.0 mmol) was dissolved in ethanol. Sodium borohydride (1.44 g, 38.0 mmol) was added and the reaction was stirred at ambient temperature for 30 min before being quenched with water. The mixture was concentrated in vacuo and partitioned between water and methylene chloride. The organic layer was dried over magnesium sulfate and evaporated in vacuo to give a solid (9.10 grams, 91%). Mp 86–87 °C.

***O,O*-Diethyl-2-cyclohexylthio-5-nitrobenzylphosphonate (14)**. The alcohol **13** (3.77 g, 14.0 mmol) was dissolved in dichloromethane (50 mL). Phosphorus tribromide (0.66 mL, 7.00 mmol) was added and the reaction was stirred under nitrogen for 16 h after which time the mixture was evaporated in vacuo to an oil. Triethyl phosphite (4.80 mL, 28.0 mmol) was added and this was heated to 130°C for 2H. The excess phosphite was distilled under vacuum and the crude product was chromatographed on a silica gel

column eluted with ethyl acetate:hexane 1:1 giving 2.90 g (54%) of **14** as an oil.

2-Cyclohexylthio-5-nitrobenzylphosphonic acid (15). The diethyl ester **14** (2.84 g, 7.33 mmol) was dissolved in dichloromethane (30 mL). Bromotrimethylsilane (3.87 L, 29.3 mmol) was added and this was allowed to stand for 16 h before being evaporated in vacuo. The residue was then dissolved in ethanol–ether and cyclohexylamine (0.84 mL) was added. The solid was filtered and washed with ether giving 1.55 g of the phosphonic acid (49.3%). An analytical sample was prepared by recrystallization from ethanol. Mp 208–210 °C; ^{1}H NMR (DMSO-d_6) δ 2.82 (d, 2H, PCH) calcd for $C_{19}H_{31}N_2O_5PS$: C, 53.01 H, 7.26 N, 6.51. Found: C, 53.18H, 7.22 N, 6.43.

***O,O*-Diethyl-2-cyclohexylthio-5-nitrophenylhydroxymethylphosphonate (16).** Diethyl phosphite (0.94 mL, 7.27 mmol) was mixed with 2-cyclohexylthio-5-nitrobenzaldehyde (1.93 g, 7.27 mmol), dichloromethane (5 mL), adsorbed onto basic alumina and allowed to stand for 16 h. The product was then extracted with dichloromethane and evaporated in vacuo. The residue was chromatographed on a silica gel column eluted with ethyl acetate:hexane (1:1) to give 1.85 g (63%) of **16**. Mp 145–146 °C; MS (CI) 404 (MH^+); ^{1}H NMR (DMSO-d_6) δ 8.37 (s, 1H), 8.12 (d, 1H), 7.73 (d, 1H), 6.65 (dd, 1H, OH), 5.40 (dd, 1H, PCH), 4.00 (m, 4H, OCH_2), 1.16 (t, 6H, CH_3); calcd for $C_{17}H_{26}NO_6PS$: C, 50.61 H, 6.50 N, 3.47. Found: C, 50.50 H, 6.57 N, 3.43.

***O,O*-Diethyl-*N*-phthalimido-(2-cyclohexylthio-5-nitro)-phenylmethyl phosphonate (17).** The ester **16** (5.67 g, 14.09 mmol) was dissolved in tetrahydrofuran (75 mL). To it was added triphenyl phosphine (4.69 grams, 17.89 mmol), phthalimide (2.07 grams, 14.09 mmol), and DEAD (2.68 mL, 17.04 mmol). The resulting reddish-yellow solution was stirred under nitrogen at ambient temperature for 20 h. The mixture was then evaporated in vacuo to an oil which was chromatographed on a silica gel column. Elution with ethyl acetate:hexane (1:1) afforded **17** (0.91 g, 12.13%). MS (CI) 533 (MH^+).

***O,O*-Diethyl-amino-2-cyclohexylthio-5-nitrophenylmethylphosphonate (18).** The phthalimide compound **17** (0.91 g, 1.71 mmol) was partially dissolved in methanol (35 mL). Hydrazine hydrate (0.25 mL) was added and the mixture was refluxed for 6 h. Upon cooling a solid formed which was filtered. The filtrate was evaporated in vacuo and the residue was triturated with ether. The insoluble material was filtered and the filtrate was evaporated in vacuo to give the product (0.63 g, 91.6%) as a yellow gum. MS (CI) 403 (MH^+).

Amino-2-cyclohexylthio-5-nitrophenymethyl phosphonic acid (19). The ester **18** (0.63 g, 1.56 mmol) was dissolved in dichloromethane (20 mL), bromotrimethylslane (1.24 mL, 9.39 mmol) was added and the resulting yellow solution was stirred under nitrogen for 5 h after which time the mixture was evaporated in vacuo. The residue was dissolved in ethanol (10 mL) and treated with propylene oxide (1 mL). The solution was evaporated in vacuo and triturated with ether to give **19** (0.38 g, 70%). Mp 190–200 °C; MS (CI) 347 (MH^+); ^{1}H NMR (DMSO-d_6) δ 8.66 (s, 1H), 8.07 (d, 1H), 7.69 (d, 1H), 4.68 (d, 1H, PCH), 3.72–3.25 (m, 1H, SCH).

***O,O*-Diethyl-2-cyclohexylthio-5-nitrophenylfluoromethyl phosphonate (20).** The starting material (2.80 g, 6.94 mmol) was dissolved in dichloromethane (40 mL) and cooled to −78 °C. DAST (1.06 mL, 8.00 mmol) was added and the dry-ice bath was removed. The reaction mixture was stirred to ambient temperature for an additional 2.5 h. Water was added and the layers were separated. The organic layer was dried over magnesium sulfate and concentrated in vacuo to an oil that was chromatographed on a silica gel column eluted with ethyl acetate:hexane (1:1) to give 1.86 g (66%) as an oil. MS (CI) 406 (MH^+); ^{1}H NMR (DMSO-d_6) δ 8.25 (m, 2H), 7.84 (d, 1H), 6.35 (dd, 1H, PCH), 4.07 (m, 4H, OCH_2), 3.62 (m, 1H, SCH), 1.22 (dt, 6H, CH_3).

2-Cyclohexylthio-5-nitrophenylfluoromethylphosphonic acid cyclohexylammonium salt (21). The ester **20** (1.85 g, 4.56 mmol) was dissolved in dichloromethane (30 mL). Bromotrimethylsilane (2.41 mL, 18.24 mmol) was added and the mixture stood under nitrogen for 16 h, after which time the mixture was concentrated in vacuo to an oil that was dissolved in methanol (15 mL) and water (15 mL). Addition of propylene oxide (1 mL) followed by cyclohexylamine (0.53 mL, 4.56 mol) caused precipitation of a solid which was filtered and washed with water to give 1.15 g of **21** (58%). Mp 207–208 °C. ^{1}H NMR (DMSO-d_6) δ 8.46 (s, 1H), 8.08 (d, 1H), 7.67 (d, 1H), 5.85 (dd, 1H, PCH); FABMS 348 (M-H); calcd for $C_{19}H_{30}FN_2O_5PS$: C, 50.88 H, 6.74 N, 6.25. Found: C, 50.74 H, 6.81 N, 6.13.

Synthesis of compounds 23a–f. The synthesis of compound **23b** is typical of the procedure used. Aldehyde **22b** (11.33 g, 72.0 mmol) was dissolved in THF (150 mL). To this was added ttmsp (24.1 mL, 72.0 mmol). After being stirred for 3h the mixture was concentrated to an oil that was dissolved in ethanol. Cyclohexylamine (16.5 mL, 144 mmol) was added and the solid which formed was filtered and washed with ethanol to give 19.40 g (80%) of **23b**. An analytical sample was prepared by recrystallization from ethanol–water. Calcd for $C_{11}H_{19}N_2O_6PS$: C, 39.05 H, 5.66 N, 8.28. Found: C, 39.03 H, 5.67 N, 8.15. Melting points and ^{1}H NMR data for compounds **23a–f** are listed in Table 2.

1-(5-Nitro)thien-2-yl-1-hydroxyethanephosphonic acid cyclohexylammonium salt (27). The aldehyde **26** (Maybridge) 2.27 g (13.3 mmol) was dissolved in THF (50 mL). Ttmsp (4.43 mL, 13.3 mmol) was added and

the reaction was stirred for 16 h under nitrogen. The mix was then evaporated to an oil that was dissolved in ethanol–ether and 1:52 mL (13.3 mmol) of cyclohexylamine was added. The product was filtered and washed with ether to give 2.60 g (55%) of **27**. Mp 185–188 °C (dec). ^{1}H NMR (DMSO-d_6) δ 7.92 (d, 1H), 6.98 (d, 1H), 1.60 (d, 3H). FABMS 252.2 (M-H). Calcd for $C_{12}H_{21}NO_6PS{\cdot}0.25\ H_2O$: C, 40.39 H, 6.07 N, 7.85. Found: C, 40.42 H, 6.34 N, 7.59.

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