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Bis[(para-methoxy)benzyl] phosphonate prodrugs with improved stability and enhanced cell penetration

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Abstract—A series of substituted bis[(para-methoxy)benzyl] (bisPMB) esters of 1-naphthalenemethylphosphonate (NMPA) were synthesized and evaluated as phosphonate prodrugs. BisPMB NMPA esters (**4b** and **4c**) with significantly improved aqueous stability were identified that also resulted in increased intracellular levels of NMPA following prodrug incubation with primary rat hepatocytes.

Phosphonic acids represent a functional group that is rarely found amongst potential drug candidates largely because at physiological pH phosphonic acids are negatively charged and therefore often exhibit low oral bioavailability and poor cell penetration.[1] To circumvent one or both of these limitations, efforts have been ongoing over the past twenty years to identify suitable phosphonate prodrugs.[2] Most phosphonate prodrug classes depend on carboxylic acid esterases to initiate a multi-step cleavage mechanism that results in the hydrolysis of the phosphonate ester. Examples include the acyloxyalkyl esters,[3,4] S-acyl-2-thioethyl (SATE) esters[5] and more recently a series of monoamidates[6,7] and bisamidates.[8] While highly successful in improving cell penetration and in some cases oral bioavailability, some of these prodrugs often fail to deliver high levels of the phosphonic acid to tissues in large part because prodrug cleavage is fast and as a consequence circulating prodrug levels are low.

Efforts to find phosphonate prodrugs that cleave by a non-esterase dependent mechanism have led to the discovery of two classes of prodrugs that have advanced into human clinical trials, namely bisphenyl esters[9] and HepDirect prodrugs.[10,11] Bisbenzyl esters have also been explored but the simple unsubstituted benzyl ester cleaves too slowly to be of use as a prodrug. Efforts to enhance the rate of prodrug cleavage led to bisbenzyl prodrugs containing a para-*O*-acyl[12,13] substituent that is rapidly cleaved by esterases to the corresponding phenol which in turn undergoes rapid, non-enzymatic breakdown to the phosphonic acid.

To discover phosphonate prodrugs with increased chemical stability that cleave by a non-esterase-mediated mechanism, we investigated various 4-alkyloxybenzyl esters as potential phosphonate prodrugs, since *O*-dealkylation of alkoxyphenyl derivatives occurs in the liver and is mediated by cytochrome P(450) (CYP) enzymes.[14,15] As outlined in Scheme 1, bisPMB phosphonate esters are expected to enter hepatocytes by passive diffusion and then undergo rapid CYP-mediated dealkylation to generate the corresponding phenol, which then undergoes elimination of quinone methide to generate the monophosphonic acid. Cleavage of the second PMB group generates the parent phosphonic acid. The by-product generated resulting from prodrug release, a quinone methide or its derivatives, is expected to react rapidly with glutathione, a thiol that exists at high concentrations inside the liver and is known to rapidly detoxify electrophiles.[16]

To investigate the potential of bisPMB phosphonate esters as prodrugs, our strategy was to first test this concept in rat hepatocytes using a model phosphonic acid.

Keywords: Phosphonate; Prodrug; Bis[(para-methoxy)benzyl] phosphonate ester.
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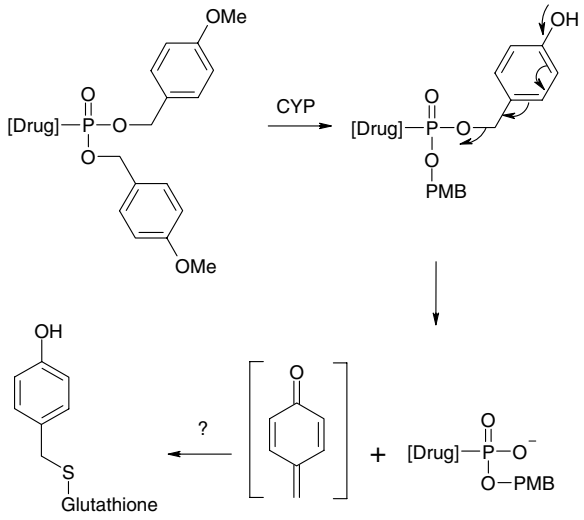

Scheme 1. Activation of bisPMB phosphonate prodrugs.

Should a bisPMB phosphonate ester be identified with desired properties such as good intracellular activation, good chemical and plasma stability, then the hope was that it could be applied to phosphonic acids with biological activity (e.g., a FBPase inhibitor) and that a pharmacological readout (e.g., glucose lowering) could then be used to test in vivo activity. Previously, we reported that studies with 1-naphthalenemethylphosphonate (NMPA, **1**) as the model phosphonic acid allowed us to identify a series of 3-phthalidyl esters as potential prodrugs.[4] Herein, various NMPA bisPMB esters (**4a**–**m**, Fig. 1) were synthesized and evaluated for prodrug activation in primary rat hepatocytes and prodrug stability in both aqueous solutions and rat plasma.

BisPMB NMPA esters (**4a**–**m**) were synthesized via a two-step procedure entailing conversion of NMPA to its corresponding dichloridate using oxalyl chloride in the presence of a catalytic amount of *N*,*N*-dimethylformamide (DMF) followed by reaction of the dichloridate with various benzyl alcohols in the presence of pyridine.[17] Isolated yields are shown in Table 1.

NMPA bisPMB esters (**4a**–**m**) were evaluated for aqueous and rat plasma stability as well as activation in cells, Table 1. Aqueous stability was determined in Krebs bicarbonate buffer at 37 °C; while plasma stability was determined by using procedures previously described.[4] NMPA bisPMB esters (**4a**–**m**) were also evaluated for activation in primary rat hepatocytes by measurement of intracellular levels of NMPA following 1 h of exposure to prodrug (100 μM). Cells were separated from medium by centrifugation through an oil layer and extracted with methanol (60%). Analysis of prodrug and NMPA levels in aqueous media or cells was performed by reverse phase HPLC using a Beckman Ultrasphere column (5 μ, 4.6 × 250 mm). A gradient was run from 10% to 80% acetonitrile in 20 mM potassium phosphate, pH 6.4, buffer over 20 min. The elution time of NMPA was ~4.6 min.

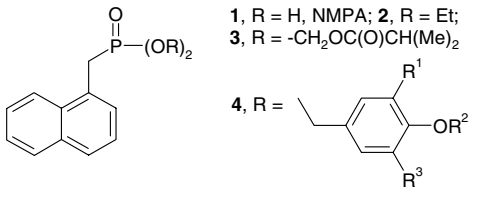

Figure 1. NMPA esters as potential prodrugs.

As expected, NMPA (**1**) and its simple diethyl ester **2** did not produce detectable intracellular levels of NMPA (entries **1**–**2**, Table 1). On the other hand, bis[(para-methoxy)benzyl]NMPA (**4a**) was converted to NMPA inside rat hepatocytes, and more importantly it generated similar NMPA levels (91 μM) compared to bis(isobutyryloxymethyl)NMPA (**3**, 85 μM), indicating an excellent degree of prodrug activation. However, **4a** exhibited a short half-life of 9 min in Krebs bicarbonate confirming the lack of chemical stability. Consequently, two approaches were taken to further stabilize **4a**. First, changing the electronics of the phenyl ring was investigated via exploration of both electron-donating and electron-withdrawing substituents. Introduction of electron-donating groups was detrimental to stability, as 3-methyl and 3-methoxy analogues of **4a**, although detectable by TLC, were too unstable to be isolated. In contrast, electron-withdrawing groups such as fluoro and chloro groups led to a significant improvement in aqueous stability. For example, the 3-fluoro (**4b**), 3-chloro (**4c**) and 3-bromo (**4d**) analogues showed much improved half-lives in Krebs bicarbonate: $t_{1/2} > 120$ min. More importantly, there is no diminution in prodrug activation for **4b** and **4c** judging by the high levels of NMPA generated in hepatocytes. These bisPMB esters (**4a**–**4d**) were also tested for plasma stability and they showed the exact profiles as in the aqueous stability assay. Therefore, for all other compounds the plasma stability assay was used to evaluate both chemical and plasma stability and results are summarized in Table 1. The second approach was to alter the methoxy group on the phenyl ring of **4a**. The *O*-ethyl (**4e**) and *O*-propyl (**4f**) analogues of **4c** also gave greater stability than **4a** due to the presence of the 3-chloro group, but **4e** generated twofold less NMPA relative to **4c**; while **4f** was not converted to NMPA at all, suggesting a lack of prodrug activation. Other **4a** analogues, in which the 4-methoxy group was replaced by methyl, methylthio, chloro and other groups, failed to generate NMPA inside hepatocytes, which is consistent with the prodrug activation mechanism shown in Scheme 1. Other para-methoxy analogues with electron withdrawing groups such as NO_2, CN, CF_3 and Ac (entries **10**–**13**, Table 1) also exhibited improved stabilities ($t_{1/2} > 120$ min), but unfortunately they also showed decreased prodrug activation (ca. twofold less). Interestingly, 3,5-disubstituted analogues (entries, **14**–**16**, Table 1) showed no activation in hepatocytes, except **4m** which is chemically unstable, suggesting that the enzymatic activation of these prodrugs may be sensitive to steric factors around the methoxy group.

Numerous reports indicate that *O*-demethylation of aryl methoxy groups is commonly observed in both rodents

Table 1. Yield, stability and activation of bisPMB NMPA esters

Entry	R^1	R^2	R^3	No.[a]	Yield[b] (%)	$t_{1/2}$[c]	NMPA[d]
1				**1**		ND[e]	0
2				**2**		ND	0
3				**3**		0.5	85
4	**H**	**Me**	**H**	**4a**	24	9	91
5	**F**	**Me**	**H**	**4b**	15	>120	125
6	**Cl**	**Me**	**H**	**4c**	28	>120	130
7	**Br**	**Me**	**H**	**4d**	15	>120	76
8	**Cl**	**Et**	**H**	**4e**	18	>120	54
9	**Cl**	**Pr**	**H**	**4f**	18	ND	0
10	**NO_2**	**Me**	**H**	**4g**	11	>120	60
11	**CN**	**Me**	**H**	**4h**	20	>120	73
12	**CF_3**	**Me**	**H**	**4i**	28	>120	46
13	**Ac**	**Me**	**H**	**4j**	12	ND	0
14	**Cl**	**Me**	**Cl**	**4k**	41	ND	0
15	**Br**	**Me**	**Br**	**4l**	15	ND	0
16	**OMe**	**Me**	**OMe**	**4m**	20	ND	26

[a] Compound numbers, Figure 1.
[b] Isolated yields for the conversion of **1** to **4a–m**.
[c] $t_{1/2}$ represents half-lives (minutes) in rat plasma at 37 °C.
[d] NMPA represents intracellular concentration (nmoles/g cells) of NMPA in isolated rat hepatocytes.
[e] Not determined.

and humans.[18–28] These observations strengthened our belief that if we were able to demonstrate prodrug activation in rat hepatocytes then this prodrug approach would have potential utility to deliver drugs to humans. Encouraged by the good activation of bisPMB NMPA esters shown in Table 1, compounds **4b–d** were tested in human liver microsomes for activation to generate NMPA, and the results are summarized in Figure 2 and Table 2.

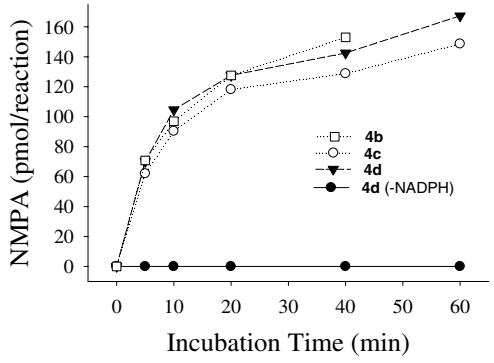

Figure 2. Activation of NMPA esters **4b–d** in human liver microsomes.

All three bisPMB esters tested (**4b–d**) were converted to NMPA by either rat or human microsomes (Fig. 2, Table 2). Consistent with the similar levels of NMPA generated by **4b–d** in rat hepatocytes (Table 1), the rates of activation of these esters in rat microsomes were similar. The NADPH-dependence of the in vitro activation of **4d** in rat and human microsomes confirms the involvement of a microsomal oxidoreductase such as a cytochrome P450 enzyme. It is encouraging to observe that bisPMB esters **4b–d** showed higher activation rates (~2-fold) in human relative to rat liver microsomes. This suggests bisPMB esters could have utility for drug delivery in humans.

In summary, a variety of bisPMB NMPA esters were synthesized to test their suitability as phosphonate prodrugs. Several bisPMB NMPA esters generated high intracellular levels of NMPA, thereby demonstrating both enhanced cellular penetration relative to the phosphonic acid and rapid prodrug cleavage in rat hepatocyte. NMPA bisPMB esters **4b** and **4c** have significantly improved chemical and plasma stability over **4a** while maintaining a high degree of prodrug activation. The stability of **4b** and **4c** to esterases may offer enhanced oral bioavailability relative to acyloxyalkyl prodrugs such as **3**, as a result of resistance to ester-

Table 2. Liver microsome activation rates of bisPMB NMPA esters[a]

Entry	R^1	R^2	R^3	No.	NADPH[b]	Human[c]	Rat[c]
1	**F**	**Me**	**H**	**4b**	Yes	5.23	2.94
2	**Cl**	**Me**	**H**	**4c**	Yes	4.52	1.58
3	**Br**	**Me**	**H**	**4d**	Yes	4.85	2.76
4	**Br**	**Me**	**H**	**4d**	No	0.00	0.00[d]

[a] Rate of NMPA appearance during incubation of bisPMB NMPA esters in NADPH-fortified rat and human liver microsomes (2 mg/mL, 37 °C) is measured.
[b] NADPH was added for entries **1–3**, but not for entry **4**.
[c] Rate of NMPA generation using either human or rat liver microsomes is reported as pmol/min/mg.
[d] NADPH was added but microsomes were omitted.

ase-mediated degradation in the intestine. In vitro prodrug activation of compounds **4b–d** was demonstrated with human liver microsomes, which suggests potential utility for drug delivery in humans. In addition, bisPMB prodrugs may result in liver-targeting similar to HepDirect prodrugs[10] based on their likely CYP-mediated activation mechanism. Although the preliminary results presented here suggest that bisPMB esters can be potentially used as phosphonate prodrugs to enhance cell penetration, further in vivo experiments are needed to assess the feasibility of this prodrug approach as a method for oral delivery of phosphonate drugs.[29]

Acknowledgments

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References and notes

1. Kornberg, R. D.; McNamee, M. G.; McConnell, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 1508.
2. For a review on phosphonate prodrugs, Krise, J. P.; Stella, V. J. *Adv. Drug Delivery Rev.* **1996**, *19*, 287.
3. Farquhar, D.; Srivastva, D. N.; Kattesch, N. J.; Saunders, P. P. *J. Pharm. Sci.* **1983**, *72*, 324.
4. Dang, Q.; Brown, B. S.; van Poelje, P. D.; Colby, T. J.; Erion, M. D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1505.
5. Lefebvre, I.; Perigaud, C.; Pompon, A.; Aubertin, A. M.; Girardet, J. L.; Kirn, A.; Gosselin, G.; Imbach, J. L. *J. Med. Chem.* **1995**, *38*, 3941.
6. McGuigan, C.; Pathirana, R. N.; Mahmood, N.; Devine, K. G.; Hay, A. J. *Antiviral. Res.* **1992**, *17*, 311.
7. Saboulard, D.; Naesens, L.; Cahard, D.; Salgado, A.; Pathirana, R.; Velazquez, S.; McGuigan, C.; De Clercq, E.; Balzarini, J. *Mol. Pharmacol.* **1999**, *56*, 693.
8. Erion, M. D.; van Poelje, P. D.; Dang, Q.; Kasibhatla, S. R.; Potter, S. C.; Reddy, M. R.; Reddy, K. R.; Jiang, T.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 7970.
9. De Lombaert, S.; Erion, M. D.; Tan, J.; Blanchard, L.; el-Chehabi, L.; Ghai, R. D.; Sakane, Y.; Berry, C.; Trapani, A. J. *J. Med. Chem.* **1994**, *37*, 498.
10. Erion, M. D.; Reddy, K. R.; Boyer, S. H.; Matelich, M. C.; Gomez-Galeno, J.; Lemus, R. H.; Ugarkar, B. G.; Colby, T. J.; Schanzer, J.; Van Poelje, P. D. *J. Am. Chem. Soc.* **2004**, *126*, 5154.
11. Erion, M. D.; van Poelje, P. D.; Mackenna, D. A.; Colby, T. J.; Montag, A. C.; Fujitaki, J. M.; Linemeyer, D. L.; Bullough, D. A. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 554.
12. Mitchell, A. G.; Thomson, W.; Nicholls, D.; Irwin, W. J.; Freeman, S. *J. Chem. Soc. Perkin. Trans.* **1992**, 2345.
13. Serafinowska, H. T.; Ashton, R. J.; Bailey, S.; Harnden, M. R.; Jackson, S. M.; Sutton, D. *J. Med. Chem.* **1995**, *38*, 1372.
14. Klutch, A.; Bordun, M. *J. Med. Chem.* **1967**, *10*, 861.
15. Mitchell, A. G.; Nicholls, D.; Irwin, W. J.; Freeman, S. *J. Chem. Soc. Perkin. Trans.* **1992**, *2*, 1145.
16. Lu, S. C. *FASEB J.* **1999**, *13*, 1169.
17. NMPA was purchased from Lancaster and used as received. General procedures for the synthesis of bis(4-methoxybenzyl) NMPA esters (**4a–m**): A suspension of NMPA (**1**, 2.25 mmol) and DMF (0.23 mmol) in anhydrous methylenechloride (25 mL) was treated with oxalyl chloride (4.95 mmol) under nitrogen, and the resulting reaction mixture was heated to reflux for 2 h. The resulting clear solution was evaporated to NMPA dichloridate as a yellow solid. The crude dichloridate was dissolved in anhydrous methylenechloride (10 mL), cooled to 0 °C under nitrogen and treated with a solution of 4-methoxybenzyl alcohol (4.95 mmol) and pyridine (7.43 mmol) in anhydrous methylenechloride (9 mL). The resulting solution was stirred at room temperature for 4 h, quenched with saturated ammonium chloride (20 mL). The layers were separated and the aqueous phase was extracted with methylenechloride (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$), evaporated and the residue was purified by flash chromatography (SiO_2, 2 × 15 cm, 55% EtOAc–hexane) to give the desired product (**4a**) as sticky yellow solid (245 mg, 24%). ^{1}H NMR (DMSO-d_6) δ 8.20–6.85 (15H, m), 4.83 (4H, d, J = 8 Hz, 2×–CH_2Ar), 3.77 (2H, d, J = 22 Hz, –CH_2), 3.74 (6H, s, 2×–CH_3). Anal. ($C_{27}H_{27}O_5P$ + 0.75H_2O) C, H.
Bis[(3-fluoro-4-methoxy)benzyl] NMPA (**4b**). A brown oil. ^{1}H NMR (DMSO-d_6) 8.20–6.90 (13H, m., 4.86 4H, d, J = 8.1 Hz, 2×–CH_2Ar), 3.83 (2H, d, J = 22.1 Hz, –CH_2), 3.82 (6H, s, 2×–CH_3). Anal. ($C_{27}H_{25}F_2O_5P$) C, H. Bis[(3-chloro-4-methoxy)benzyl] NMPA (**4c**). A brown oil. ^{1}H NMR (DMSO-d_6) 8.20–6.90 (13H, m), 4.86 (4H, d, J = 8.1 Hz, 2×–CH_2Ar), 3.83 (2H, d, J = 22.1 Hz, –CH_2), 3.82 (6H, s, 2×–CH_3). Anal. ($C_{27}H_{25}Cl_2O_5P$) C, H.
Bis[(3-chloro-4-ethoxy)benzyl] NMPA (**4d**). A yellow foam. ^{1}H NMR (DMSO-d_6) 8.20–7.03 (13H, m), 4.86 (4H, d, J = 8.1 Hz, 2×–CH_2Ar), 4.11 (4H, q, J = 6.9 Hz, 2×–CH_2CH_3), 3.84 (2H, d, J = 22.1 Hz, –CH_2), 1.36 (6H, t, J = 6.9 Hz, 2×–CH_2CH_3). Anal. ($C_{29}H_{29}Cl_2O_5P$) C, H.
Bis[(3-chloro-4-propyloxy)benzyl] NMPA (**4e**). A yellow foam. ^{1}H NMR (DMSO-d_6) 8.20–7.03 (13H, m), 4.86 (4H, d, J = 8.1 Hz, 2×–CH_2Ar), 4.01 (4H, t, J = 6.5 Hz, 2×–$CH_2CH_2CH_3$), 3.84 (2H, d, J = 22.1 Hz, –CH_2), 1.76 (4H, m, 2×–$CH_2CH_2CH_3$), 1.01 (6H, t, J = 6.5 Hz, 2×–$CH_2CH_2CH_3$). Anal. ($C_{31}H_{33}Cl_2O_5P$) C, H. Bis[(3-bromo-4-methoxy)benzyl] NMPA (**4f**). A sticky foam. ^{1}H NMR (DMSO-d_6) 8.20–7.03 (13H, m), 4.86 (4H, d, J = 8.1 Hz, 2×–CH_2Ar), 3.83 (6H, s, 2×–CH_3), 3.82 (2H, d, J = 22.1 Hz, –CH_2). Anal. ($C_{27}H_{25}Br_2O_5P$) C, H.
Bis[(3-nitro-4-methoxy)benzyl] NMPA (**4g**). A yellow solid. mp 148–151 °C; ^{1}H NMR (DMSO-d_6) 8.20–7.23 (13H, m), 4.96 (4H, d, J = 8.1 Hz, 2×–CH_2Ar), 3.91 (6H, s, 2×–CH_3), 3.88 (2H, d, J = 22.1 Hz, –CH_2).Anal. ($C_{27}H_{25}N_2O_9P$) C, H.
Bis[(3-cyano-4-methoxy)benzyl] NMPA (**4h**). A white solid. mp 137–140 °C; ^{1}H NMR (DMSO-d_6) 8.20–7.10 (13H, m), 4.91 (4H, d, J = 8.1 Hz, 2×–CH_2Ar), 3.92 (6H, s, 2×–CH_3), 3.86 (2H, d, J = 22.1 Hz, –CH_2). Anal. ($C_{29}H_{25}N_2O_5P$) C, H.
Bis[(3-trifluoromethyl-4-methoxy)benzyl] NMPA (**4i**). A brown foam. ^{1}H NMR (DMSO-d_6) 8.20–7.15 (13H, m), 4.93 (4H, d, J = 8.1 Hz, 2×–CH_2Ar), 3.87 (6H, s, 2×–CH_3), 3.85 (2H, d, J = 22.1 Hz, –CH_2). Anal. ($C_{29}H_{25}F_6O_5P$) C, H.
Bis[(3-acetyl-4-methoxy)benzyl] NMPA (**4j**). A brown foam. ^{1}H NMR ($CDCl_3$) 8.04–6.80 (13H, m), 4.78 (2H, d, J = 8.1 Hz, –CH_2Ar), 4.76 (2H, d, J = 8.1 Hz, –CH_2Ar), 3.90 (6H, s, 2×–CH_3), 3.64 (2H, d, J = 22.1 Hz, –CH_2), 2.58 (6H, s, 2×–CH_3). Anal. ($C_{31}H_{31}O_7P$ + 0.5H_2O) C, H.
Bis[(3-dichloro-4-methoxy)benzyl] NMPA (**4k**). A white sticky solid. ^{1}H NMR(DMSO-d_6) 8.20–7.30 (11H, m), 4.93 (4H, d, J = 8.1 Hz, 2×–CH_2Ar), 3.94 (2H, d, J = 22.1 Hz, –CH_2), 3.79 (6H, s, 2×–CH_3). Anal. ($C_{27}H_{23}Cl_4O_5P$) C, H.
Bis[(3,5-dibromo-4-methoxy)benzyl] NMPA (**4l**). A white solid. mp 115–118 °C; ^{1}H NMR (DMSO-d_6) 8.20–7.40 (11H, m), 4.92 (4H, d, J = 8.1 Hz, 2×–CH_2Ar), 3.93 (2H, d, J = 22.1 Hz, –CH_2), 3.78 (6H, s, 2×–CH_3). Anal. ($C_{27}H_{23}Br_4O_5P$) C, H.

18. Hubbard, J. W.; Midha, K. K.; Cooper, J. K. *Drug Metab. Dispos.* **1977**, *5*, 329.
19. Shigematsu, H.; Yamano, S.; Yoshimura, H. *Chem. Pharm. Bull. (Tokyo)* **1977**, *25*, 825.
20. Fry, J. R. *Biochem. Pharmacol.* **1981**, *30*, 1915.
21. Canivenc-Lavier, M. C.; Brunold, C.; Siess, M. H.; Suschetet, M. *Xenobiotica* **1993**, *23*, 259.
22. Busse, D.; Cosme, J.; Beaune, P.; Kroemer, H. K.; Eichelbaum, M. *Naunyn. Schmiedebergs. Arch. Pharmacol.* **1995**, *353*, 116.
23. Miners, J. O.; Coulter, S.; Tukey, R. H.; Veronese, M. E.; Birkett, D. J. *Biochem. Pharmacol.* **1996**, *51*, 1003.
24. Nakajima, M.; Inoue, T.; Shimada, N.; Tokudome, S.; Yamamoto, T.; Kuroiwa, Y. *Drug Metab. Dispos.* **1998**, *26*, 261.
25. Molden, E.; Asberg, A.; Christensen, H. *Eur. J. Clin. Pharmacol.* **2000**, *56*, 575.
26. Staack, R. F.; Theobald, D. S.; Paul, L. D.; Springer, D.; Kraemer, T.; Maurer, H. H. *Drug Metab. Dispos.* **2004**, *32*, 379.
27. Laugesen, S.; Enggaard, T. P.; Pedersen, R. S.; Sindrup, S. H.; Brosen, K. *Clin. Pharmacol. Ther.* **2005**, *77*, 312.
28. Wang, M. Z.; Saulter, J. Y.; Usuki, E.; Cheung, Y. L.; Hall, M.; Bridges, A. S.; Loewen, G.; Parkinson, O. T.; Stephens, C. E.; Allen, J. L.; Zeldin, D. C.; Boykin, D. W.; Tidwell, R. R.; Parkinson, A.; Paine, M. F.; Hall, J. E. *Drug Metab. Dispos.* **2006**, *34*, 1985.
29. For a review on phosphonic acids as drug candidates, Dang, Q. *Exp. Opin. Therapeutic Patents* **2006**, *16*, 343.