Methoxy-phenyl groups reduce the cytotoxicity and increase the aqueous solubility of phosphonium zwitterions and salts

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Abstract

The ability of phosphonium cations to act as intracellular transport vectors is well-established. Previous research has demonstrated that phosphonioalkylthiosulfate zwitterions, and ω-thioacetylalkylphosphonium salts are useful precursors for the formation of phosphonium-functionalised gold nanoparticles and enable the nanoparticles to be transported into cells for diagnostic and therapeutic purposes.

In this report we describe the synthesis and characterisation of a series of phosphonioalkylthiosulfate zwitterions, and ω-thioacetylalkylphosphonium salts derived from the methoxy-phenylphosphines tris(2,4,6-trimethoxyphenyl)phosphine, tris(2,6-dimethoxyphenyl)phosphine and tri(4-methoxyphenyl)phosphine. The methoxyphenyl-substituted phosphonium compounds show greater solubility in aqueous systems than the corresponding phenyl derivatives and cytotoxicity studies reveal that the compounds are significantly less toxic than the related triphenylphosphonium derivatives.

The solid-state structures of the tris(2,4,6-trimethoxyphenyl)- and tris(2,6-dimethoxyphenyl)-phosphoniopropylthiosulfate zwitterions have been investigated by single crystal X-ray crystallography. The differences in the molecular packing of the compounds may account for greater solubility of these zwitterions in aqueous solutions.

Keywords

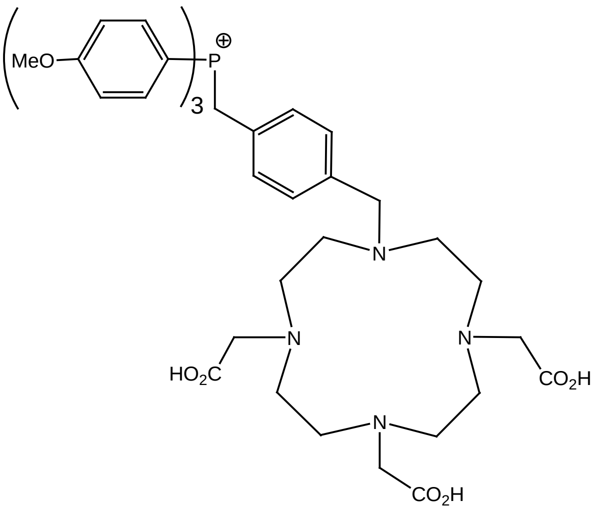
Phosphonium, zwitterion, crystal structure, mitochondria, cytotoxicity

1.0 Introduction

The lipophilic characteristics of organophosphonium cations, and their ability to be transported across cell membranes and accumulate in mitochondria, have led to widespread interest in their use as medical probes and therapeutics [1,2]. Consequently phosphonium moieties, especially triphenylphosphonium groups, have been conjugated to a wide range of molecules.

Our own work has focused on the synthesis and biological properties of alkylthiosulfate zwitterions [3-6], and alkylthioacetate salts [3,7], conjugated with triphenylphosphine and other trialkyl- and triaryl-phosphines [3-7], and also arsines [8], which can be used as precursors for the formation of phosphonium- or arsonium-functionalized gold nanoparticles [4,8], potentially useful species in mitochondria-targeted pharmaceutical nanotechnology. Other groups have developed and applied our methodology [9,10]. Although triphenylphosphonium-functionalized nanoparticles are soluble in water and biological media [4,10] and are taken-up by cells [4,9] the parent triphenylphosphonioalkylthiosulfate zwitterions are insoluble in aqueous media [3,10]. This observation has prompted us to investigate alternative triarylphosphonium groups in an attempt to improve the aqueous solubility of the zwitterions. Tris(2,4,6-trimethoxyphenyl)phosphine is a very unusual tertiary aryl phosphine [11]. The presence of the methoxy groups increases the basicity of the phosphine and also increases the steric bulk of the compound [12]. Tris(2,6-dimethoxyphenyl)phosphine has a lower basicity, but similar steric properties to tris(2,4,6-trimethoxyphenyl)-phosphine, whereas tris(4-methoxyphenyl)phosphine has a lower basicity and lower steric bulk than tris(2,4,6-trimethoxyphenyl)phosphine. Previous work by Liu and coworkers [13,14], showed that tri-(4-methoxyphenyl)- and tris(2,4,6-trimethoxyphenyl)phosphonium compounds can be used to functionalize macrocyclic derivatives **1** and **2**, that are soluble in biological media and which are readily taken up by cells. Their results showed that both the 4-methoxy- and the 2,4,6-trimethoxy- compounds are more effective at mitochondria-targeting than the analogous triphenylphosphonium derivatives. Furthermore, compound **1** is more effective at mitochondria-targeting than the 2,4,6-trimethoxy species **2**. Another important biological application of tris(2,4,6-trimethoxyphenyl)phosphonium compounds is in the field of proteomics. Cations such as the *S*-pentafluorophenylacetate (**3**) [15-19], alkylcarboxylates (**4**) [20], and the *N*-succinimidyloxycarbonylmethyl-derivative (**5**) [21-23], are used to derivatise small molecules, including amines and carboxylic acids, alcohols, aldehydes and ketones, and large biomolecules such as proteins and peptides, to enhance their detection by mass spectrometry.

We have exploited the unusual properties of tris(2,4,6-trimethoxyphenyl)phosphine and its analogues by incorporating them into phosphoniumalkylthiosulfate zwitterions and alkylthioacetate salts and report here our investigations into the chemistry and cytotoxicity of these compounds.



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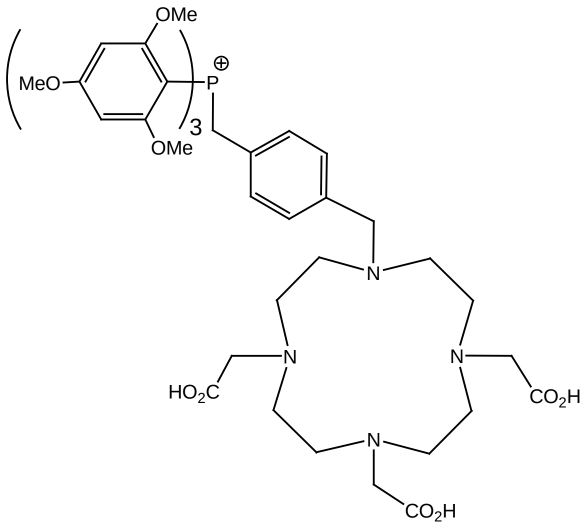

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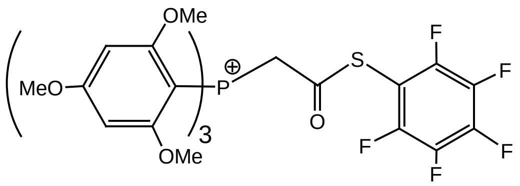

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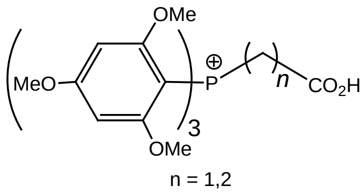

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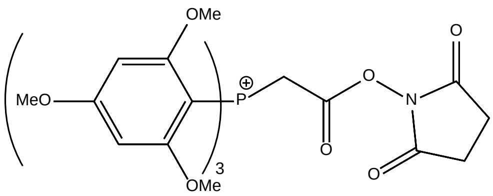

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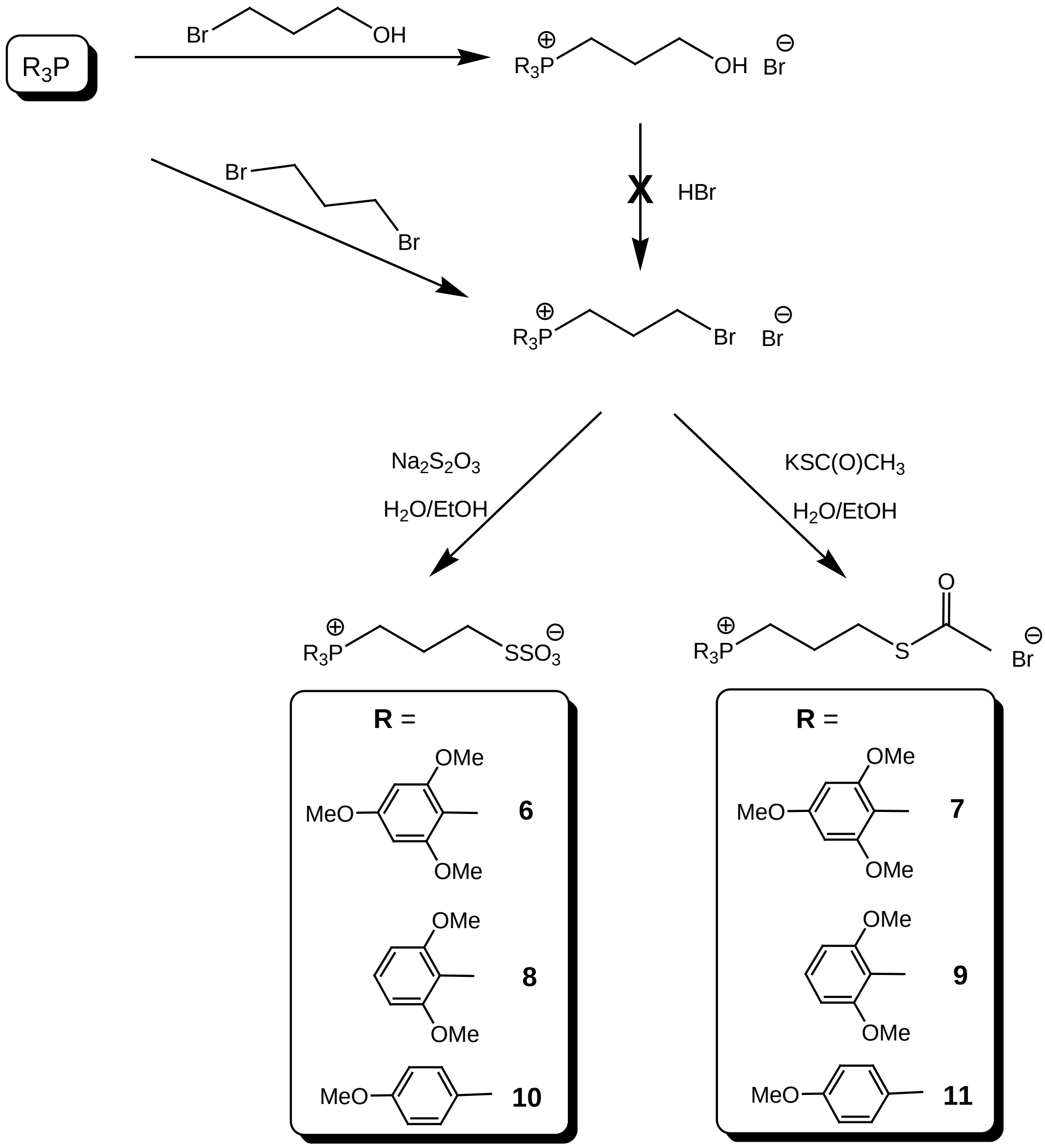
Chart 1. The structures of compounds 1 - 5

2.0 Results and Discussion

The established method for preparing triarylphosphonioalkylthiosulfate zwitterions, and the associated phosphonioalkylthioacetate salts is to reflux the parent tertiary phosphine with a bromoalcohol, such as bromopropanol, as shown in Scheme 1. The resulting (3-hydroxypropyl)triarylphosphonium bromide salt is then refluxed with hydrobromic acid to generate a (3-bromopropyl)triarylphosphonium bromide that can be converted into the alkylthiosulfate zwitterion or the thioacetate salt. Unfortunately, this synthetic route is not possible for methoxyphenylphosphines because the hydrobromic acid used in the second step preferentially attacks the ether substituents. Consequently, an alternative route has been developed, shown in scheme 1. This involves treating the methoxyphenylphosphine with an α,ω-dibromoalkane, such as 1,3-dibromopropane, which leads to good yields of the ω-bromoalkylphosphonium compounds although care has to be exercised in the initial step to avoid the methoxyphenyl phosphine reacting with both ends of the α,ω–dibromoalkane. This is achieved by using a significant excess of the α,ω-dibromoalkane and adding the methoxyphenylphosphine in small amounts over an extended period of time. This leads directly to the (3-bromopropyl)methoxyphenyl phosphonium bromide that can be converted into the alkylthiosulfate zwitterion or the thioacetate salt by refluxing with sodium thiosulfate or potassium thioacetate, respectively.

The structures of the compounds prepared in this study and their numbering, are shown in scheme 1. Regarding the length of the alkyl chain in the phosphonioalkylthiosulfate zwitterions, and ω-thioacetylalkylphosphonium salts, previous studies have shown that a propyl chain is the ideal length. Longer alkyl chains tend to produce compounds that form as waxy solids or oils that are difficult to handle. Shorter alkyl chains would be less useful for forming functionalized nanoparticles. Therefore, in this study we have focused on the propyl derivatives.

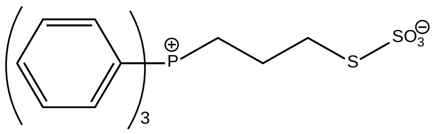


Scheme 1. Synthetic procedures employed in this study together with a summary of the compounds prepared and their numbering.

All compounds have been fully characterized by ^{31}P and ^{1}H NMR spectroscopy, ESI mass spectrometry and IR spectroscopy. The results correspond with the proposed structures of **6** - **11** and are consistent with published data. Compounds **6** - **11** dissolve readily in a range of solvents including dichloromethane, water and aqueous media. This makes them suitable for cell biology studies. The solubility of zwitterions **6**, **8** and **10** in aqueous media is notable. Previous research from our own group [3], and others [10], has shown that triarylphosphoniopropylthiosulfate zwitterions prepared from triphenyl-, tri(4-fluorophenyl)- and tri(4-tolyl)-phosphine are not so soluble in aqueous media. In contrast, the corresponding ω-thioacetylpropyl(triaryl)phosphonium bromide salts are soluble in aqueous media. This difference in solubility between the phosphonium zwitterions and phosphonium salts was attributed to strong electrostatic interactions that exist between the zwitterions in the solid state. These interactions are not present in the corresponding ω-thioacetylpropyltri(aryl)phosphonium salts. The solubility of the phosphonium zwitterions reported here can be attributed to the electronic and steric effects of the methoxy-substituents.

2.1 Single crystal X-ray analysis of tris(2,4,6-trimethoxyphenyl) phosphoniopropylthiosulfate and tris(2, 6-dimethoxyphenyl) phosphoniopropylthiosulfate

Single crystals of **6** and **8** were grown by slow diffusion of diethyl ether into a dichloromethane solution of the compound, resulting in the formation of colorless crystals. The Bricklebank group has previously reported the structure of the triphenylphosphoniopropylthiosulfate zwitterion (**12**) [5], together with those of the tri(4-fluorophenyl)phosphoniopropylthiosulfate (**13**) [3], and tributylphosphonio-thiosulfate (**14**) [3], zwitterions. The other crystallographically-characterized thiosulfate zwitterions are the ammonium derivative *S*-[4-(trimethylammonio)phenyl] thiosulfate (**15**) [24], and the triphenylarsoniopropylthiosulfate zwitterion (**16**) [8].



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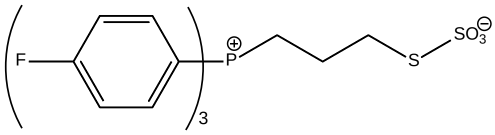

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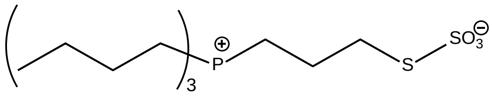

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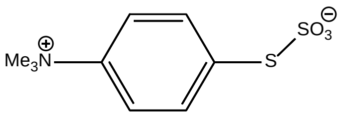

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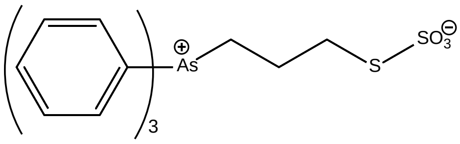

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Chart 2. The structures of compounds 12 - 16

The molecular structure of **6** is shown in Figure 1 and selected bond lengths and angles in Table 1. The asymmetric unit of **6** contains two independent molecules along with a diethyl ether solvent molecule of crystallisation. The molecule containing atoms P1, S1 and S2 is referred to as **6A**, and that containing atoms P41, S41 and S42 is **6B**. Both molecules of **6** are highly disordered which is not unusual for derivatives of tris(2,4,6-trimethoxyphenyl)phosphine (All quoted values are for the major component). The molecular structure of **8** is shown in Figure 2 and selected bond lengths and angles in Table 2. Unlike **6**, the structure of **8** is not disordered. The asymmetric unit contains one independent molecule together with water of crystallisation.

The bond lengths and angles in the aryl rings of **6** and **8** are unremarkable and are similar to those in the parent phosphines, $[2,4,6\text{-}(MeO)_3C_6H_2]_3P$ [11], and $[2,6\text{-}(MeO)_3C_6H_3]_3P$ [25]. The phosphorus atoms are tetrahedrally coordinated with mean C-P-C bond angles of 109.45^o in **6A**,109.48^o in **6B** and 109.51^o in **8**. The corresponding values for the triphenyl-, tri-4-fluorophenyl- and tributyl- analogues, **12**, **13** and **14** are 109.47^o, 109.46^o and 109.47^o respectively. The mean C-P-C angles in **6** and **8** are larger than those in the parent phosphines [11,25], but are identical to that in the phosphonium salt methyltris(2,4,6-trimethoxyphenyl)phosphonium iodide [26]. Other workers [26,27], have observed intramolecular P…O interactions between the oxygen of an *ortho*-methoxy group and the phosphorus atom in derivatives of both $[2,4,6\text{-}(MeO)_3C_6H_2]_3P$ and $[2,6\text{-}(MeO)_3C_6H_3]_3P$. Both structures have similar intramolecular P…O distances with one oxygen generally much closer than its aryl ring equivalent (Table 3).

The S-S bond length in **8** [2.1024(5)Å] is similar to the S-S bond length in **6A** [2.080(3)Å] which is shorter than that in **6B** [2.145(6)Å]. All of the S-S bonds in the phosphonium thiosulfate zwitterions are appreciably shorter than the S-S bond in the monoanion of thiosulfuric acid, $HSSO_3^-$ [2.155] [28]. The S-O bonds in **6A**, which lie in the range 1.391(11)Å - 1.441(11)Å, are similar to those in **6B**, which range from 1.399(12)Å to 1.423(9)Å. However, the S-O bonds in **6A** and **6B** are shorter than those in **8** [1.4404(13)Å - 1.4493(12)Å] and **12** - **14**. The reason for this difference is unclear.

The packing of molecules of **6** (Figure 3) and **8** (Figure 4) show no significant intermolecular interactions between the cationic phosphonium centres and the thiosulfate anions. The structure of **6** shows that the zwitterions pack together in a loose head-to-tail manner with the thiosulfate anions surrounded by methoxy ligands, whereas for **8** the solvent water helps bridge thiosulfate anions together. As noted above, the favorable aqueous solubility of zwitterions **6** - **8** possibly results from weaker electrostatic interactions between molecules in the solid state.

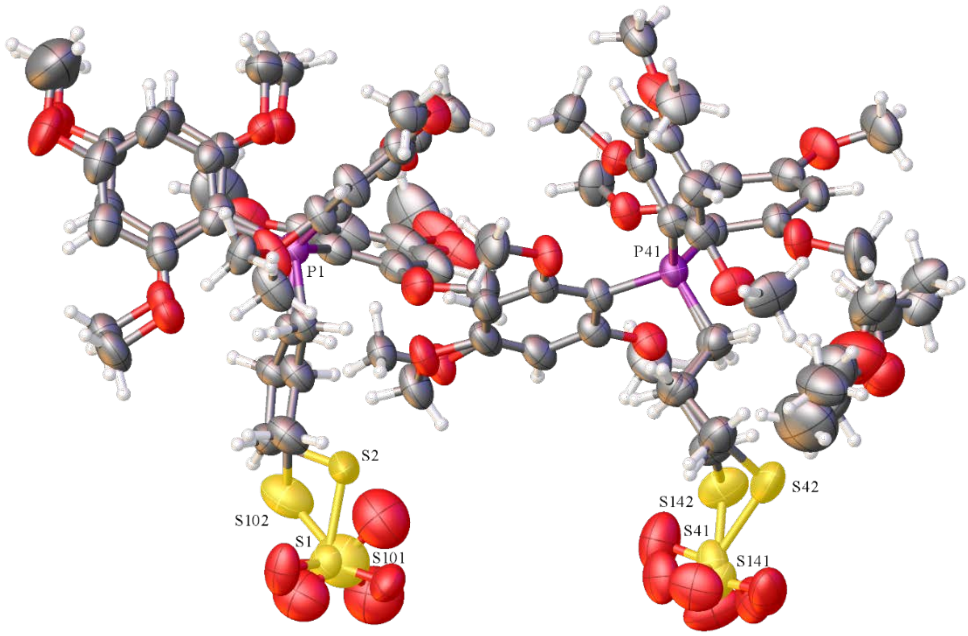


Figure 1 Thermal ellipsoid representation of the structure of compound **6**

Table 1 Selected bond lengths [Å] and angles [°] in compound **6**.

6A			
C1–P1	1.835(6)	O31–S1	1.432(4)
C11–P1	1.801(4)	O32–S1	1.397(5)
C21–P1	1.798(4)	O33–S1	1.433(4)
C31–P1	1.815(5)	S101–S102	2.093(10)
P1–C131	1.801(16)	O131–S101	1.432(11)
P1–C101	1.804(5)	O132–S101	1.391(11)
S1–S2	2.080(3)	O133–S101	1.441(11)
C11–P1–C21	110.6(2)	O33–S1–O32	118.3(3)
C11–P1–C131	99.4(10)	O33–S1–O31	114.9(3)
C21–P1–C131	104.8(11)	O32–S1–O31	109.7(4)
C11–P1–C31	110.7(2)	O33–S1–S2	98.7(2)
C11–P1–C101	106.2(2)	O32–S1–S2	108.5(3)
C21–P1–C31	103.9(3)	O31–S1–S2	105.1(3)
C21–P1–C101	111.4(5)	O133–S101–O132	112(2)
C131–P1–C101	123.5(15)	O133–S101–O131	113.8(19)
C11–P1–C1	109.3(3)	O132–S101–O131	115.6(19)
C21–P1–C1	115.2(5)	O133–S101–S102	98.9(15)
C31–P1–C1	107.0(4)	O132–S101–S102	108.1(16)
C33–S2–S1	100.27(19)	O131–S101–S102	106.6(16)
		C133–S102–S101	97.8(12)
6B			
C41–P41	1.798(4)	O71–S41	1.408(8)
C51–P41	1.790(4)	O72–S41	1.413(10)
C61–P41	1.798(4)	O73–S41	1.423(9)
C71–P41	1.827(10)	S41–S42	2.145(6)
P41–C171	1.813(11)	O171–S141	1.409(9)
S141–S142	2.105(7)	O172–S141	1.399(12)
		O173–S141	1.422(10)
C51–P41–C61	107.3(2)	O72–S41–S42	107.3(6)
C51–P41–C41	113.2(2)	O71–S41–S42	101.9(5)
C61–P41–C41	112.36(19)	O73–S41–S42	106.3(6)
C51–P41–C171	113.7(10)	C73–S42–S41	102.4(6)
C61–P41–C171	109.0(8)	O172–S141–O173	118.3(9)
C41–P41–C171	101.4(10)	O172–S141–O171	117.9(9)
C51–P41–C71	110.5(10)	O173–S141–O171	110.7(10)
C61–P41–C71	107.4(9)	O172–S141–S142	99.5(7)
C41–P41–C71	106.1(9)	O173–S141–S142	100.8(6)
O72–S41–O71	114.6(8)	O171–S141–S142	106.4(5)
O72–S41–O73	110.7(8)	C173–S142–S141	100.1(5)
O71–S41–O73	114.9(8)		

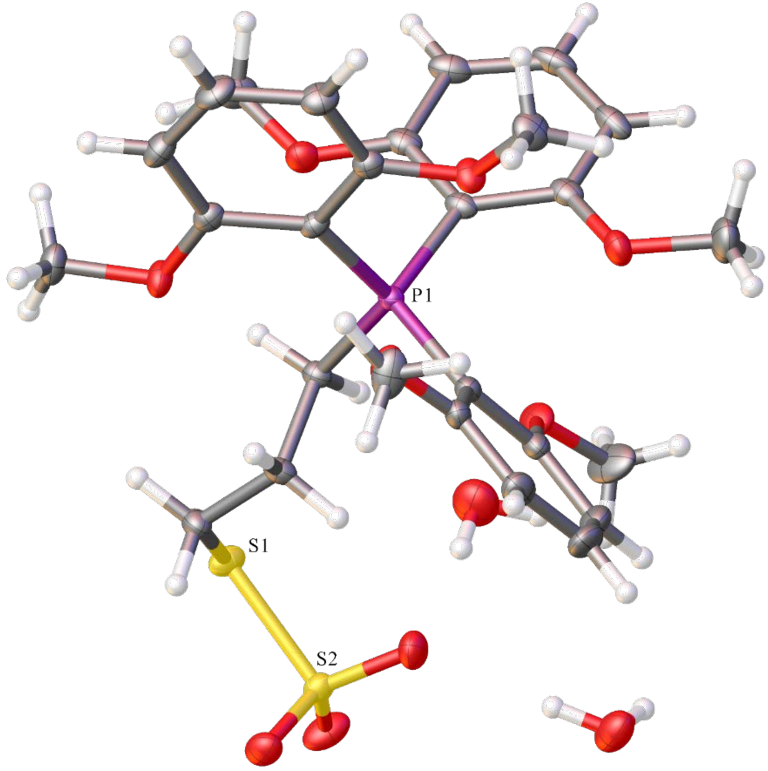


Figure 2 Thermal ellipsoid representation of the structure of compound **8**

Table 2 Selected bond lengths [Å] and angles [°] in compound **8**.

C1-P1	1.8152(15)	S1-S2	2.1024(5)
C4-P1	1.8074(16)	O1-S2	1.4469(12)
C12-P1	1.8020(15)	O2-S2	1.4493(12)
C20-P1	1.8073(15)	O3-S2	1.4404(13)
C3-S1	1.8122(17)		
C12-P1-C20	104.60(7)	O3-S2-O1	114.40(8)
C12-P1-C4	112.06(7)	O3-S2-O2	114.37(8)
C20-P1-C4	114.60(7)	O1-S2-O2	112.65(8)
C12-P1-C1	113.25(7)	O3-S2-S1	101.89(5)
C20-P1-C1	108.84(7)	O1-S2-S1	106.66(5)
C4-P1-C1	103.66(7)	O2-S2-S1	105.52(5)
C3-S1-S2	100.79(6)		

Table 3 Selected intramolecular P…O contacts [Å] in compounds **6** and **8**.

6A			
P1…O1	2.75(2)	P1…O21	3.071(42)
P1…O3	3.13(2)	P1…O23	2.828(4)
P1…O11	3.099(3)	P1…O101	2.768(18)
P1…O13	2.801(3)	P1…O103	3.130(15)
6B			
P41…O41	3.085(3)	P1…O53	2.736(3)
P41…O43	2.816(4)	P1…O61	3.103(3)
P41…O51	3.115(4)	P1…O63	2.790(4)
8			
P1…O4	2.7991(12)	P1…O7	3.0853(12)
P1…O5	3.0746(12)	P1…O8	2.8003(12)
P1…O6	2.7572(12)	P1…O9	3.0774(12)

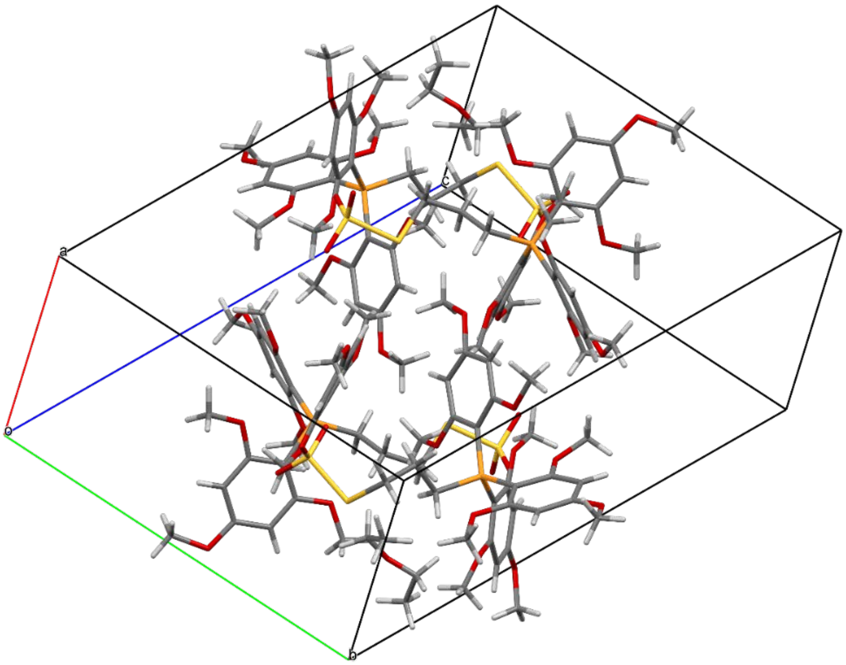


Figure 3. Molecular packing in **6**

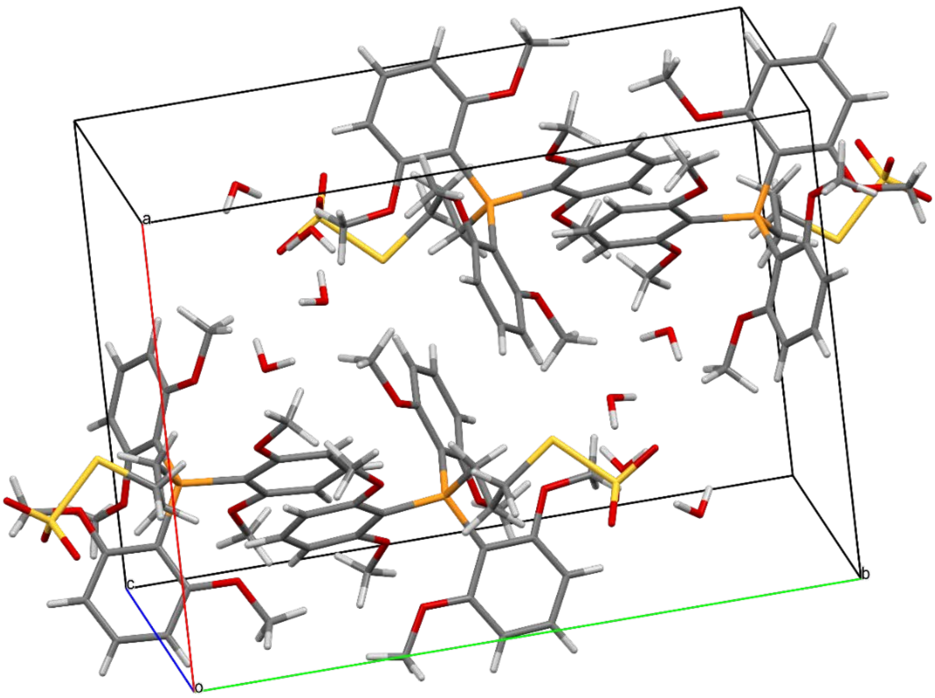


Figure 4. Molecular packing in **8**

2.2 Cytotoxicity screening of 6 - 11.

The use of phosphonium cations to transport a variety of species, including drugs, diagnostic probes, and nanomaterials, is well-established [1,2]. The advantages of phosphonium systems include their ease of synthesis but also, more importantly, their stability and lack of reactivity towards cellular components. For medical applications it is desirable that the transport vector, in this case the phosphonium group, is not reactive or toxic towards cells. Although the biological behaviour of phosphonium compounds has been widely investigated, especially their mitochondria-targeting properties and antiproliferative effects, perhaps surprisingly, few studies of the cytotoxicity of the compounds have been reported [1,2,29]. Previous research into triphenylphosphonium-conjugated compounds indicated that the toxicity was associated with the triphenylphosphonium moiety rather than the side chain [30]. To the best of our knowledge there are no reports of cytotoxicity studies into methoxyphenylphosphonium derivatives.

Cell viability studies on compounds **6- 11** were performed against the PC3 prostate cancer cell line using MTT and CellTitre-Glo assays. MTT measures mitochondrial activity to determine the *in vitro* cytotoxic effects of chemical entities whereas CellTitre-Glo assay uses luminescence to determine the number of viable cells based on a quantification of adenosine triphosphate levels. The results are shown in Figure 5 and summarised in Table 4. The data from both assays show a similar trend with zwitterions **6**, **8**, **10** displaying greater toxicity to cells after 72 hours than the corresponding thioacetate salts **7**, **9**, **11**. The IC_{50} values for **6** - **11** compare very favorably with those of the analogous ω-thioacetylpropyl(triphenyl)phosphonium salt **17** and ω-thioacetylpropyl(4-fluorophenyl)phosphonium salt **18** reported previously by us [3] and show much lower cytoxicity than the triphenylphosphonium derivative. This indicated that the methoxyphenylphosphonium compounds would be potentially useful species for transporting drug and diagnostic moieties into cells.

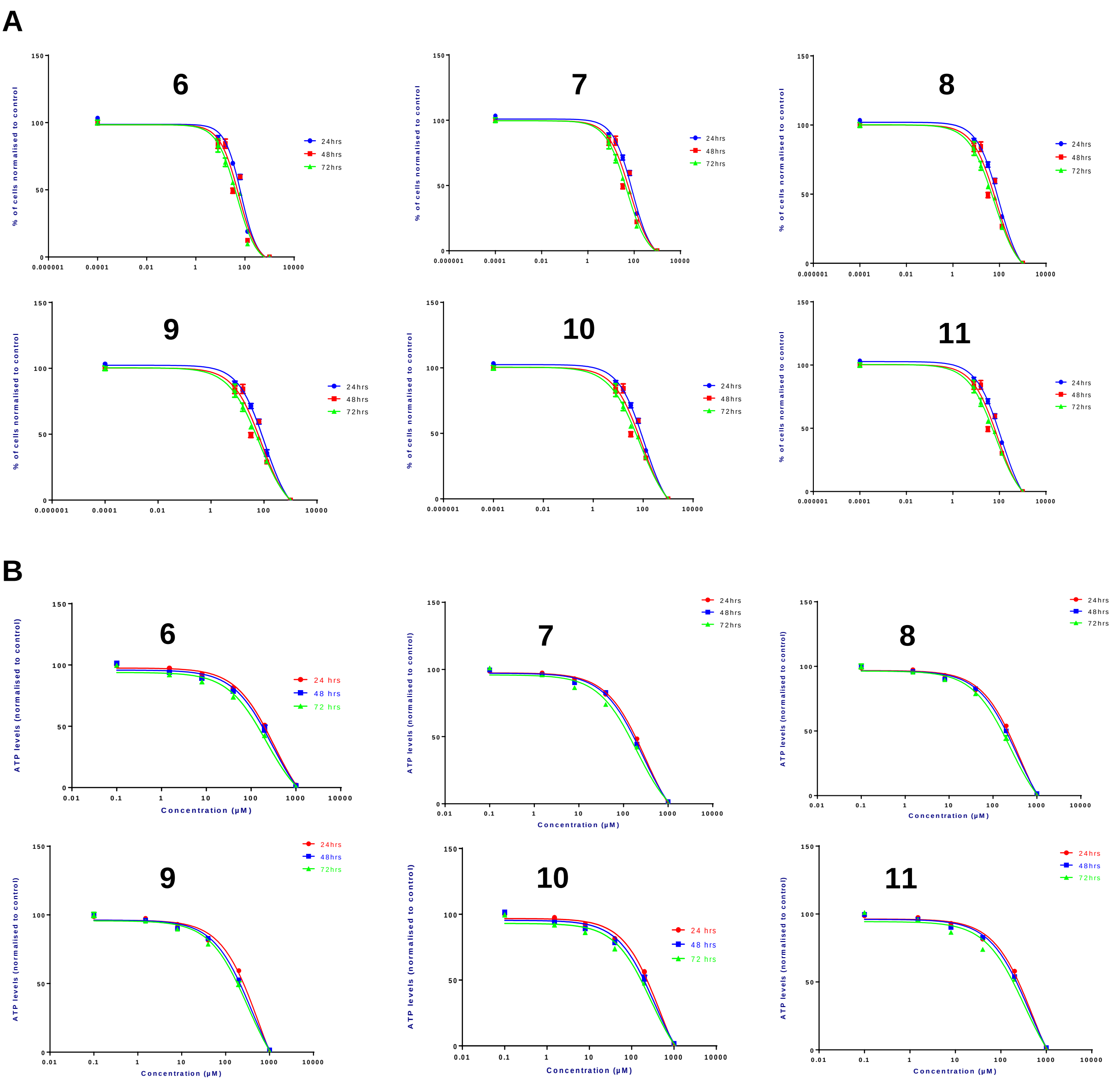
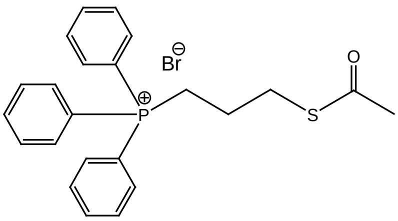


Figure 5. PC3 cells treated with **6 - 11** for 24, 48, 72 hours. **A** Cell proliferation determined by the MTT assay. **B** Cell proliferation determined by the CellTiter-Glo luminescent cell viability assay kit. All data are expressed as a percentage of living cells normalized to control.

Table 4. IC_{50} data for **6** - **11** and related phosphonium compounds after 72 hours. All values are µM.

Compound	CellTiter-Glo	MTT
6	218.3	45.65
7	289.6	66.09
8	198.1	47.80
9	328.7	74.00
10	249.9	58.70
11	319.3	68.22
17*	67.1	
18*	252.6	

NOTES
*Data reported in reference 3



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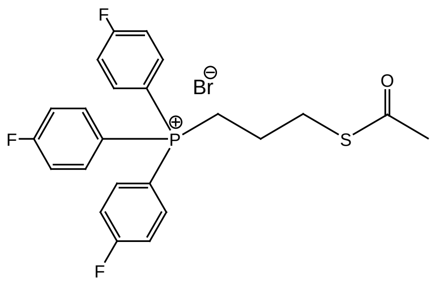

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3. Conclusion

The aim of the work reported in this paper was to synthesize methoxyphenylphosphoniopropylthiosulfate zwitterions and ω-thioacetylpropyl (methoxyphenyl)phosphonium bromide salts (**6 - 11**) and determine their cytotoxicity towards PC3 cells. All compounds are easily prepared and, unlike other triarylphosphoniopropylthiosulfate zwitterions, they are soluble in water and aqueous media. Cell viability results show the IC_{50} values for the methoxy-phenylphosphonium compounds to be much higher than the analogous triphenylphosphonium species and compounds **6** - **11** are only cytotoxic towards cells at very high concentrations making them well-suited as transport vectors for biological applications. These results indicate that methoxy-phenylphosphonium compounds offer advantages compared to their phenyl congeners and could be ideal for the surface-functionalization of gold nanoparticles for applications in the area of mitochondria-targeted pharmaceutical nanotechnology.

4. Materials and Methods

***Synthesis of* 6 - 11.**

All chemicals and solvents were purchased from Sigma-Aldrich or Acros Organics and used as received. All ^{1}H and ^{31}P NMR spectra were recorded on a Brucker AVANCE III (400 MHz). IR spectra were recorded on a Brucker ALPHA platinum ATR spectrometer. Melting points were determined on a Stuart SMP3 melting point apparatus and are uncorrected. Electrospray Ionisation Mass spectrometry was performed on a Thermo Finnigan LCQ classic in positive ion mode. Samples were dissolved in a mixture of ethanol and deionised water (50:50) to a concentration of approximately 1 mg/mL for molecular ion determination. Elemental analyses were performed by MEDAC Ltd, Chobham, Surrey, UK.

All six compounds were prepared in a similar manner, as exemplified by the preparation of compounds **6** and **7**.

Tris(2,4,6-trimethoxyphenyl)phosphine (1.0g, 1.878×10^{-4} mol), dissolved in acetonitrile (20 mL), was added dropwise to a 1,3-dibromopropane (5 mL, 1.245×10^{-2} mol) under a nitrogen atmosphere. The mixture was refluxed for 18 hours. The product, 3-bromopropyl[tris(2,4,6-trimethoxyphenyl)]phosphonium bromide was isolated by diluting the reaction mixture with deionised water (20 mL) followed by liquid-liquid extraction using dichloromethane (3 x 10 mL). The dichloromethane extracts were combined, dried over $MgSO_4$, and the solvent removed by rotary evaporation yielding the product as a white solid. To produce zwitterion **6**, 3-bromopropyl tris(2,4,6-trimethoxyphenyl)phosphonium bromide (0.250 g, 3.82 x 10^{-4} mol) and $Na_2S_2O_3$ (0.212 g, 8.56 x 10^{-4} mol) were heated under reflux in aqueous ethanol under a nitrogen atmosphere for 18 hours. The thioacetate salt **7** was produced by refluxing 3-bromopropyl tris(2,4,6-trimethoxyphenyl)phosphonium bromide (0.250 g, 3.82 x 10^{-4} mol) and $KSC(O)CH_3$ (0.098 g, 8.56 x 10^{-4} mol) in aqueous ethanol overnight. Both compounds were isolated from the reaction mixtures by extraction with dichloromethane (3 x 20 mL). Purification of the products, which form as white microcrystalline powders, was achieved by triturating with diethyl ether and recrystallizing from dichloromethane/diethyl ether. The progress of all of the reactions was monitored by TLC using a mobile phase of 80% dichloromethane : 20% methanol.

Tris(2,4,6-trimethoxyphenyl)phosphoniopropylthiosulfate (**6**)

White solid, M.P. 218 **°**C. Elemental Analysis: found: C, 52.76%; H, 5.86%; S, 9.29% requires: C, 52.47%; H, 5.67%; S 9.30%. ^{1}H NMR**:** δ 1.23 (2H, m, P-CH_2), 1.89 (2H, m, S-CH_2), 3.22 (2H, m, CH_2-CH_2-CH_2), 3.91 (18H, s, *o*-OCH_3), 3.65 (9H, s, *p*-OCH_3), 6.09 (6H, d, C_6H_2) ppm. ^{31}P NMR ($CDCl_3$) = 5.24 ppm. IR υ_{max}/cm^{-1} 2912 (CH), 1483, 1438, 1209 (SO), 1082, 1010 (SO), 744, 688, 623, 523, 466. ESI-MS (*m/z*)**:** 686.4 [$((2,4,6\text{-MeO})_3C_6H_2)_3P(CH_2)_3S_2O_3$], 708.86 [$((2,4,6\text{-MeO})_3C_6H_2)_3P\text{-}(CH_2)_3S_2O_3+Na^+$].

ω-thioacetylpropyltris(2,4,6-trimethoxyphenyl)phosphonium bromide (**7**)

White solid, M.P. 243 °C. Elemental Analysis: found: C, 53.83%; H, 5.62%; S 5.03%, requires: C, 53.62%; H, 5.54%; S 5.03%. ^{1}H NMR**:** δ 1.24 (2H, m, P-C*H*$_2$), 1.61 (3H, s, C(O)CH$_3$), 1.89 (2H, m, S-C*H*$_2$), 3.57 (2H, m, CH$_2$-C*H*$_2$-CH$_2$), 3.67 (18H, s, *o*-OC*H*$_3$), 3.03 (9H, s, *p*-OC*H*$_3$), 6.16 (6H, d, C$_6$*H*$_2$) ppm. ^{31}P NMR (CDCl$_3$) = 5.27 ppm. IR υ_{max}/cm^{-1} 2912 (CH), 1483, 1438, 1209 (SO), 1082, 1010 (SO), 744, 688, 623, 523, 466. ESI-MS (*m/z*)**:** 649.33 [((2,4,6-MeO)$_3$C$_6$H$_2$)$_3$P(CH$_2$)$_3$SC(O)CH$_3$], 650.38 [((2,4,6-MeO)$_3$C$_6$H$_2$)$_3$P(CH$_2$)$_3$SC(O)CH$_3$+H$^+$].

Tris(2,6-dimethoxyphenyl)phosphoniopropylthiosulfate (**8**)

White solid, M.P. 237 °C. Elemental Analysis: found: C, 52.42%; H, 5.51%; S 10.19%, requires: C, 52.34%; H, 5.53%; S 10.33%. ^{1}H NMR**:** δ 1.25 (2H, m, P-C*H*$_2$), 1.84 (2H, m, S-C*H*$_2$), 3.12 (2H, m, CH$_2$-C*H*$_2$-CH$_2$), 3.66 (18H, s, *o*-OC*H*$_3$), 6.61 - 7.61 (9H, m, C$_6$*H*$_3$) ppm. ^{31}P NMR (CDCl$_3$) = 7.54 ppm. IR υ_{max}/cm^{-1} 2912 (CH), 1483, 1438, 1209 (SO), 1082, 1010 (SO), 744, 688, 623, 523, 466. ESI-MS (*m/z*)**:** 596.4 [((2,6-MeO)$_2$C$_6$H$_3$)$_3$P(CH$_2$)$_3$S$_2$O$_3$], 619.33 [((2,6-MeO)$_2$C$_6$H$_3$)$_3$P(CH$_2$)$_3$S$_2$O$_3$+Na$^+$].

ω-thioacetylpropyltri(2,6-dimethoxyphenyl)phosphonium bromide (**9**)

White solid, M.P. 226 °C. Elemental Analysis: found: C, 54.31%; H, 5.90%; S 4.98%, requires: C, 54.46%; H, 5.63%; S 5.00%.^{1}H NMR**:** δ 1.18 (2H, m, P-C*H*$_2$), 1.72 (3H, s, C(O)CH$_3$), 1.65 (2H, m, S-C*H*$_2$), 3.22 (2H, m, CH$_2$-C*H*$_2$-CH$_2$), 3.64 (18H, s, *o*-OC*H*$_3$), 6.61 - 7.61 (9H, m, C$_6$*H*$_3$) ppm. ^{31}P NMR (CDCl$_3$) = 7.31 ppm. IR υ_{max}/cm^{-1} 2912 (CH), 1483, 1438, 1209 (SO), 1082, 1010 (SO), 744, 688, 623, 523, 466. ESI-MS (*m/z*)**:** 559.37 [((2,6-MeO)$_2$C$_6$H$_3$)$_3$P(CH$_2$)$_3$SC(O)CH$_3$], 560.38 [((2,6-MeO)$_2$C$_6$H$_3$)$_3$P(CH$_2$)$_3$ SC(O)CH$_3$+H$^+$].

Tri(4-methoxyphenyl)phosphoniopropylthiosulfate **(10)**

White solid, M.P. 195 °C. Elemental Analysis: found: C, 57.11%; H, 5.64%; S 10.2%, requires: C, 57.01%; H, 5.33%; S 10.62%. ^{1}H NMR**:** δ 2.15 (2H, m, P-CH_2), 3.25 (2H, m, S-CH_2), 3.45 (2H, m, CH_2-CH_2-CH_2), 3.9 (9H, s, OCH_3), 7.1–7.6 (12H, m, C_6H_4) ppm. ^{31}P NMR ($CDCl_3$) = 21.28 ppm. IR υ_{max}/cm^{-1} 2912 (CH), 1483, 1438, 1209 (SO), 1082, 1010 (SO), 744, 688, 623 (CS), 523, 466. ESI-MS (*m/z*)**:** 507.2 [$(4\text{-}MeOC_6H_4)_3P(CH_2)_3S_2O_3$], 529.2 [$(4\text{-}MeOC_6H_4)_3P(CH_2)_3S_2O_3+Na^+$].

ω-thioacetylpropyltri(4-methoxyphenyl)phosphonium bromide (**11**)

White solid, M.P. 207 °C. Elemental Analysis: found: C, 57.06%; H, 5.53%; S 5.88%, requires*:* C, 56.83%; H, 5.28%; S 5.82%. ^{1}H NMR**:** δ 2.17 (2H, m, P-*CH_2),* 2.3 (3H, s, C(O)*CH_3*), 3.22 (2H, m, S-CH_2), 3.37 (2H, m, CH_2-CH_2-CH_2), 3.90 (9H, s, OCH_3), 7.1–7.6 (12H, m, C_6H_4) ppm ^{31}P NMR ($CDCl_3$) = 21.33 ppm. IR υ_{max}/cm^{-1} 2912 (CH), 1483, 1438, 1209 (SO), 1082, 1010 (SO), 744, 688, 623, 523, 466. ESI-MS (*m/z*)**:** 469.34 [$(4\text{-}MeOC_6H_4)_3P(CH_2)_3SC(O)CH_3$], 471.35 [$(4\text{-}MeOC_6H_4)_3P(CH_2)_3SC(O)CH_3$ $+H^+$].

Cytotoxicity assay

Cytotoxicity was assessed using a CellTiter-Glo luminescent cell viability assay kit (Promega Corporation, Southampton, Hampshire, UK). PC3 cells were grown in DMEM (Dulbecco's Modified Eagle's medium) supplemented with 10% Foetal calf serum (Invitrogen) at 37ºC in 5% $C0_2$. Cells were seeded in opaque-walled 96 well plates at a density of 10,000 cells/well and allowed to adhere overnight. Cells were subsequently treated with the corresponding phosphonium ligand (0-1000μm) for 24, 48, 72 hours. After each incubation period, cell viability was measured according to the manufacturer's instructions. In brief, plates were equilibrated at room temperature for 30 mins, 100μl of assay reagent was added to each well, placed on an orbital shaker for 2mins, left to stand at room temperature for 10 minutes and read on a Wallac Victor2 1420 multilabel counter (PerkinElmer, Cambridge, Cambridgeshire, UK).

Cytotoxicity studies were also done to assess IC_{50} using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as a measure of succinate dehydrogenase activity in live cells. Cells were seeded in a 96 well plate with the corresponding ligand (0-1000 μm) for 24, 48, 72 hours. MTT was added to each well to give a final concentration of 0.3 mg/mL MTT and cells incubated with MTT for 3-4 hours at 37^{o}C. The growth medium was then removed and 100 μL DMSO was added and incubated for 30 mins prior to reading the absorbance at 570 nm.

All plates contained control wells and all measurements were performed in quadruplicates, and three independent experiments were conducted (n=12). Data are expressed as a percentage of live cell succinate dehydrogenase activity normalized to control. The average, standard deviation and IC_{50} values were plotted and calculated using GraphPad Prism (GraphPad software, La Jolla, California, USA).

X-ray crystallography

Crystal Data for **6**. $C_{32}H_{44}O_{12.5}PS_2$, M_r = 723.76, triclinic, P-1, a = 12.2703(4) Å, b = 15.4855(4) Å, c = 19.4439(3) Å, α = 72.212(4)°, β = 85.475(5)°, γ = 84.543(5)°, V = 3497.16(18) Å^3, T = 120(2) K, Z = 4, Z' = 2, λ(Mo K_α) = 0.255, 53536 reflections measured, 15979 unique (R_{int} = 0.056) which were used in all calculations. The final wR_2 was 0.2882 (all data) and R_1 was 0.1086 (I > 2(I)).

Crystal Data for **8**. $C_{27}H_{35.33}O_{10.17}PS_2$, M_r = 617.65, monoclinic, $P2_1/c$, a = 13.9991(2) Å, b = 18.4241(2) Å, c = 11.28730(10) Å, β = 99.1340(10)°, α = γ = 90°, V = 2874.31(6) Å^3, T = 120(2) K, Z = 4, Z' = 1, λ(MoK_α) = 0.297, 58735 reflections measured, 6571 unique (R_{int} = 0.0540) which were used in all calculations. The final wR_2 was 0.0917 (all data) and R_1 was 0.0363 (I > 2(I)).

Suitable crystals were selected and data collected following a standard method [32]. For compound 6 on a Rigaku SPIDER RAPID diffractomer at 120K with an image plate detector. Cell determination and data collection, data reduction, cell refinement and absorption correction were carried out using CrystalClear [33]. For compound **8** on a Nonius Kappa CCD diffractometer at 120K controlled by the Collect [34] software package. The data were processed using Denzo [35] and semi-empirical absorption corrections were applied using SADABS [36]. Using Olex2 [37] both structures were solved using SHELXT [38] and models refined with SHELXL [39].

All non-hydrogen atoms were refined anisotropically, with all hydrogen atoms placed geometrically using standard riding models

CCDC1826927 and 1826926 contain the supplementary crystallographic data for compounds **6** and **8** respectively for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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