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Research Article

Phosphate and Thiophosphate Biphenyl Analogs as Steroid Sulfatase Inhibitors

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ABSTRACT In the present work, we report convenient methods for the synthesis and biological evaluation of phosphate and thiophosphate biphenyl derivatives exhibiting steroid sulfatase (STS) activity. The described synthesis is based on straightforward preparation of biphenyl-4-ol and 4′-hydroxybiphenyl-4-carboxylic acid ethyl ester modified with various phosphate or thiophosphate moieties. The inhibitory effects of these compounds were tested on STS isolated from human placenta and led to two compounds of interest, **5a** and **5d** with IC_{50} values of 28.0 and 22.1 μM, respectively and that had interesting new binding modes in the STS active site. Drug Dev Res 76 : 94–104, 2015.

Key words: steroid sulfatase; STS inhibitors; breast cancer; biphenyls

INTRODUCTION

Biphenyl, an aromatic hydrocarbon occurs naturally in coal tar, crude oil, and natural gas. It forms an incomplete combustion of mineral oil and coal. Historically, some biphenyls were widely used as intermediates in chemical syntheses that resulted in a wide range of pesticides including the polychlorinated biphenyls (PCBs). Emerging trends in synthetic chemistry have led a number of substituted biphenyl derivatives prepared by various coupling reactions that therapeutic potential [Jain et al., 2013]. These derivatives have angiotensin receptor (Losartan, Telmisartan) [Zhu et al., 2004], antihypertensive (biphenyl carboxylic benzimidazole derivatives) [Kumar et al., 2006], diuretic (N-{(substituted)1,3-benzothiazol-2-yl}-1,1-biphenyl-4-carboxamides) [Yar and Ansari, 2009], anti-inflammatory (biphenyl-4-carboxylic acid 5-(arylidene)-2-(aryl)-4-oxothiazolidin-3-yl amides) [Deep et al., 2010], antipsychotic and anxiolytic (biphenylindanone A) [Ruggero et al., 2006] and antimicrobial (biphenyl hydrazide-hydrazone) [Deep et al., 2010] activities. Of particular interest is the use of biphenyl scaffolds in designing new steroid sulfatase (STS) inhibitors based on phosphate and thiophosphate derivatives that may be potential new therapeutics for the treatment of estrogen- and androgen-dependent

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disorders (especially breast cancer) [Nussbaumer and Billich, 2004]. Among females inindustrialized countries, breast cancer is one of the most frequently diagnosed diseases with estimates for 2014 (according to National Cancer Institute data) of more than 230,000 new cases and more than 40,000 deaths from this disease in the United States. For this reason, research work on the synthesis of efficient and selective STS inhibitors is of particular importance.

METHODS AND MATERIALS

Melting points (uncorrected) were determined with a Stuart Scientific SMP30 apparatus. NMR spectra were recorded on a Varian Gemini 500 MHz spectrometer. Chemical shifts are reported in ppm relative to the residual solvent peak ($CDCl_3$ = 7.26 ppm for ^1H, 77.0 ppm for ^{13}C, DMSO-d_6 = 2.49 ppm for ^1H, 39.5 ppm for ^{13}C) or to an external standard (85% H_3PO_4 = 0 for ^{31}P). Coupling constants are given in Hertz. IR spectra were measured on a Nicolet 8700. Elemental analysis was performed using CHNS-Carlo Erba EA-1108. Column chromatography was performed using silica gel 60 (230–400 mesh, Merck). Preparative thin-layer chromatography was performed with Polygram SIL G/UV254 silica gel (Macherey-Nagel).

Synthesis

General method for the synthesis of biphenyl derivatives modified by ethyl or methyl phosphoroamidate groups

To an ice-cooled solution of phosphorus oxychloride (0.900 g, 5.87 mmol) in dry tetrahydrofuran (THF; 20 mL) was added a solution of the corresponding biphenyl-4-ol (5.87 mmol) in THF dropwise, followed by triethylamine (0.594 g, 5.87 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 1 h. the triethylamine hydrochloride precipitate removed by filtration, and sodium alkoxide (5.87 mmol) (freshly prepared by the addition of absolute ethanol or methanol to 60% NaH dispersed in mineral oil) was added. The reaction mixture was stirred for 0.5 h, and a precipitate of NaCl formed. After 30 min, a solution of NH_3 in methanol (5 mL) was added and the reaction mixture concentrated under vacuum. The resulting residue was dispersed in cold methanol and filtered, and solvent was evaporated under vacuum. The crude product was crystallized from ethyl acetate.

Biphenyl-4-yl methyl phosphoroamidate 3a

Yield 44%, mp 173–175 °C; IR (neat, ν, cm^{-1}): 3325, 3247, 1603, 1573, 1520, 1485, 1225, 1189, 922, 806; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 3.67 (3H, d, J = 11.7, CH_3), 5.11 (2H, d, $J_{P\text{-}N}$ = 6.8, NH_2), 7.28 (2H, d, J = 7.8, Ar-H), 7.34 (1H, t, J = 7.3, Ar-H), 7.44 (2H, t, J = 7.3, Ar-H), 7.61–7.67 (4H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 151.4 (d, $J_{P\text{-}C}$ = 6.1), 140.2, 137.0, 129.6, 128.5, 128.0, 127.2, 121.4 (d, $J_{P\text{-}C}$ = 4.8), 53.5 (d, $J_{P\text{-}C}$ = 5.7); ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 9.50. Anal. Calcd for: $C_{13}H_{14}NO_3P$: C, 59.32; H, 5.36; N, 5.32. Found: C, 59.40; H, 5.41; N, 5.38.

4′-(ethoxycarbonyl)biphenyl-4-yl methyl phosphoroamidate 3b

Yield 25%, mp 134–136 °C; IR (neat, ν, cm^{-1}): 3331, 3251, 1703, 1601, 1568, 1527, 1493, 1272, 1233, 1186, 1110, 921, 806; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 1.32 (3H, t, J = 6.8, CH_3), 3.67 (3H, d, J = 11.2, CH_3), 4.32 (2H, q, J = 6.8, CH_2), 5.14 (2H, d, $J_{P\text{-}N}$ = 6.8, NH_2), 7.31 (2H, d, J = 8.3, Ar-H), 7.71–7.80 (4H, m, Ar-H), 8.01 (2H, d, J = 8.3, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 166.2, 152.2 (d, $J_{P\text{-}C}$ = 6.1), 144.6, 135.5, 130.5, 129.2, 128.9, 127.4, 121.5 (d, $J_{P\text{-}C}$ = 4.4), 61.4, 53.5 (d, $J_{P\text{-}C}$ = 5.7), 14.9; ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 9.45. Anal. Calcd for: $C_{16}H_{18}NO_5P$: C, 57.31; H, 5.41; N, 4.18. Found: C, 57.37; H, 5.46; N, 4.26.

Biphenyl-4-yl ethyl phosphoroamidate 3c

Yield 15%, mp 126–128 °C; IR (neat, ν, cm^{-1}): 3328, 3248, 1604, 1572, 1520, 1486, 1221, 1191, 1036, 954, 841, 770; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 1.25 (3H, t, J = 7.3, CH_3) 4.01–4.08 (2H, m, CH_2), 5.07 (2H, d, $J_{P\text{-}N}$ = 6.3, NH_2), 7.27 (2H, d, J = 7.8, Ar-H), 7.34 (1H, t, J = 7.3, Ar-H), 7.44 (2H, t, J = 7.8, Ar-H), 7.61–7.66 (4H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 151.5 (d, $J_{P\text{-}C}$ = 6.6), 140.2, 136.8, 129.6, 128.5, 127.9, 127.2, 121.4 (d, $J_{P\text{-}C}$ = 4.4), 62.6 (d, $J_{P\text{-}C}$ = 5.7), 16.8 (d, $J_{P\text{-}C}$ = 7.0); ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 8.20. Anal. Calcd for: $C_{14}H_{16}NO_3P$: C, 60.65; H, 5.82; N, 5.05. Found: C, 60.58; H, 5.89; N, 5.09.

4′-(ethoxycarbonyl)biphenyl-4-yl ethyl phosphoroamidate 3d

Yield 25%, mp 143–145 °C; IR (neat, ν, cm^{-1}): 3343, 3253, 1707, 1606, 1564, 1525, 1492, 1396, 1277, 1224, 1184, 1032, 947, 837, 773; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 1.23–1.34 (6H, m, CH_3), 4.02–4.08 (2H, m, CH_2), 4.32 (2H, q, J = 7.3, CH_2), 5.10 (2H, d, $J_{P\text{-}N}$ = 5.8, NH_2), 7.31

(2H, d, $J = 8.3$, Ar-H), 7.72–7.80 (4H, m, Ar-H), 8.01 (2H, d, $J = 7.8$, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 166.2, 152.2 (d, $J_{P\text{-}C} = 6.1$), 144.6, 135.4, 130.4, 129.2, 128.9, 127.4, 121.5 (d, $J_{P\text{-}C} = 4.4$), 62.6 (d, $J_{P\text{-}C} = 5.3$), 61.4, 16.8 (d, $J_{P\text{-}C} = 7.0$), 14.9; ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 8.14. Anal. Calcd for: $C_{17}H_{20}NO_5P$: C, 58.45; H, 5.77; N, 4.01. Found: C, 58.55; H, 5.83; N, 4.11.

General method for the synthesis of biphenyl derivatives modified by phosphoric acid diethyl or dimethyl ester groups

To an ice-cooled solution of phosphorus oxychloride (0.900 g, 5.87 mmol) in dry THF (20 mL) was added a solution of the corresponding biphenyl-4-ol (5.87 mmol) in THF dropwise, followed by triethylamine (0.594 g, 5.87 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 1 h. The triethylamine hydrochloride precipitate was removed by filtration, and sodium alkoxide (11.74 mmol) (freshly prepared by the addition of absolute ethanol or methanol to 60% NaH dispersed in mineral oil) was added. The reaction mixture was stirred for 1 h, and a precipitate of NaCl formed. The solution was filtered, and the solvent was evaporated. The resulting residue was purified by column chromatography using hexane:ethyl acetate 3:2 as an eluent to give the desired products.

Biphenyl-4-yl dimethyl phosphate 3e

Yield 45%, mp 44–45 °C; IR (neat, ν, cm^{-1}): 1603, 1519, 1485, 1281, 1221, 1194, 1009, 928, 841, 765; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.89 (6H, d, $J = 11.2$, CH_3), 7.29 (2H, d, $J = 7.8$, Ar-H), 7.33–7.46 (3H, m, Ar-H), 7.53–7.57 (4H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 150.3 (d, $J_{P\text{-}C} = 7.0$), 140.4, 138.6, 129.1, 128.7, 127.6, 127.3, 120.4 (d, $J_{P\text{-}C} = 4.8$), 55.2 (d, $J_{P\text{-}C} = 5.7$); ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): -2.87. Anal. Calcd for: $C_{14}H_{15}O_4P$: C, 60.43; H, 5.43. Found: C, 60.49; H, 5.50.

4′-(ethoxycarbonyl)biphenyl-4-yl dimethyl phosphate 3f

Yield 65%, mp 59–60 °C; IR (neat, ν, cm^{-1}): 1705, 1608, 1523, 1493, 1279, 1217, 1183, 1023, 937, 842, 774; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.38–1.42 (3H, m, CH_3), 3.89 (6H, dd, $J = 11.2$, CH_3), 4.36–4.41 (2H, m, CH_2), 7.31 (2H, d, $J = 8.3$, Ar-H), 7.57–7.61 (4H, m, Ar-H), 8.09 (2H, d, $J = 8.3$, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 166.6, 150.9 (d, $J_{P\text{-}C} = 6.6$), 144.6, 137.4, 130.3, 129.6, 128.9, 127.1, 120.6 (d, $J_{P\text{-}C} = 4.8$), 61.5, 55.2 (d, $J_{P\text{-}C} = 6.1$), 14.6; ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): -2.95. Anal. Calcd for: $C_{17}H_{19}O_6P$: C, 58.29; H, 5.47. Found: C, 58.34; H, 5.51.

Biphenyl-4-yl diethyl phosphate 3g

Yield 40%, oil; IR (neat, ν, cm^{-1}): 1604, 1516, 1485, 1272, 1219, 1018, 927, 842, 764, 696; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.36–1.40 (6H, m, CH_3) 4.20–4.30 (4H, m, CH_2), 7.30 (2H, d, $J = 7.3$, Ar-H), 7.32–7.37 (1H, m, Ar-H), 7.44 (2H, t, $J = 7.3$, Ar-H), 7.54–7.57 (4H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 150.5 (d, $J_{P\text{-}C} = 7.0$), 140.5, 138.3, 129.0, 128.6, 127.6, 127.2, 120.5 (d, $J_{P\text{-}C} = 4.8$), 64.9 (d, $J_{P\text{-}C} = 5.7$), 16.4 (d, $J_{P\text{-}C} = 6.6$); ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): -5.07. Anal. Calcd for: $C_{16}H_{19}O_4P$: C, 62.74; H, 6.25. Found: C, 62.70; H, 6.29.

4′-(ethoxycarbonyl)biphenyl-4-yl diethyl phosphate 3h

Yield 46%, mp 36–37 °C; IR (neat, ν, cm^{-1}): 1701, 1604, 1523, 1494, 1276, 1221, 1187, 1101, 1019, 931, 838, 769, 698; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.35–1.42 (9H, m, CH_3), 4.20–4.28 (4H, m, CH_2), 4.39 (2H, q, $J = 6.8$, CH_2), 7.31 (2H, d, $J = 7.8$, Ar-H), 7.57–7.62 (4H, m, Ar-H), 8.10 (2H, d, $J = 8.3$, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 166.7, 151.1 (d, $J_{P\text{-}C} = 6.6$), 144.7, 137.1, 130.3, 129.5, 128.8, 127.1, 120.7 (d, $J_{P\text{-}C} = 4.8$), 64.9 (d, $J_{P\text{-}C} = 5.7$), 61.2, 16.4 (d, $J_{P\text{-}C} = 6.6$), 14.6; ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): -5.18. Anal. Calcd for: $C_{19}H_{23}O_6P$: C, 60.31; H, 6.13. Found: C, 60.40; H, 6.16.

General method for the synthesis of biphenyl derivatives modified by phosphorodiamidate group

To an ice-cooled solution of phosphorus oxychloride (0.900 g, 5.87 mmol) in dry THF (20 mL) was added a solution of the corresponding biphenyl-4-ol (5.87 mmol) in THF dropwise, followed by triethylamine (0.594 g, 5.87 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 1 h. The triethylamine hydrochloride precipitate was removed by filtration and a solution of NH_3 in methanol (5 mL) was added. After concentration under vacuum, the crude product was crystallized from methanol to give the desired product.

Biphenyl-4-yl phosphorodiamidate 3i

Yield 34%, mp 233–235 °C; IR (neat, ν, cm^{-1}): 3341, 3223, 1597, 1560, 1518, 1483, 1404,

1241, 1167, 1019. 946, 839, 758; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 4.32 (4H, d, $J_{P\text{-}N} = 4.4$, NH_2), 7.26 (2H, d, $J = 7.8$, Ar-H), 7.30–7.36 (1H, m, Ar-H), 7.43 (2H, t, $J = 7.8$, Ar-H), 7.58–7.63 (4H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 152.4 (d, $J_{P\text{-}C} = 6.6$), 140.4, 136.0, 129.6, 128.1, 127.8, 127.2, 121.9 (d, $J_{P\text{-}C} = 4.4$); ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 15.70. Anal. Calcd for: $C_{12}H_{13}N_2O_2P$: C, 58.07; H, 5.28; N, 11.29. Found: C, 58.01; H, 5.34; N, 11.37.

4′-(ethoxycarbonyl)biphenyl-4-yl phosphorodiamidate 3j

Yield 20%, mp 240–242 °C; IR (neat, v, cm^{-1}): 3331, 3229, 1712, 1599, 1558 1525, 1492, 1396, 1271, 1242, 1170, 1110, 1019, 948, 833, 765; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 1.31–1.34 (3H, m, CH_3), 4.32 (2H, q, $J = 6.8$, CH_2), 4.41 (4H, d, $J_{P\text{-}N} = 3.9$, NH_2), 7.30 (2H, d, $J = 8.3$, Ar-H), 7.68–7.80 (4H, m, Ar-H), 7.99–8.02 (2H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 166.2, 153.2 (d, $J_{P\text{-}C} = 6.6$), 144.9, 134.6, 130.4, 129.0, 128.6, 127.3, 122.1 (d, $J_{P\text{-}C} = 4.4$), 61.4, 14.9; ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 15.75. Anal. Calcd for: $C_{15}H_{17}N_2O_4P$: C, 56.25; H, 5.35; N, 8.75. Found: C, 56.32; H, 5.39; N, 8.83.

General method for the synthesis of phosphoric acid derivatives

To an ice-cooled solution of derivative (**3e**) or (**3f**) (1 mmol) in dry DCM (7 mL) was added TMSBr (4 mmol) dropwise. The reaction mixture was stirred under a nitrogen atmosphere for 1 h. After concentration under vacuum, 5 mL of methanol was added. All solvents were then evaporated, and the crude product was crystallized from ethyl acetate to give the desired product.

Biphenyl-4-yl dihydrogen phosphate 3k

Yield 37%, mp 185–187 °C; IR (neat, v, cm^{-1}): 2770, 1600, 1483, 1403, 1165, 971, 842, 757; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 7.25 (2H, d, $J = 8.3$, Ar-H), 7.32 (1H, t, $J = 7.3$, Ar-H), 7.43 (2H, t, $J = 7.3$, Ar-H), 7.60–7.64 (4H, m, Ar-H), 7.8–9.8 (2H, br s, OH); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 151.9 (d, $J_{P\text{-}C} = 6.6$), 140.2, 136.6, 129.6, 128.5, 127.9, 127.2, 121.2 (d, $J_{P\text{-}C} = 4.8$); ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): -5.04. Anal. Calcd for: $C_{12}H_{11}O_4P$: C, 57.61; H, 4.43. Found: 57.71; H, 4.50.

4′-(ethoxycarbonyl)biphenyl-4-yl dihydrogen phosphate 3l

Yield 53%, mp 154–158 °C; IR (neat, v, cm^{-1}): 2762, 1710, 1600, 1490, 1399, 1272, 1165, 974, 836, 767; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 1.33 (3H, t, $J = 7.0$, CH_3), 4.33 (2H, q, $J = 7.0$, CH_2), 5.00–7.40 (2H, br s, OH), 7.28 (2H, d, $J = 8.7$, Ar-H), 7.70–7.82 (4H, m, Ar-H), 8.01 (2H, d, $J = 8.3$, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 166.2, 152.6, 144.6, 135.2, 130.5, 129.1, 128.9, 127.4, 121.3 (d, $J_{P\text{-}C} = 4.8$), 61.4, 14.9; ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): -5.12. Anal. Calcd for: $C_{15}H_{15}O_6P$: C, 55.91; H, 4.69. Found: C, 55.99; H, 4.74.

General method for the synthesis of biphenyl derivatives modified by ethyl or methyl chlorothiophosphate groups

To an ice-cooled solution of thiophosphoryl chloride (0.994 g, 5.87 mmol) in dry THF (20 mL) was added a solution of the corresponding biphenyl-4-ol (5.87 mmol) in THF dropwise, followed by triethylamine (0.594 g, 5.87 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 1 h. The triethylamine hydrochloride precipitate was removed by filtration, and sodium alkoxide (5.87 mmol) (freshly prepared by the addition of absolute ethanol or methanol to 60% NaH dispersed in mineral oil) was added. The reaction mixture was stirred for 1 h, and a precipitate of NaCl formed. The solution was filtered, and the solvent was evaporated. The resulting residue was purified by column chromatography using petroleum ether:$CHCl_3$ 3:1 as an eluent to give the desired products.

Biphenyl-4-yl methyl chlorothiophosphate 4a′

Yield 60%, mp 70–73 °C; IR (neat, v, cm^{-1}): 1600, 1519, 1483, 1218, 1186, 1016, 931, 844, 760; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.05 (3H, dd, $J = 16.1$, CH_3), 7.26–7.49 (5H, m, Ar-H), 7.56–7.64 (4H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 149.8 (d, $J_{P\text{-}C} = 9.7$), 140.2, 139.7, 129.1, 128.8, 127.8, 127.3, 121.7 (d, $J_{P\text{-}C} = 5.3$), 56.3 (d, $J_{P\text{-}C} = 7.5$); ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 66.94. Anal. Calcd for: $C_{13}H_{12}ClO_2PS$: C, 52.27; H, 4.05; S, 10.73. Found: C, 52.33; H, 4.12; S, 10.79.

4′-(ethoxycarbonyl)biphenyl-4-yl methyl chlorothiophosphate 4b′

Yield 40%, mp 49–51 °C; IR (neat, v, cm^{-1}): 1701, 1608, 1523, 1491, 1275, 1210, 1182, 1106,

1023, 921, 834, 771; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.42 (3H, t, $J = 6.8$, CH_3), 4.04 (3H, d, $J = 16.6$, CH_3), 4.41 (2H, q, $J = 7.3$, CH_2), 7.35–7.38 (2H, m, Ar-H), 7.62–7.65 (4H, m, Ar-H), 8.12 (2H, d, $J = 8.3$, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 166.6, 150.4 (d, $J_{P\text{-}C} = 9.7$), 144.4, 138.5, 130.4, 129.8, 128.9, 127.2, 121.9 (d, $J_{P\text{-}C} = 5.3$), 61.3, 56.4 (d, $J_{P\text{-}C} = 7.5$), 14.6; ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 66.81. Anal. Calcd for: $C_{16}H_{16}ClO_4PS$: C, 51.83; H, 4.35; S, 8.65. Found: C, 51.92; H, 4.40; S, 8.72.

Biphenyl-4-yl ethyl chlorothiophosphate 4c′

Yield 62%, mp 61–64 °C; IR (neat, ν, cm^{-1}): 1601, 1518, 1482, 1215, 1159, 1032, 930, 839, 762; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.48–1.53 (3H, m, CH_3) 4.41–4.51 (2H, m, CH_2), 7.34–7.39 (3H, m, Ar-H), 7.43–7.48 (2H, m, Ar-H), 7.57–7.64 (4H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 149.8 (d, $J_{P\text{-}C} = 9.7$), 140.2, 139.6, 129.1, 128.7 (d, $J_{P\text{-}C} = 1.8$), 127.8, 127.4, 121.7 (d, $J_{P\text{-}C} = 4.8$), 67.2 (d, $J_{P\text{-}C} = 7.5$), 15.9 (d, $J_{P\text{-}C} = 8.8$); ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 64.86. Anal. Calcd for: $C_{14}H_{14}ClO_2PS$: C, 53.76; H, 4.51; S, 10.25. Found: C, 53.70; H, 4.57; S, 10.33.

4′-(ethoxycarbonyl)biphenyl-4-yl ethyl chlorothiophosphate 4d′

Yield 58%, mp 58–60 °C; IR (neat, ν, cm^{-1}): 1709, 1608, 1519, 1491, 1276, 1208, 1166, 1101, 1025, 932, 837, 772; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.42 (3H, t, $J = 7.3$, CH_3), 1.50 (3H, t, $J = 7.3$, CH_3), 4.38–4.50 (4H, m, CH_2), 7.36–7.39 (2H, m, Ar-H), 7.63 (4H, d, $J = 7.3$, Ar-H), 8.12 (2H, d, $J = 8.3$, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 166.6, 150.4 (d, $J_{P\text{-}C} = 9.7$), 144.4, 138.4, 130.4, 129.7, 128.9, 127.2, 121.9 (d, $J_{P\text{-}C} = 4.8$), 67.2 (d, $J_{P\text{-}C} = 7.0$), 61.3, 15.9 (d, $J_{P\text{-}C}$=8.3), 14.6; ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 64.72. Anal. Calcd for: $C_{17}H_{18}ClO_4PS$: C, 53.06; H, 4.71; S, 8.33. Found: C, 53.13; H, 4.80; S, 8.36.

General method for the synthesis of biphenyl derivatives modified by ethyl or methyl thiophosphoroamidate groups

To an ice-cooled solution of corresponding monochloride derivatives (**4a′, 4b′, 4c′** or **4d′**) (2.94 mmol) in dry THF (5 mL) a solution of NH_3 in methanol (2 mL) was added. After concentration under vacuum, the resulting residue was purified by column chromatography using $CHCl_3$:petroleum ether 3:1 as an eluent to give the desired products.

Biphenyl-4-yl methyl thiophosphoroamidate 3a′

Yield 59%, mp 125–127 °C; IR (neat, ν, cm^{-1}): 3387, 3296, 1600, 1516, 1483, 1221, 1187, 912, 763, 695; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 3.70 (3H, d, $J = 13.7$, CH_3), 5.73 (2H, d, $J_{P\text{-}N} = 6.8$, NH_2), 7.28–7.36 (3H, m, Ar-H), 7.45 (2H, t, $J = 7.8$, Ar-H), 7.62–7.67 (4H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 151.2 (d, $J_{P\text{-}C} = 7.0$), 140.1, 137.3, 129.6, 128.4 128.0, 127.3, 122.3 (d, $J_{P\text{-}C} = 4.8$), 53.8 (d, $J_{P\text{-}C} = 5.3$); ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 72.93. Anal. Calcd for: $C_{13}H_{14}NO_2PS$: C, 55.90; H, 5.05; N, 5.02; S, 11.48. Found: C, 55.98; H, 5.11; N, 5.10; S, 11.54.

4′-(ethoxycarbonyl)biphenyl-4-yl methyl thiophosphoroamidate 3b′

Yield 25%, mp 78–80 °C; IR (neat, ν, cm^{-1}): 3366, 3282, 1716, 1602, 1523, 1493, 1281, 1219, 1185, 1108, 908, 769, 709; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.41 (3H, t, $J = 6.8$, CH_3), 3.47 (2H, s, NH_2), 3.86 (3H, d, $J = 14.2$, CH_3), 4.40 (2H, q, $J = 6.8$, CH_2), 7.32–7.35 (2H, m, Ar-H), 7.58–7.63 (4H, m, Ar-H), 8.10 (2H, d, $J = 8.3$, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 166.7, 151.2 (d, $J_{P\text{-}C} = 7.0$), 144.8, 137.3, 130.3, 129.5, 128.6, 127.1, 121.8 (d, $J_{P\text{-}C} = 4.8$), 61.3, 54.4 (d, $J_{P\text{-}C} = 5.3$), 14.6; ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 71.13. Anal. Calcd for: $C_{16}H_{18}NO_4PS$: C, 54.69; H, 5.16; N, 3.99; S, 9,13. Found: C, 54.77; H, 5.23; N, 4.02; S, 9,18.

Biphenyl-4-yl ethyl thiophosphoroamidate 3c′

Yield 70%, mp 82–83 °C; IR (neat, ν, cm^{-1}): 3425, 3285, 1600, 1541, 1514, 1483, 1389, 1217, 1165, 1036, 942, 905, 840, 762, 626; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 1.40 (3H, t, $J = 6.8$, CH_3), 3.44 (2H, s, NH_2), 4.21–4.29 (2H, m, CH_2), 7.30–7.37 (3H, m, Ar-H), 7.41–7.46 (2H, m, Ar-H), 7.56 (4H, d, $J = 8.8$, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 150.6 (d, $J_{P\text{-}C} = 7.5$), 140.6, 138.4, 129.0, 128.4, 127.5, 127.3, 121.7 (d, $J_{P\text{-}C} = 4.4$), 64.3 (d, $J_{P\text{-}C}$ 5.3), 16.2 (d, $J_{P\text{-}C} = 8.3$); ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 69.23. Anal. Calcd for: $C_{14}H_{16}NO_2PS$: C, 57.33; H, 5.50; N, 4.78; S, 10.93. Found: C, 57.29; H, 5.53; N, 4.73; S, 10.98.

4′-(ethoxycarbonyl)biphenyl-4-yl ethyl thiophosphoroamidate 3d′

Yield 46%, mp 72–75 °C; IR (neat, ν, cm^{-1}): 3415, 3311, 1706, 1600, 1537, 1523, 1493, 1386, 1279, 1215, 1170, 1035, 950, 910, 837, 769, 637; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz):

1.37–1.43 (6H, m, CH_3), 3.48 (2H, s, NH_2), 4.21–4.28 (2H, m, CH_2), 4.37–4.42 (2H, m, CH_2), 7.33 (2H, d, $J = 8.3$, Ar-H), 7.57–7.63 (4H, m, Ar-H), 8.01 (2H, d, $J = 7.3$, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 166.7, 151.3 (d, $J_{P\text{-}C} = 7.5$), 144.8, 137.2, 130.3, 129.4, 128.6, 127.1, 121.9 (d, $J_{P\text{-}C} = 4.8$), 64.3 (d, $J_{P\text{-}C} = 4.8$), 61.3, 16.2 (d, $J_{P\text{-}C} = 8.3$), 14.6; ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 69.21. Anal. Calcd for: $C_{17}H_{20}NO_4PS$: C, 55.88; H, 5.52; N, 3.83; S, 8.78. Found: C, 55.95; H, 5.60; N, 3.92; S, 8.85.

General method for the synthesis of biphenyl derivatives modified by thiophosphoric acid diethyl or dimethyl ester groups

To an ice-cooled solution of corresponding monochloride derivatives (**4a′**, **4b′**, **4c′** or **4d′**) (2.94 mmol) in dry THF (5 mL) sodium alkoxide (2.94 mmol) (freshly prepared by the addition of absolute ethanol or methanol to 60% NaH dispersed in mineral oil) was added. The reaction mixture was stirred for 1 h, and a precipitate of NaCl formed. The solution was filtered, and the solvent was evaporated. The resulting residue was purified by column chromatography using hexane:ethyl acetate 2:1 as an eluent to give the desired products.

Biphenyl-4-yl dimethyl thiophosphate 3e′

Yield 30%, mp 36–37 °C; IR (neat, ν, cm^{-1}): 1601, 1518, 1481, 1219, 1182, 1014, 932, 827, 762, 693; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.89 (6H, d, $J = 14.2$, CH_3), 7.25–7.28 (2H, m, Ar-H), 7.33–7.38 (1H, m, Ar-H), 7.44 (2H, t, $J = 8.3$, Ar-H) 7.55–7.59 (4H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 150.3 (d, $J_{P\text{-}C} = 7.5$), 140.4, 138.7, 129.0, 128.6, 127.6, 127.3, 121.3 (d, $J_{P\text{-}C} = 4.8$), 55.5 (d, $J_{P\text{-}C} = 5.7$); ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 67.70. Anal. Calcd for: $C_{14}H_{15}O_3PS$: C, 57.13; H, 5.14; S, 10.90. Found: C, 57.21; H, 5.20; S, 10.98.

4′-(ethoxycarbonyl)biphenyl-4-yl dimethyl thiophosphate 3f′

Yield 48%, mp 54–56 °C; IR (neat, ν, cm^{-1}): 1710, 1602, 1523, 1492, 1279, 1219, 1179, 1103, 1022, 931, 829, 771, 700; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.41 (3H, t, $J = 7.3$, CH_3), 3.89 (6H, d, $J = 13.7$, CH_3), 4.40 (2H, q, $J = 7.3$, CH_2), 7.26–7.29 (2H, m, Ar-H), 7.59–7.63 (4H, m, Ar-H), 8.10 (2H, d, $J = 8.3$, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 166.7, 151.0, 144.7, 137.5, 130.3, 129.6, 128.8, 127.1, 121.6 (d, $J_{P\text{-}C} = 4.8$), 61.3, 55.5 (d, $J_{P\text{-}C} = 5.7$), 14.6; ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 67.65. Anal. Calcd for: $C_{17}H_{19}O_5PS$: C, 55.73; H, 5.23; S, 8.75. Found: C, 55.79; H, 5.28; S, 8.82.

Biphenyl-4-yl diethyl thiophosphate 3g′

Yield 58%, oil; IR (neat, ν, cm^{-1}): 1604, 1514, 1483, 1213, 1165, 1017, 921, 842, 762, 696; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.38–1.41 (6H, m, CH_3) 4.24–4.31 (4H, m, CH_2), 7.25–7.28 (2H, m, Ar-H), 7.33–7.37 (1H, m, Ar-H), 7.42–7.46 (2H, m, Ar-H) 7.56 (2H, d, $J = 8.3$, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 150.5 (d, $J_{P\text{-}C} = 7.9$), 140.5, 138.5, 129.0, 128.5, 127.6, 127.3, 121.4 (d, $J_{P\text{-}C} = 4.8$), 65.4 (d, $J_{P\text{-}C} = 5.7$), 16.2 (d, $J_{P\text{-}C} = 7.5$); ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 64.16. Anal. Calcd for: $C_{16}H_{19}O_3PS$: C, 59.61; H, 5.94; S, 9.95. Found: C, 59.69; H, 5.98; S, 10.01.

4′-(ethoxycarbonyl)biphenyl-4-yl diethyl thiophosphate 3h′

Yield 48%, mp 44–47 °C; IR (neat, ν, cm^{-1}): 1701, 1605, 1521, 1493, 1276, 1215, 1182, 1103, 1016, 928, 836, 770, 703; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.37–1.43 (9H, m, CH_3), 4.23–4.31 (4H, m, CH_2), 4.40 (2H, q, $J = 7.3$, CH_2), 7.26–7.30 (2H, m, Ar-H), 7.58–7.64 (4H, m, Ar-H), 8.10 (2H, d, $J = 8.3$, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 166.7, 151.1 (d, $J_{P\text{-}C} = 7.5$), 144.7, 137.3, 130.3, 129.5, 128.7, 127.1, 121.7 (d, $J_{P\text{-}C} = 4.8$), 65.4 (d, $J_{P\text{-}C} = 5.7$), 61.2, 16.2 (d, $J_{P\text{-}C} = 7.5$), 14.6; ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 64.11. Anal. Calcd for: $C_{19}H_{23}O_5PS$: C, 57.86; H, 5.88; S, 8.13. Found: C, 57.93; H, 5.92; S, 8.20.

General method for the synthesis of biphenyl derivatives modified by thiophosphorodiamidate group

To an ice-cooled solution of thiophosphoryl chloride (0.994 g, 5.87 mmol) in dry THF (20 mL) was added a solution of the corresponding biphenyl-4-ol (5.87 mmol) in THF dropwise, followed by triethylamine (0.594 g, 5.87 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 3 h. The triethylamine hydrochloride precipitate was removed by filtration and a solution of NH_3 in methanol (5 mL) was added. After concentration under vacuum, the crude product was crystallized from methanol to give the desired product.

Biphenyl-4-yl thiophosphorodiamidate 3i′

Yield 51%, mp 187–190 °C; IR (neat, ν, cm^{-1}): 3383, 3240, 1599, 1514, 1480, 1403, 1219, 1186, 1167, 1016, 885, 841, 773; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 4.88 (4H, d, $J_{P\text{-}N} = 3.4$, NH_2),

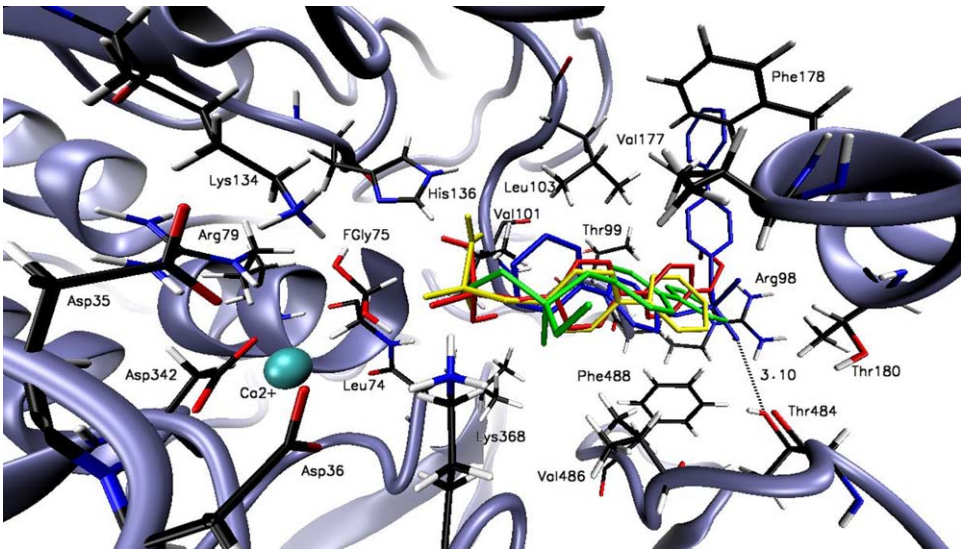

Figure 1. Docked binding modes for compounds **3l** (red), **3f** (green), **5d** (blue) and biphenyl-4-yl sulfamate (yellow). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

7.22–7.35 (3H, m, Ar-H), 7.42 (2H, t, $J = 7.3$, Ar-H), 7.60–7.64 (4H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 151.9 (d, $J_{P\text{-}C} = 7.9$), 140.4, 136.6, 129.6, 128.0, 127.8, 127.2, 123.0 (d, $= J_{P\text{-}C}$ 4.8); ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 70.44. Anal. Calcd for: $C_{12}H_{13}N_2OPS$: C, 54.54; H, 4.96; N, 10.60; S, 12.13. Found: C, 54.62; H, 5.03; N, 10.66; S, 12.19.

4′-(ethoxycarbonyl)biphenyl-4-yl thiophosphorodiamidate 3j′

Yield 44%, mp 183–185 °C; IR (neat, ν, cm^{-1}): 3379, 3242, 1710, 1599, 1523, 1489, 1397, 1273, 1221, 1186, 1168, 1116, 1022, 884, 834, 776; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 1.31–1.34 (3H, t, $J = 6.8$, CH_3), 4.32 (2H, q, $J = 6.8$, CH_2), 4.90 (4H, d, $J_{P\text{-}N} = 3.9$, NH_2), 7.31–7.34 (2H, m, Ar-H), 7.70–7.81 (4H, m, Ar-H), 8.01 (2H, d, $J = 8.8$ Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 166.2, 152.7 (d, $J_{P\text{-}C} = 7.6$), 144.8, 135.1, 130.4, 129.1, 128.4, 127.4, 123.1 (d, $J_{P\text{-}C} = 4.8$), 61.4, 14.9; ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 70.46. Anal. Calcd for: $C_{15}H_{17}N_2O_3PS$: C, 53.56; H, 5.09; N, 8.33; S, 9.53. Found: C, 53.50; H, 5.12; N, 8.37; S, 9.62.

General method for the synthesis of biphenyl-4-yl dichlorothiophosphate 2a′

To an ice-cooled solution of thiophosphoryl chloride (0.848 g, 0.05 mol) in dry THF (20 mL) was added a solution of biphenyl-4-ol (0.05 mol) in THF dropwise, followed by triethylamine (0.505 g, 0.05 mol). The reaction mixture was stirred under a nitrogen atmosphere for 3 h. The triethylamine hydrochloride precipitate was removed by filtration, and the solvent was evaporated. The resulting residue was purified by column chromatography using CH_2Cl_2:hexane 1:6 as eluent to give the desired products.

Biphenyl-4-yl dichlorothiophosphate 2a′

Yield 68%, mp 65–67 °C; IR (neat, ν, cm^{-1}): 1599, 1516, 1482, 1210, 1189, 1159, 1015, 915, 838, 761; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.35–7.67 (9H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 149.9 (d, $J_{P\text{-}C} = 14.0$), 140.5, 139.9, 129.2, 128.9 (d, $J_{P\text{-}C} = 10.6$), 128.0, 127.4, 121.9 (d, $J_{P\text{-}C} = 5.7$); ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 55.08.

General method for the synthesis of di(biphenyl-4-yl) chlorothiophosphate 5a

To a solution of biphenyl-4-yl dichlorothiophosphate (**2a′**) (1.0 mmol) in dry THF (15 mL) was added the solution of biphenyl-4-ol (1.0 mmol) followed by triethylamine (1.0 mmol). The reaction mixture was stirred under a nitrogen atmosphere for 24 h. The triethylamine hydrochloride precipitate was removed by filtration, and the solvent was evaporated. The resulting residue was washed with hot ethyl acetate to give the desired products.

Yield 55%, mp 210–212 °C; IR (neat, ν, cm^{-1}): 1599, 1518, 1481, 1213, 1186, 1157, 1016, 945, 932, 841, 760; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.37–7.48 (10H, m, Ar-H), 7.57–7.65 (8H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ,

ppm): 150.0 (d, $J_{P\text{-}C} = 10.1$), 140.2, 139.9, 129.1, 128.8, 127.8, 127.4, 121.8 (d, $J_{P\text{-}C} = 5.3$); ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 59.97. Anal. Calcd for: $C_{24}H_{18}ClO_2PS$: C, 65.98; H, 4.15; S, 7.34. Found: C, 65.93; H, 4.22; S, 7.40.

General method for the synthesis of di(biphenyl-4-yl) methyl thiophosphate 5b

To a solution of di(biphenyl-4-yl) chlorothiophosphate (**5a**) (1.0 mmol) in dry THF (15 mL) was added methanol (1.0 mmol) followed by potassium carbonate (1.0 mmol). The reaction mixture was kept under reflux for 24 h and filtered. After concentration under vacuum, the resulting residue was purified by column chromatography using CH_2Cl_2:hexane 1:2 as eluent to give the desired products.

Yield 34%, mp 123–125 °C; IR (neat, ν, cm^{-1}): 1597, 1518, 1482, 1216, 1188, 1165, 1015, 913, 844, 764; PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.02 (3H, dd, $J = 11.7$, CH_3) 7.25–7.38 (6H, m, Ar-H), 7.45 (4H, t, $J = 7.8$, Ar-H), 7.54–7.63 (8H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 150.3 (d, $J_{P\text{-}C} = 7.9$), 140.4, 139.0, 129.1, 128.6, 127.7, 127.3, 121.5 (d, $J_{P\text{-}C} = 4.8$), 56.0 (d, $J_{P\text{-}C} = 5.7$); ^{31}P NMR spectrum (202 MHz, $CDCl_3$, δ, ppm): 61.08. Anal. Calcd for: $C_{25}H_{21}O_3PS$: C, 69.43; H, 4.89; S, 7.41. Found: C, 69.51; H, 4.94; S, 7.47.

General method for the synthesis of di(biphenyl-4-yl) thiophosphoroamidate 5c

To a solution of di(biphenyl-4-yl) chlorothiophosphate (5a) (1.0 mmol) in THF (15 mL) was added a methanol solution of NH_3 (1 mL). The reaction mixture was stirred for 1 h. After concentration under vacuum, the resulting residue was purified by column chromatography using $CHCl_3$:AcOEt 10:1 as eluent to give the desired products.

Yield 67%, mp 239–242 °C; IR (neat, ν, cm^{-1}): 3404, 3230, 1601, 1535, 1513, 1482, 1217, 1184, 1162, 1015, 915, 841, 760; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 6.21 (2H, t, $J_{P\text{-}N} = 6.8$, NH_2) 7.34–7.39 (6H, m, Ar-H), 7.46 (4H, t, $J = 7.3$, Ar-H), 7.65–7.72 (8H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 151.0 (d, $J_{P\text{-}C} = 7.0$), 140.1, 137.6, 129.7, 128.5, 128.1, 127.3, 122.4 (d, $J_{P\text{-}C} = 4.4$); ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 67.33. Anal. Calcd for: $C_{24}H_{20}NO_2PS$: C, 69.05; H, 4.83; N, 3.36; S, 7.68. Found: C, 69.12; H, 4.89; N, 3.43; S, 7.70.

General method for the synthesis of di(biphenyl-4-yl) hydrogen thiophosphate 5d

To a solution of di(biphenyl-4-yl) chlorothiophosphate (5a) (1.0 mmol) in THF (15 mL) was added a water solution of potassium carbonate (0.5 mL). The reaction mixture was stirred for 24 h. After concentration under vacuum, the resulting residue was purified by column chromatography using AcOEt:MeOH 20:1 as eluent to give the desired products.

Yield 48%, mp 267 °C with decomposition; IR (neat, ν, cm^{-1}): 3382, 1603, 1514, 1483, 1205, 1166, 1105, 1009, 903, 834, 757; PMR spectrum (500 MHz, DMSO, δ, ppm, J/Hz): 7.27–7.32 (6H, m, Ar-H), 7.42 (4H, t, $J = 7.8$, Ar-H), 7.55–7.63 (8H, m, Ar-H); ^{13}C NMR spectrum (125 MHz, DMSO, δ, ppm): 153.4 (d, $J_{P\text{-}C} = 7.9$), 140.6, 135.3, 129.6, 127.9, 127.6, 127.7, 121.8 (d, $J_{P\text{-}C} = 5.3$); ^{31}P NMR spectrum (202 MHz, DMSO, δ, ppm): 45.51. Anal. Calcd for: $C_{24}H_{19}O_3PS$: C, 68.89; H, 4.58; S, 7.66. Found: C, 68.94; H, 4.51; S, 7.72.

Enzyme Purification

STS was extracted from human placenta and purified to homogeneity following a multistep chromatography protocol as previously described [Hernandez-Guzman et al., 2001].

In vitro STS assay

The reaction mixture, at a final volume of 100 µL, contained 20 µM Tris-HCl pH 7.4, 1 mM NPS (p-nitrocatechol sulfate), various concentrations of inhibitor (0.1–500 µM) and 5 U of purified enzyme (1 U is the amount of enzyme that hydrolyzes 100 µM of NPS in 1 h at 37 °C). The reaction was performed at 37 °C for 15 min and was halted by the addition of 100 µL of 1 M NaOH. Ten samples containing various concentration of inhibitor (0.1–500 µM) were performed in triplicate. The absorbance of the released p-nitrophenol was measured at 405 nm using a Microplate Reader Biotek ELx800 (SERVA). IC_{50} values were calculated using GraphPad Prism (version 4.0) software. In the first step the concentration values were transformed to log(concentration). To compute the IC_{50} values, the options were selected as follows: Nonlinear Regression (Curve Fit)/Classic equation/Sigmoidal dose–response (variable slope).

RESULTS AND DISCUSSION

Molecular Modeling

The X-ray structure of human STS was taken from the Protein Databank (Protein Data Bank accession code 1P49). After standard preparation of enzyme structure (including conversion of the catalytic amino acid FGly75 (formylglycine) to the

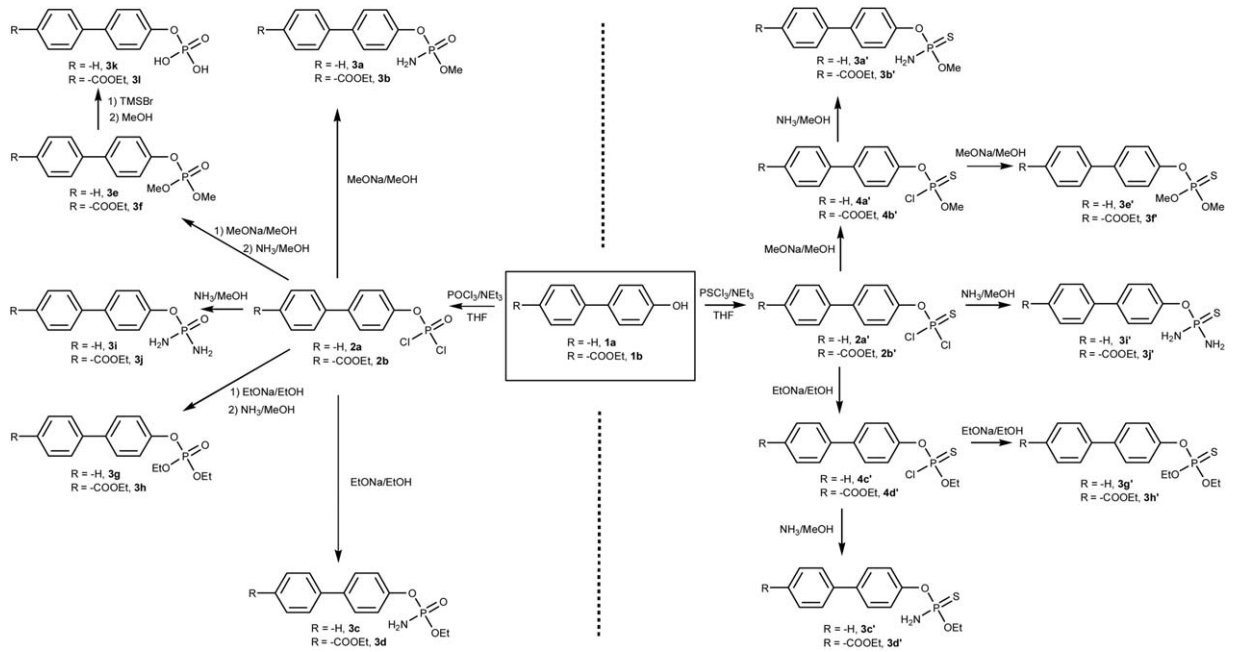

Figure 2. Synthesis of biphenyl-4-yl phosphate and thiophosphate derivatives.

gem – diol form) the docking analysis was carried out using the Autodock Vina 1.1.2 (The Molecular Graphics Laboratory, Scripps Research Institute) software. Docking studies were performed 10 times for each compound with the aim of studying the capability of the designed inhibitors to interact with the ligand binding domain (LBD) of STS (Fig. 1).

All the newly designed compound candidates theoretically bound STS. Analysis of the docking studies for compounds (**3a–3l**) and (**3a′–4d′**) showed that these STS inhibitors could adopt substrate-like poses in active site of STS in a similar manner to the mode of reported biphenyl-4-yl sulfamate (used as reference) with the phosphate or thiophosphate groups directed toward the catalytic cavity of STS (predicted binding energies from -4.5 to -6.8 kcal mol^{-1}). The best docking results were obtained for the di(biphenyl-4-yl) thiophosphates (**5a–5d**) which exhibited slightly different docking modes (not previously described) and led to values within the lowest energy range (from -7.1 to -8.0 kcal mol^{-1}). In this case, one of the nonpolar biphenyl skeleton was oriented in the centre of the active site and underwent nonpolar interaction with side chains of the hydrophobic pocket formed by Leu74, Arg98, Arg99, Val101, Leu103, Val177, Phe178, Thr180, Thr484, Val486, and Phe488. Another part of biphenyl scaffolds is located outside the entrance to the STS active site. For compound (**5d**) a short distance of the OH group to Thr484 (3.10 Å) could favor the binding via hydrogen bonding and may have a significant impact for its activity.

Chemistry

Substituted biphenyls can be prepared synthetically by various methods include: coupling reactions (Hiyama [Cornelissen et al., 2014], Hiyama-Denmark [Denmark and Tymonko, 2005], Kumada [Ackermann et al., 2010], Negishi [Liu et al., 2013], Suzuki [Dreher et al., 2009] or Stille coupling [Mee et al., 2004]), Ullmann reaction [Zhang et al., 2015], reaction of Grignard reagents with bromobenzene [Huynh and Jothibasu, 2009] or by reactions of phenyl lithium with fluorobenzene [Zhu and Wang, 2014] or benzyne [Burdon et al., 1979] Figure 2.

As a result of the theoretical study, we decided to synthesize phosphate and thiophosphate derivatives based on two biphenyl scaffolds. 4-phenylphenol (**1a**) was commercially available, analog (**1b**) was obtained *via* Fisher esterification of the 4′-hydroxybiphenyl-4-carboxylic acid with ethanol in the presence of sulfuric acid as an acidic catalyst. In the first step, compounds (**1a**) and (**1b**) were treated with phosphoryl or thiophosphoryl chloride in the presence of triethylamine at 0 °C to yield phosphoryl or thiophosphoryl dichloride derivatives (**2a**, **2b**) and (**2a′**, **2b′**). The progress of reactions was monitored

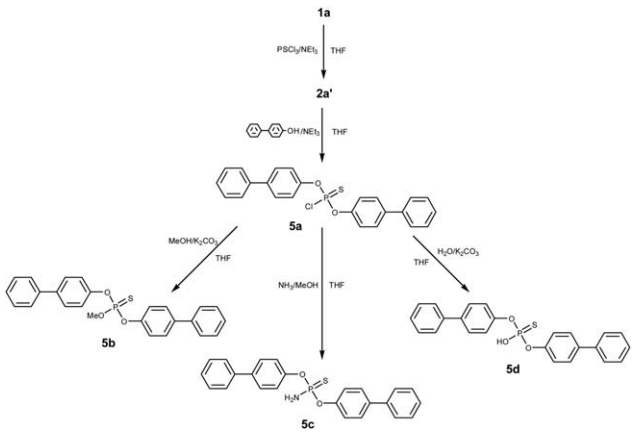

Figure 3. Synthesis of di(biphenyl-4-yl) thiophosphates (**5a-5d**).

by ^{31}P NMR spectra. After removal of precipitated triethylamine hydrochloride, raw phosphoryl or thiophosphoryl dichlorides (**2a**, **2b**) and (**2a′**, **2b′**) were treated at 0 °C with nucleophilic agents including MeONa, EtONa or NH_3/MeOH in different stoichiometric ratios to obtain the corresponding phosphate and thiophosphate biphenyl derivatives in good yield (Figure 2). Finally, biphenyl dimethyl phosphates (**3e**, **3f**) were transformed into the corresponding dihydrogenphosphate analogs (**3k**, **3l**) by treatment with bromotrimethylsilane (TMSBr). To further study the structure-activity relationships, we synthesized additional thiophosphate analogs (**5a–5d**), which exhibited very promising binding modes in molecular modeling studies. The detailed synthesis is shown in Figure 3. In this case, thiophosphoryl dichloride (**2a′**) was treated with 4-phenylphenol (**1a**) in the presence of triethylamine, obtaining the compound (**5a**) with good yield. Next, methylthiophosphate (**5b**), thiophosphoroamidate (**5c**) and hydrogenthiophosphate analogs (**5d**) were prepared *via* nucleophilic substitution reactions using $MeOH/K_2CO_3$, NH_3/MeOH or H_2O/K_2CO_3, respectively (Fig. 3).

STS Enzyme Assays

The structure-activity relationship of the synthesized biphenyl derivatives with respect to the STS enzyme was evaluated. The affinity of all synthesized compounds for STS was determined using an in vitro STS assay [Vaccaro et al., 1987; Woo et al., 2010]. Table 1 shows a summary of the results where it is clearly shown that phosphate and thiophosphate biphenyl derivatives (**3a–3l**) exhibit moderate STS inhibitor activities. We found good inhibitory potency in case of chlorothiophosphate derivatives (**4a′–4d′**) with IC_{50} values in the range of 46.8 to 53.5 μM.

TABLE 1. Activities of the Synthesized Compounds in STS Enzyme Assays

Lp.	IC_{50} [μM]	Lp.	IC_{50} [μM]
3a	98.0 ± 10.0	**3h′**	108.6 ± 13.9
3b	132.4 ± 16.8	**3i**	125.6 ± 14.9
3a′	86.6 ± 6.4	**3j**	139.6 ± 20.9
3b′	128.2 ± 15.4	**3i′**	97.2 ± 9.0
3c	134.1 ± 15.3	**3j′**	117.7 ± 14.1
3d	115.9 ± 11.5	**3k**	101.7 ± 13.9
3c′	95.6 ± 8.6	**3l**	69.3 ± 7.8
3d′	106.7 ± 13.7	**4a′**	51.2 ± 4.7
3e	101.2 ± 12.3	**4b′**	53.5 ± 5.8
3f	113.5 ± 15.6	**4c′**	48.0 ± 5.2
3e′	69.2 ± 6.4	**4d′**	46.8 ± 6.6
3f′	161.0 ± 20.3	**5a**	28.0 ± 4.3
3g	103.8 ± 10.8	**5b**	58.0 ± 6.8
3h	77.5 ± 8.9	**5c**	51.2 ± 6.2
3g′	82.3 ± 7.3	**5d**	22.1 ± 3.9
		Biphenyl-4-yl sulfamate	91.4 ± 7.9

A comparison of the biphenyl chlorothiophosphates (**4a′–4d′**) with biphenyl dialkoxy phosphates (**3e–3h**) and thiophosphates (**3e′–3h′**) indicated that their STS-inhibitory activity was attenuated. A simialr effect was observed when introducing the NH_2 moiety into the phosphorus atom. Among all synthesized compounds the highest STS inhibition occurred in a series of di(biphenyl) thiophosphate derivatives (**5a–5d**), which is in agreement with the data of molecular modeling studies. Two compounds, (**5a**) and its derivative (**5d**) had IC_{50} values of 28.0 and 22.1 μM respectively as compared to the IC_{50} value of 91.4 μM for biphenyl-4-yl sulfamate used as reference. Furthermore, increased biological activity of (**5d**) may result from the formation of hydrogen bonds with Thr484 residue in the STS active site, which may be critical for the stability of the enzyme-inhibitor complex and lead to a more effective inactivation of STS.

CONCLUSIONS

A series of biphenyl phosphates and thiophosphates have been synthesized, and their affinity for STS have been determined by means of in vitro STS assays. Docking studies on these compounds have demonstrated that the new STS inhibitors could adopt substrate-like poses in active site of STS in a similar manner to the mode of reported biphenyl-4-yl sulfamate. Moreover, we found that di(biphenyl) thiophosphates exhibited a slightly different docking modes (not previously described) to that of the reported sulfamate-based STS inhibitor and led to

increase of inhibitory potency. In the course of our investigation, the greatest inhibitory effect of STS was observed with compound (**5d**) which had an IC_{50} value of 22.1 μM. This outcome suggests that the identified hydrogen bond between thiophosphate moieties and Thr484 could favor binding and may have a significant impact on enzyme–ligand complex stability for STS.

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