Original Article

DOI: 10.5582/ddt.2017.01016

Sulfonyl phosphonic 1,4-dithia-7-azaspiro[4,4]nonane derivatives as matrix metalloproteinase inhibitors: Synthesis, a docking study, and biological evaluation

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Summary **A series of novel sulfonyl phosphonic 1,4-dithia-7-azaspiro[4,4]nonane derivatives were designed, synthesized, and assayed for their activity against matrix metalloproteinase-2 (MMP-2). Results indicated that all of the compounds exhibited moderate inhibitory activity against MMP-2 compared to LY52 (the control) (IC_{50} = 0.95 ± 0.09 μM). Several selected compounds were also examined for their antiproliferative activity against SKOV3, HL60, and A549 cells. Notably, all of the tested compounds had slightly lower antiproliferative activity against SKOV3 cells than that of LY52. Compound 6d displayed the greatest inhibitory activity in an enzymatic assay and a cell-based assay, which means that this compound is a good candidate for further development of phosphonate-based MMP inhibitors.**

Keywords: Matrix metalloproteinase-2, 1,4-dithia-7-azaspiro[4,4]nonane derivatives, inhibitors, synthesis

1. Introduction

The integrity of the extracellular matrix (ECM), a complex network of proteins and polysaccharides surrounding each cell, is a prerequisite for the normal functioning and survival of an organism. Alterations of the ECM are performed by a family of structurally and functionally related zinc-dependent endopeptidases called matrix metalloproteinases (MMPs) that play important roles in physiological and pathological processes such as development, ovulation, wound healing, and angiogenesis (*1,2*). To date, at least 26 members of the MMP family have been identified in humans, and MMPs can be mainly grouped into five classes: collagenases, gelatinases, stromelysins, membrane-type MMPs (MT-MMPs), and matrilysin (*3*). MMPs are minimally expressed and strictly regulated at multiple levels to ensure proper functioning in physiological processes, whereas their overexpression and excessive activity have been implicated in a variety of pathological disorders ranging from cardiovascular disease to cancer (*4-7*). Of all of the identified MMP subtypes, MMP-2, also known as gelatinase A due to its close correlation with tumor progression (*8-10*), has been considered an attractive target for structure-based drug design, and research on MMP-2 inhibitors is a very promising strategy for cancer therapy and development of anticancer drugs (*11*).

The rapid increase in research on the solution and crystal structures of MMP-inhibitor complexes has led to a detailed depiction of the structure of MMPs. Briefly, except for Zn^{2+} in the conserved catalytic center of the MMP-2 enzyme, MMPs have two hydrophobic domains (S_1' and S_2' pockets, respectively) that are located in proximity to the catalytic zinc center. The S_1' pocket, a deep and narrow channel, is the most prominent domain with which to distinguish the selectivity of various MMPs, and this pocket is responsible for most of the observed substrate specificity of a given MMP, while the S_2' pocket is a solvent-exposed cleft (*12,13*). Effective MMP inhibitors

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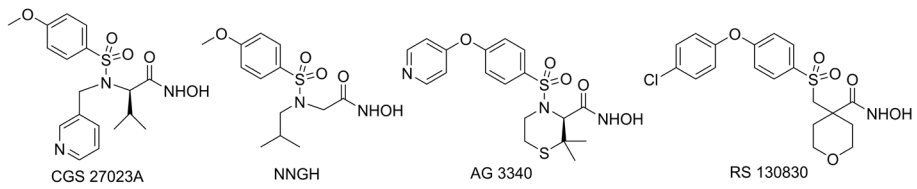


Figure 1. The Structures of CGS27023A, NNGH, AG3340, and RS 130830.

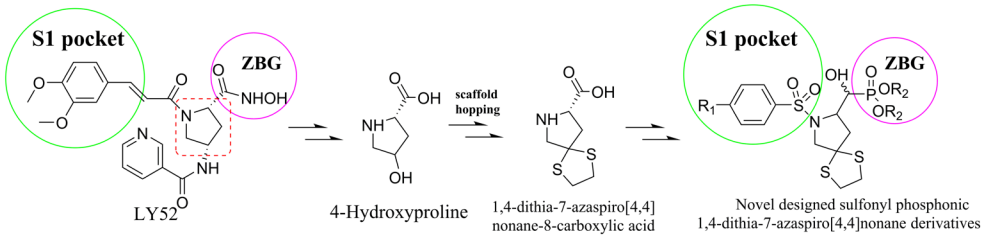


Figure 2. Construction of sulfonyl phosphonic 1,4-dithia-7-azaspiro [4,4] nonane derivatives.

are characterized by: *i*) a "warhead" for chelating with Zn^{2+}, also known as a zinc-binding group (ZBG); *ii*) one or more side chains effectively interacting with active subsites, the primary of which is the S_1'pocket; and *iii*) functional groups providing hydrogen bond interactions with the enzyme backbone (*14,15*).

The discovery of CGS 27023A (Figure 1) opened up a new avenue in the design and development of novel *N*-arylsulfonyl MMPs inhibitors (*16*). Other sulfonamide-based derivatives, including NNGH, AG 3340, and RS 130830, have also been shown in Figure 1 (*16-18*). The vast body of relevant literature indicates that the sulfonamide group was incorporated into MMP inhibitors for the following reasons: *i*) the sulfonyl group can improve enzyme-inhibitor binding by forming effective hydrogen bonds; *ii*) the sulfonyl group can properly anchor and orient the hydrophobic substituent to the S_1' groove *via* a gauche conformation, enabling it to plunge deep into the enzyme-binding domain (*18*).

The current authors' and their colleagues have recently endeavored to identify pyrrolidine derivatives as effective MMP inhibitors, exemplified by LY52 (Figure 2) (*19-22*). Moreover, there are more than 60% hydroxyproline (Hyp) and glycine (Gly) residues among the amino acids in the primary structure of collagen (*23*), which is the specific substrate of gelatinases. Buoyed by these findings, a new class of heterocyclic skeleton, 1,4-dithia-7-azaspiro[4,4]nonane-8-carboxylic acid (Figure 2), was chosen since derivatives or analogues of 4-hydroxyproline hold the promise of recognizing its substrate and subsequently interacting with the active sites of MMPs in a competitive manner. In particular, a 1,3-dithiane ring was reported to have an enormous impact on the *in vivo* efficacy of some antitumor molecules (*24*). Based on the "molecular hybridization principle," a reasonable conjecture was made that such attributes might potentially result in a synergistic effect on MMP-2 inhibition. Pursuant to this hypothesis and in light of the role of the sulfonyl group in MMP inhibitors, the current authors therefore designed sulfonyl phosphonate 1,4-dithia-7-azaspiro[4,4] nonane derivatives, wherein the arylsulfonyl group is incorporated at the 1-N position and the phosphonate group or phosphoric acid is incorporated as a zinc-binding group (ZBG).

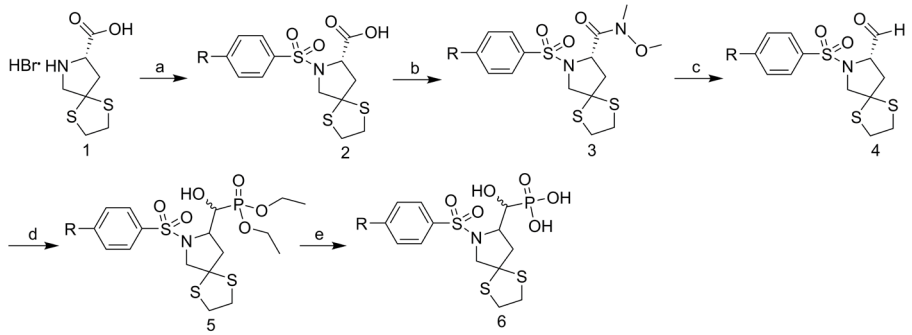
The current study describes the synthesis and biological activity of all of these sulfonyl phosphonate 1,4-dithia-7-azaspiro[4,4]nonane derivatives as well as docking studies of their interactions. Their structure-activity relationships have also been discussed.

2. Materials and Methods

2.1. *Chemicals and general procedures*

Unless otherwise noted, all of the materials, including reagents and solvents, were commercially available and used without further purification. All reactions were monitored by TLC with 0.25-mm silica gel plates (60GF-254) and were visualized with UV light or iodine vapor. Flash column chromatography was performed using 200-300-mesh silica gel. Melting points were determined on an electrothermal melting point apparatus (uncorrected). Proton NMR spectra were determined on a Brucker DRX spectrometer (300 MHz), with δ in parts per million and *J* in Hertz, using TMS as an internal standard. Measurements were made in DMSO-d_6 solutions. ESI-MS spectra were determined on an API 4000 spectrometer. HR-MS spectra were determined on an Agilent Q-TOF-6250 spectrometer at the Shandong Analysis and Test Center in Ji'nan, China. Anhydrous reactions were carried out in over-dried glassware in a nitrogen atmosphere.

The target compounds were efficiently synthesized following the procedures as illustrated in Scheme 1. The chemical structures of the target compounds were



Scheme 1. Reagents and conditions: (a) $RC_6H_4SO_2Cl$, DMAP, Et_3N; (b) TBTU, Et_3N, DCM; (c) $LiAlH_4$, THF; (d) Diethyl phosphite, Al_2O_3; (e) TMSBr, $CHCl_3$.

analytically confirmed with ^{1}H-NMR, ^{1}P-NMR, and HR-MS (see the Experimental Section).

Starting with a commercially available compound (**1**) as a chiral hydrobromide salt, sulfonamide intermediates (**2a-e**) were prepared *via* sulfonation with various sulfonyl chlorides and 4-*N*,*N*-dimethylaminopyridine (DMAP) as a catalyst and triethylamine (TEA) as a base. Condensation of **2a-e** with *N*-methoxymethanamine in dichloromethane (DCM) yielded the intermediates **3a-e**, which were then reduced with lithium tetrahydridoaluminate ($LiAlH_4$) to their aldehyde derivatives **4a-e** in anhydrous tetrahydrofuran (THF). Solvent-free nucleophilic addition of **4a-e** with diethyl phosphite and Al_2O_3 as a catalyst and medium produced α-hydroxyphosphonates **5a-e** (*25*), each of which was a mixture of two isomers that produced NMR spectra. The ethyl group of compounds **5a-d** was removed to obtain compounds **6a-d**, each of which was also a mixture of two isomers.

2.2. *In vitro MMP-2 inhibition assay*

IC_{50} values against MMP-2 were determined using succinylated gelatin as a substrate and MMP-2 (Gelatinase A, Sigma) as an enzyme or the supernatant of SKOV-3 cells in PBS (1×10^5/well). The enzyme and inhibitors were dissolved in sodium borate (pH 8.5, 50 mmol/L) and incubated in 96-well microtiter plates for 10 min at 37°C. The substrate was added and the mixture was incubated for another 30 min at 37°C. Then 0.03% TNBS was added and the mixture was incubated for an additional 20 min. The OD450 values of the resulting solution were determined at a wavelength of 450 nm with a plate reader (Varioskan, Thermo). Data were analyzed using OriginPro 7.5 software and IC_{50} values were determined.

2.3. *In vitro MMP-9 inhibition assay*

Active human MMP-9 full length protein was purchased from Abcam and the fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2 was purchased from AnaSpec. The inhibition of MMP-9 by the test compounds (**6a-d**) was fluorometrically assayed at excitation and emission wavelengths of 328 and 393 nm using 384-well plates and a plate reader (Varioskan, Thermo). Substrate hydrolysis was monitored for 15 min in a buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$, 0.01% Brij-35, and 1% DMSO) containing 10 μM substrate. For those compounds displaying > 50% inhibitory activity at a concn of 10 μM, their IC_{50} values were determined based on dose-response measurements using an inhibitor range of concentrations (1 nM-10 μM) and an enzyme concentration equal to 3 nM. The enzyme was preincubated with the inhibitor 2 h before assessment of activity. Data were analyzed using the software OriginPro 7.5.

2.4. *MTT assay*

Cell lines were grown in RPMI1640 medium containing 10% FBS at 37°C in a humidified incubator containing 5% CO_2. Cell proliferation was determined using a 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2*H*-tertazolium bromide (MTT) assay. Briefly, cells were plated on 96-well plates (10,000/well) and cultured for 4 h in complete growth medium and then treated with various concentrations of the test compounds. The plates were incubated for an additional 48 h, and then 0.5% MTT was added to each well. Four hours later, formazan formed from MTT was dissolved with DMSO for 15 min. Finally, the optical density values were determined at 570 nm using an ELISA reader.

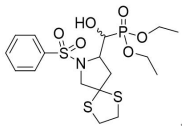
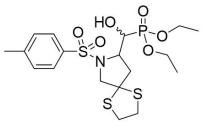
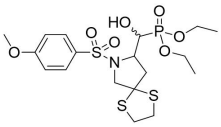
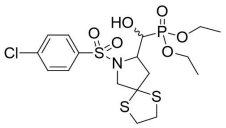
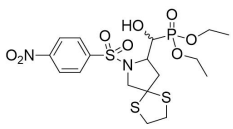
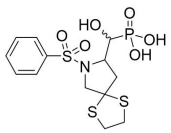
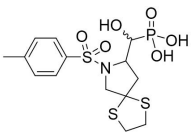
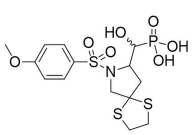
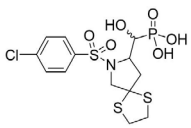
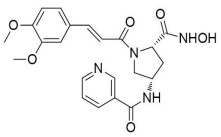
2.5. *Computational docking assay*

A docking study was conducted as follows: the selected compound was constructed with the Sybyl/Sketch module and its geometry was optimized with the Tripos force field and the Powell conjugate gradient algorithm with the convergence criterion set at 0.05 kcal/mol Å, and charges were assigned using the Gasteiger-Hückel method. The docking study of the selected compound with the active site of MMP-2 was performed using the Sybyl/ FlexX module. The active site was defined as a circle with a radius of 10.0 Å around Zn^{2+} (PDB: 1HOV).

3. Results

The newly synthesized sulfonyl phosphonic 1,4-dithia-7-azaspiro[4,4]nonane derivatives were assayed for their inhibitory activity against MMP-2, and **LY52** served as the positive control. Compounds **5a-d** and **6a-d** had IC_{50} values in the micromole range and displayed moderate inhibitory activity compared to **LY52** (the control) (IC_{50} = 0.95 ± 0.09 μM).

Table 1. The structures of the target compounds and their inhibitory activity against MMP-2

Compd	Structure	IC_{50} [a](μM)	
		MMP-2	MMP-9
5a		80.39 ± 2.52	ND
5b		63.16 ± 2.24	ND
5c		56.81 ± 1.79	ND
5d		38.24 ± 1.15	ND
5e		45.73 ± 1.28	ND
6a		14.58 ± 0.23	26.32 ± 0.20
6b		13.87 ± 0.21	25.75 ± 0.18
6c		10.25 ± 0.18	22.47 ± 0.23
6d		8.46 ± 0.14	15.26 ± 0.16
LY52		0.95 ± 0.09	1.72 ± 0.12

ND: not determined. [a]IC_{50} values are the mean of three experiments and the standard deviation is shown.

Compounds **6a-d** displayed greater inhibitory activity against MMP-2 and were thus assayed for their activity against MMP-9. Those compounds displayed moderate inhibitory activity against MMP-9 compared to **LY52** (the control) (IC_{50} = 1.72 ± 0.12 μM). Inhibition results are summarized in Table 1.

Furthermore, compounds **6a-d** were assayed for their inhibitory activity against human MMP-2 derived from cultured SKOV3 human ovarian carcinoma cells expressing a high level of MMP-2. As is apparent in Figure 3, all of the tested compounds exhibited moderate inhibitory activity against MMP-2 from SKOV3 cells compared to **LY52** (IC_{50} = 43.75 ± 1.12 μM).

Additionally, the MTT assay was used to evaluate compounds **6a-d** for their *in vitro* antiproliferative activity against a human ovarian tumor cell line (SKOV3), a leukemia cell line (HL60), and a lung cancer cell line (A549). HL60 and A549 cells over-expressed APN while SKOV3 cells over-expressed MMP-2. The results are shown in Table 2. Compounds **6a-d** had greater antiproliferative activity against SKOV3 cells than against HL60 and A549 cells, which may be due to the higher level of MMP-2 expression by SKOV3 cells than by the other two types of cells. However, a noteworthy finding was that compounds **6a-d** had slightly lower antiproliferative activity against SKOV3 cells than that of **LY52** (with respective IC_{50} values of 415.76, 346.82, 281.39, 173.58, and 697.14 μM), which was not consistent with the previous results of enzyme inhibition. This result could have been caused by several

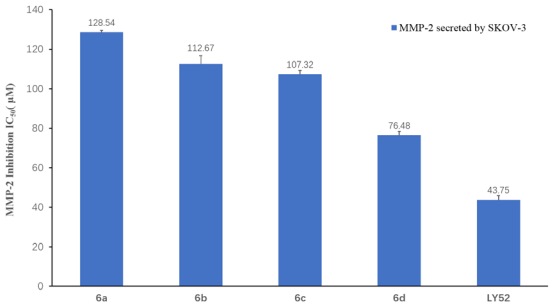


Figure 3. Inhibitory activity of compounds 6a, 6b, 6c, 6d, and LY52 against MMP-2 in a supernatant of SKOV-3 cells. Data are expressed as the mean values of three experiments.

Table 2. Anti-proliferative activity of compounds 6a, 6b, 6c, 6d, and LY52 against SKOV3, HL60, and A549 cells

Compd	IC_{50} [a](μM)		
	SKOV3	HL60	A549
6a	415.76	>1000	>1000
6b	346.82	>1000	>1000
6c	281.39	>1000	>1000
6d	173.58	>1000	>1000
LY52	697.14	>1000	>1000

[a]Mean values and the standard deviation of three experiments are shown.

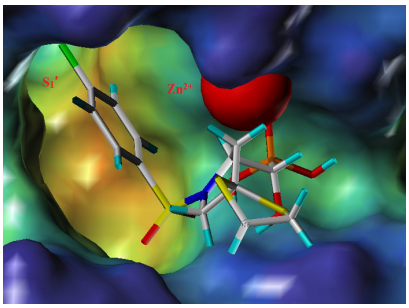


Figure 4. The FlexX docking of Compound 6d with MMP-2.

factors, such as cell membrane permeability, metabolic stability, subcellular localization, and cell mechanisms of exporting xenobiotics.

A docking analysis of the most potent compound, **6d**, was performed using Sybyl 8.0 from Tripos. The interaction of the compound with MMP-2 (PDB: 1HOV) is depicted in Figure 4 and results of the analysis suggested that the phosphinate group chelates Zn^{2+}, which is a crucial catalytic active site, while the arylsulfonyl group is incorporated into the S_1' pocket. Although the computational results partially supported this contention, the exact mode by which compound **6d** binds with MMP-2 needs to be determined in further X-ray crystal studies.

4. Discussion

All of the tested compounds displayed moderate inhibitory activity against MMP-2 and MMP-9 compared to **LY52** (the control). There was no obvious subtype selectivity between MMP-2 and MMP-9 for these sulfonyl phosphonic 1,4-dithia-7-azaspiro[4,4]nonane derivatives.

Compounds **6a-d** were more potent than compounds **5a-e**, which might be attributed to the ZBG. Phosphoric acid and phosphonate are the respective ZBGs for **6a-d** and **5a-e**, and both can chelate the zinc ion in the catalytic center of the enzyme. However, the phosphoric acid group was a more potent ZBG than the phosphonate group.

Among compounds **5a-e**, compounds **5b-e** contained a substituted arylsulfonyl group and displayed more potent inhibitory activity compared to benzenesulfonyl derivative **5a**. In particular, the chloro-substituted compound **5d** had greater inhibitory activity than the other compounds. Moreover, methyl substitution or methoxy substitution of the arylsulfonyl group at the C-4 position did not markedly affect inhibitory activity, but a compound with methoxy substitution displayed slightly greater inhibitory activity. A similar finding was noted for compounds **6a-d**.

In summary, this study has described the synthesis and biological evaluation of a series of sulfonyl phosphonic 1,4-dithia-7-azaspiro[4,4]nonane derivatives as MMP-2 inhibitors. All of the target compounds displayed moderate inhibitory activity against MMP-2 compared to **LY52** (the control). Several selected compounds were also assayed for their antiproliferative activity against SKOV3, HL60, and A549 cells. Compound **6d**, which displayed the greatest inhibitory activity in both an enzymatic assay and a cell-based assay, could be used as a candidate for further structural optimization to develop MMPIs in the future.

Acknowledgements

This work was supported by the Natural Science Foundation of Shandong Province (BS2015YY016) and the Doctoral Foundation of Weifang Medical University.

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(Received March 2, 2017; Revised June 19, 2017; Accepted June 20, 2017)

Appendix

1.

7-(Phenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-carboxylic acid (2a)

1,4-Dithia-7-azaspiro[4,4]nonane-8-carboxylic acid (14.3 g, 50 mmol) was dissolved in a solution of water/dioxane (1:1, 200 mL), and then triethylamine (Et_3N, 17.5 mL, 125 mmol) and 4-(dimethylamino)pyridine (DMAP, 0.61 g, 5 mmol) were successively added. After the addition of benzenesulfonyl chloride (9.73 g, 55 mmol) in several portions below 0°C in an ice-salt bath, the mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under a vacuum and the resulting residue was partitioned between EtOAc and 1 N aqueous HCl. The organic layer was separated and washed with 1 N HCl (3 × 50 mL) and then washed with brine (2 × 50 mL), and the organic layer was then dried over anhydrous Na_2SO_4, filtered, and concentrated in a vacuum to yield target compound **2a**. The crude product was purified *via* recrystallization in 75% ethanol/H_2O to yield 12.80 g of **2a** as white powder (74.1%). *m.p*. 178-180°C, ESI-MS *m/z*: 344.7 $[M-H]^+$.

Compounds **2b-e** were synthesized following the general procedure described above.

7-Tosyl-1,4-dithia-7-azaspiro[4,4]nonane-8-carboxylic acid (2b):

White powder, yield 68.4%, *m.p*. 147-149°C. ESI-MS *m/z*: 359.3 $[M-H]^+$.

7-*p*-Methoxyphenylsulfonyl-1,4-dithia-7-azaspiro[4,4]nonane-8-carboxylic acid (2c)

White powder, yield 78.2%, *m.p*. 143-145°C. ESI-MS *m/z*: 375.2 $[M-H]^+$.

7-*p*-Chlorophenylsulfonyl-1,4-dithia-7-azaspiro[4,4]nonane-8-carboxylic acid (2d)

White powder, yield 75.6%, *m.p*. 149-151°C. ESI-MS *m/z*: 378.9 $[M-H]^+$.

7-p-Nitrophenylsulfonyl-1,4-dithia-7-azaspiro[4,4]nonane-8-carboxylic acid (2e)

White powder, yield 71.5%, *m.p*. 170-172°C. ESI-MS *m/z*: 389.5 $[M-H]^+$.

2.

***N*-methoxy-*N*-methyl-7-(phenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-carboamide (3a)**

Compound **2a** (3.45 g, 10 mmol) was dissolved in 100 mL anhydrous DCM with Et_3N (3.5 mL, 11 mmol) and then treated with 3.53 g (11 mmol) of O-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) at 0°C. After 30 minutes, *N*-methoxymethanamine was added and the mixture was stirred at room temperature for 12 h. The mixture was washed with 1 M HCl (3 × 50 mL), saturated $NaHCO_3$ solution (3 × 50 mL), and brine (2 × 50 mL) and then dried over Na_2SO_4. Evaporation of DCM yielded a pale yellow solid (57.3%). *m.p*.: 91~93°C, ESI-MS *m/z*: 389.4 $[M+H]^+$.

Compounds **3b-e** were synthesized following the

general procedure described above.

***N*-methoxy-*N*-methyl-7-tosyl-1,4-dithia-7-azaspiro [4,4]nonane-8-carboamid (3b)**

Pale yellow solid, yield 67.8%, *m.p*. 102-104°C. ESI-MS *m/z*: 403.5 $[M+H]^+$.

***N*-methoxy-N-methyl-7-(*p*-methoxyphenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-carboamide (3c)**

Pale yellow solid, yield 72.6%, *m.p*. 121-123 °C. ESI-MS *m/z*: 419.4 $[M+H]^+$.

***N*-methoxy-*N*-methyl-7-(*p*-chlorophenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-carboamide (3d)**

Pale yellow solid, yield 74.1%, *m.p*. 125-127 °C. ESI-MS *m/z*: 423.3 $[M+H]^+$.

***N*-methoxy-*N*-methyl-7-(*p*-nitrophenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-carboamide (3e)**

Yellow solid, yield 54.2%, *m.p*. 140-142 °C. ESI-MS *m/z*: 434.5 $[M+H]^+$.

3.

7-(Phenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-carbaldehyde (4a)

Compound **3a** (3.88 g, 10 mmol) was dissolved in anhydrous THF below 0°C in an ice-salt bath and treated with LiAlH4 (3.5 mL, 10 mmol) in several portions. After 30 minutes, the ice bath was removed and the resulting mixture was stirred at room temperature for 6 h. The reaction was quenched with 1 M NaOH and filtered through a thin layer of Celite. The resulting mixture was diluted with EtOAc (100 mL) and separated. The organic phase was washed successively with H_2O (2 × 50 mL), 1 M citric acid (2 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), and brine (50 mL), and the organic phase was dried over anhydrous Na2SO4. Evaporation of EtOAc yielded a pale yellow oil (**4a**). ESI-MS *m/z*: 330.3 $[M+H]^+$.

Compounds **4b-e** were synthesized following the general procedure described above.

7-Tosyl-1,4-dithia-7-azaspiro[4,4]nonane-8-carbaldehyde (4b)

Pale yellow oil. ESI-MS *m/z*: 344.3 $[M+H]^+$.

7-(*p*-Methoxyphenylsulfonyl)-1,4-dithia-7-azaspiro [4,4]nonane-8-carbaldehyde (4c)

Pale yellow oil. ESI-MS *m/z*: 360.4 $[M+H]^+$.

7-(*p*-Chlorophenylsulfonyl)-1,4-dithia-7-azaspiro[4,4] nonane-8-carbaldehyde (4d)

Yellow oil. ESI-MS *m/z*: 364.9 $[M+H]^+$.

7-(*p*-Nitrophenylsulfonyl)-1,4-dithia-7-azaspiro[4,4] nonane-8-carbaldehyde (4e)

Yellow oil. ESI-MS *m/z*: 375.4 $[M+H]^+$.

4.

Diethyl(hydroxyl(7-(phenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-yl) methyl)phosphonate (5a)

The crude oil **4a** (about 5 mmol), diethyl phosphate (0.64 mL, 5 mmol), and Al_2O_3 (1.5 g) were stirred at room temperature for 2 h. The mixture was extracted with DCM (30 mL × 3). The organic phase was dried over Na_2SO_4 and filtered through a thin layer of Celite to remove the solid. The filtered solution was concentrated under a vacuum to yield the crude product, which was purified using flash chromatography on silica gel (PE:EA = 2:1 to 1:2) to yield a pale yellow solid. Yield 47.3%, *m.p*.: 94-97°C; HRMS *m/z*: calcd. for C17H26NO6PS3 $[M+H]^+$ 468.0738, found 468.0734; ^{1}H NMR: (DMSO-d_6, ppm) δ: 1.253 (t, *J* = 3.6 Hz, 3H, CH_3), 1.288 (t, *J* = 3.6 Hz, 3H, CH_3), 2.131-2.293 (m, 1H, CH), 2.665-2.743 (m, 1H, CH), 3.014-3.090 (m, 2H, SCH_2), 3.193-3.229 (m, 2H, SCH_2), 3.608 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.752 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.847-3.902 (m, 1H, CH), 3.965-4.020 (m, 2H, OCH_2), 4.031-4.127 (m, 2H, OCH_2), 4.470-4.665 (m, 1H, CH, CH-$PO(OEt)_2$), 6.194-6.252 (m, 1H, OH), 7.628 (t, *J* = 7.2 Hz, 2H, ArH), 7.711 (t, *J* = 7.2 Hz, 1H, ArH), 7.828-7.875 (m, 2H, ArH). ^{31}P NMR: (DMSO-d_6, ppm) δ: 21.784, 22.967.

Compounds **5b-e** were synthesized following the general procedure described above.

Diethyl(hydroxyl(7-tosy -1,4-dithia-7-azaspiro[4,4] nonane-8-yl) methyl)phosphonate (5b)

Pale yellow solid, yield 51.6%, *m.p*. 100-103°C; HRMS *m/z*: calcd for C18H28NO6PS3 $[M+H]^+$ 482.0895, found 482.0892; ^{1}H NMR: (DMSO-d_6, ppm) δ: 1.255 (t, *J* = 3.3 Hz, 3H, CH_3), 1.286 (t, *J* = 3.3 Hz, 3H, CH_3), 2.131-2.293 (m, 1H, CH), 2.402 (s, 3H , $ArCH_3$), 2.656-2.733 (m, 1H, CH), 3.090-3.161 (m, 2H, SCH_2), 3.228-3.285 (m, 2H, SCH_2), 3.602 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.702 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.820-3.874 (m, 1H, CH), 4.002-4.048 (m, 2H, OCH_2), 4.063-4.120 (m, 2H, OCH_2), 4.434-4.656 (m, 1H, CH, CH-$PO(OEt)_2$), 5.895-6.224 (m, 1H, OH), 7. 406 (d, *J* = 6.6 Hz, 1H, ArH), 7.431 (d, *J* = 6.6 Hz, 1H, ArH), 7.689 (d, *J* = 8.1 Hz, 1H, ArH), 7.735 (d, *J* = 8.1 Hz, 1H, ArH). ^{31}P NMR: (DMSO-d_6, ppm) δ: 21.865, 22.979.

Diethyl(hydroxyl(7-(p-methoxyphenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-yl) methyl) phosphonate (5c)

Pale yellow solid, yield 45.8%, *m.p*. 81-84°C; HRMS *m/z*: calcd. for C18H28NO7PS3 $[M+H]^+$ 498.0844, found 498.0837; ^{1}H NMR: (DMSO-d_6, ppm) δ: 1.243 (t, *J* = 3.6 Hz, 3H, CH_3), 1.290 (t, *J* = 3.6 Hz, 3H, CH_3), 2.138-2.297 (m, 1H, CH), 2.513-2.735 (m, 1H, CH),

3.156-3.215 (m, 2H, SCH_2), 3.231-3.288 (m, 2H, SCH_2), 3.599 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.719 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.816-3.842 (m, 1H, CH), 3.850 (s, 3H, OCH_3), 4.031-4.054 (m, 2H, OCH_2), 4.066-4.127 (m, 2H, OCH_2), 4.475-4.662 (m, 1H, CH, CH-PO(OEt)$_2$), 5.895-6.220 (m, 1H, OH), 7. 113 (d, *J* = 6 Hz, 1H, ArH), 7.142 (d, *J* = 6 Hz, 1H, ArH), 7.742 (d, *J* = 9 Hz, 1H, ArH), 7.790 (d, *J* = 9 Hz, 1H, ArH). ^{31}P NMR: (DMSO-d_6, ppm) δ: 21.926, 23.060.

Diethyl(hydroxyl(7-(p-chlorophenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-yl) methyl) phosphonate (5d)

Pale yellow solid, yield 54.2%, *m.p*. 86-88°C; HRMS *m/z*: calcd. for C17H25NO6PS3Cl $[M+H]^+$ 502.0348, found 502.0342; ^{1}H NMR: (DMSO-d_6, ppm) δ: 1.252(t, *J* = 3.6 Hz, 3H, CH_3), 1.286 (t, *J* = 3.6 Hz, 3H, CH_3), 2.151-2.316 (m, 1H, CH), 2.679-2.757 (m, 1H, CH), 3.139-3.173 (m, 2H, SCH_2), 3.235-3.261 (m, 2H, SCH_2), 3.601 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.713 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.834-3.890 (m, 1H, CH), 4.022-4.054 (m, 2H, OCH_2), 4.078-4.127 (m, 2H, OCH_2), 4.416-4.618 (m, 1H, CH, CH-PO(OEt)$_2$), 5.952-6.267 (m, 1H, OH), 7. 681 (d, *J* = 6.9 Hz, 1H, ArH), 7.709 (d, *J* = 6.9 Hz, 1H, ArH), 7.824 (d, *J* = 9.0 Hz, 1H, ArH), 7.880 (d, *J* =9.0 Hz, 1H, ArH). ^{31}P NMR: (DMSO-d_6, ppm) δ: 21.644, 22.880.

Diethyl(hydroxyl(7-(p-nitrophenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-yl) methyl) phosphonate (5e)

Yellow solid, yield 39.4%, *m.p*. 97-99°C; HRMS *m/z*: calcd. for C17H25N2O8PS3 $[M+H]^+$ 513.0589, found 513.0586; ^{1}H NMR: (DMSO-d_6, ppm) δ: 1.258(t, *J* = 3.6 Hz, 3H, CH_3), 1.292 (t, *J* = 3.6 Hz, 3H, CH_3), 2.147-2.215 (m, 1H, CH), 2.693-2.776 (m, 1H, CH), 3.130-3.187 (m, 2H, SCH_2), 3.193-3.249 (m, 2H, SCH_2), 3.601 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.732 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.846-3.947 (m, 1H, CH), 4.029-4.059 (m, 2H, OCH_2), 4.078-4.133 (m, 2H, OCH_2), 4.392-4.571 (m, 1H, CH, CH-PO(OEt)$_2$), 5.952-6.301 (m, 1H, OH), 8. 101 (d, *J* = 6.9 Hz, 1H, ArH), 8.156 (d, *J* = 6.9 Hz, 1H, ArH), 8.397 (d, *J* = 9.0 Hz, 1H, ArH), 8.460 (d, *J* =9.0 Hz, 1H, ArH). ^{31}P NMR: (DMSO-d_6, ppm) δ: 21.389, 22.721.

5.

(Hydroxyl(7-(phenylsulfonyl)-1,4-dithia-7-azaspiro [4,4]nonane-8-yl)methyl)phosphonic acid (6a)

Compound **5a** (0.47g, 1 mmol) in 10 mL anhydrous DCM was dealkylated in the presence of bromotrimethylsilane for 2 h at room temperature. The solvent was removed under a vacuum to yield the crude product, which was purified using reversed phase column chromatography to yield compound 6a (H_2O:MeOH = 100% to 65:35). Pale yellow semisolid: yield 37.6%. HRMS *m/z*: calcd. for C13H18NO6PS3 $[M+H]^+$ 412.0112, found 412.0105; ^{1}H NMR: (DMSO-d_6, ppm) δ: 2.153-2.265 (m, 1H, CH), 2.666-2.765 (m, 1H, CH), 3.090-3.147 (m, 2H, SCH_2), 3.187-3.223 (m, 2H, SCH_2), 3.584 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.720 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.975-4.046 (m, 1H, CH), 4.203-4.535 (m, 1H, CH, CH-PO(OEt)$_2$), 7.592 (t, *J* = 7.5 Hz, 2H, ArH), 7.625-7.718 (m, 1H, ArH), 7.815-7.865 (m, 2H, ArH). ^{31}P NMR: (DMSO-d_6, ppm) δ: 18.303, 18.678.

Compounds **6b-d** were synthesized following the general procedure described above.

(Hydroxyl(7-tosyl-1,4-dithia-7-azaspiro[4,4]nonane-8-yl)methyl) phosphonic acid (6b)

Pale yellow semisolid: yield 41.8%. HRMS *m/z*: calcd for C14H20NO6PS3 $[M+H]^+$ 426.0269, found 426.0261; ^{1}H NMR: (DMSO-d_6, ppm) δ: 2.164-2.263 (m, 1H, CH), 2.408 (s, 3H, $ArCH_3$), 2.654-2.767 (m, 1H, CH), 3.096-3.155 (m, 2H, SCH_2), 3.193-3.239 (m, 2H, SCH_2), 3.576 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.712 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.933-3.975 (m, 1H, CH), 4.170-4.456 (m, 1H, CH, CH-PO(OEt)$_2$), 7.412 (d, *J* = 7.2 Hz, 2H, ArH), 7.785 (d, *J* = 7.2 Hz, 2H, ArH). ^{31}P NMR: (DMSO-d_6, ppm) δ: 18.394, 18.705.

(Hydroxyl(7-(p-methoxyphenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-yl) methyl)phosphonic acid (6c)

Yellow semisolid: yield 35.2%. HRMS *m/z*: calcd. for C14H20NO7PS3 $[M+H]^+$ 442.0218, found 442.0214; ^{1}H NMR: (DMSO-d_6, ppm) δ: 2.176-2.261 (m, 1H, CH), 2.648-2.727 (m, 1H, CH), 3.077-3.137 (m, 2H, SCH_2), 3.192-3.236 (m, 2H, SCH_2), 3.570 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.706 (d, *J* = 12 Hz, 1H, CH_2-N-), 3.838 (s, 3H, $ArOCH_3$), 3.914-3.971 (m, 1H, CH), 4.163-4.451 (m, 1H, CH, CH-PO(OEt)$_2$), 7.107 (d, *J* = 9.0 Hz, 2H, ArH), 7.750 (d, *J* = 9.0 Hz, 2H, ArH). ^{31}P NMR: (DMSO-d_6, ppm) δ: 18.447, 18.738.

(Hydroxyl(7-(p-chlorophenylsulfonyl)-1,4-dithia-7-azaspiro[4,4]nonane-8-yl) methyl)phosphonic acid (6d)

Yellow semisolid: yield 43.7%. HRMS *m/z*: calcd. for C13H17ClNO6PS3 $[M+H]^+$ 445.9722, found 445.9716; ^{1}H NMR: (DMSO-d_6, ppm) δ: 2.312-2.377 (m, 1H, CH), 2.801-2.879 (m, 1H, CH), 3.080-3.142 (m, 2H, SCH_2), 3.199-3.268 (m, 2H, SCH_2), 3.706 (d, *J* = 12.3 Hz, 1H, CH_2-N-), 3.799 (d, *J* = 12.3 Hz, 1H, CH_2-N-), 4.074-4.130 (m, 1H, CH), 4.727-4.770 (m, 1H, CH, CH-PO(OEt)$_2$), 7.616 (d, *J* = 6.6 Hz, 2H, ArH), 7.908 (d, *J* = 6.6 Hz, 2H, ArH). ^{31}P NMR: (DMSO-d_6, ppm) δ: 20.202.