RSC Medicinal Chemistry

ROYAL SOCIETY OF CHEMISTRY

RESEARCH ARTICLE

View Article Online
View Journal | View Issue

Cite this: *RSC Med. Chem.*, 2026, **17**, 452

Identification of spirodioxolane nsP2 helicase inhibitors with antialphaviral activity

Hans J. Oh, [ab] John D. Sears, [bc] Bose Muthu Ramalingam, [ab] Rahman Shah Zaib Saleem, [d] Zachary W. Davis-Gilbert, [ab] Mohammed Anwar Hossain, [ab] Stella R. Moorman, [bc] Durbadal Ohja, [bc] Sabian A. Martinez, [be] Jane E. Burdick, [be] Rafael M. Couñago, [abf] Nathaniel J. Moorman, [bc] Mark T. Heise, [bce] Matthew H. Todd [d] and Timothy M. Willson *[ab]

Received 2nd September 2025,
Accepted 24th October 2025

DOI: 10.1039/d5md00772k

rsc.li/medchem

We describe the design, synthesis, and antialphaviral activity of spirodioxolane inhibitors targeting the alphavirus nsP2 helicase (nsP2hel). The spirodioxolanes are a new series of direct-acting antivirals that retain key molecular features required for inhibition of nsP2hel, including a highly substituted piperidine acetamide with its associated conformational isomerism and thermal mobility. Unlike the related oxaspiropiperidine nsP2hel inhibitors, the spirodioxolanes showed no enantioselectivity in their antiviral activity. The spirodioxolanes demonstrated antialphaviral activity against the Old World alphavirus Chikungunya virus, with some analogs also showing activity against the New World alphavirus Venezuelan equine encephalitis virus. Importantly, certain spirodioxolane analogs, such as **6b**, maintained activity against viral mutants that displayed resistance to first-generation oxaspiropiperidine inhibitors, indicating their potential for optimization as a new class of broad-spectrum antialphaviral drugs.

Introduction

Alphaviruses are a genus of arboviruses that cause a wide range of diseases in humans, from debilitating arthralgia and rash to severe encephalitis.[1,2] These viruses are broadly categorized into three main clades: Old World alphaviruses (*e.g.*, Chikungunya virus, Ross River virus, and O'nyong'nyong virus), New World alphaviruses (*e.g.*, Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, and Western equine encephalitis virus), and Sindbis-like alphaviruses (*e.g.*, Sindbis virus and Semliki Forest virus).[3] The expanding geographic ranges of these viruses and the potential for large-scale outbreaks, driven by the continued spread of their mosquito vectors into highly populated regions, highlight the urgent need for effective direct-acting antiviral (DAA) drugs to combat these emerging and re-emerging pathogens.[2,4,5] There are currently no drugs approved by a national regulatory authority for any alphavirus infection and treatment options are largely supportive, underscoring the critical gap in therapeutic interventions.

The non-structural protein 2 (nsP2) of alphaviruses has emerged as a particularly promising target for development of DAA.[6] nsP2 is a multifunctional enzyme possessing

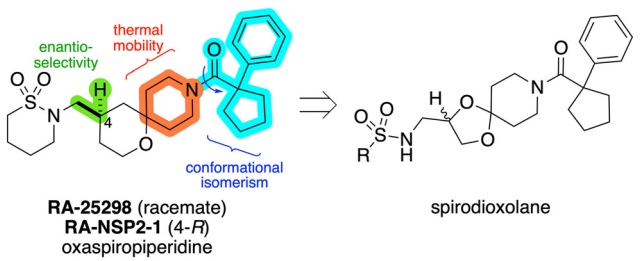


Fig. 1 Design of the spirodioxolane nsP2hel inhibitor chemotype. Key features of the oxaspiropiperidines **RA-25298** and **RA-NSP2-1** are: enantioselectivity at C-4 (green), conformational isomerism of the amide (blue), and thermal mobility of the piperidine (red). The spirodioxolane has the potential to display all the features required for potent nsP2hel inhibition.

[a] *Structural Genomics Consortium, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. E-mail: tim.willson@unc.edu*
[b] *READDI AViDD Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA*
[c] *Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA*
[d] *Structural Genomics Consortium, Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University College London, London WC1N 1AX, UK*
[e] *Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA*
[f] *Center of Medicinal Chemistry, Center for Molecular Biology and Genetic Engineering, University of Campinas, 13083-886-Campinas, SP, Brazil*

protease, NTPase, and helicase activities, all of which are essential for alphaviral replication.[7] Its conservation across various alphavirus species makes it an attractive target for development of broad-spectrum DAA. Recent breakthroughs in this area include the discovery of direct-acting nsP2 helicase (nsP2hel) inhibitors, including **RA-25298** and its (*R*)-enantiomer **RA-NSP2-1** (Fig. 1).[8–10] These nsP2hel inhibitors have demonstrated antiviral activity against multiple alphaviruses in infected cells and, importantly, have shown efficacy in a mouse model of alphavirus disease, offering a potential avenue for the development of much-needed therapeutic drugs.[8–10]

Structure–activity studies of the oxaspiropiperidine chemotype represented by **RA-25298** and **RA-NSP2-1** identified three key features that were required for potent nsP2hel inhibition and antialphaviral activity.[8,9] The oxaspiropiperidines showed enantioselectivity at C-4 with only the (*R*)-isomer retaining potent nsP2hel inhibition (Fig. 1, green).[9] The unusual 2-cyclopentyl-2-phenylacetamide (Fig. 1, blue) was required for nsP2hel inhibition and showed conformational isomerism around its highly substituted amide bond, leading to thermal mobility in the spiropiperidine ring (Fig. 1, red).[8,9] Thermal motion in the spiropiperidine ring was diagnosed by broad signals in the ^{13}C-NMR spectrum recorded at room temperature. Remarkably, analogs with modification of the cyclopentane ring resulted in sharp ^{13}C-NMR signals for the spiropiperidine carbons but were not active as nsP2hel inhibitors.[9] Thus, thermal motion in the spiropiperidine ring appeared to be required for inhibition of the helicase enzyme.

The landmark discovery of **RA-25298** and **RA-NSP2-1** demonstrated the therapeutic potential of direct-acting nsP2hel inhibitors as antialphaviral therapeutics.[10] However, two potential limitations of the oxaspiropiperidine chemotype have emerged: a) classical resolution of the spirocyclic core was required for synthesis of the active enantiomers,[9] which hindered the rapid optimization of improved analogs for clinical development, and b) emergence of resistance mutants upon repeated passage of virus in the presence of inhibitor (*e.g.* CHIKV F185L)[10] that might limit efficacy if it were to emerge during antiviral drug therapy.[11] For these reasons we sought to design new chemotypes of nsP2hel inhibitors by modifying the oxaspiropiperidine core while retaining the key features required for nsP2hel inhibition. A spirodioxolane chemotype was proposed as an alternative core which would retain the critical piperidine 2-cyclopentyl-2-phenylacetamide but replace the oxetane with a 5-membered ring that could be formed by a simple ketalization reaction (Fig. 1). The availability of 3-amino-1,2-propanediol as a chiral building block from renewable natural sources[12] would also remove the need for a classical resolution to produce analogs as single enantiomers. In this report we describe the expedient and convergent synthesis of spirodioxolane nsP2hel inhibitors with antialphaviral activity that also retain activity against viruses with resistance to first the generation inhibitors **RA-25298** and **RA-NSP2-1**.

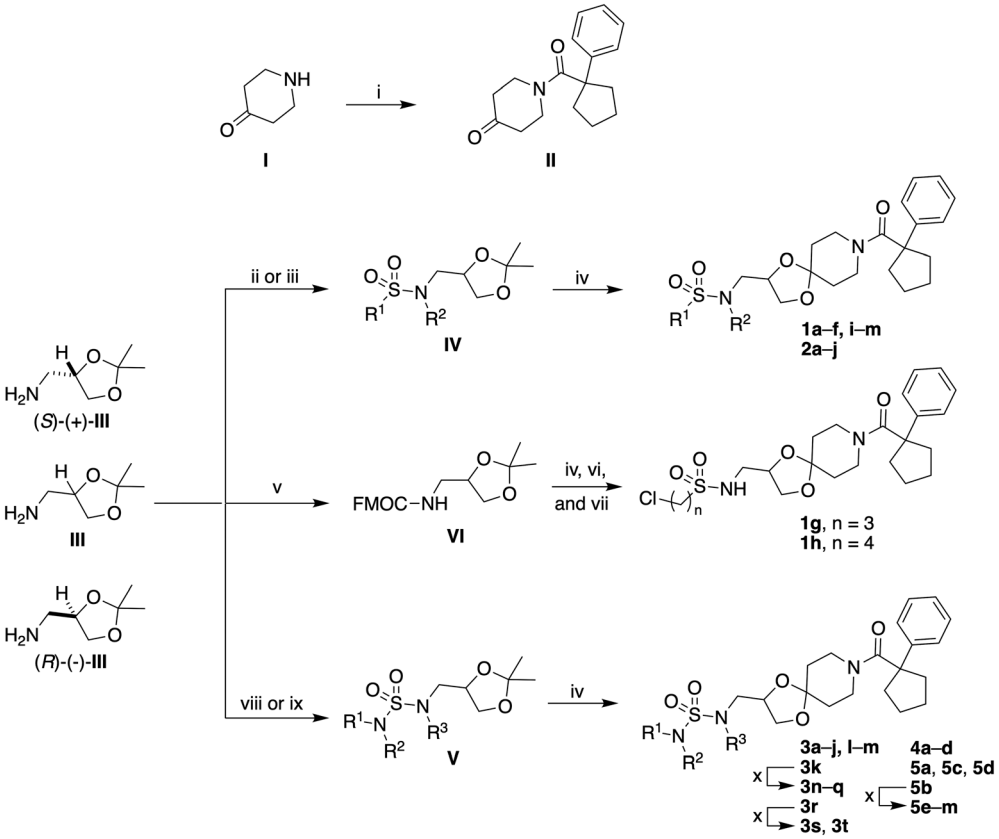
Results and discussion

Chemistry

Synthesis of spirodioxolanes. A convergent synthesis of the spirodioxolane core was achieved by the condensation of a substituted piperidin-4-one with a 3-amino-1,2-propanediol synthon (Scheme 1). The initial step involved amide coupling of unsubstituted piperidin-4-one (**I**) with commercially available 2-cyclopentane phenylacetic acid to yield the key intermediate **II**. For the 3-amino-1,2-propanediol synthon (2,2-dimethyl-[1,3]-dioxolan-4-yl)-methylamine (**III**) was functionalized by multiple routes (Scheme 1). To generate alkyl and aryl sulfonamides (**1a–f**, **1i–k**, and **2a–j**) racemic **III** was reacted with a wide range of sulfonyl chlorides in the presence of Et_3N to yield **IV** (R^2 = H) which underwent acid-catalyzed ketalization to yield the final compounds. When **III** was reacted with 3-chloropropane-1-sulfonyl chloride or 4-chloropropane-1-sulfonyl chloride with NaH as a base the cyclic thiazinane and isothiazolidine dioxides were formed by intramolecular displacement of the terminal chloride. The resulting cyclic sulfonamides **IV** (R^1, R^2 = –$(CH_2)_n$–) were reacted with **II** to yield **1l** and **1m**. Synthesis of the uncyclized chloroalkyl analogs **1g** and **1h** used a modified route in which the primary amine of **III** was protected with Fmoc as **VI**. Deprotection of **VI** with piperidine followed by reaction with 3-chloropropane-1-sulfonyl chloride or 4-chloropropane-1-sulfonyl chloride in the presence of Et_3N as the base at 0 °C yielded **1g** and **1h**. Reaction of **III** with sulfamoyl chlorides in the presence of Et_3N afforded sulfamate intermediate **V**, which was used to synthesize alkyl, cyclic, piperidine, and benzyl sulfamates (**3a–m**; **3r**; **4a–b**, **d**; and **5a–b**, respectively) by spiroketal formation with **II**. For a few analogs (*e.g.* **4c**, **5c**, and **5d**) where the sulfamoyl chloride was not commercially available, intermediate **V** was prepared by reaction of **III** with the required amine in the presence of sulfuryl chloride. In some cases (*e.g.* **3k**, **3r**, and **5b**), the sulfamate spirodioxolanes were further functionalized on the inboard or outboard nitrogen through reaction with an alkyl halide or cyanogen bromide to generate **3n–q**, **3s–t**, and **5e–m**.

To synthesize the spirodioxolanes **3a**, **3k**, **3r**, **4b**, and **5b** as single enantiomers, the commercially available (*S*)-(+)-(2,2-dimethyl-[1,3]-dioxolan-4-yl)-methylamine (*S*)-**III** or (*R*)-(−)-(2,2-dimethyl-[1,3]-dioxolan-4-yl)-methylamine (*R*)-**III** were employed as the initial building block in the reaction sequence (Scheme 1).

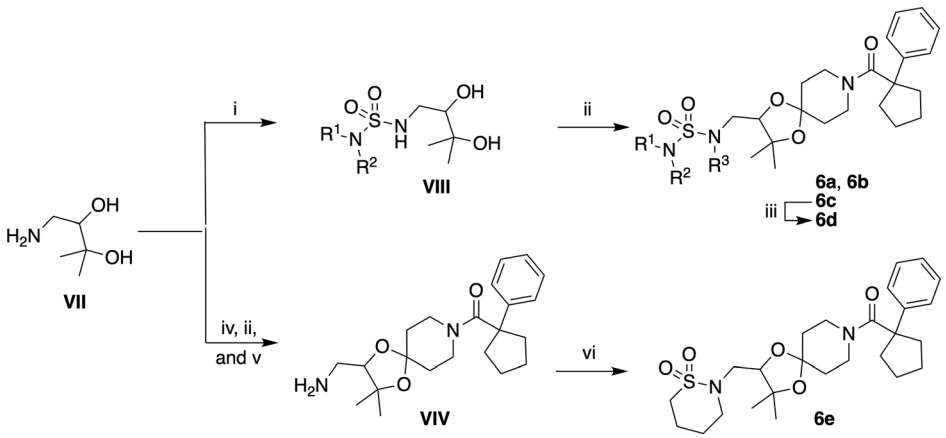
To incorporate additional methylation on the dioxolane ring, 1-amino-3-methylbutane-2,3-diol (**VII**) was functionalized with a piperidine or benzyl sulfonyl chloride to generate **VIII**, which underwent ketalization with **II** to afford compounds **6a–c** (R^3 = H) (Scheme 2). Alkylation of **6c** on the inboard sulfamate nitrogen with 2-bromo-*N*,*N*-dimethylacetamide furnished compound **6d**. To synthesize **6e**, intermediate **VII** was first protected with an Fmoc group and then subjected to ketalization with **II** and deprotected with piperidine to yield **VIV**. The free amine of **VIV** was reacted with



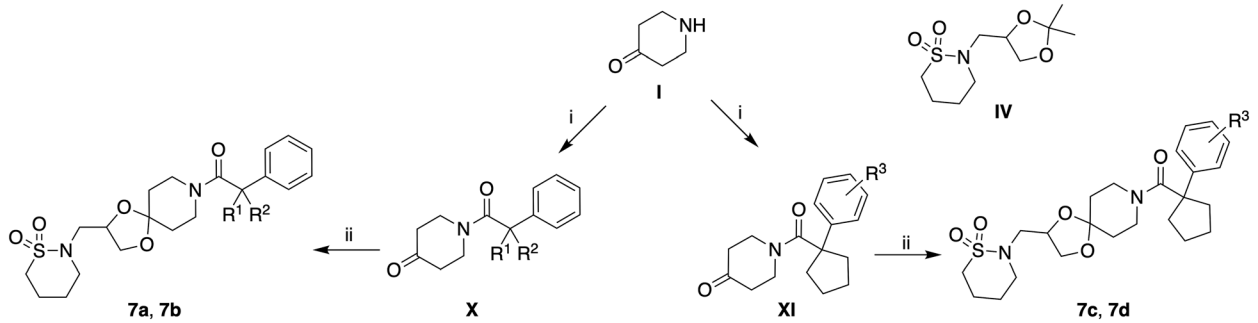
Scheme 1 Reagents and conditions: (i) 1-phenyl-1-cyclopentanecarboxylic acid, HATU, DIPEA, DMF, rt, 16 h, 70%; (ii) sulfonyl chloride, Et_3N, CH_2Cl_2, 0 °C to rt, 16 h, 40–80%; (iii) sulfonyl chloride, NaH, THF, 0–50 °C, 16 h (for **1l** and **1m**), 60–70%; (iv) intermediate **II**, *p*-TsOH, toluene, 70 °C, 16 h, 50–70%; (v) FMOC-Cl, Et_3N, CH_2Cl_2, 0 °C to rt, 16 h, 85% (vi) 20% piperidine in DMF, rt, 16 h, >95%; (vii) Et_3N, CH_2Cl_2, 0 °C, 15 min; chloroalkyl sulfonyl chloride, 0 °C to rt, 3 h, 50–60%. (viii) sulfamoyl chloride, Et_3N, CH_2Cl_2, 0 °C to rt, 16 h, 40–80%; (ix) amine, sulfuryl chloride, Et_3N, MeCN, 0 °C to rt, 8 h, 30–60%; (x) NaH, THF, 0 °C, 15 min; then alkyl halide or cyanogen bromide, 0 °C to 50 °C, 16 h, 50–80%; see Tables 1–5 for additional details of final compound structures.

4-chlorobutylsulfonyl chloride in the presence of NaH with intramolecular cyclization to yield sulfonamide **6e** (Scheme 2).

To modify the piperidine acetamide at the other end of the molecule (Scheme 3), piperidin-4-one (**I**) was coupled with either 2,2-dimethyl- or 2-cyclopropyl-phenylacetic acid,



Scheme 2 Reagents and conditions: (i) sulfonyl chloride, Et_3N, CH_2Cl_2, 0 °C to rt, 1 h, 50–80%; (ii) intermediate **II**, *p*-TsOH, toluene, 70 °C, 16 h, 50–70%; (iii) NaH, THF, 0 °C, 15 min; then 2-bromo-*N*,*N*-dimethylacetamide, 0–50 °C, 16 h, 80%; (iv): FMOC-Cl, Et_3N, CH_2Cl_2, 0 °C to rt, 1 h, 80%; (v) 20% piperidine in DMF, rt, 16 h, >95%; (vi) NaH, 0 °C, 15 min; then 4-chloro-1-butylsulfonyl chloride, 0–50 °C, 16 h, 60%. See Table 6 for additional details of final compound structures.



Scheme 3 Reagents and conditions: (i) substituted phenylacetic acid, HATU, DIPEA, DMF, rt, 16 h, 70–80%; (ii) intermediate **IV**, *p*-TsOH, toluene, 70 °C, 16 h, 50–70%. See Table 7 for additional details of final compound structures.

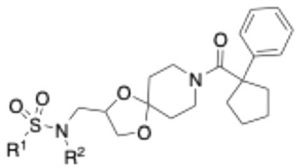
to yield intermediate **X** (R^1, R^2 = Me or *c*-Pr) or with 3-chloro- or 4-chloro-substituted analogs of 2-cyclopentyl phenylacetic acid to yield intermediate **XI** (R^3 = 3-Cl or 4-Cl). Ketalization with 2-((2,2-dimethyl-1,3-dioxolan-4-yl) methyl)-1,2-thiazinane 1,1-dioxide (**IV**, where R^1, R^2 = $-(CH_2)_3-$ in Scheme 1) provided the spirodioxolanes **7a–d**.

Biological evaluation

Inhibition of alphavirus replication. Primary structure–activity for the spirodioxolane analogs was determined using our established plate-based viral replication assays employing Chikungunya virus (CHIKV) and Venezuelan equine encephalitis virus (VEEV) with nLuc inserted into their open reading frames.[8] These cell-based reporter assays measured replication of the alphavirus mRNA genome, a process that is dependent on helicase activity. The assays were successfully used in the optimization of the oxaspiropiperidine series of nsP2hel inhibitors[8] and were less prone to protein interference[13] that has often plagued biochemical assays used in the development of other type 1 allosteric helicase inhibitors.[14] The ability of compounds to inhibit viral replication was determined in 8-point dose response from 2 nM to 40 μM. If an accurate EC_{50} could not be determined the percent inhibition at 40 μM was recorded instead (Table 1). Methyl sulfonamide (**1a**) showed inhibition of both CHIKV and VEEV only at high micromolar doses (Table 1). Extension of the methyl substituent to *n*-butyl (**1b**) gave an analog with similar activity, but additional extension to *n*-pentyl (**1c**) improved antiviral potency. The trifluoropentyl (**1d**) and ethoxyethyl (**1e**) mono-fluoropentyl (**1f**) failed to further improve in antiviral activity. However, chloroalkyl analogs appeared to be more active; the *n*-chloropropyl analog **1g** had $EC_{50} \leq 10$ μM on both CHIKV and VEEV, while elongation of the chloroalkyl chain to **1h** resulted in the first spirodioxolane analog with CHIKV $EC_{50} \leq 1$ μM.

The cyclopropyl sulfonamide (**1i**) was slightly less active (Table 1). Addition of a methylene spacer in **1j** gave no

Table 1 Antiviral activity of alkyl sulfonamide spirodioxolanes **1a–m** determined by alphavirus-nLuc reporter assays. Potency is expressed as the negative log of the 50% effective concentration



Compound	R^1	R^2	CHIKV-nLuc (pEC_{50} ± SD)	VEEV-nLuc (pEC_{50} ± SD)
1a	CH_3	H	4.6 ± 0.3	<4.4 (80)[a]
1b	$(CH_2)_3CH_3$	H	<4.4 (95)[a]	4.7 ± 0.3
1c	$(CH_2)_4CH_3$	H	5.3 ± 0.3	5.0 ± 0.1
1d	$(CH_2)_2CF_3$	H	4.7 ± 0.3	4.8 ± 0.3
1e	$(CH_2)_2OCH_2CH_3$	H	4.5 ± 0.3	4.5 ± 0.2
1f	$(CH_2)_2CH_2F$	H	4.8 ± 0.3	<4.4 (85)[a]
1g	$(CH_2)_3Cl$	H	5.6 ± 0.1	5.0 ± 0.1
1h	$(CH_2)_4Cl$	H	6.2 ± 0.2	5.3 ± 0.3
1i	*c*-C_3H_5	H	5.3 ± 0.4	5.2 ± 0.5
1j	CH_2*c*-C_3H_5	H	5.3 ± 0.4	5.2 ± 0.2
1k	*c*-C_6H_{11}	H	5.1 ± 0.1	4.8 ± 0.2
1l	$-(CH_2)_3-$		5.8 ± 0.3	5.3 ± 0.2
1m	$-(CH_2)_4-$		5.1 ± 0.1	5.4 ± 0.2

[a] Value in parentheses is % inhibition at 40 μM ± 10%. All data $n = 3$.

improvement in activity against either CHIKV or VEEV. The cyclohexyl sulfonamide (**1k**) was less active than the *n*-alkyl analogs. In the oxaspiropiperidinone series[8] intramolecular cyclization of chloroalkyl sulfonamides to their cyclic analogs resulted in 10 to 100-fold improvement in antiviral potency. However, in the spirodioxolane series the five-membered cyclic sulfonamide **1l** showed no improvement compared to its chloroalkyl precursor **1g**, and the six-membered cyclic sulfonamide **1m** was much less potent on CHIKV than **1h**. This initial series of sulfonamide and sulfamate analogs showed that it was possible to identify potent CHIKV inhibitors in the spirodioxolane series (*e.g.* **1h**), but the structure–activity diverged from that previously seen in the oxaspiropiperidine series of nsP2hel inhibitors.[8]

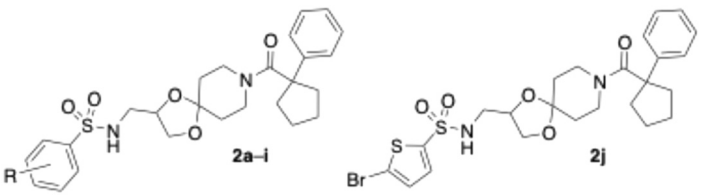
The effect of introducing aryl substituents into sulfonamide analogs **2a–j** was explored (Table 2). The addition of a *p*-tolyl group in **2a** resulted in moderate CHIKV inhibition but weaker VEEV activity. Biphenyl sulfonamide **2b** had poor antiviral activity. The 2-fluoro (**2c**), 3-bromo (**2d**), and 3-nitro (**2e**) analogs showed improved potency on CHIKV. However, the 3-amido substituent in **2f** had only modest activity. In a series of di-substituted phenyl analogs, **2g** with 3-cyano and 4-chloro substituents showed moderate antiviral activity, while analogs **2h** and **2i** with 5-nitro combined with 2-chloro or 2-methyl substituents showed improved activity against CHIKV. The 2-bromothiophene **2j** had similar activity to the 3-bromophenyl analog **2d**. From this series of aryl sulfonamides, we found that analogs with small 3- or 5-position electron withdrawing substituents showed small but consistent improvement in antiviral activity. The 5-nitro-2-tolyl analog **2i** was notable for its selective inhibition of CHIKV compared to VEEV.

A series of sulfamate analogs **3a–t** was tested for antiviral activity (Table 3). The parent *N*,*N*-dimethyl analog (**3a**) demonstrated moderate activity against both CHIKV and VEEV. Substitution of the *N*,*N*-dimethyl group with other straight-chain, branched, or cyclic alkyl groups (**3b–3i**) failed to consistently improve antiviral activity. The pyrrolidine (**3j**), piperidine (**3k**), azapane (**3l**), and morpholine (**3m**) analogs also showed only modest antiviral activity. In the piperidine sulfamate series, substitution on the inboard nitrogen with methyl (**3n**) or nitrile (**3o**) was tolerated but did not improve potency. However, *n*-hexyl substitution in **3p** led to a loss in activity and addition of a basic *N*,*N*-dimethylaminoethyl substituent gave a compound (**3q**) that showed cell toxicity. The six-membered cyclic sulfamate **3r** showed similar activity to cyclic sulfonamide **1m**. Additional *N*-methylation to generate **3s** improved activity against both CHIKV and VEEV, although extension to an *n*-hexyl group (**3t**) showed no further benefit.

Four analogs (**4a–d**) were synthesized with additional substitution on the piperidine of the sulfamate (Table 4). Addition of a 4-methyl group in analog **4a** slightly increased CHIKV activity which was further enhanced in 4-cyano analog **4b**. The 4-phenyl (**4c**) and 3,3-difluoro (**4d**) substituents resulted in similar improvements. Although the alkyl sulfamates (Tables 3 and 4) showed no systematic potency advantage as antivirals compared to the sulfonamides (Tables 1 and 2), the 4-cyanopiperidine **4b** emerged as one of the better CHIKV inhibitors in the series.

An additional series of sulfamates (**5a–m**) incorporating *N*-benzyl substituents was tested (Table 5). The parent analog (**5a**) showed promising antiviral activity on CHIKV. Notably, *N*-methylation of the outboard nitrogen resulted in an analog (**5b**) with slightly improved activity against CHIKV but only weak activity on VEEV. The ethyl (**5c**) and cyclopropyl (**5d**) analogs showed similar CHIKV-selective antiviral activity. Additional substitution on the inboard nitrogen of the *N*-benzyl-*N*-methyl sulfamate by methyl (**5e**) or cyano (**5f**) was also tolerated but the *n*-hexyl analog (**5g**)

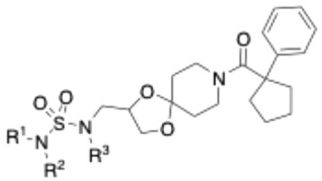
Table 2 Antiviral activity of aryl sulfonamide spirodioxolanes **2a–j** determined by alphavirus-nLuc reporter assays. Potency is expressed as the negative log of the 50% effective concentration



Compound	R	CHIKV-nLuc (pEC_{50} ± SD)	VEEV-nLuc (pEC_{50} ± SD)
2a	4-CH_3	5.2 ± 0.2	<4.4 (75)[a]
2b	4-Ph	<4.4 (75)[a]	<4.4 (65)[a]
2c	2-F	5.3 ± 0.3	4.6 ± 0.3
2d	3-Br	5.8 ± 0.6	4.8 ± 0.1
2e	3-NO_2	6.0 ± 0.4	4.8 ± 0.1
2f	3-C(O)$NHCH_3$	5.3 ± 0.3	4.5 ± 0.1
2g	3-CN, 4-Cl	5.6 ± 0.3	5.2 ± 0.3
2h	2-Cl, 5-NO_2	5.8 ± 0.4	4.8 ± 0.4
2i	2-CH_3, 5-NO_2	5.9 ± 0.3	<4.4 (90)[a]
2j	—	5.9 ± 0.3	4.7 ± 0.3

[a] Value in parentheses is % inhibition at 40 μM ± 10%. All data $n = 3$.

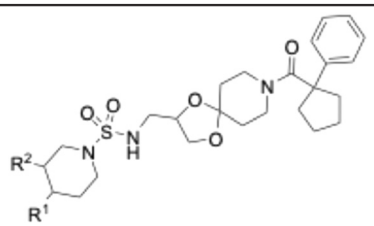
Table 3 Antiviral activity of alkyl sulfamate spirodioxolanes **3a–t** determined by alphavirus-nLuc reporter assays. Potency is expressed as the negative log of the 50% effective concentration



Compound	R^1	R^2	R^3	CHIKV-nLuc (pEC_{50} ± SD)	VEEV-nLuc (pEC_{50} ± SD)
3a	CH_3	CH_3	H	5.1 ± 0.1	5.0 ± 0.3
3b	CH_2CH_3	H	H	4.6 ± 0.4	4.9 ± 0.1
3c	CH_2CH_3	CH_3	H	5.3 ± 0.5	4.9 ± 0.2
3d	CH_2CH_3	CH_2CH_3	H	5.1 ± 0.1	4.6 ± 0.2
3e	$(CH_2)_2OCH_3$	$(CH_2)_2OCH_3$	H	5.1 ± 0.2	<4.4 (80)[a]
3f	$CH_2CH(CH_3)_2$	H	H	5.2 ± 0.5	4.7 ± 0.2
3g	$CH(CH_3)_2$	CH_3	H	5.4 ± 0.2	4.7 ± 0.2
3h	*c*-C_6H_{11}	H	H	4.9 ± 0.1	<4.4 (65)[a]
3i	*c*-C_6H_{11}	CH_3	H	5.7 ± 0.2	4.9 ± 0.1
3j	$-(CH_2)_4-$		H	5.0 ± 0.1	5.3 ± 0.2
3k	$-(CH_2)_5-$		H	5.5 ± 0.2	5.2 ± 0.2
3l	$-(CH_2)_6-$		H	5.5 ± 0.4	4.9 ± 0.1
3m	$-((CH_2)_2O(CH_2)_2)-$		H	5.2 ± 0.3	4.7 ± 0.1
3n	$-(CH_2)_5-$		CH_3	5.2 ± 0.2	4.6 ± 0.2
3o	$-(CH_2)_5-$		CN	4.6 ± 0.3	5.2 ± 0.3
3p	$-(CH_2)_5-$		$(CH_2)_5CH_3$	4.6 ± 0.3	5.1 ± 0.2
3q	$-(CH_2)_5-$		$(CH_2)_2N(CH_3)_2$	Toxic[b]	Toxic[b]
3r	H	$-(CH_2)_3-$		5.0 ± 0.1	5.3 ± 0.4
3s	CH_3	$-(CH_2)_3-$		5.7 ± 0.4	5.7 ± 0.2
3t	$(CH_2)_5CH_3$	$-(CH_2)_3-$		5.4 ± 0.4	5.2 ± 0.1

[a] Value in parentheses is % inhibition at 40 μM ± 10%. [b] Toxicity observed at concentrations >1 μM by CellTiter-Glo assay. All data $n = 3$.

Table 4 Antiviral activity of piperidine sulfamate spirodioxolanes **4a–d** determined by alphavirus-nLuc reporter assays. Potency is expressed as the negative log of the 50% effective concentration



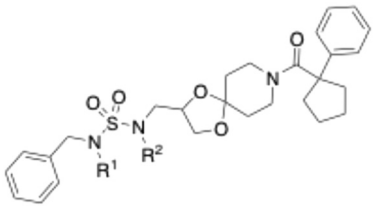
Compound	R^1	R^2	CHIKV-nLuc (pEC_{50} ± SD)	VEEV-nLuc (pEC_{50} ± SD)
4a	CH_3	H	5.4 ± 0.2	5.0 ± 0.1
4b	CN	H	5.8 ± 0.2	4.8 ± 0.3
4c	Ph	H	5.7 ± 0.2	5.0 ± 0.1
4d	H	di-F	5.7 ± 0.2	5.0 ± 0.1

All data $n = 3$.

lost CHIKV activity. The *N*,*N*-dimethylethyl analog (**5h**) was toxic, as had been seen in the sulfonamide series. However, lowering the basicity of the substituent with a morpholinoethyl group resulted in non-toxic analog **5i** with selective CHIKV inhibition. The *N*,*N*-dimethylacetamide analog (**5j**) was one of the most potent CHIKV inhibitors with EC_{50} = 500 nM. The corresponding acetamides incorporating pyrrolidine (**5k**), piperidine (**5l**), and morpholine (**5m**) were also active against CHIKV but were progressively less potent. However, the *N*-benzyl sulfamates (Table 5) showed some of the most consistent CHIKV inhibition in the spirodioxolane template.

The effect of *gem*-dimethylation substitution on the dioxolane ring was studied in analogs **6a–e** (Table 6). Compared to the corresponding parent analogs (**3k**, **4b**, **5b**, **5j**, and **1m**, respectively) the potency of CHIKV inhibition was maintained or even enhanced (*e.g.* **6b**), indicating that the *gem*-dimethyl substitution was well tolerated. In the case of analog **6d**, *gem*-dimethyl substitution led to a >10-fold increase in VEEV potency, resulting in the only

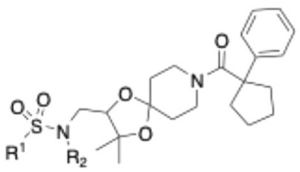
Table 5 Antiviral activity of *N*-benzyl sulfamate spirodioxolanes **5a–m** determined by alphavirus-nLuc reporter assays. Potency is expressed as the negative log of the 50% effective concentration



Compound	R^1	R^2	CHIKV-nLuc (pEC_{50} ± SD)	VEEV-nLuc (pEC_{50} ± SD)
5a	H	H	5.6 ± 0.1	4.8 ± 0.1
5b	CH_3	H	5.9 ± 0.2	<4.4 (90)[a]
5c	CH_2CH_3	H	5.8 ± 0.1	<4.4 (90)[a]
5d	*c*-C_3H_7	H	6.0 ± 0.1	4.7 ± 0.3
5e	CH_3	CH_3	5.8 ± 0.2	4.8 ± 0.3
5f	CH_3	CN	5.9 ± 0.3	<4.4 (75)[a]
5g	CH_3	$(CH_2)_5CH_3$	<4.4 (70)	<4.4 (75)[a]
5h	CH_3	$(CH_2)_2N(CH_3)_2$	Toxic[b]	Toxic[b]
5i	CH_3	$(CH_2)_2N((CH_2)_2O(CH_2)_2)$	5.8 ± 0.2	<4.4 (95)[a]
5j	CH_3	$CH_2C(O)N(CH_3)_2$	6.3 ± 0.2	4.7 ± 0.2
5k	CH_3	$CH_2C(O)N((CH_2)_4)$	6.0 ± 0.1	4.6 ± 0.3
5l	CH_3	$CH_2C(O)N((CH_2)_5)$	5.8 ± 0.2	4.8 ± 0.3
5m	CH_3	$CH_2C(O)N((CH_2)_2O(CH_2)_2)$	5.3 ± 0.2	<4.4 (50)[a]

[a] Value in parentheses is % inhibition at 40 μM ± 10%. [b] Toxicity observed at concentrations >1 μM by CellTiter-Glo assay. All data $n = 3$.

Table 6 Antiviral activity of 3,3-dimethyl spirodioxolanes **6a–e** determined by alphavirus-nLuc reporter assays. Potency is expressed as the negative log of the 50% effective concentration



Compound	R^1	R^2	CHIKV-nLuc (pEC_{50} ± SD)	VEEV-nLuc (pEC_{50} ± SD)
6a	$N(CH_2)_5$	H	5.6 ± 0.5	<4.4 (90)[a]
6b	$N(CH_2)_5$(4-CN)	H	6.2 ± 0.2	<4.4 (80)[a]
6c	$N(CH_3)CH_2Ph$	H	5.7 ± 0.2	<4.4 (80)[a]
6d	$N(CH_3)CH_2Ph$	$CH_2C(O)N(CH_3)_2$	6.0 ± 0.2	5.9 ± 0.3
6e	$-(CH_2)_4-$		5.0 ± 0.2	<4.4 (70)[a]

[a] Value in parentheses is % inhibition at 40 μM ± 10%. All data $n = 3$.

spirodioxolane that showed dual inhibition of CHIKV and VEEV with EC_{50} ~1 μM.

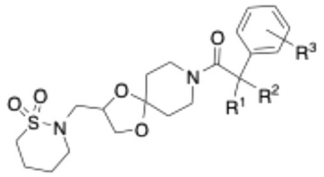
The spirodioxolane chemotype contains a single chiral center. To assess the influence of stereochemistry on antiviral activity, the (*R*)- and (*S*)-enantiomers of five CHIKV inhibitors representing the sulfonamide (**1m**) and the alkyl (**3a** and **3k**), piperidine (**4b**), and *N*-benzyl (**5b**) sulfamate series were assayed (Table 7). In the related oxaspiropiperidinone series there was a strict enantioselectivity for nsP2hel and viral inhibition, with activity residing almost exclusively in the (*R*)-enantiomer.[9] However, in the spirodioxolane series no corresponding enantioselectivity was seen (Table 7). In each case, the potency for CHIKV inhibition by the (*R*)- and (*S*)-enantiomer was close to the racemate, with no clear trend for either isomer. Thus, unlike the clear enantioselectivity seen in the sulfonamide oxaspiropiperidines, the sulfamate spirodioxolanes showed no consistent preference for either enantiomer.

Table 7 Antiviral activity of enantiomers of spirodioxolanes **1m**, **3a**, **3k**, **4b**, and **5b** determined by alphavirus-nLuc reporter assays. Potency is expressed as the negative log of the 50% effective concentration

	CHIKV-nLuc (pEC_{50} ± SD)		
Compound	Racemate	(*R*)-Isomer	(*S*)-Isomer
1m	5.1 ± 0.1	5.3 ± 0.2	5.0 ± 0.1
3a	5.1 ± 0.1	5.1 ± 0.2	5.2 ± 0.2
3k	5.4 ± 0.4	5.7 ± 0.4	5.1 ± 0.6
4b	5.8 ± 0.2	6.1 ± 0.3	5.8 ± 0.1
5b	5.9 ± 0.2	5.9 ± 0.3	6.2 ± 0.2

All data $n = 3$.

Table 8 Antiviral activity of acetamide spirodioxolanes **7a–d** determined by alphavirus-nLuc reporter assays. Potency is expressed as the negative log of the 50% effective concentration



Compound	R_1	R_2	R_3	CHIKV-nLuc (pEC_{50} ± SD)	VEEV-nLuc (pEC_{50} ± SD)
7a	CH_3	CH_3	H	<4.4 (40)[a]	<4.4 (40)[a]
7b	$-(CH_2)_2-$		H	<4.4 (25)[a]	<4.4 (20)[a]
7c	$-(CH_2)_4-$		3-Cl	5.8 ± 0.3	5.6 ± 0.3
7d	$-(CH_2)_4-$		4-Cl	<4.4 (55)[a]	<4.4 (55)[a]

[a] Value in parentheses is % inhibition at 40 μM ± 10%. All data $n = 3$.

Although there were clear differences in structure–activity and enantioselectivity for CHIKV inhibition between the spirocyclic cores of the oxaspiropiperidine and spirodioxolane series, one area of strict concordance was the piperidine acetamide (Table 8). Modification of the cyclopentyl group adjacent to the carbonyl to *gem*-dimethyl (**7a**) or cyclopropyl (**7b**) resulted in a loss in antiviral activity. Remarkably, as was previously seen in the oxaspiropiperidine series,[8] the ^{13}C-NMR resonances of the piperidine carbons in the active spirodioxolane analog **1m** were too broad to be seen at room temperature (Fig. S1). However, in the inactive analogs **7a** and **7b** the piperidine carbons were well resolved in their ^{13}C-NMR spectra (Fig. S1). We previously explained this phenomenon as evidence of conformational isomerism in the cyclopentane phenylacetamide, which imparted thermal mobility in the piperidine ring.[9] In the oxaspiropiperidine series this property was required for inhibition of the dynamic viral helicase enzyme. The molecular basis for the connection between thermal mobility of the ligand and enzyme inhibition has not yet been determined. However, observation of the same phenomenon in the spirodioxolane series lends credence to the importance of conformation isomerism for allosteric nsP2hel inhibition. Other key structure–activity in the amide pharmacophore was also conserved,[8] with the *m*-chlorophenyl substitution (**7c**) enhancing activity but the *p*-chlorophenyl (**7d**) analog losing CHIKV and VEEV inhibition (Table 8). In both the oxaspiropiperidine and spirodioxolane series, the phenyl group of the piperidine acetamide appeared to fit into a tight lipophilic pocket where only a small meta-substituent was tolerated.

Evidence of nsP2hel inhibition. The concordance of conformational isomerism and structure–activity of the piperidine acetamide across the oxaspiropiperidine and spirodioxolane series strongly supports a common molecular mechanism of action, namely direct inhibition of the nsP2hel enzyme. As additional evidence of direct nsP2hel inhibition, five inhibitors of CHIKV replication with pEC_{50} 5.8–6.2 were tested for inhibition of nsP2 ATPase activity (Table 9); one sulfonamide analog (**1h**), three analogs (**4b**, **5b**, and **5j**) with diverse sulfamate substitutions, and one analog with *gem*-dimethyl substitution on the core (**6b**). When assayed for inhibition of nsP2 ATPase biochemical activity the five analogs showed pIC_{50} between 5.1–5.8, demonstrating reductions in ATP consumption as would be expected with their action as direct acting nsP2hel inhibitors.

To further confirm that the antiviral activity of the spirodioxolanes was due to inhibition of CHIKV nsP2hel, the four most active analogs from the ATPase assay (**4b**, **5b**, **5j**, and **6b**) were tested on viruses harboring point mutations in their helicase RecA domain (Table 10). The CHIKV F185L point mutant was observed with high frequency following continuous passage of the virus with high concentrations of **RA-25298** and showed strong resistance to the antiviral activity of **RA-25298** and **RA-NSP2-1**.[10] The point mutants G285T and F286P were identified in an independent study of nsP2 function and had previously shown moderate resistance to inhibition by **RA-25298**.[10] The wild type and point mutant CHIKV-nLuc assays were performed in parallel to minimize variability (Table 10). Oxaspiropiperidine **RA-25298** and the four spirodioxolanes (**4b**, **5b**, **5j**, and **6b**) demonstrated robust inhibition of wild type CHIKV-nLuc but showed >10-fold decreased potency against one or more of the nsP2hel RecA domain point mutants, consistent with a mechanism of direct inhibition of the enzyme. However, key differences were seen in the degree of resistance of each spirodioxolane analog across the panel of CHIKV point mutants. For

Table 9 Inhibition of nsP2 enzyme function by spirodioxolanes **4b**, **5b**, **5j**, and **6b** determined by ATPase assay. Potency is expressed as the negative log of the 50% inhibitory concentration

Compound	nsP2 ATPase (pIC_{50} ± SD)
1h	5.1 ± 0.1
4b	5.4 ± 0.3
5b	5.4 ± 0.3
5j	5.7 ± 0.2
6b	5.8 ± 0.1

All data $n = 3$.

Table 10 Antiviral activity of oxaspiropiperidine **RA-25298** and spirodioxolanes **4b**, **5b**, **5j**, and **6b** on wild type and mutant CHIKV-nLuc reporter assays. Potency is expressed as the negative log of the 50% effective concentration

Compound	CHIKV-nLuc (pEC_{50} ± SD)[a]			
	WT	F185L	G285T	F286P
RA-25298	6.5 ± 0.2	4.5 ± 0.1	5.4 ± 0.2	5.0 ± 0.1
4b	5.7 ± 0.1	5.1 ± 0.1	4.9 ± 0.5	4.6 ± 0.3
5b	5.8 ± 0.2	4.7 ± 0.2	5.0 ± 0.1	4.8 ± 0.2
5j	5.9 ± 0.4	4.9 ± 0.1	5.0 ± 0.1	5.0 ± 0.1
6b	5.9 ± 0.3	5.3 ± 0.2	5.0 ± 0.2	4.6 ± 0.3

[a] pEC_{50} were color coded using a green-to-red scale in Excel. All data $n = 3$.

4-cyanopiperidine sulfamate **4b**, strongest resistance was seen with the F286P mutant but the F185L mutant had only a moderate effect. Notably, this resistance profile was different to that observed with oxaspiropiperidine **RA-25298**. The *N*-benzyl sulfamate **5b** showed strongest resistance to the F185L and F286P mutants, but less with the G285T mutant. However, analog **5j**, in which the *N*-benzyl sulfamate contained an additional acetamide on the inboard nitrogen, was not as severely affected by either the F185L or F286P mutants. The 4-cyanopiperidine sulfamate with additional *gem*-dimethyl substitution on the dioxolane core (**6b**) showed strong resistance with only the F286P mutant, suggesting that the additional substitution on the core could overcome resistance to either F185L or G285T point mutations. Thus, in addition to confirming that antialphaviral activity was due to engagement of nsP2hel, the structure-dependent resistance profiles demonstrated that modification of the inhibitor core could partially overcome resistance due to specific point mutations. These results underscore the value of the spirodioxolanes as a new series of nsP2hel inhibitors with different resistance profiles to the first generation oxaspiropiperidines.

Antialphaviral activity. Finally, to demonstrate the therapeutic potential of the spirodioxolanes nsP2hel inhibitors, *N*-benzyl sulfamate **5b** and 4-cyanopiperidine sulfamate **6b** were tested against infectious isolates of genetically diverse alphaviruses. The alphavirus family can be divided into three distinct clades based on the sequence and structure of nsP2hel: Old Word (CHIKV), New World (VEEV), and Sindbis-like (SINV) alphaviruses.[3] Both **5b** and **6b** were effective at reducing viral titer of CHIKV but showed minimal to no effect on VEEV or SINV (Fig. 2). The results were consistent with the profile measured in the nLuc reporter viral replication assays, where both **5b** and **6b** were much more potent on CHIKV compared to VEEV. While most of the spirodioxolanes were predominantly CHIKV-selective at least two examples (**6d** and **7c**) demonstrated strong VEEV inhibition. Thus, although the current leads are predominantly selective inhibitors of CHIKV, it may be possible to identify new spirodioxolane nsP2hel inhibitors that have broad spectrum antialphaviral activity by additional structure-guided optimization.

Conclusions

We have developed a second series of spirocyclic nsP2hel inhibitors that demonstrate antialphaviral activity. The spirodioxolanes share many of the key features of the oxaspiropiperidine nsP2hel inhibitors; however, their structure–activity diverges in several ways. Most notably, there is no clear enantioselectivity in the spirodioxolane core, with the individual (*R*)- and (*S*)-isomers showing equivalent activity to the racemic mixtures. Sulfamate analogs were generally more potent than sulfonamides and the optimal substituents were unique to the spirodioxolane series.

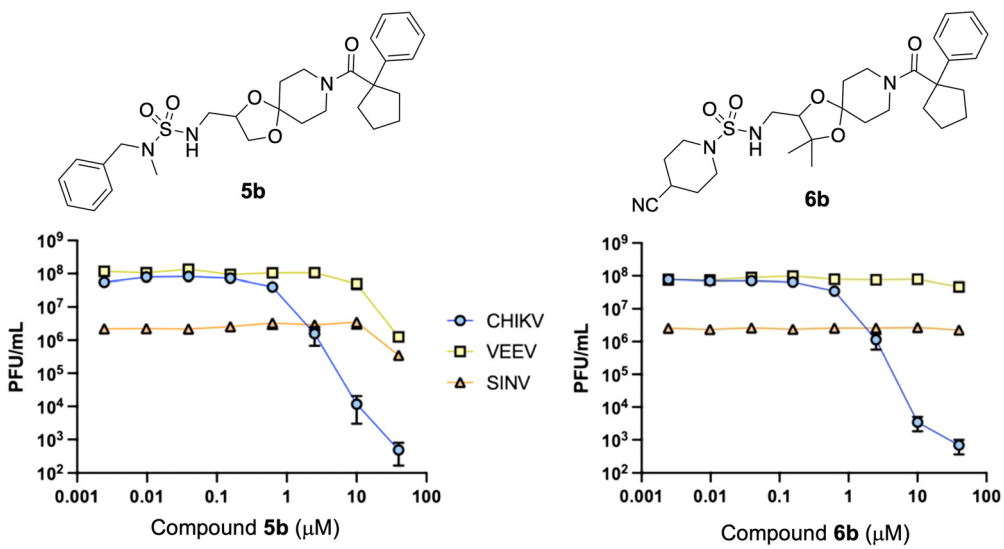


Fig. 2 Antiviral activity of **5b** and **6b** against infectious isolates of CHIKV (circles), VEEV (squares), or SINV (triangles). Viral titer measured by plaque assay in Vero cells, $n = 3 \pm$ SD.

Despite these differences, the highly substituted piperidine acetamide remained a strict requirement in both spirocyclic series, suggesting that they bind to the same pocket in the nsP2hel RecA domain and require the conformational isomerism and thermal mobility of the piperidine ring to be active as helicase inhibitors. Most of the spirodioxolanes were selective for the Old World alphavirus CHIKV and had only limited activity on New World or Sindbis-like viruses. However, there were some analogs that showed robust inhibition of VEEV, suggesting that additional optimization of the spirodioxolane chemotype may yield analogs with broad spectrum antiviral activity. Importantly, analogs such as **6b** containing a *gem*-dimethyl substitution on the dioxolane core retained activity against the CHIKV F185L point mutant that was resistant to inhibition by **RA-NSP2-1**. These results augur well for further optimization of spirodioxolane nsP2hel inhibitors as an alternative series of antialphaviral drugs.

Experimental protocols

Chemistry

All reactions were conducted in oven-dried glassware under a dry nitrogen atmosphere unless otherwise specified. All reagents and solvents were obtained from commercial sources and used without further purification. No unexpected safety hazards were encountered during the syntheses. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel plates (200 μm, F_{254} indicator), visualized under UV light or by staining with iodine and $KMnO_4$. Column chromatography utilized pre-loaded silica gel cartridges on a Biotage automated purification system. ^1H and ^{13}C NMR spectra were recorded in CD_3OD at 500/400 and 126/101 MHz, respectively, on a Bruker spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane for ^1H NMR, with major peaks designated as s (singlet), d (doublet), t (triplet), dd (doublet of doublets), and m (multiplet). The absence of carbon peaks due to low intensity or broad signals have been noted. High-resolution mass spectrometry (HRMS) analyses were performed at the UNC Department of Chemistry Mass Spectrometry Core Laboratory using a Q Exactive HF-X mass spectrometer. Liquid chromatography-mass spectrometry (LCMS) was conducted on an Agilent 1290 Infinity II LC System with an Agilent Infinity Lab PoroShell 120 EC-C18 column (30 °C, 2.7 μm, 2.1 × 50 mm), employing a 5–95% CH_3CN in water as eluent, with 0.2% (v/v) formic acid as the modifier and a flow rate of 1 mL min^{-1}. Preparative high-performance liquid chromatography (HPLC) was executed using an Agilent 1260 Infinity II LC System equipped with a Phenomenex C18 column (PhenylHexyl, 30 °C, 5 μm, 75 × 30 mm), with a 5–95% CH_3CN in water as eluent and 0.05% (v/v) trifluoroacetic acid as the modifier and at a flow rate of 30 mL min^{-1}. Analytical HPLC data were recorded on a Waters Alliance instrument with a PDA detector or an Agilent 1260 Infinity II series instrument with a PDA detector (EC-C18, 100 mm × 4.6 mm, 3.5 μm), using a 10–90% CH_3CN in water as eluent at a flow rate of 0.5 mL min^{-1}. The final compounds were confirmed to be >95% pure by HPLC analysis. Chiral HPLC data were recorded on an Agilent 1260 Infinity II series instrument with a PDA detector using a Diacel chiralpak IA column (250 mm × 4.6 mm, 5 μm), using 90% iPrOH in water as eluent at a flow rate of 1.0 mL min^{-1}. (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine, 1-amino-3-methylbutane-2,3-diol, carboxylic acids, sulfonyl chlorides, and sulfamoyl chlorides were obtained from commercial vendors unless otherwise noted. (*R*)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methanamine and (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine were obtained from AmBeed (Buffalo Grove, Illinois) at an enantiopurity of 97% ee and 95% ee, respectively.

General procedure 1 for synthesis of spirodioxolanes. To a solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (1.0 eq.) in CH_2Cl_2 at a concentration of 0.1 M was added Et_3N (3.0 eq.) at 0 °C. The mixture was stirred in an ice bath for 30 min, followed by the addition of a sulfonyl chloride (1.2–1.4 eq.) or sulfamoyl chloride (1.2–1.4 eq.). The reaction was stirred for 16 h at room temperature. Upon completion of the reaction based on TLC and LCMS, the mixture was poured into saturated aq. $NaHCO_3$ solution, extracted with CH_2Cl_2 (3×), washed with brine, and the combined organic layers dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude sulfonamide or sulfamate of (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine was used without further purification. To a scintillation vial with a rubber septum was added 1-(1-phenylcyclopentane-1-carbonyl)piperidin-4-one (intermediate **II**, 1.0 eq.), *p*-TsOH (0.5 eq.), and the crude sulfonamide or sulfamate of (2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (1.0–2.0 eq.) in toluene at a concentration of 0.1 M. A syringe needle was inserted through the rubber septum, positioned above the mixture, to allow pressure equalization during the reaction. The mixture was heated for 16 h at 70 °C. Upon completion of the reaction based on TLC and LCMS, the mixture was poured into saturated aq. $NaHCO_3$, extracted with EtOAc (3×), and the combined organic layers dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified by chromatography (Biotage, eluted with 50–60% EtOAc in hexane). Fractions containing the product were pooled, concentrated, dissolved in MeOH, and further purified by preparative HPLC if necessary. Fractions containing the product were collected in scintillation vials and dried for 16 h under a stream of air to provide the product as a gum. The gum was dissolved in EtOAc and washed with aq. $NaHCO_3$. The EtOAc layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. Et_2O (2 mL) was added to the resulting gum and the solution was evaporated under a stream of air and then under high vacuum to deliver the spirodioxolane as a white foam or solid.

General procedure 2 for synthesis of sulfamate spirodioxolanes. To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (1.0 eq.) in MeCN at a concentration of 0.1 M was added Et_3N (10 eq.) and an amine

(1.0 eq.) at 0 °C. 1 M SO_2Cl_2 in CH_2Cl_2 (3 eq.) was added dropwise over 5 min. The mixture was stirred at 0–20 °C for 1 h. Upon completion of the reaction based on TLC and LCMS, the mixture was poured into saturated aq. $NaHCO_3$, extracted with EtOAc (3×), washed with brine, and the combined organic layers dried over anhydrous $MgSO_4$, filtered, and concentrated. The resulting sulfamate of (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine was used without further purification for the synthesis of spirodioxolanes as described in General procedure 1.

General procedure 3 for alkylation of sulfamate spirodioxolanes. A scintillation vial containing a sulfamate spirodioxolane (1.0 eq.) in THF at a concentration of 0.1 M was cooled to 0 °C, followed by the portion-wise addition of sodium hydride (60% in mineral oil, 5.0 eq.). After stirring for 10 min at 0 °C, an alkyl bromide (1.2–3.0 eq.) or alkyl iodide (2.5–3.0 eq.) was added portion-wise. The mixture was stirred at room temperature for 1 h and then heated at 50 °C for 16 h. Upon completion of the reaction based on TLC and LCMS, the alkylated sulfamate spirodioxolane product was isolated and purified as described in General procedure 1.

General procedure 4 for synthesis of chloroalkyl sulfonamide spirodioxolanes. To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (1.0 eq.) in CH_2Cl_2 at a concentration of 0.1 M at 0 °C was added Fmoc-Cl (2.0 eq.). The mixture was stirred at room temperature for 5 h. Upon completion based on TLC and LCMS, the reaction was quenched with saturated aq. $NaHCO_3$, extracted with CH_2Cl_2 (3×), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude Fmoc-protected amine (1.0 eq.) was reacted with 1-(1-phenylcyclopentane-1-carbonyl)piperidin-4-one (intermediate **II**, 1.0 eq.) following general procedure 1. The Fmoc-protected spirodioxolane was dissolved in 20% piperidine in DMF at a concentration of 0.1 M and stirred for 16 h at room temperature. The mixture was concentrated under reduced pressure and the resulting amine used without further purification. In a scintillation vial was added the crude amine (1.0 eq.), Et_3N (3.0 eq.), and CH_2Cl_2 at a concentration of 0.1 M. The mixture was cooled to 0 °C before addition of a chloroalkyl sulfonyl chloride (1.2–1.3 eq.) dropwise over 5 min. The mixture was allowed to warm to room temperature and stirred for 3 h. Upon completion based on TLC and LCMS, the reaction was quenched with saturated aq. $NaHCO_3$, extracted with CH_2Cl_2 (3×), dried over anhydrous $MgSO_4$, filtered, and concentrated. The chloroalkyl sulfonamide spirodioxolane was purified as described in General procedure 1.

General procedure 5 for synthesis of cyclic sulfonamide spirodioxolanes. A solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (1.0 eq.) in dry THF at a concentration of 0.1 M was cooled to 0 °C, then NaH (60% in mineral oil, 3.5 eq.) was added portion-wise. After 10 min, a chloroalkyl sulfonyl chloride (1.1 eq.) was added dropwise. The mixture was stirred at room temperature for 1 h and then at 50 °C for 16 h. Upon completion of the reaction based on TLC and LCMS, the mixture was poured into saturated aq. $NaHCO_3$, extracted with EtOAc (3×), washed with brine, and the combined organic layers dried over anhydrous $MgSO_4$, filtered, and concentrated. The resulting cyclic sulfonamide of (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine was used for synthesis of spirodioxolanes following General procedure 1.

General procedure 6 for synthesis of 3,3-dimethyl spirodioxolanes. To a scintillation vial was added 1-amino-3-methylbutane-2,3-diol (1.0 eq.) and Et_3N (2.0 eq.) in CH_2Cl_2 at a concentration of 0.1 M. After cooling to 0 °C, a sulfonyl chloride (0.9 eq.) was added dropwise. The mixture was allowed to warm to room temperature over 1 h. Upon completion of the reaction based on TLC and LCMS, the mixture was poured into saturated aq. $NaHCO_3$, extracted with CH_2Cl_2 (3×), washed with brine (1×), and the combined organic layers dried over anhydrous $MgSO_4$, filtered, and concentrated. The resulting sulfonamide of 1-amino-3-methylbutane-2,3-diol was used for synthesis of 3,3-dimethyl spirodioxolanes following General procedure 1.

General procedure 7 for synthesis of acetamide spirodioxolanes. To a scintillation vial was added piperidin-4-one (1.0 eq.), a carboxylic acid (1.3 eq.), HATU (1.5 eq.), DIPEA (3.0 eq.), and DMF at a concentration of 0.1 M. The mixture was stirred at room temperature for 16 h. Upon completion of the reaction based on TLC and LCMS, the mixture was poured into saturated aq. $NaHCO_3$, extracted with CH_2Cl_2 (3×), washed with brine, and the combined organic layers dried over anhydrous $MgSO_4$, filtered, and concentrated. The acetamide of piperidin-4-one (1.0 eq.) was reacted with 2-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1,2-thiazinane 1,1-dioxide (from General procedure 5, 1.0 eq.) in THF at a concentration of 0.1 M and the acetamide spirodioxolane isolated and purified using the procedure described in General procedure 1.

1-(1-Phenylcyclopentane-1-carbonyl)piperidin-4-one ***II.*** A solution of 1-phenylcyclopentane-1-carboxylic acid (2.5 g, 13 mmol, 1 eq.), piperidin-4-one hydrochloride (2.1 g, 16 mmol, 1.2 eq.) HATU (9.1 g, 24 mmol, 1.5 eq.), DIPEA (4.6 mL, 26 mmol, 2 eq.) in DMF (250 mL) was stirred at room temperature for 16 h. Upon completion of the reaction based on TLC and LCMS, the mixture was poured into saturated aq. $NaHCO_3$, extracted with CH_2Cl_2 (3×), washed with brine, and the combined organic layers dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified by chromatography (Biotage, eluted with 35% EtOAc in hexane). Fractions containing the product were pooled, concentrated, and dried to give **I** as a beige powder (2.5 g, 70%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.39–7.28 (m, 3H), 7.25–7.21 (m, 2H), 3.84–3.44 (m, 3H), 3.17–3.14 (m, 1H), 2.51–2.28 (m, 3H), 2.11–2.01 (m, 2H), 1.77–1.59 (m, 6H), 1.16–1.07 (s, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 209.2, 177.0, 176.6, 146.6, 146.1, 130.1, 129.9, 127.8, 127.5, 126.3, 126.2, 96.4, 60.0, 59.9, 44.7, 41.4, 39.2, 35.9, 35.5, 26.0; MS (ESI) m/z (%): 272.4 $[M + H]^+$; HPLC purity (254 nm): >99%.

N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)methanesulfonamide ***1a.*** The compound was synthesized from intermediate **II** (0.27 g, 1.0 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (200 mg, 1.5 mmol, 1.5 eq.), and methanesulfonyl chloride (0.14 mL, 1.8 mmol, 1.8 eq.) following General procedure 1 to yield **1a** as a white foam (0.30 g, 72%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.31 (m, 2H), 7.26–7.20 (m, 3H), 4.15–3.96 (m, 2H), 3.80–3.49 (m, 3H), 3.30–3.00 (m, 4H), 2.92 (s, 3H), 2.43–2.32 (m, 2H), 2.11–1.99 (m, 2H), 1.77–1.53 (m, 6H), 1.08–0.89 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.4, 129.9, 127.6, 127.5, 126.2, 109.0, 76.4, 67.6, 59.9, 47.9, 46.8, 46.2, 45.3, 42.1, 40.1, 39.3, 36.8, 35.3, 26.0; MS (ESI) *m*/*z* (%): 423.4 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{21}H_{30}N_2O_5S$ $[M + H]^+$ 423.1948, found: 423.1950.

N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)butane-1-sulfonamide ***1b.*** The compound was synthesized from intermediate **II** (0.12 g, 0.44 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (80 mg, 0.61 mmol, 1.4 eq.), and butane-1-sulfonyl chloride (0.10 mL, 0.79 mmol, 1.8 eq.) following General procedure 1 to yield **1b** as a white foam (0.12 g, 59%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.30 (m, 2H), 7.25–7.21 (m, 3H), 4.21–3.92 (m, 2H), 3.80–3.53 (m, 3H), 3.30–2.93 (m, 6H), 2.45–2.28 (m, 2H), 2.15–1.96 (m, 2H), 1.78–1.49 (m, 8H), 1.50–1.39 (m, 2H), 1.15–0.98 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.0, 76.5, 67.7, 59.9, 53.0, 46.2, 45.3, 42.1, 39.5, 36.8, 35.3, 26.8, 25.9, 22.5, 14.0; MS (ESI) *m*/*z* (%): 465.3 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{36}N_2O_5S$ $[M + H]^+$ 465.2418, found: 465.2417.

N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)pentane-1-sulfonamide ***1c.*** The compound was synthesized from intermediate **II** (0.14 g, 0.52 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (90 mg, 0.69 mmol, 1.3 eq.), and pentane-1-sulfonyl chloride (0.13 mL, 0.89 mmol, 1.7 eq.) following General procedure 1 to yield **1c** as a white foam (0.17 g, 70%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.31 (m, 2H), 7.26–7.21 (m, 3H), 4.21–3.91 (m, 2H), 3.82–3.52 (m, 3H), 3.30–2.96 (m, 6H), 2.42–2.30 (m, 2H), 2.11–1.97 (m, 2H), 1.78–1.56 (m, 8H), 1.42–1.33 (m, 4H), 1.15–0.97 (m, 2H), 0.93 (t, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.5, 146.4, 129.9, 127.5, 126.2, 108.9, 75.9, 72.1, 67.9, 59.9, 59.0, 46.3, 45.2, 42.1, 39.5, 36.8, 35.3, 25.9; MS (ESI) *m*/*z* (%): 479.2 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{25}H_{38}N_2O_5S$ $[M + H]^+$ 479.2574, found: 479.2573.

3,3,3-Trifluoro-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)propane-1-sulfonamide ***1d.*** The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (150 mg, 1.1 mmol, 1.8 eq.), and 3,3,3-trifluoropropane-1-sulfonyl chloride (0.19 mL, 1.5 mmol, 2.5 eq.) following General procedure 1 to yield **1d** as a white foam (0.16 g, 55%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.30 (m, 2H), 7.25–7.21 (m, 3H), 4.22–3.92 (m, 2H), 3.83–3.51 (m, 3H), 3.29–3.06 (m, 6H), 2.71–2.57 (m, 2H), 2.41–2.30 (m, 2H), 2.12–1.96 (m, 2H), 1.75–1.57 (m, 6H), 1.20–0.92 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.4, 130.0, 127.6, 126.2, 109.1, 76.5, 67.6, 59.9, 46.4, 46.3, 30.0, 29.8, 25.9 with two carbons not observed; MS (ESI) *m*/*z* (%): 505.4 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{23}H_{31}F_3N_2O_5S$ $[M + H]^+$ 505.1979, found: 505.1975.

2-Ethoxy-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)ethane-1-sulfonamide ***1e.*** The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (150 mg, 1.1 mmol, 1.8 eq.) and 2-ethoxyethane-1-sulfonyl chloride (0.14 mL, 1.1 mmol, 1.8 eq.) following General procedure 1 to yield **1e** as a white foam (0.12 g, 42%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.31 (m, 2H), 7.26–7.21 (m, 3H), 4.21–3.94 (m, 2H), 3.81–3.60 (m, 5H), 3.54–3.46 (m, 2H), 3.31–3.05 (m, 6H), 2.41–2.32 (m, 2H), 2.10–1.99 (m, 2H), 1.75–1.55 (m, 6H), 1.20–0.98 (m, 5H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.0, 76.4, 67.7, 67.5, 65.7, 59.9, 53.0, 46.3, 45.2, 42.0, 39.3, 36.8, 35.3, 25.9, 15.4; MS (ESI) *m*/*z* (%): 481.2 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{36}N_2O_6S$ $[M + H]^+$ 481.2367, found: 481.2367.

3-Fluoro-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)propane-1-sulfonamide ***1f.*** The compound was synthesized from intermediate **II** (0.15 g, 0.53 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (90 mg, 0.69 mmol, 1.3 eq.) and 3-fluoropropane-1-sulfonyl chloride (0.10 mL, 0.89 mmol, 1.7 eq.) following General procedure 1 to yield **1f** as a white foam (0.15 g, 62%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.31 (m, 2H), 7.26–7.20 (m, 3H), 4.57 (t, J = 5.8 Hz, 1H), 4.47 (t, J = 5.8 Hz, 1H), 4.22–3.92 (m, 2H), 3.79–3.51 (m, 3H), 3.29–3.04 (m, 6H), 2.41–2.30 (m, 2H), 2.18–1.95 (m, 5H), 1.77–1.51 (m, 6H), 1.20–0.93 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 210.0, 176.6, 146.5, 129.9, 127.5, 126.2, 109.0, 83.6, 82.2, 76.5, 67.6, 59.9, 46.2, 45.2, 42.0, 39.3, 36.8, 30.7, 26.3, 26.2 (d, J = 21.4 Hz), 25.9; MS (ESI) *m*/*z* (%): 469.2 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{23}H_{33}FN_2O_5S$ $[M + H]^+$ 469.2167, found: 469.2165.

3-Chloro-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)propane-1-sulfonamide ***1g.*** The compound was synthesized from benzyl ((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)carbamate (75 mg, 0.22 mmol, 1.0 eq.) and 3-chloropropane-1-sulfonyl chloride (0.032 mL, 0.26 mmol, 1.2 eq.) following General procedure 4 using to yield a white foam (36 mg, 34%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.33 (m, 2H), 7.26–7.20 (m, 3H), 4.21–3.94 (m, 2H), 3.70–3.67 (m, 5H), 3.26–3.08 (m, 6H), 2.37–2.35 (m, 2H), 2.22–2.16 (m, 2H), 2.06–2.01 (m, 2H), 1.75–1.53 (m, 6H), 1.15–0.93 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.1, 76.5, 67.7, 59.9, 50.7, 46.2, 43.8, 28.2, 25.9 with three carbons not observed; MS (ESI) *m*/*z* (%): 485.2 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF

MS ES$^+$): calcd. $C_{23}H_{33}ClN_2O_5S$ $[M + H]^+$ 485.1872, found: 485.1869.

4-Chloro-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)butane-1-sulfonamide ***1h***. The compound was synthesized from benzyl ((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)carbamate (80 mg, 0.23 mmol, 1.0 eq.) and 4-chlorobutane-1-sulfonyl chloride (0.042 mL, 0.30 mmol, 1.3 eq.) following General procedure 4 to yield **1h** as a white foam (35 mg, 30%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.33 (m, 2H), 7.25–7.21 (m, 3H), 4.23–3.94 (m, 2H), 3.73–3.57 (m, 5H), 3.24–3.05 (m, 6H), 2.40–2.33 (m, 2H), 2.09–2.01 (m, 2H), 1.92–1.87 (m, 4H), 1.75–1.59 (m, 6H), 1.19–0.98 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.0, 76.5, 67.7, 59.9, 52.4, 46.2, 45.1, 39.4, 32.1, 25.9, 22.3 with two carbons not observed; MS (ESI) *m*/*z* (%): 499.6 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{35}ClN_2O_5S$ $[M + H]^+$ 499.2028, found: 499.2028.

N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)cyclopropanesulfonamide ***1i***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (150 mg, 1.1 mmol, 1.8 eq.) and cyclopropanesulfonyl chloride (0.15 mL, 1.5 mmol, 2.5 eq.) following General procedure 1 to yield **1i** as a white foam (0.15 g, 56%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.32 (m, 2H), 7.25–7.20 (m, 3H), 4.21–3.92 (m, 2H), 3.78–3.54 (m, 3H), 3.27–3.11 (m, 4H), 2.55–2.48 (m, 1H), 2.41–2.32 (m, 2H), 2.10–1.98 (m, 2H), 1.75–1.55 (m, 6H), 1.10–0.92 (m, 6H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.0, 76.4, 67.7, 59.9, 46.2, 39.2, 30.6, 25.9, 25.9, 5.5 with one carbon not observed; MS (ESI) *m*/*z* (%): 449.4 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{23}H_{32}N_2O_5S$ $[M + H]^+$ 449.2105, found: 499.2103.

1-Cyclopropyl-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)methanesulfonamide ***1j***. The compound was synthesized from intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (150 mg, 1.1 mmol, 2.0 eq.) and cyclopropylmethanesulfonyl chloride (0.11 mL, 1.4 mmol, 2.5 eq.) following General procedure 1 using to yield **1j** as a white foam (0.16 g, 64%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.39–7.31 (m, 2H), 7.28–7.20 (m, 3H), 4.22–3.91 (m, 2H), 3.78–3.54 (m, 3H), 3.29–3.07 (m, 4H), 3.01–2.91 (m, 2H), 2.43–2.30 (m, 2H), 2.11–1.96 (m, 2H), 1.78–1.56 (m, 6H), 1.15–0.93 (m, 3H), 0.68–0.58 (m, 2H), 0.41–0.29 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.0, 76.6, 67.7, 59.9, 58.2, 46.3, 45.3, 42.1, 39.2, 36.8, 35.3, 25.9, 6.2, 4.8; MS (ESI) *m*/*z* (%): 463.3 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{34}N_2O_5S$ $[M + H]^+$ 463.2261, found: 463.2258.

N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)cyclohexanesulfonamide ***1k***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (150 mg, 1.1 mmol, 1.9 eq.) and cyclohexane sulfonyl chloride (0.22 mL, 1.4 mmol, 2.4 eq.) following General procedure 1 using to yield **1k** as a white foam (0.15 g, 53%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.31 (m, 2H), 7.25–7.20 (m, 3H), 4.17–3.92 (m, 2H), 3.80–3.53 (m, 3H), 3.28–3.08 (m, 4H), 2.94–2.92 (m, 1H), 2.41–2.30 (m, 2H), 2.13–1.99 (m, 4H), 1.89–1.82 (m, 2H), 1.75–1.60 (m, 7H), 1.50–1.39 (m, 2H), 1.35–1.21 (m, 3H), 1.14–0.98 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.0, 76.7, 67.7, 62.0, 59.9, 46.4, 42.1, 27.6, 26.4, 26.2, 25.9 with two carbons not observed; MS (ESI) *m*/*z* (%): 491.7 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{38}N_2O_5S$ $[M + H]^+$ 491.2574, found: 491.2572.

(2-((1,1-Dioxidoisothiazolidin-2-yl)methyl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)(1-phenylcyclopentyl)methanone ***1l***. The compound was synthesized from (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (200 mg, 1.5 mmol, 1.0 eq.) and 3-chloropropane-1-sulfonyl chloride (0.20 mL, 1.7 mmol, 1.1 eq.) following General procedure 5 to yield **1l** as a white foam (0.35 g, 52%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.33 (m, 2H), 7.28–7.20 (m, 3H), 4.30–3.92 (m, 2H), 3.69–3.63 (m, 3H), 3.44–3.35 (m, 2H), 3.29–3.07 (m, 5H), 3.04–3.00 (m, 1H), 2.41–2.25 (m, 4H), 2.07–2.02 (m, 2H), 1.76–1.58 (m, 6H), 1.07–0.96 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.1, 76.0, 67.9, 59.9, 47.1, 45.3, 42.1, 39.3, 36.8, 35.3, 25.9, 20.0; MS (ESI) *m*/*z* (%): 449.4 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{23}H_{32}N_2O_5S$ $[M + H]^+$ 449.2105, found: 449.2101.

(2-((1,1-Dioxido-1,2-thiazinan-2-yl)methyl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)(1-phenylcyclopentyl)methanone ***1m***. The compound was synthesized from (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (100 mg, 0.76 mmol, 1.0 eq.) and 4-chlorobutane-1-sulfonyl chloride (0.12 mL, 0.84 mmol, 1.1 eq.) following General procedure 5 to yield **1m** as a white foam (0.11 g, 40%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.31 (m, 2H), 7.27–7.19 (m, 3H), 4.26–3.91 (m, 2H), 3.72–3.60 (m, 3H), 3.50–3.39 (m, 2H), 3.28–3.14 (m, 4H), 3.07–2.96 (m, 2H), 2.42–2.32 (m, 2H), 2.18–1.99 (m, 4H), 1.77–1.58 (m, 8H), 1.15–0.91 (m, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.0, 76.4, 67.7, 59.9, 52.6, 50.4, 45.3, 42.1, 39.3, 36.2, 25.9, 25.0, 21.8; MS (ESI) *m*/*z* (%): 463.04 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{34}N_2O_5S$ $[M + H]^+$ 463.2261, found: 463.2265.

4-Methyl-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)benzenesulfonamide ***2a***. The compound was synthesized from intermediate **II** (0.10 g, 0.37 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (100 mg, 0.76 mmol, 2.0 eq.) and *p*-toluene sulfonyl chloride (190 mg, 0.99 mmol, 2.7 eq.) following General procedure 1 to yield **2a** as a white foam (0.12 g, 67%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.70 (d, J = 7.8 Hz, 2H), 7.35–7.32 (m, 4H), 7.25–7.19 (m, 3H), 4.12–3.87 (m, 2H), 3.65–3.61 (m, 3H), 3.24–3.06 (m, 2H), 2.91–2.81 (m, 2H), 2.41 (s, 3H), 2.38–2.32 (m, 2H), 2.05–2.00 (m, 2H), 1.75–1.68 (m, 4H), 1.60–1.37 (m, 2H), 1.13–0.86 (m, 2H);

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.5, 145.6, 143.7, 136.6, 129.8, 128.8, 127.0, 126.3, 125.0, 108.3, 73.9, 66.2, 60.4, 58.5, 38.3, 25.2, 21.5, 14.2; MS (ESI) *m*/*z* (%): 499.2 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{27}H_{34}N_2O_5S$ $[M + H]^+$ 499.2261, found: 499.2258.

N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)-[1,1′-biphenyl]-4-sulfonamide ***2b***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (100 mg, 0.76 mmol, 1.3 eq.) and [1,1′-biphenyl]-4-sulfonyl chloride (270 mg, 1.1 mmol, 1.9 eq.) following General procedure 1 to yield **2b** as a white foam (0.23 g, 68%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.89 (d, J = 6.8 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 7.70–7.67 (m, 2H), 7.54–7.47 (m, 2H), 7.46–7.42 (m, 1H), 7.32–7.29 (m, 2H), 7.22–7.15 (m, 3H), 4.16–3.87 (m, 2H), 3.73–3.46 (m, 3H), 3.17–2.87 (m, 4H), 2.35–2.24 (m, 2H), 2.03–1.98 (m, 2H), 1.71–1.49 (m, 6H), 1.15–0.93 (m, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.6, 146.4, 140.6, 130.2, 129.9, 129.6, 128.6, 128.6, 128.3, 127.5, 126.2, 108.9, 75.9, 67.7, 59.9, 49.6, 46.4, 39.3, 25.9 with two carbons not observed; MS (ESI) *m*/*z* (%): 561.2 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{32}H_{36}N_2O_5S$ $[M + H]^+$ 561.2418, found: 561.2421.

2-Fluoro-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)benzenesulfonamide ***2c***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (150 mg, 1.1 mmol, 1.9 eq.) and 2-fluorobenzenesulfonyl chloride (190 mg, 0.99 mmol, 1.7 eq.) following General procedure 1 to yield **2c** as a white foam (0.20 g, 68%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.87–7.80 (m, 1H), 7.65–7.63 (m, 1H), 7.38–7.20 (m, 7H), 4.12–3.87 (m, 2H), 3.69–3.52 (m, 3H), 3.20–2.97 (m, 4H), 2.40–2.31 (m, 2H), 2.07–1.98 (m, 2H), 1.76–1.69 (m, 4H), 1.62–1.53 (m, 2H), 1.07–0.82 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 161.3 (d, J = 264.6 Hz), 146.5, 136.2 (d, J = 8.8 Hz), 131.1, 130.2, 130.1 (d, J = 13.9 Hz), 127.6, 126.2, 125.6 (d, J = 3.8 Hz), 118.2 (d, J = 21.4 Hz), 108.9, 75.9, 67.7, 59.9, 46.3, 45.1, 42.0, 39.3, 36.2, 34.6, 25.9; MS (ESI) *m*/*z* (%): 503.6 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{31}FN_2O_5S$ $[M + H]^+$ 503.2011, found: 503.2014.

3-Bromo-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)benzenesulfonamide ***2d***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (150 mg, 1.1 mmol, 1.9 eq.) and 3-bromobenzenesulfonyl chloride (250 mg, 0.99 mmol, 1.7 eq.) following General procedure 1 using to yield **2d** as a white foam (0.30 g, 90%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.98 (s, 1H), 7.84–7.72 (m, 2H), 7.46 (t, J = 7.9 Hz, 1H), 7.37–7.31 (m, 2H), 7.28–7.20 (m, 3H), 4.17–3.87 (m, 2H), 3.71–3.63 (m, 3H), 3.26–3.07 (m, 2H), 3.05–2.83 (m, 2H), 2.39–2.33 (m, 2H), 2.07–2.00 (m, 2H), 1.75–1.50 (m, 6H), 1.03–0.85 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 144.3, 136.5, 132.1, 130.7, 130.0, 127.6, 126.7, 126.2, 123.8, 109.0, 75.8, 67.6, 59.9, 46.3, 39.4, 25.9 with two carbons not observed; MS (ESI) *m*/*z* (%): 563.1 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{31}BrN_2O_5S$ $[M + H]^+$ 563.1210, found: 563.1209.

3-Nitro-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)benzenesulfonamide ***2e***. The compound was synthesized from intermediate **II** (0.22 g, 0.69 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (90 mg, 0.69 mmol, 1.0 eq.) and 3-nitrobenzenesulfonyl chloride (0.20 g, 0.89 mmol, 1.3 eq.) following General procedure 1 using to yield **2e** as a white foam (0.22 g, 58%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.64 (s, 1H), 8.45 (d, J = 7.6 Hz, 1H), 8.20 (d, J = 7.7 Hz, 1H), 7.88–7.75 (m, 1H), 7.37–7.30 (m, 2H), 7.25–7.20 (m, 3H), 4.17–3.87 (m, 2H), 3.73–3.52 (m, 3H), 3.21–2.93 (m, 4H), 2.38–2.30 (m, 2H), 2.07–1.99 (m, 2H), 1.74–1.68 (m, 4H), 1.63–1.46 (m, 2H), 1.06–0.87 (m, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 149.8, 146.5, 144.4, 133.6, 131.9, 130.0, 127.9, 127.6, 126.2, 122.9, 109.0, 75.9, 67.6, 59.9, 46.4, 39.3, 25.9 with two carbons not observed; MS (ESI) *m*/*z* (%): 530.2 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{31}N_3O_7S$ $[M + H]^+$ 530.1956, found: 530.1960.

N-Methyl-3-(N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)sulfamoyl)benzamide ***2f***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (100 mg, 0.76 mmol, 1.3 eq.) and 3-(methylcarbamoyl)benzenesulfonyl chloride (0.25 g, 1.1 mmol, 1.9 eq.) following General procedure 1 to yield **2f** as a white foam (0.20 g, 62%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.27 (s, 1H), 8.03–8.00 (m, 1H), 7.98–7.96 (m, 1H), 7.64 (t, J = 7.9 Hz, 1H), 7.35–7.32 (m, 2H), 7.23–7.21 (m, 3H), 4.07–3.94 (m, 2H), 3.64–3.61 (m, 3H), 3.21–3.08 (m, 2H), 3.01–2.98 (m, 1H), 2.95–2.92 (m, 4H), 2.39–2.30 (m, 2H), 2.05–2.01 (m, 2H), 1.76–1.70 (m, 4H), 1.60–1.53 (m, 2H), 1.07–0.96 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 168.8, 146.5, 142.9, 136.7, 131.9, 130.6, 129.9, 127.6, 126.8, 126.2, 108.9, 75.9, 67.7, 59.9, 46.3, 45.2, 42.1, 39.4, 36.7, 34.7, 27.0, 25.9; MS (ESI) *m*/*z* (%): 542.2 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{28}H_{35}N_3O_6S$ $[M + H]^+$ 542.2320, found: 542.2316.

4-Chloro-3-cyano-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)benzenesulfonamide ***2g***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (100 mg, 0.76 mmol, 1.3 eq.) and 4-chloro-3-cyanobenzenesulfonyl chloride (250 mg, 1.1 mmol, 1.9 eq.) following General procedure 1 to yield **2g** as a white foam (0.18 g, 56%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.25 (s, 1H), 8.06 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.35–7.32 (m, 2H), 7.24–7.20 (m, 3H), 4.15–3.89 (m, 2H), 3.71–3.54 (m, 3H), 3.21–2.91 (m, 4H), 2.38–2.33 (m, 2H), 2.10–1.98 (m, 2H), 1.74–1.51 (m, 6H), 1.08–0.86 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 142.6, 141.4, 133.8, 133.5, 132.2, 130.0, 127.6, 126.2, 115.7, 115.2, 109.0, 75.9, 67.5, 59.9, 46.3, 39.4, 26.0, 25.9 with two carbons not observed; MS (ESI) *m*/*z* (%): 544.2 $[M + H]^+$; HPLC purity (254 nm): >99%;

HRMS (TOF MS ES$^+$): calcd. for $C_{27}H_{30}ClN_3O_5S$ [M + H]$^+$ 544.1668, found: 554.1664.

2-Chloro-5-nitro-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)benzenesulfonamide ***2h***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (100 mg, 0.76 mmol, 1.3 eq.) and 2-chloro-5-nitrobenzenesulfonyl chloride (270 mg, 1.1 mmol, 1.9 eq.) following General procedure 1 to yield **2h** as a white foam (0.25 g, 75%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.78 (s, 1H), 8.39 (dd, J = 8.9, 2.7 Hz, 1H), 7.82 (dd, J = 8.7 Hz, 1H), 7.38–7.31 (m, 2H), 7.27–7.19 (m, 3H), 4.06–3.99 (m, 2H), 3.63–3.58 (m, 3H), 3.20–2.98 (m, 4H), 2.40–2.28 (m, 2H), 2.07–1.98 (m, 2H), 1.72 (m, 4H), 1.50–1.40 (m, 2H), 0.92–0.90 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 147.7, 146.5, 141.6, 139.5, 134.4, 130.0, 128.9, 127.6, 126.7, 126.2, 109.0, 76.0, 67.6, 59.9, 46.6, 39.5, 39.2, 26.0 with one carbon not observed; MS (ESI) m/z (%): 564.2 [M + H]$^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{30}ClN_3O_7S$ [M + H]$^+$ 564.1566, found: 564.1566.

2-Methyl-5-nitro-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)benzenesulfonamide ***2i***. The compound was synthesized from intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (90 mg, 0.69 mmol, 1.3 eq.) and 2-methyl-5-nitrobenzenesulfonyl chloride (0.19 g, 0.82 mmol, 1.5 eq.) following General procedure 1 to yield **2i** as a white foam (0.12 g, 40%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.68 (s, 1H), 8.30 (d, J = 7.3 Hz, 1H), 7.59 (d, J = 8.3 Hz, 1H), 7.38–7.31 (m, 2H), 7.26–7.20 (m, 3H), 4.12–3.87 (m, 2H), 3.74–3.51 (m, 3H), 3.18–2.93 (m, 4H), 2.73 (s, 3H), 2.39–2.30 (m, 2H), 2.06–1.97 (m, 2H), 1.76–1.68 (m, 4H), 1.57–1.40 (m, 2H), 1.04–0.89 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 147.3, 146.5, 146.2, 142.2, 135.0, 130.0, 127.7, 127.6, 126.2, 124.7, 109.0, 75.9, 67.6, 59.9, 46.4, 41.9, 39.4, 26.0, 20.5 with one carbon not observed; MS (ESI) m/z (%): 544.3 [M + H]$^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{27}H_{33}N_3O_7S$ [M + H]$^+$ 544.2112, found: 544.2111.

5-Bromo-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)thiophene-2-sulfonamide ***2j***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (100 mg, 0.76 mmol, 1.3 eq.) and 5-bromothiophene-2-sulfonyl chloride (280 mg, 1.1 mmol, 1.9 eq.) following General procedure 1 using to yield **2j** as a white foam (0.28 g, 83%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.31 (m, 3H), 7.26–7.20 (m, 3H), 7.20–7.14 (m, 1H), 4.19–3.90 (m, 2H), 3.75–3.51 (m, 3H), 3.30–2.93 (m, 4H), 2.41–2.32 (m, 2H), 2.08–1.99 (m, 2H), 1.75–1.47 (m, 6H), 1.16–1.00 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 144.3, 133.2, 132.0, 130.0, 127.6, 126.2, 120.0, 109.0, 75.8, 67.7, 59.9, 46.5, 45.2, 39.4, 25.9 with one carbon not observed; MS (ESI) m/z (%): 571.0 [M + H]$^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{29}BrN_2O_5S_2$ [M + H]$^+$ 569.0774, found: 569.0770.

2-[(Dimethylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***3a***. The compound was synthesized from intermediate **II** (0.31 g, 1.1 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (250 mg, 1.9 mmol, 1.7 eq.) and dimethylsulfamoyl chloride (0.27 mL, 2.5 mmol, 2.3 eq.) following General procedure 1 using to yield **3a** as a white foam (0.32 g, 48%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.35–7.32 (m, 2H), 7.26–7.19 (m, 3H), 4.21–3.91 (m, 2H), 3.80–3.52 (m, 3H), 3.30–3.00 (m, 4H), 2.73 (s, 6H), 2.41–2.29 (m, 2H), 2.12–1.95 (m, 2H), 1.76–1.51 (m, 6H), 1.18–0.90 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 76.0, 67.8, 59.9, 46.7, 45.3, 42.1, 39.3, 38.3, 36.3, 34.7, 25.9; MS (ESI) m/z (%): 452.3 [M + H]$^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{22}H_{33}N_3O_5S$ [M + H]$^+$ 452.2214, found: 452.2208.

{2-[(Ethylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***3b***. The compound was synthesized from intermediate **II** (0.12 g, 0.44 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (80 mg, 0.61 mmol, 1.4 eq.) and ethylsulfamoyl chloride (0.081 mL, 0.79 mmol, 1.8 eq.) following General procedure 1 to yield **3b** as a white foam (0.10 g, 51%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.31 (m, 2H), 7.25–7.21 (m, 3H), 4.23–3.93 (m, 2H), 3.79–3.57 (m, 3H), 3.28–3.11 (m, 2H), 3.03–2.93 (m, 4H), 2.41–2.33 (m, 2H), 2.09–1.99 (m, 2H), 1.75–1.57 (m, 6H), 1.17–0.98 (m, 5H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 76.0, 68.0, 59.9, 46.3, 39.3, 38.7, 25.9, 15.2 with two carbons not observed; MS (ESI) m/z (%): 452.1 [M + H]$^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{22}H_{33}N_3O_5S$ [M + H]$^+$ 452.2214, found: 452.2211.

{2-[(N-Ethyl-N-methylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***3c***. The compound was synthesized from intermediate **II** (0.12 g, 0.44 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (80 mg, 0.61 mmol, 1.4 eq.) and ethyl(methyl)sulfamoyl chloride (0.098 mL, 0.79 mmol, 1.8 eq.) following General procedure 1 to yield **3c** as a white foam (0.12 g, 59%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.30 (m, 2H), 7.28–7.19 (m, 3H), 4.22–3.90 (m, 2H), 3.81–3.57 (m, 3H), 3.27–3.08 (m, 4H), 3.05–2.96 (m, 2H), 2.74 (s, 3H), 2.40–2.33 (m, 2H), 2.09–1.99 (m, 2H), 1.75–1.54 (m, 6H), 1.18–0.98 (m, 5H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 129.9, 127.6, 126.2, 108.9, 76.0, 67.9, 59.9, 46.5, 46.2, 45.3, 42.1, 39.3, 34.4, 25.9, 13.2; MS (ESI) m/z (%): 466.5 [M + H]$^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{23}H_{35}N_3O_5S$ [M + H]$^+$ 466.2370, found: 466.2376.

{2-[(Diethylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***3d***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (100 mg, 0.76 mmol, 1.3 eq.) and diethylsulfamoyl chloride (18 mg, 1.1 mmol, 1.9 eq.) following General procedure 1 to yield **3d** as a white foam (0.19 g, 68%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.31 (m, 2H), 7.27–7.19 (m, 3H), 4.21–3.93 (m, 2H), 3.82–3.52 (m, 3H), 3.29–3.14 (m, 6H), 3.02–2.91 (m, 2H), 2.42–2.30 (m, 2H), 2.05–2.01 (m, 2H), 1.77–1.55 (m, 6H),

1.18–0.99 (m, 8H); ^{13}C NMR (126 MHz, CD_3OD) δ 175.2, 145.1, 128.6, 126.2, 124.8, 107.5, 74.5, 66.6, 58.5, 48.2, 45.0, 43.4, 41.4, 38.0, 24.5, 12.7; MS (ESI) *m*/*z* (%): 480.2 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{37}N_3O_5S$ $[M + H]^+$ 480.2527, found: 480.2527.

{2-{[1-(3-Oxabutyl)-4-oxa-1-azapentylsulfonylamino]methyl}-1,4-dioxa-8-aza-8-spiro[4.5]decyl)(1-phenylcyclopentyl)methanone ***3e***. The compound was synthesized from intermediate **II** (0.14 g, 0.52 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (90 mg, 0.69 mmol, 1.3 eq.) and bis(2-methoxyethyl)sulfamoyl chloride (0.21 g, 0.89 mmol, 1.7 eq.) following General procedure 1 to yield **3e** as a colorless oil (0.15 g, 55%). ^{1}H NMR (500 MHz, DMSO) δ 7.36–7.31 (m, 2H), 7.23–7.17 (m, 3H), 4.11–3.87 (m, 2H), 3.71–3.51 (m, 2H), 3.42 (t, J = 6.0 Hz, 5H), 3.27–3.22 (m, 10H), 3.12–2.78 (m, 4H), 2.39–2.18 (m, 2H), 2.04–1.80 (m, 2H), 1.67–1.31 (m, 6H), 1.06–0.73 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 129.9, 127.6, 126.2, 108.9, 75.9, 72.1, 67.9, 59.9, 59.0, 46.3, 45.3, 42.0, 39.5, 36.3, 34.7, 25.9; MS (ESI) *m*/*z* (%): 540.6 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{41}N_3O_7S$ $[M + H]^+$ 540.2738, found: 540.2734.

{2-[(Isobutylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***3f***. The compound was synthesized from intermediate **II** (0.12 g, 0.44 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (80 mg, 0.61 mmol, 1.4 eq.) and isobutylsulfamoyl chloride (0.14 g, 0.79 mmol, 1.8 eq.) following General procedure 1 to yield **3f** as a white foam (99 mg, 47%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.30 (m, 2H), 7.27–7.19 (m, 3H), 4.24–3.91 (m, 2H), 3.80–3.52 (m, 3H), 3.30–3.08 (m, 2H), 3.06–3.01 (m, 2H), 2.77–2.69 (m, 2H), 2.42–2.32 (m, 2H), 2.09–1.94 (m, 2H), 1.81–1.52 (m, 7H), 1.15–0.94 (m, 2H), 0.92 (d, J = 6.7 Hz, 6H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 129.9, 127.6, 126.2, 108.9, 76.0, 68.0, 59.9, 51.4, 46.2, 45.3, 42.1, 36.9, 35.3, 29.6, 25.9, 20.5; MS (ESI) *m*/*z* (%): 480.2 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{37}N_3O_5S$ $[M + H]^+$ 480.2527, found: 480.2523.

{2-[(N-Isopropyl-N-methylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***3g***. The compound was synthesized from intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (90 mg, 0.69 mmol, 1.3 eq.) and isopropyl(methyl)sulfamoyl chloride (0.11 mL, 0.82 mmol, 1.5 eq.) following General procedure 1 to yield ***3g*** as a white foam (0.20 g, 75%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.30 (m, 2H), 7.27–7.20 (m, 3H), 4.23–3.93 (m, 3H), 3.81–3.52 (m, 3H), 3.30–3.05 (m, 2H), 3.01–2.89 (m, 2H), 2.64 (s, 3H), 2.43–2.29 (m, 2H), 2.10–1.96 (m, 2H), 1.75–1.51 (m, 6H), 1.15 (d, J = 6.7 Hz, 6H), 1.12–0.90 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 129.9, 127.6, 126.2, 108.9, 75.9, 67.9, 59.9, 50.2, 46.4, 45.3, 42.1, 39.3, 36.3, 35.3, 27.8, 25.9, 20.1;MS (ESI) *m*/*z* (%): 480.3 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{37}N_3O_5S$ $[M + H]^+$ 480.2527, found: 480.2531.

{2-[(Cyclohexylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***3h***. The compound was synthesized from intermediate **II** (0.12 g, 0.44 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (80 mg, 0.61 mmol, 1.4 eq.) and cyclohexylsulfamoyl chloride (0.12 mL, 0.79 mmol, 1.8 eq.) following General procedure 1 to yield **3g** as a white foam (0.11 g, 49%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.31 (m, 2H), 7.27–7.19 (m, 3H), 4.25–3.91 (m, 2H), 3.81–3.54 (m, 3H), 3.30–2.94 (m, 5H), 2.44–2.30 (m, 2H), 2.10–1.91 (m, 4H), 1.79–1.54 (m, 9H), 1.37–0.97 (m, 8H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 76.0, 68.0, 59.9, 53.7, 46.4, 35.0, 26.5, 26.3, 25.9 with three carbons not observed; MS (ESI) *m*/*z* (%): 506.4 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{39}N_3O_5S$ $[M + H]^+$ 506.2683, found: 506.2683.

{2-[(N-Cyclohexyl-N-methylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***3i***. The compound was synthesized from intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (100 mg, 0.76 mmol, 1.4 eq.) and cyclohexyl(methyl)sulfamoyl chloride (210 mg, 0.99 mmol, 1.8 eq.) following General procedure 1 to yield **3i** as a white foam (0.20 g, 70%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.31 (m, 2H), 7.26–7.20 (m, 3H), 4.22–3.92 (m, 2H), 3.83–3.51 (m, 4H), 3.28–3.10 (m, 2H), 3.00–2.89 (m, 2H), 2.68 (s, 3H), 2.42–2.29 (m, 2H), 2.11–1.97 (m, 2H), 1.84–1.59 (m, 11H), 1.54–1.42 (m, 2H), 1.40–1.26 (m, 2H), 1.17–0.93 (m, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 75.9, 67.9, 59.9, 58.5, 46.4, 31.5, 29.3, 27.0, 26.5, 25.9 with three carbons not observed; MS (ESI) *m*/*z* (%): 520.5 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{27}H_{41}N_3O_5S$ $[M + H]^+$ 520.2840, found: 520.2839.

N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)pyrrolidine-1-sulfonamide ***3j***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (100 mg, 0.76 mmol, 1.4 eq.) and pyrrolidine-1-sulfonyl chloride (0.12 mL, 1.1 mmol, 1.9 eq.) following General procedure 1 to yield **3j** as a white foam (0.12 g, 44%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.32 (m, 2H), 7.25–7.20 (m, 3H), 4.23–3.91 (m, 2H), 3.81–3.53 (m, 3H), 3.27–3.14 (m, 6H), 3.12–2.98 (m, 2H), 2.43–2.29 (m, 2H), 2.11–1.98 (m, 2H), 1.92–1.87 (m, 4H), 1.78–1.53 (m, 6H), 1.15–0.90 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 76.1, 67.9, 59.9, 46.6, 45.3, 39.4, 26.5, 25.9 with two carbons not observed; MS (ESI) *m*/*z* (%): 478.4 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{35}N_3O_5S$ $[M + H]^+$ 478.2370, found: 478.2374.

N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide ***3k***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (100 mg, 0.76 mmol, 1.4 eq.) and piperidine-1-sulfonyl chloride (0.15 mL, 1.1 mmol, 1.9 eq.) following General procedure 1 to yield **3k** as a white foam (0.18 g, 61%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.31 (m, 2H), 7.25–7.21 (m,

3H), 4.22–3.92 (m, 2H), 3.78–3.59 (m, 3H), 3.25–3.02 (m, 8H), 2.41–2.32 (m, 2H), 2.09–2.00 (m, 2H), 1.76–1.71 (m, 4H), 1.66–1.53 (m, 8H), 1.15–0.92 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 76.0, 67.9, 59.9, 47.9, 46.7, 26.4, 25.9, 24.8; MS (ESI) *m*/*z* (%): 492.1 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{25}H_{37}N_3O_5S$ $[M + H]^+$ 492.2527, found: 492.2525.

N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)azepane-1-sulfonamide ***3l***. The compound was synthesized from intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (90 mg, 0.69 mmol, 1.3 eq.) and azepane-1-sulfonyl chloride (0.16 g, 0.82 mmol, 1.5 eq.) following General procedure 1 to yield **3l** as a white foam (0.19 g, 68%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.32 (m, 2H), 7.27–7.21 (m, 3H), 4.21–3.90 (m, 2H), 3.81–3.57 (m, 3H), 3.30–3.11 (m, 4H), 3.05–2.91 (m, 2H), 2.41–2.30 (m, 2H), 2.10–1.99 (m, 2H), 1.80–1.54 (m, 16H), 1.17–0.94 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 76.0, 68.0, 46.3, 45.3, 42.1, 39.3, 36.2, 34.7, 30.3, 28.0, 25.9; MS (ESI) *m*/*z* (%): 506.6 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{39}N_3O_5S$ $[M + H]^+$ 506.2683, found: 506.2683.

N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)morpholine-4-sulfonamide ***3m***. The compound was synthesized from intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.), (2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (100 mg, 0.76 mmol, 1.4 eq.) and morpholine-4-sulfonyl chloride (200 mg, 1.1 mmol, 1.9 eq.) following General procedure 1 to yield **3m** as a white foam (0.20 g, 69%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.31 (m, 2H), 7.26–7.21 (m, 3H), 4.22–3.95 (m, 2H), 3.74–3.62 (m, 7H), 3.25–3.06 (m, 8H), 2.41–2.33 (m, 2H), 2.09–1.98 (m, 2H), 1.75–1.56 (m, 6H), 1.15–0.92 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.0, 76.0, 67.8, 67.3, 59.9, 47.3, 46.8, 39.2, 25.9 with two carbons not observed; MS (ESI) *m*/*z* (%): 494.1 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{35}N_3O_6S$ $[M + H]^+$ 494.2320, found: 494.2317.

N-Methyl-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide ***3n***. The compound was synthesized from **3k** (180 mg, 0.36 mmol, 1.0 eq.) and iodomethane (0.056 mL, 0.90 mmol, 2.5 eq.) following General procedure 3 to yield **3n** as a colorless oil (0.12 g, 68%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.31 (m, 2H), 7.26–7.21 (m, 3H), 4.30–3.94 (m, 2H), 3.78–3.55 (m, 3H), 3.24–3.11 (m, 7H), 2.84 (s, 3H), 2.41–2.32 (m, 2H), 2.09–2.00 (m, 2H), 1.74–1.51 (m, 13H), 1.16–0.91 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.0, 76.1, 67.9, 59.9, 54.2, 48.1, 45.3, 42.1, 39.3, 37.1, 26.6, 25.9, 24.8; MS (ESI) *m*/*z* (%): 506.2 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{39}N_3O_5S$ $[M + H]^+$ 506.2683, found: 506.2682.

N-Cyano-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide ***3o***. The compound was synthesized from **3k** (30 mg, 0.061 mmol, 1.0 eq.) and cyanogen bromide (13 mg, 0.12 mmol, 2.0 eq.) following General procedure 3 to yield **3o** as a white solid (15 mg, 48%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.32 (m, 2H), 7.26–7.21 (m, 3H), 4.45–3.96 (m, 2H), 3.87–3.44 (m, 4H), 3.41–3.39 (m, 5H), 3.30–3.08 (m, 2H), 2.44–2.30 (m, 2H), 2.14–1.95 (m, 2H), 1.77–1.57 (m, 12H), 1.16–0.92 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.4, 130.0, 127.6, 126.2, 111.4, 109.7, 74.9, 67.1, 59.9, 54.4, 45.2, 42.1, 39.5, 36.1, 26.3, 26.0, 24.3; MS (ESI) *m*/*z* (%): 517.2 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{36}N_4O_5S$ $[M + H]^+$ 517.2479, found: 517.2482.

N-Hexyl-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide ***3p***. The compound was synthesized from **3k** (100 mg, 0.020 mmol, 1.0 eq.) and 1-iodohexane (0.075 mL, 0.51 mmol, 2.5 eq.) following General procedure 3 to yield **3p** as a colorless oil (45 mg, 28%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.30 (m, 2H), 7.27–7.20 (m, 3H), 4.33–3.95 (m, 2H), 3.82–3.53 (m, 3H), 3.42–3.31 (m, 2H), 3.30–3.06 (m, 8H), 2.45–2.29 (m, 2H), 2.11–1.95 (m, 2H), 1.78–1.52 (m, 14H), 1.34–1.25 (m, 6H), 1.16–0.97 (m, 2H), 0.91 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.1, 76.4, 68.2, 59.9, 51.9, 50.6, 48.0, 45.4, 42.1, 39.2, 34.8, 32.7, 29.1, 27.5, 26.5, 26.0, 24.8, 23.7, 14.4; MS (ESI) *m*/*z* (%): 576.4 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{31}H_{49}N_3O_5S$ $[M + H]^+$ 576.3466, found: 576.3465.

N-(2-(Dimethylamino)ethyl)-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide ***3q***. The compound was synthesized from **3k** (35 mg, 0.71 mmol, 1.0 eq.) and 2-bromo-*N*,*N*-dimethylethylamine hydrobromide (20 mg, 0.085 mmol, 1.2 eq.) following General procedure 3 to yield **3q** as a white solid (19 mg, 47%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.30 (m, 2H), 7.27–7.20 (m, 3H), 4.40–4.17 (m, 1H), 4.12–3.79 (m, 2H), 3.66–3.43 (m, 5H), 3.25–2.99 (m, 9H), 2.66 (s, 6H), 2.48–2.25 (m, 2H), 2.21–1.89 (m, 2H), 1.78–1.56 (m, 12H), 1.33–0.87 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.2, 76.6, 68.1, 59.9, 58.4, 52.7, 48.0, 45.7, 45.4, 42.1, 39.8, 36.9, 35.4, 26.5, 25.9, 24.8; MS (ESI) *m*/*z* (%): 563.6 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{29}H_{46}N_4O_5S$ $[M + H]^+$ 563.3262, found: 563.3264.

(2-((1,1-Dioxido-1,2,6-thiadiazinan-2-yl)methyl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)(1-phenylcyclopentyl)methanone ***3r***. The compound was synthesized from 2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (150 mg, 1.1 mmol, 1.0 eq.) and (3-chloropropyl)sulfamoyl chloride (0.16 mL, 1.3 mmol, 1.1 eq.) following General procedure 5 to yield **3r** as a white solid (0.10 g, 45%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.30 (m, 2H), 7.27–7.19 (m, 3H), 4.29–3.90 (m, 2H), 3.84–3.51 (m, 3H), 3.49–3.35 (m, 4H), 3.28–2.98 (m, 4H), 2.43–2.31 (m, 2H), 2.11–1.97 (m, 2H), 1.79–1.48 (m, 8H), 1.18–0.89 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.5, 146.4, 129.9, 127.5, 126.2, 108.8, 76.0, 67.9, 59.9, 53.0, 51.5, 45.9, 45.2, 42.1, 39.3, 36.8, 34.8, 25.9, 23.5; MS (ESI) *m*/*z* (%): 464.4 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{23}H_{33}N_3O_5S$ $[M + H]^+$ 464.2214, found: 464.2212.

(2-((6-Methyl-1,1-dioxido-1,2,6-thiadiazinan-2-yl)methyl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)(1-phenylcyclopentyl)methanone ***3s***. The compound was synthesizing from **3r** (20 mg, 0.043 mmol, 1.0 eq.) and iodomethane (0.0081 mL, 0.13 mmol, 3.0 eq.) following General procedure 3 to yield **3s** as a white solid (14 mg, 67%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.32 (m, 2H), 7.27–7.20 (m, 3H), 4.30–3.91 (m, 2H), 3.78–3.42 (m, 5H), 3.29–3.06 (m, 4H), 2.71 (s, 3H), 2.41–2.31 (m, 2H), 2.08–2.00 (m, 2H), 1.89–1.39 (m, 9H), 1.33–1.27 (m, 1H), 1.11–0.96 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 76.2, 67.8, 59.9, 53.6, 52.6, 51.6, 45.2, 42.1, 39.3, 37.0, 30.7, 25.9, 20.4; MS (ESI) *m*/*z* (%): 478.2 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{35}N_3O_5S$ $[M + H]^+$ 478.2370, found: 478.2369.

(2-((6-Hexyl-1,1-dioxido-1,2,6-thiadiazinan-2-yl)methyl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)(1-phenylcyclopentyl)methanone ***3t***. The compound was synthesizing from **3r** (20 mg, 0.043 mmol, 1.0 eq.) and iodohexane (0.019 mL, 0.13 mmol, 3.0 eq.) following General procedure 3 to yield **3t** as a white solid (10 mg, 42%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.32 (m, 2H), 7.26–7.20 (m, 3H), 4.28–3.93 (m, 2H), 3.81–3.42 (m, 5H), 3.29–2.97 (m, 6H), 2.41–2.31 (m, 2H), 2.10–2.00 (m, 2H), 1.82–1.70 (m, 6H), 1.67–1.48 (m, 4H), 1.37–1.26 (m, 8H), 1.12–0.97 (m, 2H), 0.92–0.87 (m, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 76.2, 67.8, 59.9, 52.6, 51.7, 50.5, 39.3, 32.5, 30.7, 28.9, 27.4, 25.9, 23.6, 20.8, 14.4 with two carbons not observed; MS (ESI) *m*/*z* (%): 548.2 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{29}H_{45}N_3O_5S$ $[M + H]^+$ 548.3153, found: 548.3151.

4-Methyl-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine 1-sulfonamide ***4a***. The compound was synthesized from intermediate **II** (0.13 g, 0.48 mmol, 1.0 eq.), 2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (90 mg, 0.69 mmol, 1.4 eq.) and 4-methylpiperidine-1-sulfonyl chloride (0.13 mL, 0.89 mmol, 1.9 eq.) following General procedure 1 to yield **4a** as a white foam (0.11 g, 45%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.31 (m, 2H), 7.26–7.20 (m, 3H), 4.14–3.99 (m, 2H), 3.81–3.53 (m, 5H), 3.22–3.04 (m, 4H), 2.73–2.62 (m, 2H), 2.38–2.35 (m, 2H), 2.11–1.98 (m, 2H), 1.77–1.54 (m, 8H), 1.52–1.41 (m, 1H), 1.24–0.99 (m, 4H), 0.95 (d, J = 6.6 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 76.0, 67.9, 59.9, 47.4, 46.7, 45.3, 42.2, 39.4, 34.7, 31.6, 25.9, 22.0; MS (ESI) *m*/*z* (%): 506.4 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{39}N_3O_5S$ $[M + H]^+$ 506.2683, found: 506.2684.

4-Cyano-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide ***4b***. The compound was synthesized from intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.), 2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (90 mg, 0.69 mmol, 1.3 eq.) and 4-cyanopiperidine-1-sulfonyl chloride (0.17 g, 0.82 mmol, 1.5 eq.) following General procedure 1 to yield **4b** as a white foam (0.15 g, 52%). ^{1}H NMR (500 MHz, MeOfD) δ 7.37–7.31 (m, 2H), 7.27–7.20 (m, 3H), 4.22–3.93 (m, 2H), 3.68–3.61 (m, 3H), 3.39–3.31 (m, 2H), 3.30–3.01 (m, 6H), 2.97–2.87 (m, 1H), 2.42–2.32 (m, 2H), 2.13–1.94 (m, 4H), 1.89–1.79 (m, 2H), 1.76–1.55 (m, 6H), 1.08–1.04 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 122.3, 109.0, 76.0, 67.8, 59.9, 46.7, 45.3, 39.2, 29.3, 26.6, 25.9; MS (ESI) *m*/*z* (%): 517.2 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{36}N_4O_5S$ $[M + H]^+$ 517.2479, found: 517.2481.

4-Phenyl-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide ***4c***. The compound was synthesized from 2,2-dimethyl-1,3-dioxolan-4-yl)methanamine 1.0 g, 7.6 mmol, 1.0 eq.) and 4-phenylpiperidine (1.23 g, 7.6 mmol, 1.0 eq.) following General procedure 2 to yield **4c** as a white foam (80 mg, 1.9%). ^{1}H NMR (400 MHz, DMSO) δ 7.37–7.26 (m, 5H), 7.27–7.17 (m, 6H), 7.03 (s, 1H), 4.16–4.05 (m, 1H), 4.01–3.92 (m, 1H), 3.72–3.60 (m, 3H), 3.53–3.15 (m, 4H), 3.05–2.93 (m, 3H), 2.84–2.72 (m, 2H), 2.65–2.61 (m, 1H), 2.39–2.27 (m, 2H), 1.98–1.80 (m, 4H), 1.71–1.60 (m, 6H), 1.33–1.23 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 173.0, 145.4, 128.6, 128.4, 126.6, 126.2, 124.8, 107.2, 74.1, 66.3, 57.9, 46.1, 45.4, 40.9, 38.0, 34.8, 33.8, 32.2, 24.8 with two carbons not observed; MS (ESI) *m*/*z* (%): 568.5 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{31}H_{41}N_3O_5S$ $[M + H]^+$ 568.2840, found: 568.2834.

3,3-Difluoro-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide ***4d***. The compound was synthesized from intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.), 2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (90 mg, 0.69 mmol, 1.3 eq.) and 3,3-difluoropiperidine-1-sulfonyl chloride (0.18 g, 0.82 mmol, 1.5 eq.) following General procedure 1 to yield **4d** as a white foam (0.17 g, 58%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.30 (m, 2H), 7.27–7.19 (m, 3H), 4.23–3.92 (m, 2H), 3.67–3.55 (m, 3H), 3.39–3.31 (m, 2H), 3.28–3.12 (m, 4H), 3.04 (s, 2H), 2.43–2.31 (m, 2H), 2.08–1.91 (m, 4H), 1.83–1.55 (m, 8H), 1.16–0.90 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 122.5, 120.6, 118.7, 109.0, 75.9, 67.8, 59.9, 52.1 (t, J = 30.5 Hz), 46.6, 46.1, 45.2, 42.0, 35.3, 32.7 (t, J = 22.7 Hz), 25.9, 22.8 (t, J = 5 Hz). MS (ESI) *m*/*z* (%): 528.2 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{25}H_{35}F_2N_3O_5S$ $[M + H]^+$ 528.2338, found: 528.2342.

{2-[(Benzylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***5a***. The compound was synthesized from intermediate **II** (0.12 g, 0.44 mmol, 1.0 eq.), 2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (80 mg, 0.61 mmol, 1.4 eq.) and benzylsulfamoyl chloride (0.12 mL, 0.79 mmol, 1.3 eq.) following General procedure 1 to yield **5a** as a white foam (0.18 g, 81%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.32 (m, 6H), 7.30–7.23 (m, 4H), 4.11–3.98 (m, 4H), 3.80–3.53 (m, 3H), 3.28–3.07 (m, 2H), 3.02–2.92 (m, 2H), 2.42–2.33 (m, 2H), 2.04–2.03 (m, 2H), 1.76–1.56 (m, 6H), 1.16–0.95 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 182.5, 154.8, 147.8, 138.2, 137.7, 137.1, 136.5, 135.6, 134.3, 116.6, 83.4, 76.0, 67.4, 55.2, 54.2, 34.3 with two carbons not observed; MS (ESI) *m*/*z* (%): 514.5 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{27}H_{35}N_3O_5S$ $[M + H]^+$ 514.2370, found: 514.2373.

{2-[(N-Benzyl-N-methylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***5b***. The compound was synthesized from intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.), 2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (100 mg, 0.76 mmol, 1.4 eq.) and benzyl(methyl)sulfamoyl chloride (0.16 mL, 0.99 mmol, 1.8 eq.) following General procedure 1 to yield **5b** as a white foam (0.15 g, 50%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.27 (m, 7H), 7.26–7.19 (m, 3H), 4.25–3.99 (m, 4H), 3.79–3.54 (m, 3H), 3.30–2.99 (m, 4H), 2.62 (s, 3H), 2.42–2.30 (m, 2H), 2.12–1.98 (m, 2H), 1.76–1.50 (m, 6H), 1.15–0.87 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 138.1, 130.0, 129.6, 129.5, 128.8, 127.6, 126.2, 108.9, 76.0, 67.9, 59.9, 55.2, 46.5, 45.2, 42.0, 39.3, 36.9, 35.3, 34.8, 25.9; MS (ESI) *m/z* (%): 528.4 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{28}H_{37}N_3O_5S$ $[M + H]^+$ 528.2527, found: 528.2528.

{2-[(N-Benzyl-N-ethylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***5c***. The compound was synthesized from (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (0.97 g, 7.4 mmol, 1.0 eq.) and *N*-benzylethanamine (1.0 g, 7.4 mmol, 1.0 eq.) following General procedure 2 using to yield **5c** as a white foam (0.17 g, 34%). ^{1}H NMR (400 MHz, DMSO) δ 7.42–7.24 (m, 7H), 7.24–7.16 (m, 3H), 4.32–4.24 (m, 2H), 4.12–4.02 (m, 1H), 3.98–3.88 (m, 1H), 3.66–3.58 (m, 1H), 3.42–3.29 (m, 4H), 3.13–3.01 (m, 2H), 2.98–2.84 (m, 2H), 2.35–2.27 (m, 2H), 1.99–1.86 (m, 2H), 1.70–1.54 (m, 4H), 1.35–1.11 (m, 4H), 1.01–0.89 (m, 3H); ^{13}C NMR (101 MHz, DMSO) δ 173.0, 145.3, 137.4, 128.7, 128.4, 128.0, 127.3, 126.1, 124.8, 107.2, 74.0, 66.3, 57.9, 49.9, 45.0, 43.4, 41.6, 34.9, 24.8, 12.8 with one carbon not observed; MS (ESI) *m/z* (%): 542.4 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{29}H_{39}N_3O_5S$ $[M + H]^+$ 542.2683, found: 542.2680.

{2-[(N-Benzyl-N-cyclopropylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***5d***. The compound was synthesized from (2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (0.89 g, 6.8 mmol, 1.0 eq.) and *N*-benzylcyclopropanamine (1.0 g, 6.8 mmol, 1.0 eq.) following General procedure 2 to yield **5d** as a white foam (40 mg, 16%). ^{1}H NMR (400 MHz, DMSO) δ 7.37–7.18 (m, 10H), 4.30–4.25 (m, 2H), 4.11–4.00 (m, 1H), 3.97–3.86 (m, 1H), 3.66–3.56 (m, 1H), 3.32–3.29 (m, 4H), 2.95–2.87 (m, 2H), 2.37–2.28 (m, 3H), 1.97–1.85 (m, 2H), 1.67–1.57 (m, 4H), 1.29–1.14 (m, 4H), 0.59–0.49 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 173.0, 145.4, 137.8, 128.7, 128.3, 128.2, 127.2, 126.2, 124.8, 107.2, 74.1, 66.3, 57.9, 53.9, 45.1, 29.9, 24.8, 6.9 with three carbons not observed; MS (ESI) *m/z* (%): 554.5 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{30}H_{39}N_3O_5S$ $[M + H]^+$ 554.2683, found: 554.2687.

(2-{[N-Methyl(N-benzyl-N-methylaminosulfonyl)amino]methyl}-1,4-dioxa-8-aza-8-spiro[4.5]decyl)(1-phenylcyclopentyl)methanone ***5e***. The compound was synthesized from **5b** (0.14 g, 0.26 mmol, 1.0 eq.) and iodomethane (0.041 mL, 0.65 mmol, 2.5 eq.) following General procedure 3 to yield **5e** as a white foam (56 mg, 40%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.27 (m, 7H), 7.25–7.19 (m, 3H), 4.34–4.19 (m, 3H), 4.07–3.94 (m, 1H), 3.76–3.48 (m, 3H), 3.30–3.08 (m, 4H), 2.87 (s, 3H), 2.64 (s, 3H), 2.41–2.30 (m, 2H), 2.08–1.98 (m, 2H), 1.77–1.51 (m, 6H), 1.25–0.89 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.4, 137.9, 129.9, 129.7, 129.4, 128.9, 127.6, 126.2, 109.1, 75.9, 67.9, 59.9, 55.3, 54.2, 45.3, 42.1, 39.3, 36.8, 36.2, 35.4, 34.9, 25.9; MS (ESI) *m/z* (%): 542.4 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{29}H_{39}N_3O_5S$ $[M + H]^+$ 542.2683, found: 542.2692.

2-{[N-cyano(N-benzyl-N-methylaminosulfonyl)amino]methyl}-8-[(1-phenylcyclopentyl)carbonyl]-1,4-dioxa-8-azaspiro[4.5]decane ***5f***. The compound was synthesized from **5b** (0.047 g, 0.089 mmol, 1 eq.) and cyanogen bromide (0.019 g, 0.18 mmol, 2.0 eq.) following General procedure 3 to yield **5f** as a white foam (27 mg, 55%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.42–7.31 (m, 7H), 7.26–7.18 (m, 3H), 4.55–4.29 (m, 3H), 4.14–3.96 (m, 1H), 3.85–3.42 (m, 5H), 3.30–3.08 (m, 2H), 2.88 (s, 3H), 2.44–2.27 (m, 2H), 2.12–1.97 (m, 2H), 1.78–1.58 (m, 6H), 1.16–0.92 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.4, 136.3, 130.0, 129.5, 127.6, 126.2, 111.2, 109.8, 74.9, 67.1, 59.9, 56.1, 54.3, 49.6, 39.7, 35.9, 25.9; MS (ESI) *m/z* (%): 553.5 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{29}H_{36}N_4O_5S$ $[M + H]^+$ 553.2479, found: 553.2478.

(2-{[N-Hexyl(N-benzyl-N-methylaminosulfonyl)amino]methyl}-1,4-dioxa-8-aza-8-spiro[4.5]decyl)(1-phenylcyclopentyl)methanone ***5g***. The compound was synthesized form **5b** (0.14 g, 0.26 mmol, 1.0 eq.) and iodohexane (0.097 mL, 0.65 mmol, 2.5 eq.) following General procedure 3 to yield **5g** as a colorless oil (56 mg, 35%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.28 (m, 7H), 7.24–7.19 (m, 3H), 4.37–4.21 (m, 3H), 4.06–4.02 (m, 1H), 3.78–3.54 (m, 3H), 3.42–3.33 (m, 1H), 3.29–3.09 (m, 5H), 2.61 (s, 3H), 2.40–2.30 (m, 2H), 2.08–1.96 (m, 2H), 1.76–1.55 (m, 8H), 1.33–1.26 (m, 6H), 1.05–0.99 (m, 2H), 0.91 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.4, 138.0, 130.0, 129.7, 129.5, 128.9, 127.6, 126.2, 109.1, 76.2, 68.2, 59.9, 55.3, 51.9, 50.4, 45.3, 42.1, 39.3, 36.3, 35.3, 34.9, 32.7, 29.0, 27.5, 26.0, 23.7, 14.4; MS (ESI) *m/z* (%): 612.6 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{34}H_{49}N_3O_5S$ $[M + H]^+$ 612.3466, found: 612.3464.

[2-({[2-(Dimethylamino)ethyl](N-benzyl-N-methylaminosulfonyl)amino}methyl)-1,4-dioxa-8-aza-8-spiro[4.5]decyl][(1-phenylcyclopentyl)methanone ***5h***. The compound was synthesized from **5b** (30 mg, 0.057 mmol, 1.0 eq.) and 2-bromo-*N*,*N*-dimethylethylamine hydrobromide (16 mg, 0.068 mmol, 1.2 eq.) following General procedure 3 to yield **5h** as a white foam (24 mg, 71%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.27 (m, 7H), 7.25–7.18 (m, 3H), 4.38–4.19 (m, 3H), 4.08–3.95 (m, 1H), 3.77–3.34 (m, 6H), 3.30–3.11 (m, 3H), 2.69–2.69 (m, 5H), 2.39–2.21 (m, 8H), 2.14–1.90 (m, 2H), 1.79–1.49 (m, 6H), 1.14–0.94 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 175.2, 145.1, 136.5, 128.6, 128.3, 128.1, 127.5, 126.2, 124.8, 75.0, 66.7, 58.5, 56.8, 53.9, 51.3, 48.1, 46.4, 44.3, 40.7, 38.2, 34.9, 33.5, 24.5; MS (ESI) *m/z* (%): 599.7 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{32}H_{46}N_4O_5S$ $[M + H]^+$ 599.3262, found: 599.3259.

(2-{[(2-Morpholinoethyl)(N-benzyl-N-methylaminosulfonyl)amino]methyl}-1,4-dioxa-8-aza-8-

spiro[4.5]decyl)(1-phenylcyclopentyl)methanone ***5i***. The compound was synthesized from **5b** (50 mg, 0.095 mmol, 1 eq.) and 4-(2-bromoethyl)morpholine hydrobromide (34 mg, 0.12 mmol, 1.3 eq.) following General procedure 3 to yield **5i** as a white foam (45 mg, 74%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.26 (m, 7H), 7.26–7.18 (m, 3H), 4.36–4.27 (m, 3H), 4.09–3.95 (m, 1H), 3.74–3.53 (m, 7H), 3.48–3.34 (m, 3H), 3.28–3.07 (m, 3H), 2.68–2.58 (m, 5H), 2.45–2.35 (m, 6H), 2.13–1.91 (m, 2H), 1.75–1.49 (m, 6H), 1.14–0.92 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 138.0, 130.0, 129.7, 129.5, 128.9, 127.6, 126.2, 109.2, 76.3, 68.1, 67.8, 59.9, 57.9, 55.3, 54.9, 52.5, 46.9, 45.3, 42.1, 39.8, 36.8, 34.9, 25.9; MS (ESI) *m*/*z* (%): 641.7 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{34}H_{48}N_4O_6S$ $[M + H]^+$ 641.3368, found: 641.3367.

2-((N-Benzyl-N-methylsulfamoyl)((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)amino)-N,N-dimethylacetamide ***5j***. The compound was synthesized from **5b** (50 mg, 0.095 mmol, 1.0 eq.) and 2-bromo-*N*,*N*-dimethylacetamide (0.021 mL, 0.19 mmol, 2.0 eq.) following General procedure 3 to yield **5j** as a white foam (50 mg, 86%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.27 (m, 7H), 7.26–7.19 (m, 3H), 4.35–4.21 (m, 5H), 4.10–4.01 (m, 1H), 3.78–3.51 (m, 4H), 3.30–3.03 (m, 3H), 3.01–2.91 (m, 6H), 2.66 (s, 3H), 2.36–2.01 (m, 4H), 1.78–1.49 (m, 6H), 1.14–0.86 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 170.3, 146.5, 138.1, 130.0, 129.6, 129.5, 128.8, 127.6, 126.2, 109.1, 76.5, 68.1, 59.9, 55.3, 52.6, 50.8, 45.3, 42.1, 40.0, 36.7, 36.1, 35.0, 25.9; MS (ESI) *m*/*z* (%): 613.7 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{32}H_{44}N_4O_6S$ $[M + H]^+$ 613.3055, found: 613.3052.

2-[(N-Benzyl-N-methylaminosulfonyl)({8-[(1-phenylcyclopentyl)carbonyl]-1,4-dioxa-8-aza-2-spiro[4.5]decyl}methyl)amino]-1-(1-pyrrolidinyl)-1-ethanone ***5k***. The compound was synthesized from **5b** (25 mg, 0.047 mmol, 1.0 eq.) and 2-bromo-1-(pyrrolidin-1-yl)ethan-1-one (27 mg, 0.14 mmol, 3.0 eq.) following General procedure 3 to yield **5k** as a white foam (50 mg, 86%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.35–7.27 (m, 7H), 7.25–7.20 (m, 3H), 4.36–4.14 (m, 5H), 4.07–3.96 (m, 1H), 3.79–3.47 (m, 4H), 3.45–3.36 (m, 5H), 3.29–3.06 (m, 2H), 2.67 (s, 3H), 2.41–2.28 (m, 2H), 2.10–1.95 (m, 4H), 1.90–1.80 (m, 2H), 1.75–1.55 (m, 6H), 1.15–0.95 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 168.9, 146.4, 138.1, 130.0, 129.6, 129.5, 128.8, 127.6, 126.2, 109.1, 76.6, 68.1, 59.9, 55.3, 52.7, 47.2, 46.9, 45.3, 42.0, 35.0, 27.1, 25.9, 25.0; MS (ESI) *m*/*z* (%): 639.6 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{34}H_{46}N_4O_6S$ $[M + H]^+$ 639.3211, found: 639.3216.

2-[(N-Benzyl-N-methylaminosulfonyl)({8-[(1-phenylcyclopentyl)carbonyl]-1,4-dioxa-8-aza-2-spiro[4.5]decyl}methyl)amino]-1-piperidino-1-ethanone ***5l***. The compound was synthesized from **5b** (22 mg, 0.042 mmol, 1.0 eq.) and 2-bromo-1-(piperidin-1-yl)ethan-1-one (17 mg, 0.083 mmol, 2.0 eq.) following General procedure 3 to yield **5l** as a white foam (12 mg, 44%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.35–7.28 (m, 7H), 7.25–7.21 (m, 3H), 4.34–4.24 (m, 5H), 4.10–4.01 (m, 1H), 3.79–3.38 (m, 8H), 3.27–3.06 (m, 2H), 2.66 (s, 3H), 2.39–2.30 (m, 2H), 2.16–2.03 (s, 2H), 1.75–1.51 (m, 13H), 1.21–0.90 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 168.7, 146.5, 130.0, 127.6, 126.2, 122.3, 109.1, 76.6, 68.0, 59.9, 52.7, 51.2, 47.3, 46.9, 45.4, 42.1, 38.9, 36.3, 34.8, 29.4, 27.1, 26.6, 25.9, 25.0; MS (ESI) *m*/*z* (%): 653.2 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{35}H_{48}N_4O_6S$ $[M + H]^+$ 653.3368, found: 653.3364.

1-Morpholino-2-[(N-benzyl-N-methylaminosulfonyl)({8-[(1-phenylcyclopentyl)carbonyl]-1,4-dioxa-8-aza-2-spiro[4.5]decyl}methyl)amino]-1-ethanone ***5m***. The compound was synthesized from **5b** (22 mg, 0.042 mmol, 1.0 eq.) and 2-bromo-1-morpholinoethan-1-one (17 mg, 0.083 mmol, 2.0 eq.) following General procedure 3 to yield **5m** as a white foam (20 mg, 74%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.26 (m, 7H), 7.27–7.19 (m, 3H), 4.35–4.21 (m, 5H), 4.13–4.02 (m, 1H), 3.73–3.37 (m, 13H), 3.26–3.00 (m, 2H), 2.66 (s, 3H), 2.46–2.26 (m, 2H), 2.19–1.87 (m, 2H), 1.77–1.49 (m, 6H), 1.20–0.84 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 169.0, 146.5, 138.1, 130.0, 129.6, 129.5, 128.8, 127.6, 126.2, 109.1, 76.5, 68.1, 67.8, 67.6, 59.9, 55.3, 52.7, 50.9, 46.3, 45.3, 43.4, 42.2, 38.9, 36.8, 35.0, 25.9; MS (ESI) *m*/*z* (%): 655.7 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{34}H_{46}N_4O_7S$ $[M + H]^+$ 655.3160, found: 655.3165.

N-((3,3-Dimethyl-8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide ***6a***. The compound was synthesized according to General procedure 6 using 1-amino-3-methylbutane-2,3-diol (100 mg, 0.84 mmol, 1.0 eq.) and piperidine-1-sulfonyl chloride (0.11 mL, 0.76 mmol, 0.90 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.) afforded 6a as a white foam (0.14 g, 49%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.30 (m, 2H), 7.27–7.19 (m, 3H), 3.92–3.50 (m, 3H), 3.27–3.06 (m, 8H), 2.42–2.32 (m, 2H), 2.10–2.00 (m, 2H), 1.74–1.54 (m, 12H), 1.30–1.21 (m, 3H), 1.13–0.98 (m, 5H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 129.9, 127.5, 126.2, 106.8, 82.9, 81.1, 59.9, 47.9, 44.0, 42.0 39.3, 26.7, 26.5, 25.9, 24.9, 23.3; MS (ESI) *m*/*z* (%): 520.2 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{27}H_{41}N_3O_5S$ $[M + H]^+$ 520.2840, found: 520.2838.

4-Cyano-N-((3,3-dimethyl-8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide ***6b***. The compound was synthesized according to General procedure 6 using 1-amino-3-methylbutane-2,3-diol (110 mg, 0.92 mmol, 1.0 eq.) and 4-cyanopiperidine-1-sulfonyl chloride (173 mg, 0.83 mmol, 0.90 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.) afforded **6b** as a white foam (0.16 g, 53%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.37–7.30 (m, 2H), 7.26–7.19 (m, 3H), 3.89–3.51 (m, 3H), 3.41–3.32 (m, 2H), 3.30–3.02 (m, 6H), 2.99–2.88 (m, 1H), 2.42–2.31 (m, 2H), 2.08–1.92 (m, 4H), 1.89–1.78 (m, 2H), 1.75–1.49 (m, 6H), 1.30–1.22 (m, 3H), 1.15–0.84 (m, 5H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.5, 122.3, 106.9, 82.9, 81.1, 59.9, 45.3, 45.2, 44.1, 42.0, 39.3, 38.5, 36.6, 29.3, 26.6, 26.0, 23.3; MS (ESI) *m*/*z* (%): 545.4 $[M + H]^+$;

HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{28}H_{40}N_4O_5S$ $[M + H]^+$ 545.2792, found: 545.2799.

{2,2-Dimethyl-3-[(N-benzyl-N-methylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl}(1-phenylcyclopentyl)methanone ***6c***. The compound was synthesized according to General procedure 6 using 1-amino-3-methylbutane-2,3-diol (110 mg, 0.92 mmol, 1.0 eq.) and benzyl(methyl)sulfamoyl chloride (0.12 mL, 0.83 mmol, 0.90 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.) afforded **6c** as a white foam (0.19 g, 62%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.35–7.28 (m, 7H), 7.24–7.20 (m, 3H), 4.31–4.21 (m, 2H), 3.95–3.42 (m, 3H), 3.16–3.06 (m, 3H), 2.65 (s, 3H), 2.40–2.29 (m, 2H), 2.07–1.98 (m, 2H), 1.74–1.54 (m, 6H), 1.29–1.20 (m, 3H), 1.11–0.96 (m, 5H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 138.1, 129.9, 129.7, 129.5, 128.8, 127.5, 126.2, 106.8, 82.8, 81.1, 59.9, 55.1, 45.2, 43.9, 42.0, 39.3, 37.8, 36.6, 34.9, 26.6, 25.9, 23.3; MS (ESI) *m*/*z* (%): 556.4 $[M + H]^+$; HPLC purity (254 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{30}H_{41}N_3O_5S$ $[M + H]^+$ 556.2840, found: 556.2842.

2-((N-Benzyl-N-methylsulfamoyl)((3,3-dimethyl-8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)amino)-N,N-dimethylacetamide ***6d***. The compound was synthesized from **6c** (15 mg, 0.027 mmol, 1.0 eq.) and 2-bromo-*N*,*N*-dimethylacetamide (9.0 mg, 0.054 mmol, 2.0 eq.) following General procedure 3 to yield **6d** as a white foam (8.0 mg, 47%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.35–7.28 (m, 7H), 7.26–7.19 (m, 3H), 4.35–4.21 (m, 4H), 4.06–3.70 (m, 2H), 3.60–3.54 (m, 1H), 3.30–3.15 (m, 2H), 3.00–2.82 (m, 7H), 2.69 (s, 3H), 2.40–2.30 (m, 2H), 2.14–1.92 (m, 3H), 1.76–1.52 (m, 6H), 1.25–1.18 (m, 3H), 1.10–0.93 (m, 5H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 170.4, 146.5, 138.1, 130.0, 129.7, 129.5, 128.8, 127.6, 126.2, 107.1, 83.5, 81.2, 59.9, 55.2, 51.0, 45.2, 42.1, 39.4, 36.7, 36.1, 35.0, 26.0, 23.2; MS (ESI) *m*/*z* (%): 641.3 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{34}H_{48}N_4O_6S$ $[M + H]^+$ 641.3368, found: 641.3363.

(3-((1,1-Dioxido-1,2-thiazinan-2-yl)methyl)-2,2-dimethyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)(1-phenylcyclopentyl)methanone ***6e***. To a stirred solution of 1-amino-3-methylbutane-2,3-diol (0.30 g, 2.5 mmol, 1.0 eq.) in CH_2Cl_2 was added Fmoc-Cl (0.59 g, 2.2 mmol, 0.90 eq.) at 0 °C. The mixture was stirred at room temperature for 5 h. Upon completion based on TLC and LCMS, the reaction was quenched with saturated aq. $NaHCO_3$, extracted with CH_2Cl_2 (3×), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude Fmoc-protected amine (0.58 g, 1.7 mmol, 1.0 eq.) was subjected to ketalization with intermediate **II** (0.45 g, 1.7 mmol, 1.0 eq.) by General procedure 1. Upon completion based on TLC and LCMS, the reaction was quenched with saturated aq. $NaHCO_3$, extracted with CH_2Cl_2 (3×), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified by chromatography (Biotage, 60–70% EtOAc in hexanes) to afford (9*H*-fluoren-9-yl)methyl((3,3-dimethyl-8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)carbamate (0.62 g, 63%), which was then dissolved in 20% piperidine in DMF (5 mL) and stirred at room temperature for 16 h. The mixture was concentrated under reduced pressure and the resulting amine, ((3-(aminomethyl)-2,2-dimethyl-1,4-dioxa-8-azaspiro-[4.5]decan-8-yl)(1-phenylcyclopentyl)methanone), was used in the next step without purification. In a scintillation vial was added (3-(aminomethyl)-2,2-dimethyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)(1-phenylcyclopentyl)methanone (100 mg, 0.27 mmol, 1.0 eq.) and THF (2 mL). The mixture was stirred at 0 °C for 15 min before the addition of NaH (60% in mineral oil, 26 mg, 1.1 mmol, 4.0 eq.) The mixture was stirred at 0 °C for 15 min before addition of 4-chloro-1-butylsulfonyl chloride (0.041 mL, 0.030 mmol, 1.1 eq.) dropwise over 5 min. The mixture was stirred at 50 °C for 3 h until completion based on TLC and LCMS. The reaction was quenched with saturated aq. $NaHCO_3$, extracted with CH_2Cl_2 (3×), dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified as stated in General procedure 1 to yield **6e** as a white foam (86 mg, 65%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.31 (m, 2H), 7.28–7.19 (m, 3H), 3.97–3.71 (m, 2H), 3.55–3.40 (m, 4H), 3.29–3.02 (m, 5H), 2.44–2.30 (m, 2H), 2.16–1.97 (m, 4H), 1.77–1.58 (m, 8H), 1.27–1.21 (m, 3H), 1.14–0.96 (m, 5H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 106.9, 83.5, 81.3, 59.9, 51.8, 45.2, 42.2, 26.3, 25.9, 25.0, 23.3, 22.0. (missing two carbons); MS (ESI) *m*/*z* (%): 491.2 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{38}N_2O_5S$ $[M + H]^+$ 491.2574, found: 491.2577.

1-(2-((1,1-Dioxido-1,2-thiazinan-2-yl)methyl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-2-methyl-2-phenylpropan-1-one ***7a***. The compound was synthesized from 2-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1,2-thiazinane 1,1-dioxide (0.10 g, 0.40 mmol, 1.0 eq.), piperidin-4-one (50 mg, 0.50 mmol, 1.3 eq.), and 2-methyl-2-phenylpropanoic acid (0.11 g, 0.66 mmol, 1.7 eq.) following General procedure 7 to yield **7a** as a white solid (78 mg, 65%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.39–7.36 (m, 2H), 7.27–7.24 (m, 3H), 4.20–3.99 (m, 2H), 3.82–3.50 (m, 3H), 3.49–3.43 (m, 2H), 3.27–3.02 (m, 6H), 2.18–2.10 (m, 2H), 1.75–1.56 (m, 4H), 1.52 (s, 6H), 1.32–0.96 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 173.2, 114.8, 130.0, 127.7, 126.5, 109.0, 76.5, 67.8, 66.9, 52.6, 50.5, 44.9, 41.5, 36.7, 35.3, 30.5, 20.5, 21.8, 15.6; MS (ESI) *m*/*z* (%): 437.4 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{22}H_{32}N_2O_5S$ $[M + H]^+$ 437.2105, found: 437.2103.

(2-((1,1-Dioxido-1,2-thiazinan-2-yl)methyl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)(1-phenylcyclopropyl)methanone ***7b***. The compound was synthesized from 2-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1,2-thiazinane 1,1-dioxide (0.10 g, 0.40 mmol, 1.0 eq.), piperidin-4-one (50 mg, 0.50 mmol, 1.3 eq.), and 1-phenylcyclopropane-1-carboxylic acid (0.17 g, 0.66 mmol, 1.7 eq.) following General procedure 7 to yield **7b** as a white solid (88 mg, 73%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.36–7.29 (m, 2H), 7.26–7.18 (m, 3H), 4.30–3.98 (m, 2H), 3.81–3.43 (m, 7H), 3.30–3.16 (m, 2H), 3.10–2.97 (m, 2H), 2.19–2.07 (m, 2H), 1.75–1.53 (m, 4H), 1.42–1.19 (m, 6H); ^{13}C NMR (126 MHz, CD_3OD) δ 173.2, 141.8, 130.0, 127.7, 126.5,

108.9, 76.5, 67.8, 52.6, 50.5, 45.0, 41.5, 36.6, 35.3, 30.5, 25.0, 21.8, 15.6; MS (ESI) *m*/*z* (%): 435.4 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{22}H_{30}N_2O_5S$ $[M + H]^+$ 435.1948, found: 435.1952.

(1-(3-Chlorophenyl)cyclopentyl)(2-((1,1-dioxido-1,2-thiazinan-2-yl)methyl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)methanone ***7c***. The compound was synthesized from 2-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1,2-thiazinane 1,1-dioxide (0.10 g, 0.40 mmol, 1.0 eq.), piperidin-4-one (50 mg, 0.50 mmol, 1.3 eq.), and 1-(3-chlorophenyl)cyclopentane-1-carboxylic acid (0.15 g, 0.66 mmol, 1.7 eq.) following General procedure 7 to yield **7c** as a white foam (90 mg, 60%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.36–7.33 (m, 1H), 7.27–7.22 (m, 2H), 7.21–7.14 (m, 1H), 4.32–3.93 (m, 2H), 3.80–3.51 (m, 3H), 3.50–3.41 (m, 2H), 3.30–3.09 (m, 4H), 3.07–2.98 (m, 2H), 2.42–2.34 (m, 2H), 2.16–1.98 (m, 4H), 1.75–1.58 (m, 8H), 1.28–0.99 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 175.8, 149.0, 135.9, 131.5, 127.7, 126.4, 124.7, 108.9, 76.5, 67.8, 59.8, 52.6, 50.4, 39.5, 26.0, 25.0, 21.9 with three carbons not observed; MS (ESI) *m*/*z* (%): 497.3 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{33}ClN_2O_5S$ $[M + H]^+$ 497.1872, found: 497.1871.

(1-(4-Chlorophenyl)cyclopentyl)(2-((1,1-dioxido-1,2-thiazinan-2-yl)methyl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)methanone ***7d***. The compound was synthesized from 2-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1,2-thiazinane 1,1-dioxide (0.10 g, 0.40 mmol, 1.0 eq.), piperidin-4-one (50 mg, 0.50 mmol, 1.3 eq.), and 1-(4-chlorophenyl)cyclopentane-1-carboxylic acid (0.15 g, 0.66 mmol, 1.7 eq.) following General procedure 7 to yield **7d** as a white foam (90 mg, 60%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.34 (m, 2H), 7.25–7.22 (m, 2H), 4.25–3.95 (m, 2H), 3.77–3.56 (m, 3H), 3.50–3.42 (m, 2H), 3.25–3.18 (m, 4H), 3.06–2.99 (m, 2H), 2.40–2.34 (m, 2H), 2.16–2.09 (m, 2H), 2.01–1.94 (m, 2H), 1.75–1.62 (m, 8H), 1.29–1.02 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ 176.5, 145.3, 133.3, 130.0, 128.0, 108.9, 76.5, 67.8, 59.6, 52.6, 50.4, 39.4, 25.9, 25.0, 21.9 with three carbons not observed; MS (ESI) *m*/*z* (%): 497.1 $[M + H]^+$; HPLC purity (220 nm): >99%; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{33}ClN_2O_5S$ $[M + H]^+$ 497.1872, found: 497.1879.

*(R)-(2-((1,1-Dioxido-1,2-thiazinan-2-yl)methyl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)(1-phenylcyclopentyl)methanone (R)-****1m***. The compound was synthesized following General procedure 1 using (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (100 mg, 0.76 mmol, 1.0 eq.) and 4-chlorobutane-1-sulfonyl chloride (0.12 mL, 0.84 mmol, 1.1 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.) afforded (*R*)-**1m** as a white foam (0.19 g, 70%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.32 (m, 2H), 7.25–7.21 (m, 3H), 4.25–3.93 (m, 2H), 3.78–3.55 (m, 3H), 3.49–3.39 (m, 2H), 3.29–3.10 (m, 4H), 3.06–2.96 (m, 2H), 2.42–2.31 (m, 2H), 2.16–2.01 (m, 4H), 1.74–1.58 (m, 8H), 1.15–0.93 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.0, 76.4, 67.7, 59.9, 52.6, 50.4, 45.3, 42.1, 39.3, 34.8, 25.9, 25.0, 21.8; MS (ESI) *m*/*z* (%): 463.4 $[M + H]^+$; HPLC purity (220 nm): >99%; chiral HPLC (220 nm) 99.9:0.1; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{34}N_2O_5S$ $[M + H]^+$ 463.2261, found: 463.2258.

*(S)-(2-((1,1-Dioxido-1,2-thiazinan-2-yl)methyl)-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)(1-phenylcyclopentyl)methanone (S)-****1m***. The compound was synthesized following General procedure 1 using (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (100 mg, 0.76 mmol, 1.0 eq.) and 4-chlorobutane-1-sulfonyl chloride (0.12 mL, 0.84 mmol, 1.1 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.16 g, 0.59 mmol, 1.0 eq.) afforded (*S*)-**1m** as a white foam (0.15 g, 55%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.32 (m, 2H), 7.27–7.21 (m, 3H), 4.26–3.94 (m, 2H), 3.78–3.54 (m, 3H), 3.49–3.39 (m, 2H), 3.27–3.14 (m, 4H), 3.05–2.97 (m, 2H), 2.39–2.33 (m, 2H), 2.17–2.00 (m, 4H), 1.74–1.54 (m, 8H), 1.14–0.91 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 109.0, 76.4, 67.7, 59.9, 52.6, 50.4, 45.2, 42.2, 39.3, 36.8, 25.9, 25.0, 21.8; MS (ESI) *m*/*z* (%): 463.4 $[M + H]^+$; HPLC purity (220 nm): >99%; chiral HPLC (220 nm) 95.0:5.0; HRMS (TOF MS ES$^+$): calcd. for $C_{24}H_{34}N_2O_5S$ $[M + H]^+$ 463.2261, found: 463.2265.

*[(R)-2-[(Dimethylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl](1-phenylcyclopentyl)methanone (R)-****3a***. The compound was synthesized following General procedure 1 using (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (250 mg, 1.9 mmol, 1.0 eq.) and dimethylsulfamoyl chloride (0.27 mL, 2.5 mmol, 1.3 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.32 g, 1.2 mmol, 1.0 eq.) afforded (*R*)-**3a** (0.21 g, 31%) as a white foam. ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.32 (m, 2H), 7.26–7.20 (m, 3H), 4.15–3.99 (m, 2H), 3.79–3.53 (m, 3H), 3.21–3.06 (m, 4H), 2.73 (s, 6H), 2.40–2.34 (m, 2H), 2.06–2.02 (m, 2H), 1.77–1.49 (m, 6H), 1.17–0.84 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.4, 129.9, 127.5, 126.2, 108.9, 76.0, 67.8, 59.9, 46.7, 45.2, 42.1, 39.3, 38.3, 36.8, 35.3, 25.9; MS (ESI) *m*/*z* (%): 452.1 $[M + H]^+$; HPLC purity (254 nm): >99%; chiral HPLC (220 nm) 99.1:0.9; HRMS (TOF MS ES$^+$): calcd. for $C_{22}H_{33}N_3O_5S$ $[M + H]^+$ 452.2214, found: 452.2216.

*[(S)-2-[(Dimethylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl](1-phenylcyclopentyl)methanone (S)-****3a***. The compound was synthesized following General procedure 1 using (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (250 mg, 1.9 mmol, 1.0 eq.) and dimethylsulfamoyl chloride (0.27 mL, 2.5 mmol, 1.3 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.32 g, 1.2 mmol, 1.0 eq.) afforded (*S*)-**3a** as a white foam (0.19 g, 28%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.32 (m, 2H), 7.26–7.20 (m, 3H), 4.21–3.93 (m, 2H), 3.79–3.54 (m, 3H), 3.29–3.00 (m, 4H), 2.73 (s, 6H), 2.41–2.31 (m, 2H), 2.05–2.01 (m, 2H), 1.75–1.55 (m, 6H), 1.15–0.90 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 76.1, 67.9, 59.9, 46.7, 45.3, 42.0, 39.4, 38.3, 36.2, 35.3, 25.9; MS (ESI) *m*/*z* (%): 452.3 $[M + H]^+$; HPLC purity (254 nm): >99%; chiral HPLC (220 nm) 98.6:1.4; HRMS (TOF MS ES$^+$): calcd. for $C_{22}H_{33}N_3O_5S$ $[M + H]^+$ 452.2214, found: 452.2210.

*(R)-N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide (R)-****3k***.

The compound was synthesized following General procedure 1 using (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (100 mg, 0.76 mmol, 1.0 eq.) and piperidine-1-sulfonyl chloride (0.13 mL, 0.92 mmol, 1.2 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.15 g, 0.54 mmol, 1.0 eq.) afforded (*R*)-**3k** as a white foam (0.14 g, 53%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.32 (m, 2H), 7.27–7.20 (m, 3H), 4.24–3.94 (m, 2H), 3.83–3.53 (m, 3H), 3.30–3.02 (m, 8H), 2.43–2.30 (m, 2H), 2.12–1.99 (m, 2H), 1.77–1.52 (m, 12H), 1.23–0.89 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 108.9, 76.0, 67.9, 59.9, 47.9, 46.7, 45.3, 42.1, 39.3, 36.8, 35.3, 26.4, 25.9, 24.8; MS (ESI) *m*/*z* (%): 492.4 $[M + H]^+$; HPLC purity (220 nm): >99%; chiral HPLC (220 nm) 98.9:1.1; HRMS (TOF MS ES$^+$): calcd. for $C_{25}H_{37}N_3O_5S$ $[M + H]^+$ 492.2527, found: 492.2523.

*(S)-N-((8-(1-Phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide (S)-**3k**.* The compound was synthesized following General procedure 1 using (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanamine (100 mg, 0.76 mmol, 1.0 eq.) and piperidine-1-sulfonyl chloride (0.13 mL, 0.92 mmol, 1.2 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.15 g, 0.54 mmol, 1.0 eq.) afforded (*S*)-**3k** as a white foam (0.12 g, 45%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.31 (m, 2H), 7.26–7.20 (m, 3H), 4.23–3.92 (m, 2H), 3.81–3.49 (m, 3H), 3.30–2.97 (m, 8H), 2.47–2.29 (m, 2H), 2.11–1.97 (m, 2H), 1.77–1.48 (m, 12H), 1.23–0.82 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.5, 146.4, 129.9, 127.5, 126.2, 108.9, 76.0, 67.9, 59.9, 47.9, 46.6, 45.2, 42.1, 39.4, 36.8, 35.3, 26.4, 25.9, 24.8; MS (ESI) *m*/*z* (%): 492.4 $[M + H]^+$; HPLC purity (220 nm): >99%; chiral HPLC (220 nm) 99.4:0.6; HRMS (TOF MS ES$^+$): calcd. for $C_{25}H_{37}N_3O_5S$ $[M + H]^+$ 492.2527, found: 492.2526.

*(R)-4-Cyano-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide (R)-**4b**.* The compound was synthesized following General procedure 1 using (*R*)-(2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (80 mg, 0.61 mmol, 1.0 eq.) and 4-cyanopiperidine-1-sulfonyl chloride (0.15 g, 0.73 mmol, 1.2 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.13 g, 0.48 mmol, 1.0 eq.) afforded (*R*)-**4b** as a white foam (90 mg, 36%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.31 (m, 2H), 7.27–7.20 (m, 3H), 4.22–3.92 (m, 2H), 3.79–3.52 (m, 3H), 3.40–3.32 (m, 2H), 3.29–2.99 (m, 6H), 2.97–2.88 (m, 1H), 2.43–2.30 (m, 2H), 2.11–1.93 (m, 4H), 1.89–1.79 (m, 2H), 1.77–1.50 (m, 6H), 1.15–0.84 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 122.3, 109.0, 76.0, 67.8, 59.9, 46.7, 45.3, 42.2, 39.2, 36.8, 36.3, 29.3, 26.6, 25.9; MS (ESI) *m*/*z* (%): 517.6 $[M + H]^+$; HPLC purity (220 nm): >99%; chiral HPLC (220 nm) 99.2:0.8; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{36}N_4O_5S$ $[M + H]^+$ 517.2479, found: 517.2480.

*(S)-4-Cyano-N-((8-(1-phenylcyclopentane-1-carbonyl)-1,4-dioxa-8-azaspiro[4.5]decan-2-yl)methyl)piperidine-1-sulfonamide (S)-**4b**.* The compound was synthesized following General procedure 1 using (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (80 mg, 0.61 mmol, 1.0 eq.) and 4-cyanopiperidine-1-sulfonyl chloride (0.15 g, 0.73 mmol, 1.2 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.13 g, 0.48 mmol, 1.0 eq.) afforded (*S*)-**4b** as a white foam (0.12 g, 48%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.31 (m, 2H), 7.27–7.20 (m, 3H), 4.22–3.92 (m, 2H), 3.83–3.52 (m, 3H), 3.40–3.32 (m, 2H), 3.30–2.98 (m, 6H), 2.97–2.88 (m, 1H), 2.43–2.31 (m, 2H), 2.10–1.93 (m, 4H), 1.89–1.79 (m, 2H), 1.75–1.50 (m, 6H), 1.15–0.94 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 130.0, 127.6, 126.2, 122.3, 109.0, 76.0, 67.8, 59.9, 46.7, 45.3, 42.1, 39.2, 36.9, 34.7, 29.3, 26.6, 25.9; MS (ESI) *m*/*z* (%): 517.4 $[M + H]^+$; HPLC purity (220 nm): >99%; chiral HPLC (220 nm) 99.3:0.7; HRMS (TOF MS ES$^+$): calcd. for $C_{26}H_{36}N_4O_5S$ $[M + H]^+$ 517.2479, found: 517.2479.

*[(S)-2-[(N-Benzyl-N-methylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl](1-phenylcyclopentyl)methanone (S)-**5b**.* The compound was synthesized following General procedure 1 using (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (100 mg, 0.76 mmol, 1.0 eq.) and benzyl(methyl) sulfamoyl chloride (0.14 mL, 0.92 mmol, 1.2 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (0.15 g, 0.55 mmol, 1.0 eq.) afforded (*S*)-**5b** as a white foam (0.17 g, 58%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.36–7.26 (m, 7H), 7.25–7.19 (m, 3H), 4.25–4.17 (m, 3H), 4.05–3.92 (m, 1H), 3.78–3.51 (m, 3H), 3.28–3.01 (m, 4H), 2.62 (s, 3H), 2.40–2.30 (m, 2H), 2.09–1.97 (m, 2H), 1.74–1.49 (m, 6H), 1.14–0.86 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 138.1, 130.0, 129.7, 129.5, 128.8, 127.6, 126.2, 108.9, 76.0, 67.9, 59.9, 55.2, 46.6, 45.3, 42.1, 39.3, 36.3, 35.3, 34.8, 26.0; MS (ESI) *m*/*z* (%): 528.4 $[M + H]^+$; HPLC purity (220 nm): >99%; chiral HPLC (220 nm) 98.2:1.8; HRMS (TOF MS ES$^+$): calcd. for $C_{28}H_{37}N_3O_5S$ $[M + H]^+$ 528.2527, found: 528.2525.

*[(R)-2-[(N-Benzyl-N-methylaminosulfonylamino)methyl]-1,4-dioxa-8-aza-8-spiro[4.5]decyl](1-phenylcyclopentyl)methanone (R)-**5b**.* The compound was synthesized following General procedure 1 using (*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl) methanamine (100 mg, 0.76 mmol, 1.0 eq.) and benzyl(methyl) sulfamoyl chloride (0.14 mL, 0.92 mmol, 1.2 eq.) for preparation of the sulfamate intermediate. Ketalization with intermediate **II** (100 mg, 0.37 mmol, 1.0 eq.) afforded (*R*)-**5b** as a white foam (0.14 g, 48%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.37–7.26 (m, 7H), 7.27–7.18 (m, 3H), 4.25–4.16 (m, 3H), 4.06–3.93 (m, 1H), 3.69–3.60 (m, 3H), 3.30–3.02 (m, 4H), 2.62 (s, 3H), 2.41–2.30 (m, 2H), 2.10–1.97 (m, 2H), 1.76–1.55 (m, 6H), 1.15–0.84 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.6, 146.5, 138.1, 130.0, 129.7, 129.6, 128.8, 127.6, 126.2, 108.9, 76.0, 67.9, 59.9, 55.2, 46.6, 45.3, 42.1, 39.4, 36.9, 35.3, 34.8, 26.0; MS (ESI) *m*/*z* (%): 528.4 $[M + H]^+$; HPLC purity (220 nm): >99%; chiral HPLC (220 nm) 98.3:1.7; HRMS (TOF MS ES$^+$): calcd. for $C_{28}H_{37}N_3O_5S$ $[M + H]^+$ 528.2527, found: 528.2524.

Biological evaluation

Antiviral assays

Alphavirus replication assays. Plate-based assays using CHIKV-nLuc reporter virus constructed in the 181/25 strain

background (CHIKV 181/25-nLuc-Capsid) or VEEV-nLuc reporter virus constructed in the TC-83 strain background (VEEV TC83-nLuc-nsP3) were performed as previously described.[8,15]

Alphavirus titer reduction assays. MRC-5 fibroblasts were infected with alphaviruses (CHIKV-181/25, VEEV-TC83, or SINV-G100) at an MOI of 0.1 for 1 h and treated with inhibitor concentrations spanning a 12-point 4-fold dose curve starting at 40 μM. Infectious virus in the supernatants were quantified by plaque assay. Ten-fold serial dilutions of collected supernatant were plated on Vero CL-81 cells (ATCC). After adsorption for 1 h at 37 °C, cells were overlaid with 2 mL of media containing carboxymethylcellulose (1 : 1 mixture of 2× α-MEM containing 6% FBS, 2% penicillin/streptomycin, 2% L-glutamine, 2% HEPES, and 2.5% carboxymethylcellulose). Cells were incubated at 37 °C for 2 d (SINV and VEEV) or 3 d (CHIKV), then fixed by the addition of 4% paraformaldehyde followed by 0.25% crystal violet. Plaques were visually counted to determine the number of plaque forming units (PFU) per mL.

Helicase ATPase assay. CHIKV nsP2 ATPase activity was measured using the Promega ADP-Glo assay kit in a 384-well format (Corning) following manufacturer's instructions. In brief, 1 nM of CHIKV nsP2 was incubated with inhibitor compound resuspended in DMSO and diluted in an 8-point 3-fold dose response starting at 250 μM for 30 min. Following initial incubation, 30 μM Ultra Pure ATP (Promega) was added to each reaction and incubated for 1 h at room temperature on a plate shaker at 250 rpm. 2 μL of ADP-Glo reagent (Promega) was added and the reaction was incubated for 40 min. 2 μL kinase detection reagent was added, and plates were incubated for an additional 30 min. Luminescence was measured using a Tecan Spark and % inhibition was calculated by comparing relative luminescence values to DMSO control reactions.

Author contributions

HJO, JDS, MHT, and TMW conceived the project. HJO, BMR, RSZS, ZWD, MAH, MHT, and TMW designed and synthesized the compounds. JDS, SRM, DO, SAM, JEB, RMC, NJM, and MTH supervised and performed the biological studies. HJO and TMW took the lead in writing the manuscript. All authors have provided critical feedback and approved the final manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). These SI files contain Fig. S1, ^{1}H and ^{13}C NMR and HPLC traces of tested compounds.

Supplementary information is available. See DOI: https://doi.org/10.1039/d5md00772k.

Acknowledgements

The Structural Genomics Consortium (SGC) is a registered charity (no: 1097737) that receives funds from Bayer AG, Boehringer Ingelheim, Bristol Myers Squibb, Genentech, Genome Canada, through Ontario Genomics Institute [OGI-196], EU/EFPIA/OICR/McGill/KTH/Diamond Innovative Medicines Initiative 2 Joint Under-taking [EUbOPEN grant 875510], Janssen, Merck KGaA (also known as EMD in Canada and the US), Pfizer, and Takeda. The research reported in this publication was supported by NIH grant 1U19AI171292-01 (READDI-AViDD Center) and in part by the NC Biotech Center Institutional Support Grant 2018-IDG-1030 and by NIH grant S10OD032476 for upgrading the 500 MHz NMR spectrometer in the UNC Eshelman School of Pharmacy NMR Facility. We gratefully acknowledge the support of Piramal Pharma Solutions for assistance in the synthesis of intermediates and final compounds and Matthew Binder (UNC) for assistance in collecting chiral HPLC data.

References

1 E. A. Gould, B. Coutard, H. Malet, B. Morin, S. Jamal, S. Weaver, A. Gorbalenya, G. Moureau, C. Baronti, I. Delogu, N. Forrester, M. Khasnatinov, T. Gritsun, X. de Lamballerie and B. Canard, *Antiviral Res.*, 2010, **87**, 111–124.
2 S. R. Azar, R. K. Campos, N. A. Bergren, V. N. Camargos and S. L. Rossi, *Microorganisms*, 2020, **8**, 1167.
3 A. M. Powers, A. C. Brault, Y. Shirako, E. G. Strauss, W. Kang, J. H. Strauss and S. C. Weaver, *J. Virol.*, 2001, **75**, 10118–10131.
4 M. Bassetto and A. Brancale, in *Annual Reports in Medicinal Chemistry*, ed. K. Seley-Radtke, Academic Press, 2021, vol. 57, pp. 133–151.
5 W. M. de Souza, G. S. Ribeiro, S. T. S. de Lima, R. de Jesus, F. R. R. Moreira, C. Whittaker, M. A. M. Sallum, C. V. F. Carrington, E. C. Sabino, U. Kitron, N. R. Faria and S. C. Weaver, *Lancet Reg. Health Am.*, 2024, **30**, 100673.
6 D. S. Metibemu, O. S. Adeyinka, J. Falode, O. Crown and I. V. Ogungbe, *ACS Infect. Dis.*, 2024, **10**, 2507–2524.
7 S. Wang, S. Mahalingam and A. Merits, *Rev. Med. Virol.*, 2025, **35**, e70030.
8 B. M. Ramalingam, J. D. Sears, K. M. Talbot, Y.-W. N. Su, S. Houliston, M. A. Hossain, Z. W. Davis-Gilbert, C. Zhao, H. J. Oh, P. J. Brown, M. K. Sanders, S. R. Moorman, D. Ojha, J. E. Burdick, I. Law, N. L. Morales, S. A. Martinez, P. Loppnau, J. G. Perez, A. M. Drobish, T. E. Morrison, Z. J. Streblow, D. N. Streblow, C. H. Arrowsmith, A. Vargason, R. M. Couñago, L. Halabelian, J. J. Arnold, C. E. Cameron, N. J. Moorman, M. T. Heise and T. M. Willson, *J. Med. Chem.*, 2025, **68**, 14514–14536.
9 B. M. Ramalingam, H. J. Oh, J. D. Sears, C.-H. Chen, A. Vala, S. Liu, K. M. Talbot, M. A. Hossain, P. J. Brown, S. Houliston, J. Garcia Perez, F. Li, M. G. Amare, P. Halfmann, J. Smith, A. Hirsch, C. H. Arrowsmith, L. Halabelian, A. M. Vargason, R. M. Counago, J. J. Arnold, C. E. Cameron, N. J.

Moorman, M. T. Heise and T. M. Willson, *ACS Infect. Dis.*, 2025, **11**, 2331–2341.

10 J. D. Sears, D. Ojha, S. Taft-Benz, P. Sylvester, Z. Streblow, A. J. Gramm, A. M. Drobish, B. M. Ramalingam, M. A. Hossain, K. Talbot, W. Sanders, C.-K. Chang, I. Law, S. A. Martinez, J. E. Burdick, R. Wiltshire, N. A. Saba, J. H. Poplarski, R. M. Couñago, P. J. Brown, G. S. Anand, T. E. Morrison, J. J. Arnold, C. E. Cameron, D. N. Streblow, T. M. Willson, M. T. Heise and N. J. Moorman, *bioRxiv*, 2025, preprint, 2025.2006.2003.657755, DOI: 10.1101/2025.06.03.657755.

11 A. N. Matthew, F. Leidner, G. J. Lockbaum, M. Henes, J. Zephyr, S. Hou, D. N. Rao, J. Timm, L. N. Rusere, D. A. Ragland, J. L. Paulsen, K. Prachanronarong, D. I. Soumana, E. A. Nalivaika, N. Kurt Yilmaz, A. Ali and C. A. Schiffer, *Chem. Rev.*, 2021, **121**, 3238–3270.

12 S.-H. Lee and O.-J. Park, *Appl. Microbiol. Biotechnol.*, 2009, **84**, 817–828.

13 A. Heuser, W. Abdul Rahman, E. Bechter, J. Blank, S. Buhr, D. Erdmann, P. Fontana, F. Mermet-Meillon, M. Meyerhofer, R. Strang, M. Schrapp, C. Zimmermann, M. Cortes-Cros, H. Mobitz and J. Hamon, *ChemMedChem*, 2024, **19**, e202300613.

14 L. Selvaratnam, T. M. Willson and M. Schapira, *J. Med. Chem.*, 2025, **68**, 4022–4039.

15 E. M. Merten, J. D. Sears, T. M. Leisner, P. B. Hardy, A. Ghoshal, M. A. Hossain, K. H. Asressu, P. J. Brown, E. G. Tse, M. A. Stashko, K. Li, J. L. Norris-Drouin, L. E. Herring, A. L. Mordant, T. S. Webb, C. A. Mills, N. K. Barker, Z. J. Streblow, S. Perveen, C. H. Arrowsmith, R. M. Counago, J. J. Arnold, C. E. Cameron, D. N. Streblow, N. J. Moorman, M. T. Heise, T. M. Willson, K. I. Popov and K. H. Pearce, *Proc. Natl. Acad. Sci. U. S. A.*, 2024, **121**, e2409166121.

