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Synthesis and anti-coronavirus activity of a series of 1-thia-4-azaspiro[4.5]decan-3-one derivatives

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Abstract

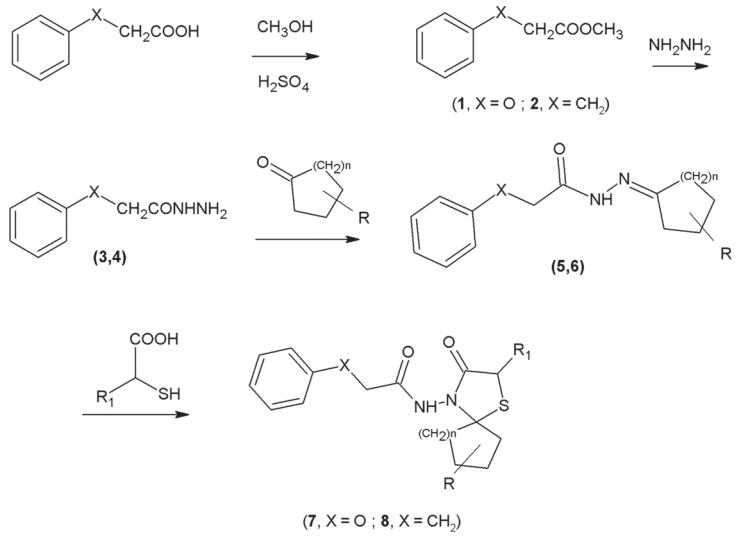
A series of 1-thia-4-azaspiro[4.5]decan-3-ones bearing an amide group at C-4 and various substitutions at C-2 and C-8 were synthesized and evaluated against human coronavirus and influenza virus. Compounds **7m**, **7n**, **8k**, **8l**, **8m**, **8n**, and **8p** were found to inhibit human coronavirus 229E replication. The most active compound was *N*-(2-methyl-8-*tert*-butyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (**8n**), with an EC_{50} value of 5.5 μM, comparable to the known coronavirus inhibitor, (*Z*)-*N*-[3-[4-(4-bromophenyl)-4-hydroxypiperidin-1-yl]-3-oxo-1-phenylprop-1-en-2-yl]benzamide (**K22**). Compound **8n** and structural analogs were devoid of anti-influenza virus activity, although their scaffold is shared with a previously discovered class of H3 hemagglutinin-specific influenza virus fusion inhibitors. These findings point to the 1-thia-4-azaspiro[4.5]decan-3-one scaffold as a versatile chemical structure with high relevance for antiviral drug development.

KEYWORDS
antimicrobial activity, cycloaddition, structure elucidation, synthesis

1 | INTRODUCTION

Coronaviruses (CoVs) are a large family of enveloped RNA viruses with positive-sense single-stranded RNA genome, affecting a wide range of animal species. Some widely spread human coronaviruses (HCoVs, i.e., strains 229E, OC43, NL63, and HKU1) are the etiologic agents in 15–30% of common cold disease,[1] a relatively benign yet highly contagious respiratory illness that can possibly be complicated by virus-induced asthma or exacerbations of chronic obstructive pulmonary disease.[2] These HCoVs can also cause high-morbidity lower respiratory tract conditions, such as pneumonia, bronchiolitis, and croup, especially in immunocompromised patients, the elderly and young children.[3–5] Besides, two highly pathogenic CoVs originating from animal reservoirs have had a major medical and socio-economic impact. In 2003, the newly discovered SARS-CoV[6] responsible for the severe acute respiratory syndrome, suddenly emerged in South-East Asia, after which it caused 8096 infections worldwide with a case-fatality (CFR) rate of 9.6% and a total societal cost estimated at 40 billion USD.[7] In 2012, the thus far unknown Middle East Respiratory Syndrome virus (MERS-CoV) appeared[6] which, to date, caused 2249 cases with a CFR of 36%.[8] The high virulence of SARS- and MERS-CoVs, combined with their sudden emergence and clear pandemic potential, has boosted the development of preventive (i.e., vaccines) or therapeutic (i.e., antiviral drugs) interventions to address serious CoV infections in humans. Thus far, except for quarantine measures, no effective intervention exists.

In our laboratory, we initiated a small-molecule screening program in HCoV-infected cells, aimed at identifying new chemical entities and CoV targets with relevance for antiviral drug development. In the present report, we describe the identification of a new class of CoV inhibitors carrying a 1-thia-4-azaspiro[4.5]decan-3-one scaffold (Scheme 1). A series of structural analogs were chemically synthesized to delineate the structure-activity relationship. Intriguingly, the same scaffold is present in a class of H3 hemagglutinin-specific influenza virus fusion inhibitors that was discovered by our team some time ago.[9] This points to the 1-thia-4-azaspiro[4.5]-decan-3-one scaffold as a versatile chemical structure with high relevance for antiviral drug development.



Compound	R	R_1	n	Compound	R	R_1	n
7a	H	H	1	**8a**	H	H	1
7b	3-CH_3	H	2	**8b**	H	H	2
7c	4-CH_3	H	2	**8c**	4-CH_3	H	2
7d	4-$C(CH_3)_3$	H	2	**8d**	4-C_2H_5	H	2
7e	4-CF_3	H	2	**8e**	4-C_3H_7	H	2
7f	4-C_6H_5	H	2	**8f**	4-$C(CH_3)_3$	H	2
7g	H	H	3	**8g**	4-CF_3	H	2
7h	H	CH_3	1	**8h**	4-C_6H_5	H	2
7i	H	CH_3	2	**8i**	H	CH_3	1
7j	3-CH_3	CH_3	2	**8j**	H	CH_3	2
7k	4-CH_3	CH_3	2	**8k**	4-CH_3	CH_3	2
7l	4-$C(CH_3)_3$	CH_3	2	**8l**	4-C_2H_5	CH_3	2
7m	4-CF_3	CH_3	2	**8m**	4-C_3H_7	CH_3	2
7n	4-C_6H_5	CH_3	2	**8n**	4-$C(CH_3)_3$	CH_3	2
				8o	4-CF_3	CH_3	2
				8p	4-C_6H_5	CH_3	2

SCHEME 1 Synthetic route and chemical structures for compound series **7** and **8**

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Hydrazides (**3**, **4**) were obtained by stepwise reactions, firstly by esterification of acids in methanol in the presence of H_2SO_4 to give esters (**1**, **2**), and secondly hydrazinolysis of **1**, **2** to give **3**, **4**.[10] **3**, **4** were reacted with cyclic ketones to afford corresponding derivatives **5**, **6** that are commercially available or recorded earlier, except **5a** and **6a**.[11,12] Spectral and analytical data of **5a** and **6a** are given under "General procedure for the synthesis of *N*′-(cycloalkylidene)-2-phenoxyacetohydrazides (**5**) or *N*′-(cycloalkylidene)-3-phenylpropanehydrazides (**6**)". Cyclocondensation of **5** or **6** with sulphanylacetic acid or 2-sulphanylpropanoic acid led to *N*-(substituted 3-oxo-1-thia-4-azaspiro[4.4]non-4-yl/[4.5]dec-4-yl/[4.6]undec-4-yl)-2-phenoxyacetamide (**7**) or *N*-(substituted 3-oxo-1-thia-4-azaspiro[4.4]non-4-yl/[4.5]decan-4-yl)-3-phenylpropanamides (**8**). The first evidence for the formation of **7** and **8** was the observation of a new band between 1728–1697 cm^{-1} in their IR spectra due to 3-carbonyl group. The C=O stretching band of the carboxamide moiety was also observed at 1660–1693 cm^{-1}. Bands between 3468–3167 cm^{-1} region confirmed the presence of NH stretching bands. In the ^{1}H-NMR spectra of **7**, CONH protons were observed at δ 9.91–10.59 ppm as singlets. C_2–methylene protons of **7a–g** and **8a–h** resonated at δ 3.52–3.64 ppm as singlets, while C_2-methine protons of **7h–n** and **8i–p** gave quartets at δ 3.80–3.93 ppm. The ^{13}C-NMR spectra of **7a–m** and **8j** showed spirothiazolidinone C_2 carbon (δ 27.76–38.08 ppm) and spirothiazolidinone C=O carbon (δ 167.59–170.77 ppm) resonances as another evidence for the formation of a ring system. Molecular weights

(for the group coded as **7**) were verified by taking mass spectra to get another proof. ESI method was applied in positive or negative modes and $[M+H]^+$ or $[M–H]^-$ ions formed confirmed the molecular weights.

2.2 | Biological activity

The compounds were evaluated for antiviral activity against human coronavirus 229E in HEL 299 fibroblast cells, and against influenza A/H1N1, A/H3N2, and B viruses in MDCK cells. The test compounds

TABLE 1 Activity in human coronavirus 229E-infected HEL[a] cells

Compounds	Antiviral activity (EC_{50}[b])	Cytotoxicity (MCC[c])
7a	>100	>100
7b	>100	>100
7c	>100	>100
7d	>100	≥100
7e	>100	>100
7f	>100	>100
7g	>100	>100
7h	>100	>100
7i	>100	>100
7j	>100	>100
7k	>100	>100
7l	>100	>100
7m	31 ± 1	>100
7n	12 ± 1	100
8a	>100	>100
8b	>100	>100
8c	>100	>100
8d	>100	>100
8e	>100	>100
8f	>100	>100
8g	>100	>100
8h	>100	100
8i	>100	>100
8j	>100	>100
8k	28 ± 0	>100
8l	18 ± 5	≥100
8m	8.1 ± 2.2	100
8n	5.5 ± 0.7	≥100
8o	>100	>100
8p	6.1 ± 0.2	50
K22	3.3 ± 1.0	50

Data represent the averages ± SEM of 3–4 independent tests.
[a]HEL: human embryonic lung fibroblast cells.
[b]EC_{50}: compound concentration (μM) producing 50% inhibition of virus replication, as determined by microscopic scoring of the cytopathic effect (CPE).
[c]MCC: minimum cytotoxic concentration (μM), that is, compound concentration producing minimal changes in cell morphology, as estimated by microscopy.

can be classified in either Group **7**, carrying a phenoxyethyl side chain, or Group **8**, with a phenethyl side chain. Our previously discovered H3 hemagglutinin-specific influenza virus fusion inhibitors[9] have the same scaffold but carry an imidazo[2,1-b]thiazole or *o*-hydroxyphenyl side chain. The current series of molecules show no activity against influenza viruses at concentrations up to 100 μM (data not shown), probably phenoxymethyl and phenethyl side chains are not suitable for binding to influenza virus hemagglutinin.

Seven compounds, i.e. **7m**, **7n**, **8k**, **8l**, **8m**, **8n**, and **8p**, were found to inhibit human coronavirus 229E with low cytotoxicity (Table 1). The compounds showing activity all carry a methyl substituent at the C-2 position of the azaspiro[4.5]decane (R_1), whereas the unmethylated analogs (i.e., **7e**, **7f**, **8c**, **8d**, **8e**, **8f**, **8h**) were devoid of antiviral activity. The same observation was previously made for the structurally related influenza virus fusion inhibitors.[9] In addition, for compound series **8**, the anti-coronavirus activity clearly depended on the bulkiness of the C-8 substituent of the azaspiro[4.5]decane (R). No activity was seen for the unsubstituted compound **8j** (R = H). Intermediate activity was observed for **8k** and **8l** (EC_{50} = 28 and 18 μM), which carry a 4-methyl and 4-ethyl-group, respectively. A further increase in activity was seen for **8m**, **8n**, and **8p** (EC_{50} values 5.5–8.1 μM), carrying, respectively, a 4-propyl, 4-*tert*-butyl and 4-phenyl group. An exception here is **8o** (R = 4-CF_3), which did not show antiviral activity at concentrations up to 100 μM. Its direct analog belonging to Group 7 (**7m**) however, was moderately active (EC_{50} = 31 μM), and so was **7n** (EC_{50} = 12 μM), which carries the same substituent (4-phenyl) as **8p**. Molecules **7k** and **7l**, which are the counterparts of **8k** and **8n**, were not active.

3 | CONCLUSION

We report the chemical synthesis of a compound series with 4-carboxamido-3-oxo-1-thia-4-azaspiro[4.4]nonan/[4.5]decan/[4.6]undecan structure. Upon evaluation in virus-infected cell culture assays, seven compounds proved to be able to inhibit human coronavirus 229E virus replication, with the most potent analog having an EC_{50} value of 5.5 μM. The anti-coronavirus activity was found to be strongly dependent on the substituents at the C-2 and C-8 positions of the azaspiro[4.5]decane scaffold. While a series of closely related analogs was previously demonstrated to target influenza virus fusion by interfering with hemagglutinin refolding at low pH, the present molecules have no activity against human influenza A/H1N1, A/H3N2, and B viruses. Ongoing mechanistic investigations will reveal the biochemical basis for their inhibitory effect on coronavirus.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Melting points were determined in open capillary tubes with a Büchi B-540 melting point apparatus and are uncorrected. IR spectra were

recorded in KBr disks on a Shimadzu IR Affinity-1 FTIR spectrophotometer. ^{1}H and ^{13}C-NMR spectra were recorded on Varian Unity Inova (500 MHz) and Varian Mercury (400 MHz) spectrophotometers. All chemical shifts were reported as δ (ppm) values and spin-spin couplings (*J*) were expressed in Hz. Microanalyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. Electrospray ionization (ESI)/MS were determined on Agilent 6460 TripQuad LC-MS/MS and Waters 2695 Alliance Micromass ZQ LC/MS spectrometers.

The original spectra of the investigated compounds are provided as Supporting Information. The InChI codes of the investigated compounds together with some biological activity data are also provided as Supporting Information.

4.1.2 | Synthesis of methyl phenoxyacetate (1) or methyl 3-phenylpropanoate (2)

A solution of phenoxyacetic acid (0.01 mol) or 3-phenylpropionic acid (0.01 mol) and sulfuric acid (98%) (0.1 ml) in 50 ml of methanol was heated for 24 hr. The solution was neutralized with 0.01 mol of sodium bicarbonate solution, the mixture was extracted with diethyl ether and, after distillation of solvent, the ester was used without further purification.[10,13]

4.1.3 | Synthesis of 2-phenoxyacetohydrazide (3) or 3-phenylpropane-hydrazide (4)

A mixture of **1** or **2** (0.01 mol), ethanol (50 ml), and hydrazine (0.0125 mol) was refluxed for 1 hr. The solid was filtered off, washed with cold water and used without recrystallization.[10,13]

4.1.4 | General procedure for the synthesis of *N*′-(cycloalkylidene)-2-phenoxyacetohydrazides (5) or *N*′-(cycloalkylidene)-3-phenylpropanehydrazides (6)

A solution of hydrazide (**3** or **4**) and appropriate ketone (0.01 mol) in 20 ml of ethanol (96%) was refluxed for 1–3 hr and then the solution was allowed to cool. The solid thus formed was filtered off, dried and directly used in the next step.

N′-[4-(Trifluoromethyl)cyclohexylidene]-2-phenoxyacetohydrazide (5a)

Yield: 80%. mp: 140–142°C; IR (KBr) υ (cm^{-1}): 3331, 3184, 3082 (N-H), 1683 (C=O). ^{1}H-NMR (dimethyl sulfoxide [DMSO]-d_6/400 MHz): 1.27–1.53 (2H, m, cyclohexane $C_{2,6\text{-ax}}$-H), 1.86–2.10, 2.23–2.46, 2.51–2.72 (6H, 3m, $C_{2,6\text{-eq}}$-H, $C_{3,5}$-H), 2.89, 3.05 (1H, 2d, *J* = 16 Hz, C_4-H), 4.59, 4.95 (2H, 2s, OCH_2), 6.90–7.00 (3H, m, phenyl $C_{2,4,6}$-H), 7.20–7.40 (2H, m, phenyl $C_{3,5}$-H), 10.45, 10.62 (1H, 2s, NH). ^{13}C-NMR (proton decoupled) (100 MHz) (DMSO-d_6): 23.56, 24.01, 24.79, 25.03, 32.34, 32.45 (cyclohexane $C_{2,3,5,6}$), 38.87–40.12 (C-CF_3 with $(CD_3)_2SO$), 64.70, 65.91 (OCH_2), 114.35, 114.50, 114.69, 120.59, 121.01, 121.20, 129.33, 129.43 (phenyl $C_{2\text{-}6}$), 124.98 (q, $^{2}J_{C\text{-}F}$ = 277 Hz, CF_3), 153.97, 157.70, 157.87, 158.18 (phenyl C_1), 160.90, 163.97 (cyclohexane C_1), 166.69, 169.29 (CO). (ESI+) MS *m/z* (%): 337 ([M+Na]$^+$, 40), 315 ([M+H]$^+$, 100). Anal. calcd. for $C_{15}H_{17}F_3N_2O_2$ (314.30): C: 57.32, H: 5.45, N: 8.91. Found: C: 57.19, H: 5.40, N: 8.94.

N′-[4-(Trifluoromethyl)cyclohexylidene]-3-phenylpropanehydrazide (6a)

Yield: 69%. mp: 117–120°C; IR (KBr) υ (cm^{-1}): 3234, 3176 (N-H), 1668 (C=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 1.28–1.47; 1.86–2.00; 2.23–2.41; 2.59–2.62 (9H, m, cyclohexane $C_{2\text{-}6}$-H), 2.79–2.88 (4H, m, CH_2), 7.15–7.29 (5H, m, phenyl $C_{2\text{-}6}$-H), 10.17, 10.25 (1H, 2s, NH). Anal. calcd. for $C_{16}H_{19}F_3N_2O$ (312.33): C: 61.53, H: 6.13, N: 8.97. Found: C: 61.60, H: 6.21, N: 9.12.

4.1.5 | General procedure for the synthesis of *N*-(substituted 3-oxo-1-thia-4-azaspiro[4.4]non-4-yl/[4.5]dec-4-yl/[4.6]undec-4-yl)-2-phenoxyacetamides (7) and *N*-(substituted 3-oxo-1-thia-4-azaspiro[4.4]-non-4-yl/[4.5]decan-4-yl)-3-phenylpropanamides (8)

A mixture of **5** or **6** (0.005 mol) and sulfanylacetic acid or 2-sulfanylpropanoic acid (1.5 ml) in 30 ml of dried toluene was refluxed for 4–10 hr, using a Dean Stark water separator. Toluene was evaporated *in vacuo*. The residue was neutralized with saturated sodium bicarbonate and was allowed to solidify. The crude product was filtered and recrystallized from ethanol or ethanol/water mixture.

N-(3-Oxo-1-thia-4-azaspiro[4.4]non-4-yl)-2-phenoxyacetamide (7a)

Yield: 65%. mp: 113–117°C; IR (KBr) υ (cm^{-1}): 3383, 3238 (N-H), 1722 (C=O), 1687 (NHC=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 1.52–1.62 (4H, m, spirononane $C_{7,8}$-H), 1.76, 1.95–2.07 (2H, 2H, dt, *J* = 13 Hz, 6 Hz, m, spirononane $C_{6,9}$-H), 3.64 (2H, s, spirononane C_2-H), 4.68 (2H, s, OCH_2), 6.95–6.99 (3H, m, phenyl $C_{2,4,6}$-H), 7.27–7.32 (2H, m, phenyl $C_{3,5}$-H), 10.36 (1H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/125 MHz): 22.94 (spirononane $C_{7,8}$), 29.17 (spirononane C_2), 38.42 (spirononane $C_{6,9}$), 66.49 (OCH_2), 76.15 (spirononane C_5), 115.23, 121.81, 129.89 (phenyl $C_{2\text{-}6}$), 158.01 (phenyl C_1), 167.87 (NHCO), 168.13 (CO). (ESI+) MS *m/z* (%): 329 ([M+Na]$^+$, 50), 307 ([M+H]$^+$, 100), 233 (90). Anal. calcd. for $C_{15}H_{18}N_2O_3S$ (306.38): C: 58.80, H: 5.92, N: 9.14. Found: C: 58.28, H: 6.43, N: 9.18.

N-(7-Methyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-2-phenoxyacetamide (7b)

Yield: 91%. mp: 78–80°C; IR (KBr) υ (cm^{-1}): 3487, 3224 (O-H/N-H), 1714 (C=O), 1668 (NHC=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 0.62 (1H, qd, *J* = 13 Hz, 3 Hz, $C_{8\text{-ax}}$-H), 0.83 (3H, d, *J* = 6 Hz, spirodecane C_7-CH_3), 1.21–1.74 (8H, m, C_6-H, C_7-H, $C_{8\text{-eq}}$-H, C_9-H and C_{10}-H), 3.56 (2H, s, spirodecane C_2-H), 4.69 (2H, s, OCH_2), 6.95–7.00 (3H, m, phenyl $C_{2,4,6}$-H), 7.27–7.32 (2H, m, phenyl $C_{3,5}$-H), 10.29 (1 H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/125 MHz): 22.45 (spirodecane C_7-CH_3), 22.83 (spirodecane C_9), 28.35 (spirodecane C_2), 29.92 (spirodecane C_7), 33.06 (spirodecane C_8), 36.73 (spirodecane C_{10}), 45.39 (spirodecane C_6), 66.41 (OCH_2), 72.62 (spirodecane C_5),

115.29, 121.80, 129.91 (phenyl $C_{2\text{-}6}$), 158.00 (phenyl C_1), 167.84 (NHCO), 168.06 (CO). (ESI+) MS *m/z* (%): 357 ($[M+Na]^+$, 50), 335 ($[M+H]^+$, 100), 261 (40). Anal. calcd. for $C_{17}H_{22}N_2O_3S.0.5H_2O$ (343.43): C: 59.40, H: 6.70, N: 8.16. Found: C: 59.31, H: 6.36, N: 8.03.

N-(8-Methyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-2-phenoxyacetamide (7c)

Yield: 98%. mp: 122–124°C; IR (KBr) υ (cm^{-1}): 3446, 3221 (N-H), 1726 (C=O), 1681 (NHC=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 0.83 (3H, d, *J* = 6 Hz, CH_3), 1.03–1.22 (3H, m, spirodecane $C_{7\text{-}ax}$-H, C_8-H and $C_{9\text{-}ax}$-H), 1.63 (2H, d, *J* = 13 Hz, $C_{7,9\text{-}eq}$-H), 1.66–1.75 (2H, m, $C_{6,10}$-H), 3.57 (2H, s, spirodecane C_2-H), 4.68 (2H, s, OCH_2), 6.95–7.00 (3H, m, phenyl $C_{2,4,6}$-H), 7.27–7.32 (2H, m, phenyl $C_{3,5}$-H), 10.33 (1H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/100 MHz): 21.76 (C_8-CH_3), 27.76 (spirodecane C_2), 30.38 (spirodecane C_8), 31.28, 36.54 (spirodecane $C_{6,7,9,10}$), 65.95 (OCH_2), 71.99 (spirodecane C_5), 114.77, 121.27, 129.38 (phenyl $C_{2\text{-}6}$), 157.56 (phenyl C_1), 167.45 (NHCO), 167.59 (CO). (ESI−) MS *m/z* (%): 333 ($[M-H]^-$, 100), 259 (32). Anal. calcd. for $C_{17}H_{22}N_2O_3S$ (334.43): C: 61.05, H: 6.63, N: 8.38. Found: C: 60.86, H: 6.56, N: 8.32.

N-(8-*tert*-Butyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-2-phenoxyacetamide (7d)

Yield: 79%. mp: 166–170°C; IR (KBr) υ (cm^{-1}): 3466, 3234 (N-H), 1724 (C=O), 1681 (NHC=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 0.70–0.90 (1H, m, C_8-H), 0.81 (9H, s, *tert*-butyl CH_3), 1.07–1.23 (2H, m, spirodecane $C_{7,9\text{-}ax}$-H), 1.54–1.71 (6H, m, $C_{7,9\text{-}eq}$-H and $C_{6,10}$-H), 3.55 (2H, s, spirodecane C_2-H), 4.68 (2H, s, OCH_2), 6.95–7.00 (3H, m, phenyl $C_{2,4,6}$-H), 7.27–7.32 (2H, m, phenyl $C_{3,5}$-H), 10.30 (1H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/125 MHz): 24.11 (spirodecane $C_{7\text{-}9}$), 27.74 (*tert*-butyl CH_3), 28.27 (spirodecane C_2), 32.34 (spirodecane C_8-C), 37.35 (spirodecane $C_{6,10}$), 46.13 (spirodecane C_8), 66.47 (OCH_2), 72.64 (spirodecane C_5), 115.32, 121.81, 129.89 (phenyl $C_{2\text{-}6}$), 158.01 (phenyl C_1), 167.95 (NHCO), 168.02 (CO). (ESI−) MS *m/z* (%): 375 ($[M-H]^-$, 100), 301 (22). Anal. calcd. for $C_{20}H_{28}N_2O_3S$ (376.51): C: 63.80, H: 7.50, N: 7.44. Found: C: 63.86, H: 8.00, N: 7.41.

N-[3-Oxo-8-(trifluoromethyl)-1-thia-4-azaspiro[4.5]dec-4-yl]-2-phenoxyacetamide (7e)

Yield: 100%. mp: 131–135°C; IR (KBr) υ (cm^{-1}): 3468, 3167 (O-H/N-H), 1697 (C=O), 1660 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 1.36–1.52 (2H, m, spirodecane $C_{6,10\text{-}ax}$-H), 1.69–1.95 (6H, m, $C_{6,10\text{-}eq}$-H and $C_{7,9}$-H), 2.10-2.25 (1H, m, $C_{8\text{-}ax}$-H), 3.63 (2H, s, spirodecane C_2-H), 4.69 (2H, s, OCH_2), 6.97–7.03 (3H, m, phenyl $C_{2,4,6}$-H), 7.31–7.35 (2H, m, phenyl $C_{3,5}$-H), 10.38 (1H, s, NH). ^{13}C-NMR (APT) (DMSO-d_6/125 MHz): 22.06, 35.25 (spirodecane $C_{6,7,9,10}$), 28.23 (spirodecane C_2), 39.00 (q, $^2J_{C\text{-}F}$ = 26 Hz, C_8), 66.46 (OCH_2), 71.47 (spirodecane C_5), 115.31, 121.79, 129.94 (phenyl $C_{2\text{-}6}$), 124.80, 128.13 (q, $^1J_{C\text{-}F}$ = 277 Hz, CF_3), 158.04 (phenyl C_1), 167.93 (NHCO), 168.19 (CO). (ESI−) MS *m/z* (%): 387 ($[M-H]^-$, 100), 313 (20). Anal. calcd. for $C_{17}H_{19}F_3N_2O_3S.2H_2O$ (424.43): C: 48.11, H: 5.46, N: 6.60. Found: C: 48.39, H: 5.13, N: 6.79.

N-(8-Phenyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-2-phenoxyacetamide (7f)

Yield: 93%. mp: 165–169°C; IR (KBr) υ (cm^{-1}): 3460, 3253 (N-H), 1724 (C=O), 1680 (NHC = O). ^{1}H-NMR (DMSO-d_6/400 MHz): 1.55–1.70 (2H, m, spirodecane $C_{7,9\text{-}ax}$-H), 1.74–1.94 (6H, m, spirodecane $C_{7,9\text{-}eq}$-H and $C_{6,10}$-H), 2.35 (1H, tt, *J* = 13 Hz, 3 Hz, spirodecane C_8-H), 3.61 (2H, s, spirodecane C_2-H), 4.72 (2H, s, OCH_2), 6.96–7.07 (3H, m, phenoxy $C_{2,4,6}$-H), 7.14–7.22 (3H, m, phenyl C_8-H and phenoxy $C_{3,5}$-H), 7.25–7.37 (4H, m, phenyl-H), 10.36 (1H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/125 MHz): 28.34, 30.88 (spirodecane $C_{6,7,9,10}$), 37.30 (spirodecane C_2), 41.93 (spirodecane C_8), 66.52 (OCH_2), 72.17 (spirodecane C_5), 115.17, 115.35, 121.84, 129.91, 129.96 (phenoxy $C_{2\text{-}6}$), 126.59, 127.00, 128.85, (phenyl $C_{2\text{-}6}$), 146.24 (phenyl C_1), 158.05 (phenoxy C_1), 168.00 (NHCO), 168.14 (CO). (ESI−) MS *m/z* (%): 395 ($[M-H]^-$, 100), 321 (12). Anal. calcd. for $C_{22}H_{24}N_2O_3S$ (396.50): C: 66.64, H: 6.10, N: 7.07. Found: C: 66.55, H: 5.99, N: 7.05.

N-(3-Oxo-1-thia-4-azaspiro[4.6]undec-4-yl)-2-phenoxyacetamide (7g)

Yield: 60%. mp: 115–119°C; IR (KBr) υ (cm^{-1}): 3462, 3236 (N-H), 1726 (C=O), 1681 (NHC = O). ^{1}H-NMR (DMSO-d_6/500 MHz): 1.32–1.43 (4H, m, $C_{8,9}$-H), 1.43–1.68 (4H, m, $C_{7,10}$-H), 1.87 (2H, dd, *J* = 14.5 Hz, 8 Hz, $C_{6/11}$-H), 1.99 (2H, broad dd, *J* = 14.5 Hz, 8H, $C_{6/11}$-H), 3.57 (2H, s, C_2-H), 4.70 (2H, s, OCH_2), 6.96–7.01 (3H, m, phenyl $C_{2,4,6}$-H), 7.29–7.33 (2H, m, phenyl $C_{3,5}$-H), 10.33 (1H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/125 MHz): 22.62, 28.53 (spiroundecane $C_{6\text{-}11}$), 28.60 (spiroundecane C_2), 66.43 (OCH_2), 74.79 (spiroundecane C_5), 115.22, 121.79, 129.90 (phenyl $C_{2\text{-}6}$), 158.06 (phenyl C_1), 167.42 (NHCO), 168.07 (CO). (ESI+) MS *m/z* (%): 357 ($[M+Na]^+$, 100), 335 ($[M+H]^+$, 80), 261 (90). Anal. calcd. for $C_{17}H_{22}N_2O_3S$ (334.43): C: 61.05, H: 6.63, N: 8.38. Found: C: 61.15, H: 6.59, N: 8.72.

N-(2-Methyl-3-oxo-1-thia-4-azaspiro[4.4]non-4-yl)-2-phenoxyacetamide (7h)

Yield: 80%. mp: 121–125°C; IR (KBr) υ (cm^{-1}): 3448, 3230 (N-H), 1728 (C=O), 1685 (NH=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 1.41 (3H, d, *J* = 7 Hz, CH_3), 1.49–1.62 (4H, m, spirononane $C_{7,8}$-H), 1.72, 1.80 (1H, 1H, dt, *J* = 13 Hz, 6 Hz, $C_{6,9}$-H), 1.95–2.15 (2H, m, $C_{6,9}$-H), 3.92 (1H, q, *J* = 7 Hz, spirononane C_2-H), 4.68 (2H, s, OCH_2), 6.95–6.99 (3H, m, phenyl $C_{2,4,6}$-H), 7.28–7.32 (2H, m, phenyl $C_{3,5}$-H), 10.39 (1H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/125 MHz): 19.62 (CH_3), 23.03, 23.10, 38.80 (spirononane $C_{6\text{-}9}$), 38.08 (spirononane C_2), 66.50 (OCH_2), 74.68 (spirononane C_5), 115.23, 121.81, 129.89 (phenyl $C_{2\text{-}6}$), 158.02 (phenyl C_1), 168.10 (NHCO), 170.57 (CO). (ESI−) MS *m/z* (%): 319 ($[M-H]^-$, 100), 245 (8). Anal. calcd. for $C_{16}H_{20}N_2O_3S$ (320.40): C: 59.98, H: 6.29, N: 8.74. Found: C: 59.88, H: 6.21, N: 8.56.

N-(2-Methyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-2-phenoxyacetamide (7i)

Yield: 94%. mp: 134–136°C; IR (KBr) υ (cm^{-1}): 3444, 3230 (N-H), 1728 (C=O), 1687 (NHC=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 0.92 (1H, broad q, *J* = 13 Hz, spirodecane $C_{8\text{-}ax}$-H), 1.39 (3H, d, *J* = 7 Hz,

CH_3), 1.24–1.45 (2H, m, spirodecane $C_{7,9\text{-}ax}$-H), 1.50 (1H, broad d, J = 13 Hz, spirodecane $C_{8\text{-}eq}$-H), 1.56–1.78 (6H, m, $C_{6,10}$-H, $C_{7,9\text{-}eq}$-H), 3.87 (1H, q, J = 7 Hz, spirodecane C_2-H), 4.69 (2H, s, OCH_2), 6.96–7.02 (3H, m, phenyl $C_{2,4,6}$-H), 7.29–7.34 (2H, m, phenyl $C_{3,5}$-H), 10.37 (1H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/100 MHz): 19.58 (CH_3), 22.59, 23.03, 23.80, 36.57, and 36.86 (spirodecane $C_{6\text{-}10}$), 37.75 (spirodecane C_2), 65.90 (OCH_2), 70.73 (spirodecane C_5), 114.72, 121.23, 129.35 (phenyl $C_{2\text{-}6}$), 157.50 (phenyl C_1), 167.52 (NHCO), 169.97 (CO). (ESI+) MS *m/z* (%): 357 ([M+Na]$^+$, 100), 335 ([M+H]$^+$, 85), 247 (75). Anal. calcd. for $C_{17}H_{22}N_2O_3S$ (334.43): C: 61.05, H: 6.63, N: 8.38. Found: C: 61.24, H: 6.46, N: 8.50.

N-(2,7-Dimethyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-2-phenoxyacetamide (7j)

Yield: 92%. mp: 132–138°C; IR (KBr) υ (cm^{-1}): 3383, 3251 (N-H), 1712 (C=O), 1687 (NHC=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 0.62 (1H, qd, J = 13 Hz, 3 Hz, spirodecane $C_{8\text{-}ax}$-H), 0.82 (3H, d, J = 7 Hz, C_7-CH_3), 1.29–1.73 (8H, m, spirodecane C_6-H, C_7-H, $C_{8\text{-}eq}$-H, C_9-H and C_{10}-H), 1.39 (3H, d, J = 7 Hz, spirodecane C_2-CH_3), 3.86 (1H, q, J = 7 Hz, spirodecane C_2-H), 4.68 (2H, s, OCH_2), 6.95–7.00 (3H, m, phenyl $C_{2,4,6}$-H), 7.27–7.32 (2H, m, phenyl $C_{3,5}$-H), 10.33 (1H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/125 MHz): 19.90 (spirodecane C_2-CH_3), 22.54 (spirodecane C_7-CH_3), 23.12 (spirodecane C_9), 29.78 (spirodecane C_7), 37.16 (spirodecane C_2), 33.06 (spirodecane C_8), 36.78 (spirodecane C_{10}), 46.22 (spirodecane C_6), 66.41 (OCH_2), 71.24 (spirodecane C_5), 115.28, 121.79, 129.91 (phenyl $C_{2\text{-}6}$), 158.00 (phenyl C_1), 168.03 (NHCO), 170.46 (CO). (ESI+) MS *m/z* (%): 371 ([M+Na]$^+$, 40), 349 ([M+H]$^+$, 100), 261 (38). Anal. calcd. for $C_{18}H_{24}N_2O_3S$ (348.45): C: 62.04, H: 6.94, N: 8.04. Found: C: 62.26, H: 6.92, N: 8.56.

N-(2,8-Dimethyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-2-phenoxyacetamide (7k)

Yield: 100%. mp: 147–152°C; IR (KBr) υ (cm^{-1}): 3390, 3259 (N-H), 1726 (C=O), 1685 (NHC=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 0.82 (3H, d, J = 6 Hz, spirodecane C_8-CH_3), 0.96–1.25 (3H, m, spirodecane $C_{7,9\text{-}ax}$-H and C_8-H), 1.38 (3H, d, J = 7 Hz, spirodecane C_2-CH_3), 1.56–1.85 (6H, m, spirodecane $C_{6,10}$-H, $C_{7,9\text{-}eq}$-H), 3.86 (1H, q, J = 7 Hz, spirodecane C_2-H), 4.68 (2H, s, OCH_2), 6.93–6.99 (3H, m, phenyl $C_{2,4,6}$-H), 7.26–7.32 (2H, m, phenyl $C_{3,5}$-H), 10.31 (1H, s, NH). ^{13}C-NMR (proton decoupled; DMSO-d_6/100 MHz): 19.65 (spirodecane C_2-CH_3), 21.81 (spirodecane C_8-CH_3), 30.38 (spirodecane C_8), 31.13, 31.62, 37.53 (spirodecane $C_{6,7,9,10}$), 36.65 (spirodecane C_2), 65.96 (OCH_2), 70.65 (spirodecane C_5), 114.69, 114.78, 121.20, 121.29, 129.42 (phenyl $C_{2\text{-}6}$), 157.58 (phenyl C_1), 166.69, 167.59 (NHCO), 170.11 (CO). (ESI−) MS *m/z* (%): 347 ([M–H]$^-$, 100), 259 (25). Anal. calcd. for $C_{18}H_{24}N_2O_3S$ (348.45): C: 62.04, H: 6.94, N: 8.04. Found: C: 61.61, H: 6.89, N: 8.15.

N-(2-Methyl-8-*tert*-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-2-phenoxyacetamide (7l)

Yield: 100%. mp: 155–158°C; IR (KBr) υ (cm^{-1}): 3444, 3251 (N-H), 1722 (C=O), 1693 (NHC=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 0.70–0.89 (1H, m, spirodecane C_8-H), 0.80 (9H, s, *tert*-butyl CH_3), 1.04–1.24 (2H, m, spirodecane $C_{7,9\text{-}ax}$-H), 1.38 (3H, d, J = 7 Hz, spirodecane CH_3), 1.54–1.79 (6H, m, spirodecane $C_{7,9\text{-}eq}$-H, $C_{6,10}$-H), 3.85 (H, q, J = 7 Hz, spirodecane C_2-H), 4.68 (2H, s, OCH_2), 6.94–7.00 (3H, m, phenyl $C_{2,4,6}$-H), 7.27–7.31 (2H, m, phenyl $C_{3,5}$-H), 10.34 (1H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/100 MHz): 19.50 (spirodecane C_2-CH_3), 23.38, 23.82, 36.90, and 37.60 (spirodecane $C_{6,7,9,10}$), 27.16 (*tert*-butyl CH_3), 31.77 (*tert*-butyl C), 36.60 (spirodecane C_2), 45.50 (spirodecane C_8), 65.90 (OCH_2), 70.73 (spirodecane C_5), 114.61, 114.76, 121.24, 129.33 (phenyl $C_{2\text{-}6}$), 157.47, 157.50 (phenyl C_1), 166.50, 167.43 (NHCO), 170.04 (CO). (ESI+) MS *m/z* (%): 413 ([M+Na]$^+$, 75), 391 ([M+H]$^+$, 100), 303 (40). Anal. calcd. for $C_{21}H_{30}N_2O_3S$ (390.53): C: 64.58, H: 7.74, N: 7.17. Found: C: 64.04, H: 7.75, N: 7.27.

N-[2-Methyl-3-oxo-8-(trifluoromethyl)-1-thia-4-azaspiro[4.5]-dec-4-yl]-2-phenoxyacetamide (7m)

Yield: 100%. mp: 78–81°C; IR (KBr) υ (cm^{-1}): 3444, 3196 (O-H/N-H), 1716 (C=O), 1685 (NHC=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 1.29–1.53 (2H, m, spirodecane $C_{6,10\text{-}ax}$-H), 1.39 (3H, d, J = 7 Hz, spirodecane C_2-CH_3), 1.66–1.99 (6H, m, spirodecane $C_{6,10\text{-}eq}$-H and $C_{7,9}$-H), 2.08–2.25 (1H, m, C_8-H), 3.92 (1H, q, J = 7 Hz, spirodecane C_2-H), 4.66 (2H, s, OCH_2), 6.94–7.00 (3H, m, phenyl $C_{2,4,6}$-H), 7.28–7.32 (2H, m, phenyl $C_{3,5}$-H), 10.40 (1H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/100 MHz): 19.51 (CH_3), 21.43, 21.86, 34.90, 35.71 (spirodecane $C_{6,7,9,10}$), 36.73 (spirodecane C_2), 39.49 (q,$^2J_{C\text{-}F}$ = 26 Hz, spirodecane C_8), 65.99 (OCH_2), 69.67 (spirodecane C_5), 114.83, 121.32, 129.47 (phenyl $C_{2\text{-}6}$), 127.65 (q,$^1J_{C\text{-}F}$ = 277 Hz, CF_3), 157.57 (phenyl C_1), 167.71 (NHCO), 170.08 (CO). (ESI+) MS *m/z* (%): 425 ([M+Na]$^+$, 32), 403 ([M+H]$^+$, 100), 315 (72). Anal. calcd. for $C_{18}H_{21}F_3N_2O_3S.0.5H_2O$ (411.43): C: 52.49, H: 5.34, N: 6.80. Found: C: 52.34, H: 5.52, N: 6.75.

N-(2-Methyl-8-phenyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)-2-phenoxyacetamide (7n)

Yield: 100%. mp: 78–80°C; IR (KBr) υ (cm^{-1}): 3334, 3197 (N-H), 1712 (C=O), 1676 (NHC=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 1.43 (3H, d, J = 8 Hz, spirodecane C_2-CH_3), 1.48–1.71 (2H, m, spirodecane $C_{7,9\text{-}ax}$-H), 1.73–2.03 (6H, m, spirodecane $C_{7,9\text{-}eq}$-H, $C_{6,10}$-H), 2.35 (1H, broad t, J = 13 Hz, spirodecane C_8-H), 3.93 (1H, q, J = 8 Hz, spirodecane C_2-H), 4.73 (2H, s, OCH_2), 6.95–7.06 (3H, m, phenoxy $C_{2,4,6}$-H), 7.14–7.21 (3H, m, phenoxy $C_{3,5}$-H and C_8-phenyl-H), 7.25–7.37 (4H, m, phenyl-H), 10.44 (1H, s, NH). ^{13}C-NMR (proton decoupled) (DMSO-d_6/100 MHz): 18.46 (spirodecane C_2-CH_3), 30.12, 30.68, 36.85, 37.71 (spirodecane $C_{6,7,9,10}$), 36.69 (spirodecane C_2), 41.36 (spirodecane C_8), 65.96 (OCH_2), 70.26 (spirodecane C_5), 114.79, 121.28, 129.40 (phenoxy $C_{2\text{-}6}$), 126.03, 128.30 (phenyl $C_{2\text{-}6}$), 145.72 (phenyl C_1), 157.52 (phenoxy C_1), 167.57 (NHCO), 170.07 (CO). (ESI−) MS *m/z* (%): 409 ([M–H]$^-$, 100), 321 (10). Anal. calcd. for $C_{23}H_{26}N_2O_3S.C_2H_5OH$ (456.59): C: 65.76, H: 7.06, N: 6.14. Found: C: 65.48, H: 6.79, N: 6.50.

N-(2-Methyl-3-oxo-1-thia-4-azaspiro[4.4]nonan-4-yl)-3-phenylpropanamide (8a)

Yield: 89%. mp: 190–193°C; IR (KBr) υ (cm^{-1}): 3221, 3196 (N-H), 1716 (C=O), 1670 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 1.04–1.82 (8H,

m, spirononane $C_{6\text{-}9}$-H), 2.49–2.53 (m, CH_2, and DMSO-d_6), 2.86 (2H, t, J = 7 Hz, CH_2), 3.61 (2H, s, spirononane C_2-H), 7.18–7.29 (5H, m, phenyl $C_{2\text{-}6}$-H), 9.97 (1H, s, NH). Anal. calcd. for $C_{16}H_{20}N_2O_2S$ (304.40): C: 63.13, H: 6.62, N: 9.20. Found: C: 63.08, H: 6.71, N: 9.23.

N-(3-Oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8b)

Yield: 100%. mp: 98–99°C; IR (KBr) υ (cm^{-1}): 3354, 3167 (N-H), 1703 (C=O), 1670 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 0.88–1.62 (10H, m, spirodecane $C_{6\text{-}10}$), 2.49–2.52 (m, CH_2, and DMSO-d_6), 2.86 (2H, t, J = 7 Hz, CH_2), 3.52 (2H, s, spirodecane C_2-H), 7.16–7.29 (5H, m, phenyl $C_{2\text{-}6}$-H), 9.91 (1H, s, NH). Anal. calcd. for $C_{17}H_{22}N_2O_2S$. $0.5C_2H_5OH$ (321.43): C: 63.31, H: 7.38, N: 8.20. Found: C: 62.78, H:6.81, N: 8.59.

N-(8-Methyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8c)

Yield: 100%. mp: 102–105°C; IR (KBr) υ (cm^{-1}): 3408, 3232 (N-H), 1705 (C=O), 1674 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 0.81–1.60 (9H, m, spirodecane $C_{6\text{-}10}$), 0.84 (3H, d, J = 7 Hz, CH_3), 2.49–2.51 (m, CH_2, and DMSO-d_6), 2.86 (2H, t, J = 7 Hz, CH_2), 3.53 (2H, s, spirodecane C_2-H), 7.16–7.28 (5H, m, phenyl $C_{2\text{-}6}$-H), 9.91 (1H, s, NH). Anal. calcd. for $C_{18}H_{24}N_2O_2S$. $0.5C_2H_5OH$ (355.46): C: 64.61, H: 7.79, N: 7.73. Found: C: 64.13, H: 7.35, N: 8.03.

N-(8-Ethyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8d)

Yield: 97%. mp: 145–147°C; IR (KBr) υ (cm^{-1}): 3396, 3230 (N-H), 1705 (C=O), 1674 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 0.83–1.62 (9H, m, spirodecane $C_{6\text{-}10}$), 0.84 (3H, t, J = 7 Hz, CH_3), 1.15 (2H, qu, J = 7 Hz, spirodecane C_8-CH_2), 2.49–2.52 (m, CH_2, and DMSO-d_6), 2.86 (2H, t, J = 7 Hz, CH_2), 3.52 (2H, s, spirodecane C_2-H), 7.15–7.28 (5H, m, phenyl $C_{2\text{-}6}$-H), 9.91 (1H, s, NH). Anal. calcd. for $C_{19}H_{26}N_2O_2S$. $0.5C_2H_5OH$ (369.48): C: 65.01, H:7.51, N: 7.58. Found: C: 65.16, H:7.52, N: 7.68.

N-(3-Oxo-8-propyl-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8e)

Yield: 100%. mp: 102–107°C; IR (KBr) υ (cm^{-1}): 3383, 3230 (N-H), 1705 (C=O), 1668 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 0.86 (3H, t, J = 7 Hz, CH_3), 0.99–1.61 (9H, m, spirodecane $C_{6\text{-}10}$), 1.05 (2H, t, J = 7 Hz, spirodecane C_8-CH_2), 1.23–1.31 (2H, m, spirodecane C_8-CH_2), 2.49–2.52 (m, CH_2, and DMSO-d_6), 2.87 (2H, t, J = 7 Hz, CH_2), 3.52 (2H, s, spirodecane C_2-H), 7.17–7.29 (5H, m, phenyl $C_{2\text{-}6}$-H), 9.91 (1H, s, NH). Anal. calcd. for $C_{20}H_{28}N_2O_2S$. $0.5C_2H_5OH$ (383.51): C: 65.76, H: 8.15, N: 7.30. Found: C: 65.58, H: 7.86, N: 7.29.

N-(8-*tert*-Butyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8f)

Yield: 100%. mp: 154–155°C; IR (KBr) υ (cm^{-1}): 3385, 3253 (N-H), 1701 (C=O), 1668 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 0.72–1.64 (9H, m, spirodecane $C_{6\text{-}10}$), 0.82 (9H, s, CH_3), 2.49–2.52 (m, CH_2, and DMSO-d_6), 2.87 (2H, t, J = 7 Hz, CH_2), 3.52 (2H, s, spirodecane C_2-H), 7.17–7.29 (5H, m, phenyl-CH), 9.91 (1H, s, NH). Anal. calcd. for $C_{21}H_{30}N_2O_2S$. $0.5C_2H_5OH$ (397.54): C: 66.46, H: 8.37, N: 7.05. Found: C: 66.84, H: 8.14, N: 7.04.

N-[3-Oxo-(8-trifluoromethyl)-1-thia-4-azaspiro[4.5]decan-4-yl]-3-phenylpropanamide (8g)

Yield: 94%. mp: 94–100°C; IR (KBr) υ (cm^{-1}): 3392, 3186 (N-H), 1697 (C=O), 1670 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 1.39–2.09 (9H, m, spirodecane $C_{6\text{-}10}$), 2.49–2.53 (m, CH_2 and DMSO-d_6), 2.88 (2H, t, J = 7 Hz, CH_2), 3.59 (2H, s, spirodecane C_2-H), 7.16–7.31 (5H, m, phenyl-CH), 10.00 (1H, s, NH). Anal. calcd. for $C_{18}H_{21}F_3N_2O_2S$. $0.5H_2O$ (395.43): C: 54.68, H: 5.82, N: 7.08. Found: C: 54.71, H: 5.97, N: 7.12.

N-(8-Phenyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8h)

Yield: 100%. mp: 194–196°C; IR (KBr) υ (cm^{-1}): 3300, 3223 (N-H), 1726 (C=O), 1681 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 1.04–2.31 (9H, m, spirodecane $C_{6\text{-}10}$), 2.49–2.56 (m, CH_2 and DMSO-d_6), 2.90 (2H, t, J = 7 Hz, CH_2), 3.58 (2H, s, spirodecane C_2-H), 7.19–7.34 (10H, m, phenyl-CH), 10.00 (1H, s, NH). Anal. calcd. for $C_{23}H_{26}N_2O_2S$ (394.52): C: 70.02, H: 6.64, N: 7.10. Found: C: 69.99, H: 6.58, N: 7.19.

N-(2-Methyl-3-oxo-1-thia-4-azaspiro[4.4]nonan-4-yl)-3-phenylpropanamide (8i)

Yield: 90%. mp: 169–171°C; IR (KBr) υ (cm^{-1}): 3380, 3190 (N-H), 1716 (C=O), 1668 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 1.04–1.88 (8H, m, spirononane $C_{6\text{-}9}$-H), 1.40 (3H, d, J = 7 Hz, CH_3), 2.49–2.53 (m, CH_2, and DMSO-d_6), 2.86 (2H, t, J = 7 Hz, CH_2), 3.90 (1H, q, J = 7 Hz, spirononane C_2-H), 7.18–7.29 (5H, m, phenyl-CH), 10.02 (1H, s, NH). Anal. calcd. for $C_{17}H_{22}N_2O_2S$ (318.43): C: 64.12, H: 6.96, N: 8.80. Found: C: 64.16, H: 6.80, N: 8.87.

N-(2-Methyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8j)

Yield: 100%. mp: 141–145°C; IR (KBr) υ (cm^{-1}): 3390, 3290 (N-H), 1716 (C=O), 1681 (NHC=O). ^{1}H-NMR (DMSO-d_6/400 MHz): 0.86–1.59 (10H, m, spirodecane $C_{6\text{-}10}$), 1.35 (3H, d, J = 7 Hz, C_2-CH_3), 2.46–2.54 (m, CH_2, and DMSO-d_6), 2.85 (2H, t, J = 7 Hz, CH_2), 3.80 (1H, q, J = 7 Hz, spirodecane C_2-H), 7.14–7.28 (5H, m, phenyl $C_{2\text{-}6}$-H), 9.95 (1H, s, NH). ^{13}C-NMR (DEPT) (DMSO-d_6/400 MHz): 19.68 (CH_3), 22.59, 23.40, 23.74, 30.66, 34.67 (spirodecane $C_{2\text{-}6}$ and 2CH_2), 36.62 (spirodecane C_2), 70.55 (spirodecane C_5), 125.99, 128.18, 128.35 (phenyl $C_{2\text{-}6}$), 140.52 (phenyl C_1), 170.04 (amide CO), 170.77 (spirodecane CO). Anal. calcd. for $C_{18}H_{24}N_2O_2S$ (332.46): C: 65.03, H: 7.28, N: 8.43. Found: C: 64.77, H: 7.11, N: 8.49.

N-(2,8-Dimethyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8k)

Yield: 100%. mp: 68–71°C; IR (KBr) υ (cm^{-1}): 3421, 3261 (N-H), 1701 (C=O), 1670 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 0.79–1.60 (9H, m, spirodecane $C_{6\text{-}10}$), 0.84 (3H, d, J = 7 Hz, CH_3), 1.37 (3H, d, J = 7 Hz, C_2-CH_3), 2.49–2.53 (m, CH_2, and DMSO-d_6), 2.87 (2H, t, J = 7 Hz, CH_2),

3.83 (1H, q, J = 7 Hz, spirodecane C_2-H), 7.16–7.29 (5H, m, phenyl $C_{2\text{-}6}$-H), 9.96 (1H, s, NH). Anal. calcd. for $C_{19}H_{26}N_2O_2S$. $0.5H_2O$ (346.48): C: 64.22, H: 7.88, N: 7.88. Found: C: 63.98, H: 7.55, N: 8.04.

***N*-(8-Ethyl-2-methyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8l)**

Yield: 94%. mp: 144–146°C; IR (KBr) υ (cm^{-1}): 3310, 3186 (N-H), 1720 (C=O), 1674 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 0.81–1.63 (9H, m, spirodecane $C_{6\text{-}10}$), 0.84 (3H, t, J = 7 Hz, CH_3), 1.16 (2H, qu, J = 7 Hz, spirodecane C_8-CH_2), 1.37 (3H, d, J = 7 Hz, C_2-CH_3), 2.49–2.51 (m, CH_2, and DMSO-d_6), 2.87 (2H, t, J = 7 Hz, CH_2), 3.83 (1H, q, J = 7 Hz, spirodecane C_2-H), 7.17–7.29 (5 H, m, phenyl $C_{2\text{-}6}$-H), 9.97 (1H, s, NH). Anal. calcd. for $C_{20}H_{28}N_2O_2S$ (360.51): C: 66.63, H: 7.83, N: 7.77. Found: C: 66.68, H: 7.55, N: 7.90.

***N*-(2-Methyl-3-oxo-8-propyl-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8m)**

Yield: 93%. mp: 157–159°C; IR (KBr) υ (cm^{-1}): 3388, 3188 (N-H), 1724 (C=O), 1670 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 0.86 (3H, t, J = 7 Hz, CH_3), 0.99–1.61 (9H, m, spirodecane $C_{6\text{-}10}$), 1.27 (2H, m, spirodecane C_8-CH_2), 1.37 (3H, d, J = 7 Hz, C_2-CH_3), 2.49–2.52 (m, CH_2, and DMSO-d_6), 2.86 (2H, t, J = 7 Hz, CH_2), 3.82 (1H, q, J = 7 Hz, spd C_2-H), 7.17–7.29 (5 H, m, phenyl $C_{2\text{-}6}$-H), 9.96 (1 H, s, NH). Anal. calcd. for $C_{21}H_{30}N_2O_2S$ (374.54): C: 67.34, H: 8.07, N: 7.48. Found: C: 67.21, H: 7.76, N: 7.57.

***N*-(2-Methyl-8-*tert*-butyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8n)**

Yield: 100%. mp: 122–123°C; IR (KBr) υ (cm^{-1}): 3385, 3125 (N-H), 1701 (C=O), 1668 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 0.69–1.62 (9H, m, spirodecane $C_{6\text{-}10}$), 0.82 (9H, s, CH_3), 1.37 (3H, d, J = 7 Hz, C_2-CH_3), 2.49–2.52 (m, CH_2 and DMSO-d_6), 2.87 (2H, t, J = 7 Hz, CH_2), 3.82 (1H, q, J = 7 Hz, spirodecane C_2-H), 7.17–7.29 (5H, m, phenyl $C_{2\text{-}6}$-H), 9.96 (1H, s, NH). Anal. calcd. for $C_{22}H_{32}N_2O_2S.H_2O$ (406.56): C: 64.93, H: 8.36, N: 6.88. Found: C: 64.73, H: 8.16, N: 6.98.

***N*-[2-Methyl-3-oxo-(8-trifluoromethyl)-1-thia-4-azaspiro[4.5]-decan-4-yl]-3-phenylpropanamide (8o)**

Yield: 95%. mp: 126–130°C; IR (KBr) υ (cm^{-1}): 3390, 3196 (N-H), 1697 (C=O), 1660 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 1.27–2.09 (9H, m, spirodecane $C_{6\text{-}10}$), 1.39 (3H, d, J = 7 Hz, C_2-CH_3), 2.47–2.53 (m, CH_2 and DMSO-d_6), 2.88 (2H, t, J = 7 Hz, CH_2), 3.89 (1H, q, J = 7 Hz, spirodecane C_2-H), 7.15–7.31 (5H, m, phenyl $C_{2\text{-}6}$-H), 10.06 (1H, s, NH). Anal. calcd. for $C_{19}H_{23}F_3N_2O_2S$ (418.45): C: 54.53, H: 6.02, N: 6.69. Found: C: 54.61, H: 6.11, N: 6.91.

***N*-(2-Methyl-8-phenyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-3-phenylpropanamide (8p)**

Yield: 96%. mp: 206–207°C; IR (KBr) υ (cm^{-1}): 3350, 3182 (N-H), 1720 (C=O), 1668 (NHC=O). ^{1}H-NMR (DMSO-d_6/500 MHz): 1.04–2.31 (9H, m, spirodecane $C_{6\text{-}10}$), 1.41 (3H, d, J = 7 Hz, C_2-CH_3), 2.49–2.56 (m, CH_2, and DMSO-d_6), 2.90 (2 H, t, J = 7 Hz, CH_2), 3.89 (1H, q, J = 7 Hz, spirodecane C_2-H), 7.19–7.32 (10H, m, phenyl-CH), 10.05 (1H, s, NH). Anal. calcd. for $C_{24}H_{28}N_2O_2S$ (408.55): C: 70.55, H: 6.91, N: 6.86. Found: C: 70.57, H: 6.79, N: 6.94.

4.2 | Antiviral activity assays

Cytopathic effect (CPE) reduction assays were performed to determine antiviral activity against human coronavirus 229E and influenza A/H1N1, A/H3N2, and B virus. The HCoV-229E stock was obtained from ATCC (VR-740™) and expanded in human embryonic lung (HEL) 299 fibroblast cells (ATCC CCL-137™). For the CPE reduction assay, HEL cells were seeded in 96-well plates and grown for 6 days until they reached confluence.

The influenza virus strain A/HK/7/87 (A/H3N2) was a kind gift from J. Neyts (Leuven, Belgium), whereas the clinical isolates A/Ned/378/05 (A/H1N1) and B/Ned/537/05 were generously provided by R. Fouchier (Rotterdam, The Netherlands). Virus stocks were prepared in 10-day-old embryonated hen eggs; the clinical isolates first underwent one passage in eggs. The influenza CPE reduction assays were performed in Madin-Darby canine kidney (MDCK) cells (a kind gift from M. Matrosovich, Marburg, Germany) which were seeded 1 day before infection at 7500 cells per well in 96-well plates.

The cells were infected with the respective viruses at a multiplicity of infection of 100 (HCoV-229E) or 50 (influenza virus) "50% cell culture infective doses" ($CCID_{50}$) per well in the presence of serial dilutions of the test compounds. After five (HCoV-229E) or three (influenza virus) days incubation at 35°C, virus-induced CPE and compound cytotoxicity (in uninfected cells) were determined by microscopic scoring. Antiviral activity was expressed as EC_{50} or concentration showing 50% effectivity; cytotoxicity was expressed as MCC, that is, minimum cytotoxic concentration producing minimal changes in cell morphology. For HCoV-229E, **K22** [(*Z*)-*N*-[3-[4-(4-bromophenyl)-4-hydroxypiperidin-1-yl]-3-oxo-1-phenylprop-1-en-2-yl]benzamide; purchased from ChemDiv][14] was included as a reference compound. The following reference compounds were included for influenza virus: ribavirin (Virazole from ICN Pharmaceuticals, Costa, CA), rimantadine and amantadine (both from Sigma-Aldrich, Belgium). Compounds showing anti-HCoV activity were evaluated in 3–4 independent experiments.

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CONFLICT OF INTERESTS

The authors declared that they have no conflict of interests.

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