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3,4-Dihydropyrimido(1,2-*a*)indol-10(2*H*)-ones as potent non-peptidic inhibitors of caspase-3

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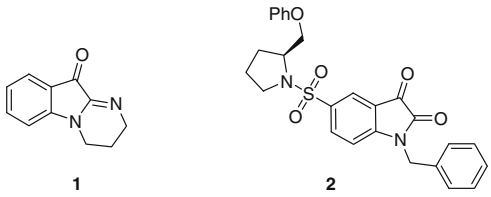
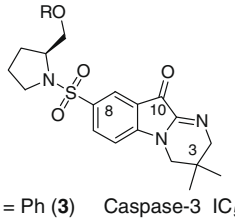
ABSTRACT

Cysteine-dependant aspartyl protease (caspase) activation has been implicated as a part of the signal transduction pathway leading to apoptosis. It has been postulated that caspase-3 inhibition could attenuate cell damage after an ischemic event and thereby providing for a novel neuroprotective treatment for stroke. As part of a program to develop a small molecule inhibitor of caspase-3, a novel series of 3,4-dihydropyrimido(1,2-*a*)indol-10(2*H*)-ones (pyrimidoindolones) was identified. The synthesis, biological evaluation and structure–activity relationships of the pyrimidoindolones are described.

1. Introduction

Cysteine-dependant aspartyl proteases (caspases) have been implicated in the signal transduction cascade leading to apoptosis. The 11 known human caspases can be divided into three sub-types based on their structure and function.[1,2] Group I caspases (1, 4, 5 and 14) are primarily involved in inflammation. Group II caspases (6, 8, 9 and 10) are primary involved in apoptosis as upstream regulators of the Group III caspases (3 and 7). The Group III caspases are effector caspases that, once activated, stimulate a signalling pathway that ultimately leads to the death of the cell. Over the past decade, evidence has emerged that caspase inhibition can provide for tissue protection and reduce infarct volume in rodent models of ischemia.[3–8] While these studies have been carried out using primarily peptide-based broad spectrum caspase inhibitors, more recently, there has been a focus on the identification of selective, non-peptidic caspase inhibitors. Multiple groups have reported their efforts toward selective, small molecule caspase-3 inhibitors[9–14] and one of these compounds has been shown to reduce tissue damage in an isolated rabbit heart model of ischemia injury.[15,16] A key structural feature of almost all known caspase inhibitors is an electrophilic group that can form a reversible or irreversible bond with the active site cysteine leading to the inactivation of the enzyme. In an effort to identify potent, selective caspase-3 inhibitors as potential therapeutic treatments for ischemic stroke, we undertook an extensive structure–activity based optimization of several key leads identified from our compound collection.





R = Ph (**3**) Caspase-3 IC_{50} = 7 nM
R = CH_3 (**4**) Caspase-3 IC_{50} = 6 nM

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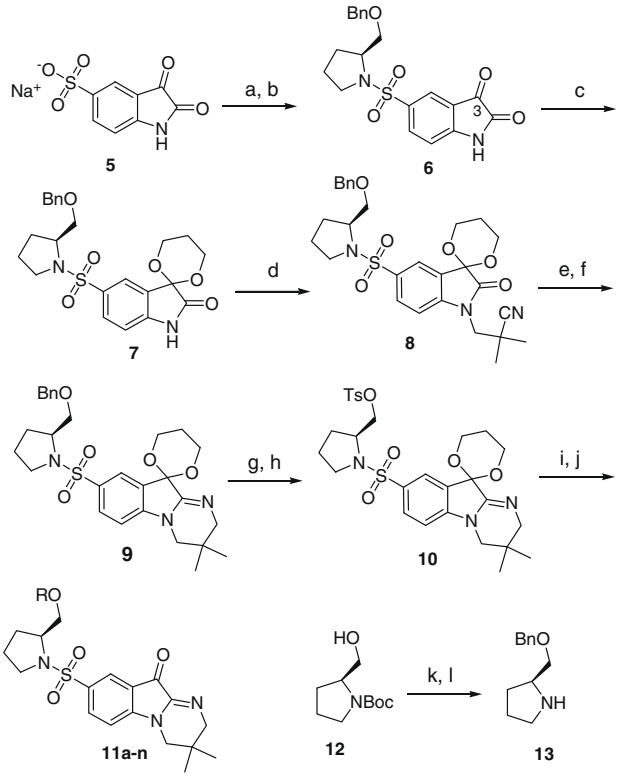
Through a high throughput screen of our compound library we identified **1** as a singleton hit for caspase-3 inhibition. Modification in our exploratory chemistry group and recognition of the possible similarity to a previously reported series of caspase-3 inhibitors (**2**)[9] allowed us to identify a lead series of novel 3,4-dihydropyrimido(1,2-*a*)indol-10(2*H*)-ones (pyrimidoindolones) (**3** and **4**) as potent and selective inhibitors of caspase-3.[17] An X-ray crystal structure of **3** bound to human caspase-3 (Fig. 1)[18] was obtained and revealed that a covalent bond forms between the electrophilic 10-position ketone of **3** and the nucleophilic active site cysteine of the activated caspase-3 in the enzyme–inhibitor complex. Using this information, we began to optimize this series of compounds. Herein, we describe the synthesis and structure–activity relationships of this novel series of pyrimidoindolones.

2. Chemistry

A general approach to the preparation of phenoxy analogs is shown in Scheme 1. 5-Chlorosulfonylisatin was prepared as previously reported[19] by treating sodium 5-isatin sulfonate **5** with phosphorus oxychloride in sulfolane at 60 °C. The resulting sulfonyl chloride was reacted with (*S*)-2-(benzyloxymethyl)-pyrrolidine (**13**) in the presence of Hunig's base to give sulfonamide **6**. The 3-position carbonyl was protected as a ketal and subsequently alkylated with 3-chloro-2,2-dimethyl-propionitrile[20] to give nitrile **8**. Hydrogenation with Raney nickel followed by heating of the resultant primary amine in a sealed tube yielded the protected pyrimidoindolone **9**. The benzyl protecting group was removed under phase transfer hydrogenolysis conditions and a tosylate group was installed to afford the common intermediate **10**, through which the first key analogs were prepared. Reaction of **10** with substituted phenols or other heterocyclic phenols followed by deprotection of the ketal gave target analogs **11a–n**. Tosylate intermediate **10** could also be used to prepare amino analogs **14a–d** as shown in Scheme 2 by heating with various amines in THF followed by the usual ketal deprotection.

In an effort to investigate the substitution of the sulfonyl moiety for a carbonyl, the construction of the corresponding amide derivatives of **3** and **4** was undertaken (Scheme 3). A similar approach as above was taken to build up the pyrimidoindolone core starting with commercially available 5-bromoisatin (**15**). An exocyclic vinyl group was then introduced using a palladium-mediated Stille coupling. Ozonolysis followed by saponification of the resultant ester yielded carboxylic acid **18**, which was then routinely coupled with



Scheme 1. Reagents and conditions: (a) $POCl_3$, sulfolane, 60 °C, 3 h, 66%; (b) **13**, DIPEA, CH_2Cl_2, rt, 1 h, 78%; (c) 1,3-propanediol, *p*TsOH, PhH, reflux, 14 h, 90%; (d) 3-chloro-2,2-dimethyl-propionitrile, KO*t*-Bu, DMSO, 132 °C, 21 h, 99%; (e) H_2, Ra/Ni, 2 M NH_3 in EtOH/THF, rt, 22 h; (f) 135 °C (sealed tube), 2 M NH_3 in EtOH, 18 h, 60% (two steps); (g) cyclohexadiene, 10% Pd/C, EtOH, reflux, 2 d, 72%; (h) *p*TsCl, DIPEA, DMAP, CH_2Cl_2, rt, 2 d, 97%; (i) NaH, ROH, THF/DMF (1/1), 100 °C, o/n; (j) methanesulfonic acid, CH_2Cl_2, 50 °C, o/n, 16–70% (two steps); (k) NaH, BnBr, THF, rt, 17 h, 86%; (l) TFA, CH_2Cl_2, 0 °C, 1 h, 92%.

(*S*)-2-(methoxymethyl)-pyrrolidine or (*S*)-2-(phenoxymethyl)-pyrrolidine.[9] Deprotection of the ketal, as before, provided the desired amides **19a–b**.

The corresponding reverse sulfonamide analogs were prepared starting from 5-nitroisatin (**20**) as shown in Scheme 4. Once again, the 3-carbonyl was protected and the nitrogen was alkylated with 3-chloro-2,2-dimethyl-propionitrile. Hydrogenation under Raney

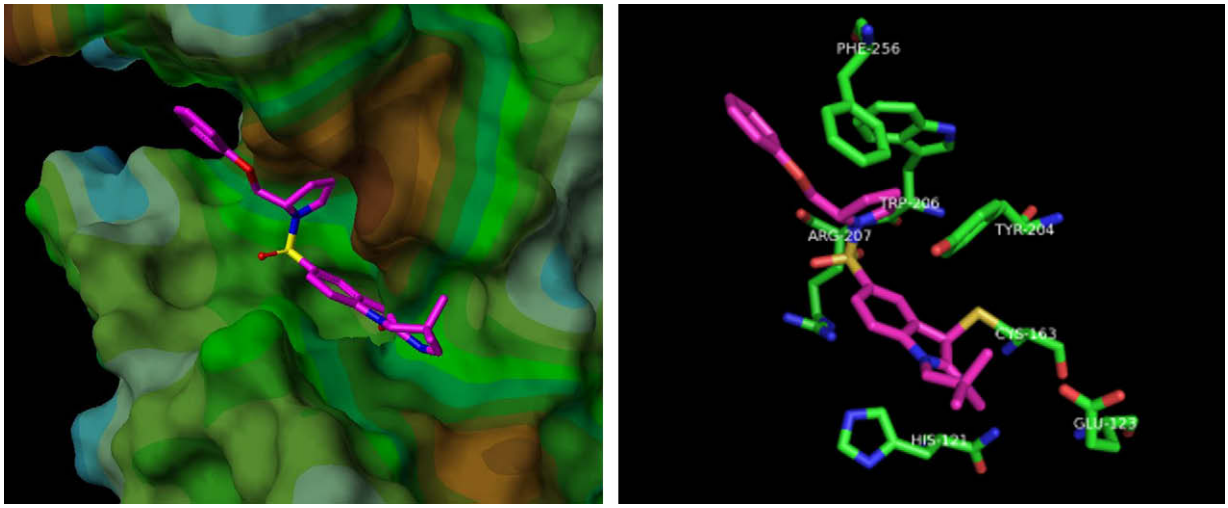
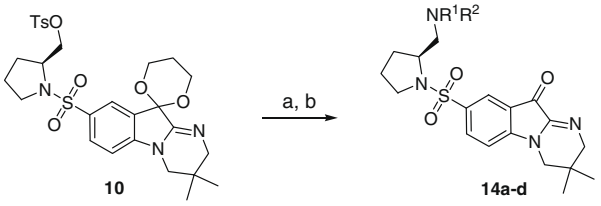
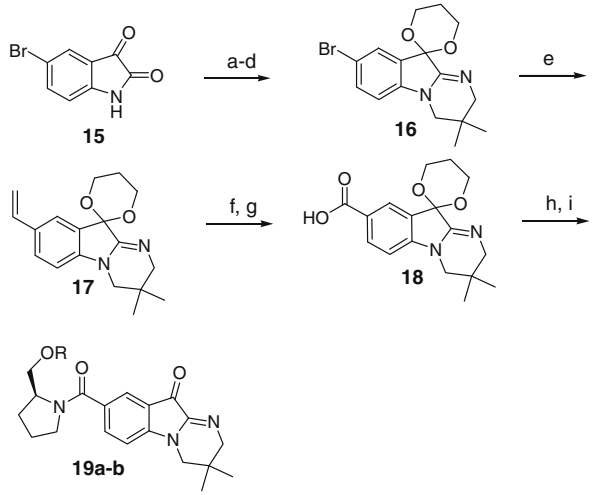


Figure 1. X-ray co-crystal of **3** with human caspase-3 (left) and with the residues within 5 Å illustrated (right).

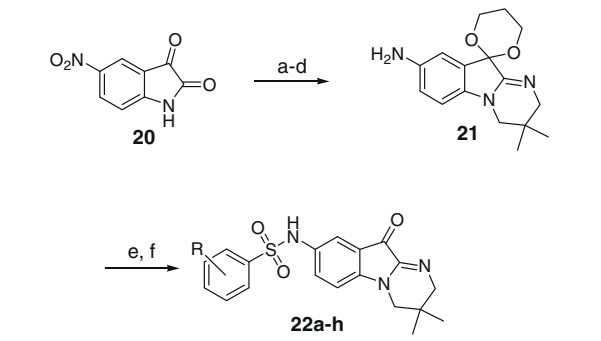
nickel catalysis transformed the nitrile to the primary amine with concomitant reduction of the 5-nitro group. Heating in a sealed tube in 2 M NH_3 in EtOH provided the protected pyrimidoindolone **21**. Compound **21** could be treated with a variety of sulfonyl chlorides to give the corresponding sulfonamides. The target reverse sulfonamides **22a–h** were obtained following the usual ketal deprotection.



Scheme 2. Reagents and conditions: (a) NHR^1R^2, THF, 100 °C, 2 d; (b) methanesulfonic acid, CH_2Cl_2, rt, o/n, 36–76% (two steps).



Scheme 3. Reagents and conditions: (a) 1,3-propanediol, *p*TsOH, PhH, reflux, 14 h, 85%; (b) 3-chloro-2,2-dimethyl-propionitrile, KO*t*-Bu, DMSO, 135 °C, 21 h, 82%; (c) H_2, Ra/Ni, 2 M NH_3 in EtOH/THF, 22 h; (d) 135 °C (sealed tube), 2 M NH_3 in EtOH, 18, 85% (two steps); (e) vinyl Sn(*n*Bu)$_3$, Pd(PPh$_3$)$_4$, dioxane, 100 °C, 6 h, 79%; (f) O_3, 2.5 N NaOH, MeOH, −60 °C, 15 m, 54%; (g) 1 N NaOH, H_2O, THF/EtOH, reflux, 1 h, 50%; (h) (*S*)-2-(methoxymethyl)-pyrrolidine or (*S*)-2-(phenoxymethyl)-pyrrolidine, DCC, HOBT, TEA, CH_2Cl_2, rt, o/n; (i) methanesulfonic acid, CH_2Cl_2, rt, o/n, 20–26% (two steps).



Scheme 4. Reagents and conditions: (a) 1,3-propanediol, *p*TsOH, PhH, reflux, 14 h, 96%; (b) 3-chloro-2,2-dimethyl-propionitrile, KO*t*-Bu, DMSO, 180 °C, 21 h, 90%; (c) H_2, Ra/Ni, 2 M NH_3 in EtOH/THF, 22 h; (d) 135 °C (sealed tube), 2 M NH_3 in EtOH, 18 h, 67% (two steps); (e) R-PhSO$_2$Cl, Et$_3$N, CH_2Cl_2, rt, 1 h; (f) methanesulfonic acid, CH_2Cl_2, rt, 15 h, 15–58% (two steps).

Spiroannulated pyrimidoindolone derivatives (**25a–f**) were prepared as shown in Scheme 5 by methods very similar to those described previously. The key step was the alkylation of **23** with the appropriate 2,2-spiroalkyl-3-chloro-propanenitriles (**26a–c**).[20]

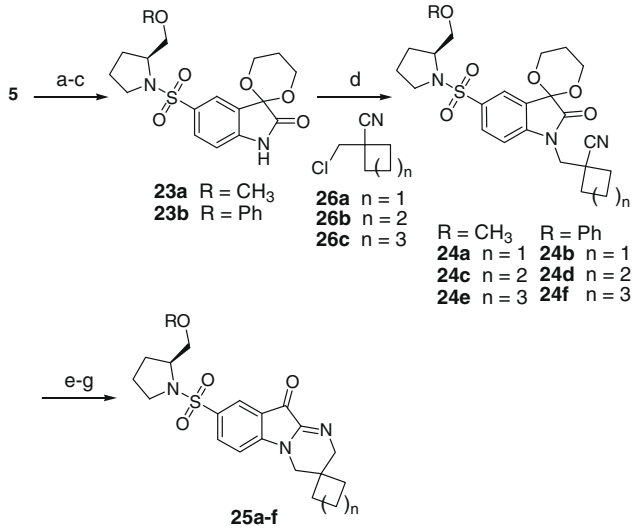
Once synthesized, all compounds were tested in a fluorescence based recombinant human caspase-3 assay for their ability to inhibit substrate (AcDEVD-AFC) cleavage by caspase-3 using a procedure that has been described previously.[21–23] Data are shown in Tables 1–5.

3. Results and discussion

Early in our caspase program, our SAR studies examined the sulfonamide region of the molecule with the goals of improving binding affinity and modifying the physicochemical properties of the lead compound. As our program became more advanced, we identified an issue with the aqueous stability of the pyrimidoindolone ring system itself and then focused on improvement of this moiety. These efforts are detailed below.

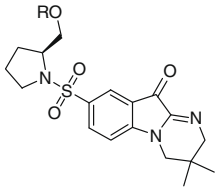
Initially, our SAR studies focused on the substitution at the phenoxy substituent of the pyrrolidine. Overall, multiple groups were generally well tolerated as seen in Table 1. From this examination, the optimal aryl groups identified were phenyl (**3**, IC_{50} = 7 nM), or the 4-substituted derivatives; 4-F-phenyl (**11a**, IC_{50} = 10.77 nM) and 4-CH_3O-phenyl (**11b**, IC_{50} = 13.83 nM). However, bulky groups at the 4-position, such as *t*-butyl (**11e**), resulted in a 10-fold reduction in potency from the parent compound **3**. Disubstituted analogs were prepared (entries **11f–g**), but, unfortunately, were 2–6-less potent as compared to the corresponding 4-monosubstituted analogs (e.g., **11a** vs **11f**, **11b** vs **11g**). In an attempt to improve the physical chemical properties, the 2- and 3-pyridyl (**11h–n**) analogs were prepared and were shown to be very potent with the advantage of increased solubility.[24]

Replacement of the phenoxy moiety with simple amines was also examined (Table 2) with the goal of improving solubility. These changes were also well tolerated with morpholino (**14a**) or *N*-carboxyethyl-piperazine (**14b**) proving to be the optimal substituents for enzyme inhibition. Similar to the pyridyl analogs, these amino-based analogs demonstrated improved physicochemical



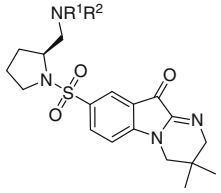
Scheme 5. Reagents and conditions: (a) $POCl_3$, sulfolane, 60 °C, 3 h, 66%; (b) (*S*)-2-(methoxymethyl)-pyrrolidine or (*S*)-2-(phenoxymethyl)-pyrrolidine, DIPEA, CH_2Cl_2, rt, 1 h, 62–93%; (c) 1,3-propanediol, *p*TsOH, PhH, reflux, 14 h, 45–62%; (d) **26a–c**, KO*t*-Bu, DMSO, 185 °C, 21 h, 72–92%; (e) H_2, Ra/Ni, 2 M NH_3 in EtOH/THF, 22 h; (f) 135 °C (sealed tube), 2 M NH_3 in EtOH, 18 h, 64–87% (two steps); (g) methanesulfonic acid, CH_2Cl_2, rt, 14 h, 48–68%.

Table 1
Phenoxy analogs



Compound	R	IC_{50} (nM)	*c* log *D* @ pH 7.4	Solubility @ pH 7.4 (μg/mL)
3	Phenyl	7	4.84	11
11a	4-F-phenyl	10.77 ± 0.49	5.06	0
11b	4-OCH_3-phenyl	13.83 ± 1.42	4.91	17
11c	4-Cl phenyl	37 ± 3	5.58	5
11d	4-Ac phenyl	30 ± 3	4.43	39
11e	4-*t*-Bu phenyl	81 ± 6	6.91	1
11f	4-F-3-CH_3 phenyl	23 ± 2	5.57	1
11g	4-OCH_3-2-Cl phenyl	82 ± 6	5.65	7
11h	2-Pyridyl	7.81 ± 0.55	3.47	13
11i	5-Cl 2-pyridyl	10.24 ± 0.52	4.21	28
11j	6-CH_3 2-pyridyl	12.57 ± 0.61	3.99	55
11k	3-Pyridyl	2.40 ± 0.13	3.47	>100
11l	5-Cl 3-pyridyl	4.43 ± 0.51	4.21	30
11m	2-CH_3 3-pyridyl	7.18 ± 0.59	3.99	66
11n	5-CO_2CH_3 3-pyridyl	10.14 ± 0.72	3.56	>100

Table 2
Amino analogs



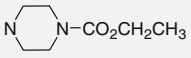
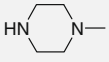
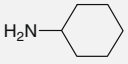
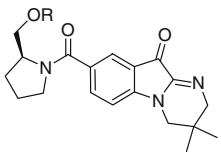
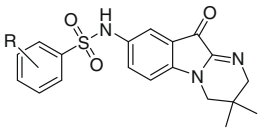
Compound	NR^1R^2	IC_{50} (nM)	*c* log *D* @ pH 7.4	Solubility @ pH 7.4 (μg/mL)
14a		4.99 ± 1.30	1.56	34
14b		14.32 ± 1.17	0.82	45
14c		43.63 ± 2.68	1.44	47
14d		59 ± 8	1.98	55

Table 3
Amide analogs



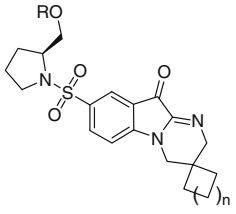
Compound	R	% Inhibition
19a	Ph	25% @ 50 μM
19b	CH_3	10% @ 1 μM

Table 4
Reverse sulfonamide analogs



Compound	R	IC_{50} (nM)
22a	H	364 ± 23
22b	2-F	148 ± 6
22c	3-CF_3	191 ± 9
22d	3-OCH_3	350 ± 58
22e	4-Cl	1338 ± 92
22f	4-OCH_3	1503 ± 159
22g	4-OCF_3	2524 ± 95
22h	4-F	2394 ± 140

Table 5
Spirocyclic analogs



Compound	R	*n*	IC_{50} (nM)	Stability: % Parent @ 24 h
25a	CH_3	1	3.30 ± 0.28	74
25b	Ph	1	1.29 ± 0.51	69
25c	CH_3	2	14.83 ± 1.68	87
25d	Ph	2	2.32 ± 0.32	89
25e	CH_3	3	13.61 ± 0.24	71
25f	Ph	3	24.63 ± 4.96	72

properties (solubility,[24] lower calculated log *D*[25]) than the parent compound, **3**.

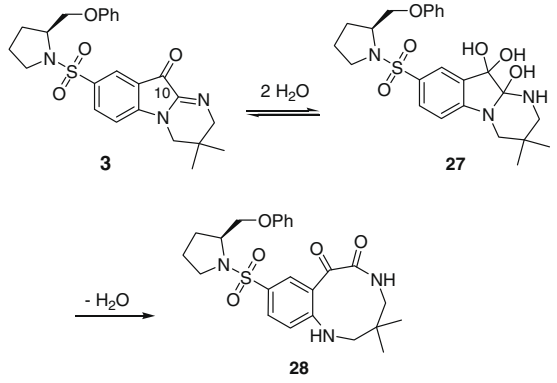
After determining that the phenoxy region was amenable to a variety of changes, we examined the role of the sulfonamide

moiety. The amide analogs of **3** (**19a**) or **4** (**19b**) (Table 3) were considerably less potent than the corresponding sulfonamides, thus limiting the breadth of this substitution, and further attempts to alter this moiety were halted.

3.1. Stability studies of compound 3

As work focused on the pyrimidoindolone series, the apparent stability of these compounds was questioned as key experiments showed the potency of several analogs substantially decreased over time under standard aqueous conditions.[26] Extensive NMR studies determined that the conversion (shown in Scheme 6) was occurring under neutral conditions and that as much as 30% of **3** rearranged after 24 h (Fig. 2). Under these conditions, it is hypothesized that the parent structure **3** undergoes the addition of two molecules of water to form the dihydrate intermediate **27**. Subsequent expulsion of a water molecule and opening to the nine-membered keto-amide structure **28** completes the transformation.

It was initially postulated that we could improve the stability of this class of molecules by modifying the electrophilicity of the carbonyl group of the isatin. However, this carbonyl is key to the inhibitory activity and must remain sufficiently electrophilic in order for the active site cysteine to form a covalent bond leading to inactivation of the enzyme. As observed with most caspase inhibitors, the formation of a covalent bond between an electrophilic group and the active site cysteine plays the primary role in the compound's ability to inhibit caspase-3. In exploring the electrophilic nature of this key moiety, we turned to an examination of the energetic characteristics of the carbonyl. The reactivity of the carbonyl in parent compound **3** is dependent on its LUMO energy. The lower the LUMO energy, the easier it is for nucleophiles such as water and cysteine sulfur to share electrons with the carbonyl carbon, thereby resulting in chemical reaction. The calculated LUMO of the 10-carbonyl group of the parent compound **3** is −30 kcal/mol. By proposing a series of reverse sulfonamides, we strove to retain the non-covalent interactions between the sulfonamide portion of the molecule and the enzyme while reducing the electrophilicity of the 10-carbonyl. The hope was that by modulating the electrophilicity of the ketone, the new entity would be resistant to intermolecular attack by water and subsequent ring expansion, but could still form a bond with the active site cysteine. Molecular modelling calculations determined the calculated LUMO of the ketone in the reverse sulfonamide **22a** to be −22 kcal/mol, a change of 8 kcal/mol. Modelling analysis of an overlay of compounds **3** and **22a** (Fig. 3) shows that the sulfonyl groups of both molecules can form similar hydrogen bond interactions with Arg-207. This study also suggests that the reverse sulfonamide can make additional favourable interactions in the binding pocket



Scheme 6. Conversion of **3** to **28** under standard aqueous conditions.

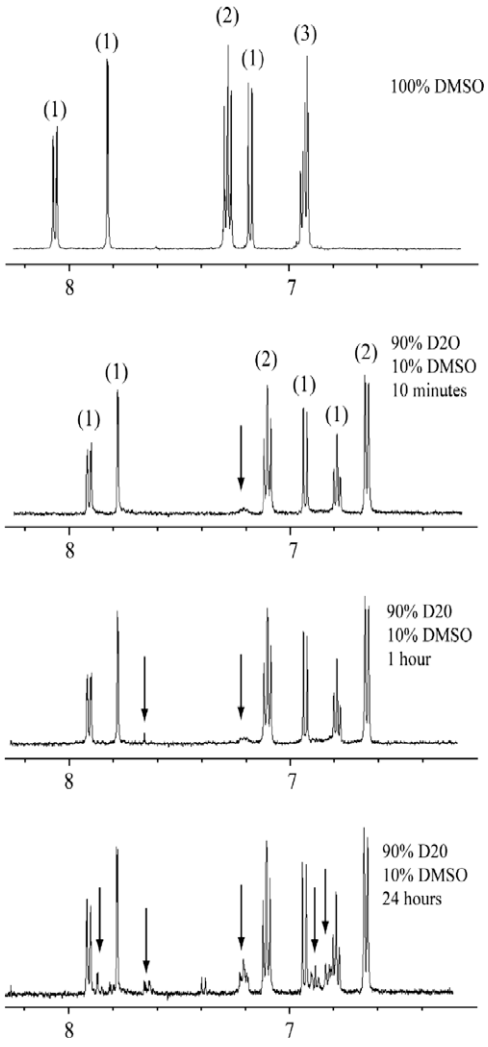


Figure 2. The time course NMR study illustrating the conversion of **3** to **28** via the intermediate **27**. Shown is the aromatic region of the proton NMR spectra with the integration values (in parenthesis) for each resonance in DMSO and for the first time interval of the time course study. The arrows indicate resonances arising from the formation of **28**. Resonances from other trace byproducts can also be observed.

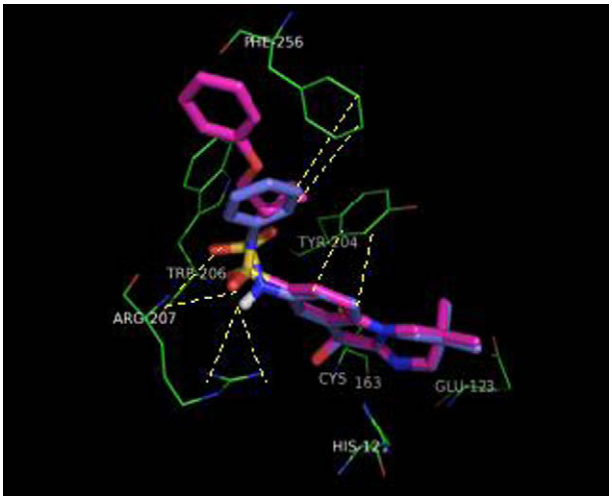


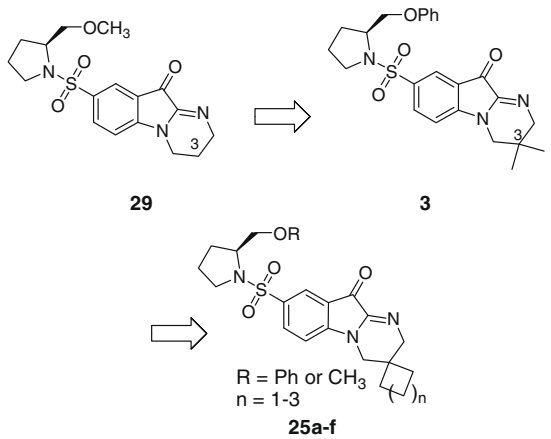
Figure 3. Molecular modelling overlay of compound **3** (magenta) and **22a** (violet).

such as an edge to face π–π interaction[27] between the phenyl ring of **22a** and Phe-256 and a hydrogen bond between the sulfonamide NH with the guanidine group of Arg-207.

Based on this modelling analysis, a series of reverse sulfonamides **22a–h** (Table 4) was prepared and evaluated. Although these compounds were not as potent as some of our earlier leads, we were gratified to see that we could dramatically reduce the electrophilicity of the carbonyl and maintain a reasonable level of caspase-3 inhibition. This study also revealed a clear preference for *ortho* or *meta* substitution on the aryl ring of the reverse sulfonamide. The most potent compounds from this series were the 2-F (**22b**, IC_{50} = 148 nM) and the 3-CF_3 (**22c**, IC_{50} = 191 nM) analogs. *para*-Substituted compounds **22e–h** showed a dramatic reduction in activity. It seems likely that substitution at the 4-position of the phenyl ring cannot be easily accommodated in the active site, leading to an unfavourable interaction and the observed reduction in binding affinity as compared to *ortho* or *meta* substitution. Selected members of the reverse sulfonamide series were chosen for NMR stability studies, however, these compounds showed no improvement in ring stability over the earlier lead **3**. This work indicated that despite dramatically reducing the electrophilicity of the 10-carbonyl group, hydration in the reversed sulfonamide series is still a facile event which ultimately leads to nine-member ring formation.

Based on the disappointing results for the reverse sulfonamides, we explored a different approach to improve the stability of the pyrimidoindolones (Scheme 7). NMR studies on early program leads that were unsubstituted at the 3-position, such as **29**, indicated that these compounds were extremely unstable in aqueous media, with none of the parent structure remaining after 24 h. Introduction of a gem-dimethyl group imparted improved stability at 24 h that we observed for compound **3**.[17] In an attempt to increase stability of the ring system by substitution at this position and to avoid metabolic stability liabilities, we chose to tie the methyl groups together to afford the spiroannulated structure **25**. A series of spiroannulated analogs (**25a–f**) investigating the effects of ring size were prepared and are shown in Table 5.

Spirocyclobutyl analogs with either methoxy-methyl pyrrolidine (**25a**) or phenoxy-methyl pyrrolidine (**25b**) sulfonamide substituents were some of the most potent compounds prepared with IC_{50} values of 3.30 and 1.29 nM, respectively. Unfortunately, they showed a stability profile similar to that of **3** with 69–74% of the parent structure remaining after 24 h. However, the spirocyclopentyl analogs with either methoxy (**25c**) or phenoxy (**25d**) substitution showed an improved stability profile with 87–89% of the parent structure remaining after 24 h in the NMR aqueous stability studies. Furthermore, these analogs maintained low nanomolar capsase-3 inhibitory activity. Somewhat surprisingly, the next ring size increment, the spirocyclohexyl analogs (**25e** or **25f**), while maintaining potency, did not improve stability over earlier leads.



Scheme 7. Spiroannulated analogs.

Attempts to determine the reason for the improved stability associated with the spirocyclopentyl analogs by molecular modelling calculations of the energy differences between the parents, the hydrated species and the open nine-membered degradation products have not yet led to definitive conclusions. However, we are continuing studies to order to understand this effect. Nevertheless, with the results of the SAR program and our ability to favourably impart stability to the ring system, this pyridoindolone lead series shows excellent promise as caspase-3 inhibitors.

4. Conclusion

In summary, we have described the synthesis and examined the structure–activity relationships for a series of pyrimidoindolone capsase-3 inhibitors. We determined that the phenoxy region was amenable to variety of substituents, which can be used to modify the physicochemical properties of the molecules. Additionally, the stability of this series under aqueous biological assay conditions was improved by the preparation of spiropentacyclic analogs **25c** and **25d** while maintaining low nanomolar caspase-3 inhibitory activity.

5. Experimental

5.1. General methods: chemistry

Melting points were determined on a Thomas–Hoover capillary or an Electrothermal melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Unity Plus 400 or a Varian 500 Unity INOVA spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) downfield from zero relative to the residual DMSO signal (2.49 ppm). Coupling constants are reported in hertz (Hz). Mass spectra were recorded on a Hewlett–Packard 5995A, a Finnigan Trace MS or a Micromass LCT spectrometer. C,H,N combustion analyses were determined on either a Perkin–Elmer 2400 analyzer or were performed by Robertson Microlit (Madison, NJ). Unless otherwise noted, reagents were obtained from commercial sources and were used without further purification. Chromatographic purifications were performed by flash chromatography using Baker 40-μm silica gel.

5.1.1. (*S*)-2-(Benzyloxymethyl)-pyrrolidine (13)

Step 1: To a slurry of sodium hydride (60%) (2.18 g, 54.50 mmol, 1.1 equiv) in THF (100 mL) was added (*S*)-1-(*tert*-butoxycarbonyl)-2-pyrrolidinemethanol (10 g, 49.68 mmol, 1 equiv) in THF (100 mL) and the mixture was stirred at room temperature for 1 h. Benzyl bromide (8.86 mL, 74.5 mmol, 1.5 equiv) was added and the reaction was stirred at room temperature overnight. It was then poured into brine and extracted with ethyl acetate. The combined organics were washed with brine, dried over magnesium sulfate and concentrated. The crude residue purified by column chromatography using ethyl acetate/hexanes (10/90) as an eluent to give (*S*)-*tert*-butyl 2-(benzyloxymethyl) pyrrolidine-1-carboxylate as a yellow oil (12.41 g, 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.32–7.21 (m, 5H) 4.44 (s, 2H) 3.82–3.71 (m, 1H) 3.49–3.39 (m, 1H) 3.33–3.23 (m, 1H) 3.20–3.1 (m, 2H) 1.90–1.63 (m, 4H) 1.23–1.40 (m, 9H).

Step 2: A solution of (*S*)-*tert*-butyl 2-(benzyloxymethyl) pyrrolidine-1-carboxylate (5.57 g, 19.11 mmol, 1 equiv) in CH_2Cl_2 (15 mL) was cooled to 0 °C and TFA (15 mL) was added. The reaction was stirred at room temperature for 1 h and then poured carefully into

1 N NaOH. The pH was adjusted to basic with 2.5 N NaOH and it was extracted with CH_2Cl_2. The combined organics dried over sodium sulfate and concentrated to afford the title compound as yellow oil (3.35 g, 92%), which was used crude in the following reaction. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.35–7.21 (m, 5H) 4.45 (s, 2H) 3.30–3.20 (m, 2H) 3.18–3.10 (m, 1H) 2.78–2.65 (m, 2H) 2.33 (br s, 1H) 1.73–1.66 (m, 1H) 1.61–1.52 (m, 2H) 1.34–1.27 (m, 1H).

5.1.2. General Procedure A—amidation—5′-({(2*S*)-2-[(benzyloxy)methyl]pyrrolidin-1-yl}sulfonyl)-1*H*-indole-2,3-dione (6)

Step 1: A mixture of isatin-5-sulfonic acid sodium salt dihydrate (**5**) (10.00 g, 35.1 mmol, 1 equiv) and phosphorous oxychloride (18.5 mL, 198 mmol, 5.6 equiv) in tetramethylene sulfone (50 mL) was heated at 60 °C for 3 h under a dry N_2 atmosphere. The reaction was cooled in an ice bath to 0 °C and water was cautiously added drop-wise, keeping the internal temperature below 6 °C. The resulting green solid was collected by filtration and was washed well with water. The solid was dissolved in ethyl acetate (200 mL) and washed again with water (3 × 50 mL), dried over magnesium sulfate, filtered and concentrated. The crude product was recrystallized from ethyl acetate/hexanes with hot filtration to give 2,3-dioxo-2,3-dihydro-1*H*-indole-5-sulfonyl chloride (5.81 g, 66%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.09 (s, 1H) 7.77 (dd, J = 8.1, 1.7 Hz, 1H) 7.55 (d, J = 1.6 Hz, 1H) 6.84 (d, J = 8.1 Hz, 1H).

Step 2: To a suspension of 2,3-dioxo-2,3-dihydro-1*H*-indole-5-sulfonyl chloride (9.33 g, 38.0 mmol, 1 equiv) in a 1:1 mixture of $CHCl_3$/THF (410 mL) was added drop-wise a solution of (*S*)-2-(benzyloxymethyl)-pyrrolidine (8.00 g, 41.8 mmol, 1.1 equiv) and *N,N*-diisopropylethylamine (12.2 mL, 70.0 mmol, 1.8 equiv) in $CHCl_3$ (63 mL) over 1.25 h with cooling in an ice bath under a dry N_2 atmosphere. The reaction was complete (by TLC) after stirring for 1 h at room temperature. The reaction was concentrated and purified on silica gel eluting with 50/50 pet ether/EtOAc to give the title compound as a bright orange solid (11.8 g, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.40 (br s, 1H) 7.98 (dd, J = 8.5, 2.0 Hz, 1H) 7.73 (d, J = 1.7 Hz, 1H) 7.36–7.24 (m, 5H) 7.04 (d, J = 8.2 Hz, 1H) 4.48 (s, 2H) 3.71–3.67 (m, 1H) 3.58–3.53 (m, 1H) 3.42–3.38 (m, 1H) 3.30–3.25 (m, 1H) 3.08–3.02 (m, 1H) 1.80–1.71 (m, 2H) 1.55–1.47 (m, 2H).

5.1.3. General Procedure B—ketalization—preparation of 5′-({(2*S*)-2-[(Benzyloxy)methyl]pyrrolidin-1-yl}sulfonyl)spiro[1,3-dioxane-2,3′-indol]-2′(1′*H*)-one (7)

A suspension of **6** (11.8 g, 29.4 mmol, 1 equiv), *p*-toluenesulfonic acid monohydrate (2.24 g, 11.8 mmol, 0.4 equiv) and 1,3-propanediol (8.63 mL, 119.4 mmol, 4 equiv) in benzene (527 mL) was refluxed for 14 h with a Dean Stark Trap. After cooling to room temperature, the reaction was washed with satd aq $NaHCO_3$ (3×), water (3×) and brine (3×), dried over Na_2SO_4, filtered and concentrated. The crude product was purified on silica gel eluting with 60/40 pet ether/EtOAc to give the title compound as a yellow foam (12.25 g, 90%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.88 (s, 1H) 7.77–7.72 (m, 1H) 7.57 (d, J = 1.9 Hz, 1H) 7.43 (d, J = 7.9 Hz, 1H) 7.33–7.25 (m, 5H) 4.74–4.67 (m, 2H) 4.47 (s, 2H) 3.96–3.87 (m, 2H) 3.67–3.60 (m, 1H) 3.55–3.52 (m, 1H) 3.40–3.30 (m, 1H) 3.27–3.20 (m, 1H) 3.00–2.90 (m, 1H) 2.25–2.10 (m, 1H) 1.80–1.70 (m, 2H) 1.65–1.57 (m, 1H) 1.50–1.40 (m, 2H). MS: (ES+) m/z 459.1 [M+H].

5.1.4. General Procedure C—alkylation—preparation of 3-[5′-({(2*S*)-2-[(Benzyloxy)methyl]pyrrolidin-1-yl}sulfonyl)-2′-oxo spiro[1,3-dioxane-2,3′-indol]-1′(2′*H*)-yl]-2,2-dimethyl propanenitrile (8)

To a solution of potassium *t*-butoxide (3.58 g, 31.9 mmol, 1.2 equiv) in anhydrous DMSO (72 mL) was added **7** (12.20 g, 26.6 mmol, 1 equiv) all at one time under a dry N_2 atmosphere. After stirring 20 min, 3-chloro-2,2-dimethylpropionitrile[20] (9.38 g, 79.8 mmol, 3 equiv) was added drop-wise and the reaction was heated at 132 °C for 21 h. After cooling in an ice bath, the reaction mixture was poured into H_2O and extracted with Et_2O. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The crude product was purified on Biotage KP silica gel using a step gradient of CH_2Cl_2/CH_3OH/NH_4OH (99.25/0.5/0.25 to 96/2/1) to give the title compound as a yellow solid (14.21 g, 99%yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.89 (dd, J = 8.5, 1.8 Hz, 1H) 7.68 (d, J = 1.8 Hz, 1H) 7.53 (d, J = 8.2 Hz, 1H) 7.40–7.23 (m, 5H) 4.73 (m, 2H) 4.50 (s, 2H) 4.01–3.91 (m, 4H) 3.75–3.65 (m, 1H) 3.57 (dd, J = 9.5, 4.0 Hz, 1H) 3.42 (dd, J = 9.3, 7.8 Hz, 1H) 3.29 (m, 1H) 3.09–2.99 (m, 1H) 2.33–2.18 (m, 1H) 1.83–1.73 (m, 2H) 1.68 (m, 1H) 1.56–1.45 (m, 2H) 1.38 (s, 6H).

5.1.5. General Procedure D—reduction/cyclization—8′-({(2*S*)-2-[(benzyloxy)methyl]pyrrolidin-1-yl}sulfonyl)-3′,3′-dimethyl-3′,4′-dihydro-2′*H*-spiro[1,3-dioxane-2,10′-pyrimido[1,2-*a*]indole] (9)

A mixture of **8** (3.00 g, 5.56 mmol, 1 equiv), wet Raney nickel (3.18 g) in 2 M NH_3 in EtOH (180 mL) and THF (30 mL) was hydrogenated at 54 psi for 22 h. After the reaction was filtered through Celite, the filtrate was poured into a sealed tube and heated to 132 °C for 18 h. After cooling to room temperature, the reaction was concentrated and purified on Biotage KP silica gel eluting with CH_2Cl_2/CH_3OH/NH_4OH (98.5/1/0.5) to give the title compound as a white solid (1.77 g, 60%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.77 (dd, J = 8.3, 1.9 Hz, 1H) 7.56 (d, J = 1.8 Hz, 1H) 7.44–7.13 (m, 5H) 6.91 (d, J = 8.2 Hz, 1H) 5.04 (m, 2H) 4.50 (s, 2H) 3.85–3.75 (m, 2H) 3.69–3.60 (m, 1H) 3.57 (dd, J = 9.3, 3.7 Hz, 1H) 3.40 (m, 1H) 3.28–3.18 (m, 5H) 3.05–2.90 (m, 1H) 2.17 (m, 1H) 1.82–1.68 (m, 2H) 1.57 (m, 1H) 1.53–1.42 (m, 2H) 0.95 (s, 6H).

5.1.6. {(2*S*)-1-[(3′,3′-Dimethyl-3′,4′-dihydro-2′*H*-spiro[1,3-dioxane-2,10′-pyrimido[1,2-*a*]indol]-8′-yl)sulfonyl]pyrrolidin-2-yl}methyl 4-methylbenzenesulfonate (10)

Step 1: A mixture of **9** (0.250 g, 0.480 mmol, 1 equiv) and 10% Pd/C (0.250 g) in EtOH (10 mL) was degassed for 20 min, then 1,4 cyclohexadiene (5 mL) was added and the mixture was refluxed for 2 days. The reaction was filtered through Celite and the filtrate was concentrated. The crude product was purified on Biotage KP silica gel eluting with acetone/hexane (30/70) to give {(2*S*)-1-[(3′,3′-dimethyl-3′,4′-dihydro-2′*H*-spiro[1,3-dioxane-2,10′-pyrimido[1,2-*a*]indol]-8′-yl)sulfonyl]pyrrolidin-2-yl}methanol as a white foam (0.150 g, 72%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.77 (dd, J = 8.2, 1.8 Hz, 1H) 7.57 (d, J = 1.8 Hz, 1H) 6.94 (d, J = 8.2 Hz, 1H) 5.10–4.99 (m, 2H) 4.83 (t, J = 5.8 Hz, 1H) 3.84–3.76 (m, 2H) 3.59–3.51 (m, 1H) 3.48–3.40 (m, 1H) 3.31–3.22 (m, 6H) 3.00–2.91 (m, 1H) 2.25–2.12 (m, 1H) 1.83–1.68 (m, 2H) 1.59 (m, 1H) 1.53–1.42 (m, 1H) 1.42–1.32 (m, 1H) 0.96 (s, 6H). MS: (ES+) m/z 436.1 [M+H].

Step 2: A solution of {(2*S*)-1-[(3′,3′-dimethyl-3′,4′-dihydro-2′*H*-spiro[1,3-dioxane-2,10′-pyrimido[1,2-*a*]indol]-8′-yl)sulfonyl]pyrrolidin-2-yl}methanol (0.100 g, 0.23 mmol, 1 equiv), *p*-toluenesulfonyl chloride (0.070 g, 0.34 mmol, 1.5 equiv), *N,N*-diisopropylethylamine (0.100 mL, 0.58 mmol, 2.5 equiv) and 4-(dimethylamino)pyridine (0.020 g, 0.07 mmol, 0.3 equiv) in CH_2Cl_2 (5 mL) was stirred at rt for 2 days. The reaction was poured into brine and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated to give the title compound as a white foam (0.132 g, 97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.81 (d, J = 8.3 Hz, 2H) 7.71 (dd, J = 8.4, 2.0 Hz, 1H) 7.54 (d, J = 2.0 Hz, 1H) 7.50 (d, J = 8.3 Hz, 2H) 6.92 (d, J = 8.4 Hz, 1H) 5.03 (m, 2H) 4.16–3.97 (m, 2H) 3.85–3.75 (m, 2H) 3.71–3.60 (m, 1H) 3.31 (s, 2H) 3.27–3.12 (m, 3H) 2.99–2.86 (m, 1H) 2.43 (s,

3H) 2.25–2.08 (m, 1H) 1.78–1.36 (m, 5H) 0.95 (s, 6H). MS: (ES+) *m*/*z* 590.3 [M+H].

5.1.7. 8-({(2*S*)-2-[(4-Methoxyphenoxy)methyl]pyrrolidin-1-yl} sulfonyl)-3,3-dimethyl-3,4-dihydropyrimido[1,2-*a*]indol-10 (2*H*)-one (11b)

To a solution of 4-methoxy-phenol (0.104 g 0.838 mmol, 1.4 equiv) in THF (5 mL) was added NaH (60% dispersion in mineral oil) (0.048 g, 1.200 mmol, 2 equiv) and the reaction was stirred at rt for 1 h. Compound **10** (0.352 g, 0.59 mmol, 1 equiv) in 1/1 THF /DMF (6 mL) was then added and the reaction heated overnight at 100 °C. The reaction was quenched with water and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered, concentrated and purified on silica gel eluting with acetone/hexane (30/70) to give the ketal intermediate as a white foam (0.258 g, 80%). 0.149 g of the resulting intermediate was dissolved in CH_2Cl_2 (5 mL), methanesulfonic acid (5 mL) was added and the solution was stirred at 50 °C overnight. The reaction mixture was poured onto ice, basified to pH 11 with ammonium hydroxide and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered, concentrated and purified on silica gel eluting with acetone/hexane (30/70) to give the title compound as a light yellow foam (0.064 g, 48%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.05 (dd, J = 8.5, 1.8 Hz, 1H) 7.81 (d, J = 1.8 Hz, 1H) 7.17 (d, J = 8.5 Hz, 1H) 6.84 (s, 4H) 4.02 (dd, J = 9.3, 2.9 Hz, 1H) 3.95–3.78 (m, 2H) 3.68 (s, 3H) 3.45 (s, 2H) 3.42–3.33 (m, 3H) 3.19–3.04 (m, 1H) 1.90–1.80 (m, 2H) 1.73–1.46 (m, 2H) 0.97 (s, 6H). MS: (ES+) *m*/*z* 484.1 [M+H]. HRMS calcd for $C_{25}H_{29}N_3O_5S$ [M+H]: 484.1900; found: 484.1898.

5.1.8. 8-({(2*S*)-2-[(4-Fluorophenoxy)methyl]pyrrolidin-1-yl} sulfonyl)-3,3-dimethyl-3,4-dihydropyrimido[1,2-*a*]indol-10 (2*H*)-one (11a)

The title compound was prepared as yellow foam in 55% yield from **10** and 4-fluorophenol according to the procedure for **11b**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.05 (dd, J = 8.5, 2.0 Hz, 1H) 7.81 (d, J = 2.0 Hz, 1H) 7.17 (d, J = 8.5 Hz, 1H) 7.14–7.04 (m, 2H) 6.98–6.85 (m, 2H) 4.05 (dd, J = 9.4, 3.2 Hz, 1H) 3.98–3.91 (m, 1H) 3.90–3.81 (m, 1H) 3.45 (s, 2H) 3.40 (s, 2H) 3.39–3.33 (m, 1H) 3.18–3.07 (m, 1H) 1.95–1.75 (m, 2H) 1.73–1.50 (m, 2H) 0.98 (s, 6H).MS: (ES+) *m*/*z* 472.2 [M+H]. HRMS calcd for $C_{24}H_{26}FN_3O_4S$ [M+H]: 472.1700; found: 472.1697.

5.1.9. 8-({(2*S*)-2-[(4-Chlorophenoxy)methyl]pyrrolidin-1-yl} sulfonyl)-3,3-dimethyl-3,4-dihydropyrimido[1,2-*a*]indol-10 (2*H*)-one (11c)

The title compound was prepared as brown foam in 52% yield from **10** and 4-chlorophenol according to the procedure for **11b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.06 (dd, J = 8.5, 1.6 Hz, 1H) 7.82 (d, J = 1.5 Hz, 1H) 7.31 (d, J = 8.8 Hz, 2H) 7.17 (d, J = 8.5 Hz, 1H) 6.94 (d, J = 8.8 Hz, 2H) 4.06 (dd, J = 9.6, 3.5 Hz, 1H) 3.97 (dd, J = 7.0, 2.4 Hz, 1H) 3.93–3.81 (m, 1H) 3.46 (s, 2H) 3.40 (s, 2H) 3.39–3.34 (m, 1H) 3.21–3.09 (m, 1H) 1.98–1.76 (m, 2H) 1.75–1.52 (m, 2H) 0.98 (s, 6H). MS: (ES+) *m*/*z* 488.1 [M+H]. HRMS calcd for $C_{24}H_{26}ClN_3O_4S$ [M+H]: 488.1405; found: 488.1408.

5.1.10. 8-({(2*S*)-2-[(4-Acetylphenoxy)methyl]pyrrolidin-1-yl} sulfonyl)-3,3-dimethyl-3,4-dihydropyrimido[1,2-*a*]indol-10(2*H*)-one (11d)

The title compound was prepared as yellow foam in 46% yield from **10** and 4-acetylphenol according to the procedure for **11b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.06 (dd, J = 8.5, 1.8 Hz, 1H) 7.90 (d, J = 8.8 Hz, 2H) 7.81 (d, J = 1.8 Hz, 1H) 7.16 (d, J = 8.5 Hz, 1H) 7.01 (d, J = 8.8 Hz, 2H) 4.28–4.02 (m, 2H) 3.99–3.82 (m, 1H) 3.44 (s, 2H) 3.42–3.34 (m, 3H) 3.21–3.10 (m, 1H) 2.50 (s, 3H) 2.01–1.78 (m, 2H) 1.77–1.52 (m, 2H) 0.99 (s, 3H) 0.98 (s, 3H). MS: (ES+) *m*/*z* 496.1 [M+H]. HRMS calcd for $C_{26}H_{29}N_3O_5S$: 496.1901; found: 496.1902.

5.1.11. 8-({(2*S*)-2-[(4-*tert*-Butylphenoxy)methyl]pyrrolidin-1-yl}sulfonyl)-3,3-dimethyl-3,4-dihydropyrimido[1,2-*a*]indol-10 (2*H*)-one (11e)

The title compound was prepared as yellow foam in 40% yield from **10** and 4-*t*-butylphenol according to the procedure for **11b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.07 (dd, J = 8.4, 2.0 Hz, 1H) 7.83 (d, J = 1.8 Hz, 1H) 7.30 (d, J = 8.8 Hz, 2H) 7.20 (d, J = 8.5 Hz, 1H) 6.86 (d, J = 8.8 Hz, 2H) 4.08 (dd, J = 9.6, 3.5 Hz, 1H) 3.93 (m, 1H) 3.86 (m, 1H) 3.46 (s, 2H) 3.43–3.38 (m, 3H) 3.17–3.05 (m, 1H) 1.82 (m, 2H) 1.70–1.52 (m, 2H) 1.25 (s, 9H) 0.98 (s, 6H). MS: (ES−) *m*/*z* 508.2 [M−H]. HRMS calcd for $C_{28}H_{35}N_3O_4S$ [M+H]: 510.2421; found: 510.2420.

5.1.12. 8-({(2*S*)-2-[(4-Fluoro-3-methylphenoxy)methyl] pyrrolidin-1-yl}sulfonyl)-3,3-dimethyl-3,4-dihydropyrimido [1,2-*a*]indol-10(2*H*)-one (11f)

The title compound was prepared as light brown foam in 70% yield from **10** and 4-fluoro, 3-methylphenol according to the procedure for **11b**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.06 (dd, J = 8.5, 2.0 Hz, 1H) 7.82 (d, J = 2.0 Hz, 1H) 7.18 (d, J = 8.5 Hz, 1H) 7.03 (dd, J = 9.1, 9.1 Hz, 1H) 6.85 (dd, J = 6.1, 3.0 Hz, 1H) 6.79–6.64 (m, 1H) 4.00–4.12 (m, 1H) 3.97–3.92 (m, 2H) 3.47 (s, 2H) 3.44–3.35 (m, 3H) 3.21–3.08 (m, 1H) 2.20 (s, 3H) 1.99–1.76 (m, 2H) 1.75–1.51 (m, 2H) 0.99 (s, 6H). MS: (ES+) *m*/*z* 486.2 [M+H]. HRMS calcd for $C_{25}H_{28}FN_3O_4S$ [M+H]: 486.1857; found: 486.1855.

5.1.13. 8-({(2*S*)-2-[(2-Chloro-4-methoxyphenoxy)methyl] pyrrolidin-1-yl}sulfonyl)-3,3-dimethyl-3,4-dihydropyrimido [1,2-*a*]indol-10(2*H*)-one (11g)

The title compound was prepared as light brown foam in 33% yield from **10** and 2-chloro 4-methoxyphenol according to the procedure for **11b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.04 (dd, J = 8.5, 1.8 Hz, 1H) 7.80 (d, J = 1.8 Hz, 1H) 7.14 (d, J = 8.5 Hz, 1H) 7.04 (d, J = 9.0 Hz, 1H) 6.99 (d, J = 3.0 Hz, 1H) 6.85 (dd, J = 9.0, 2.9 Hz, 1H) 4.12–4.04 (m, 1H) 4.03–3.96 (m, 1H) 3.96–3.85 (m, 1H) 3.72 (s, 3H) 3.46 (s, 2H) 3.44–3.35 (m, 3H) 3.23–3.13 (m, 1H) 2.10–1.83 (m, 2H) 1.80–1.54 (m, 2H) 0.98 (s, 3H) 0.97 (s, 3H). MS: (ES+) *m*/*z* 518.1 [M+H]. HRMS calcd for $C_{25}H_{28}ClN_3O_5S$ [M+H]: 518.1511; found: 518.1507.

5.1.14. 3,3-Dimethyl-8-({(2*S*)-2-[(pyridin-2-yloxy)methyl] pyrrolidin-1-yl}sulfonyl)-3,4-dihydropyrimido[1,2-*a*]indol-10(2*H*)-one (11h)

The title compound was prepared as yellow foam in 20% yield from **10** and 2-pyridinol according to the procedure for **11b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.17 (m, 1H) 8.06 (dd, J = 8.6, 2.0 Hz, 1H) 7.84 (d, J = 1.7, 1H) 7.69 (m, 1H) 7.18 (d, J = 8.5, 1H) 6.97 (m, 1H) 6.77 (d, J = 8.4 Hz, 1H) 4.42 (dd, J = 10.7, 4.0 Hz, 1H) 4.18 (dd, J = 10.7, 7.4 Hz, 1H) 4.00–3.82 (m, 1H) 3.46 (s, 2H) 3.43 (s, 2H) 3.42–3.34 (m, 1H) 3.21–3.10 (m, 1H) 1.92–1.75 (m, 2H) 1.71–1.47 (m, 2H) 0.98 (s, 6H). MS: (ES−) *m*/*z* 453.2 [M−H]. HRMS calcd for $C_{23}H_{26}N_4O_4S$ [M+H]: 455.1745; found: 455.1748.

5.1.15. 8-[((2*S*)-2-{[(5-Chloropyridin-2-yl)oxy]methyl} pyrrolidin-1-yl)sulfonyl]-3,3-dimethyl-3,4-dihydropyrimido [1,2-*a*]indol-10(2*H*)-one (11i)

The title compound was prepared as a yellow foam in 16% yield from **10** and 5-chloro-2-pyridinol according to the procedure for **11b**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.21 (d, J = 2.7 Hz, 1H) 8.05 (dd, J = 8.5, 2.0 Hz, 1H) 7.83 (d, J = 1.9 Hz, 1H) 7.79 (dd, J = 8.8, 2.7 Hz, 1H) 7.17 (d, J = 8.5 Hz, 1H) 6.84 (d, J = 8.84 Hz, 1H) 4.39 (dd, J = 10.7, 4.1 Hz, 1H) 4.21 (dd, J = 10.7, 7.1 Hz, 1H) 3.93 (m, 1H) 3.46 (s, 2H) 3.40 (s, 2H) 3.37 (m, 1H) 3.17 (m, 1H) 1.83

(m, 2H) 1.68 (m, 1H) 1.57 (m, 1H) 0.97 (s, 6H). MS: (ES+) *m*/*z* 489.2 [M+H]. HRMS calcd for $C_{23}H_{25}ClN_4O_4S$ [M+H]: 489.1358; found: 489.1358.

5.1.16. 3,3-Dimethyl-8-[((2*S*)-2-{[(6-methylpyridin-2-yl) oxy]methyl}pyrrolidin-1-yl)sulfonyl]-3,4-dihydropyrimido[1,2-*a*]indol-10(2*H*)-one (11j)

The title compound was prepared as yellow foam in 32% yield from **10** and 6-methyl-2-pyridinol according to the procedure for **11b**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.06 (dd, J = 8.5, 2.0 Hz, 1H) 7.83 (d, J = 2.0 Hz, 1H) 7.55 (m, 1H) 7.18 (d, J = 8.5 Hz, 1H) 6.82 (d, J = 7.1 Hz, 1H) 6.55 (d, J = 8.3 Hz, 1H) 4.40 (dd, J = 10.5, 3.9 Hz, 1H) 4.14 (dd, J = 10.5, 7.6 Hz, 1H) 4.03–3.85 (m, 1H) 3.47 (s, 2H) 3.45–3.36 (m, 3H) 3.22–3.10 (m, 1H) 2.40 (s, 3H) 1.97–1.74 (m, 2H) 1.73–1.49 (m, 2H) 0.99 (s, 6H). MS: (ES+) *m*/*z* 469.2 [M+H]. HRMS calcd for $C_{24}H_{28}N_4O_4S$ [M+H]: 469.1904; found: 469.1902.

5.1.17. 3,3-Dimethyl-8-({(2*S*)-2-[(pyridin-3-yloxy)methyl] pyrrolidin-1-yl}sulfonyl)-3,4-dihydropyrimido[1,2-*a*]indol-10(2*H*)-one (11k)

The title compound was prepared as yellow foam in 39% yield from **10** and 3-pyridinol according to the procedure for **11b**. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.26 (d, J = 2.7 Hz, 1H) 8.16 (dd, J = 4.5, 1.3 Hz, 1H) 8.06 (dd, J = 8.5, 1.9 Hz, 1H) 7.82 (dd, J = 1.8 Hz, 1H) 7.39 (ddd, J = 8.4, 2.9, 1.4 Hz, 1H) 7.32 (dd, J = 8.4, 4.5 Hz, 1H) 7.17 (d, J = 8.5 Hz, 1H) 4.14 (dd, J = 9.8, 4.7 Hz, 1H) 4.06 (dd, J = 9.8, 6.9 Hz, 1H) 3.92 (m, 1H) 3.46 (s, 2H) 3.40 (s, 2H) 3.37 (m, 1H) 3.14 (m, 1H) 1.92–1.77 (m, 2H) 1.66 (m, 1H) 1.55 (m, 1H) 0.96 (s, 6H). MS: (ES−) *m*/*z* 453.2 [M−H]. HRMS calcd for $C_{23}H_{26}N_4O_4S$ [M+H]: 455.1745; found: 455.1748.

5.1.18. 8-[((2*S*)-2-{[(5-Chloropyridin-3-yl)oxy]methyl} pyrrolidin-1-yl)sulfonyl]-3,3-dimethyl-3,4-dihydropyrimido [1,2-*a*]indol-10(2*H*)-one (11l)

The title compound was prepared as yellow foam in 62% yield from **10** and 5-chloro-3-pyridinol according to the procedure for **11b**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.22 (dd, J = 11.7, 2.2 Hz, 2H) 8.06 (dd, J = 8.5, 2.0 Hz, 1H) 7.82 (d, J = 1.7 Hz, 1H) 7.59 (m, 1H) 7.17 (d, J = 8.5 Hz, 1H) 4.25–4.04 (m, 2H) 4.01–3.86 (m, 1H) 3.46 (s, 2H) 3.43–3.33 (m, 3H) 3.21–3.11 (m, 1H) 1.98–1.78 (m, 2H) 1.76–1.54 (m, 2H) 0.98 (s, 6H). MS: (ES+) *m*/*z* 489.1 [M+H]. HRMS calcd for $C_{23}H_{25}ClN_4O_4S$ [M+H]: 489.1358; found: 489.1357.

5.1.19. 3,3-Dimethyl-8-[((2*S*)-2-{[(2-methylpyridin-3-yl)oxy] methyl}pyrrolidin-1-yl)sulfonyl]-3,4-dihydropyrimido[1,2-*a*] indol-10(2*H*)-one (11m)

The title compound was prepared as yellow foam in 50% yield from **10** and 2-methyl-3-pyridinol according to the procedure for **11b**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.05 (dd, J = 8.5, 2.0 Hz, 1H) 7.98 (dd, J = 4.8, 1.1 Hz, 1H) 7.81 (d, J = 2.0 Hz, 1H) 7.29 (dd, J = 8.3, 1.2 Hz, 1H) 7.20–7.09 (m, 2H) 4.13–4.06 (m, 1H) 4.06–3.91 (m, 2H) 3.46 (s, 2H) 3.43–3.35 (m, 3H) 3.24–3.13 (m, 1H) 2.31 (s, 3H) 2.01–1.83 (m, 2H) 1.72 (m, 1H) 1.60 (m, 1H) 0.98 (s, 6H). MS: (ES+) *m*/*z* 468.2 [M+H]. HRMS calcd for $C_{24}H_{28}N_4O_4S$ [M+H]: 469.1904; found: 469.1903.

5.1.20. Methyl 5-({(2*S*)-1-[(3,3-dimethyl-10-oxo-2,3,4,10-tetrahydropyrimido[1,2-*a*]indol-8-yl)sulfonyl]pyrrolidin-2-yl} methoxy)nicotinate (11n)

The title compound was prepared as yellow foam in 40% yield from **10** and 5-hydroxynicotinic acid methyl ester according to the procedure for **11b**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.68 (d, J = 1.7 Hz, 1H) 8.49 (d, J = 2.9 Hz, 1H) 8.06 (dd, J = 8.5, 2.0 Hz, 1H) 7.84 (d, J = 2.0 Hz, 1H) 7.77 (dd, J = 2.9, 1.7 Hz, 1H) 7.14 (d, J = 8.5 Hz, 1H) 4.32–4.08 (m, 2H) 3.98–3.91 (m, 1H) 3.89 (s, 3H) 3.45 (s, 2H) 3.43–3.36 (m, 3H) 3.22–3.09 (m, 1H) 2.02–1.43 (m, 4H) 0.97 (s, 6H). MS: (ES+) *m*/*z* 513.2 [M+H]. HRMS calcd for $C_{25}H_{28}N_4O_6S$ [M+H]: 513.1802; found: 513.1799.

5.1.21. 8-({(2*S*)-2-[(Cyclohexylamino)methyl]pyrrolidin-1-yl} sulfonyl)-3,3-dimethyl-3,4-dihydropyrimido[1,2-*a*]indol-10 (2*H*)-one (14d)

To a solution of **10** (0.10 g, 0.17 mmol, 1 equiv) in THF (2 mL) was added cyclohexylamine (0.034 g, 0.34 mmol, 2 equiv) and the reaction was stirred at 70 °C overnight. Additional cyclohexylamine (0.088 g, 0.85 mmol, 5 equiv) was added to the reaction and stirred at 100 °C for 2 days. The reaction was quenched with water and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered, concentrated and purified on Biotage Si 12+M cartridge silica gel eluting with acetone/hexane (35/65). The resulting product was dissolved in CH_2Cl_2 (2 mL), methanesulfonic acid (2 mL) was added and the solution was stirred at rt overnight. The reaction mixture was poured onto ice, basified to pH 11 with ammonium hydroxide and extracted with EtOAc (3×). The combined organic extracts were dried over Na_2SO_4, filtered, concentrated and purified on Biotage Si 12+M cartridge silica gel eluting with acetone/hexane (35/65) to give the title compound as a yellow foam (0.036 g, 46%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.02 (d, J = 8.5 Hz, 1H) 7.80 (s, 1H) 7.20 (d, J = 8.5 Hz, 1H) 3.58–3.50 (m, 1H) 3.46 (s, 2H) 3.42 (s, 2H) 3.27–3.21 (m, 1H) 3.13–3.03 (m, 1H) 2.81–2.69 (m, 1H) 2.53 (m, 1H) 2.35 (m, 1H) 1.85–1.59 (m, 6H) 1.58–1.40 (m, 3H) 1.28–1.00 (m, 5H) 0.98 (s, 6H) NH proton not distinctively observed. MS: (ESI+) *m*/*z* 459.3 [M+H]. HRMS calcd for $C_{24}H_{34}N_4O_3S$ [M+H]: 459.2424; found: 459.2423.

5.1.22. 3,3-Dimethyl-8-{[(2*S*)-2-(morpholin-4-ylmethyl) pyrrolidin-1-yl]sulfonyl}-3,4-dihydropyrimido[1,2-*a*]indol-10 (2*H*)-one (14a)

The title compound was prepared as yellow foam in 65% yield from **10** and morpholine according to the procedure for **14d**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.04 (dd, J = 8.5, 1.9 Hz, 1H) 7.81 (d, J = 1.8 Hz, 1H) 7.18 (d, J = 8.5 Hz, 1H) 3.76–3.65 (m, 1H) 3.54 (m, 4H) 3.45 (s, 2H) 3.42 (s, 2H) 3.28–3.22 (m, 1H) 3.10–2.97 (m, 1H) 2.47–2.41 (m, 3H) 2.40–2.27 (m, 3H) 1.85–1.68 (m, 2H) 1.59–1.42 (m, 2H) 0.98 (s, 6H). MS: (ES+) *m*/*z* 447.2 [M+H]. HRMS calcd for $C_{22}H_{30}N_4O_4S$ [M+H]: 447.2061; found: 447.2062.

5.1.23. Ethyl 4-({(2*S*)-1-[(3,3-dimethyl-10-oxo-2,3,4,10-tetrahydropyrimido[1,2-*a*]indol-8-yl)sulfonyl]pyrrolidin-2-yl} methyl)piperazine-1-carboxylate (14b)

The title compound was prepared as yellow foam in 36% yield from **10** and ethyl piperazine-1-carboxylate according to the procedure for **14d**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.05 (dd, J = 8.4, 2.0 Hz, 1H) 7.82 (d, J = 2.0 Hz, 1H) 7.18 (d, J = 8.5 Hz, 1H) 4.02 (q, J = 7.1 Hz, 2H) 3.71 (m, 1H) 3.46 (s, 2H) 3.42 (s, 2H) 3.32 (m, 4H) 3.28–3.23 (m, 1H) 3.10–3.00 (m, 1H) 2.47–2.41 (m, 3H) 2.39–2.29 (m, 3H) 1.83–1.70 (m, 2H) 1.60–1.45 (m, 2H) 1.17 (t, J = 7.2 Hz, 3H) 0.98 (s, 6H). MS: (ES+) *m*/*z* 518.2 [M+H]. HRMS calcd for $C_{25}H_{35}N_5O_5S$ [M+H]: 518.2432; found: 518.2430.

5.1.24. 3,3-Dimethyl-8-({(2*S*)-2-[(4-methylpiperazin-1-yl) methyl]pyrrolidin-1-yl}sulfonyl)-3,4-dihydropyrimido[1,2-*a*] indol-10(2*H*)-one (14c)

The title compound was prepared as yellow foam in 76% yield from **10** and *n*-methyl piperazine according to the procedure for **14d**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.04 (dd, J = 8.5, 2.0 Hz, 1H) 7.81 (d, J = 2.0 Hz, 1H) 7.19 (d, J = 8.5 Hz, 1H) 3.77–3.59 (m, 1H) 3.46 (s, 2H) 3.42 (s, 2H) 3.28–3.20 (m, 1H) 3.11–2.97 (m, 1H) 2.47–2.17 (m, 10H) 2.13 (s, 3H) 1.84–1.63 (m, 2H) 1.51–1.43 (m,

2H) 0.97 (s, 6H). MS: (ES+) *m*/*z* 460.2 [M+H]. HRMS calcd for $C_{23}H_{33}N_5O_3S$ [M+H]: 460.2377; found: 460.2373.

5.1.25. 8′-Bromo-3′,3′-dimethyl-3′,4′-dihydro-2′*H*-spiro[1,3-dioxane-2,10′-pyrimido[1,2-*a*]indole] (16)

Step 1: 5′-Bromospiro[1,3-dioxane-2,3′-indol]-2′(1′*H*)-one was synthesized from 5-bromo isatin in 85% yield as a white solid according to General Procedure B. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.58 (br s, 1H) 7.52–7.39 (m, 2H) 6.77 (d, *J* = 8.2 Hz, 1H) 4.76–4.64 (m, 2H) 3.95–3.86 (m, 2H) 2.20–2.05 (m, 1H) 1.70–1.58 (m, 1H). MS: (ES) *m*/*z* 282.0 [M−H].

Step 2: 3-(5′-Bromo-2′-oxospiro[1,3-dioxane-2,3′-indol]-1′(2′*H*)-yl)-2,2-dimethylpropanenitrile was synthesized from 5′-bromospiro[1,3-dioxane-2,3′-indol]-2′(1′*H*)-one in 82% yield as a white solid according to General Procedure C. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.61 (dd, *J* = 8.5, 2.1 Hz, 1H) 7.52 (d, *J* = 2.1 Hz, 1H) 7.31 (d, *J* = 8.5 Hz, 1H) 4.76–4.59 (m, 2H) 3.99–3.91 (m, 2H) 3.87 (s, 2H) 2.25–2.08 (m, 1H) 1.73–1.63 (m, 1H) 1.36 (s, 6H). MS: (EI) *m*/*z* 364.0 [M+].

Step 3: The title compound was synthesized from 3-(5′-bromo-2′-oxospiro[1,3-dioxane-2,3′-indol]-1′(2′*H*)-yl)-2,2-dimethylpropanenitrile in 85% yield as a light brown solid according to General Procedure D. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.46 (dd, *J* = 8.4, 2.0 Hz, 1H) 7.37 (d, *J* = 1.8 Hz, 1H) 6.71 (d, *J* = 8.5 Hz, 1H) 5.01 (m, 2H) 3.76 (m, 2H) 3.21 (s, 2H) 3.18 (s, 2H) 2.23–1.98 (m, 1H) 1.57 (m, 1H) 0.93 (s, 6H). MS: (EI) *m*/*z* 350 [M−H].

5.1.26. 3′,3′-Dimethyl-8′-vinyl-3′,4′-dihydro-2′*H*-spiro[1,3-dioxane-2,10′-pyrimido[1,2-*a*]indole] (17)

To the stirred solution of **16** (2.576 g, 7.33 mmol, 1 equiv) in dioxane (70 mL) was added tributyl(vinyl) tin (3.490 g, 11.00 mmol, 1.5 equiv) and the solution was degassed with N_2 for 15 min. $Pd(PPh_3)_4$ (0.507 g, 0.439 mmol, 0.06 equiv) was added and the mixture was stirred at 100 °C for 6 h. The reaction was diluted with EtOAc, washed with brine and concentrated. The resulting residue was dissolved in EtOAc and stirred with 1 M aq potassium fluoride solution (20 mL) overnight. The reaction was filtered and the filtrate was washed with brine. The organics were dried over Na_2SO_4, concentrated and purified on a silica gel column eluting with acetone/hexane (10/90) to give the title compound as white solid (1.720 g, 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.37–7.32 (m, 2H) 6.67–6.59 (m, 2H) 7.63 (d, *J* = 17.5 Hz, 1H) 5.05–5.00 (m, 3H) 3.75–3.71 (m, 2H) 3.21 (s, 2H) 3.18 (s, 2H) 2.15–2.05 (m, 1H) 1.55–1.50 (m, 1H) 0.91 (s, 6H).

5.1.27. 3′,3′-Dimethyl-3′,4′-dihydro-2′*H*-spiro[1,3-dioxane-2,10′-pyrimido[1,2-*a*]indole]-8′-carboxylic acid (18)

Step 1: To a solution of **17** (0.687 g, 2.303 mmol, 1 equiv) in CH_2Cl_2 (15 mL), was added 2.5 N NaOH (7.5 mL) and MeOH (7.5 mL) and the solution was cooled to −60 °C. A stream of O_3 was bubbled into the reaction for 15 min until TLC indicated starting material was consumed. H_2O (20 mL) was then added to the reaction and it was extracted with EtOAc. The combined organics were dried over Na_2SO_4, filtered, concentrated and purified on a silica gel column eluting with acetone/hexane (10/90) to give methyl 3′,3′-dimethyl-3′,4′-dihydro-2′*H*-spiro[1,3-dioxane-2,10′-pyrimido[1,2-*a*]indole]-8′-carboxylate as a white solid (0.410 g, 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.94 (dd, *J* = 8.3, 1.7 Hz, 1H) 7.80 (d, *J* = 1.5 Hz, 1H) 6.85 (d, *J* = 8.5 Hz, 1H) 5.04 (m, 2H) 3.81 (s, 3H) 3.80–3.75 (m, 2H) 3.26 (s, 2H) 3.24 (s, 2H) 2.23–2.10 (m, 1H) 1.59 (m, 1H) 0.95 (s, 6H). MS: (ES+) *m*/*z* 331.2 [M+H].

Step 2: To a solution of methyl 3′,3′-dimethyl-3′,4′-dihydro-2′*H*-spiro[1,3-dioxane-2,10′-pyrimido[1,2-*a*]indole]-8′-carboxylate (0.200 g, 0.610 mmol, 1 equiv) in 1/1 THF /EtOH (8 mL) was added 1 N NaOH (2 mL) and H_2O (2 mL) and the reaction was refluxed for 1 h. The reaction was quenched with 1 N HCl and extracted with EtOAc (3×). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated to give the title compound as white solid (0.097 g, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.23 (br s, 1H) 7.91 (dd, *J* = 8.3, 1.7 Hz, 1H) 7.79 (d, *J* = 1.7 Hz, 1H) 6.81 (d, *J* = 8.3 Hz, 1H) 5.04 (m, 2H) 3.78 (m, 2H) 3.25 (s, 2H) 3.23 (s, 2H) 2.25–2.04 (m, 1H) 1.59 (m, 1H) 0.95 (s, 6H). MS: (ES+) *m*/*z* 317.3 [M+H].

5.1.28. 3,3-Dimethyl-8-{[(2*S*)-2-(phenoxymethyl)pyrrolidin-1-yl]carbonyl}-3,4-dihydropyrimido[1,2-*a*]indol-10(2*H*)-one (19a)

To a solution of **18** (0.090 g, 0.284 mmol, 1 equiv) in CH_2Cl_2 (7 mL) was added (*S*)-2-(phenoxymethyl)-pyrrolidine[9] (0.75 g, 0.423 mmol, 1.5 equiv), DCC (0.082 g, 0.397 mmol, 1.4 equiv), HOBT (0.038 g, 0.284 mmol, 1 equiv) and Et_3N (0.045 mL, 0.323 mmol, 1.1 equiv) and the solution was stirred overnight at rt. The reaction was filtered through 1 cm of silica gel washing with EtOAc. The filtrate was dried over Na_2SO_4, filtered, concentrated and purified on silica gel eluting with acetone/hexane (30/70). The resulting white foam was dissolved in CH_2Cl_2 (1.5 mL), methanesulfonic acid (1 mL) was added and the solution was stirred at rt overnight. The reaction was poured onto ice, basified to pH 11 with ammonium hydroxide and extracted with EtOAc (3×). The combined organic extracts were dried over Na_2SO_4, filtered, concentrated and purified on Biotage Si 12+M cartridge silica gel eluting with acetone/hexane (35/65) to give the title compound as a yellow foam (0.021 g, 20%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.74 (dd, *J* = 8.3, 1.2 Hz, 1H) 7.60 (d, *J* = 1.1 Hz, 1H) 7.26 (m, 2H) 7.01 (d, *J* = 8.3 Hz, 1H) 6.98–6.77 (m, 3H) 4.41 (m, 1H) 4.30–3.94 (m, 2H) 3.58–3.48 (m, 1H) 3.43 (m, 3H) 3.37 (s, 2H) 2.17–1.61 (m, 4H) 0.97 (s, 6H). (Resonance broadening due to amide rotamers). MS: (ES+) *m*/*z* 418.3 [M+H] HRMS calcd for $C_{25}H_{27}N_3O_3$ [M+H]: 418.2125; found: 418.2125.

5.1.29. 8-{[(2*S*)-2-(Methoxymethyl)pyrrolidin-1-yl]carbonyl}-3,3-dimethyl-3,4-dihydropyrimido[1,2-*a*]indol-10(2*H*)-one (19b)

The title compound was prepared as an orange foam in 26% yield from **18** and (*S*)-2-(methoxymethyl)-pyrrolidine according to the procedure for **17a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.76 (dd, *J* = 8.2, 1.6 Hz, 1H) 7.61 (d, *J* = 1.5 Hz, 1H) 7.04 (d, *J* = 8.3 Hz, 1H) 4.26–4.11 (m, 1H) 3.51–3.39 (m, 4H) 3.37–3.30 (s, 2H) 3.29–3.15 (m, 5H) 2.04–1.57 (m, 4H) 0.97 (s, 6H) (Resonance broadening due to amide rotamers). MS: (ES+) *m*/*z* 356.2 [M+H]. HRMS calcd for $C_{20}H_{25}N_3O_3$ [M+H]: 356.1969; found: 356.1967.

5.1.30. 3′,3′-Dimethyl-3′,4′-dihydro-2′*H*-spiro[1,3-dioxane-2,10′-pyrimido[1,2-*a*]indol]-8′amine (21)

Step 1: 5′-Nitrospiro[1,3-dioxane-2,3′-indol]-2′(1′*H*)-one was synthesized from 5-nitro isatin in 96% yield according to General Procedure B. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.17 (s, 1H) 8.26 (dd, *J* = 8.7, 2.4 Hz, 1H) 8.08 (d, *J* = 2.6 Hz, 1H) 7.02 (d, *J* = 8.6 Hz, 1H) 4.72 (m, 2H) 3.95 (m, 2H) 2.31–2.10 (m, 1H) 1.76–1.59 (m, 1H).

Step 2: 3-(5′-Nitro-2′-oxospiro[1,3-dioxane-2,3′-indol]-1′(2′*H*)-yl)-2,2-dimethylpropanenitrile was prepared in 90% yield from 5′-nitrospiro[1,3-dioxane-2,3′-indol]-2′(1′*H*)-one and 3-chloro-2,2-dimethylpropionitrile[20] following General Procedure C. ^{1}H NMR (400Mz, DMSO-d_6): δ ppm 8.35 (dd, *J* = 8.8, 2.4 Hz, 1H) 8.10 (d, *J* = 2.3 Hz, 1H) 7.57 (d, *J* = 8.8 Hz, 1H) 4.72–4.65 (m, 2H) 3.97 (m, 2H) 3.95 (s, 2H) 2.23 (m, 1H) 1.69 (m, 1H) 1.36 (s, 6H).

Step 3: The title compound was prepared in 67% yield from 3-(5′-nitro-2′-oxospiro[1,3-dioxane-2,3′-indol]-1′(2′*H*)-yl)-2,2-dimethylpropanenitrile following General Procedure D. ^{1}H NMR (400Mz, DMSO-d_6): δ ppm 6.62 (d, *J* = 2.2 Hz, 1H) 6.45 (dd, *J* = 8.1, 2.3 Hz, 1H) 6.37 (d, *J* = 8.1 Hz, 1H) 5.05 (m, 2H) 4.63 (s,

2H) 3.68 (m, 2H) 3.08 (s, 2H) 3.06 (s, 2H) 2.04 (m, 1H) 1.52 (m, 1H) 0.89 (s, 6H).

5.1.31. *N*-(3,3-Dimethyl-10-oxo-2,3,4,10-tetrahydropyrimido [1,2-*a*]indol-8-yl)benzenesulfonamide (22a)

To a solution of **21** (0.060 g, 0.21 mmol, 1 equiv) in CH_2Cl_2 (2 mL) was added Et_3N (0.060 mL, 0.43 mmol, 2.05 equiv) and benzenesulfonyl chloride (0.030 mL, 0.23 mmol, 1.07 equiv). The reaction was stirred at rt 1 h and concentrated. The crude residue purified by column chromatography using acetone/hexanes (30/70) as an eluent. The resulting white solid was dissolved in CH_2Cl_2 (4 mL) and methanesulfonic acid (2 mL) was added. The reaction was stirred at rt 15 h. and then poured into brine. It was basified to pH 10, saturated with solid NaCl and extracted with EtOAc. The combined organics were dried over Na_2SO_4 and concentrated. The crude residue purified by column chromatography using acetone/hexanes (40/60) as an eluent to give the title compound as a yellow foam (0.032 g, 58%). ^{1}H NMR (500Mz, DMSO-d_6): δ ppm 10.14 (br s, 1H) 7.70 (m, 2H) 7.61 (m, 1H) 7.55 (m, 2H) 7.13 (dd, J = 8.6, 2.2 Hz, 1H) 7.27 (d, J = 2.1 Hz, 1H) 6.91 (d, J = 8.5 Hz, 1H) 3.36 (s, 2H) 3.25 (s, 2H) 0.92 (s, 6H). MS: (ES−) m/z 368 [M−H]. HRMS calcd for $C_{19}H_{19}N_3O_3S$ [M+H]: 370.1220; found: 370.1217.

5.1.32. *N*-(3,3-Dimethyl-10-oxo-2,3,4,10-tetrahydropyrimido [1,2-*a*]indol-8-yl)-2-fluorobenzenesulfonamide (22b)

The title compound was prepared as a yellow foam in 17% yield from **21** and 2-fluorobenzenesulfonyl chloride according to the procedure for **22a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.45 (br s, 1H) 7.74 (m, 1H) 7.71–7.61 (m, 1H) 7.41 (m, 1H) 7.37–7.27 (m, 2H) 7.17 (d, J = 2.0 Hz, 1H) 6.91 (d, J = 8.5 Hz, 1H) 3.36 (s, 2H) 3.25 (s, 2H) 0.92 (s, 6H). MS: (APPI) m/z 386 [M−H]. HRMS calcd for $C_{19}H_{18}FN_3O_3S$ [M+H]: 388.1125; found: 388.1120.

5.1.33. *N*-(3,3-Dimethyl-10-oxo-2,3,4,10-tetrahydropyrimido [1,2-*a*]indol-8-yl)-3-(trifluoromethyl)benzenesulfonamide (22c)

The title compound was prepared as a yellow foam in 15% yield from **21** and 3-trifluoromethyl benzenesulfonyl chloride according to the procedure for **22a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.29 (br s, 1H) 8.03 (d, J = 7.6 Hz, 1H) 7.98–7.93 (m, 2H) 7.81 (dd, J = 8.5, 2.2 Hz, 1H) 7.27 (dd, J = 8.4, 2.1 Hz, 1H) 7.11 (d, J = 2.2 Hz, 1H) 6.94 (d, J = 8.6 Hz, 1H) 3.38 (s, 2H) 3.27 (s, 2H) 0.94 (s, 6H). MS: (APPI) m/z 436 [M−H]. HRMS calcd for $C_{20}H_{18}F_3N_3O_3S$ [M+H]: 438.1094; found: 438.1087.

5.1.34. *N*-(3,3-Dimethyl-10-oxo-2,3,4,10-tetrahydropyrimido [1,2-*a*]indol-8-yl)-3-methoxybenzenesulfonamide (22d)

The title compound was prepared as a yellow foam in 51% yield from **21** and 3-methoxy benzenesulfonyl chloride according to the procedure for **22a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.12 (br s, 1H) 7.45 (m, 1H) 7.28 (dd, J = 8.5, 2.2 Hz, 1H) 7.26–7.11 (m, 4H) 6.91 (d, J = 8.5 Hz, 1H) 3.75 (s, 3H) 3.37 (s, 2H) 3.25 (s, 2H) 0.92 (s, 6H). MS: (APPI) m/z 398 [M−H]. HRMS calcd for $C_{20}H_{21}N_3O_4S$ [M+H]: 400.1325; found: 400.1328.

5.1.35. 4-Chloro-*N*-(3,3-dimethyl-10-oxo-2,3,4,10-tetrahydro pyrimido[1,2-*a*]indol-8-yl)benzenesulfonamide (22e)

The title compound was prepared as a yellow solid in 17% yield from **21** and 4-chloro benzenesulfonyl chloride according to the procedure for **22a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.21 (br s, 1H) 7.69 (d, J = 8.8 Hz, 2H) 7.63 (d, J = 8.8 Hz, 2H) 7.26 (dd, J = 8.5, 2.4 Hz, 1H) 7.14 (d, J = 2.2 Hz, 1H) 6.92 (d, J = 8.5 Hz, 1H) 3.37 (s, 2H) 3.26 (s, 2H) 0.93 (s, 6H). MS: (APPI) m/z 402 [M−H]. HRMS calcd for $C_{19}H_{18}ClN_3O_3S$ [M+H]: 404.0830; found: 404.0829. Mp: 161.1–162.4 °C.

5.1.36. *N*-(3,3-Dimethyl-10-oxo-2,3,4,10-tetrahydropyrimido [1,2-*a*]indol-8-yl)-4-methoxybenzenesulfonamide (22f)

The title compound was prepared as a yellow foam in 29% yield from **21** and 4-methoxy benzenesulfonyl chloride according to the procedure for **22a**. ^{1}H NMR (400Mz, DMSO-d_6): 1H ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.99 (s, 1H) 7.59 (d, J = 8.9 Hz,, 2H) 7.27 (dd, J = 8.5, 2.2 Hz, 1H) 7.14 (d, J = 2.2 Hz, 1H) 7.05 (d, J = 9.0 Hz, 2H) 6.91 (d, J = 8.5 Hz, 1H) 3.78 (s, 3H) 3.37 (s, 2H) 3.25 (s, 2H) 0.93 (s, 6H). MS: (APPI) m/z 398 [M−H]. HRMS calcd for $C_{20}H_{21}N_3O_4S$ [M+H]: 400.1325; found: 400.1325.

5.1.37. *N*-(3,3-Dimethyl-10-oxo-2,3,4,10-tetrahydropyrimido [1,2-*a*]indol-8-yl)-4-(trifluoromethoxy)benzenesulfonamide (22g)

The title compound was prepared as a yellow foam in 17% yield from **21** and 4-trifluoromethoxy benzenesulfonyl chloride according to the procedure for **22a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.26 (br s, 1H) 7.82 (d, J = 8.8 Hz, 2H) 7.55 (d, J = 8.8 Hz, 2H) 7.27 (dd, J = 8.5, 2.2 Hz, 1H) 7.14 (d, J = 2.0 Hz, 1H) 6.93 (d, J = 8.5 Hz, 1H) 3.37 (s, 2H) 3.26 (s, 2H) 0.93 (s, 6H). MS: (APPI) m/z 452 [M−H]. HRMS calcd for $C_{20}H_{18}F_3N_3O_4S$ [M+H]: 454.1043; found: 454.1048.

5.1.38. *N*-(3,3-Dimethyl-10-oxo-2,3,4,10-tetrahydropyrimido [1,2-*a*]indol-8-yl)-4-fluorobenzenesulfonamide (22h)

The title compound was prepared as a yellow foam in 17% yield from **21** and 4-fluoro benzenesulfonyl chloride according to the procedure for **22a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.15 (br s, 1H) 7.75 (m, 2H) 7.46–7.33 (m, 2H) 7.26 (dd, J = 8.7, 2.1 Hz, 1H) 7.14 (d, J = 2.2 Hz, 1H) 6.91 (d, J = 8.5 Hz, 1H) 3.37 (s, 2H) 3.26 (s, 2H) 0.93 (s, 6H). MS: (APPI) m/z 386 [M−H]. HRMS calcd for $C_{19}H_{18}FN_3O_3S$ [M+H]: 388.1126; found: 388.1120.

5.1.39. 5′-{[(2*S*)-2-(Methoxymethyl)pyrrolidin-1-yl]sulfonyl} spiro[1,3-dioxane-2,3′-indol]-2′(1′*H*)-one (23a)

The title compound was prepared as a white solid in 61% yield from isatinsulfonic acid sodium salt hydrate and (*S*)-2-(methoxymethyl)-pyrrolidine according to General Procedures A and B. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.93 (s, 1H) 7.80 (dd, J = 8.2, 1.7 Hz, 1H) 7.61 (d, J = 1.5 Hz, 1H) 7.01 (d, J = 8.2 Hz, 1H) 4.74 (m, 2H) 3.93 (m, 2H) 3.63–3.55 (m, 1H) 3.44 (dd, J = 9.4, 3.7 Hz, 1H) 3.28 (s, 2H) 3.26 (s, 3H) 3.04–2.94 (m, 1H) 2.22 (m, 1H) 1.83–1.59 (m, 3H) 1.52–1.40 (m, 2H). MS: (ESI) m/z 383 [M+H]. Mp: 127–128 °C.

5.1.40. 5′-{[(2*S*)-2-(Phenoxymethyl)pyrrolidin-1-yl]sulfonyl} spiro[1,3-dioxane-2,3′-indol]-2′(1′*H*)-one (23b)

The title compound was prepared as a white solid in 58% yield from isatinsulfonic acid sodium salt hydrate and (*S*)-2-(phenoxymethyl)-pyrrolidine[9] according to General Procedures A and B. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.94 (br s, 1H) 7.83 (dd, J = 8.3, 2.0 Hz, 1H) 7.65 (d, J = 1.7 Hz, 1H) 7.26–7.34 (m, 2H) 7.01 (d, J = 8.3 Hz, 1H) 6.98–6.91 (m, 3H) 4.74 (m, 2H) 4.10 (dd, J = 9.5, 3.7 Hz, 1H) 3.98–3.88 (m, 2H) 3.82 (dd, J = 7.3, 3.9 Hz, 1H) 3.36 (dd, J = 6.8, 3.4 Hz, 2H) 3.08–2.98 (m, 1H) 2.28–2.13 (m, 1H) 1.94–1.77 (m, 2H) 1.70–1.46 (m, 3H). MS: (ESI) m/z 445 [M+H]. Mp: 201–203 °C.

5.1.41. 1-{[5′-{[(2*S*)-2-(Methoxymethyl)pyrrolidin-1-yl] sul fonyl}-2′-oxospiro[1,3-dioxane-2,3′-indol]-1′(2′*H*)-yl] methyl} cyclobutanecarbonitrile (24a)

The title compound was prepared as a white solid in 96% yield from **23a** and 1-chloromethyl-cyclobutanecarbonitrile[20] according to General Procedure C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.91 (dd, J = 8.4, 2.0 Hz, 1H) 7.67 (d, J = 1.8 Hz, 1H) 7.51 (d, J = 8.4 Hz, 1H) 4.73 (dd, J = 11.4, 9.7 Hz, 2H) 4.15 (s, 2H) 3.96 (dd, J = 11.5,

3.7 Hz, 2H) 3.65 (m, 1H) 3.50–3.40 (m, 1H) 3.33–3.28 (m, 2H) 3.25 (s, 3H) 3.03 (m, 1H) 2.43–2.33 (m, 4H) 2.32–2.20 (m, 1H) 2.13–1.97 (m, 2H) 1.83–1.62 (m, 3H) 1.56–1.39 (m, 2H). MS: (ES+) *m*/*z* 476.2 [M+H]. Mp: 130.0–131.1 °C.

5.1.42. 1-{[2′-Oxo-5′-{[(2*S*)-2-(phenoxymethyl)pyrrolidin-1-yl] sulfonyl}spiro[1,3-dioxane-2,3′-indol]-1′(2′*H*)-yl]methyl} cyclobutanecarbonitrile (24b)

The title compound was prepared as a white foam in 80% yield from **23b** and 1-chloromethyl-cyclobutanecarbonitrile[20] according to General Procedure C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.94 (dd, *J* = 8.5, 2.0 Hz, 1H) 7.70 (d, *J* = 2.0 Hz, 1H) 7.51 (d, *J* = 8.5 Hz, 1H) 7.34–7.23 (m, 2H) 7.00–6.88 (m, 3H) 4.72 (m, 2H) 4.15 (s, 2H) 4.10 (dd, *J* = 9.6, 3.5 Hz, 1H) 4.00–3.91 (m, 3H) 3.90–3.80 (m, 1H) 3.42–3.33 (m, 1H) 3.08 (m, 1H) 2.43–2.33 (m, 4H) 2.31–2.17 (m, 1H) 2.11–2.01 (m, 2H) 1.94–1.79 (m, 2H) 1.73–1.45 (m, 3H). MS: (ES+) *m*/*z* 538.2 [M+H].

5.1.43. 1-{[5′-{[(2*S*)-2-(Methoxymethyl)pyrrolidin-1-yl] sulfonyl}-2′-oxospiro[1,3-dioxane-2,3′-indol]-1′(2′*H*)-yl] methyl} cyclopentanecarbonitrile (24c)

The title compound was prepared as a white solid in 77% yield from **23a** and 1-chloromethyl-cyclopentanecarbonitrile[20] according to General Procedure C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.91 (dd, *J* = 8.3, 2.0 Hz, 1H) 7.67 (d, *J* = 2.0 Hz, 1H) 7.54 (d, *J* = 8.3 Hz, 1H) 4.81–4.63 (m, 2H) 4.02 (s, 2H) 4.00–3.91 (m, 2H) 3.70–3.58 (m, 1H) 3.44 (dd, *J* = 9.4, 3.8 Hz, 1H) 3.34–3.28 (m, 2H) 3.26 (s, 3H) 3.09–2.98 (m, 1H) 2.36–2.18 (m, 1H) 2.05–1.95 (m, 2H) 1.92–1.83 (m, 2H) 1.83–1.64 (m, 7H) 1.54–1.39 (m, 2H). MS: (ES+) *m*/*z* 490.2 [M+H]. Mp: 70.3–72.1 °C.

5.1.44. 1-{[2′-Oxo-5′-{[(2*S*)-2-(phenoxymethyl)pyrrolidin-1-yl] sulfonyl}spiro[1,3-dioxane-2,3′-indol]-1′(2′*H*)-yl]methyl} cyclopentanecarbonitrile (24d)

The title compound was prepared as a white solid in 79% yield (2 steps) from **23b** and 1-chloromethyl-cyclopentanecarbonitrile[20] according to General Procedure C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.94 (dd, *J* = 8.5, 2.0 Hz, 1H) 7.70 (d, *J* = 2.0 Hz, 1H) 7.54 (d, *J* = 8.5 Hz, 1H) 7.35–7.23 (m, 2H) 6.99–6.88 (m, 3H) 4.73 (m, 2H) 4.10 (dd, *J* = 9.5, 3.7 Hz, 1H) 4.01 (s, 2H) 3.99–3.92 (m, 3H) 3.86 (m, 1H) 3.44–3.33 (m, 2H) 3.16–3.02 (m, 1H) 2.34–2.15 (m, 1H) 2.06–1.95 (m, 1H) 1.93–1.65 (m, 8H) 1.65–1.45 (m, 2H) 1.28–1.18 (m, 1H). MS: (ES+) *m*/*z* 552.22 [M+H]. Mp: 82.2–83.9 °C.

5.1.45. 1-{[5′-{[(2*S*)-2-(Methoxymethyl)pyrrolidin-1-yl] sulfonyl}-2′-oxospiro[1,3-dioxane-2,3′-indol]-1′(2′*H*)-yl] methyl}cyclohexanecarbonitrile (24e)

The title compound was prepared as a white foam in 76% yield from **23a** and 1-chloromethyl-cyclohexanecarbonitrile[20] according to General Procedure C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.89 (dd, *J* = 8.4, 1.9 Hz, 1H) 7.67 (d, *J* = 2.0 Hz, 1H) 7.54 (d, *J* = 8.5 Hz, 1H) 4.81–4.60 (m, 2H) 4.01–3.95 (m, 2H) 3.94 (s, 2H) 3.69–3.60 (m, 1H) 3.44 (dd, *J* = 9.3, 3.9 Hz, 1H) 3.34–3.29 (m, 2H) 3.26 (s, 3H) 3.10–2.96 (m, 1H) 2.36–2.16 (m, 1H) 1.94–1.84 (m, 2H) 1.81–1.60 (m, 7H) 1.57–1.30 (m, 6H). MS: (ES+) *m*/*z* 504.2 [M+H].

5.1.46. 1-{[2′-Oxo-5′-{[(2*S*)-2-(phenoxymethyl)pyrrolidin-1-yl] sulfonyl}spiro[1,3-dioxane-2,3′-indol]-1′(2′*H*)-yl]methyl} cyclohexanecarbonitrile (24f)

The title compound was prepared as a white foam in 94% yield from **23b** and 1-chloromethyl-cyclohexanecarbonitrile[20] according to General Procedure C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.92 (dd, *J* = 8.4, 1.8 Hz, 1H) 7.70 (d, *J* = 1.7 Hz, 1H) 7.54 (d, *J* = 8.5 Hz, 1H) 7.34–7.25 (m, 2H) 6.98–6.91 (m, 3H) 4.72 (m, 2H) 4.10 (dd, *J* = 9.5, 3.7 Hz, 1H) 4.00–3.94 (m, 2H) 3.94 (s, 3H) 3.87 (m, 1H) 3.42–3.34 (m, 1H) 3.09 (m, 1H) 2.33–2.17 (m, 1H) 1.95–1.79 (m, 4H) 1.77–1.30 (m, 11H). MS: (ES+) *m*/*z* 566.2 [M+H].

5.1.47. 8′-{[(2*S*)-2-(Methoxymethyl)pyrrolidin-1-yl]sulfonyl} spiro[cyclopentane-1,3′-pyrimido[1,2-*a*]indol]-10′(2′*H*)-one (25c)

Step 1: 8′-{[(2*S*)-2-(methoxymethyl)pyrrolidin-1-yl]sulfonyl}-2′*H*-dispiro[cyclopentane-1,3′-pyrimido[1,2-*a*]indole-10′,2″-[1,3] dioxane] was prepared as a white solid in 91% yield from **24c** according to General Procedure D. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.79 (dd, *J* = 8.3, 1.8 Hz, 1H) 7.56 (d, *J* = 1.8 Hz, 1H) 6.95 (d, *J* = 8.3 Hz, 1H) 4.96–5.10 (m, 2H) 3.73–3.84 (m, 2H) 3.58 (m, 1H) 3.46 (dd, *J* = 9.4, 3.8 Hz, 1H) 3.40 (s, 2H) 3.34 (s, 2H) 3.28–3.32 (m, 2H) 3.27 (s, 3H) 2.90–3.05 (m, 1H) 2.18 (m, 1H) 1.53–1.81 (m, 7H) 1.34–1.50 (m, 6H). MS: (ES+) *m*/*z* 476.2 [M+H].

Step 2: To a solution of 8′-{[(2*S*)-2-(methoxymethyl)pyrrolidin-1-yl]sulfonyl}-2′*H*-dispiro[cyclopentane-1,3′-pyrimido[1,2-*a*]indole-10′,2″-[1,3]dioxane] (0.220 g, 0.46 mmol, 1 equiv) in CH_2Cl_2 (12 mL) was added methanesulfonic acid (4 mL). The reaction was stirred at rt 14 h. and poured into brine. It was basified to pH 13 with NH_4OH and extracted with EtOAc. The combined organics were dried over sodium sulfate and concentrated. The crude residue purified by column chromatography using acetone/hexane (25/75) as an eluent to give the title compound as a yellow solid (0.112 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.04 (dd, *J* = 8.5, 2.0 Hz, 1H) 7.80 (d, *J* = 2.0 Hz, 1H) 7.22 (d, *J* = 8.5 Hz, 1H) 3.66 (m, 1H) 3.57 (s, 2H) 3.50 (s, 2H) 3.45 (dd, *J* = 9.4, 3.8 Hz, 1H) 3.37–3.28 (m, 2H) 3.27 (s, 3H) 3.06 (m, 1H) 1.83–1.60 (m, 6H) 1.58–1.32 (m, 6H). Anal. Calcd for $C_{21}H_{27}N_3O_4S$: C, 60.41; H, 6.52; N, 10.06. Found: C, 60.24; H, 6.42; N, 9.98. MS: (ES−) *m*/*z* 416 [M−H]. HRMS calcd for $C_{21}H_{27}N_3O_4S$ [M+H]: 418.1795; found: 418.1794. Mp: 171.2–171.9 °C.

5.1.48. 8′-{[(2*S*)-2-(Methoxymethyl)pyrrolidin-1-yl]sulfonyl} spiro[cyclobutane-1,3′-pyrimido[1,2-*a*]indol]-10′(2′*H*)-one (25a)

The title compound was prepared as a yellow solid in 54% yield (two steps) from **24a** according to the procedure for **25c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.06 (dd, *J* = 8.5, 1.9 Hz, 1H) 7.79 (d, *J* = 1.7 Hz, 1H) 7.24 (d, *J* = 8.5 Hz, 1H) 3.73 (s, 2H) 3.70 (s, 2H) 3.61–3.69 (m, 1H) 3.45 (dd, *J* = 9.4, 3.8 Hz, 1H) 3.34–3.28 (m, 2H) 3.27 (s, 3H) 3.06 (m, 1H) 2.06–1.92 (m, 2H) 1.91–1.81 (m, 4H) 1.81–1.65 (m, 2H) 1.50 (m, 2H). MS: (ES−) *m*/*z* 402 [M−H]. HRMS calcd for $C_{20}H_{25}N_3O_4S$ [M+H]: 404.1639; found: 404.1640. Mp: 146.8–147.4 °C.

5.1.49. 8′-{[(2*S*)-2-(Phenoxymethyl)pyrrolidin-1-yl]sulfonyl} spiro[cyclobutane-1,3′-pyrimido[1,2-*a*]indol]-10′(2′*H*)-one (25b)

The title compound was prepared as a light yellow foam in 29% yield (two steps) from **24b** according to the procedure for **25c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.08 (dd, *J* = 8.5, 2.0 Hz, 1H) 7.81 (d, *J* = 2.0 Hz, 1H) 7.31–7.24 (m, 2H) 7.22 (d, *J* = 8.5 Hz, 1H) 6.98–6.87 (m, 3H) 4.08 (dd, *J* = 9.8, 3.4 Hz, 1H) 4.00–3.93 (m, 1H) 3.89 (m, 1H) 3.73 (s, 2H) 3.69 (s, 2H) 3.37 (m, 1H) 3.14 (m, 1H) 2.05–1.92 (m, 2H) 1.93–1.78 (m, 6H) 1.73–1.64 (m, 1H) 1.63–1.52 (m, 1H). MS: (ES−) *m*/*z* 464 [M−H]. HRMS calcd for $C_{25}H_{27}N_3O_4S$ [M+H]: 466.1795; found: 466.1797.

5.1.50. 8′-{[(2*S*)-2-(Phenoxymethyl)pyrrolidin-1-yl]sulfonyl} spiro[cyclopentane-1,3′-pyrimido[1,2-*a*]indol]-10′(2′*H*)-one (25d)

The title compound was prepared as a yellow solid in 29% yield (two steps) from **24d** according to the procedure for **25c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.06 (dd, *J* = 8.5, 2.0 Hz, 1H) 7.82 (d, *J* = 2.0 Hz, 1H) 7.32–7.24 (m, 2H) 7.21 (d, *J* = 8.5 Hz, 1H) 6.97–

6.87 (m, 3H) 4.08 (dd, J = 9.5, 3.4 Hz, 1H) 4.00–3.92 (m, 1H) 3.89 (m, 1H) 3.57 (s, 2H) 3.49 (s, 2H) 3.37 (m, 1H) 3.14 (m 1H) 1.97–1.76 (m, 2H) 1.73–1.54 (m, 6H) 1.50–1.32 (m, 4H). Anal. Calcd for $C_{26}H_{29}N_3O_4S$: C, 65.11; H, 6.09; N, 8.76. Found: C, 64.85; H, 5.98; N, 8.60. MS: (ES−) m/z 478 [M−H]. HRMS calcd for $C_{26}H_{29}N_3O_4S$ [M+H]: 480.1952. Found: 480.1956. Mp: 142.9.8–144.5 °C.

5.1.51. 8′-{[(2*S*)-2-(Methoxymethyl)pyrrolidin-1-yl]sulfonyl} spiro[cyclohexane-1,3′-pyrimido[1,2-*a*]indol]-10′(2′*H*)-one (25e)

The title compound was prepared as a light yellow foam in 41% yield (two steps) from **24e** according to the procedure for **25c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.04 (dd, J = 8.5, 1.9 Hz, 1H) 7.79 (d, J = 1.8 Hz, 1H) 7.31 (d, J = 8.5 Hz, 1H) 3.67 (m, 1H) 3.53 (s, 4H) 3.45 (dd, J = 9.3, 3.9 Hz, 1H) 3.34–3.28 (m, 2H) 3.27 (s, 3H) 3.07 (m, 1H) 1.82–1.64 (m, 2H) 1.58–1.42 (m, 7H) 1.41–1.22 (m, 5H). Anal. Calcd for $C_{22}H_{29}N_3O_4S$: C, 60.32; H, 6.69; N, 9.55. Found: C, 60.69; H, 6.84; N, 9.52. MS: (ES−) m/z 430 [M−H]. HRMS calcd for $C_{22}H_{29}N_3O_4S$ [M+H]: 432.1952; found: 432.1950.

5.1.52. 8′-{[(2*S*)-2-(Phenoxymethyl)pyrrolidinyl]sulfonyl}spiro [cyclohexane-1,3′-pyrimido[1,2-*a*]indol]-10′(2′*H*)-one (25f)

The title compound was prepared as a light yellow foam in 31% yield (two steps) from **24f** according to the procedure for **25c**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.06 (dd, J = 8.5, 2.0 Hz, 1H) 7.81 (d, J = 2.0 Hz, 1H) 7.29 (m, 3H) 6.99–6.85 (m, 3H) 4.08 (dd, J = 9.4, 3.3 Hz, 1H) 3.99–3.93 (m, 1H) 3.89 (m, 1H) 3.53 (s, 2H) 3.52 (s, 2H) 3.38 (m, 1H) 3.14 (m, 1H) 1.96–1.79 (m, 2H) 1.73–1.65 (m, 1H) 1.64–1.55 (m, 1H) 1.52–1.42 (m, 5H) 1.41–1.25 (m, 5H). MS: (ES−) m/z 492 [M−H]. HRMS calcd for $C_{27}H_{31}N_3O_4S$ [M+H]: 494.2108; found: 494.2112.

5.2. Preparation of active caspase-3

Caspase-3 was expressed intracellularly in *Escherichia coli* with a c-terminal His tag. Fermentation was performed at 25 °C in a B. Braun Biotech Biostat C 10 litre bioreactor vessel. The culture was collected in 1 L bottles and centrifuged in Komspin KA-7.1000 rotors at approximately 8000 RCF (Relative Centrifugal Force). The cell pellets were re-suspended in 20 mM Tris pH 8.0, 500 mM NaCl and 5 mM imidazole. The cell suspension was disrupted by passing 5 times through a microfluidizer Model 110Y (Microfluidics Corp, Newton, Mass). After centrifugation (13 kg, 30 min at 4C), the supernatant was applied to a column of Nickle-NTA agarose. The caspase-3 was eluted with a gradient of 5–150 mM imidazole in the above buffer. Fractions containing caspase-3 were pooled and concentrated with a Millipore Ultrafree filtration device. The concentrated caspase-3 solution was loaded unto a TSK gel G3000sw column (Tosoh Bioseph LLC), equilibrated with a buffer of 20 mM PIPES pH 7.2, 100 mM NaCl, 1 mM EDTA and 5 mM Cysteine. Fractions containing caspase-3 were pooled and concentrated. Sometimes CHAPS was added to 0.1% and sucrose to 10% into the protein sample. The caspase-3 obtained with this method shows two subunits of 17 and 13 kD on reduced SDS–PAGE and aliquots stored at −80 °C.

5.3. Caspase-3 Inhibition assay

This standard pharmacological test procedure to assess the inhibition of recombinant caspase-3 activity of selected compounds was adapted from previously reported procedures.[21–23] The procedure used and results obtained are briefly described below.

Caspase-3 was assayed at 23 °C (room temp) in 96-well plates using the internally quenched tetrapeptide substrate *N*-acetyl-aspartyl-glutamyl-valyl-aspartate-7-amino-4-trifluoromethyl coumarin (Ac-DEVD-AFC purchased from Biomol). The assays are conducted at pH 7.2 in a buffered system containing 20 mM PIPES, 100 mM NaCl, 1 mM EDTA, 0.1% CHAPS, 10% sucrose and 5 mM L-cysteine. The final concentration of the substrate is 25 μM. Enzymic cleavage between the aspartate and the AFC fluorophore liberates 7-amino-4-trifluoromethyl coumarin which is detected using an excitation wavelength of 400 nm and an emission wavelength of 505 nm in a SpectraMax GeminiXS plate reader operated at room temperature. A steady state rate of substrate cleavage is obtained for analysis.

For IC_{50} determination, typically 11 concentrations ranging from 20 μM to 20 nM were freshly prepared by serial dilution with assay buffer containing no cysteine with 80 μL of 31.25 μM substrate added to the assay well. Once substrate and inhibitor were added to the assay plate, the reaction was initiated by addition of 10 μL of 2.5 nM enzyme, prepared in assay buffer containing 50 mM Cysteine, to the assay mixture (final concentration 0.25 nM). After the reaction was initiated with the addition of enzyme, AFC production was monitored continuously for 90 min by exciting at 400 nm and measuring the emission at 505 nm every 42 s. The progress curves generated were fitted by computer to Eq. 1 to generate an IC_{50} value. Eq. 1: $y = B_{max}{}^{*}(1 - (x^n/(K^n + x^n)))$, where B_{max} is rate in the absence of inhibitor.

5.4. Modelling

Starting with the X-ray co-crystal structure of compound **3** bound to human caspase-3 a molecular mechanics optimization of the whole structure was performed using Molecular Operating Environment (MOE)[28] and the MMFF94 potential.[29] Using this optimized complex compound **1** was then stripped down to its maximum common substructure with compound **22a** (pyrimidoindolone fused heterocycle with attached gem-dimethyl). Then compound **22a** was grown into the binding pocket one torsion group at a time. After each added torsion group a full rotor search was performed to set the optimal torsion angle. Once compound **22a** was completely grown into the pocket a full minimization of **22a** and all caspase-3 residues within 5 Å was performed. The optimized structures of compounds **3** and **22a** bound to caspase-3 were then used to analyze the interactions between the compounds and various residues in the caspase-3 binding site. Compounds **3** and **22a** were then removed from the caspase-3 binding pocket and optimized to their nearest local minima using the MMFF94 potential. The LUMO energies of compounds **3** and **22a** were then calculated in the PM3 approximation using MOPAC.[30] The SYBYL[31] interface was used to perform these computations.

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