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Original article

Discovery of novel 2,6-disubstituted pyridazinone derivatives as acetylcholinesterase inhibitors

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ABSTRACT

2,6-Disubstituted pyridazinone **4** was identified by HTS as a novel acetylcholinesterase (AChE) inhibitor. Under SAR development, compound **17e** stood out as displaying high AChE inhibitory activity and AChE/butyrylcholinesterase (BuChE) selectivity in vitro. Docking studies revealed that **17e** might interact with the catalytic active site (CAS) and the peripheral anionic site (PAS) simultaneously. Based on this novel binding information, 6-ortho-tolylamino and *N*-ethyl-*N*-isopropylacetamide substituted piperidine were disclosed as new PAS and CAS binders.

1. Introduction

Alzheimer's disease (AD) is a complex neurodegenerative disorder of the central nervous system. It is estimated that nearly 36 million people worldwide are now suffering from AD, and the figure would be increased to about 66 million by 2030 if no breakthroughs were made. Acetylcholinesterase (AChE), a serine protease, is responsible for acetylcholine hydrolysis and plays a fundamental role in impulse transmission by terminating the action of the neurotransmitter acetylcholine at the cholinergic synapses and neuromuscular junction [1]. Among the various approaches for treating AD, inhibition of AChE is still prevailing in treating or alleviating the symptoms of AD. Tacrine (**1**, Fig. 1), a nonselective AChE/butyrylcholinesterase (BuChE) inhibitor, was the first drug approved by FDA in 1993. Other selective inhibitors, such as donepezil (**2**, Fig. 1), also reached the market sequentially. In recent years, novel AChE inhibitors are continuingly discovered from natural resources or by synthetic approaches, bearing such as berberine [2–6], coumarin [7,8], benzofuran [9], β-carboline [10], quinoline [11–13], benzophenone [14,15], triazin [16], ferulic acid [17] and naphthyridine [18] frameworks as the primary pharmacophoric scaffolds.

The most interesting structural character of AChE protein is the presence of a deep narrow gorge, at the bottom of which a triad lies in the catalytic anionic site (CAS). Besides, the peripheral anionic site (PAS) lies at the entrance of the gorge as a regulatory site. Based on these findings, Pang et al. first reported a tacrine dimer **3** (Fig. 1) as a bivalent AChE inhibitor which interacts with CAS and PAS simultaneously [19]. This compound displayed much higher AChE inhibitory activity and specificity over BuChE than the parent compound **1**. Through this strategy, the second generation AChE inhibitors are developed by utilizing one or two known scaffolds of AChE inhibitor and most of them possess elevated potency and improved AChE/BuChE selectivity profile [20–28].

Through an in vitro HTS campaign with diverse compounds libraries inhouse, pyridazinone derivative **4** (Fig. 1) stood out as a potential AChE inhibitor with an IC_{50} of 1.66 μM. To our best knowledge, only a few compounds bearing this scaffold were reported with a liner pyridazine-donepezil hybrid **5** (Fig. 1) in the literature [29–31], which behaved as a dual site binding inhibitor. By optimizing the 3,6-substitutions on the pyridazine ring, high AChE affinity of the compound was achieved. However, this type of molecules displayed low AChE/BuChE selectivity, which might lead

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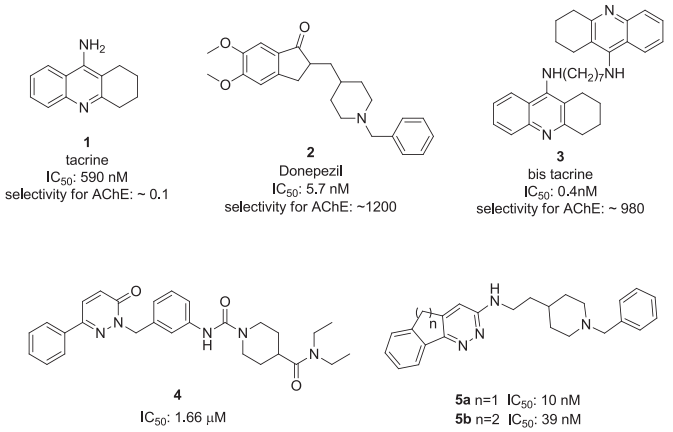


Fig. 1. Representative AChE inhibitors.

to undesirable peripheral side effects. As the compound **4** bears different substituents on pyridazinone ring from **5a**/**5b** and does not share any structural similarity with donepezil, we realized that the SAR of **4** might not parallel with that of **5a**/**5b** or donepezil, thus leading this de novo pyridazinone molecule to be worth of optimization for finding diverse AChE inhibitors with high potency and AChE/BuChE selectivity.

2. Results and discussion

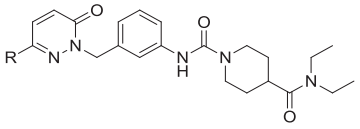
2.1. Chemistry

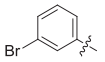
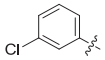
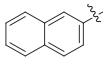
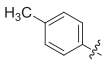
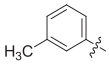
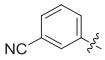
The general synthetic route to build the focused AChE-targeted inhibitor library is illustrated in Scheme 1. Compound **9** could be obtained by a sequential process involving alkylation of corresponding pyridazinone (**6**) [32–34] and followed by reduction of the nitro group under Fe/HOAc in refluxing EtOH. The final products were obtained by **9** condensing the substituted piperidines in the presence of triphosgene.

2.2. SAR study and docking study

Structural inspections of the compounds in our HTS library revealed that the terminal 4-diethyl amide piperidine in **4** may be essential for activity. Firstly, we commenced with our work by changing the substituents on the pyridazinone ring (Table 1). Replacing the phenyl in **4** by a single hydrogen (**10**) caused a slight loss in activity, which indicated that the phenyl might function as a hydrophobic pharmacophore. As the AChE binding pocket is a narrow hydrophobic gorge, a small hydrophobic methyl group was added to the phenyl (**11a**–**c**) for modulating the binding

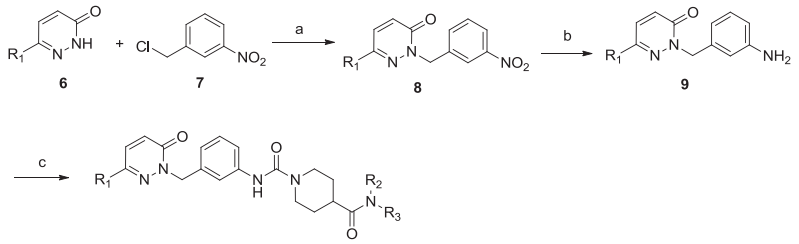
Table 1
AChE inhibitory activity of 6-aryl substituted pyridazinones.



No.	R	IC_{50} (μM)	No.	R	IC_{50} (μM)
4		1.66	**11d**		0.623
10	H	4.90	**11e**		0.648
11a		2.61	**12a**		0.462
11b		2.01	**12b**		0.188
11c		0.746	**12c**		0.220

affinity. Compound **11c**, a *meta*-methyl substituted pattern, displayed a slight enhancement in activity with an IC_{50} of 0.746 μM. Besides, other *meta*-halo substituted analogs (**11d**–**e**) remained biological activity at enzymatic level. When the conjugated system in **4** was extended by replacing the phenyl with naphthyl (**12a**–**b**), or by adding a cyano group on *meta* position (**12c**), the potency of compounds was evidently enhanced. The most promising compound **12b** displayed a potent activity with an IC_{50} of 0.188 μM, which was about 9 folds more potent than the no-conjugation extended compound **4**.

In order to get a comprehensive understanding of the SAR, docking study was performed. Crystal complex (code: 1EVE) of donepezil and AChE protein was downloaded from Protein Data Bank, in which the positively charged piperidine nitrogen in donepezil achieves a caution-π interaction with Phe330 and the terminal benzyl ring involves in π–π stacking with aromatic Trp84. Docking results showed that **12b** may function as a bi-functional AChE inhibitor with novel binding conformation, which the naphthyl occupies in the PAS pocket and the 4-diethyl amide piperidine is located at the CAS near the bottom of the gorge. The carbonyl oxygen on pyridazinone ring possibly takes part in hydrogen bonding interaction with Phe288 and the 2-benzyl on pyridazinone involves in π–π stacking with Tyr334 (Fig. 2). No direct hydrogen bond interaction was observed in the urea motif of **12b**, however, just as donepezil complex, a water bridged hydrogen bonding might occur in biological circumstances.



[α]Conditions: (a) Cs_2CO_3, DMF, 40-50°C, 3h; (b) Fe, HOAc, 95% EtOH, 120°C reflux, 5h;(c) triphosgene, Et_3N, DCM, 0°C, 5-10min, then 4-substituted piperidine, overnight

Scheme 1. Synthesis of analogues[α].

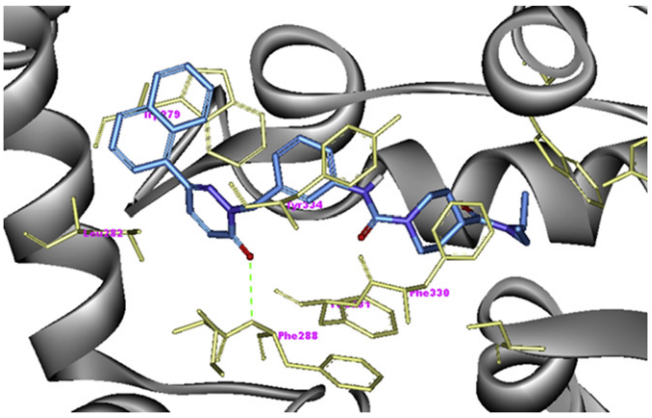


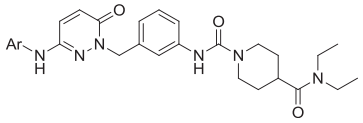
Fig. 2. Model of compound **12a** bound to AChE. The naphthyl and 4-substituted piperidine interact with PAS and CAS respectively.

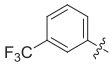
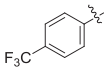
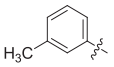
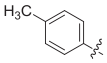
As shown in Fig. 3, docking results revealed that the naphthyl for PAS binder in **12b** not only acts as a hydrophobic group involving in the van der Waals interactions with the protein, but also functions as a π–π interaction element, which mainly works by the extended aromatic ring B. Considering the hydrophobic effects induced by the phenyl A in **12b** and the methyl in **11c**, a novel 6-aniline pyridazinone scaffold was designed, in which the ring B is remained as a π–π interaction motif and ring A is broken by a single nitrogen linker. In this new scaffold, π–π stacking efficiency of ring B may also be elevated due to the increased rotation freedom.

Based on above analyses, 6-substituted aniline pyridazinones were synthesized. As shown in Table 2, when the naphthyl (hydrophobic-π motif) in **12b** was replaced by a single aniline (nitrogen-π motif, **13**), the AChE inhibitory activity of **13** decreased drastically (about 17 folds). However, the activity of **14** was restored when the aniline was changed to more hydrophobic naphthalen-1-amine, which indicated that the hydrophobic substitutions on the aniline ring played a great part in binding affinity. Subsequently, other analogs with different substituted anilines were synthesized (**15a**–**f**). The activity of compounds was decreased in the presence of *para* substitutions (**15c**, **f**). The *ortho* substitutions significantly increased the binding affinity probably due to a similar hydrophobic effect induced by ring A in **12b**. In addition, a prevailing 'Magic Methyl' effect emerged in these pyridazinone compounds [35], a single 2-methyl aniline derivative (**15a**) showed the prominent inhibitory potency with an IC_{50} of 0.049 μM. It was about 34 and 4-fold more potent than **4** and **12b**, respectively. Further enlarging the steric hindrance to dimethyl, ethyl or isopropyl (**16a**–**c**) decreased the activity.

Docking studies showed that **15a** displays a similar binding mode as **12b** (Fig. 4A). Overlapping compounds **15a**, **4** and **12b** (Fig. 4B) revealed that changing the phenyl in **4** with naphthyl (**12b**) or adding a conjugated group (**12c**) made it possible for π–π stacking with Trp279, which led to an increase in activity. In the case of **15a**, the 2-methyl aniline does act as a bi-functional group: the aromatic phenyl stacks with Trp279 while the terminal methyl fit within the PAS hydrophobic pocket perfectly.

Table 2
AChE inhibitory activity of 6-aniline substituted pyridazinones.



No.	Ar	IC_{50} (μM)	No.	Ar	IC_{50} (μM)
13		3.28	**15d**		0.119
14		0.126	**15e**		0.135
15a		0.049	**15f**		4.84
15b		1.42	**16a**		0.607
15c		19.8	**16b**		0.188
			16c		1.50

After the modification of the active compounds for PAS binding site, we optimized the CAS binding motifs to synthesize compounds **17a**–**f** (Table 3). Cyclic analog **17a** with the terminal morpholine motif lost the activity totally. Among the acyclic amide series (**17b**–**f**), the inhibitory activity of the compounds strongly paralleled with the hydrophobic effect of the substitutions. Among them, the *N*-ethyl-*N*-isopropyl amide derivative **17e** showed slightly more potent than **15a**. This phenomenon is consistent with the hydrophobic environment of the CAS binding pocket.

2.3. Selectivity profile for AChE/BuChE

Moreover, the most potent and representative compounds were selected for evaluation of AChE/BuChE selectivity profile. As shown in Table 4, compared with the reported 3-amino-6-aryl pyridazine series [29,30], 2,6-disubstituted pyridazin-3-one series displayed about 200-fold or higher AChE/BuChE selectivity. For this scaffold, AChE inhibitory activity of the compounds could be enhanced by modifying PAS binding motif without affecting the BuChE affinity by comparing **4** with **12b**, or **13** with **15a**. In the catalytic binding pocket, increasing the hydrophobic groups of the amide (**17d**–**e**) lowered the IC_{50}s for AChE accompanying with slight increasing for the binding affinities of BuChE, However, the selectivity profiles were still maintained.

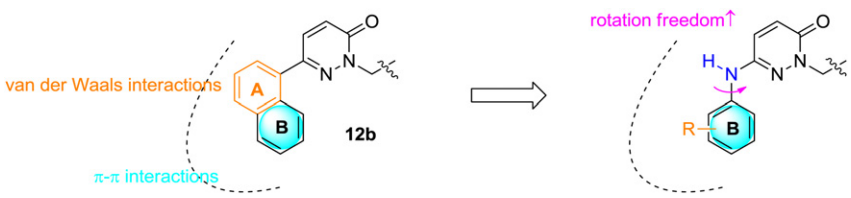


Fig. 3. Designing strategy for aniline series.

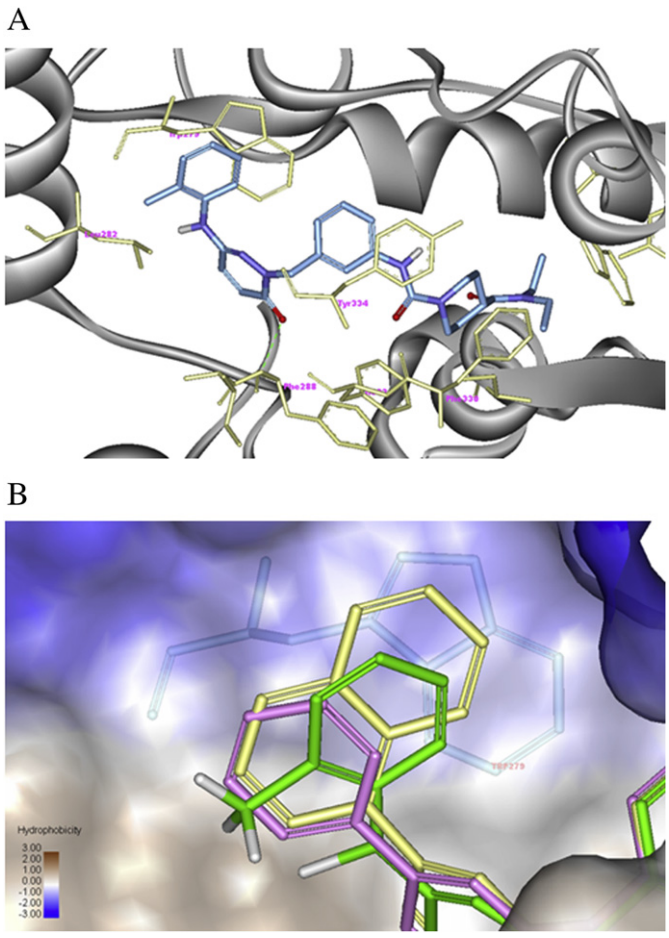


Fig. 4. (A) Model of compound **15a** bound to AChE. The *ortho*-tolylamino and 4-substituted piperidine interact with PAS and CAS, respectively. (B) Overlap of **4** (magenta), **12b** (yellow), and **15a** (green). The naphthyl in **12b** and ortho-methyl amine in **15a** function as π−π stacking element and involve in PAS van der Waals interaction.

3. Conclusion

In this report, a series of novel 2,6-disubstituted pyridazinone derivatives for AChE inhibition was optimized. Under the SAR development, high AChE affinity of the compounds was achieved by optimizing different substituents on the pyridazinone ring, without sacrificing the AChE/BuChE selectivity profile.

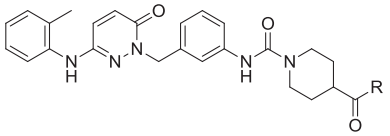
Docking study revealed that these pyridazinones might behave as dual binding site inhibitors with novel binding conformation. Utilizing this information, a delicate SAR study was performed at PAS binding site and 6-ortho-tolylamino substitution was optimized for involving in both π−π stacking and "Magic Methyl" hydrophobic interaction. Moreover, rather than the prevailing *N*-benzyl piperidine reported in donepezil, a novel hydrophobic 4-substituted piperidine motif for catalytic pocket binder was disclosed, which also provided a new platform for designing AChE inhibitor. Further biological evaluations of these pyridazinone series are ongoing.

4. Experimental section

4.1. General information

Reagents were purified prior to use unless otherwise stated. Column chromatography was carried out on silica gel (200–300 mesh). ^{1}H NMR and ^{13}C NMR spectral data were recorded in DMSO-d_6, CD_3OD or acetone-d_6 on Varian Mercury 500, 400 or 300 NMR spectrometer and Chemical shifts (δ) were reported in parts per million (ppm), and the signals were described as brs (broad singlet), d (doublet), dd (doublet of doublet), m (multiple), q (quarter), s (singlet), and t (triplet). Coupling constants (J values) were given in Hz. Low-resolution mass spectra (ESI) was obtained using Agilent HPLC-MS (1260-6120B) and high-resolution mass spectra (ESI) were obtained using Waters Q-Tof Ultima apparatus.

Table 3
SAR study at catalytic binding site.



No.	R	IC_{50}(μM)
17a		NA
17b		7.60
17c		0.473
17d		0.046
15a		0.050
17e		0.028
17f		0.116

4.2. General procedures

To a solution of pyridazinone derivative [32–34] (0.5 mmol) in DMF (10 mL) was added 1-(chloromethyl) 3-nitrobenzene (0.52 mmol) and Cs_2CO_3 (0.55 mmol), the resulting reaction mixture was stirred at 40–50 °C until no starting materials was detected by TLC (about 3 h). The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (30 mL), washed with brine (3 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was dissolved in 95% ethanol (50 mL) containing 10 mmol acetic acid. Iron powder (2 mmol) was added and the resulting mixture

Table 4
Selectivity profile of representative compounds for AChE versus BuChE.

No.	IC_{50}(μM)		Ratio of IC_{50} (BuChE/AChE)
	AChE	BuChE	
4	1.66	>40	>24
12b	0.188	>40	>212
13	3.28	>40	>12
15a	0.049	>40	>816
17c	0.473	>40	>84
17d	0.046	10.0	217
17e	0.028	5.50	196

was stirred for 5 h. After cooled to room temperature, the reaction mixture was filtered through celite and the filter cake was washed with 95% ethanol (3 × 15 mL). The combined ethanol layers were evaporated in vacuo and the residue was re-dissolved in ethyl acetate (30 mL). The organic layer was washed with brine (3 × 10 mL) and 2 M NaOH (10 mL) sequentially. The organic layer was dried over anhydrous Na_2SO_4, evaporated in vacuo to afford the crude 2-aminobenzyl-6-substituted-pyridazin-3(2*H*)-ones, which were used without further purification.

To a stirred solution of 2-aminobenzyl-6-substituted-pyridazin-3(2*H*)-one and triphosgene (1 mmol) in dry dichloromethane (5 mL) was added triethylamine (2 mmol) under nitrogen atmosphere. A solution of the corresponding piperidine (1 mmol) in dichloromethane (5 mL) was added 5–10 min later and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane (15 mL) and washed with water (3 × 20 mL). The organic phases were separated, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography to afford the products.

4.2.1. N^4,N^4-Diethyl-N^1-(3-((6-oxo-3-phenylpyridazin-1(6H)-yl) methyl)phenyl)piperidine-1,4-dicarboxamide (**4**)

Yield: 81%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.03–7.87 (m, 4H), 7.61–7.38 (m, 5H), 7.19 (t, J = 7.9 Hz, 1H), 7.04 (d, J = 7.7 Hz, 1H), 6.99 (d, J = 9.9 Hz, 1H), 5.32 (s, 2H), 4.30–4.15 (m, 2H), 3.47–3.37 (m, 2H), 3.32 (d, J = 6.8 Hz, 2H), 2.99–2.74 (m, 3H), 1.68 (s, 4H), 1.18 (t, J = 7.0 Hz, 3H), 1.03 (t, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.67, 160.25, 156.20, 145.43, 139.91, 136.53, 134.31, 131.02, 129.41, 129.22, 128.51, 128.35, 125.74, 122.58, 120.41, 120.20, 55.33 (−$\underline{C}H_2$−Ph−), 43.31 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.84 (−CON($\underline{C}H_2CH_3)_2$), 40.29 (−CON($\underline{C}H_2CH_3)_2$), 38.32 (−NHCON$(CH_2CH_2)_2\underline{C}$HCO−), 28.39 (−NHCON$(CH_2\underline{C}H_2)_2$CHCO−), 13.85 (−CON$(CH_2\underline{C}H_3)_2$), 11.90 (−CON$(CH_2\underline{C}H_3)_2$); HRMS(ESI): m/z [M + H]$^+$ 488; HRMS(ESI) calcd for $C_{28}H_{33}N_5O_3Na$ [M + Na]$^+$ 510.2481, found 510.2492.

4.2.2. N^4,N^4-Diethyl-N^1-(3-((6-oxopyridazin-1(6H)-yl)methyl) phenyl)piperidine-1,4-dicarboxamide (**10**)

Yield: 58%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.06 (s, 1H), 7.82 (dd, J = 3.6, 1.4 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.47 (s, 1H), 7.33 (dd, J = 9.5, 3.8 Hz, 1H), 7.16 (t, J = 7.9 Hz, 1H), 6.94 (d, J = 7.7 Hz, 1H), 6.87 (dd, J = 9.5, 1.6 Hz, 1H), 5.21 (s, 2H), 4.29–4.15 (m, 2H), 3.43 (q, J = 7.1 Hz, 2H), 3.32 (q, J = 7.0 Hz, 2H), 2.98–2.77 (m, 3H), 1.73–1.59 (m, 4H), 1.18 (t, J = 7.1 Hz, 3H), 1.03 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.73, 161.04, 156.17, 139.92, 137.38, 136.56, 132.36, 129.08, 128.25, 122.32, 120.22, 120.04, 54.88 (−$\underline{C}H_2$−Ph−), 43.29 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.81 (−CON($\underline{C}H_2CH_3)_2$), 40.25 (−CON($\underline{C}H_2CH_3)_2$), 38.28 (−NHCON$(CH_2CH_2)_2\underline{C}$HCO−), 28.39 (−NHCON$(CH_2\underline{C}H_2)_2$CHCO−), 13.73 (−CON$(CH_2\underline{C}H_3)_2$), 11.78 (−CON$(CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z 412 [M + H]$^+$; HRMS(ESI) calcd for $C_{22}H_{30}N_5O_3$ [M + H]$^+$ 412.2349, found 412.2331.

4.2.3. Diethyl-N^1-(3-((6-oxo-3-(o-tolyl)pyridazin-1(6H)-yl)methyl) phenyl)piperidine-1,4-dicarboxamide (**11a**)

Yield: 69%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.01 (s, 1H), 7.53 (t, J = 8.3 Hz, 3H), 7.41 (d, J = 7.1 Hz, 1H), 7.35–7.23 (m, 3H), 7.17 (t, J = 7.7 Hz, 1H), 7.03–6.92 (m, 2H), 5.27 (s, 2H), 4.31–4.16 (m, 2H), 3.42 (q, J = 7.0 Hz, 2H), 3.34–3.26 (m, 2H), 2.87 (ddd, J = 21.2, 14.9, 7.1 Hz, 3H), 2.32 (s, 3H), 1.75–1.61 (m, 4H), 1.17 (t, J = 7.1 Hz, 3H), 1.02 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.72, 159.99, 156.17, 147.71, 139.97, 136.68, 135.94, 134.90, 134.25, 130.60, 128.91, 128.80, 128.71, 128.29, 125.72, 122.49, 120.40, 120.06, 55.02 (−$\underline{C}H_2$−Ph−), 43.30 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.80 (−CON($\underline{C}H_2CH_3)_2$), 40.25 (−CON($\underline{C}H_2CH_3)_2$), 38.29 (−NHCON$(CH_2CH_2)_2\underline{C}$HCO−), 28.39 (−NHCON$(CH_2\underline{C}H_2)_2$CHCO−), 19.20 (−NH−Ph−$\underline{C}H_3$), 13.73 (−CON$(CH_2\underline{C}H_3)_2$), 11.79 (−CON$(CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z [M + H]$^+$ 502; HRMS(ESI) calcd for $C_{29}H_{35}N_5O_3Na$ [M + Na]$^+$ 524.2638, found 524.2657.

4.2.4. N^4,N^4-Diethyl-N^1-(3-((6-oxo-3-(p-tolyl)pyridazin-1(6H)-yl) methyl)phenyl)piperidine-1,4-dicarboxamide(**11b**)

Yield: 61%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.02 (s, 1H), 7.90 (d, J = 9.7 Hz, 1H), 7.78 (d, J = 8.3 Hz, 2H), 7.53 (d, J = 10.8 Hz, 2H), 7.27 (d, J = 8.0 Hz, 2H), 7.17 (t, J = 7.8 Hz, 1H), 7.02 (d, J = 7.4 Hz, 1H), 6.95 (d, J = 9.7 Hz, 1H), 5.29 (s, 2H), 4.30–4.15 (m, 2H), 3.42 (q, J = 7.2 Hz, 2H), 3.31 (q, J = 7.0 Hz, 2H), 2.96–2.73 (m, 3H), 2.35 (s, 3H), 1.71–1.58 (m, 4H), 1.17 (t, J = 7.1 Hz, 3H), 1.02 (t, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, MeOD-d_4) δ 174.72, 160.21, 156.21, 145.44, 139.94, 139.53, 136.66, 131.53, 130.93, 129.26, 129.11, 128.29, 125.62, 122.50, 120.31, 120.13, 55.18 (−$\underline{C}H_2$−Ph−), 43.28 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.79 (−CON($\underline{C}H_2CH_3)_2$), 40.24 (−CON($\underline{C}H_2CH_3)_2$), 38.28 (−NHCON$(CH_2CH_2)_2\underline{C}$HCO−), 28.37 (−NHCON($\underline{C}H_2\underline{C}H_2)_2$CHCO−), 19.80 (−NH−Ph−$\underline{C}H_3$), 13.71 (−CON$(CH_2\underline{C}H_3)_2$), 11.77 (−CON$(CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z [M + H]$^+$ 502; HRMS(ESI) calcd for $C_{29}H_{35}N_5O_3Na$ [M + Na]$^+$ 524.2638, found 524.2622.

4.2.5. N^4,N^4-Diethyl-N^1-(3-((6-oxo-3-(m-tolyl)pyridazin-1(6H)-yl) methyl)phenyl)piperidine-1,4-dicarboxamide (**11c**)

Yield: 73%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.08 (s, 1H), 7.88 (d, J = 9.8 Hz, 1H), 7.72 (s, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.61 (s, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.32 (t, J = 7.7 Hz, 1H), 7.22 (d, J = 7.6 Hz, 1H), 7.17 (t, J = 7.9 Hz, 1H), 7.01 (d, J = 7.5 Hz, 1H), 6.94 (d, J = 9.7 Hz, 1H), 5.29 (s, 2H), 4.28–4.15 (m, 2H), 3.47–3.28 (m, 4H), 3.00–2.71 (m, 3H), 2.37 (s, 3H), 1.75–1.55 (m, 4H), 1.22–1.10 (t, J = 7.0 Hz, 3H), 1.01 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.66, 160.15, 156.08, 145.40, 140.02, 138.35, 136.63, 134.19, 131.01, 129.91, 129.23, 128.40, 128.33, 126.24, 122.88, 122.43, 120.22, 120.00, 55.24 (−$\underline{C}H_2$−Ph−), 43.28 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.80 (−CON($\underline{C}H_2CH_3)_2$), 40.24 (−CON($\underline{C}H_2CH_3)_2$), 38.25 (−NHCON$(CH_2CH_2)_2\underline{C}$HCO−), 28.39 (−NHCON$(CH_2\underline{C}H_2)_2$CHCO−), 20.13 (−NH−Ph−$\underline{C}H_3$−), 13.79 (−CON$(CH_2\underline{C}H_3)_2$), 11.86 (−CON$(CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z [M + H]$^+$ 502; HRMS(ESI) calcd for $C_{29}H_{35}N_5O_3Na$ [M + Na]$^+$ 524.2638, found 524.2637.

4.2.6. N^1-(3-((3-(3-Bromophenyl)-6-oxopyridazin-1(6H)-yl) methyl)phenyl)-N^4,N^4-diethylpiperidine-1,4-dicarboxamide (**11d**)

Yield: 86%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.08 (s, 1H), 8.03–7.93 (m, 2H), 7.90 (d, J = 8.0 Hz, 1H), 7.60 (s, 2H), 7.51 (d, J = 8.1 Hz, 1H), 7.42 (t, J = 7.7 Hz, 1H), 7.18 (t, J = 7.7 Hz, 1H), 7.00 (t, J = 9.3 Hz, 2H), 5.31 (s, 2H), 4.28–4.15 (m, 2H), 3.42 (d, J = 7.0 Hz, 2H), 3.31 (d, J = 7.2 Hz, 2H), 3.01–2.76 (m, 3H), 1.67 (s, 4H), 1.17 (t, J = 6.8 Hz, 3H), 1.02 (t, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.72, 160.14, 156.16, 143.77, 139.99, 136.47, 132.03, 130.76, 130.25, 129.50, 128.54, 128.33, 124.49, 122.54, 120.30, 120.12, 55.28 (−$\underline{C}H_2$−Ph−), 43.28 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.80 (−CON($\underline{C}H_2CH_3)_2$), 40.25 (−CON($\underline{C}H_2CH_3)_2$), 38.27 (−NHCON$(CH_2CH_2)_2\underline{C}$HCO−), 28.38 (−NHCON($\underline{C}H_2\underline{C}H_2)_2$CHCO−), 13.72 (−CON$(CH_2\underline{C}H_3)_2$), 11.78 (−CON$(CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z [M + H]$^+$ 566; HRMS(ESI) calcd for $C_{28}H_{32}N_5O_3NaBr$ [M + Na]$^+$ 588.1586, found 588.1569.

4.2.7. N^1-(3-((3-(3-Chlorophenyl)-6-oxopyridazin-1(6H)-yl) methyl)phenyl)-N^4,N^4-diethylpiperidine-1,4-dicarboxamide (**11e**)

Yield: 92%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.06 (s, 1H), 7.96 (d, J = 9.8 Hz, 2H), 7.85 (d, J = 7.1 Hz, 1H), 7.63 (s, 1H), 7.55–7.43 (m, 3H), 7.18 (t, J = 7.8 Hz, 1H), 7.02 (t, J = 8.1 Hz, 2H), 5.32 (s, 2H), 4.30–4.19 (m, 2H), 3.50–3.25 (m, 4H), 3.01–2.75 (m, 3H), 1.78–1.60 (m, 4H), 1.18 (dd, J = 8.3, 5.8 Hz, 3H), 1.03 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.72, 160.16, 156.18, 143.90, 139.98, 136.47, 136.29, 134.54, 130.79, 130.03, 129.50, 129.05, 128.32, 125.61, 124.07, 122.55, 120.33, 120.13, 55.28 (−$\underline{C}H_2$−Ph−), 43.27 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.79 (−CON($\underline{C}H_2CH_3)_2$), 40.24 (−

CON($\underline{C}H_2CH_3)_2$), 38.27 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 28.37 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 13.71 (−CON($CH_2\underline{C}H_3)_2$), 11.77 (−CON($CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z $[M + H]^+$ 522; HRMS(ESI) calcd for $C_{28}H_{32}N_5O_3NaCl$ $[M + Na]^+$ 544.2091, found 544.2084.

4.2.8. N^4,N^4-Diethyl-N^1-(3-((3-(naphthalen-2-yl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) piperidine-1,4-dicarboxamide (**12a**)

Yield: 88%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.39 (s, 1H), 8.25–7.70 (m, 6H), 7.68 (s, 1H), 7.53 (d, J = 6.3 Hz, 3H), 7.19 (t, J = 7.8 Hz, 1H), 7.06 (d, J = 7.6 Hz, 1H), 6.98 (d, J = 9.8 Hz, 1H), 5.33 (s, 2H), 4.30–4.15 (m, 2H), 3.44–3.29 (m, 4H), 2.98–2.73 (m, 3H), 1.68 (m, 4H), 1.23–1.09 (t, J = 7.0 Hz, 3H), 1.02 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.69, 160.16, 156.18, 145.07, 140.00, 136.63, 133.66, 133.14, 131.52, 130.89, 129.24, 128.33, 128.22, 128.20, 127.23, 126.61, 126.21, 125.33, 122.82, 122.57, 120.33, 120.09, 55.24 (−$\underline{C}H_2$−Ph−), 43.27 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.78 (−CON($\underline{C}H_2CH_3)_2$), 40.23 (−CON($\underline{C}H_2CH_3)_2$), 38.25 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 28.36 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 13.70 (−CON($CH_2\underline{C}H_3)_2$), 11.77 (−CON($CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z $[M + H]^+$ 538; HRMS(ESI) calcd for $C_{32}H_{35}N_5O_3Na$ $[M + Na]^+$ 560.2638, found 560.2643.

4.2.9. N^4,N^4-Diethyl-N^1-(3-((3-(naphthalen-1-yl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) piperidine-1,4-dicarboxamide (**12b**)

Yield: 76%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.19–7.87 (m, 4H), 7.72–7.46 (m, 7H), 7.22 (t, J = 7.8 Hz, 1H), 7.03 (q, J = 8.7 Hz, 2H), 5.34 (s, 2H), 4.30–4.16 (m, 2H), 3.47–3.27 (m, 4H), 3.15–2.25 (m, 3H), 1.80–1.50 (m, 4H), 1.23–1.12 (t, J = 7.0 Hz, 3H), 1.03 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 174.74, 160.15, 156.21, 147.00, 140.08, 136.73, 134.90, 133.93, 132.83, 130.49, 129.56, 129.22, 128.46, 128.20, 127.13, 126.62, 125.93, 124.87, 124.70, 122.86, 120.70, 120.26, 55.04 (−$\underline{C}H_2$−Ph−), 43.35 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.88 (−CON($\underline{C}H_2CH_3)_2$), 40.34 (−CON($\underline{C}H_2CH_3)_2$), 38.36 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 28.46 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 13.86 (−CON($CH_2\underline{C}H_3)_2$), 11.91 (−CON($CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z $[M + H]^+$ 538; HRMS(ESI) calcd for $C_{32}H_{35}N_5O_3Na$ $[M + Na]^+$ 560.2638, found 560.2631.

4.2.10. N^1-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl) methyl)phenyl)-N^4,N^4-diethyl piperidine-1,4-dicarboxamide (**12c**)

Yield: 79%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.31 (s, 1H), 8.23 (d, J = 7.8 Hz, 1H), 8.05 (s, 1H), 8.01 (s, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.70–7.65 (m, 2H), 7.48 (d, J = 7.9 Hz, 1H), 7.19 (t, J = 7.8 Hz, 1H), 7.05 (d, J = 7.7 Hz, 1H), 7.00 (d, J = 9.8 Hz, 1H), 4.30–4.17 (m, 2H), 3.43–3.25 (m, 4H), 3.00–2.80 (m, 3H), 1.70–1.60 (m, 4H), 1.12 (t, J = 7.0 Hz, 3H), 1.03 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.71, 160.04, 156.10, 143.14, 140.06, 136.39, 135.65, 132.42, 130.56, 130.07, 129.64, 129.27, 128.36, 122.58, 120.29, 120.01, 117.87, 112.71, 55.26 (−$\underline{C}H_2$−Ph−), 43.29 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.80 (−CON($\underline{C}H_2CH_3)_2$), 40.25 (−CON($\underline{C}H_2CH_3)_2$), 38.27 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 28.39 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 13.74 (−CON($CH_2\underline{C}H_3)_2$), 11.80 (−CON($CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z $[M + H]^+$ 513; HRMS(ESI) calcd for $C_{29}H_{32}N_6O_3Na$ $[M + Na]^+$ 535.2434, found 535.2439.

4.2.11. N^4,N^4-Diethyl-N^1-(3-((6-oxo-3-(phenylamino)pyridazin-1(6H)-yl)methyl)phenyl) piperidine-1,4-dicarboxamide (**13**)

Yield: 64%: ^{1}H NMR (300 MHz, CD_3OD-d_4) δ 7.47 (d, J = 8.2 Hz, 2H), 7.41 (s, 1H), 7.33 (d, J = 8.7 Hz, 1H), 7.25–7.07 (m, 4H), 7.08 (d, J = 7.6 Hz, 1H), 6.92 (t, J = 7.8 Hz, 2H), 5.20 (s, 2H), 4.30–4.15 (m, 2H), 3.50–3.30 (m, 4H), 3.05–2.72 (m, 3H), 1.80–1.64 (m, 4H), 1.23 (t, J = 7.0 Hz, 3H), 1.10 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.74, 158.48, 156.24, 145.41, 140.36, 139.85, 137.09, 130.15, 128.22, 128.17, 122.72, 121.03, 120.72, 120.05, 117.66, 101.92, 54.02 (−$\underline{C}H_2$−Ph−), 43.28 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.79 (−CON($\underline{C}H_2CH_3)_2$), 40.24 (−CON($\underline{C}H_2CH_3)_2$), 38.30 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 28.38 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 13.69 (−CON($CH_2\underline{C}H_3)_2$), 11.75 (−CON($CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z $[M + H]^+$ 503; HRMS(ESI) calcd for $C_{28}H_{34}N_6O_3Na$ $[M + Na]^+$ 525.2590, found 525.2618.

4.2.12. N^4,N^4-Diethyl-N^1-(3-((3-(naphthalen-1-ylamino)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) piperidine-1,4-dicarboxamide (**14**)

Yield: 81%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.23 (d, J = 8.8 Hz, 1H), 8.16 (s, 1H), 8.03 (s, 1H), 7.99–7.91 (m, 1H), 7.88 (d, J = 4.7 Hz, 1H), 7.63–7.38 (m, 7H), 7.18 (t, J = 7.8 Hz, 1H), 6.99 (d, J = 7.8 Hz, 1H), 6.87 (d, J = 9.8 Hz, 1H), 5.10 (s, 2H), 4.30–4.15 (m, 2H), 3.48–3.39 (m, 2H), 3.39–3.25 (m, 2H), 3.00–2.18 (m, 3H), 1.79–1.62 (m, 4H), 1.19 (t, J = 6.8 Hz, 3H), 1.04 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.84, 158.86, 156.34, 146.75, 139.88, 137.14, 135.47, 134.45, 130.31, 128.29, 128.10, 128.03, 127.27, 125.56, 125.40, 125.33, 123.40, 122.83, 121.69, 120.85, 120.16, 117.56, 54.11 (−$\underline{C}H_2$−Ph−), 43.39 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.91 (−CON($\underline{C}H_2CH_3)_2$), 40.36 (−CON($\underline{C}H_2CH_3)_2$), 38.41 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 28.51 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 13.82 (−CON($CH_2\underline{C}H_3)_2$), 11.89 (−CON($CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z $[M + H]^+$ 553; HRMS(ESI) calcd for $C_{32}H_{36}N_6O_3Na$ $[M + Na]^+$ 575.2747, found 575.2739.

4.2.13. N^4,N^4-Diethyl-N^1-(3-((6-oxo-3-(o-tolylamino)pyridazin-1(6H)-yl)methyl)phenyl) piperidine-1,4-dicarboxamide (**15a**)

Yield: 80%: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (s, 1H), 7.93 (s, 1H), 7.60 (d, J = 9.0 Hz, 1H), 7.38 (m, 2H), 7.31–7.05 (m, 3H), 6.96–6.79 (m, 3H), 4.20–4.12 (m 2H), 3.40–3.24 (m, 4H), 2.94–2.69 (m, 3H), 2.16 (s, 3H), 1.67–1.44 (m, 4H), 1.13 (t, J = 7.0 Hz, 3H), 0.99 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.73, 158.67, 156.21, 146.39, 139.77, 137.96, 137.05, 130.17, 130.02, 129.93, 128.15, 127.87, 125.84, 123.29, 122.65, 121.85, 120.75, 120.04, 53.87 (−$\underline{C}H_2$−Ph−), 43.28 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.80 (−CON($\underline{C}H_2CH_3)_2$), 40.25 (−CON($\underline{C}H_2CH_3)_2$), 38.30 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 28.40 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 16.77 (−NH−Ph−$\underline{C}H_3$), 13.71 (−CON($CH_2\underline{C}H_3)_2$), 11.77 (−CON($CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z $[M + H]^+$ 517; HRMS(ESI) calcd for $C_{29}H_{36}N_6O_3Na$ $[M + Na]^+$ 539.2747, found 539.2742.

4.2.14. N^4,N^4-Diethyl-N^1-(3-((6-oxo-3-(m-tolylamino)pyridazin-1(6H)-yl)methyl)phenyl) piperidine-1,4-dicarboxamide (**15b**)

Yield: 78%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.15 (s, 1H), 8.01 (s, 1H), 7.56 (s, 2H), 7.40 (s, 1H), 7.34 (d, J = 7.9 Hz, 1H), 7.26–7.10 (m, 3H), 7.07 (d, J = 7.5 Hz, 1H), 6.83 (d, J = 9.8 Hz, 1H), 6.74 (d, J = 6.8 Hz, 1H), 5.15 (s, 2H), 4.30–4.20 (m, 2H), 3.44 (t, J = 7.0 Hz, 2H), 3.34 (q, J = 7.0 Hz, 2H), 3.00–2.92 (m, 3H), 2.28 (s, 3H), 1.75–1.64 (m, 4H), 1.20 (d, J = 7.0 Hz, 3H), 1.05 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.66, 158.38, 156.14, 145.39, 140.29, 139.92, 138.00, 137.23, 129.97, 128.30, 128.19, 128.14, 122.79, 121.82, 120.80, 120.09, 118.22, 114.84, 53.96 (−$\underline{C}H_2$−Ph−), 43.28 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.81 (−CON($\underline{C}H_2CH_3)_2$), 40.25 (−CON($\underline{C}H_2CH_3)_2$), 38.26 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 28.41 (−NHCON($CH_2\underline{C}H_2)_2$CH CO−), 20.41 (−NH−Ph−$\underline{C}H_3$), 13.80 (−CON($CH_2\underline{C}H_3)_2$), 11.87 (−CON($CH_2\underline{C}H_3)_2$); LCMS(ESI) m/z $[M + H]^+$ 517; HRMS(ESI) calcd for $C_{29}H_{36}N_6O_3Na$ $[M + Na]^+$ 593.2747, found 593.2744.

4.2.15. N^4,N^4-Diethyl-N^1-(3-((6-oxo-3-(p-tolylamino)pyridazin-1(6H)-yl)methyl)phenyl) piperidine-1,4-dicarboxamide (**15c**)

Yield: 82%: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.49 (s, 1H), 7.45 (s, 1H), 7.40–7.32 (m, 3H), 7.20–7.13 (m, 2H), 7.02 (d, J = 8.5 Hz, 2H), 6.88–6.78 (m, 2H), 4.15–4.05 (m, 2H), 3.30–3.19 (m, 4H), 2.90–2.70 (m, 3H), 2.19 (s, 3H), 1.65–1.40 (m, 4H), 1.11 (t, J = 7.1 Hz, 3H), 0.97 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 174.79, 158.51, 156.32, 145.63, 139.90, 137.90, 137.17, 130.55, 130.06, 128.74, 128.28, 128.22, 122.79, 120.78, 120.11, 117.87, 54.12 (−$\underline{C}H_2$−Ph−), 43.36 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 41.86 (−CON($\underline{C}H_2CH_3)_2$), 40.31(−CON($\underline{C}H_2CH_3)_2$), 38.36 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 28.46

(−NHCON($CH_2\underline{C}H_2$)$_2$CHCO−), 19.40 (−NH−Ph−$\underline{C}H_3$), 13.77 (−CON($CH_2\underline{C}H_3$)$_2$), 11.83 (−CON($CH_2\underline{C}H_3$)$_2$); LCMS(ESI) *m/z* [M + H]$^+$ 517; HRMS(ESI) calcd for $C_{29}H_{36}N_6O_3Na$ [M + Na]$^+$ 593.2747, found 593.2745.

4.2.16. N^4,N^4-Diethyl-N^1-(3-((6-oxo-3-((2-(trifluoromethyl)phenyl) amino)pyridazin-1(6H)-yl)methyl)phenyl)piperidine-1,4-dicarboxamide (**15d**)

Yield: 75%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.06 (s, 1H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 7.9 Hz, 1H), 7.61−7.53 (m, 2H), 7.50 (d, *J* = 8.1 Hz, 1H), 7.39 (d, *J* = 9.8 Hz, 2H), 7.25−7.10 (m, 2H), 6.97 (d, *J* = 7.6 Hz, 1H), 6.88 (d, *J* = 9.8 Hz, 1H), 5.07 (s, 2H), 4.30−4.15 (m, 2H), 3.50−3.40 (m, 2H), 3.40−3.25 (m, 2H), 2.90−2.77 (m, 3H), 1.78−1.63 (m, 4H), 1.23−1.14 (t, *J* = 7.0 Hz, 3H), 1.04 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.68, 158.74, 156.12, 145.73, 139.89, 137.65, 136.99, 132.35, 130.67, 128.23, 127.90, 125.92, 125.88, 125.10, 123.35, 122.67, 121.71, 121.48, 120.76, 120.03, 53.98 (−$\underline{C}H_2$−Ph−), 43.30 (−NHCON($\underline{C}H_2CH_2$)$_2$CHCO−), 41.82 (−CON($\underline{C}H_2CH_3$)$_2$), 40.25 (−CON($\underline{C}H_2CH_3$)$_2$), 38.29 (−NHCON(CH_2CH_2)$_2\underline{C}$HCO−), 28.44 (−NHCON($CH_2\underline{C}H_2$)$_2$CHCO−), 13.80 (−CON($CH_2\underline{C}H_3$)$_2$), 11.86 (−CON($CH_2\underline{C}H_3$)$_2$); LCMS(ESI) *m/z* [M + H]$^+$ 571; HRMS(ESI) calcd for $C_{29}H_{33}N_6O_3NaF_3$ [M + Na]$^+$ 593.2464, found 593.2485.

4.2.17. N^4,N^4-Diethyl-N^1-(3-((6-oxo-3-((3-(trifluoromethyl)phenyl) amino)pyridazin-1(6H)-yl) methyl)phenyl)piperidine-1,4-dicarboxamide (**15e**)

Yield: 74%: ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (s, 1H), 7.57 (d, *J* = 9.2 Hz, 1H), 7.46 (s, 1H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.21 (t, *J* = 7.8 Hz, 1H), 7.12 (d, *J* = 7.7 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 1H), 7.00 (d, *J* = 9.7 Hz, 1H), 6.76 (d, *J* = 9.7 Hz, 1H), 5.12 (s, 2H), 4.18 (d, *J* = 13.2 Hz, 2H), 3.45−3.34 (m, 2H), 3.30 (t, *J* = 7.1 Hz, 2H), 2.99−2.82 (m, 2H), 2.82−2.73 (m, 1H), 1.69 (dd, *J* = 19.6, 8.4 Hz, 4H), 1.17 (t, *J* = 7.1 Hz, 3H), 1.05 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.63, 158.28, 156.16, 144.83, 141.02, 139.97, 137.01, 130.45, 129.02, 128.36, 127.95, 125.36, 123.20, 123.03, 121.08, 120.67, 120.38, 117.01, 113.70, 54.15 (−$\underline{C}H_2$−Ph−), 43.31 (−NHCON($\underline{C}H_2CH_2$)$_2$CHCO−), 41.80 (−CON($\underline{C}H_2CH_3$)$_2$), 40.24 (−CON($\underline{C}H_2CH_3$)$_2$), 38.26 (−NHCON(CH_2CH_2)$_2\underline{C}$HCO−), 28.40 (−NHCON($CH_2\underline{C}H_2$)$_2$CHCO−), 13.80 (−CON($CH_2\underline{C}H_3$)$_2$), 11.87 (−CON($CH_2\underline{C}H_3$)$_2$); LCMS(ESI) *m/z* [M + H]$^+$ 571; HRMS(ESI) calcd for $C_{29}H_{33}N_6O_3NaF_3$ [M + Na]$^+$ 593.2464, found 593.2449.

4.2.18. N^4,N^4-Diethyl-N^1-(3-((6-oxo-3-((4-(trifluoromethyl)phenyl) amino)pyridazin-1(6H)-yl) methyl)phenyl)piperidine-1,4-dicarboxamide (**15f**)

Yield: 58%: ^{1}H NMR (300 MHz, CD_3OD-d_4) δ 7.62 (d, *J* = 8.6 Hz, 2H), 7.54−7.45 (m, 3H), 7.35−7.29 (m, 1H), 7.26 (t, *J* = 7.7 Hz, 1H), 7.18 (d, *J* = 9.7 Hz, 1H), 7.09 (d, *J* = 7.2 Hz, 1H), 6.94 (d, *J* = 9.7 Hz, 1H), 4.25−4.15 (m, 2H), 3.50−3.31 (m, 4H), 3.32−2.78 (m, 3H), 1.77−1.66 (m, 3H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.09 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.40, 157.48, 155.21, 155.14, 144.53, 143.87, 143.78, 141.31, 141.21, 137.65, 131.83, 128.75, 128.37, 128.30, 126.40, 126.14, 123.99, 122.11, 120.87, 120.61, 119.99, 119.89, 119.01, 118.91, 117.30, 117.24, 53.73 (−$\underline{C}H_2$−Ph−), 43.71 (−NHCON($\underline{C}H_2CH_2$)$_2$ CHCO−), 41.48 (−CON($\underline{C}H_2CH_3$)$_2$), 39.76 (−CON($\underline{C}H_2CH_3$)$_2$), 37.86 (−NHCON(CH_2CH_2)$_2\underline{C}$HCO−), 28.93 (−NHCON($\underline{C}H_2CH_2$)$_2$CHCO−), 15.34 (−CON($CH_2\underline{C}H_3$)$_2$), 13.39 (−CON($CH_2\underline{C}H_3$)$_2$); LCMS(ESI) *m/z* [M + H]$^+$ 571; HRMS(ESI) calcd for $C_{29}H_{33}N_6O_3NaF_3$ [M + Na]$^+$ 593.2464, found 593.2482.

4.2.19. N^1-(3-((3-((2,6-Dimethylphenyl)amino)-6-oxopyridazin-1(6H)-yl)methyl)phenyl)-N^4,N^4-diethylpiperidine-1,4-dicarboxamide (**16a**)

Yield: 76%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.01 (s, 1H), 7.51 (d, *J* = 9.1 Hz, 1H), 7.31 (d, *J* = 7.0 Hz, 2H), 7.16−7.02 (m, 5H), 6.79 (d, *J* = 9.7 Hz, 2H), 4.92 (s, 2H), 4.3−4.15 (m, 2H), 3.51−3.31 (m, 4H), 3.10−2.25 (m, 3H), 2.14 (s, 6H), 1.77−1.63 (m, 4H), 1.19 (t, *J* = 7.0 Hz, 3H), 1.04 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.74, 158.58, 156.17, 147.34, 139.60, 136.96, 135.97, 135.60, 130.43, 128.02, 127.62, 126.56, 125.95, 122.47, 120.84, 120.01, 53.60 (−$\underline{C}H_2$−Ph−), 43.28 (−NHCON($\underline{C}H_2CH_2$)$_2$CHCO−), 41.81 (−CON($\underline{C}H_2CH_3$)$_2$), 40.25 (−CON($\underline{C}H_2CH_3$)$_2$), 38.32 (−NHCON(CH_2CH_2)$_2\underline{C}$HCO−), 28.43 (−NHCON($CH_2\underline{C}H_2$)$_2$CHCO−), 17.07, 13.72 (−CON($\underline{C}H_2CH_3$)$_2$), 11.78 (−CON($CH_2\underline{C}H_3$)$_2$); HRMS(ESI) *m/z* [M + H]$^+$ 531; HRMS(ESI) calcd for $C_{30}H_{38}N_6O_3Na$ [M + Na]$^+$ 553.2903, found 553.2876.

4.2.20. N^4,N^4-Diethyl-N^1-(3-((3-((2-ethylphenyl)amino)-6-oxopyridazin-1(6H)-yl)methyl) phenyl)piperidine-1,4-dicarboxamide (**16b**)

Yield: 70%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.07 (s, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.36 (s, 1H), 7.26 (t, *J* = 6.6 Hz, 1H), 7.21−7.08 (m, 3H), 6.99 (m, 2H), 6.85−6.76 (m, 1H), 5.06 (s, 2H), 4.24 (m, 2H), 3.50−3.28 (m, 4H), 3.10−2.28 (m, 3H), 2.66 (q, *J* = 7.5 Hz, 2H), 1.78−1.62 (m, 4H), 1.24−1.09 (m, 6H), 1.15−1.10 (m, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.73, 158.68, 156.21, 146.84, 139.75, 137.22, 137.06, 136.55, 130.19, 128.25, 128.13, 127.68, 125.83, 123.96, 123.11, 122.64, 120.75, 120.04, 53.86 (−$\underline{C}H_2$−Ph−), 43.28 (−NHCON($\underline{C}H_2CH_2$)$_2$CHCO−), 41.80 (−CON($\underline{C}H_2CH_3$)$_2$), 40.25 (−CON($\underline{C}H_2CH_3$)$_2$), 38.31 (−NHCON(CH_2CH_2)$_2\underline{C}$HCO−), 28.41 (−NHCON(CH_2CH_2)$_2\underline{C}$HCO−), 23.76 (−NH−Ph−$\underline{C}H_2CH_3$), 13.71 (−CON($CH_2\underline{C}H_3$)$_2$), 13.16 (−NH−Ph−$CH_2\underline{C}H_3$), 11.78 (−CON($CH_2\underline{C}H_3$)$_2$); HRMS(ESI) *m/z* [M + H]$^+$ 531; HRMS(ESI) calcd for $C_{30}H_{38}N_6O_3Na$ [M + Na]$^+$ 553.2903, found 553.2903.

4.2.21. N^4,N^4-Diethyl-N^1-(3-((3-((2-isopropylphenyl)amino)-6-oxopyridazin-1(6H)-yl)methyl) phenyl)piperidine-1,4-dicarboxamide (**16c**)

Yield: 89%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.10 (s, 1H), 7.49 (m, 4H), 7.27 (d, *J* = 7.4 Hz, 1H), 7.22 (d, *J* = 9.8 Hz, 1H), 7.18−7.01 (m, 3H), 6.91 (d, *J* = 7.5 Hz, 1H), 6.79 (d, *J* = 9.8 Hz, 1H), 5.03 (s, 2H), 4.30−4.15 (m, 2H), 3.50−3.25 (m, 5H), 2.99−2.73 (m, 3H), 1.75−1.61 (m, 4H), 1.25−1.05 (m, 9H), 1.02 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.73, 158.71, 156.21, 147.50, 142.44, 139.71, 137.06, 136.38, 130.20, 128.11, 127.46, 125.67, 125.31, 124.83, 124.61, 122.46, 120.59, 120.00, 53.92 (−$\underline{C}H_2$−Ph−), 43.27 (−NHCON($\underline{C}H_2CH_2$)$_2$CHCO−), 41.80 (−CON($\underline{C}H_2CH_3$)$_2$), 40.24 (−CON($\underline{C}H_2CH_3$)$_2$), 38.30 (−NHCON(CH_2CH_2)$_2\underline{C}$HCO−), 28.40 (−NHCON($\underline{C}H_2CH_2$)$_2$CHCO−), 27.21 (Ph−$\underline{C}H(CH_3)_2$−), 22.22 (Ph−CH($\underline{C}H_3$)$_2$−), 13.71 (−CON($CH_2\ \underline{C}H_3$)$_2$), 11.77 (−CON($CH_2\underline{C}H_3$)$_2$); HRMS(ESI) *m/z* [M + H]$^+$ 545; HRMS(ESI) calcd for $C_{31}H_{40}N_6O_3Na$ [M + H]$^+$ 567.3060, found 567.3058.

4.2.22. 4-(Morpholine-4-carbonyl)-N-(3-((6-oxo-3-(o-tolylamino) pyridazin-1(6H)-yl)methyl) phenyl)piperidine-1-carboxamide (**17a**)

Yield: 89%: ^{1}H NMR (300 MHz, acetone-d_6) δ 7.65 (s, 1H), 7.37 (d, *J* = 7.7 Hz, 1H), 7.20−7.15 (d, *J* = 9.2 Hz, 2H), 6.92 (d, *J* = 9.6 Hz, 2H), 6.85−6.70 (m, 3H), 6.64−6.51 (m, 2H), 6.46 (d, *J* = 9.5 Hz, 1H), 3.90−3.75 (m, 2H), 3.30−3.20 (s, 4H), 3.19−3.08 (m, 2H), 2.57−2.48 (m, 5H), 1.88 (s, 3H), 1.75−1.60 (m, 4H); ^{13}C NMR (126 MHz, MeOD-d_4) δ 174.05, 158.78, 156.32, 146.49, 139.86, 138.07, 137.17, 130.29, 130.13, 130.03, 128.28, 127.99, 125.95, 123.39, 122.78, 121.95, 120.89, 120.16, 66.61 (CON($CH_2\underline{C}H_2$)$_2$O), 66.41(CON($CH_2\underline{C}H_2$)$_2$O), 53.99 (−$\underline{C}H_2$−Ph−), 45.83 (CON($\underline{C}H_2CH_2$)$_2$O), 43.35 (−NHCON($\underline{C}H_2CH_2$)$_2$CHCO−), 42.05 (CON($\underline{C}H_2CH_2$)$_2$ O), 37.75 (−NHCON($\underline{C}H_2CH_2$)$_2\underline{C}$HCO−), 28.21 (−NHCON($CH_2\underline{C}H_2$)$_2$CHCO−); LCMS(ESI) *m/z* [M + H]$^+$ 531; HRMS(ESI) calcd for $C_{29}H_{34}N_6O_4Na$ [M + Na]$^+$ 553.2539, found 553.2514.

4.2.23. N^4,N^4-Dimethyl-N^1-(3-((6-oxo-3-(o-tolylamino)pyridazin-1(6H)-yl)methyl)phenyl) piperidine-1,4-dicarboxamide(**17b**)

Yield: 93%: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.04 (s, 1H), 7.72 (d, *J* = 8.7 Hz, 1H), 7.51 (m, 2H), 7.33 (s, 1H), 7.28 (d, *J* = 9.8 Hz, 1H),

7.21–7.09 (m, 3H), 7.00–6.70 (m, 2H), 6.82 (d, J = 9.8 Hz, 1H), 5.07 (s, 2H), 4.30–4.15 (m, 2H), 3.10 (s, 3H), 2.92–2.80 (m, 3H), 2.86 (s, 3H), 2.24 (s, 3H), 1.76–1.54 (m, 4H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 175.45, 158.78, 156.32, 146.49, 139.89, 138.07, 137.17, 130.29, 130.14, 130.04, 128.27, 128.00, 125.95, 123.40, 122.75, 121.96, 120.88, 120.14, 53.98 (−$\underline{C}H_2$−Ph−), 43.40 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 38.25 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 36.19 (−CON($\underline{C}H_3)_2$), 34.65 (−CON($\underline{C}H_3)_2$), 28.01 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 16.90; LCMS(ESI) m/z [M + H]$^+$ 489; HRMS(ESI) calcd for $C_{27}H_{32}N_6O_3Na$ [M + Na]$^+$ 511.2434, found 511.2453.

4.2.24. N^4-Ethyl-N^4-methyl-N^1-(3-((6-oxo-3-(o-tolylamino) pyridazin-1(6H)-yl)methyl) phenyl)piperidine-1,4-dicarboxamide(**17c**)

Yield: 75%: ^{1}H NMR (300 MHz, CD_3OD) δ 7.48 (d, J = 8.0 Hz, 1H), 7.31 (m, 2H), 7.22 (t, J = 7.9 Hz, 2H), 7.18–7.05 (m, 2H), 6.96 (t, J = 7.8 Hz, 2H), 6.91–6.82 (m, 1H), 5.09 (s, 2H), 4.25–4.10 (m, 2H), 3.52–3.27 (m, 2H), 3.08 (s, 1.5H), 3.03–2.78 (m, 4.5H), 2.18 (s, 3H), 1.80–1.58 (m, 4H), 1.21 (t, J = 7.1 Hz, 1.5H), 1.08 (t, J = 7.1 Hz, 1.5H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 175.27, 174.89, 158.78, 156.32, 146.48, 139.89, 138.08, 137.18, 130.29, 130.14, 130.01, 128.27, 127.98, 125.96, 123.39, 122.76, 121.94, 120.86, 120.15, 53.98 (−$\underline{C}H_2$−Ph−), 44.14 (−CON(CH_3)$\underline{C}H_2CH_3$), 43.41 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 43.38 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 42.53 (−CON(CH_3)$\underline{C}H_2CH_3$), 38.45 (−NHCON($CH_2CH_2)_2\underline{C}$HCO−), 38.20 (−NHCON($CH_2CH_2)_2$ $\underline{C}$HCO−), 33.79 (−CON($\underline{C}H_3$)CH_2CH_3), 32.17 (−CON($\underline{C}H_3$)CH_2CH_3), 28.54 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 27.95 (−NHCON($CH_2\underline{C}H_2)_2$ CHCO−), 16.89, 13.02 (−CON(CH_3)$CH_2\underline{C}H_3$), 11.04 (−CON(CH_3) $CH_2\underline{C}H_3$); LCMS(ESI) m/z [M + H]$^+$ 503; HRMS(ESI) calcd for $C_{28}H_{34}N_6O_3Na$ [M + Na]$^+$ 525.2590, found 525.2600.

4.2.25. N^4-Isopropyl-N^4-methyl-N^1-(3-((6-oxo-3-(o-tolylamino) pyridazin-1(6H)-yl)methyl) phenyl)piperidine-1,4-dicarboxamide (**17d**)

Yield: 68%: ^{1}H NMR (300 MHz, CD_3OD) δ 7.49 (d, J = 8.0 Hz, 1H), 7.33–7.27 (m, 2H), 7.27–7.19 (m, 2H), 7.19–7.06 (m, 2H), 6.97 (t, J = 7.7 Hz, 2H), 6.88 (d, J = 9.7 Hz, 1H), 5.10 (s, 2H), 4.80–4.71 (m, 0.5H), 4.39–4.24 (m, 0.5H), 4.25–4.12 (m, 2H), 3.04–2.81 (m, 4.5H), 2.77 (s, 1.5H), 2.19 (s, 3H), 1.82–1.59 (m, 4H), 1.24 (d, J = 6.6 Hz, 3H), 1.10 (d, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 174.92, 174.88, 158.78, 156.33, 146.49, 139.88, 138.07, 137.17, 130.28, 130.13, 130.04, 128.26, 127.97, 125.95, 123.40, 122.76, 121.97, 120.87, 120.16, 53.97 (−$\underline{C}H_2$−Ph−), 44.38 (−CON(CH_3)$\underline{C}H(CH_3)_2$), 43.44 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 43.40 (−NHCON ($\underline{C}H_2CH_2)_2$CH CO−), 39.00 (−NHCON($CH_2CH_2)_2\underline{C}$HCO−), 38.43 (−NHCON(CH_2 $CH_2)_2$ $\underline{C}$HCO−), 28.65 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 27.99 (−NHCON ($CH_2\underline{C}H_2)_2$CHCO−), 27.24 (−CO N($\underline{C}H_3$)$CH(CH_3)_2$), 25.43 (−CON ($\underline{C}H_3$)$CH(CH_3)_2$), 19.49 (−CON(CH_3)$CH(\underline{C}H_3)_2$), 18.10 (−CON(CH_3) $CH(\underline{C}H_3)_2$), 16.88 (−NH−Ph−$\underline{C}H_3$); LCMS(ESI) m/z [M + H]$^+$ 517; HRMS(ESI) calcd for $C_{29}H_{36}N_6O_3Na$ [M + Na]$^+$ 539.2747, found 539.2733.

4.2.26. N^4-Ethyl-N^4-isopropyl-N^1-(3-((6-oxo-3-(o-tolylamino) pyridazin-1(6H)-yl)methyl) phenyl)piperidine-1,4-dicarboxamide(**17e**)

Yield: 55%: ^{1}H NMR (300 MHz, CD_3OD) δ 7.49 (d, J = 8.0 Hz, 1H), 7.33 (d, J = 7.8 Hz, 2H), 7.29–7.19 (m, 2H), 7.19–7.05 (m, 2H), 6.98 (d, J = 7.6 Hz, 2H), 6.88 (d, J = 9.7 Hz, 1H), 5.10 (s, 2H), 4.65–4.50 (m, 0.5H), 4.30–4.12 (m, 2.5H), 3.43–3.20 (m, 2H), 3.10–2.75 (m, 3H), 2.19 (s, 3H), 1.90–1.75 (m, 4H), 1.28–1.08 (m, 9H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 175.20, 174.34, 158.63, 156.15, 146.35, 139.79, 137.97, 137.03, 130.16, 130.06, 129.80, 128.19, 127.93, 125.87, 123.24, 122.62, 121.74, 120.81, 120.03, 53.98 (−$\underline{C}H_2$−Ph−), 45.73 (−CON(CH_3) $\underline{C}H(CH_3)_2$), 43.32 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 42.02 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 39.25 (−NHCON($CH_2CH_2)_2$ $\underline{C}$HCO−), 38.43 (−NHCON($CH_2CH_2)_2$ $\underline{C}$HCO−), 37.02 (−CON($\underline{C}H_2CH_3$) $CH(CH_3)_2$), 35.27 (−CON($\underline{C}H_2CH_3$)$CH(CH_3)_2$), 28.52 (−NHCO N($CH_2\underline{C}H_2)_2$CHCO−), 28.50 (−NHCON($CH_2\underline{C}H_2)_2$ CHCO−), 20.27 (−CON($\underline{C}H_2CH_3$)$CH(\underline{C}H_3)_2$), 19.13 (−CON($CH_2\underline{C}H_3$)$CH(\underline{C}H_3)_2$), 16.87 (−NH−Ph−$\underline{C}H_3$), 16.17 (−CON($CH_2\underline{C}H_3$)$CH(CH_3)_2$), 13.71 (−CON($\underline{C}H_2\underline{C}H_3$)$CH(CH_3)_2$); LCMS(ESI) m/z [M + H]$^+$ 531; HRMS(ESI) calcd for $C_{30}H_{38}N_6O_3Na$ [M + Na]$^+$ 553.2903, found 553.2926.

4.2.27. N^4,N^4-Diisopropyl-N^1-(3-((6-oxo-3-(o-tolylamino) pyridazin-1(6H)-yl)methyl) phenyl)piperidine-1,4-dicarboxamide (**17f**)

Yield: 62%: ^{1}H NMR (300 MHz, acetone-d_6) δ 7.97 (s, 1H), 7.77–7.66 (m, 1H), 7.51 (m, 2H), 7.34–7.21 (m, 2H), 7.21–7.10 (m, 3H), 6.99–6.70 (m, 2H), 6.81 (d, J = 9.8 Hz, 1H), 5.06 (s, 2H), 4.25–4.11 (m, 4H), 3.55 (s, 1H), 3.01–2.74 (m, 3H), 2.23 (s, 3H), 1.74–1.62 (m, 4H), 1.36–1.19 (m, 12H); ^{13}C NMR (125 MHz, MeOD-d_4) δ 158.80, 156.36, 146.51, 139.88, 138.07, 137.17, 130.29, 130.12, 130.08, 128.25, 127.97, 125.94, 123.41, 122.76, 122.00, 120.85, 120.16, 53.97 (−$\underline{C}H_2$−Ph−), 45.77 (−CON($\underline{C}H(CH_3)_2)_2$), 43.43 (−NHCON($\underline{C}H_2CH_2)_2$CHCO−), 39.84 (−NHCON($CH_2CH_2)_2\underline{C}$HCO−), 28.56 (−NHCON($CH_2\underline{C}H_2)_2$CHCO−), 19.52 (−CON(CH($\underline{C}H_3)_2)_2$), 16.86; LCMS(ESI) m/z [M + H]$^+$ 545; HRMS(ESI) calcd for $C_{31}H_{40}N_6O_3Na$ [M + Na]$^+$ 567.3060, found 567.3073.

5. Materials and methods

5.1. Biological assays

Cholinesterase (ChE) activity was evaluated using modified Ellman's spectrophotometric method [36]. Individual compounds were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 10 mM and diluted to appropriate concentrations in double-distilled water. For in vitro tests of inhibition, 4 mL reaction mixtures consisting 0.1 mL of test compound, selective substrate [0.6 mL acetylthiocholine iodide for AChE (2 mM) or 0.8 mL *S*-butyrylthiocholine iodide for BuChE (2 mM)], 1 mL of phosphate buffered solution (0.1 mM), and 0.1 mL of enzyme (homogenate of rat cerebral cortex for AChE, rat serum for BuChE) were incubated at 37 °C for 8 min, and the reaction was terminated with 1 mL of 3% (w/v) sodium dodecylsulfate (SDS). Finally, 1 mL of 0.2% (w/v) 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) was added, and the yellow anion, 5-thio-2-nitrobenzate, was measured at 440 nm. All samples were assayed in duplicate.

5.2. Docking

The crystal structure of donepezil–AChE complex (code ID: 1EVE) was downloaded from the Protein Data Bank. Further preparation of the protein included deleting the waters, addition of hydrogen atoms and applying CHARMm forcefield. The 3D Structures of **4**, **12b** and **15a** were built and performed geometry optimization by molecular mechanics.

Docking studies were performed using the CDOCKER protocol of Discovery Studio 2.1 program. The binding sphere was generated at the center of the donepezil with a radius of 11.5 Å. Then the ligand donepezil was deleted and the docking results were obtained using the default settings except the value of random conformations was set to 300 and the value of top hits was set to 20. The docking results were analyzed using Discovery Studio 3.5 Visualizer.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.01.056.

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