Synthesis and preliminary biological evaluation of new phthalazinone derivatives with PARP-1 and cholinesterase inhibitory activities

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Abstract

The inhibition of Poly (ADP-ribose) polymerases-1 (PARP-1) has a potentially therapeutical value for AD. In order to search for a new agent based on multitarget-directed ligands (MTDLs) strategy, a series of 21 novel compounds incorporated the respective pharmacophores of two marketed drugs, namely 4-benzyl phthalazinone moiety of PARP-1 inhibitor Olaparib and N-benzylpiperidine moiety of AChE inhibitor Donepezil, into one molecule was synthesized. The inhibitory activities of all the synthesized compounds against the enzymes PARP-1, AChE and BChE were evaluated. Among them, **30** exhibited the most potent inhibitory effect on PARP-1 enzyme (8.18 ± 2.81 nM) and moderate BChE inhibitory activity ($1.63 \pm 0.52 \mu\text{M}$), while its AChE inhibitory activity ($13.48 \pm 2.15 \mu\text{M}$) was weaker than Donepezil ($0.04 \pm 0.01 \mu\text{M}$). Further molecular docking studies revealed that four hydrogen bonds were formed between **30** and PARP-1 which were similar with the interactions between Olaparib and PARP-1. **30** interacted with the critical residues His438 and Trp82 of huBChE through hydrogen bond and hydrophobic interaction which were necessary for huBChE inhibitory potency. Our research gave a clue to search for new agents based on PARP-1 and cholinesterase dual-inhibited activities to treat Alzheimer's disease.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, characterized by cognitive impairment and behavioral abnormalities. It is the most common form of dementia affecting elderly people, which can lead to long term memory loss, disorientation, and ultimately cause death [1]. AD has become a growing public health concern due to the rapid increase in life expectancy especially in developed country. Effectively therapeutic approaches for treatment of AD are urgently needed. Although the precise pathogenetic mechanisms of AD remain to be elucidated, various hypotheses have been proposed during the past few decades, such as the cholinergic hypothesis, β -amyloid ($A\beta$) peptide theory, tau hyperphosphorylation hypothesis, imbalanced inflammatory changes, dysregulation on energy metabolism, involvement of oxidative stress and calcium dyshomeostasis [2–5]. However, only several drugs succeeded clinically that based on those theories for the treatment of AD. Currently, there are four drugs in the market for the palliative management of AD: three acetylcholinesterase (AChE) inhibitors (donepezil, rivastigmine, and galantamine) and an antagonist of the N-methyl-D-aspartate (NMDA) receptor (memantine) [6]. These drugs show limited clinical results and cannot halt the disease progression. Thus, it is imperative to develop new drugs based on new mechanisms with improved efficacy.

The nuclear PARP-1 is a pivotal enzyme ubiquitously expressed in eukaryotic cells and its most known function is DNA repair. When DNA is damaged, PARP-1 catalyses the transfer of ADP-ribose residues from nicotinamide adenine dinucleotide (NAD^+) onto itself or the other target substrates to build poly(ADP-ribose) (PAR) chains [7], which then attracts multiple DNA repair proteins, including DNA ligase III, DNA polymerase β (pol β), and XRCC1, to the damage site to repair DNA. Inhibition of PARP-1 activities will result in a death of a cell with damaged DNA. In cancerous cells, some drugs can kill them by breaking DNA, but which can be repaired by PARP-1, inhibition of PARP-1 activities will enhance the drugs'

antitumor effects. Besides the PARP-1 dependent repair pathway, the damaged DNA can also be repaired by homologous recombination (HR) pathway, whose function is executed mainly through key proteins encoded by breast cancer gene 1 (BRCA1) or breast cancer gene 2 (BRCA2). In BRCA1/2-mutant tumor cells, only treatment with PARP-1 inhibitor can lead efficiently to the death of tumor cell without combining with the other anticancer drugs [8–10]. So PARP-1 has become an important target for anticancer drugs in recent years, and five PARP-1 inhibitors have been marketed as anticancer drugs now.

In addition to DNA repair, the role of PARP-1 in AD has received increasing attention recently [11–13]. PARP-1 hyperactivation will lead to cellular depletion of NAD^+ and ATP, which further result in mitochondrial dysfunction and cell necrosis. Free PAR chains can exit the nucleus and diffuse to cytosol where they incorporate with apoptosis-inducing factor 1 (AIF) released from damaged mitochondria, then, which induce nuclear DNA fragmentation and a controlled nerve cell death. This PARP-related specific neurodegenerative mechanism was termed as parthanatos [14–15]. Meanwhile, PARP-1 hyperactivation triggers the expression of pro-inflammatory factors such as several NF- κ B-dependent cytokines, chemokines, adhesion molecules [16]. The chronic inflammatory conditions cause dysregulation of mechanisms to clear misfolded or damaged neuronal proteins and concomitantly lead to tau-associated impairments of axonal integrity and transport, which are also implicated in the pathogenesis of AD [17]. On the other hand, some PARP-1 inhibitors, such as PJ34, nicotinamide, have been proved to be beneficial for the treatment of AD [18–19]. Taken all together, PARP-1 has shown its pathological role in AD and it might be a great potential target for drugs to treat AD [15, 20].

Considering the highly complex pathophysiology of AD that many biological factors concur simultaneously, search for agents based on MTDLs strategy to treat AD may be more promising than the traditional “one drug-one target” strategy [21, 22]. In light of the roles of PARP-1 and cholinesterases played in the pathologic process of AD, especially, which both are drugable targets, the PARP-1 and cholinesterase dual-targeted inhibitors for AD are worthy to be investigated. Olaparib is the first-in-class PARP-1 inhibitor marketed in 2014 as monotherapy for advanced BRCA-deficient ovarian cancer. The reported structure-activity relationship (SAR) revealed that 4-benzyl phthalazinone was the core scaffold mainly responsible for PARP-1-inhibiting potency [23, 24]. The N-benzylpiperidine fragment from donepezil was well recognized as an excellent fit responsible for its adequate recognition by AChE in the catalytic anionic site of AChE (CAS) [25]. Therefore, we envisioned that the incorporation of 4-benzyl phthalazinone and N-benzylpiperidine into one molecule with the hope that the molecular hybrids based on the combination of the pharmacophore of both drugs can exert a synergetic effect on the treatment of AD.

In this work, we reported the design and synthesis of a series of new hybrid compounds containing 4-benzyl phthalazinone and N-benzylpiperidine as cholinesterase and PARP-1 enzyme inhibitors (Figure. 1). Their preliminary effects on inhibition of AChE, BuChE and PARP-1 enzymes were evaluated. Meanwhile, molecular dockings were conducted to investigate their interaction fashion with the corresponding proteins.

Results And Discussion

Chemistry

The route adopted for the preparation of the new phthalazinone derivatives is depicted in Schemes 1. 2-Formyl benzoic acid **1** reacted with dimethyl phosphate to afford the dimethyl (1, 3-dihydro-3-oxoisobenzofuran-1-yl)-1-phosphonate **2**, and then, the Wittig reaction of **2** with 2-fluoro-5-formylbenzonitrile was conducted to give compound **3**. The key intermediate **4** was finally obtained after hydrolysis of **3**. The N-Boc protected piperidinyl amines **5** and **9** were alkylated with various substituted 1-(bromomethyl)benzenes **6** and **10** to give corresponding compounds **7** and **11**, in turn, which were deprotected with trifluoroacetic acid to afford multiple primary amines **8** and **12**. The piperidin-4-ol **13** performed a N-alkylation reaction with substituted 1-(bromomethyl)benzenes **14** to yield compounds **15**. Finally, intermediates **4** carried out an esterification reaction between **4** and **8**, **12**, and an amide reaction between **4** and **15** to provide the target compounds **16-34** and **35-36**, respectively.

The structures of newly synthesized target compounds **16-36** were confirmed by various spectral techniques. The IR spectra of **16-36** exhibited absorption bands at around 3400 cm^{-1} which was assigned to NH group stretching vibration. In the ^1H NMR spectra of **16-36** the N-H from CONH-N= group appeared at a δ range 10.54-12.58 ppm, while another N-H from amide group of **16-34** appeared in the region 7.20-7.50 ppm. The two characteristically aliphatic CH_2 showed the signals at 4.30 ppm ($\text{PhCH}_2\text{C}=\text{N}$) and a δ range 3.50-3.80 ppm (PhCH_2). Before used for biological assays, all synthesized compounds **16-36** were determined their purity (all >97%) by HPLC.

Biological evaluation

In vitro PARP-1 inhibitory activity

The *in vitro* PARP-1 enzyme inhibitory activities of all the target compounds were evaluated and their IC_{50} values were determined. The results are summarized in Table 1. From the obtained results, it was explicated that most of the prepared compounds displayed inhibitory activities against the PARP-1 enzyme to some degree. Compound **30** was found to be the most potent derivative among the synthesized ones ($\text{IC}_{50}=8.18\pm2.81\text{ nM}$) but weaker than Olaparib ($\text{IC}_{50}=7.13\pm1.18\text{ nM}$). Concerning SAR, it appeared that (piperidin-4-yl)methanamine had improved potency than piperidin-4-amine, as **30** and **21**, **27** and **22**, **26** and **19** had similarly substituted pattern, but **30**, **27**, **26** with (piperidin-4-yl)methanamine moiety exhibited higher inhibition rates. Comparisons of **31-34** respectively with **16**, **17**, **21**, **20** indicated that the location of the piperidine nitrogen seemed to have no effects on PARP-1 inhibitory activities, as **31** and **34** with a *meta*-nitrogen orientation showed higher inhibition rates while **17** and **21** with a *para*-nitrogen orientation exhibited higher inhibition rates. The replacement of amide group with ester group decreased PARP-1 inhibitory activity obviously.

Table 1
PARP-1 inhibitory activities of compounds **16-36**

Compd.	IC ₅₀ (nM) ^a	Compd.	IC ₅₀ (nM)	Compd.	IC ₅₀ (nM)
Olaparib	7.13±1.18	23	ND	31	58.69±7.64
16	ND ^b	24	76.87±9.68	32	106.75±8.79
17	36.86±5.62	25	134.82±10.37	33	136.43±16.46
18	81.06±7.53	26	12.36±3.55	34	16.13±1.68
19	72.34±8.12	27	19.93±4.13	35	197.57±24.31
20	124.53±15.36	28	102.54±9.83	36	213.98±21.56
21	74.47±11.36	29	157.41±17.26		
22	57.92±4.85	30	8.18±2.81		
a) Data were expressed as Mean±SEM, n=3; b) ND: not determined.					

In vitro AChE and BuChE inhibitory activity

The *in vitro* inhibitory effects of all target compounds on Electrophorus electricus AChE (EeAChE) and equine serum butyrylcholinesterase (eqBChE) were investigated according to the method [26]. Two drugs, Donepezil and Rivastigmine, were used as the standards. The results are summarized in Table 2.

Table 2
AChE and BChE inhibitory activities of compounds **16-36**

Compd.	IC ₅₀ (μM) ^a		Compd.	IC ₅₀ (μM) ^a	
	AChE	BuChE		AChE	BuChE
Donepezil	0.04±0.01	2.20±0.03	26	>200	3.82±1.112
Rivastigmine	4.37±1.26	0.67±0.04	27	>200	>200
16	2.01±0.59	19.67±1.34	28	13.01±1.02	16.18±1.22
17	2.78±1.00	167.77±2.37	29	>200	>200
18	>200	>200	30	13.48±2.15	1.63±0.52
19	49.57±2.117	>200	31	16.12±1.37	8.43±1.18
20	>200	>200	32	11.15±2.10	6.57±0.60
21	161.07±3.13	140.25±4.45	33	12.69±2.08	3.78±0.71
22	>200	>200	34	13.26±3.73	16.71±4.20
23	>200	>200	35	3.13±1.04	2.82±1.33
24	18.85±1.23	9.18±1.85	36	5.73±1.26	>200
25	4.01±0.86	11.48±1.25			
a) Data were expressed as Mean±SEM, n=3; b) AChE from electric eel and BChE from equine serum were used.					

In order to investigate the influences of a link distance between carbonyl and piperidinyll group and the location of the piperidine nitrogen on the inhibitory potencies against PARP-1 and cholinesterases, three serials of compounds (**16-23**, **24-30**, **31-34**) were synthesized. As shown in Table 2, the IC₅₀ values suggested that most of the synthesized compounds exhibited little to moderate inhibitory activities against cholinesterases. Compound **16** was the most potent AChE inhibitor (IC₅₀=2.01±0.59μM) but much weaker than Donepezil (IC₅₀=0.04±0.01μM). Compound **30** exhibited the most potent inhibitory effect on BuChE (IC₅₀=1.63±0.52μM), being little weaker than Rivastigmine (IC₅₀=0.67±0.04μM). Comparisons of **24-26** respectively with **16**, **17**, **19** revealed that (piperidin-4-yl)methanamine resulted in decreased AChE inhibition activity but increased BuChE inhibition activity than piperidin-4-amine. The similar trends were observed about the influences of the location of the piperidine nitrogen, and the *meta*-nitrogen orientation derivatives (**31**, **32**) showed decreased AChE inhibition activities but increased BuChE inhibition activities than the *para*-nitrogen orientation derivatives (**16**, **17**). Additionally, in the *para*-nitrogen orientation serial, except for compound **34**, the other three compounds (**31-33**) showed better inhibitions of BChE than AChE.

AChE and BChE are two important cholinesterases which can hydrolyze acetylthiocholine (ACh) in the synaptic gap of neuronal cells. ACh is dominantly decomposed by AChE instead of BuChE in a healthy brain. However, in an advanced AD brain, the level of AChE declines to 55-67% of normal values, as a compensating for its loss, BuChE increases to 120% of normal levels [27]. It indicates that BuChE plays a critical role for ACh hydrolysis in the late stage of AD. Besides that, it has been shown that BChE (over-)expression is associated with senile plaques and the transformation of nonfibrillar to fibrillary A β plaques, inhibition of BChE can decrease those plaques. Hence, inhibition of BChE may be more therapeutically valuable than inhibition of AChE in later stages of AD and researches on selective BChE inhibitors are paid wide attention to in recent years[28]. In this study, most of the synthesized compounds exhibited inhibitory activities against cholinesterases, although the molecules were incorporated within the pharmacophore of an AChE inhibitor Donepezil, surprisingly, some of which showed selectivity over BChE. Compound **16** had potent inhibitory effect on AChE but poor effect on PARP-1 inhibition while compound **30** showed potent inhibitory activities against both PARP-1 and BChE, therefore, **30** was chosen to carry on molecular docking with PARP-1 and BChE proteins to obtain an insight into the molecular level of the ligand binding.

Molecular Docking Of Parp-1 And hubche By 30

To gain insight into the binding mode of **30** inside the PARP-1 (PDB ID: 5DS3) [29] and the huBChE (PDB ID: 5NN0) [30], molecular docking were performed. The results were illustrated in the 2D diagrams plotted by LigPlot⁺ (Figure 2a). Docking simulation of **30** showed that three hydrogen bonds in **30**-PARP-1 complex were formed in the phthalazinone ring which was well recognized as a scaffold mainly responsible for PARP-1 inhibitory activity (Figure 2a): the carbonyl group as H-bond acceptor with the Gly863 A-NH group (2.84 Å) and Ser904 A-OH group (2.85 Å), the -NH group as H-bond donor with Gly863 A-C=O group (2.93 Å). The fourth identical hydrogen bond was established between the C=O group from amide moiety and the Tyr896 A-NH group (3.20 Å). Besides the interaction of hydrogen bond, compound **30** formed hydrophobic interactions with residues Tyr907, Lys903, Phe897, Ala898, His862, Glu988, Gly888, Leu877, Ser864 and Asn868.

The crystal structure of the recombinant human BChE (PDB ID: 5NN0) was chosen to be used for docking to analyze the key binding interactions of the synthesized compound **30** for huBChE inhibition, as its ligand shows high selectivity and an extremely potent inhibitory effect on huBChE with picomolar affinity [30]. As illustrated in the 2D diagram plotted by LigPlot⁺ (Figure 2b), the molecular docking result revealed the carbonyl group of **30** as H-bond acceptor established a hydrogen bond with the His438 A-NH group (2.84 Å). Meanwhile, compound **30** formed hydrophobic interactions with various residues Gly116, Phe398, Gly439, Glu197, Trp82, Tyr332, Ala328 and Phe329. Especially, the Trp82 is an important residue located in the choline-binding pocket of huBChE with which strong interaction could result in an excellently inhibitory effect on huBChE. The fluorobenzyl group of **30** formed hydrophobic interactions with residue Trp82. The His438 is another important residue. Although in the crystalline complex 5NN0 there is no hydrogen

bonding related with His438, in another reported crystal structure of the recombinant human BChE (PDB ID: 4BDS) complexed with Tacrine, the hydrogen bonding between the aromatic nitrogen N7 of Tacrine and the main chain carbonyl of His438 was considered to be very significant for Tacrine to exert BChE inhibitory effect [31]. Generally, the results of respective docking of **30** with PARP-1 and huBChE indicated that **30** could interact well to some degree with the active sites of PARP-1 and huBChE protein, which provided a basis and valuable information for further studies aimed at identifying inhibitors of both PARP-1 and huBChE.

Conclusions

As the inhibition of PARP-1 has a potentially therapeutic value for AD and the AChE inhibitor Donepezil has been used to treat AD, in order to search for a new agent based on MTDLs strategy, a series of 21 novel compounds was synthesized which incorporated the pharmacophores of two marketed drugs, namely 4-benzyl phthalazinone moiety of Olaparib and N-benzylpiperidine moiety of Donepezil, into one molecule. The inhibitory activities of all the synthesized compounds against the enzymes PARP-1, AChE and BChE were evaluated. Among them, **30** exhibited the most potent inhibitory effect on PARP-1 enzyme (8.18 ± 2.81 nM), which was just a little weaker than Olaparib. Meanwhile, **30** displayed moderate BChE inhibitory activity (1.63 ± 0.52 μ M) but its AChE inhibitory activity (13.48 ± 2.15 μ M) was weaker than Donepezil (0.04 ± 0.01 μ M). Further molecular docking studies revealed that three hydrogen bonds were formed between **30** and PARP-1 which were similar with the interactions between Olaparib and PARP-1. At the same time **30** interacted with the critical residues His438 and Trp82 of huBChE through hydrogen bond and hydrophobic interaction which were necessary for huBChE inhibitory potency. The original intention of this study was to search for molecule which can synergistically exhibit PARP-1 and AChE inhibitory activities, interestingly, most of the target compounds showed inhibition selectivity for BChE over AChE to some degree. In light of the therapeutic effects of BChE inhibitor on AD, further studies on anti-AD effects of **30** are going on. It is maybe more efficacious to launch investigation of this class of hybrids directly based on both PARP-1 and BChE inhibition as potential drug candidates for the treatment of AD.

Experimental Section

Chemistry

General methods

All reagents and solvents were obtained from commercially available sources and were used without further purification. Reaction progress was monitored using analytical thin layer chromatography (TLC) on precoated silica gel GF254 (Qingdao Haiyang Chemical Plant, Qing-Dao, China) plates and spots were detected under UV light (254 nm). Melting points were determined on a WRS-2B digital melting point apparatus and uncorrected. IR spectra were recorded using a Nicolet 380 Fourier-transform infrared (FTIR) spectrophotometer (Thermo, USA) from KBr pellets. ^1H NMR and ^{13}C NMR spectra were recorded

on a Bruker AVANCE III-600 NMR spectrometer (Bruker Biospin Co., Switzerland) with tetramethylsilane (TMS) as the internal standard and CDCl₃ or DMSO-d₆ as solvent and known chemical shifts of residual proton signals of deuterated solvents (¹H NMR: δ: 2.50 for DMSO-d₆ and δ: 3.33 for H₂O) or carbon signals of deuterated solvents (¹³C NMR: δ: 39.52 for DMSO-d₆) as internal standard. MS (ESI) measurement was conducted on an Agilent 1100 LC-MS spectrometer (Agilent, Palo Alto, USA).

Dimethyl (3-oxo-1, 3-dihydro-isobenzofuran-1-yl)-phosphonate (2)

Compounds **2**, **3** and **4** were prepared according to the literature procedure [32]. A mixture of 2-formylbenzoic acid **1** (10.0 g, 66.7 mmol) and dimethyl phosphate (16.8 g, 152.7 mmol) was heated to 100 °C in oil bath and stirred for 8 h. Subsequently, the mixture was cooled to room temperature and then poured into ice-cold water (50 mL), which was followed by extraction with dichloromethane (3×60 mL). The combined organic extracts were dried over MgSO₄, and were evaporated in vacuum to produce a white solid which was crystallized in ethanol to yield 10.1g compound **2**. Yield: 62.5%, mp: 96.1-98.2°C. ¹H-NMR (600 MHz, CDCl₃), δ: 7.93 (d, J=7.68Hz, 1H, ArH), 7.76-7.70 (m, 2H, ArH), 7.58 (t, J=7.56Hz, 1H, ArH), 5.70 (d, J=10.98Hz, 1H, CH), 3.91 (d, J=10.92Hz, 3H, OCH₃), 3.58 (d, J=10.62Hz, 3H, OCH₃).

2-fluoro-5-[(3-oxo-2-benzofuran-1-ylidene) methyl] benzonitrile (3)

To a mixture of **2** (4.3 g, 17.8 mmol) and 2-fluoro-5-formylbenzonitrile (2.6 g, 17.5 mmol) in anhydrous THF (40 mL) was added triethylamine (1.8 mL, 13mmol) dropwise in 30 min, and the temperature was maintained below 15°C. The reaction mixture was slowly warmed to room temperature. The progress of the reaction was monitored by TLC. After completion of the reactions, the reaction mixture was then concentrated in vacuo. The residue was slurried in water (50 mL) for 30 min, and the solid was collected by filtration, was washed with diethyl ether (2×10 mL), and was dried to afford a white solid which was crystallized in ethanol to yield 3.6g compound **3**. Yield: 92.6%, mp: 217.2-220.4°C. ¹H-NMR (600MHz, DMSO-d₆) δ: 8.13-8.10 (m, 1H, ArH), 8.03(dd, J=6.06, 2.28Hz, 1H, ArH), 7.97(d, J=7.68Hz, 1H, ArH), 7.76 (td, J=8.06, 5.94Hz, 2H, ArH), 7.61 (td, J=6.06, 2.16Hz, 1H, ArH), 7.25 (dd, J=13.05, 8.70Hz, 1H, ArH), 6.32 (s, 1H, =CH).

2-Fluoro-5-[(4-oxo-3H-phthalazin-1-yl) methyl] benzoic Acid (4)

To a stirred suspension of **3** (1.0 g, 3.8 mmol) in water (10 mL) was added aqueous NaOH (10 mol/L, 1.8 mL) with subsequent heating to 90°C for 1 h. The reaction mixture was cooled to 70°C into which hydrazine hydrate (2.7 mL, 47.0 mmol) was added, and the mixture was stirred for 18 h at 70°C before TLC indicated the reaction was completed. The mixture was cooled to ambient temperature and was acidified with HCl (4 mol/L) to pH 4, then the suspension was filtered and was washed with diethyl ether and dried to get 1.1 g of **4** as a light red powder. Yield: 93.7% mp: 208.4-210.1°C ¹H-NMR (600 MHz, DMSO-d₆) δ: 13.05 (s, 1H, COOH), 12.56 (s, 1H, NH), 8.26-8.20 (m, 1H, ArH), 7.94 (d, J = 8.0 Hz, 1H, ArH), 7.91-7.84 (m, 1H, ArH), 7.83-7.75 (m, 2H, ArH), 7.58-7.50 (m, 1H, ArH), 7.20 (m, 1H, ArH), 4.32 (s, 2H, CH₂).

General procedure for the synthesis of compounds (7a-7o)

To a mixture of various substituted bromomethyl benzene **6** (4.8 mmol) and *tert*-butyl (piperidin-4-yl)methylcarbamate or *tert*-butyl piperidin-4-ylcarbamate **5** (4.8 mmol) in anhydrous DMF (10 mL) was added potassium carbonate (1.0 g, 7.2 mmol). The reaction mixture was stirred and warmed to 70°C. The progress of the reaction was monitored by TLC. After completion of the reactions, the reaction mixture was poured into ice-cold water (30 mL) and stirred for 30 min. The solid was collected by filtration, and then it was dried to afford a white solid which was crystallized in ethanol to yield **7(7a-7o)**.

tert-butyl 1-benzylpiperidin-4-ylcarbamate (7a)

A white solid, yield: 80.3%, mp: 118.2-119.3 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.32-7.29 (m, 3H, ArH), 7.28-7.23 (m, 2H, ArH), 4.44 (d, *J* = 6.66 Hz, 1H, NH), 3.49 (s, 2H, PhCH₂N), 3.49-3.48(m, 1H, NCH), 2.81 (t, *J* = 9.36 Hz, 2H, NCH₂), 2.09 (t, *J* = 10.56 Hz, 2H, NCH₂), 1.90 (d, *J* = 11.6 Hz, 2H, CH₂), 1.43 (s, 9H, C(CH₃)₃), 1.43-1.40(m, 2H, CH₂).

tert-butyl 1-(2-fluorobenzyl)piperidin-4-ylcarbamate (7b)

A white solid, yield: 78.5%, mp: 121.7-122.9 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.35(td, *J* = 7.44, 1.14Hz, 1H, ArH), 7.25-7.21(m, 1H, ArH), 7.10(td, *J* = 7.50, 0.96Hz, 1H, ArH), 7.02(td, *J* = 8.34, 0.78Hz, 1H, ArH), 4.43 (d, *J* = 6.66 Hz, 1H, NH), 3.56 (s, 2H, PhCH₂N), 3.48-3.46(m, 1H, NCH), 2.82 (d, *J* = 9.6 Hz, 2H, NCH₂), 2.15 (t, *J* = 10.9 Hz, 2H, NCH₂), 1.90 (d, *J* = 11.5 Hz, 2H, CH₂), 1.46-1.43(m, 2H, CH₂), 1.43 (s, 9H, C(CH₃)₃).

tert-butyl 1-(2-nitrobenzyl)piperidin-4-ylcarbamate (7c)

A light yellow solid, yield: 79.8%, mp: 118.5-119.9 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.81 (d, *J* = 7.38 Hz, 1H, ArH), 7.63(t, *J* = 3.18 Hz, 1H, ArH), 7.55(t, *J* = 7.26 Hz, 1H, ArH), 7.41(t, *J* = 7.68 Hz, 1H, ArH), 4.44 (d, *J* = 6.18 Hz, 1H, NH), 3.83(s, 2H, PhCH₂N), 3.51-3.47(t, m, 1H, NCH), 2.76 (t, *J* = 14.94 Hz, 2H, NCH₂), 2.21 (t, *J* = 14.64 Hz, 2H, NCH₂), 1.89(d, *J* = 11.94 Hz, 2H, CH₂), 1.46-1.43(m, 2H, CH₂), 1.43 (s, 9H, C(CH₃)₃).

tert-butyl 1-(3-methoxybenzyl)piperidin-4-ylcarbamate (7d)

A white solid, yield: 80.4%, mp: 95.6-96.0 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.22 (t, *J* = 7.68 Hz, 1H, ArH), 6.89(d, *J* = 7.32 Hz, 2H, ArH), 6.80(d, *J* = 7.14 Hz, 1H, ArH), 4.44 (d, *J* = 6.48 Hz, 1H, NH), 3.80(s, 3H, OCH₃), 3.48(s, 3H, PhCH₂N & NCH), 2.82(t, *J* = 14.94 Hz, 2H, NCH₂), 2.11(t, *J* = 9.72 Hz, 2H, NCH₂), 1.91(d, *J* = 11.52 Hz, 2H, CH₂), 1.46-1.43(m, 2H, CH₂), 1.43 (s, 9H, C(CH₃)₃).

tert-butyl 1-(4-methylbenzyl)piperidin-4-ylcarbamate (7e)

A white solid, yield: 74.7%, mp: 98.7-98.9 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.19 (d, *J* = 7.50 Hz, 2H, ArH), 7.12 (d, *J* = 7.74 Hz, 2H, ArH), 4.44 (d, *J* = 6.06 Hz, 1H, NH), 3.47 (s, 3H, PhCH₂N & NCH), 2.83(t, *J* =

5.28 Hz, 2H, NCH₂), 2.33(s, 3H, CH₃), 2.08(t, *J* = 9.18 Hz, 2H, NCH₂), 1.90 (dd, *J* = 11.4, 0.78 Hz, 2H, CH₂), 1.47-1.43(m, 2H, CH₂), 1.43 (s, 9H, C(CH₃)₃).

tert-butyl 1-(4-fluorobenzyl)piperidin-4-ylcarbamate (7f)

A white solid, yield: 75.3%, mp: 130.0-130.5 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.28(t, *J* = 8.64 Hz, 2H, ArH), 6.99(t, *J* = 8.64 Hz, 2H, ArH), 4.47-4.43(m, 1H, NH), 3.48(s, 2H, PhCH₂N), 3.48-3.45(m, 1H, NCH), 2.79(t, *J* = 10.96 Hz, 2H, NCH₂), 2.08(t, *J* = 11.34 Hz, 2H, NCH₂), 1.90(d, *J* = 11.46 Hz, 2H, CH₂), 1.47-1.43(m, 2H, CH₂), 1.43 (s, 9H, C(CH₃)₃).

tert-butyl 1-(4-(trifluoromethyl)benzyl)piperidin-4-ylcarbamate (7g)

A white solid, yield: 89%, mp: 122.5-123.7 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.68(d, *J* = 7.84 Hz, 2H, ArH), 7.52(d, *J* = 7.88 Hz, 2H, ArH), 4.47-4.44(m, 1H, NH), 3.48(s, 2H, PhCH₂N), 3.48-3.46(m, 1H, NCH), 2.71(t, *J* = 11.30 Hz, 2H, NCH₂), 2.04(t, *J* = 10.78 Hz, 2H, NCH₂), 1.89(d, *J* = 11.36 Hz, 2H, CH₂), 1.47-1.43(m, 2H, CH₂), 1.43 (s, 9H, C(CH₃)₃).

tert-butyl 1-(4-(trifluoromethoxy)benzyl)piperidin-4-ylcarbamate (7h)

A white solid, yield: 88%, mp: 103.9-104.7 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.78 (d, *J* = 7.86 Hz, 2H, ArH), 7.37(d, *J* = 7.62 Hz, 2H, ArH), 4.46-4.43(m, 1H, NH), 3.47(s, 2H, PhCH₂N), 3.47-3.45(m, 1H, NCH), 2.69(t, *J* = 11.36 Hz, 2H, NCH₂), 2.05(t, *J* = 10.78 Hz, 2H, NCH₂), 1.90(d, *J* = 11.36 Hz, 2H, CH₂), 1.47-1.43(m, 2H, CH₂), 1.43 (s, 9H, C(CH₃)₃).

tert-butyl (1-benzylpiperidin-4-yl)methylcarbamate (7i)

A white solid, yield: 85%, mp: 76.2-76.6 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.31-7.29 (m, 3H, ArH), 7.25 (d, *J* = 7.4 Hz, 2H, ArH), 4.59 (s, 1H, NH), 3.51(s, 2H, PhCH₂N), 3.01(t, *J* = 5.82 Hz, 2H, NCH₂), 2.91 (d, *J* = 11.1 Hz, 2H, NCH₂), 1.97(t, *J* = 10.44 Hz, 2H, NCH₂), 1.65 (d, *J* = 12.66 Hz, 2H, CH₂), 1.43 (s, 9H, C(CH₃)₃), 1.32-1.29 (m, 2H).

tert-butyl (1-(2-fluorobenzyl)piperidin-4-yl)methylcarbamate (7j)

A white solid, yield: 79.3%, mp: 73.6-74.1 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.36 (t, *J* = 7.44 Hz, 1H, ArH), 7.23-7.20(m, 1H, ArH), 7.09 (t, *J* = 7.44 Hz, 1H, ArH), 7.01 (t, *J* = 9.00 Hz, 1H, ArH), 4.58 (s, 1H, NH), 3.57 (s, 2H, PhCH₂N), 3.00 (t, *J* = 5.94 Hz, 2H, NCH₂), 2.90 (d, *J* = 11.46 Hz, 2H, NCH₂), 2.00 (t, *J* = 11.46 Hz, 2H, CH₂), 1.77-1.74(m, 1H, CH), 1.65(d, *J* = 12.90 Hz, 2H, CH₂), 1.42(s, 9H, C(CH₃)₃), 1.30-1.24(m, 2H, CH₂).

tert-butyl (1-(3-methoxybenzyl)piperidin-4-yl)methylcarbamate (7k)

A white solid, yield: 89.3%, mp: 72.3-73.5 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.21(t, *J* = 7.74 Hz, 1H, ArH), 6.90-6.89(m, 2H, ArH), 6.80(d, *J* = 7.14 Hz, 1H, ArH), 4.56 (s, 1H, NH), 3.80(s, 3H, OCH₃), 3.55 (s, 2H,

PhCH₂N), 3.16(t, *J* = 6.00 Hz, 2H, NCH₂), 2.92(d, *J* = 10.50 Hz, 2H, NCH₂), 1.99(t, *J* = 10.80 Hz, 2H, NCH₂), 1.72 (d, *J* = 8.82 Hz, 2H, CH₂), 1.77-1.74(m, 1H, CH), 1.42(s, 9H, C(CH₃)₃), 1.38-1.34(m, 2H, CH₂).

tert-butyl (1-(4-(trifluoromethyl)benzyl)piperidin-4-yl)methylcarbamate (7l)

A white solid, yield: 75.6%, mp: 98.7-99.7 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.58 (d, *J* = 8.04 Hz, 2H, ArH), 7.49 (d, *J* = 7.14 Hz, 2H, ArH), 4.64 (s, 1H, NH), 3.64 (s, 2H, PhCH₂N), 3.02(t, *J* = 6.12 Hz, 2H, NCH₂), 2.95(t, *J* = 4.26 Hz, 2H, NCH₂), 2.08-2.05(m, 2H, CH₂), 1.69(d, *J* = 12.06 Hz, 2H, CH₂), 1.53-1.50(m, 1H, CH), 1.42(s, 9H, C(CH₃)₃), 1.40-1.38 (m, 2H, CH₂).

tert-butyl (1-(4-methylbenzyl)piperidin-4-yl)methylcarbamate (7m)

A white solid, yield: 74.6%, mp: 81.2-83.1 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.19 (d, *J* = 7.74 Hz, 2H, ArH), 7.12 (d, *J* = 7.74 Hz, 2H, ArH), 4.58 (s, 1H, NH), 3.48(s, 2H, PhCH₂N), 3.01(t, *J* = 6.18 Hz, 2H, NCH₂), 2.89(d, *J* = 10.8 Hz, 2H, NCH₂), 2.33(s, 3H, CH₃), 1.94(t, *J* = 11.94 Hz, 2H, CH₂), 1.65(d, *J* = 12.30 Hz, 2H, CH₂), 1.53-1.43(m, 1H, CH), 1.43(s, 9H, C(CH₃)₃), 1.30-1.23 (m, 2H, CH₂).

tert-butyl (1-(4-(trifluoromethoxy)benzyl)piperidin-4-yl)methylcarbamate (7n)

A white solid, yield: 79.8%, mp: 88.4-88.9 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.34 (d, *J* = 8.04 Hz, 2H, ArH), 7.15 (d, *J* = 8.10 Hz, 2H, ArH), 4.59 (s, 1H, NH), 3.48(d, *J* = 5.52 Hz, 2H, PhCH₂N), 3.01(t, *J* = 6.36 Hz, 2H, NCH₂), 2.89(d, *J* = 10.86 Hz, 2H, NCH₂), 1.93(t, *J* = 11.94 Hz, 2H, CH₂), 1.66(d, *J* = 12.48 Hz, 2H, CH₂), 1.43(s, 9H, C(CH₃)₃), 1.43-1.41(m, 1H, CH), 1.30-1.23 (m, 2H, CH₂).

tert-butyl (1-(4-fluorobenzyl)piperidin-4-yl)methylcarbamate(7o)

A white solid, yield: 79.2%, mp: 86.4-87.3 °C, ¹H NMR (600 MHz, CDCl₃) δ: 7.26(t, *J* = 8.22 Hz, 2H, ArH), 6.98(t, *J* = 8.64 Hz, 2H, ArH), 4.59 (s, 1H, NH), 3.45 (s, 2H, PhCH₂N), 3.01(t, *J* = 6.12 Hz, 2H, NCH₂), 2.86(d, *J* = 11.22 Hz, 2H, NCH₂), 1.93(t, *J* = 11.34 Hz, 2H, CH₂), 1.66(d, *J* = 12.66 Hz, 2H, CH₂), 1.53-1.43(m, 1H, CH), 1.43(s, 9H, C(CH₃)₃), 1.29-1.25(m, 2H, CH₂)

General procedure for the synthesis of intermediates 8

Compound **7** (12.0 mmol) was dissolved and stirred in dichloromethane (DCM, 30 mL) which was cooled by ice-cold water, and then trifluoroacetic acid (8.90 g, 78.0 mmol) was added dropwise in 10 min. Subsequently, the temperature was slowly warmed to room temperature and maintained for 12 h. Methanol (20 mL) was added to the mixture, followed by basification with saturated sodium hydroxide solution to pH 10. The mixture was stirred for 30min, and then extracted with DCM (3×20 mL). The combined organic layers were dried over Na₂SO₄ and concentrated and purified by silica gel column chromatography (hexanes/EtOAc (v/v)= (6-3): 1) to give **8 (8a-8o)** as colorless or light yellow oil. The material **8** was carried forward without further purification

General procedure for the synthesis of compounds (11a-11d)

Compounds **11a-11d** were synthesized by following a similar procedure for the synthesis of **7**, while using *tert*-butyl piperidin-3-ylcarbamate to replace *tert*-butyl piperidin-4-yl-carbamate.

tert-butyl 1-benzylpiperidin-3-yl-carbamate (11a)

A white solid, yield: 75.2%, mp: 83.8-84.2 °C, ¹H NMR (600 MHz, DMSO-d₆) δ: 7.30-7.25(m, 4H, ArH), 7.22(t, *J* = 7.02 Hz, 1H, ArH), 6.66 (d, *J* = 7.74 Hz, 1H, NH), 3.42 (s, 2H, PhCH₂N), 3.39-3.36(m, 1H, NCH), 2.71 (d, *J* = 8.22 Hz, 1H, NCH₂H), 2.59 (d, *J* = 9.54 Hz, 1H, NCH₂H), 1.81(t, *J* = 9.48 Hz, 1H, NCH₂H), 1.72(t, *J* = 8.58 Hz, 1H, NCH₂H), 1.67-1.65(m, 1H, CH₂H), 1.59-1.57(m, 1H, CH₂H), 1.43-1.38(m, 1H, CH₂H), 1.33(s, 9H, C(CH₃)₃), 1.12-1.09(m, 1H, CH₂H).

tert-butyl 1-(2-fluorobenzyl)piperidin-3-yl-carbamate (11b)

A white solid, yield: 69.7%, mp: 59.5-61.7 °C, ¹H NMR (600 MHz, DMSO-d₆) δ: 7.37(t, *J* = 6.96 Hz, 1H, ArH), 7.29(q, *J* = 7.26 Hz, 1H, ArH), 7.16-7.11(m, 2H, ArH), 6.66 (d, *J* = 7.98 Hz, 1H, NH), 3.39 (s, 2H, PhCH₂N), 3.35-3.34(m, 1H, NCH), 2.73 (d, *J* = 8.76 Hz, 1H, NCH₂H), 2.69 (d, *J* = 9.48 Hz, 1H, NCH₂H), 1.85(t, *J* = 10.56 Hz, 1H, NCH₂H), 1.75(t, *J* = 8.94 Hz, 1H, NCH₂H), 1.65-1.64(m, 1H, CH₂H), 1.59-1.57(m, 1H, CH₂H), 1.44-1.38(m, 1H, CH₂H), 1.34(s, 9H, C(CH₃)₃), 1.11-1.06(m, 1H, CH₂H).

tert-butyl 1-(4-fluorobenzyl)piperidin-3-yl-carbamate (11c)

A white solid, yield: 74.7%, mp: 75.2-76.3 °C, ¹H NMR (600 MHz, DMSO-d₆) δ: 7.35 (dd, *J* = 31.24, 7.6 Hz, 4H, ArH), 6.65 (d, *J* = 7.84 Hz, 1H, NH), 3.38 (s, 2H, PhCH₂N), 3.35-3.34(m, 1H, NCH), 2.74 (d, *J* = 8.84 Hz, 1H, NCH₂H), 2.69 (d, *J* = 9.56 Hz, 1H, NCH₂H), 1.84(t, *J* = 10.84 Hz, 1H, NCH₂H), 1.74(t, *J* = 8.94 Hz, 1H, NCH₂H), 1.65-1.64(m, 1H, CH₂H), 1.59-1.57(m, 1H, CH₂H), 1.44-1.39(m, 1H, CH₂H), 1.34(s, 9H, C(CH₃)₃), 1.12-1.07(m, 1H, CH₂H).

tert-butyl 1-(4-methylbenzyl)piperidin-3-yl-carbamate (11d)

A white solid, yield: 79.6%, mp: 90.6-91.3 °C, ¹H NMR (600 MHz, DMSO-d₆) δ: 7.11(dd, *J* = 25.80, 7.8 Hz, 4H, ArH), 6.65 (d, *J* = 7.98 Hz, 1H, NH), 3.49 (s, 2H, PhCH₂N), 3.34-3.33(m, 1H, NCH), 2.69 (d, *J* = 8.64 Hz, 1H, NCH₂H), 2.58 (d, *J* = 10.44 Hz, 1H, NCH₂H), 2.25(s, 3H, CH₃), 1.79(t, *J* = 10.68 Hz, 1H, NCH₂H), 1.67(t, *J* = 10.62 Hz, 2H, CH₂), 1.58-1.55(m, 1H, CH₂H), 1.33(s, 9H, C(CH₃)₃), 1.31-1.25(m, 1H, CH₂H), 1.09-1.08(m, 1H, CH₂H).

General procedure for the synthesis of intermediates 12a-12d

Compounds **12a-12d** were synthesized by following a similar procedure for the synthesis of **8**. The intermediates **12a-12d** were got by silica gel column chromatography (hexanes/EtOAc(v/v) = 3: 1) as colorless oil, which were carried forward without further purification.

General procedure for the synthesis of intermediates 15a-15b

The piperidin-4-ol **13** (1.0g, 9.8mmol) and the substituted bromomethyl benzene **14** (9.8mmol) were dissolved and stirred in anhydrous DMF (10mL), and then anhydrous K₂CO₃ (2.07g, 14.7mmol) was added. The mixture was stirred at 70°C for 1 h, and then cooled to room temperature, followed by pouring into ice-water (30 mL). The solution was stirred for 10 min, and then extracted with ethyl acetate (3×30 mL). The combined organic layers were dried over Na₂SO₄ and concentrated and purified by silica gel column chromatography (hexanes/EtOAc(v/v) = 3: 1) to give **15a-15b** as colorless oil. The material **15** was carried forward without further purification.

General procedure for the synthesis of target compounds 16-36

Compound **4** (1.00g, 3.35mmol), DIPEA (0.65g, 5.03mmol) and HATU (1.90g, 5.03mmol) were dissolved and stirred in DCM (10mL) at 0°C for 10 min, and then **8**, **12** or **15** (5.03mmol) were added. The mixture was warmed to room temperature and stirred for 2 h, followed by pouring into ice-water (30 mL). The solution was stirred for 10 min, and then extracted with DCM (3×30 mL). The combined organic layers were dried over Na₂SO₄ and concentrated and purified by silica gel column chromatography (hexanes/EtOAc(v/v) =(2-1): 1) to give **16-36**.

N-(1-benzylpiperidin-4-yl)-2-fluoro-5-((1, 2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide (16)

A white solid, yield: 60%, mp: 196.6-197.4 °C, ¹H NMR (600 MHz, CDCl₃) δ: 11.24(s, 1H, CONH-N=), 8.46(d, *J* = 6.78 Hz, 1H, ArH), 8.07(d, *J* = 6.0 Hz, 1H, ArH), 7.73-7.72(m, 3H, ArH), 7.31-7.26(m, 6H, CONH & ArH), 7.01(t, *J* = 10.20 Hz, 1H, ArH), 6.66(t, *J* = 10.68 Hz, 1H, ArH), 4.31(s, 2H, PhCH₂C=N), 4.05-4.04(m, 1H, CH), 3.52(s, 2H, PhCH₂), 2.83(t, *J* = 7.80 Hz, 2H, NCH₂), 2.22 (d, *J* = 8.52 Hz, 2H, NCH₂), 2.02-2.00(m, 2H, CH₂), 1.62-1.57(m, 2H, CH₂); ¹³C NMR (150 MHz, CDCl₃) δ: 162.27, 160.62, 162.25, 160.63, 160.31, 158.68, 145.66, 138.19, 133.60, 131.94, 131.47, 129.13, 128.26, 127.11, 127.07, 125.06, 121.24, 121.16, 116.51, 116.35, 63.06, 52.09, 46.96, 37.83, 32.01; IR (KBr, cm⁻¹): 3455, 3262, 3071, 2939, 1653, 1559, 1489; ESI-MS: 471.22 [M+H]⁺.

N-(1-(2-fluorobenzyl)piperidin-4-yl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide (17)

A white solid, yield: 55%, mp: 182.3-183.3 °C, ¹H NMR (600 MHz, CDCl₃) δ: 11.15(s, 1H, CONH-N=), 8.45(d, *J* = 6.96 Hz, 1H, ArH), 8.06(d, *J* = 5.94 Hz, 1H, ArH), 7.73(d, *J* = 6.42 Hz, 3H, ArH), 7.37(t, *J* = 6.90 Hz, 1H, ArH), 7.31(s, 1H, CONH), 7.24(d, *J* = 6.24 Hz, 1H, ArH), 7.10(t, *J* = 7.32 Hz, 1H, ArH), 7.01(dd, *J* = 18.96, 9.96 Hz, 2H, ArH), 6.67(t, *J* = 8.04 Hz, 1H, ArH), 4.30(s, 2H, PhCH₂C=N), 4.03-4.02(m, 1H, CH), 3.60(s, 2H, PhCH₂), 2.86(t, *J* = 4.38 Hz, 2H, NCH₂), 2.27(t, *J* = 10.38 Hz, 2H, NCH₂), 2.02-2.00(m, 2H, CH₂), 1.63-1.58(m, 2H, CH₂); ¹³C NMR (150 MHz, CDCl₃) δ: 162.32, 162.19, 160.60, 160.30, 158.67, 145.70, 134.22, 133.63, 133.13, 131.91, 131.58, 131.49, 129.50, 128.86, 128.27, 127.10, 125.06, 123.88, 121.22, 116.52, 115.34, 55.25, 51.84, 46.80, 37.80, 31.91; IR (KBr, cm⁻¹): 3453, 3054, 2946, 1667, 1548; ESI-MS: 489.21 [M+H]⁺.

N-(1-(2-nitrobenzyl)piperidin-4-yl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide(18)

A light yellow solid, yield: 50%, mp: 192.0-193.1 °C, ¹H NMR (600 MHz, CDCl₃) δ: 10.40(s, 1H, CONH-N=), 8.45(d, *J* = 6.84 Hz, 1H, ArH), 8.05(d, *J* = 5.88 Hz, 1H, ArH), 7.82(d, *J* = 6.66 Hz, 1H, ArH), 7.77-7.71(m, 3H, ArH), 7.55(t, *J* = 6.30 Hz, 2H, ArH), 7.41(t, *J* = 9.36 Hz, 1H, ArH), 7.33(s, 1H, CONH), 7.03(td, *J* = 10.11, 2.82 Hz, 1H, ArH), 6.67-6.64(m, 1H, ArH), 4.30(s, 2H, PhCH₂C=N), 4.07-4.02(m, 1H, CH), 3.85(s, 2H, PhCH₂), 2.78(t, *J* = 9.54 Hz, 2H, NCH₂), 2.23(t, *J* = 13.62 Hz, 2H, NCH₂), 2.03-1.98(m, 2H, CH₂), 1.76-1.66(m, 2H, CH₂); ¹³C NMR (150 MHz, DMSO-d₆) δ: 163.45, 159.81, 158.95, 157.31, 150.04, 145.38, 134.64, 133.95, 133.52, 132.99, 131.99, 130.28, 129.49, 128.91, 128.31, 126.49, 125.97, 125.06, 124.56, 116.42, 58.72, 52.46, 47.02, 36.87, 31.71; IR (KBr, cm⁻¹): 3343, 3016, 2911, 2804, 1662, 1615, 1522; ESI-MS:516.20 [M+H]⁺.

N-(1-(3-methoxybenzyl)piperidin-4-yl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide (19)

A white solid, yield: 65%, mp: 193.0-194.3 °C, ¹H NMR (600 MHz, CDCl₃) δ: 11.15(s, 1H, CONH-N=), 8.45(d, *J* = 7.38 Hz, 1H, ArH), 8.08(d, *J* = 6.90 Hz, 1H, ArH), 7.76-7.71(m, 3H, ArH), 7.32(s, 1H, CONH), 7.22(t, *J* = 8.04 Hz, 1H, ArH), 7.01(td, *J* = 10.11, 2.58 Hz, 1H, ArH), 6.90(s, 2H, ArH), 6.79(d, *J* = 7.98 Hz, 1H, ArH), 6.66(dd, *J* = 12.36, 8.04 Hz, 1H, ArH), 4.30(s, 2H, PhCH₂C=N), 4.04-4.03(m, 1H, CH), 3.80(s, 3H, OCH₃), 3.50(s, 2H, PhCH₂), 2.83(t, *J* = 7.80 Hz, 2H, NCH₂), 2.20(t, *J* = 9.84 Hz, 2H, NCH₂), 2.02-2.00(m, 2H, CH₂), 1.62-1.57(m, 2H, CH₂); ¹³C NMR (150 MHz, CDCl₃) δ:162.27, 160.62, 160.31, 159.60, 158.68, 145.67, 134.25, 133.61, 133.11, 131.94, 131.48, 129.51, 129.17, 128.29, 127.11, 125.06, 121.41, 121.23, 116.52, 114.56, 112.47, 62.95, 55.19, 52.10, 46.95, 37.83, 32.01; IR (KBr, cm⁻¹): 3452, 3177, 3069, 2943, 2802, 1649, 1559; ESI-MS:501.23[M+H]⁺.

N-(1-(4-methylbenzyl)piperidin-4-yl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide (20)

A white solid, yield: 55%, mp: 201.4-202.2 °C, ¹H NMR (600 MHz, CDCl₃) δ: 11.01(s, 1H, CONH-N=), 8.45(dd, *J* = 6.06, 1.44 Hz, 1H, ArH), 8.07(dd, *J* = 7.26, 2.16 Hz, 1H, ArH), 7.76-7.71(m, 3H, ArH), 7.32(s, 1H, CONH), 7.20(d, *J* = 7.86 Hz, 2H, ArH), 7.12(d, *J* = 7.80 Hz, 2H, ArH), 7.01(td, *J* = 10.05, 3.06 Hz, 1H, ArH), 6.66(dd, *J* = 12.72, 7.92 Hz, 1H, ArH), 4.30(s, 2H, PhCH₂C=N), 4.04-4.03(m, 1H, CH), 3.49(s, 2H, PhCH₂), 2.83(t, *J* = 5.34 Hz, 2H, NCH₂), 2.33(s, 3H, CH₃), 2.19(t, *J* = 10.68 Hz, 2H, NCH₂), 2.02-2.00(m, 2H, CH₂), 1.62-1.57(m, 2H, CH₂); ¹³C NMR (150 MHz, DMSO-d₆) δ:163.47, 159.82, 158.94, 157.30, 145.37, 134.69, 133.95, 132.60, 132.00, 130.26, 129.49, 129.23, 128.31, 126.50, 125.96, 125.06, 124.95, 116.58, 116.43, 61.93, 52.12, 46.89, 36.87, 31.35, 21.15; IR (KBr, cm⁻¹): 3456, 3071, 2939, 1651, 1559; ESI-MS:485.23 [M+H]⁺.

N-(1-(4-fluorobenzyl)piperidin-4-yl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide (21)

A white solid, yield: 54%, mp: 201.0-202.3 °C, ¹H NMR (600 MHz, CDCl₃) δ: 10.09(s, 1H, CONH-N=), 8.44(d, *J* = 7.08 Hz, 1H, ArH), 8.05(d, *J* = 6.24 Hz, 1H, ArH), 7.76-7.71(m, 3H, ArH), 7.37-7.33(m, 3H, CONH & ArH),

7.06-7.02(m, 3H, NH & ArH), 6.67(dd, J = 7.26, 4.02 Hz, 1H, ArH), 4.30(s, 2H, PhCH₂C=N), 4.09-4.02(m, 1H, CH), 3.58(s, 2H, PhCH₂), 2.93(t, J = 7.56 Hz, 2H, NCH₂), 2.30(t, J = 8.70 Hz, 2H, NCH₂), 2.06-2.04(m, 2H, CH₂), 1.66-1.60(m, 2H, CH₂); ¹³C NMR (150 MHz, DMSO-d₆) δ : 163.43, 159.81, 158.93, 157.29, 145.37, 135.22, 134.69, 133.96, 132.56, 132.01, 130.97, 130.26, 129.49, 128.31, 126.50, 125.98, 125.10, 116.58, 115.37, 61.62, 52.29, 47.20, 36.86, 31.75; IR (KBr, cm⁻¹): 3455, 3070, 2942, 1656, 1504; ESI-MS: 489.21 [M+H]⁺.

N-((1-(4-trifluoromethyl)benzyl)piperidin-4-yl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide(22)

A white solid, yield: 56%, mp: 229.3-230.3 °C, ¹H NMR (600 MHz, DMSO-d₆) δ : 12.58(s, 1H, CONH-N=), 8.24(d, J = 7.98 Hz, 1H, ArH), 8.19(d, J = 6.30 Hz, 1H, ArH), 7.95(d, J = 7.92 Hz, 1H, ArH), 7.87(td, J = 7.26, 1.26 Hz, 1H, ArH), 7.81(td, J = 7.50, 0.96 Hz, 1H, ArH), 7.67(d, J = 7.80 Hz, 2H, ArH), 7.51(d, J = 7.80 Hz, 2H, ArH), 7.47(dd, J = 6.30, 1.80 Hz, 1H, ArH), 7.41(s, 1H, CONH), 7.17(td, J = 9.36, 1.08 Hz, 1H, ArH), 4.30(s, 2H, PhCH₂C=N), 3.72-3.68(m, 1H, CH), 3.53(s, 2H, PhCH₂), 2.74(t, J = 10.80 Hz, 2H, NCH₂), 2.04(t, J = 14.34 Hz, 2H, NCH₂), 1.77-1.73(m, 2H, CH₂), 1.52-1.47(m, 2H, CH₂); ¹³C NMR (150 MHz, DMSO-d₆) δ : 163.46, 159.82, 158.93, 157.30, 145.38, 134.69, 133.95, 132.58, 132.00, 130.28, 129.72, 129.48, 128.30, 128.10, 127.88, 127.50, 126.50, 125.97, 125.48, 125.90, 123.90, 116.58, 61.78, 52.40, 47.08, 36.86, 31.70; IR (KBr, cm⁻¹): 3266, 2944, 1651, 1563; ESI-MS: 539.20 [M+H]⁺.

N-((1-(4-trifluoromethoxy)benzyl)piperidin-4-yl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide(23)

A white solid, yield: 45%, mp: 227.1-228.0 °C, ¹H NMR (600 MHz, CDCl₃) δ : 10.68(s, 1H, CONH-N=), 8.45(dd, J = 7.17, 1.98 Hz, 1H, ArH), 8.07(dd, J = 7.20, 1.68 Hz, 1H, ArH), 7.76-7.71(m, 3H, ArH), 7.37(d, J = 7.62 Hz, 2H, ArH), 7.33(s, 1H, CONH), 7.16(d, J = 8.16 Hz, 2H, ArH), 7.17(td, J = 10.08, 3.00 Hz, 1H, ArH), 6.66(dd, J = 12.66, 9.36 Hz, 1H, ArH), 4.30(s, 2H, PhCH₂C=N), 4.06-4.05(m, 1H, CH), 3.53(s, 2H, PhCH₂), 2.84(t, J = 4.62 Hz, 2H, NCH₂), 2.23(t, J = 7.86 Hz, 2H, NCH₂), 2.04-2.02(m, 2H, CH₂), 1.66-1.60(m, 2H, CH₂); ¹³C NMR (150 MHz, DMSO-d₆) δ : 163.49, 159.81, 158.93, 157.29, 145.38, 134.70, 133.95, 132.60, 132.00, 131.00, 130.25, 129.49, 128.31, 126.49, 125.97, 125.07, 124.97, 121.38, 119.68, 116.43, 54.02, 52.21, 42.27, 36.86, 31.62; IR (KBr, cm⁻¹): 3457, 2945, 1650, 1566, 1495; ESI-MS: 555.19 [M+H]⁺.

N-((1-benzyl)piperidin-4-yl)methyl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide(24)

A white solid, yield: 65%, mp: 191.1-192.2 °C, ¹H NMR (600 MHz, CDCl₃) δ : 11.07(s, 1H, CONH-N=), 8.45(d, J = 7.44, Hz, 1H, ArH), 8.07(dd, J = 7.32, 1.98 Hz, 1H, ArH), 7.76-7.71(m, 3H, ArH), 7.34-7.32(m, 1H, CONH), 7.30-7.28(m, 4H, ArH), 7.25-7.22(m, 1H, ArH), 7.02(td, J = 10.02, 3.00 Hz, 1H, ArH), 6.84-6.80(m, 1H, ArH), 4.30(s, 2H, PhCH₂C=N), 3.50(s, 2H, PhCH₂), 3.36(t, J = 6.06 Hz, 2H, NCH₂), 2.91(d, J = 11.4 Hz, 2H, NCH₂), 1.96(t, J = 11.4 Hz, 2H, NCH₂), 1.72-1.70(m, 2H, CH₂), 1.64-1.60(m, 1H, CH), 1.39-1.33(m, 2H, CH₂); ¹³C NMR (150 MHz, DMSO-d₆) δ : 164.06, 160.85, 159.81, 158.99, 157.37, 145.77, 134.73, 133.97, 132.66,

131.98, 131.03, 130.38, 129.50, 128.31, 126.49, 125.96, 124.83, 116.59, 115.37, 61.77, 53.75, 45.07, 36.77, 35.93, 29.74; IR (KBr, cm^{-1}): 3288, 2926, 1665, 1540, 1493; ESI-MS: 485.23 $[\text{M}+\text{H}]^+$.

N-((1-(2-fluorobenzyl)piperidin-4-yl)methyl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide(25)

A white solid, yield: 55%, mp: 178.9-180.4 °C, ^1H NMR (600 MHz, CDCl_3) δ : 11.20(s, 1H, CONH-N=), 8.45(d, J = 7.74, Hz, 1H, ArH), 8.07(dd, J = 7.32, 2.10 Hz, 1H, ArH), 7.76-7.71(m, 3H, ArH), 7.36(t, J = 6.66 Hz, 1H, ArH), 7.34-7.31(m, 1H, CONH), 7.23-7.20(m, 1H, ArH), 7.08(t, J = 7.32 Hz, 1H, ArH), 7.00(td, J = 8.82, 1.68 Hz, 2H, ArH), 6.85-6.81(m, 1H, ArH), 4.30(s, 2H, $\text{PhCH}_2\text{C}=\text{N}$), 3.59(s, 2H, PhCH_2), 3.45(t, J = 6.06 Hz, 2H, NCH_2), 2.93(d, J = 11.46 Hz, 2H, NCH_2), 2.04(t, J = 10.08 Hz, 2H, NCH_2), 1.72 (d, J = 12.54 Hz, 2H, CH_2), 1.62-1.58(m, 1H, CH), 1.40-1.33(m, 2H, CH_2); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ : 163.15, 162.18, 160.62, 160.31, 158.67, 145.64, 134.29, 133.60, 133.13, 131.98, 131.67, 131.46, 129.51, 128.74, 128.29, 127.10, 125.05, 123.80, 121.14, 116.49, 115.23, 55.35, 52.96, 45.52, 37.81, 35.82, 29.89; IR (KBr, cm^{-1}): 3330, 2927, 1662, 1547, 1490; ESI-MS: 503.22 $[\text{M}+\text{H}]^+$.

N-((1-(3-methoxybenzyl)piperidin-4-yl)methyl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide(26)

A white solid, yield: 59%, mp: 169.4-170.3 °C, ^1H NMR (600 MHz, CDCl_3) δ : 10.33(s, 1H, CONH-N=), 8.45(dd, J = 6.36, 1.74 Hz, 1H, ArH), 8.08(dd, J = 7.32, 1.98 Hz, 1H, ArH), 7.78-7.72(m, 3H, ArH), 7.35-7.32(m, 1H, CONH), 7.21(t, J = 7.74 Hz, 1H, ArH), 7.03(td, J = 10.08, 3.12 Hz, 1H, ArH), 6.90-6.89(m, 2H, ArH), 6.82-6.79(m, 2H, ArH), 4.30(s, 2H, $\text{PhCH}_2\text{C}=\text{N}$), 3.80(s, 3H, OCH_3), 3.50(s, 2H, PhCH_2), 3.37(t, J = 6.00 Hz, 2H, NCH_2), 2.92(d, J = 10.50 Hz, 2H, NCH_2), 1.99(t, J = 10.80 Hz, 2H, NCH_2), 1.72 (d, J = 8.82 Hz, 2H, CH_2), 1.66-1.60(m, 1H, CH), 1.41-1.36(m, 2H, CH_2); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ : 163.13, 160.34, 159.56, 158.70, 145.66, 134.22, 133.65, 133.15, 132.02, 131.52, 129.50, 129.11, 128.31, 127.12, 125.06, 121.54, 121.12, 116.52, 116.35, 114.65, 112.49, 63.13, 55.20, 53.23, 45.53, 37.80, 35.89, 29.84; IR (KBr, cm^{-1}): 3300, 2922, 1665, 1603, 1542; ESI-MS: 515.24 $[\text{M}+\text{H}]^+$.

N-((1-(4-(trifluoromethyl)benzyl)piperidin-4-yl)methyl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide(27)

A white solid, yield: 56%, mp: 215.5-216.6 °C, ^1H NMR (600 MHz, CDCl_3) δ : 10.96(s, 1H, CONH-N=), 8.45(d, J = 7.62 Hz, 1H, ArH), 8.07(d, J = 7.02 Hz, 1H, ArH), 7.76-7.72(m, 3H, ArH), 7.55(d, J = 7.92 Hz, 2H, ArH), 7.43(d, J = 7.86 Hz, 2H, ArH), 7.34-7.33(m, 1H, CONH), 7.02(td, J = 10.05, 2.70 Hz, 1H, ArH), 6.85-6.82(m, 1H, ArH), 4.30(s, 2H, $\text{PhCH}_2\text{C}=\text{N}$), 3.54(s, 2H, PhCH_2), 3.37(t, J = 6.12 Hz, 2H, NCH_2), 2.88(d, J = 11.16 Hz, 2H, NCH_2), 1.99(t, J = 11.34 Hz, 2H, NCH_2), 1.72 (d, J = 12.42 Hz, 2H, CH_2), 1.66-1.62(m, 1H, CH), 1.39-1.34(m, 2H, CH_2); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ : 164.07, 159.81, 158.99, 157.35, 145.37, 134.74, 133.93, 132.66, 131.98, 130.38, 129.50, 128.32, 127.50, 126.49, 125.97, 125.45, 124.83, 124.72, 123.90,

116.60, 61.97, 53.31, 45.06, 36.87, 35.86, 30.02; IR (KBr, cm^{-1}): 3287, 2928, 1664, 1541, 1495 ESI-MS: 553.22 $[\text{M}+\text{H}]^+$.

N-((1-(4-methylbenzyl)piperidin-4-yl)methyl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide

(28)

A white solid, yield: 55%, mp: 194.3-195.3 °C, ^1H NMR (600 MHz, CDCl_3) δ : 10.53(s, 1H, CONH-N=), 8.45(dd, J = 7.59, 1.62 Hz, 1H, ArH), 8.08(dd, J = 7.26, 1.92 Hz, 1H, ArH), 7.77-7.72(m, 3H, ArH), 7.34-7.33(m, 1H, CONH), 7.19(d, J = 7.80 Hz, 2H, ArH), 7.11(d, J = 7.74 Hz, 2H, ArH), 7.02(td, J = 10.08, 3.00 Hz, 1H, ArH), 6.82-6.79(m, 1H, ArH), 4.30(s, 2H, $\text{PhCH}_2\text{C}=\text{N}$), 3.48(s, 2H, PhCH_2), 3.36(t, J = 5.94 Hz, 2H, NCH_2), 2.91(d, J = 10.92 Hz, 2H, NCH_2), 2.33(s, 3H, CH_3), 1.96(t, J = 10.74 Hz, 2H, NCH_2), 1.71 (d, J = 12.72 Hz, 2H, CH_2), 1.64-1.60(m, 1H, CH), 1.39-1.33(m, 2H, CH_2); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ : 164.06, 159.81, 158.98, 157.34, 145.38, 134.73, 133.95, 132.65, 131.99, 130.37, 129.49, 129.30, 129.15, 128.31, 126.49, 125.97, 124.83, 124.73, 116.60, 62.32, 53.17, 45.06, 36.86, 35.93, 29.98, 21.15; IR (KBr, cm^{-1}): 3326, 2917, 1683, 1645, 1543, 1493 ESI-MS: 499.25 $[\text{M}+\text{H}]^+$.

N-((1-(4-trifluoromethoxybenzyl)piperidin-4-yl)methyl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl) benzamide(29)

A white solid, yield: 56%, mp: 213.3-214.3 °C, ^1H NMR (600 MHz, CDCl_3) δ : 10.65(s, 1H, CONH-N=), 8.45(d, J = 7.68 Hz, 1H, ArH), 8.08(d, J = 5.94 Hz, 1H, ArH), 7.77-7.72(m, 3H, ArH), 7.36-7.35(m, 3H, CONH & ArH), 7.15(d, J = 7.98 Hz, 2H, ArH), 7.03(td, J = 10.08, 2.76 Hz, 1H, ArH), 6.85-6.81(m, 1H, ArH), 4.30(s, 2H, $\text{PhCH}_2\text{C}=\text{N}$), 3.53(s, 2H, PhCH_2), 3.37(t, J = 5.88 Hz, 2H, NCH_2), 2.92(d, J = 9.60 Hz, 2H, NCH_2), 2.02(t, J = 11.34 Hz, 2H, NCH_2), 1.74 (d, J = 12.36 Hz, 2H, CH_2), 1.67-1.65(m, 1H, CH), 1.42-1.39(m, 2H, CH_2); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ : 164.06, 159.81, 159.04, 157.39, 147.60, 145.37, 138.77, 134.73, 133.94, 132.64, 131.98, 130.76, 130.37, 129.50, 128.32, 126.49, 125.97, 121.14, 116.59, 116.44, 61.79, 53.31, 45.11, 36.86, 35.99, 30.10; IR (KBr, cm^{-1}): 3288, 2930, 1664, 1542, 1503 ESI-MS: 569.21 $[\text{M}+\text{H}]^+$.

N-((1-(4-fluorobenzyl)piperidin-4-yl)methyl)-2-fluoro-5-((1, 2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide

(30)

A white solid, yield: 55%, mp: 153.4-155.4 °C, ^1H NMR (600 MHz, CDCl_3) δ : 12.58(s, 1H, CONH-N=), 8.45(d, J = 5.94 Hz, 1H, ArH), 8.08(d, J = 4.86 Hz, 1H, ArH), 7.75-7.73(m, 3H, ArH), 7.34-7.29(m, 3H, CONH & ArH), 7.05-6.99(m, 3H, ArH), 6.83-6.81(m, 1H, ArH), 4.30(s, 2H, $\text{PhCH}_2\text{C}=\text{N}$), 3.51(s, 2H, PhCH_2), 3.37(t, J = 5.88 Hz, 2H, NCH_2), 2.92(d, J = 9.60 Hz, 2H, NCH_2), 2.02-2.00(m, 2H, NCH_2), 1.74 (d, J = 10.80 Hz, 2H, CH_2), 1.68-1.62(m, 1H, CH), 1.44-1.37(m, 2H, CH_2); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ : 164.06, 162.46, 160.85, 159.81, 158.99, 157.35, 145.37, 134.73, 133.93, 132.66, 131.98, 130.38, 129.50, 128.32, 126.49, 125.96,

124.83, 116.59, 115.34, 61.76, 53.15, 54.07, 36.87, 35.93, 29.99; IR (KBr, cm^{-1}): 3287, 2932, 1663, 1542, 1504; ESI-MS: 503.22 $[\text{M}+\text{H}]^+$.

N-(1-benzylpiperidin-3-yl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide(31)

A white solid, yield: 60%, mp: 199.9-201.1°C, ^1H NMR (600 MHz, DMSO-d_6) δ : 12.58(s, 1H, CONH-N=), 8.24(d, $J=7.80$ Hz, 1H, ArH), 8.06(d, $J=6.30$ Hz, 1H, ArH), 7.95(d, $J=7.98$ Hz, 1H, ArH), 7.87(t, $J=7.38$ Hz, 1H, ArH), 7.80(t, $J=7.56$ Hz, 1H, ArH), 7.52(d, $J=5.58$ Hz, 1H, ArH), 7.42(s, 1H, CONH), 7.30-7.28(m, 4H, ArH), 7.22-7.16(m, 2H, ArH), 4.30(s, 2H, $\text{PhCH}_2\text{C}=\text{N}$), 3.92-3.87(m, 1H, CH), 3.45(s, 2H, PhCH_2), 2.72-2.70(m, 1H, NCHH), 2.57-2.53(m, 1H, NCHH), 1.99(d, $J=11.58$ Hz, 2H, NCH_2), 1.71-1.69(m, 1H, CHH), 1.65-1.62(m, 1H, CHH), 1.48-1.44(m, 1H, CHH), 1.34-1.29(m, 1H, CHH); ^{13}C NMR (150MHz, DMSO-d_6) δ : 163.11, 159.80, 159.12, 157.49, 145.36, 138.75, 134.76, 133.96, 132.84, 132.00, 130.50, 129.49, 129.11, 128.58, 128.31, 127.32, 126.50, 125.97, 116.63, 62.52, 58.16, 53.24, 46.65, 36.86, 29.89, 23.73; IR (KBr, cm^{-1}): 3434, 2795, 1653, 1549, 1494; ESI-MS: 471.22 $[\text{M}+\text{H}]^+$.

N-(1-(2-fluorobenzyl)piperidin-3-yl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide(32)

A white solid, yield: 55%, mp: 163.9-165.7°C, ^1H NMR (600 MHz, DMSO-d_6) δ : 12.59(s, 1H, CONH-N=), 8.24(d, $J=7.74$ Hz, 1H, ArH), 8.06(d, $J=6.06$ Hz, 1H, ArH), 7.95(d, $J=8.04$ Hz, 1H, ArH), 7.86(t, $J=7.20$ Hz, 1H, ArH), 7.80(t, $J=7.56$ Hz, 1H, ArH), 7.53(d, $J=5.28$ Hz, 1H, ArH), 7.43-7.38(m, 2H, CONH & ArH), 7.30-7.27(m, 1H, ArH), 7.18(dd, $J=9.51, 1.14$ Hz, 1H, ArH), 7.16-7.12(m, 2H, ArH), 4.30(s, 2H, $\text{PhCH}_2=\text{N}$), 3.90-3.88(m, 1H, CH), 3.52(s, 2H, PhCH_2), 2.75(d, $J=8.94$ Hz, 1H, NCHH), 2.56(d, $J=8.04$ Hz, 1H, NCHH), 2.02(t, $J=10.50$ Hz, 2H, NCH_2), 1.70-1.68(m, 1H, CHH), 1.64-1.62(m, 1H, CHH), 1.46(td, $J=13.68, 10.20$ Hz, 1H, CHH), 1.32-1.27(m, 1H, CHH); ^{13}C NMR (150MHz, DMSO-d_6) δ : 163.16, 162.00, 160.38, 159.81, 159.12, 157.48, 145.36, 134.77, 133.95, 132.85, 131.99, 130.51, 129.49, 128.32, 126.50, 125.96, 124.91, 124.56, 124.41, 116.48, 115.48, 57.99, 54.98, 52.98, 46.67, 36.87, 29.77, 23.74; IR (KBr, cm^{-1}): 3436, 2940, 1668, 1541, 1493; ESI-MS: 489.21 $[\text{M}+\text{H}]^+$.

N-(1-(4-fluorobenzyl)piperidin-3-yl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide(33)

A white solid, yield: 56%, mp: 137.9-140.0°C, ^1H NMR (600 MHz, DMSO-d_6) δ : 12.57(s, 1H, CONH-N=), 8.24(d, $J=7.86$ Hz, 1H, ArH), 8.07-8.05(m, 1H, ArH), 7.95(d, $J=8.04$ Hz, 1H, ArH), 7.87(td, $J=7.68, 0.96$ Hz, 1H, ArH), 7.80(t, $J=7.50$ Hz, 1H, ArH), 7.52(d, $J=5.70$ Hz, 1H, ArH), 7.43(s, 1H, CONH), 7.32(dd, $J=1.50, 0.66$ Hz, 2H, ArH), 7.18(t, $J=9.66$ Hz, 1H, ArH), 7.16-7.12(m, 2H, ArH), 4.30(s, 2H, $\text{PhCH}_2=\text{N}$), 3.93-3.88(m, 1H, CH), 3.45(s, 2H, PhCH_2), 2.72(d, $J=8.88$ Hz, 1H, NCHH), 2.53(d, $J=9.90$ Hz, 1H, NCHH), 1.99(t, $J=7.74$ Hz, 2H, NCH_2), 1.72-1.64(m, 2H, CH_2), 1.50-1.45(m, 1H, CHH), 1.35-1.29(m, 1H, CHH); ^{13}C NMR (150MHz, DMSO-d_6) δ : 163.14, 162.45, 160.85, 159.81, 159.11, 157.48, 145.36, 134.75, 133.95, 132.84, 132.00, 130.90, 130.50, 129.49, 128.31, 126.50, 125.97, 116.63, 115.36, 61.56, 58.07, 53.07, 46.66, 36.87, 29.89, 23.74; IR (KBr, cm^{-1}): 3430, 2940, 1658, 1520; ESI-MS: 489.21 $[\text{M}+\text{H}]^+$.

N-(1-(4-methylbenzyl)piperidin-3-yl)-2-fluoro-5-((1,2-dihydro-1-oxophthalazin-4-yl)methyl)benzamide(34)

A white solid, yield: 51%, mp: 187.9-189.1°C, ^1H NMR (600 MHz, DMSO- d_6) δ : 12.57(s, 1H, CONH-N=), 8.24(d, J =7.86 Hz, 1H, ArH), 8.04(d, J =6.12Hz, 1H, ArH), 7.95(d, J =8.04Hz, 1H, ArH), 7.87(td, J =7.68, 0.96Hz, 1H, ArH), 7.80(t, J =7.38Hz, 1H, ArH), 7.53 (d, J =6.54Hz, 1H, ArH), 7.43-7.41(m, 1H, CONH), 7.19(d, J = 10.08Hz, 1H, ArH), 7.16(d, J = 7.98Hz, 2H, ArH), 7.09(d, J = 7.74Hz, 2H, ArH), 4.30(s, 2H, PhCH₂=N), 3.89-3.88(m, 1H, CH), 3.41(s, 2H, PhCH₂), 2.68(d, J =7.38Hz, 1H, NCHH), 2.53(d, J =7.80Hz, 1H, NCHH), 2.25(s, 3H, PhCH₃), 2.02-1.95 (m, 2H, NCH₂), 1.70-1.69(m, 1H, CHH), 1.63-1.61(m, 1H, CHH), 1.49-1.43(m, 1H, CHH), 1.34-1.28 (m, 1H, CHH); ^{13}C NMR (150MHz, DMSO- d_6) δ : 163.09, 159.81, 159.13, 157.49, 145.36, 136.33, 135.59, 134.77, 133.95, 132.86, 131.99, 130.53, 129.49, 129.16, 128.31, 126.50, 125.96, 116.63, 116.48, 62.26, 58.05, 53.21, 46.63, 36.87, 29.91, 23.73, 21.13; IR (KBr, cm⁻¹): 3439, 2931, 1660, 1531, 1492; ESI-MS: 485.23 [M+H]⁺.

1-benzylpiperidin-4-yl 2-fluoro-5-((1, 2-dihydro-1-oxophthalazin-4-yl)methyl)benzoate (35)

A white solid, yield: 50%, mp: 182.1-183.4°C, ^1H NMR (600 MHz, DMSO- d_6) δ : 12.61(s, 1H, CONH-N=), 8.25(d, J =7.86 Hz, 1H, ArH), 7.96(d, J =8.04Hz, 1H, ArH), 7.88(td, J =7.68, 0.96Hz, 1H, ArH), 7.82(t, J =7.26Hz, 1H, ArH), 7.77 (dd, J =6.48, 1.44Hz, 1H, ArH), 7.60 (td, J =4.26, 1.44Hz, 1H, ArH), 7.31-7.25(m, 5H, ArH), 7.23-7.21(m, 1H, ArH), 4.94-4.93(m, 1H, OCH), 4.36(s, 2H, PhCH₂=N), 3.43(s, 2H, PhCH₂), 2.47(t, J = 8.82Hz, 2H, NCH₂), 2.28(t, J = 9.30Hz, 2H, NCH₂), 1.88-1.85(m, 2H, CH₂), 1.66-1.64(m, 2H, CH₂); ^{13}C NMR (150MHz, DMSO- d_6) δ : 163.24, 159.80, 159.32, 145.26, 138.94, 135.86, 134.92, 133.98, 132.02, 131.97, 129.51, 129.13, 128.58, 128.29, 127.28, 126.52, 125.88, 117.67, 117.52, 71.27, 62.45, 50.16, 36.67, 30.68; IR (KBr, cm⁻¹): 3403, 2946, 1677, 1608, 1494; ESI-MS: 472.20 [M+H]⁺.

1-(4-fluorobenzyl)piperidin-4-yl 2-fluoro-5-((1, 2-dihydro-1-oxophthalazin-4-yl)methyl)benzoate (36)

A white solid, yield: 49%, mp: 156.7-157.1°C, ^1H NMR (600 MHz, CDCl₃) δ : 11.45(s, 1H, CONH-N=), 8.49(d, J =6.66 Hz, 1H, ArH), 7.87(d, J =4.92Hz, 1H, ArH), 7.76-7.71(m, 3H, ArH), 7.40-7.39(m, 1H, ArH), 7.28-7.26(m, 2H, ArH), 7.04(t, J =9.12Hz, 1H, ArH), 6.98(t, J =8.10Hz, 2H, ArH), 5.12-5.04(m, 1H, OCH), 4.31(s, 2H, PhCH₂=N), 3.48(s, 2H, PhCH₂), 2.64(t, J = 8.92Hz, 2H, NCH₂), 2.36(t, J = 9.10Hz, 2H, NCH₂), 2.03-1.92(m, 2H, CH₂), 1.89-1.79(m, 2H, CH₂); ^{13}C NMR (150MHz, CDCl₃) δ :163.75, 162.76, 161.68, 160.80, 159.96, 145.58, 134.37, 133.61, 133.33, 131.92, 131.52, 130.54, 128.29, 127.17, 124.96, 119.28, 117.31, 114.92, 109.99, 71.09, 62.15, 50.24, 37.71, 30.63; IR (KBr, cm⁻¹): 3420, 2950, 1713, 1667, 1607, 1501; ESI-MS: 490.19 [M+H]⁺.

Biological Activity

PARP inhibition assay

PARP enzyme inhibition was measured using an HT F Homogeneous 96-well PARP Inhibition Assay Kit (Trevigen, Cat# 4690-096-K), according to the manufacturer's protocol. The various synthesized compounds were dissolved in DMSO and then serially diluted to the required concentrations with distilled water, keeping the final concentration of DMSO lower than 1%. Olaparib was used as positive control. Fluorescence values under the condition of excitation wavelength (544 nm) and emission wavelength (590 nm) were measured using a multi-well spectrophotometer (Molecular Devices SpectraMax M5 microplate reader). Then the standard curve was drawn and the inhibition rate of each test compound was calculated. IC₅₀ value of each compound was calculated using GraphPad Prism 6 software according to the above results.

Anti-cholinesterase activity assays

The acetylcholinesterase (eeAChE, from the electric eel), butyrylcholinesterase (eqBChE, from equine serum), 5, 5'-dithiobis-2-nitrobenzoic acid (Ellman's reagent, DTNB), S-butyrylthiocholine iodide (BTCl), acetylthiocholine iodide (ATCl), and Donepezil hydrochloride and Rivastigmine methyl sulfate were purchased from Sigma Aldrich.

The capacity of the test compounds **16-36** to inhibit the AChE and BChE activities were assessed by the Ellman's method in 96-well plates with slight modification [26]. The Donepezil and Rivastigmine were used as reference drugs. The test compounds were dissolved in a minimum volume of DMSO (1%) and were diluted using sodium phosphate buffer (0.1 M, pH 7.4). A reaction mixture containing 20 μ L sodium phosphate buffer, 100 μ L DTMB (1 mM) and 40 μ L AChE (0.2 U/MI)/BChE (0.3 U/mL) were incubated with 20 μ L of various concentrations of test compounds at 37°C for 15 min followed by the addition of the substrate (20 μ L) acetylthiocholine iodide (1 mM) or S-butyrylthiocholine iodide (1 mM) and incubation for another 3 min. The activities were determined by using a Varioskan Flash Multimode Reader at 412 nm. The inhibition percent was calculated by the following expression: $(1-A_i/A_c) \times 100$, where A_i and A_c were the absorbance obtained for AChE/BChE in the presence and absence of inhibitors, respectively. The concentration of compound producing 50% of enzyme activity inhibition (IC₅₀) was calculated by nonlinear regression analysis of the response-concentration (log) curve, using the Graph-Pad Prism program package (Graph Pad Software; San Diego, CA). Results are expressed as the mean $\pm$ SEM of at least three different experiments performed in triplicate.

Molecular docking

Molecular docking studies were performed using the AutoDock Vina 1.1.2 [33] along with the AutoDockTools 1.5.6 (ADT) and AutoGridFR 1.0. The structure of compound **30** was drawn and then subjected to energy minimization with the molecular mechanics (MM2) using the ChemBioOffice 13.0 (CambridgeSoft, Perkin Elmer Inc, MA, USA). The ligand was further processed and saved in the pdbqt format for docking by ADT program. The X-ray crystallographic structures of PARP-1 (PDB Code 5DS3) and hBChE (PDB Code 5NN0) were obtained from the online PDB database (RCSB) (<http://www.rcsb.org>). Both receptors were firstly prepared using the Acceryls Discovery Studio 2017 R2

(Accelrys Inc, San Diego, CA, USA) involving removal of water molecules, non-bonded inhibitors and cofactors. Subsequently, both proteins were edited using the ADT program to add the polar hydrogen atoms and Kollman charges as means to rigidify protein structures and also saved in the same pdbqt format as the ligand **30**. The torsions for ligand and receptors were set to be rotatable and rigid respectively. The AutoGridFR software was applied to determine the binding pocket and the grid coordinate [34]. After that, docking was performed using the default settings of Vina and only the minimum predicted Gibbs binding energy pose was considered for each case. The molecular docking protocols were validated through re-docking of the co-crystallized ligands in the vicinity of the PARP-1 and hBChE active site. Except for the hydrogen bonds mediated by water molecule it reproduced all the other key interactions accomplished by the co-crystallized ligand with the key amino acids in the active site as shown in the crystal, indicating the suitability of the used setup for the docking study. Analysis and visualization of the docking results were done using the LigPlot⁺ version 2.2.4 [35] for 2D ones.

Declarations

Disclosure

The authors report no conflicts of interest in this work.

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Schemes

Scheme1 is available as a download in the Supplemental Files section

Figures

Figure 1

Structures of Olaparib, Donepezil and the designed target compounds 16-36

Figure 2

(a) Ligplot image showing the interaction of 30 with the enzyme PARP-1. The commonly interacting amino acid residues in both interactions were encircled in red circles. Arcs with red lines represented amino acid hydrophobic contacts; green dashed lines represented hydrogen bonds. (b) Ligplot image showing the interaction of the 30 with the enzyme huBChE.

Supplementary Files

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