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Intepirdine Derivatives Possessing Dual 5HT6 Antagonism / HDAC6 Inhibitory Activity

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Dedicated to Professor Philip Charles Bulman Page on the occasion of his 70th birthday

Alzheimer's Disease (AD) impacts significantly the quality of life of people aged over 65 years old, while millions more suffer from other types of dementia, yet no effective drugs exist. The few approved drugs in this area address mostly the associated symptoms, while several selective agents acting on promising targets have failed in clinical trials. The complexity of neurodegenerative diseases has prompted multitargeting ligands as the new paradigm, where more than one biological mechanism may be perturbed synergistically. In this context, we explored the design and synthesis of dual 5-HT_6 antagonists / HDAC6 inhibitors since these actions have been individually demonstrated to elicit cognitive-enhancing effects. Prototypes with this dual action on a GPCR and an enzyme were designed and synthesized by tethering an aryl-hydroxamic acid unit, an established pharmacophore for HDAC6 inhibition, to the piperazine of intepirdine, a potent 5-HT_6 antagonist. A new gram-scale synthesis of intepirdine was developed followed by attaching different types of arylhydroxamic acids. Derivative RG-283AG emerged to possess sub-micromolar potency toward HDAC6 inhibition (IC_{50} 0.54 $\mu\mu$), nanomolar affinity (K_i 0.7 nM) for 5-HT_6 receptor and favorable BBB penetration capacity, thus constituting the first example of a dual-acting 5-HT_6 / HDAC6 ligand with potential cognitive-enhancing properties.

1. Introduction

Alzheimer's disease (AD) is ranked 4th among 369 diseases in terms of incidence, prevalence, mortality, years of life lost (YLLs), and disability-adjusted life-years (DALYs) in people aged over 75 years old.[1] AD is a neurodegenerative disease that represents the predominant contributor to all types of dementia affecting 55 million people over the age of 65, and its occurrence is estimated to triple globally by 2050.[2] According to a recent report, the cost for treatment and care of patients suffering from AD, is currently estimated at $305 billion and is expected to increase to over $1 trillion by 2050.[3]

Furthermore, an increasing number of people, even under the age of 65, suffer from other types of dementia, such as vascular, frontotemporal, Lewy body, or secondary dementias (e.g., due to Parkinson's disease, Huntighton's disease, traumatic brain injury, Wernicke-Korsakoff syndrome caused by alcohol use disorder etc.). In addition, cognitive impairment is frequently present in various psychiatric disorders such as schizophrenia, bipolar disorder, and major depressive disorder.[4] Despite the diverse phenomenology and pathophysiology of dementias, there are approved medications only for AD.[5]

With the exception of two monoclonal antibodies approved recently,[6] there are only four small molecule drugs that were approved more than 20 years ago for AD; the acetyl cholinesterase (AChE) inhibitors donepezil (1996), rivastigmine (also approved for mild to moderate dementia due to Parkinson's disease) (1997), and galantamine (2001) for mild to moderate AD and the antagonist of the glutamate NMDA receptors memantine (2003) for moderate to severe AD. These drugs, when efficacious, only alleviate some of the cognitive symptoms in AD without halting neurodegeneration, whilst their efficacy fades over time. In addition, due to the wide expression of

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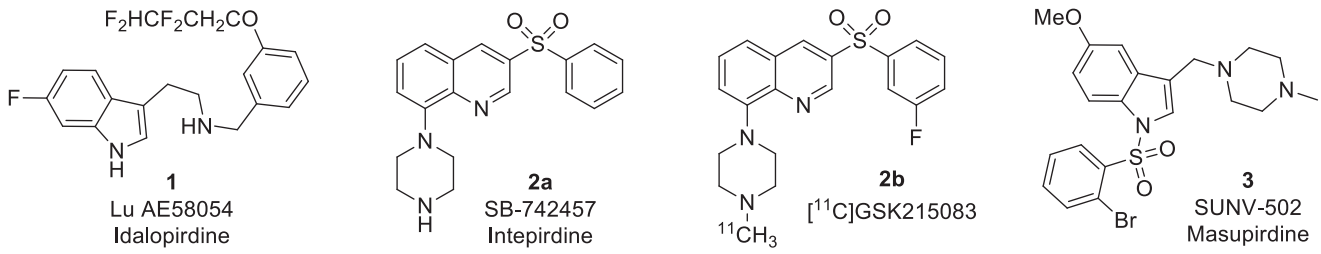


Scheme 1. 5HT6 inhibitors that have progressed in clinical trials for AD.

AChE in the periphery, AChE inhibitors cause adverse effects such as gastrointestinal disturbances, bradycardia, and excessive salivation. Another therapeutic approach has been the development of secretase and tau aggregation inhibitors aiming at the management of amyloid plaques and neurofibrillary tangles, respectively. Despite the intense research efforts and numerous clinical trials conducted by several pharmaceutical companies, none of the molecules associated with these mechanisms have succeeded in demonstrating benefits in AD to date nevertheless, understanding the association of tauopathies with memory and cognitive decline remains one of the main areas of research in AD.[7]

The 5-HT_6 receptor is a GPCR, the latest identified member of 5HT receptors, that is exclusively localized in brain regions, including the olfactory tubercle, therefore, in contrast to AChEIs, 5-HT_6 modulation would be more relevant to cognition and due to the absence of 5HT6 receptors from the periphery would exhibit limited, if any, side effects. The 5-HT_6 receptor signaling is complex yet, studies over the last two decades have established significant correlations between 5-HT_6 receptor blockade and AD. These arise primarily due to 5-HT_6 modulation of several neurotransmitters including acetylcholine and the Fyn-dependent modulation of $pERK_1$, both of which are involved in the phosphorylation of tau protein.[8–13] Consequently, pharmaceutical companies have pursued drug discovery programs for AD by targeting potent and selective 5-HT_6 receptor antagonists, several of which have progressed into clinical trials (Scheme 1).[14–16] Idalopirdine and intepirdine, despite positive results in Phase II studies, failed narrowly in demonstrating a statistically significant benefit in AD in Phase III clinical trials.

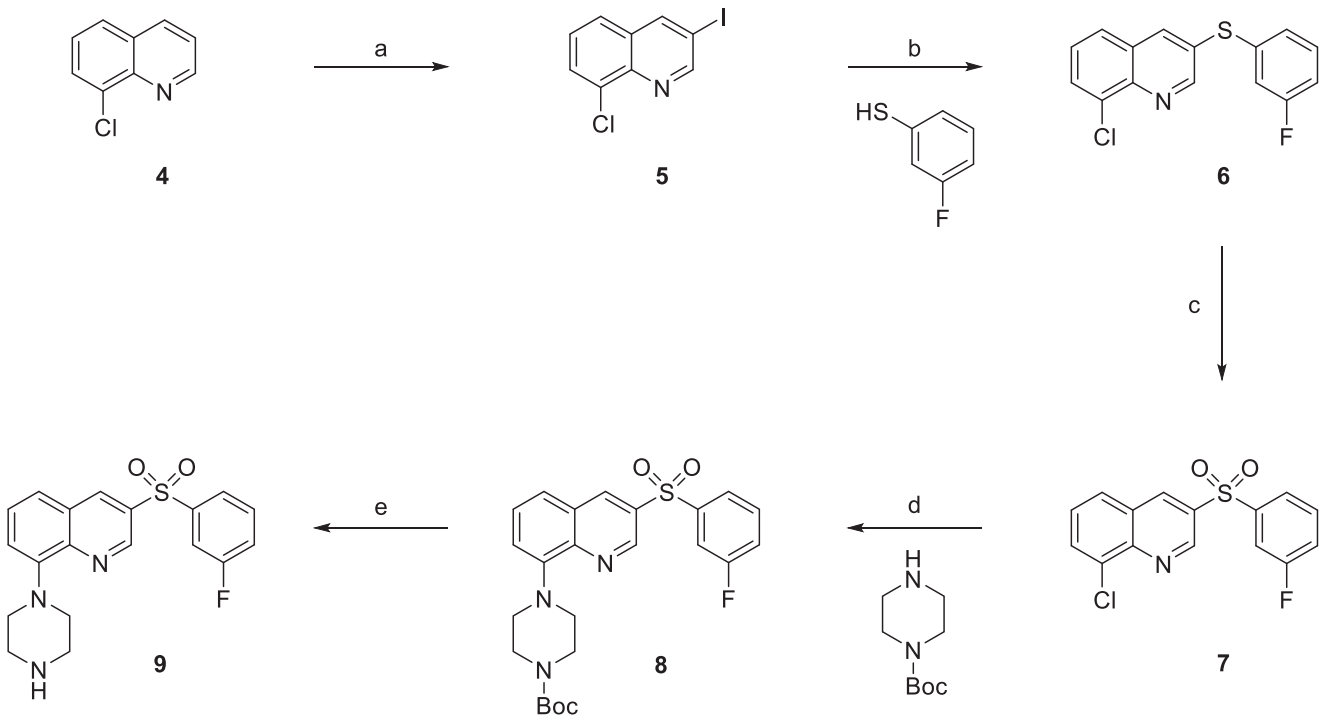
The pathophysiology and progression of cognitive decline are complex and not fully understood nevertheless, neurotransmitter imbalance, neuroinflammation, vascular pathology, and mitochondrial dysfunction appear to be the factors with the most significant association.[17] This multifactorial nature of AD has prompted the scientific community in pursuing multi-targeting ligands so that therapeutic intervention to several relevant biological mechanisms is achieved by a single molecular entity.[18,19] Oddly enough, almost invariably,[20,21] these efforts have involved the outdated AChE inhibition despite the general consensus of its inability to modify dementias and the abandonment by pharmaceutical companies of further investment in AChE inhibitors. In terms of exploring the potential of 5HT6 antagonists in conjunction with additional modes of action, there have been several reports on dual 5-HT_6/5-HT_3 and 5-HT_6/D_3, antagonists for improving cognitive function and also addressing other CNS disorders.[22–26] In this context we decided to pursue HDAC6 inhibition as an alternative complementary target to 5-HT_6 antagonism, thus generating a novel class of multi-targeting directed ligands for treating cognitive decline.

There are eleven subtypes of HDACs, which have been categorized into four distinctive groups: group I (HDACs 1–3 and 8), group II (HDACs 4, 5, 7, and 9), group III (HDACs 6 and 10), and group IV (HDAC 11).[27] HDAC6 in particular has been linked strongly with CNS disorders such as depression and cognitive decline.[28,29] In animal models, the inhibition of HDAC6 supports the reduction of Tau phosphorylation and the deacetylation of a-tubulin, improvement in the pathology of amyloid plaques, hence the potential enhancement of memory and learning functions.[30–33] Moreover, HDAC6 knockout in mice results in a healthy and viable phenotype thus, inhibition of 5-HT6 supports a safe pharmacological intervention for potential therapies in AD and other dementias.[34]

Based on these findings, we decided to explore dual-acting 5-HT_6/HDAC6 ligands so that two validated mechanisms for cognitive enhancement, one associated with a GPCR and the other one with an enzyme, may be engaged independently by a single molecule. We chose to use one of the most effective and selective 5-HT_6 inhibitors in the literature, intepirdine and its related PET tracer (**2a** and **2b** in Scheme 1)[35] as the 5-HT_6 scaffold and attach to it established pharmacophore units associated with selective and potent HDAC6 inhibition.[36]

1.1. Synthesis of Intepirdine-Related Scaffold Conferring 5-HT6 Antagonism

The original preparation of **2a, 2b** and related derivatives according to an early GlaxoSmithKline patent, utilized 8-nitro quinoline as the starting material, which is subjected to a selective iodination at the 3-position using NIS in AcOH.[35a,b] Installation of the phenylsulfone at the 3-position is accomplished by the direct displacement of the iodide with sodium benzenesulfinate under copper catalysis followed by reduction of the nitro group and treating the resulting 8-amino-(3-phenylsulfone)-quinoline with bis-(2-chloroethyl)amine, produces **2a** (and **2b** after appropriate methylation). This route is hampered by low yields, tedious work-up procedures, and extensive chromatographic purifications. In a subsequent GlaxoSmithKline patent related to analogues of intepirdine (**2a**) the route was modified to employ 8-chloro- and 8-fluoro-quinoline as the starting material so that the piperazine could be introduced by a Buchwald-Hartwig coupling or



Scheme 2. a) TBHP (aq), I_2, MeCN, reflux, 71%; b) CuI, Cs_2CO_3, 1,1,1-tris(hydroxymethyl)ethane, DMF/dioxane (1:9), 110 °C, 16 hours; 90% c) H_2O_2, $Na_2WO_4{\cdot}2H_2O$, $PhPO_3H_2$, tetrabutylammonium hydrogen sulfate, DMAc, 65 °C, 16 hours; 85% d) 1-Boc-piperazine, $Pd(OAc)_2$, XPhos, NaO^tBu, dioxane, 90 °C, 16 hours; 85% e)TFA, DCM, rt, 1 hour then NaOH (aq); 76%.

nucleophilic aromatic substitution, respectively. Furthermore, the low-yielding direct formation of the sulfone moiety using benzenesulfinate was replaced with a two-step process involving the formation of the corresponding diaryl sulfide followed by oxidation to the sulfone. Despite the chromatographic purifications required, we decided to follow this process and attempt to improve it as appropriate.

Accordingly, the iodination of 8-chloroquinoline **4** was initially attempted using N-iodosuccinimide in acetic acid as per GlaxoSmithKline's procedure,[35a,b] however, in addition to the moderate yield for the desired **5** (40–45%), significant amounts of bis-iodinated byproducts were generated. Column chromatography was necessary to remove the impurities and isolate pure **5** in 36% yield. In order to avoid byproduct formation and chromatographic purification, we tested alternative iodination conditions and achieved the direct and regioselective iodination at C3 using TBHP (70 wt.% aqueous solution) and iodine according to the method of Sharma et al. (Scheme 2, conditions b).[37] Credit to those authors, this reaction is essentially single peak to single peak by HPLC, and after workup and recrystallization from water/ethanol, **5** was isolated in excellent quality and 71% yield. The reaction was reproducible even at a scale of five grams, although occasionally additional TBHP (1–1.5 eq.) and reaction time (4–8 hours) were required. With iodo-quinoline **5** in hand, we examined the direct sulfonylation reaction with benzenesulfinic acid using catalytic copper iodide and *N,N′*-dimethyl-ethylendiamine as a ligand as reported by GlaxoSmithKline for the 8-fluoroquinoline substrate.[35b]

Nevertheless, this protocol suffered from reproducibility issues and low yields (<30%) of the expected sulfone (des-fluoro **7**) with **5** remaining mostly unreacted. A brief screening was conducted with respect to the copper catalyst, ligand, base, solvent, and temperature, however, none of the conditions investigated gave improved yields, if any product at all. The same outcome was obtained using the conditions and ligand reported by Zhao et al. in a similar synthesis of intepirdine.[38] Since the direct synthesis of heteroaryl sulfone **6** was unsuccessful, a stepwise approach was pursued by coupling quinoline iodide **5** with 3-fluorothiophenol followed by oxidation of the resulting sulfide **6** to the corresponding sulfone **7**.

In the first attempt, diaryl sulfide **6** was produced from the reaction of quinolone **3** with 3-fluorothiophenol in 1,2-ethanediol at 80 °C in the presence of potassium phosphate and catalytic copper iodide, as reported by GlaxoSmithKline.[35a,b] This reaction worked exceptionally well even at a scale of five grams, delivering **6** in 95% yield and in excellent quality. This protocol was used to supply the quantities of **6** needed initially for exploring and optimizing the synthetic steps downstream however, it presented reproducibility issues with respect to full conversion and quality of the isolated sulfide. A screen of several protocols with copper and palladium catalysts for this Ullmann-type coupling was conducted, exemplifying as the optimum the method reported by Chen and Chen, as it delivers **6** robustly and reproducibly in 90% yield even at a 5 g scale.[39] This protocol utilizes 1,1,1-tris(hydroxymethyl)ethane as the CuI ligand and has been used successfully in our laboratory in similar heteroatom/aryl coupling reactions. Regarding the subsequent oxidation of **6** to the corresponding diaryl sulfone **7**, the method reported by GlaxoSmithKline with $MMPP{\cdot}6H_2O$ in DCM/MeOH also worked in our hands delivering successfully the desired

product **7** in a pure state and 74% yield even on multigram scale. Nevertheless, a greener and safer alternative to the stoichiometric peracid oxidant was considered therefore a catalytic method reported by Noyori was tested. This protocol involves aqueous H_2O_2 (30 wt%), $Na_2WO_4{\cdot}2H_2O$ catalyst with $PhPO_3H_2$ ligand/additive and tetraoctylammonium chloride phase transfer catalyst in MeCN.[40] In using the reported conditions the oxidation reaction of sulfide **6** stalled at a 1:1 sulfoxide/sulfone mixture (HPLC a/a) and did not complete even after further additions of hydrogen peroxide and/or at elevated temperature. Therefore, an optimization effort took place encompassing almost all reaction variables which demonstrated that the mode of addition and concentration of reactants are of paramount importance. The more concentrated the reaction the higher the tendency to stall, whereas all modes of hydrogen peroxide addition where brisk gas effervescence was manifested (catalase activity of the catalyst), led to inefficient oxidation of the substrate. The solvent switch from MeCN to DMAc allowed for a homogeneous solution of **6,** which is added at ambient temperature over 15 minutes to an aqueous solution of $NaWO_4{\cdot}2H_2O$ (5 mol%), $PhPO_3H_2$ (5 mol%), tetrabutylammonium hydrogen sulfate (2.5 mol%) (the later allowing also for the pH to be adjusted to 2.5–3) and hydrogen peroxide (5 eq.) (Scheme 2). The reaction is initially warmed to 50 °C and within less than one hour a 15:85 sulfoxide/sulfone mixture (HPLC a/a) is achieved, reaching 5:95 after 16 hours at 65 °C. Complete conversion of the sulfoxide intermediate to the desired sulfone **7** proved challenging and essentially unresponsive to additional amounts of catalyst and oxidant. Isolation at this point by simple filtration affords 85% of the crude sulfone contaminated with $<$ 5% sulfoxide, which is however inconsequential since it is purged in the next step.

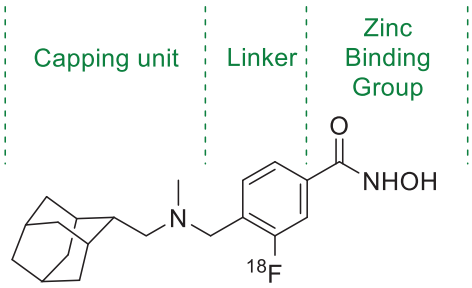
The synthesis of piperazine derivative **8** was performed through a Buchwald-Hartwig *N*-arylation reaction of **7** and 1-Boc-piperazine (Scheme 2). This transformation was also employed by GlaxoSmithKline in the synthesis of intepirdine analogues, using the corresponding 8-iodoquinoline intermediate with Pd_2dba_3 and DavePhos, although moderate yields were reported including the need for chromatographic purification.[35a,b] We decided to improve this key step hence tested several of the most effective combinations of palladium/ ligand systems and conditions reported in the literature for similar processes.[41] In this context, the less expensive and more stable $Pd(OAc)_2$ precatalyst was tested in combinations with BINAP, DavePhos, ^{t}BuDavePhos, RuPhos, XPhos, and CM-Phos in dioxane solvent and potassium *tert*-butoxide as the base. XPhos, RuPhos, and CM-Phos proved to be significantly better in delivering **8** (80–85% isolated yield) than the other ligands (incomplete reaction after 16 hours), with RuPhos and XPhos becoming the ligands of choice due to their lower cost in comparison to CM-Phos. The final step in the synthesis of our 5-HT_6 ligand (**10**) was the removal of the Boc group from the piperazine nitrogen atom using our previously reported method of methanesulfonic acid in EtOAc, which has proved superior to the TFA/DCM method on several challenging occasions,[42] and provided **9** in 58% yield after neutralization of the initially isolated mesylate salt. In this case however, the standard TFA/DCM protocol allowed, within one hour, for a greater isolated yield of the corresponding TFA salt of **9** (86%), which after neutralization, gave free base **9** in excellent quality and 76% isolated yield (from **8**). Overall, this synthesis provides pure **9** in 35% yield over five steps in a robust and reproducible manner, without the need for chromatography, and was demonstrated on a five gram scale, thus enabling **9** to be accessed in multigram quantities and derivatized further with an HDAC6 inhibitory pharmacophore.

1.2. 5-HT6 / HDAC6 Dual-Acting Ligands

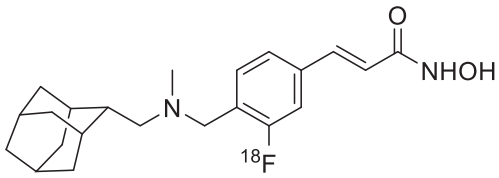
Our design of the intended dual-acting ligands pivoted on attaching to the basic/nucleophilic piperazine nitrogen atom of the 5HT6 antagonism pharmacophore **9** (fluoro-intepirdine or des-methyl GSK215083) an established HDAC6 inhibitory pharmacophore, namely a 4-methylene-benzohydroxamic acid. Considering the design of hybrid HDAC6 inhibitors with an additional pharmacophore/mode of action stemming from the capping unit (Scheme 3), it should be noted that selective and potent HDAC6 inhibitors possessing nitrogenous tethers between the capping and the linker/zinc binding domains, mostly involve a nonbasic nitrogen atom, typically associated with an amide, indole, or an aniline-type nitrogen atom.[43] To the best of our knowledge, bavorastat,[44] (including its ^{18}F analogue) is the only exception to this trend among selective HDAC6 inhibitors, namely bearing a basic nitrogen atom, and perhaps the less HDAC6/1 selective CM-695 (Scheme 3) although this has been designed to engage both HDAC6 and PDE9.[45]

Interestingly, ^{18}F-martinostat (MGS3),[46] the cinnamic analogue of bavarostat, is a more selective inhibitor toward HDAC1, 2, and 3 which convinced us to focus on bavorastat's benzohydroxamic acid pharmacophore as the zinc-binding domain, which is also consistently utilized in the most potent and selective HDAC6 inhibitors.[47] Related variations in the *m*-position of the aryl linker have exemplified the beneficial role of fluorine atoms in increasing HDAC6 selectivity, and to a lesser extent potency, whereas the impact of replacing one of the *m*-carbon atoms with a nitrogen atom (pyridine analogues) has been less predictable (Scheme 3).[48] Accordingly, we decided to prepare the novel intepirdine derivatives RG-281AG, '283 and '285 (Scheme 3) in order to assess the impact of the variation in the arylhydroxamic acid on the HDAC6 inhibitory activity and if 5HT6 potency is affected by attaching the additional pharmacophore.

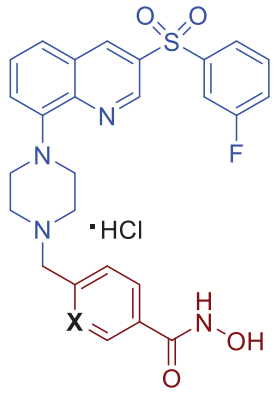
N-alkylation of **9** with **10a-c** in the presence of potassium carbonate in acetonitrile led to intermediates **11a-c** in 88–93% yields (Scheme 4). Next, we tried to prepare the hydroxamic acid group directly from the corresponding ester in one step according to literature procedures.[49] Nevertheless, as confirmed by NMR and mass spectroscopy, we isolated primarily the corresponding acid instead of the desired hydroxamic derivative, which forced us to follow a stepwise approach toward hydroxamic acid derivatives RG-281AG, '283, and '285. Firstly, the hydrolysis of esters **11a-c** was achieved with aqueous 3 M NaOH in MeOH/THF (4:1) in 60–70% yields. The resulting carboxylic acids **12a-c** were subsequently



^{18}F-Bavarostat



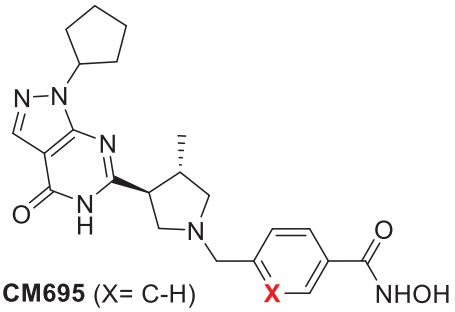
^{18}F-Martinostat



This work

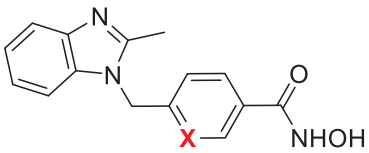
5HT6 antag. / HDAC6 inhib. hybrids

RG-281AG X= C-H
RG-283AG X= C-F
RG-285AG X= N

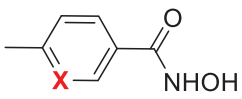


CM695 (X= C-H)

X	HDAc6 IC_{50}(nM)	HDAC6/1 selectivity
C-H	40	14.8
C-F	62	32.2
N	143	13.4



X	HDAc6 IC_{50}(nM)	HDAC6/1 selectivity
C-H	9.2	955
C-F	3.0	1440
N	77	362



X	HDAc6 IC_{50}(nM)	HDAC6/1 selectivity
C-H	140	40
C-F	49	140
N	70	162

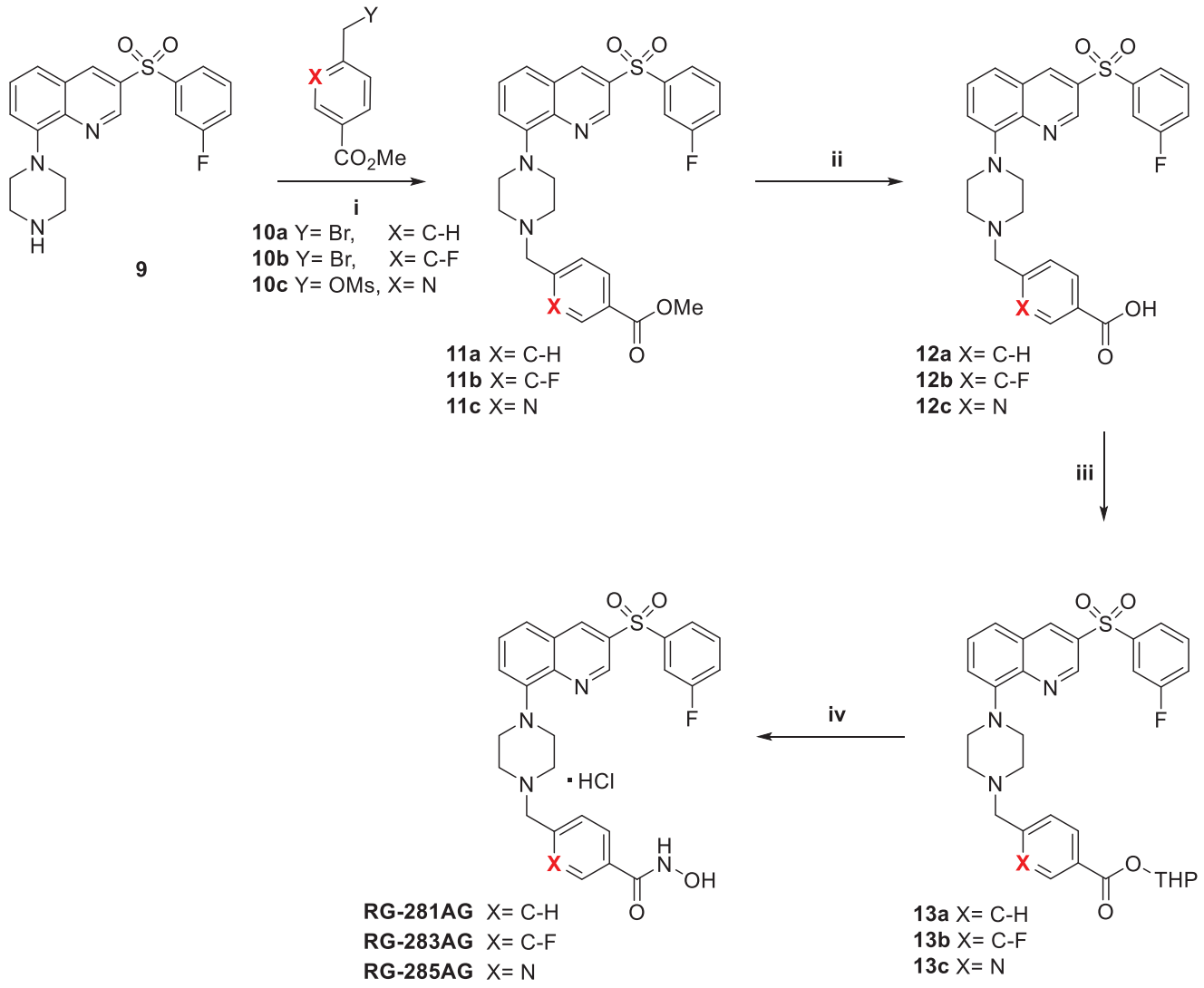
Scheme 3. Examples of selective HDAC6 inhibitors with a non-amide nitrogen atom linking the arylhydroxamic acid and capping domains.

condensed with O-THP hydroxylamine using our preferred protocol based on T3P (50 wt.% solution in EtOAc %), as in our hands it has proved superior to other amide coupling agents in challenging cases.[50] Application of the T3P protocol allowed for simple isolation of intermediates **13a-c** in 57–65% yields. Deprotection of the THP group was achieved by transacetalization in methanolic solution of **13a-c** with added anhydrous 6 M HCl solution in isopropanol and stirring at ambient temperature overnight (Scheme 4).

Removal of most of the solvent followed by addition of diethyl ether caused the hydroxamic acid products **RG-281AG**, **'283,** and **'285** to crystallize in the form of hydrochloride salts in 69–78% yields. In each of these last four steps the crude intermediates **11a,b,c** -**13a,b,c** (90–95% purity) were progressed into the next step, and all side products were purged in the crystallization of the **RG-281AG**, **'283,** and **'285** HCl salts. Combined with the five-step synthesis of intermediate **9**, this process constitutes overall a chromatography-free and amenable to-scale-up, nine-step synthesis for each of the targeted derivatives **RG-281AG**, **RG-283AG,** and **RG-281AG** (Scheme 4).

1.3. Biological Activity and Pharmacokinetic Considerations

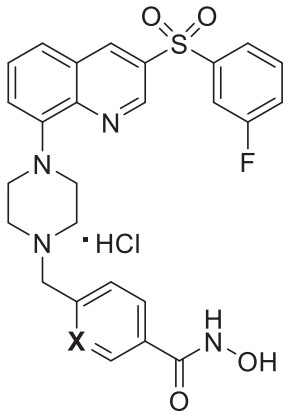
Rewardingly, biological evaluation of these hybrid molecules revealed that all three intepirdine derivatives, **RG-283AG**, **RG-281AG,** and **RG-285AG,** maintain nanomolar affinity for the 5-HT_6 receptor, while the HDAC6 inhibitory potency appears sensi-



Scheme 4. General synthesis of 5-HT_6 – HDAC6 analogs, reagents, and conditions: (i) K_2CO_3, MeCN, rt, 16 hours, 88–93%; (ii) aq.3 M NaOH, MeOH/THF 4:1, 62–71%; (iii) O-(tetrahydro-2H-pyran-2-yl)hydroxylamine, T3P in EtOAc (50 wt%), Et_3N, DCM, 16 hours, 57–65%; (iv) 6 M HCl in isopropanol, MeOH, rt, 69–78%.

tive to the substitution of the aryl core of the hydroxamic acid domain (Scheme 6). More specifically, the fluoro-analogue **RG-283AG** demonstrated submicromolar (0.54 $\mu\mu$) potency for HDAC6 inhibition, thus constituting the first example of a potent, dual-acting 5-HT_6 antagonist / HDAC6 inhibitor. The fluoro-substitution in the arylhydroxamic acid domain in this scaffold increases HDAC6 inhibitory potency but not selectivity over HDAC1, with the pyridyl analogue **RG-285AG** being the most selective in that respect. This trend was also observed with other scaffolds related to HDAC6 inhibitors, although most exhibit the reverse pattern (Scheme 5).[28,48] Despite the low HDAC6/1 selectivity in this series, compounds such as **RG-283AG** could prove valuable tools in deciphering the role of HDAC1 inhibition in Alzheimer's and related disorders since there have been conflicting reports regarding the benefit of HDAC1 inhibition in these indications.[51] **RG-283AG** also possesses promising pharmacokinetic attributes such as *c*LogP, PSA, aqueous solubility, and gastrointestinal absorption based on the respective calculated values generated by the SwissADME software.[52]

Given that these new chemical entities aim at operating in the brain, the best derivative, **RG-283 AG**, was assessed for its potential to cross the blood-brain barrier. **RG-283 AG** exhibits a Chromatography Hydrophobicity Index (CHI-IAM) ≈ 34 which is within the 30–45 window typical for well-balanced blood-brain barrier-penetrant drugs (e.g., propranolol CHI ≈ 35, risperidone ≈ 38).[53] A log k_IAM ≈ 1.2 is known to translate to a Clark log BB of roughly 0.1–0.3, that is, brain concentrations comparable to, or slightly higher than, their total plasma counterparts.[54] This is consistent with the parent intepirdine scaffold for which the brain uptake in humans has been verified by PET studies. No secondary peak shoulders or tailing were observed, indicating minimal ionic interaction under the chosen ionic strength, and the closely matching k′ values across duplicate runs (<5 % RSD) support the robustness of the measurement. Taken together, the IAM data strengthen the prediction that **RG-283 AG** crosses the blood–brain barrier despite the slight deviation from the recommended TPSA value (111 vs. 90 for CNS), and hold promise that it will achieve pharmacologically relevant exposure in vivo, justify-



		5HT6 K_i	HDAc6 IC_{50}	HDAc6/1 selectivity	cLogP	PSA
RG-281AG	X= C-H	0.9 nM	4.0 μM	2	3.37	111
RG-283AG	X= C-F	0.7 nM	0.54 μM	1.5	3.67	111
RG-285AG	X= N	1.6 nM	4.1 μM	5	2.57	124

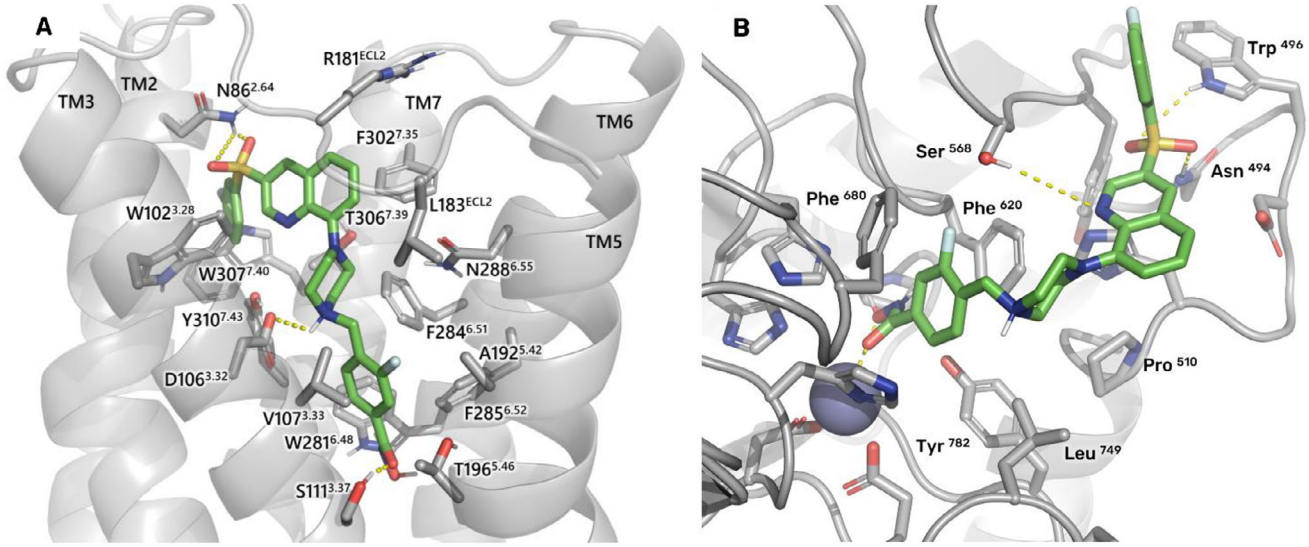
Scheme 5. Biochemical assessment and pharmacokinetic attributes of dual 5-HT_6 antagonists/HDAC6 inhibitors synthesized in this work. HDAC6/1 selectivity was determined at 10 μM for all compounds; cLog P and PSA values were generated using the SwissADME software.[52].

ing its further evaluation in cognition models and, potentially, in modifications for PET-tracer application.

1.4. Molecular Docking Studies

The most potent derivative, **RG-283AG**, was docked to the inactive state model of the 5-HT_6 receptor in order to gain a better understanding of the binding mode associated with antagonists (Scheme 6A; superscripts denote the Ballesteros–Weinstein numbering for Class A GPCRs aminoacid residues important for the serotonin-5HT6 binding, as shown by recent structural data).[55] Toward the extracellular domain of the receptor, the quinoline ring forms several hydrophobic interactions with the upper TM3, TM6, and ECL2 residues, more specifically with $W102^{3.28}$, $F302^{7.35}$, and $R181^{ECL2}$. The sulfone oxygen atoms form hydrogen bonding with the sidechain of $N86^{2.64}$ and the fluorophenyl sulfone ring appears to be engaged in π stacking interactions with $R181^{ECL2}$. As expected, the positively charged, protonated piperazine nitrogen atom forms a salt bridge with $D106^{3.32}$. The fluorophenyl group bearing the hydroxamic acid moiety is positioned in a hydrophobic space, interacting with $F284^{6.51}$ and $V107^{3.33}$, while the hydroxamic acid itself develops hydrogen bonding with $S111^{3.37}$. **RG-283AG** and related analogues **RG-281AG** and **RG-285AG** proved potent ligands for the 5-HT_6 receptor exemplifying for the first time that *N*-substitution at the piperazine terminus of intepirdine, with longer and/or larger groups than methyl, is readily accommodated without compromising its high affinity for the 5-HT_6 receptor.

Regarding the aryl-hydroxamic acid moiety, all three derivatives showed similar binding interactions with the catalytic Domain 2 of HDAC6.[56] The hydroxamic acid functionality is a well-established zinc-binding group and interacts with the zinc ion present in the binding pocket (Scheme 6B, purple sphere), while the aromatic ring (linker) participates in hydrophobic interactions with Phe^{620} and Phe^{680}. The presence of the fluorine atom in the aryl group appears to facilitate further the insertion of the aryl ring into the hydrophobic region between



Scheme 6. A) Docking of RG-283AG (green) in the A) 5-HT_6 binding site and B) in the HDAC6 Catalytic Domain 2 (PDB code 5EDU).[53] Dashed yellow lines indicate polar interactions.

Phe[620] and Phe[680] and enhance the binding of **RG-283AG**. In comparison, the des-fluoro (less hydrophobic) and pyridyl (polar/hydrophilic) analogues, **RG-281AG** and **RG-2835 G,** respectively, exhibit reduced potency with respect to HDAC6 inhibition. Regarding the positioning of 5HT6 pharmacophore domain of **RG-283AG** in the HDAC6 active site, the piperazine ring is involved in hydrophobic interactions with Pro[510] and the oxygen atoms of the sulfone group develop hydrogen bonding interactions with Trp[496] and Asn[494]. This arrangement sets up a hydrogen bonding interaction between the hydroxyl group of Ser[568] and the nitrogen atom of the quinoline ring, (Scheme 6B). This docking study supports our design hypothesis that the intepirdine 5-HT$_6$ pharmacophore is compatible as a capping unit to the generic HDAC6 inhibition pharmacophore and overall corroborates the dually active capacity of **RG-283AG**.

2. Conclusion

There is a significant lag in developing effective treatments for AD and related dementia and cognitive disorders, which continue to be unmet medical needs and impact significantly the quality of life of millions of people globally. We reasoned that the 5HT6 antagonist intepirdine that reached PhIII clinical trials could be equipped with an additional mode of action, thus leading to the next generation of cognitive enhancers with improved efficacy and suitability over a wider dementia phenomenology. In the context of the paradigm of multitargeting molecules, we decided to explore the design and synthesis of new chemical entities engaging an unprecedented combination of targets concerning the GCPR 5-HT$_6$ and the enzyme HDAC6. Both our design and synthesis were successfully implemented and delivered for the first time, multitargeting molecules possessing potent 5HT6 antagonism and HDAC6 inhibitory activity with favorable PK and BBB penetration attributes. In this effort we developed a new synthetic process for the interpirdine scaffold that supersedes that reported by GlaxoSmithKline due to its robustness, scalability, efficiency, and chromatography-free aspect. The intepirdine / 5-HT$_6$ antagonist domain, was then linked via its nucleophilic piperazine nitrogen atom to 4-(methylene)-aryl-hydroxamic acid fragments namely established pharmacophore for selective HDAC6 inhibition. Three derivatives were prepared in an overall nine-step chromatography-free synthesis allowing for late-stage variation of the aryl group. Comparison of the activities of the three compounds showed that the fluoro-substituted analogue **RG-283AG** exhibits sub-micromolar potency toward HDAC6 inhibition (IC$_{50}$ 0.54 $\mu\mu$) and nanomolar affinity (K$_i$ 0.7 nM) for the 5HT6 receptor, thus constituting the first reported dual 5-HT$_6$ antagonist/HDAC6 inhibitor. Work is ongoing to develop more potent and selective analogues over other HDACs and assess the potential of dual 5-HT$_6$ and HDAC6 inhibition in animal models of dementias and cognitive decline.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: 5HT6 antagonists · Alzheimer Disease · Buchwald-Hartwig N-arylation · cognitive decline · dementia · HDAC6 inhibitors · hydroxamic acids · intepirdine · multi-targeting directed ligands · quinoline Ullmann-type coupling · sulfide oxidation to sulfone · T3P amide synthesis

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