HHS Public Access

Author manuscript

*Bioorg Med Chem*. Author manuscript; available in PMC 2021 August 01.

Published in final edited form as:

*Bioorg Med Chem*. 2020 August 01; 28(15): 115578. doi:10.1016/j.bmc.2020.115578.

# Structural manipulation of aporphines via C10 nitrogenation leads to the identification of new 5-HT$_{7A}$R ligands

**Anupam Karki**[a,b], **Hari K. Namballa**[a], **Ian Alberts**[c], **Wayne W. Harding**[a,b,d]

[a]Department of Chemistry, Hunter College, City University of New York, 695 Park Avenue, NY 10065, USA

[b]Ph.D. Program in Biochemistry, CUNY Graduate Center, 365 5th Avenue, New York, NY 10016, USA

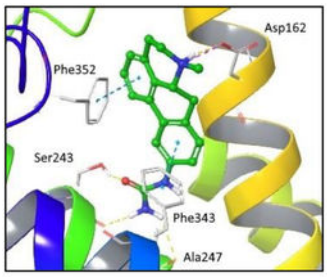
[c]LaGuardia Community College, Department of Chemistry, 31-10 Thompson Avenue, LIC, NY 11104, USA

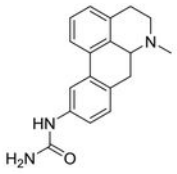
[d]Ph.D. Program in Chemistry, CUNY Graduate Center, 365 5th Avenue, New York, NY 10016, USA

## Abstract

Aporphine alkaloids containing a C10 nitrogen motif were synthesized and evaluated for affinity at 5-HT$_{1A}$R, 5-HT$_{2A}$R, 5-HT$_{6}$R and 5-HT$_{7A}$R. Three series of racemic aporphines were investigated: 1,2,10-trisubstituted, C10 *N*-monosubstituted and compounds containing a C10 benzofused aminothiazole moiety. The 1,2,10-trisubstituted series of compounds as a group displayed modest selectivity for 5-HT$_{7A}$R and also had moderate 5-HT$_{7A}$R affinity. Compounds from the C10 *N*-monosubstituted series generally lacked affinity for 5-HT$_{2A}$R and 5-HT$_{6}$R and showed strong affinity for 5-HT$_{1A}$ or 5-HT$_{7A}$R. Compounds in this series that contained an *N*6-methyl group were up to 27-fold selective for 5-HT$_{7A}$R over 5-HT$_{1A}$R, whereas compounds with an *N*6-propyl substituent showed a reversal in this selectivity. The C10 benzofused aminothiazole analogues showed a similar binding profile as the C10 *N*-monosubstituted series i.e. strong affinity for 5-HT$_{1A}$R or 5-HT$_{7A}$R, with selectivity between the two receptors being similarly influenced by *N*6-methyl or *N*6-propyl substituents. Compounds **29** and **34a** exhibit high 5-HT$_{7A}$R affinity, excellent selectivity versus dopamine receptors and function as antagonists in 5-HT$_{7A}$R cAMP-based assays. Compounds **29** and **34a** have been identified as new lead molecules for further tool and pharmaceutical optimization.

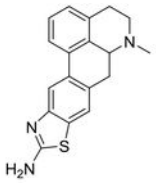
## Graphical Abstract



**43**

$K_i$ @ 5-$HT_{7A}$ = 4.5 nM
$IC_{50}$ = 1.25 μM
selective vs 5-$HT_{1A}$ and DRs



**48a**

$K_i$ @ 5-$HT_{7A}$ = 6.5 nM
$IC_{50}$ = 0.26 μM
selective vs 5-$HT_{1A}$ and DRs

**Keywords**

5-$HT_7$; 5-$HT_{1A}$; aporphine; serotonin; dopamine; CNS; SAR

## 1. Introduction

The neurotransmitter serotonin (5-HT) is involved in numerous physiological functions. Seven 5-HT receptors types are known (5-$HT_1$R – 5-$HT_7$R) of which all except 5-$HT_3$R (a ligand-gated ion channel) are metabotropic G-protein coupled receptors (GPCRs). In all, there are at least 14 5-HT receptors owing to the occurrence of sub-types for some of the receptors. The 5-$HT_7$R is the most recently described serotonergic receptor [1–3] and it is involved in various pathophysiological functions such as regulation of circadian rhythm, body temperature and sleep. [4–6] There are four known 5-$HT_7$R isoforms: 5-$HT_{7A}$R, 5-$HT_{7B}$R and 5-$HT_{7C}$R isoforms are found in rats whereas 5-$HT_{7A}$R, 5-$HT_{7B}$R and 5-$HT_{7D}$R are present in humans [7]. The 5-$HT_{7A}$R is a full-length receptor comprising 445 amino acid residues, while the 5-$HT_{7B}$R is a truncated variant with 432 amino acid residues. The 5-$HT_{7D}$R is a distinct isoform containing 479 amino acid residues. The 5-$HT_{7A}$R is the most abundant of the human isoforms. 5-$HT_7$Rs signal via $G_\alpha$s; agonist activation of 5-$HT_7$Rs leads to adenylate cyclase activation and cAMP production. [8]

5-$HT_7$R antagonists appear to be useful agents to remediate neuropsychiatric conditions including obsessive-compulsive disorder, schizophrenia, anxiety, depression and substance abuse. [9–12] Agonists of the 5-$HT_7$R are able to restore deficient locomotory activity and may be useful as therapeutics for spinal cord injury, amyotrophic lateral sclerosis and sleep apnea. The role of 5-$HT_7$R in the regulation of nociception, mitogenesis, gastrointestinal disorders, cardiopulmonary disorders and cognition has been less clear as both agonists and antagonists have shown positive outcomes in animal studies.[11]

A number of scaffolds have yielded potent 5-$HT_7$R ligands.[9] Among these are the so-called “long chain” arylpiperazines (e.g. **1**, Fig. 1), arylsulfonamides (e.g. **2**), biarylalkylamines (e.g. **3**), azepines (e.g. **4**) and aminotriazines (e.g. **5**). However, selectivity of 5-$HT_7$R ligands especially versus the closely related 5-$HT_{1A}$ receptor has been challenging. The 5-$HT_7$R antagonist JNJ-18038683 (**4**) is currently undergoing clinical trials for the treatment of cognitive impairment in bipolar disorder.[9] JNJ-1803863 is the only selective 5-$HT_7$R ligand that has been evaluated in clinical trials. The discovery of new potent and selective 5-$HT_7$R ligands promises to expand our armamentarium of useful

pharmacological tools and drugs pertinent to various neuropsychiatric disorders. Thus, the identification of new 5-$HT_7$R ligands remains an area of current interest.

Aporphine alkaloids are known to have affinity across a range of serotonergic and dopaminergic receptors. In particular, structure-affinity relationship studies on aporphines have resulted in the identification of potent and selective ligands for serotonin 5-$HT_{1A}$R [13–16] and dopamine $D_2$R [17, 18] as well as compounds with interesting multi-receptor profiles [19–22]. In contrast to their evaluation as 5-$HT_{1A}$ receptor ligands, the evaluation of aporphines as 5-$HT_7$R ligands has been little studied. In that regard, Johannson et al have described a number of atropisomeric 11-phenyl aporphines and 1,11-methylene aporphines (e.g. compounds **6** and **7**, Fig. 2) with 5-$HT_7$R receptor potency and selectivity versus the 5-$HT_{1A}$R. [23, 24] Recently we conducted an SAR study on the aporphine alkaloid *N*-methyllaurotetanine and identified a number of compounds with moderate 5-$HT_7$R affinity and selectivity. (e.g. compound **8**, Figure 2). [25]

We regard the aporphine template as a privileged scaffold for the discovery of new CNS receptor targeting agents and envisage that structural modification of the aporphine core may lead to ligands that selectively target individual or multiple CNS receptors. Although numerous SAR studies have been performed on aporphines in relation to their affinity and activity at CNS receptors, there have been no previous SAR studies examining the effect of a C-10 nitrogen substituent in this context. Thus, in this exploratory SAR study, we were curious to determine the extent to which C10 nitrogen substituents would impact affinity and selectivity of the scaffold across a range of serotonin receptors, including 5-$HT_7$R. In this manuscript we describe the synthesis and pharmacological evaluation of novel aporphines that bear a C10 nitrogen substituent group at a subset of 5-HT receptors which has led to the identification of novel selective 5-$HT_7$R ligands. In addition, we describe computational docking experiments that shed light on the observed 5-$HT_7$R affinity of the newly identified ligands.

## 2. Results and discussion

### Synthesis

As mentioned earlier in this study we sought to examine compounds that contain 1,2,10-trisubstituted and 10-substituted patterns on the racemic aporphine core. Compounds with 9,10- and 10,11- benzo-fused aminothiazole moieties were also targeted for synthesis and evaluation as these represented cyclized C10 nitrogen motifs. Additionally, we were interested in examining the impacts of *N*-methyl and *N*-propyl variations on 5-$HT_7$R affinity and selectivity of the aporphines as such *N*-substituent groups have featured prominently in the selectivity of aporphines at other CNS receptor targets. [17, 18, 26, 27]

The synthesis of compounds bearing 1,2,10-trisubstituted and 10-monosubstituted patterns required the preparation of the corresponding C10 aniline substrates. These aniline precursors were prepared as we have previously described (in a study evaluating the activity of C10 nitro-, amide- and aniline-substituted aporphine analogues at dopamine receptors). [28] Synthesis of the 1,2,10-trisubstituted analogues was accomplished by treatment of the aniline precursors **9a-e** with various carboxylic anhydrides, methanesulfonic anhydride and

potassium cyanate to give the corresponding amides (**11**-**14**), sulfonamides (**15**-**18**) and urea analogues (**19**-**21**) respectively (Scheme 1). Reductive methylation of aniline precursors gave the *N,N*-dimethylated analogues (**22**-**24**). In a similar fashion to synthesis of the 1,2,10-trisubstituted analogues, aniline precursors **10a** and **10b** were transformed into the corresponding 10-monosubstituted amide, sulfonamide, urea and *N,N*-dimethylated analogues **25–26**, **27–28**, **29–30** and **31–32** respectively.

Compounds **10a** and **10b** were reacted with potassium thiocyanate and bromine to afford the C10/C11 benzofused aminothiazole derivatives **33a** and **33b** and their respective C9/C10 regioisomers **34a** and **34b** (Scheme 2). In each case, the pair of regioisomers was present in an approximately 1:1 ratio after the reaction (as determined by $^{1}$H NMR). The tedious purification of each pair of regioisomers was accomplished by prep TLC. The 9/10 regioisomers were slightly more polar than their corresponding 10/11 congeners.

## Biological Evaluation

Table 1 summarizes data from evaluation of compounds in the 1,2,10-trisubstituted series of compounds. As a group, the analogues in Table 1 showed low affinity for the 5-HT receptors evaluated. The highest affinity observed at the 5-$HT_{1A}$R was for compound **16** (385 nM). Compounds with C1 methoxy groups in tandem with C10 methanesulfonamide or urea groups were poorly tolerated for affinity at the 5-$HT_{1A}$R (i.e. compounds **15** and **19** – no affinity in a primary assay). Compounds with a C10 butanamide group (e.g. **11**, **12b**, **13**, **14**) or a C10 methanesulfonamide group (e.g. **16**-**18**) show slightly better affinity for the 5-$HT_{1A}$R than compounds with a C10 urea motif (e.g. **19**-**21**). A similar situation ensued at the 5-$HT_{2A}$R as was evident from the lack of affinity of compounds **15** and **19** for the 5-$HT_{2A}$R. For compounds with C10 amide, sulfonamide or urea groups, C1 allyl or cyclopropylmethyl groups are better tolerated than C1 propargyloxy groups (e.g. compare **13** versus **14**; **16** versus **18**; **20** versus **21**). Whereas a C1 methyl group was not beneficial for 5-$HT_{1A}$R and 5-$HT_{2A}$R affinity, affinity for the 5-$HT_{6}$R was maintained (e.g. compound **15**, 86 nM at 5-$HT_{6}$R; no affinity at 5-$HT_{1A}$R and 5-$HT_{2A}$R). Although the affinities of the compounds for the 5-$HT_{7A}$R was moderate, in most cases individual compounds displayed higher affinity for the 5-$HT_{7A}$R than for the other receptors tested indicating selectivity of the group of compounds on a whole for the 5-$HT_{7A}$R.

Data for evaluation of the C10 monosubstituted analogues are compiled in Table 2. As a group the compounds display stronger affinity for 5-$HT_{1A}$R and 5-$HT_{7A}$R than for 5-$HT_{2A}$R and 5-$HT_{6}$R. In fact, compound **31** was the only compound in this series to display affinity for 5-$HT_{2A}$R and 5-$HT_{6}$R (this being albeit fairly weak affinity). In this series, the compounds with a C10 amide motif seem to prefer binding to 5-$HT_{1A}$R over 5-$HT_{7A}$R (e.g. **25** and **26**). The *N*-propyl-containing compound **25** showed good 5-$HT_{1A}$R affinity ($K_i$ = 21 nM) and was selective over all other 5-HT receptors tested (no affinity at other 5-HT receptors). The C10 sulfonamide-containing compound **28** which also bears an *N*-propyl group, also showed selectivity for 5-$HT_{1A}$R over 5-$HT_{7A}$R; the C10 urea-containing *N*-propyl analogue **30** similarly displayed 5-$HT_{1A}$R versus 5-$HT_{7A}$R selectivity. Overall, it appears that irrespective of the C10 functionality, an *N*-propyl group is more favored for binding to the 5-$HT_{1A}$R than 5-$HT_{7A}$R in this series (e.g. compare the *N*-propyl analogues

**25**, **26**, **28**, **30** and **32** with the *N*-methyl analogues **27**, **29** and **31**). The compound with the highest 5-$HT_{7A}$R affinity in this series was compound **29** ($K_i$ = 4.5 nM); **29** is 10- fold selective for 5-$HT_{7A}$R versus 5-$HT_{1A}$R and is among the most potent aporphinoid 5-$HT_7$R ligands identified to date.

Data for evaluation of the benzofused aminothiazole analogues is shown in Table 3. As with the 10-monosubstituted derivatives, the aminothiazole analogues showed stronger affinity for 5-$HT_{1A}$R and 5-$HT_{7A}$R than for 5-$HT_{2A}$R and 5-$HT_6$R. The compounds all lacked affinity for the 5-$HT_6$R. Another similarity in the SAR of the benzofused aminothiazole series with the C10-monosubstituted series is that an *N*6-methyl moiety confers 5-$HT_{7A}$R selectivity versus 5-$HT_{1A}$R, whereas an *N*6-propyl moiety is preferred for 5-$HT_{1A}$R selectivity. Thus, the *N*-methyl analogues **33a** and **34a** are approximately 19- and 4-fold selective respectively for 5-$HT_{7A}$R, whereas the *N*-propyl analogues **33b** and **34b** are roughly 2-and 17-fold more selective respectively for the 5-$HT_{1A}$R.

The compounds with the highest 5-$HT_{7A}$R and 5-$HT_{1A}$ affinity (**29**, **33a**, **34a** and **34b**) were further profiled for affinity at dopamine receptors ($D_1$R - $D_5$R). Table 4 shows these results. Compound **29** lacked affinity for all receptors except $D_3$R, where low affinity was seen ($K_i$ = 2460 nM). Compound **33a** showed low affinity for $D_1$R, $D_2$R, $D_3$R and $D_4$R ($K_i$ = 1150, 1740, 381 and 481 nM respectively) and had no affinity for $D_5$R. Compound **34a** lacked affinity for all dopamine receptors. Similar dopamine receptor binding studies on the 5-$HT_{1A}$R selective compound **34b** revealed a lack of affinity for $D_1$R, $D_2$R, $D_4$R and $D_5$R; low affinity ($K_i$ = 2110 nM) was observed for $D_3$R

Compounds **29** and **34a** were evaluated for 5-$HT_7$R functional activity using Hit Hunter® agonist and antagonist cAMP assays at Eurofins Pharma Discovery Services. Data from these assays is provided in Table 5. Both compounds were found to be 5-$HT_7$R antagonists with $EC_{50}$ values of 0.125 and 0.26 μM respectively for **29** and **34a**, which was comparable to the positive control spiperone; the compounds lacked agonist activity in this assay.

## Docking studies

Computational docking simulations were performed in order to rationalize the high measured 5-$HT_7$R binding affinities for compounds **29**, **33a** and **34a**. In this context, we investigated the docked ligand poses and identified key receptor-ligand interactions that influence binding to the 5-$HT_7$R and provide a deeper appreciation of the observed high affinity towards this receptor system. A homology model of the 5-$HT_7$R was generated from the high-resolution crystal structure of the human serotonin 5-$HT_{1B}$ G protein-coupled receptor with pdb code 4IAQ [29]. This approach involved utilization of the Schrödinger Prime Structure Prediction and Glide software modules and manual intervention to support the generation of known key receptor-ligand interactions. The model for the 5-$HT_7$R structure, therefore, comprised suitable backbone and side-chain orientations within the binding site. The docking simulations of compounds **29**, **33a** and **34a** into the 5-$HT_7$R binding site exploited the Schrödinger Induced Fit and Glide methodologies in Standard Precision (SP) mode. Using this docking protocol, the Glidescore scoring function was used to give an estimate of the ligand binding affinities for the highest ranked poses of

compounds **29**, **33a** and **34a** in the 5-HT$_7$R target. The ligand binding poses are depicted in Figure 3.

Compounds **29** and **34a** give very similar binding poses in the serotonin 5-HT$_7$R binding pocket as shown in Figures 3A and C. The bound structures with these ligands comprise the key salt bridge between the quaternary N atom and Asp162, hydrogen bonding interactions to the Ser243 side chain and Ala247 backbone and π-stacking interactions between the ligand aromatic rings and receptor Phe343 and Phe352 residues. Compound **34a** gives a better predicted binding energy (−10.0 kcal/mol) compared to **29** (−8.9 kcal/mol), due to a slightly stronger protonated N-Asp salt bridge and better π-π interactions involving the fused thiazole ring in **34a**.

For compound **33a**, in order to accommodate the modified position of the fused thiazole ring, the best binding pose identified by induced fit docking involves a refined 5-HT7 receptor structure with a rotated Asp162 side-chain orientation. As depicted in Figure 3B, key receptor-ligand interactions include the quaternary N – Asp162 salt bridge, H-bonds to Ser234 and Ser243 and a π-cation interaction between the same quaternary N and Phe352. As a consequence of the modified binding pose, the binding energy for compound **33a** (−8.7 kcal/mol) is worse than that for **34a** (−10.0kcal/mol) primarily due to the lack of complementary π-π interactions involving the ligand aromatic rings in **33a**.

## 3. Conclusions

In this study, we sought to evaluate the extent to which C10 nitrogen substituents on the aporphine core impact affinity and selectivity across a range of 5-HT receptors, including 5-HT$_{7A}$R. Aporphines with 1,2,10-trisubstituted and 10-substituted substitution patterns as well as ring D aminothiazole moieties were investigated.

We found that affinities of the 1,2,10-trisubstituted analogues across the four receptors evaluated (5-HT$_{1A}$R, 5-HT$_{2A}$R, 5-HT$_6$R and 5-HT$_{7A}$R) was low to moderate; C10 *N*-substitution here did not result in pronounced high affinity and selectivity for any of the 5-HT receptors, although there was generally a slight preference for 5-HT$_7$R binding.

In contrast, the C10 monosubstituted group of compounds as well as the benzofused aminothiazole group of compounds were found to selectively target 5-HT$_{1A}$R and 5-HT$_{7A}$R and largely lacked affinity for 5-HT$_{2A}$R and 5-HT$_6$R. Taken together with the results from the trisubstituted series, it would appear that C1 and C2 alkoxy substituents on the aporphine core are not required for affinity to 5-HT$_{7A}$R and 5-HT$_{1A}$R. Furthermore, the SAR results indicate that in general, an *N*6-methyl substituent engenders 5-HT$_{7A}$R selectivity over 5-HT$_{1A}$R whereas this selectivity is reversed with an *N*6-propyl substituent.

Overall, our study provides valuable SAR information by revealing that the inclusion of a C10 nitrogen substituent group on the ring A unsubstituted aporphine core, allows for selective binding of the scaffold to 5-HT$_7$R and 5-HT$_{1A}$R. Compounds **29** and **34a** were found to function as 5-HT$_7$R antagonists in assays that measured cAMP production. These compounds do not display any appreciable affinity for dopamine receptors, have moderate selectivity versus 5-HT$_{1A}$R and represent good starting points from which to further

optimize C10 nitrogenated aporphines as selective 5-$HT_7$R antagonists. The impact of the configuration of the chiral center of the aporphines on affinity and selectivity will need to be investigated in future. Optimization of the ligands for 5-$HT_7$R affinity is expected to be challenging given the 5-$HT_{1A}$R affinity of the series, but is nevertheless a promising dimension for future studies.

## 4. Experimental

### 4.1 Synthetic experimental procedures

**General Procedures:** All glass apparatus was oven-dried prior to use. HRESIMS spectra were obtained using an Agilent 6520 Q-TOF instrument. $^1$H NMR and $^{13}$C NMR spectra were recorded using a Bruker DPX-500 spectrometer (operating at 500 and 600 MHz for $^1$H; 125 and 150 MHz, respectively, for $^{13}$C) using $CDCl_3$ as solvent. Tetramethylsilane (δ 0.00 ppm) served as an internal standard in $^1$H NMR and $^{13}$C NMR unless stated otherwise. Chemical shift (δ 0.00 ppm) values are reported in parts per million and coupling constants in Hertz (Hz). Splitting patterns are described as singlet (s), doublet (d), triplet (t), and multiplet (m). Reactions were monitored by TLC with Whatman Flexible TLC silica gel G/UV 254 precoated plates (0.25 mm). TLC plates were visualized in UV light (254 nm) and by staining with phosphomolybdate spray reagent, vanillin or iodine. Flash column chromatography was performed with silica gel 60 (EMD Chemicals, 230–400 mesh, 0.04–0.063 mm particle size). Preparative thin layer chromatography was performed with silica gel GF plates (Analtech, catalog # 02003). All chemicals and reagents were obtained from Sigma-Aldrich and Fischer Scientific (USA) in reagent grade and were used without further purification. Yields reported are after purification.

**General procedure for synthesis of amide analogues:** To a stirred solution of the corresponding aniline (0.2 mmol) in DCM (10 mL) was added the respective anhydride (0.4 mmol) and the resulting reaction mixture was stirred at rt for 2 h under nitrogen atmosphere. After completion of the reaction (monitored by TLC) the reaction mixture was concentrated *in-vacuo* and a solution of NaOH (0.2 g) in methanol (10 mL) was added and stirred for another 30 min at the same temperature. The reaction mixture was concentrated *in-vacuo* and the obtained crude mass was dissolved in water and extracted with DCM (3×10 mL). The organic layer was dried over anhydrous $Na_2SO_4$, filtered and concentrated *in-vacuo* to give crude product, which was purified by preparative TLC purification eluting in 5% MeOH/DCM.

**N-(1,2-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)butyramide (11):** 37% yield. Yellow oil. $^1$H NMR (600 MHz, $CDCl_3$): δ 8.25 (d, $J$ = 8.6 Hz, 1H), 7.66 (s, 1H), 7.28 (s, 1H), 7.18 – 7.13 (m, 1H), 6.54 (s, 1H), 3.81 (s, 3H), 3.56 (s, 3H), 3.12 – 3.05 (m, 1H), 3.02 (m, 1H), 2.99 – 2.92 (m, 2H), 2.61 (m, 1H), 2.54 (m, 1H), 2.48 – 2.41 (m, 4H), 2.29 (t, $J$ = 7.4 Hz, 2H), 1.74 – 1.67 (m, 2H), 0.95 (t, $J$ = 7.4 Hz, 3H) ppm. $^{13}$C NMR (150 MHz, $CDCl_3$): δ 171.4, 152.0, 144.8, 137.6, 136.9, 128.9, 128.8, 128.1, 127.5, 126.6, 119.0, 117.9, 110.8, 62.2, 60.2, 55.8, 53.3, 44.0, 39.8, 35.2, 29.2, 19.2, 13.8 ppm. HRESIMS *m/z* 381.2173 $[M+H]^+$ (calcd. for $C_{23}H_{28}N_2O_3$, 381.2177)

**N-(1-(cyclopropylmethoxy)-2-methoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)acetamide (12a).:** 48% yield. Dark yellow oil. $^{1}$H NMR (600 MHz, $CDCl_3$): δ 8.29 (d, *J* = 8.6 Hz, 1H), 7.62 – 7.51 (m, 1H), 7.45 (s, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 6.45 (s, 1H), 3.72 (s, 3H), 3.55 (m, 1H), 3.25 (m, 1H), 3.18 – 3.07 (m, 2H), 3.07 – 2.99 (m, 1H), 2.98 – 2.91 (m, 1H), 2.64 – 2.52 (m, 3H), 2.48 (s, 3H), 2.05 (s, 3H), 0.98 (m, 1H), 0.34 – 0.27 (m, 2H), 0.03 – −0.07 (m, 2H) ppm.$^{13}$C NMR (125 MHz, $CDCl_3$): δ 168.3, 152.4, 143.9, 136.9, 136.9, 129.6, 128.4, 128.4, 128.0, 127.1, 118.9, 117.8, 110.8, 77.8, 62.2, 55.8, 53.2, 43.4, 34.8, 29.7, 24.8, 11.0, 3.4, 3.1 ppm. HRESIMS *m/z* 393.2179 $[M+H]^+$ (calcd. for $C_{24}H_{28}N_2O_3$, 393.2170)

**N-(1-(cyclopropylmethoxy)-2-methoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)butyramide (12b):** 40% yield. White solid. Melting point: 89.1 °C – 90.1 °C. $^{1}$H NMR (500 MHz, $CDCl_3$): δ 8.31 (d, *J* = 8.6 Hz, 1H), 7.57 (s, 1H), 7.20 (s, 1H), 7.11 (dd, *J* = 8.5, 1.8 Hz, 1H), 6.46 (s, 1H), 3.72 (s, 3H), 3.56 (m, 1H), 3.25 (m, 1H), 3.08 – 2.98 (m, 1H), 2.98 – 2.87 (m, 3H), 2.55 (m, 1H), 2.48 (m, 1H), 2.43 – 2.36 (m, 4H), 2.23 (t, *J* = 7.4 Hz, 2H), 1.65 (m, 2H), 1.03 – 0.94 (m, 1H), 0.89 (t, *J* = 7.4 Hz, 3H), 0.30 (m, 2H), −0.02 (m, 2H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 171.4, 152.1, 143.7, 137.4, 136.8, 129.6, 128.5, 128.4, 127.2, 127.1, 118.9, 117.6, 110.8, 77.8, 62.2, 55.8, 53.3, 43.9, 39.8, 35.2, 29.1, 19.1, 13.8, 11.0, 3.4, 3.0 ppm. HRESIMS *m/z* 421.2490. $[M+H]^+$ (calcd. for $C_{26}H_{33}N_2O_3$, 421.2486)

**N-(1-(allyloxy)-2-methoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)butyramide (13):** 55% yield. Brown solid. Melting point: 99.1 °C – 99.6 °C. $^{1}$H NMR (500 MHz, $CDCl_3$): δ 8.27 (d, *J* = 8.5 Hz, 1H), 7.65 (s, 1H), 7.18–7.10 (m, 2H), 6.53 (s, 1H), 5.88 (m, 1H), 5.16 (m, 1H), 5.05 (m, 1H), 4.28 (m, 1H), 4.09 (m, 1H), 3.79 (s, 3H), 3.10 (s, 1H), 3.05 – 2.93 (m, 3H), 2.61 (m, 1H), 2.54 (m, 1H), 2.45 (m, 4H), 2.29 (t, *J* = 7.4 Hz, 2H), 1.71 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm. $^{13}$C NMR (150 MHz, $CDCl_3$) δ 171.3, 152.1, 143.3, 137.5, 136.9, 134.2, 129.3, 128.7, 128.7, 128.2, 127.0, 118.9, 117.6, 117.5, 110.8, 73.7, 62.2, 55.9, 53.3, 43.9, 39.8, 35.1, 29.1, 19.2, 13.8 ppm. HRESIMS *m/z* 407.2329 $[M+H]^+$ (calcd. for $C_{25}H_{30}N_2O_3$, 407.2177)

**N-(2-methoxy-6-methyl-1-(prop-2-yn-1-yloxy)-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)butyramide (14):** 52% yield. Yellow oil. $^{1}$H NMR (500 MHz, $CDCl_3$): δ 8.27 (d, *J* = 8.6 Hz, 1H), 7.67 (s, 1H), 7.19 (s, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.54 (s, 1H), 4.46 (dd, *J* = 15.0, 2.3 Hz, 1H), 4.33 (dd, *J* = 15.0, 2.4 Hz, 1H), 3.80 (s, 3H), 3.16 – 3.06 (m, 1H), 3.05 – 2.95 (m, 3H), 2.62 (m, 1H), 2.55 (m, 1H), 2.51 – 2.43 (m, 4H), 2.31 – 2.25 (m, 3H), 1.72 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 171.3, 152.0, 142.3, 137.5, 137.1, 129.4, 128.0, 127.3, 127.2, 118.9, 117.8, 110.9, 79.2, 74.9, 62.1, 59.6, 55.9, 53.2, 43.8, 39.8, 35.1, 29.0, 19.1, 13.8 ppm. HRESIMS *m/z* 405.2173 $[M+H]^+$ (calcd. for $C_{25}H_{28}N_2O_3$, 405.2177)

**N-(6-propyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)acetamide (25):** 73% yield. Off-white solid. Melting point: 99.8 °C – 100.3 °C. $^{1}$H NMR (600 MHz, $CDCl_3$): δ 7.56 (d, *J* = 8.4 Hz, 1H), 7.51 (s, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.30 (m, 1H), 7.22 (t, *J* = 11.5 Hz, 1H), 7.15 (t, *J* = 7.6 Hz, 1H), 6.98 (d, *J* = 7.5 Hz, 1H), 3.44 (m, 1H), 3.18 – 3.09

(m, 2H), 3.07 (m, 1H), 2.87 (m, 1H), 2.72 – 2.67 (m, 1H), 2.63 (m, 1H), 2.52 – 2.40 (m, 2H), 2.11 (s, 3H), 1.63 – 1.48 (m, 2H), 0.90 (t, $J$ = 7.4 Hz, 3H) ppm.$^{13}$C NMR (125 MHz, $CDCl_3$): δ 168.4, 137.3, 136.1, 133.6, 133.3, 130.4, 127.7, 127.0, 124.3, 121.6, 119.5, 119.4, 118.7, 59.3, 56.2, 49.4, 33.9, 28.9, 24.7, 18.9, 12.0 ppm. HRESIMS *m/z* 329.1964 $[M+H]^+$ (calcd. for $C_{21}H_{25}N_2O$, 321.1961)

**N-(6-propyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)butyramide (26):** 50% yield. Pale yellow solid. Melting point: 104.9 °C – 106.5 °C. $^1$H NMR (600 MHz, $CDCl_3$): δ 7.66 (d, $J$ = 7.4 Hz, 2H), 7.51 (d, $J$ = 7.6 Hz, 1H), 7.34 (d, $J$ = 7.0 Hz, 1H), 7.25 (t, $J$ = 7.6 Hz, 1H), 7.07 (d, $J$ = 7.4 Hz, 1H), 3.61 – 3.50 (m, 1H), 3.31 – 3.15 (m, 3H), 3.01 – 2.92 (m, 1H), 2.84 – 2.71 (m, 2H), 2.66 – 2.52 (m, 2H), 2.37 (t, $J$ = 7.4 Hz, 2H), 1.80 (m, 2H), 1.74 – 1.59 (m, 2H), 1.05 (t, $J$ = 7.3 Hz, 3H), 1.00 (t, $J$ = 7.3 Hz, 3H) ppm.$^{13}$C NMR (150 MHz, $CDCl_3$): δ 171.5, 137.3, 136.2, 133.5, 133.2, 133.1, 130.4, 127.7, 126.9, 124.4, 121.5, 119.6, 118.6, 61.9, 53.5, 53.4, 43.9, 39.7, 34.2, 29.7, 28.9, 19.1, 13.8 ppm. HRESIMS *m/z* 349.2279 $[M+H]^+$ (calcd. for $C_{23}H_{29}N_20$, 349.2274)

**General procedure for preparation of methanesulfonamide analogues:** To a stirred solution of corresponding aniline (0.1 mmol) in DCM (10 mL), methanesulfonic anhydride (0.25 mmol) and triethylamine (0.3 mmol) were added. The resulting mixture was stirred at rt for 12h under nitrogen atmosphere. After completion of the reaction, the reaction mixture was concentrated *in-vacuo* and a solution of NaOH (0.2 g) in methanol (10 mL) was added to the flask and stirring was continued for another 30 min. The reaction mixture was concentrated *in-vacuo*, dissolved in water and extracted with DCM (3×10 mL). The organic layer was dried over anhydrous $Na_2SO_4$, filtered and concentrated *in-vacuo*. The crude product thus obtained was purified using preparative TLC eluting in 5% MeOH/DCM to give respective sulfonamide analogues.

**N-(1,2-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)methanesulfonamide (15):** 45% yield. Off-white solid. Melting point: 153.0 °C – 154.6 °C. $^1$H NMR (600 MHz, $CDCl_3$): δ 8.26 (d, $J$ = 8.5 Hz, 1H), 7.09 (s, 1H), 7.05 – 7.02 (m, 1H), 6.55 (s, 1H), 3.80 (s, 3H), 3.59 (s, 3H), 3.13 – 3.04 (m, 1H), 3.03 – 2.93 (m, 6H), 2.64 – 2.59 (m, 1H), 2.52 (m, 1H), 2.48 – 2.41 (m, 4H) ppm. $^{13}$C NMR (150 MHz, $CDCl_3$): δ 152.0, 144.9, 138.3, 135.6, 129.7, 129.3, 128.8, 127.3, 126.1, 119.6, 118.7, 111.3, 62.0, 60.2, 55.8, 53.2, 43.9, 39.6, 35.1, 29.1 ppm. HRESIMS *m/z* 389.1529 $[M+H]^+$ (calcd. for $C_{20}H_{25}N_2O_4S$, 389.4895)

**N-(1-(cyclopropylmethoxy)-2-methoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)methanesulfonamide (16):** 46% yield. Brown solid. Melting point: 140.3 °C – 141.5 °C. $^1$H NMR (500 MHz, $CDCl_3$): δ 8.41 (d, $J$ = 8.5 Hz, 1H), 7.18 (s, 1H), 7.08 (s, 1H), 7.03 (d, $J$ = 7.0 Hz, 1H), 6.53 (s, 1H), 3.78 (s, 3H), 3.63 (m, 1H), 3.34 (m, 1H), 3.16 – 3.06 (m, 2H), 3.06 – 2.97 (m, 5H), 2.63 (m, 2H), 2.58 – 2.44 (m, 4H), 1.08 – 0.99 (m, 1H), 0.37 (d, $J$ = 8.1 Hz, 2H), 0.10 – −0.03 (m, 2H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$) δ 152.2, 143.8, 137.9, 135.4, 130.4, 129.7, 128.7, 128.1, 126.6, 119.4, 118.4, 111.1, 77.9, 62.0, 55.8, 53.5, 53.2, 39.6, 35.0, 11.0, 3.4, 3.1 ppm.

**N-(1-(allyloxy)-2-methoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)methanesulfonamide (17):** 33% yield. Light-yellow oil. $^{1}$H NMR (500 MHz, $CDCl_3$): δ 8.30 (d, *J* = 8.5 Hz, 1H), 7.10 (d, *J* = 2.0 Hz, 1H), 7.01 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.55 (s, 1H), 5.88 (ddt, *J* = 16.4, 10.4, 5.9 Hz, 1H), 5.17 (dd, *J* = 17.2, 1.5 Hz, 1H), 5.06 (dd, *J* = 10.4, 1.2 Hz, 1H), 4.31 (dd, *J* = 12.0, 5.9 Hz, 1H), 4.12 (dd, *J* = 12.0, 5.9 Hz, 1H), 3.80 (s, 3H), 3.14 – 3.07 (m, 1H), 3.07 – 2.95 (m, 6H), 2.63 (dd, *J* = 16.3, 3.1 Hz, 1H), 2.55 – 2.44 (m, 4H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 152.2, 143.5, 138.2, 135.5, 134.2, 130.1, 129.4, 128.8, 127.1, 126.5, 119.4, 118.4, 117.6, 111.3, 73.8, 62.0, 55.9, 53.2, 43.8, 39.6, 35.0, 29.0 ppm. HRESIMS *m/z* 415.1690 [M+H]+ (calcd. for $C_{22}H_{26}N_2O_4S$, 415.1686)

**N-(2-methoxy-6-methyl-1-(prop-2-yn-1-yloxy)-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)methanesulfonamide (18):** 33% yield. Yellow oil. $^{1}$H NMR (500 MHz, $CDCl_3$): δ 8.29 (d, *J* = 8.5 Hz, 1H), 7.10 (d, *J* = 1.9 Hz, 1H), 7.05 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.55 (s, 1H), 4.50 (m, 1H), 4.37 (m, 1H), 3.80 (s, 3H), 3.12 – 3.04 (m, 1H), 3.02 – 2.92 (m, 6H), 2.62 (m, 1H), 2.49 (m, 5H), 2.26 (t, *J* = 2.4 Hz, 1H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 152.0, 142.3, 138.3, 135.7, 130.1, 129.6, 129.3, 127.4, 126.9, 119.5, 118.7, 111.2, 79.1, 75.01, 62.0, 59.8, 55.9, 53.2, 44.0, 39.5, 35.1, 29.1 ppm. HRESIMS *m/z* 413.1532 [M+H]+ (calcd. for $C_{22}H_{24}N_2O_4S$, 413.1530)

**N-(6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)methanesulfonamide (27):** 48% yield. Light brown oil. $^{1}$H NMR (500 MHz, $CDCl_3$): δ 7.61 (d, *J* = 8.1 Hz, 1H), 7.44 (d, *J* = 7.7 Hz, 1H), 7.21 – 7.17 (m, 1H), 7.12 – 7.07 (m, *J* = 8.5 Hz, 2H), 7.02 (d, *J* = 7.6 Hz, 1H), 3.18 (m, 2H), 3.14 – 3.02 (m, 2H), 2.99 (s, 3H), 2.74 – 2.67 (m, 1H), 2.63 (m, 1H), 2.56 – 2.47 (m, 4H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 135.9, 135.0, 132.6, 132.1, 131.7, 130.6, 127.2, 126.2, 124.1, 120.7, 119.5, 118.6, 60.8, 52.4, 42.9, 38.5, 33.0, 27.8 ppm. HRESIMS *m/z* 329.1322 $[M+H]^{1+}$ (calcd. for $C_{18}H_{21}N_2O_2S$, 329.1318)

**N-(6-propyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)methanesulfonamide (28):** 38% yield. Dark brown oil. $^{1}$H NMR (500 MHz, $CDCl_3$): δ 7.61 (d, *J* = 8.3 Hz, 1H), 7.42 (d, *J* = 7.7 Hz, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.10 (s, 1H), 7.07 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 1H), 3.39 (dd, *J* = 14.0, 3.8 Hz, 1H), 3.17 – 3.05 (m, 3H), 3.00 (s, 3H), 2.86 (m, 1H), 2.69 (m, 1H), 2.59 (m, 1H), 2.47 – 2.35 (m, 2H), 1.63 – 1.48 (m, 2H), 0.91 (t, *J* = 7.4 Hz, 3H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 136.2, 134.9, 133.0, 131.9, 130.9, 127.7, 127.2, 126.0, 124.1, 120.7, 119.4, 118.6, 58.2, 55.4, 48.4, 38.5, 33.2, 28.1, 18.3, 11.1 ppm. HRESIMS *m/z* 357.1636 $[M+H]^{+}$ (calcd. for $C_{20}H_{25}N_2O_2S$, 357.1631)

**General procedure for synthesis of urea analogues:** To a magnetically stirred solution of amine (100 mg, 1 equiv.) in 10% acetic acid solution (10 mL) (v/v), a solution of potassium cyanate (2 equiv.) in water (5 mL) was added. The reaction was stirred at rt for 2h. After completion of the reaction (monitored by TLC), the reaction was quenched with saturated sodium bicarbonate and extracted with EtOAc (3×15 mL). The combined organic layer was dried over $Na_2SO_4$, filtered, concentrated under reduced pressure and the crude product thus obtained was purified using preparative TLC, eluting in 5% MeOH/DCM to give the respective urea analogues.

**1-(1,2-dimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)urea(19):** 40 % yield. Brown solid. Melting point: 208 °C – 209 °C. $^{1}$H NMR (600 MHz, $CDCl_3$): δ 8.15 (d, *J* = 8.5 Hz, 1H), 7.53 (s, 1H), 7.22 (s, 1H), 7.13 (d, *J* = 8.3 Hz, 1H), 6.50 (s, 1H), 5.12 (bs, 2H), 3.77 (s, 3H), 3.53 (s, 3H), 3.15 – 3.06 (m, 1H), 3.00 (m, 2H), 2.93 (m, 1H), 2.59 (m, 1H), 2.53 (m, 1H), 2.45 (m, 4H). $^{13}$C NMR (150 MHz, $CDCl_3$): δ 156.8, 152.2, 144.8, 137.9, 137.1, 129.1, 128.3, 127.2, 126.6, 126.3, 119.5, 118.8, 110.8, 62.0, 60.2, 55.8, 53.0, 43.3, 34.7, 28.5 ppm. HRESIMS *m/z* 354.1811 [M+H]+ (calcd. for $C_{20}H_{24}N_3O_3$, 354.4295)

**1-(1-(cyclopropylmethoxy)-2-methoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)urea (20):** 30 % yield. Brown oil. $^{1}$H NMR (600 MHz, $CDCl_3$): δ 8.26 (d, *J* = 8.5 Hz, 1H), 8.22 (s, 1H), 7.28 – 7.22 (m, 1H), 6.46 (s, 1H), 5.48 (bs, 2H), 3.75 (s, 3H), 3.57 – 3.52 (m, 1H), 3.34 – 3.28 (m, 1H), 3.22 – 3.08 (m, 3H), 2.94 (m, 1H), 2.62 (m, 3H), 2.54 (s, 3H), 1.01 (m, 1H), 0.34 (d, *J* = 6.5 Hz, 2H), 0.07 – −0.04 (m, 2H) ppm. $^{13}$C NMR (150 MHz, $CDCl_3$): δ 157.1, 152.6, 143.7, 138.49, 136.1, 129.5, 128.6, 127.2, 126.6, 124.6, 118.7, 118.0, 110.4, 77.8, 61.9, 55.8, 52.8, 42.4, 34.1, 27.7, 11.0, 3.4, 3.2 ppm. HRESIMS *m/z* 394.2123 $[M+H]^+$ (calcd. for $C_{23}H_{28}N_3O_3$, 394.4945).

**1-(2-methoxy-6-methyl-1-(prop-2-yn-1-yloxy)-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)urea (21):** 30 % yield. Light brown solid. Melting point: 172.4 – 172.8 °C. $^{1}$H NMR (600 MHz, Acetone): δ 8.25 (d, *J* = 8.6 Hz, 1H), 8.09 (s, 1H), 7.55 (s, 1H), 7.32 (d, *J* = 8.3 Hz, 1H), 6.70 (s, 1H), 5.44 (bs, 2H), 4.60 (m, 1H), 4.44 (m, 1H), 3.86 (s, 3H), 3.08 – 3.04 (m, 2H), 2.98 (m, 1H), 2.87 – 2.83 (m, 2H), 2.64 (m, 1H), 2.47 (s, 3H), 2.41 (m, 2H) ppm. $^{13}$C NMR (150 MHz, Acetone): δ 155.7, 152.1, 142.1, 139.9, 137.5, 129.6, 128.9, 127.7, 127.4, 125.6, 117.3, 116.1, 110.8, 79.5, 75.3, 62.6, 59.6, 59.1, 55.3, 53.1, 43.4, 35.3 ppm. HRESIMS *m/z* 378.1819 [M+H]+ (calcd. for $C_{22}H_{24}N_3O_3$, 378.4515).

**1-(6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)urea (29):** 83% yield. White solid. Melting point: 125.6 °C – 126.9 °C. $^{1}$H NMR (500 MHz, $CDCl_3$): δ 7.56 (d, *J* = 8.2 Hz, 1H), 7.42 (d, *J* = 7.5 Hz, 1H), 7.34 (bs, 1H), 7.26 (s, 1H), 7.20 (m, 2H), 7.04 (d, *J* = 7.5 Hz, 1H), 5.12 (bs, 2H), 3.25 – 3.16 (m, 2H), 3.08 (m, 2H), 2.74 (m, 1H), 2.65 (m, 1H), 2.54 (m, 4H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 156.7, 137.9, 136.0, 133.1, 133.0, 132.5, 130.0, 127.7, 127.2, 124.6, 121.5, 120.6, 119.9, 61.7, 53.3, 43.5, 33.8, 28.6 ppm. HRESIMS *m/z* 294.1604 $[M+H]^+$ (calcd. for $C_{18}H_{19}N_3O$, 294.1601)

**1-(6-propyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-yl)urea (30):** 86% yield. Brown Solid. Melting point: 114.5 °C – 114.9 °C. $^{1}$H NMR (600 MHz, Acetone-$d_6$) δ 8.26 (dd, *J* = 2.2, 1.0 Hz, 1H), 8.20 (ddd, *J* = 8.6, 2.5, 0.9 Hz, 1H), 8.05 (d, *J* = 8.6 Hz, 1H), 7.75 (d, *J* = 7.7 Hz, 1H), 7.34 (t, *J* = 7.7 Hz, 1H), 7.23 (d, *J* = 7.6 Hz, 1H), 3.60 – 3.55 (m, 1H), 3.44 (m, 1H), 3.24 (m, 1H), 3.08 (m, 1H), 3.02 (m, 1H), 2.90 (bs, 2H), 2.79 (m, 1H), 2.62 (m, 1H), 2.50 – 2.42 (m, 2H), 1.69 – 1.56 (m, 2H), 0.99 (t, *J* = 7.4 Hz, 3H) ppm. $^{13}$C NMR (125 MHz, Acetone-$d_6$): δ 147.7, 141.8, 138.4, 136.5, 135.6, 132.5, 130.8, 128.0, 125.6, 124.3, 123.9, 123.3, 59.9, 56.8, 50.0, 34.6, 30.1, 20.5, 12.2 ppm. HRESIMS *m/z* 322.1922 $[M+H]^+$ (calcd. for $C_{20}H_{24}N_3O$, 322.4315)

**General procedure for synthesis of N,N-dimethylated analogues:** To a stirred solution of the corresponding aniline (0.1 mmol) in DCM (10 mL) was added formaldehyde (37% aqueous solution, 5 μL, 0.1 mmol) and the reaction mixture was stirred at room temperature for 1h. $NaBH(OAc)_3$ (200 mg, 1 mmol) was added and the resulting reaction mixture was stirred at rt for 12h. After completion of the reaction (monitored by TLC) the reaction mixture was quenched with saturated sodium bicarbonate solution and extracted with DCM (3×15 mL). The combined organic layer was dried over $Na_2SO_4$, filtered and concentrated *in-vacuo*. The residue thus obtained was purified using preparative TLC eluting in 5% MeOH/DCM to give the *N*-methylated analogues.

**1,2-dimethoxy-N,N,6-trimethyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-amine (22):** 30% yield. Light purple solid. Melting point: 150.1 °C – 150.9 °C. $^1$H NMR (600 MHz, $CDCl_3$): δ 8.20 (d, *J* = 8.8 Hz, 1H), 6.62 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.54 (d, *J* = 2.5 Hz, 1H), 6.46 (s, 1H), 3.80 (s, 3H), 3.58 (s, 3H), 3.15 – 3.07 (m, 1H), 3.05 – 2.95 (m, d H), 2.94 (s, 6H), 2.65 – 2.57 (m, 2H), 2.50–2.52 (m, 4H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 152.0, 149.6, 144.2, 137.7, 129.4, 128.5, 127.5, 127.0, 120.6, 111.3, 110.9, 109.5, 62.6, 60.0, 55.8, 53.4, 44.1, 40.4, 35.9, 29.3 ppm. HRESIMS *m/z* 339.2065 [M+H]+ (calcd. for $C_{21}H_{27}N_2O_2$, 339.2067)

**1-(cyclopropylmethoxy)-2-methoxy-N,N,6-trimethyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-amine (23):** 40% yield. Orange oil. $^1$H NMR (500 MHz, $CDCl_3$): δ 8.24 (d, *J* = 8.8 Hz, 1H), 6.53 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.44 (d, *J* = 2.6 Hz, 1H), 6.35 (s, 1H), 3.69 (s, 3H), 3.48 (m, 1H), 3.27 (m, 1H), 3.03 – 2.96 (m, 1H), 2.90 – 2.86 (m, 3H), 2.84 (s, 6H), 2.53 – 2.45 (m, 2H), 2.39 (s, 3H), 2.39 – 2.33 (m, 1H), 1.07 – 0.98 (m, 1H), 0.34 – 0.27 (m, 2H), 0.03 – −0.04 (m, 2H) ppm. $^{13}$C NMR (150 MHz, $CDCl_3$): δ 152.1, 149.5, 143.1, 137.5, 129.9, 128.3, 127.9, 126.9, 121.0, 111.2, 110.7, 109.5, 77.4, 62.6, 55.8, 53.4, 44.0, 40.4, 35.9, 29.2, 11.1, 3.3, 3.1 ppm. HRESIMS *m/z* 379.2378 [M+H] + (calcd. for $C_{24}H_{31}N_2O_2$, 379.2380)

**1-(allyloxy)-2-methoxy-N,N,6-trimethyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-amine (24):** 48% yield. Brown oil. $^1$H NMR (600 MHz, $CDCl_3$): δ 8.22 (d, *J* = 8.8 Hz, 1H), 6.59 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.53 (d, *J* = 2.2 Hz, 1H), 6.45 (s, 1H), 5.94 (ddt, *J* = 10.7, 5.8 Hz, 1H), 5.19 (dd, *J* = 17.2, 1.4 Hz, 1H), 5.06 (d, *J* = 10.4 Hz, 1H), 4.25 (dd, *J* = 12.0, 5.9 Hz, 1H), 4.14 (dd, *J* = 12.0, 5.7 Hz, 1H), 3.78 (s, 3H), 3.14 – 3.06 (m, 1H), 3.03 – 2.95 (m, 3H), 2.92 (s, 6H), 2.58 – 2.62 (m, 2H), 2.48 (m, 4H) ppm. $^{13}$C NMR (150 MHz, $CDCl_3$): δ 152.2, 149.6, 142.9, 137.4, 134.6, 129.7, 128.3, 127.9, 126.6, 120.7, 117.2, 111.3, 110.8, 109.6, 73.4, 62.5, 55.8, 53.3, 43.8, 40.4, 35.7, 29.0 ppm. HRESIMS *m/z* 365.2222 [M+H]+ (calcd. for $C_{23}H_{29}N_2O_2$, 365.2224)

**N,N,6-trimethyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-amine (31):** 63% yield. Brown oil. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.52 (d, *J* = 8.6 Hz, 1H), 7.38 (d, *J* = 7.7 Hz, 1H), 7.12 (t, *J* = 7.6 Hz, 1H), 6.89 (d, *J* = 7.5 Hz, 1H), 6.61 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.54 (d, *J* = 2.2 Hz, 1H), 3.17 – 3.08 (m, 2H), 3.07 – 3.02 (m, 1H), 3.02 – 2.96 (m, 1H), 2.91 (s, 6H), 2.69 – 2.65 (m, 1H), 2.63 (m, 1H), 2.50 (s, 3H), 2.46 (m, 1H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 150.1, 136.4, 134.1, 133.3, 132.6, 126.8, 126.3, 124.8, 123.1, 120.6, 111.9,

111.5, 62.4, 53.6, 44.2, 40.6, 35.0, 29.2 ppm. HRESIMS *m/z* 279.1861 $[M+H]^+$ (calcd. for $C_{19}H_{23}N_2$, 279.1856)

**N,N-dimethyl-6-propyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-10-amine (32):** 62%. Light brown solid. Melting point: 164.5 °C – 165.8 °C. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.63 (d, *J* = 8.6 Hz, 1H), 7.48 (d, *J* = 7.7 Hz, 1H), 7.22 (t, *J* = 7.6 Hz, 1H), 7.00 (d, *J* = 7.5 Hz, 1H), 6.73 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.66 (d, *J* = 2.4 Hz, 1H), 3.59 – 3.51 (m, 1H), 3.29 – 3.22 (m, 1H), 3.20 – 3.12 (m, 2H), 3.02 (s, 6H), 3.01 – 2.95 (m, 1H), 2.80 (s, 1H), 2.76 (s, 1H), 2.58 (m, 2H), 1.77 – 1.58 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 150.1, 136.6, 134.3, 133.6, 133.1, 126.7, 126.3, 124.8, 123.2, 120.6, 111.9, 111.5, 59.6, 56.4, 49.4, 40.6, 34.9, 29.2, 19.2, 12.2 ppm. HRESIMS *m/z* 307.2168 $[M+H]^+$ (calcd. for $C_{21}H_{27}N_2$, 307.2169)

**General Procedure for synthesis of benzofused aminothiazole analogues - compounds 33–34 (a-b):** To a magnetically stirred solution of respective aniline (100 mg, 1 equiv.) in acetic acid was added potassium thiocyanate (2 equiv.) at 0 °C. This was followed by addition of a solution of bromine (1.2 equiv, vol?.) in acetic acid (1 mL) in a dropwise manner. The resulting mixture was allowed to warm to rt and stirred at the same temperature for 12h. After completion of the reaction, the reaction mixture was concentrated *in-vacuo*, treated with saturated sodium bicarbonate solution and extracted with EtOAc (3×20 mL). The combined organic layer was dried using anhydrous $Na_2SO_4$ and concentrated *in-vacuo*. The crude compounds thus obtained were purified by preparative TLC using 5% MeOH/ DCM.

**7-methyl-6a,7,8,9-tetrahydro-6H-benzo[de]thiazolo[4',5':5,6]benzo[1,2-g]quinolin-2-amine (33a):** 30% yield. Orange solid. Melting point: 215 °C – 216 °C. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.65 (d, *J* = 8.5 Hz, 1H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.22 – 7.16 (m, 1H), 6.99 (d, *J* = 7.3 Hz, 1H), 5.30 (bs, 2H), 3.29 (d, *J* = 10.4 Hz, 1H), 3.20 – 3.12 (m, 1H), 3.08 – 2.97 (m, 2H), 2.78 (m, 1H), 2.70 (m, 1H), 2.61 – 2.47 (m, 4H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 165.6, 151.8, 133.6, 133.5, 132.4, 131.8, 128.5, 128.1, 127.6, 127.1, 122.3, 121.8, 118.2, 61.6, 53.4, 44.1, 34.2, 29.0 ppm. HRESIMS *m/z* 308.1219 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_3S$, 308.1216)

**6-methyl-5,6,6a,7-tetrahydro-4H-benzo[de]thiazolo[5',4':4,5]benzo[1,2-g]quinolin-10-amine (33b):** 32% yield. Light yellow oil. $^1$H NMR (600 MHz, $CDCl_3$): δ 7.80 (s, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.32 (s, 1H), 7.14 (t, *J* = 7.6 Hz, 1H), 6.96 (d, *J* = 7.5 Hz, 1H), 5.86 (s, 2H), 3.41 (d, *J* = 11.8 Hz, 1H), 3.13 (dd, *J* = 13.8, 4.1 Hz, 2H), 3.11 – 3.03 (m, 1H), 2.88 (m, 1H), 2.67 (m, 2H), 2.49 – 2.38 (m, 2H), 1.55 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H) ppm. $^{13}$C NMR (150 MHz, $CDCl_3$): δ 166.8, 151.5, 134.1, 134.0, 133.9, 133.7, 130.6, 129.5, 127.6, 126.8, 121.6, 118.4, 116.2, 59.5, 56.3, 49.3, 34.5, 29.1, 19.2, 12.2 ppm. HRESIMS *m/z* 336.1582 $[M+H]^+$ (calcd. for $C_{20}H_{22}N_3S$, 336.1529)

**7-propyl-6a,7,8,9-tetrahydro-6H-benzo[de]thiazolo[4',5':5,6]benzo[1,2-g]quinolin-2-amine (34a):** 35% yield. Brownish-white solid. Melting point: 198.1 °C – 199.5 °C. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.84 (s, 1H), 7.45 (d, *J* = 7.7 Hz, 1H), 7.34 (s, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 6.99 (d, *J* = 7.5 Hz, 1H), 5.50 (bs, 2H), 3.17 (m, 3H), 3.03 (m, 1H), 2.69 (m,

2H), 2.52 – 2.49 (m, 4H) ppm. $^{13}$C NMR (125 MHz, $CDCl_3$): δ 166.4, 151.6, 133.9, 133.6, 133.5, 133.5, 130.9, 129.4, 127.6, 127.0, 121.6, 118.6, 116.3, 62.2, 53.4, 44.0, 34.6, 29.1 ppm. HRESIMS *m/z* 308.1219 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_3S$, 308.1216)

**6-propyl-5,6,6a,7-tetrahydro-4H-benzo[de]thiazolo[5',4':4,5]benzo[1,2-g]quinolin-10-amine (34b):** 35% yield. Off-yellow solid. Melting point: 156.6 °C – 157.5 °C. $^1$H NMR (600 MHz, $CDCl_3$): δ 7.80 (s, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.32 (s, 1H), 7.14 (t, *J* = 7.6 Hz, 1H), 6.96 (d, *J* = 7.5 Hz, 1H), 5.86 (s, 2H), 3.41 (d, *J* = 11.8 Hz, 1H), 3.17 – 3.11 (m, 2H), 3.07 (m, 1H), 2.88 (m, 1H), 2.66 (m, 2H), 2.49 – 2.38 (m, 2H), 1.55 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H) ppm. $^{13}$C NMR (150 MHz, $CDCl_3$): δ 166.8, 151.5, 134.1, 134.0, 133.9, 133.7, 130.6, 129.5, 127.6, 126.8, 121.6, 118.4, 116.2, 59.5, 56.3, 49.3, 34.5, 29.1, 19.2, 12.2 ppm. HRESIMS *m/z* 336.1534 $[M+H]^+$ (calcd. for $C_{20}H_{22}N_3S$, 336.1529)

## 4.2 Biological assay procedures

### 4.2.1 Receptor binding assays—

All receptor binding assays were performed by the Psychoactive Drug Screening Program (PDSP). Complete details of the assays performed may be found online in the PDSP assay protocol book. (http://pdsp.med.unc.edu/PDSP%20Protocols%20II%202013-03-28.pdf).

### 4.2.2 5-HT$_7$R Hit Hunter® cAMP assays

**Cell Handling:** cAMP Hunter cell lines were expanded from freezer stocks according to standard procedures. Cells were seeded in a total volume of 20 μL in white-walled 384-well microplates and incubated at 37 °C for the appropriate time prior to testing. cAMP modulation was determined using the DiscoverX Hit Hunter cAMP XS+ assay

**Gs agonist format:** For agonist determination, cells were incubated with sample to induce response. Media was aspirated from cells and replaced with 15 μL 2:1 HBSS/10 mM Hepes: cAMP XS + Ab reagent. Intermediate dilution of sample stocks was performed to generate 4X sample in assay buffer. 5 μL of 4X sample was added to cells and incubated at 37 °C or room temperature for 30 or 60 minutes. Vehicle concentration was 1%.

**Gs antagonist format:** For antagonist determination, cells were pre-incubated with sample followed by agonist challenge at the $EC_{80}$ concentration. Media was aspirated from cells and replaced with 10 μL 1:1 HBSS/HEPES:cAMP XS + Ab reagent. 5 μL of 4X compound was added to the cells and incubated at 37 °C or room temperature for 30 minutes. 5 μL of 4X $EC_{80}$ agonist was added to the cells and incubated at 37 °C or room temperature for 30 or 60 minutes

**Signal detection:** After appropriate compound incubation, assay signal was generated through incubation with 20 μL cAMP XS + ED/CL lysis cocktail for one hour followed by incubation with 20 μL cAMP XS + EA reagent for three hours at room temperature. Microplates were read following signal generation with a Perkin-Elmer Envision TM instrument for chemiluminescent signal detection.

**Data analysis:** Compound activity was analyzed using CBIS data analysis suite (ChemInnovation, CA). For Gs agonist mode assays, percentage activity is calculated using the following formula: %Activity = 100% × (mean RLU of test sample – mean RLU of vehicle control)/(mean RLU of MAX control – mean RLU of vehicle control). For Gs antagonist mode, percentage inhibition is calculated using the following formula: %Inhibition =100%×(1-(mean RLU of test sample – mean RLU of vehicle control)/(mean RLU of EC80 control – mean RLU of vehicle control)).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

This publication was made possible by Grant Number 1SC1DA049961-01 from the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or its divisions. $K_i$ determinations, and receptor binding profiles were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda MD, USA. For experimental details please refer to the PDSP website http://pdsp.med.unc.edu/ and click on "Binding Assay" or "Functional Assay" on the menu bar.

## References

[1]. Bard JA, Zgombick J, Adham N, Vaysse P, Branchek TA, Weinshank RL, Cloning of a novel human serotonin receptor (5-HT7) positively linked to adenylate cyclase, J Biol Chem, 268 (1993) 23422–23426. [PubMed: 8226867]

[2]. Lovenberg TW, Baron BM, de Lecea L, Miller JD, Prosser RA, Rea MA, Foye PE, Racke M, Slone AL, Siegel BW, et al., A novel adenylyl cyclase-activating serotonin receptor (5-HT7) implicated in the regulation of mammalian circadian rhythms, Neuron, 11 (1993) 449–458. [PubMed: 8398139]

[3]. Ruat M, Traiffort E, Leurs R, Tardivel-Lacombe J, Diaz J, Arrang JM, Schwartz JC, Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT7) activating cAMP formation, Proc Natl Acad Sci U S A, 90 (1993) 8547–8551. [PubMed: 8397408]

[4]. Sprouse J, Li X, Stock J, McNeish J, Reynolds L, Circadian rhythm phenotype of 5-HT7 receptor knockout mice: 5-HT and 8-OH-DPAT-induced phase advances of SCN neuronal firing, J Biol Rhythms, 20 (2005) 122–131. [PubMed: 15834109]

[5]. Faure C, Mnie-Filali O, Scarna H, Debonnel G, Haddjeri N, Effects of the 5-HT7 receptor antagonist SB-269970 on rat hormonal and temperature responses to the 5-HT1A/7 receptor agonist 8-OH-DPAT, Neurosci Lett, 404 (2006) 122–126. [PubMed: 16759802]

[6]. Monti JM, Jantos H, The role of serotonin 5-HT7 receptor in regulating sleep and wakefulness, Rev Neurosci, 25 (2014) 429–437. [PubMed: 24681431]

[7]. Heidmann DE, Metcalf MA, Kohen R, Hamblin MW, Four 5-hydroxytryptamine7 (5-HT7) receptor isoforms in human and rat produced by alternative splicing: species differences due to altered intron-exon organization, J Neurochem, 68 (1997) 1372–1381. [PubMed: 9084407]

[8]. Giulietti M, Vivenzio V, Piva F, Principato G, Bellantuono C, Nardi B, How much do we know about the coupling of G-proteins to serotonin receptors?, Mol Brain, 7 (2014) 49. [PubMed: 25011628]

[9]. Thirumaran SL, Lepailleur A, Rochais C, Structure-activity relationships of serotonin 5-HT7 receptors ligands: A review, Eur J Med Chem, 183 (2019) 111705. [PubMed: 31581003]

[10]. Balcer OM, Seager MA, Gleason SD, Li X, Rasmussen K, Maxwell JK, Nomikos G, Degroot A, Witkin JM, Evaluation of 5-HT7 receptor antagonism for the treatment of anxiety, depression,

and schizophrenia through the use of receptor-deficient mice, Behav Brain Res, 360 (2019) 270–278. [PubMed: 30543903]

[11]. Nikiforuk A, Targeting the Serotonin 5-HT7 Receptor in the Search for Treatments for CNS Disorders: Rationale and Progress to Date, CNS Drugs, 29 (2015) 265–275. [PubMed: 25721336]

[12]. Mnie-Filali O, Lambas-Senas L, Scarna H, Haddjeri N, Therapeutic potential of 5-HT7 receptors in mood disorders, Curr Drug Targets, 10 (2009) 1109–1117. [PubMed: 19702552]

[13]. Si YG, Gardner MP, Tarazi FI, Baldessarini RJ, Neumeyer JL, R-(−)-N-alkyl-11-hydroxy-10-hydroxymethyl- and 10-methyl-aporphines as 5-HT1A receptor ligands, Bioorg Med Chem Lett, 17 (2007) 4128–4130. [PubMed: 17543523]

[14]. Gafner S, Dietz BM, McPhail KL, Scott IM, Glinski JA, Russell FE, McCollom MM, Budzinski JW, Foster BC, Bergeron C, Rhyu MR, Bolton JL, Alkaloids from Eschscholzia californica and their capacity to inhibit binding of [3H]8-Hydroxy-2-(di-N-propylamino)tetralin to 5-HT1A receptors in Vitro, J Nat Prod, 69 (2006) 432–435. [PubMed: 16562853]

[15]. Hedberg MH, Jansen JM, Nordvall G, Hjorth S, Unelius L, Johansson AM, 10-substituted 11-oxygenated (R)-aporphines: synthesis, pharmacology, and modeling of 5-HT1A receptor interactions, J Med Chem, 39 (1996) 3491–3502. [PubMed: 8784447]

[16]. Cannon JG, Moe ST, Long JP, Enantiomers of 11-hydroxy-10-methylaporphine having opposing pharmacological effects at 5-HT1A receptors, Chirality, 3 (1991) 19–23. [PubMed: 2039679]

[17]. Si YG, Gardner MP, Tarazi FI, Baldessarini RJ, Neumeyer JL, Synthesis and binding studies of 2-O- and 11-O-substituted N-alkylnoraporphines, Bioorg Med Chem Lett, 18 (2008) 3971–3973. [PubMed: 18585036]

[18]. Hedberg MH, Linnanen T, Jansen JM, Nordvall G, Hjorth S, Unelius L, Johansson AM, 11-substituted (R)-aporphines: synthesis, pharmacology, and modeling of D2A and 5-HT1A receptor interactions, J Med Chem, 39 (1996) 3503–3513. [PubMed: 8784448]

[19]. Zhang H, Ye N, Zhou S, Guo L, Zheng L, Liu Z, Gao B, Zhen X, Zhang A, Identification of N-propylnoraporphin-11-yl 5-(1,2-dithiolan-3-yl)pentanoate as a new anti-Parkinson's agent possessing a dopamine D2 and serotonin 5-HT1A dual-agonist profile, J Med Chem, 54 (2011) 4324–4338. [PubMed: 21591752]

[20]. Zhao R, Lu W, Fang X, Guo L, Yang Z, Ye N, Zhao J, Liu Z, Jia J, Zheng L, Zhao B, Zhang A, Zhen X, (6aR)-11-amino-N-propyl-noraporphine, a new dopamine D2 and serotonin 5-HT1A dual agonist, elicits potent antiparkinsonian action and attenuates levodopa-induced dyskinesia in a 6-OHDA-lesioned rat model of Parkinson's disease, Pharmacol Biochem Behav, 124 (2014) 204–210. [PubMed: 24955866]

[21]. Liu Z, Chen X, Yu L, Zhen X, Zhang A, Synthesis and pharmacological investigation of novel 2-aminothiazole-privileged aporphines, Bioorg Med Chem, 16 (2008) 6675–6681. [PubMed: 18562201]

[22]. Liu Z, Chen X, Sun P, Yu L, Zhen X, Zhang A, N-Propylnoraporphin-11-O-yl carboxylic esters as potent dopamine D(2) and serotonin 5-HT(1A) receptor dual ligands, Bioorg Med Chem, 16 (2008) 8335–8338. [PubMed: 18783955]

[23]. Linnanen T, Brisander M, Unelius L, Rosqvist S, Nordvall G, Hacksell U, Johansson AM, Atropisomeric derivatives of 2',6'-disubstituted (R)-11-phenylaporphine: selective serotonin 5-HT(7) receptor antagonists, J Med Chem, 44 (2001) 1337–1340. [PubMed: 11311055]

[24]. Linnanen T, Brisander M, Unelius L, Sundholm G, Hacksell U, Johansson AM, Derivatives of (R)-1,11-methyleneaporphine: synthesis, structure, and interactions with G-protein coupled receptors, J Med Chem, 43 (2000) 1339–1349. [PubMed: 10753471]

[25]. Madapa S, Harding WW, Semisynthetic Studies on and Biological Evaluation of NMethyllaurotetanine Analogues as Ligands for 5-HT Receptors, J Nat Prod, 78 (2015) 722–729. [PubMed: 25695425]

[26]. Si YG, Gardner MP, Tarazi FI, Baldessarini RJ, Neumeyer JL, Synthesis and dopamine receptor affinities of N-alkyl-11-hydroxy-2-methoxynoraporphines: N-alkyl substituents determine D1 versus D2 receptor selectivity, J Med Chem, 51 (2008) 983–987. [PubMed: 18251489]

[27]. Gao YG, Ram VJ, Campbell A, Kula NS, Baldessarini RJ, Neumeyer JL, Synthesis and structural requirements of N-substituted norapomorphines for affinity and activity at dopamine D-1, D-2, and agonist receptor sites in rat brain, J Med Chem, 33 (1990) 39–44. [PubMed: 2136919]

[28]. Karki A, Juarez R, Namballa HK, Alberts I, Harding WW, Identification of C10 nitrogen-containing aporphines with dopamine D1 versus D5 receptor selectivity, Bioorg Med Chem Lett, 30 (2020) 127053. [PubMed: 32107165]

[29]. Wang C, Jiang Y, Ma J, Wu H, Wacker D, Katritch V, Han GW, Liu W, Huang XP, Vardy E, McCorvy JD, Gao X, Zhou XE, Melcher K, Zhang C, Bai F, Yang H, Yang L, Jiang H, Roth BL, Cherezov V, Stevens RC, Xu HE, Structural basis for molecular recognition at serotonin receptors, Science, 340 (2013) 610–614. [PubMed: 23519210]

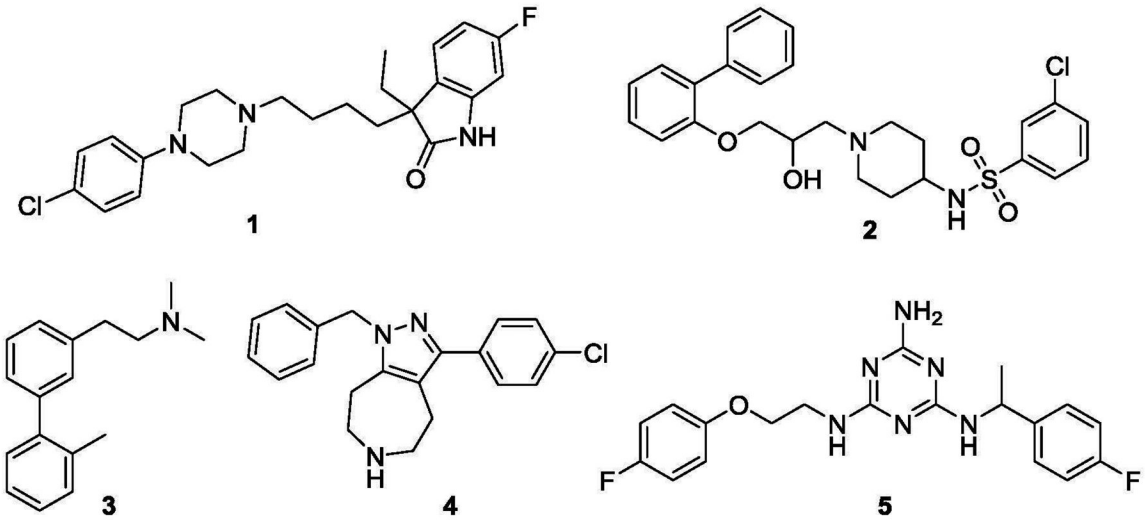



**Figure 1.**

Structures of typical 5-$HT_7$R ligands with “long chain” arylpiperazine (**1**), arylsulfonamide (**2**), biarylalkylamine (**3**), azepine (**4**, JNJ-18038683) and aminotriazine (**5**) scaffolds.

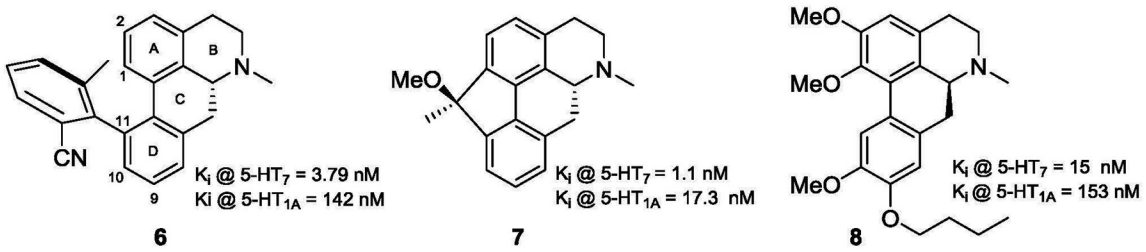



**Figure 2.**
Structures of aporphine 5-HT7 receptor ligands

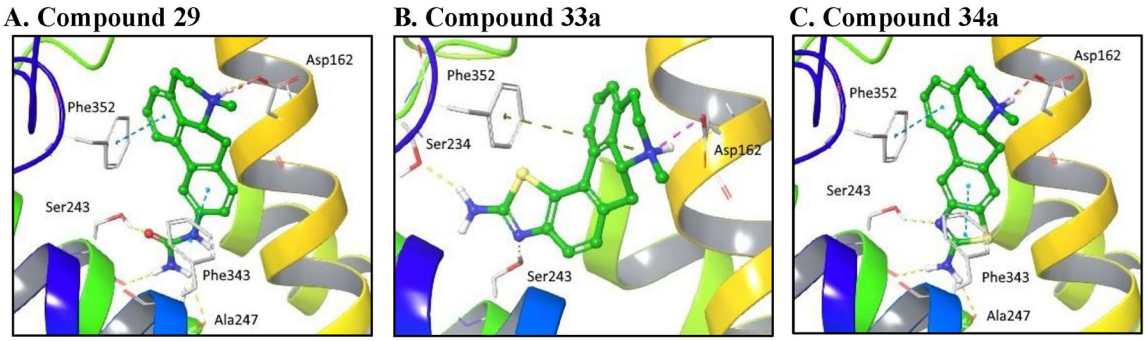
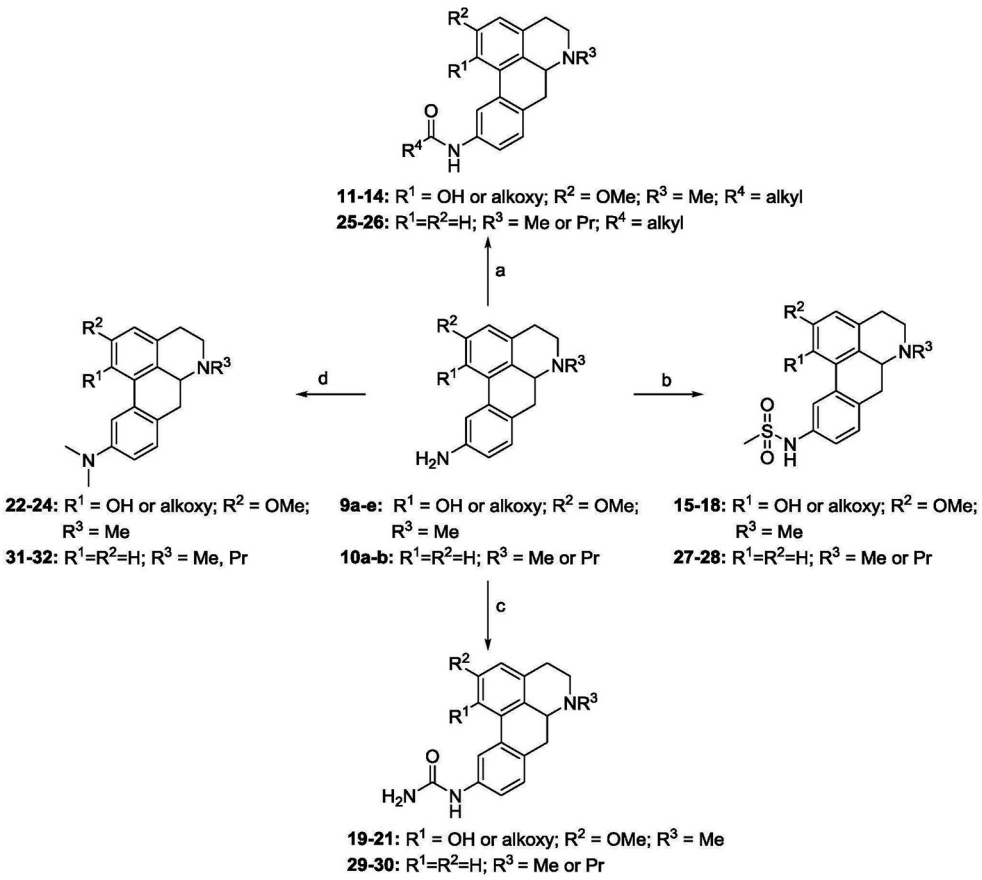


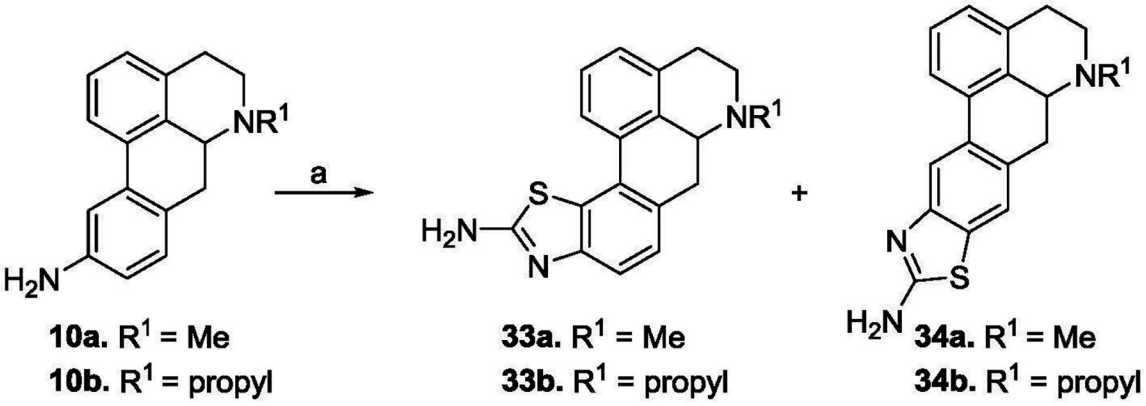

**Figure 3.**

Docked poses of **A.** compound **29** (with binding energy of −8.9 kcal/mol), **B.** compound **33a** (with binding energy of −8.7 kcal/mol) and **C.** compound **34a** (with binding energy of −10.0 kcal/mol) shown by green carbon atoms in the serotonin 5-HT7 receptor target shown by secondary structure elements and grey carbon atoms for select residues. Key quaternary N – Asp salt bridges are depicted by the pink dashed lines, H-bonding interactions by the yellow dashed lines, π-π stacking by the blue dashed lines and corresponding π-cation interactions by the green dashed lines.




**Scheme 1. Reagents and conditions:**

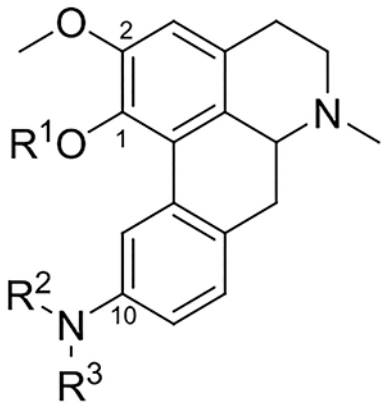
(a) corresponding anhydride, triethylamine, DCM, 2h-14h, rt, 37–73%; (b) methanesulfonic anhydride, triethylamine, DCM, rt, 14h, 33–48%; (c) potassium cyanate, acetic acid, water, 1h, rt, 30–86%; (d) formaldehyde, $NaBH(OAc)_3$, DCM, rt, 18h, 30–63%

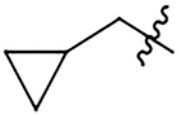
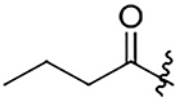
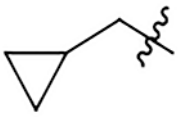
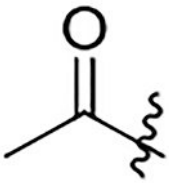
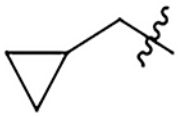
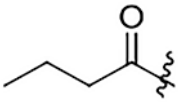
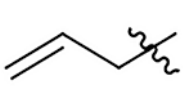
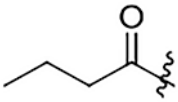


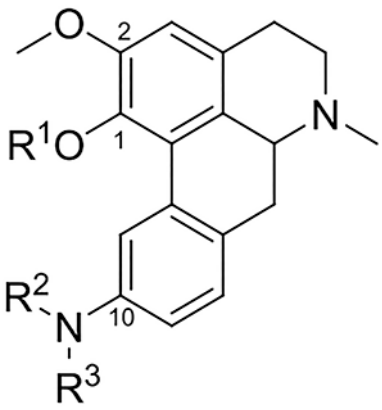

**Scheme 2. Reagents and conditions:**
KSCN, $Br_2$, acetic acid, 0 °C - rt, 12h, 30–35%

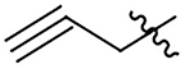
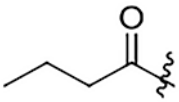
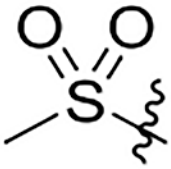
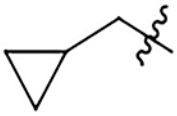
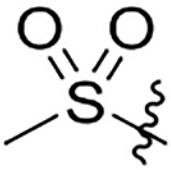
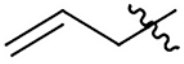
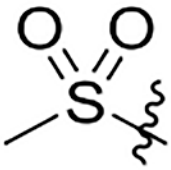
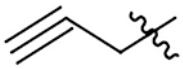
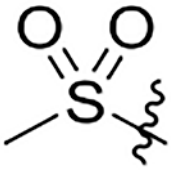
**Table 1.**

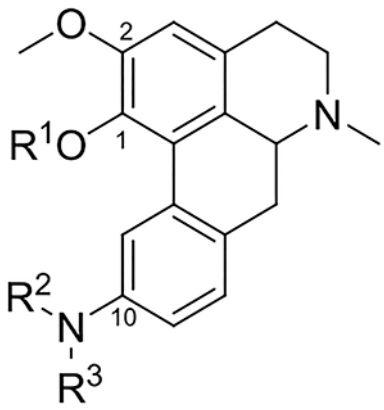
Affinity of 1,2,10-trisubstituted analogues at 5-HT receptors

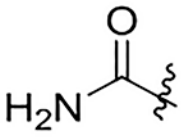
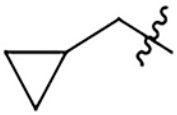
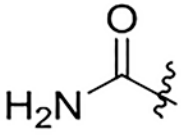
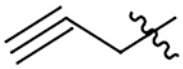
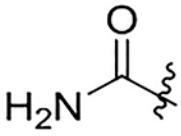
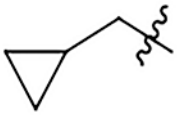
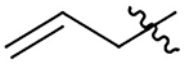



| Cmpd. # | $R^1$ | $R^2$ | $R^3$ | $K_i$ (nM)[a] 5-$HT_{1A}R$[b] | 5-$HT_{2A}R$[c] | 5-$HT_6R$[d] | 5-$HT_{7A}R$[e] |
|---|---|---|---|---|---|---|---|
| **9c** |  | H | H | na[f] | 110±14 | 132±17 | 104±13 |
| **11** | Me |  | H | 469±61 | 417±54 | 356±46 | 67±8.6 |
| **12a** |  |  | H | 1540±200 | 974±130 | 1500±190 | 636±82 |
| **12b** |  |  | H | 429±55 | 665±86 | 322±42 | 395±51 |
| **13** |  |  | H | 463±60 | 443±57 | 198±26 | 188±24 |




| Cmpd. # | $R^1$ | $R^2$ | $R^3$ | $K_i$ (nM)[a] 5-$HT_{1A}R$[b] | 5-$HT_{2A}R$[c] | 5-$HT_6R$[d] | 5-$HT_{7A}R$[e] |
|---|---|---|---|---|---|---|---|
| **14** |  |  | H | 661±85 | 1038±130 | 354±46 | 455±59 |
| **15** | Me |  | H | na | na | 86±11 | 89±11 |
| **16** |  |  | H | 385±50 | 433±56 | 505±65 | 114±15 |
| **17** |  |  | H | 403±52 | 668±86 | 590±76 | 42±5.4 |
| **18** |  |  | H | 511±66 | 1331±170 | 218±28 | 31±4.0 |




| Cmpd. # | R[1] | R[2] | R[3] | $K_i$ (nM)[a] 5-HT$_{1A}$R[b] | 5-HT$_{2A}$R[c] | 5-HT$_6$R[d] | 5-HT$_{7A}$R[e] |
|---|---|---|---|---|---|---|---|
| **19** | Me |  | H | na | na | 415±54 | 289±37 |
| **20** |  |  | H | 1280±170 | 857±110 | 527±68 | 353±46 |
| **21** |  |  | H | 911±120 | na | 410±53 | 123±16 |
| **22** | Me | Me | Me | 1570±200 | 295±38 | 127±16 | 73±9.4 |
| **23** |  | Me | Me | 1440±190 | 878±110 | 329±42 | 752±97 |
| **24** |  | Me | Me | na | 318±41 | 102±13 | 309±40 |
| **8-OH DPAT** | | | | 0.8 ±0.01 | | | |
| **Clozapine** | | | | | 3.0±0.4 | 4.0±0.5 | 11.4±1.5 |

[a] Experiments carried out in triplicate;

[b] [3H]WAY100635 used as radioligand;

[c] [3H]Ketanserin used as radioligand;

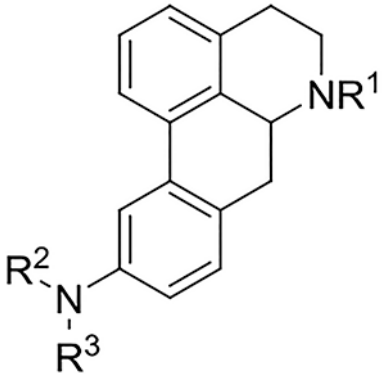
[d] [3H]LSD used as radioligand;

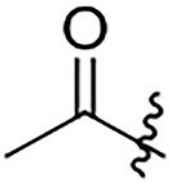
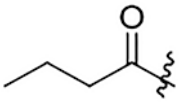
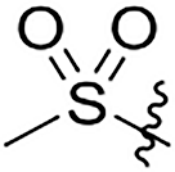
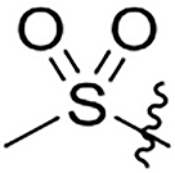
[e] [3H]LSD used as radioligand;

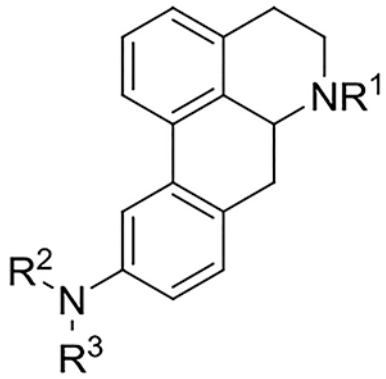
[f] na – not active (compounds displayed < 50% inhibition in a primary assay)

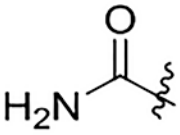
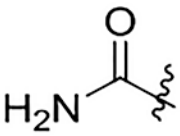
**Table 2.**

Affinity of 10-monosubstituted analogues at 5-HT receptors




| | | | | $K_i$ (nM)[a] | | | | |
|---|---|---|---|---|---|---|---|---|
| **Cmpd #** | **$R^1$** | **$R^2$** | **$R^3$** | **5-$HT_{1A}R$[b]** | **5-$HT_{2A}R$[c]** | **5-$HT_6R$[d]** | **5-$HT_{7A}R$[d]** | **clogP[e]** |
| **10b** | Pr | H | H | 30±3.8 | na[f] | na | 63±8.2 | 3.6 |
| **25** | Pr |  | H | 21±2.7 | na | na | na | 3.8 |
| **26** | Pr |  | H | 15±2.0 | na | na | 311±40 | 4.9 |
| **27** | Me |  | H | 133±17 | na | na | 27±3.5 | 2.6 |
| **28** | Pr |  | H | 23±3.0 | na | na | 199±26 | 3.6 |




| | | | | $K_i$ (nM)[a] | | | | |
|---|---|---|---|---|---|---|---|---|
| **Cmpd #** | **$R^1$** | **$R^2$** | **$R^3$** | **5-$HT_{1A}R$[b]** | **5-$HT_{2A}R$[c]** | **5-$HT_6R$[d]** | **5-$HT_{7A}R$[d]** | **clogP[e]** |
| **29** | Me |  | H | 49±6.3 | na | na | 4.5±0.6 | 2.8 |
| **30** | Pr |  | H | 236±30 | na | na | na | 3.8 |
| **31** | Me | Me | Me | 458±59 | 566±73 | 1588±200 | 17±2.2 | 3.9 |
| **32** | Pr | Me | Me | 256±33 | na | na | 984±130 | 5.0 |
| **8-OH DPAT** | | | | 0.8 ±0.01 | | | | |
| **Clozapine** | | | | | 3.0±0.4 | 4.0±0.5 | 11.4±1.5 | |

[a] Experiments carried out in triplicate;

[b] [3H]WAY100635 used as radioligand;

[c] [3H]Ketanserin used as radioligand;

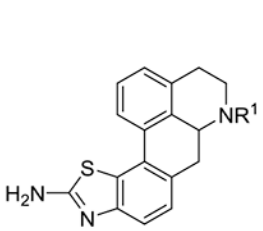
[d] [3H]LSD used as radioligand;

[e] calculated with ChemDraw Professional v. 16.0.1.4;

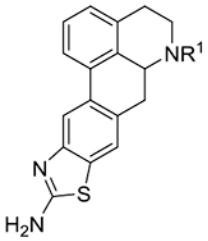
[f] na – not active (compounds displayed < 50% inhibition in a primary assay)

**Table 3.**

Affinity of aminothiazole analogues at 5-HT receptors




33




34

| | | $K_i$ (nM)[a] | | | | |
|---|---|---|---|---|---|---|
| **Cmpd #** | **$R^1$** | **5-$HT_{1A}R$[b]** | **5-$HT_{2A}R$[c]** | **5-$HT_6R$[d]** | **5-$HT_{7A}R$[d]** | **clogP[e]** |
| **33a** | Me | 187±24 | 1040±130 | na[f] | 9.9±1.3 | 3.5 |
| **33b** | Pr | 93±12 | na | na | 150±19 | 4.5 |
| **34a** | Me | 23±3.0 | na | na | 6.5±0.8 | 3.5 |
| **34b** | Pr | 7.5±0.97 | na | na | 132±17 | 4.5 |
| **8-OH DPAT** | | 0.8 ±0.01 | | | | |
| **Clozapine** | | | 3.0±0.4 | 4.0±0.5 | 11.4±1.5 | |

[a] Experiments carried out in triplicate;

[b] [3H]WAY100635 used as radioligand;

[c] [3H]Ketanserin used as radioligand;

[d] [3H]LSD used as radioligand;

[e] calculated with ChemDraw Professional v. 16.0.1.4;

[f] na – not active (compounds displayed < 50% inhibition in a primary assay)

**Table 4.**

Affinity of **29**, **33a**, **34a** and **34b** at dopamine receptors

| | Ki (nM)[a] | | | | |
|---|---|---|---|---|---|
| **Cmpd #** | **$D_1R$**[b] | **$D_2R$**[c] | **$D_3R$**[c] | **$D_4R$**[c] | **$D_5R$** |
| **29** | na[d] | na | 2460±320 | na | na |
| **33a** | 115 0±150 | 1740±220 | 381±49 | 481±62 | na |
| **34a** | na | na | na | na | na |
| **34b** | na | na | 2110±270 | na | na |
| **(+)-butaclamol** | 4.30±0.55 | | | | |
| **Haloperidol** | | 5.58±0.72 | | | |
| **Nemonapride** | | | 1.18±0.15 | 0.86±0.11 | |

[a] Experiments carried out in triplicate;

[b] [3H]SCH23390 used as radioligand;

[c] [3H]N-Methylspiperone used as radioligand;

[d] na – not active (compounds displayed < 50% inhibition in a primary assay)

**Table 5.**

Functional activity of compounds **29** and **34a** in cAMP assays

| Compound | Assay mode | Agonist[a] $EC_{50}$(μM) | Antagonist[a] $IC_{50}$ (μM) |
|---|---|---|---|
| **29** | agonist | >10 | - |
| **29** | antagonist | - | 0.125 |
| **34a** | agonist | >10 | - |
| **34a** | antagonist | - | 0.26 |
| **Serotonin** | agonist | 0.07 | - |
| **Spiperone.HCl** | antagonist | - | 0.31 |

[a]Experiments performed in duplicate