NOVEL DERIVATIVES OF 1*H*-PYRROLO[3,2-*C*]QUINOLINE AS DUAL 5-HT$_6$/D$_3$R ANTAGONISTS WITH PROCOGNITIVE PROPERTIES

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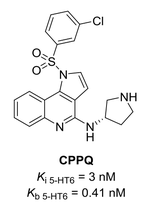
Introduction

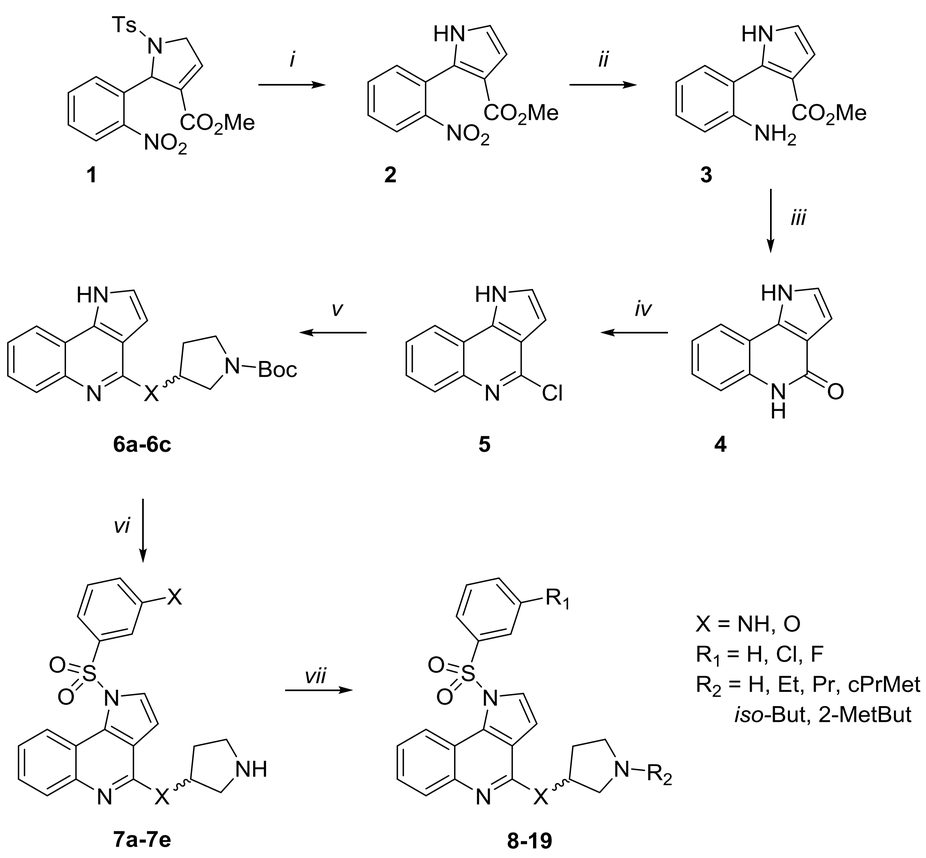
Cognitive impairment, which involves memory and attention disturbances, constitutes a common feature of various central nervous system disorders such as schizophrenia and Alzheimer's disease.[1] Although various procognitive drug candidates have been investigated in clinical trials for cognitive dysfunction, most of them failed to display clinically relevant effects. In the recent years serotonin 5-HT$_6$Rs and dopamine D$_3$Rs have attracted considerable interest as pharmacological target for the treatment of cognitive decline. Both 5-HT$_6$ and D$_3$Rs belong to GPCRs, coupled to adenyl cyclase. Additionally, their modulation engage mammalian target of rapamycin (mTOR) which play role in cognition and neurogenesis process. It has been demonstrated that 5-HT$_6$R and D$_3$R antagonists modify the transmission of acetylcholine, glutamate, noradrenaline and dopamine. Moreover, the results of advanced preclinical and clinical trials indicate the role of serotonin 5-HT$_6$ and dopamine D$_3$ receptor antagonists, in the control of cognitive functions.

We have recently described compound CPPQ, a neutral 5-HT$_6$R antagonist. Since derivative CPPQ displayed low affinity for D$_3$Rs in the screening procedure, in the presented study it was used as a chemical template for the development of dual 5-HT$_6$/D$_3$ receptors antagonists. Herein, we report chemical synthesis of novel *N*-alkylated analogs of CPPQ, their biological evaluation, followed by determination of neuroprotective properties and evaluation of procognitive properties in novel object recognition test (NOR) in rats.



CPPQ
$K_{i\ 5\text{-}HT6}$ = 3 nM
$K_{b\ 5\text{-}HT6}$ = 0.41 nM

Synthesis



X = NH, O
R_1 = H, Cl, F
R_2 = H, Et, Pr, cPrMet
iso-But, 2-MetBut

Reagents and conditions: (*i*) *t*-BuONa, DMF, RT, 2 h; (*ii*) H_2, Pd/C, MeOH, RT, 2 h; (*iii*) AcOH, *sec*-BuOH, 60 °C, 3 h; (*iv*) $POCl_3$, 105 °C, 4 h; (*v*) (*R*)-3-amino-1-Boc-pyrrolidine or (*S*)-3-amino-1-Boc-pyrrolidine, MeCN, MW 140 °C 7h or: 1. benzyl bromide, Cs_2CO_3, DMF, RT, 30 min, 2. (*S*)-3-hydroxy-1-Boc-pyrrolidine, $Pd_2(dba)_3$, BINAP, *t*-BuOK, toluene, 3. O_2, DMSO, 70 °C, 1h; (*vi*) 1. arylsulfonyl chloride, BTPP, DCM, 0 °C – RT, 3 h, 2. 1M HCl/MeOH RT, 5h; (*vii*) aldehyde, $NaBH_3CN$, EtOH, RT, 12 h.

Biological results

Obtained compounds were evaluated in the radioligand binding assays for their affinity for 5-HT$_6$Rs. Selected derivatives were tested in the screening procedure for their affinity for D$_3$ sites.

Cmpd	R_1	X	R_2	R/S	K_i [nM][a] 5-HT$_6$	%inh binding[b] D$_3$
CPPQ	3-Cl	-NH-	H	S	3[C]	69
8	3-Cl	-NH-	Et	R	NT	NT
9	3-Cl	-NH-	Et	S	11	77
10	3-Cl	-NH-	Pr	R	61	NT
11	3-Cl	-NH-	Pr	S	17	87
12	3-Cl	-O-	Pr	S	21	80
13	3-Cl	-NH-	*c*PrMet	R	106	NT
14	3-Cl	-NH-	*c*PrMet	S	41	NT
15	3-Cl	-NH-	*iso*But	R	56	NT
16	**3-Cl**	**-NH-**	***iso*But**	**S**	**27**	**98**
17	3-Cl	-NH-	2-MetBut	S	30	NT
18	3-F	-NH-	Pr	S	4	48
19	H	-NH-	Pr	S	51	NT

[a]Mean K_i values, based on three independent binding experiments (SEM ≤ 36%).
[b]Percentage displacement values at 10^{-6} M; performed at Eurofins Cerep.[C]ref. [2]

Derivative **16** was further evaluated in NG108-15 neuroblastoma cell line transiently expressing 5-HT$_6$Rs, in order to determine its influence on receptors constitutive activity at Gs signalling (Fig. 1). Additionally, compound **16** was tested for its antagonist properties at D$_3$ sites in the screening procedure.

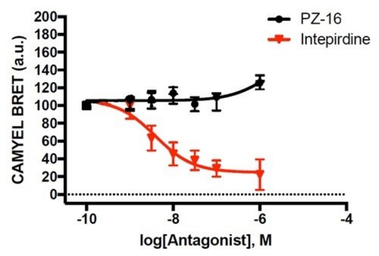
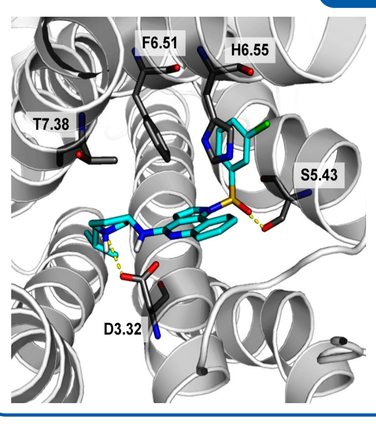


Figure 1. Influence of compound **16** and Intepirdine on the 5-HT$_6$R constitutive activity at Gs signalling.

Cmpd	K_i [nM][a]		antagonist effect[c]	K_i [nM][a]			
	5-HT$_6$	D$_3$	D$_3$	5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_7$	D$_2$
16	27	30	82%	6155	1378	1437	296

[a]Mean K_i values, based on three independent binding experiments (SEM ≤ 36%). [b]Mean K_b values (SEM ≤ 22%) [c]Percentage displacement values at 10^{-6} M; performed at Eurofins Cerep.

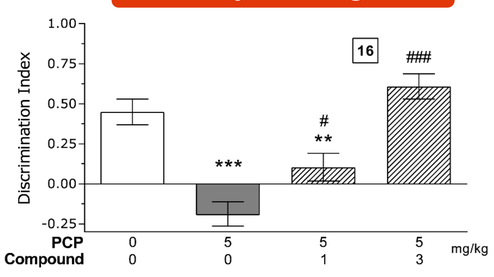
Docking studies



The crystal structure of D$_3$ receptor (PDB ID: 3PBL) was optimized using the induced-fit docking procedure and structure of compound **16**. Among all obtained complexes, several showing proper binding mode were selected and further optimized using QM/MM calculations (ligand and closest amino acid side chains were described by B3LYP/6-31++G*, the remaining part of the receptor was described by OPLS3 force field). The binding mode indicated the protonated pyrrolidine moiety created a salt bridge with D3.32, the 1*H*-pyrrolo[3,2-*c*]quinoline ring formed CH–π interaction with F6.51, the sulfonamide group formed a hydrogen bond with S5.43, and the terminal 3-chlorophenyl ring formed π– π interaction with H6.55.

Behavioral studies

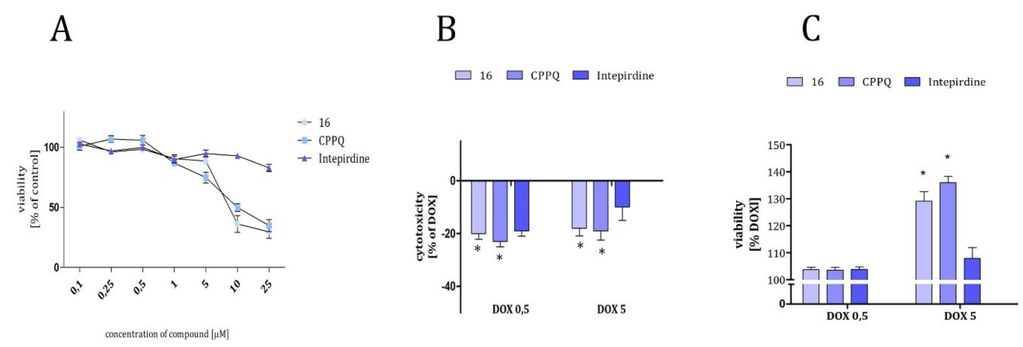
Novel object recognition



This paradigm is based on the spontaneous exploration of novel and familiar objects. Successful object recognition is indicated when an animal spends more time interacting with the novel object in the retention trial.
Since 5-HT$_6$Rs and D$_3$Rs antagonists reverse memory decline in animal models, procognitive properties of compound **16** were assesed in NOR task.

Neuroprotection

The neuroprotective effect of compounds: **16**, CPPQ and reference – intepirdine was evaluated by means of DOX-induced toxicity in astrocytes model (CRL-2547). Treatment of astrocytes with the **16** and CPPQ affected the cytotoxicity caused by DOX exposition and prevented cells' viability reduction.



The cytotoxicity studies were performed using two independent viability assays, analyzing two different physiological parameters - LDH(B) and MTT(C).

Conclusions

- Introduction of alkyl chains on the pyrrolidine nitrogen atom of CPPQ maintained high affinity fo 5-HT$_6$Rs and increased affinity for D$_3$ sites.
- The study allowed for the identification of compound **16**, classified as neutral antagonist of 5-HT$_6$Rs, which displayed antagonist properties at D$_3$ sites.
- Derivative **16** showed good selectivity over other GPCRs tested.
- Compound **16** as well as CPPQ prevented astrocytes against cell membrane damage induced by DOX and significantly increased its viability.
- Compound **16** and CPPQ protected cells from the DOX-induced cellular metabolism impairment.
- Following single administration, derivative **16** reversed phencyclidine (PCP) induced memory deficits in NOR test in rats at doses 1-3 mg/kg.
- Obtained results support therapeutic potential of dual 5-HT$_6$/D$_3$Rs antagonists in cognitive disorders.

Acknowledgements

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References

[1] Millan, M.J. *et. al*. *Nat. Rev. Drug Discov.* 11 (2012) 141–168. [2] Grychowska, K. *et al*. *ACS Chem. Neurosci* 20 (2016) 972–983