Probing of 2-Amino-6-Chloro-3,4-Dihydroquinazoline Hydrochloride At 5-HT3 Receptor

Ahmed S. Abdelkhalek, Mohamed E. El-Sadek, Sobhy M. El-Adl, Samy M. Ibrahim, Mahmoud M. Sebaiy[*]

Department of Medicinal Chemistry, Faculty of pharmacy, Zagazig University, Egypt

Corresponding author: *Sebaiy MM, Department of Medicinal Chemistry, Faculty of pharmacy, Zagazig University, 44519, Egypt. E-mail: mmsebaiy@zu.edu.eg; sebaiym@gmail.com.*

ABSTRACT

2-amino-6-chloro-3,4-dihydroquinazoline Hydrochloride (2) is 5-HT3 receptor antagonist. The results viewed that mono-substitution and di-substitution of terminal amine lead to abolish binding affinity at 5-HT3 receptors and this was consistent with molecular docking studies. Even quternization showed reduction in affinity.

Keywords: Dihydroquinazolines, Binding affinities, Molecular docking.

INTRODUCTION

5-HT$_3$ receptor is nonselective Na$^+$, K$^+$ and Ca^{+2} ion channel receptors. There is no therapeutic use for 5-HT$_3$ agonist while 5-HT$_3$ antagonist, ondansetron (**1**) is clinically used as antiemetic for post-operative nausea and vomiting. Recently, 2-amino-6-chloro-3,4-dihydroquinazoline Hydrochloride (**2**) was recently identified as novel 5-HT$_3$ antagonist [1]. It is conformationally restricted analog of 4-chlorophenylguanidine (**3**) [2]. *N*-alkylation of 2-amino-6-chloro-3,4-dihydroquinazo- line HCl (6-Chloro-2-(*N*-ethylamino)-3,4-dihydroquinazoline Hydroiodide; **4**) and 3,4-dihydroquinazolin-2-amine hydrochloride (**5**) bind 48-fold less than the lead compound (**2**) [1] (Figure1).
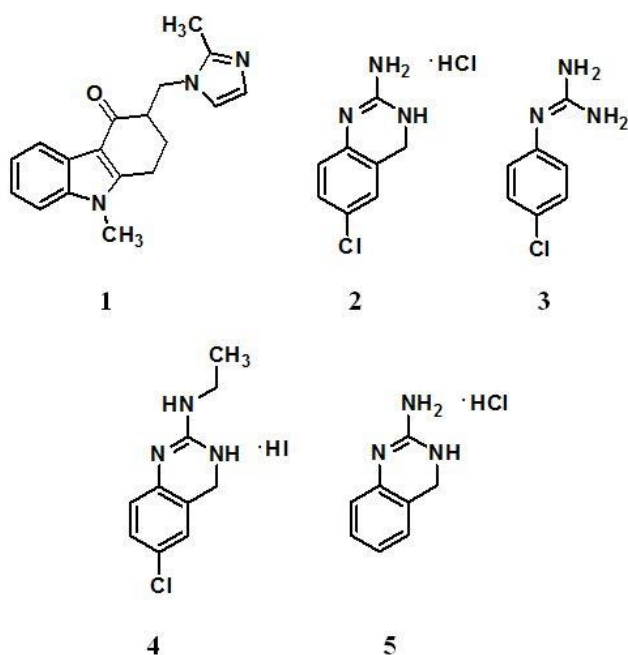
Figure 1: Chemical structures of ondansetron and dihydroquinazoline analog **(1-5).**

The aim of the study is to explore the effect of terminal amine substitution on the binding affinity at 5-HT$_3$ receptor. To determine the effect of quaternization of 2-Amino-6-chlorotetraline on 5-HT$_3$ receptor binding affinity

MATERIALS AND METHODS

Chemistry

The lead compound **2** was synthesized according to a previously reported procedure [1-3]. Secondary amine derivatives of compound; **2** (**4**) was obtained by a four steps reaction. In brief, 6-Chloro-2-(*N,N*-diethylamino)-3,4-dihydroquinazoline hydrochloride (**6**) could be obtained by the following procedure (**Scheme I**). *N,N*-diethylthiourea (**10**) was synthesized by adding dimethylamine to a previously stirred solution of ethylchloroformate and potassium thioscyanate in acetone, followed by acid-base workup [4]. *S*-methyl-*N,N*-diethylisothiourea hydroiodide (**11**) was prepared by alkylation of *N,N*-diethylthiourea (**10**) with iodomethane in excellent yield, followed by condensation reaction with 5-chloro-isatoic anhydride in dioxane for 24 h to afford 6-Chloro-2- (*N,N*-diethylamino)-3,4-dihydro- quinazolin-4(3H)-one (**12**). Reduction of 6-Chloro-2- (*N,N*-diethylamino)-3,4-dihydroquinazolin-4(3H)-one (**12**) with BH$_3$. THF, followed by stirring with HCl gas in EtOH to give the corresponding compound (**6**).
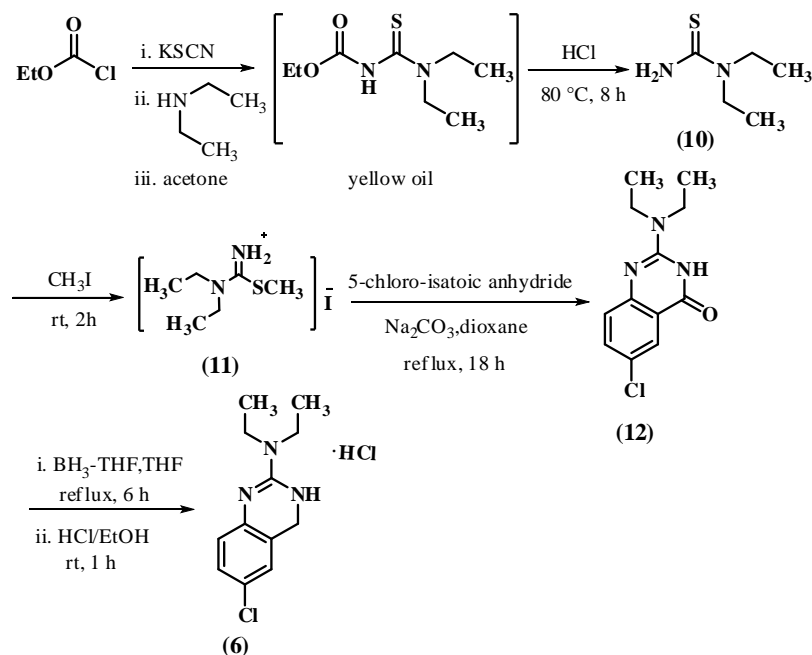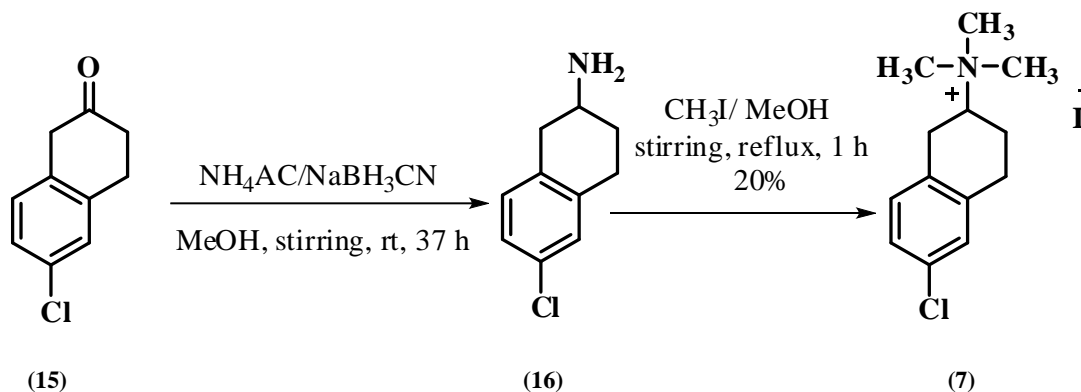
Scheme I: Synthesis of 6-Chloro-2-(*N,N*-diethylamino)-3,4-dihydroquinazoline hydrochloride (**6**).

However, there is a trial to obtain compound (**6**) by direct reaction of 6- Chloro-2-(methylthio)-3,4-dihydroquinazoline Hydroiodide (**13**) with diethylamine using ethanolic condition; the results showed that instead of formation of the target (**6**), 6-chloro-2-ethoxy-3,4-dihydroquinazoline (**14**) was obtained which was confirmed by [1]Hnmr (**Scheme II**). [1]Hnmr showed three peaks at aliphatic region; triplet peak at 1.36-1.40 ppm due to CH_3 of ethoxy group, quartet peak at 4.53-4.58 ppm due to CH_2 of ethoxy group and singlet peak at 4.69 ppm due to methylene bridge.
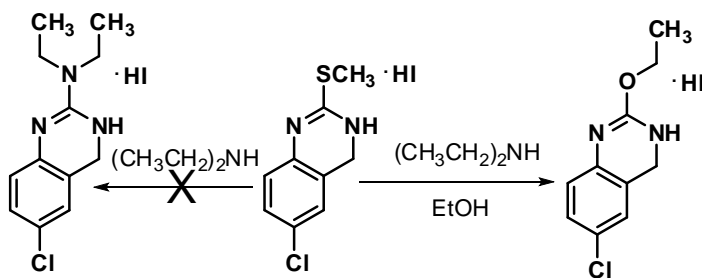
Scheme II: Synthesis of 6-chloro-2-ethoxy-3,4-dihydroquinazoline hydroiodide (**14**).

Target compound, 6-Chloro-2-trimethyl aminotetralin Hydroiodide (**7**) was synthesized by methylation of 2-Amino-6-chlorotetraline (**16**) with iodomethane according to procedure outlined in **Scheme III.**

Scheme III: Synthesis of 6-Chloro-2-trimethyl aminotetralin Hydroiodide (**7**).

Radioligand binding assay

Table 1: Binding affinity for compounds (2,4,6, and 7).

Compound	$1^\square$ binding (% inhibition)	$2^\square$ binding	
		K_i (nM)	PK_i
2-amino-6-chloro-3,4-dihydroquinazoline Hydrochloride (**2**)	89.5	209	6.7 ±0.1
N-ethyl analog of 2-amino-6-chloro-3,4-dihydroquinazoline Hydrochloride (**4**)	23.5	> 10,000	--
N,N-diethyl analog of 2-amino-6-chloro-3,4-dihydroquinazoline Hydrochloride (**6**)	-5.4	> 10,000	--
6-Chloro-2-trimethyl aminotetralin Hydroiodide (**7**)	21	> 10,000	--

Monosubstituted, disubstituted analogs, and 6-Chloro-2-trimethyl aminotetralin Hydroiodide of 2-amino-6-chloro-3,4-dihydroquinazoline Hydrochloride (**2**) displayed not more than 25% inhibition in primary binding assay.

Molecular dockings

X-ray crystal structure of the m5-HT3 receptor retrieved from the Protein Data Bank (PDB ID: 4PIR). Only two subunits (protomers) of the receptor (extracelluar domain) were selected and energy minimized for the docking studies. The ligands were sketched and energy minimized using TriposForce Field and Gasteiger-Hückel charges in SYBYL-X 2.1 (Tripos International). Molecules were docked within 10 Å radius around amino acid W183 in the orthosteric binding site using GoldSuite 5.4. The obtained solutions were analyzed with GOLD score and the selected ligand-receptor complexes were visualized and energy minimized. PyMol 1.3 (Schrödinger, LLC) was used to obtain high resolution images. 2-amino-6-chloro-3,4-dihydroquinazoline Hydrochloride (**2**) (**Figure 2**) showed four hydrogen bond (HB) interactions with amino acid residues E236, T181, and S182. N$_1$ forms a salt bridge with E236, and the phenyl ring is involved in face-to-

face interaction with F226 and face-to-edge interaction with W90. While, *N*-ethyl and *N,N*-diethyl analogs docking studies showed that structure completely flipped over preventing formation of crucial Hydrogen bonds and ionic interactions (**Figure 3**).
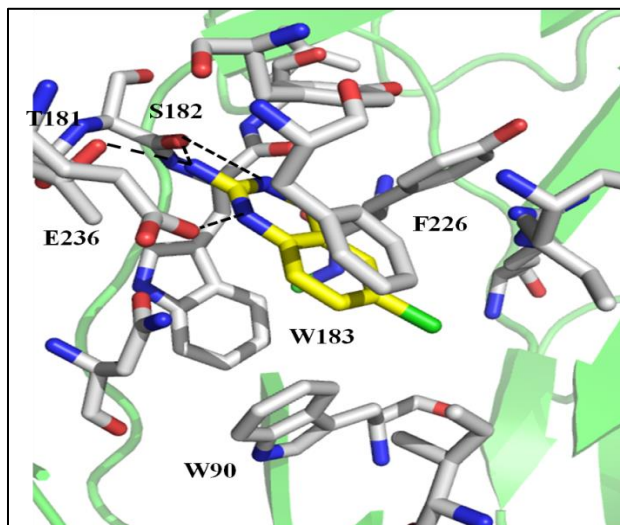

Figure 2: Proposed binding mode of the 2-amino-6-chloro-3,4-dihydroquinazoline Hydrochloride (**2**) to the m5-HT3A receptor. The ligands and amino acid residues are displayed as yellow capped sticks and pale white-capped sticks, respectively. The dashed black lines indicate a Hydrogen bond interaction between the N-atoms of the guanidinium moiety and the amino acids E236, T181, and S182.
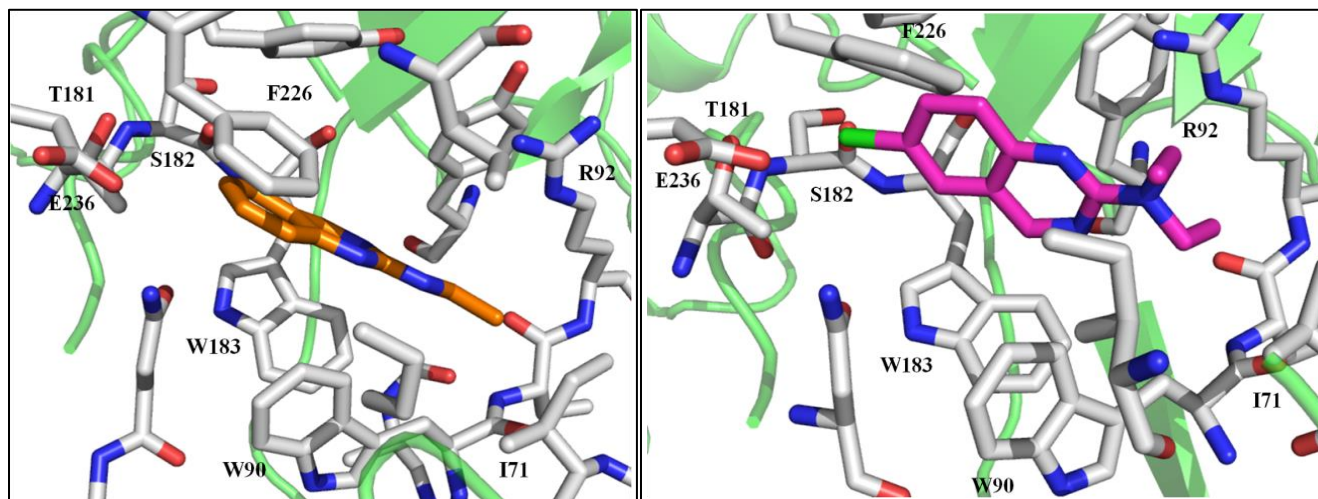

Figure 3: Proposed binding mode of the *N*-ethyl and *N,N*-diethyl analog of compound (**2**) to the m5-HT3A receptor. The ligands and amino acid residues are displayed as (orange; *N*-ethyl and magenta; *N*-diethyl) capped sticks and pale white-capped sticks, respectively.

32

EXPERIMENTAL PROCEDURE

Chemistry

Synthesis: Melting points (°C) were taken in glass capillary tubes on a Mel-Temp or Thomas Hoover apparatus and are uncorrected.^{1}H NMR were recorded with a Bruker ARX 400 MHz or Bruker AVANCE III 400 MHz spectrometer, and peak positions are given in parts per million (ppm) downfield from tetramethylsilane as internal standard. IR spectra were determined using Thermo Nicolet iS10 FT-IR. MS was obtained using a Waters Acquity TQD (tandem quadrapole) spectrometer utilizing electrospray ionization in positive ion mode. Microanalyses were performed by Atlantic Microlab Inc. (Norcross, GA) for the indicated elements, and results are within 0.4% of calculated values. Chromatographic separations were performed on silica gel columns (silica gel 62,60−200 mesh, Sigma-Aldrich). Reactions were monitored by thin-layer chromatography (TLC) on silica gel GHLF plates (250 μm, 2.5×10 cm^2; Analtech Inc.,Newark, DE).

N,N-Diethylthiourea (10)

Compound (**10**) was prepared according to a literature procedure[4]. A solution of ethylchloroformate (7.82 g, 72.06 mmol) in acetone (25 mL) was added to a solution of potassium thiocyanate (7.00 g, 72.06 mmol) in acetone (25 mL). The reaction mixture was heated at reflux for 2 h, allowed to cool to room temperature, Et$_2$NH (8.94 g, 122.13 mmol) was added in a dropwise manner at room temperature to the reaction mixture and stirred at room temperature for 1 h. The reaction mixture was acidified with HCl (6N, to pH~1) and extracted with EtOAc (3×25 mL). The combined organic portion was dried (MgSO$_4$) and evaporated to dryness under reduced pressure to give a yellow-colored oil. A mixture of the yellow-colored oil (0.15 mol) and conc. HCl (12 mL) was heated at 80°C for 8 h. The mixture was cooled to 0°C (ice-bath), neutralized with (NH$_4$)$_2$CO$_3$ (to pH~7) and extracted with EtOAc (3×25 mL). The combined organic portion was evaporated to dryness under reduced pressure to give crude white-colored solid which was recrystallized from EtOH to yield 1.40 g (46%) as a white-colored powder. mp 96-98°C (lit. 101-102°C); ^{1}H NMR (DMSO-d_6) δ 1.07 (t, *J*=7.2 Hz, 6H, 2-CH$_3$), 3.57 (q, 4H, 2-CH$_2$), 7.12 (br s, 2H, NH$_2$).

S-Methyl-N,N-diethylisothiourea Hydroiodide (11)

CH$_3$I (0.73 mL, 11.47 mmol) was added to a solution of **10** (1.40 g, 10.58 mmol) in absolute EtOH (5 mL), and the reaction was stirred at 0°C (ice-bath) for 2 h. The solvent was evaporated to dryness under reduced pressure to give a yellow-colored oil which upon recrystallization from Et$_2$O afford 2.80 g (96%) of **11** as white-colored powder. m.p 86-88°C; ^{1}HNMR (DMSO-d_6) δ 1.17 (t, *J*=7.2 Hz, 6H, 2-CH$_3$), 2.67 (s, 3H, SCH$_3$), 3.58 (q, *J*=7.2 Hz, 4H, 2-CH$_2$), 8.68 (br s, 2H, NH$_2^+$).

6-Chloro-2-(N,N-diethylamino)-3,4-dihydroquinazolin-4(3H)-one (12)

A solution of 5-chloroisatoic anhydride (1.00 g, 3.65 mmol) in anhydrous dioxane (10 mL) was added to a stirred solution of **11** (1.38 g, 3.65 mmol) and Na$_2$CO$_3$ (0.60 g, 5.66 mmol) in anhydrous dioxane (20 mL) and the resulting suspension was heated at reflux for 18 h. The reaction mixture was allowed to cool to room temperature for 1.5 h. The resulting yellow-colored precipitate was stirred with H$_2$O for 2 h and collected by filtration. The yellow-colored solid was dried under reduced pressure and recrystallized from THF to yield 0.90 g (48%) of **12** as a white-colored solid. mp 264-266°C; ^{1}H NMR (DMSO-d_6) δ 1.13 (t, *J*=7.2 Hz, 6H, 2-CH$_3$), 3.56 (q, *J*=7.2 Hz, 4H, 2-CH$_2$), 6.80-6.86 (m, 1H, ArH), 7.24-7.54 (m, 1H, ArH), 7.80 (d, *J*=2.8 Hz, 1H, ArH), 11.19 (br s, 1H, NH).

6-Chloro-2-(N, N-diethylamino)-3,4-dihydroquinazoline Hydrochloride (6)

A complex of BH_3.THF (1M, 8 mL) was added in a dropwise manner to a stirred solution of 12 (0.50 g, 1.98 mmol) in anhydrous THF (6 mL) under an N_2 atmosphere at 0 ^{o}C (ice-bath). The stirred reaction mixture was heated at reflux for 6 h, cooled to room temperature, and quenched by addition of HCl (6N, to pH~1). The mixture was basified with NaOH (6N, to pH~13) and extracted with hot $CHCl_3$ (3 × 25 mL).The combined organic portion was dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow-colored oil 0.24 g (48%) as a crude product.

A solution of the yellow-colored oil (0.15 g, 0.63 mmol) in EtOH (10 mL) was stirred at 0°C (ice-bath). A saturated solution of HCl gas in absolute EtOH (10 mL) was added, and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated to yield a white-colored solid which upon recrystallization from $EtOH/Et_2O$ gave 0.08 g (47%) of **6** as a white-colored solid: mp 190-192°C. ^{1}H NMR (DMSO-d_6): δ 1.17 (t, *J*=7.2 Hz, 6H, 2-CH_3), 3.56 (q, *J*=7.2 Hz, 4H, 2-CH_2), 4.45 (s, 2H, CH_2), 7.33-7.37 (m, 2H, ArH), 7.49 (d, *J*=8.4 Hz, 1H, ArH), 8.63 (s, 1H, NH, D_2O ex), 10.51 (s, 1H, NH^+, D_2O ex); Anal. Calcd for ($C_{12}H_{16}ClN_3 \cdot HCl \cdot 0.15\ H_2O$) C, 52.05; H, 6.29; N, 15.17. Found: C, 52.00; H, 6.14; N, 14.98.

6-Chloro-2-trimethyl aminotetralin Hydroiodide (7)

Sodium cyanoborohydride (0.08 g, 1.33 mmol) was added to a solution of 6-chloro-β-tetralone **15** (0.20 g, 1.11 mmol) and NH_4OAc (0.85 g, 11.07 mmol) in MeOH (15 mL) at room temperature. The resulting yellow-colored solution was allowed to stir for 37 h. The reaction mixture was acidified with 10% HCl to pH ~2, concentrated under reduced pressure, and then extracted with CH_2Cl_2 (2 × 75 mL). The aqueous portion was basified with 6 N NaOH to pH ~10 and extracted with CH_2Cl_2 (3 × 75 mL). The combined organic portion was dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.11 g (58%) as a greenish grey-colored oil.

In a sealed tube, a solution of CH_3I (0.10 mL, 1.94 mmol) in anhydrous Et_2O (1.00 mL) was added to the grey-colored oil **16** (0.03 g, 0.16 mmol) in anhydrous MeOH (1.00 mL), and the reaction mixture was heated at reflux for 1 h. The reaction mixture was allowed to cool to room temperature and evaporated under reduced pressure to yield 0.02 g (24%) of **7** as a white-colored solid. mp 240-242°C; ^{1}H NMR (DMSO-d_6) δ 1.72-1.82 (m, 1H, CH), 2.38-2.44 (m, 1H, CH), 2.79-2.88 (m, 1H, CH), 2.97-3.02 (m, 1H, CH), 3.05-3.07 (m, 1H, CH), 3.11 (s, 9H, 3-CH_3), 3.21-3.27 (m, 1H, CH), 3.71-3.79 (m, 1H, CH), 7.17-7.19 (d, *J*=8 Hz, 1H, ArH), 7.23-7.27 (m, 2H, ArH); Anal. Calcd ($C_{13}H_{19}ClIN \cdot 0.2H_2O$) C, 43.95; H, 5.50; N, 3.94. Found: C, 43.60; H, 5.27; N, 3.94.

RESULTS AND DISCUSSION

Radioligand binding assay

Membrane preparation: HEK293T cells are subcultured into 15-cm dish containing 18 µg DNA, 100 µl of 2.5 M CaCl2, 100 µl of TE (1 mM Tris HCl, 0.1 mM EDTA, pH 7.60) and diluted into a final volume of 1 ml water. The mixture is added in a dropwise manner to an equal volume of 2x HBS to cells.The cells are incubated overnight in fresh growth medium. Medium containing dialyzed FBS is added overnight prior to harvesting after 2-3 days of transfection [5]. The cells are rinsed with PBS, scraped off into 50 ml conical tubes, and pelleted by centrifugation (1000 × g, 10 min at 4°C). The cell pellet is resuspended in chilled (4°C) lysis buffer (50 mM Tris HCl buffer, pH 7.4) and triturated gently for hypotonic lysis. The suspension is then centrifuged at 21,000 × g for 20 min (4°C) to obtain a crude membrane fraction pellet. The fresh membrane pellet is then resuspended in cold lysis buffer with 3x volumes of the pellet size and is

34

immediately subjected to the Bradford protein assay to determine protein concentration, followed by a saturation binding assay. Fresh membrane suspensions are stored at -80°C in small aliquots (enough for one 96-well plate to have at least 500 cpm/well when assayed at 0.5 – 1.0x Kd value of the hot ligand) depending on the receptor expression level and the Kd value [6].

Saturation binding: Saturation binding assay is carried out to measure receptor expression level (Bmax) and binding affinity (Kd) values of a selected radioligand. Saturation binding assays are carried out in 96-well plates in a final volume of 125 μl per well. Radioligand (25 μL) is added to a 96-well plate; followed by addition of 25 μl binding buffer (total binding) or 25 μl reference compound (non specific binding) at a final concentration of 10 μM. Fresh membrane protein (75 μl) is added and the reaction is incubated at room temperature for 90 min in the dark. The reaction is stopped by vacuum filtration onto cold 0.3% polyethyleneimine (PEI) soaked 96-well filter mats using a 96-well Packard Filtermate harvester, followed by three washes with cold wash buffers. Scintillation cocktail is then melted onto microwave-dried filters on a hot plate and radioactivity is counted in a Microbeta counter. Total binding and nonspecific binding results are analyzed in Prism v5.0 by fitting results to the following equations [6].

$$\text{Nonspecific binding} = NS \times X + \text{Background}$$

$$\text{Total binding} = \text{Nonspecific binding} = + B_{max} \times X$$

$$(X + K_d)$$

sPrimary and secondary radioligand binding assay [6,7]

Selected compounds are subjected to primary radioligand binding assays at 5-HT3 receptors. In the primary binding assays, compounds are usually tested at 10 μM in quadruplicate n 96-well plates and % inhibition could be obtained from the following equation:

$$\% \text{ Inhibition} = 100 - \frac{\text{Sample cpm-non specific cpm}}{\text{Total cpm-non specific cpm}} \times 100$$

In the secondary binding assays, selected compounds that showed 50% inhibition in primary binding assay are usually tested at 11 concentrations (0.1, 0.3, 1, 3, 10, 30, 100, 300 nM, 1, 3, 10 μM), in triplicate. K_i values are calculated according to Cheng-Prusoff equation:

$$K_i = \frac{IC50}{1 + \dfrac{L}{kd}}$$

Both primary and secondary radioligand binding assays are carried out in a final of volume of 125 μl per well in appropriate binding buffer (50 mM Tris HCl, 10 mM MgCl2, 0.1 mM EDTA, pH 7.4, RT). The target compound concentration is close to the K_d. Total binding and nonspecific binding are determined in the absence and presence of 10 μM appropriate reference compound, respectively. In summary, plates are usually incubated at room temperature and in the dark for 90 min.

35

CONCLUSION

Reactions are stopped by vacuum filtration onto 0.3% polyethyleneimine (PEI) soaked 96-well filter mats using a 96-well filtermate harvester, followed by three washes with cold wash buffer (50 mM Tris HCl, pH 7.4, cold). Scintillation cocktail is then melted onto the microwave-dried filters on a hot plate and radioactivity is counted in a Microbeta counter. Terminal nitrogen (N2) is crucial for the binding affinity (e.g. N-alkyl group diminish the binding affinity). Quaternary compound were not tolerated by 5-HT3 receptor.

ACKNOWLEDGEMENT

REFERENCES

1. Abdelkhalek, AS., et al. "Methylene bridge" to 5-HT3 receptor antagonists: Conformationally-constrained phenylguanidines". *ACS Chem. Neurosci*. **2018.**

2. Dukat, M., 5-HT3 serotonin receptor agonist: a pharmacophore journey. *Curr. Med. Chem*. **2004.** 4: 77-94.

3. Dukat, M., et al. 2-Amino-6-chloro-3, 4-dihydroquinazoline: A novel 5-HT3 receptor antagonist with antidepressant character. *Bioorg. med. chem. lett*. **2013.** 23 (21): 5945-5948.

4. Yokoyama, M., et al. Synthesis of mesoionic triazoline nucleosides. *J. Chem. Soc., Perkin Trans.* **1990.** 12: 3243-3247.

5. Jordan, M., et al. Transfecting mammalian cells: Optimization of critical parameters affecting calcium-phosphate precipitate formation. *Nucleic acids Res.* **1996.** 24 (4): 596-601.

6. Roth, B., National Institute of Mental Health Psychoactive Drug Screening Program (NIMH PDSP). Assay protocol book, Version II, Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC **2018.** 27599.

7. Besnard, J., et al. Automated design of ligands to polypharmacological profiles. . *Nature*, **2012.** 492: 215-220.