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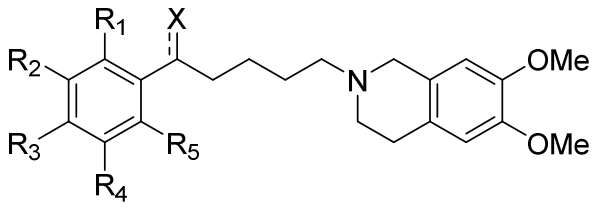
Graphical abstract



3b R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, X = O;
3e R_1,R_3 = OMe, R_2,R_4,R_5 = H, X = O;
4b R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, X = OH;
4e R_1,R_3 = OMe, R_2,R_4,R_5 = H, X = OH.

Analog	Kiσ_1 (nM)	Kiσ_2 (nM)	Huh-7 (μM)	KYSE-140 (μM)
3b	> 1000	5.1 ± 1.5		
3e	32.9 ± 4.1	5.8 ± 0.8		
4b	> 1000	5.2 ± 1.0	12.50 ± 0.36	14.86 ± 0.90
4e	> 1000	6.2 ± 1.6		

Synthesis and pharmacological evaluation of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives as sigma-2 receptor ligands

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Abstract

Increasing evidences have implicated that sigma-2 receptor is a biomarker and significantly over-expressed in many proliferative cancer cells with no or low expression in normal cells. Sigma-2 receptor selective ligands have been successfully used as valuable tools to study its pharmacological functions, tumor imaging, and cancer therapeutics or adjuvants. 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolinylalkyl benzamides are among a few categories of structures that have demonstrated high affinities and selectivities for sigma-2 receptor and been used extensively as study tools in various tumor imaging and therapy. As a continuous effort, we have synthesized a new series of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivatives and evaluated their affinities for both sigma-1 and sigma-2 receptors. Most of these newly developed analogs showed good to excellent binding affinities for sigma-2 receptor with no or low affinities for sigma-1 receptor. In particular, compounds **3b**, **3e**, **4b**, and **4e** demonstrated K_i values of 5-6 nM affinities and excellent selectivities for sigma-2 receptor. In addition, these analogs also demonstrated moderate anticancer activities against human liver Huh-7 tumor cells and human esophagus KYSE-140 cancer cells. But their cytotoxicities seem not to be correlated with their sigma-2 receptor affinities.

Keywords: Sigma receptor, ligands, 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, affinity

1. Introduction

Sigma receptors have attracted extensive interests in the scientific fields of pharmacology, oncology, and medicinal chemistry due to their possible involvement in diverse roles of physiological and neurological disorders as well as diseases such as cancer, Alzheimer's disease, schizophrenia, memory loss or damaged cognition [1-4]. Sigma receptors are currently classified into two subtypes, termed sigma-1 (σ_1) and sigma-2 (σ_2) [5]. Their physiological functions have not yet been clearly elucidated and their corresponding natural ligands are still not known, though some candidates such as some neurosteroids and *N*,*N*-dimethyltryptamine (DMT) were indicated as potential endogenous ligands for sigma-1 receptor [1,4]. Both receptors are widely expressed in the central nervous system (CNS) as well as in many peripheral tissues such as in the liver, kidney, prostate, etc [4,6]. Accumulating evidences indicate that sigma-1 receptor is involved in CNS regulated cognition and movement, while the sigma-2 receptor may play important roles in cell morphology, survival and differentiation [1,4,7,8]. Sigma-1 receptor was cloned in 1996 and proposed to be a transmembrane protein with a molecular weight of ~25.3 KDa [9]. Despite the confirmed presence of a 21.5 kDa protein by photoaffinity labeling, the sigma-2 receptor was not able to be cloned for a very long time [10]. Due to its similarity to progesterone receptor membrane component 1 (PGRMC1) protein in association with tumorigenesis and coexistence in cellular environment [11,12], the sigma-2 receptor was widely suggested to be or a component of the PGRMC1 protein complex [13]. But more recent studies have indicated that PGRMC1 protein and sigma-2 receptor are two different bio-molecules [14-17]. Most recently, Alon and coworkers have been able to purify the sigma-2 protein by an affinity column and identify the gene for the sigma-2 receptor [18]. Therefore, they have provided more convincing evidence to distinguish sigma-2 receptor from PGRMC1 protein. According to their study, the sigma-2 receptor is the same as TMEM97 (an endoplasmic reticulum-resident transmembrane protein that regulates the sterol transporter NPC1) and contains 4 transmembrane segments and 176 amino acids with Asp 29 and Asp 56 being the essential ligand binding residues. The successful cloning and revealing of the sigma-2 receptor will accelerate the research toward better understanding and potential application of this receptor in drug discovery.

An increasing interest in sigma-2 receptor is largely due to its involvement in oncogenesis and cancer cell proliferation [19,20]. Sigma-2 receptor has been identified to be a cancer cell proliferative biomarker that has almost 10 folds higher densities in proliferative cancer cells than in quiescent tumor tissue [21-24]. Various radio- or fluorescent labeled sigma-2 ligands have demonstrated to specifically bind to the tumor tissues and thus are potentially useful for cancer diagnosis [25-34]. Another developing field is that sigma-2 ligands (agonists) bind to siamg-2 receptor and inhibit cancer cell proliferation and trigger cancer cell death via apoptosis pathway [1,4]. Many sigma-2 ligands showed strong inhibitory effects against various

cancer cell lines both *in vitro* and in animal models [35-37]. In addition, sigma-2 ligands also demonstrated strong potentiation or synergistic effects when combined with an anticancer agent [38-40]. Yet another newly developing field is to use sigma-2 receptor ligands as carriers that specifically deliver anticancer agents to the tumor sites and thus to kill the cancer cells with no or minimum effects to normal cells [41-45]. Though the exact mechanism of action of sigma-2 receptor and its ligands is unknown, some evidence points to the coupling of sigma-2 receptor to cell signaling pathways that may involve caspases, kinases, Ca^{2+} channels, K^{+} channels, reactive oxygen species (ROS), etc [1,3,4].

The structural moiety of piperidine is the hallmark pharmacophore for both sigma-1 and sigma-2 receptor binding pockets [1,46,47]. Though the sigma-1 receptor has a wide variety of structural tolerance with various alkylamines binding to the sigma-1 receptor with high affinities, the piperidine derived cycloalkylamine analogs demonstrated extremely potent affinities for sigma-1 receptor [46-48]. On the other hand, most reported sigma-2 ligands more or less contain the piperidine structure or some modified form of it (Scheme 1). Among the four reported categories of sigma-2 receptor pharmacophores, compounds containing the 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline moiety demonstrated the overall highest selectivity for sigma-2 receptor over sigma-1 receptor [1,49-52]. Thus, in order to be sigma-2 selective, the piperidine structure must be modified with more bulky elements incorporated. We discovered, for the first time, that 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline was an ideal piperidine analog and that compounds containing this moiety bound to sigma-2 receptor with high affinities and selectivities [53]. Since our initial publication, many 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline containing benzamide analogs have been synthesized and reported to have good to excellent affinities and selectivities for sigma-2 receptor [1,54-59]. Recently, some hybrid analogs containing an indole moiety and a 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline structure linked with an alkyl chain showed excellent affinities and selectivities for sigma-2 receptor [50,51,60,61]. Some of these analogs demonstrated good anticancer activity against multidrug resistant tumors [60,62]. In an effort to further explore the binding requirement and discover novel and more selective sigma-2 ligands, we synthesized a new series of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline analogs without the benzamide moiety and evaluated their binding affinities for both sigma receptors. Among them, four analogs demonstrated nanomolar affinities for sigma-2 receptor and moderate or no significant affinities for sigma-1 receptor. These compounds are among the most potent and selective sigma-2 ligands reported and thus potentially useful as pharmacological tools to study the functions of sigma-2 receptor or can be developed as cancer diagnostic agents. In addition, these analogs showed moderate anticancer activities against human liver Huh-7 cancer cells and human esophagus KYSE-140 cancer cells.

2. Results and discussion

2.1. Chemistry

The benzamide structure was once believed to be involved in the binding to the sigma-2 receptor, but later indicated not to be a required sigma-2 receptor pharmacophore because a number of non-benzamide analogs, such as indole-6,7-dimethoxy-1,2,3,4-tetrahydroisoquine hybrid analogs without the amide moiety, were reported to have high affinities and selectivities for sigma-2 receptor [50,60]. Efforts toward the modifications of the 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline structure did not improve the binding affinity or selectivity for the sigma-2 receptor. For example, the removal, replacement, or cyclolization of the 6,7-dimethoxy substituents resulted in reduced or loss of activity; the opening or enlargement of the piperidine moiety, or changing the nitrogen position on the piperidine ring of the 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline moiety resulted in significantly decreased or loss of activity; the contraction of the piperidine ring to form 5,6-dimethoxyindoline maintained binding activity for sigma-2 receptor [54-56].

The strategy for this study was to synthesize a new series of hybrid compounds containing 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline and an aromatic moiety of electron rich or deficiency with an alkyl chain linkage between them. The syntheses of these analogs were very straightforward and outlined in Schemes 2 and Scheme 3. An appropriately substituted benzene was carried out Friedel-Crafts acylation with a chloroalkylacyl chloride, followed by substitution of the chloro atom by 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline to give the phenyl ketone products **3a**-**3f**, and subsequently undergone reduction with sodium borohydride to afford the hydroxyl product **4a**-**4e** (Scheme 2). A series of quinazoline substituted analogs were prepared according to the well known established methods, substituted at the 3-N position with a chloroalkyl bromide and followed by replacement of the chloro atom by 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline to give the products **8a**-**8e** (Scheme 3). All the novel intermediates were characterized by ^{1}H NMR unless otherwise notified and all the test analogs were confirmed by ^{1}H NMR, ^{13}C NMR, and elemental analysis. Both the ^{1}H NMR and ^{13}C NMR results indicated that analog **3f** lost one methyl group of the tetramethoxyl substituted aromatic ring. This tetramethoxyl substituted aromatic series of analogs are being further investigated and will be reported elsewhere.

2.2. Pharmacology

Compounds **3a**-**3f**, **4a**-**4e**, and **8a**-**8e** were evaluated for their binding affinities for both sigma-1 and sigma-2 receptors according to methods as previously described [48,58]. Sigma-1 receptor competitive binding assays were conducted in guinea pig brain membrane homogenates with [^{3}H](+)-pentazocine as the radioligand. Sigma-2 receptor binding assays were conducted in rat liver membrane homogenates with [^{3}H]di-o-tolylguanidine (DTG) as the radioligand and in the presence of 100 nM of (+)-pentazocine (to mask the sigma-1 sites). All test compounds were dissolved in ethanol, diluted to various concentrations ranging from 0.1 nM to 1000 nM in 50 mM Tris-HCl (pH 8.0), added to the incubation at 25°C and terminated by the addition of

ice-cold 10 mM Tris-HCl (pH 8.0). Initially, all the test compounds were subjected to a displacement assay at 1 μM concentration and only those with significant displacement of the [^{3}H](+)-pentazocine (sigma-1) or [^{3}H]-DTG (sigma-2) were identified as "hits" (greater than 50% displacement). The hits were then undergone full assays and were measured for their binding K_i values for sigma-1 and sigma-2 receptors. Nonspecific bindings were determined in the presence of 10 μM haloperidol for sigma-1 receptor and 5 μM DTG for sigma-2 receptor. All binding experiments were repeated at least three times and the binding results are shown in Table 1.

The binding results in Table 1 indicated that all the tested compounds, except for **3f**, **8c**, **8d**, and **8e**, showed moderate to excellent affinities for sigma-2 receptor. Only two analogs, **3a** and **3e**, showed moderate binding affinities for sigma-1 receptor with $K_{i(\sigma_1)}$ values of 13.3 and 32.9 nM, respectively, and the rest of the analogs had no measurable affinities for sigma-1 receptor. In particular, analogs **3b**, **3e**, **4b** and **4e** demonstrated the highest binding affinities with $K_{i(\sigma_2)}$ values of 5.1, 5.8, 5.2 and 6.2 nM, respectively, for sigma-2 receptor. Since analogs **3b**, **4b** and **4e** had no measurable affinities for sigma-1 receptor, these three analogs displayed the highest selectivities of >196, >192, and >161, respectively, for sigma-2 over sigma-1 receptor. Analogs **3a**, **3c**, **3d**, **4a**, **4c**, and **4d** also demonstrated moderate affinities with $K_{i(\sigma_2)}$ values of 23.7, 53.8, 68.1, 18.2, 62.0, and 30.2 nM, respectively, for sigma-2 receptor. It is interesting to note that all the hydroxyl series of analogs (**4a**-**4e**) did not show any measurable affinities for sigma-1 receptor, whereas two of the corresponding ketone analogs, **3a** and **3e**, showed moderate affinities for sigma-1 receptor. Therefore, the reduction of the ketone moiety (**3a**-**3e**) to form the corresponding hydroxyl group (**4a**-**4e)** has no harmful effect or slightly positive effect for sigma-2 receptor affinity and has a negative effect for sigma-1 receptor binding. This may attribute to the conjugation between the carbonyl group and the aromatic moiety and the electron withdrawing effect of the carbonyl group, resulting in a reduced electron density on the aromatic moiety that is projected to bind to the primary hydrophobic binding pocket of the sigma-2 receptor [1,58]. Analog **3f** was designed as a tetramethoxyl substituted aromatic ketone and expected to show at least some sigma-2 receptor affinity. As a result, one of the methoxyl group (most likely the one adjacent to the carbonyl group or its *para*- position) was demethylated (very likely during the Friedel-Crafts reaction) and the resultant trimethoxyphenol ketone did not show any measurable affinity for both sigma receptors. The exact reason for the lack of affinity of **3f** toward either sigma receptor is not clear. However, it is well known that both sigma-1 and sigma-2 receptors' binding pocket consist at least one primary hydrophobic binding site, one ionizable nitrogen binding site, and maybe an additional hydrophobic binding site (even more true for sigma-2 receptor) [1]. Thus the lack of binding affinity of **3f** for both sigma receptors is probably due to an increased hydrophilicity that may have a detrimental effect toward the binding of the phenol aromatic moiety to the primary hydrophobic binding pocket of the receptor protein.

A number of quinazoline linked 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline analogs **8a**-**8e** were also synthesized and measured their affinities for both sigma receptors. Only **8a** and **8b** showed moderate affinities with Ki values of 13.9 and 43.8 nM, respectively, for sigma-2 receptor and all these quinazoline analogs did not display any affinities for sigma-1 receptor. Quinazoline is an electron deficient aromatic moiety and therefore does not favorably bind to the primary hydrophobic binding pocket of the sigma receptor. However, when the quinazoline ring is substituted with two methoxyl groups, the resultant analogs **8a** and **8b** bind to the sigma-2 receptor with moderate affinities, which may be explained that the electron donating methoxyl groups reverse the electron deficiency and help the substituted quinazoline moiety bind to the sigma-2 receptor's primary hydrophobic binding site. On the other hand, 6-nitro-7-chloro substituted quinazoline moiety is even more electron deficient than the quinazoline ring itself. As expected, the corresponding analogs **8c**-**8e** did not show any binding affinities for either receptor.

These analogs were tested their anticancer activities against human liver Huh-7 cancer cells and human esophagus KYSE-140 cancer cells. Several of them showed moderate cytotoxicities agsinst either or both cell lines. Particularly, analog **4b** displayed IC_{50} values of 12.5 μM and 14.86 μM against the growth of Huh-7 and KYSE-140 cells, respectively, which are comparable to 15.31 μM and 21.34 μM against Huh-7 and KYSE-140 cells, respectively, for cisplatin in the same assay. Analogs **3a**, **3d**, **3f**, and **4d** demonstrated moderate anticancer activities with IC_{50} values of 42.83, 75.33, 81.63, and 45.89 μM, respectively, against Huh-7 cell growth. Analogs **3a**, **3e**, **3f**, and **4d** also demonstrated moderate activities with IC_{50} values of 60.11, 88.32, 93.73, 26.10 μM, respectively, against KYSE-140 cell growth. These analogs's anticancer activities are comparable to the IC_{50} values for several recently published sigma ligands (though against different cancer cell lines) [63]. Analog **4b** was one of the best sigma-2 ligands in this study and also had the most potent anticancer activity against both Huh-7 and KYSE-140 cells, but the other two potent and selective sigma-2 ligands **3b** and **3e** did not show similar potent anticancer activities against Huh-7 cells or KYSE-140 cells (Note: analog **4e** was not available at the time of anticancer screening and not tested in this assay). Therefore, the anticancer activities of these analogs may not attribute to their sigma-2 receptor interactions. Another possibility is that analog **4b** is an agonist for sigma-2 receptor, whereas, analogs **3b** and **3e** are antagonists for sigma-2 receptor. Thus, it is necessary to further investigate the agnostic/antagonistic profile and mechanism of action for these analogs in future studies.

3. Conclusions

A series of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline analogs were synthesized and evaluated for both sigma-1 and sigma-2 receptor affinities. Many of these analogs showed good to excellent affinities for sigma-2 receptor and no or moderate affinities for sigma-1 receptor. Among them, analogs **3b**, **3e**, **4b** and **4e** showed the highest affinities and analogs **3b**, **4b** and **4e** had the highest selectivities

for sigma-2 receptor. The common structural feature among these four analogs is that all of them have 5 carbon units between the 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline moiety and the aromatic moiety. These four analogs are among the most potent and selective sigma-2 ligands reported and thus are potentially useful for the development of cancer diagnostic agents, functional study tools, and anticancer adjuvants. Analog **4b** has cytotoxicities that are comparable to those of cisplatin against both liver Huh-7 and esophagus KYSE-140 cancer cells and thus may be worth further investigation of its mechanism of anticancer action and potentially useful for drug development against liver or esophageal cancer.

4. Experimental Section

4.1. Chemistry

All reagents or solvents purchased commercially were used directly without further purification. Reactions were monitored by analytical thin-layer chromatography (TLC) on silica gel F254 glass plates and visualized under UV light (254 and 365 nm) and temperatures were recorded using regular thermometer without correction. Flash column chromatography was performed on silica gel (200–300 mesh). Melting points were measured using capillary method without correction. ^{1}H NMR spectra were recorded with a Bruker Avance III 400 MHz NMR spectrometer at room temperature. Chemical shifts were recorded as parts per million (ppm) downfield to tetramethylsilane (TMS). The following abbreviations are used for multiplicity of NMR signals: s) singlet, d) doublet, t) triplet, q) quartet, m) multiplet, dd) doublet of doublets, td) triplet of doublets; dt) doublet of triplets, dq) doublet of quartets, b) broad, ^{13}C NMR or DEPT-^{13}C (distortionless enhancement by polarization transfer ^{13}C-NMR) spectra were recorded with Bruker Avance III 400 MHz NMR spectrometer (100 MHz).

4.1.1. 3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(4-methoxy-2-methylphenyl)propan-1-one (3a)

To a 100 mL flask was added dichloromethane (50 mL), 3-methylanisole (5 g, 40.9 mmol), $AlCl_3$ (6.5 g, 48.7 mmol). The mixture was stirred at stirred at 0 ^{o}C for 30 min and added 3-chloropropinoyl chloride (5.2 g, 40.9 mml). The reaction was stirred at 0 ^{o}C for 6 hr and then was poured into ice-water, extrcted with dichloromethane, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed and the product was recrystallized from ethyl acetate and petroleum to give **2a** as a white solid (in freezer) that quickly became an oil in room temperature (7.6 g, yield 87%). ^{1}H NMR (DMSO-d_6, ppm): δ 7.68-7.70 (d, 1H), 6.73-6.80 (m, 2H), 3.81-3.84 (m, 5H), 3.31-3.34 (t, 2H), 2,53 (s, 3H).

The above intermediate **2a** (5 g, 23.5 mmol)was dissolved in dichloromethane (25 mL) and was added 6,7-dimethoxy1,2,3,4-tetrahydroisoquinoline hydrochloride (5.4 g, 23.5 mmol) and triethylamine (4 mL, 28.8 mmol). The reaction mixture was stirred at rt overnight and then poured into ice-water, extracted with dichloromethane, washed

with brine and dried over Na_2SO_4. The solvent was removed and the crude product was purified by silica gel colum to afford **3a** as a light yellow oil (6.8 g, yield 78%, HPLC 97.9%). ^{1}H NMR ($CDCl_3$, ppm): δ 7.75-7.78 (t, 1H), 6.74-6.77 (m, 2H), 6.59 (s, 1H), 6.52 (s, 1H), 3.82-3.83 (m, 9H), 3.61 (s, 2H), 3.18-3.22 (t, 2H), 2.93-2.97 (t, 2H), 2.80-2.82 (t, 2H), 2.75-2.77 (t, 2H), 2.54 (s, 3H). ^{13}C NMR ($CDCl_3$, ppm): δ 200.89, 161.82, 147.51, 147.18, 142.04, 131.54, 129.99, 126.34, 125.98, 117.53, 111.31, 110.61, 109.41, 55.90, 55.87, 55.70, 55.30, 53.39, 51.11, 38.83, 28.67, 22.38. Anal: C 71.28, H 7.23, N 3.66 (Calcd: C 71.52, H 7.37, N 3.79).

4.1.2. 3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(4-methoxy-2-methylphenyl)propan-1-ol (4a)

A solution of **3a** (4.5 g, 12.2 mmol) in methanol (15 mL) and $NaBH_4$ (0.5 g, 13.2 mmol) was stirred at rt for 6 hr. The mixture was poured into water and extracted with ethyl acetate, washed with brine and dried over Na_2SO_4. The solvent was removed in vacuum and the product was purified by silica gel column to give **4a** as a white solid product (3.8 g, yield 84%, HPLC 98.0%). mp 113-115℃; ^{1}H NMR ($CDCl_3$, ppm): δ 7.48-7.50 (d, 1H), 6.78-6.81 (dd, 1H), 6.70-6.71 (d, 1H), 6.63 (s,1H), 6.57 (s, 1H), 5.10-5.13 (m, 1H), 3.87 (6H, 2O**Me**), 3.78-3.83 (4H, C**H**-O + O**Me**), 3.62-3.78 (m, 2H), 2.74-2.94 (m, 6H), 2.32 (s, 3H), 1.88-1.93 (m, 2H); ^{13}C NMR ($CDCl_3$, ppm): δ 158.24, 147.67, 147.31, 135.44, 135.19, 126.77, 125.98, 125.87, 115.77, 111.30, 111.07, 109.41, 72.06, 56.95, 55.97, 55.94, 55.87, 55.19, 50.81, 32.70, 28.56, 19.20; Anal: C 70.91, H 7.68, N 3.59 (Calcd: C 71.13, H 7.87, N 3.77).

4.1.3. 5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(4-methoxy-2-methylphenyl)pentan-1-one (3b)

5-Chloro-1-(4-methoxy-2-methylphenyl)pentan-1-one (**2b**) was prepared according to the procedure of **2a** and abtained as a white solid in 80% yield. mp 97-98℃; ^{1}H NMR ($CDCl_3$, ppm): δ 7.71-7.74 (d, 1H), 6.75-6.78(m, 2H), 3.85 (s, 3H), 3.56-3.59 (m, 2H), 2.88-2.96 (m, 2H), 2.55 (s, 3H), 1.84-1.87 (m, 4H).

5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(4-methoxy-2-methylphenyl)pentan-1-one (**3b**) was prepared according to the procedure of **3a** and obtained as an oil in 78% yield (HPLC 98.0%). ^{1}H NMR ($CDCl_3$, ppm): δ 7.72-7.74 (d, 1H), 6.68-6.74 (m, 2H), 6.58 (s, 1H), 6.51 (s, 1H). 3.81-3.83 (t, 9H), 3.54 (s, 2H), 2.90-2.94 (t, 2H), 2.79-2.82 (t, 2H), 2.68-2.71 (t, 2H), 2.51-2.55 (m, 5H), 1.73-1.79 (m, 2H), 1.64-1.70 (m, 2H); ^{13}C NMR ($CDCl_3$, ppm): δ 202.12, 161.86, 147.43, 147.13, 141.86, 131.61, 130.08, 126.70, 126.22, 117.47, 111.31, 110.50, 109.45, 58.06, 55.90, 55.87, 55.83, 55.27, 51.03, 40.58, 28.71, 26.86, 22.79, 22.42; Anal: C 72.56, H 7.77, N 3.61 (Calcd: C 72.52, H 7.86, N 3.52).

4.1.4. 5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(4-methoxy-2-methylphenyl)pentan-1-ol (4b)

5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(4-methoxy-2-methylphenyl)pentan-1-ol (**4b**) was prepared according to the procedure of **4a** to give a white solid in 86% yield (HPLC 98.8%). mp 131-132℃; ^{1}H NMR ($CDCl_3$, ppm): δ

7.35-7.37 (d, 1H), 6.72-6.75 (dd, 1H), 6.66-6.67 (d, 1H), 6.58 (s, 1H), 6.49 (s, 1H), 4.81-4.84 (m, 1H), 3.79-3.85 (m, 10H, C**H**-O + 3O**Me**), 3.53 (s, 2H), 2.80-2.83 (t, 2H), 2.70-2.73 (t, 2H), 2.49-2.53 (t, 2H), 2.29 (s, 3H), 1.54-1.77 (m, 6H); ^{13}C NMR ($CDCl_3$, ppm): δ 158.34, 147.50, 147.17, 135.95, 135.58, 126.51, 126.19, 126.01, 115.76, 111.27, 111.23, 109.40, 70.03, 58.03, 55.89 (2C), 55.59, 55.16, 50.96, 37.95, 28.34, 26.80, 23.94, 19.30; Anal: C 71.92, H 8.19, N 3.66 (Calcd: C 72.15, H 8.33, N 3.45).

4.1.5. 2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)ethanone (3c)

2-Chloro-1-(2,4-dimethoxyphenyl)ethanone (**2c**) was prepared according to the procedure of **2a** and obtained as a white solid in 87.5% yield. mp 116-118 ^{o}C; ^{1}H NMR (DMSO-d_6, ppm): δ 7.95-7.98 (d, 1H), 6.57-6.60 (dd, 1H), 6.47-6.48 (d, 1H), 4.77 (s, 2H), 3.94 (s, 3H), 3.88 (s, 3H).

2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)ethanone (**3c**) was prepared according to the procedure of **3a** and was obtained as a light yellow solid in 79% yield (HPLC 98.2%). mp 117-119 ^{o}C; ^{1}H NMR ($CDCl_3$, ppm): δ 7.90-7.93 (d, 1H), 6.46-6.60 (m, 4H), 3.76-3.99 (m, 16H), 2.86-2.92 (m, 4H); ^{13}C NMR-DEPT135 ($CDCl_3$, ppm): δ 132.86, 111.33, 109.37, 105.32, 98.19, 67.88, 55.88, 55.58, 55.50, 55.38, 51.18, 28.51; Anal: C 68.06, H 6.65, N 3.71 (Calcd: C 67.91, H 6.78, N 3.77).

4.1.6. 2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)ethanol (4c)

2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)ethanol (**4c**) was prepared according to the procedure of **4a** to give a white solid in 92% yield (HPLC 99.1%). mp 119-120 ^{o}C; ^{1}H NMR ($CDCl_3$, ppm): δ 7.46-7.49 (d, 1H), 6.64 (s, 1H), 6.53-6.57 (m, 2H), 6.46-6.47 (d, 1H), 5.18-5.21 (m, 1H), 3.83-3.88 (4O**Me**+CH_2 + C**H**-O, 15H), 3.63-3.66 (d, 1H), 3.01-3.07 (m, 1H), 2.74-2.95 (m, 4H), 2.57-2.62 (m, 1H); ^{13}C NMR-DEPT135 ($CDCl_3$, ppm): δ 127.10, 111.36, 109.38, 104.26, 98.31, 64.27, 63.89, 55.97, 55.94, 55.49, 55.40, 55.37, 51.02, 28.62; Anal: C 64.37, H 7.31, N 3.54 (Calcd (+H_2O): C 64.43, H 7.47, N 3.58).

4.1.7. 3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)propan-1-one (3d)

3-Chloro-1-(2,4-dimethoxyphenyl)propan-1-one (**2d**) was prepared according to the procedure of **2a** and obtained as a white solid in 92% yield. mp 98-99 ^{o}C; ^{1}H NMR (DMSO-d_6, ppm): δ 7.69-7.72 (d, 1H), 6.60-6.65 (m, 2H), 3.84-3.90 (m, 8H), 3.36-3.39 (t, 2H).

3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)propan-1-one (**3d**) was prepared according to the procedure of **3a** and obtained as a light yellow oil in 97.7% yield (HPLC 97.8%). ^{1}H NMR (DMSO-d_6, ppm): δ 7.73-7.75 (d, 1H), 6.56-6.62 (m, 4H), 3.90 (s, 3H), 3.83 (s, 3H), 3.72 (s, 6H), 3.50 (s, 2H), 3.19 (b, 2H), 2.83 (b, 2H), 2.71 (b, 2H), 2.65 (b, 2H); ^{13}C NMR (DMSO-d_6, ppm): δ 198.54,

164.60, 160.98, 147.72, 147.47, 132.38, 126.98, 126.27, 120.88, 112.02, 110.27, 106.10, 98.67, 55.97, 55.77 (2Cs), 55.71, 55.52, 53.62, 51.09, 41.41, 28.86; Anal: C 68.42, H 6.94, N 3.49 (Calcd: C 68.55, H 7.06, N 3.63).

4.1.8. 3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)propan-1-ol (4d)

3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)propan-1-ol (**4d**) was prepared according to the procedure of **4a** to give a white solid in 93% yield (HPLC 99.2%). mp 114-116 ^{o}C; ^{1}H NMR (DMSO-d_6, ppm): δ 7.31-7.33 (d, 1H), 6.63-6.65 (d, 2H), 6.51-6.52 (m, 2H), 4.91-4.94 (m, 1H), 3.69-3.77 (m, 13H, C**H**-O + 4O**Me**), 3.44-3.46 (m, 2H), 2.70-2.72 (m, 2H), 2.55-2.63 (m, 4H), 1.63-1.89 (m, 2H); ^{13}C NMR (DMSO-d_6, ppm): δ 159.62, 156.78, 147.60, 147.34, 127.10, 127.07, 126.98, 126.31, 112.15, 110.39, 104.83, 98.38, 65.71, 55.91, 55.88, 55.77, 55.68, 55.54, 55.45, 51.19, 35.42, 28.78; Anal: C 68.07, H 7.28, N 3.55 (Calcd: C 68.20, H 7.54, N 3.61).

4.1.9. 5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)pentan-1-one (3e)

5-Chloro-1-(2,4-dimethoxyphenyl)pentan-1-one (**2e**) was prepared according to the procedure of **2a** and obtained as a white solid in 89% yield. mp 58-59 ^{o}C; ^{1}H NMR ($CDCl_3$, ppm): δ 7.80-7.22 (d, 1H), 5.52-5.55 (dd, 2H), 5.46-5.47 (d, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 3.56-3.59 (m, 2H), 2.97-3.00 (m, 2H), 1.83-1.85 (m, 4H).

5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)pentan-1-one (**3e**) prepared according to the procedure of **3a** and obtained as a white solid in 73% yield (HPLC 97.7%). mp 71-72 ^{o}C; ^{1}H NMR ($CDCl_3$, ppm): δ 7.78-7.80 (d, 1H), 6.58 (s, 1H), 6.51-6.52 (m, 2H), 6.44-6.45 (d, 1H), 3.82-3.87 (4OMe, 12H), 3.54 (s, 2H), 2.97-3.01 (t, 2H), 2.79-2.82 (t, 2H), 2.68-2.71 (t, 2H), 2.51-2.54 (t, 2H), 1.63-1.76 (m, 4H); ^{13}C NMR ($CDCl_3$, ppm): δ 200.50, 164.24, 160.65, 147.44, 147.14, 132.64, 126.55, 126.16, 121.24, 111.29, 109.44, 105.03, 98.35, 58.24, 55.84, 55.80, 55.77, 55.45, 55.41, 50.99, 43.45, 28.61, 26.97, 22.59; ^{13}C NMR-DEPT135 ($CDCl_3$, ppm): δ 132.64, 111.28, 109.43, 105.03, 98.34, 58.32, 55.90, 55.87, 55.82, 55.51, 55.46, 51.04, 43.47, 28.72, 27.03, 22.62;. Anal: C 69.59, H 7.45, N 3.26 (Calcd: C 69.71, H 7.56, N 3.39).

4.1.10. 5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)pentan-1-ol (4e)

5-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2,4-dimethoxyphenyl)pentan-1-ol (**4e**) was prepared according to the procedure of **4a** to give a white solid in 93% yield (HPLC 99.0%). mp 91-92 ^{o}C; ^{1}H NMR ($CDCl_3$, ppm): δ 7.21-7.23 (dd, 1H), 6.59 (s, 1H), 6.51 (s, 1H), 6.45-6.47 (m, 2H), 4.81-4.85 (m, 1H), 3.81-3.85 (4OMe, 12H), 3.54 (s, 2H), 2.95 (b, 1H), 2.81-2.84 (t, 2H), 2.68-2.71 (t, 2H), 2.48-2.52 (t, 2H), 1.77-1.84 (m, 2H), 1.61-1.68 (m, 2H), 1.49-1.56 (m, 1H), 1.37-1.45 (m, 1H); ^{13}C NMR-DEPT135 ($CDCl_3$, ppm): δ 127.46, 111.28, 109.42, 104.00, 98.58, 69.98, 58.27, 55.90, 55.88, 55.78, 55.37, 55.28, 51.07, 37.10, 28.60, 26.98, 24.03;

Anal: C 69.59, H 7.45, N 3.26 (Calcd: C 69.71, H 7.56, N 3.39).

4.1.11. 3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-1-(2-hydroxy-3,4,5-trimethoxy-6-methylphenyl)propan-1-one (3f)

A solution of 1,2,3,4-tetramethoxy-5-methylbenzene (1.0 g, 4.7 mmol) in dichloromethane (25 mL) was added with $AlCl_3$ (0.75 g, 5.6 mmol) and stirred at 0 ^oC. A solution of 3-chloropropanoyl chloride (0.65 g, 5.1 mmol) was added dropwise at 0 ^oC and then the reaction was stirred at rt overnight. The reaction mixture was poured onto 10% HCl ice-water, extracted with dichloromethane, washed with brine and dried, solvent removed to give **2f** (1.05 g, crude yield 74%) as an oil product that decomposed quickly upon standing or column purification, therefore was used directly in the next reaction.

To a solution of **2f** (0.4 g, 1.3 mmol) in dichloromethane (10 mL) and was added 6,7-dimethoxy1,2,3,4-tetrahydroisoquinoline hydrochloride (0.3 g, 1.3 mmol) and triethylamine (0.4 g, 4.0 mmol). The reaction was stirred at rt for 6 hr and extrated with dichloromethane, eashed with brine, dried over anhydrious sodium sulfate. The crude product was purified by silica gel column to give **3f** as a light yellow solid (0.21 g, 36%). mp 87-90 ^oC; ^1H NMR ($CDCl_3$, ppm): δ 6.58 (s, 1H), 6.51 (s, 1H), 3.96 (s, 3H), 3.82-3.85 (m, 9H), 3.75 (s, 3H), 3.69 (s, 2H), 3.05-3.10 (m, 4H), 2.86 (s, 4H), 2.17 (s, 3H); ^{13}C NMR ($CDCl_3$, ppm): δ 206.89, 148.48, 147.75, 147.38, 145.73, 144.51, 139.67, 125.50, 125.10, 123.68, 123.63, 111.29, 109.40, 61.04, 61.03, 60.82, 55.92, 55.91, 55.12, 53.70, 50.88, 42.55, 27.49, 12.76; Anal: C 64.73, H 6.97, N 3.08 (Calcd: C 64.70, H 7.01, N 3.14).

4.1.12. 3-(3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)propyl)-6,7-dimethoxyquinazolin-4(3H)-one (8a)

To a 100 mL flask was added 3,4-dimethoxybenzoic acid (10g, 54.9 mmol) and nitric acid (50 mL, 20%) in an ice-bath. The reaction mixture was then stirred at 60 ^oC for 6 hr. After cooling to rt, the mixture was poured onto ice-water. The solid was filtered, washed with water, dried. The crude was purified by a silica gel column to afford 2-nitro-4,5-dimethoxybenaoic acid as a light yellow solid (9.6 g, yield 77%). mp 195-197 ^oC; ^1H NMR ($CDCl_3$, ppm): δ 7.42 (s, 1H), 7.26 (s, 1H), 4.03 (s, 3H), 4.02 (s, 3H).

A solution of 2-nitro-4,5-dimethoxybenaoic acid (8 g, 35.2 mmol) in EtOH (50 mL) was added with ammonium formate (8.8 g, 139.7 mmol) and 5% Pd-C (0.19 g) and wad heated to reflux for 6 hr. The reaction was cooled to rt and filtered off the solid. The solution was concentrated to dryness that was then purified by silica gel column to give 2-amino-4,5-dimethoxy benzoic acid (**5a**) as a white solid (6 g, yield 65%). mp 156-157 ^oC; ^1H NMR ($CDCl_3$, ppm): δ 7.36 (s, 1H), 6.17 (s, 1H), 3.91 (s, 3H), 3.86 (s, 3H).

6,7-dimethoxyquinazolin-4(3H)-one (**6a**) was prepared according to a similar procedure of Luth and Lowe [62], with formamide in replace of formamidine acetate. Briefly, a solution of **5a** (5 g, 25.4 mmol) in formamide (30 mL) was heated to reflux for 4 hr, cooled to rt and poured onto ice-water, extracted with ethyl acetate, washed

with brine and dried over Na_2SO_4, After removal of the solvent, the residue was purified by silica gel column to give 6,7-dimethoxyquinazolin-4(3H)-one (**6a**) as a white solid (4.5 g, yield 86%). mp 310-312 ^oC (reference mp 300 ^oC [64]).

To a 100 mL flask was added **6a** (2 g, 9.7 mmol), DMF (20 mL) and 1-bromo-3-chloropentane (3 g, 19 mmol). The solution was stirred at rt and added NaH (0.47 g, 19.6 mmol) and then heated to 80 ^oC for 6 hr. After cooled to rt, the solution was poured onto ice-water, extracted with dichloromethane, washed with brine and dried over Na_2SO_4. The crude product was purified by silica gel column to afford 3-(3-chloropropyl)-6,7-dimethoxyquinazolin-4(3H)-one (**7a**) as an oil (1.7 g, yield 62%). ^1H NMR ($CDCl_3$, ppm): δ 8.28 (s, 1H), 6.83-6.85 (m, 1H), 6.68-6.71 (m, 1H), 3.83-3.88 (m, 8H), 3.48-3.52 (t, 2H). 1.97-2.07 (m, 2H).

A solution of **7a** (1.0 g, 3.5 mmol), DMF (10 mL), K_2CO_3 (1 g, 7.2 mmol), and 1,2,3,4-tetrahydroisoquinoline hydrochloride (0.9 g, 3.9 mmol) was stirred at 85 ^oC for 8 hr. After cooled to rt, the mixture was poured onto ice-water, extracted with dichloromethane, washed with brine, and dried over Na_2SO_4. The crude product was purified by a silica gel column to afford **8a** as an oil (1.1 g, yield 71%, HPLC 97.1%). ^1H NMR ($CDCl_3$, ppm): δ 8.30 (s, 1H), 6.82-6.86 (m, 1H), 6.69-6.74 (m, 1H), 6.56 (s, 1H), 6.46 (s, 1H), 3.81-3.88 (m, 14H), 3.47-3.48 (s, 2H), 2.75-2.79 (m, 2H), 2.62-2.65 (t, 2H), 2.49-2.53 (t, 2H), 1.81-1.85 (m, 2H); ^{13}C NMR ($CDCl_3$, ppm): δ 162.49, 149.60, 148.20, 147.48, 147.16, 134.06, 126.30, 126.01, 117.39, 111.39, 111.27, 109.36, 108.70, 56.10, 56.04, 55.89, 55.88, 55.62, 55.33, 50.97, 43.85, 28.56, 25.36; Anal: C 62.87, H 6.67, N 9.07 (Calcd (+H_2O): C 63.00, H 6.83, N 9.18).

4.1.13. 3-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-6,7-dimethoxyquinazolin-4(3H)-one (8b)

3-(4-chlorobutyl)-6,7-dimethoxyquinazolin-4(3H)-one (**7b**) was prepared according to the procedure of **7a** and obtained as an light yellow oil in 63% yield. ^1H NMR ($CDCl_3$, ppm): δ 8.28-8.30 (d, 1H), 6.86-6.88 (m, 1H), 6.67-6.68 (d, 1H), 3.87-3.89 (m, 6H), 3.77-3.80 (t, 2H), 3.52-3.55 (t, 1H), 3.39-3.42 (t, 1H), 1.72-1.92 (m, 4H).

3-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-6,7-dimethoxyquinazolin-4(3H)-one (**8b**) was prepared according to the procedure of **8a** and obtained as an oil in 72% yield (HPLC 97.3%). ^1H NMR ($CDCl_3$, ppm): δ 8.30 (s, 1H), 6.83-6.86 (m, 1H), 6.73-6.75 (m, 1H), 6.58 (s, 1H), 6.51 (s, 1H), 3.79-3.92 (m, 14H), 3.56 (s, 1H), 2.80-2.83 (t, 2H), 2.70-2.73 (t, 2H), 2.51-2.55 (t, 2H), 1.61-1.63 (m, 4H); ^{13}C NMR ($CDCl_3$, ppm): δ 162.55, 149.60, 148.24, 147.57, 147.24, 133.87, 125.88, 117.49, 111.39, 111.28, 109.40, 108.77, 57.44, 56.10, 55.92, 55.89, 55.70, 55.50, 50.79, 45.07, 28.28, 25.47, 24.10; Anal: C 64.90, H 6.81, N 8.94 (Calcd (+1/2H_2O): C 64.92, H 6.97, N 9.08).

4.1.14. 7-Chloro-3-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-6-nitroquinazolin-4(3H)-one (8c)

A solution of 2-amino-4-chlorobenaoic acid (20 g, 116.5 mmol) in formamide (50 mL) was heated to 130 ^oC under stirring for 30 min and then at 160 ^oC for 4 hr. after

cooling to rt, the mixture was poured onto ice-water and the solid wad filtered, washed and dried, and recrystallized from ethyl acetate to give 7-chloroquinazolin-4(3H)-one (**6b**) as a white solid (19.1 g, yield 91%). ^{1}H NMR (DMSO-d_6, ppm): δ 12.41 (b, 1H), 8.11-8.15 (m, 2H), 7.73 (s, 1H), 7.55-7.57 (m, 1H).

To a 100-mL flask was added concentrated sulfuric acid (10 mL), concentrated nitric acid (5 mL) and **6b** (10 g, 55.4 mmol) under stirring at 0 ^{o}C. The mixture was heated and stirred at 90 ^{o}C for 3 hr. After cooling to rt, the mixture was poured onto ice-water, filtered, washed with water, dried. The crude product was recrystallized from acetic acid to afford 7-chloro-6-nitroquinazolin-4(3H)-one (**6c**) as a light yellow solid (9 g, yield 72%). mp 315-316 ^{o}C; ^{1}H NMR (DMSO-d_6, ppm): δ 12.79 (b, 1H), 8.67 (s, 1H), 8.31 (s, 1H), 8.01 (s, 1H).

A solution of **6c** (2 g, 8.8 mmol) in DMF (20 mL), 1-bromo-2-chloroethane (1.9 g, 13.2 mmol) and NaH (0.4 g, 17.9 mmol) was stirred at 80 ^{o}C for 6 hr, cooled, poured onto ice-water. The solid was filtered, washed with water, dried, to give 7-chloro-3-(2-chloroethyl)-6-nitroquinazolin-4(3H)-one (**7c**) as a light yellow solid (2.5 g, yield 98%). mp 186-187 ^{o}C; ^{1}H NMR ($CDCl_3$, ppm): δ 8.83 (s, 1H), 8.21 (s, 1H), 7.93 (s, 1H), 4.41-4.44 (m, 1H), 4.34-4.37 (m, 1H), 3.93-3.96 (m, 1H), 3.79-3.82 (m, 1H).

A solution of **7c** (1 g, 3.47 mmol), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (0,88 g, 3.8 mmol) and K_2CO_3 (1 g, 7.2 mmol) in DMF (20 mL) was stirred at 80 ^{o}C for 6 hr. After cooling to rt, the mixture was poured onto ice-water, filtered, washed and dried. The crude product was purified by a silica gel column to afford **8c** as a white solid (0.96 g, yield 62%, HPLC 98.6%). mp 179-180 ^{o}C; ^{1}H NMR ($CDCl_3$, ppm): δ 8.69 (s, 1H), 8.13 (s, 1H), 7.30 (s, 1H), 6.67 (s, 1H), 6.62 (s, 1H), 4.34 (s, 2H), 4.28-4.31 (t, 2H), 3.90-3.93 (t, 2H), 3.87-3.88 (d, 6H), 3.52-3.55 (t, 2H), 2.95-2.98 (t, 2H); ^{13}C NMR ($CDCl_3$, ppm): δ 159.55, 151.00, 149.61, 148.76, 148.04, 147.80, 139.75, 126.88, 126.16, 124.48, 114.11, 112.51, 111.30, 109.03, 56.04, 55.99, 51.66, 49.16, 48.99, 41.96, 27.82; Anal: C 56.54, H 4.62, N 12.37 (Calcd: C 56.70, H 4.76, N 12.59).

4.1.15. 7-Chloro-3-(3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)propyl)-6-nitroquinazolin-4(3H)-one (8d)

7-Chloro-3-(3-chloropropyl)-6-nitroquinazolin-4(3H)-one (**7d**) was prepared according to the procedure of **7a** to afford a light yellow solid in 95% yield, mp 150-151 ^{o}C. ^{1}H NMR ($CDCl_3$, ppm): δ 8.81 (s, 1H), 8.22-8.24 (d, 1H), 7.91 (s, 1H), 4.21-4.25 (m, 2H), 3.61-3.64 (t, 1H), 3.45-3.48 (t, 1H), 2.38-2.44 (m, 1H), 2.30-2.36 (m, 1H).

7-Chloro-3-(3-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)propyl)-6-nitroquinazolin-4(3H)-one (**8d**) was prepared according to the procedure of **8c** as a light yellow solid in 63% yield (HPLC 98.2%). mp 177-179 ^{o}C; ^{1}H NMR ($CDCl_3$, ppm): δ 8.67 (s, 1H), 8.08 (s, 1H), 7.26 (s, 1H), 6.67 (s, 1H), 6.61 (s, 1H), 4.32 (s, 2H), 4.14-4.17 (t, 2H), 3.87-3.88 (d, 6H), 3.59-3.62 (t, 2H), 3.51-3.54 (t, 2H), 2.94-2.97 (t, 2H), 2.26-2.32 (m, 2H); ^{13}C NMR ($CDCl_3$, ppm): δ 159.82, 151.43, 149.37, 148.62,

148.02, 147.78, 139.80, 126.75, 126.17, 124.54, 114.36, 112.94, 111.31, 109.03, 56.03, 55.98, 51.65, 49.20, 44.52, 41.51, 30.98, 27.83; Anal: C 57.41, H 4.89, N 12.03 (Calcd: C 57.58, H, 5.05, N 12.21).

4.1.16. 7-Chloro-3-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-6-nitroquinazolin-4(3H)-one (8e)

7-Chloro-3-(4-chlorobutyl)-6-nitroquinazolin-4(3H)-one (**7e**) was prepared according to the procedure of **7a** to afford a light yellow solid in 91% yield, mp 153-154 ^{o}C. ^{1}H NMR ($CDCl_3$, ppm): δ 8.82 (s, 1H), 8.17 (s, 1H), 7.91 (s, 1H), 4.07-4.11 (m, 2H), 3.47-3.64 (m, 2H), 1.88-2.05 (m, 4H).

7-Chloro-3-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-6-nitroquinazolin-4(3H)-one (**8e**) was prepared according to the procedure of **8c** as a light yellow solid in 65% yield (HPLC 98.8%). mp 175-176 ^{o}C; ^{1}H NMR ($CDCl_3$, ppm): δ 8.66 (s, 1H), 8.02 (s, 1H), 7.24 (s, 1H), 6.66 (s, 1H), 6.61 (s, 1H), 4.32 (s, 2H), 3.98-4.02 (t, 2H), 3.86-3.87 (d, 6H), 3.57-3.60 (t, 2H), 3.49-3.52 (t, 2H), 2.93-2.96 (t, 2H), 1.92-2.00 (m, 2H), 1.82-1,89 (m, 2H); ^{13}C NMR ($CDCl_3$, ppm): δ 159.72, 151.39, 149.11, 148.57, 148.00, 147.76, 139.79, 126.81, 126.17, 124.56, 114.35, 112.99, 111.30, 109.04, 56.03, 55.97, 51.63, 49.24, 46.08, 44.15, 29.40, 27.84, 26.92; Anal: C 58.24, H 5.26, N 11.69 (Calcd: C 58.41, H 5.33, N 11.85).

4.2. Biological Assays

4.2.1. Tissue Source and Radioligands

Sigma-1 receptor binding was labeled with [^{3}H]-(+)-pentazocine (DuPont-NEN, Billerica, MA) in guinea pig brain membranes (Rockland Biological, Gilbertsville, PA) according to published procedures [10]. Sigma-2 receptor binding was labeled with [^{3}H]-DTG (DuPont-NEN, Boston, MA) in rat liver membranes in the presence of (+)-pentazocine.

4.2.2. Membrane Preparation

Quinea pig brain membrane homogenates were prepared from frozen guinea pig brains without cerebellum. Liver membrane homogenates were prepared from the livers of male Sprague-Dawley rats (300-350 g) according to reported procedures [47].

4.2.3. Sigma-1 Binding Assay

Guinea pig brain membrane homogenates (100 μL, 300 μg) were incubated with 50 μL of 6 nM [^{3}H]-(+)-pentazocine (~42 Ci/mmol) and 50 μL of each test compound solution in 50 mM Tris-HCl (pH 8.0) for a total volume of 200 μL at 25 °C for 120 min. Test compounds were dissolved in ethanol and then diluted in buffer and added in concentrations ranging from 0.1 to 1000 nM. Incubations were terminated by the addition of ice-cold 10 mM Tris-HCl (pH 8.0) and followed by rapid filtration through Whatman GF/B glass fiber filters (presoaked in 0.5% polyethylenimine) using a Brandel cell harvester (Gaithersburg, MD). Filters were washed 3 times with 5 mL of ice cold buffer. Each filter was removed with forceps, added into 4 mL vials, added 3

mL microscintillation 20, and counted in microbeta counter with 45% efficiency. Nonspecific binding was determined in the presence of 10 μM (+)-pentazocine.

4.2.4. Sigma-2 Binding Assay

Rat liver membrane homogenates (100 μL, 300 μg) were incubated with 50 μL of 5.65 nM [^{3}H]DTG (~82 Ci/ mmol), 50 μL of each test compound solution, and 50 μL of 100 nM (+)-pentazocine in 50 mM Tris-HCl (pH 8.0) for a total volume of 250 μL at 25 °C for 120 min. Test compounds were dissolved in ethanol and then diluted in buffer and added in concentrations ranging from 0.1 to 1000 nM. Incubations were terminated by the addition of ice-cold 10 mM Tris-HCl (pH 8.0) and followed by rapid filtration through Whatman GF/B glass fiber filters (presoaked in 0.5% polyethylenimine) using a Brandel cell harvester (Gaithersburg, MD). Filters were washed 3 times with 5 mL of ice-cold buffer. Each filter was removed with forceps, added into 4 mL vials, added 3 mL microscintillation 20, and counted in microbeta counter with 45% efficiency. Nonspecific binding was determined in the presence of 5 μM DTG.

4.2.5. Data Analysis

The IC_{50} values for sigma receptors were generally determined in triplicate from nonlinear regression of binding data as analyzed by Prism (GraphPad Software, Inc., La Jolla, CA), using -10 concentrations of each compound. K_i values were calculated using the method of Cheng-Prusoff and represent mean values.

4.2.6. Cytotoxicity Assay

CCK-8 assays were carried out on Huh7 hepatocellular carcinoma cells and KYSE-140 esophageal squamous carcinoma cells to primarily determine the anti-cancer activities of these drugs. Briefly, Huh7 and KYSE-140 cells with exponential growth were seeded into 96-well culture plates at a density of 4,000 cells/well and allowed to adhere overnight. The cells were then treated with a series of 6 concentrations of the test compounds for 48 h. Cells were treated with an equal volume of DMSO as the control. Thereafter, 10 μL CCK-8 solutions (Dojindo Laboratories) were added to each well and the plates were incubated at 37 °C for 2 h in the dark. The UV absorbance was measured at 450nm with an enzyme immunoassay reader (Bio-Rad). Cell viability was calculated as (optical density of experimental sample/optical density of control) ×100%. The IC_{50} values were calculated using the Prism 6.0 program (GraphPad).

Supporting Information

Spectroscopic data (^{1}H and ^{13}C NMR) for the intermediates and the test compounds are attached at the end of this manuscript.

Acknowledgments

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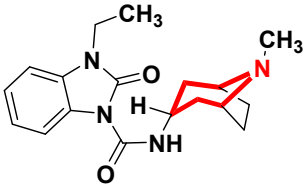
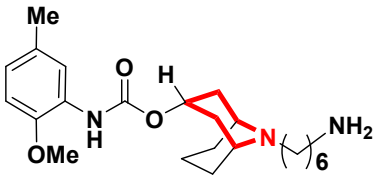
Representative tropane and granatane sigma-2 analogs

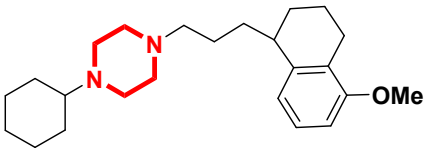


BIMU-1 K_i (σ_1): 6300 nM
K_i (σ_2): 32 nM

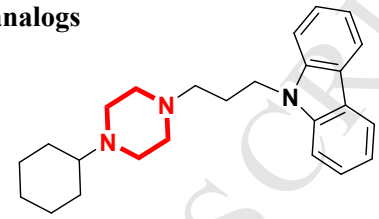


SV119 K_i (σ_1): 1418 ± 439 nM
K_i (σ_2): 5.19 ± 0.8 nM

Representative cyclohexylpiperazine sigma-2 analogs

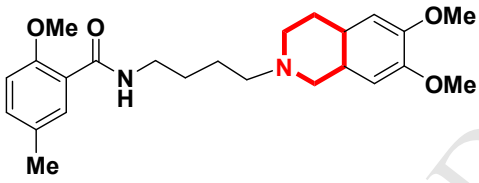


PB28 K_i (σ_1): 13.6 ± 1.9 nM
K_i (σ_2): 0.34 ± 0.02 nM

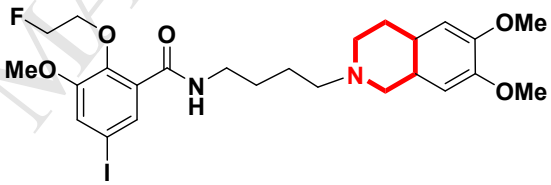


F281 K_i (σ_1): 3450 ± 1660 nM
K_i (σ_2): 12.6 ± 3.2 nM

Representative 6,7-dimethoxytetrahydroisoquinoline benzamide sigma-2 analogs

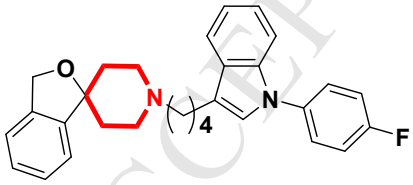


RMH-1 K_i (σ_1): 3078 ± 86.89 nM
K_i (σ_2): 10.33 ± 1.46 nM

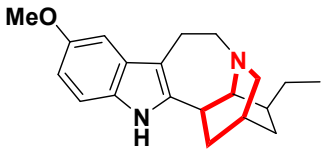


[18F]ISO-1 K_i (σ_1): 2150 ± 410 nM
K_i (σ_2): 0.26 ± 0.07 nM

Representative indole sigma-2 analogs



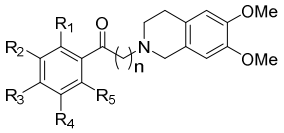
Siramesine
Lu28-179 K_i (σ_1): 17 nM
K_i (σ_2): 0.12 nM



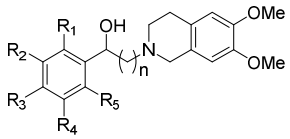
Ibogaine K_i (σ_1): 9310 ± 630 nM
K_i (σ_2): 90.4 ± 10.1 nM

Scheme 1. Representative sigma-2 selective ligands from 4 major structural classes. Binding data were extracted from reference [1].

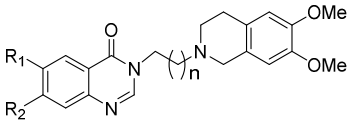
Table 1. Sigma receptor binding profiles in cell membranes and cytotoxicities against liver Huh-7 and KYSE-140 cancer cells for analogs **3a-3f**, **4a-4e**, **8a-8e**.



3a R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, n = 2
3b R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, n = 4
3c R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 1
3d R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 2
3e R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 4
3f R_1= OH, R_2,R_3,R_4 = OMe, R_5 = Me, n = 2



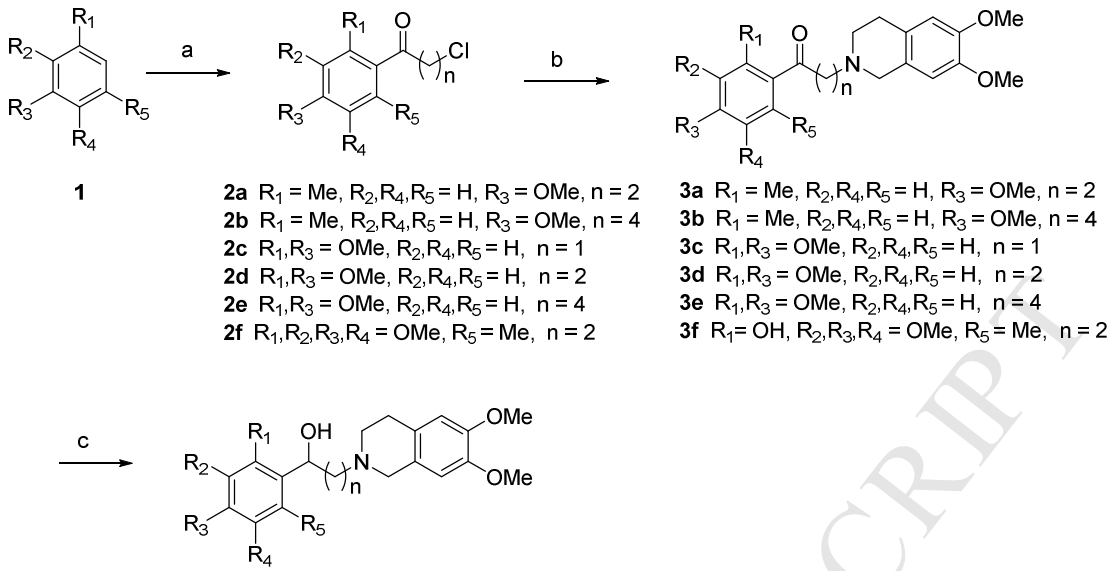
4a R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, n = 2
4b R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, n = 4
4c R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 1
4d R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 2
4e R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 4



8a R_1,R_2 = OMe, n = 2
8b R_1,R_2 = OMe, n = 3
8c R_1 = NO_2, R_2 = Cl, n = 1
8d R_1 = NO_2, R_2 = Cl, n = 2
8e R_1 = NO_2, R_2 = Cl, n = 3

	Ki (nM ± SEM) [a]			IC50 (μM ± SEM)[e]	
Analog	σ_1[b]	σ_2[c]	σ_1/σ_2[d]	Huh-7	KYSE-140
3a	13.3 ± 1.6	23.7 ± 7.4	0.5	42.83 ± 4.37	60.11 ± 2.61
3b	> 1000	5.1 ± 1.5	> 196	NA	NA
3c	> 1000	53.8 ± 1.9	> 18	NA	NA
3d	> 1000	68.1 ± 21.2	> 14	75.33 ± 5.81	NA
3e	32.9 ± 4.1	5.8 ± 0.8	5.6	NA	88.32 ± 1.80
3f	> 1000	> 1000		81.63 ± 2.85	93.73 ± 5.53
4a	> 1000	18.2 ± 3.9	> 55	NA[f]	NA
4b	> 1000	5.2 ± 1.0	> 192	12.50 ± 0.36	14.86 ± 0.90
4c	> 1000	62.0 ± 9.3	> 16	NA	NA
4d	> 1000	30.2 ± 3.9	> 33	45.89 ± 5.36	26.10 ± 3.22
4e	> 1000	6.2 ± 1.6	> 161	NT[g]	NT
8a	> 1000	13.9 ± 2.9	> 71.9	NA	NA
8b	> 1000	43.8 ± 0.3	> 22.8	NA	NA
8c	> 1000	> 1000		NA	NA
8d	> 1000	> 1000		NA	NA
8e	> 1000	> 1000		NA	NA
DTG	58.5 ± 5.8	19.4 ± 1.2			
Cisplatin				15.31 ± 0.17	21.34 ± 1.08

[a] Mean (SEM), *K*i values were determined by at least three experiments. Each inhibition curve consisted of 10 points from each binding assay. [b] *K*i values for σ_1 receptor were measured on guinea pig brain membranes using [^{3}H](+)-pentazocine as the radioligand. [c] *K*i values for σ_2 receptor were measured on rat liver membranes using [^{3}H]-DTG as the radioligand in the presence of (+)-pentazocine. [d] *K*i value for σ_1 receptor/*K*i value for σ_2 receptor. [e] IC50 values were determined by at least three experiments. Each inhibition curve consisted of7 points from each inhibition assay against the growth of Huh-7 liver cancer cells. [f] NA indicates cytotoxicity IC50 > 100 nM or not accurately determined. [g] NT indicates not tested.



1

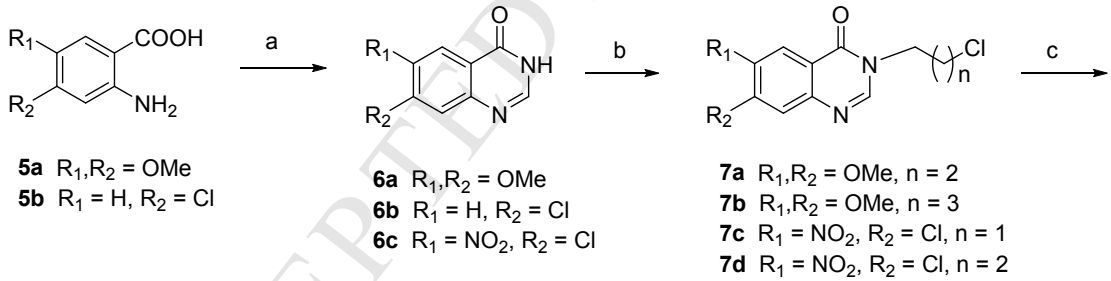
2a R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, n = 2
2b R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, n = 4
2c R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 1
2d R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 2
2e R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 4
2f R_1,R_2,R_3,R_4 = OMe, R_5 = Me, n = 2

3a R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, n = 2
3b R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, n = 4
3c R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 1
3d R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 2
3e R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 4
3f R_1= OH, R_2,R_3,R_4 = OMe, R_5 = Me, n = 2

4a R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, n = 2
4b R_1 = Me, R_2,R_4,R_5 = H, R_3 = OMe, n = 4
4c R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 1
4d R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 2
4e R_1,R_3 = OMe, R_2,R_4,R_5 = H, n = 4

Scheme 2. Synthesis of analogs **3a-3f** and **4a-4e**.

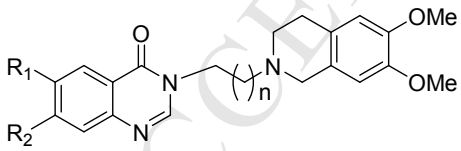
Reagents and conditions: a) chloroalkyl chloride/$AlCl_3$, 0 ^oC; b) 6,7-dimethoxy-1,2,3,4-tetrahydro –isoquinoline HCl/Et_3N, rt; c) $NaBH_4$, rt.



5a R_1,R_2 = OMe
5b R_1 = H, R_2 = Cl

6a R_1,R_2 = OMe
6b R_1 = H, R_2 = Cl
6c R_1 = NO_2, R_2 = Cl

7a R_1,R_2 = OMe, n = 2
7b R_1,R_2 = OMe, n = 3
7c R_1 = NO_2, R_2 = Cl, n = 1
7d R_1 = NO_2, R_2 = Cl, n = 2
7e R_1 = NO_2, R_2 = Cl, n = 3



8a R_1,R_2 = OMe, n = 2
8b R_1,R_2 = OMe, n = 3
8c R_1 = NO_2, R_2 = Cl, n = 1
8d R_1 = NO_2, R_2 = Cl, n = 2
8e R_1 = NO_2, R_2 = Cl, n = 3

Scheme 3. Synthesis of analogs **8a-8e**.

Reagents and conditions: a) formamide, reflux; b) bromoalkyl chloride, 80 ^oC; c) 6,7-dimethoxy-1,2,3,4-tetrahydro –isoquinoline HCl/Et_3N, 85 ^oC.

Highlights

- A total of 16 of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline analogs were synthesized and their structures were confirmed by ^{1}H NMR, ^{13}C NMR, elemental analysis.
- These analogs were assayed for sigma-1 and sigma-2 receptor binding with 4 of them showing excellent affinities and selectivities for sigma-2 receptor.
- These four analogs are among the most potent sigma-2 ligands and are potentially useful for further development as cancer diagnostic agents, anticancer adjuvants, or pharmacological tools for the study of sigma-2 receptor.
- These analogs were also screened for their cytotoxicities against liver cancer Huh-7 and esophagus KYSE-140 cancer cells and analog **4b** showed promising anticancer activities against both Huh-7 and esophagus KYSE-140 cancer cells, which are comparable to those of cisplatin in the same assay.