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Identification of N3-Substituted oxazolidinones as subtype selective 5-HT2b ligands

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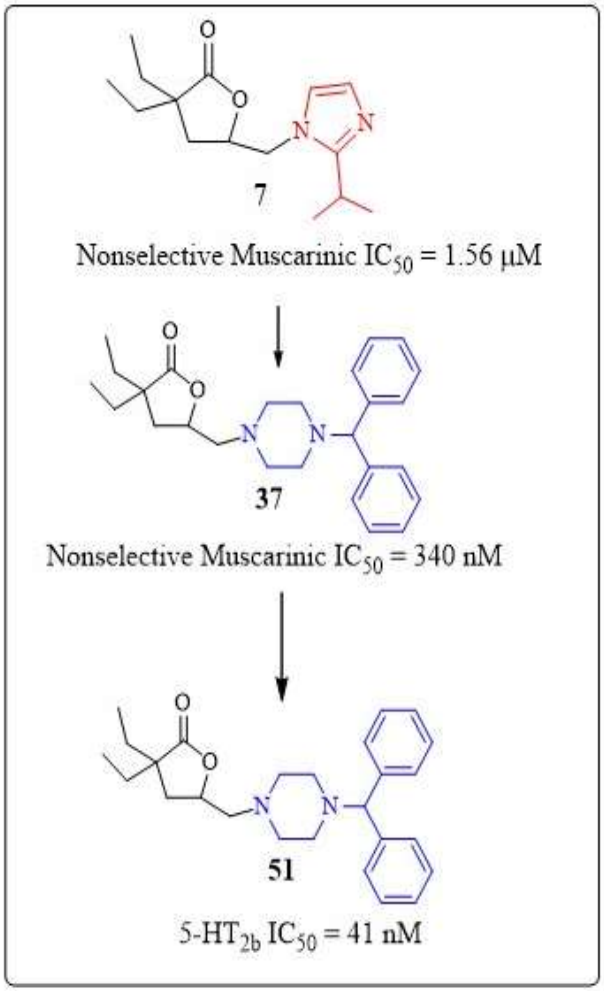
Graphical Abstract



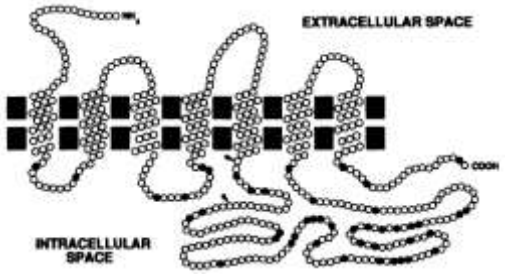
Abstract: The purpose of this study was to evaluate the binding of N3-substituted oxazolidinone-based compounds against a panel of receptor subtypes. Literature reports suggests that the oxazolidinone nucleus is a suitable fragment for binding to GPCR receptors including adenosine, muscarinic and serotonin receptors. Previous studies involving oxazolidinone-based and Chromone-based compounds previously synthesized in our laboratory exhibited low to moderate affinity for muscarinic receptors. Based on these reports, we decided to screen our oxazolidinone and chromone based compounds in GPCR receptor panels using a receptor radioligand binding assay. Test compounds were evaluated for affinity in the binding assays and those ligands exhibiting % specific inhibition >50% were selected for further evaluation (IC_{50} and ultimately subtype selectivity). Preliminary binding evaluation of oxazolidinone-based compounds indicate that the oxazolidinone nucleus represents a novel chemical entity in serotonergic (5-HT) ligands. The novel ligands evaluated for 5-HT subtype selectivity were found to be selective towards the serotonin subtype 2b. A cyclopentyl substituted oxazolidinone-based ligand containing a diphenylmethylpiperazine fragment (Compound 51) was identified as a 5-HT2b ligand with an IC_{50} of 41 nM. The synthesis and evaluation of our novel oxazolidinone-based 5-HT ligands will be presented along with a discussion of the structure-activity relationship data for the series. The compounds reported herein represent an interesting series of novel 5-HT ligands that warrant further study. The data provided herein will assist in the design of future 5-HT2b ligands possessing improved affinity and selectivity. Such compounds will be useful research tools with which to better understand the physiological role of the 5-HT2b receptor subtype.

Keywords: GPCR, Oxazolidinones, Chromones, 5-HT2b

Introduction

G-protein coupled receptors (GPCRs)

- The G-protein coupled receptor (GPCR) superfamily is one of the largest families of proteins found in nature.
- Designing ligands for GPCRs for therapeutic purposes has been fruitful with more than 50 % of drugs in the market.
- The GPCR superfamily is characterized by the presence of seven transmembrane helical domains (TM I - VII) which are connected by three intracellular and three extracellular loops.
- The human GPCRs can be classified into five distinct families: rhodopsin, glutamate receptor, adhesion receptor, taste2 receptor and secretin receptor.

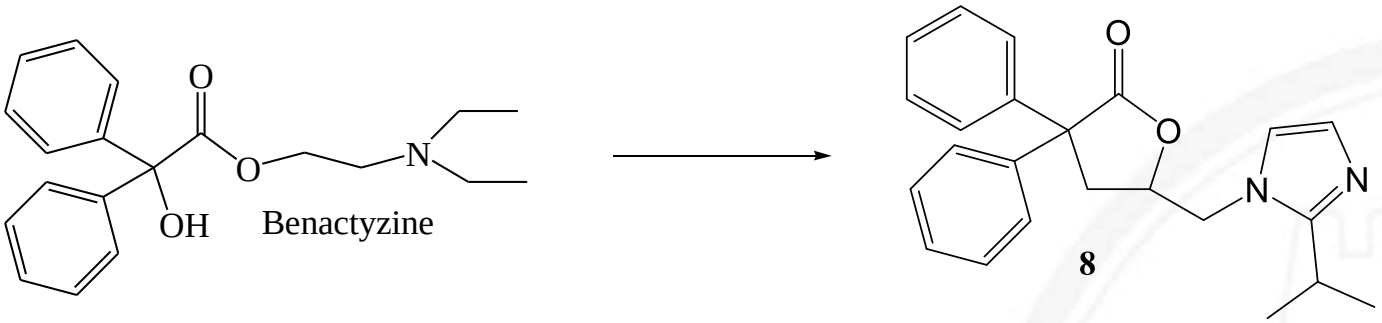


Distribution and functional role of muscarinic receptors

Peripheral	Central
Bladder: M_2, M_3	Brain: M_1: neocortex, hippocampus and neostriatum M_2: throughout the brain M_3: cortex, hippocampus M_4: neostriatum, hippocampus and cortex M_5: striatum, substantia nigra
Salivary glands: M_1, M_3	
Gastrointestinal tract: M_2, M_3	
Heart: M_2, M_3, M_1	

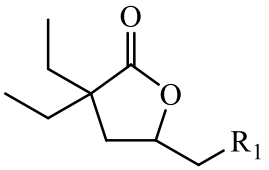
Disease/ disorder	Target receptor
Alzheimer's disease and cognitive impairments	M_1, M_2, M_5
Schizophrenia	M_2, M_4
Pain	M_2
COPD/bronchial asthma	M_3
Parkinson's disease	M_4, M_5
Impairment in salivary secretions	M_3
Gastrointestinal (GI) and Urinary bladder disorder	M_3
Peptic ulcer	M_1, M_3, M_5
Type 2 diabetes	M_3

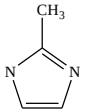
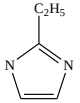
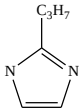
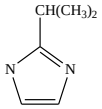
Lactone based muscarinic ligands designed by Kaiser



Antimuscarinic activity		
Vas deferens rabbit *K*i (nM) M_1	atria, guinea pig *K*i (nM) M_2	ileum, guinea pig *K*i (nM) M_3
133	115	8

Structure of representative lactone - based muscarinic ligands reported by our lab



		% Initial inhibtion at 10 μM[a,b]		
Compound #	R_1	M_1 [^{3}H]-pirenzepine (*K*i = 2.2 nM)	M_2 [^{3}H]-AF-DX 384 (*K*i = 6.4 nM)	M_3 [^{3}H]-NMS (*K*i = 1.4 nM)
1		63.2	**83.8**	53.2
2		17	**58.7**	38.6
3		17.8	**64.4**	19.1
4		60.36	35.36	0.1
5		**53.32**	32.56	15.59
6		**66.86**	22.31	13.96
7		**81.65**	38.62	17.89

In-vivo functional analysis revealed these lactones to be M_1 agonist

Challenges in developing subtype selective ligands

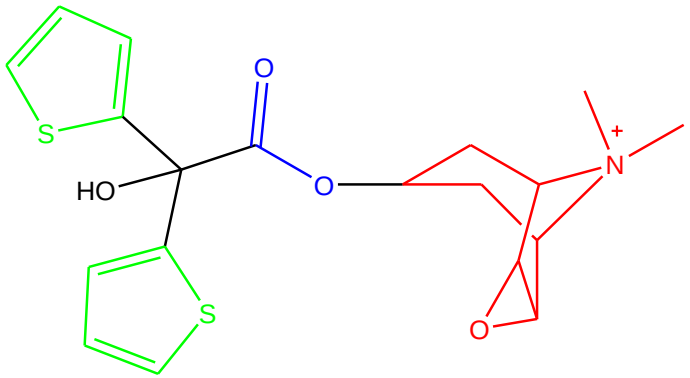
- No truly selective ligands on which to base design
- Homology of subtypes
- Crystal structure based on rhodopsin until recent availability of M_2 and M_3 crystal structure

But…….

- SAR data from available ligands may be useful in design
- Pharmacophore/homology model
- Use of crystal structure for the design of new ligands

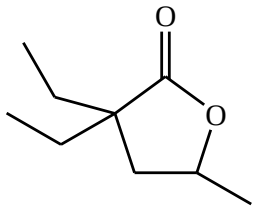
Pharmacophoric requirements

- Suitable hydrogen bonding center
- Quaternary ammonium group or its equivalent
- Presence of suitably located lipophilic/alkyl groups



Tiotropium

Proposed ligand scaffold



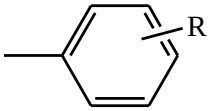
Hydrogen bonding center

CH_2

Linker

Piperazine,
Piperidine or
N containing heterocycles
[Fragments from literature]

Cationic center



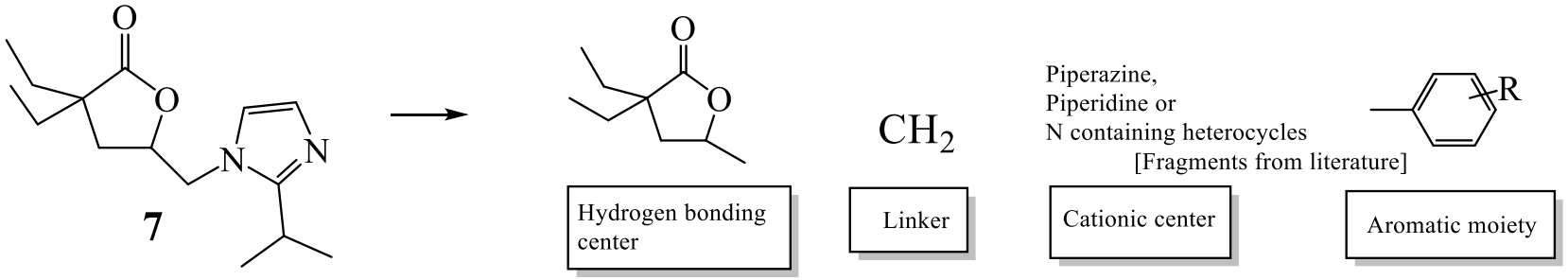
Aromatic moiety

Hypothesis:
appropriately positioned functional groups (eg. substituted and unsubstituted aromatic rings and N-containing heterocycles) exhibiting a wide range of physicochemical properties on lead molecules may provide improved affinity towards muscarinic receptor

An important distinction between the newly designed ligands and the lead lactones is the presence of substituted or unsubstituted aromatic systems that provide opportunities for interactions with auxiliary binding sites of mAChR's

Specific aims

1:Modification of lead lactone **7** by incorporating aromatic and heteroaromatic ring systems to improve the affinity and evaluate higher affinity ligands for subtype selectivity

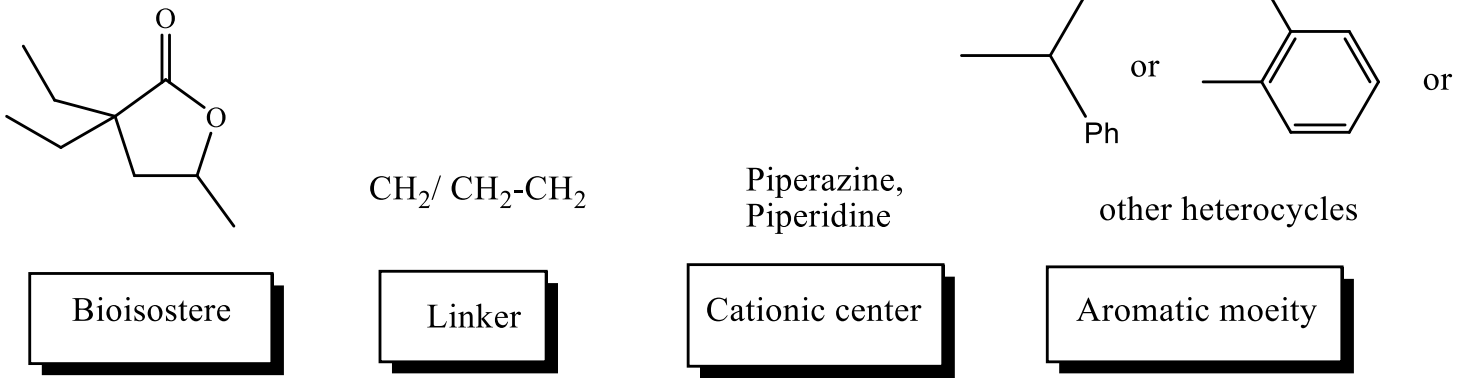


2: Modification of lead lactone by evaluation of bioisosteric replacements for the hydrogen bonding lactone fragment

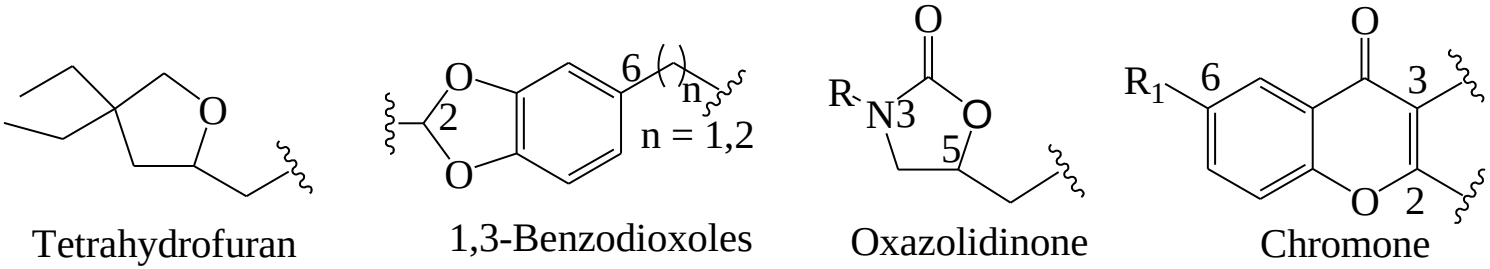
3: *In-vitro* evaluation of target ligands in muscarinic receptor binding (CEREP, France) assay/subtype selectivity assays and solubility assays

4: Use the information from the preliminary data in the design of new ligands

Proposed bioisostere based scaffold



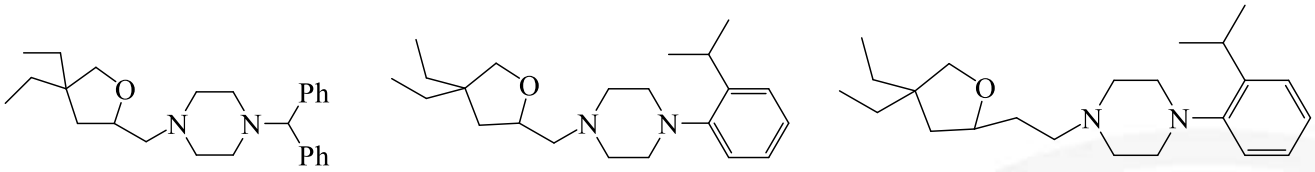
Proposed bioisosteres for lactone ring



Tetrahydrofuran | 1,3-Benzodioxoles | Oxazolidinone | Chromone

Proposed lactone bioisostere based ligands

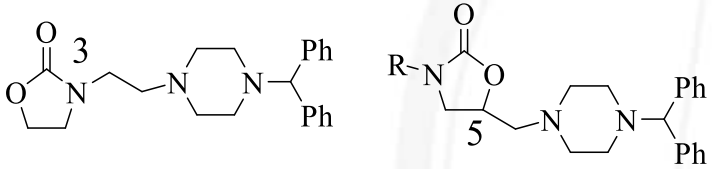
Tetrahydrofuran



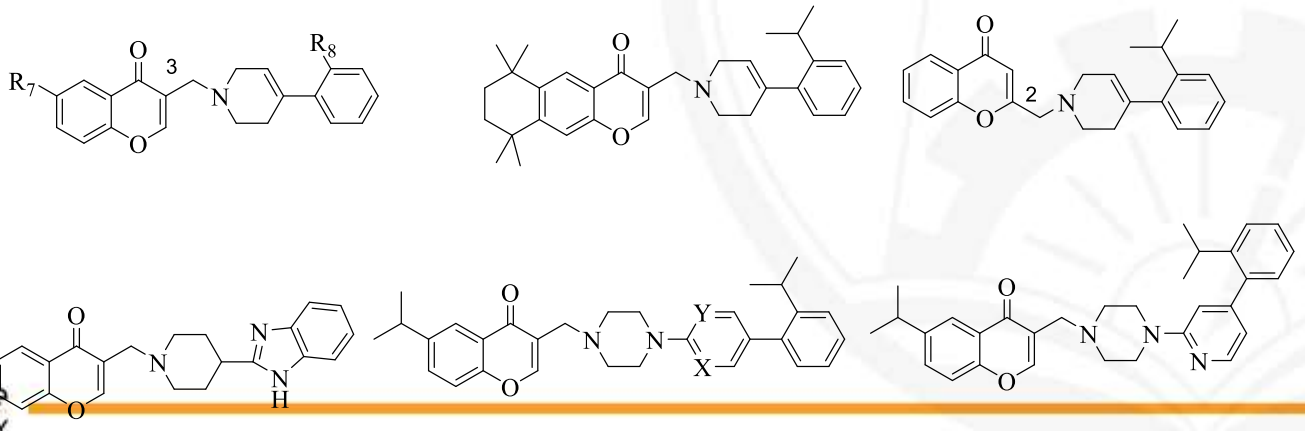
1,3-Benzodioxole

n= 1,2

Oxazolidinone



Chromone



In-vitro evaluation of target ligands

- The target compounds were evaluated in radioligand binding assays performed by CEREP, France using rat cerebral cortex membranes expressing muscarinic receptor subtypes M_1 - M_5

- The test compounds were evaluated at a concentration of 10 μM

- For interpretation of this type of preliminary data, CEREP suggests the following guidelines:

  i. 50 % inhibition or higher represent significant effects (i.e. 50 % is a common cut-off value for further investigation).

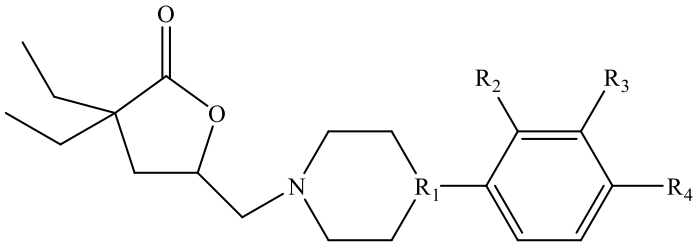
  ii. Results showing an inhibition between 20 and 50 % indicate weak to moderate effects.

  iii. inhibition less than 20 % are considered inactive

- Solubility assay

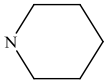
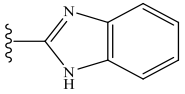
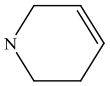
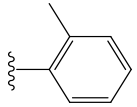
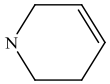
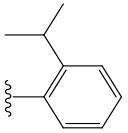
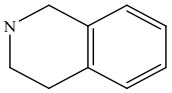
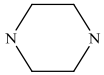
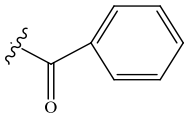
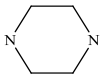
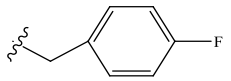
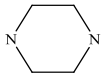
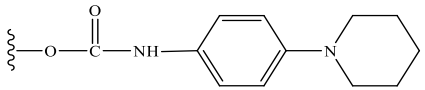
Results and discussion

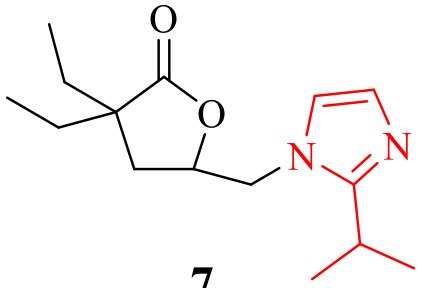
Preliminary binding data



Compound #	R_1	R_2	R_3	R_4	% specific inhibition at 10 μM
20	N	H	H	OCH_3	26
21	N	H	H	CN	18
22	N	H	H	NO_2	18
23	N	H	H	OH	7
24	N	H	H	H	16
25	N	OCH_3	H	H	32
26	N	H	OCH_3	H	9
27	N	OH	H	H	**46**
28	N	CN	H	H	31
29	C	H	H	H	**68**
30	C	CH_3	H	H	**57**

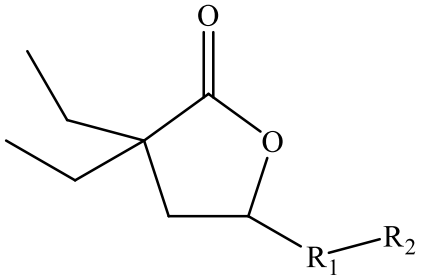
Preliminary binding data

Compound #	R_1	R_2	% specific inhibition at 10 µM
31			46
32			57
33			67
34			44
35			5
36			28
37			**97**
38			31



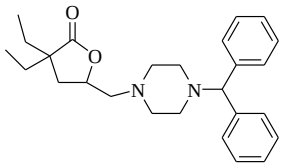
7

IC_{50} = 1.56 µM

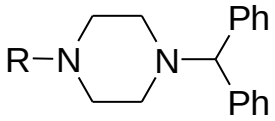


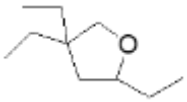
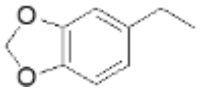
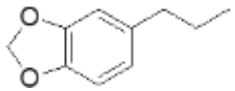
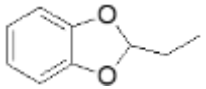
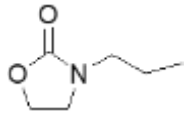
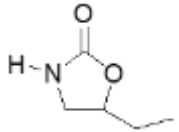
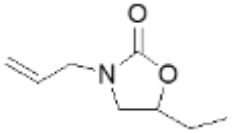
IC_{50} for 37 = 340 nM

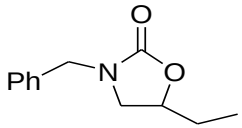
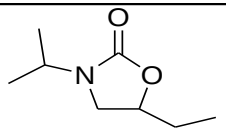
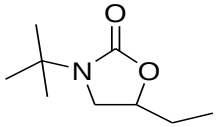
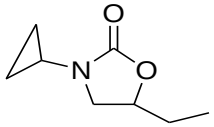
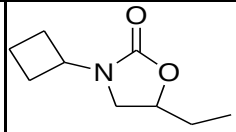
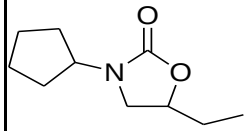
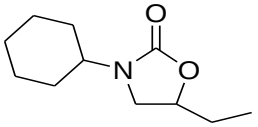
Preliminary binding data



% inhibition of **97 %**; IC_{50} = **340 nM**

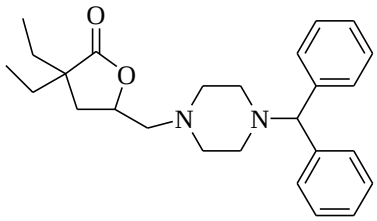


Compound #	R	% inhibition at 10 µM
39		**98**
40		**98**
41		**97**
42		17
43		**81**
44		**87**
45		53

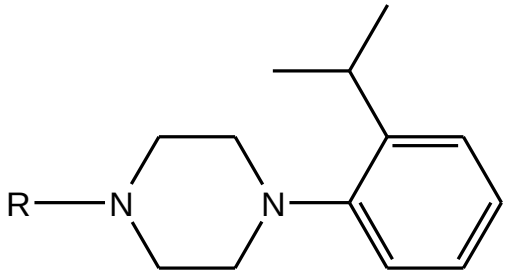
Compound #	R	% inhibition at 10 µM
46		66
47		58
48		74
49		46
50		65
51		**80**
52		73

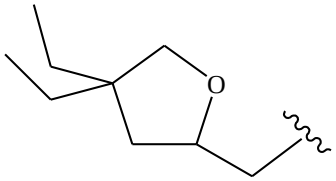
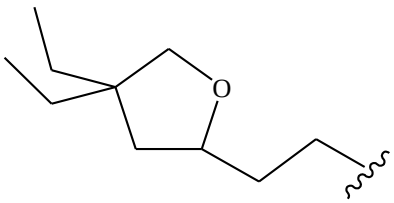
IC_{50} for **40** = 280 nM

Preliminary binding data

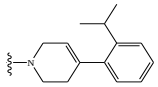
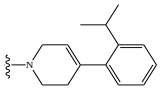
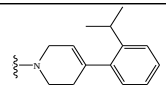
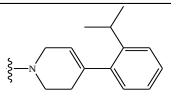
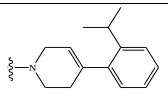
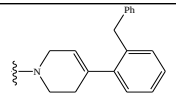
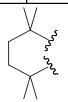
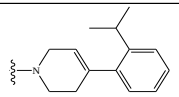
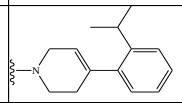
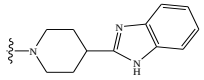
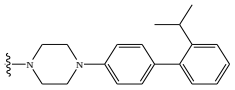
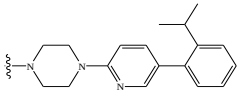
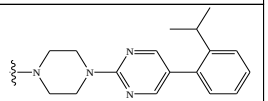


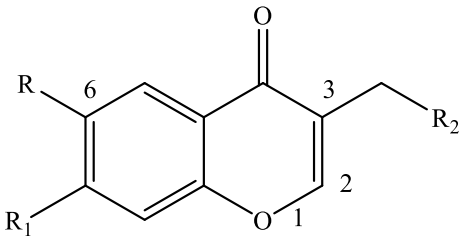
% inhibition of **97 %**; IC_{50} = **340 nM**



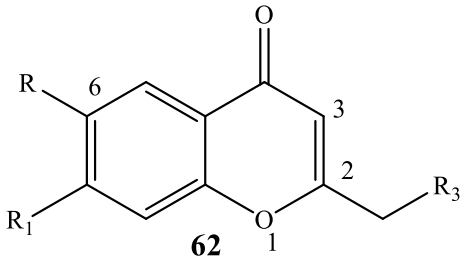
Compound #	R	% inhibition at 10 µM
53		54
54		89

Preliminary binding data

Compound #	R	R_1	R_2	R_3	% specific inhibition at 10 μM
55	H	H			82
56	CH_3	H			82
57	C_2H_5	H			75
58	$(CH_3)_2CH$	H			**90**
59	NO_2	H			80
60	$(CH_3)_2CH$	H			61
61					74
62	H	H			50
63	H	H			23
64	$(CH_3)_2CH$	H			14
65	$(CH_3)_2CH$	H			33
66	$(CH_3)_2CH$	H			38

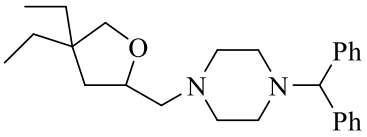


55-61, 63-66

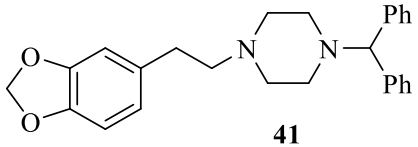


62

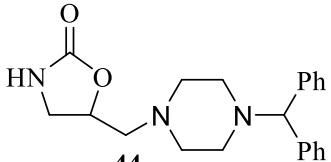
Subtype selectivity



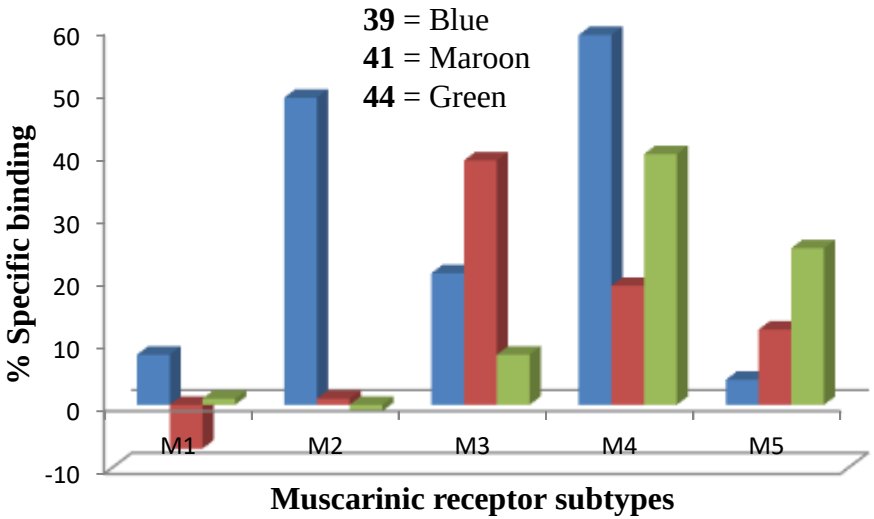
39

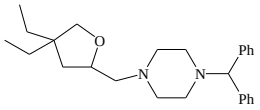
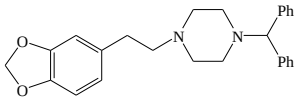
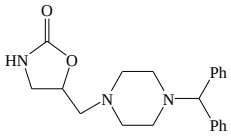


41



44



Compound #	Structure	% specific inhibition				
		M_1	M_2	M_3	M_4	M_5
39		8	49	21	59	4
41		7	1	39	19	12
44		1	-1	8	40	25

Compounds **146**, **148** and **151** were tested at concentration of 500, 100 and 500 nM, respectively

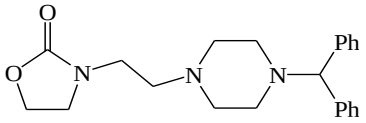
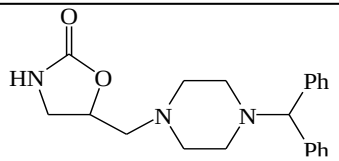
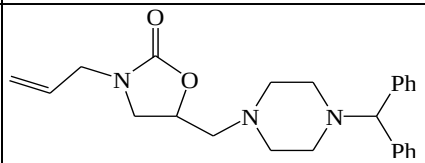
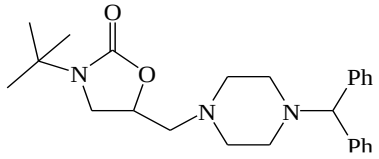
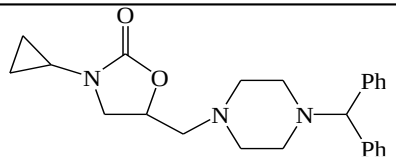
Solubility assay

- The test compounds were evaluated in solubility assay (kinetic) to determine the influence of solubility on binding characteristics.

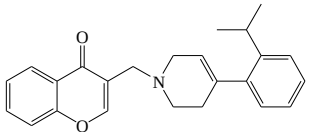
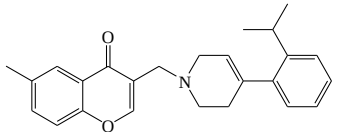
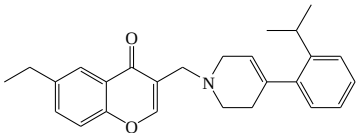
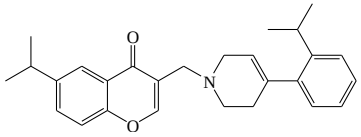
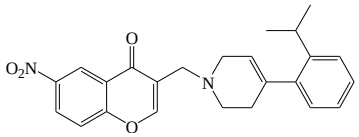
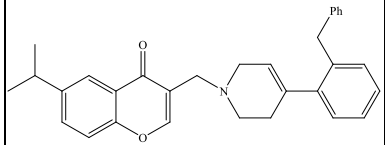
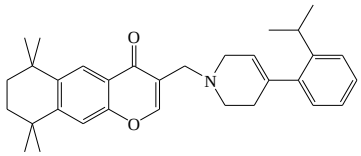
- The test compounds are dissolved in DMSO and diluted with universal buffer (45 mM ethanolamine, 45 mM potassium dihydrogen phosphate and 45 mM potassium acetate) at pH 7.4.

- After 90 mins, the solution is filtered and the sample concentration is analyzed by UV or Mass spec.

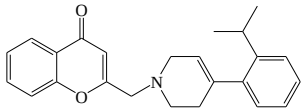
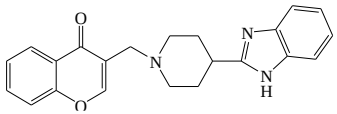
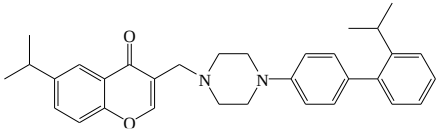
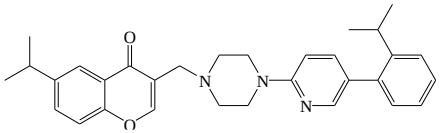
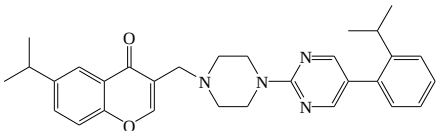
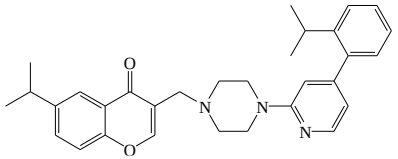
Solubility profile for oxazolidinone based compounds

Compound #	Structure	% specific inhibition at 10 µM	Solubility (µM)
43		81	83
44		87	200
45		53	198
48		74	14.2
49		46	200

Solubility profile for chromone based compounds

Compound #	Structure	% specific inhibition at 10 µM	Solubility (µM)
55		82	43.9
56		82	20
57		75	17.1
58		90	7.9
59		80	57.6
60		61	5.5
61		74	4

Solubility profile for chromone based compounds

Compound #	Structure	% specific inhibition at 10 µM	Solubility (µM)
62		50	52.2
63		23	200
64		14	4
65		33	3
66		38	2
67		15	3

PDSP Analysis

- Compounds were chosen from the solubility study and sent to Psychoactive Drug Screening Laboratoty (PDSP) at University of North Carolina, Chapel Hill.
- Compounds having solubility of more than 10 µM were chosen.
- Oxazolidinones and chromones were screened against serotonin subtypes.
- Compounds were initially screened at a dose of 10 µM in the preliminary binding assay.
- Compounds with more than 50% inhibition in the preliminary binsing assay were evaluated for secondary binding and Ki was determined.

Oxazolidinones & Chromones* evaluated against a panel of Serotonin Receptors at PDSP Lab

Cmpd #	5-HT1A	5-HT1B	5-HT1D	5-HT1e	5-HT2A	5-HT2B	5-HT2C	5-HT3	5-HT4	5-HT5a	5-HT6	5-HT7
43	-	-	-	-	102	156	-	-	-	-	1362	-
44	-	-	-	-	-	201	-	-	-	-	-	4498
45		-	-	-	937	126	-	-	-	502	-	353
46	-	-	>10000	-	915	125	-	-	-	-	2345	305
47	-	-	-	-	-	274	-	-	-	873	-	881
48	-	-	-	-	-	1871	-	-	-		-	-
49	-	-	-	-	-	298	-	-	-	420	-	1267
50	162	-	2967	-	824	190	-	-	-	756	-	349
51	-	-	-	-	1211	41	-	-	-	1227	-	216
52	2386	>10000	-	-	159	237	-	-	-	-	-	-

cmpd #	5-HT1A	5-HT1B	5-HT1D	5-HT1e	5-HT2A	5-HT2B	5-HT2C	5-HT3	5-HT4	5-HT5a	5-HT6	5-HT7
55	819	-	-	-	-	387	278	-	-	-	-	489
56	1723	-	-	-	>10000	113	>10000	-	-	-	-	870
57	-	-	-	-	-	153	-	-	-	-	-	2580
63	-	-	-	-	601	101	-	-	-	-	-	1586

*(Values are indicator of IC_{50} in nM; **"-"** indicates % inhibition in preliminary binding assay to be less than 50%)

Conclusions

- Oxazolidinone **51** having cyclopropyl substituent was found to be the most potent compound having an affinity of 41nM and having better affinity over other 5-HT subtypes.
- Among chromones, compound **56** was found to have affinity of 113 nM and better selectivity over other subtypes.
- Further studies needs to be to done to assess the binding characteristics to understand the influence of other substituents attached to nitrogen of oxazolidinone.

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