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Studies toward the discovery of the next generation of antidepressants. Part 5: 3,4-Dihydro-2*H*-benzo[1,4]oxazine derivatives with dual 5-HT$_{1A}$ receptor and serotonin transporter affinity

Dahui Zhou,[a,*] Boyd L. Harrison,[a] Uresh Shah,[a] Terrance H. Andree,[b] Geoffrey A. Hornby,[b] Rosemary Scerni,[b] Lee E. Schechter,[b] Deborah L. Smith,[b] Kelly M. Sullivan[b] and Richard E. Mewshaw[a,*]

[a]*Chemical and Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08543-8000, USA*
[b]*Neuroscience, Discovery Research, Wyeth Research, CN 8000, Princeton, NJ 08543-8000, USA*

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Abstract—The design, synthesis, and structure–activity relationship of two novel classes of benzoxazine derivatives with dual selective serotonin reuptake inhibitors and 5-HT$_{1A}$ receptor activities are described.

Selective serotonin reuptake inhibitors (SSRIs) have achieved great success in treating depression and related illnesses. SSRIs work by blocking the neuronal reuptake of serotonin (5-HT), increasing the concentration of synaptic 5-HT and thus, increasing the activation of postsynaptic 5-HT receptors. Unfortunately, long-term treatment is required before clinical efficacy is achieved. This is speculated to be the result of acute stimulation of the somatodendritic 5-HT$_{1A}$ autoreceptors that decrease neuron firing to release serotonin in the forebrain. Only after desensitization of these autoreceptors, the serotonergic neurons resume normal firing and the eventual increase in 5-HT levels in the major forebrain areas.[1–3]

It has been proposed that addition of a 5-HT$_{1A}$ antagonist component to the action of an SSRI can limit the negative feedback through blockage of the autoreceptor, allowing an immediate increase in synaptic 5-HT. Preclinical evidence has shown that acute SSRI-induced increases in forebrain 5-HT can be significantly augmented by co-treatment with either pindolol or the more selective 5-HT$_{1A}$ antagonist WAY-100635.[4,5] Furthermore, co-administration of fluoxetine and WAY-100635 produces an immediate increase in 5-HT levels in rat frontal cortex using in vivo microdialysis, whereas this effect cannot be seen with fluoxetine treatment alone.[6] In support of this hypothesis, clinical trials performed by Artigas et al.[7] and Blier and Bergeron[8] demonstrated that combination of (±)-pindolol, a non-selective 5-HT$_{1A}$ antagonist, with the SSRI paroxetine shortened the onset of antidepressant action to a period of 3–7 days in contrast to the 2–3 weeks required with the SSRI alone. Therefore, incorporating both 5-HT$_{1A}$ antagonism and 5-HT reuptake inhibition within a single molecule should provide an immediate increase in 5-HT in frontal cortex, resulting in a rapid onset antidepressant. This dual-action feature would thus form the basis of the next generation of antidepressant therapy.[9–13]

Previous reports from our laboratories have revealed two novel series capable of modulating both SSRI and 5-HT$_{1A}$ receptor activities by linking an aryloxyethylamine with a 3-indoletetrahydropyridine (**1**) or a 3-indolealkylamine (**2**) through a common basic nitrogen.[12,13] In both series, aryloxyethylamines have been successfully utilized to produce potent 5-HT$_{1A}$ ligands, while the indoletetrahydropyridine or 3-indolealkylamine moieties have served as robust 5-HT uptake inhibitor

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* Corresponding authors. Tel.: +1 732 274 4423; fax: +1 732 274 4505; e-mail: zhoud@wyeth.com

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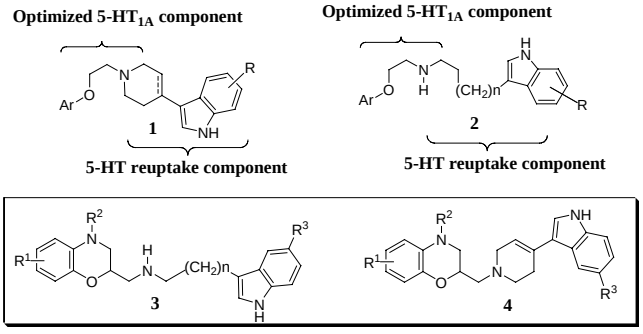
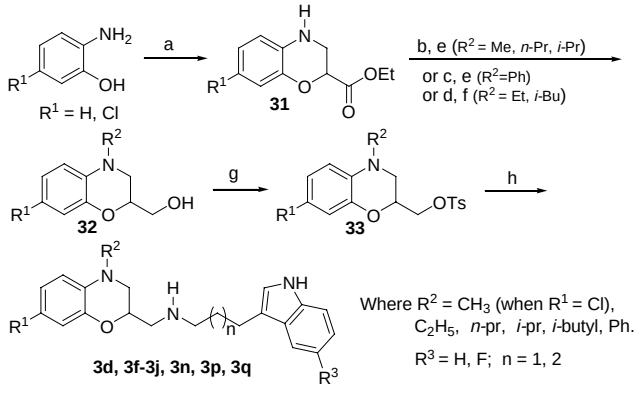
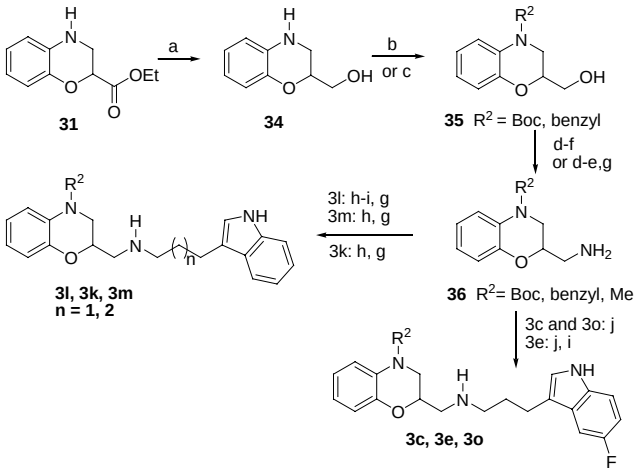
moieties. However, shortcomings were observed in both series, that is, lack of selectivity over the α_1 receptor. In order to expand upon the type of structures that can potentially maintain both desired activities, we decided to constrain the 5-HT_{1A} pharmacophore within a cyclic ring, benzoxazine ring, in a manner similar to that previously exemplified with benzodioxan moiety.[14] We are particularly interested in the benzoxazine system, since from a synthetic point of view, the benzoxazine analog offered the advantage of constraining the aryloxyethylamine moiety, as well as allowing us to probe another region in space via functionalization of the nitrogen of the oxazine ring. We now report the syntheses and the structure–activity relationships (SARs) of two new classes of benzoxazine 3-indolealkylamines **3a**–**q** and benzoxazine 3-indoletetrahydropiridine analogs **4a**–**e**.



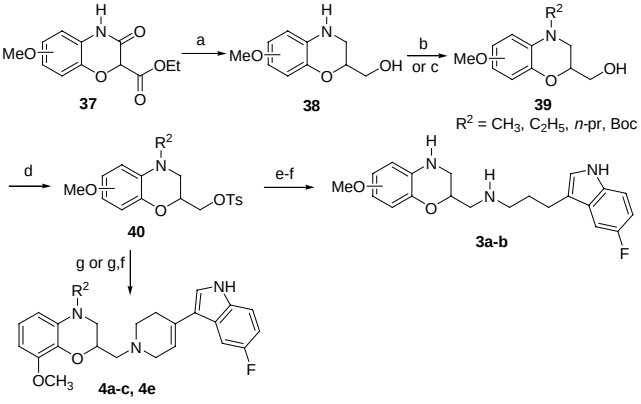
Schemes 1–4 show the syntheses of target molecules **3a**–**q** and **4a**–**e**. The condensation of commercially available 2-amino-phenol and 2,3-dibromopropionic acid ethyl ester afforded ethyl 3,4-dihydro-2*H*-benzo[1,4]-oxazine-2-carboxylate **31** in moderate yield (33%). The nitrogen of the oxazine ring was alkylated under standard conditions and the ester reduced to generate benzoxazine derivative **32**. Alcohols **32** were converted to the tosylates **33** using *p*-toluenesulfonyl chloride in pyridine, and coupling of tosylates **33** with different 3-indolepropylamines[13] afforded the desired products **3d**,**f**–**j**,**n**,**p**, and **q**.



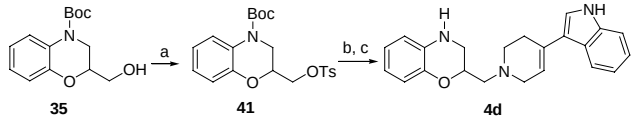
Scheme 1. Reagents and conditions: (a) $BrCH_2CHBrCO_2Et/K_2CO_3$/acetone, reflux; (b) R^2Br or $(R^2I)/Et_3N/CH_3CN$, reflux; (c) 1,4-*cyclo*-hexanedione/*p*-toluenesulfonic acid/toluene, reflux; (d) $(R^2CO)_2O$/THF, reflux; (e) $LiBH_4$; (f) $LiAlH_4$/THF, rt; (g) TsCl/py, rt; (h) 3-indoealkylamines/Et_3N/DMSO, 90 °C.



Scheme 2. Reagent and conditions: (a) $LiBH_4$/THF, rt; (b) $(t\text{-Boc})_2O$/THF, reflux; (c) BnBr/Et_3N/CH_3CN, rt; (d) TsCl/Py, rt; (e) NaN_3/DMF, 60 °C; (f) Ph_3P/THF–H_2O, rt; (g) $LiAlH_4$/THF, reflux; (h) 3-indolealkanoic acid/DMAPC/CH_2Cl_2, rt; (i) TFA/CH_2Cl_2, rt; (j) 5-fluoro-3-indolepropylbromide/Et_3N/DMSO, 90 °C.



Scheme 3. Reagent and conditions: (a) $LiBH_4$/THF, rt; (b) $(t\text{-Boc})_2O$/THF, reflux; (c) $R^2Br/Et_3N/CH_3CN$, rt; (d) TsCl/Py, rt; (e) 5-fluoro-3-indolepropylamine/Et_3N/DMSO, 90 °C; (f) TFA/CH_2Cl_2, rt; (g) 5-fluoro-3-indolepyridine/Et_3N/DMSO, 90 °C.



Scheme 4. Reagent and conditions: (a) TsCl/Py, rt; (b) 3-indolepyridine/Et_3N/DMSO, 90 °C; (c) TFA/CH_2Cl_2, rt.

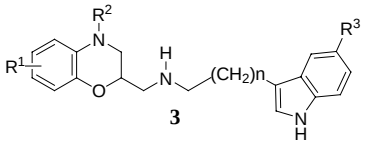
In Scheme 2, reduction of ester **31** with $LiBH_4$ led to alcohols **34**, which were then treated either with di-*tert*-butyldicarbonate or benzylbromide to give alcohols **35**. Alcohols **35** were converted to amines **36** using a standard protocol.[13] Coupling of **36** to the appropriate 3-indolealkanoic acids, followed by reduction and deprotection, afforded target compounds **3l**,**k**, and **m**. Treatment of amines **36** with 5-fluoro-3-indolepropylbromide[13] resulted in target compounds **3c**,**e**, and **o**.

In Scheme 3, reduction of 3-oxo-benzoxazine derivative **37** with $LiBH_4$ gave alcohol **38**, which was then treated either with di-*tert*-butyldicarbonate or different halides to afford **39**. The target compounds **3a** and **b** were prepared by heating the corresponding tosylates **40** and 5-fluoro-3-indolepropylamine, followed by deprotection in TFA. Coupling the tosylates **40** with 5-fluoro-3-indolepyridine[12] gave target compounds **4a**–**c** and **e**.

The preparation of the benzoxazine analog **4d** is shown in Scheme 4. Alkylation of tosylate **41** with 3-indole tetrahydropyridine[12] gave the desired product **4d** in good yield.

All compounds listed in Tables 1 and 2 were the racemates.[19] Serotonin transporter (r-5-HT-T) and 5-HT_{1A} receptor (h-5-HT_{1A}) and α_1 affinities, as well as 5-HT_{1A} intrinsic activity (GTPγS E_{max}) are tabulated in Tables 1 and 2.

Table 1. Dual SSRI and 5-HT_{1A} receptor activities for compounds **3a**–**q**[a]



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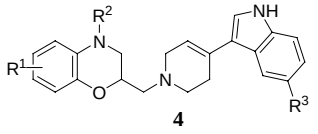
Compound	R^1	R^2	R^3	*n*	r-5-HT-T K_i (nM)[b]	h-5-HT_{1A} K_i (nM)[c]	GTPS E_{max} (EC_{50} nM)[d]	α_1 K_i (nM)[e]
3a	8-OCH_3	H	F	1	3.13 ± 1.73	9.31 ± 1.25	66 ± 9.2 (52.2)	7.6 ± 3.6
3b	7-OCH_3	H	F	1	11.70 ± 4.67	103.00 ± 15.34	56 ± 17.7 (181.5)	44.5 ± 16.3
3c	H	Me	F	1	12.43 ± 9.51	8.46 ± 2.27	80 ± 0.7 (167.5)	26% at 100 nM
3d	H	Et	F	1	15.28 ± 0.39	15.31 ± 2.20	100 ± 0 (90.0)	—
3e	H	H	F	1	15.98 ± 0.04	10.98 ± 3.14	100 ± 0 (155.0)	70.0 ± 1.4
3f	H	*i*-Pr	F	1	21.13 ± 5.48	2.81 ± 0.10	90 ± 7.1 (190.5)	—
3g	H	*n*-Pr	H	1	22.73 ± 4.63	12.76 ± 2.84	100 ± 0 (196.5)	481 ± 229.8
3h	H	*i*-Pr	H	2	23.78 ± 5.34	90.42 ± 29.12	88 ± 12.7 (329.0)	—
3i	7-Cl	Me	F	1	42.58 ± 2.02	75.18 ± 20.45	64 ± 13.4 (336)	607 ± 272.9
3j	H	*i*-Bu	F	1	51.48 ± 47.41	8.15 ± 0.70	88 ± 12.7 (329)	—
3k	H	Me	H	1	88.50 ± 33.23	5.86 ± 1.12	95 ± 7.1 (33.6)	28% at 100 nM
3l	H	H	H	1	89.75 ± 15.20	6.91±0.52	96 ± 6.4 (93.5)	26% at 100 nM
3m	H	Bn	H	2	108.75 ± 39.24	76.14 ± 20.62	64 ± 7.8 (1385)	—
3n	H	Ph	F	1	43% at 100 nM	7.14 ± 0.22	—	65% at 100 nM
3o	H	Bn	F	1	23% at 100 nM	7.25 ± 1.52	—	—
3p	H	Ph	H	1	23% at 100 nM	30.21 ± 10.96	—	—
3q	H	Ph	H	2	7% at 100 nM	75.77 ± 8.90	—	—

[a] K_i, E_{max}, and EC_{50} values are means of at least two experiments ± SEM (performed in triplicate, determined from nine concentrations).
[b] Binding affinity at rat cortical 5-HT reuptake sites labeled with [^{3}H]-paroxetine.[15]
[c] Binding affinity at human 5-HT_{1A} receptors in CHO cells labeled with [^{3}H]-8-OH-DPAT.[16]
[d] Stimulation of [^{35}S]-GTPγS binding in CHO cells expressing 5-HT_{1A} receptor;[17] E_{max} refers to maximal agonist effect relative to 5-HT.
[e] Binding affinity at rat cortical a1 adrenergic receptors labeled with [3H]-prazosin.[18]

Table 2. Dual SSRI and 5-HT_{1A} receptor activities for compounds **4a**–**e**[a]



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Compound	R^1	R^2	R^3	r-5-HT-T K_i (nM)[b]	h-5-HT_{1A} K_i (nM)[c]	GTPγS E_{max} (EC_{50} nM)[d]	α_1 K_i (nM)[e]
4a	8-OCH_3	H	F	6.94 ± 4.61	154.85 ± 3.15	35 ± 15.6 (386)	14.5 ± 3.6
4b	8-OCH_3	Me	F	12.58 ± 5.76	139.9 ± 8.56	93 ± 9.9 (190)	96.5 ± 29.0
4c	8-OCH_3	Et	F	13.15 ± 1.63	136.45 ± 18.00	54 ± 26.9 (418)	229.0 ± 33.9
4d	H	H	H	8.37 ± 10.79	804.80 ± 123.33	62 ± 0.7 (1131)	16.6 ± 18.1
4e	8-OCH_3	*n*-Pr	F	22.58 ± 3.64	362.80 ± 46.95	92 ± 0.7 (135)	505.5 ± 222.7

[a] K_i, E_{max}, and EC_{50} values are means of at least two experiments ± SEM (performed in triplicate, determined from nine concentrations).
[b] Binding affinity at rat cortical 5-HT reuptake sites labeled with [^{3}H]-paroxetine.[15]
[c] Binding affinity at human 5-HT_{1A} receptors in CHO cells labeled with [^{3}H]-8-OH-DPAT.[16]
[d] Stimulation of [^{35}S]-GTPγS binding in CHO cells expressing the 5-HT_{1A} receptor;[17] E_{max} refers to maximal agonist effect relative to 5-HT.
[e] Binding affinity at rat cortical a1 adrenergic receptors labeled with [3H]-prazosin.[18]

Shown in Table 1 are the biological results of target molecules **3a**–**q**. Consistent with our previous paper (Part 3),[13] substitution of the 5-fluoro moieties to the indole ring resulted in a significant improvement in 5-HT transporter affinity but little effect on the affinity for 5-HT_{1A} receptor (**3c** vs **k**). Interestingly, incorporation of substitutents at the nitrogen of the oxazine ring had a minor effect on affinity for the 5-HT_{1A} receptor, indicating a large tolerance of the 5-HT_{1A} receptor toward structural variation in this region of the molecule. However, 5-HT transporter affinity dramatically decreased as the substitutent on the nitrogen of the oxazine ring increased in size (**3c** vs **d**,**f**,**j**,**n**, and **o**). A 5-fold increase in 5-HT transporter affinity was manifested when the methoxy group was attached at the 8-position (**3a** vs **e**). Surprisingly, when the methoxy group was moved to the 7-position, a 9-fold loss in affinity for 5-HT_{1A} receptor was found as well as ~4-fold loss in affinity for 5-HT transporter (**3a** vs **b**). In contrast to our previous studies (Parts 2 and 3), three compounds (**3c**,**k**, and **l**) in this series had very low α_1 affinity. Unfortunately, all compounds from this study were found to be either 5-HT_{1A} agonists or partial agonists as measured by the GTPγS assay.

Table 2 depicts the investigation of the indole tetrahydropyridinyl group utilized as a transporter moiety in the benzoxazine series. Comparing compound **3a** to **4a**, a 16-fold loss in 5-HT_{1A} affinity was observed. A slight trend toward lower 5-HT transporter affinity was observed when the nitrogen of the oxazine ring was alkylated (**4a**–**c** and **e**). A 2-fold loss in affinity 5-HT_{1A} receptor was found when R^2 was an isopropyl group (**4a** vs **e**). Surprisingly, though we previously showed that compounds employing the tetrahydropyridinyl moiety had 5-HT_{1A} antagonism,[12] all the compounds listed in Table 2 were found to be either full or partial 5-HT_{1A} agonists in the GTPγS assay in this report. In contrast to most of the compounds in Table 1, the benzoxazine indole tetrahydropyridinyl derivatives in Table 2 had higher affinity for α_1 receptor (i.e., **4a**,**b**, and **d** were more potent for α_1 receptor than the 5-HT_{1A} receptor).

In conclusion, we have discovered that the benzoxazine moiety can be utilized to embrace both the 5-HT_{1A} pharmacophore along with the 5-HT transporter moiety to access dual SSRI and 5-HT_{1A} receptor activities. The selectivity over α_1 receptor[20] was improved in several compounds, though most of the compounds in these two classes were found to function as 5-HT_{1A} receptor agonists. Studies in our laboratories are continuing to further understand the 5-HT transporter and 5-HT_{1A} pharmacophoric criteria within a single molecule and to identify novel agents that can represent the next generation of rapid onset antidepressants.

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