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Relating the Structure, Activity, and Physical Properties of Ultrashort-Acting Benzodiazepine Receptor Agonists

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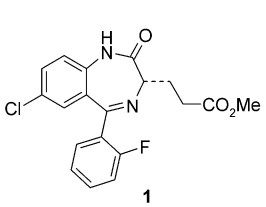
Abstract—The ultrashort-acting benzodiazepine (USA BZD) agonists reported previously have been structurally modified to improve aqueous solubility. Lactam-to-amidine modifications, replacement of the C5-haloaryl ring, and annulation of heterocycles are presented. These analogues retain BZD receptor potency and full agonism profiles.

Our earlier work[1] had identified shorter-acting anxiolytic-sedatives exemplified by benzodiazepinone **1**. We considered that a neuropharmacological profile of agonism would be preserved through binding to the $GABA_A$ receptor with a benzodiazepine (BZD) core and diminution of activity would be controlled via rapid degradation of the appended carboxylic ester by non-specific esterases. The predictable, rapid onset of and recovery from sedation that characterize these compounds represent an alternative to midazolam (**2**).[2,3] The entities described previously, however potent or selective, lacked the physical properties required of an adjunct for total intravenous anesthesia.

We directed our attention towards the discovery of agents with pharmacology comparable to **1** and with improved aqueous solubility. Initial efforts focused on the incorporation of additional functionalities into the benzodiazepinone nucleus of **1** and targeted aqueous solubilities of >1 mg/mL at pH 3 for the purpose of formulating an injectable solution. Clearly these changes could neither disrupt the high-affinity binding to the BZD receptor nor alter the full agonism profile. We rationalized that a heteroatom-based moiety would provide additional sites for coordination with solvent and/or salt formation. One approach explored the use of substituted amidines, as exemplified by the structure of the hallmark benzodiazepine agonist, chlordiazepoxide (**3**), not only to introduce these properties but also to provide a point of diversification in the context of SAR development. We also considered replacement of the C5-aryl group; while the 2′-haloaryl subunit confers potency, 2′-pyridyl variants, such as bromazepam (**4**),[4] display reasonable activities. Finally, fused heterocyclic systems, as exemplified by **2** itself, were examined.



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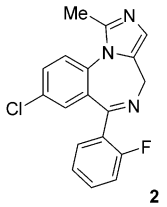

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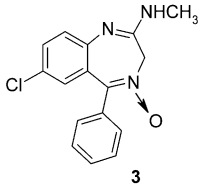

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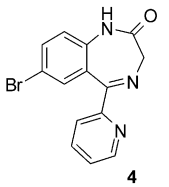

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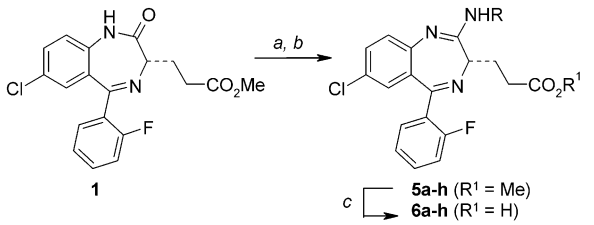
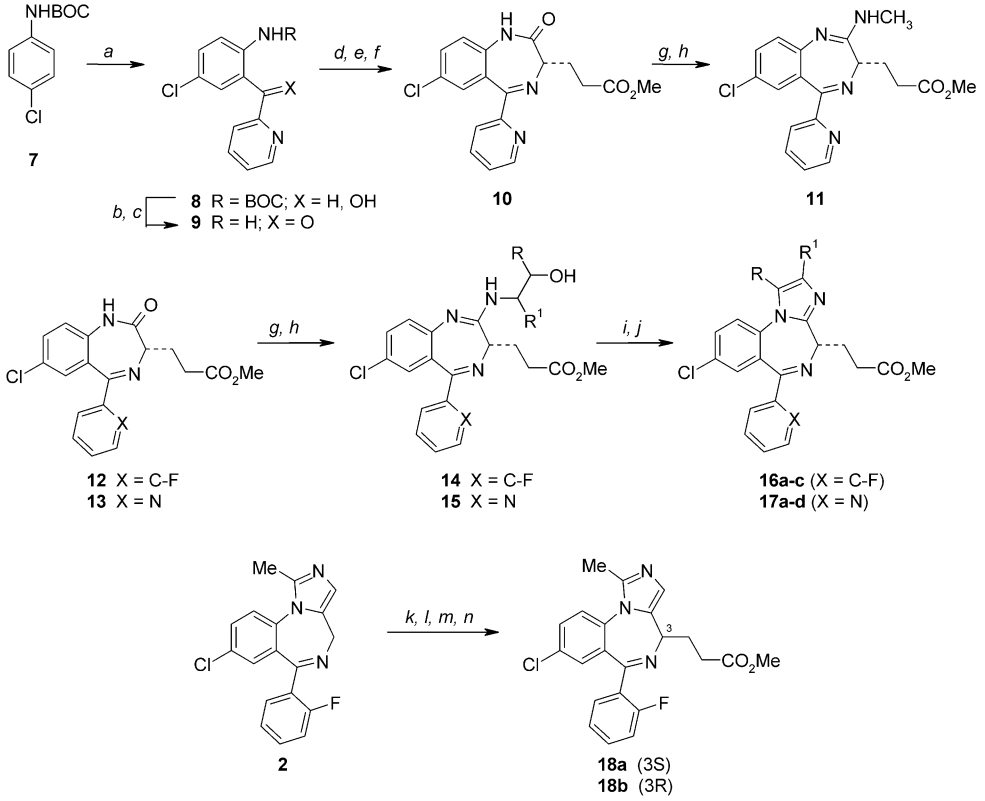


Scheme 1. Reagents and conditions: (a) Lawesson's reagent, $PhCH_3$, reflux; (b) RNH_2, THF or 1,4-dioxane, reflux; (c) LiOH, THF/MeOH/H_2O.

Amidines **5a**–**h** were prepared using the two-step process shown in Scheme 1. In the event, lactam **1** was reacted with Lawesson's reagent[5] to provide, after removal of inorganic materials and trituration, the corresponding thiolactam in 65% yield. Other thionation procedures (P_2S_5;[6] P_2S_5, *n*-BuLi[7]) were explored, but they required tedious purification and provided thiolactam in poor chemical yield. Attempts to activate the lactam via conversion to its chloroimine ($POCl_3$; PCl_5) returned starting lactam or led to intractable mixtures of materials. With a reactive intermediate in hand, we turned to displacements with primary amines. These transformations proceeded smoothly at reflux in THF or 1,4-dioxane to give the desired amidines in moderate to good yield (30–85%) after chromatography.[8] Saponification of the esters delivered the corresponding carboxylic acids.

Syntheses of the C5-pyridyl target compounds **10** and **11** were achieved using the sequence outlined in Scheme 2. Directed metalation[9] of **7** at low temperature and reaction of the derived lithio species with 2-pyridinecarboxaldehyde provided alcohol **8**. Oxidation with MnO_2 gave the BOC-protected aminobenzophenone and subsequent deprotection with HCl gave **9** in 54% yield over these three steps. As before,[1] amide bond formation, deprotection, and intramolecular cyclization delivered **10** in 45% overall yield. Lactam **10** was converted into amidine **11** using the aforementioned two-step protocol.

Analogues with a fused imidazole ring were prepared as shown in Scheme 2. Lactams **12** and **13** were converted into the requisite amidines via the two-step procedure described above using appropriately substituted 2-aminoalcohols. Swern oxidation of the free alcohol present in **14** and **15** was followed by acid-catalyzed intramolecular cyclization to give **16a**–**c** and **17a**–**d** in good (50–75%) yield. The regioisomeric imidazoles, (*3S*)-**18a** and its enantiomer (*3R*)-**18b**, were available from midazolam (**2**) directly. In the event, a novel Michael addition was employed to install the propionate sidechain; the use of *t*-butyl acrylate optimally provided the desired racemic adduct.[10,11] The sequence of protecting group removal and alkylation of the intermediate acid delivered (±)-**18**. Chiral preparative HPLC separation[12] gave enantiopure[13] methyl esters (*3S*)-**18a** and (*3R*)-**18b**. Saponification of these esters provided the corresponding carboxylic acids.



Scheme 2. Reagents and conditions: (a) (i) *t*-BuLi, THF, −78 to −20 °C; (ii) 2-pyridinecarboxaldehyde, −78 °C; (b) MnO_2, $CHCl_3$; (c) 4 M HCl/dioxane; (d) FMOC-Glu(OMe)-Cl, $CHCl_3$; (e) piperidine, DMF; (f) HOAc, DMF; (g) Lawesson's reagent, $PhCH_3$, reflux; (h) RNH_2, THF or 1,4-dioxane, reflux; (i) Swern oxidation; (j) *p*-TsOH, DMF; (k) KO*t*-Bu, CH_2CHCO_2t-Bu, THF/*t*-BuOH, −10 °C; (l) TFA; (m) K_2CO_3, MeI, DMF; (n) chiral HPLC separation.

The lead benzodiazepinones[1] were quite potent when evaluated in our receptor-binding assay, however, the amidine-containing analogues of **1** generally displayed reduced potencies (Table 1). A modest preference was noted for smaller appendages in the series of hydrophobic analogues. For example, compounds **5a** and **5b** were somewhat less potent than **1** while incorporation of a phenyl group (**5c**) or a branched alkyl (**5d**) into the amidine decreased binding affinity dramatically. This trend was evident with hydrophilic analogues as well. Compounds **5e** and **5f** were markedly weaker binders than **1**, but, again, the bulkier heterocycle-containing **5g** and **5h** were considerably less potent than **1**. As satisfactory performance in the receptor binding assay was a prerequisite for continued investigation, only simply substituted amidines offered an alternative to the lactam **1** and amidine **5a** emerged as the lead in this series. Receptor affinity for **5a** had decreased by 20-fold relative to **1**, but **5a** now displayed an improved solubility profile (13 mg/mL at pH 3) relative to both midazolam (**2**, $\geq$8 mg/mL at pH 3) and **1** (<3 mg/mL at pH 3 (estimated value)). Furthermore, this amidine elicited a full agonism response in the rat loss-of-righting reflex (LRR)[14] assessment (Table 2) to confirm that structural changes had not altered pharmacology. Also noteworthy are the recovery times (initial = 15 min, total = 22 min) observed with this compound in the LRR. An identical response was observed when **5a** was dosed in water adjusted to pH3. These data positioned the amidines, specifically **5a**, as a potential replacement for **1** and broadly supported the hypothesis of lactam-to-amidine modification.

Concurrently, we explored the effects of replacing the C5-haloaryl moiety with a C5-pyridyl appendage. Biological evaluation of **10** showed a significant drop in binding affinity as well as selectivity, relative to the parent C5-haloaryl BZD, but a full agonism profile was retained. While the recovery times (initial = 9 min, total = 16 min) observed in the LRR were noteworthy and provided a significant advantage over both **1** and **5a**, the unanticipated drop in its pH-dependent solubility (1 mg/mL at pH 3) was disappointing. Interestingly, combining the amidine and C5 replacement strategies in the form of **11** offered no advantage in terms of receptor affinity.

Table 1. Receptor binding affinity of BZDs

Compd	R	R^1	K_i[a]	Selectivity[b]
1			7	984
2			2	
3			438	
5a	CH_3		96	156
5b	CH_2CH_3		146	
5c	CH_2Ph		875	
5d	$CH_2CH(CH_3)_2$		1012	
5e	CH_2CH_2OH		303	
5f	$(CH_2)_2$-4-imidazolyl		208	
5g	$(CH_2)_2$-4-pyridyl		792	
5h	CH_2-4-pyridyl		1230	
10			139	46
11			1075	
16a	H	H	7	64
16b	H	CH_3	7	156
16c	CH_3	H	8	74
17a	H	H	79	176
17b	H	CH_3	60	156
17c	CH_3	H	98	147
17d	CH_3	CH_3	130	40
18a			11	226
18b			3572	28

[a]Rat BZD receptor, nM.
[b]Selectivity = K_i (acid)/K_i (ester).

Table 2. Comparative data for BZDs

Compd	Solubility[a]	Dose[b]	LRR Recovery[c]	
			Initial	Total
1	<3[d]	25	9	24
2	≥8	30	23	121
5a	13	25	15	22
10	1	50	9	16
16a	ND	25	17	23
16b	ND	25	6	21
16c	<1	25	23	33
17a	ND	25	7	10
17b	2	25	13	15
17c	13	25	9	11
18a	ND	25	20	24

[a]Determined at pH 3;[15] mg/mL; ND = not determined.
[b]Bolus injection, mg/kg; vehicle = 50% PEG/25% EtOH/25% saline.
[c]Recovery time in min. A compound was identified as inactive in this model if LRR was not observed within 5 min following injection.
[d]Estimated value.

Both amidine- and C5-pyridyl-containing BZDs displayed reasonable pharmacological profiles, but neither series provided entities that combined these properties with aqueous solubility. We speculated that incorporation of an additional heterocycle into the BZD scaffold might improve solubility without impacting other attributes. Towards that end, **16a–c**, **17a–d**, and **18a** and **b** were synthesized and evaluated. Receptor binding affinities paralleled those of the respective parent BZDs, and the aforementioned preference[1] for the (*S*)-stereoisomer prevailed with the midazolam-derived esters, **18a** and **b**, as well. Moderate selectivity (i.e., receptor potency of ester versus acid) was observed with these series, so compounds were progressed to the in vivo LRR assessment to confirm their full agonism profiles. Importantly, total recovery times observed with each of these compounds was significantly shorter than the recovery time observed in animals dosed with **2**. The decreased aqueous solubility of **16c**, as compared to **2**, suggested that related methyl group positional isomers in the 2′-haloaryl series, especially **18a**, would possess solubility profiles similar to **16c**. This observation together with the synthetic protocol required to deliver **18a** prompted its removal from further consideration despite its performance in the LRR assessment. In contrast, compound **17c** presented a level of in vivo efficacy and aqueous solubility commensurate with our objectives. However, like compound **5a**, its relatively weak receptor affinity would likely necessitate administration of inconveniently higher doses, and dose volumes, and reduce its clinical utility.

Improving the physical properties of our lead benzodiazepinones was achieved through various manipulations of the BZD scaffold. The three strategies of lactam-to-amidine modification, C5-haloaryl replacement, and

heterocycle annulation provided novel alternatives to **1**, however the lead compounds from these efforts, **5a** and **17c**, lacked the receptor potency that was required. Our work to combine all attributes (potency, agonism, solubility) into a single chemical series will be the subject of future disclosures.

Acknowledgements

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References and Notes

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12. Chiral HPLC purification was performed using a Daicel AD Column (5×50 cm, 20 μm packing). Elution with 20% *i*-PrOH/hexanes, 50 mL/min flow rate and UV (270 nM) detection gave retention times of 42 min for **18a** and 72 min for **18b**.
13. Absolute configurations were assigned using vibrational circular dichroism (VCD) spectroscopy. Spectra were measured with a Bomem ChiralIR™ VCD spectrometer. Conformational searching was performed on model structures using the Molecular Visualization Program (MVP).[16] Ab initio predictions of VCD spectra for model structures were performed using Gaussian '94.
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15. Solubility was determined by visual inspection or UV assay of a saturated sample. In both types of determinations, solubility at pH 3 was verified using solubility at other pH and spectrophotometric p*K*a values.
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