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Identification and Structure–Activity Studies of Novel Ultrashort-Acting Benzodiazepine Receptor Agonists

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Abstract—The synthesis and evaluation of novel ultrashort-acting benzodiazepine (USA BZD) agonists is described. A BZD scaffold was modified by incorporation of amino acids and derivatives. The propionate side chain of glutamic acid tethers an enzymatically labile functionality where the metabolite carboxylic acid displays markedly reduced BZD receptor affinity. The USA BZDs were characterized by full agonism profiles.

Benzodiazepines (BZDs) have been widely used to provide anxiolysis and sedation in numerous clinical settings since their discovery over four decades ago.[1] BZDs are known to exert their broad pharmacological effects through specific binding to a subunit on the γ-aminobutyric acid ($GABA_A$) receptor, the native target for the primary inhibitory neurotransmitter GABA, that is referred to as the BZD receptor. Despite their utility, oversedation and prolonged recovery periods often confound the therapeutic effects of currently marketed drugs; this latter property can be particularly problematic with agents that are administered by continuous intravenous (iv) infusion in intensive care units.[2] These observations together with growing trends towards ambulatory care both in the United States and in Europe have demanded shorter-acting sedating agents that provide for rapid onset, deep sedation, and full, rapid emergence from the effects of anaesthesia. Thus, our goal became the discovery of an iv-deliverable, ultrashort-acting BZD (USA BZD) that retains the broad pharmacology of a BZD full agonist, such as midazolam (**1**)[3] or diazepam (**2**) (Fig. 1).[4] Such an agent would be expected to provide useful alternatives in both contemporary anaesthesiology and ambulatory care.[5]

The strategy for providing a short-acting disposition to a BZD emanated from our development of the ultrashort-acting opioid analgesic, remifentanil.[6] The pharmacologically active form of remifentanil is a carboxylic ester which, by virtue of its lability to nonspecific plasma and tissue esterases, undergoes rapid metabolism to a pharmacologically null carboxylic acid.[7] Translating this model to BZD agonism required: (a) incorporation of a carboxylic ester into the BZD scaffold without compromising receptor binding affinity, (b) demonstration that the corresponding carboxylic acid displays significantly reduced binding affinity for the BZD receptor and, (c) retention of a full agonism profile. Such pharmacology together with an organ-independent elimination mechanism is expected to provide more predictable and reproducible pharmacodynamic and pharmacokinetic profiles than BZDs in current clinical practice.

The synthesis of benzodiazepinone **11a** shown in Scheme 1 exemplifies preparation of the compounds described in this paper. Condensation between the glutamate-derived acid chloride **3**[8] and an appropriate 2-aminobenzophenone (**4**) yielded anilide **5** in excellent yield without detectable racemization.[9] Removal of the

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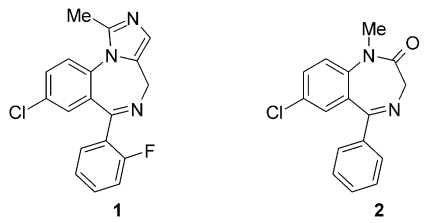
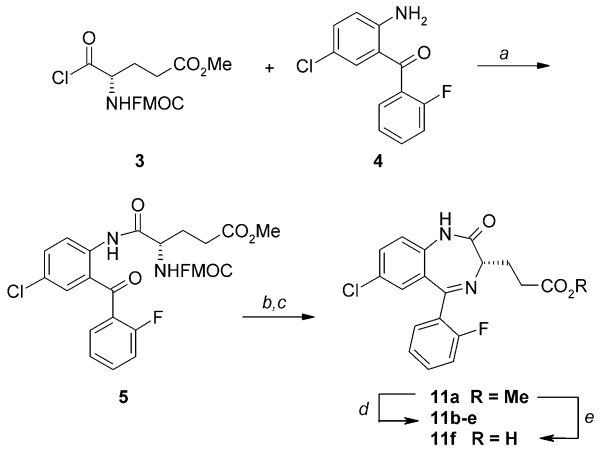


Figure 1. Structures of midazolam (**1**) and diazepam (**2**).

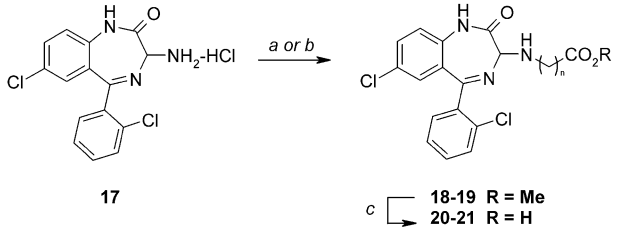


Scheme 1. Reagents and conditions: (a) $CHCl_3$, reflux; (b) Et_3N, CH_2Cl_2, reflux; (c) 5% HOAc in $(CH_2Cl)_2$, 60 °C; (d) [$(n\text{-}Bu_2Sn(Cl)]_2O$, ROH, $PhCH_3$, reflux; (e) LiOH, THF, MeOH, H_2O.

FMOC protecting group was achieved by treatment with triethylamine in dichloromethane. The solvent was removed and replaced with 5% acetic acid in dichloroethane; the resulting solution was warmed to effect cyclodehydration and provide **11a** in 55% yield.[10] Transesterifications were executed using the protocol reported by Otera[11] to deliver **11b–e**. Saponification provided acid **11f**.

Analogues **18a–c** and **19a–c** were prepared according to Scheme 2. In the event, 3-amino-BZD (**17**)[12] was reacted with methyl bromoacetate or methyl acrylate after which chiral HPLC was utilized to supply the adducts in enantiomerically pure form. Subsequently (3*S*)-amino-BZD[13] was used to synthesize analogues of interest. As before, saponification of the esters afforded the corresponding acids.

Our project required esters with levels of potency comparable to clinically relevant BZDs, targeted as $K_i \leq 50$ nM as measured in our BZD radioligand binding



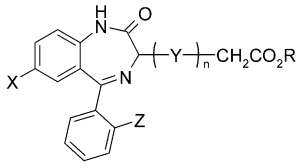
Scheme 2. Reagents and conditions: (a) methyl bromoacetate, Et_3N, THF; (b) methyl acrylate, EtOH; (c) LiOH, THF, MeOH, H_2O.

assay.[14] Attachment of the ester functionality at C3 provided entities with potency/selectivity and the opportunity to map SAR as a function of changes in the tether (Table 1). Based on tether length alone, potent compounds were identified from each group of analogues, and heteroatom-containing linkers delivered compounds equipotent to carbon analogues. Interestingly, the configuration at the C3-stereogenic center proved important regardless of the tether length; natural l-amino acids conferred greater potency than their unnatural d-counterparts, and this trend continued with heteroatom linkers as well. In the end, the potency of the glutamate-based analogues (**11a**, **11d**, **13**, and **15**) versus the single atom-spaced versions (**8b–d**), the costliness of the aminoadipate analogue (**16**), and the relatively cumbersome synthesis of the amine-linked analogues (**18b** and **19b**) afforded advantages to this glutamate-based series.

The strategy called for esters that bind with high affinity to the BZD receptor whereas their corresponding acids would display reduced affinity. Thus, subsequent comparisons of ester-acid pairs were performed and are represented by K_i (acid)/K_i (ester) ratio (Table 1). A ratio of $\geq$100-fold in relative activities was targeted. This filter confirmed the utility of the glutamate-based analogues and prompted an investigation of selectivity as a function of the identity of the ester. Because of the lipophilic character of the BZD receptor,[15] aliphatic and simple aralkyl esters were evaluated. The methyl ester **11a** emerged as a particularly interesting compound owing to its excellent receptor binding affinity and the remarkable separation in binding affinity relative to its corresponding acid **11f**, which binds the human BZD receptor with a K_i value of 6.2 μM.

Neither high affinity binding of a ligand to the benzodiazepine receptor nor selectivity within an ester–acid pair characterizes the intrinsic efficacy (full agonist, inverse agonist, antagonist) of a BZD receptor ligand. Therefore, determination of intrinsic efficacy was assessed by the ability of a compound to cause loss of the righting reflex (LRR) in rats, an effect associated with benzodiazepine full agonism.[16] Compounds producing LRR were evaluated by three measures: (1) time to onset of LRR, (2) initial recovery from LRR (animal rights itself three consecutive times after losing its righting reflex), and (3) total recovery time (the animal walks without ataxia *and* pulls itself up three consecutive times when suspended from a horizontal wire). Several compounds were evaluated in the LRR assay and the recovery behaviors were compared to the clinical standards **1** and **2**. Results are tabulated in Table 1. Compound **11a** presented the desired pharmacodynamic profile offering rapid, uneventful sedation and significantly shorter recovery times (initial = 9 min, total = 24 min) relative to **1** and **2**. The finding that the acid **11f** is the only identifiable metabolite from withdrawn plasma samples bolsters the hypothesis that the observed pharmacodynamic profile is attributed to the plasma and/or tissue degradation of the BZD ester. In addition, in vitro incubation of **11a** in rat plasma gives rise to **11f** with a half-life <1 min.

Table 1. Comparative data for USA BZDs



Compd	X	Y	Z	*n*	R	C3	K_i[a]	Ratio[b]	Dose[c]	LRR recovery[d]	
										Initial	Total
1							2		30	23	121
2							14		15	5	82
6	Cl		H	0	CH_2Ph	S	1447		25	4	14
7	Cl		H	0	CH_2Ph	R	100,000				
8a	Cl		F	0	CH_2(4-pyridyl)	S	289				
8b	Cl		F	0	CH_3	S	81	165			
8c	Cl		F	0	CH_2CH_3	S	45	297			
8d	Cl		F	0	CH_2Ph	S	176	76	25	13	17
9a	H	CH_2	F	1	CH_3	S	92				
9b	H	CH_2	F	1	CH_2Ph	S	163				
10	Cl	CH_2	H	1	CH_2Ph	S	200		70	15	22
11a	Cl	CH_2	F	1	CH_3	S	7	984	25	9	24
11b	Cl	CH_2	F	1	$CH(CH_3)_2$	S	39	108			
11c	Cl	CH_2	F	1	$(CH_2)_3CH_3$	S	242	17	25	15	25
11d	Cl	CH_2	F	1	CH_2Ph	S	29	143	25	8[e]	19
11e	Cl	CH_2	F	1	CH_2(4-pyridyl)	S	34	124			
12	Cl	CH_2	F	1	CH_3	R	741		25	2	4
13	Br	CH_2	F	1	CH_3	S	3	431	25	22	30
14a	O_2N	CH_2	H	1	CH_3	S	118	95	25	2	14
14b	O_2N	CH_2	H	1	CH_2Ph	S	124	91	25	2	3
15	O_2N	CH_2	F	1	CH_3	S	7				
16	Cl	CH_2	F	2	CH_3	S	3	186			
18a	Cl	NH	Cl	1	CH_3	RS	53	35			
18b	Cl	NH	Cl	1	CH_3	S	23	172			
18c	Cl	NH	Cl	1	CH_3	R	480	9			
19a	Cl	$NHCH_2$	Cl	1	CH_3	RS	17	17			
19b	Cl	$NHCH_2$	Cl	1	CH_3	S	3	130			
19c	Cl	$NHCH_2$	Cl	1	CH_3	R	189	1			

[a]Affinity for rat BZD receptor, nM.
[b]K_i (acid)/K_i (ester).
[c]Bolus injection, mg/kg; vehicle = 50% PEG/25% EtOH/25% saline.
[d]Recovery time in min. A compound was identified as inactive in this model if LRR was not observed within 5 min following injection.
[e]Delayed onset of sedation.

The work described herein had incorporated an enzymatically labile ester functionality into a BZD scaffold to identify **11a** as our lead USA BZD compound. Consistent with our strategy, the corresponding acid **11f** was considerably less potent in our receptor binding assay and lacked the ability to provoke a pharmacological response in the LRR assay, even at a dose of 100 mg/kg. Albeit possessing all of the desired in vitro properties, **11a** lacked sufficient aqueous solubility for optimum use as an injectable in clinical settings. The succeeding manuscript describes our efforts to address this shortcoming.

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14. Membrane homogenates were prepared from male Sprague–Dawley rat brain according to the methods described (Marangos, P. J.; Martino, A. M. *Mol. Pharmacol.* **1981**, *20*, 16.) Test compounds were prepared in 100% DMSO at a concentration of 20–55 mM. Compounds were diluted in assay buffer such that the first well contained 100 μM (final concentration). Eleven 3-fold serial dilutions were prepared in buffer to complete a 12-point concentration–response curve for each test compound. Each concentration was tested in triplicate and compounds of interest were tested on at least three separate occasions. The final concentration of DMSO in each well did not exceed 0.4%. Nonspecific binding was defined in the presence of 10 μM 2′-chlorodiazepam ($K_i = 0.5$ nM). The final concentrations of ^{3}H-flunitrazepam were 2 nM. Concentration–response curves for midazolam, chlordiazepoxide or flumazenil were conducted as controls with each assay run. Radioligand, test compounds and membrane homogenates were incubated for 90 min at 4 °C in buffer consisting of 50 mM Tris–HCl, pH 7.4, containing 150 mM NaCl. All assays were conducted in 96-well plates in a total assay volume of 200 μL. Protein concentrations were 12 μg/well. The reaction was terminated by rapid filtration (Packard Filtermate-196) through 96-well GF/B filter plates (Packard # 6005177). The filters were washed, 8 times with 200 μL/well, with ice-cold Tris 50 mM, pH 7.4 (~1.6 mL total). Plates were counted using a Packard TopCount microtiter plate scintillation counter. K_i values were calculated, see: Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
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