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STRUCTURE-ACTIVITY RELATIONSHIPS IN 3-OXO-1,4-BENZODIAZEPINE-2-ACETIC ACID GPIIb/IIIa ANTAGONISTS. THE 2-BENZAZEPINE SERIES

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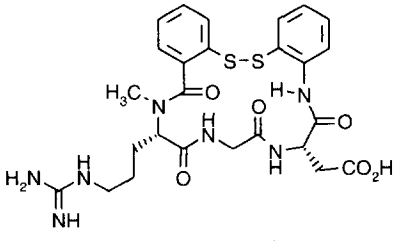
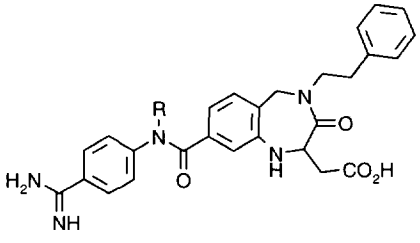
Abstract: In an investigation of the contribution of N-1 to the binding, antiaggregatory, and oral activity in 3-oxo-1,4-benzodiazepine-2-acetic acid based GPIIb/IIIa antagonists, a series of 2-benzazepine analogs, wherein N-1 of the 1,4-benzodiazepine nucleus has been replaced by a methylene group, was examined.

Platelet aggregation has been shown to be mediated, at least in part, by the GPIIb/IIIa receptor complex on the platelet plasma membrane surface.[1] This receptor, also known as $\alpha_{IIb}\beta_3$ or the fibrinogen receptor, is a member of the integrin superfamily of adhesion receptors, and is known to recognize the Arg-Gly-Asp (RGD) tripeptide sequence. Platelet aggregation occurs when fibrinogen, a natural ligand for GPIIb/IIIa which contains the RGD sequence, binds to GPIIb/IIIa on adjacent activated platelets. Disruption of this binding interaction by small, RGD-containing linear peptides,[2] such as Ac-Arg-Gly-Asp-Ser-NH_2, and by cyclic RGD-containing peptides,[3] such as SK&F 107260, has been demonstrated to inhibit platelet aggregation. More recently, small molecule, nonpeptide RGD mimetics have also been shown to inhibit platelet aggregation.[1b] Inhibition of platelet aggregation represents an attractive therapeutic approach for the treatment of thrombotic disorders, such as myocardial infarction and stroke.



SK&F 107260

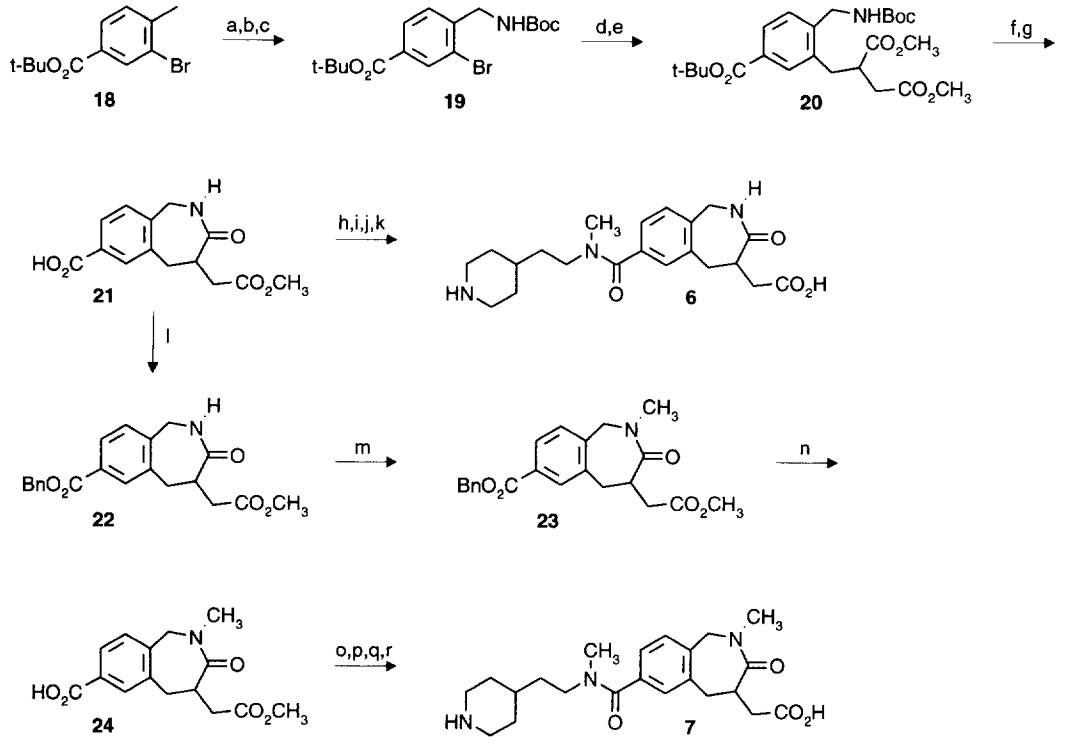


1 R = H
2 R = CH_3

In a previous report from these laboratories,[4] we described the direct design of 1,4-benzodiazepine **1** from the constrained RGD peptide SK&F 107260,[3a,b] and showed **1** to be a potent GPIIb/IIIa antagonist. In subsequent studies, we methylated the linking amide of **1** to afford the potent GPIIb/IIIa antagonist **2**, which was orally active in the conscious dog following intraduodenal (id) administration.[5] In continuing our efforts to identify potent, orally bioavailable GPIIb/IIIa antagonists, we have undertaken a detailed SAR study of the 1,4-benzodiazepine system.[6,7] In this communication, we report binding affinity and antiaggregatory activity for a series of GPIIb/IIIa antagonists based on the 3-oxo-2-benzazepine template, wherein N-1 of the 3-oxo-1,4-

benzodiazepine system has been replaced by a methylene group. We also describe the results of testing of selected members of this series for oral activity.

Scheme 1



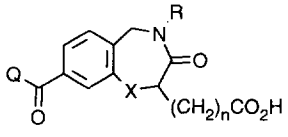
(a) NBS, $(PhCO)_2O_2$, CCl_4, reflux; (b) $MeO_2CN(K)CO_2t$-Bu, DMF, 60 °C (61% for two steps); (c) 1 N NaOH, MeOH (95%); (d) dimethyl itaconate, $Pd(OAc)_2$, P(*o*-tol)$_3$, (i-Pr)$_2$NEt, CH_3CH_2CN, reflux; (e) H_2 (50 psi), 10% Pd/C, 1:1 EtOAc/MeOH, then H_2 (50 psi), $Pd(OH)_2$/C, MeOH (75% for two steps); (f) 1:1 TFA/CH_2Cl_2; (g) NaOMe, MeOH, reflux (94% for two steps); (h) 4-(2-methylaminoethyl)pyridine, EDC, HOBt · H_2O, (i-Pr)$_2$NEt, DMF (91%); (i) H_2 (balloon), PtO_2, 1 N HCl, MeOH; (j) 1 N NaOH, MeOH; (k) TFA, 1:1 CH_3CN/H_2O, 0 °C (47% for three steps); (l) BnOH, EDC, DMAP, (i-Pr)$_2$NEt, DMF (83%); (m) NaH, CH_3I, 1:1 THF/DMF (100%); (n) H_2 (balloon), 10% Pd/C, MeOH (93%); (o) 4-(2-methylaminoethyl)pyridine, EDC, HOBt · H_2O, (i-Pr)$_2$NEt, DMF (97%); (p) H_2 (balloon), PtO_2, 1 N HCl, MeOH; (q) 1 N NaOH, MeOH; (r) TFA, 1:1 CH_3CN/H_2O, 0 °C (74% for three steps).

The benzazepine derivatives required for this study were prepared either according to our reported route[8] to the 2-benzazepine ring system or by the minor modifications of this route described in Scheme 1 for the synthesis of **7**.[9] The known[8] *tert*-butyl ester **18** was brominated at the benzylic position, and the corresponding bromide was reacted with the potassium salt of methyl *tert*-butyl iminodicarboxylate.[10] Partial hydrolysis of the resulting imide with 1 N NaOH in methanol gave Boc derivative **19**. Reaction of **19** with dimethyl itaconate in a Heck reaction,[11] followed by hydrogenation of the resulting olefinic products, gave **20**. Simultaneous deprotection of both the *tert*-butyl ester and Boc groups with trifluoroacetic acid, followed by cyclization, gave the 2-benzazepine **21**. This was converted to **6** via standard manipulations. Alternatively, **21** could be converted to its benzyl ester **22**, which proved to be a suitable substrate for alkylation of the seven-membered

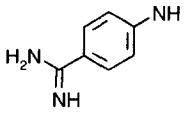
ring amide. In this regard, treatment of **22** with NaH and an alkylating agent, for instance methyl iodide, gave **23**. Hydrogenolysis gave **24**, which was converted to **7** as described above for the synthesis of **6**.

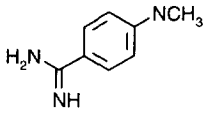
Benzazepine **3** was prepared as previously described,[8] and **5** was prepared similarly, except that dimethyl 2-methylene-1,5-pentanoate[12] was substituted for dimethyl itaconate in the Heck reaction. In the case of thiophene derivative **15**, hydrogenation of the olefinic products obtained from the Heck reaction resulted in desulfurization. To circumvent this problem, we performed the reduction with sodium borohydride in the presence of nickel (II) chloride.[13] Compound **12** was obtained as a byproduct in the preparation of **4**.

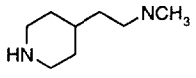
Table 1. In Vitro Activity for Benzazepine Derivatives



No.	X	n	Q[a]	R	method[b]	Binding Inhibition[c] human GPIIb/IIIa K_i (nM)	Antiaggregation[d] human PRP/ADP IC_{50} (nM)
1[e]	NH	1	A	CH_2CH_2Ph	---	2.8 ± 0.1	8 ± 2
2[e]	NH	1	B	CH_2CH_2Ph	---	1.6 ± 0.2	28 ± 2
3[f]	CH_2	1	B	CH_2CH_2Ph	synthesis	1.5 ± 1	12 ± 4
4	CH_2	1	C	CH_2CH_2Ph	synthesis	3.0 ± 0.2	13 ± 2
5	CH_2	2	C	CH_2CH_2Ph	synthesis	650 ± 50	7790 ± 820
6	CH_2	1	C	H	synthesis	3.2 ± 0.1	71 ± 20
7	CH_2	1	C	CH_3	alkylation	1.5 ± 0.1	33 ± 8
8	CH_2	1	C	$(CH_2)_3CH_3$	alkylation	1.5 ± 0.1	31 ± 1
9	CH_2	1	C	$CH(CH_3)_2$	synthesis	2.0 ± 0.1	37 ± 1
10	CH_2	1	C	cyclo-C_6H_{11}	synthesis	1.3 ± 0.1	49 ± 16
11	CH_2	1	C	$(CH_2)_2CH(CH_3)_2$	synthesis	1.5 ± 0.1	32 ± 2
12	CH_2	1	C	$(CH_2)_2$cyclo-C_6H_{11}	synthesis	2.0 ± 0.1	27 ± 7
13	CH_2	1	C	$(CH_2)_2C(CH_3)_3$	synthesis	2.0 ± 0.1	27 ± 5
14	CH_2	1	C	CH_2Ph	alkylation	1.0 ± 0.1	36 ± 2
15	CH_2	1	C	$(CH_2)_2$(2-thienyl)	synthesis	2.5 ± 0.1	18 ± 5
16	CH_2	1	C	$(CH_2)_2$(4-F-C_6H_4)	synthesis	1.7 ± 0.1	27 ± 8
17	CH_2	1	C	$(CH_2)_5Ph$	alkylation	2.0 ± 0.1	8 ± 3

[a]Arg mimetics: A = 

B = 

C = 

[b]Method of preparation: synthesis = prepared by the previously reported procedure (see text); alkylation = prepared via alkylation of **22** (see text). [c]Inhibition of ^{3}H-SK&F 107260 binding to GPIIb/IIIa purified from human platelets, reconstituted in liposomes. The K_i values represent the means of values determined in two to three separate experiments. [d]Inhibition of platelet aggregation in human platelet-rich plasma induced by ADP. The IC_{50} values represent the means of values determined in a minimum of three separate experiments. [e]Ref 5. [f]Ref 8.

The results of in vitro evaluation[14,15] of the benzazepine analogs of the 3-oxo-1,4-benzodiazepine-2-acetic acid system are summarized in Table 1. In the design[4] of benzodiazepine **1**, nitrogen was incorporated at position 1 as a mimic of the Asp-N of SK&F 107260. In principle, the nitrogen at position 1 of the benzodiazepine could be involved in a binding interaction with GPIIb/IIIa, either as a hydrogen bond acceptor via the nitrogen lone pair, or as a hydrogen bond donor via the N-H bond. If either or both of these type of interactions are required for high affinity binding to GPIIb/IIIa, then benzazepine **3** would be expected to lose activity relative to benzodiazepine **2**. However, benzazepine **3** showed binding affinity and antiaggregatory activity fully comparable to that of benzodiazepine **2**. Therefore, neither the nitrogen lone pair nor the N-H bond at position 1 of the benzodiazepine is required for high affinity binding to GPIIb/IIIa. This result is independent of the Arg mimetic at the 7-position, as **4**, with the piperidinylethyl group,[6] was essentially equipotent to both **2** and **3**.

Previously, peptide SAR studies established that the Asp side chain, or an equivalent, was absolutely essential for biological activity.[3] In a brief study at this position, we investigated the effects of homologation of the acetic acid side chain on biological activity. The propionate derivative **5**, which might be viewed as an Arg-Gly-Glu mimetic, showed greatly decreased activity. Although this result does not unequivocally establish the acetic acid side chain as optimal in the benzazepine series, it is in accordance with the peptide SAR, and suggests that the acetic acid side chain has a critical binding function in benzazepine GPIIb/IIIa antagonists.

In the original design,[4] we incorporated a phenethyl group at position 4 of the benzodiazepine, as the peptide SAR had revealed that a lipophilic group following Asp appeared to increase activity.[3] In order to investigate whether the phenethyl group provided optimal activity in the benzazepine series, we varied this substituent while maintaining the Arg mimetic as the piperidinylethyl group.[6] A wide range of lipophilic groups was found to be tolerated at position 2 of the benzazepine (which is equivalent to position 4 in the benzodiazepine series) with minimal effects on binding affinity or antiaggregatory activity. However, when this site remains unsubstituted, as in benzazepine **6**, antiaggregatory activity appears to fall off slightly.

The oral activity of selected benzazepines was assessed in mongrel dogs using a 3 mg/kg dose administered as an id bolus.[5] Under these conditions, benzodiazepine **2** produced a rapid onset of inhibition of ex vivo platelet aggregation which reached about 80% at 60 min, returning to 50% of control after 90 min and to baseline after 4 h.[5] In contrast, id administration of the corresponding benzazepine (**3**) produced no significant inhibition of ex vivo platelet aggregation over a period of 5 h (data not shown). Thus, replacement of N-1 of benzodiazepine **2** by a CH_2 group might appear to have an adverse effect on oral activity. However, id administration of benzazepine **4**, which contains the piperidinylethyl Arg mimetic rather than the benzamidine, produced a rapid onset of inhibition of ex vivo platelet aggregation, which returned to control after about 3 h (Figure 1). Although the duration of action of **4** was relatively short, the results were encouraging, as oral activity had been detected. Following id administration, the corresponding 2-methyl analog **7** showed a lower level of activity than **4** at early time points, but tended towards somewhat greater activity at later time points, suggesting an improved duration of action. Intraduodenal administration of **6**, wherein the seven-membered ring amide is unsubstituted, produced a rapid onset of inhibition of ex vivo platelet aggregation which reached about 80% at 90 min, returning to 50% of control after 240 min. At the end of the experiment (7 h), ex vivo platelet aggregation had not returned to control levels, revealing that **6** had an extended duration of action. Comparison of the iv and id curves for **6** gave an apparent oral bioavailability of approximately 10% (Figure 2).

The results of id testing of selected benzazepine GPIIb/IIIa antagonists suggest that the nature of both the Arg mimetic and the substituent at position 2 have a strong influence on oral activity. The piperidinylethyl Arg mimetic appears to be better than the benzamidine Arg mimetic at imparting oral activity, and an unsubstituted amide in the seven-membered ring appears to be better than substituted amides both in imparting oral activity and in prolonging the oral duration of action. The lower molecular weight of **6** might be partly responsible for the improved absorption, but we suggest that a sensitive balance of lipophilicity and

hydrophilicity may be responsible for the oral activity in both the benzazepine and benzodiazepine series. Further investigation is required to address this issue. The prolonged duration of action of **6** may result from a decrease in metabolic lability relative to the substituted analogs.

Figure 1. Id Curves for Compounds **4**, **6**, and **7**

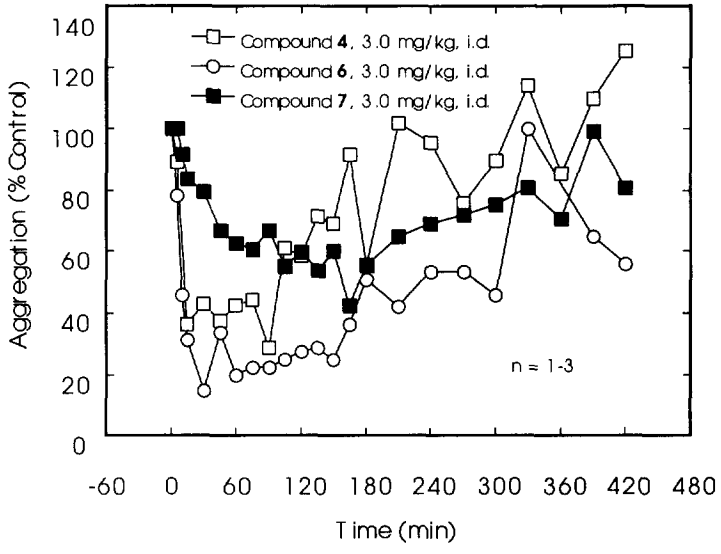
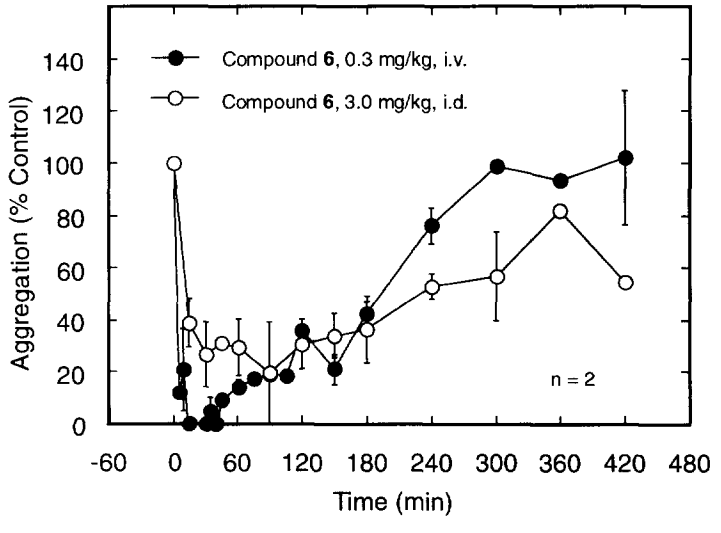


Figure 2. Iv and Id Curves for Compound **6**



In summary, we have investigated the binding affinity and antiaggregatory activity for a series of 2-benzazepine analogs, and conclude that N-1 of the 3-oxo-1,4-benzodiazepine system is not involved in binding interactions with GPIIb/IIIa. We have also examined several benzazepine analogs for oral activity in mongrel dogs, and have identified benzazepine **6** as an orally active member of this series.

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