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NONPEPTIDE GLYCOPROTEIN IIb/IIIa INHIBITORS. 13. DESIGN AND SYNTHESIS OF AN ORALLY ACTIVE PYRAZOLOPIPERAZINONE NONPEPTIDE FIBRINOGEN RECEPTOR ANTAGONIST.

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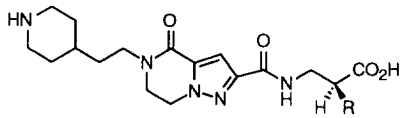
Abstract: The synthesis and antiplatelet activity of a series of pyrazolopiperazinone nonpeptide fibrinogen receptor antagonists is reported. The sulfonamide analog **6** (L-734,115), significantly inhibited ex vivo platelet aggregation 24 h after oral administration at doses of 1.0 and 2.0 mg/kg to dogs and rhesus monkeys, respectively. © 1997 Elsevier Science Ltd.

The activation, adherence, and subsequent aggregation of circulating platelets has been shown to play an important role in various vasoocculsive disorders such as unstable angina, myocardial infarction, transient ischemic attacks, and stroke.[3] The final common pathway for platelet aggregation, regardless of the activating signal, is the binding of the plasma protein fibrinogen to the glycoprotein receptor GPIIb/IIIa on the surface of activated platelets.[4] This binding is mediated in part by Arg-Gly-Asp (RGD) sequences present in fibrinogen, therefore, small molecules and peptidomimetics that feature elements of this tripeptide sequence have been pursued as inhibitors of platelet aggregation.[5]

Previously, we described the design and synthesis of the constrained pyrazolopiperazinone analog **1**.[6] We now report modifications resulting in the identification of **6** (L-734,115) a potent, integrin selective, nonpeptide fibrinogen receptor antagonist that exhibits significant oral activity in dogs and monkeys.



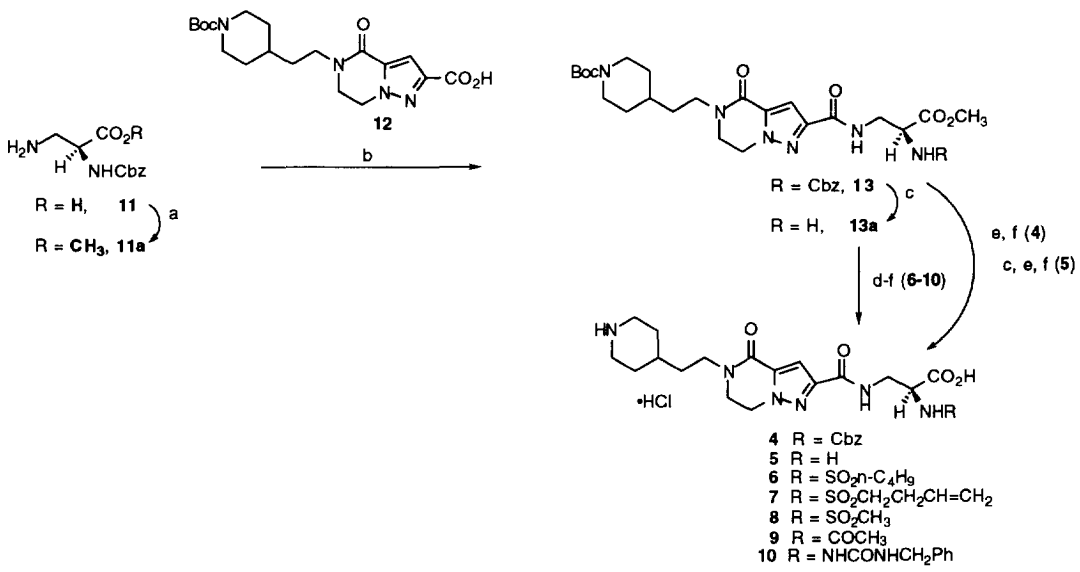
1 R = H
6 R = $NHSO_2n\text{-}C_4H_9$ (L-734,115)

Chemistry

The synthesis of the compounds listed in Table 1 is depicted in Scheme 1.[7] Compounds **1–3** were prepared by coupling **12** with the appropriate β-alanine analog followed by ester hydrolysis and removal of the Boc protecting group with HCl.[6] The 3-substituted β-alanine analogs used to synthesize **2** and **3** were prepared as previously described.[8] Commercially available N-α-(S)-Cbz-diaminopropionic acid (**11**) served as the starting material for 2-substituted analogs, **4–10**. Following treatment of **11** with methanolic HCl, the resulting ester **11a** was coupled with **12** using EDC and HOBt to afford **13**, which was treated successively with aqueous LiOH and HCl in ethyl acetate to give **4**. The α-Cbz moiety of **13** was removed by catalytic hydrogenolysis and the resulting amino ester **13a** was treated with the appropriate electrophile and then deprotected to yield

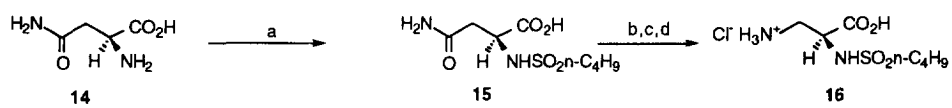
compounds **6–10** (Table 1) in high yield (Scheme 1). Since 10–15% racemization occurred durring the ester hydrolysis step in the synthesis of **6** depicted in Scheme 1, a non-racemizing route was developed.[9] This alternate synthesis of **6** is illustrated in Schemes 2 and 3. Sulfonylation of L-asparagine with *n*-butyl sulfonyl chloride afforded **15** in 50% yield. Treatment of **15** with bromine in aqueous NaOH afforded the 2-sulfonamido propionate that was Boc protected in situ to facilitate its isolation. Deprotection with HCl gave **16** in 75% yield. The mixed anhydride obtained following activation of **12** with isobutyl chloroformate was condensed with **16** and the resulting product was deprotected with HCl, neutralized on an ion exchange resin, and crystallized from water to afford optically pure **6** in 65% yield over the 4 steps.[9]

Scheme 1



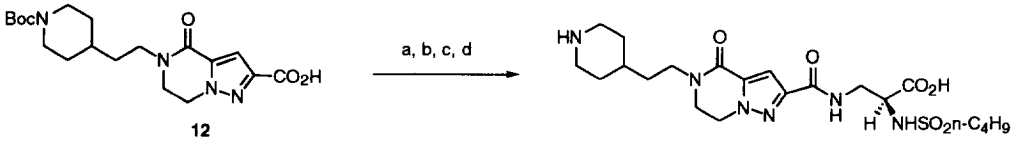
(a) HCl, MeOH, (100%); (b) **11a**, EDC, HOBt, DMF, (95%); (c) H_2, 10% Pd/C, EtOH ,(100%); (d) RSO_2Cl, RCOCl, or RNCO, CH_2Cl_2, Et_3N, (65-100%); (e) LiOH, THF, H_2O, (100%); (f) HCl, EtOAc, 0°, (85-98%).

Scheme 2



(a) *n*-$BuSO_2Cl$, 50% aqueous dioxane, (65%); (b) Br_2, NaOH; (c) Boc_2O, THF, (85%); (d) HCl, EtOAc, (98%)

Scheme 3



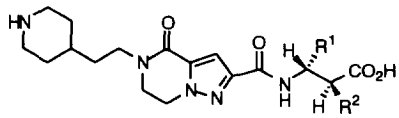
(a) i-BuCOCl, N-methylmorpholine, THF, 0°, (98%); (b)**16**, THF/H_2O, 0°, (83%); (c) HCl, EtOAc, 0° , (100%); (d) ion exchange chromatography, Dowex 50X8-200, (85%).

Results and Discussion

Efforts in these laboratories have focused on the identification of GPIIb/IIIa selective, orally active fibrinogen receptor antagonists that inhibit platelet aggregation at concentrations below 50 nM.[6, 8a,10] Using the pyrazolopiperazinone **1** as a constrained lead, we sought to achieve the desired 5- to 10-fold potency enhancement by placing substituents on the β-alanine moiety. Researchers at these and other laboratories have reported that substitution at the 2-, or 3-positions of β-alanine (R^1 or R^2) containing antagonists led to potency increases.[8,10c] Although 3-substituents have previously been shown to increase potency in other structural classes,[8] incorporation of a methyl or ethynyl moiety at the 3-position resulted in compounds **2** and **3**, respectively, whose potencies were similar to **1** (Table 1). Incorporation of amino derivatives at the 2-position of the β-alanine moiety (R^2) increased in vitro potency of **1** by 5- to 50-fold. The carbamoyl and sulfonamido analogs **4** and **6** had IC_{50} values < 10 nM in the platelet aggregation assay, and were the most potent analogs in this series.

In addition to its increased in vitro potency, compound **6** demonstrated profound antiaggregatory activity in dogs following oral administration at 0.2 and 1.0 mg/kg while **1** was not orally active when dosed at 2.0 mg/kg.[11] The effects of oral administration of the 1.0 mg/kg dose of **6** on ADP and collagen mediated ex vivo platelet aggregation are depicted in Figure 1. ADP mediated platelet aggregation was inhibited by >90% for 8 h following the 1.0 mg/kg dose and remained inhibited by 83% 24 h after dosing. Likewise, the aggregatory response to collagen was inhibited by greater than 85% at 8 h, and remained 78% inhibited at 24 h following the 1.0 mg/kg dose. Following the 0.20 mg/kg dose, both collagen and ADP mediated aggregation were inhibited by >80% between 40 and 200 min with 20 and 30% inhibition observed at 8 h for ADP and collagen, respectively (data not shown).

Table 1. In Vitro Antiplatelet Activity



compound	R^1	R^2	IC_{50}[a] (nM)
1	H	H	250
2	CH_3	H	260
3	CCH	H	120
4	H	NHCBZ	5
5	H	NH_2	45
6 (L-734,115)	H	$NHSO_2nBu$	9
7	H	$NHSO_2CH_2CH_2CH{=}CH$	11
8	H	$NHSO_2CH_3$	15
9	H	$NHCOCH_3$	25
10	H	$NHCONHCH_2Ph$	22

[a]Platelet aggregation of human gel-filtered platelets (GFP) was measured by light transmittance at 37 °C with 2 x 10^8 platlets/mL, 0.1 mg/mL of human fibrinogen and 1 mM $CaCl_2$. Aggregation was initiated by adding 10 mM ADP after all other components were added. The rate of aggregation in the absence of inhibitor served as control, and values reported are the concentration necessary to inhibit the rate of aggregation by 50%. At least two determinations were made for each compound, and values typically varied by <20%.

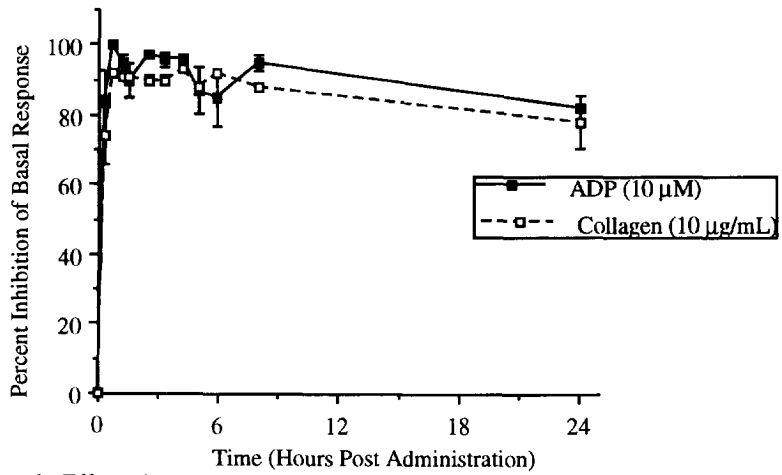


Figure 1. Effect of **6**, 1.0 mg/kg po by gastric gavage in conscious dogs, on the rate and extent of ex vivo platelet aggregation in response to collagen (10 µg/mL + 1 µM epinephrine) and ADP (10 µM + 1 µM epinephrine), respectively.

The oral antiplatelet activity of compound **6** was also evaluated in a primate model (Figure 2) in which four conscious rhesus monkeys[11] were given a 2.0 mg/kg dose of **6** by nasal gastric tube. Platelet aggregation was inhibited by >90% for 6 h with 86% and 31% inhibition at 24 h in response to ADP and collagen, respectively.

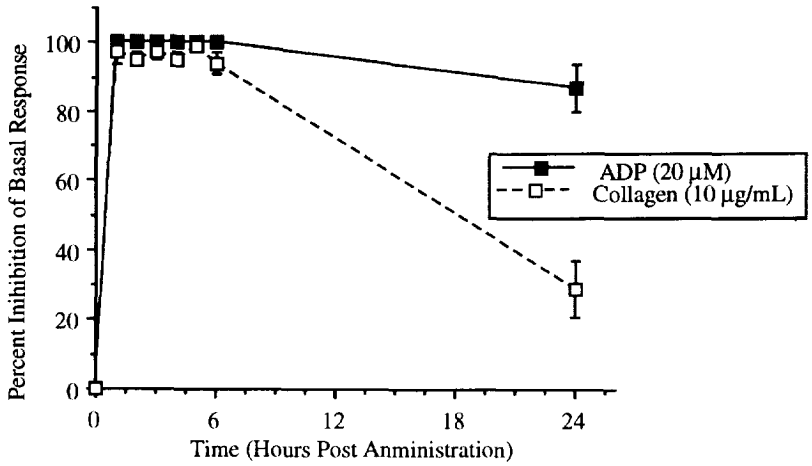


Figure 2. Effect of **6** (2.0 mg/kg) on ex vivo platelet aggregation in conscious rhesus monkeys (n = 4)

Specificity for GPIIb/IIIa over other integrins that recognize RGD ligands is believed to be an important requirement for safety in an oral antiplatelet agent. The specificity of **6** was evaluated by comparing its ability to inhibit platelet aggregation with its ability to inhibit the binding of human umbilical vein endothelial cells (HUVEC) to fibrinogen, vitronectin and fibronectin coated surfaces.[12] Less than 20% inhibition of HUVEC binding was observed with **6** at a concentration of 300 mM, indicating >33,000-fold selectivity for GPIIb/IIIa.

In summary, we describe modifications of previously disclosed pyrazolopiperazinone analog **1** that provided the potent, selective, nonpeptide fibrinogen receptor antagonist **6** (L-734,115), which produces a profound antiaggregatory response 24 h after its oral administration to dog and rhesus monkeys at doses of 1.0 and 2.0 mg/kg, respectively. The on-going in vivo and in vitro characterization of this series of compounds will be the subject of subsequent reports.

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9. The optical purity of **6** was determined by HPLC using β-cyclodextrin based chiral column; mp 283 °C; ^{1}H NMR (DMSO-d_6) δ 8.95 (br s, 1H) 8.33 (t, J = 5.7 Hz, 1H) 7.64 (d, J = 9 Hz, 1H) 7.02 (s, 1H) 4.35 (t, J = 5.1 Hz, 2H) 4.10 (m, 1H); 3.81 (t, J = 5.2 Hz, 2H) 3.6-3.4 (m, 4H) 3.21 (d, J= 10.5 Hz, 2H) 2.95 (t, J = 7.8 Hz, 2H) 2.81 (br m, 2H); 1.96 (d, J = 11Hz, 2H) 1.62-1.2 (overlaping multiplets, 9H) 0.80 (t, J = 7.3Hz, 2H). MS (FAB$^+$) *m/z* 499; Anal calcd for $C_{21}H_{34}N_6O_6S$•0.25 H_2O: C, 50.13; H, 6.91; N, 16.17 ; found: C, 50.17; H, 6.81; N, 16.38.
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