Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2073–2078

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

STRUCTURE-ACTIVITY RELATIONSHIPS OF PYRROLOQUINAZOLINES AS THROMBIN RECEPTOR ANTAGONISTS

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Received 25 March 1999; accepted 8 June 1999

Abstract: A series of pyrroloquinazolines has been discovered that represent novel small molecule inhibitors of the intramolecular ligand of the thrombin receptor. Analogs were prepared to study the structure-activity relationships of substitution at the N1, N3, and N7 positions of the heterocycle. Compounds **4e** and **4f** have been identified with IC_{50}'s of 56 and 52 nM, respectively.

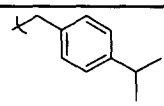
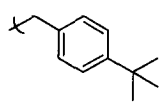
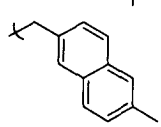
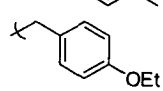
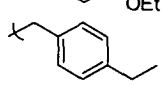
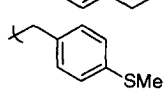
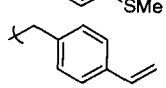
The inhibition of platelet aggregation in humans is predicted to have beneficial cardiovascular effects, particularly in instances where this aggregation is known to be problematic, such as restenosis following balloon angioplasty.[1] Thrombin is known to activate platelets causing aggregation and is also an integral component of the blood clotting pathway, cleaving fibrinogen to fibrin. These effects and others are the result of the proteolytic activity of thrombin. Thus inhibition of this proteolytic activity not only inhibits aggregation, but also presents potential liabilities, such as bleeding. The protease activated thrombin receptor (PAR-1) potentially holds the key to the desired selectivity of function.[2,3] This thrombin receptor is activated by an intramolecular ligand that is revealed by the proteolytic action of thrombin. While peptidic[4] and peptidomimetic[5] antagonists of the thrombin receptor have been reported, our efforts have focused on the identification of small molecule inhibitors of the intramolecular ligand of the thrombin receptor.[6] In this communication, we report the discovery and refinement of a series of pyrroloquinazolines (Scheme 1) that behave as effective antagonists of the thrombin receptor. This paper details our efforts to improve the SAR at three critical sites of functionality - N1, N3, and N7.

Our initial lead in this program, pyrroloquinazoline (PQ) **2a**, came from high-throughput screening of our corporate files. The parent PQ **1** was prepared according to established procedures.[7,8] The initial derivatization of the pyrrole nitrogen (N7) was pursued in a parallel synthesis fashion by simple alkylation. Thus PQ **1** was treated with cesium carbonate in DMF followed by the appropriate alkylating agent. The reactions were quenched by addition of a bulk quantity of water, followed by filtration of the precipitated product.[9] While benzylations afforded analogues with significant inhibition of a high affinity thrombin receptor agonist peptide (ha-TRAP, Ala-Phe(p-F)-Arg-ChA-HArg-Tyr-NH_2)[10] binding to the thrombin receptor (Table 1), other simple alkylations, acylations, and sulfonylations produced compounds with negligible receptor affinity.[11]

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00339-X

Scheme 1. Derivatization of N7 of the Pyrroloquinazolines

Table 1. Biological Activity of N7-Substituted Pyrroloquinazoline Derivatives[11]

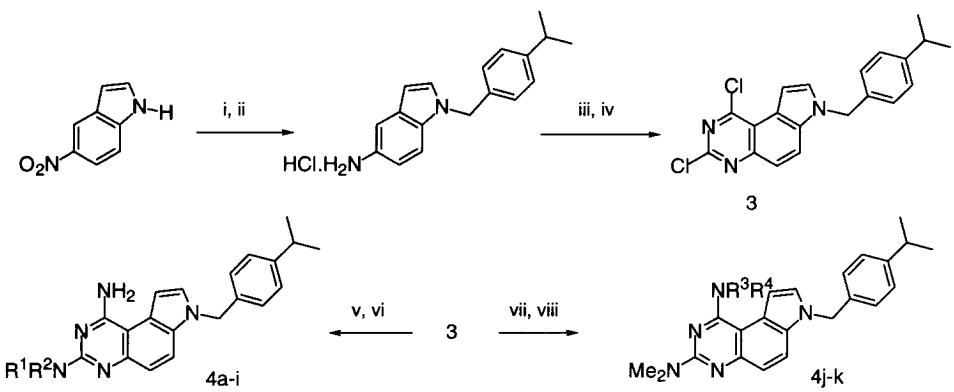
Compd	R	TR IC_{50} (nM)	Aggregation IC_{50} (nM)*
2a**		300	3000
2b		1230	NT
2c		1750	NT
2d		2625	NT
2e		4500	NT
2f		6875	NT
2g		9600	NT

* Aggregation induced by 300 nM ha-TRAP

Compound **2a did not inhibit 0.1 U/mL thrombin induced platelet aggregation at 10 μM.

Derivatizations at N1 and N3 of the PQ nucleus were carried out with (4-isopropyl)benzyl substitution at N7. The dichloro derivative **3** served as a common intermediate for these modifications, and was prepared from 5-nitroindole as shown in Scheme 2. Reaction of **3** with ammonia followed by a primary or secondary amine afforded N3 modified analogues. Modification of N1 was achieved by displacement of the C1 chloro substituent of **3** with a primary or secondary amine, followed by displacement of the C3 chloro by dimethylamine.[12,13]

Scheme 2. Derivatization of N1 and N3 of the Pyrroloquinazolines

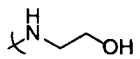
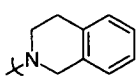
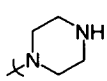


Conditions: (i) 4-isopropylbenzyl chloride, $CsCO_3$, DMF, rt; (ii) H_2 30 psi, 10% Pd/C, MeOH, then aq HCl; (iii) Cl_3CNCO, 1,4-dioxane, rt, 16 h; (iv) $POCl_3$, 1,2-dichloroethane, reflux, 24 h; (v) liq. NH_3, rt, 24 h; (vi) NHR^1R^2, NMP, 120 °C; (vii) NHR^3R^4, CH_2Cl_2, rt; (viii) 2.0 M Me_2NH/THF, 150 °C.

Derivatization of the C3 amino group as a small mono- or dialkylamino substituent was well tolerated (Table 2). Of the groups examined, cyclopropylamino substitution, as in **4e** and **4f**, was optimal. Increased steric demand or introduction of additional polar functionality into the C3 substituent resulted in decreased potency. Replacement of the C1 amino group by methylamino or dimethylamino also resulted in loss of potency.

Several compounds were evaluated for function in a washed platelet aggregation assay using ha-TRAP or thrombin to induce aggregation.[14] The results are shown in Tables 1 and 2. When 300 nM ha-TRAP was used to initiate aggregation, the rank order of potency correlated well with the binding data, although IC_{50}'s were consistently higher for inhibition of aggregation. Binding data were obtained using 10 nM ha-TRAP as the ligand, a concentration close to its Kd of 15 nM, while aggregation experiments used 300 nM ha-TRAP, the EC_{50} for aggregation.[11] Use of 30-fold higher ligand concentration may explain the higher IC_{50} values.

Table 2. Biological Activity of N1- and N3-Derivatized Pyrroloquinazoline Analogues[11]

Compd[b]	NR^1R^2	NR^3R^4	TR IC_{50} (nM)	ha-TRAP Induced Aggregation IC_{50} (nM)*	Thrombin Induced Aggregation IC_{50} (nM)**
4a		NH_2	870	600	NA @ 10 μM
4b		NH_2	100	2000	NA @ 10 μM
4c		NH_2	524	600	NA @ 10 μM
4d		NH_2	4900	NA @ 10 μM	NA @ 10 μM
4e		NH_2	56	300	3000
4f		NH_2	52	150	700
4g		NH_2	100	600	NA @ 10 μM
4h		NH_2	NA @ 1 μM	NT	NA @ 10 μM
4i		NH_2	17% inh. @ 1 μM	NA @10 μM	NA @ 10 μM
4j			3200	ND	NA @ 10 μM
4k			1250	40% Inh. @ 1 0μM	NA @ 10 μM

* Aggregation induced by 300 nM ha-TRAP

** Aggregation induced by 0.1 U/mL Thrombin. IC_{50} determined at 2 min.

The same compounds were tested at concentrations ranging up to 10 μM for their ability to inhibit thrombin induced platelet aggregation. Only the two most potent compounds, **4e** and **4f**, were able to block aggregation induced by 0.1 U/mL thrombin, and these compounds could not block aggregation induced by 1.0 U/mL thrombin. In contrast to the results with ha-TRAP, where inhibition was constant for the entire time course of the assay (7 min), inhibition of thrombin induced aggregation was transient. IC_{50}'s were determined 2 minutes after the initiation of aggregation, but in all cases, aggregation proceeded to completion by 5 minutes. Platelets are known to have multiple thrombin receptors, including a second protease activated receptor, PAR-4.[15, 16] None of these compounds inhibited platelet aggregation induced by 1 mM of the PAR-4 receptor ligand peptide, Gly-Tyr-Pro-Gly-Gln-Val-NH_2 (data not shown). Therefore, the delayed aggregation in the presence of 0.1 U/mL thrombin and the aggregation in response to 1 U/mL thrombin could be mediated by PAR-4, which is known to require higher concentrations of thrombin for activation than PAR-1.[15, 16] Alternatively, the tethered ligand of PAR-1 produced by thrombin cleavage, could compete more successfully with the compounds than with the free

peptide ligand due to its entropic advantage. A contribution from other thrombin mechanisms, such as Glycoprotein Ib, cannot be ruled out by these studies.

The selectivity of these compounds for PAR-1 was first demonstrated by the aforementioned PAR-4 experiment. Selectivity was confirmed by testing against two other platelet activating compounds, 100 μM ADP and 5 μM collagen. At 10 μM, none of the compounds tested blocked either of these activators. The compounds do not appear to block the proteolytic action of thrombin, since active site inhibitors, such as D-Phe-Pro-Arg-chloromethylketone, completely block thrombin induced aggregation under these assay conditions (data not shown).

Conclusion

The results herein describe a novel series of non-peptidic, selective thrombin receptor antagonists that was developed from the lead compound, **2a**. The behavior of the compounds as functional thrombin receptor antagonists was determined by their ability to inhibit ha-TRAP induced platelet induced aggregation. This ability correlated well with the compound's binding affinity for the PAR-1 receptor. The two most potent analogues that were identified, **4e** (IC_{50} = 56 nM) and **4f** (IC_{50} = 52 nM), were also shown to transiently inhibit platelet aggregation induced by thrombin. The pharmocological significance of thrombin receptor antagonism by nonpeptidic small molecules is currently under investigation.

Acknowledgments: The authors would like to thank Drs. Brian McKittrick, Henry Vaccaro, and William Greenlee for helpful chemical discussions.

References and Notes

1. Baykal, D.; Schmedtje, J. F.; Runge, M. S. *Am. J. Cardiol.* **1995**, *75*, 72B.
2. Vu, T. K. H.; Hung, D.; Wheaton, V. I.; Coughlin, S. R. *Cell* **1991**, *64*, 1057.
3. Rasmussen, U. B.; Vouret-Craviari, V.; Jallat, S.; Schlesinger, Y.; Pagés, G.; Pavirani, A.; Lecocq, J.-P.; Pouysségur, J.; Van Obberghen-Schiling, E. *FEBS Lett.* **1991**, *288*, 123.
4. Bernatowicz, M.S.; Klimas, C. E.; Hartl, K. S.; Peluso, M.; Allegretto, N. J.; Seiler, S. M. *J. Med. Chem.* **1996**, *39*, 4879.
5. Hoekstra, W. J.; Hulshizer, B. L.; McComsey, D. F.; Andrade-Gordon, P.; Kauffman, J. A.; Addo, M. F.; Oksenberg, D.; Scarborough, R. M.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1649.
6. A Fujisawa group has reported a small molecule inhibitor of the thrombin receptor: Kato, Y.; Kita, Y.; Nishio, M.; Ito, K. Yamanaka, T.; Seki, J. 70th Annual Meeting of the Japanese Pharmacological Society, Chiba, Japan, March 22-25, 1997.
7. Ledig, K. W. US Patent 4,118,561 (1978); K. W. Ledig, US Patent 4,208,520 (1980).
8. Jones, M. L.; Kuyper, L. F.; Styles, V. L.; Caddell, J. M. *J. Heterocyclic Chem.* **1994**, *31*, 1681.

9. All products were washed with water and hexane. Product purities were assessed by TLC and LC/MS where possible. NMR confirmed the product structure on a sampling of reactions. The analogs thus prepared were >90% pure. The most active analogs were resynthesized and purified for confirmation.

10. Feng, D.-M.; Veber, D. F.; Connolly, T. M.; Condra, C.; Tang, M.-J.; Nutt, R. F. *J. Med. Chem.* **1995**, *38*, 4125.

11. The thrombin receptor binding assay was carried out by the published procedure. Ahn, H-S.; Foster, C.; Boykow, G.; Arik, L.; Smith-Torhan, A.; Hesk, D.; Chatterjee, M. *Mol. Pharm.* **1997**, *51*, 350.

12. Attempts to displace the C3 chloro with NH_3 resulted in concomitant displacement of the substituent at C1.

13. All compounds were purified by silica-gel chromatography and characterized by ^1H NMR and MS. Analytical data for **4e**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, 1H, J = 8.9 Hz), 7.41 (d, 1H, J = 8.9 Hz), 7.34 (d, 1H, J = 2.8 Hz), 7.21 (m, 2H), 7.07 (m, 2H), 6.75 (d, 1H, J = 2.8 Hz), 5.69 (bs, 2H), 5.42 (s, 2H), 5.40 (bs, 1H), 2.95–2.90 (m, 2H), 1.26 (d, 6H, J = 5.5 Hz), 0.86 (m, 2H), 0.62 (m, 2H). MS (FAB) *m/e* 372 $(M+H)^+$. Anal. calcd for $C_{23}H_{25}N_5$: C, 74.36; H, 6.78; N, 18.85%. Found: C, 74.53; H, 7.08; N, 18.44%.

14. The platelet aggregation assay was carried out by the published procedure. Bednar, B.; Condra, C.; Gould, R. J.; Connolly, R. M. *Thrombosis Res.* **1995**, *77*, 453.

15. Kahn, M. L.; Nakanishi-Matsui, M.; Shapiro, M. J.; Ishihara, H.; Coughlin, S. *J. Clin. Invest.* **1999**, *103*, 879-887.

16. Xu, W.-F.; Anderson, H.; Whitmore, T. E.; Presnell, S. R.; Yee, D. P.; Ching, A.; Gilbert, T.; Davie, E. W.; Foster, D. C. *Proc. Nat Acad. Sci. USA* **1998**, *95*, 6642-6646.