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Quinoline-Carboxylic Acids are Potent Inhibitors that Inhibit the Binding of Insulin-Like Growth Factor (IGF) to IGF-Binding Proteins

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Abstract—4-Benzylquinolines **5**, based on a series of isoquinolines **1**, were prepared and tested as inhibitors of the IGF/IGFBP-3 complex based on their ability to displace IGF-I from its binding to IGF-binding protein-3. SAR studies on the 6,7-dihydroxy moiety of the quinoline **5a** showed that the catecol moiety could be replaced with other functional groups. Computational modeling of the **5a**/mini-IGFBP-5 complex revealed the possible binding site of **5a** on IGFBP-5.

The small polypeptide hormones insulin-like growth factors (IGF-I and II) regulate cell proliferation, cell differentiation, cell death, and cell metabolic activities.[1] The mitogenic and metabolic actions of the IGFs are mediated by their binding and activation of the cell surface IGF-I receptor, a $\alpha_2\beta_2$ heterotetramer closely related to the insulin receptor.[2] However, the concentration of freely circulating IGF in blood and interstitial fluids, including the cerebrospinal fluid, is exceedingly low because most of the IGFs are bound to one or more of six high affinity IGF-binding proteins (the major binding protein in circulation is IGFBP-3), which inhibit their interaction with the IGF-I receptor.[3]

Previous communications from our laboratory reported the discovery and SAR of isoquinoline-3-carboxylic acids and 3-hydroxy isoquinolines as potent non-peptidyl IGFBP inhibitors (Fig. 1).[4] Isoquinolines **1** exhibit excellent in vitro potency in both binding and functional assays.[4] This is an alternative approach to develop an orally active compound that can potentiate the action of endogenous IGF-I by displacing the bound IGF-I from the large pool of IGF/IGFBP complexes in the body fluids.

Notable discoveries within the isoquinoline-3-carboxlyic acid class of IGFBP inhibitors included the potency-enhancing 3-carboxylic group and the 1-(3,4-dihydroxybenzoyl) substituents. Methylation of one or more of the hydroxy groups of compound **1a** to replace metabolically labile catechol group resulted in less active analogues.[5] In this paper we report the synthesis, SAR and computational docking studies on a series of quinoline analogues, represented by **5a** (Fig. 1), as potent IGFBP inhibitors.

IGFBPs are proteins of 219–289 residues, with mature IGFBP-5 consisting of 252 residues and all IGFBPs share a common domain organization.[6] Although the three-dimensional structures of IGF-I and IGF-II are known,[7] there is little structural information on any protein of the IGFBP family. The only available information on IGFBP-3 is a domain resolution model for the IGF binding to IGFBP-3.[8] Very recently, the only high-affinity binding site of the entire IGF-BP5 protein for IGFs was identified to reside between residues 40–92 (mini-IGFBP-5).[9] The crystal structure of IGF-I complex with mini-IGF-BP5 has been solved.[10]

The IGF-I binding sites of the IGFBP-5 and IGFBP-3 are nearly identical (Fig. 2) with only two residues lining the binding cavity being different.[11] Val49/Ala55 in IGFBP-5 are equivalent to Ile57/Gly63 in IGFBP-3.

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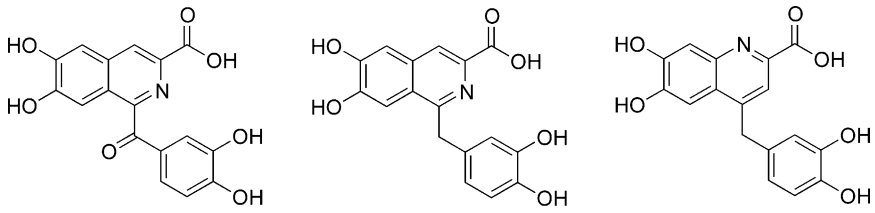


1a, K_i = 5.6 nM **1b**, K_i = 7.2 nM **5a**

Figure 1. IGFBP-3 Inhibitors.

IGFBP-5	43	**E**	**G**	**Q**	**A**	**C**	**G**	**V**	**Y**	**T**	**E**	**R**	**C**	**A**	**Q**	**G**	**L**	**R**	**C**	**L**	**P**	**R**	**Q**	**D**	**E**	**E**	**K**	**P**	**L**	**H**	**A**	**L**	**L**	**H**	**G**	76
IGFBP-3	50	**E**	**G**	**Q**	**P**	**C**	**G**	**I**	**Y**	**T**	**E**	**R**	**C**	**G**	**S**	**G**	**L**	**R**	**C**	**Q**	**P**	**S**	**P**	**D**	**E**	**A**	**R**	**P**	**L**	**Q**	**A**	**L**	**L**	**D**	**G**	83

Figure 2. The amino acid sequences of the IGF-binding region of *h*IGFBP-5 and *h*IGFBP-3. The boxed residues in *h*IGFBP-5 and the corresponding sites in hIGFBP-3.

Since compound **5a** and its close analogues also bind to IGFBP-5 with high potency (K_i for IGFBP-5: 13 nM, for IGFBP-3: 8 nM), we selected the crystal structure of the ligand binding domain of IGFBP-5 to dock compound **5a**. The energy-minimized conformation of **5a** was manually docked into the IGF-I binding cavity of mini-IGFBP-5 after IGF-I was removed from the crystal complex. As shown in Figure 3 of the final mini-IGFBP-5–**5a** complex, which resulted from the computational study using ***Docking*** and ***Affinity*** software provided by Accelrys.[12] Four amino acid residues from the IGFBP-5 are identified that may potentially involve in key interactions with **5a**. Those residues are Arg59, Arg53, Leu73, and Val49 (highlighted by colored balls, whereas **5a** is shown in the style of colored ball and stick, oxygen atoms are colored in red, nitrogen in blue, and carbon in green, respectively).

4-Benzylquinolines bearing a 2-carboxylic acid (**5**) were synthesized by cyclization of a substituted aniline **2** with acetylenedicarboxylate under heating conditions, and the resulting hydroxylquinolines were then converted to the corresponding 4-chloroquinolines **3**. Replacement of the chlorine in 3 with 3,4-dimethoxyphenylacetonitrile promoted by sodium hydride in THF gave quinolines **4**.[13] Acidic hydrolysis of **4** with 48% HBr resulted in saponification of the methyl ester, hydrolysis and decarboxylation of the nitrile, and deprotection of the methoxy groups to afford benzylquinolines **5** (Scheme 1).

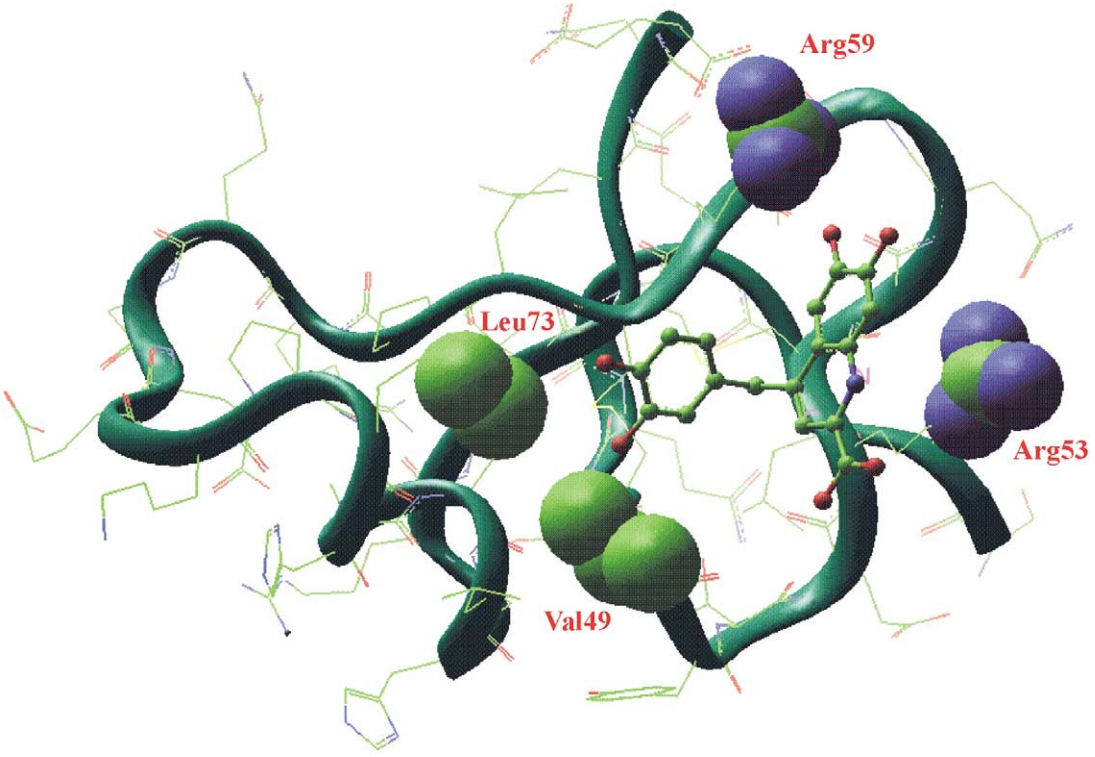
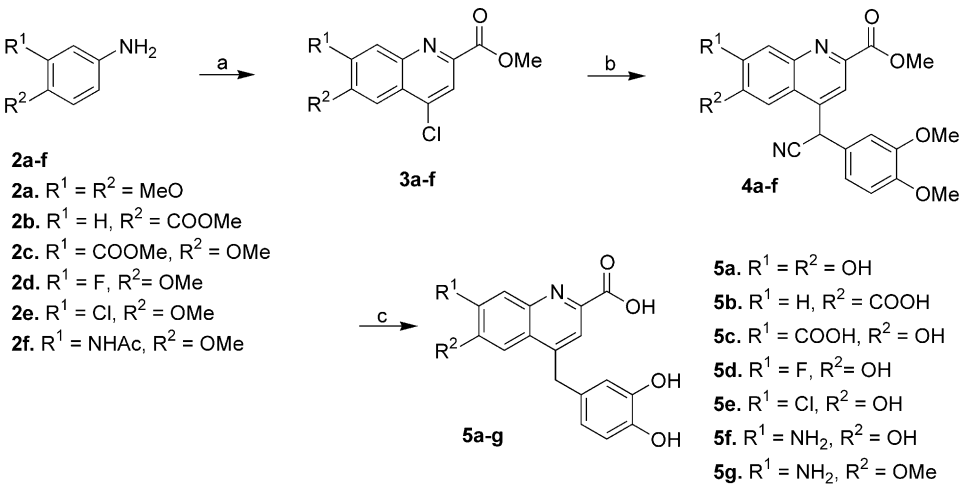


Figure 3. Compound **5a** binding to the mini-IGFBP-5. The four residues (Val49, Arg53, Arg59, Leu73) that contacted with **5a** are highlighted.



Scheme 1. Reagents and conditions: (a) (i) $MeO_2CC{\equiv}CCO_2Me$/heat; (ii) $POCl_3$/reflux; (b) 3,4-$(MeO)_2PhCH_2CN$/NaH/THF; (c) 48% HBr/reflux.

Table 1. SAR of substituted quinolines **5**

Compd	R^1	R^2	K_i (nM)
5a	OH	OH	13
5b	H	COOH	>10,000
5c	COOH	OH	110
5d	F	OH	53
5e	Cl	OH	240
5f	NH_2	OH	52
5g	NH_2	OMe	5300

There is no obvious interaction of the 2-nitrogen of the isoquinoline **5a** with the IGFBP-5 protein. That may explain why quinoline **5a** had very similar activity as its isomeric isoquinoline **1b** (**1b**, $K_i = 7.2$ nM, **5a**, $K_i = 13$ nM). From this binding model, there is clearly a charge-charge interaction between the 3-carboxlyic group of isoquinoline **1** or quinoline **5**, with the Arg53 residue of the IGFBP-5. The 6,7-dihydroxy moiety of the isoquinoline **5** is proposed to interact with another basic residue, Arg59. Arg53 and Arg59 have strong interaction with Asp20 and Glu58 of IGF-I, respectively, in the IGF-I/mini-IGFBP-5 complex. Attempts to replace this catechol moiety with a carboxylic acid at the 6-position of the quinoline core resulted in a totally inactive analogue (**5b**). It is rather difficult to interpret this result since the arginine residue is located on the surface of the IGFBP-5 protein and should be flexible. Replacing the 7-hydroxy group with a carboxylic acid reduced binding activity (**5c**), possibly because of a steric clash with Arg59. Interestingly, the 6-hydroxy-7-fluoro compound **5d** was more active than its 7-chloro analogue **5e** (K_i 53 nM for **5d**; 240 nM for **5e**). In these two cases, the acidity of the 6-hydroxy group seems to play an important role, because the fluoro analogue should be more acidic due to high electron-withdrawing effect of the fluorine atom. Finally, the 7-amino compound **5f** only lost about 4-fold binding affinity ($K_i = 52$ nM), but its 6-methoxy analogue was much less active (**5g**, $K_i = 5300$ nM). This result again emphasizes the importance of an acidic hydroxy group in this region as we had previously reported in the isoquinoline series that replacement of the 6,7-dihydroxy moiety with a

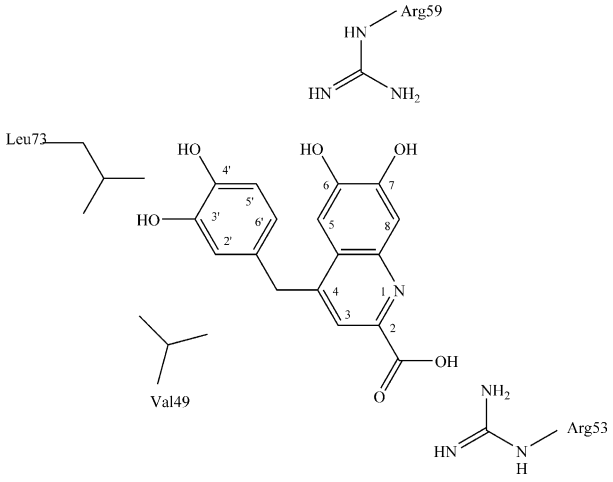


Figure 4. Illustration of the interaction of compound **5a** with IGFBP-5.

6-methylsulfonamido-7-hydroxy group resulted in a much less active compound (Table 1).[14]

Based on our docking model, the benzyl group of **5a** interacts with several lipophilic residues including Val49 and Leu73 of IGFBP-5 (Fig. 4), depending on the size and shape of the substituted phenyl group. A bigger group such as 3-phenoxyphenyl (see previous paper) may also interact with Pro62 and Leu70 based on this model. The Thr51 residue may have a hydrogen-bonding interaction with the benzoyl carbonyl group of **1a**. This may explain why some benzyl alcohols were more potent inhibitors.[12] This binding cavity is the host of Phe16 of IGF-I based on crystal structure.[8] One can then predict that replacement of the benzyl with a suitable aliphatic group may also generate potent IGFBP antagonists.

In conclusion, we have discovered that quinolines **5** bearing a carboxylic acid are potent inhibitors of IGF-I binding to IGFBP-3 and IGFBP-5. While the catechol moiety on the quinoline ring seems to be important in binding, it could be mimicked with other functional group to generate potent but more stable analogues. The 3,4-dihydroxybenzyl group should be replaced by

different substituted benzyl moieties, possibly an aliphatic group, as we observed in the isoquinone series.[5] This discovery may help to design compounds with better physicochemical properties. Lastly, the binding model may guide the design of potent inhibitors with selectivity among the six different IGF-binding proteins.

References and Notes

1. (a) For recent reviews, see: Roith, D. L.; Bondy, C.; Yakar, S.; Liu, J.; Butler, A. *Endocr. Rev.* **2001**, *22*, 53. (b) Jones, J. I.; Clemmons, D. R. *Endocr. Rev.* **1995**, *16*, 3.
2. Ullrich, A.; Gray, A.; Tam, A. W.; Yang-Fang, T.; Tsubokawa, M.; Collins, C.; Henzel, W.; Le Bon, T.; Kathuria, S.; Chen, E.; Jacobs, S.; Francke, U.; Ramachandran, J.; Fujita-Yamaguchi, Y. *EMBO J.* **1986**, *5*, 2503.
3. (a) Shimasaki, S.; Ling, N. *Progress in Growth Factor Res.* **1992**, *3*, 243. (b) Shimasaki, S.; Shimonaka, M.; Zhang, H.; Ling, N. *J. Biol. Chem.* **1991**, *266*, 10646.
4. Liu, X.-J.; Xie, Q.; Zhu, Y.-F.; Chen, C.; Ling, N. *J. Biol. Chem.* **2001**, *276*, 32419.
5. Chen, C.; Zhu, Y.-F.; Liu, X.-J.; Lu, Z.-X.; Xie, Q.; Ling, N. *J. Med. Chem.* **2001**, *44*, 4001.
6. Rajaram, S.; Baylink, D. J.; Mohan, S. J. *Endocrinol. Rev.* **1997**, *18*, 801.
7. (a) Cooke, R. M.; Harvey, T. S.; Campbell, I. D. *Biochemistry* **1991**, *30*, 5484. (b) Terasawa, H.; Kohda, D.; Hatanaka, H.; Nagata, K.; Higashihashi, N.; Fujiwara, H.Sakano; Inagaki, F. *EMBO J.* **1994**, *13*, 5590.
8. Spenser, E. M.; Chan, K. *Prog. Growth Factor Res.* **1995**, *6*, 209.
9. Kalus, W.; Zweckstetter, M.; Renner, C.; Grol, M.; Demuth, D.; Schumacher, R.; Dony, C.; Lang, K.; Holak, T. A. *EMBO J.* **1998**, *17*, 6558.
10. Zestawski, W.; Beisel, H.-G.; Kamionka, M.; Kalus, W.; Engh, R. A.; Huber, R.; Lang, K.; Holak, T. A. *EMBO J.* **2001**, *20*, 3638.
11. Hong, J.; Zhang, G.; Dong, F.; Rechler, M. M. *J. Biol. Chem.* **2002**, *277*, 10489.
12. ***Docking*** and ***Affinity*** molecular modeling software are provided by Accelrys.[15] In docking, the interaction energy is computed by summing the energy contributions between all atoms of the two molecules. The objective of a docking type calculation is to evaluate the interaction energies of many orientations of one molecule relative to the other, while searching for the orientations that result in low interaction energies. A more advanced simulation module ***Affinity*** is employed that automatically moves the small molecule, evaluates energies, and checks if the structure is acceptable by energy evaluation. In our simulation, the small molecule and the side chains of mini-IGFBP are flexible during the search, whereas the backbone of IGFBP is fixed.
13. Cutler, M. *J. Am. Chem. Soc.* **1949**, *71*, 3375.
14. Zhu, Y. F.; Wilcoxen, K.; Gross, T.; Connors, P.; Strack, N.; Gross, R.; Huang, C. Q.; McCarthy, J. R.; Xie, Q.; Ling, N.; Chen, C. *Bioorg. Med. Chem. Lett.* See previous paper. doi: 10.1016/50960-894X(03)00321-4.
15. Luty, B. A.; Wasserman, Z. R.; Stouten, P. F. W.; Hodge, C. N.; Zacharias, M.; McCammon, J. A. *J. Comp. Chem.* **1995**, *16*, 454.