Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 2, pp. 113-116, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(96)00589-6

COVALENT BINDING OF CATECHOLS TO SRC FAMILY SH2 DOMAINS

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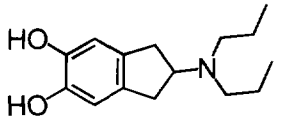
Abstract: Src family SH2 domains contain an uncoupled cysteine residue, that, when arylated with air oxidized catechols, inhibits the protein from binding to otherwise high affinity peptide ligands. No inhibition is observed for Crk, Abl, or Grb2 SH2 domains.

Protein-protein interactions mediated through SH2 domains[1] occur in a variety of signal transduction pathways.[2] Molecules that interrupt a specific SH2 domain binding event in vivo would potentially be useful for the treatment of cell-proliferative disorders.[3] So far, there has been no report of a suitable molecule,[4] either discovered or designed, despite the efforts of many who have proposed and initiated[5] peptidomimetic discovery programs and high-throughput screens to capitalize on this promising opportunity.

In the course of screening our chemical compound library in an enzyme-linked immunosorbent assay (ELISA),[6] we found that the conformationally restricted dopamine analog **1**[7,8] inhibits the binding of Src family[9] SH2 domains to high affinity peptide ligands. Of greater importance, however, is the fact that under identical assay conditions **1** caused no inhibition of binding of Crk, Abl, or Grb2 SH2 domains to their high affinity ligands. In an attempt to understand this observation, we examined structural models of SH2-peptide complexes and, concurrently, began to synthesize[10] compounds that would probe the importance of the different functionalities of **1**. Both strategies proved to be worthwhile.



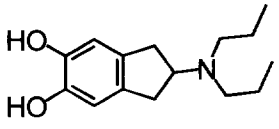
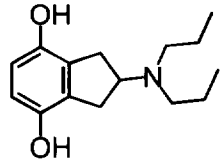
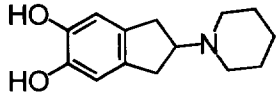
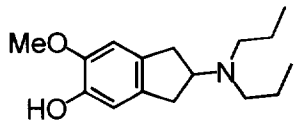
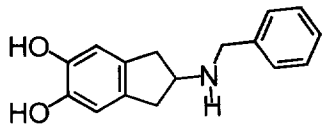
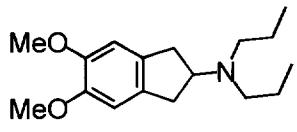
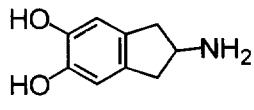
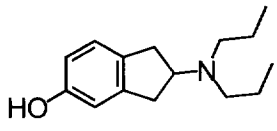
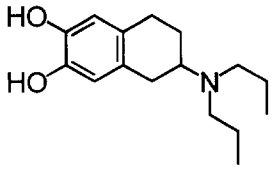
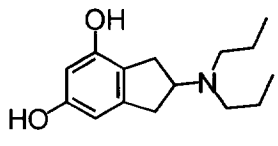
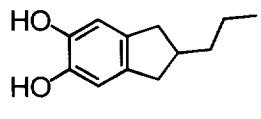
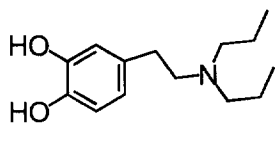
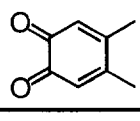
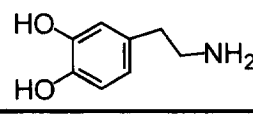
1

From the model we were alerted to the presence of unique cysteine residues in the amino acid sequence of Src family SH2 domains. In the ELISA for SH2 binding inhibition of Fyn, a stable prototypical Src family member, IC_{50} values for an authentic sample of **1** and the set of analogs (see Table) varied over a large range;[11] whereas, all were inactive against Grb2.

We propose, consistent with these data, a possible mechanism involving initial air oxidation of the catechol to an *o*-quinone that then reacts with sulfhydryl groups on the SH2 domain, and the resulting steric changes in the protein-*o*-quinone adduct prevents ligand binding. The evidence for this mechanism mounts.

Table

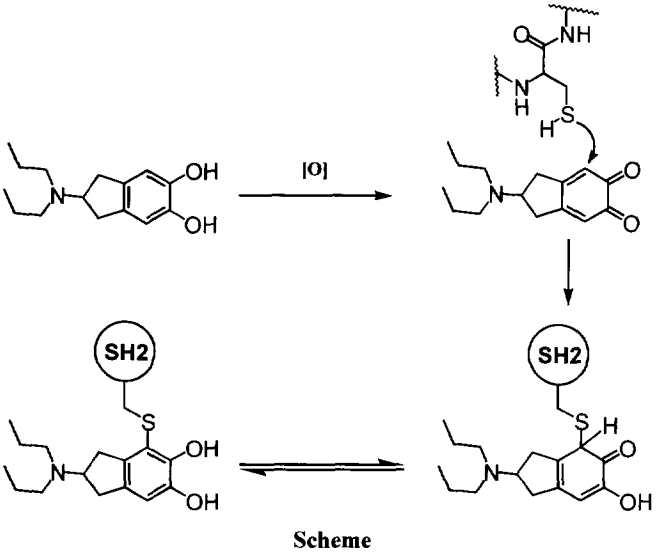
IC_{50} values† of Fyn SH2

		IC_{50} (μM)			IC_{50} (μM)
1		10	8		50
2		10	9		>100
3		10	10		>100
4		10	11		>100
5		50	12		>100
6		1	13		>100
7		1	14		>100

>100 means no inhibition was detected at 100 μM

†IC_{50} values (triplicate determinations) represent the concentration of compound required to inhibit 50% of the maximum signal generated in the absence of compound from the binding of Fyn SH2 to the peptide EPQ(pY)EEIPI.

To wit, (1) substituted catechols are known to rapidly undergo autoxidation to *o*-quinones.[12] The inhibitory activity attributable to **1** is abolished when antioxidants (dithiothreitol or $Na_2S_2O_5$) are added to the assay buffer.[13] Examination of the IC_{50} values for the compounds shown in the table reveals that a catechol functional group is necessary for inhibitory activity. The guaicol, veratrol, and resorcinol type analogs **9**, **10**, and **12** are inactive, as is the phenol **11**, for none undergoes significant air oxidation during the assay. The hydroquinone derivative **8** is capable of undergoing oxidation and does show moderate inhibitory activity. Substitution on the nitrogen has little effect on reactivity, but a compound without nitrogen, **6**, decomposes if stored on the bench over a period of a few days. Nonetheless, freshly prepared samples of **6** display the highest activity, comparable only to 4,5-dimethyl-*o*-quinone (**7**).[14] (2) Quinones are known to react rapidly with



Scheme

sulfhydryl groups to generate aromatic systems (see Scheme).[15] For example, urushiol, the active principle of poison ivy, forms covalent adducts with human serum albumin.[16] Likewise, the natural product stypoldione, which bears a stable *o*-quinone, has been shown to form covalent bonds with proteins and small molecules that contain sulfhydryl groups.[17] (3) Inhibitory activity for **1** is only observed on SH2 domains that contain a cysteine residue (Src family). Stypoldione has an identical activity pattern against SH2 domains as does **1**. (4) Covalent modification of Src SH2 domain by iodoacetamide is sufficient to prevent ligand binding.[18]

Dopamine (**14**) was shown to be inactive in the assay. The inactivity can be reasoned that upon oxidation **14** cyclizes to form an indoline ring before it can react with the protein.[19] However, *N,N*-dipropyldopamine (**13**) was also inactive; indole formation in that case is unlikely. Alternatively, autoxidation of **13** and **14** to *o*-quinones may not be occurring. Autoxidation rates (of catechols) are known to decrease as

methyl substitution of the ring is reduced.[20]

By synthesizing and testing a judicious set of analogs we have explained how a class of not-so-obvious compounds behaves in an important biological model. Whether or not this particular mechanism will operate in cells, understanding the chemistry of proteins with small organic molecules is a first step in unraveling the complexities of biological systems that are the targets for new therapeutic agents.

Acknowledgment
The authors wish to thank Larry Feldberg and Robert Mallon for compound testing, and Carlo Nalin and Virginia Estevez for helpful discussions.

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(*Received in USA* 15 *October* 1996; *accepted* 2 *December* 1996)