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Imidazole-based Ligands of the Src SH_2 Protein

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Abstract—Starting from known Src SH2 inhibitors incorporating five-membered heterocycles or benzamide scaffolds, we prepared tetrasubstituted imidazole compounds able to interact with the pY, pY + 1 and pY + 3 binding sites of the Src SH2 protein. The synthesis and biological data are presented.

The $pp60^{c\text{-}src}$ (Src) is a nonreceptor tyrosine kinase involved in intracellular signal transduction.[1] The observation that osteopetrosis is the major phenotype in Src−/−mice [2] incited us to develop a program for the inhibition of the Src protein via its SH2 domain for the treatment of osteoporosis. Src SH2 domain[3] contains approximately 100 amino acids and binds to phosphotyrosine (pTyr) containing peptides.

Extensive crystallographic studies[4,5] between the pY-E-E-I tetrapeptide and the Src SH2 protein have revealed the presence of two major binding pockets, one interacting with the pTyr and the other with the pY + 3 Ileu residue. From rational drug design, new potent and less peptidic inhibitors[6–11] have been found, based on various scaffolds which are able to interact positively with the protein and also to deliver their side chains into the respective pY and pY + 3 pockets. Among these scaffolds, benzamide[8] is potent, the phenyl group being in contact with the hydrophobic surface generated by the Tyr βD5 and the carboxamide function taking the place of a structural water molecule and thus interacting directly with Lys 60. Five-membered ring scaffolds (thiazole, oxadiazole) have also been disclosed recently[10,11] and proved to efficiently deliver the side chains to their respective pockets. However, these heterocycles are lacking the interaction generated by the carboxamide of the benzamide scaffold, and thus the inhibitors designed from them appeared to be a slightly less active than pYEEI and those derived from the benzamide scaffold.

In this paper, we would like to disclose the synthesis and biological evaluation of Src SH2 inhibitors based on the imidazole scaffold, incorporating a carboxamide moiety susceptible to displace the water molecule. This should enhance the entropic contribution to binding when compared to the five-membered rings already described.[10,11]

The target compound identified for synthesis is the tetrasubstituted imidazole **15b**. The cyclohexyl moiety was introduced to interact with the hydrophobic pocket while the 5-CONH2 was intended to displace the structural water molecule. Moreover, the substitution at the 2-position of the imidazole was designed to pick up the pY as well as the pY + 1 interactions (originally pY-E on the tetrapeptide pYEEI). However, Buchanan et al.[10] at Ariad showed that simple alkyl side chains can be acceptable replacements for the (pY + 1) Glu residue within the five-membered ring oxadiazole series. This was also the case in the benzamide series developed by Parke Davies.[8] Thus, aware of the importance of reducing the overall charge of the molecule but also for synthetic feasibility, we decided to replace the original Glu residue by a methyl group.

Compound **7** was the key intermediate for the synthesis of **15b** and was prepared via a multigram four-step sequence with an adaptation of the pathway developed for the synthesis of angiotensin II antagonists.[12,13] Compound **7** proved to be a versatile scaffold which allowed easy functionalization of positions 2, 4 and 5 of the imidazole ring. Thus several compounds could be derived from imidazole **7** via chemical modifications of the 2-carboxaldehyde, the 4-masqued thiol and 5-carboxyester, as shown with the synthesis of the target compounds **15a–c** (Scheme 1).

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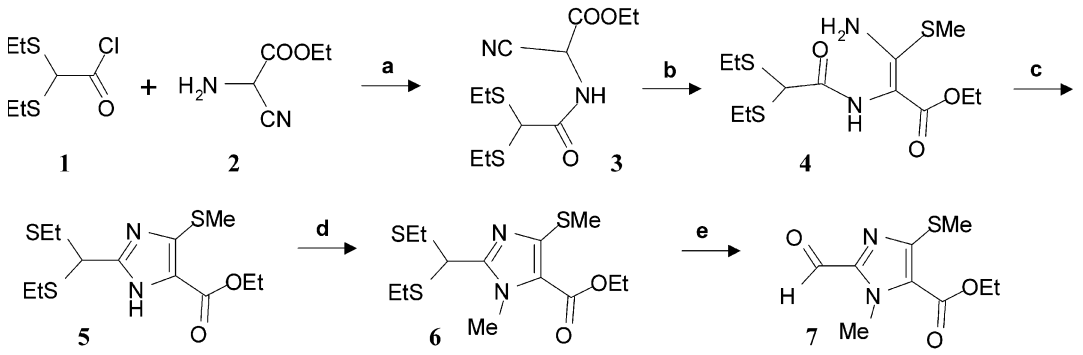


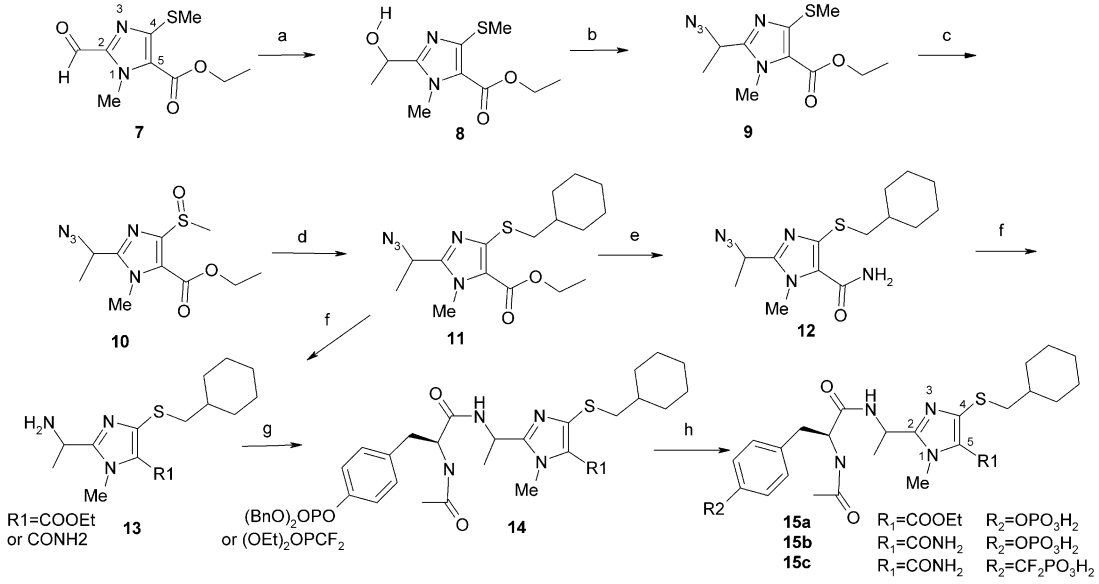
Scheme 1. Synthesis of the tetrasubstituted imidazole scaffold **7.** Reagents and conditions: (a) pyridine, THF, 2 h, 62%; (b) MeSH excess in EtOH, TEA, overnight 5 °C, 84%; (c) propane phosphonic anhydride, AcOEt, 30 mn rt then reflux 2 h, 79%; (d) K_2CO_3, DMF, 1.1 equiv MeI 3 h, 90%; (e) 50 equiv of HCOCOOH, H_2O in CH_3COOH HCl_{conc} (50 equiv), 30 h, rt, 89%.

Condensation of dithioethylacetylchloride[14] **1** with ethyl aminocyanoacetate **2** led to intermediate **3**. Addition of methylthiol onto the nitrile gave rise to the amino compound **4** which could easily undergo an intramolecular cyclization using propane phosphonic anhydride in 75% yield. Alkylation of the resulting imidazole **5** with methyl iodide afforded N–Me imidazole **6** as the major regioisomer (90% yield). Final deprotection of the dithioacetal proved to be rather difficult and attempts using $HgCl_2$–HgO in CH_2Cl_2, $HgCl_2$ with $CaCO_3$ in CH_3CN, HCl in MeOH or $SOCl_2$ with SiO_2 in CH_2Cl_2 were unsuccessful. Finally, the use of glyoxylic acid which gave smooth displacement of the dithioethyl group was the method of choice[15] and led to the desired 2-carboxaldehyde in 89% yield (Scheme 2).

With this scaffold **7** in hand, we could functionalize the 2- and 4-positions of the imidazole ring in order to deliver the pY and pY + 3 side chains into their respective pockets. This was done by construction of the desired fragment from the aldehyde and generation of the thiol from the thiomethyl group.

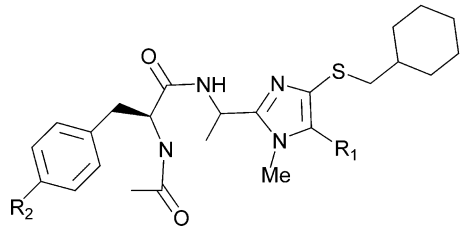
Modification of the aldehyde was first achieved by introduction of the pY + 1 side chain as a methyl group by addition of methyl Grignard. Then, in order to prepare the required amine, the resulting alcohol **8** was converted into azido derivative **9** using DPPA in toluene with a base. This reaction was more efficient with DBU as a base than using TEA (85% yield vs 20%).

The azido group of compound **9** was then used as an amino protecting group during the functionalization of the 4-thiomethyl moiety. Conversion of thiomethyl to another thioalkyl (methylcyclohexyl in this paper) was performed according to a straightforward two-step procedure developed recently in our team.[16] The first step consists of oxidation of the sulfur atom with mCPBA at 0 °C. The resulting sulfoxide **10** was then submitted to Pummerer reaction with trifluoroacetic



Scheme 2. Synthesis of imidazole based Src SH_2 ligands **15a**–**c**. Reagents and conditions: (a) MeMgBr, THF, 0 °C 15 min, 55%; (b) DPPA, toluene, DBU 24 h, 85%; (c) mCPBA, CH_2Cl_2 0 °C; 15 min, 85%; (d) (i) TFAA, CH_2Cl_2 15 min, then evaporation; (ii) degassed MeOH, TEA 5 min, followed by addition of ICH_2cHexyl, overnight at rt, 81%; (e) (i) 5 equiv of 2 N NaOH, EtOH, overnight (91%); (ii) EDC, HOBT, NMM in THF 1 h, then NH_4OH 30% 3 h (63%); (f) H_2, Pd/C AcOEt (100%); (g) EDC, HOBt, RCOOH, 3 h, 88%; (h) H2, Pd/C MeOH, 70% [for $PO(OBn)_2$ or TMSBr in CH_2Cl_2], 3 h at 0 °C [for $PO(OEt)_2$].

Table 1. Src SH_2 binding affinities



Compd	R1	R2	IC_{50} (μM)[18]
15a	COOEt	OPO_3H_2	6.9
15b	$CONH_2$	OPO_3H_2	8.6
15c	$CONH_2$	$CF_2PO_3H_2$	5.3

anhydride to generate the intermediate protected thiol SCH_2OCOCF_3. Basic treatment with TEA in MeOH liberated in situ the thiol which was immediately converted *one pot* in the desired thiomethylcyclohexyl **11** by addition of the corresponding iodide (81% yield for the two steps from sulfoxide).

Final functional group modifications led to the desired compound. Thus, introduction of the carboxamide group was achieved via saponification of the ester group followed by a EDC coupling of the resulting acid with ammonia. Functionalization of the 2-position of the imidazole was achieved after conversion of the azido to the amino group by hydrogenation (**13**) and EDC coupling with dibenzyl protected phosphotyrosine (or F2PMP tyrosine[17]). Final deprotection (hydrogenolysis in EtOH for debenzylation of the dibenzylphosphate or TMSBr in CH_2Cl_2 for removal of the ethyl groups from the $CF_2PO(OEt)_2$ fragment afforded the final desired compounds **15b–c**. To evaluate the influence of the substitution in the position 5 of the imidazole (ester vs carboxamide), the intermediate 5-ethyl carboxylate ester imidazole **11** was also coupled to phosphotyrosine after hydrogenation of the azido group to finally give **15a**.

These three compounds **15a–c** have been evaluated using a competition assay (Scintillation Proximity assay, SPA)[18] and the biological results are indicated in Table 1. The μM level of activity indicates that some positive interactions between the protein and these ligands exist. However, these results are not as promising as expected because the prepared compounds are around 30 times less active than the reference peptide pYEEI in our assay (150 nM). Moreover, there is no difference in binding between compound **15a** and **15b** (5-COOEt vs 5-CONH2). By comparison with the benzamide scaffold,[8] the carboxamide imidazole scaffold appears to be less attractive. This suggests that our ligand are not interacting by displacement of the water molecule as expected.

References and Notes

1. (a) Brown, M. T.; Cooper, J. A. *Biochim. Biophys. Acta* **1996**, *1287*, 121. (b) Liu, D.; Wang, L.-H. *J. Biomed. Sci.* **1994**, *1*, 65. (c) Thomas, S. M.; Brugge, J. S. *Annu. Rev. Cell Dev. Biol.* **1997**, 13513.
2. Soriano, P.; Montgomery, C.; Geske, R.; Bradley, A. *Cell* **1991**, *64*, 693.
3. (a) Sawyer, T. K. *Biopolymers* **1998**, *47*, 243. (b) Pawson, T. *Nature* **1994**, *373*, 573.
4. Waksman, G.; Shoelson, S. E.; Pant, N.; Cowburn, D.; Kuriyan, J. *Cell* **1993**, *72*, 779.
5. (a) Eck, M. J.; Shoelson, S. E.; Harrison, S. C. *Nature* **1993**, *362*, 87. (b) Eck, M. J.; Atwell, S. K.; Shoelson, S. E.; Harrison, S. C. *Nature* **1994**, *368*, 764.
6. Charifson, P. S.; Shewchuk, L. M.; Rocque, W.; Hummel, C. W.; Jordan, S. R.; Mohr, C.; Pacofsky, G. J.; Peel, M. R.; Rodriguez, M.; Sternbach, D. D.; Consler, T. G. *Biochemistry* **1997**, *36*, 6283.
7. Lunney, E. A.; Para, K. S.; Rubin, J. R.; Humblet, C.; Fergus, J. H.; Marks, J. S.; Sawyer, T. K. *J. Am. Chem. Soc.* **1997**, *119*, 12471.
8. (a) Plummer, M. S.; Holland, D. R.; Shahripour, A.; Lunney, E. A.; Fergus, J. H.; Marks, J. S.; McConnel, P.; Mueller, W. T.; Sawyer, T. K. *J. Med. Chem.* **1997**, *40*, 3719. (b) Lunney, E. A.; Para, K. S.; Rubin, J. R.; Humblet, C.; Fergus, J. H.; Marks, J. S.; Sawyer, T. K. *J. Am. Chem. Soc.* **1997**, *119*, 12471.
9. Beaulieu, P. L.; Cameron, D. R.; Ferland, J.-M.; Gauthier, J.; Ghiro, E.; Gillard, J.; Gorys, V.; Poirier, M.; Rancourt, J.; Wernic, D.; Llinas-Brunet, M. *J. Med. Chem.* **1999**, *42*, 1757.
10. Buchanan, J. L.; Vu, C. B.; Merry, T. J.; Corpuz, E. G.; Pradeepan, S. G.; Mani, U. N.; Yang, M.; Plake, H. R.; Varkhedkar, V. M.; Lynch, B. A.; MacNeil, I. A.; Loiacono, K. A.; Tiong, C. L.; Holt, D. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2359.
11. Buchanan, J. L.; Bohacek, R. S.; Luke, G. P.; Hatada, M.; Lu, X.; Dalgarno, D. S.; Narula, S. S.; Yuan, R. Y.; Holt, D. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2353.
12. Caille, J. C.; Didierlaurent, S.; Lefrancois, D.; Lelièvre, M. H.; Surry, C.; Aszodi, J. *Synthesis* **1995**, *6*, 635.
13. Deprez, P.; Guillaume, J.; Becker, R.; Corbier, A.; Didierlaurent, S.; Fortin, M.; Frechet, D.; Hamon, G.; Heckmann, H. H.; Kleemann, H.-W.; Vevert, J.-P.; Vincent, J.-C.; Wagner, A.; Zhang, J. *J. Med. Chem.* **1995**, *38*, 2357.
14. Bates, S. *J. Chem. Soc., Chem. Commun.* **1979**, 161.
15. Muxfeldt, H.; Unterweger, W. D.; Helmchen, G. *Synthesis* **1976**, 694.
16. Deprez, P.; Vevert, J. P. *J. Fluorine Chem.* **1996**, *80*, 159.
17. Burke, T. R.; Smyth, M. S.; Otaka, A.; Roller, P. R. *Tetrahedron Lett.* **1993**, *34*, 4125.
18. (a) Mandine, E.; Gofflo, D.; Jean-Baptiste, V.; Gaillard-Kelly, M.; Zoller, M.; Baron, R.; Lesuisse, D. *JMBM* **1998**, *23*, S221. (b) Mandine, E.; Gofflo, D.; Jean-Baptiste, V.; Sarubbi, E.; Touyer, G.; Deprez, P.; Lesuisse, D. *J. Mol. Recog.* **2001**, *14*, 254.