Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 6, pp. 707-712, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00097-2

THE IDENTIFICATION OF CYCLOOXYGENASE-1 INHIBITORS FROM 4-THIAZOLIDINONE COMBINATORIAL LIBRARIES

Gary C. Look,* John R. Schullek, Christopher P. Holmes,
Jason P. Chinn, Eric M. Gordon, and Mark A. Gallop

Affymax Research Institute, 3410 Central Expressway,
Santa Clara, California 95051

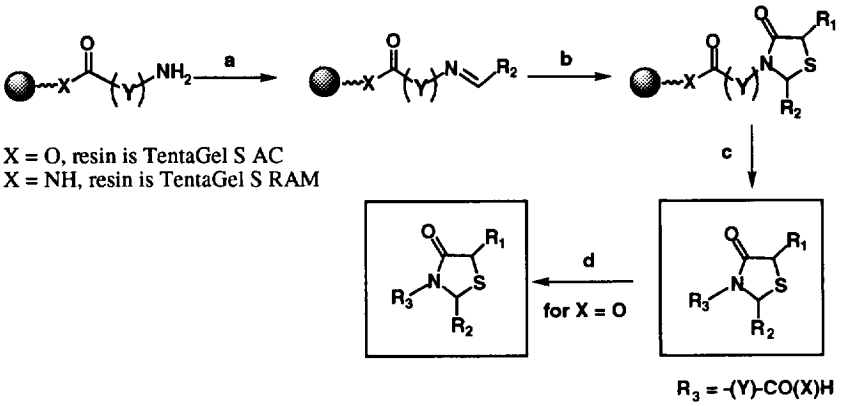
Abstract: Three 4-thiazolidinone libraries, each containing up to 540 compounds, were prepared and assayed for inhibition of the enzyme cyclooxygenase-1 (COX-1). Deconvolution analysis led to the identification of a compound that is equipotent with the commercial COX-1 inhibitors ibuprofen and phenylbutazone.

The rapidly developing field of small molecule combinatorial chemistry is an area of research which has the potential to be a rich source of compounds for the discovery of drug candidates.[1] Of particular appeal to the medicinal chemist is the ease with which small heterocyclic compound libraries can be synthesized.[2] As the repertoire of small molecule chemistries which have been adapted for synthesis on solid supports increases, attention is increasingly being focused on the biological attributes of molecules isolated from combinatorial libraries.[2b,3]

An important application of small molecule libraries is the preparation of a directed or focused combinatorial library for assay against a specific biological target. In this context, the synthesis of libraries centered on a known lead compound are of value for the evaluation of the chemical integrity of libraries as well as for obtaining preliminary structure-activity relationships. Previously, we have reported the synthesis of 4-thiazolidinones on solid supports.[4] We now report the preparation of three 4-thiazolidinone libraries, each containing 125 members plus their stereoisomers (potentially 540 compounds), which were subsequently screened for inhibition of the enzyme cyclooxygenase-1 (COX-1).

Prostaglandin endoperoxide synthase-1 (PGHS-1, COX-1) is a key enzyme in the conversion of arachidonic acid to prostaglandins, important mediators of inflammation. Many commonly used non-steroidal anti-inflammatory drugs (NSAIDs) derive their therapeutic effect through the inhibition of COX-1. Two isozymes of PGHS have been identified, the constitutively expressed COX-1 form, and a second form, COX-2, which is induced by several stimuli.[5]

A recent patent described structure–activity relationships of approximately 100 thiazolidinones as inhibitors of COX-1.[6] We chose to apply our solid supported synthesis technique in the creation of several 4-thiazolidinone libraries focused around one of the more potent of these compounds.[6] The synthesis of these libraries employed the Furka split synthesis method[7] and the elucidation of the active components of the libraries was performed by deconvolution. The general route for the synthesis of the thiazolidinone libraries is shown in Scheme 1 and Figure 1. With the appropriate linker used for the attachment of the amino acid building block to the resin, either the acid or amide could be released from the solid support. The free acid-based library was esterified after cleavage from the resin to afford the corresponding methyl ester library.[8] The building blocks employed in the construction of libraries were chosen after extensive reactivity studies in thiazolidinone formation.[4]



Scheme 1. General route for the solid-supported synthesis of thiazolidinone combinatorial libraries. Resin is TentaGel AC or RAM: (a) R_2CHO in $CH(OMe)_3$; (b) Mercaptoacid, $CH(OMe)_3$, 70 °C; (c) 33% TFA in CH_2Cl_2; (d) CH_2N_2, EtOH.

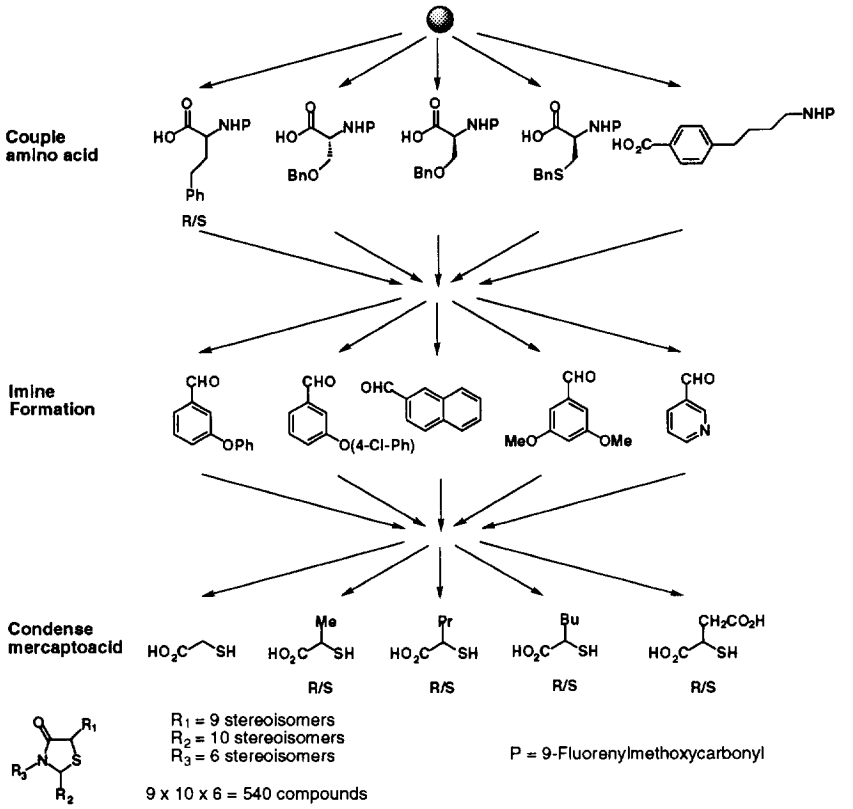


Figure 1. Split/Pool Combinatorial Synthesis of Primary Thiazolidinone Libraries on Solid Support.

The carboxylic acid, carboxamide, and methyl ester libraries were each screened at an initial pool concentration of approximately 10 μM and the only significant activity against COX-1 was found with the ester library.[9,10] The identity of active inhibitors was deduced by deconvolution with the building block affording the most active pool being used in the subsequent sublibrary resynthesis and testing. The results of the study are presented in Figure 2. For the purposes of this study, the thiazolidinones were assayed as racemic mixtures of their *trans* and *cis* diastereomers where typical diastereomeric ratios are less than 2:1 *trans* to *cis* as determined by HPLC.[9]

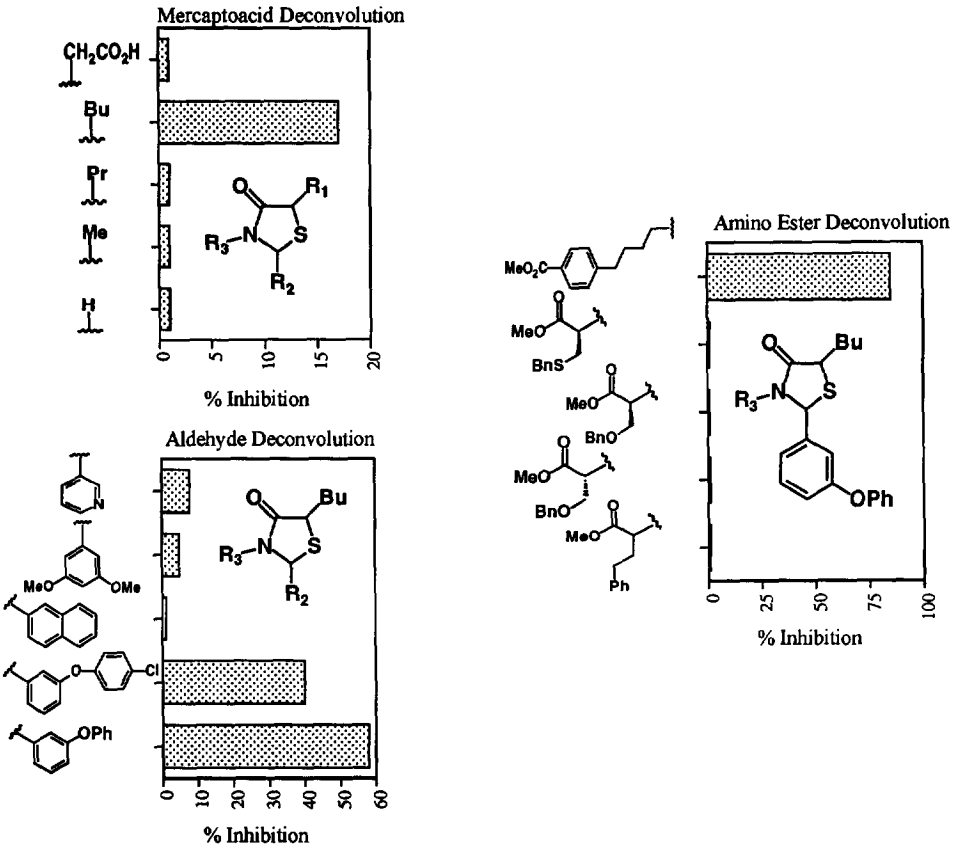
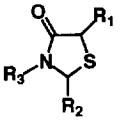


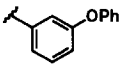
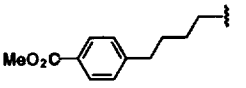
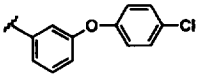
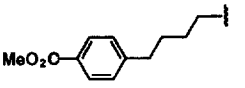
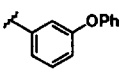
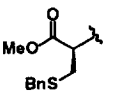
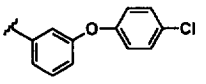
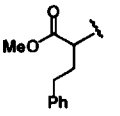
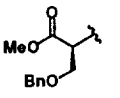
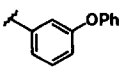
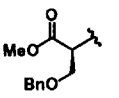
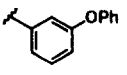
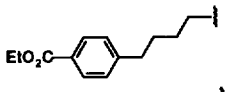
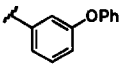
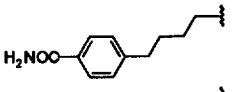
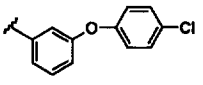
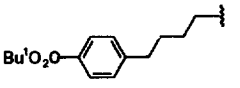
Figure 2. Deconvolution data for the methyl ester thiazolidinone library. The pool concentration of each library was estimated at approximately 10 μM based upon the loading of the amino acid as determined by Fmoc deprotection and spectrophotometric measurement and assuming a high degree of conversion to the thiazolidinone consistent with our previous studies.[4]

Three rounds of deconvolution and resynthesis led to compound **1** being identified as a particularly active component of the ester library. Thiazolidinone **1** was therefore prepared on a preparative scale and the cis and trans diastereomers were separated for structure verification and biological testing (Table 1).[11] Additional members of the libraries also were prepared for IC_{50} determination.[11] These compounds included thiazolidinone

2, an analog of **1** containing the second most potent aldehyde, the Walsh thiazolidinone **7**, and the analogous amide **8**. Thiazolidinone **1** was found to be the most potent of the library compounds which were prepared, in agreement with the published SAR data. The *cis* and *trans* diastereomers of **1** were found to be equipotent to one another.

Table 1. Inhibition of COX-1 by 4-thiazolidinones and control compounds.



Compound	R_1	R_2	R_3	IC_{50}[a]
1	Bu			3.7 ± 1.4 μM[b]
2	Bu			34.4 ± 2.4 μM
3	H			no inh. at 10 μM[c]
4	Me			no inh. at 10 μM[c]
5	Me			no inh. at 10 μM[c]
6	Bu			no inh. at 10 μM[c]
7	Bu			2.8 ± 0.4 μM[d]
8	Bu			6% inh. at 50 μM
9	Bu			9.4 μM[e]
ibuprofen	-	-	-	6.3 ± 1.6 μM[f]
phenylbutazone	-	-	-	3.0 ± 1.3 μM[g]

[a]Assays were performed according to References 13 and 14 on independently prepared and purified compounds. [b]The cis and trans diastereomers exhibited similar activities. [c]Exhibited no inhibition of COX-1 at this concentration. [d]Lit. 50-70 nM (Ref. 6, 15). [e]Based on a nine point titration curve. [f]Lit. 2.6 ± 0.4 μM (Ref. 16). [g]Lit. 75 μM (Ref. 17).

Interestingly, when **2** was converted to the tertiary butyl ester **9**, a modest increase in activity was observed.[12] The intermediate free acid exhibited no activity at 10 μM concentration. The *in vitro* potency of thiazolidinone **1** was found to be equivalent to two commercially available COX-1 inhibitors, ibuprofen, and phenylbutazone (Table 1 and Figure 3).[13] Although a compound more potent than the original lead was not identified, it was gratifying that the combinatorial synthesis/deconvolution process was able to efficiently reproduce the Walsh data by selecting **1** as the most active compound.

In summary, three thiazolidinone libraries, each containing potentially 540 compounds, have been screened versus the COX-1 enzyme and the biologically active compounds identified through deconvolution. The most active member correlates to a known inhibitor of COX-1 while the second most active member is a close analog. These experiments serve to demonstrate the utility of thiazolidinone combinatorial libraries in drug discovery.

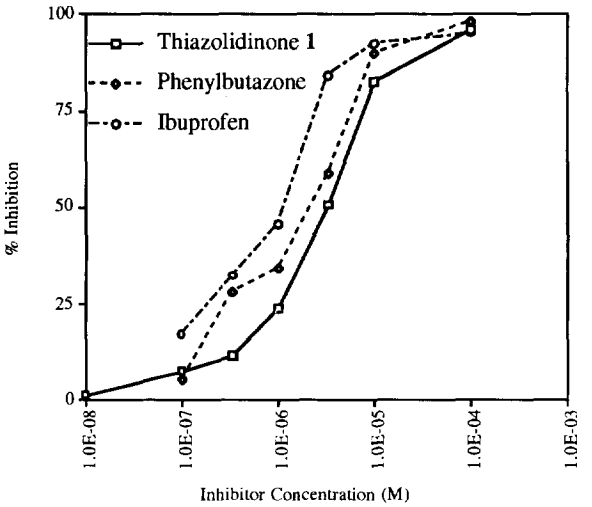


Figure 3. Inhibitor Concentrations versus COX-1.

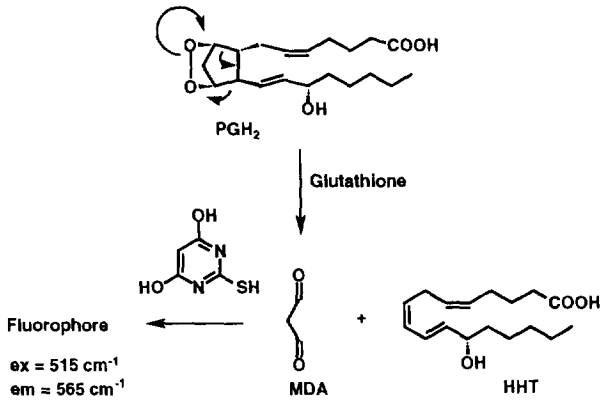
Acknowledgment: The authors would like to thank Olga Chernov for her technical help with the COX-1 assays. We would also like to thank Dr. Peter Barker for helpful discussions during the preparation of this manuscript.

References and Notes:

1. For recent reviews on combinatorial chemistry, see: (a) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135. (b) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233. (c) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385.
2. For representative examples, see: (a) Ruhland, B.; Bhandari, A.; Gordon, E. M.; Gallop, M. A. *J. Am. Chem. Soc.* **1996**, *118*, 253. (b) Murphy, M. M.; Schullek, J. R.; Gordon, E. M.; Gallop, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 7029. (c) Gordeev, M. F.; Patel, D. V.; Gordon, E. M. *J. Org. Chem.*, in press. (d) Campbell, D. A.; Bermak, J. C.; Burkoth, T. S.; Patel, D. V. *J. Am. Chem. Soc.* **1995**, *117*, 5381. (e) DeWitt, S. H.; Kiely, J. S.; Stankovic, C. J.; Schroeder, M. C.; Reynolds Cody, D. M.; Pavia, M. R. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 6909. (f) Plunkett, M. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 3306. (g) Chen, C.; Ahlberg Randell, L. A.; Miller, R. B.; Jones, A. D.; Kurth, M. J. *J. Am. Chem. Soc.* **1994**, *116*, 2661. (h) Gordon, D. W.; Steele, J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 47. (i) Carell, T.; Wintner, A. J.; Sutherland, A. J.; Dunayevskiy, Y. M.; Vouros, P. *Chem. Biol.* **1995**,

2, 171. (j) Pátek, M.; Drake, B.; Lebl, M. *Tetrahedron Lett.* **1995**, *36*, 2227. (k) Baldwin, J. J.; Burbaum, J. J.; Henderson, I.; Ohlmeyer, M. H. J. *J. Am. Chem. Soc.* **1995**, *117*, 5588. (l) Green, J. *J. Org. Chem.* **1995**, *60*, 4287.

3. (a) Wang, G. T.; Li, S.; Wideburg, N.; Krafft, G. A.; Kempf, D. J. *J. Med. Chem.* **1995**, *38*, 2995. (b) Zuckermann, R. N.; Martin, E. J.; Spellmeyer, D. C.; Stauber, G. B.; Shoemaker, K. R.; Kerr, J. M.; Figliozzi, G. M.; Goff, D. A.; Siani, M. A.; Simon, R. J.; Banville, S. C.; Brown, E. G.; Wang, L.; Richter, L. S.; Moos, W. H. *J. Med. Chem.* **1994**, *37*, 2678.
4. Holmes, C. P.; Chinn, J. P.; Look, G. C.; Gordon, E. M.; Gallop, M. A. *J. Org. Chem.* **1995**, *60*, 7328.
5. For recent work on the preparation of COX-1 and COX-2 inhibitors, see: (a) Huang, H.-C.; Chamberlain, T. S.; Seibert, K.; Kobolt, C. M.; Isakson, P. C.; Reitz, D. B. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2377. (b) Reitz, D. B.; Li, J. J.; Norton, M. B.; Reinhard, E. J.; Collins, J. T.; Anderson, G. D.; Gregory, S. A.; Koboldt, C, M.; Perkins, W. E.; Seibert, K.; Isakson, P. C. *J. Med. Chem.* **1994**, *37*, 3878. (c) Backes, B. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11171.
6. Walsh, D. A.; Uwaydah, I. M. U.S. Patent 5 061 720, 1991.
7. For a review on the split synthesis method, see: Furka, Á. *Drug Dev. Res.* **1995**, *36*, 1.
8. The methylation procedure for the thiazolidinones was validated by HPLC studies of the conversion of the free acid thiazolidinones to their methyl ester analogs.
9. This correlates well with the data reported in the Walsh patent where thiazolidinone acids are typically less potent than the esters. Additionally, the Walsh patent shows no great variation in the biological activity between the cis and trans diastereoisomers of a given thiazolidinone.
10. The loading of the TentaGel S AC resin used was 280 μmol/g whereas the yield after amino acid loading was 30-60 μmol/g due to inefficient ester formation. These low yields were not expected to compromise the integrity of the libraries as the free unreacted alcohol sites of the resin would not participate in subsequent reactions during the thiazolidinone synthesis.
11. The compounds used for IC_{50} determinations were independently synthesized in solution and purified by silica gel chromatography. The structures of the purified compounds were confirmed by NMR and MS analysis.
12. Prepared with the di-*tert*-butyl acetal of *N,N*-dimethylformamide.
13. The assay that was used measured the glutathione-mediated decomposition of PGH_2 (see figure below and ref. 14). This assay differed from that employed by Walsh and Uwaydah which measured oxygen uptake in the cyclooxygenase mediated formation of PGG_2.[6]



14. (a) Yagi, K. *Biochem. Med.* **1976**, *15*, 212. (b) Shimizu, T.; Kondo, K.; Hayashi, O. *Arch. Biochem. Biophys.* **1981**, *206*, 271.
15. Using the assay described in Ref. 13, compound **7** was determined here to be 40-fold less potent than reported by Walsh (3 μM vs 70 nM). However, we have run assays using known literature standards (eg. ibuprofen and phenylbutazone) and our numbers are consistent with the published data.
16. Barnett, J.; Chow, J.; Ives, D.; Chiou, M.; Mackenzie, R.; Osen, E.; Nguyen, B.; Tsing, S.; Bach, C.; Freire, J.; Chan, H.; Sigal, E.; Ramesha, C. *Biochem. Biophys. Acta* **1994**, *1209*, 130. The IC_{50}'s were determined by measuring the oxygen uptake.
17. Tam, S. S. C.; Lee, D. H. S.; Wang, E. Y.; Munroe, D. G.; Lau, C. Y. *J. Biol. Chem.* **1995**, *270*, 13948.

(*Received in USA* 11 *January* 1996; *accepted* 13 *February* 1996)