Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 10, pp. 1099-1104, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00180-1

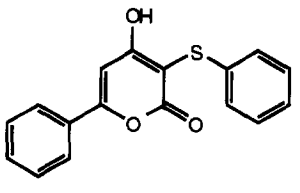
A TOPLISS TREE ANALYSIS OF THE HIV-PROTEASE INHIBITORY ACTIVITY OF 6-PHENYL-4-HYDROXY-PYRAN-2-ONES

Bruce A. Steinbaugh,* Harriet W. Hamilton, J.V.N. Vara Prasad, Kimberly S. Para, Peter J. Tummino, Donna Ferguson, Elizabeth A. Lunney, and C. John Blankley

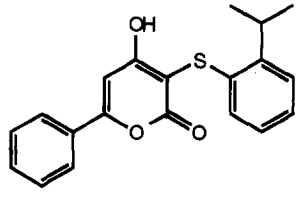
Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company
Ann Arbor, Michigan 48105

Abstract In a study of 4-hydroxy-pyran-2-ones as possible inhibitors of HIV protease, a series of compounds were synthesized following the Topliss operational scheme for substitution on a phenyl group at the 6 position of the pyrone. In addition, a number of compounds with polar substituents were made.

HIV infection and the resulting development of AIDS has remained a therapeutic challenge over the last decade. An important target for inhibition has been the HIV protease enzyme which is required for the activation of newly formed viral particles in the last stages of replication.[1] As such, a number of groups have been active in the search for HIV protease inhibitors.[2] Our own work has been based on the nonpeptide lead of the 4-hydroxy-3-thiosubstituted-pyran-2-ones, as previously reported.[3] The mass screening lead, **1**, has led to a potent inhibitor, **2**, with an IC_{50} of 37 nM.[4] These thiosubstituted pyrones are similar in structure to the carbon branched compounds of Upjohn,[5] but are generally more potent and do not have a chiral center in the 3 position.



1



2

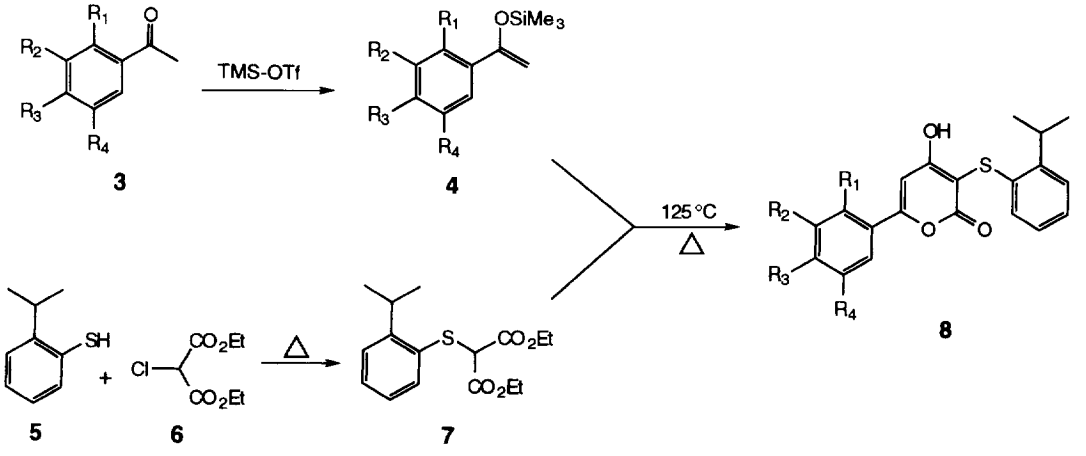
In this analysis we sought to optimize substitution of a phenyl ring in the 6 position of the pyrone, **2**, using the Topliss operational scheme or tree.[6,7] This method is based upon the physicochemical parameters relating to hydrophobic (π), electronic (σ), and steric (E_s) effects and is designed as a decision tree. A number of additional compounds incorporating polar functionalities were also synthesized in order to possibly gain additional binding with the enzyme.

Chemistry

The pyrone compounds for this study were synthesized using the methods shown in Scheme I.[8] The reaction of a substituted acetophenone **3** with trimethylsilyl trifluoromethanesulfonate resulted in the formation of the corresponding silyl enol ether **4**. The substituted malonate **7** was made by reacting 2-isopropylthiophenol

5 with diethyl chloromalonate **6** under reflux. The resulting malonate **7** was condensed neat with the silyl enol ethers **4** at ~125 °C to afford the desired pyrones **8** with yields in the 5 to 70% range. Differences in this sequence were required only in the case of a carboxylic acid substituent on the phenyl ring. This was cyclized as the ethyl ester and then saponified in a subsequent step. Compounds which were sensitive to an acid-base workup (such as phenols) were purified by flash chromatography. In most other cases it was found that trituration with ether after the extractive workup gave pyrone of satisfactory purity.

Scheme I



Biochemistry

The *in vitro* IC_{50} values for HIV-1 protease inhibition were determined as previously reported.[9] The compounds were tested against affinity-purified HIV-1 protease (BH10) at a pH of 4.7 and a final enzyme concentration of 0.45-1.1 nM. Analyses were conducted in duplicate. The data for those compounds from the Topliss tree are shown in Figure I, while the complete data for all of the compounds are shown in Table I. Compound **9** is included as a reference and is a close analog of Ro 31-8959 (Saquinavir) in which the decahydro-3-isoquinolinecarbonyl has been replaced by piperidine-2-carbonyl.[10]

Results

This study sought to optimize stereoelectronic effects of the 6-phenyl ring of pyrones in HIV protease enzyme inhibition by following the logic of a Topliss tree analysis. For this purpose, the remainder of the molecule was held constant as the 4-hydroxy-3-[(isopropyl-phenyl)-thio]-pyran-2-one. The Topliss tree operational scheme starts with the unsubstituted phenyl compound **2**, which was found to have an IC_{50} value of 37 nM (Figure I). The first compound to be synthesized according to the scheme was the *p*-Cl phenyl derivative

FIGURE I

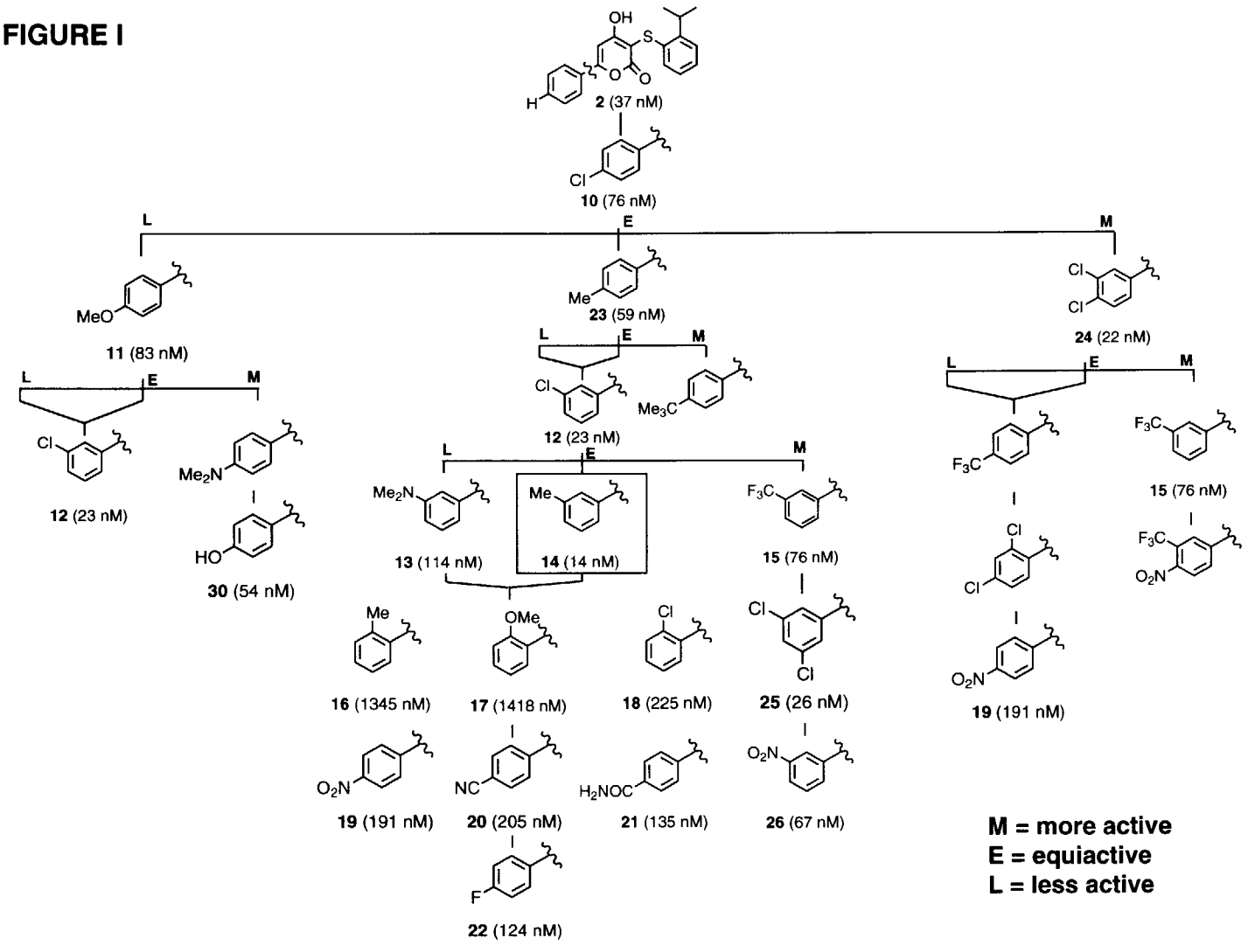
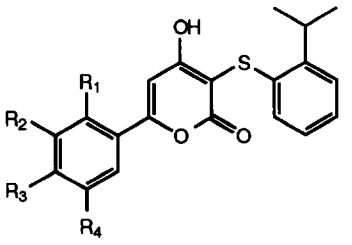

2 (37 nM)
10 (76 nM)
L
E
M
11 (83 nM)
23 (59 nM)
24 (22 nM)
12 (23 nM)
30 (54 nM)
13 (114 nM)
14 (14 nM)
15 (76 nM)
16 (1345 nM)
17 (1418 nM)
18 (225 nM)
25 (26 nM)
19 (191 nM)
20 (205 nM)
21 (135 nM)
26 (67 nM)
22 (124 nM)
M = more active
E = equiactive
L = less active

Table I



Cmpd	R_1	R_2	R_3	R_4	IC_{50} (nM)	Yield
9					**10** ± 3	
2	H	H	H	H	**37** ± 15	45%
10	H	H	Cl	H	**76** ± 7	27%
11	H	H	OMe	H	**83** ± 19	22%
12	H	Cl	H	H	**23** ± 7	70%
13	H	NMe_2	H	H	**114** ± 11	7%
14	H	Me	H	H	**14** ± 3	62%
15	H	CF_3	H	H	**76** ± 5	33%
16	Me	H	H	H	**1345** ± 90	20%
17	OMe	H	H	H	**1418** ± 85	24%
18	Cl	H	H	H	**225** ± 26	5%
19	H	H	NO_2	H	**191** ± 18	5%
20	H	H	CN	H	**205** ± 48	21%
21	H	H	$CONH_2$	H	**135** ± 13	-[a]
22	H	H	F	H	**124** ± 4	26%
23	H	H	Me	H	**59** ± 4	27%
24	H	Cl	Cl	H	**22** ± 6	13%
25	H	Cl	H	Cl	**26** ± 4	70%
26	H	NO_2	H	H	**67** ± 7	13%
27	H	Me	H	Me	**15** ± 3	12%
28	H	Et	H	H	**262** ± 58	25%
29	H	Ph	H	H	**38** ± 1	31%
30	H	H	OH	H	**54** ± 7	5%
31	H	OH	H	H	**89** ± 10	8%
32	H	H	COOEt	H	**35** ± 15	31%
33	H	COOEt	H	H	**1934** ± 95	20%
34	H	H	COOH	H	**68** ± 8	-[b]
35	H	COOH	H	H	**151** ± 46	-[c]
36	H	COOEt	OH	H	**148** ± 36	27%
37	H	COOMe	OH	H	**62** ± 15	-[d]
38	H	COOH	OH	H	**292** ± 10	-[e]
39	H	COOMe	H	H	**80** ± 7	5%

[a]Hydrolysis of **20** with KOH/tBuOH gave **21** in a 56% yield. [b]Saponification of **32** with NaOH gave **34** in a 100% yield. [c]Saponification of **33** with NaOH gave **35** in a 100% yield. [d]Transesterification of **36** with NaOH/MeOH gave **37** in a 35% yield. [e]Saponification of **37** with NaOH gave **38** in a 45% yield.

10. Because this was less active than the parent unsubstituted phenyl (76 nM vs. 37 nM), the *p*-OMe phenyl compound **11** was made next. This was discovered to be equipotent with the *p*-Cl (83 nM vs. 76 nM), leading

to synthesis of the *m*-Cl phenyl compound **12** which had an IC_{50} of 23 nM. Following a shift to the middle branch of the Topliss tree where the *m*-Cl compound **12** also is found, the *m*-$N(Me)_2$, *m*-Me, and *m*-CF_3 phenyl analogs (**13** to **15**) were made, and were found to have IC_{50} values of 114 nM, 14 nM, and 76 nM respectively. Following down the tree from the most potent *m*-Me phenyl compound **14** led to the *o*-Me, *o*-OMe, *o*-Cl, *p*-NO_2, *p*-CN, *p*-$CONH_2$, and *p*-F phenyl derivatives, **16** to **22**. None of these analogs resulted in any enhancement of activity when compared to the *m*-Me phenyl compound, which remained the most active compound, with an IC_{50} of 14 nM.

The *m,m*-dimethyl phenyl compound **27** (Table I) was synthesized to examine whether further improvements in activity could be made by bis substitution. This analog was found to be equipotent with the mono-substituted compound (15 nM vs. 14 nM). Similar results were seen with the mono- and bis-*meta* chloro derivatives **12** and **25** (23 nM and 26 nM, respectively). The size of *meta* substituents was examined further by the synthesis of the ethyl compound **28**. A reduction in activity from 14 nM to 262 nM was observed. However, a smaller drop in activity was observed with the larger *m*-phenyl analog **29** (38 nM) suggesting that more than just size is having an effect in the *meta* position.

Several carboxylic acids, esters, and hydroxyl substituted phenyl analogs were made to explore the activity of compounds with these functionalities (**30** to **39**). The introduction of this polarity was found to be better tolerated in the *para* than in the *meta* position (**30** vs. **31**, **32** vs. **33**, and **34** vs. **35**), however, none of these compounds resulted in an improvement over the simple alkyl compound **14**.

Discussion

The Topliss tree can be useful in synthetic planning when a compound contains a phenyl group, but in this case the small enhancement in activity found would be difficult to attribute to following the tree. The increase in potency from H to the most potent compound (37 nM to 14 nM) is less than a log unit in difference. *Ortho* substitution was found not to be tolerated. Steric limitations within the enzyme cavity or a change in the torsional angle between the phenyl group and the pyrone ring may explain these results.

Although not directly suggested by the Topliss tree, carboxylic acid and ester substituents on the phenyl ring were synthesized with the aim of possibly altering physiochemical parameters. Empirically these were preferred in the *para* position, although the difference between the *meta* esters (**33** and **39**) and acid **35** is somewhat surprising. A steric limitation may be involved in the loss of activity seen with **33**.

An exploratory QSAR analysis was carried out with the data generated from this study. This analysis showed no significant trends, but did tend to support the preferences for *meta* and *para* substitution as described. The failure of the Topliss tree to indicate a favorable direction for activity improvement suggested that the factors that went into its development (namely lipophilicity, electronic, and size parameters), play a minor role in determining the activity variation within this set of compounds. Another QSAR analysis using these and additional steric parameters for the individual *meta* and *para* position substituents also failed to give any significant correlations. This could mean that there is more than one binding mode or that the enzyme changes conformations when binding with an inhibitor. It also suggests that additional potency would appear to depend

more on increasing enzyme/inhibitor interactions generated by filling more than just the S1 pocket than the electronics of the 6-phenyl ring of the pyrone structure.

Conclusions

This analysis of 6-phenyl substituents resulted in a relatively flat SAR. Only a slight improvement in enzyme binding affinity (2-fold) was observed. The most active compound tested with an IC_{50} of 14 nM was the *m*-Me phenyl substituted pyrone **14**. The lack of significant trends in the QSAR analysis further supports the notion that electronic parameters play a lesser role than steric/lipophilic interactions between the inhibitors and the enzyme. Future synthesis would be better directed using the X-ray crystal structures of the enzyme-inhibitor complexes.

References and Notes

1. Meek, T. D. *J. Enzyme Inhibition* **1992**, *6*, 65.
2. Redshaw, S. *Exp. Opin. Invest. Drugs* **1994**, *3*, 273.
3. Vara Prasad, J. V. N.; Para, K. S.; Lunney, E. A.; Ortwine, D. F.; Dunbar, J. B., Jr.; Ferguson, D.; Tummino, P. J.; Hupe, D.; Tait, B. D.; Domagala, J. M.; Humblet, C.; Bhat, T. N.; Liu, B.; Guerin, D. M. A.; Baldwin, E. T.; Erickson, J. W.; Sawyer, T. K. *J. Am. Chem. Soc.* **1994**, *116*, 6989.
4. Vara Prasad, J. V. N.; Lunney, E. A.; Ferguson, D.; Tummino, P. J.; Rubin, J. R.; Reyner, E. L.; Stewart, B. H.; Guttendorf, R. J.; Domagala, J. M.; Suvorov, L. I.; Gulnik, S. V.; Topol, I. A.; Bhat, T. N.; Erickson, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 11070.
5. Skulnick, H. I.; Johnson, P. D.; Howe, W. J.; Tomich, P. K.; Chong, K. T.; Watenpaugh, K. D.; Janakiraman, M. N.; Dolak, L. A.; McGrath, J. P.; Lynn, J. C.; Horng, M. M.; Hinshaw, R. R.; Zipp, G. L.; Ruwart, M. J.; Schwende, F. J.; Zhong, W. Z.; Padbury, G. E.; Dalga, R. J.; Shiou, L.; Possert, P. L.; Rush, B. D.; Wilkinson, K. F.; Howard, G. M.; Toth, L. N.; Williams, M. G.; Kakuk, T. J.; Cole, S. L.; Zaya, R. M.; Lovasz, K. D.; Morris, J. K.; Romines, K. R.; Thaisrivongs, S.; Aristoff, P. A. *J. Med. Chem.* **1995**, *38*, 4968.
6. Topliss, J. G. *J. Med. Chem.* **1972**, *15*, 1006.
7. Topliss, J. G.; Martin, Y. C. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1975; Vol. 5, pp 1-21.
8. Para, K. S.; Ellsworth, E. L.; Vara Prasad, J. V. N. *J. Heterocyclic Chem.* **1994**, *31*, 1619.
9. Tummino, P. J.; Ferguson, D.; Hupe, L.; Hupe, D. *Biochem. Biophys. Res. Commun.* **1994**, *200*, 1658.
10. Compound XIV in Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Krohn, A.; Lambert, R. W.; Merrett, J. H.; Mills, J. S.; Parkes, K. E. B.; Redshaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. J.; Machin, P. J. *Science* **1990**, *248*, 358.

(*Received in USA* 23 *January* 1996; *accepted* 17 *April* 1996)