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# *O*-ALKYLCARBOXYLATE OXIME AND *N*-HYDROXYUREA ANALOGS OF SUBSTITUTED INDOLE LEUKOTRIENE BIOSYNTHESIS INHIBITORS

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**Abstract**. Reference FLAP inhibitors **1** and **2** were converted into the corresponding *O*-acetic acid oxime congeners **8** and **11a**, respectively, resulting in potent, orally active, leukotriene biosynthesis inhibitors. An attempt to create a dual FLAP and direct 5-LO inhibitor by replacing the carboxylate group in **1** with the *N*-hydroxyurea pharmacophore did not provide superior inhibitors. 

**Background**. Leukotriene (LT) biosynthesis inhibitors represent a promising therapeutic modality for the treatment of a variety of conditions including, asthma, ulcerative colitis, and rhinitis.[1] This report describes the results of an investigation with the objective to design and optimize a series of orally bioavailable leukotriene biosynthesis inhibitors. Two experimental approaches were explored: (1) to design compounds with both five-lipoxygenase activating protein (FLAP) and 5-lipoxygenase (5-LO) inhibitory activity, and (2) to identify orally active FLAP inhibitors with potent *in vivo* pharmacologic properties.

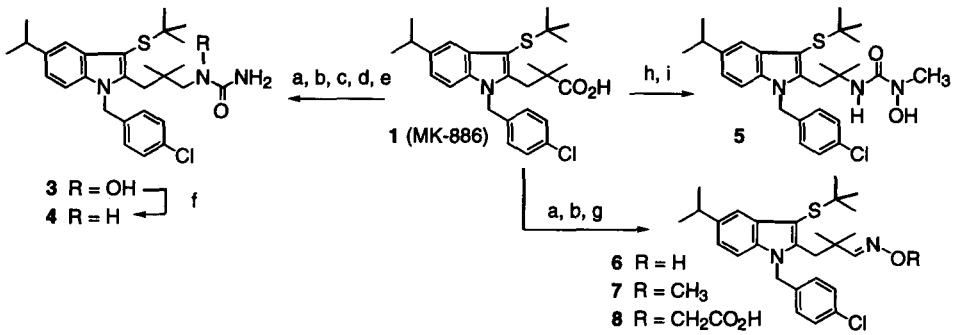
Merck-Frosst scientists discovered five-lipoxygenase activating protein (FLAP) and MK-886 (**1**), a compound that interfered with FLAP, thus introducing a new modality for blocking leukotriene biosynthesis.[2] FLAP is currently believed to function as an arachidonate presenting protein[3] that enhances the catalytic activity of 5-LO.[4] FLAP is localized at the inner nuclear membrane, and both 5-lipoxygenase (5-LO) and the cytosolic phospolipase $A_2$ translocate to this site.[5] A better understanding of the complex regulatory mechanisms for 5-LO and FLAP and the significance of the nuclear site of interaction could lead to future therapeutic opportunities.

Clinical studies with **1** did not provide the degree of LT inhibition measured in both stimulated blood samples and in urine that was expected for an inhibitor with such potent biochemical activity.[6] An even more potent analog, MK-0591 (**2**), was discovered with FLAP binding activity ($IC_{50}$ = 2 nM) and LT inhibition in intact human neutrophils ($IC_{50}$ = 3 nM) and in stimulated human blood ($IC_{50}$ = 500 nM).[7] Clinical studies with **2** demonstrated efficacy for the FLAP modality of LT intervention.[8] This report describes our initial research to identify a FLAP inhibitor suitable for clinical development.

**Biological Testing Protocol**. The biological testing protocol[9] involved: (1) a broken cell assay consisting of a lysate of rat basophilic leukemia cells to evaluate direct 5-LO catalytic inhibition (FLAP inhibitors would be inactive); (2) an intact cell assay involving calcium ionophore stimulated $LTB_4$ synthesis in human neutrophils to evaluate both direct 5-LO and FLAP inhibitors; (3) a whole blood assay involving calcium ionophore stimulated $LTB_4$ synthesis in human blood to evaluate both direct 5-LO and FLAP inhibitors in the presence of blood components; (4) a rat anaphylaxis model involving antigen-antibody stimulated leukotriene generation in the peritonium to evaluate *in vivo* activity after oral administration; and (5) measuring the time course of *ex vivo* inhibition of calcium ionophore stimulated $LTB_4$ formation in blood samples from orally dosed dogs and monkeys.[10]

***N*-Hydroxyurea Congeners.** We proposed that replacing the carboxylate group in the FLAP inhibitor standard, **1** with a *N*-hydroxyurea group might provide an expeditious route to dual FLAP/5-LO inhibitors. Previous studies had clearly shown that the *N*-hydroxyurea group was a useful pharmacophore providing selective inhibitors of 5-LO catalysis.[11] A possible favorable outcome would be additive or synergistic inhibitory activity. The *N*-hydroxyurea congener (**3**) was synthesized in a straightforward manner, as shown in Scheme 1. The corresponding urea (**4**) was prepared by deoxygenation of **3**. The regioisomeric *N*-hydroxyurea (**5**) was prepared by reaction of *N*-methylhydroxylamine with the isocyanate derived from **1** (Scheme 1).

**Scheme 1.**[a]




[a]Reagents: (a) $BH_3$•DMS, THF, 0 °C→rt; 17 h; (b) Swern Oxidation: $(COCl)_2$, DMSO, $CH_2Cl_2$, -63 °C, 1 h; (c) $H_2NOH$•HCl, EtOH/pyr, rt, 16 h; (d) $BH_3$•pyr, EtOH, rt, 2 h; (e) $(CH_3)_3SiNCO$, THF, rt, 2.5 h; (f) $TiCl_3$, NaOAc, MeOH, 45 °C, 24 h; (g) $H_2NOR$, EtOH/pyr, rt, 16 h; (h) DPPA, TEA, benzene, reflux, 1 h; (i) $HONHCH_3$, TEA, $H_2O$, reflux, 2 h.

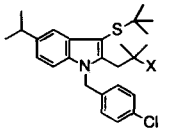
The *N*-hydroxyureas **3** and **5** were found to be inferior LT inhibitors *in vitro* and *in vivo* compared to **1** (Table 1). Both **3** and **5** were weak inhibitors of 5-LO catalysis in the broken cell assay with similar $IC_{50}$'s of 7.5 μM. The urea **4** was inactive in the broken cell assay as expected since the *N*-hydroxyurea pharmacophore was previously established as important for direct inhibition of 5-LO catalysis.[11] All three congeners (**3**, **4**, and **5**) were effective inhibitors of calcium ionophore stimulated $LTB_4$ formation in human neutrophils with similar $IC_{50}$'s of 170, 150, and 180 nM, respectively. These compounds also showed similar inhibitory activity in human whole blood with $IC_{50}$'s from 1-1.6 μM. Incorporating the *N*-hydroxyurea pharmacophore did not provide significant additional biochemical activity attributable to direct 5-LO inhibition. However, by inference these analogs retained inhibitory activity against FLAP. This approach to design dual FLAP/5-LO inhibitors was not pursued further. Hutchinson and coworkers[12] have reported thiopyranol[2,3,4-*c,d*]indoles which have dual FLAP/5-LO activity.

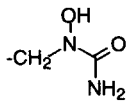
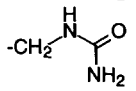
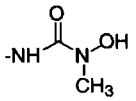
**Oxime Insertion Congeners.** The second avenue of investigation was to identify a FLAP inhibitor with optimized *in vivo* properties suitable for clinical development. We proposed that inserting an oxime moiety in the 2-alkylcarboxylate substituent in **1** would provide FLAP inhibitors with improved *in vivo* pharmacological properties. The oxime congeners (**6**-**8**) were prepared by reaction of the requisite hydroxylamine with the aldehyde intermediate derived from **1** as shown in Scheme 1. The biological activity of these analogs is summarized in Table 1 and described as follows. The oxime analogs **6** and **7** were about ten-fold less potent than **1** in the human neutrophil assay. Appending the carboxylate group as in the *O*-acetic acid oxime congener

**8** provided a very promising compound with *in vitro* inhibitory activity comparable to **1**. More important was the observation of significantly improved oral activity in the rat anaphylaxis model where **8** had an $ED_{50}$ of 3 μmol/kg that was about five-fold better than that found for **1**. With the discovery that an oxime moiety could be inserted into the alkylcarboxylate substituent to provide promising indole FLAP inhibitors, a more extensive structure-activity study was conducted.

Alternative heteroarylmethoxy substituents on the indole template were examined and the results are summarized in Table 2. The preparation of these compounds was accomplished by alkylation of the 5-hydroxyindole intermediate **9** as shown in Scheme 2. Most of the previously described indole compounds were often >ten-fold more potent against the human neutrophil than the human blood assay. A possible explanation for this difference was that plasma protein binding in the human blood assay was attenuating the activity of the inhibitors. Since increased plasma protein binding could be attributed to lipophilicity, we investigated less lipophilic heteroarylmethoxy substituents to replace the quinolylmethoxy group in an attempt to improve potency in human blood. The 4-thiazolyl (**10b**) and 2-pyridyl (**10c**) congeners were found to have four to five-fold improved potency over **2** in the human blood assay. Furthermore, **10b** had comparable activity to **2** in the rat anaphylaxis model whereas the 2-pyridyl analog **10c** was ten-fold more potent with an $ED_{50}$ of 0.16 μmol/kg.

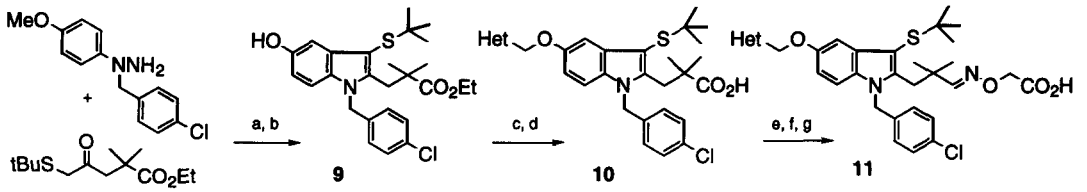
**Table 1. Indole LT Inhibitors Replacing the Carboxylate of 1 (MK-886)**



| Compound[e] (mol.wt.) X = | | Broken RBL Cell[a] $IC_{50}$ μM | Human neutrophil[b] $IC_{50}$ μM | Human blood[c] $IC_{50}$ μM | *In vivo* rat[d] $ED_{50}$ μmol/kg or % I @ μmol/kg |
|---|---|---|---|---|---|
| **1** (472.10) | $CO_2H$ | NA @ 32 μM[f] | 0.03 (0.2-0.5) | 0.34 (0.26-0.41) | 16 (3-30) |
| **3** (516.15) |  | 7.5 (6.4-8.7) | 0.17 (0.12-0.26) | 1.6 (0.94-2.4) | 46 (29-93) |
| **4** (500.15) |  | NA @ 50 μM[f] | 0.18 (0.12-0.26) | 0.97 (0.57-1.3) | 11 (4.7 -18) |
| **5** (516.15) |  | 7.5 (6.3-8.9 | 0.15 (0.09-0.40) | 1.3 (1.0-1.6) | 49% @ 60 μmol/kg |
| **6** (471.11) | -CH=NOH | -- | 0.27 (0.20-0.37) | 2.2 (1.9-2.5) | 46% @ 100 μmol/kg |
| **7** (485.14) | $-CH=NOCH_3$ | -- | 0.34 (0.11-0.99) | 11.0 (6.3-33) | -- |
| **8** (529.15) | $-CH=NOCH_2CO_2H$ | -- | 0.10 (0.09-0.12) | 0.68 (0.47-0.95) | 3 (2-5) |

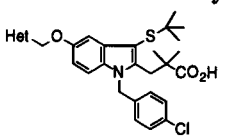
[a]Inhibition of 5-LO activity in a broken cell supernatant from rat basophilic leukemia cells; $IC_{50}$ with 95% confidence limits shown in parenthesis. [b]Inhibition of calcium ionophore (A23187) stimulated $LTB_4$ formation in human neutrophils; $IC_{50}$ with 95% confidence limits shown in parenthesis. [c]Human whole blood was stimulated with calcium ionophore (A23187) and $LTB_4$ was measured by enzyme immunoassay; 95% confidence limits shown in parenthesis. [d]Rat anaphylaxis in the peritoneal cavity; oral dose response determination $ED_{50}$ or % I of cysteinyl LT's at a given oral dose. [e]Compounds gave satisfactory spectral and analytical data. [f]NA@, not active at concentration tested.

**Scheme 2.**[a]




[a]Reagents: (a) Fischer indole synthesis: toluene, HOAc, dark, rt, 4 d; (b) $AlCl_3$, tBuSH, $CH_2Cl_2$, 0 °C → rt, 3 h; (c) Het-$CH_2Cl$, $K_2CO_3$, DMF, rt, 4 d; (d) LiOH, THF/MeOH, reflux, 3 h; (e) $BH_3$•DMS, THF, rt, 12 h; (f) Swern Oxidation: $(COCl)_2$, DMSO, $CH_2Cl_2$, -78 °C → rt, 1 h; (g) $H_2NOCH_2CO_2H$, EtOH/pyridine, 16 h.

**Table 2. 5-Heteroarylmethoxy Substituted Indole LT Inhibitors**

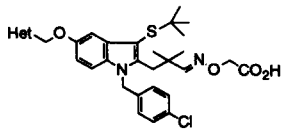



| Compound[d] (mol. wt.) | Het = | Human neutrophil[a] $IC_{50}$ nM | Human blood[b] $IC_{50}$ μM | *In vivo* rat[c] $ED_{50}$ μmol/kg or % I @ μmol/kg |
|---|---|---|---|---|
| **2** (587.19) | 2-quinolyl | 8 (7-10) | 0.20 (0.014-0.029) | 1.6 (0.8-2.5) |
| **10a** (586.20) | 2-naphthyl | 12 (10-20) | 4.42 (3.4-6.0) | NA @ 100 μmol/kg[e] |
| **10b** (543.15) | 4-thiazolyl | 4 (1-10) | 0.04 (0.03-0.08) | 1.8 (0.8-6.8) |
| **10c** (543.15) | 2-thiazolyl | 7 (1-10) | 41% @ 0.05 μM | 16% @ 1 μmol/kg |
| **10d** (593.21) | 2-benzothiazolyl | 10 (5-20) | 0.36 (0.34-0.38) | 71% @ 1 μmol/kg |
| **10e** (537.13) | 2-pyridyl | 100% @ 50 nM | 0.05 (0.04-0.06) | 0.16 (0.08-0.25) |
| **10f** (537.13) | 3-pyridyl | 17 (10-40) | 1.09 (0.40-1.91) | 4% @ 100 μmol/kg |
| **10g** (537.13) | 4-pyridyl | 100% @ 100 nM | 0.84 (0.65-1.11) | NA @ 100 μmol/kg[e] |
| **10h** (571.57) | 2-(6-chloropyridyl) | 17 (10-30) | 0.11 (0.9-0.13) | 1.2 (1.0-1.5) |

[a]Inhibition of calcium ionophore (A23187) stimulated $LTB_4$ formation in human neutrophils; 95% confidence limits shown in parenthesis. [b]Human whole blood was stimulated with calcium ionophore (A23187) and $LTB_4$ was measured by enzyme immunoassay; 95% confidence limits shown in parenthesis. [c]Rat anaphylaxis in the peritoneal cavity; oral dose response determination $ED_{50}$ or % I of cysteinyl LT's at a given oral dose. [d]Compounds gave satisfactory spectral and analytical data. eNA@, not active at concentration tested.

The oxime ether insertion modification was then applied to the more potent carboxylate inhibitors from the previous study (Table 2) to provide the oxime congeners (**11a-11d**) (Table 3). These inhibitors were prepared as previously described (Scheme 2) and were found to have potent inhibitory activity. It was interesting to find that the insertion of the oxime moiety did not dramatically alter the *in vitro* inhibitory potency for these four congeners compared to the carboxylate precursors in the assays studied. The 2-quinolylmethoxy (**11a**) and 2-pyridylmethoxy (**11b**) analogs were particularly potent in the rat anaphylaxis model.

**Table 3. 5-Heteroarylmethoxy Substituted *O*-Alkyl Carboxylate-Oxime Indole LT Inhibitors**

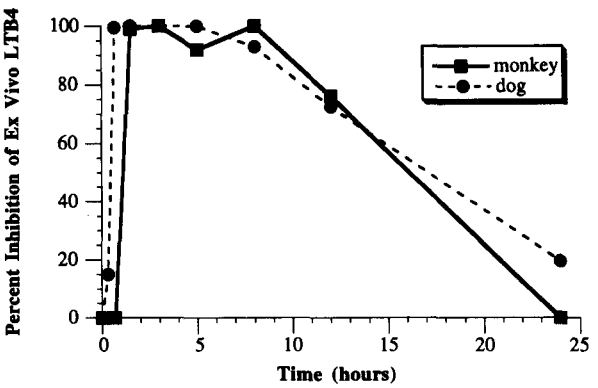


| Compound[d] (mol. wt.) | Het = | Human neutrophil[a] $IC_{50}$ nM | Human blood[b] $IC_{50}$ μM | *In vivo* rat[c] $ED_{50}$ μmol/kg or % I @ μmol/kg |
|---|---|---|---|---|
| **11a** (644.24) | 2-quinolyl | 12 (10-20) | 0.60 (0.54-0.65) | 0.24 (0.05-0.50) |
| **11b** (594.18) | 2-pyridyl | 13 (10-20) | 0.60 (0.43-0.77) | 0.12 (0.01-0.40) |
| **11c** (628.62) | 2-(6-chloropyridyl) | 10 (9-11) | 0.23 (0.20-0.26) | 2.6 (1.6 - 5.1) |
| **11d** (600.20) | 4-thiazolyl | 11 (10-11) | 0.18 (0.13-0.25) | 4.4 (2.7 - 7.0) |

[a]Inhibition of calcium ionophore (A23187) stimulated $LTB_4$ formation in human neutrophils; 95% confidence limits shown in parenthesis. [b]Human whole blood was stimulated with calcium ionophore (A23187) and $LTB_4$ was measured by enzyme immunoassay; 95% confidence limits shown in parenthesis. [c]Rat anaphylaxis in the peritoneal cavity; oral dose response determination $ED_{50}$ or % I of cysteinyl LT's at a given oral dose. [d]Compounds gave satisfactory spectral and analytical data.

The oxime analogs (**11a**, **11b**, and **11d**) were evaluated orally (10 mg/kg dose) in cynomologus monkeys for drug plasma levels and for *ex vivo* inhibition of calcium ionophore stimulated $LTB_4$ formation in blood samples taken over a 24 h period.[10] The 2-pyridyl analog (**11b**) gave no detectable drug plasma levels at 0.3 to 24 h post dose and the 4-thiazolyl analog (**11d**) had low plasma levels ($T_{max}$ 0.7 h, $C_{max}$ 2.0 μM). In the monkey, the quinolylmethoxy analog (**11a**) gave the highest plasma levels ($T_{max}$ 3 h, $C_{max}$ 5.6 μM) and provided >90% *ex vivo* inhibition of $LTB_4$ in blood samples over an 8 h period, as shown in Figure 1. In the dog, **11a** given orally at 10 mg/kg provided >90% *ex vivo* inhibition of $LTB_4$ over an 8 h period (Figure 1). The oxime congener (**11a**) proved to have the best overall profile of oral bioavailability in rat, dog and monkey of the compounds evaluated in this study.

**Figure 1. *Ex Vivo* Inhibition of Calcium Ionophore Stimulated $LTB_4$ Formation in Blood Samples from Monkey and Dog Orally Dosed with 10 mg/kg of 11a**



**Summary**. Replacing the carboxylate with the *N*-hydroxyurea pharmacophore in the FLAP indole series as exemplified by congeners **3** and **5** resulted in compounds which retained FLAP inhibitory activity but had only weak direct 5-LO inhibitory activity. Insertion of an oxime function into the alkylcarboxylate substituent of the FLAP indole standards **1** and **2**, led to congeners with promising oral activity. The 2-quinolylmethoxy substituted indole oxime congener **11a** (A-81834) demonstrated excellent inhibitory activity in rat, dog and monkey by oral administration. A-81834 represents an optimized FLAP inhibitor with properties appropriate for further pharmacological studies as an antileukotriene drug.

**References.**

1. (a) Samuelsson, B. *Science* **1983**, *220*, 568. (b) Lewis, R. A.; Austen, K. F.; Soberman, R. J. *New Eng. J. Med.* **1990**, *323*, 645.

2. (a) Vickers, P. J. *J. Lipid Med. Cell Signalling* **1995**, *12*, 185. (b) Gillard, J. W.; Ford-Hutchinson, A. W.; C., C.; Charleson, S.; Denis, D.; Foster, A.; Fortin, R.; Léger, S.; McFarlane, C. S.; Morton, H.; Piechuta, H.; Riendeau, D.; Rouzer, C. A.; Rokach, J.; Young, R. N.; MacIntyre, D. E.; Peterson, L.; Bach, T.; Eiermann, G.; Hopple, S.; Humes, J.; Hupe, D.; Luell, S.; Metzger, J.; Meurer, R.; Miller, D. K.; Opas, E.; Pacholok, S. *Can. J. Physiol. Pharmacol.* **1989**, *67*, 456.

3. Mancini, J. A.; Abramovitz, M.; Cox, M. E.; Wong, E.; Charleson, S.; Perrier, H.; Wang, Z.; Prasit, P.; Vickers, P. J. *FEBS Lett.* **1993**, *318*, 277.

4. Abramovitz, M.; Wong, E.; Cos, M. E.; Richardson, C. D.; Li, C.; Vickers, P. J. *Eur. J. Biochem.* **1993**, *215*, 105.

5. (a) Woods, J. W.; Evans, J. F.; Ethier, D.; Scott, S.; Vickers, P. J.; Hearn, L.; Heibein, J. A.; Charleson, S.; Singer, I. I. *J. Exp. Med.* **1993**, *178*, 1935. (b) Peters-Golden, M.; McNish, R. W. *Biochem. Biophys. Res. Commun.* **1993**, *196*, 147.

6. Bel, E. H.; Tanaka, W.; Spector, R.; Friedman, B.; Von de Veen, J. H.; Dijkman, J. H.; Sterk, P. J. *Am. Rev. Respir. Dis.* **1990**, *141*, A31.

7. Brideau, C.; Chan, C.; Denis, D.; Evans, J. F.; Ford-Hutchinson, A. W.; Fortin, R.; Gillard, J. W.; Guay, J.; Hutchinson, J.; Jones, T.; Léger, S.; Mancini, J. A.; McFarlane, C. S.; Pickett, C.; Piechuta, H.; Prasit, P.; Riendeau, D.; Rouzer, C. A.; Tagari, P.; Vickers, P.; Young, R. N. *Can. J. Physiol. Pharmacol.* **1992**, *70*, 799.

8. (a) Chapman, K. R.; Freidman, B. S.; Shingo, S.; Heyse, J.; Reiss, T.; Spector, R. *Am. J. Respir. Crit. Care Med.* **1994**, *149*, A215. (b) Storms, W.; Friedman, B. S.; Zhang, J.; Santanello, N.; Allegar, N.; Appel, D.; Beaucher, W.; Bronsky, F.; Busse, W.; Chervinsky, P.; Dockhorn, R.; Edwards, T.; Goldstein, M.; Grossman, J.; Hendeles, L.; Kemp, J.; Memon, N.; Noonan, M.; Owens, G.; Shapiro, G.; Spirn, I.; Strek, M.; Stricker, W.; Tinkelman, D.; Townley, R.; Wanderer, A.; Weisberg, S.; Winder, J.; Woehler, T. *Am. J. Respir. Crit. Care Med.* **1995**, *151*, A377.

9. For the biological assay methods see: Carter, G. W.; Young, P. R.; Albert, D. H.; Bouska, J.; Dyer, R.; Bell, R. L.; Summers, J. B.; Brooks, D. W. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 929.

10. For the *ex vivo* stimulated blood $LTB_4$ assay method see: Brooks, C. D. W.; Stewart, A. O.; Basha, A.; Bhatia, P.; Ratajczyk, J. D.; Martin, J. G.; Craig, R. A.; Kolasa, T.; Bouska, J. B.; Lanni, C.; Harris, R. R.; Carter, G. W.; Bell, R. L. *J. Med. Chem.* **1995**, *38*, 4768.

11. Brooks, D. W.; Carter, G. W. In *The Search for Anti-Inflammatory Drugs*; Merluzzi, V. J. and Adams, J., Eds.; Birkhauser: Boston, **1995**; Chapter 5, pp 129-160.

12. Hutchinson, J. H.; Charleson, S.; Evans, J. F.; Falgueyret, J.-P.; Hoogsteen, K.; Jones, T. R.; Kargman, S.; Macdonald, D.; McFarlane, C.S.; Nicholson, D. W.; Piechuta, H.; Riendeau, D.; Scheigetz, J.; Thérien, M.; Girard, Y. *J. Med. Chem.* **1995**, *38*, 4538.

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