Bull. Korean Chem. Soc. 2014, Vol. 35, No. 8 2581
http://dx.doi.org/10.5012/bkcs.2014.35.8.2581

Design, Synthesis, and Molecular Docking Study of Flavonol Derivatives as Selective JAK1 Inhibitors

Mi Kyoung Kim,[a] Onnuri Bae,[a] and Youhoon Chong*

*Department of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea. *E-mail: chongy@konkuk.ac.kr*

Received April 24, 2014, Accepted April 28, 2014

Key Words : Flavonol, Janus kinase 1 (JAK1), Selective inhibitor, Propargyl alcohol

The Janus kinase family members of intracellular non-receptor tyrosine kinases (JAK1, JAK2, JAK3 and TYK2) play key roles in transmitting inflammatory and proliferative signals through the JAK-STAT (signal transducer and activator of transcription) pathway. Thus, the JAK kinases have been highlighted as targets of therapeutic intervention for cancer as well as inflammatory diseases.[1-3] Among the JAK isozymes, JAK1 has been investigated as an attractive target for the treatment of immunologic disorders such as rheumatoid arthritis (RA).[4-8] Several JAK1 inhibitors have been discovered as potential therapeutic agents for treatment of RA,[9,10] but selective inhibition of JAK1 over other JAK isozymes needs to be achieved to provide efficacy against RA and to minimize side effects.[11]

Nevertheless, design of the JAK isozyme-selective inhibitors has been hampered by the significant overall sequence homology among the four isozymes.[12] In this study, based on the slight structural differences around the ATP-binding sites of the JAK isozymes, we intended to design a novel inhibitor which binds at the hereto unexplored binding site. In general, as shown in the binding mode of the pan-JAK inhibitor, tofacitinib (CP-690,550), to the ATP-binding site of JAK1 (PDB ID: 3EYG),[13] JAK inhibitors occupy the binding pocket composed of three binding regions, A, B, and C (Fig. 1); hydrogen bonding with the hinge motif (A), hydrophobic interaction with the activation segment (B) and the glycine-rich loop (C). However, the lower part of the pocket (D, Fig. 1) has rarely been shown to be involved in binding interactions with the inhibitors. Thus, we reasoned that a linear substituent attached to the JAK kinase inhibitor scaffolds (A, B, and C) might enable the resulting inhibitor to differentiate the JAK isozymes through formation of additional binding interactions (D) with the target enzyme.

In this study, natural flavonoids which are well known for kinase inhibiting activity[14,15] were used as a novel scaffold for JAK kinase inhibitors. The flavonoid β-hydroxyketo group behaves as a hinge-binding motif, which could be nicely superimposed with the pyrrolopyrimidine core of tofacitinib. More interestingly, the methyl and cyanomethyl functionalities of tofacitinib, which play key roles in binding of tofacitinib with the JAKs (B and C, Fig. 1), were well matched with the 2- and 5- positions of the flavonoid B-ring. Therefore, the flavonoid structure could be modified to mimic tofacitinib by introduction of 2-methyl and/or 5-cyanomethyl functionalities at the flavonol B-ring (**1a-1c**, Fig. 2). In addition, as a linear tether to bind the empty binding region of the ATP-binding site (D, Fig. 1), a propargyl alcohol group was attached to the 4-position of the flavonol B-ring to give a series of flavonol derivatives **2a-2c** (Fig. 2).

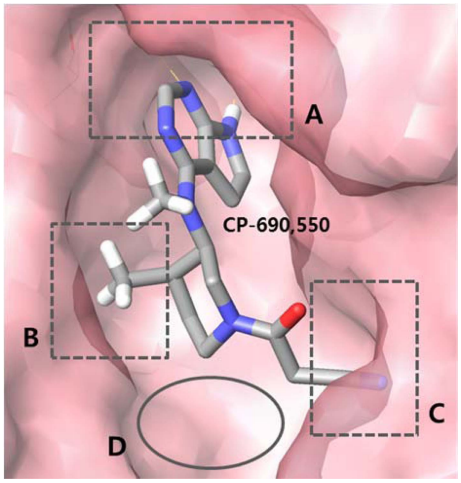


Figure 1. Binding mode of tofacitinib (CP-690,550) to the active site of JAK1 (PDB ID: 3EYG). A: hydrogen-bonding with the hinge motif, B: hydrophobic binding pocket around the methyl group, C: hydrophobic binding pocket around the cyanomethyl group, D: empty binding region.

Herein, we report synthesis of a series of flavonol derivatives and evaluation of their JAK1-selective inhibitory activity.

The title compounds were prepared by employing a well-known synthetic routine composed of condensation of 2′-hydroxyacetophenone (**4**) with benzoic acid (**5** or **6**) followed by ring closure (Scheme 1).[16] 2′-Hydroxyacetophenone (**4**) was prepared by debenzylation followed by THP-protection of **3** which was obtained by base-promoted fragmentation of perbenzylated quercetin.[16] EDC-condensation of **4** with **5a-5c** provided the corresponding esters (**7**) which were immediately subjected to TBAB-promoted cyclization followed by THP-deprotection conditions to give the flavonol scaffolds. Reduction of 4-bromo functionality was perform-

[a]These authors contributed equally to this work.

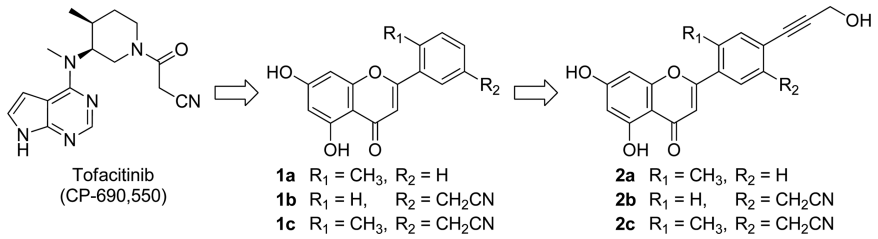


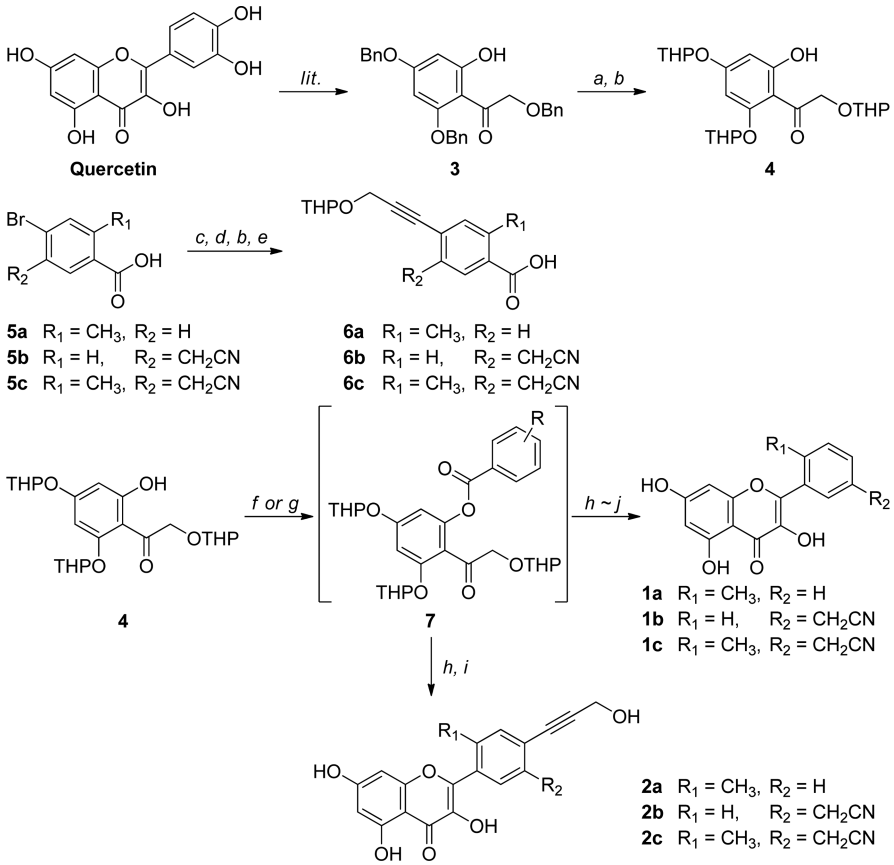
Figure 2. Design of flavonol derivatives substituted with the characteristic binding groups of the tofacitinib (CP-690,550) (**1a-1c**) and straight aryl substituent (**2a-2c**).

ed by LAH[17] to give the desired flavonols **1a-1c**. On the other hand, 4-bromobenzoic acids **5a-5c** were converted into 4-alkynyl benzoic acids **6a-6c** *via* esterification followed by Sonogashira coupling with propargyl alcohol,[18] THP-protection, and hydrolysis. 4-Alkynyl benzoic acids **6a-6c** were then coupled with **4** to give the corresponding esters which were transformed into the flavonols **2a-2c** after cyclization and global THP-deprotection.

In vitro inhibitory activity of the flavonol derivatives on the JAK isozymes was determined by Z′-LYTE™ Kinase Assay Kit-Tyr 6 Peptide (JAK1-JAK3) and Tyr 3 Peptide (Tyk2) (Invitrogen) according to the manufacturer's instruction. IC_{50} values of the flavonol derivatives (**1a-1c**, **2a-2c**) against JAK isozymes are summarized in Table 1.

The most striking feature of the JAK isozyme inhibitory activity of the synthesized flavonol derivatives was the lack of inhibition against JAK2 as well as Tyk2. In particular, due to the lack of JAK2-inhibition, JAK1-selectivities of the compounds **1c** and **2b** were more than 7.6 and 12.0 fold, respectively, which were significantly higher than that of the reported JAK1-selective inhibitor (6.4 fold).[10]

Also noteworthy was the JAK1-selectivity of **2b** over JAK3. In general, moderate to potent inhibitions of JAK1 and JAK3 by the flavonol derivatives were observed. Even though structure-activity relationship is not clear, it can be assured that certain combinations of the aromatic substituents



Reagent and Conditions: a) Pd/C, H_2, MeOH/THF, rt; b) DHP, PPTS, CH_2Cl_2, rt; c) H_2SO_4, MeOH, 65 °C; d) propargyl alcohol, $Pd(PPh_3)_4$, CuI, TEA, THF, 65 °C; e) LiOH, THF/H_2O, 65 °C; f) **5a-5c**, EDC, DMAP, CH_2Cl_2, rt; g) **6a-6c**, EDC, DMAP, CH_2Cl_2, rt. h) TBAB, K_2CO_3, Toluene, 90 °C; i) TsOH, MeOH, rt; j) $LiAlH_4$, THF, 0 °C to rt;

Scheme 1. Synthesis of the title compounds (**1a-1c** and **2a-2c**).

Table 1. Inhibitory activity (IC_{50}) of the flavonol derivatives against JAK isozymes

Compounds	IC_{50} (μM)[a]				Selectivity[b]	
	JAK1	JAK2	JAK3	Tyk2	JAK1/JAK2	JAK1/JAK3
1a	22.6	97.2	27.7	>100	4.3	1.2
1b	72.4	>100	74.2	>100	>1.4	1.0
1c	13.2	>100	10.6	>100	>7.6	0.8
2a	56.0	>100	50.2	>100	>1.8	0.9
2b	8.3	>100	86.4	>100	>12.0	10.4
2c	35.2	>100	76.8	>100	>2.8	2.2
Inh[c] (nM)	4.3 (3.3)[d]	1.3 (2.5)[d]	12.6 (10.9)[d]	5.1 (3.6)[d]	0.3	2.9

[a]Each experiment was repeated at least three times. [b]Selectivity = IC_{50} against JAK2 or JAK3/IC_{50} against JAK1. [c]Pan-JAK inhibitor, 'pyridine 6'[19]. [d]IC_{50} values reported in reference 19

are the keys to the potency as well as selectivity of the inhibitory activities of the flavonol derivatives. For example, without the 4-propargyl alcohol substituent, the flavonol derivatives **1a-1c** showed similar IC_{50} values against JAK1 and JAK3, and the 2′-methyl-5′-cyanomethyl-substituted analogue (**1c**) was the most potent inhibitor in this series. In the case of the flavonol derivatives with a 4′-propargyl alcohol substituent (**2a-2c**), however, the 5′-cyanomethyl substituent significantly potentiated the flavonol's inhibitory activity against JAK1 to provide **2b** with the most potent and JAK1-selective inhibitory activity (10.4-fold).

The JAK1-selectivity of **2b** was then addressed by molecular docking study. The compound **2b** was therefore docked into the ATP-binding site of JAK1 (PDB ID = 4E5W),[9] JAK2 (PDB ID = 2B7A),[20] and JAK3 (PDB ID = 3LXK)[21] by using the flexible ligand docking software Glide incorporated into the Schrödinger molecular modeling software suite. Although the ligand-binding sites of JAK1-JAK3 share almost the same features, the best docking poses of **2b** for the three enzymes showed subtle, but significant, differences (Fig. 3). Flavonol **2b** is shown to fit nicely into the ATP-binding site of JAK1 (Fig. 3(a)); the size of **2b** is well matched to that of the binding pocket, and its 3-OH, 4′-$CCCH_2OH$, and 3′-CH_2CN functionalities form hydrogen bonds with either the backbone carbonyl (Leu881 and Glu883) or backbone NH (Glu883) group. In particular, the 4′-propargyl alcohol substituent is well positioned at the bottom of the binding pocket to form a hydrogen bond with the backbone carbonyl group of Glu883. In contrast, flavonol **2b** binds to JAK2 with its B-ring located out of the binding pocket due to the presence of a salt bridge between Lys857 and Arg980, which dissects the pocket (Fig. 3(b)). The binding pocket of JAK3 is somewhat smaller than that of JAK1 due to formation of a hydrogen bond between Asn832 and Asp949 at the bottom of the pocket (Fig. 3(c)). As a result, the flavonol **2b** does not occupy the binding pocket of JAK3 but binds above the pocket (Fig. 3(d)) to experience loss of hydrogen bonding as well as van der Waals interaction with the pocket.

In summary, in order to develop JAK1-selective inhibitors, we designed a novel flavonol-based scaffold by mimicking the pan-JAK inhibitor, tofacitinib, through introduction of 2-methyl and/or 5-cyanomethyl functionalities at the flavonoid B-ring. Selective inhibition of JAK1 was pursued by further substitution of the flavonol B-ring with a linear propargyl alcohol group, which was anticipated to bind to the hereto unexplored lower position of the ATP-binding sites of the JAK isozymes. Among the synthesized flavonol series, the propargyl alcohol-substituted derivative **2b** showed remarkable JAK1 selectivity (>12.0 times over JAK2 and 10.4 times over JAK3), and molecular docking study revealed that the linear tether played an important role in differentiating the ATP-binding site of JAK1 from those of other isozymes.

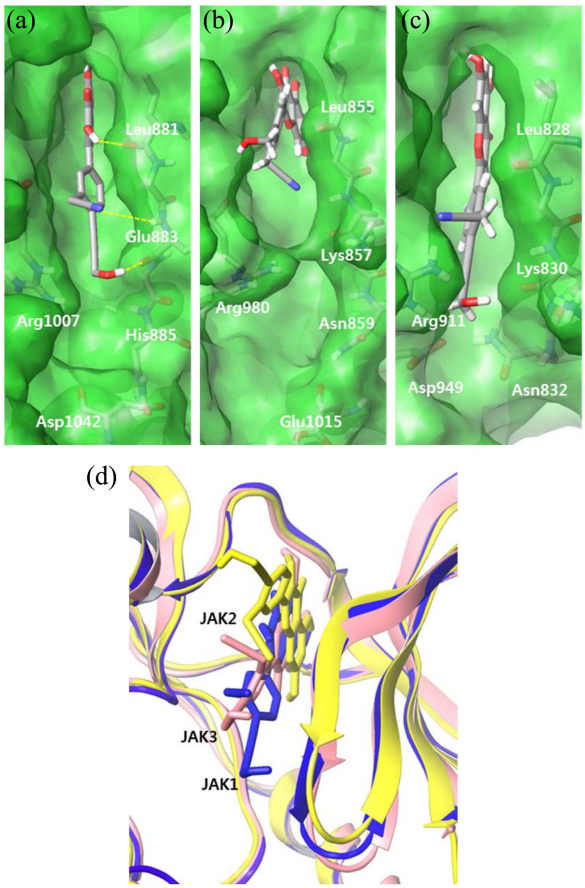


Figure 3. Docked structures of flavonol **2b** into (a) JAK1, (b) JAK2, and (c) JAK3, and (d) their superimposed structures.

Experimental Section

Preparation of the Title Compound 2b. To a stirred suspension of 2′-hydroxyacetophenone (**4**) (1 g, 2.3 mmol) in dichloromethane (10 mL) was added EDC (881 mg, 4.6 mmol) followed by DMAP (562 mg, 4.6 mmol). Upon complete dissolution, benzoic acid **6b** (2.3 mmol) was added. The mixture was stirred at rt for 4 h and concentrated under reduced pressure. The ester (**7**) (2.3 mmol) thus obtained was dissolved in toluene (10 mL), and the resulting solution was treated with K_2CO_3 (636 mg, 4.6 mmol) and tetra-*n*-butylammonium bromide (741 mg, 2.3 mmol). After stirring for 3 h at 90 °C, solid materials were removed by filtration, and the filtrate was concentrated under reduced pressure. The crude compound obtained above was dissolved in MeOH (5 mL), and the resulting solution was treated with *p*-TsOH (10 mmol) at 0 °C. The reaction mixture was stirred for 4 h at room temperature, and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (2:1 = Hex:Acetone) to give 2-(2-(3-hydroxyprop-1-yn-1-yl)-5-(3,5,7-trihydroxy-4-oxo-4*H*-chromen-2-yl)phenyl) acetonitrile (**2b**) as yellow powder: ^{1}H NMR (400 MHz, Acetone-d_6) δ 12.8 (s, OH), 7.42 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 2.0 Hz, 1H), 7.12 (dd, J = 2.1, 8.4 Hz, 1H), 6.44 (d, J = 2.1 Hz, 1H), 6.40 (d, J = 2.2 Hz, 1H), 4.42 (s, 2H), 4.12 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.1, 166.7, 161.8, 160.3, 147.2, 136.6, 133.5, 130.2, 128.1, 126.9, 125.9, 122.3, 117.8, 104.8, 99.5, 95.1, 98.6, 88.4, 52.6, 23.5.

Acknowledgments. This research was supported by a grant from the Korean Health Technology R&D project, Ministry of Health & Welfare, Republic of Korea (A111698), by the Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093824), and by a grant from Next Generation Bio-Green 21 PJ007982 (Korea Rural Development Administration).

References

1. (a) O'Shea, J. J. *Immunity* **1997**, *7*, 1. (b) Pesu, M.; Laurence, A.; Kishore, N.; Wzillich, S. H.; Chan, G.; O'Shea, J. J. *Immunol. Rev.* **2008**, *223*, 132.
2. O'Shea, J. J.; Pesu, M.; Borie, D. C.; Changelian, P. S. *Nat. Rev. Drug Discovery* **2004**, *3*, 555.
3. (a) Clark, S. C.; Kamen, R. *Science* **1987**, *236*, 1229. (b) Metcalf, D. *Nature* **1989**, *339*, 27.
4. Rodig, S. J.; Meraz, M. A.; White, J. M.; Lampe, P. A.; Riley, J. K.; Arthur, C. D.; King, K. L.; Sheehan, K. C.; Yin, L.; Pennica, D.; Johnson, E. M., Jr.; Schreiber, R. D. *Cell* **1998**, *93*, 373.
5. Shimoda, K.; Kouji, K.; Aoki, K.; Matsuda, T.; Miyamoto, A.; Shibamori, M.; Yamashita, M.; Numata, A.; Takase, K.; Kobayashi, S.; Shibata, S.; Asano, Y.; Gondo, H.; Sekiguchi, K.; Nakayama, K.; Nakayama, T.; Okamura, T.; Okamura, S.; Niho, Y.; Nakayama, K. *Immunity* **2000**, *13*, 561.
6. Parganas, E.; Wang, D.; Stravopodis, D.; Topham, D. J.; Marine, J. C.; Teglund, S.; Vanin, E. F.; Bodner, S.; Colamonici, O. R.; van Deursen, J. M.; Grosveld, G.; Ihle, J. N. *Cell* **1998**, *93*, 385.
7. Smolen, J. S.; Beaulieu, A.; Rubbert-Roth, A.; Ramos-Remus, C.; Rovensky, J.; Alecock, E.; Woodworth, T.; Alten, R. *Lancet* **2008**, *371*, 987.
8. Haan, C.; Rolvering, C.; Raulf, F.; Kapp, M.; Druckes, P.; Thoma, G.; Behrmann, I.; Zerwes, H. G. *Chem. Biol.* **2011**, *18*, 314.
9. Kulagowski, J. J.; Blair, W.; Bull, R. J.; Chang, C.; Deshmukh, G.; Dyke, H. J.; Eigenbrot, C.; Ghilardi, N.; Gibbons, P.; Harrison, T. K.; Hewitt, P. R.; Liimatta, M.; Hurley, C. A.; Johnson, A.; Johnson, T.; Kenny, J. R.; Kohli, P. B.; Maxey, R. J.; Mendonca, R.; Mortara, K.; Murray, J.; Narukulla, R.; Shia, S.; Steffek, M.; Ubhayakar, S.; Ultsch, M.; van Abbema, A.; Ward, S. I.; Waszkowycz, B.; Zak, M. *J. Med. Chem.* **2012**, *55*, 5901.
10. Zak, M.; Mendonca, R.; Balazs, M.; Barrett, K.; Bergeron, P.; Blair, W. S.; Chang, C.; Deshmukh, G.; DeVoss, J.; Dragovich, P. S.; Eigenbrot, C.; Ghilardi, N.; Gibbons, P.; Gradl, S.; Hamman, C.; Hanan, E. J.; Harstad, E.; Hewitt, P. R.; Hurley, C. A.; Jin, T.; Johnson, A.; Johnson, T.; Kenny, J. R.; Koehler, M. F. T.; Kohli, P. B.; Kulagowski, J. J.; Labadie, S.; Liao, J.; Liimatta, M.; Lin, J.; Lupardus, P. J.; Maxey, R. J.; Murray, J. M.; Pulk, R.; Rodriguez, M.; Savage, S.; Shia, S.; Steffek, M.; Ubhayakar, S.; Ultsch, M.; van Abbema, A.; Ward, S. I.; Xiao, L.; Xiao, Y. *J. Med. Chem.* **2012**, *55*, 6176.
11. Vardiman, J. W.; Harris, N. L.; Brunning, R. D. *Blood*. **2002**, *100*, 2292.
12. Wilks, A. F. *Semin. Cell Dev. Biol.* **2008**, *19*, 319.
13. Williams, N. K.; Bamert, R. S.; Patel, O.; Wang, C.; Walden, P. M.; Wilks, A. F.; Fantino, E.; Rossjohn, J.; Lucet, I. S. *J. Mol. Biol.* **2009**, *387*, 219.
14. Agullo, G.; Gamet-Payrastre, L.; Manenti, S.; Viala, C.; Rémésy, C.; Chap, H.; Payrastre, B. *Biochem. Pharmacol.* **1997**, *53*, 1649.
15. Hou, D. X.; Kumamoto, T. *Antioxid. Redox Signal* **2010**, *13*, 691.
16. Caldwell, S. T.; Crozier, A.; Hartley, R. C. *Tetrahedron* **2000**, *56*, 4101.
17. Han, B. H.; Boudjouk, P. *Tetrahedron Lett*. **1982**, *23*, 1643.
18. Sonogashira, K. *Organomet. Chem.* **2002**, *653*, 46.
19. Thompson, J. E.; Cubbon, R. M.; Cummings, R. T.; Wicker, L. S.; Frankshun, R.; Cunningham, B. R.; Cameron, P. M.; Meinke, P. T.; Liverton, N.; Weng, Y.; DeMartino, J. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1219.
20. Lucet, I. S.; Fantino, E.; Styles, M.; Bamert, R.; Patel, O.; Broughton, S. E.; Walter, M.; Burns, C. J.; Treutlein, H.; Wilks, A. F.; Rossjohn, J. *Blood* **2006**, *107*, 176.
21. Chrencik, J. E.; Patny, A.; Leung, I. K.; Korniski, B.; Emmons, T. L.; Hall, T.; Weinberg, R. A.; Gormley, J. A.; Williams, J. M.; Day, J. E.; Hirsch, J. L.; Kiefer, J. R.; Leone, J. W.; Fischer, H. D.; Sommers, C. D.; Huang, H. C.; Jacobsen, E. J.; Tenbrink, R. E.; Tomasselli, A. G.; Benson, T. E. *J. Mol. Biol*. **2010**, *400*, 413.