Bioorganic & Medicinal Chemistry Letters 23 (2013) 2298–2301

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of pyrrolo[2,3-*g*]indazoles as new Pim kinase inhibitors

Laurent Gavara, Virginie Suchaud, Lionel Nauton, Vincent Théry, Fabrice Anizon *, Pascale Moreau

Clermont Université, Université Blaise Pascal, Institut de Chimie de Clermont-Ferrand, BP 10448, 63000 Clermont-Ferrand, France
CNRS, UMR 6296, ICCF, BP 80026, 63171 Aubière, France

ARTICLE INFO

Article history:
Received 31 January 2013
Revised 12 February 2013
Accepted 14 February 2013
Available online 26 February 2013

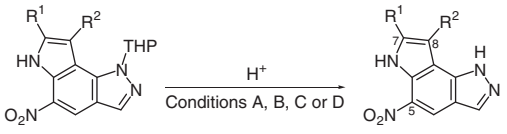
Keywords:
Pyrrolo[2,3-g]indazole
Indazole
Indole
Pim kinase inhibition

ABSTRACT

The synthesis and Pim kinase inhibition potency of a new series of pyrrolo[2,3-*g*]indazole derivatives is described. The results obtained in this preliminary structure–activity relationship study pointed out that sub-micromolar Pim-1 and Pim-3 inhibitory potencies could be obtained in this series, more particularly for compounds **10** and **20**, showing that pyrrolo[2,3-*g*]indazole scaffold could be used for the development of new potent Pim kinase inhibitors. Molecular modeling experiments were also performed to study the binding mode of these compounds in Pim-3 ATP-binding pocket.

Indole and indazole ring systems are considered as highly valuable heterocyclic scaffolds in drug discovery. The chemistry of indole nucleus has been abundantly described, as well as numerous biological applications leading to pharmaceutically active molecules. Indazole nucleus is an indole bio-isoster. Several recent reports in organic synthesis and medicinal chemistry confirmed the great interest of this scaffold.[1–8] Particularly, indazole derivatives have been described as protein kinase inhibitors.[9–12] Accordingly, in our ongoing research aiming at developing new Pim kinase inhibitors,[13–18] we were interested in the development of novel scaffolds containing the indazole heterocyclic system. Pim (Provirus integration site for Moloney murine leukaemia virus) family is represented by three highly homologous Ser/Thr kinases (Pim-1, Pim-2 and Pim-3) involved in cell survival and malignant transformation.[19–24] Accordingly, Pim kinases are considered as important targets in the field of drug discovery against cancer. Therefore, in continuation of our study on the use of indazole derivatives as Pim inhibitors, we herein report the synthesis and biological evaluation of a series of diversely substituted dihydropyrrolo[2,3-*g*]indazoles as well as the evaluation of their potencies toward the three Pim kinase isoforms.

We recently reported the synthesis of N1-protected 1,6-dihydropyrrolo[2,3-*g*]indazoles **1**–**7** (Scheme 1).[25] These compounds can be considered as valuable synthetic intermediates that could easily lead to deprotected derivatives and their corresponding amino analogues. Thus, compound **1** was treated by acetic acid in THF/H_2O in order to perform THP cleavage. Two products were obtained: the deprotected derivative **8** was isolated in 52% yield, as well as its desilylated analogue **9** in 48% yield. Deprotection of compound **2** needed stronger acidic conditions. By using *p*-tolu-



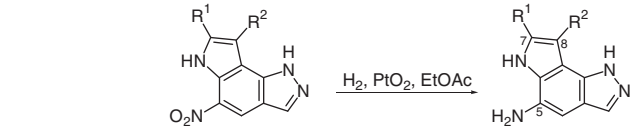
1–7 8–18

Compd	R^1	R^2	Conditions	Product	R^1	R^2	% Yield
1	TMS	H	A	**8**	TMS	H	52
				9	H	H	48
2	Et	Ph	B	**10**	Et	Ph	97
3[a]	H	CH_2OBn	B	**11**	H	CH_2OEt	22
4	H	$CH(OEt)_2$	A	**12**	H	CHO	42
5	CO_2Et	Me	C	**13**	H	Me	41
			B	**14**	CO_2Et	Me	38
6	CO_2Me	*n*-Pr	C	**15**[b]	H	*n*-Pr	55
			B	**16**	CO_2Me	*n*-Pr	86
7	CO_2Et	Ph	D	**17**[b]	H	Ph	62
			B	**18**	CO_2Et	Ph	88

Scheme 1. Synthesis of compounds **8**–**18**. Reagents and conditions: (A) AcOH, THF/H_2O, (B) PTSA, EtOH or MeOH, H_2O, (C) concd HCl, (D) concd HBr. [a]Compound **3** contains ~30% of the 7-CH_2OBn regioisomer, [b]See Ref. 24.

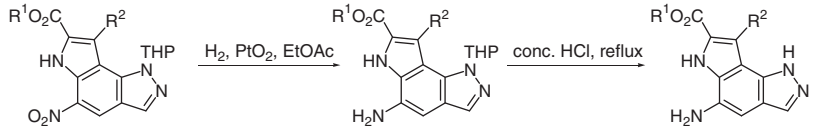
* Corresponding author. Tel.: +33 (0) 4 73 40 53 64; fax: +33 (0) 4 73 40 77 17.
E-mail address: Fabrice.ANIZON@univ-bpclermont.fr (F. Anizon).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.02.074



8–10, 13–18 **19–27**

Compd	Product	R^1	R^2	% Yield
8	**19**	TMS	H	76
9	**20**	H	H	43
10	**21**	Et	Ph	82
13	**22**	H	Me	20
14	**23**	CO_2Et	Me	–
15	**24**	H	*n*-Pr	63
16	**25**	CO_2Me	*n*-Pr	–
17	**26**	H	Ph	78
18	**27**	CO_2Et	Ph	82



5, 6 **28**, 70% **29**, 77% **23**, 61% **25**, 23%

5, **28**, **23** R^1 = Et, R^2 = Me
6, **29**, **25** R^1 = Me, R^2 = *n*-Pr

Scheme 2. Synthesis of compounds **19**–**29**.

Table 1
Kinase inhibitory potencies: % of residual kinase activity at 10 and 1 μM

Compd	Kinase inhibition—% of residual kinase activity									
	Pim-1		Pim-2		Pim-3					
	10 μM	1 μM	10 μM	1 μM	10 μM	1 μM	Compd	R^1	R^2	R^3
8	17 ± 0	63 ± 2	64 ± 13	103 ± 18	9 ± 0 (0.47 ± 0.02)	51 ± 4	**8**	TMS	H	NO_2
9	30 ± 2	102 ± 33	80 ± 2	92 ± 5	18 ± 2	78 ± 5	**9**	H	H	NO_2
10	51 ± 10 (0.23 ± 0.03)	43 ± 4	84 ± 11	77 ± 6	36 ± 5 (0.119 ± 0.006)	31 ± 1	**10**	Et	Ph	NO_2
11	26 ± 1	82 ± 1	63 ± 2	109 ± 10	19 ± 2	71 ± 11	**11**	H	CH_2OEt	NO_2
12	27 ± 3	97 ± 25	70 ± 17	117 ± 31	25 ± 1	76 ± 2	**12**	H	CHO	NO_2
13	26 ± 6	68 ± 4	61 ± 2	99 ± 1	26 ± 1	77 ± 10	**13**	H	Me	NO_2
14	72 ± 6	78 ± 6	97 ± 6	105 ± 3	47 ± 2	72 ± 7	**14**	CO_2Et	Me	NO_2
15	54 ± 4	78 ± 11	27 ± 10	66 ± 9	6 ± 0 (0.26 ± 0.02)	24 ± 4	**15**	H	*n*Pr	NO_2
16	39 ± 2	110 ± 37	85 ± 8	113 ± 52	24 ± 0 (0.546 ± 0.009)	50 ± 3	**16**	CO_2Me	*n*Pr	NO_2
17	28 ± 3 (1.0 ± 0.7)	52 ± 6	70 ± 7	82 ± 8	21 ± 1 (0.53 ± 0.04)	38 ± 5	**17**	H	Ph	NO_2
18	55 ± 1	86 ± 7	113 ± 21	92 ± 15	90 ± 23	73 ± 11	**18**	CO_2Et	Ph	NO_2
19	52 ± 10	94 ± 6	111 ± 16	93 ± 27	24 ± 1	67 ± 13	**19**	TMS	H	NH_2
20	17 ± 2 (0.46 ± 0.05)	63 ± 19	104 ± 8	99 ± 11	4 ± 0 (0.033 ± 0.002)	20 ± 2	**20**	H	H	NH_2
21	44 ± 4	92 ± 4	75 ± 11	91 ± 9	23 ± 0	72 ± 3	**21**	Et	Ph	NH_2
22	63 ± 1	91 ± 3	88 ± 13	105 ± 8	17 ± 4	65 ± 2	**22**	H	Me	NH_2
23	61 ± 3	93 ± 4	97 ± 10	102 ± 17	26 ± 4	93 ± 17	**23**	CO_2Et	Me	NH_2
24	57 ± 15	104 ± 17	93 ± 3	90 ± 6	39 ± 2	79 ± 0	**24**	H	*n*Pr	NH_2
25	26 ± 8	71 ± 2	78 ± 11	105 ± 5	12 ± 1	82 ± 14	**25**	CO_2Me	*n*Pr	NH_2
26	51 ± 10	111 ± 0	92 ± 3	103 ± 20	26 ± 1	68 ± 7	**26**	H	Ph	NH_2
27	29 ± 9	74 ± 9	74 ± 18	101 ± 0	20 ± 3	72 ± 4	**27**	CO_2Et	Ph	NH_2

IC_{50} values (in brackets) were determined when the remaining kinase activity was found to be inferior to 50% when the compounds were tested at 1 μM.

enesulfonic acid (PTSA) in EtOH/H_2O, **10** was isolated in 97% yield. From compound **3**, the use of PTSA and ethanol cleaved the benzyloxy moiety and produced the ethoxy-substituted derivative **11**. Formyl derivative **12** was obtained from **4** by concomitant indazole nitrogen deprotection and acetal hydrolysis (Scheme 1).

As previously described for compounds **6** and **7**,[25] compound **5** was simultaneously deprotected and decarboxylated in refluxing concentrated HCl, leading to compound **13** in 41% yield. To avoid the decarboxylation, **5**–**7** were treated by PTSA leading to THP-deprotected indazole alkyl carboxylates **14**, **16** and **18** in 38–88% yields. Low yield observed for compound **14** could be explained by purification difficulties due to low solubility (Scheme 1).

Amino analogues were next prepared by catalytic hydrogenation of the nitro function. Thus, compounds **8**–**10** and **13**–**18** were hydrogenated in the presence of PtO_2. Due to the difficulty to isolate **11** in satisfactory quantity and due to the very poor solubility of compound **12**, the corresponding amino derivatives were not synthesized. Compounds **19**–**22**, **24**, **26** and **27** were obtained from their nitro analogues in 20–82% yields (Scheme 2). However, in the case of alkyl indazole carboxylates **14** and **16**, we met some difficulties to proceed to this reduction step. Accordingly, the reduction of the nitro function was performed prior to the THP-deprotection step, leading to compounds **28** and **29** in 70% and 77% yield, respectively (Scheme 2). Deprotection of N-1 indazole nitrogen in concentrated HCl finally led to expected compounds **23** and **25** with moderate yields which could be explained by partial hydrolysis of the ester function.

The kinase inhibitory potencies of pyrroloindazoles **8**–**27** were evaluated at 10 and 1 μM concentrations in duplicate assays against Pim-1, Pim-2 and Pim-3. Kinase assays were performed by the International Centre for Kinase Profiling (Dundee, UK).[26] The percentages of kinase residual activities are reported in Table 1. When the remaining kinase activity was found to be inferior to 50% when the compounds were tested at 1 μM, IC_{50} values were also determined.

According to the results reported in Table 1, Pim-3 is the isoform that was the most inhibited by the tested compounds. In contrast, Pim-2 was poorly inhibited. Regarding Pim-1, the IC_{50} values were determined only for three compounds (**10**, **17** and **20**). Compounds **17** and **20** were moderate Pim-1 inhibitors, with IC_{50} values of 1.0 and 0.46 μM, respectively. The best result toward Pim-1 was found for compound **10**, bearing a nitro group at the C5 position, an ethyl group at the C7 position and a phenyl group at the C8 position, with an IC_{50} value of 0.23 μM.

Regarding Pim-3 inhibition, compounds **8**, **10**, **15**–**17**, and **20** are the most potent with IC_{50} values in the sub-micromolar range (Table 1). Interestingly, with the exception of compound **20**, all these active compounds were bearing a nitro group at the C5 position and were either mono-substituted at the position C7 (**8**) or C8 (**15**, **17**), or di-substituted at these positions (**10**, **16**). Among the nitro derivatives, **10** exhibited the best inhibitory potency toward Pim-3, showing that in this nitro series C7 and C8 positions can be substituted by hydrophobic groups to inhibit both Pim-1 and Pim-3 kinases. Nevertheless, the best inhibitory potency toward Pim-3 was found for C7/C8 unsubstituted amino derivative **20**, with an IC_{50} value of 33 nM, showing that contrarily to what was observed for nitro analogues, the substitution of positions C7 and/or C8 was detrimental to the inhibitory activity. These results suggest that the interaction of 5-nitro and 5-amino analogues with Pim-3 ATP-binding site may involve different binding modes.

Thus, we next performed molecular modeling experiments in order to study the putative binding mode of compounds **10** and **20** in the ATP-binding pocket of Pim-3. These two pyrroloindazoles are the best Pim-3 inhibitors in the series and are bearing a nitro or an amino group at the C5 position, respectively. We also studied the Pim-3 binding modes of their amino/nitro counterparts;

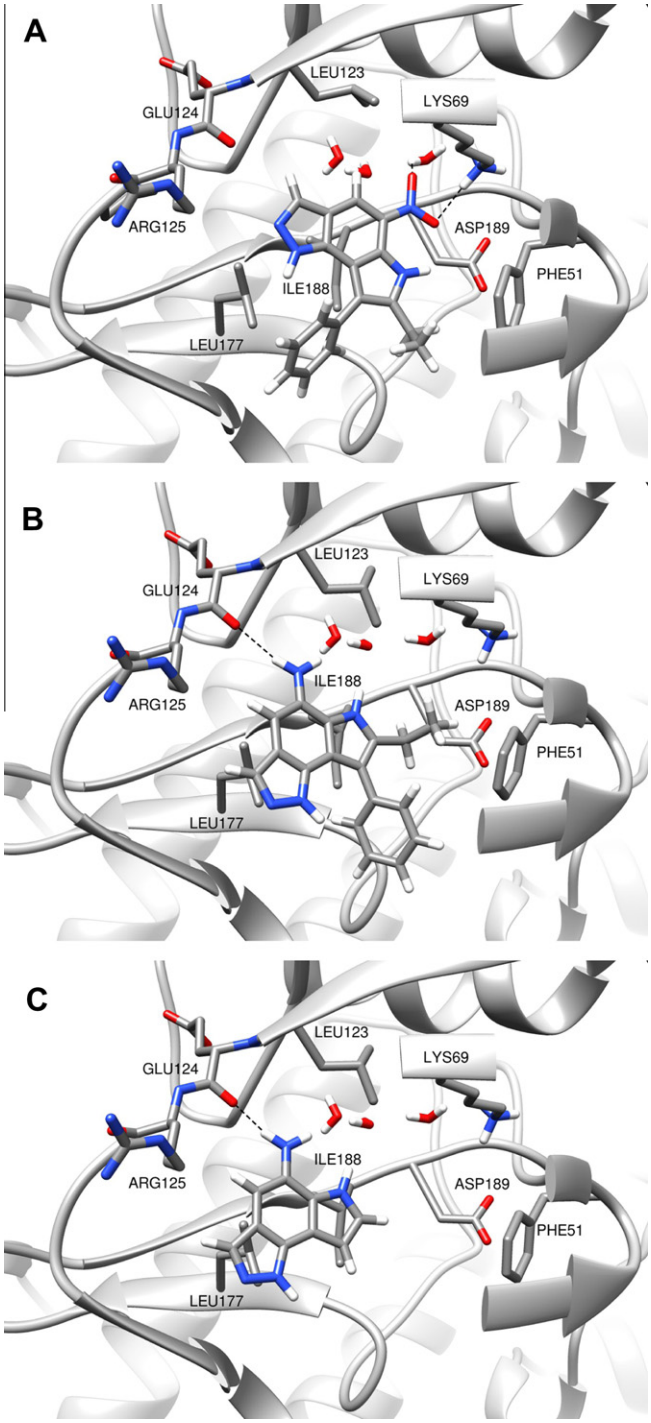


Figure 1. Docking models of compounds **10** (A), **21** (B) and **20** (C) bound to the Pim-3 ATP binding site. Hydrogen bonds are indicated as dashed lines. Molecular graphics images were produced using UCSF Chimera.[27]

compounds **21** and **9**, respectively. To the best of our knowledge, no Pim-3 X-ray crystal structure has been solved so far. Therefore, as Pim-3 presents a high sequence identity with Pim-1, we generated a Pim-3 homology model using the same method we previously reported.[13] Thus, we generated a Pim-3 model using Modeller9V11 and UCSF Chimera,[27–30] Sybylx2.0[31] software and Pim-1 1XWS crystal structure available in the Protein Data Bank (PDB). Docking experiments were then performed using Sybylx2.0 for compounds **9**, **10**, **20** and **21**, and the best docking solution for each compound was minimized. The docking solutions found for

compounds **10**, **20** and **21** are depicted in Figure 1. Concerning compound **9**, docking experiments did not allow us to observe any major interaction with Pim-3 ATP-binding pocket.

As shown in Figure 1A and B, compounds **10** and **21**, bearing ethyl and phenyl substituents at the C7 and C8 positions, are inserted in Pim-3 ATP-binding cleft but showed different binding modes. Compound **10** is placed deep in the pocket and interacts via its nitro group with protonated Lys69 side chain, as well as with a conserved water molecule. However, this compound does not establish hydrogen bond with the hinge region. On the other hand, the amino function of compound **21** is turned toward the Glu124 backbone carbonyl leading to the formation of a weak hydrogen bond (2.24 Å) with the hinge. An additional hydrophobic interaction was found for **21** between the methyl group of C7 ethyl substituent and Phe51 side chain. Regarding compound **20** (Fig. 1C), a similar orientation of the pyrroloindazole scaffold to that of **21** was found. However, in this case, a strong hydrogen bond can be established between the amino group of the inhibitor and Glu124 backbone carbonyl (1.84 Å). This strong interaction might explain the potent inhibitory potency of compound **20** toward Pim-3. This interaction is weaker in the case of compound **21** probably due to the presence of the substituents at the C7 and C8 positions that prevent the amino group to get closer to Glu124 backbone carbonyl. Finally, this molecular modeling study showed that a different binding mode could probably be involved in the interaction with Pim-3 of pyrroloindazoles substituted at the C5 position by nitro or amino groups. In the amino series, a hydrogen bond with the hinge region is possible but is strongly influenced by the substitution at the C7 and C8 positions. These results will be used to further optimize the biological profile of this 1,6-dihydropyrrolo[2,3-*g*]indazole series.

In conclusion, a structure–activity relationship study was performed on a series of 1,6-dihydropyrrolo[2,3-*g*]indazoles regarding their Pim kinase inhibitory potencies. These results identified 1,6-dihydropyrrolo[2,3-*g*]indazole as a promising scaffold for the development of new potent Pim kinase inhibitors. We found that compounds **10** and **20** exhibited interesting Pim-1 and Pim-3 inhibitory properties. More particularly, compound **20** demonstrated a nanomolar activity against Pim-3. We also performed molecular modeling experiments that enable us to propose a binding mode of compounds **10** and **20** in Pim-3 ATP-binding pocket. Due to its high inhibitory potency toward Pim-3, compound **20** could be used as an interesting tool to study the biological role of Pim-3 compared to the one of Pim-1 and Pim-2.

Acknowledgments

The authors are grateful for the financial support of ANR (ANR-08-JCJC-0131-CSD 3) and thank Bertrand Légeret for mass spectra analysis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.074.

References and notes

1. Hattori, K.; Yamaguchi, K.; Yamaguchi, J.; Itami, K. *Tetrahedron* **2012**, *68*, 7605.
2. Sun, F.; Feng, X.; Zhao, X.; Huang, Z.-B.; Shi, D.-Q. *Tetrahedron* **2012**, *68*, 3851.
3. Li, P.; Wu, C.; Zhao, J.; Rogness, D. C.; Shi, F. *J. Org. Chem.* **2012**, *77*, 3149.
4. Xiong, X.; Jiang, Y.; Ma, D. *Org. Lett.* **2012**, *14*, 2552.
5. Yong, F.-F.; Teo, Y.-C. *Synlett* **2012**, 2106.
6. El Kaïm, L.; Grimaud, L.; Purumandla, S. R. *Synlett* **2012**, 295.
7. Schmidt, A.; Beutler, A.; Snovydovych, B. *Eur. J. Org. Chem.* **2008**, 4073.
8. Cerecetto, H.; Gerpe, A.; González, M.; Arán, V. J.; de Ocáriz, C. O. *Mini-Rev. Med. Chem.* **2005**, *5*, 869.
9. Lukasik, P. M.; Elabar, S.; Lam, F.; Shao, H.; Liu, X.; Abbas, A. Y.; Wang, S. *Eur. J. Med. Chem.* **2012**, *57*, 311.
10. Li, R.; Martin, M. P.; Liu, Y.; Wang, B.; Patel, R. A.; Zhu, J.-Y.; Sun, N.; Pireddu, R.; Lawrence, N. J.; Li, J.; Haura, E. B.; Sung, S.-S.; Guida, W. C.; Schonbrunn, E.; Sebti, S. M. *J. Med. Chem.* **2012**, *55*, 2474.
11. Fuchi, N.; Iura, Y.; Kaneko, H.; Nitta, A.; Suyama, K.; Ueda, H.; Yamaguchi, S.; Nishimura, K.; Fujii, S.; Sekiya, Y.; Yamada, M.; Takahashi, T. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4358.
12. Deng, X.; Zhou, W.; Weisberg, E.; Wang, J.; Zhang, J.; Sasaki, T.; Nelson, E.; Griffin, J. D.; Jänne, P. A.; Gray, N. S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4579.
13. Giraud, F.; Akué-Gédu, R.; Nauton, L.; Candelon, N.; Debiton, E.; Théry, V.; Anizon, F.; Moreau, P. *Eur. J. Med. Chem.* **2012**, *56*, 225.
14. Letribot, B.; Akué-Gédu, R.; Santio, N. M.; El-Ghozzi, M.; Avignant, D.; Cisnetti, F.; Koskinen, P. J.; Gautier, A.; Anizon, F.; Moreau, P. *Eur. J. Med. Chem.* **2012**, *50*, 304.
15. Akué-Gédu, R.; Letribot, B.; Saugues, E.; Debiton, E.; Anizon, F.; Moreau, P. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3807.
16. Akué-Gédu, R.; Nauton, L.; Théry, V.; Bain, J.; Cohen, P.; Anizon, F.; Moreau, P. *Bioorg. Med. Chem.* **2010**, *18*, 6865.
17. Gavara, L.; Saugues, E.; Alves, G.; Debiton, E.; Anizon, F.; Moreau, P. *Eur. J. Med. Chem.* **2010**, *45*, 5520.
18. Akué-Gédu, R.; Rossignol, E.; Azzaro, S.; Knapp, S.; Filippakopoulos, P.; Bullock, A. N.; Bain, J.; Cohen, P.; Prudhomme, M.; Anizon, F.; Moreau, P. *J. Med. Chem.* **2009**, *52*, 6369.
19. Anizon, F.; Shtil, A. A.; Danilenko, V. N.; Moreau, P. *Curr. Med. Chem.* **2010**, *17*, 4114.
20. Morwick, T. *Expert Opin. Ther. Pat.* **2010**, *20*, 193.
21. Brault, L.; Gasser, C.; Bracher, F.; Huber, K.; Knapp, S.; Schwaller, J. *Haematologica* **2010**, *95*, 1004.
22. Magnuson, N. S.; Wang, Z.; Ding, G.; Reeves, R. *Future Oncol.* **2010**, *6*, 1461.
23. Isaac, M.; Siu, A.; Jongstra, J. *Drug Resist. Update* **2011**, *14*, 203.
24. Aho, T. L.; Sandholm, J.; Peltola, K. J.; Mankonen, H. P.; Lilly, M.; Koskinen, P. J. *FEBS Lett.* **2004**, *571*, 43.
25. Gavara, L.; Anizon, F.; Moreau, P. *Tetrahedron* **2011**, *67*, 7330.
26. Bain, J.; Plater, L.; Elliott, M.; Shpiro, N.; Hastie, J.; McLauchlan, H.; Klervernic, I.; Arthur, S. C.; Alessi, D. R.; Cohen, P. *Biochem. J.* **2007**, *408*, 297.
27. Sali, A.; Blundell, T. L. *J. Mol. Biol.* **1993**, *234*, 779.
28. Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605.
29. Meng, E. C.; Pettersen, E. F.; Couch, G. S.; Huang, C. C.; Ferrin, T. E. *BMC Bioinform.* **2006**, *7*, 339.
30. Yang, Z.; Lasker, K.; Schneidman-Duhovny, D.; Webb, B.; Huang, C. C.; Pettersen, E. F.; Goddard, T. D.; Meng, E. C.; Sali, A.; Ferrin, T. E. *J. Struct. Biol.* **2012**, *179*, 269.
31. Sybylx2.0, Tripos International, 1699 South Hanley Rd., St. Louis, Missouri 63144, USA.