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Design, synthesis, and structure–activity relationships of pyrazole derivatives as potential FabH inhibitors

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ABSTRACT

Fatty acid biosynthesis is essential for bacterial survival. FabH, β-ketoacyl-acyl carrier protein (ACP) synthase III, is a particularly attractive target, since it is central to the initiation of fatty acid biosynthesis and is highly conserved among Gram-positive and -negative bacteria. Fifty-six 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives were synthesized and developed as potent inhibitors of FabH. This inhibitor class demonstrates strong antibacterial activity. *Escherichia coli* FabH inhibitory assay and docking simulation indicated that the compounds 1-(5-(4-fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (**12**) and 1-(5-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (**13**) were potent inhibitors of *E. coli* FabH.

The emergence of bacterial resistance to most of all antibiotics poses a threat to health care, and novel therapeutics are needed. Recently, the research has been focused toward development of new antibacterial agents with novel target so as to prevent this serious medical problem. A promising target is the fatty acid synthase (FAS) pathway in bacteria. Fatty acid biosynthesis (FAB) is an essential metabolic process for prokaryotic organisms and is required for cell viability and growth.[1] β-Ketoacyl-acyl carrier protein (ACP) synthase III, also known as FabH or KAS III, plays an essential and regulatory role in bacterial FAB.[2,3] The enzyme initiates the fatty acid elongation cycles,[4,5] and is involved in the feedback regulation of the biosynthetic pathway via product inhibition.[6] Therefore, it represents a promising target for the design of novel antimicrobial drugs. Because of this, various kinds of compounds were screened by enzymatic assays to generate leads that were co-crystallized with various pathogenic FabH proteins and subsequently optimized using structure guided drug design methods.[7–11]

Many pyrazole derivatives are acknowledged to possess a wide range of bioactivities. The pyrazole motif makes up the core structure of numerous biologically active compounds. Thus, some representatives of this heterocycle exhibit anti-viral/anti-tumor,[12–14] antibacterial,[15–18] antiinflamatory,[19] analgesic,[20] fungistatic,[21] and anti-hyperglycemic activity.[22,23] Much attention was paid to pyrazole as a potential antimicrobial agent after the discovery of the natural pyrazole *C*-glycoside, pyrazofurin which demonstrated a broad spectrum of antimicrobial activity.[24]

However, to our knowledge, few reports have been dedicated to the synthesis and FabH inhibitory activity of 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives. Herein, in continuation to extend our research on antibacterial compounds with FabH inhibitory activity,[25,26] we report in the present work the synthesis and structure–activity relationships of a series of pyrazole derivatives. This combination was suggested in an attempt to investigate the inhibitory activity against *Escherichia coli* FabH. Biological evaluation indicated that compounds **12** and **13** displayed not only significant antibacterial activity against *E. coli* ATCC 35218, but also favorable activity against other five bacterial strains, indicating that they possessing broad-spectrum antibacterial activity. Besides, further *E. coli* FabH inhibitory assay was undertaken and the results suggested that compounds **12** and **13** were potent *E. coli* FabH inhibitors. In addition, docking simulation were performed to position compound **12** into the *E. coli* FabH active site to determine the probable binding conformation and the results confirmed that compound **12** was potential inhibitor of *E. coli* FabH.

Fifty-six 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives were synthesized to evaluate their antibacterial activity and inhibitory activity against *E. coli* FabH. The synthesis of compounds **4–59** followed the general pathway outlined in Scheme 1. They are prepared in two steps. Firstly, the chalcones were obtained by direct condensation between the aromatic aldehydes and the substituted acetophenone, using 20% potassium

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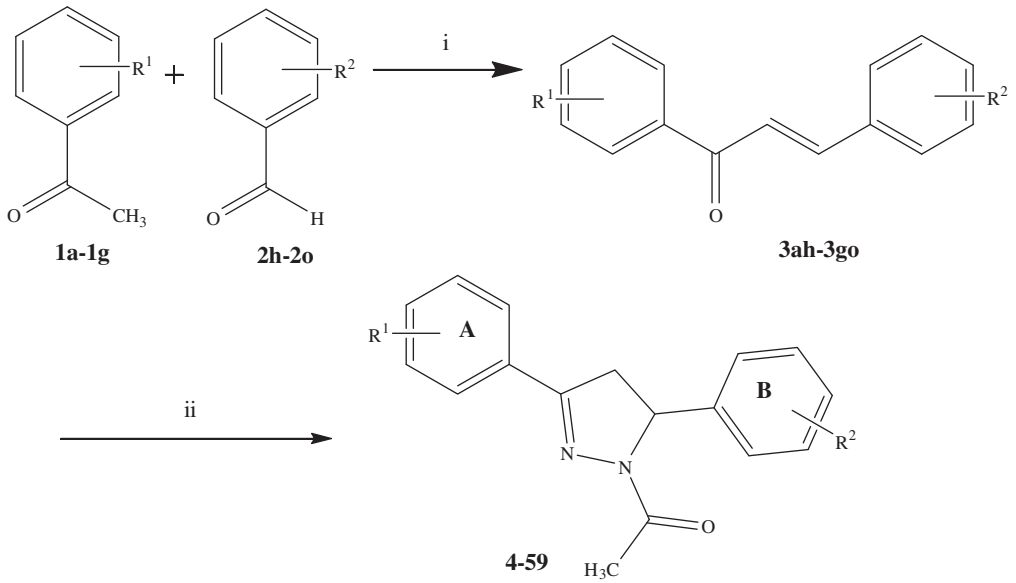

R^1= 4-Cl (**a**), 4-Br (**b**), 4-OCH_3 (**c**), 2-F (**d**), 3,4-2Cl (**e**), 3,4-2CH_3 (**f**), 4-H (**g**);

R^2= 4-F (**h**), 4-Cl (**i**), 4-Br (**j**), 4-CH_3 (**k**), 4-OCH_3 (**l**), 2-Cl (**m**), 4-H (**n**), 3,5-2OCH_3 (**o**).

Scheme 1. Reagents and conditions. (i) NaOH, CH_3CH_2OH, 25 °C, 4–10 h; (ii) $N_2H_4{\cdot}H_2O$, CH_3COOH, 120 °C, reflux, 12 h.

hydroxide as catalyst in ethanol. Secondly, a solution of chalcones (5 mmol) in 30 mL of acetic acid was added dropwise to 0.6 mL of hydrazine hydrate (12.5 mmol) and kept under stirring at 120 °C for 24 h. The mixture was then poured into ice-water, obtaining the 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives pyrazole derivatives **4–59**. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

All the synthesized compounds were screened for antibacterial activity against three Gram positive bacterial strains and three Gram negative bacterial strains by MTT method. The MICs (minimum inhibitory concentrations) of the compounds against six strains are presented in Table 1. The antibacterial activity of reference compounds kanamycin B and penicillin G were also included. Studies were performed by modification of the A ring and B ring of the parent compounds to determine how the substituents of the subunits affected the antibacterial activities. As shown in Table 1, compounds **12** and **13** displayed potent activity with MIC values of 0.39 μg/mL and 0.78 μg/mL against *E. coli* ATCC 35218, which were superior to the positive control kanamycin B. Compound **14** exhibited significant activity with MIC values of 1.562 μg/mL against *E. coli* ATCC 35218, which was comparable to the positive control kanamycin B. Based on the data obtained, we found that compounds bearing a methoxy group at 4-position at A ring (compounds **12**–**19**) showed potent antibacterial activities against *E. coli* ATCC 35218. Among them, a comparison of the substitution on B-ring demonstrated that 4-position-substituted derivatives with halogen atoms have more potent activity against *E. coli* ATCC 35218. Most significantly, the stronger electron-withdrawing substituents the compound contained on B-ring at 4-position, the more potent it showed (compounds **12**, **13**, and **14**), which was illustrated by the potency order F > Cl > Br. Meanwhile, the derivatives which have electron-donating substituents (such as CH_3, OCH_3) on B-ring exhibited less potent activity against *E. coli* ATCC 35218, and their MICs values range from 3.125 μg/mL to 25 μg/mL (compounds **15**, **16**, and **19**).

In addition, compounds **36–43** which bear two chlorine atoms at 3- and 4-position at A ring exhibited significant activity against *Bacillus subtilis* ATCC 6633. For example, Compounds **37** showed potent activity with MIC values of 0.78 μg/mL, which were superior to the positive control penicillin G. Compounds **36** and **38** suggested favorable activity with MIC values of 1.562 μg/mL, which was comparable to the positive control penicillin G. Compounds **39–43** showed moderate activity with MIC values ranging from 3.125 μg/mL to 12.5 μg/mL against *B. subtilis* ATCC 6633. Besides, Compounds **4**, **12**, **20**, and **36** displayed potent activity with MIC values of 1.562 μg/mL against *Streptococcus faecalis* ATCC 9790, which were comparable to the positive control penicillin G.

Then, the *E. coli* FabH inhibitory potency of the selected compounds **12**, **13**, **14**, **15**, **16**, **17**, and **18** was examined and the results are summarized in Table 2. As shown in Table 2, among the tested compounds, compounds **12** and **13** showed potent inhibitory activity with IC_{50} of 4.2 μM and 7.6 μM, respectively. Other tested compounds displayed moderate inhibitory activity with IC_{50} ranging from 10.4 μM to 46.7 μM.

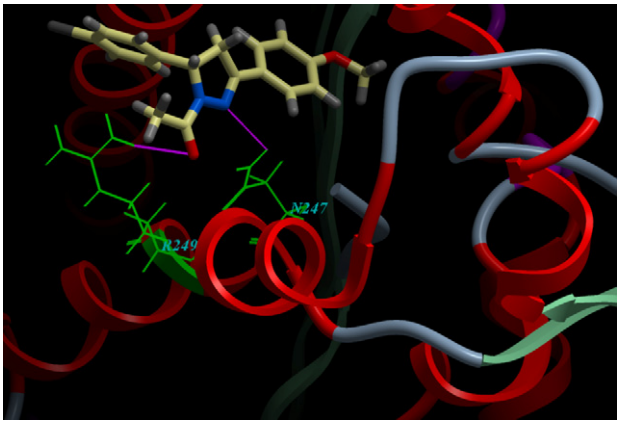

Figure 1. Binding model of compound **12** and *E. coli* FabH. For clarity, only interacting residues are displayed. The H-bond (purple) is displayed as line. Asn247 forms hydrogen bond with the nitrogen atom of the pyrazole ring of compound **12**, meanwhile, Arg249 forms hydrogen bond with the oxygen atom of the carbonyl group of compound **12**.

Table 1
Physical properties and MICs (minimum inhibitory concentrations) (μg/mL) of compounds **4**–**59**

Compounds			Microorganisms					
			Gram positive			Gram negative		
Entry	R_1	R_2	*B. subtilis* ATCC 6633	*S. aureus* ATCC 6538	*S. faecalis* ATCC 9790	*P. aeruginosa* ATCC 13525	*E. coli* ATCC 35218	*E. cloacae* ATCC 13047
4	4-Cl	4-F	12.5	12.5	1.562	12.5	25	25
5	4-Cl	4-Cl	12.5	25	6.25	6.25	12.5	25
6	4-Cl	4-Br	6.25	25	6.25	>50	12.5	>50
7	4-Cl	4-CH_3	6.25	12.5	25	12.5	12.5	12.5
8	4-Cl	4-OCH_3	12.5	>50	25	25	25	>50
9	4-Cl	2-Cl	6.25	25	50	12.5	12.5	12.5
10	4-Cl	4-H	12.5	>50	25	>50	25	12.5
11	4-Cl	3,5-2OCH_3	6.25	6.25	50	25	12.5	>50
12	4-OCH_3	4-F	6.25	6.25	1.562	12.5	0.39	12.5
13	4-OCH_3	4-Cl	6.25	25	6.25	25	0.78	25
14	4-OCH_3	4-Br	12.5	12.5	3.125	12.5	1.562	>50
15	4-OCH_3	4-CH_3	25	6.25	25	12.5	6.25	3.125
16	4-OCH_3	4-OCH_3	6.25	6.25	12.5	>50	3.125	12.5
17	4-OCH_3	2-Cl	25	>50	12.5	>50	6.25	6.25
18	4-OCH_3	4-H	6.25	>50	50	12.5	25	>50
19	4-OCH_3	3,5-2OCH_3	6.25	12.5	50	12.5	12.5	6.25
20	4-Br	4-F	25	1.562	1.562	3.125	12.5	12.5
21	4-Br	4-Cl	25	3.125	6.25	3.125	25	12.5
22	4-Br	4-Br	12.5	3.125	25	6.25	25	25
23	4-Br	4-CH_3	25	6.25	50	1.562	25	6.25
24	4-Br	4-OCH_3	25	12.5	6.25	1.562	50	3.125
25	4-Br	2-Cl	25	25	50	6.25	12.5	3.125
26	4-Br	4-H	50	6.25	25	12.5	12.5	>50
27	4-Br	3,5-2OCH_3	50	6.25	25	3.125	50	12.5
28	2-F	4-F	25	>50	50	12.5	50	>50
29	2-F	4-Cl	12.5	>50	12.5	12.5	25	>50
30	2-F	4-Br	25	25	25	12.5	25	12.5
31	2-F	4-CH_3	25	>50	25	>50	25	12.5
32	2-F	4-OCH_3	6.25	25	12.5	25	12.5	25
33	2-F	2-Cl	6.25	12.5	25	12.5	12.5	12.5
34	2-F	4-H	12.5	25	25	25	>50	>50
35	2-F	3,5-2OCH_3	6.25	25	12.5	12.5	12.5	>50
36	3,4-2Cl	4-F	1.562	12.5	1.562	12.5	12.5	12.5
37	3,4-2Cl	4-Cl	0.78	25	6.25	25	25	>50
38	3,4-2Cl	4-Br	1.562	>50	3.125	25	12.5	12.5
39	3,4-2Cl	4-CH_3	6.25	>50	12.5	25	>50	25
40	3,4-2Cl	4-OCH_3	3.125	25	6.25	12.5	>50	25
41	3,4-2Cl	2-Cl	3.125	25	12.5	12.5	12.5	25
42	3,4-2Cl	4-H	6.25	>50	12.5	25	12.5	25
43	3,4-2Cl	3,5-2OCH_3	12.5	25	50	12.5	>50	12.5
44	3,4-2CH_3	4-F	25	1.562	12.5	6.25	25	12.5
45	3,4-2CH_3	4-Cl	>50	1.562	50	6.25	12.5	6.25
46	3,4-2CH_3	4-Br	25	12.6	50	12.5	25	3.125
47	3,4-2CH_3	4-CH_3	12.5	3.125	12.5	3.125	>50	>50
48	3,4-2CH_3	4-OCH_3	12.5	3.125	12.5	3.125	25	12.5
49	3,4-2CH_3	2-Cl	>50	25	50	6.25	25	>50
50	3,4-2CH_3	4-H	>50	25	25	12.5	>50	>50
51	3,4-2CH_3	3,5-2OCH_3	12.5	>50	12.5	3.125	>50	25
52	4-H	4-F	25	25	50	12.5	25	12.5
53	4-H	4-Cl	>50	12.5	12.5	12.5	25	25
54	4-H	4-Br	25	>50	12.5	25	12.5	12.5
55	4-H	4-CH_3	>50	>50	6.25	12.5	25	25
56	4-H	4-OCH_3	>50	12.5	50	12.5	>50	>50
57	4-H	2-Cl	25	>50	25	25	>50	25
58	4-H	4-H	25	25	12.5	12.5	25	12.5
59	4-H	3,5-2OCH_3	12.5	25	25	25	25	>50
Penicillin G	—	—	1.562	6.25	1.562	6.25	3.125	3.125
Kanamycin B	—	—	0.39	3.125	3.125	3.125	1.562	1.562

Table 2
E. coli FabH inhibitory activity of the selected compounds

Compounds	*E. coli* FabH IC_{50} (μM)	Hemolysis LC_{30}[a] (mg/mL)
12	4.2	>10
13	7.6	>10
14	10.4	>10
15	17.3	>10
16	19.6	>10
17	31.4	>10
18	46.7	>10

[a] Lytic concentration 30%.

Furthermore, molecular docking of compound **12** and *E. coli* FabH were performed on the binding model based on the *E. coli* FabH–CoA complex structure (1HNJ.pdb).[27] The binding model of compounds **12** and *E. coli* FabH are depicted in Figure 1. It can be seen from Figure 1 that, in the binding model of compound **12** and *E. coli* FabH, there are two hydrogen bonds. Asn247 forms hydrogen bond with the nitrogen atom of the pyrazole ring of compound **12**, meanwhile, Arg249 forms hydrogen bond with the oxygen atom of the carbonyl group of compound **12**. These results, along with the data of *E. coli* FabH inhibitory activity assay

indicated that compounds **12** would be potential inhibitors of *E. coli* FabH with potent antibacterial activity.

In conclusion, 56 1-acetyl-3,5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives were synthesized and evaluated for their inhibitory activity against *E. coli* FabH. Data obtained indicated that compounds **12** and **13** displayed potent antibacterial activity against *E. coli* ATCC 35218. Further *E. coli* FabH inhibitory activity assay suggested that they are potent *E. coli* FabH inhibitors. Besides, docking simulation were performed to position compound **12** into the *E. coli* FabH active site to determine the probable binding conformation and the results confirmed that compound **12** was potential inhibitor of *E. coli* FabH.

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References and notes

1. Cronan, J. E., Jr.; Rock, C. O. In *Escherichia coli and Salmonella typhimurium*; Neidhardt, F. C., Ingraham, I. L., Low, K. B., et al., Eds.; Cellular and Molecular Biology; American Society of Microbiology: Washington, DC, 1996; pp 612–638.
2. Khandekar, S. S.; Daines, R. A.; Lonsdale, J. T. *Curr. Protein Pept. Sci.* **2003**, *4*, 21.
3. Heath, R. J.; Rock, C. O. *Nat. Prod. Rep.* **2002**, *19*, 581.
4. Tsay, J. T.; Oh, W.; Larson, T. J.; Jakowski, S.; Rock, C. O. *J. Biol. Chem.* **1992**, *267*, 6807.
5. Clough, R. C.; Matthis, A. L.; Barnum, S. R.; Jaworski, J. G. *J. Biol. Chem.* **1992**, *267*, 20992.
6. Heath, R. J.; Rock, C. O. *J. Biol. Chem.* **1996**, *271*, 1833.
7. Lee, P. J.; Bhonsle, J. B.; Gaona, H. W.; Huddler, D. P.; Heady, T. N.; Kreishman-Deitrick, M.; Bhattacharjee, A.; McCalmont, W. F.; Gerena, L.; Lopez-Sanchez, M.; Roncal, N. E.; Hudson, T. H.; Johnson, J. D.; Prigge, S. T.; Waters, N. C. *J. Med. Chem.* **2009**, *52*, 952.
8. Nie, Z.; Perretta, C.; Lu, J.; Su, Y.; Margosiak, S.; Gajiwala, K. S.; Cortez, J.; Nikulin, V.; Yager, K. M.; Appelt, K.; Chu, S. *J. Med. Chem.* **2005**, *48*, 1596.
9. Daines, R. A.; Pendrak, I.; Sham, K.; Aller, G. S. V.; Konstantinidis, A. K.; Lonsdale, J. T.; Janson, C. A.; Qiu, X.; Brandt, M.; Khandekar, S. S.; Silverman, C.; Head, M. S. *J. Med. Chem.* **2003**, *46*, 5.
10. Asheka, A.; Cho, S. J. *Bioorg. Med. Chem.* **2006**, *14*, 1474.
11. Singh, S.; Soni, L. K.; Gupta, M. K.; Prabhakar, Y. S.; Kaskhedikar, S. G. *Eur. J. Med. Chem.* **2008**, *43*, 1071.
12. Manfredini, S.; Bazzanini, R.; Baraldi, P. G.; Guarneri, M.; Simoni, D.; Marongiu, M. E.; Pani, A.; Tramontano, E.; La Colla, P. *J. Med. Chem.* **1992**, *35*, 917.
13. Manfredini, S.; Bazzanini, R.; Baraldi, P. G.; Bonora, M.; Marangoni, M.; Simoni, D.; Pani, A.; Scintu, F.; Pinna, E. *Anti-Cancer Drug Des.* **1996**, *11*, 193.
14. Park, H.-A.; Lee, K.; Park, S.-J.; Ahn, B.; Lee, J.-C.; Cho, H. Y.; Lee, K.-I. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3307.
15. Tanitame, A.; Oyamada, Y.; Ofuji, K.; Fujimoto, M.; Iwai, N.; Hiyama, Y.; Suzuki, K.; Ito, H.; Wachi, M.; Yamagishi, J. *J. Med. Chem.* **2004**, *47*, 3693.
16. Küçükgüzel, S. G.; Rollas, S.; Erdeniz, H.; Kiraz, M.; Ekinci, A. C.; Vidin, A. *Eur. J. Med. Chem.* **2000**, *35*, 761.
17. Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem.* **2000**, *43*, 953.
18. Bekhit, A. A.; Fahmy, H. T. Y.; Rostom, S. A. F.; Baraka, A. M. *Eur. J. Med. Chem.* **2003**, *38*, 27.
19. Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347.
20. Menozzi, G.; Mosti, L.; Fossa, P.; Mattioli, F.; Ghia, M. *J. Heterocycl. Chem.* **1997**, *34*, 963.
21. Sridhar, R.; Perumal, P. T.; Etti, S.; Shanmugam, G.; Ponnuswamy, M. N.; Prabavathy, V. R.; Mathivanan, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6035.
22. Kees, K. L.; Fitzgerald, J. J.; Steiner, K. E.; Mattes, J. F.; Mihan, B.; Tosi, T.; Mondoro, D.; McCaleb, M. L. *J. Med. Chem.* **1996**, *39*, 3920.
23. Bebernitz, G. R.; Argentieri, G.; Battle, B.; Brennan, C.; Balkan, B.; Burkey, B. F.; Eckhardt, M.; Gao, J.; Kapa, P.; Strohschein, R. J.; Schuster, H. F.; Wilson, M.; Xu, D. D. *J. Med. Chem.* **2001**, *44*, 2601.
24. Comber, R. N.; Gray, R. J.; Secrist, J. A. *Carbohydr. Res.* **1992**, *216*, 441.
25. Li, H.-Q.; Shi, L.; Li, Q.-S.; Liu, P.-G.; Luo, Y.; Zhao, J.; Zhu, H.-L. *Bioorg. Med. Chem.* **2009**, *17*, 6264.
26. Lv, P.-C.; Wang, K.-R.; Yang, Y.; Mao, W.-J.; Chen, J.; Xiong, J.; Zhu, H.-L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6750.
27. Qiu, X.; Janson, C. A.; Smith, W. W.; Head, M.; Lonsdale, J.; Konstantinidis, A. K. *J. Mol. Biol.* **2001**, *307*, 341.