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Synthesis and Bioactivity of Pyrazole and Triazole Derivatives as Potential PDE4 Inhibitors

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Abstract: A series of pyrazole and triazole derivatives containing 5-phenyl-2-furan functionality were designed and synthesized as phosphodiesterase type 4 (PDE4) inhibitors. The bioassay results showed that title compounds exhibited considerable inhibitory activity against PDE4B and blockade of LPS-induced TNFα release. Meanwhile, the activity of compounds containing 1,2,4-triazole (series **II**) was higher than that of pyrazole-attached derivatives (series **I**). The primary structure–activity relationship study and docking results showed that the 1,2,4-triazole moiety of compound **IIk** played a key role to form integral hydrogen bonds and π-π stacking interaction with PDE4B protein while the rest part of the molecule extended into the catalytic domain to block the access of cAMP and formed the foundation for inhibition of PDE4. Compound **IIk** would be great promise as a hit compound for further study based on the preliminary structure-activity relationship and molecular modeling studies.

Key words: synthesis; 5-phenyl-2-furan; pyrazole and triazole derivatives; PDE4 inhibitor; molecular simulation

Phosphodiesterases (PDEs) play a key role in catalyzing the hydrolysis of the secondary signal messengers, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), which are able to regulate the function of airway smooth muscle, inflammatory cells, and immune cells.[1-3] The PDE4, as one of the 11-membered PDEs, specifically targets the second messenger cAMP and is expressed predominantly in inflammatory and immune cells including eosinophils, lymphocytes, macrophages, and neutrophils.[4,5] When PDE4 is inhibited, the resultant elevation of intracellular cAMP levels leads to an activation of specific protein phosphorylation cascades, which elicit a variety of functional responses in the inflammatory cells such as suppression of TNFα production.[6-8] Therefore, the development of PDE4 inhibitors as anti-inflammatory drugs for the treatment of asthma and chronic obstructive pulmonary disease (COPD) has made a long standing research effort.[9-14]

PDE4 inhibitors have been extensively studied as anti-inflammatory drugs since the discovery of rolipram (**Fig.1**) and piclamilast (**Fig.1**) in the 1990s. A detailed structure-activity relationship (SAR) study revealed that the 4-(3,4-dialkoxyphenyl) moiety of catechol (**Fig.1**) was important for PDE4 inhibition where two alkoxy groups occupied each different lipophilic pocket and the catechol ether oxygens constructed H-bond to the purine-selective glutamine residue, which is surrounded by the P-clamp.[15,16] Further structural modification suggested that the 8-methoxyquinoline-5- carboxamides (such as SCH 365351) showed excellent PDE4 inhibitory activity. Modeling studies on the 8-methoxyquinoline-5-carboxamide related compounds demonstrated that the quinoline moiety binds to the adenosine recognition site, while the amide portion served as a linker to anchor a group containing a polar atom which provided favorable interactions with the metal ion binding site of PDE4.[17-19] Five-membered heterocyclic

oxazole moiety was explored as possible linker to replace the amide portion, whcih was found to be a highly versatile linker and the derivatives exhibited significantly potent PDE4 inhibitory activity.[20-22] In this study, the oxazole was replaced by furan ring, and pyrazole and triazole were introduced to form a new combination as PDE4-inhibitor pharmacophores (**Fig.1**).

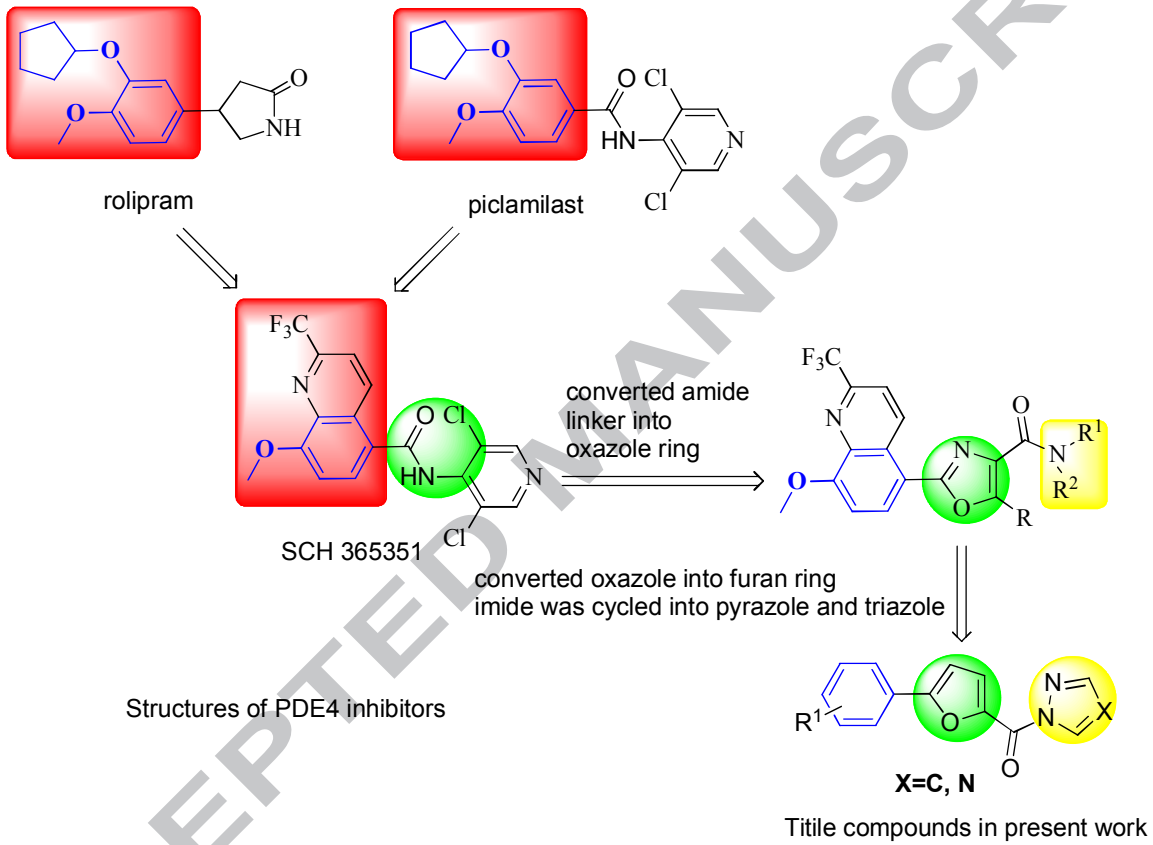
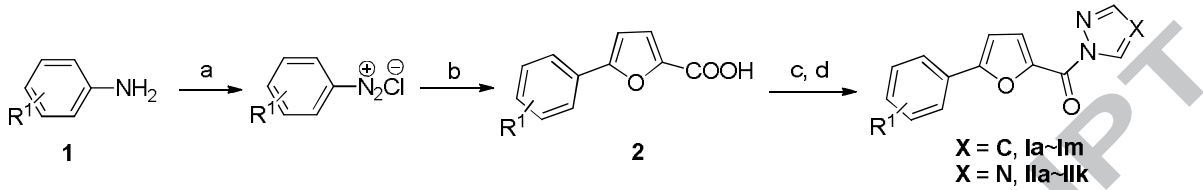


Figure 1. The designed strategy for the title compounds.

The synthetic route of title compounds **I** and **II** was shown in **Scheme 1**. The key intermediate **2** was synthesized from substituted aniline by Meerwein arylation reaction according to the reported procedure.[23,24] A mixture of 5-substituted phenyl-2-furancarboxylic acid **2** and thionyl chloride was refluxed in anhydrous toluene for 3 h to afford the 5-phenyl-2-furancarbonyl chloride, which was added into pyrazole or 1, 2,

4-triazole in anhydrous dichloromethane to react and obtain the title compounds **I** and **II** as solid (see the supplementary data for the details).



1 2

R^1= **Ia:** 4-Cl; **Ib:** 2-NO_2; **Ic:** 2-Cl; **Id:** 3-Cl; **Ie:** 3-F; **If:** 4-F; **Ig:** 2.4-di-F; **Ih:** 2.6-di-F; **Ii:** H; **Ij:** 4-CH_3; **Ik:** 4-OCH_3; **Il:** 3-NO_2; **Im:** 2-F; **IIa:** 4-Cl; **IIb:** 2-NO_2; **IIc:** 2-Cl; **IId:** 3-Cl; **IIe:** 3-F; **IIf:** 4-F; **IIg:** 2.4-di-F; **IIh:** 2.6-di-F; **IIi:** H; **IIj:** 4-CH_3; **IIk:** 4-OCH_3

Scheme 1: The synthetic route of the title compounds **I** and **II.** Reagents and conditions: (a) $NaNO_2$, hydrochloric acid, 0-5 ^oC, 3 h; (b) furoic acid, $CuCl_2$ (cat.), acetone-H_2O, r.t., 5 h; (40-65%, two steps) (c) $SOCl_2$, anhydrous toluene, reflux, 3 h; (d) pyrazole or 1,2,4-triazole, anhydrous dichloromethane, reflux, 4 h (75-91%, two steps).

In vitro data for the inhibition of PDE4B and blockade of LPS-induced TNFα release were listed in **Table 1**. Rolipram was chosen as the positive control. Generally, the activity of title compounds containing 1,2,4-triazole (series **II**) was better than that of compounds containing pyrazole (series **I**), except compounds **e** and **h**. Among the title compounds, the IC_{50} value of **IIk** was 1.2±0.1 μM and 9.8±0.7 μM respectively against PDE4B and TNFα, which showed comparable or better activity than rolipram (1.5±0.1 μM and 12.5±1.1 μM) (**Table 1**). Compound **Ik** displayed comparable IC_{50} values (2.8±0.3 μM agaisnt PDE4B and 22.7±2.4 μM against TNFα) to that of rolipram. In addition, compounds **Ia** and **IIa, Ig** and **IIg** also showed favorable activity. A primary structure–activity relationship study showed that the position of the substituted group played a key role in the bioactivity. Activity with respect to substitution at the benzene

ring follows the trend: 4->2,4->3->2,6->2-. The compounds **Ii** and **IIi** without any substituted group showed the poorest activity.

Table 1 Impact on enzymatic potency (PDE4B) and inhibition of TNFα release from human blood mononuclear cells stimulated with lipopolysaccharide [a]

Compd.	X	R^1	PDE4B IC_{50}(μM)	TNFα IC_{50}(μM)	Compd.	X	R^1	PDE4B IC_{50}(μM)	TNFα IC_{50}(μM)
Ia	C	4-Cl	5.6±0.3	36.7±3.1	**IIa**	N	4-Cl	3.9±0.5	28.4±1.6
Ib	C	2-NO_2	75.8±3.1	256.1±9.8	**IIb**	N	2-NO_2	56.8±2.7	194.5±8.1
Ic	C	2-Cl	68.1±2.9	172.4±7.9	**IIc**	N	2-Cl	47.8±1.9	159.7±7.4
Id	C	3-Cl	29.4±1.8	78.1±3.4	**IId**	N	3-Cl	20.7±1.2	64.1±3.1
Ie	C	3-F	20.1±1.6	80.4±3.2	**IIe**	N	3-F	27.9±1.9	81.5±3.2
If	C	4-F	10.2±0.9	53.8±2.8	**IIf**	N	4-F	8.7±0.6	21.4±1.5
Ig	C	2.4-di-F	6.4±0.7	24.1±1.3	**IIg**	N	2.4-di-F	4.8±0.3	15.7±0.9
Ih	C	2.6-di-F	21.7±2.5	120.4±6.2	**IIh**	N	2.6-di-F	36.7±2.1	152.4±8.1
Ii	C	H	78.1±3.2	217.6±8.6	**IIi**	N	H	65.7±3.1	189.7±9.5
Ij	C	4-CH_3	18.7±1.8	89.5±5.8	**IIj**	N	4-CH_3	5.6±0.3	28.9±2.1
Ik	C	4-OCH_3	2.8±0.3	22.7±2.4	**IIk**	N	4-OCH_3	1.2±0.1	9.8±0.7
Il	C	3-NO_2	16.8±1.7	57.2±3.7	rolipram			1.5 ±0.1	12.5±1.1
Im	C	2-F	65.1±2.8	168.9±8.7					

[a] Results are the average of at least three assays.

Considering the inhibitory activity of title compounds, it was of interest to explore the binding to the PDE4 structure. The bioassay results demonstrated that compounds containing *para*-methoxy group (**Ik** and **IIk**) showed the best activity among all the title compounds. Therefore docking simulation of compound **Ik** and **IIk** at PDE4B (PDB ID: 1XMY) was conducted using Surflex-Dock in Sybyl 8.0 (see the supplementary data for the method),[16,25] and the docking contour maps were shown in **Fig. 3**. The docking orientation demonstrated that the five-membered heterocyclic moiety as the pivotal pharmacophore formed integral hydrogen bonds with the conserved glutamine residue

(Gln443) (**Fig**. **3**) and the heterocyclic ring was evidently positioned between the phenylalanine (Phe446) and isoleucine (Ile410) (**Fig**. **3C** and **3E**), which formed the cavity accommodating the hydrophobic moiety of compounds **Ik** and **IIk**. Compared with the pyrazole derivative **Ik**, the 1,2,4-triazole moiety of **IIk** formed obvious π-π stacking interaction with benzene ring (3.26 Å, **Fig**. **3E** and 3**F**) in the phenylalanine (Phe446), which could enhance the binding affinity with the enzyme. That could be the reason why the activity of most title compounds containing 1,2,4-triazole (series **II**) was better than that of compounds containing pyrazole. The remainder of the molecule was displayed to extend into the catalytic domain in close to both the Zn^{2+} and Mg^{2+} cations (**Fig**. **3D** and 3**F**), which played important roles in the catalytic mechanism of cAMP hydrolysis. The *para*-methoxy group formed coordinate bond with the Zn^{2+} (2.23 Å, **Ik**, **Fig**. **3C** and **3D**) and Mg^{2+} (1.92 Å, **IIk**, **Fig**. **3E** and **3F**) cations. Such orientation and interactions would block the access of cAMP to the catalytic domain and formed the foundation for inhibition of PDE4.

In summary, the design and synthesis of pyrazole and triazole derivatives containing 5-phenyl-2-furan moiety were reported in this letter. Their bioactivity against phosphodiesterase type 4 and TNF*α* were evaluated. Compound **IIk** showed the best inhibitory activity against PDE4B and blockade of LPS-induced TNF*α* release among all the title compounds. The bioactivity showed that compounds containing 1,2,4-triazole (series **II**) was better than that of compounds containing pyrazole (series **I**). The primary structure–activity relationship study and docking results suggested that **IIk** interacted well with PDE4B protein where the 1,2,4-triazole played a key role in formation of integral hydrogen bond and π-π stacking interaction while the rest part of the molecule extended into the catalytic domain to block the access of cAMP, which formed the

foundation for inhibition of PDE4. The formation of hydrogen bonds, π-π stacking interactions and the hydrophobic interactions in the ligand-receptor complex were vital for the binding affinity. Such efforts were helpful to develop additional small molecules with enhanced activity as novel and effective PDE4 inhibitors.

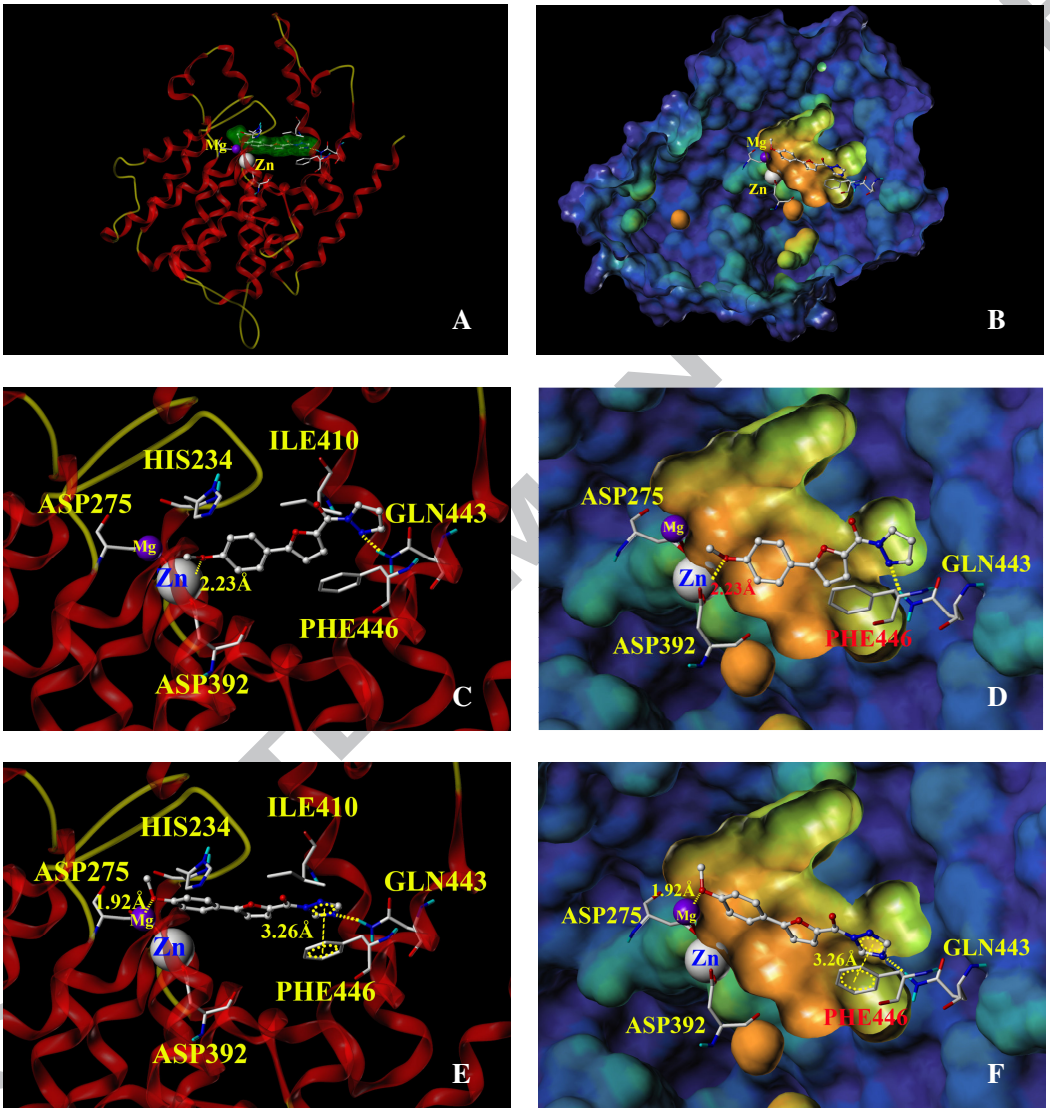


Figure 3. Model of PDE4 and docking of compounds **Ik** and **IIk**. (A, B) The entire PDE4B structure (N-terminal domain, a catalytic domain and a C-terminal domain) bound to **IIk**. (C, D) The catalytic domain bound to **Ik**. (E, F) The catalytic domain bound to **IIk**.

Acknowledgements

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References and notes

1. Conti, M.; Richter, W.; Mehats, C.; Livera, G.; Park J. Y.; Jin, C. *J. Biol. Chem*. **2003**, *278*, 5493.
2. Guo, L.; Luo, L. F.; Ju, R.; Chen, C.; Zhu, L.; Li, J.; Yu, X. L.; Ye, C. Y.; Zhang, D. G. *Eur. J. Pharmacol.* **2015**, *746*, 14.
3. Joergensen, C.; Yasmeen, S.; Iversen, H. K.; Kruuse, C. *J Neurol. Sci.* **2015**, *359*, 266.
4. Beavo, J. A.; Reifsnyder, D. H. *Trends Pharmacol. Sci*. **1990**, *11*, 150.
5. Tasken, K.; Aandahl, E. M. *Physiol. Rev*. **2004**, *84*, 137.
6. Caron, S.; Vazquez, E. *Org. Process Res. Dev*. **2001**, *5*, 587.
7. Kadam, R. U.; Garg, D.; Chavan, A.; Roy, N. *J. Chem. Inf. Model.* **2007**, *47*, 1188.
8. Felding, J.; Soerensen, M. D.; Poulsen, T. D.; Larsen, J.; Andersson, C.; Refer, P.; Engell, K.; Ladefoged, L. G.; Thormann, T.; Vinggaard, A. M.; Hegardt, P.; Soehoel, A.; Nielsen, S. F. *J. Med. Chem*. **2014**, *57*, 5893.
9. Le Roux, J.; Leriche, C.; Chamiot-Clerc, P.; Feutrill, J.; Halley, F.; Papin, D.;

Derimay, N.; Mugler, C.; Grepin, C.; Schio, L. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 454.

10. Huang, L.; Shan, W. J.; Zhou, Q.; Xie, J. X.; Lai, K. F.; Li, X. S. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 249.
11. Boland, S.; Alen, J.; Bourin, A.; Castermans, K.; Boumans, N.; Panitti, L.; Vanormelingen, J.; Leysen, D.; Defert, O. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4594.
12. Gewald, R.; Grunwald, C.; Egerland, U. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4308.
13. Gorja, D. R.; Shiva Kumar, K.; Kandale, A.; Meda, C. L. T.; Parsa, K. V. L.; Mukkanti, K.; Pal, M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2480.
14. Bihel, F. J. J.; Justiniano, H.; Schmitt, M.; Hellal, M.; Ibrahim, M. A.; Lugnier, C.; Bourguignon, J. J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6567.
15. Houslay, M. D.; Schafer, P.; Zhang, K. Y. J. *Drug. Discov. Today* **2005**, *10*, 1503.
16. Song, G. P.; Zhao, D. S.; Hu, D. K.; Li, Y. S.; Jin, H. W.; Cui, Z. N. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4610.
17. Buckley, G. M.; Cooper, N.; Dyke, H. J.; Galleway, F.; Gowers, L.; Haughan, A. F.; Kendall, H. J.; Lowe, C.; Maxey, R.; Montana, J. G.; Naylor, R.; Oxford, J.; Peake, J. C.; Picken, C. L.; Runcie, K. A.; Sabin, V.; Sharpe, A.; Warneck, J. B. H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1613.
18. Billah, M.; Buckley, G. M.; Cooper, N.; Dyke, H. J.; Egan, R.; Ganguly, A.; Gowers, L.; Haughan, A. F.; Kendall, H. J.; Lowe, C.; Minnicozzi, M.; Montana, J. G.; Oxford, J.; Peake, J. C.; Picken, C. L.; Piwinski, J. J.; Naylor, R.; Sabin, V.; Shih, N.-Y.; Warneck, J. B. H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1617.
19. Billah, M.; Cooper, N.; Cuss, F.; Davenport, R. J.; Dyke, H. J.; Egan, R.; Ganguly, A.;

198 Gowers, L.; Hannah, D. R.; Haughan, A. F.; Kendall, H. J.; Lowe, C.; Minnicozzi,
199 M.; Montana, J. G.; Naylor, R.; Oxford, J.; Peake, J. C.; Piwinski, J. J.; Runcie, K. A.;
200 Sabin, V.; Sharpe, A.; Shih, N.-Y.; Warneck, J. B. H. *Bioorg. Med. Chem. Lett*. **2002**,
201 *12*, 1621.
202 20. Kuang, R.; Shue, H.-J.; Blythin, D. J.; Shih, N.-Y.; Gu, D.; Chen, X.; Schwerdt, J.;
203 Lin, L.; Ting, P. C.; Zhu, X.; Aslanian, R.; Xiao, L.; Prelusky, D.; Wu, P.; Zhang, J.;
204 Zhang, X.; Celly, C. S.; Minnicozzi, M.; Billah, M.; Wang, P. *Bioorg. Med. Chem.*
205 *Lett.* **2007**, *17*, 5150.
206 21. Kuang, R.; Shue, H.-J.; Xiao, L.; Blythin, D. J.; Shih, N.-Y.; Chen, X.; Gu, D.;
207 Schwerdt, J.; Ling, L.; Ting, P. C.; Cao, J.; Aslanian, R.; Piwinski, J. J.; Prelusky, D.;
208 Wu, P.; Zhang, J.; Zhang, X.; Celly, C. S.; Billah, M.; Wang, P. *Bioorg. Med. Chem.*
209 *Lett.* **2012**, *22*, 2594.
210 22. Ting, P. C.; Lee, J. F.; Kuang, R.; Cao, J.; Gu, D.; Huang, Y.; Liu, Z.; Aslanian, R. G.;
211 Feng, K.-I.; Prelusky, D.; Lamca, J.; House, A.; Phillips, J. E.; Wang, P.; Wu, P.;
212 Lundell, D.; Chapman, R. W.; Celly, C. S. *Bioorg. Med. Chem. Lett*. **2013**, *23*, 5528.
213 23. Cui, Z. N.; Li, X.; Tian, F.; Yan, X. J. *Int. J. Mol. Sci.* **2014**, *15*, 8941.
214 24. Cui, Z. N.; Li, Y.; Ling, Y.; Huang, J.; Cui, J. R.; Wang, R.; Yang, X L. *Eur. J. Med.*
215 *Chem.* **2010**, *45*, 5576.
216 25. Song, G. P.; Li, S. M.; Si, H. Z.; Li, Y. B.; Li, Y. S.; Fan, J. H; Liang, Q. Q.; He, H. B.;
217 Ye, H. M.; Cui, Z. N. *RSC Adv.* **2015**, *5*, 36092.
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Graphical Abstract

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