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Development of novel proteasome inhibitors based on phthalazinone scaffold

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ABSTRACT

In this study we designed a series of proteasome inhibitors using pyridazinone as initial scaffold, and extended the structure with rational design by computer aided drug design (CADD). Two different synthetic routes were explored and the biological evaluation of the phthalazinone derivatives was investigated. Most importantly, electron positive triphenylphosphine group was first introduced in the structure of proteasome inhibitors and potent inhibition was achieved. As **6c** was the most potent inhibitor of proteasome, we examined the structure–activity relationship (SAR) of **6c** analogs.

The ubiquitin proteasome pathway (UPP) is essential for intracellular protein degradation in eukaryotic cells and is involved in many vital cellular processes such as signal transduction, apoptosis, cell cycling and antigen processing for appropriate immune responses.[1] The 26S proteasome is known as a large multicatalytic protein complex, consisting of two 19S regulatory particles and one 20S catalytic core. The active sites of the 20S proteasome are located on the β_1, β_2 and β_5 subunits with caspase-like (C-L), trypsin-like (T-L), and chymotrypsin-like (ChT-L) activities, respectively. The proteasome is a validated drug target for treatment of cancer, and three proteasome inhibitors bortezomib, carfilzomib, and marizomib have recently been approved by FDA for the treatment of multiple myeloma. Besides, several second-generation proteasome inhibitors are currently in clinical trials.[2,3]

In terms of the molecular structure or scaffold, current proteasome inhibitors are mainly based on peptides and peptidomimetics, which mimic the substrates binding to threonine of β_5 subunit. For instance, we previously reported a series of furan-based proteasome β_5 subunit inhibitors, which are simply di- or tri-peptide derivatives.[4,5] However, many novel proteasome inhibitors have been developed in the past two decades and various small-molecule proteasome inhibitors with different scaffolds have been investigated.[6–12]

Pyridazinone has been proved as a superior pharmacophore for building a broad range of chemicals with versatile functions.[13–29] When the C4 and C5 of pyridazinone are conjugated with a benzene ring, the π conjugation is extended and the new structure is formed as phthalazinone. Phthalazinone is another important pharmacophore contained in several drugs including Olaparib (Fig. 1a), the first poly ADP-ribose polymerase (PARP) inhibitor approved to treat advanced ovarian cancer in women with defective BRCA genes.[30] However, neither pyridazinone, nor phthalazinone has ever been reported in designing proteasome inhibitors.

Our group is devoted to discover proteasome inhibitors with novel scaffolds. In this study, we designed a series of proteasome inhibitors using pyridazinone as initial scaffold and extended the structure through rational design. Two different synthetic routes were explored and the biological evaluation of the phthalazinone derivatives was investigated. Most importantly, positively charged triphenylphosphine group was first introduced into a proteasome inhibitor and potent activity has been achieved.

Based on our previous results, we proposed that three important positions of the pyridazinone scaffold could be modified and the stepwise extension strategy was shown in Figure 1b. In order to occupy the small S2 pocket of the proteasome β_5 site, we introduced a benzene ring conjugated to the C4 and C5 positions of pyridazinone yielding phthalazinone. Then we introduced a *N*,*N*-diethylaniline group targeting S1 pocket, and a bromobutyl group extending to S3 pocket. Thus the compound **5a** was first designed and the corresponding docking model showed that **5a** was able to fully occupy all three pockets. Unfortunately, compound **5a** did not show any observable proteasome inhibition (Table 1). We speculated that perhaps the S3 pocket may have low affinity to

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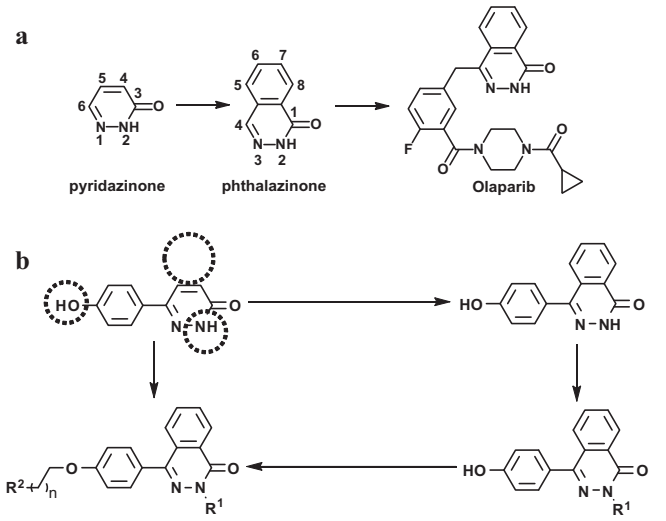


Figure 1. (a) Phthalazinone structure in drug. (b) The design of phthalazinone derivatives by stepwise extension of the pyridazinone scaffold.

the bromobutyl chain. Thus, we considered to substitute it with triphenylphosphine group, which is much more bulky, to bind to the wider S3 pocket. As we expected, the biochemical evaluation result showed that the new compound **6a** exhibited significant enhancement of proteasome inhibition with a single modification (Fig. 2).

To further optimize our design, we synthesized a series phthalazinone **6a** derivatives, and evaluated their proteasome inhibition activity. As shown in Figure 3, we designed **7a**, **7b** and **7c** by eliminating the extended moieties of phthalazinone scaffold. As we expected, when the phthalazinone scaffold was replaced by phenyl or pyridazinone, no activity has been observed, which proved the phthalazinone structure is irreplaceable. Moreover, **7c** showed no activity that confirmed the necessary modification of phenol group by alkyl chain. Furthermore, we investigated the substituted position and length of alkyl chain. When the alkyl was linked to the *meta*-phenol of **7d**, the activity diminished due to the altered trend of alkyl chain toward the S3 pocket. In the case of **7e**, the length of alkyl chain was prolonged while the activity maintained.

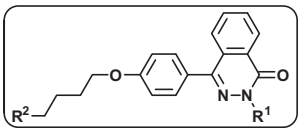
With the results above, we summarized the first round SAR information. It is confirmed that both the phthalazinone and positively charged triphenylphosphine group are irreplaceable. The substituted position of alkyl on the phenol group is also crucial for the proteasome inhibition activity but the length is not important.

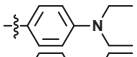
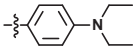
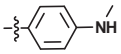
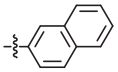
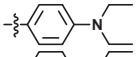
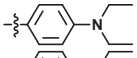
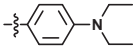
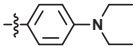
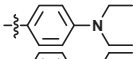
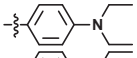
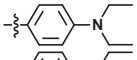
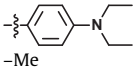
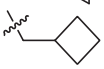
In the second round optimization, a series of phthalazinone derivatives were designed and synthesized with some common features including (1) retention of the phthalazinone and triphenylphosphine groups; (2) modification of 2-NH of phthalazinone by various aromatic and aliphatic groups; (3) fixation of –OH on the *para*-position of the phenyl group; (4) installation of the alkyl on the –OH group is *n*-butyl.

Depending on the different N-substitution of the phthalazinone, two synthetic routes were carried out.[33–37] In one case, the aromatic group was introduced to the 2-NH of phthalazinone by copper(I) catalyzed C–N bond coupling reaction. As shown in Scheme 1, compound **6** can be synthesized efficiently by 5 steps. The starting material 2,3-dihydrophthalazine-1,4-dione **1** was refluxed with $POBr_3$ for 24 h. After cooling, the white precipitation can be filtered and subsequently refluxed in acetic acid for 2 h. After these two steps, a white product 4-bromophthalazin-1(2*H*)-one **2** can be filtered out for next step without purification. Suzuki coupling reaction was employed to prepare **3** with 4-hydroxyphenylboronicacid. Compound **4** can be obtained by copper(I) catalyzed C-N bond coupling reaction between **3** and various aromatic halides. Next, compound **5** was easily obtained by reflux of **4** and 1,4-dibromobutane in acetonitrile. Finally, **6** was prepared by nucleophilic substitution of **5** by different nucleophiles such as triphenylphosphine. The total yields were ranging from 18% to 30%.

In another case, different aliphatic groups were introduced to the 2-NH of phthalazinone. Thus the synthetic route was altered.

Table 1
Structure and proteasome ChT-L inhibitory activity by enzyme assays[a]



Compd	R^1	R^2	Inhib. [%][b]	IC_{50} [μM]
5a		–Br	NI[c]	ND[d]
6a		$-PPh_3^+$	99.1 ± 0.1	10.25 ± 0.45
6b		$-PPh_3^+$	93.2 ± 0.3	17.63 ± 0.56
6c		$-PPh_3^+$	99.5 ± 0.6	9.88 ± 0.21
6d		$-PPh_3^+$	94.3 ± 2.0	14.23 ± 0.15
6e		$-PPh_3^+$	98.7 ± 0.2	13.76 ± 0.22
6f		$-P(Cy)_3^+$	98.5 ± 1.6	13.34 ± 0.08
6g			8.6 ± 0.4	ND
6h			5.8 ± 0.6	ND
6i			10.3 ± 0.9	ND
6j		$-PPh_2$	19.6 ± 2.1	ND
6k			5.1 ± 2.0	ND
6l			8.0 ± 1.3	ND
6m			13.2 ± 2.6	ND
8a	–Me	$-PPh_3^+$	NI	ND
8b	–Et	$-PPh_3^+$	NI	ND
8c	*n*-Propyl	$-PPh_3^+$	12.1 ± 1.4	ND
8d	*n*-Butyl	$-PPh_3^+$	35.8 ± 1.1	ND
8e		$-PPh_3^+$	52.4 ± 0.4	26.05 ± 0.11
8f		$-PPh_3^+$	48.2 ± 2.8	23.05 ± 0.27
8g		$-PPh_3^+$	70.5 ± 0.7	21.54 ± 0.09
8h		$-PPh_3^+$	84.9 ± 0.5	16.14 ± 0.29
8i		$-PPh_3^+$	80.9 ± 2.4	11.86 ± 0.17
MG-132[e]	—	—		37.0 ± 3.2 nM

[a] Values represent the mean ± SD of three independent experiments, each based on four biological replicates.
[b] Percent inhibition at 25 μg mL^{-1}.
[c] NI: no inhibition.
[d] ND: not determined.
[e] *N*-(Benzyloxycarbonyl)-leucinyl-leucinyl-leucinal: a potent proteasome inhibitor, used as positive control.

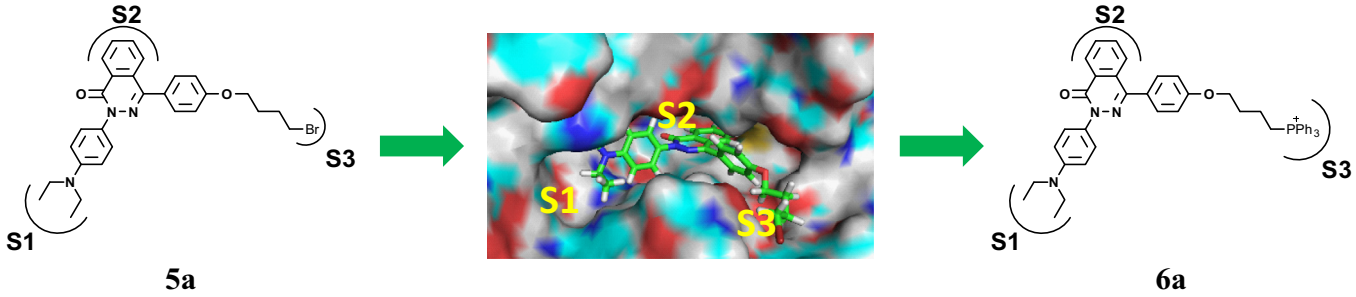


Figure 2. Docking of compound **5a** in proteasome β_5 site and the further optimization of the terminal group. Gold 3.0 was used to dock the inhibitors into the β_5 subunit of the 20S yeast proteasome, the structure of which was obtained from the RCSB Protein Data Bank (PDB ID: 3SHJ).[9] The structure of the human 20S proteasome is quite similar to that of the yeast 20S proteasome, and the chymotrypsin active site is highly conserved.[31] Before docking, the protein and ligands were prepared in Discovery Studio 2.5.[32]

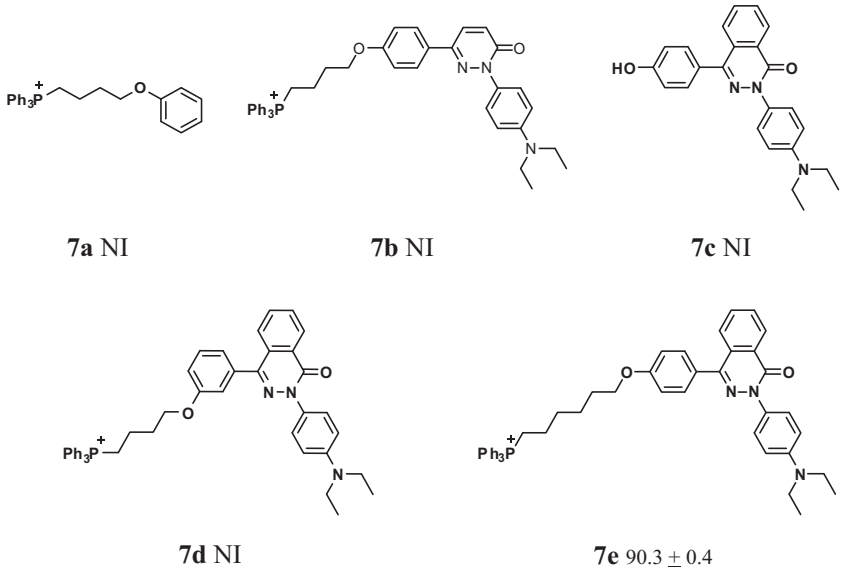
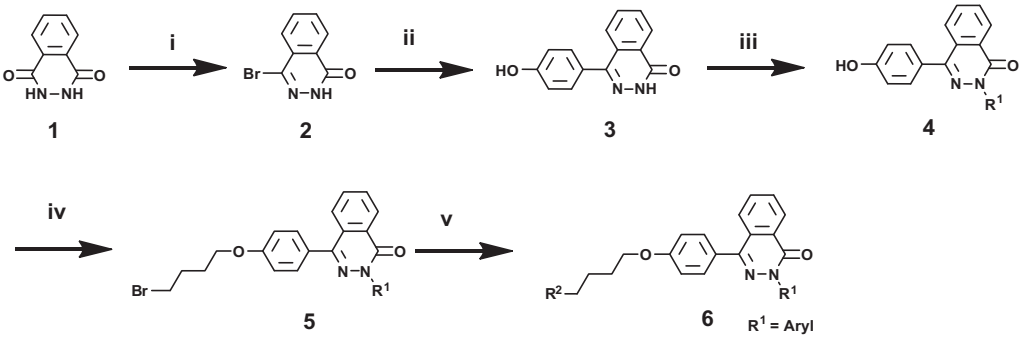


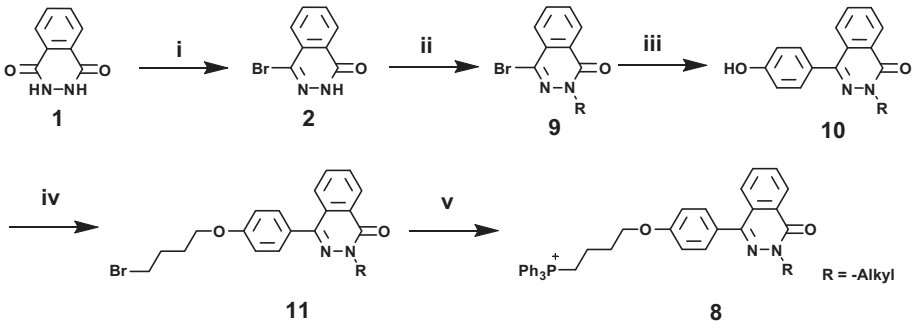
Figure 3. Structures and proteasome ChT-L inhibitory activity of compounds **7a–e**. Values represent the mean ± SD of three independent experiments, each based on four biological replicates. Percent inhibition at 25 μg mL^{-1}. NI: no inhibition. *N*-(Benzyloxycarbonyl)-leucinyl-leucinyl-leucinal: a potent proteasome inhibitor, used as positive control.



Scheme 1. Synthesis of compound **6**. *Reagents and conditions:* (i) step 1: 1,2-dichloroethane, $POBr_3$, reflux for 24 h, cooling and filtration; step 2: acetic acid, reflux for 2 h, cooling and filtration; (ii) 4-hydroxyphenylboronicacid, $Pd(PPh_3)_4$, Na_2CO_3, DMF, H_2O, 120 °C, 48 h; (iii) 4-bromo-*N*,*N*-diethylaniline, CuI, Cs_2CO_3, DMF, 120 °C, 36 h; (iv) 1,4-dibromobutane, K_2CO_3, CH_3CN, reflux for 18 h; (v) R (R = PPh_3 or other nucleophiles), CH_3CN, reflux for 24 h.

As outlined in Scheme 2, the final products can be synthesized in 5 steps with total yields from 20% to 37%. Compound **2** was prepared in the similar procedure as depicted in Scheme 1. In order to prepare **9**, compound **2** was mixed with various alkyl halides and sodium hydride in DMF, the reaction was accomplished under room temperature for 24 h. Then Suzuki coupling reaction was utilized to construct **10**. Finally, **8** was obtained via the same procedure as described in Scheme 1.

After the second round synthesis, totally 23 phthalazinone derivatives were obtained for biochemical evaluation. As shown in Table 1, we investigated the effect of *N*-substituted group. Various aromatic groups including *N*,*N*-diethylamino **6a**, 2-thiophene



Scheme 2. Synthesis of compound **8**. *Reagents and conditions:* (i) step 1: 1,2-dichloroethane, $POBr_3$, reflux for 24 h, cooling and filtration; step 2: acetic acid, reflux for 2 h, cooling and filtration; (ii) NaH, alkyl halide, DMF, 0 °C – rt, 24 h; (iii) 4-hydroxyphenylboronicacid, $Pd(PPh_3)_4$, Na_2CO_3, DMF, H_2O, 120 °C, 48 h; (iv) 1,4-dibromobutane, K_2CO_3, CH_3CN, reflux for 18 h; (v) PPh_3, CH_3CN, reflux for 24 h.

6b, phenyl **6c**, *p*-methylaminophenyl **6d**, and naphthyl **6e**, were introduced and obvious activity were observed. The best result was achieved by **6c** which showed IC_{50} less than 10 μM. **6a** also exhibited considerable proteasome activity with IC_{50} 10 μM. To investigate whether the PPh_3 could be replaced by other positively charged groups, we synthesized several compounds with different positively charged groups including $P(Cy)_3$ **6f**, pyridine **6g**, methyl imidazole **6h** and triethylamine **6i**. However, only **6f** showed competitive activity probably due to the similar structure with triphenylphosphine. **6g**, **6h** and **6i** showed poor inhibition activity which indicating that positive charge might not be the only essential factor, the size of terminal group also plays important role. Furthermore, we investigated electroneutral terminal groups such as bromine **5a**, diphenylphosphine **6j**, morpholine **6k**, 6-*H*-pyridine **6l**, 4-*H*-pyrrole **6m**, all of them exhibited negligible inhibition activity.

Besides, the effect of alkyl N-substitution has also been studied. As shown in Table 1, alkyl groups such as methyl **8a**, ethyl **8b**, *n*-propyl **8c**, and *n*-butyl **8d** showed poor contribution to proteasome inhibition activity due to the small size. To circumvent this problem, bulky groups such as isobutyl **8e**, cyclopropyl **8f**, cyclobutyl **8g**, cyclohexyl **8h**, and benzyl **8i** were introduced to the 2-NH of phthalazinone. To our delight, these compounds showed obvious inhibition activity due to improved occupation of the bulky group in the S1 pocket. Among these compounds, **8i** showed the most potent proteasome inhibition activity with IC_{50} approximately to 10 μM.

Based on these results, the study of SAR was focused on the scaffold and the substituted groups. It is revealed both the phthalazinone scaffold and positively charged triphenylphosphine group are irreplaceable. Besides, the 2-NH position of phthalazinone should be modified by bulky aromatic or aliphatic group such as phenyl, 4-*N*,*N*-diethylaniline, or benzyl group. The substituted position of alkyl on the phenol group should be *para* which is crucial for the proteasome inhibition activity, but the length of alkyl is not restricted.

In summary, a series of phthalazinone derivatives were designed, synthesized and evaluated as human 20S proteasome inhibitors. In this study, electron positive triphenylphosphine group was first introduced in the structure of proteasome inhibitors and potent inhibition was achieved. Several target compounds showed moderate proteasome inhibitory effect in vitro and most of these compounds were found to have IC_{50} around 10 μM on proteasome β_5 subunit. Compound **6c** was the most potent inhibitor of proteasome with IC_{50} less than 10 μM. We also examined the structure-activity relationship (SAR) of these phthalazinone derivatives that is valuable for development of novel proteasome inhibitors. Further optimization and mechanistic study are in progress in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.04.067.

References and notes

1. Borissenko, L.; Groll, M. *Chem. Rev.* **2007**, *107*, 687.
2. (a) Huber, E. W.; Groll, M. *Angew. Chem., Int. Ed.* **2012**, *51*, 8708; (b) Rentsch, A.; Landsberg, D.; Broadmann, T.; Bülow, L.; Girbig, A.-K.; Kalesse, M. *Angew. Chem., Int. Ed.* **2013**, *52*, 5450; (c) Buckley, D. L.; Crews, C. M. *Angew. Chem., Int. Ed.* **2014**, *53*, 2312.
3. Buac, D.; Shen, M.; Schmitt, S.; Kona, F. R.; Deshmukh, R.; Zhang, Z.; Dudas, C. N.; Mitra, B.; Dou, Q. P. *Curr. Pharm. Des.* **2013**, *19*, 4025.
4. Fu, Y. Q.; Xu, B.; Zou, X. M.; Ma, C.; Yang, X. M.; Mou, K.; Fu, G.; Lv, Y.; Xu, P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1102.
5. Sun, Q.; Xu, B.; Niu, Y.; Xu, F.; Liang, L.; Wang, C.; Yu, J.; Yan, G.; Wang, W.; Jin, H.; Xu, P. *ChemMedChem* **2015**, *10*, 498.
6. Feling, R. H.; Buchanan, G. O.; Mincer, T. J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Angew. Chem., Int. Ed.* **2003**, *42*, 355.
7. Basse, N.; Montes, M.; Marechal, X.; Qin, L.; Bouvier-Durand, M.; Genin, E.; Vidal, J.; Villoutreix, B. O.; Reboud-Ravaux, M. *J. Med. Chem.* **2010**, *53*, 509.
8. Ge, Y.; Kazi, A.; Marsilio, F.; Luo, Y.; Jain, S.; Brooks, W.; Daniel, K. G.; Guida, W. C.; Sebti, S. M.; Lawrence, H. R. *J. Med. Chem.* **2012**, *55*, 1978.
9. Gallastegui, N.; Beck, P.; Arciniega, M.; Huber, R.; Hillebrand, S.; Groll, M. *Angew. Chem., Int. Ed.* **2012**, *51*, 247.
10. Beck, P.; Lansdell, T. A.; Hewlett, N. M.; Tepe, J. J.; Groll, M. *Angew. Chem., Int. Ed.* **2015**, *54*, 2830.
11. Miller, Z.; Kim, K.-S.; Lee, D.-M.; Kasam, V.; Baek, S. E.; Lee, K. H.; Zhang, Y.-Y.; Ao, L.; Carmony, K.; Lee, N.-R.; Zhou, S.; Zhao, Q.; Jang, Y.; Jeong, H.-Y.; Zhan, C.-G.; Lee, W.; Kim, D.-E.; Kim, K. B. *J. Med. Chem.* **2015**, *58*, 2036.
12. Yang, G.; Sun, Q.; Wang, C.; Liang, L.; Xu, F.; Niu, Y.; Xu, P. *J. Chin. Pharm. Sci.* **2014**, *23*, 626.
13. Goldberg, A. D.; Nicklas, J.; Goldstein, S. *Am. J. Cardiol.* **1991**, *68*, 631.
14. Barrett, J. A.; Meacham, R. H.; Kelley, M. F., Jr.; Graney, W. F.; Perrone, M. H. *Cardiovasc. Drug Rev.* **1990**, *8*, 323.
15. Kauffman, R. F.; Robertson, D. W.; Franklin, R. B.; Sandusky, G. E., Jr.; Dies, F.; McNay, J. L.; Hayes, J. S. *Cardiovasc. Drug Rev.* **1990**, *8*, 303.
16. Hudkins, R.; Raddatz, R.; Tao, M.; Mathiasen, J.; Aimone, L.; Becknell, N.; Prouty, C.; Knutsen, L.; Yazdanian, M.; Moachon, G.; Ator, M.; Mallamo, J.; Marino, M.; Bacon, E.; Williams, M. *J. Med. Chem.* **2011**, *54*, 4781.
17. Ukena, D.; Rentz, K.; Reiber, C.; Sybrecht, G. W. *Respir. Med.* **1995**, *89*, 441.
18. Ovais, S.; Javed, K.; Yaseen, S.; Bashir, R.; Rathore, P.; Yaseen, R.; Hameed, A. D.; Samim, M. *Eur. J. Med. Chem.* **2013**, *67*, 352.
19. Tan, O. U.; Ozadali, K.; Yogeeswari, P.; Sriram, D.; Balkan, A. *Med. Chem. Res.* **2012**, *21*, 2388.
20. Bristol, J. A.; Sircar, I.; Moos, W. H.; Evans, D. B.; Weishaar, R. E. *J. Med. Chem.* **1984**, *27*, 1099.
21. Sircar, I.; Duell, B. L.; Bobowski, G.; Bristol, J. A.; Evans, D. B. *J. Med. Chem.* **1985**, *28*, 1405.

22. Corsano, S.; Scapicchi, R.; Strappaghetti, G.; Marucci, G.; Paparelli, F. *Eur. J. Med. Chem.* **1995**, *30*, 71.
23. Costas, T.; Besada, P.; Piras, A.; Acevedo, L.; Yañez, M.; Orallo, F.; Laguna, R.; Terán, C. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6624.
24. Thota, S.; Bansal, R. *Med. Chem. Res.* **2010**, *19*, 808.
25. Saeed, M. M.; Khalil, N. A.; Ahmed, E. M.; Eissa, K. I. *Arch. Pharm. Res.* **2012**, *35*, 2077.
26. Özadalı, K.; Özkanlı, F.; Jain, S.; Rao, P. P. N.; Velázquez-Martínez, C. A. *Bioorg. Med. Chem.* **2012**, *20*, 2912.
27. Sweeney, Z. K.; Dunn, J. P.; Li, Y.; Heilek, G.; Dunten, P.; Elworthy, T. R.; Han, X.; Harris, S. F.; Hirschfeld, D. R.; Hogg, J. H.; Huber, W.; Kaiser, A. C.; Kertesz, D. J.; Kim, W.; Mirzadegan, T.; Roepel, M. G.; Saito, Y. D.; Silva, T. M. P. C.; Swallow, S.; Tracy, J. L.; Villasenor, A.; Vora, H.; Zhou, A. S.; Klumpp, K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4352.
28. Tao, M.; Aimone, L. D.; Huang, Z.; Mathiasen, J.; Raddatz, R.; Lyons, J.; Hudkins, R. *J. Med. Chem.* **2012**, *55*, 414.
29. Raddatz, R.; Hudkins, R. L.; Mathiasen, J. R.; Gruner, J. A.; Flood, D. G.; Aimone, L. D.; Le, S.; Schaffhauser, H.; Duzic, E.; Gasior, M.; Bozyczko-Coyne, D.; Marino, M. J.; Ator, M. A.; Bacon, E. R.; Mallamo, J. P.; Williams, M. *J. Pharmacol. Exp. Ther.* **2012**, *340*, 124.
30. Deeks, E. D. *Drugs* **2015**, *75*, 231.
31. Kisselev, A. F.; Goldberg, A. L. *Chem. Biol.* **2001**, *8*, 739.
32. Discovery Studio version 2.5, Accelrys: accelrys.com/products/discoverystudio.
33. Wang, W.; Liang, L.; Xu, F.; Huang, W.; Niu, Y.; Sun, Q.; Xu, P. *Eur. J. Org. Chem.* **2014**, 6863.
34. Liang, L.; Wang, W.; Wu, J.; Xu, F.; Niu, Y.; Xu, B.; Xu, P. *Chem. Eur. J.* **2013**, *19*, 13774.
35. Liang, L.; Wang, W.; Xu, F.; Niu, Y.; Sun, Q.; Xu, P. *Org. Lett.* **2013**, *15*, 2770.
36. Liang, L.; Yang, G.; Xu, F.; Niu, Y.; Sun, Q.; Xu, P. *Adv. Syn. Cat.* **2013**, *355*, 1284.
37. Liang, L.; Yang, G.; Xu, F.; Niu, Y.; Sun, Q.; Xu, P. *Eur. J. Org. Chem.* **2013**, 6130.

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