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Development of a new class of proteasome inhibitors with an epoxyketone warhead: Rational hybridization of non-peptidic belactosin derivatives and peptide epoxyketones

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ABSTRACT

Proteasome inhibitors are currently a focus of increased attention as anticancer drug candidates. We recently performed systematic structure–activity relationship studies of the peptidic natural product belactosin A and identified non-peptidic derivative **2** as a highly potent proteasome inhibitor. However, the cell growth inhibitory effect of **2** is only moderate, probably due to the biologically unstable β-lactone warhead. Peptide epoxyketones are an important class of proteasome inhibitors exhibit high potency in cellular systems based on the efficient α,β-epoxyketone warhead. Importantly, belactosin derivatives bind primarily to the primed binding site, while peptide epoxyketones bind only to the non-primed binding site of proteasome, suggesting that hybridization of them might lead to the development of a new class of proteasome inhibitors. Thus, we successfully identified a novel chemotype of proteasome inhibitors **3** and **4** by rational structure-based design, which are expected to bind to both the primed and non-primed binding sites of proteasome.

1. Introduction

The major pathway for systematic degradation of intracellular proteins is the ubiquitin–proteasome system,[1] which is involved in many physiologically important cellular processes, such as signal transduction,[2] immune responses,[3] the unfolded protein response (UPR),[4] and cell cycle progression.[5] Proteasome inhibition causes cell cycle arrest and induces apoptosis, making proteasomes attractive target molecules for drugs to fight cancer and autoimmune disorders.[6] In fact, the US Food and Drug Administration recently approved the proteasome inhibitors bortezomib and carfilzomib (Fig. 1) for the treatment of multiple myeloma,[7] and several other proteasome inhibitors are currently in clinical trials.[8]

Belactosin A, comprising L-alanine, 3-(*trans*-2-aminocyclopropyl)-L-alanine (*trans*-3,4-methano-L-ornithine), and a chiral carboxy-β-lactone moiety, is a naturally occurring tripeptide metabolite produced by *Streptomyces* sp. (Fig. 1),[9] It inhibits proteasome chymotrypsin-like (ChT-L) activity[10] by acylating the active site Thr residue via its strained β-lactone-opening, as confirmed by X-ray crystallographic analysis of belactosin derivatives in complex with proteasome (Fig. 2a).[11] Importantly, belactosin A and its derivatives are the only known proteasome inhibitors that bind to both the primed and non-primed substrate binding sites of proteasome[11] and they are thus attractive lead compounds for the development of unique proteasome inhibitors.

As shown in Figure 3, we performed systematic structure–activity relationship studies of belactosin A and developed the highly potent derivative **1**.[11a] Furthermore, by the topology-based scaffold hopping of **1** based on our structure–activity relationship (SAR) and binding-mode analyses results,[12] we identified significantly simplified non-peptide inhibitor **2**.[13] Despite its significant proteasome inhibitory activity, however, the cell growth inhibitory effects were only moderate. We hypothesized that these contradictory results were due to its unstable β-lactone warhead under biological

Abbreviations: AMC, aminomethylcoumarin; Boc, *t*-butoxycarbonyl; ChT-L, chymotrypsin-like; DIEA, *N*,*N*-diisopropylethylamine; HBTU, *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; Piv, pivaloyl; Suc, succinyl; TFA, trifluoroacetic acid; UPR, unfolded protein response.

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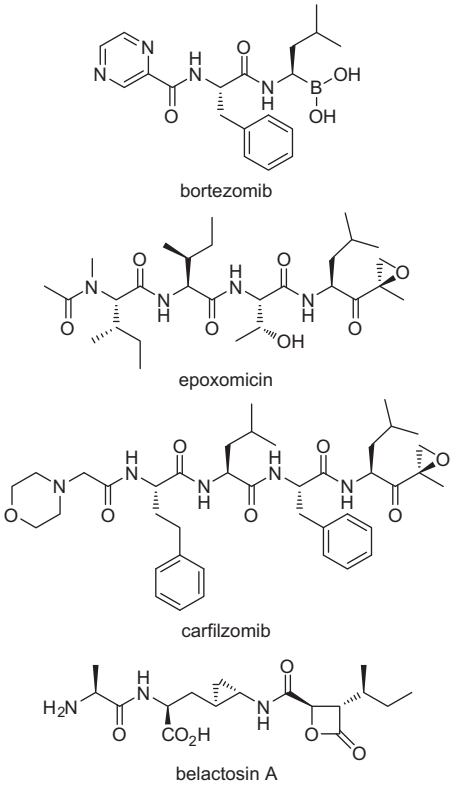


Figure 1. Known proteasome inhibitors.

conditions,[14] and therefore, planned to replace it with a more stable and irreversible warhead to develop potent cell growth inhibitors.

Peptide proteasome inhibitors with an α,β-epoxyketone as the reactive warhead comprise one of the most extensively studied classes of proteasome inhibitors, which inhibit proteasome covalently by forming a morpholino adduct (Fig. 2b).[6a,15] This class of proteasome inhibitors binds only to the non-primed binding site of the proteasome and exhibits remarkable cell growth inhibitory effects, as represented by the clinical drug carfilzomib.[16,14] We planned to produce a new class of non peptidic proteasome inhibitors by replacing the β-lactone moiety of the non-peptidic belactosin A derivatives with an α,β-epoxyketone residue. These hybrid compounds were expected to bind to both the primed and non-primed binding sites of proteasomes like belactosin A and to exhibit potent cell growth inhibitory effects like carfilzomib due to the α,β-epoxyketone warhead. Here, we describe the design, synthesis, and biological activity of this new class of proteasome inhibitors.

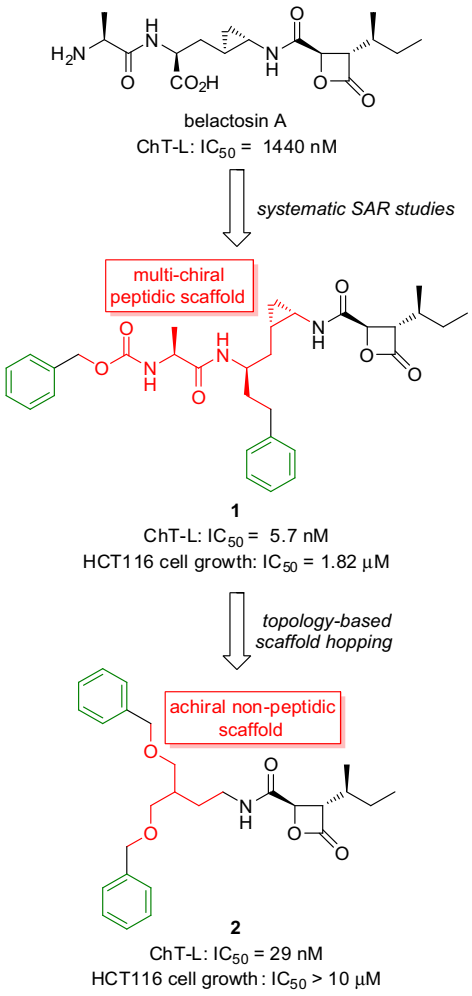


Figure 3. Belactosin A derivatives previously developed by us.

2. Results and discussion

2.1. Design of compounds

Figure 4 is a superposition of the two X-ray crystal structures of proteasome in complex with epoxomicin,[17] a peptidic proteasome inhibitor with an α,β-epoxyketone, and belactosin A derivative **1**,[11a] which clearly shows that epoxomicin binds to the non-primed binding site and its P2 side-chain is directed to the vacant primed binding site of proteasome. Therefore, we assumed that elongating

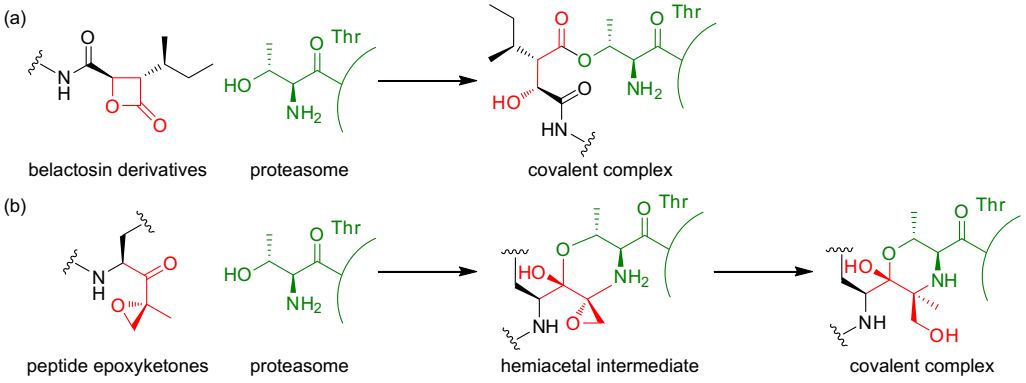


Figure 2. Inhibitory mechanism of covalent proteasome inhibitors: (a) belactosin derivatives (b) peptide epoxyketones.

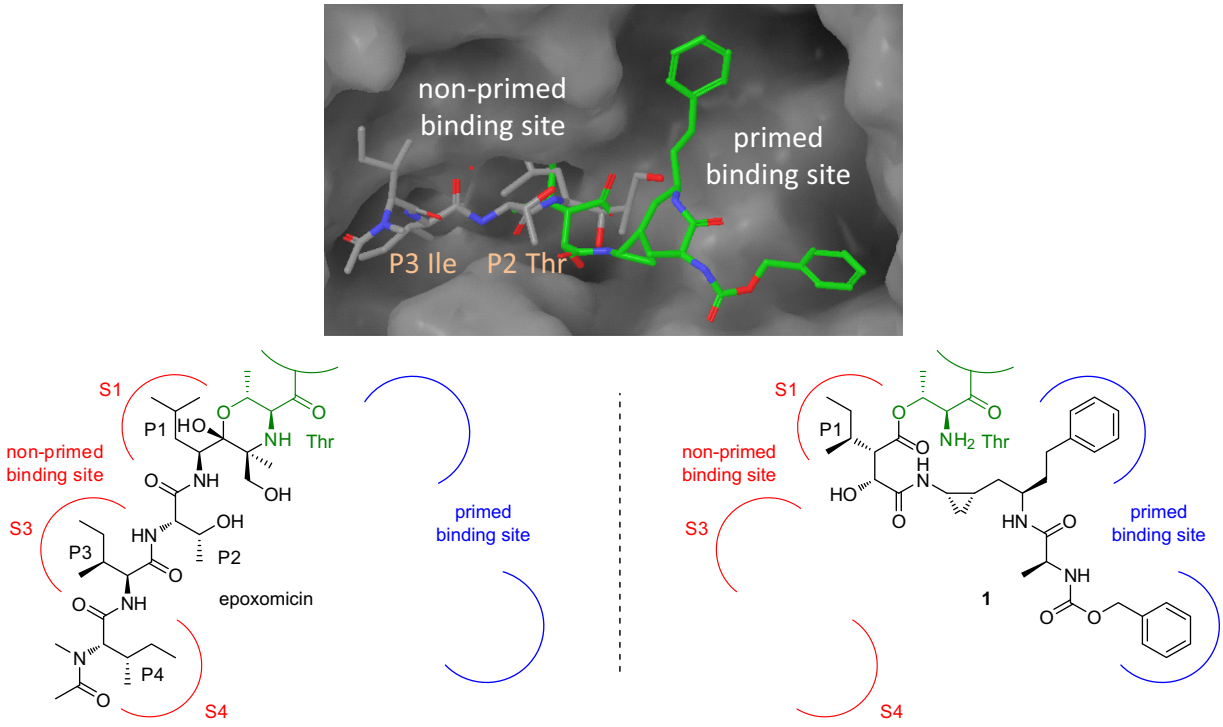


Figure 4. Superposition of the X-ray crystal structures of epoxomicin (gray tube; PDB code, 1G65)[17] and belactosin A derivative **1** (green tube; PDB code, 4J70)[11a] in complex with proteasome.

the P2 side-chain would allow us to design unique hybrid compounds of the peptide epoxyketones and the non-peptide belactosin A derivatives that could bind to both the primed and non-primed biding sites. Although all of the peptide epoxyketone proteasome inhibitors known to date have a P3 side-chain accommodated in the proteasome S3 pocket,[18] we postulated that interactions with the primed binding site would compensate for the lack of interaction with the S3 pocket, and thus designed hybrid compounds **3** and **4** without a P3 side-chain, as shown in Figure 5.

To confirm the molecular design, we performed computational simulations to predict binding modes of the designed hybrids **3** and **4**. As shown in Figure 6, in the simulated binding mode, the moiety derived from the non-peptidic belactosin A derivative ligated with the P2 side-chain is effectively accommodated in the primed binding site, as expected. Therefore, we synthesized these molecules and evaluated their biological effects.

2.2. Synthesis

Designed compounds **3** and **4** were synthesized as shown in Scheme 1. Boc-Asp-OBn **5** was condensed with **6**[13] or **7**[19] by a mixed anhydride method to yield compounds **8** or **9**, respectively. The Boc group of **8** and **9** was removed by treatment with TFA/CH_2Cl_2, and subsequent acetylation of the products with acetic anhydride gave **10** and **11**, respectively. The Bn group of **10** and **11** was removed under hydrogen transfer conditions using 1,4-cyclohexadiene, and the resulting carboxylic acids were subsequently condensed with the known epoxyketone unit prepared by deprotection of **12**[20] to yield the target compounds **3** and **4**, respectively.

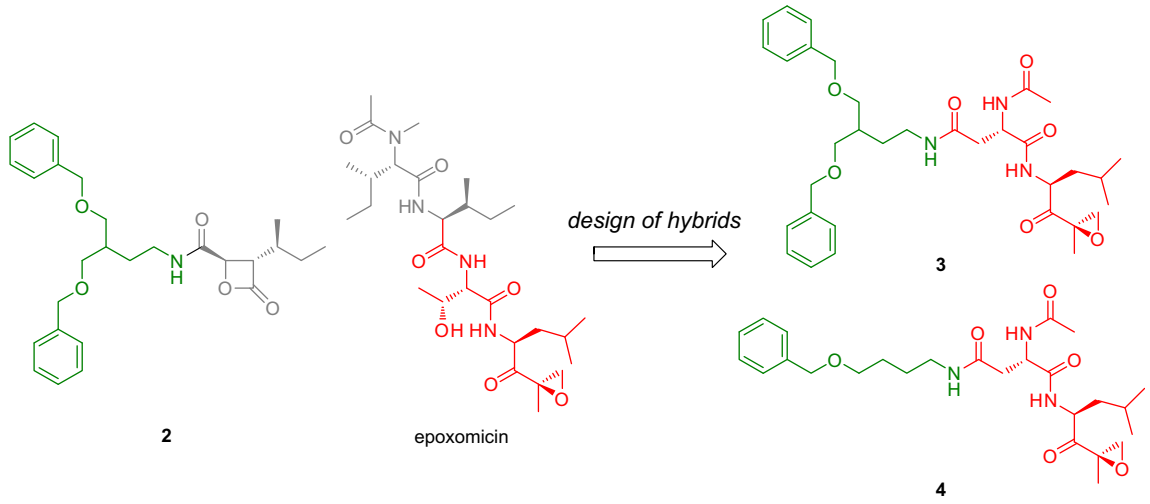


Figure 5. Design of hybrids of non-peptide belactosin A derivative and peptide epoxyketone.

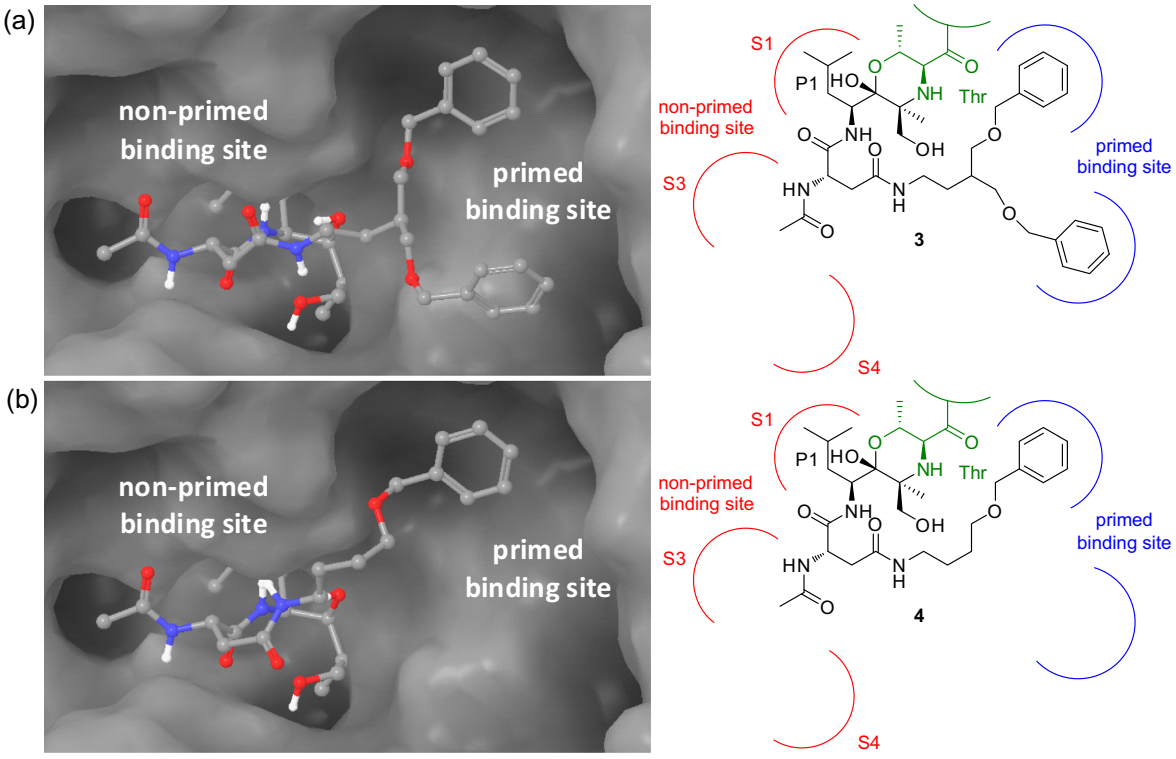
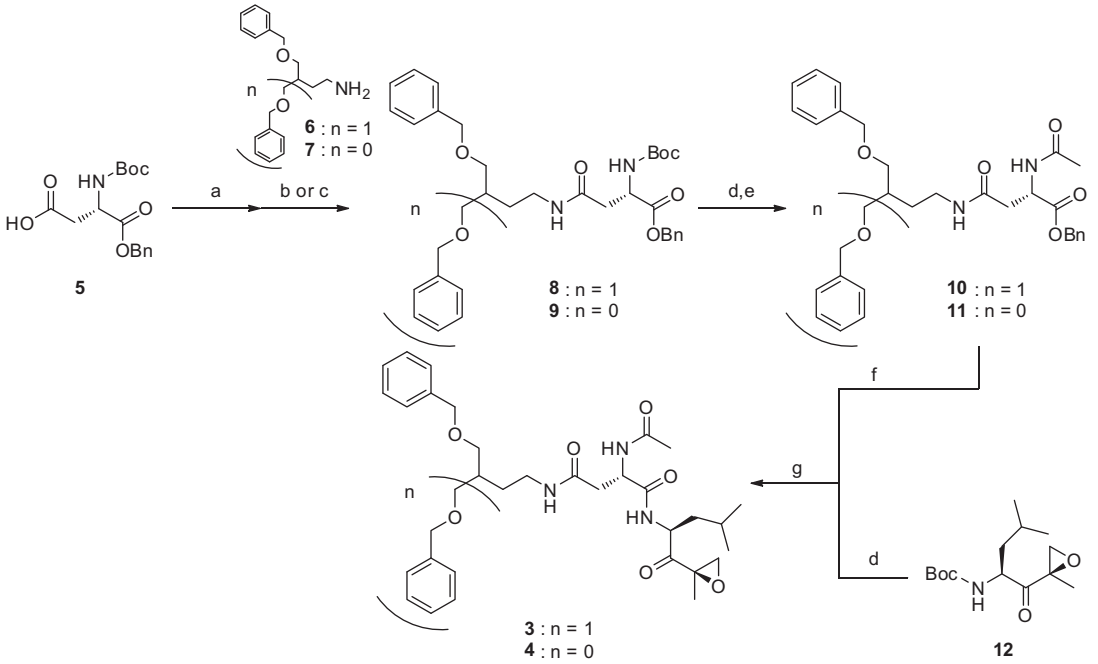


Figure 6. Computationally predicted binding mode of compounds **3** (a) and **4** (b).



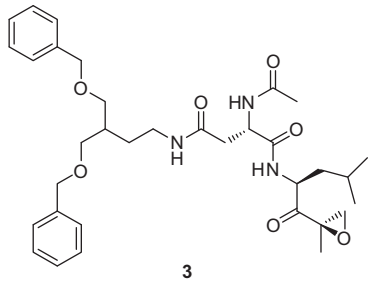
Scheme 1. Synthesis of target compounds **3** and **4**. Reagents and conditions: (a) PivCl, Et_3N, CH_2Cl_2, 0 °C to rt; (b) **6**, Et_3N, CH_2Cl_2, 0 °C to rt; (c) **7**, Et_3N, CH_2Cl_2, 0 °C to rt; (d) TFA, CH_2Cl_2; (e) Ac_2O, Et_3N, CH_2Cl_2, 0 °C to rt; (f) 1,4-cyclohexadiene, Pd/C, EtOH; (g) HBTU, HOBt, DIEA, THF, −5 °C, 60% for **3** (6 steps), 60% for **4** (6 steps).

2.3. Pharmacological effects

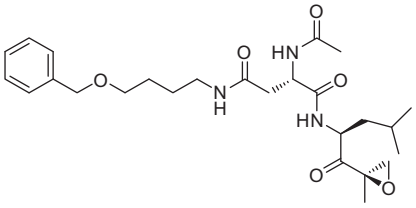
The inhibitory effects of compounds on the ChT-L activity of human 20S proteasomes were evaluated using the chromophoric substrate Suc-LLVY-AMC. As summarized in Table 1, compounds **3** and **4** inhibited ChT-L activity with an IC_{50} of 2.3 μM and 5.7 μM, respectively, despite the absence of a P3 side-chain.

We also evaluated the cell growth inhibitory effects of these compounds on HCT116 cells, and the results are summarized in Table 1. Compounds **3** and **4** showed definite cell growth inhibitory effects with an IC_{50} value of 1.9 μM and 2.9 μM, respectively, in spite of the lack of their P3 side-chain, which suggests that the interactions with the proteasome primed binding site compensated for the lack of the interactions with proteasome S3 pocket.

Table 1
Inhibitory effects of **3** and **4** on proteasome ChT-L activity and HCT116 cell growth



3



4

Compound number	IC_{50}[a] [μM]	
	ChT-L activity	HCT116 cell growth
3	2.3 ± 0.1	1.9
4	5.7 ± 0.4	2.9

[a] Based on three experiments.

Interestingly, compound **4** with one hydrophobic moiety showed proteasome inhibitory activity comparable to that of compound **3** with two hydrophobic moieties. These results are consistent with our previous results on the hybrid compounds of peptide boronates and non-peptide belactosin derivatives, in which the contribution of the hydrophobic interaction due to the moieties derived from non-peptidic belactosin derivatives to their proteasome inhibitory activity were robustly shown.[19] To our knowledge, these are the first peptide epoxyketone proteasome inhibitors without a P3 side-chain. Importantly, as we expected, the cell growth inhibitory effects were comparable to the proteasome inhibition effects, in stark contrast with the effects of their parent compound **2** that has a biologically unstable β-lactone warhead, of which HCT116 cell growth inhibitory effect (IC_{50} >10 μM) is drastically weak compared with its proteasome inhibitory effect (IC_{50} = 29 nM).

Thus, these structurally novel hybrid compounds **3** and **4** are attractive lead compounds for the development of a new class of proteasome inhibitors, having the epoxyketone warhead and binding to both of the primed and non-primed binding sites.

3. Conclusions

In summary, we successfully developed novel epoxyketone-type proteasome inhibitors **3** and **4** by a structure-based hybridization strategy with non-peptide belactosin A derivatives and epoxomicin, a peptide epoxyketone. Because the binding mode of these compounds seems to be different from that of known peptide epoxyketone inhibitors due to their structural novelty, they are attractive lead compounds for further development of potent proteasome inhibitors.

Acknowledgments

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Supplementary data

Supplementary data (experimental details of synthesis, biological evaluations, computational simulations, and a table listing combustion analysis data for target compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.04.032.

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