Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Cyclobutane Derivatives As Novel Nonpeptidic Small Molecule Agonists of Glucagon-Like Peptide-1 Receptor

Qing Liu,[†] Na Li,[†,#] Yunyun Yuan,[†,▽] Huili Lu,[†] Xiaoyan Wu,[†] Caihong Zhou,[†,‡] Min He,[†,‡] Haoran Su,[†,○] Meng Zhang,[†] Jia Wang,[†] Bao Wang,[§] You Wang,[§] Dawei Ma,[§] Yang Ye,[‡] Hans-Christoph Weiss,[∥] Ernst R. F. Gesing,[⊥] Jiayu Liao,[†,◆] and Ming-Wei Wang*[,†,‡]

[†]The National Center for Drug Screening, 189 Guo Shou Jing Road, Shanghai 201203, China

[‡]The State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China

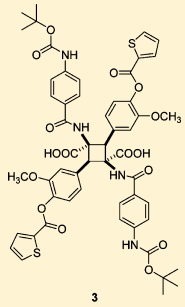
[§]Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Ling Ling Road, Shanghai 200032, China

[∥]Currenta GmbH & Co. OHG, Leverkusen, Amtsgericht Köln HR A 20833, Germany

[⊥]Bayer CropScience Aktiengesellschaft, Alfred-Nobel-Straße 50, D-40789 Monheim, Germany

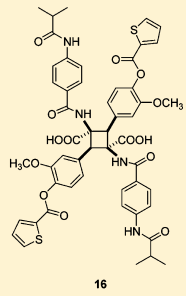
Supporting Information

ABSTRACT: A novel cyclobutane class of nonpeptidic glucagon-like peptide-1 (GLP-1) receptor agonists, exemplified by **3**, was identified using receptor binding and multiple response element/cAMP response element (MRE/CRE)-driven reporter gene assays. The structures of **3** and its three isomers were elucidated by NMR, HRESIMS, and X-ray crystallography. A series of structural modifications were also made based on the core structure of **3** with different substitution groups at the west and east ends. Among these analogues, compound **16** was found to be 4- to 5-fold more potent than **3** both in vitro and in vivo.



3

EC_{50}: 846.8±287.3 nM



16

EC_{50}: 202.5±143.9 nM

INTRODUCTION

Type 2 diabetes is emerging as one of the largest health issues worldwide, with an estimated 23.6 million children and adults (7.8% of the population) affected in the United States alone.[1] Glucagon-like peptide-1 (GLP-1) has proven to be an efficacious agent to combat this serious and life-long disease.[2,3] GLP-1 as a therapeutic approach, however, is limited by two major drawbacks: (i) the half-life of the native peptide is very short (2−3 min in the circulation) due to its cleavage by dipeptidyl peptidase-4 (DPP-4),[4] and (ii) GLP-1 peptidic mimetics resistant to DPP-4 such as Exenatide[5,6] and Liraglutide[7] require twice or once daily injections, respectively.[8] Therefore, suppression of the degradation of endogenous GLP-1 has become an alternative strategy to prolong the beneficial effects of GLP-1 in glucose homeostasis. Although DPP-4 inhibitors (e.g., Sitagliptin, Vildagliptin, and Saxagliptin)[9−11] are effective in glycemic control, their benefits to body weight changes are neutral.[12]

In an attempt to discover nonpeptidic small molecule GLP-1 receptor (GLP-1R) agonists, we screened a diverse library of 48160 synthetic and natural compounds against HEK293 cells stably transfected with a rat GLP-1R expression vector and a multiple response element/cAMP response element (MRE/CRE)-driven luciferase reporter plasmid (HEK293-rGLP-1R cells). From the high-throughput screening (HTS) campaign, (*Z*)-4-((2-(4-(acetamido)phenyl)-5-oxooxazol-4(5*H*)-ylidene)-methyl)-2-ethoxyphenyl thiophene-2-carboxylate (SH14800)[13] was confirmed to invoke luciferase activity, to elevate cAMP and to displace [^{125}I] GLP-1(7−36) amide from the receptors. After minor structural modification, we obtained two derivatives of (*Z*)-4-((2-(4-((cyclopentanecarbonyl)amino)-phenyl)-5-oxooxazol-4(5*H*)-ylidene)methyl)-2-methoxyphenylthiophene-2-carboxylate (NC133908)[13] and (*Z*)-4-((2-(4-((*t*-butoxycarbonyl)amino)phenyl)-5-oxooxazol-4(5*H*)-ylidene)methyl)-2-methoxyphenylthiophene-2-carboxylate (NC133909)[13] (Figure 1) that displayed sporadic, but greater luciferase activity. Further research indicated that the observed bioactivities resided not in these analogues themselves but resulted from a polymerization process in DMSO solution occurring under natural light and led to the formation of the substituted cyclobutane, 1,2-*cis*-2,3-*cis*-3,4-*cis*-1,3-bis(4-((cyclopentanecarbonyl)amino)benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic acid (S4P)[13] and 1,2-*cis*-2,3-*cis*-3,4-*cis*-1,3-bis(4-((*t*-butoxycarbonyl)amino)benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic acid

Received: August 29, 2011
Published: November 21, 2011

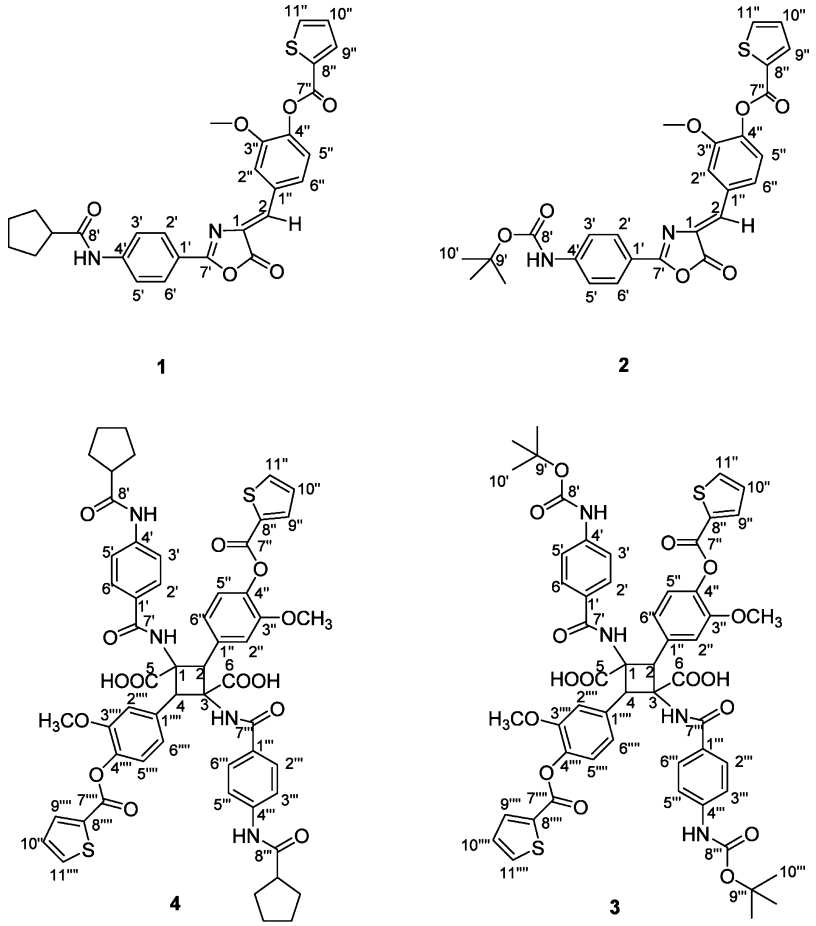


Figure 1. Structures of **3**, **4**, and their respective monomers.

(Boc5),[13] derived from **1** (NC133908) and **2** (NC133909), respectively. Compound **3** (Boc5) was synthesized subsequently using the ultraviolet high-pressure mercury lamp (350−450 nm) and has been extensively pharmacologically characterized in both monogenetic and polygenetic rodent models of diabetes and obesity.[13−15]

In this report, we described in detail the chemical characterization of **3** and its stereoisomers, the structure−activity relationship (SAR) analyses, and agonist activities of its analogues both in vitro and in vivo. Guided by a set of bioassays, our chemistry efforts have yielded a more potent analogue, compound **16**, with consistently better in vitro and in vivo performance than **3**.

RESULTS AND DISCUSSION

Characterization of 3, Its Isomers, and Dehydrated Product. Structures were elucidated on the basis of NMR and MS spectra. The HRMS (ESI) of **3** gave the quasimolecular ion peak at m/z 1099.2756, consistent with the molecular formula of $C_{54}H_{52}N_4O_{16}NaS_2$ $[M + Na]^+$. Comparing to **2**, the similar ^1H and ^{13}C NMR signals indicated the existence of 4-((*t*-butoxycarbonyl)amino)phenyl group and 3-methoxy-4-(thiophene-2-carbonyloxy)phenyl group (Figure 1 and Table 1). Except for the above signals, two carbonyl carbons at δ 172.8 (1/3-COOH) and 166.8 (C-7′/7‴), one quaternary carbon at δ 63.1 (C-1/3) and one methine at δ 48.5 (C-2/4) in the ^{13}C NMR, as well as a singlet proton at δ 4.94 (H-2/4) and an active proton at δ 8.55 (7′/7‴-CONH) in the ^1H NMR were remaining. In the HMBC spectrum, the singlet proton at δ 4.94 correlated with the carbonyl group at δ 172.8, the quaternary carbon (δ 63.1), and C-1″ and C-2″/6″ of 3-methoxy-4-(thiophene-2-carbonyloxy)phenyl group; the active proton at δ 8.55 correlated with the carbonyl groups at δ 166.8 and 172.8, quaternary carbon at δ 63.1, and H-2′/6′ of 4-((*t*-butoxycarbonyl)amino)phenyl group correlated with carbonyl carbon at δ 166.8, which suggested that 4-((*t*-butoxycarbonyl)-amino)phenyl group and 3-methoxy-4-(thiophene-2-carbonyloxy)phenyl group were connected by −CO-NH-C(COOH)-CH− (Figure 2). All proton and carbon signals were assigned to the above moiety with a molecular weight of 538, half of the MS value. This led to our proposition that the compound was a symmetrical dimer composed of two above moieties, implying the existence of a four-membered ring. When **3** was formed by the polymerization of its monomer **2** in the solution of DMSO, there existed two possible conformations, i.e., *anti* head-to-tail and *syn* head-to-head. Thus, the planar structure of **3** was deduced to be 1,3-bis(4-((*t*-butoxycarbonyl)amino)benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic acid or 1,2-bis(4-((*t*-butoxycarbonyl)amino)benzamido)-3,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy) phenyl)cyclobutane-1,3-dicarboxylic acid.

Theoretically, each conformation should possess four chiral carbon atoms which consequently formed 16 conformers. After eliminating the repetitive conformers, there remained five possible relative configurations including three symmetrical and two unsymmetrical ones for each constitutional isomer. Crystals of **3** were carefully obtained from a mixed solution

dx.doi.org/10.1021/jm201150j | J. Med. Chem. 2012, 55, 250−267

Table 1. ^{1}H and ^{13}C NMR Data for 2 and 3 (DMSO-d_6)[a]

	δ_C		δ_H (J Hz)	
no.	2	3	2	3
1	132.9	63.1		
2	128.8	48.5	7.29 (1H, brs)	4.94 (2H, s)
1,3-COOH	167.0	172.8		12.95 (2H, brs)
1′	117.9	128.4		
2′	129.3	128.1	8.03 (1H, d, J = 8.8)	7.32 (2H, d, J = 8.5)
3′	118.0	117.0	7.71 (1H, d, J = 8.7)	7.42 (2H, d, J = 8.5)
4′	144.9	142.3		
5′	118.0	117.0	7.71 (1H, d, J = 8.7)	7.42 (2H, d, J = 8.5)
6′	129.3	128.1	8.03 (1H, d, J = 8.8)	7.32 (2H, d, J = 8.5)
7′	163.0	166.8		
8′	152.4	152.5		
9′	80.0	79.5		
10′	28.0	28.1	1.50 (9H, s)	1.41 (18H, s)
1″	133.4	133.6		
2″	115.5	112.6	8.23 (1H, brs)	7.26 (2H, brs)
3″	151.0	150.1		
4″	140.8	137.7		
5″	123.5	122.5	7.40 (1H, d, J = 8.2)	7.23 (2H, d, J= 8.3)
6″	125.4	122.1	7.90 (1H, brd, J = 8.1)	7.17 (2H, brd, J = 8.1)
7″	159.3	159.4		
8″	131.2	131.7		
9″	135.6	135.1	8.12 (1H, d, J = 4.3)	8.04 (2H, dd, J = 4.9, 1.1)
10″	128.3	128.7	7.33 (1H, t, J = 4.2)	7.27 (2H, dd, J = 4.9, 3.9)
11″	135.5	135.1	8.04 (1H, overlap)	7.99 (2H, dd, J = 3.9, 1.1)
3″-OCH_3	56.0	54.9	3.89 (3H, s)	3.23 (6H, s)
8′-CONH			9.97 (1H, s)	9.52 (2H, s)
7′-CONH				8.55 (2H, s)

[a]Signals were assigned from the HMQC, HMBC, COSY, and NOESY spectra.

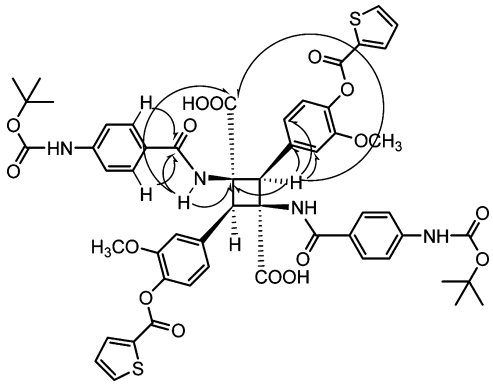


Figure 2. Selected HMBC correlations for **3**.

of methanol and water, and the structure was unambiguously confirmed by X-ray crystallography (Figure 3). Unexpectedly, the X-ray analysis revealed **3** as a 1,2-*cis*-2,3-*cis*-3,4-*cis* configuration in which the four side chains of the core pointed to the same direction, like four legs of a stool (Figure 4).

Compound **4** (S4P) had similar NMR data as **3**, except that the five multiplets in the high field at δ 1.53 (4H), 1.65 (4H), 1.70 (4H), 1.82 (4H), and 2.74 (2H) in ^{1}H NMR, two methylenes at δ 25.7 and 30.0, and one methine at δ 45.3 in the ^{13}C NMR were assigned to cyclopentyl group. HRMS(ESI) of **4** was at m/z 1091.2856, consistent with the molecular formula of $C_{56}H_{52}N_4O_{14}NaS_2$ $[M + Na]^+$. Hence, compound **4** was assigned as 1,2-*cis*-2,3-*cis*-3,4-*cis*-1,3-bis(4-((cyclopentanecarbonyl)amino)benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic acid.

Three isomers (**3a**, **3b**, and **3c**) and one dehydrated product **3d** of compound **3** (Figure 4) were isolated at the same time. The same quasimolecular ion peaks of these three isomers indicated that they were isomers of **3**. Different to **3**, two sets of ^{1}H and ^{13}C NMR signals of 3-methoxy-4-(thiophene-2-carbonyloxy)phenyl groups were observed for **3a**, while the proton and carbon signals of (4-(*t*-butoxycarbonyl)amino)phenyl group were almost identical (Table 2). Moreover, the proton and carbon signals of two methine groups on the cyclobutane were also different, which indicated that the orientation of one (3-methoxy-4-(thiophene-2-carbonyloxy))phenyl group was changed. Therefore, the relative configuration of **3a** could be deduced to be 1,2-*trans*-2,3-*trans*-3,4-*cis*. Compound **3c**, however, presented an opposite picture. The proton and carbon signals of (3-methoxy-4-(thiophene-2-carbonyloxy))phenyl group basically remained the same as that of **3**, while two sets of proton and carbon NMR signals of (4-(*t*-butoxycarbonyl)-amino)phenyl group were observed, indicating that the orientation of one (4-(*t*-butoxycarbonyl)amino)phenyl group was changed. Consequently, the relative configuration of **3c** was proposed to be 1,2-*cis*-2,3-*trans*-3,4-*trans*.

Similar to **3**, only half proton and carbon signals were detected for **3b**, which strongly suggested a symmetrical structure, but the chemical shifts of most protons were different. Theoretically, it has two possible relative configurations, i.e., centrosymmetric (I) and axial symmetrical (II) structures (Figure 4). We tried to grow single crystals of **3b** and were able to obtain a small crystal eventually. However, it was of poor quality with diffraction properties to higher angles. One of the reasons may be due to the high flexibility of the "outer" parts of the molecule. Nevertheless, the crystal system and the space groups of **3b** could be determined without doubts as the centrosymmetric configuration (I) while the hetero atoms remained unassigned (data not shown).

Compound **3d** (Figure 4) had similar proton signals as **3** and the HRMS(ESI) of **3d** gave the quasimolecular ion peak at m/z 1063.2528, consistent with the molecular formula of $C_{54}H_{48}N_4O_{14}NaS_2$ $[M + Na]^+$. Comparing to **3**, the disappearance of ^{1}H NMR signals at δ 8.55 (7′-CONH) and 12.95 (1/3-COOH), and the reduced molecular weight ($-2H_2O$) indicated the existence of oxazol-5(4*H*)-one group, implying that it was a dehydrated product of **3**.

Chemistry. While both **3** and **4** were capable of eliciting bioactivities in vitro and in vivo, compound **3** behaved like a full agonist.[13] Thus, compound **3** had been extensively studied as a therapeutic agent in both monogenetic and polygenetic mouse models of diabetes and obesity.[14,15] To improve the potency and efficacy and to gain a further understanding of SAR for this chemotype as a tool molecule, we carried out a series of chemistry efforts aiming at optimizing the core, west (W), and east (E) ends of the molecule, which led to the identification of a more potent analogue **16**.

Compound **3** is a dimer constituted by a cyclobutane core and two pairs of symmetrical side chains. It was formed during the illuminating process of monomer **2**. The structure of **2** is similar to **3** except that it contains a double bond (sp^2 hybrid orbits) in the middle of the structure while **3** contains a cyclobutane structure (sp^3 hybrid orbits). At the outset, we modified the cyclobutane core into a cyclopropane core with

dx.doi.org/10.1021/jm201150j | J. Med. Chem. 2012, 55, 250−267

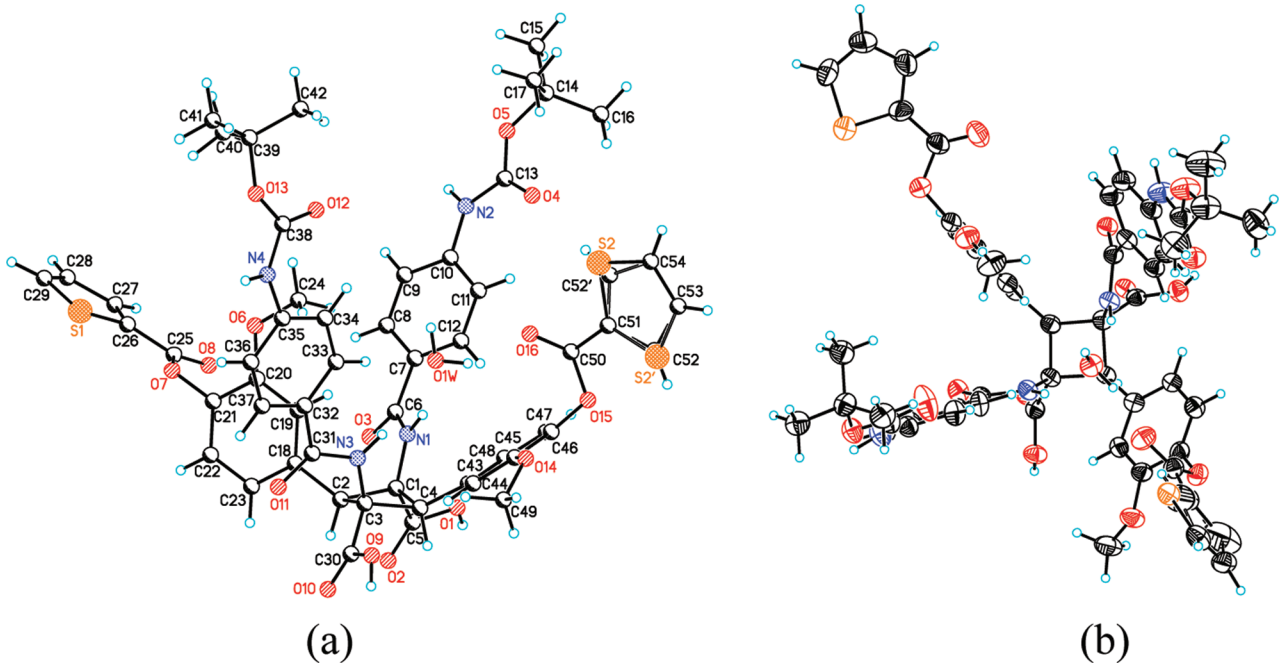


Figure 3. ORTEP drawing of the X-ray crystal structure of **3**. (a) ORTEP plot (50%); (b) ORTEP plot (50%), different orientation.

two side chains and one carboxylic acid to explore the necessity of the cyclobutane core (Supporting Information Scheme S1). The central cyclopropane restricted the rotation of the C_α–C_β bond so that the α- and β-functionality were fixed in space. Our synthesis of the (*Z*) target compound was accomplished by using, in the early stages, a reaction sequence already published in the literatures.[16,17] The synthesis of (*Z*) compound **5** proceeded through a known intermediate, cyclopropane analogue **6**, which was subsequently undergone alcoholysis and debenzylation by hydrogenation.

Next, we evaluated the contributions of the two carboxylic acids to the potency and efficacy on the central cyclobutane while retaining the configuration of the west and east ends with the same substitutes as those of **3** (Scheme 1). The amide analogue **8** was obtained through reacting **3** with ethyl chloroformate, followed by amination with anhydrous ammonia.[18] The ester analogue **9** was also synthesized using diazomethane as the methylation agent (data not shown).

Then the Boc groups of **3** were first removed with the trifluoroacetic acid to form its corresponding amine, which was subsequently acylated with various types of acyl chlorides to obtain **10**–**21**. The amine intermediate was also condensed with isothiocyanate/isocyanate to form thiourea (**22**–**23**) or urea (**24**–**25**), or with *N*-Boc-alanine to give **26**. Compound **19** was debenzylated by reacting with BF_3–Et_2O in ethanethiol to give **28** (Scheme 2).

Finally, we explored the effects of different substitution on the east end of **3**. Because of the limitation of the special structure of **3**, compounds **29**–**34** and **39**–**43** that did not contain a methoxy group at the meta position or an ester moiety at the para position of the phenyl could only be synthesized by illumination of the corresponding monomers. The methods for the preparation of monomers have been reported previously.[19−24] Conveniently, the ester group of **3** was hydrolyzed with 10% NaOH solution to form a phenolic intermediate, which was either alkylated to the relevant ethers (**35**–**38**) or acylated to the respective esters (**44**–**52**). Compounds **53**–**54** were obtained by the similar procedure of compound **44** using compound **16** as the starting material (Scheme 3).

Photosynthesis of 3 and Its Stability. The photochemical [2 + 2] cycloaddition of α,β unsaturated carbonyl compounds in the crystalline state for the stereospecific construction of a 1,2,3,4-tetrasubstituted cyclobutane ring has been extensively investigated.[25−31] It has been demonstrated that such reactions are strictly controlled by the packing arrangement of molecules. On the basis of studies on the cinnamic acids, it was empirically deduced that the olefins should lie in parallel planes with the double bonds oriented in the same direction with the distance between the olefins of less than 4.2 Å for dimerization to occur. However, when these "topochemical rules" are used in the practical cases, problems arise. It is difficult to control the photodimerization to form the desired type of products. We thus focused our efforts on those potential factors that may influence the photosynthesis, such as light source, makeup of reaction flask, and catalyst. First, five light sources with different power and wavelength outputs, i.e., ordinary high-pressure mercury, ultraviolet high-pressure mercury, iodine–gallium, halogen (iron mixing), and LED (light-emitting diode) lamps were tested for reaction efficiency (time and yield; Supporting Information Table S1). The results indicated that the ultraviolet high-pressure mercury lamp (350–450 nm, peak: 365 nm) and the iodine–gallium lamp (350–450 nm, peak: 417 nm) were the best lamps with the shortest reaction time and highest yields while relatively longer reaction time was required for the ordinary high-pressure mercury lamp, and no targeted products were obtained by use of the halogen lamp (iron mixing) and LED lamp, which might be caused by either too low (LED, 20 W) or too high (halogen, 1000 W) power supply.

We subsequently investigated the effects of two types of reaction flask made of glass and quartz, respectively. It appeared that the quartz bottle had better performance than the glass one in terms of reaction time and yield of the crude product. As far

dx.doi.org/10.1021/jm201150j | *J. Med. Chem.* 2012, 55, 250–267

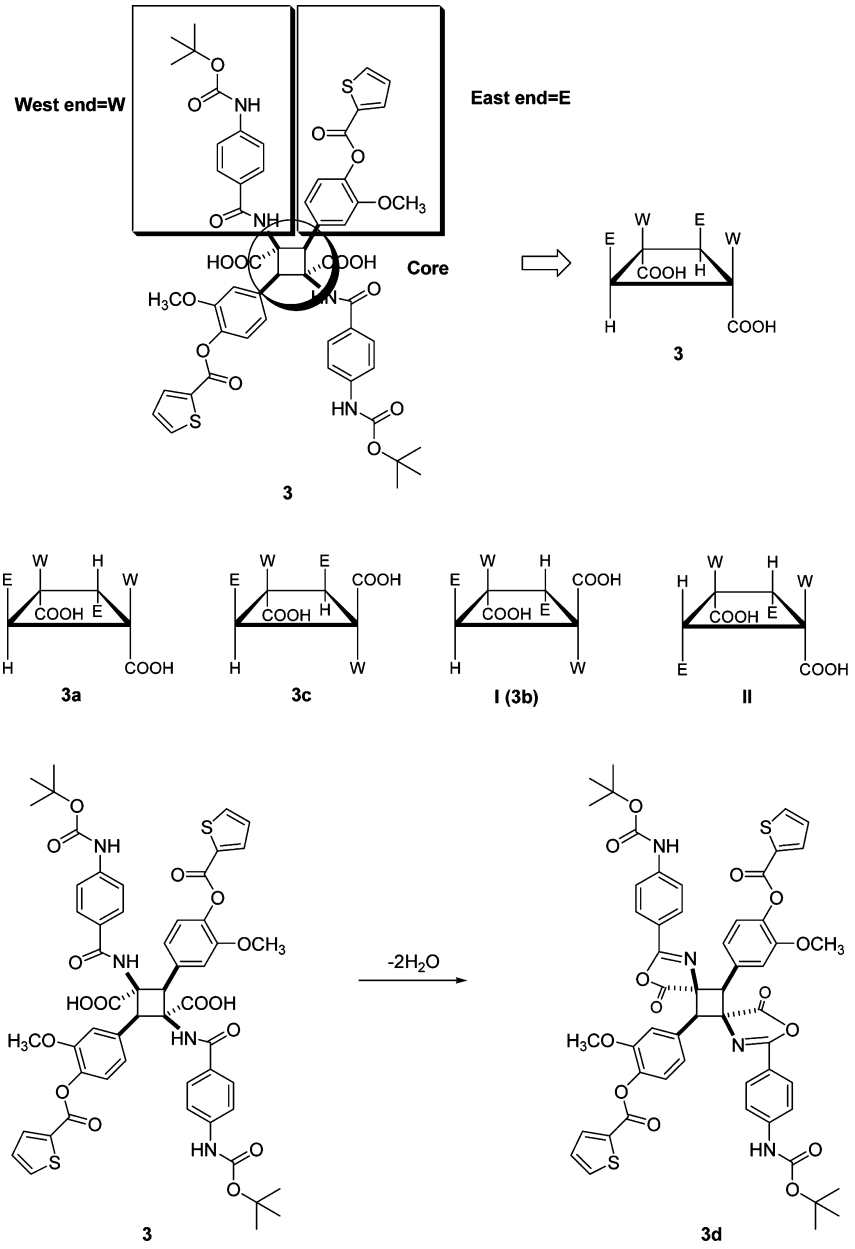


Figure 4. The relative configurations of **3**, its three isomers, and dehydrated product (**3a**–**d**).

as catalyst is concerned, photosensitizer diphenylmethanone[32] was selected for comparison, and the results showed no improvement on both reaction rate and yield.

The stability of **3** was also examined in both the solid and the solution state under the conditions of illuminating for 10 days or heating at 60 °C. The results showed that **3** was relatively stable either in solid state or solution with pH values between 1.8 and 7.0; illumination for 10 days or heated at 60 °C did not affect its stability (Supporting Information Figures S1–S3).

In Vitro Characterization of 3 and Its Analogues, SAR. The bioactivities of **3** and its three isomers differed significantly in both GLP-1R binding and MRE/CRE-driven reporter gene assays (Table 3). Among them, compound **3** was found to be the most potent GLP-1R agonist, exhibiting as high as 96.7% efficacy relative to the native peptide, GLP-1. When the configuration of one east end was reversed (**3a**), GLP-1 receptor activation was reduced to 68.2%, while reversal of one west end (**3c**) or simultaneous reversal of one east and one west end (**3b**) resulted in a dramatic decrease in agonist activities, implying that at least one east end and two west ends are essential to receptor activation. All four conformers demonstrated high GLP-1R binding affinities in competition with [^{125}I] GLP-1(7–36) amide, with **3** on the top of the list (87.2% inhibition). It is interesting that **3b** and **3c** behaved differently between receptor binding (42.0% and 64.7% inhibition, respectively) and reporter gene expression (16.5% efficacy and no activity, respectively).

Cyclopropane analogue **5** was designed and prepared to investigate the tolerance of the core structure for the receptor-binding affinity and receptor activation efficacy. However, it did not display any bioactivity in the HEK293-rGLP-1R cells, pointing to the importance of the cyclobutyl central skeleton. Likewise, the amide analogue **8** and the methyl ester analogue **9** (data not shown) were prepared to examine the role of the two carboxylic groups on the cyclobutane core. As above, no

dx.doi.org/10.1021/jm201150j | J. Med. Chem. 2012, 55, 250–267

Table 2. ^{1}H and ^{13}C NMR Data of 3 and Its Isomers 3a−3c (DMSO-d_6)[a]

	δ_C				δ_H (J Hz)			
no.	3	3a	3b	3c	3	3a	3b	3c
1/3	63.1	64.2	64.1	66.0				
2/4	48.5	54.9	51.6	48.7	4.94 (2H, s)	4.67 (1H, s)	5.32 (2H, s)	5.58 (2H, s)
		47.9				5.88 (1H, s)		
1,3-COOH	172.8	170.7	172.2	172.0	12.95 (2H, brs)	12.23 (2H, brs)	12.43 (2H, brs)	
1′/1‴	128.4	127.2	126.9	127.7				
				127.3				
2′/2‴	128.1	128.1	128.0	127.3	7.32 (2H, d, 8.5)	7.60 (2H, d, 8.5)	7.68 (2H, d, 8.7)	7.75 (1H, d, 8.8)
				127.7				7.46 (1H, d, 7.8)
3′/3‴	117.0	116.9	116.9	117.6	7.42 (2H, d, 8.5)	7.46 (2H, d, 8.5)	7.50 (2H, d, 8.6)	7.55 (1H, d, 8.3)
				117.0				7.34 (1H, d, 8.8)
4′/4‴	142.3	142.4	142.4	142.4				
				142.0				
5′/5‴	117.0	116.9	116.9	117.6	7.42 (2H, d, 8.5)	7.46 (2H, d, 8.5)	7.50 (2H, d, 8.6)	7.55 (1H, d, 8.3)
				117.0				7.34 (1H, d, 8.8)
6′/6‴	128.1	128.1	128.0	127.3	7.32 (2H, d, 8.5)	7.60 (2H, d, 8.5)	7.68 (2H, d, 8.7)	7.75 (1H, d, 8.8)
				127.7				7.46 (1H, d, 7.8)
7′/7‴	166.8	165.5	165.1	164.7				
8′/8‴	152.5	152.5	152.5	152.5				
				152.6				
9′/9‴	79.5	79.5	79.5	79.4				
				79.3				
10′/10‴	28.1	28.1	28.1	28.1	1.41 (18H, s)	1.49 (18H, s)	1.49 (18H, s)	1.45 (9H, s)
								1.42 (9H, s)
1″/1‴	133.6	135.5	134.7	134.8				
		134.4						
2″/2‴	112.6	114.0	114.1	112.7	7.26 (2H, brs)	7.25 (1H, brs)	7.25 (2H, brs)	7.46 (2H, brs)
		113.7				7.07 (1H, brs)		
3″/3‴	150.1	149.0	149.4	149.7				
		149.7						
4″/4‴	137.7	137.3	137.8	136.3				
		138.4						
5″/5‴	122.5	122.1	121.4	118.8	7.23 (2H, d, 8.3)	7.14 (1H, d, 8.3)	7.02 (2H, d, 8.2)	6.93 (2H, d, 8.3)
		121.2				6.83 (1H, d, 8.3)		
6″/6‴	122.1	122.5	122.9	121.4	7.17 (2H, brd, 8.1)	7.04 (1H, brd, 8.1)	7.14 (2H, brd, 8.3)	6.89 (2H, d, 8.3)
		123.7				6.90 (1H, brd, 8.1)		
7″/7‴	159.4	159.2	159.2	159.5				
		159.3						
8″/8‴	131.7	131.8	131.6	131.9				
		131.6						
9″/9‴	135.1	135.2	134.9	134.8	8.04 (2H, dd, 4.9, 1.1)	8.04 (1H, dd, 4.9, 1.1)	8.05 (2H, dd, 4.9, 0.8)	7.98 (2H, brd, 4.9)
		134.8				7.97 (1H, m)		
10″/10‴	128.7	128.8	128.6	128.6	7.27 (2H, dd, 4.9, 3.9)	7.27 (1H, dd, 4.9, 3.9)	7.27 (2H, dd, 4.6, 4.1)	7.21 (2H, dd, 3.9, 4.9)
		128.6				7.20 (1H, dd, 4.9, 3.9)		
11″/11‴	135.1	135.2	134.9	134.8	7.99 (2H, dd, 3.9, 1.1)	7.97 (1H, m)	7.96 (2H, dd, 3.9, 0.8)	7.90 (2H, brd, 3)
		134.8				7.87 (1H, dd, 3.6, 1.1)		
3″/3‴-OCH_3	54.9	55.5	55.4	55.5	3.23 (6H, s)	3.79 (3H, s)	3.57 (6H, s)	3.52 (6H, s)
		54.6				3.07 (3H, s)		
8′/8‴-CONH					9.52 (2H, s)	9.55 (2H, s)	9.59 (2H, s)	11.22 (brs, 1H)
								9.58 (s, 1H)
7′/7‴-CONH					8.55 (2H, s)	8.71 (2H, s)	8.95 (2H, s)	9.44 (2H, s)

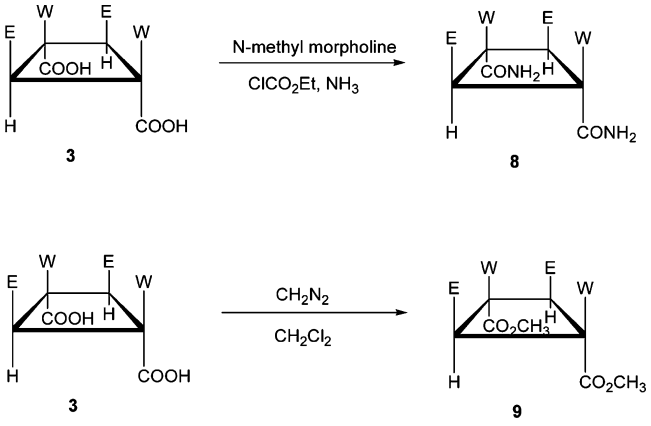
[a]Signals were assigned from the HMQC, HMBC, COSY and NOESY spectra.

cellular activity was detected for both compounds, thereby reaffirming our earlier observation that the cyclobutane core and the two carboxylic groups were essential for the bioactivity, and modifications such as decreasing the size of the ring and conversion of the acids to amides or esters were not tolerated at all.

We further tested compounds **4** and **10**−**28** to study the SAR of different substitutions on the west end of **3**. Compounds **10**−**18** were designed and synthesized to understand the influence of the length of side chain and the steric hindrance with the receptor by varying the R_1 groups (Scheme 2). As shown in Table 4, the size of cycloalkyl (**4** and **10**−**12**) and the

dx.doi.org/10.1021/jm201150j | J. Med. Chem. 2012, 55, 250−267

Scheme 1. Core Modifications of 3

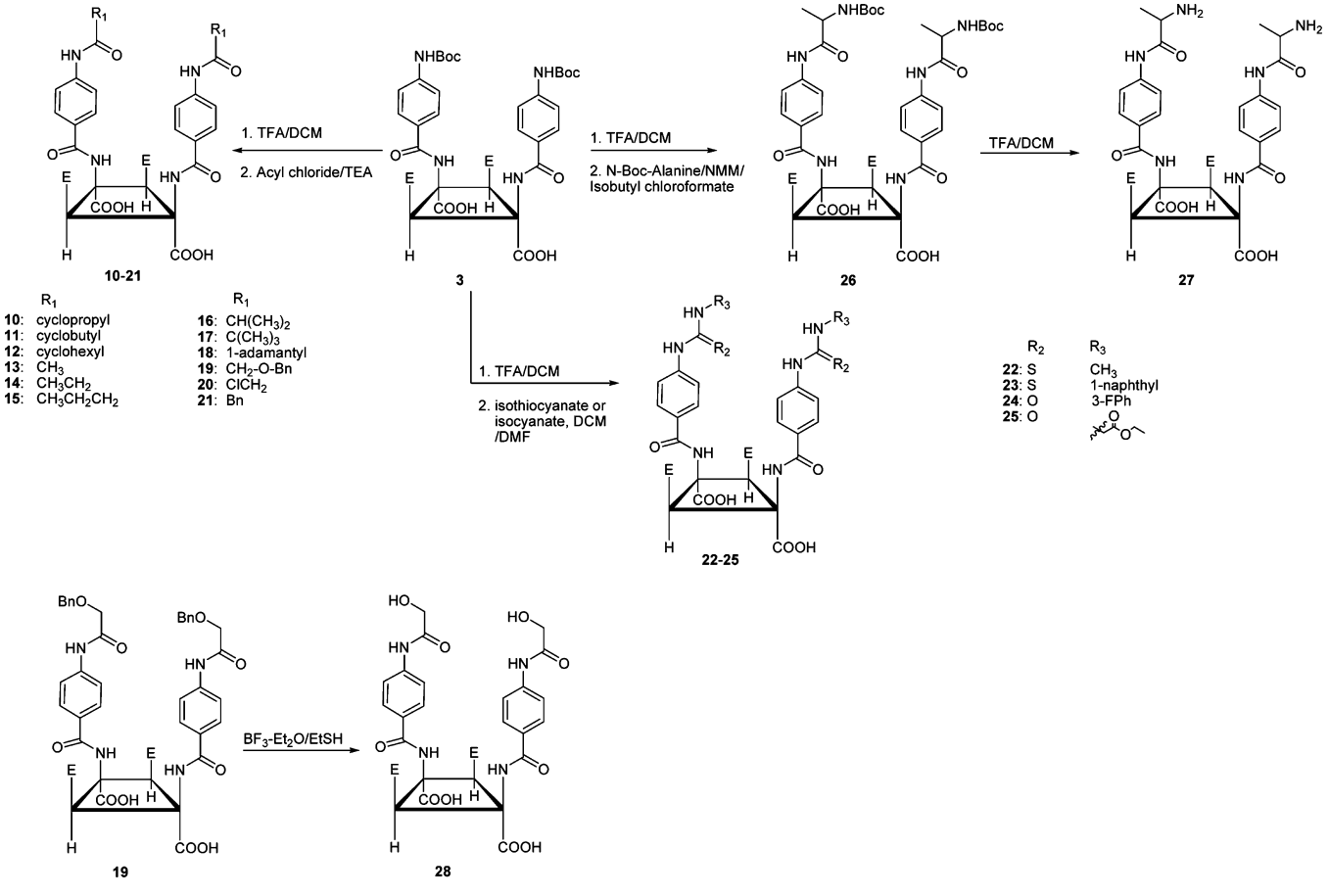


length of the side chain (**13**–**15**) as well as the steric hindrance of the R_1 group (**16**–**18**) lined up closely with the bioactivity. When the ring size was altered such as cyclopropyl (**10**), cyclobutyl (**11**), cyclopentyl (**4**), and cyclohexyl (**12**), bioactivity fluctuated with the cyclopropyl as the optimum group for both receptor binding and cellular activities. Compared with compound **10**, compound **11** was able to retain 87.5% efficacy in the reporter gene assay with a 40% decrease in receptor binding strength. Larger ring size led to an apparent decrease in the efficacy of reporter gene assay (**4**, 35.9%; **12**, 25%), indicative of a rather small binding pocket on the receptor. Changes in the length of the amido moieties (from two carbon to four carbons; compounds **13**–**15**) had little impact on the receptor binding affinity (IC_{50} = 2.3, 4.9, and 3.4 μM, respectively), and the cellular activity was better manifested when the length was two carbons (compound **13**, 61.1% efficacy; EC_{50} =118.1 nM). The effect of steric hindrance was also examined by comparing compounds **16**–**18**. It seemed that large steric hindrance did not affect the binding but reduced the cellular activity (**16** and **17** vs **18**), whereas medium steric hindrance could increase the receptor binding affinity (IC_{50} for compounds **16**, **17**, and **18** was 0.3, 0.5, and 8.0 μM, respectively). Compared with **3**, although the efficacy of compound **16** was 20% lower, it was 4- to 5-fold more potent than **3** in both the receptor binding and activation (Figure 5).

We also found that the amido groups could be replaced by thiourea or urea (**22**–**23** and **25**) without a dramatic drop in potency and efficacy. Methyl thiourea (**22**) showed a 68% activation on GLP-1R, but replacement of the methyl group with a 1-naphthyl group (**23**) resulted in 50% decrease in the reporter gene expression, implying large substituents were not well-tolerated. While compound **22** exhibited a similar binding affinity (IC_{50} = 1.4 μM) as **3**, its efficacy in stimulating cAMP release was 30% lower, suggesting a partial agonist feature. The electron-withdrawing moieties on compound **24** caused a significant loss of activity in both binding and reporter assays. Removal of this amido group (**55**) led to a complete loss of activity (Table 4 and Supporting Information Table S2).

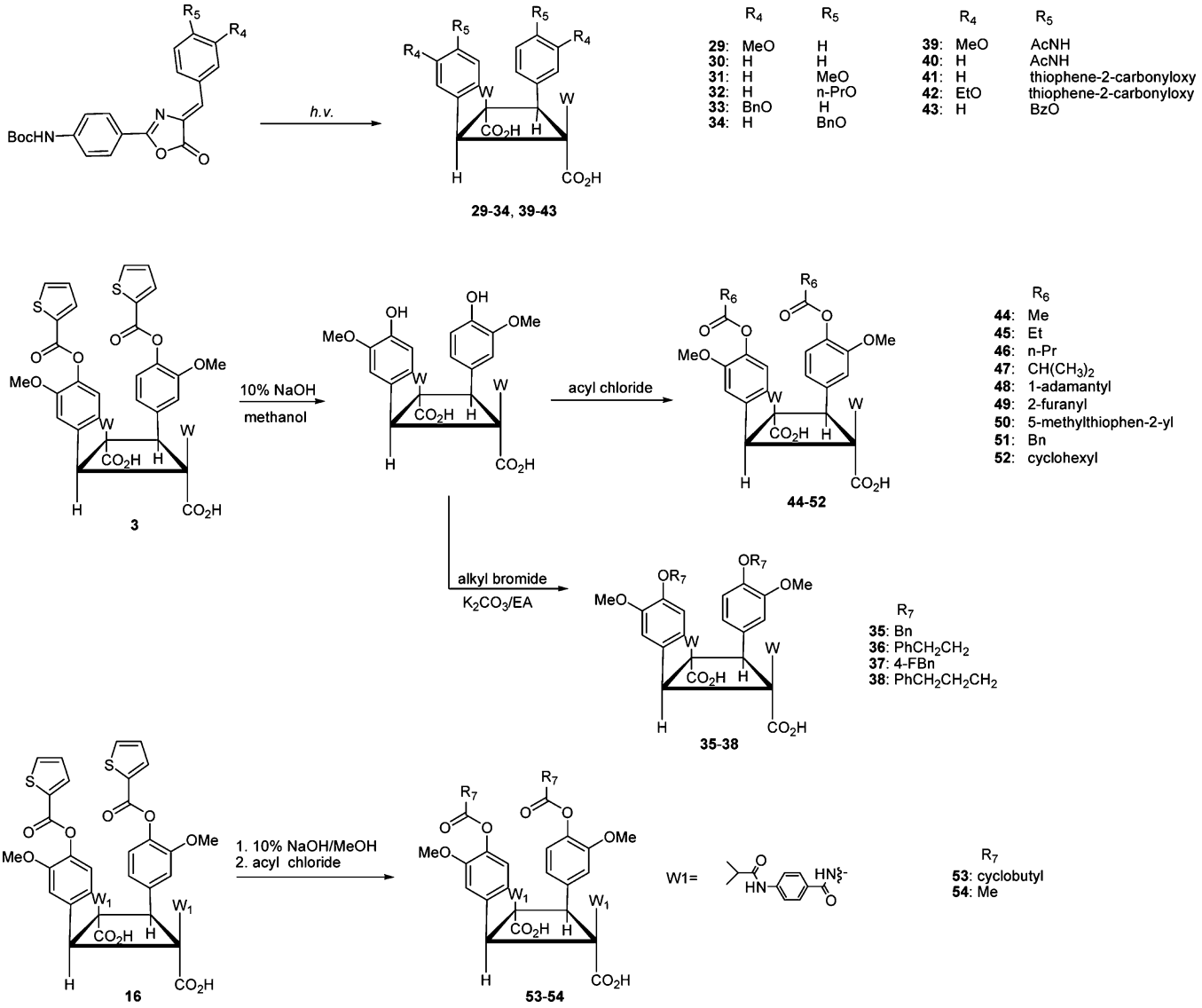
Induction of heteroatom (e.g., BocNH) improved rGLP-1R binding affinity by nearly 6-fold, but the effect on receptor activation was negligible (**26** vs **14**).

Scheme 2. Modifications of the West End



dx.doi.org/10.1021/jm201150j | J. Med. Chem. 2012, 55, 250–267

Scheme 3. Modifications of the East End



Finally, compounds **29**−**54** were prepared to inspect the necessity of ester and methoxy groups on the east end (Scheme 3 and Table 5). It was found that removal of the ester (**29**) led to the total loss of activity in terms of both receptor binding and reporter gene expression. A similar situation also applied to several other analogues (**30**−**38**), as only phenylethyl ether (**36**) exhibited 37.6% binding competition at GLP-1 receptors and benzyl ether (**34**) at the para position of the phenyl exhibited feeble receptor activation. Of the two amide analogues (**39** and **40**), compound **39** showed a weak GLP-1R binding property but none of them displayed any cellular agonist activity. This was consistent with our earlier observation that the ester group was essential for the GLP-1R bioactivity. 3-Alkanoxy groups were not necessary but they might contribute to the increased efficacy (**42** vs **41**, and **3** vs **41**). Furthermore, the 2-thiophenyl groups could be substituted by the straight alkyl chain (**44**−**46**), with three carbons as the optimal length (**45**). We also replaced the 2-thiophenyl groups with some large groups such as *i*-Pr (**47**) and 1-adamantyl (**48**). For compound **48**, the binding affinity to GLP-1R was 2-fold better than compound **47**, accompanied by an 86% efficacy in cAMP stimulation. Investigation with other heterocycles such as 2-furanyl (**49**) and 5-methylthiophen-2-yl (**50**) revealed that the replacement of 2-furanyl groups completely eliminated the receptor activation whereas the addition of methyl groups to the thiophene moieties (**50**) significantly reduced the binding affinity by 50% (**50** vs **3**).

Specificity of 3. The specificity of **3** for GLP-1R was studied in HEK293 cells transiently transfected with other related G-protein coupled receptors (GPCRs) such as GLP-2 receptor,[33] glucagon receptor[34] and glucose-dependent insulinotropic polypeptide (GIP) receptor.[35] Like GLP-1, compound **3** did not activate the MRE/CRE-driven luciferase activities in all these cells, whereas GLP-2, glucagon, and GIP could specifically induce the expression of their respective reporter genes, thereby confirming that **3** was a selective agonist of GLP-1 receptor (Figure 6).

In Vivo Activities of 16. As **3** was systematically characterized in both diabetic *db*/*db*[14] and diet-induced obese mice,[15] we continued to inspect the in vivo effects of **16** compared with **3**. In an acute experiment, compound **16** not only dose-dependently inhibited food intake at 6 h after intraperitoneal administration in male C57BL/6J mice but also demonstrated a 5.3-fold better potency than **3** (Figure 7).

dx.doi.org/10.1021/jm201150j | J. Med. Chem. 2012, 55, 250−267

Table 3. rGLP-1R Binding Assay and Reporter Gene Assay Results for 3, 3a−3c, and Core-Modified Analogues

	rGLP-1R binding assay[a]			reporter gene assay[b]	
compd	inhibition (%, competing with 40 pM [^{125}I] GLP-1 (7−36) amide)	inhibition (%, competing with 40 pM [^{125}I]Exendin(9−39))	IC_{50} (μM, competing with 40 pM [^{125}I] GLP-1(7−36) amide)	efficacy (%)	EC_{50} (nM)
GLP-1	100.0 ± 2.7	97.0 ± 2.1	1.0 ± 0.1	100.0 ± 5.1	0.09 ± 0.02
3	87.2 ± 1.3	63.1 ± 0.6	1.6 ± 0.1	96.7 ± 17.5	846.8 ± 287.3
3 (crystal)	89.8 ± 2.8	70.5 ± 2.3	0.6 ± 0.3	120.8[c]	370.7[c]
3a	73.4[c]	ND	1.3[c]	68.2 ± 13.7	ND
3b	42.0 ± 25.5	56.3[c]	4.3[c]	16.5 ± 4.5	ND
3c	64.7 ± 3.0	55.9 ± 3.7	3.6 ± 0.9	NA	NA
5	NA	NA	NA	NA	NA
8	NA	NA	NA	NA	NA
9	NA	NA	NA	NA	NA

[a]The compounds were evaluated for their binding abilities to rGLP-1R in competition with [^{125}I]GLP-1(7−36) amide and [^{125}I]Exendin(9−39) in HEK293-rGLP-1R cells, respectively.[13] [b]Agonist activities were assessed in HEK293-rGLP-1R cells.[13] The maximum response of GLP-1 at 10 nM was assigned as 100%. [c]The value was obtained from a single experiment. ND, not dertermined. NA, not active, defined as efficacy <15%, or potency >20 μM. Each value represents mean ± SEM of two independent experiments. The crystal of **3** used for X-ray crystallography was reconstituted in the solution before assaying in a manner similar to that of the dry powder.

Table 4. rGLP-1R Binding Assay and Reporter Gene Assay Results for the West End-Modified Analogues of 3

	rGLP-1R binding assay[a]			reporter gene assay[b]	
compd	inhibition (%, competing with 40 pM [^{125}I] GLP-1(7−36) amide)	inhibition (%, competing with 40 pM [^{125}I] Exendin(9−39))	IC_{50} (μM, competing with 40 pM [^{125}I] GLP-1(7−36) amide)	efficacy (%)	EC_{50} (nM)
GLP-1	100.0 ± 2.7	97.0 ± 2.1	0.001 ± 0.0001	100.0 ± 5.1	0.09 ± 0.02
3	87.2 ± 1.3	63.1 ± 0.6	1.6 ± 0.1	96.7 ± 17.5	846.8 ± 287.3
4	39.3 ± 3.3	37.9 ± 8.8	0.3 ± 0.2	35.9 ± 1.1	657.0 ± 422.8
10	86.4 ± 7.3	67.8 ± 5.2	1.2 ± 0.3	84.2[c]	147.8[c]
11	53.6 ± 8.6	45.6 ± 9.5	1.2 ± 0.6	87.5 ± 14.5	749.0 ± 635.0
12	58.9 ± 5.9	54.8 ± 3.0	1.0 ± 0.4	25.0[c]	ND
13	55.4[c]	30.6[c]	2.31[c]	61.1 ± 2.2	118.1 ± 112.9
14	75.2[c]	36.1[c]	4.9[c]	48.4 ± 19.1	695.8 ± 195.5
15	52.4 ± 12.2	48.2 ± 9.3	3.4 ± 0.6	50.0[c]	ND
16	58.2 ± 14.1	49.7 ± 14.6	0.3 ± 0.2	73.6 ± 17.5	202.5 ± 143.9
17	47.6[c]	33.4[c]	0.5[c]	60.8 ± 24.6	1114.5 ± 1085.1
18	50.7 ± 3.4	46.8 ± 5.1	8.0 ± 1.5	6.0[c]	ND
19	57.5 ± 11.2	45.9 ± 9.6	1.9 ± 0.6	12.0[c]	ND
20	43.8 ± 4.5	32.9 ± 5.7	8.5 ± 0.8	58.0[c]	3801.0[c]
21	45.7 ± 3.3	33.4 ± 3.6	3.2 ± 0.6	32.0[c]	ND
22	72.7[c]	70.1[c]	1.4[c]	68.0[c]	9120.0[c]
23	57.4[c]	52.3[c]	3.0[c]	29.8[c]	2125.0[c]
24	3.2 ± 1.3	4.7 ± 1.8	ND	7.7[c]	2706.0[c]
25	43.8 ± 7.2	37.9 ± 6.2	4.3 ± 1.5	33.7[c]	4496.0[c]
26	77.3 ± 5.9	69.7 ± 4.4	0.9 ± 0.8	49.0[c]	ND
27	35.2 ± 4.3	27.9 ± 2.7	16.2 ± 0.9	48.0[c]	ND
28	59.3 ± 4.5	52.7 ± 5.1	8.1 ± 0.6	38.0[c]	ND

[a]Analogues of **3** were evaluated for their abilities to compete binding of [^{125}I] GLP-1(7−36) amide and [^{125}I] Exendin(9−39) to rGLP-1R in HEK293-rGLP-1R cells, respectively.[13] [b]Agonist activities were assessed in HEK293-rGLP-1R cells.[13] The maximum response of GLP-1 at 10 nM was assigned as 100%. [c]Data obtained from one experiment. ND, not dertermined; NA, not active, defined as efficacy <15%, or potency >20 μM. Each value represents mean ± SEM of two independent experiments.

The in vivo effects of **3** and **16** on the body weight reduction were also evaluated in a mouse model of diet-induced obesity. As shown in Figure 8, chronic treatment (ip) with this molecule led to a dose-dependent and significant reduction in body weight, which was sustained during the entire treatment period (28 days). The weight loss effect elicited by **16** was nearly twice that of **3** with an identical treatment regimen (3 mg), reaching to the control level where normal diet was consumed. These in vivo results clearly collaborated with those generated in vitro, indicating that compound **16** was more potent than its parent molecule **3**.

CONCLUSIONS

A novel class of nonpeptidic GLP-1R agonists, cyclobutane analogues, was discovered using receptor binding and MRE/CRE-driven reporter gene assays. A series of structural modifications were performed on and surrounding the core with different substitution groups at the west and east ends.

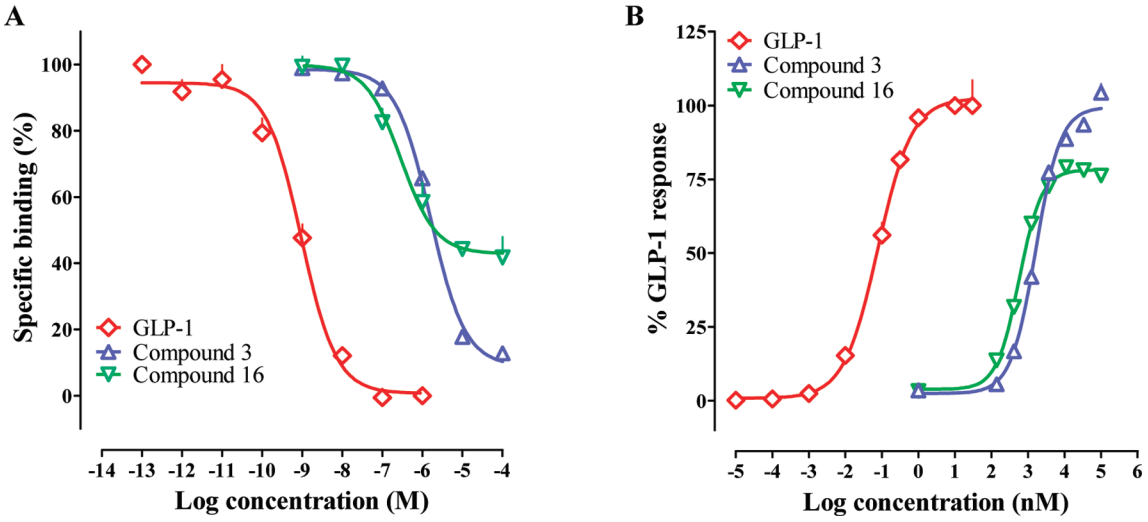


Figure 5. Bioactivities of **16** in rat GLP-1 receptor binding and reporter gene assays. (A) Competitive inhibition of [^{125}I] GLP-1(7−36) amide binding to rat GLP-1R by GLP-1, compound **3**, and compound **16**. (B) Multiple response element and cAMP response element-driven luciferase activities were induced by different concentrations of GLP-1, compound **3**, and compound **16** in the HEK293-rGLP-1R cells. Values are mean ± SEM, representative of two independent experiments.

Table 5. rGLP-1R Binding Assay and Reporter Gene Assay Results for the East End-Modified Analogues of 3

	rGLP-1R binding[a]			reporter gene assay[b]	
compd	inhibition (%, competing with 40 pM [^{125}I] GLP-1(7−36) amide)	inhibition (%, competing with 40 pM [^{125}I]Exendin(9−39))	IC_{50} (μM, competing with 40 pM [^{125}I] GLP-1(7−36) amide)	efficacy (%)	EC_{50} (nM)
GLP-1	100 ± 2.7	97 ± 2.1	0.001 ± 0.0001	100.0 ± 5.1	0.09 ± 0.02
3	87.2 ± 1.3	63.1 ± 0.6	1.6 ± 0.1	96.7 ± 17.5	846.8 ± 287.3
29	NA	NA	NA	NA	NA
30	NA	NA	NA	NA	NA
31	7.45[c]	2.4[c]	ND	7.5 ± 0.6	9404.0[c]
32	NA	NA	NA	NA	NA
33	NA	NA	NA	NA	NA
34	21.2 ± 3.7	9.6[c]	ND	24.8 ± 3.8	ND
35	10[c]	14.3[c]	ND	NA	NA
36	37.6 ± 6.4	29.8 ± 5.2	ND	5.6[c]	ND
37	NA	NA	NA	NA	NA
38	NA	NA	NA	NA	NA
39	32.5 ± 2.5	9.4 ± 3.1	ND	NA	NA
40	12.1 ± 2.4	2.8 ± 1.7	ND	NA	NA
41	63.7[c]	NA	ND	63.7 ± 7.4	ND
42	50.2[c]	NA	3.1[c]	85.6 ± 12.5	326.0 ± 80.0
43	13.5 ± 8.1	6.3 ± 1.5	ND	21.6 ± 4.4	ND
44	43.6[c]	NA	ND	43.6 ± 3.5	8310.0 ± 425.8
45	86 ± 3.7	68.3 ± 4.6	2.7 ± 0.7	91.0[c]	2499.0[c]
46	65.1 ± 9.5	53.8 ± 9.8	7.5 ± 1.1	86.0[c]	3334.0[c]
47	47.2 ± 7.6	34.8 ± 8.1	7.5 ± 0.7	65.0[c]	1380.0[c]
48	75.8 ± 8.1	63.5 ± 8.7	3.6 ± 0.6	86.0[c]	2149.0[c]
49	27.6 ± 4.4	8.3 ± 1.6	ND	NA	NA
50	40.1 ± 5.2	29.4 ± 5.8	17.5 ± 4.9	67.0[c]	19055.0[c]
51	17.5 ± 3.5	4.8 ± 1.6	ND	26.0[c]	ND
52	NA	NA	NA	NA	NA
53	47.9 ± 6.0	36.8 ± 3.3	9.6 ± 1.6	50.7[c]	15820.0[c]
54	34.1 ± 5.3	35.2 ± 6.2	ND	30.0[c]	ND

[a]The compounds were evaluated for their binding abilities to rGLP-1R in competition with [^{125}I]GLP-1(7−36) amide and [^{125}I]Exendin(9−39) in HEK293-rGLP-1R cells, respectively.[13] [b]Agonist activities were assessed in HEK293-rGLP-1R cells.[13] The maximum response of GLP-1 at 10 nM was assigned as 100%. [c]Data obtained from one experiment. ND, not dertermined; NA, not active, defined as efficacy <15% or potency >20 μM. Each value represents mean ± SEM of two independent experiments.

In vitro characterization of these analogues demonstrated that the cyclobutane core and the two carboxylic groups were essential for the bioactivity and modifications such as decreasing the size of the ring and conversion of the acids to

dx.doi.org/10.1021/jm201150j | J. Med. Chem. 2012, 55, 250−267

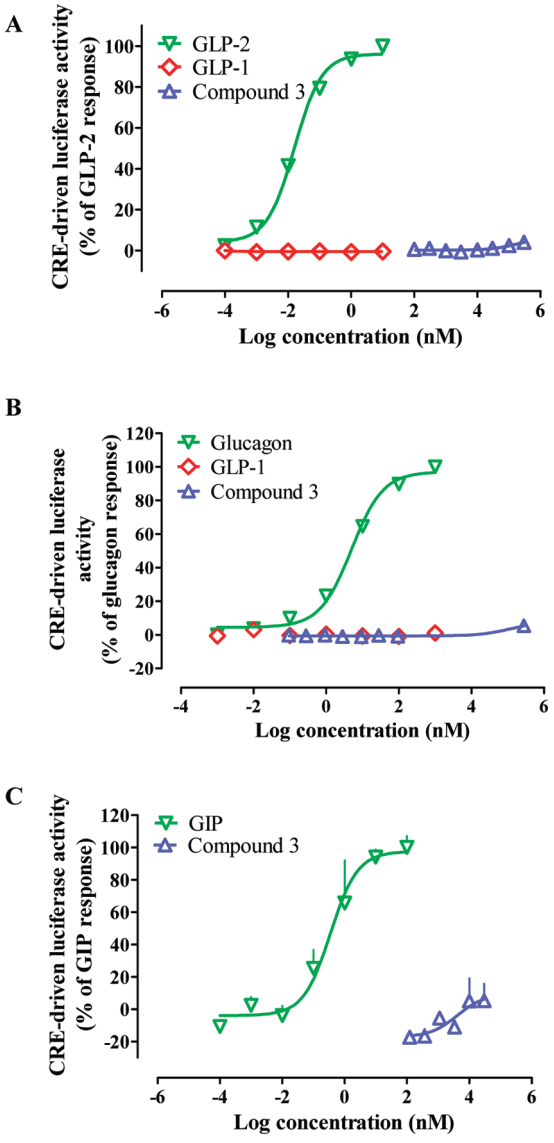


Figure 6. Interaction of compound **3** with GLP-2 (A), glucagon (B), and GIP receptors (C). Plasmids containing three copies of multiple response element and one copy of cAMP response element-driven luciferase gene were cotransfected with human GLP-2 receptor, glucagon receptor or GIP receptor expression vectors into HEK293 cells, respectively. Relative luciferase activities induced by different concentrations of compound **3** were compared with those elicited by GLP-2, glucagon, and GIP (100%), respectively. Each value represents mean ± SEM of triplicate experiments.

amide and ester led to the total loss of activity. The Boc and the 2-thiophenyl groups were well-tolerated, with **16** as the optimum that consistently displayed more potent GLP-1 activities (than **3**) both in vitro and in vivo. Preliminary structure–activity relationship studies suggested that these cyclobutane analogues may serve as a starting point for the development of new GLP-1 mimetics.

EXPERIMENTAL SECTION

Chemistry. *General Statement.* Reagents were commercial grade and were used as received unless otherwise noted. The structures of all new compounds were consistent with their ^{1}H, ^{13}C NMR, and mass spectra, and were judged to be ≥95% pure by HPLC. NMR spectra were recorded on Varian Mercury 300, Bruker AN-400, and Varian Inova 600 spectrometers. Chemical shifts were reported in

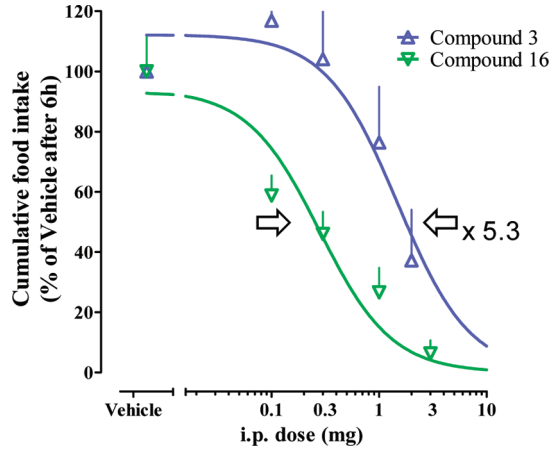


Figure 7. Effects of compound **3** and compound **16** on acute food intake. Dose–response characteristics were assessed at 6 h following an intraperitoneal injection (ip) of different doses in male C57BL/6J mice ($n \geq 6$ per dose group). The amount of food consumed was recorded every 15 or 30 min for 6 h. Control animals received vehicle treatment. Data shown are mean ± SEM.

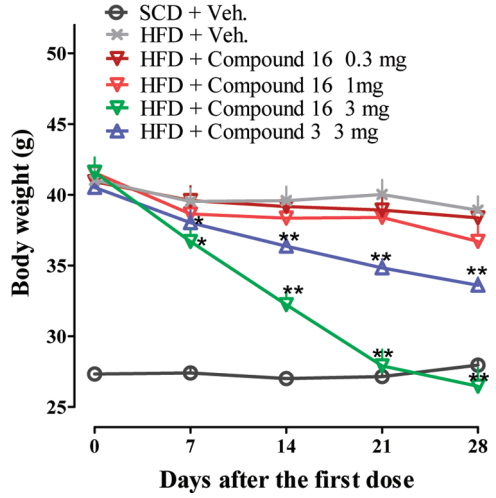


Figure 8. Effects of chronically administered (ip) compound **16** on body weight of diet-induced obese mice (n = 8 or 9 per dose group). Values given are mean ± SEM in all panels. SCD, standard chow diet; HFD, high-fat diet.

parts per million (ppm), with the solvent resonance as the internal standard (CD_3OD 3.31 ppm, DMSO-d_6 2.50 ppm for ^{1}H NMR; DMSO-d_6 39.52 ppm for ^{13}C NMR). Low resolution mass spectral data (electrospray ionization) were acquired on a Finnigan LCQ-DECA mass spectrometer. High resolution mass spectral data were collected on a Finigan MAT 95 and Waters Q-Tof of Ultima mass spectrometer. Samples were analyzed for purity on a HP1100 series equipped with a Zorbax SB-C18 column (5 μm, 4.6 mm × 250 mm). Purities of final compounds were determined using a 5 μL injection with quantitation by AUC at 210 and 254 nm (Agilent diode array detector). The HPLC retention time (RT) was recorded through an isocratic mobile phase of 0.1% formic acid-containing acetonitrile and water (65:35) over 15 min with a flow rate of 1 mL/min. Reversed-phase silica gel (ODS) (20–45 μm, Fuji Silysia Chemical Co., Ltd.) was used for column chromatography. The monomers including **1** and **2** were prepared according to the previous literatures.[19–24]

General Procedure A for the Photodimerization. Compound **2** (0.5 g) was dissolved in 12 mL of DMSO and illuminated under a 500 W ultraviolet high-pressure mercury lamp for 3 days. The reaction solution was monitored by HPLC. After the raw material disappeared,

dx.doi.org/10.1021/jm201150j | J. Med. Chem. 2012, 55, 250–267

the solution was lyophilized to remove solvent and the residue was separated with ODS column chromatography eluting with a gradient mixtures of acetonitrile and water at ratios of 40:60, 45:55, 50:50, 55:45, 60:40, 65:35, 70:30, 80:20, 90:10, and 100:0. Thus, the products were obtained as follows: **3** (55 mg, 10.6%), **3a** (22 mg, 4.2%), and a small amount of **3b**, **3c**, and dehydrated product **3d**. The total yield is 14.8%.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-((t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**3**).* The purity was 99% by HPLC analysis. RT = 5.1 min. ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) data see Table 2. HRMS (ESI) m/z calcd for $C_{54}H_{52}N_4O_{16}NaS_2$ $[M + Na]^+$ 1099.2717, found 1099.2756.

*(1,2-trans-2,3-trans-3,4-cis)-1,3-Bis(4-((t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**3a**).* The purity was 96% by HPLC analysis. RT = 5.4 min. ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) data see Table 2. HRMS (ESI) m/z calcd for $C_{54}H_{52}N_4O_{16}NaS_2$ $[M + Na]^+$ 1099.2717, found 1099.2753.

*(1,2-trans-2,3-cis-3,4-trans)-1,3-Bis(4-((t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**3b**).* The purity was 99% by HPLC analysis. RT = 5.0 min. ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) data see Table 2. HRMS (ESI) m/z calcd for $C_{54}H_{52}N_4O_{16}NaS_2$ $[M + Na]^+$ 1099.2717, found 1099.2747.

*(1,2-cis-2,3-trans-3,4-trans)-1,3-bis(4-((t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**3c**).* The purity was 96% by HPLC analysis. RT = 5.1 min. ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) data see Table 2. HRMS (ESI) m/z calcd for $C_{54}H_{52}N_4O_{16}NaS_2$ $[M + Na]^+$ 1099.2717, found 1099.2702.

*(5,6-cis-6,7-cis-7,12-cis)-2-Methoxy-4-(2,9-bis(4-(t-butoxycarbonyl)amino)phenyl)-12-(3-methoxy-4-((thiophen-2-yl)-carbonyloxy)phenyl)-4,11-dioxo-3,10-dioxa-1,8-diazadispiro-[4.1.4^7.1^5]dodeca-1,8-dien-6-yl)phenylthiophene-2-carboxylate (**3d**).* The purity was 96% by HPLC analysis. RT = 5.8 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.48 (s, 18H), 3.76 (s, 6H), 4.89 (s, 2H), 7.15 (d, J = 8.2 Hz, 2H), 7.18 (d, J = 8.2 Hz, 2H), 7.27 (t, J = 3.8 Hz, 2H), 7.57 (s, 2H), 7.65 (d, J = 8.6 Hz, 4H), 7.91 (d, J = 8.8 Hz, 4H), 7.95 (dd, J_1 = 1.3 Hz, J_2 = 3.9 Hz, 2H), 8.02 (dd, J_1 = 1.3 Hz, J_2 = 4.8 Hz, 2H), 9.68 (s, 2NH). MS (ESI) m/z 1063.2 $[M + Na]^+$. HRMS (ESI) m/z calcd for $C_{54}H_{48}N_4O_{14}NaS_2$ $[M + Na]^+$ 1063.2506, found 1063.2528.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(((4-cyclopentanecarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**4**).* Compound **4** was obtained using the general procedure A with **1** as the raw material in 10.0% yield. The purity was 96% by HPLC analysis. RT = 5.0 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.90 (brs, 2H), 10.05 (brs, 2H), 8.63 (brs, 2H), 8.09 (dd, J_1 = 4.8 Hz, J_2 = 1.2 Hz, 2H), 8.03 (dd, J_1 = 3.6 Hz, J_2 = 1.2 Hz, 2H), 7.61 (d, J = 8.4 Hz, 4H), 7.40 (d, J = 8.1 Hz, 4H), 7.31 (dd, J_1 = 4.2 Hz, J_2 = 3.0 Hz, 2H), 7.28 (brs, 2H), 7.26 (m, 2H), 7.21 (brd, J = 8.1 Hz, 2H), 4.99 (brs, 2H), 3.24 (s, 6H), 2.74 (m, 2H), 1.82 (m, 4H), 1.70 (m, 4H), 1.65 (m, 4H), 1.53 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.7, 172.8, 166.8, 159.4, 150.1, 142.1, 137.7, 135.2, 133.6, 131.6, 129.1, 128.7, 128.1, 122.5, 122.1, 118.1, 112.5, 63.2, 54.9, 48.4, 45.3, 30.0, 25.7. HRMS (ESI) m/z calcd for $C_{56}H_{52}N_4O_{14}NaS_2$ $[M + Na]^+$ 1091.2819, found 1091.2856.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-diformamide (**8**).* Compound **3** (0.5 g, 0.46 mmol) was dissolved in 20 mL anhydrous tetrahydrofuran followed by addition of *N*-methylmorpholine (0.1 mL, 0.92 mmol). The mixture was stirred and cooled to −10 °C in an ice bath. Then ethyl chloroformate (0.1 mL, 0.92 mmol) was added under nitrogen. The mixture was stirred for 30 min, and a white solid was precipitated. Ammonia was bubbled into the solution at −10 °C for 10 min, and the solution was stood overnight in refrigerator. Anhydrous tetrahydrofuran (30 mL) was then added, and the solution was filtered with suction. The cake was washed with tetrahydrofuran (10 mL × 3), and the filtrate was combined and evaporated to give a crude product, which was further crystallized with ether/ethanol to give white powder (0.45 g, 91.0%). The purity was 97% by HPLC analysis. RT = 5.2 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.44 (s, 18H), 3.32 (s, 6H), 4.93 (s, 2H), 7.16 (d, J = 8.1 Hz, 2H), 7.31 (t, J = 4.9 Hz, 2H), 7.31 (d, J = 8.9 Hz, 2H), 7.33 (s, 2H), 7.38 (d, J = 8.8 Hz, 4H), 7.42 (d, J = 8.7 Hz, 4H), 8.02 (d, J = 3.8 Hz, 2H), 8.08 (d, J = 5.0 Hz, 2H), 8.19 (s, 2H), 9.51 (s, 2H). MS (ESI) m/z 1097.4 $[M + Na]^+$. HRMS (ESI) m/z calcd for $C_{54}H_{54}N_6O_{14}NaS_2$ $[M + Na]^+$ 1097.3037, found 1097.3019.

*General Procedure B for the Compounds **10**–**21**.* Compound **3** (0.2 g, 0.19 mmol) was dissolved in 4 mL of dichloromethane, and the mixture was cooled to 0 °C in an ice bath. Trifluoroacetic acid (0.74 mL, 9.60 mmol) was added slowly at this temperature. The ice bath was removed afterward and the solution was stirred at room temperature for 2 h. The solvent was removed in vacuo, and the resultant residue was dissolved subsequently in 4 mL of dichloromethane, to which catalytic triethylamine was added. The solution was stirred and cooled to 0 °C in ice bath, and acyl chloride (4.60 mmol) was added dropwise into the mixture. The reaction was stirred at the room temperature overnight and then poured into water and extracted with dichloromethane (20 mL × 3). The combined organic layers were washed with water and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo and the residue was further stirred in 10 mL of tetrahydrofuran by addition of 10% NaOH solution (pH 10) for 30 min. After neutralization, it was condensed and purified on chromatographic plates (0.4–0.5 mm) with methanol as the developing phase.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(cyclopropanecarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**10**).* Compound **10** was prepared according to the general procedure B above, in which cyclopropanecarbonyl chloride was used as the acylating reagent, and a light-yellow powder was obtained in 40.0% yield. The purity was 96% by HPLC analysis. RT = 5.4 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.76 (s, 8H), 1.21 (s, 2H), 3.35 (s, 6H), 5.32 (s, 2H), 6.92 (d, J = 8.4 Hz, 4H), 7.23 (t, J = 8.7 Hz, 2H), 7.28 (s, 2H), 7.62–7.68 (m, 8H), 7.89 (d, J = 3.5 Hz, 2H), 8.0 (d, J = 4.9 Hz, 2H), 10.62 (s, 2NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 176.5, 172.1, 167.5, 159.5, 149.9, 142.1, 137.0, 136.5, 134.9, 133.0, 131.8, 129.4, 128.6, 127.6, 121.6, 121.1, 118.3, 112.2, 62.8, 55.1, 52.0, 14.6, 7.4.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(cyclobutanecarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**11**).* Compound **11** was prepared according to the general procedure B above, in which cyclobutanecarbonyl chloride was used as the acylating reagent, and a light-yellow powder was obtained in 42.0% yield. The purity was 97% by HPLC analysis. RT = 5.6 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.79–1.80 (m, 2H), 1.87–1.95 (m, 2H), 2.06–2.24 (m, 8H), 3.04–3.13 (m, 2H), 3.24 (s, 6H), 4.99 (s, 2H), 7.22 (d, J = 8.3 Hz, 2H), 7.28 (d, J = 8.4 Hz, 2H), 7.29 (s, 2H), 7.33 (t, J = 4.7 Hz, 2H), 7.39 (d, J = 8.7 Hz, 4H), 7.61 (d, J = 8.6 Hz, 4H), 8.04 (d, J = 3.8 Hz, 2H), 8.11 (d, J = 4.9 Hz, 2H), 8.36 (s, 2H), 9.92 (s, 2H). MS (ESI) m/z 1040.2 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(cyclohexanecarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**12**).* Compound **12** was prepared according to the general procedure above, in which cyclohexanecarbonyl chloride was used as the acylating reagent, and a light-yellow powder was obtained in 44.0% yield. The purity was 96% by HPLC analysis. RT = 5.6 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.20–1.21 (m, 12H), 1.68–1.82 (m, 8H), 2.19–2.33 (m, 2H), 3.25 (s, 6H), 4.99 (s, 2H), 7.21–7.22 (d, J = 8.0 Hz, 2H), 7.26 (s, 2H), 7.27 (d, J = 8.3 Hz, 2H), 7.33 (t, J = 4.0 Hz, 2H), 7.40 (d, J = 8.5 Hz, 4H), 7.61 (d, J = 8.7 Hz, 4H), 8.03 (d, J = 3.7 Hz, 2H), 8.10 (d, J = 5.0 Hz, 2H), 8.60 (s, 2H). MS (ESI) m/z 1097.3 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-acetamidobenzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**13**).* Compound **13** was prepared according to general procedure B above, in which acetyl chloride was used as the

dx.doi.org/10.1021/jm201150j | J. Med. Chem. 2012, 55, 250–267

acylating reagent and a light-yellow powder was obtained in 50.0% yield. The purity was 96% by HPLC analysis. RT = 5.3 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.12 (brs, 2H), 8.62 (brs, 2H), 8.10 (dd, J_1 = 4.8 Hz, J_2 = 1.2 Hz, 2H), 8.03 (dd, J_1 = 3.9 Hz, J_2 = 1.5 Hz, 2H), 7.57 (d, J = 8.4 Hz, 4H), 7.37 (d, J = 8.7 Hz, 4H), 7.32 (dd, J_1 = 3.9 Hz, J_2 = 5.1 Hz, 2H), 7.28 (m, 2H), 7.26 (m, 2H), 7.20 (brd, J = 8.1 Hz, 2H), 4.98 (brs, 2H), 3.23 (s, 6H), 2.02 (s, 6H). ^{13}C NMR (DMSO-d_6, 75 MHz,): δ 176.5, 168.8, 165.1, 159.5, 149.9, 142.0, 136.9, 136.5, 134.9, 131.7, 129.5, 128.6, 127.5, 121.6, 120.2, 118.3, 112.3, 64.9, 55.1, 52.0, 24.0. MS (ESI) m/z 983.0 $[M + Na]^+$, 961.1 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-propionamidobenzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**14**).* Compound **14** was prepared according to the general procedure B above, in which propionyl chloride was used as the acylating reagent, and a light-yellow powder was obtained in 50.0% yield. The purity was 96% by HPLC analysis. RT = 6.9 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.07 (t, J = 7.4 Hz, 6H), 2.35 (q, J = 7.4 Hz, 4H), 3.36 (s, 6H), 5.28 (s, 2H), 6.95 (brs, 4H), 7.26 (t, J = 4.6 Hz, 2H), 7.33 (s, 2H), 7.67 (brs, 8H), 7.93 (d, J = 2.9 Hz, 2H), 8.04 (d, J = 4.7 Hz, 2H), 10.24 (s, 2NH). MS (ESI) m/z 987.1 $[M - H]^-$. HRMS (ESI) m/z calcd for $C_{50}H_{43}N_4O_{14}S_2$ $[M - H]^-$ 987.2217, found 987.2211.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-butyramidobenzamido)-2,4-bis-(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**15**).* Compound **15** was prepared according to the general procedure B above, in which butyryl chloride was used as the acylating reagent, and the light-yellow powder was obtained in 48% yield. The purity was 96% by HPLC analysis. RT = 5.6 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.91 (t, J = 6.9 Hz, 6H), 1.58−1.63 (m, 4H), 2.31 (t, J = 7.1 Hz, 4H), 3.35 (s, 6H), 5.26 (s, 2H), 6.80 (s, 2H), 6.93 (brs, 2H), 6.97 (d, J = 8.8 Hz, 2H), 7.26 (t, J = 4.2 Hz, 2H), 7.67 (brs, 8H), 7.93 (d, J = 3.0 Hz, 2H), 8.04 (d, J = 5.0 Hz, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 176.1, 171.7, 165.2, 159.5, 150.0, 142.2, 136.6, 136.0, 134.9, 131.8, 129.3, 128.6, 127.6, 121.8, 120.2, 118.4, 112.1, 64.8, 55.1, 51.9, 35.0, 18.5, 13.6.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-isobutyramidobenzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**16**).* Compound **16** was prepared according to the general procedure B above, in which isobutyryl chloride was used as the acylating reagent, and a light-yellow powder was obtained in 40.0% yield. The purity was 96% by HPLC analysis. RT = 5.7 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.12 (d, J = 6.8 Hz, 12H), 2.60−2.65 (m, 2H), 3.77 (s, 6H), 4.62 (s, 2H), 7.12 (d, J = 8.2 Hz, 2H), 7.23 (s, 2H), 7.27 (d, J = 4.0 Hz, 2H), 7.63−7.64 (m, 2H), 7.85 (d, J = 8.7 Hz, 4H), 7.92 (d, J = 2.9 Hz, 2H), 8.02 (d, J = 8.8 Hz, 4H), 8.05 (d, J = 5.0 Hz, 2H), 10.28 (s, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 175.1, 172.3, 166.3, 159.0, 149.7, 141.6, 137.2, 134.7, 133.1, 131.2, 128.7, 128.3, 127.7, 122.1, 121.7, 117.7, 112.0, 62.8, 54.4, 47.9, 34.5, 18.9.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-pivalamidobenzamido)-2,4-bis-(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**17**).* Compound **17** was prepared according to the general procedure B above, in which pivaloyl chloride was used as the acylating reagent, and a light-yellow powder was obtained in 45.0% yield. The purity was 97% by HPLC analysis. RT = 5.6 min. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.23 (s, 18H), 3.38 (s, 6H), 5.39 (s, 2H), 6.90 (d, J = 8.0 Hz, 2H), 7.00 (d, J = 8.2 Hz, 2H), 7.70 (s, 2H), 7.26 (t, J = 4.8 Hz, 2H), 7.66 (brs, 4H), 7.79 (d, J = 8.8 Hz, 4H), 7.93 (dd, J_1 = 1.2 Hz, J_2 = 3.8 Hz, 2H), 8.04 (dd, J_1 = 1.2 Hz, J_2 = 5.0 Hz, 2H), 9.56 (s, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 176.9, 174.8, 165.1, 159.4, 150.0, 142.3, 137.4, 136.7, 135.3, 135.0, 131.7, 129.1, 128.6, 127.3, 121.9, 120.0, 119.5, 111.9, 64.5, 55.1, 52.1, 45.4, 27.1.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-((1-adamantane)carbonyl)-amino)benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**18**).* Compound **18** was prepared according to the general procedure B above, in which 1-adamantanecarbonyl chloride was used as the acylating reagent, and a light-yellow powder was obtained in 42.0% yield. The purity was 96% by HPLC analysis. RT = 5.6 min. ^{1}H NMR (CD_3Cl, 400 MHz): δ 1.71 (brs, 12H), 1.92 (brs, 12H), 2.02 (brs, 6H), 3.31 (s, 6H), 5.20 (s, 2H), 6.87 (d, J = 8.0 Hz, 2H), 6.99 (d, J = 8.0 Hz, 2H), 7.17 (brs, 2H), 7.26 (t, J = 5.0 Hz, 2H), 7.67 (brs, 4H), 7.75 (d, J = 8.4 Hz, 4H), 7.94 (dd, J_1 = 1.2 Hz, J_2 = 3.7 Hz, 2H), 8.04 (dd, J_1 = 1.1 Hz, J_2 = 4.9 Hz, 2H), 9.33 (s, 2NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 176.2, 174.5, 164.9, 159.4, 149.9, 142.2, 136.6, 136.2, 134.9, 131.7, 129.3, 128.6, 127.3, 121.8, 120.0, 119.4, 112.0, 64.6, 55.1, 51.7, 45.6, 41.1, 36.0, 27.6.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-(2-benzyloxy)-acetamidobenzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**19**).* Compound **19** was prepared according to the general procedure B above, in which benzyloxyacetyl chloride was used as the acylating reagent, and a light-yellow powder was obtained in 40.0% yield. The purity was 96% by HPLC analysis. RT = 5.7 min. ^{1}H NMR (CD_3Cl, 400 MHz): δ 3.35 (s, 6H), 4.12 (s, 4H), 4.62 (s, 4H), 6.95 (d, J = 8.0 Hz, 2H), 7.02 (d, J = 8.0 Hz, 2H), 7.21 (s, 2H), 7.26 (t, J = 3.9 Hz, 2H), 7.31 (t, J = 7.0 Hz, 4H), 7.37 (t, J = 7.2 Hz, 2H), 7.41 (d, J = 7.2 Hz, 4H), 7.71 (d, J = 8.0 Hz, 4H), 7.75 (d, J = 8.2 Hz, 4H), 7.94 (d, J = 4.0 Hz, 2H), 8.03 (d, J = 4.8 Hz, 2H), 10.09 (s, 2NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 176.0, 168.5, 165.2, 159.5, 150.1, 141.4, 137.7, 136.9, 136.2, 134.9, 131.8, 129.9, 128.6, 128.3, 127.9, 127.7, 127.6, 122.0, 120.5, 119.2, 112.5, 72.2, 69.5, 64.9, 55.2, 51.8.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-(2-chloroacetamido)-benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**20**).* Compound **20** was prepared according to the general procedure B above, in which 2-chloroacetyl chloride was used as the acylating reagent, and a light-yellow powder was obtained in 35.0% yield. The purity was 97% by HPLC analysis. RT = 5.7 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.76 (s, 6H), 4.31 (s, 4H), 4.63 (s, 2H), 7.11 (d, J = 8.2 Hz, 2H), 7.23 (dd, J_1 = 1.7 Hz, J_2 = 6.0 Hz, 2H), 7.25 (t, J = 4.8 Hz, 2H), 7.61 (d, J = 1.6 Hz, 2H), 7.82 (d, J = 8.7 Hz, 4H), 7.91 (dd, J_1 = 1.2 Hz, J_2 = 3.7 Hz, 2H), 8.04 (d, J = 1.8 Hz, 2H), 8.06 (d, J = 7.0 Hz, 4H), 10.73 (s, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 172.8, 168.6, 166.9, 165.0, 159.5, 150.2, 141.1, 137.8, 135.2, 133.6, 131.7, 130.2, 128.8, 128.3, 122.6, 122.2, 118.6, 112.6, 63.3, 55.0, 48.5, 45.7, 43.6.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-(phenylacetamido)benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)-cyclobutane-1,3-dicarboxylic Acid (**21**).* Compound **21** was prepared according to the general procedure B above, in which phenylacetyl chloride was used as the acylating reagent, and a light-yellow powder was obtained in 35.0% yield. The purity was 98% by HPLC analysis. RT = 5.4 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.56 (s, 4H), 3.63 (s, 6H), 4.98 (s, 2H), 7.25−7.32 (m, 18H), 7.39 (d, J = 8.5 Hz, 4H), 7.60 (d, J = 8.5 Hz, 4H), 8.02 (d, J = 3.6 Hz, 2H), 8.09 (d, J = 4.2 Hz, 2H), 8.62 (s, 2H), 10.40 (s, 2H). MS (ESI) m/z 1111.2 $[M - H]^-$.

*General Procedure C for Compounds **22**−**25**.* Compound **3** (0.16 g, 0.15 mmol) was dissolved in 6 mL of dichloromethane and cooled in iced bath. Trifluoroacetic acid (0.6 mL) was added to the solution and was stirred at room temperature for 2 h. The solvent was removed in vacuo, and the residue was dissolved in the mixture of 2 mL of dichloromethane and 3 drops of DMF. Isothiocyanate or isocyanate (0.33 mmol) was added to the solution and stirred for 2 days at 50 °C. After removal of the solvent in vacuo, the residue was purified on silica gel column to afford the product.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-(3-methyl)-thioureidobenzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**22**).* Compound **22** was prepared according to the general procedure C above, in which isothiocyanatomethane was used as the reagent (yield: 30.0%). The purity was 96% by HPLC analysis. RT = 5.5 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.84 (d, J = 3.3 Hz, 6H), 3.39 (s, 6H), 5.25 (s, 2H), 6.92−6.93 (m, 4H), 7.23 (t, J = 4.5 Hz, 2H), 7.36 (s, 2H), 7.57 (d, J = 7.5 Hz, 4H), 7.73 (d, J = 9.0 Hz, 4H), 7.92 (brs, 2H), 8.00 (d, J = 4.8 Hz, 2H), 10.36 (brs, 2H), 11.96 (brs, 2H). MS (ESI) m/z 1021.1 $[M - H]^-$. HRMS (ESI) m/z calcd for $C_{48}H_{41}N_6O_{12}S_4$ $[M - H]^-$ 1021.1665, found 1021.1707.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-(3-naphthalen-1-yl)-thioureidobenzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**23**).* Compound **23** was prepared according to the general procedure C above, in which 1-isothiocyanatonaphthalene was used as the reagent (yield: 32.0%). The purity was 96% by HPLC analysis. RT = 5.6 min.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.38 (s, 6H), 5.31 (s, 2H), 6.94 (brs, 3H), 7.23 (t, J = 3.9 Hz, 3H), 7.35−7.36 (m, 9H), 7.58−7.59 (m, 7H), 7.78−7.79 (m, 2H), 7.91−7.92 (m, 6H), 8.00−8.01 (m, 4H). MS (ESI) m/z 1247.3 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-(3-m-fluorophenyl)-ureidobenzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**24**).* Compound **24** was prepared according to the general procedure C above, in which 1-fluoro-3-isocyanatobenzene was used as the reagent (yield: 28.1%). The purity was 96% by HPLC analysis. RT = 5.7 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.35 (s, 6H), 5.33 (s, 2H), 6.68−6.69 (m, 2H), 7.06−7.07 (m, 4H), 7.23−7.24 (m, 4H), 7.37−7.38 (m, 4H), 7.53−7.54 (m, 4H), 7.65−7.66 (m, 4H), 7.77−7.78 (m, 2H), 7.93−7.94 (m, 2H), 8.01 (d, J = 4.2 Hz, 2H). MS (ESI) m/z 1149.1 $[M - H]^-$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-(3-(2-ethoxy-2-oxoethyl)ureido) benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**25**).* Compound **25** was prepared according to the general procedure C above, in which ethyl 2-isocyanatoacetate was used as the reagent (yield: 23.0%). The purity was 96% by HPLC analysis. RT = 5.5 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20−1.21 (m, 6H), 3.36 (s, 6H), 4.04−4.05 (m, 8H), 5.22 (s, 2H), 6.92 (brs, 4H), 7.23−7.24 (m, 2H), 7.30−7.31 (m, 2H), 7.42−7.43 (m, 2H), 7.47−7.48 (m, 2H), 7.56−7.57 (m, 2H), 7.65−7.66 (m, 2H), 7.91 (d, J = 2.7 Hz, 2H), 8.00 (m, 2H). MS (ESI) m/z 1135.2 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-(2-tert-butoxyamido)-propanamidobenzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**26**).* *N*-Boc-alanine (1.73 g, 9.1 mmol) was dissolved in 50 mL of tetrahydrofuran and cooled to −20 °C. *N*-methyl morpholine (3 mL, 27.3 mmol) and isobutyl chloroformate (1.2 mL, 9.2 mmol) were applied successively to the solution, and the mixture was stirred for half an hour at −20 °C. The tetrahydrofuran solution of the deprotected product of **3** (200 mg, 0.19 mmol) (see the general procedure B) was then added, and the mixture was stirred at the room temperature overnight. The solvent was then removed in vacuo and the mixture extracted with dichloromethane (20 mL × 3). The organic phase was combined, washed with water, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo to give an oil product which was subjected for LC-MS analysis. From the result of MS (ESI) m/z 1182.0 $[M + H]^+$, the possible structure could be a dehydrated product. The oil was then dissolved in tetrahydrofuran, and the solution was basified to pH 9 by 10% NaOH. The mixture was stirred at room temperature for half an hour and adjusted to pH 7 by 1 N HCl. The solvent was then removed, and the residue was separated on chromatographic plates (0.4−0.5 mm) to obtain **26** (50 mg, 21.6%) and its monohydrolyzed product (65 mg). The purity was 97% by HPLC analysis. RT = 5.5 min. ^{1}H NMR(DMSO-d_6, 400 MHz): δ 1.18 (d, J = 7.1 Hz, 6H), 1.38 (s, 18H), 3.35 (s, 6H), 4.11−4.15 (m, 2H), 5.21 (s, 2H), 6.87 (d, J = 8.9 Hz, 2H), 7.01−7.03 (d, J = 8.3 Hz, 2H), 7.10 (brs, 4H), 7.26 (t, J = 4.6 Hz, 2H), 7.68 (d, J = 7.0 Hz, 4H), 7.94 (d, J = 3.8 Hz, 2H), 8.03 (d, J = 4.2 Hz, 2H), 10.17 (s, 2NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.1, 172.3, 165.0, 159.4, 155.2, 150.0, 141.9, 136.8, 135.7, 134.9, 131.7, 129.2, 128.6, 127.7, 122.0, 120.0, 118.6, 111.9, 78.1, 64.4, 55.1, 51.5, 50.6, 28.2, 17.9.

Another structure was the monohydrolyzed product, (1,2-*cis*-2,3-*cis*-3,4-*cis*)-1,3-bis(4-((2-(*t*-butoxycarbonyl)amino)propanamido)-benzamido)-2-(4-hydroxy-3-methoxyphenyl)-4-(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic acid. The purity was 96% by HPLC analysis. RT = 11.6 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.20 (d, J = 6.9 Hz, 3H), 1.30 (dd, J_1 = 6.8 Hz, J_2 = 3.0 Hz, 3H), 1.38 (s, 9H), 1.42 (s, 9H), 3.60−3.70 (m, 1H), 4.09−4.18 (m, 1H), 5.06 (s, 1H), 5.18 (s, 1H), 6.53 (d, J = 7.8 Hz, 2H), 6.65 (d, J = 8.1 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.90 (s, 1H), 7.00 (d, J = 8.4 Hz, 1H), 7.12 (brs, 4H), 7.26 (t, J = 4.0 Hz, 1H), 7.68 (brs, 4H), 7.93 (d, J = 3.9 Hz, 1H), 8.03 (d, J = 4.8 Hz, 1H), 8.53 (s, 1H), 9.13 (s, NH), 9.20 (s, NH), 10.20 (s, 2NH). MS (ESI) m/z 1107.3 $[M - H]^-$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-(2-amino)propanamidobenzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)phenyl)-cyclobutane-1,3-dicarboxylic Acid (**27**).* Compound **26** (0.1 g) was dissolved in 6 mL of dichloromethane and cooled in iced bath. Trifluoroacetic acid (0.74 mL) was added into the solution and stirred at room temperature for 2 h. The solvent was removed in vacuo, and compound **27** was obtained as red oil (66 mg, 78.9%). The purity was 97% by HPLC analysis. RT = 5.0 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.43 (d, J = 7.0 Hz, 6H), 3.26 (s, 6H), 3.96−4.02 (m, 2H), 5.02 (s, 2H), 7.22 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.2 Hz, 2H), 7.31 (s, 2H), 7.33 (t, J = 4.7 Hz, 2H), 7.47 (d, J = 8.6 Hz, 4H), 7.62 (d, J = 8.6 Hz, 4H), 8.03 (dd, J_1 = 1.0 Hz, J_2 = 3.6 Hz, 2H), 8.10 (d, J = 5.0 Hz, 2H). MS (ESI) m/z 1019.2 $[M + H]^+$. HRMS (ESI) m/z calcd for $C_{50}H_{47}N_6O_{14}S_2$ $[M + H]^+$ 1019.2592, found 1019.2618.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(2-hydroxy)acetamido)-benzamido)-2,4-bis(3-methoxy-4-(thiophene-2-carbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**28**).* Compound **19** (0.2 g, 0.17 mmol), BF_3−Et_2O (0.4 mL, 3 mmol), and ethanethiol (1.7 mL) were dissolved in 3.4 mL of CH_2Cl_2, and the mixture was stirred overnight at room temperature. Water was introduced to terminate the reaction, and a yellow solid was precipitated. After filtration, the solid was collected and purified on chromatographic plate to give the pure product **28** (0.12 g, 71.1%). The purity was 96% by HPLC analysis. RT = 5.9 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.38(s, 6H), 4.00 (d, J = 5.8 Hz, 4H), 5.28 (s, 2H), 6.95 (brs, 4H), 7.25 (t, J = 4.8 Hz, 2H), 7.34 (brs, 2H), 7.66 (d, J = 8.8 Hz, 4H), 7.78(d, J = 8.8 Hz, 4H), 7.93 (dd, J_1 = 1.2 Hz, J_2 = 3.8 Hz, 2H), 8.03 (dd, J_1 = 1.2 Hz, J_2 = 5.0 Hz, 2H), 9.96 (s, 2NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 176.9, 172.1, 165.7, 160.1, 150.6, 141.9, 137.7, 137.2, 135.5, 132.4, 130.7, 129.3, 128.2, 122.4, 120.8, 119.6, 112.9, 65.0, 62.6, 55.8, 52.7.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**29**).* Compound **29** was prepared according to the general procedure A above, in which (*Z*)-2-((4-(*t*-butoxycarbonyl)amino)-phenyl)-4-(3-methoxy)benzylidene-5(4*H*)-oxazolone was used as the raw material, and a yellowish amorphous powder was obtained in 10.1% yield. The purity was 98% by HPLC analysis. RT = 5.6 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.45 (s, 18H), 3.48 (s, 6H), 4.89 (s, 2H), 6.80 (d, J = 7.8 Hz, 2H), 7.12−7.13 (m, 4H), 7.24 (dd, J_1 = 7.8, J_2 = 7.5 Hz, 2H), 7.32 (d, J = 8.7 Hz, 4H), 7.43 (d, J = 8.4 Hz, 4H), 8.45 (s, 2H), 9.57 (s, 2H), 12.80 (brs, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 173.0, 166.6, 158.8, 152.6, 142.3, 135.9, 129.0, 128.5, 128.2, 122.0, 117.0, 113.7, 112.9, 79.6, 63.2, 54.5, 48.8, 28.1. MS (ESI) m/z 847.0 $[M + Na]^+$, 825.1 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-diphenylcyclobutane-1,3-dicarboxylic Acid (**30**).* Compound **30** was prepared according to the general procedure A above, in which (*Z*)-2-((4-(*t*-butoxycarbonyl)amino)phenyl)-4-benzylidene-5(4*H*)-oxazolone was used as the raw material, and a white amorphous powder was obtained in 10.3% yield. The purity was 97% by HPLC analysis. RT = 5.6 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.45 (s, 18H), 4.91 (s, 2H), 7.23−7.24 (m, 6H), 7.32 (d, J = 8.4 Hz, 4H), 7.43 (d, J = 8.7 Hz, 4H), 7.53−7.54 (m, 4H), 8.43 (brs, 2H), 9.53 (brs, 2H). MS (ESI) m/z 787.0 $[M + Na]^+$, 765.1 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(4-methoxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**31**).* Compound **31** was prepared according to the general procedure A above, in which (*Z*)-2-((4-(*t*-butoxycarbonyl)amino)-phenyl)-4-(4-methoxy)benzylidene-5(4*H*)-oxazolone was used as the raw material, and a white amorphous powder was obtained in 10.2% yield. The purity was 96% by HPLC analysis. RT = 5.6 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.44 (s, 18H), 3.70 (s, 6H), 4.78 (s, 2H), 6.81 (d, J = 8.3 Hz, 4H), 7.35 (d, J = 8.3 Hz, 4H), 7.43 (d, J = 8.3 Hz, 8H), 8.32 (s, 2H), 9.57 (s, 2H), 12.70 (brs, 2H). MS (ESI) m/z 847.4 $[M + Na]^+$, 825.3 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(4-propoxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**32**).* Compound **32** was prepared according to the general procedure A above, in which (*Z*)-2-((4-(*t*-butoxycarbonyl)amino)-phenyl)-4-(4-propoxy)benzylidene-5(4*H*)-oxazolone was used as the raw material, and a white amorphous powder was obtained in 11.0% yield. The purity was 97% by HPLC analysis. RT = 5.7 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.68 (brs, 2H), 9.53 (s, 2H), 8.29 (s, 2H),

dx.doi.org/10.1021/jm201150j | J. Med. Chem. 2012, 55, 250−267

7.43−7.44 (m, 12H), 6.80 (d, J = 8.4 Hz, 4H), 4.80 (s, 2H), 3.88 (t, J = 6.3 Hz, 4H), 1.71−1.72 (m, 4H), 1.46 (s, 18H), 0.97 (t, J = 7.5 Hz, 6H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 173.1, 166.5, 157.4, 152.6, 142.1, 130.1, 128.6, 128.1, 126.1, 117.1, 113.8, 79.5, 68.8, 63.1, 48.4, 28.1, 22.1, 10.5. MS (ESI) m/z 903.0 $[M + Na]^+$, 881.1 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-benzyloxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**33**).* Compound **33** was prepared according to the general procedure A above, in which (*Z*)-2-((4-(*t*-butoxycarbonyl)amino)-phenyl)-4-(3-benzyloxy)benzylidene-5(4*H*)-oxazolone was used as the raw material, and a light-yellow amorphous powder was obtained in 10.0% yield. The purity was 99% by HPLC analysis. RT = 5.8 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.46 (s, 18H), 4.38 (s, 4H), 4.93 (s, 2H), 6.85 (d, J = 7.8 Hz, 2H), 7.1−7.2 (m, 8H), 7.2−7.4 (m, 12H), 7.46 (d, J = 8.7 Hz, 4H), 8.45 (brs, 2H), 9.59 (brs, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 172.9, 166.6, 157.9, 152.5, 142.4, 136.5, 135.5, 128.9, 128.4, 128.2, 128.1, 127.4, 127.0, 122.4, 116.9, 114.3, 113.1, 79.5, 68.9, 63.0, 48.6, 28.1. MS (ESI) m/z 999.0 $[M + Na]^+$, 977.1 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(4-benzyloxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**34**).* Compound **34** was prepared according to the general procedure A above, in which (*Z*)-2-((4-(*t*-butoxycarbonyl)amino)-phenyl)-4-(4-benzyloxy)benzylidene-5(4*H*)-oxazolone was used as the raw material, and a white amorphous powder was obtained in 12.0% yield. The purity was 98% by HPLC analysis. RT = 5.7 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.46 (s, 18H), 4.80 (s, 2H), 5.07 (s, 4H), 6.90 (d, J = 8.7 Hz, 4H), 7.2−7.5 (m, 22H), 8.31 (brs, 2H), 9.54 (brs, 2H). MS (ESI) m/z 999.0 $[M + Na]^+$, 977.0 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-acetamidophenyl)cyclobutane-1,3-dicarboxylic Acid (**39**).* Compound **39** was prepared according to the general procedure A above, in which (*Z*)-2-((4-(*t*-butoxycarbonyl)amino)phenyl)-4-(3-methoxy-4-acetamidobenzylidene)-5(4*H*)-oxazolone was used as the raw material, and a yellowish amorphous powder was obtained in 12.0% yield. The purity was 98% by HPLC analysis. RT = 5.5 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.48 (s, 18H), 1.98 (s, 6H), 3.36 (s, 6H), 5.25 (s, 2H), 6.85 (d, J = 8.0 Hz, 2H), 7.15 (s, 2H), 7.5 (d, J = 8.5 Hz, 4H), 7.60 (d, J = 6.9 Hz, 2H), 7.66 (d, J = 8.3 Hz, 4H), 8.86 (s, 2NH), 9.62 (s, 2NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.1, 164.9, 152.6, 148.5, 142.2, 133.9, 128.8, 127.6, 124.6, 120.7, 119.9, 117.3, 110.5, 79.5, 64.9, 54.9, 52.1, 28.1, 23.8.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(4-acetamidophenyl)cyclobutane-1,3-dicarboxylic Acid (**40**).* Compound **40** was prepared according to the general procedure A above, in which (*Z*)-2-((4-(*t*-butoxycarbonyl)amino)-phenyl)-4-(4-acetamidobenzylidene)-5(4*H*)-oxazolone was used as the raw material, and a yellowish amorphous powder was obtained in 10.0% yield. The purity was 97% by HPLC analysis. RT = 5.6 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.48 (s, 18H), 1.94 (s, 6H), 5.21 (s, 2H), 7.27 (brs, 8H), 7.47 (d, J = 8.5 Hz, 4H), 7.54 (d, J = 8.2 Hz, 4H), 9.58 (s, 2NH), 9.79 (s, 2NH). MS (ESI) m/z 939.4 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(4-(thiophene-2-carboxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**41**).* Compound **41** was prepared according to the general procedure A above, in which (*Z*)-2-((4-(*t*-butoxycarbonyl)amino)phenyl)-4-(4-(thiophene-2-carboxy)benzylidene)-5(4*H*)-oxazolone was used as the raw material, and a white amorphous powder was obtained in 10% yield. The purity was 97% by HPLC analysis. RT = 5.7 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.47 (s, 18H), 5.16 (s, 2H), 6.94 (d, J = 8.4 Hz, 4H), 7.26 (dd, J_1 = 4.6 Hz, J_2 = 4.1 Hz, 2H), 7.44 (d, J = 8.4 Hz, 4H), 7.47 (d, J = 8.2 Hz, 4H), 7.53 (d, J = 8.3 Hz, 4H), 7.95 (d, J = 2.9 Hz, 2H), 8.03 (d, J = 4.8 Hz, 2H), 9.49 (s, 2H), 10.2 (brs, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 175.7, 165.0, 160.0, 152.6, 147.6, 141.9, 136.1, 134.8, 132.1, 129.6, 128.5, 127.5, 120.2, 117.3, 79.3, 65.0, 51.9, 28.0. MS (ESI) m/z 1039.0 $[M + Na]^+$, 1017.2 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-ethoxy-4-(thiophene-2-carboxy)phenyl)-cyclobutane-1,3-dicarboxylic Acid (**42**).* Compound **42** was prepared according to the general procedure A above, in which (*Z*)-2-((4-(*t*-butoxycarbonyl)amino)phenyl)-4-(3-ethoxy-4-(thiophene-2-carboxy))benzylidene-5(4*H*)-oxazolone was used as the raw material, and a white amorphous powder was obtained in 10.0% yield. The purity was 97% by HPLC analysis. RT = 5.7 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.91 (t, J = 6.8 Hz, 6H), 1.42 (s, 18H), 3.34−3.35 (m, 4H), 4.93 (s, 2H), 7.16 (d, J = 8.3 Hz, 2H), 7.21 (s, 2H), 7.24 (d, J = 8.3 Hz, 2H), 7.31−7.32 (m, 6H), 7.41 (d, J = 8.3 Hz, 4H), 8.01 (d, J = 3.4 Hz, 2H), 8.08 (d, J = 4.9 Hz, 2H), 8.56 (s, 2H), 9.58 (s, 2H), 12.90 (brs, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 172.8, 166.9, 159.5, 152.6, 149.5, 142.3, 138.1, 134.9, 133.4, 131.8, 128.7, 128.4, 128.2, 122.3, 122.1, 117.0, 113.9, 79.5, 63.4, 63.2, 48.5, 28.0, 14.2. MS (ESI) m/z 1127.0 $[M + Na]^+$, 1105.0 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(4-benzoyloxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**43**).* Compound **43** was prepared according to the general procedure A above, in which (*Z*)-2-((4-(*t*-butoxycarbonyl)amino)-phenyl)-4-(4-benzoyloxy)benzylidene-5(4H)-oxazolone was used as the raw material, and a white amorphous powder was obtained in 12.0% yield. The purity was 98% by HPLC analysis. RT = 5.8 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.42 (s, 18H), 4.96 (s, 2H), 7.20 (d, J = 8.4 Hz, 4H), 7.35 (d, J = 8.4 Hz, 4H), 7.43 (d, J = 8.6 Hz, 4H), 7.60−7.61 (m, 8H), 7.74 (t, J = 7.3 Hz, 2H), 8.14 (d, J = 7.3 Hz, 4H), 8.55 (s, 2H), 9.56 (s, 2H), 12.90 (brs, 2H). MS (ESI) m/z 1027.0 $[M + Na]^+$, 1005.0 $[M + H]^+$.

*General Procedure E for Compounds **35**−**38**.* Compound **3** (0.24 g, 0.23 mmol) was dissolved in 5 mL of methanol and 1 mL of 10% NaOH solution. The mixture was stirred overnight at room temperature and pH adjusted to 7 by 1 N HCl. The solvent was removed in vacuo, and the residue was dissolved in 15 mL of ethyl acetate followed by addition of alkyl bromide (0.5 mmol), potassium carbonate (0.13 g, 0.94 mmol), and a small amount of TEBA. The mixture was stirred and heated to reflux for a day. After cooling, water was added and the solution was extracted with ethyl acetate (20 mL × 3). The organic phase was combined, washed with water, and dried over anhydrous sodium sulfate. After filtration, the solvent was removed in vacuo and the residue was separated on chromatographic plates to afford the product.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-benzyloxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**35**).* Compound **35** was prepared according to the general procedure E above, in which benzyl bromide was used as the reagent and a yellowish amorphous powder was obtained in 50.0% yield. The purity was 96% by HPLC analysis. RT = 5.7 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.45 (s, 18H), 2.99 (s, 6H), 4.92 (s, 2H), 5.12 (s, 4H), 6.69 (d, J = 8.2 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.93 (s, 2H), 7.24−7.25 (m, 10H), 7.37 (d, J = 8.6 Hz, 4H), 7.45 (d, J = 8.5 Hz, 4H), 8.46 (s, 2NH), 9.59 (s, 2NH). MS (ESI) m/z 1059.0 $[M + Na]^+$, 1037.2 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-phenylethoxyphenyl)-cyclobutane-1,3-dicarboxylic Acid (**36**).* Compound **36** was prepared according to the general procedure E above, in which 1-(2-bromoethyl)benzene was used as the reagent (yield: 55.0%). The purity was 97% by HPLC analysis. RT = 5.5 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.48 (s, 18H), 2.93 (t, J = 7.1 Hz, 4H), 3.31 (s, 6H), 4.02 (t, J = 7.0 Hz, 4H), 5.07 (s, 2H), 6.71 (d, J = 8.4 Hz, 2H), 6.75 (d, J = 8.6 Hz, 2H), 6.97 (brs, 2H), 7.17−7.22 (m, 2H), 7.27 (d, J = 4.4 Hz, 8H), 7.48 (d, J = 8.7 Hz, 4H), 7.57 (brs, 4H), 9.59 (s, 2H). MS (ESI) m/z 1087.1 $[M + Na]^+$, 1065.3 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(4-fluorobenzyloxy)phenyl)-cyclobutane-1,3-dicarboxylic Acid (**37**).* Compound **37** was prepared according to the general procedure E above, in which 4-fluorobenzyl bromide was used as the reagent (yield: 53.0%). The purity was 98% by HPLC analysis. RT = 5.2 min. ^{1}H NMR (CD_3Cl, 400 MHz): δ 1.46 (s, 18H), 3.05 (s, 6H), 4.91 (s, 2H), 5.13 (s, 4H), 6.69 (d, J = 8.1 Hz, 2H), 6.89 (d, J = 8.4 Hz, 2H), 6.94 (s, 2H), 7.12 (t, J = 8.8 Hz, 4H), 7.36 (d, J = 7.2 Hz, 4H), 7.45 (d, J = 8.5 Hz, 4H), 8.45 (s, 2H), 8.84 (s, 2H), 9.58 (s, 2H). MS (ESI) m/z 1095.3 $[M + Na]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(3-phenylpropoxy)phenyl)-cyclobutane-1,3-dicarboxylic Acid (**38**).* Compound **38** was prepared according to the general procedure E above, in which 1-(3-bromopropyl)benzene was used as the reagent (yield: 60.0%). The purity was 98% by HPLC analysis. RT = 5.2 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.50 (s, 18H), 1.82−1.86 (m, 4H), 2.02 (t, J = 7.7 Hz, 4H), 3.29 (s, 6H), 4.10 (t, J = 5.8 Hz, 4H), 5.0 (s, 2H), 7.06 (d, J = 8.0 Hz, 4H), 7.39 (d, J = 8.6 Hz, 4H), 7.46 (d, J = 8.2 Hz, 4H), 9.59 (s, 2NH). MS (ESI) m/z 1115.4 $[M + Na]^+$.

*General Procedure D for Compounds **44**−**52**.* Compound **3** (0.2 g, 0.19 mmol) was dissolved in 5 mL of methanol and 1 mL of 10% NaOH solution. The mixture was stirred overnight at room temperature. The pH of the solution was adjusted to 13 with 10% NaOH, and acyl chloride (4.6 mmol) was added. The mixture was stirred for a further one day at room temperature. The solvent was removed in vacuo, and the residue was further stirred in 10 mL of tetrahydrofuran by addition of 10% NaOH solution (pH 10) for 30 min. After neutralization, the residue was separated on chromatographic plates (0.4−0.5 mm) to afford the product.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-acetoxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**44**).* Compound **44** was prepared according to the general procedure D above, in which acetyl chloride was used as the acylating reagent, and a light-yellow amorphous powder was obtained in 30.0% yield. The purity was 98% by HPLC analysis. RT = 5.7 min. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.55 (brs, 2H), 8.53 (brs, 2H), 7.42 (d, J = 8.4 Hz, 4H), 7.28 (d, J = 8.7 Hz, 4H), 7.22 (brs, 2H), 7.14 (d, J = 9.0 Hz, 2H), 7.09 (d, J = 8.1 Hz, 2H), 4.92 (brs, 2H), 3.23 (s, 6H), 2.26 (s, 6H), 1.45 (s, 18H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 172.9, 168.5, 166.9, 152.6, 150.0, 142.3, 138.1, 133.1, 128.4, 128.2, 122.4, 122.0, 117.0, 112.4, 79.6, 63.1, 54.8, 48.4, 28.1, 20.5. MS (ESI) m/z 962.3 $[M + Na]$,$^+$ 940.2 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-propionyloxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**45**).* Compound **45** was prepared according to the general procedure D above, in which propionyl chloride was used as the acylating reagent, and a yellowish amorphous powder was obtained in 33.0% yield. The purity was 98% by HPLC analysis. RT = 5.4 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.07 (t, J = 7.4 Hz, 6H), 1.48 (s, 18H), 2.47−2.51 (m, 4H), 3.34 (s, 6H), 5.19 (s, 2H), 6.82 (brs, 4H), 7.13 (s, 2H), 7.49 (d, J = 8.4 Hz, 4H), 7.57 (brs, 4H), 9.61 (s, 2H). MS (ESI) m/z 967.3 $[M - H]^-$. HRMS (ESI) m/z calcd for $C_{50}H_{55}N_4O_{16}$ $[M - H]^-$ 967.3613, found 967.3640.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-butyryloxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**46**).* Compound **46** was prepared according to the general procedure D above, in which butyryl chloride was used as the acylating reagent, and a yellowish amorphous powder was obtained in 30.0% yield. The purity was 98% by HPLC analysis. RT = 5.3 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.91 (t, J = 7.4 Hz, 6H), 1.47 (s, 18H), 1.54−1.63 (m, 4H), 2.42 (t, J = 7.1 Hz, 4H), 3.32 (s, 6H), 5.31 (s, 2H), 6.77 (d, J = 8.0 Hz, 2H), 6.90 (d, J = 8.0 Hz, 2H), 7.20 (s, 2H), 7.50 (d, J = 8.4 Hz, 4H), 7.57 (brs, 4H), 9.64 (s, 2NH). MS (ESI) m/z 995.3 $[M - H]^-$. HRMS (ESI) m/z calcd for $C_{52}H_{59}N_4O_{16}$ $[M - H]^-$ 995.3926, found 995.3911.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-isobutyryloxyphenyl)-cyclobutane-1,3-dicarboxylic Acid (**47**).* Compound **47** was prepared according to the general procedure D above, in which isobutyryl chloride was used as the acylating reagent, and a yellowish amorphous powder was obtained in 30.0% yield. The purity was 98% by HPLC analysis. RT = 5.8 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.16 (d, J = 6.9 Hz, 12H), 1.48 (s, 18H), 2.67−2.74 (m, 2H), 3.34 (s, 6H), 5.22 (s, 2H), 6.84 (s, 4H), 7.10 (s, 2H), 7.52 (d, J = 8.2 Hz, 4H), 7.60 (brs, 4H), 9.64 (s, 2NH). MS (ESI) m/z 995.3 $[M - H]^-$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(1-adamantanecarbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**48**).* Compound **48** was prepared according to the general procedure D above, in which 1-adamantanecarbonyl chloride was used as the acylating reagent, and a yellowish amorphous powder was obtained in 30.0% yield. The purity was 98% by HPLC analysis. RT = 5.3 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.48 (s, 18H), 1.68 (s, 12H), 1.90 (s, 12H), 1.98 (s, 6H), 3.32 (s, 6H), 5.20 (s, 2H), 6.76 (d, J = 8.0 Hz, 2H), 6.84 (d, J = 8.0 Hz, 2H), 7.15 (s, 2H), 7.49 (d, J = 8.4 Hz, 4H), 7.60 (brs, 4H), 9.62 (s, 2NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.7, 165.1, 152.7, 149.9, 142.4, 137.3, 135.9, 128.5, 127.7, 121.6, 120.0, 117.4, 112.1, 79.6, 64.7, 55.3, 51.8, 38.7, 38.4, 35.9, 28.1, 27.5.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(furan-2-carbonyloxy)phenyl)-cyclobutane-1,3-dicarboxylic Acid (**49**).* Compound **49** was prepared according to the general procedure D above, in which furan-2-carbonyl chloride was used as the acylating reagent, and a yellowish amorphous powder was obtained in 30.0% yield. The purity was 98% by HPLC analysis. RT = 5.5 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.45 (s, 18H), 3.24 (s, 6H), 4.97 (s, 2H), 6.80−6.81 (m, 2H), 7.21 (d, J = 3.5 Hz, 2H), 7.26 (d, J = 8.5 Hz, 4H), 7.32 (d, J = 8.7 Hz, 4H), 7.44 (d, J = 8.6 Hz, 4H), 7.58 (d, J = 3.5 Hz, 2H), 8.12 (d, J = 0.8 Hz, 2H), 8.60 (s, 2NH), 9.59 (s, 2NH). MS (ESI) m/z 1067.1 $[M + Na]^+$, 1045.3 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(5-methyl-thiophen-2-ylcarbonyloxy)phenyl)cyclobutane-1,3-dicarboxylic Acid (**50**).* Compound **50** was prepared according to the general procedure D above, in which 5-methyl-thiophen-2-ylcarbonyl chloride was used as the acylating reagent, and a yellowish amorphous powder was obtained in 31.0% yield. The purity was 97% by HPLC analysis. RT = 5.6 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.45 (s, 18H), 2.58 (s, 6H), 3.25 (s, 6H), 4.97 (s, 2H), 7.04 (d, J = 2.9 Hz, 2H), 7.20 (s, 2H), 7.23 (d, J = 7.7 Hz, 4H), 7.33 (d, J = 8.4 Hz, 4H), 7.44 (d, J = 8.4 Hz, 4H), 7.85 (d, J = 3.6 Hz, 2H), 8.59 (s, 2NH), 9.59 (s, 2NH), 12.93 (s, 2COOH). MS (ESI) m/z 1127.6 $[M + Na]^+$, 1105.3 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(2-phenylacetoxy)phenyl)-cyclobutane-1,3-dicarboxylic Acid (**51**).* Compound **51** was prepared according to the general procedure D above, in which 2-phenylacetyl chloride was used as the acylating reagent, and a yellowish amorphous powder was obtained in 32.0% yield. The purity was 97% by HPLC analysis. RT = 5.3 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.49 (s, 18H), 3.32 (s, 6H), 3.85 (s, 4H), 5.17 (s, 2H), 6.79 (d, J = 8.1 Hz, 2H), 6.84 (d, J = 8.2 Hz, 2H), 7.3−7.4 (m, 12H), 7.47 (d, J = 8.7 Hz, 4H), 7.56 (d, J = 7.5 Hz, 4H), 9.59 (s, 2NH). MS (ESI) m/z 1093.1 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis((4-(t-butoxycarbonyl)amino)-benzamido)-2,4-bis(3-methoxy-4-(cyclohexanecarbonyloxy)-phenyl)cyclobutane-1,3-dicarboxylic Acid (**52**).* Compound **52** was prepared according to the general procedure D above, in which cyclohexanecarbonyl chloride was used as the acylating reagent, and a yellowish amorphous powder was obtained in 23.0% yield. The purity was 97% by HPLC analysis. RT = 5.3 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.27−1.28 (m, 4H), 1.48 (s, 18H), 1.68−1.69 (m, 8H), 1.84−1.85 (m, 8H), 2.81 (t, J = 6.4 Hz, 2H), 3.33 (s, 6H), 5.24 (s, 2H), 6.76 (d, J = 8.1 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 7.22 (s, 2H), 7.47 (d, J = 8.5 Hz, 4H), 7.57 (d, J = 7.8 Hz, 4H), 9.61 (s, 2H). MS (ESI) m/z 1075.4.3 $[M - H]^-$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-isobutyramidobenzamido)-2,4-bis(3-methoxy-4-cyclobutanecarboxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**53**).* Compound **16** (200 mg, 0.19 mmol) was dissolved in 5 mL of methanol and 1 mL of 10% NaOH solution. The mixture was stirred overnight at room temperature and pH adjusted to 13 with 10% NaOH before adding 4.6 mmol of cyclobutanecarbonyl chloride. The mixture was stirred for a further one day at room temperature. The solvent was removed in vacuo and the residue was separated on chromatographic plates (0.4−0.5 mm) to obtain **53** as a light-yellow amorphous powder (73 mg, 40.0%). The purity was 98% by HPLC analysis. RT = 5.3 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.10 (d, J = 6.8 Hz, 12H), 1.83−2.00 (m, 4H), 2.17−2.33 (m, 8H), 2.62−2.68 (m, 4H), 3.35 (s, 6H), 5.23 (s, 2H), 6.77 (d, J = 8.2 Hz, 2H), 6.88 (d, J = 7.9 Hz, 2H), 7.3 (s, 2H), 7.62 (d, J = 8.2 Hz, 4H), 7.67 (d, J = 8.7 Hz, 4H), 10.15 (s, 2H). MS (ESI) m/z 961.3 $[M + H]^+$.

*(1,2-cis-2,3-cis-3,4-cis)-1,3-Bis(4-isobutyramidobenzamido)-2,4-bis(3-methoxy-4-acetoxyphenyl)cyclobutane-1,3-dicarboxylic Acid (**54**).* Compound **54** was prepared according to **53** above, in which acetyl chloride was used as the acylating reagent (yield: 30.0%). The purity was 98% by HPLC analysis. RT = 5.4 min. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.10 (d, J = 6.6 Hz, 12H), 2.15 (s, 6H), 2.59−2.68 (m, 2H), 3.36 (s, 6H), 5.25 (s, 2H), 6.76 (d, J = 8.0 Hz, 2H), 6.89 (d, J = 8.2 Hz, 2H), 7.28 (s, 2H), 7.61 (d, J = 8.0 Hz, 4H), 7.65 (d, J = 8.3 Hz, 4H), 10.11 (s, 4H). MS (ESI) m/z 881.4 $[M + H]^+$.

Receptor Binding Assay. The receptor binding assay was performed as previously described.[13] Human embryonic kidney 293 cells were stably cotransfected with a rat GLP-1 receptor expression vector pCMV-Tag2B-rGLP-1R and a reporter plasmid that contains three copies of multiple response element and one copy of cAMP response element in front of the luciferase gene in pGL3 vector. Then 17 μg of membrane protein preparations made from these HEK293-rGLP-1R cells and 150 μg of FlashBlue beads (PerkinElmer, Boston, MA) were added to each well and incubated for 5 h at 4 °C. After that, 40 pM [^{125}I]GLP-1(7−36) amide or [^{125}I]Exendin(9−39) (Amersham, Piscataway, NJ), 2 μg/mL aprotinin (Merck KGaA, Darmstadt, Germany), 100 μM leupeptin (Merck), and different concentrations of analogues of **3** were added to give a final volume of 100 μL per well in a 96-well Isoplate (PerkinElmer). The plates were incubated at 4 °C overnight and counted for radioactivity the following day at a MicroBeta counter (PerkinElmer).

Reporter Gene Assay. The HEK293-rGLP-1R cells were seeded onto 96-well cell culture plates with a density of 40000 cells per well and incubated overnight. At the time of assaying, GLP-1(7−37) (Sigma, St. Louis, MO) or different concentrations of analogues dissolved in DMSO were added. After 6 h of incubation, cells were lysed and quantified for luciferase activity by using the Steady-Glo luciferase assay system (Promega Corporation, Madison, WI).

Acute Food Intake Inhibition Study. Overnight-fasted male C57BL/6J mice (6 weeks old, 18−20 g; Shanghai SLAC Laboratory Animals Co. Ltd., Shanghai, China) were injected (ip) with vehicle or 0.1, 0.3, 1, or 3 mg of **3** or **16**, dissolved in 1% DMSO and 20% PEG400 in saline, respectively. Individually caged mice were exposed to a preweighed food pellet, which was then reweighed every 15 or 30 min for 6 h to determine cumulative intake.

Chronic Weight Loss Study. Eight-week old male C57BL/6J mice (18−20 g; Shanghai SLAC Laboratory Animals) were housed (two per cage) at 22.7 ± 0.8 °C in a 12 h/12 h light:dark cycle. Animals were fed a high fat diet (HFD, D12492; 60% fat, 20% protein, and 20% carbohydrate; 5.24 kcal/g) or a standard chow diet (SCD, D12450B; 10% fat, 20% protein, and 70% carbohydrate; 3.85 kcal/g) and watered ad libitum. Both diets were supplied by Research Diets (New Brunswick, NJ). Animal experimentation was conducted in accordance with the regulations approved by the Animal Care and Use Committee, Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Experimental animals were maintained on prescribed HFD for 12 weeks and then randomly assigned into 4 treatment groups (n = 8−9 per group) with matched body weight. They were injected (ip) three times a week, in the mornings of each Monday, Wednesday and Friday, with 0 (vehicle control), 0.3, 1, or 3 mg compound **16** (1% DMSO, 20% PEG400 in saline, pH 7.4, 0.5 mL) for 28 days. A comparator group of mice eating SCD (n = 8) was used to index responses to normal values. Body weight was monitored daily. Effects of compound **3** (3 mg treatment) on body weight in diet-induced obese mice were examined in a separate experiment.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The synthetic route and preparation for the cyclopropane analogues. Factors that affect the photochemistry. Stability of **3** in solid state and solution of methanol and acetonitrile measured by HPLC chromatography. LC/MS of **3**. Bioactivities of analogues of **3** in rGLP-1R binding and reporter gene assays. Crystal and molecular structure of **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +86 21 50800598. Fax: +86 21 50800721. E-mail: wangmw@mail.shcnc.ac.cn.

Present Addresses

[#]Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Macao SAR, China

[▽]Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia, United States

[○]Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan, United States

[◆]Department of Bioengineering, University of California, Riverside, California, United States

■ ABBREVIATIONS USED

GLP-1, glucagon-like peptide-1; DPP-4, dipeptidyl peptidase-4; HTS, high-throughput screening; MRE, multiple response element; CRE, cAMP response element; HRMS (ESI), high resolution mass spectroscopy (electrospray ionization)

■ ACKNOWLEDGMENTS

We are indebted to Lilin Lin, Yingyi Su, Chong Ji, Ling Zhu, Hongquan Zhang, Guangxin Wang, Hui Hu, Yulin Hua, Jianhua Yan, Desu Chen, Ping Zhang, Qianqian Wu, and Yang Feng for technical assistance, to Simon Campbell, Edward Roberts, Peppi Prasit, Ling Zhou, Changqiang Ke, Chunping Tang, Minghua Xu, Jingkang Shen, Xin Xie, and Fajun Nan for valuable discussions, to Bing Xiong for molecular structure analysis, and to Dale E. Mais for critical review of this manuscript. This work was supported in part by grants from the Ministry of Science and Technology of China (2009ZX09302-001 and 2012ZX09304-011), the Chinese Academy of Sciences (KSCX1-YW-02-2 and KSCX2-YW-R-17), the Natural Science Foundation of China (30628024 and 30623008), Shanghai Science and Technology Development Fund (074319114, 08DZ2291300 and 09DZ2291200), and the CAS−Novo Nordisk Research Fund.

■ REFERENCES

(1) *Diabetes Overview*; National Diabetes Information Clearing House: Bethesda, MD; http://diabetes.niddk.nih.gov/dm/pubs/overview/index.htm (Accessed November 2008).

(2) Li, Y. Z.; Perry, T. A.; Kindy, M. S.; Harvey, B. K.; Tweedie, D.; Holloway, H. W.; Powers, K.; Shen, H.; Egan, J. M.; Sambamurti, K.; Brossi, A.; Lahiri, D. K.; Mattson, M. P.; Hoffer, B. J.; Wang, Y.; Greig, N. H. GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinsonism. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 1285−1290.

(3) Ahren, B.; Schmitz, O. GLP-1 receptor agonists and DPP-4 inhibitors in the treatment of type 2 diabetes. *Horm. Metab. Res.* **2004**, *36*, 867−876.

(4) Doyle, M. E.; Egan, J. M. Mechanisms of action of glucagon-like peptide 1 in the pancreas. *Pharmacol. Ther.* **2007**, *113*, 546−593.

(5) Amori, R. E.; Lau, J.; Pittas, A. G. Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. *J. Am. Med. Assoc.* **2007**, *298*, 194−206.

(6) van Genugten, R. E.; van Raalte, D. H.; Diamant, M. Does glucagon-like peptide-1 receptor agonist therapy add value in the treatment of type 2 diabetes? Focus on exenatide. *Diabetes Res. Clin. Pract.* **2009**, *86S*, S26−S34.

(7) Liraglutide (Victoza) for type 2 diabetes. *Med. Lett. Drugs Ther.* **2010**, *52*, 25-27.
(8) Siddiqui, N. I. Incretin mimetics and DPP-4 inhibitors: new approach to treatment of type 2 diabetes mellitus. *Mymensingh Med. J.* **2009**, *18*, 113−124.
(9) Rosenstock, J.; Baron, M. A.; Dejager, S.; Mills, D.; Schweizer, A. Comparison of vildagliptin and rosiglitazone monotherapy in patients with type 2 diabetes: a 24-week, double-blind, randomized trial. *Diabetes Care* **2007**, *30*, 217−223.
(10) *FDA Approves New Drug Treatment for Type 2 Diabetes*; http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm174780.htm (Accessed July 31, 2009).
(11) Daniel, J. D.; Michael, A. N. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* **2006**, *368*, 1696−1705.
(12) Wang, M. W.; Liu, Q.; Zhou, C. H. Non-peptidic glucagon-like peptide-1 receptor agonists: aftermath of a serendipitous discovery (Perspective). *Acta Pharmacol. Sin.* **2010**, *31*, 1026−1030.
(13) Chen, D. S.; Liao, J. Y.; Li, N.; Zhou, C. H.; Liu, Q.; Wang, G. X.; Zhang, R.; Zhang, S.; Lin, L. L.; Chen, K. X.; Xie, X.; Nan, F. J.; Young, A. A.; Wang, M. W. A nonpeptidic agonist of glucagon-like peptide 1 receptors with efficacy in diabetic *db*/*db* mice. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 943−948.
(14) Su, H. R.; He, M.; Li, H. M.; Liu, Q.; Wang, J.; Wang, Y. Q.; Gao, W. W.; Zhou, L.; Liao, J. Y.; Young, A. A.; Wang, M. W. Boc5, a non-peptidic glucagon-like peptide-1 receptor agonist, invokes sustained glycemic control and weight loss in diabetic mice. *PLoS One* **2008**, *3*, e2892.
(15) He, M.; Su, H. R.; Gao, W. W.; Johansson, S. M.; Liu, Q.; Wu, X. Y.; Liao, J. Y.; Young, A. A.; Bartfai, T.; Wang, M. W. Reversal of obesity and insulin resistance by a non-peptidic glucagon-like peptide-1 receptor agonist in diet-induced obese mice. *PLoS One* **2010**, *5*, e14205.
(16) King, S. W.; Riordan, J. M.; Holt, E. M.; Stammer, C. H. Synthesis of racemic (*E*)- and (*Z*)-1-amino-2-phenylcyclopropanecarboxylic acids: (*E*)- and (*Z*)-"cyclopropylphenylalanine. *J. Org. Chem.* **1982**, *47*, 3270−3273.
(17) Inmaculada, A.; Manuel, B.; Eldiberto, F. A.; Soldedad, P. Synthesis of (*E*)- and (*Z*)-1-Amino-2-aryl(methyl)-cyclopropanecarboxylic Acids via Spirooxazolones. *Synthesis* **1985**, *8*, 773−775.
(18) Almond, M. R.; Stimmel, J. B.; Thompson, E. A.; Loudon, G. M. Hofmann Rearrangement under mildly acidic conditions using [I,I-bis(trifluoroacetoxy)]iodobenzene: cyclobutylamine hydrochloride from cyclobutanecarboxamide. *Org. Synth.* **1988**, *66*, 132.
(19) Shaikh, B. M.; Chobe, S. S.; Konda, S. G.; Khandare, N. T.; Chavan, S. A.; Dawane, B. S. An efficient synthesis and in vitro antimicrobial activity of 1,2,4-triazin-6-(5*H*)-one derivatives. *Chem. Sin.* **2010**, *1*, 86−91.
(20) Palcut, M. Spectral properties of novel 1,3-oxazol-5(4*H*)-ones with substituted benzylidene and phenyl rings. *Acta Chim. Slov.* **2009**, *56*, 362−368.
(21) Johannes, S. B.; Walter, S. I. Azlactone of α-benzoylamino-β-(3,4-dimethoxyphenyl)-acrylic acid. *Org. Synth.* **1943**, *CV2*, 55.
(22) Ram, S. S.; Radhey, M. Singh. Ethanolysis of 4-(*N*,*N*-dimethylaminomethylene)-2-aryl-2-oxazolin-5-ones with sodium ethoxide in ethanol at reflux temperature: unusual formation of *N*-acyl-α-amino acids. *Indian J. Chem.* **1998**, *37B*, 1296−1299.
(23) Samet, A. V.; Coughlin, D. J.; Buchanan, A. C. III; Gakh, A. A. An improved "one-pot" procedure for synthesis of fluorinated DL-phenylalanines. *Synth. Commun.* **2002**, *32*, 941−946.
(24) Rao, Y. S. Geometric isomers of 2-aryl(aralkyl)-4-arylidene(alkylidene)-5(4*H*)-oxazolones. *Synthesis* **1975**, 749−764.
(25) Lewis, F. D.; Quillen, S. L.; Hale, P. D.; Oxman, J. D. Lewis acid catalysis of photochemical reactions. 7. Photodimerization and cross-cycloaddition of cinnamic esters. *J. Am. Chem. Soc.* **1988**, *110*, 1261−1267.
(26) Wever, E.; Hecker, M.; Csöregh, I.; Czugler, M. New host family based on small-ring compounds. *J. Am. Chem. Soc.* **1989**, *111*, 7866−7872.
(27) Ichikawa, M.; Takahashi, M.; Aoyagi, S.; Kibayashi, C. Total synthesis of (−)-Incarvilline, (+)-Incarvine C, and (−)-Incarvillateine. *J. Am. Chem. Soc.* **2004**, *126*, 16553−16558.
(28) Khan, M.; Brunklaus, G.; Enkelmann, V.; Spiess, H. W. Transient states in [2 + 2] photodimerization of cinnamic acid: correlation of solid-state NMR and X-ray analysis. *J. Am. Chem. Soc.* **2008**, *130*, 1741−1748.
(29) Chimichi, S.; Piero, S. F. Solid-state photoreactivity of ethyl (*E*)-α-cyano-2-methoxycinnamate. *J. Org. Chem.* **1987**, *52*, 5124−5126.
(30) Pattabiraman, M.; Natarajan, A.; Kaanumalle, L. S.; Ramamurthy, V. Templating photodimerization of *trans*-cinnamic acids with cucurbit[8]uril and γ-cyclodextrin. *Org. Lett.* **2005**, *7*, 529−532.
(31) Marhold, M.; Buer, A.; Hiemstra, H.; van Maarseveen, J. H.; Haufe, G. Synthesis of vinyl fluorides by ring-closing metathesis. *Tetrahedron Lett.* **2004**, *45*, 57−60.
(32) Allen, N. S.; Mallon, D.; Timms, A.; Green, A. W.; Catalina, F. Synthesis and spectroscopic properties of novel cinnamate derivatives of benzophenone: photocuring activity versus photodimerization. *Eur. Polym. J.* **1993**, *29*, 533−538.
(33) Kamboj, R. K.; Chen, H.; McCallum, K.; Martin, S. S.; Drucker, D. J.; Crivici, A. Prototypic G protein-coupled receptor for the intestinotrophic factor glucagon-like peptide 2. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1569−1573.
(34) Qureshi, S. A.; Candelore, M. R.; Xie, D.; Tota, L. M.; Ding, V. D.-H.; Li, Z. H.; Bansal, A.; Miller, C.; Cohen, S. M.; Jiang, G. Q.; Brady, E.; Saperstein, R.; Duffy, J. L.; Tata, J. R.; Chapman, K. T.; Moller, D. E.; Zhang, B. B. A novel glucagon receptor antagonist inhibits glucagon-mediated biological effects. *Diabetes* **2004**, *53*, 3267−3273.
(35) Lynn, F. C.; Pamir, N.; Ng, E. H.; McIntosh, C. H.; Kieffer, T. J.; Pederson, R. A. Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. *Diabetes* **2001**, *50*, 1004−1011.