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Toward the development of chemoprevention agents. Part II: Chemo-enzymatic synthesis and anti-inflammatory activities of a new class of 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes

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Abstract—A new series of optically pure 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes were designed and synthesized via a chemo-enzymatic combined method to develop new chemoprevention agents. Twenty-four of newly synthesized compounds significantly inhibited xylene-induced rat ear edema and exhibited comparable or better anti-inflammatory activities than the reference drug aspirin. Treatment of these anti-inflammatory agents did not prolong the tail bleeding time in rat. In addition, 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes exhibited good membrane permeability based on in vitro Caco-2 cell monolayer permeability assay. Furthermore, some preliminary structure–activity relationships were further analyzed among these compounds. Taken together, 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes may represent a new class of anti-inflammatory drugs with safer pharmacological profile.

1. Introduction

Inflammation can originate from both infectious and non-infectious processes of chronic injury or irritation.[1] The inflammatory response leads to the recruitment of mast cells and leukocytes to the site of damage with subsequent release of free radicals, including reactive oxygen species.[1] These free radicals are known to damage macromolecules, including lipids and DNA.[2,3] In addition to the cellular and genomic damage that occurs as part of free-radical activity and other by-products, the release of eicosanoids triggers cell proliferation.[4] The combination of these processes facilitates carcinogenesis.[4] Inflammation is now considered a well-established cancer risk factor. So, it is not surprising that the regular use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) is related to a decreased risk of several types of cancer.[5–7]

Chemoprevention is an exciting research field in cancer research. In recent years, aspirin and other NSAIDs show promise as chemoprevention agents.[1] Possible targets include cancers of the colon, stomach, breast, and lung. The most convincing evidence is from colorectal cancer. Compelling data from epidemiological studies suggest that NSAIDs reduce the risk of colorectal cancer by around a half.[7] For this reason, the US food and Drug Administration has approved the use of celecoxib in the prevention of colorectal polyps in patients with familial adenomatous polyposis.[6]

An ideal chemoprevention agent should be safe and non-toxic over a long term use. Current NSAIDS have serious toxicity rate that quickly exceeds any benefit from cancer chemoprevention. In contrast, low-dose aspirin is worth evaluating, especially because of the potential for simultaneous cardiovascular risk reduction. In addition, development of new NSAIDS, with less toxicity, is another approach toward making the toxicity-benefit ratio more favorable for the use of these agents for cancer chemoprevention.

1,3-Dioxane derivatives have been reported recently having anti-inflammatory, anti-cancer, and reperfusion

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injury protection effects through their anti-proliferative and anti-inflammatory activities in human neutrophils and tumor cells.[8] We have been investigating 2,5-disubstituted-1,3-dioxanes as a new class of anti-inflammatory agents.[9–12] In contrast to classical, acidic non-steroidal anti-inflammatory agents (NSAIDs), these new analogues of 1,3-dioxanes are basic. To further improve the efficiency and safety of anti-inflammatory agents, in this study a new series of optically pure 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes were synthesized via a chemo-enzymatic combined method. The in vivo anti-inflammatory activities of these compounds were evaluated using the xylene-induced mouse ear edema model. In addition, we have reported here the preliminary structure–activity relationship studies.

2. Results and discussion

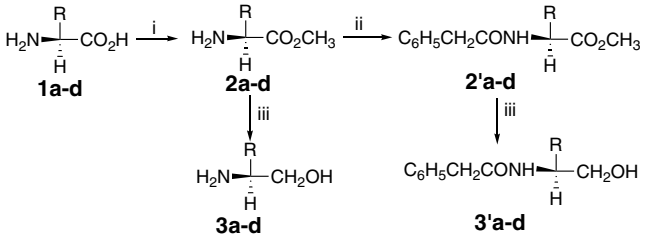
2.1. Synthesis of aminodiols[12]

Starting from the optically active amino acids **1a–d**, in the presence of thionyl chloride and methanol, the corresponding methyl esters **2a–d** were readily prepared with high yields, which then were subjected to KBH_4 reduction. Accordingly, aminodiols **3a–d** were obtained in moderate yields (39–62%). Similarly, after treatment of **2a–d** with phenylacetyl chloride, followed by KBH_4 reduction, a series of phenylacetamidodiols **3′a–d** were obtained in excellent yields (91–93%). To avoid any possible racemization all the reactions involved in the preparation of the aminodiols should be carried out at 0 °C to room temperature (Scheme 1).

2.2. Synthesis of 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes via the acetalization

Using *p*-toluenesulfonic acid as catalyst, aminodiols **3a–d** were subjected to acetalization with substituted benzaldehydes **4a–e**. As a result, a series of 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes, **5a–j**, **6** and **6′a,b**, **7** and **7′c–e**, **8** and **8′c–e**, were obtained in low to moderate yields (Scheme 2).

However, through nuclear Overhauser effect (NOE) experiments, no positive NOE signals were observed in these compounds (**5a–j**, **6** and **6′a,b**, **7** and **7′c–e**, **8** and **8′c–e**). We hypothesized that this may be due to the rapid exchange of hydrogens between both the 5-NH_2 and 2-substitution with $CHCl_3$-*d* solvent, resulting in the hydrogens unable to participate in subsequent NOE experiment.



Scheme 1. Synthetic route to **3** and **3′a–d**. Reagents and conditions: (i) $SOCl_2$ and MeOH; (ii) $C_6H_5CH_2COCl$/THF; (iii) KBH_4, THF. In **1a**, **2a**, **2′a**, **3a**, and **3′a**: R = CH_2OH; **1b**, **2b**, **2′b**, **3b**, and **3′b**: R = $CH(CH_3)OH$; **1c** R = CH_2CO_2H; **1d** R = $CH_2CH_2CO_2H$; **2c** and **2′c**: R = $CH_2CO_2CH_3$; **2d** and **2′d**: R = $CH_2CH_2CO_2CH_3$; **3c** and **3′c**: R = CH_2CH_2OH; **3d** and **3′d**: R = $CH_2CH_2CH_2OH$.

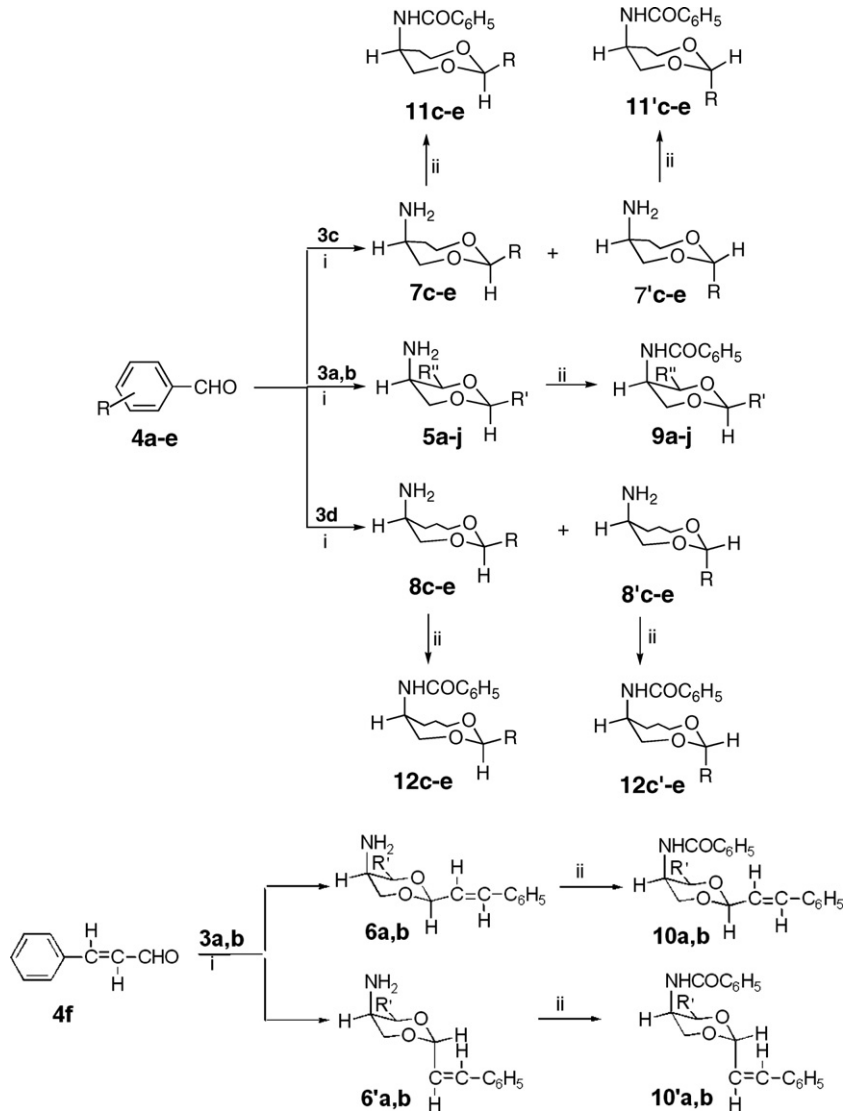
To make the stereochemistry of **5a–j** assignable they were then converted to **9a–j**, of which the benzoyllation on the 5-NH_2 group and consequently stabilize the hydrogen atom of 5-NH_2 for participation in the NOE difference spectra. For example, a positive NOE effect was observed in **9a–e** between the NH at the 5-position and the proton of the phenyl at the 2-position, indicating that these two substituents on the ring were arranged in a *syn*-configuration. Thus, based on the configuration of **9a–e**, the substituents at both 2- and 5-positions of **5a–e** were accordingly assigned in a *syn*-arrangement. Similarly, for **9f–j**, the positive NOE signals were observed between the substituents at the 2-, 4-, and 5-positions, which indicated that both substituents on the rings of **9f–j** are in *syn*-arrangement. Furthermore, based on the configuration of **9f–j**, the substituents at the 2-, 4-, and 5-positions on the ring of **5f–j** were then assigned to be in *syn*-arrangement. In a similar manner, to assign the stereochemistry of **6** and **6′a,b**, **7** and **7′c–e**, **8** and **8′c–e**, both compounds again underwent benzoylacetylation reactions to afford **10** and **10′a,b**, **11** and **11′c–e**, **12** and **12′c–e**, respectively. As a result, the stereochemistry of **6** and **6′a,b**, **7** and **7′c–e**, **8** and **8′c–e** was assigned based on the configuration of **10** and **10′a,b**, **11** and **11′c–e**, **12** and **12′c–e**, respectively.

2.3. Synthesis of 5-amino-2-substitutedphenyl-1,3-dioxane via chemo-enzymatic method

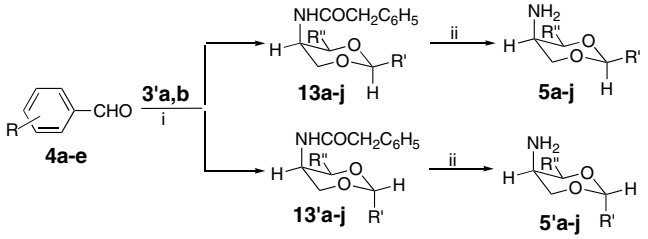
It was worth to point out, a mixture of *cis*- and *trans*-isomers was obtained when **3c,d** were treated with substituted benzaldehydes **4a–e**. In contrast, when **3a,b** were treated with aldehyde, **4a–e** exclusively gave *cis*-products **5a–j**. No more sterically demanding *trans*-products were observed even changing the mole ratio of the starting materials and the reaction conditions (e.g., catalysts and reaction temperature). To enable a more precise elucidation of the structure–activity relationship, we attempted different approaches to prepare the *trans*-products of 5-amino-2-substitutedphenyl-1,3-dioxane.

Our previous study indicated the stereoselectivity of 2,5-disubstituted-1,3-dioxanes could be tuned by the substituents of their precursors. On the basis of this finding, aminodiols **3a,b** were replaced by 2-phenylacetamido-1,3-diols **3′a,b**, which were then subjected to the acetalization with **4a–e**. As we expected, a pair of (*cis*)- and (*trans*)-products, **13** and **13′a–j**, was obtained in good yield (Scheme 3).[12]

Next, difficulties were encountered during the deprotection of *N*-phenylacetyl group of **13** and **13′a–j**. It was observed that racemization occurred to some extent under the strong basic deprotection condition. In



Scheme 2. Synthetic route to **5a**–**j**, **6** and **6′a**,**b**, **7** and **7′c**–**e**, **8** and **8′c**–**e**. Reagents and condition: (i) $CHCl_3$/THF, rt, *p*-toluenesulfonic acid (catalyst), anhydrous Na_2SO_4; (ii) PhCOCl/TEA, $CHCl_3$, 0 °C to rt. In **4a**: R = H; **4b**: R = 4-CH_3; **4c**: R = 4-Cl; **4d**: R = 4-NO_2; **4e**: R = 3-NO_2; **6a**, **6′a**, **10a** and **10′a** R′ = H; **6b**, **6′b**, **10b** and **10′b** R′ = CH_3.



Scheme 3. Synthesis of **5** and **5′a**–**j** via a chemo-enzymatic combined method. Reagents and conditions: (i) $CHCl_3$/THF, rt, *p*-toluenesulfonic acid (catalyst), anhydrous Na_2SO_4; (ii) penicillin acylase, rt, pH 6.0, 4 h.

search of a mild deprotection condition, the use of enzymatic method attracted our attention.[13–19] We attempted to remove the *N*-phenylacetyl protecting group of these compounds with enzymatic hydrolysis. We discovered that penicillin acylase is a valuable agent for the removal of *N*-phenylacetyl protection group of the dioxacycloalkanes.

Often, the substrate specificity of enzymes limits their application in synthetic organic chemistry. However, penicillin acylase from *Escherichia coli* can recognize a broad range of substrates. Besides benzylpenicillin, it accepts a range of substituted phenylacetylamides with high specificity for the phenylacetyl group but low specificity for the amine moiety. In fact, the mild and neutral enzymatic method provided reliable access to our desired compounds. It was noticed, even in the presence of organic co-solvents like methanol, penicillin acylase could recognize a broad range of *N*-phenylacetyl protected dioxacycloalkanes. Seemingly, it tolerated variations in the substituents and ring size of dioxacycloalkanes. Nevertheless, the hydrolysis rates varied with the steric and electronic factors of the 2-substitution of dioxacycloalkanes, as well the solubility of the substrates under the reaction conditions.

Upon treatment of an aqueous solution of **13** and **13′a–j** with the penicillin acylase at pH 6.0 and 37 °C, the *N*-phenylacetyl protecting group was removed smoothly to give the expected **5** and **5′a–j** without any undesired side reaction. HPLC analysis indicated no racemization occurred during the enzymatic hydrolysis.

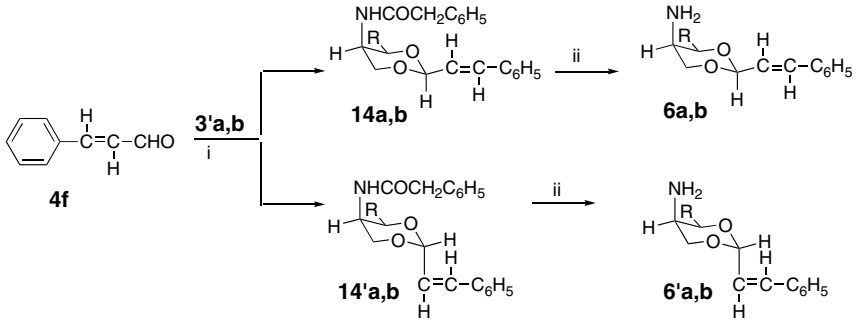
Under the similar condition, the acetalization of (*E*)-phenylvinyl aldehyde **4f** with 2-phenylacetamido-1,3-diols **3′a,b** stereoselectively provided two pairs of (*cis*)- and (*trans*)-products, namely **14** and **14′a,b** which were then subjected to enzymatic hydrolysis.[12] Accordingly, the de-protected products **6** and **6′a,b** were obtained in good yields (Scheme 4).

To test the generality of the enzymatic hydrolysis, we then expanded this method for the large scale preparation of optically pure 7- and 8-membered 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes (Scheme 5). Specifically, the acetalization of benzaldehyde **4c–e** and 2-phenylacetamido-1,4-diol **3′c** provided a series of isomers containing 7-membered dioxacycloalkanes, **15** and **15′c–e**. Under similar condition, a series of 8-membered dioxacycloalkanes, **16** and **16′c–e**, were prepared stereoselectively. Subsequently, **15** and **15′c–e** and **16** and **16′c–e** were subjected to enzymatic hydrolysis, respectively. Accordingly, **7** and **7′c–e** and **8** and **8′c–e** were obtained in good to excellent yields.

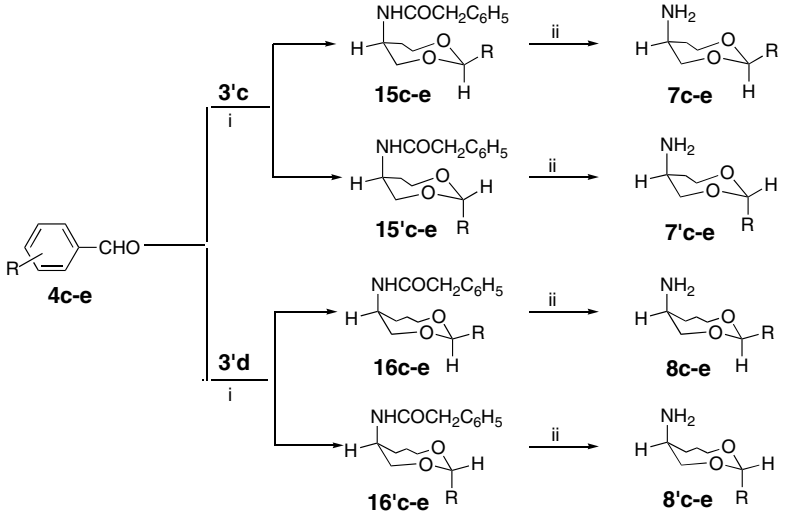
In summary, we have developed a straightforward and efficient chemo-enzymatic method to produce a series of new 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes with high optical purity and good yields. This method was easily scaled up to yield gram scale quantity of this new class of compounds. These results suggested that the enzymatic removal of the *N*-phenylacetyl protecting group of dioxacycloalkanes could advantageously be applied in heterocyclic chemistry.

2.4. In vivo anti-inflammatory activities evaluated in the ear edema model and SAR studies

All the newly synthesized 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes were then evaluated for their anti-inflammatory activity by using a xylene-induced ear edema model assay. Briefly, the in vivo assay involved the test compounds being administerted orally in 0.5% carboxymethyl cellulose (CMC) suspension. Each compound was initially tested at a concentration of 20 mg/kg. It was observed that 24 of the tested compounds showed significant inhibition against xylene-induced inflammation in mice as compared with the



Scheme 4. Synthesis of **6** and **6′a,b** via chemo-enzymatic combined method. Reagents and conditions: (i) $CHCl_3$/THF, rt, *p*-toluenesulfonic acid (catalyst), anhydrous Na_2SO_4; (ii) penicillin acylase, rt, pH 7.5, 4–5 h.



Scheme 5. Synthesis of **6a,b**, **7c–e**, **7′c–e**, **8c–e**, and **8′c–e** via chemo-enzymatic combined method. Reagents and conditions: (i) $CHCl_3$/THF, rt, *p*-toluenesulfonic acid (catalyst), anhydrous Na_2SO_4; (ii) penicillin acylase, rt, pH 7.5.

control (Table 1), indicating that these compounds possess potent anti-inflammatory activity. It was especially noted that these compounds exhibited even higher anti-inflammatory activities than that of the standard reference drug aspirin. Subsequently, those compounds with significant anti-inflammatory activity (**5** and **5′h–j**) were administerted in a series of lower concentration doses to enable a detailed pharmacological activity profile (Table 2).

Structure–activity relationship studies were also performed on a series of phenylacetaminodiol compounds to assess whether the presence of 1,3-dioxacycle rings is required for anti-inflammatory activity. Observations that phenylacetaminodiols **3′a–d** were ineffective as anti-inflammatory compounds suggested that the rigid 1,3-dioxacycle ring should be retained. This observation was consistent with our previously reported results.

To examine the importance of the spatial disposition, all of the isomers with different stereochemistries were compared. For example, compounds **5a–j** and **5′a–j**, their comparable anti-inflammatory activities indicated the stereochemical constraints of these 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes having little or no effect on their anti-inflammatory activities (**5a**: 51.6% vs **5′a**: 50.6%; **5b**: 50.0% vs **5′b**: 49.4%; **5c**: 64.1% vs **5′c**: 64.7%; **5d**: 41.0% vs **5′d**: 42.0%; **5e**: 56.7% vs **5′e**: 56.1%; **5f**: 52.9% vs **5′f**: 51.9%; **5g**: 54.5% vs **5′g**: 53.8; **5h**: 66.7% vs **5′h**: 67.9%; **5i**: 78.5% vs **5′i**: 77.9; **5j**: 61.5% vs **5′j**: 60.9%).

To investigate the effect of heterocyclic ring sizes, the anti-inflammatory activities of 6-, 7-, and 8-membered dioxacycloalkanes were compared. It was noticed that the anti-inflammatory activities of the compounds containing 7-membered dioxacycloalkanes (**7c**: 32.1%; **7d**: 21.8%; **7e**: 24.7%; **7′c**: 31.1%; **7′d**: 23.1%; **7′e**: 23.7%) were apparently decreased over their corresponding 6-membered counterparts (**5c**: 64.1%; **5d**: 41.0%; **5e**: 56.7%; **5′c**: 64.7%; **5′d**: 42.0%; **5′e**: 56.1%). Similar

Table 2. Anti-inflammatory activities of **5** and **5′h–j** at different doses against xylene-induced ear edema in mice

	Dose (mg/kg)	Edema weight (X ± SD mg)	Inhibition (%)
CMC		3.12 ± 0.55	
Aspirin	30	1.85 ± 0.72	40.7
5h	5	1.89 ± 0.48	39.4
5i	5	1.80 ± 0.51	42.3
5j	5	2.20 ± 0.56	29.4
5′h	5	2.19 ± 0.49	29.8
5′i	5	1.87 ± 0.50	40.1
5′j	5	2.21 ± 0.53	29.2
5h	10	1.42 ± 0.49[b]	54.5
5i	10	1.31 ± 0.50[b]	58.0
5j	10	1.46 ± 0.48[b]	53.2
5′h	10	1.68 ± 0.53[b]	46.2
5′i	10	1.35 ± 0.49[b]	56.7
5′j	10	1.72 ± 0.46[b]	44.9
5h	20	1.04 ± 0.29[a]	66.7
5i	20	0.67 ± 0.52[c]	78.5
5j	20	1.20 ± 0.42[a]	61.5
5′h	20	1.07 ± 0.35[c]	65.7
5′i	20	0.69 ± 0.51[c]	77.9
5′j	20	1.22 ± 0.44[a]	60.9

$N = 11$.
[a] Compare to 10 mg/kg group $P < 0.05$.
[b] Compare to 5 mg/kg group $P < 0.05$.
[c] Compare to 10 mg/kg group $P < 0.01$.

Table 1. Anti-inflammatory activities of 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes against xylene-induced ear edema in mice

Agents	Edema weight (X ± SD mg)	Inhibition (%)	Agents	Edema weight (X ± SD mg)	Inhibition (%)
CMC	3.12 ± 0.55		Aspirin	1.85 ± 0.72[b]	40.7
5a	1.51 ± 0.53[b]	51.6	**5′a**	1.54 ± 0.55[b]	50.6
5b	1.56 ± 0.21[b]	50.0	**5′b**	1.58 ± 0.54[b]	49.4
5c	1.12 ± 0.57[b,c]	64.1	**5′c**	1.10 ± 0.53[b,c]	64.7
5d	1.84 ± 0.73[b],	41.0	**5′d**	1.81 ± 0.70[b]	42.0
5e	1.35 ± 0.51[b]	56.7	**5′e**	1.37 ± 0.52[b]	56.1
5f	1.47 ± 0.50[b]	52.9	**5′f**	1.50 ± 0.52[b]	51.9
5g	1.42 ± 0.35[b]	54.5	**5′g**	1.44 ± 0.53[b]	53.8
5h	1.04 ± 0.29[b,d]	66.7	**5′h**	1.00 ± 0.35[b,d]	67.9
5i	0.67 ± 0.52[b,d]	78.5	**5′i**	0.69 ± 0.51[b,d]	77.9
5j	1.20 ± 0.42[b,c]	61.5	**5′j**	1.22 ± 0.44[b,c]	60.9
6a	1.56 ± 0.52[b]	50.0	**6′a**	1.54 ± 0.50[b],	50.6
6b	1.47 ± 0.48[b]	52.9	**6′b**	1.51 ± 0.52[b]	51.6
7c	2.12 ± 0.56[b]	32.1	**7′c**	2.15 ± 0.53[b]	31.1
7d	2.48 ± 0.53[a]	21.8	**7′d**	2.40 ± 0.55[b]	23.1
7e	2.35 ± 0.52[b]	24.7	**7′e**	2.38 ± 0.55[b]	23.7
8c	2.24 ± 0.54[b]	28.2	**8′c**	2.22 ± 0.52[b]	28.8
8d	2.38 ± 0.55[b]	23.7	**8′d**	2.41 ± 0.53[b]	22.8
8e	2.37 ± 0.56[b]	24.0	**8′e**	2.35 ± 0.54[b]	24.7

For 1,3-dioxacycloalkanes dose = 20 mg/kg, for aspirin dose = 30 mg/kg; $n = 11$.
[a] Compare to control, $P < 0.05$.
[b] Compare to control, $P < 0.01$.
[c] Compare to aspirin, $P < 0.05$.
[d] compare to aspirin, $P < 0.01$.

tendency was also observed in the case of 8-membered dioxacycloalkanes (**8c**: 28.2%; **8d**: 23.7%; **8e**: 24.0%; **8′c**: 28.8%; **8′d**: 22.8%; **8′e**: 24.7%). These observations indicated that, the anti-inflammatory activity of 1,3-dioxane was more potent than either 1,3-dioxacycloheptane or 1,3-dioxacyclooctane, suggesting that 6-membered dioxacycloalkanes cannot be further maximized.

It was of interest to observe that the substitution on the phenyl ring was important for anti-inflammatory activity. In the case of **5a**–**j** and **5′a**–**j**, it seemed that the electron-withdrawing groups on the phenyl ring endowed these compounds with good anti-inflammatory profiles (i.e., **5i**: 78.5%; **5j**: 61.5%; **5′i**: 77.9%; **5′j**: 60.9%). Interestingly, in the same series, the chlorine substitution on the phenyl ring induced a superior anti-inflammatory effect (**5c**: 64.1; **5′c**: 64.7%; **5h**: 66.7%; **5′h**: 67.9%).

In addition, it was noticed that, compared with *N*-phenylacetyl and *N*-benzyl substituted counterparts (**9a**–**j**, **10** and **10′a**,**b**, **11** and **11′c**–**e**, **13** and **13′a**–**j**, **14** and **14′a**,**b**, **15** and **15′c**–**e**, **16** and **16′c**–**e**), the free state of 5-amino of 1,3-dioxacycloalkanes (**5** and **5′a**–**j**, **6** and **6′a**,**b**) led to a significant improvement of the anti-inflammatory activity. This observation suggested that the amino group was optimal for the anti-inflammatory potency. Nevertheless, the formation of the hydrogen bond with the amino moiety might be critical during the receptor binding process; we strongly suspect the better anti-inflammatory activities of compounds result from facile protonation of the amine moieties, which, in turn, enable the molecules to easily access cellular membrane. A minor structural alteration can lead to a marked change in anti-inflammatory activity, which implied that the improved biological profile might result from a synergic effect.

Furthermore, it was noted that the most potent compounds (**5** and **5′h**–**j**) all exhibited anti-inflammatory activities much higher than that of the standard reference aspirin. The ear edema inhibition for **5** and **5′h**–**j** at the dose of 20 mg/kg is calculated to be 66.7%, 78.5%, 61.5%, 65.7%, 77.9%, and 60.9%, respectively, compared with that of aspirin 40.7% (30 mg/kg). Consequently, they were administered in a series of lower concentration doses to enable a detailed pharmacological activity profile (data summarized in Table 2).

2.5. Dose-dependent anti-inflammatory activities

Oral administration of compounds **5** and **5′h**–**j** was observed and all produced a dose-dependent anti-inflammatory response in the xylene-induced mouse ear edema test. For example, compound **5i** at doses of 5, 10.0, and 20.0 mg/kg, the anti-inflammatory effect was observed to be 42.3%, 58.0%, and 78.5%, respectively. Apparently, **5i** demonstrated a significant enhancement in its anti-inflammatory activity with doses above 5.0 mg/kg. Aspirin, by comparison, had anti-inflammatory effect of 40.7% at a dose of 30 mg/kg. Similarly, the anti-inflammatory activity for compound **5′i** was 40.1%, 56.7%, and 77.9%, at doses of 5, 10.0, and 20.0 mg/kg, respectively. Additionally, compound **5h** exhibited comparable anti-inflammatory activity with aspirin at a dose of 5.0 mg/kg. Therefore, compounds **5** and **5′h**–**j** exhibited dose-dependent anti-inflammatory action (Table 2).

2.6. Tail bleeding time measurement

The use of conventional NSAIDs is associated with a number of significant adverse events, including gastrointestinal (GI) bleeding, impaired platelet function, and prolonged bleeding time. The clinical effects of NSAID-induced platelet dysfunction consist of an increased bleeding, prolonged surgical bleeding, and additive risk of significant or life-threatening bleeding in patients taking anticoagulants. Both surgical and non-surgical studies have shown that conventional NSAIDs increase bleeding time and blood loss. To develop safer anti-inflammatory agents, it is critical to evaluate if these newly synthetic agents have deleterious effect on normal haemostasis leading to bleeding complications. The cutaneous bleeding time model is the most common method used in animal experiments to investigate bleeding potential in humans.[20–28]

Male mice (18–22 g) were orally administered with the new anti-inflammatory agents. After 30, 45, 60, and 90 min of administration, a mouse was placed in a tube holder with its tail protruding, and a 2-mm cut was made on the tail. Flowing blood until it stopped was gently wiped away with a tissue every 30 s until bleeding ceased and the time recorded. Baseline bleeding times were similar between treatment groups with an average of 113–121 s. The treatment with the new anti-inflammatory agents at the high doses (200 mg/kg) did not significantly prolong bleeding time at any time point (30, 45, 60, and 90 min) compared with NS (Table 3).

2.7. In vitro membrane permeation study

Oral administration is the most commonly used drug-dosing route. Therefore, the ability to predict the extent of absorption of drug candidates after oral administration is crucial during the preclinical evaluation. The oral bioavailability of drug candidates is influenced by many factors, including dissolution, absorption, pre-systemic and systemic metabolism, and elimination. However, the intestinal mucosa is a significant barrier to oral delivery of drugs into the systemic circulation. Caco-2 cells possess many structural and functional similarities to human enterocytes. Recently, Caco-2 cell permeability test has been generally used as a screening tool for assessing drug oral bioavailability during the early stage of drug development.[29–33]

Caco-2 cell monolayer was utilized as an in vitro model of the intestinal mucosa to assess the membrane permeability for these newly synthesized compounds. Drug permeability is difficult to measure, thus this assay provides a convenient way to measure permeability based on the apparent permeability coefficient (P_{app}). P_{app} (A → B) is the permeability from the apical to the basolateral side (intestine to blood), and P_{app} (B → A) is the permeability from the basolateral to the apical side (blood to intestine). It was

Table 3. Effect of 1,3-dioxacycloalkanes on the tail bleeding time ($\overline{X} \pm$ SDs) of mice

Compound	Before drug administration	Post-drug administration			
		30 min	45 min	60 min	90 min
NS	117.9 ± 8.2	118.8 ± 8.0	118.7 ± 7.9	119.2 ± 8.1	118.9 ± 8.6
5a	118.4 ± 8.4	119.2 ± 8.5	118.8 ± 7.6	117.6 ± 8.0	118.7 ± 8.8
5b	117.2 ± 7.9	118.3 ± 8.1	119.0 ± 8.7	118.7 ± 7.9	118.4 ± 8.0
5c	119.3 ± 8.5	118.5 ± 8.4	118.9 ± 7.9	120.9 ± 8.6	119.4 ± 8.3
5d	120.3 ± 8.6	119.4 ± 8.7	118.7 ± 8.6	120.6 ± 8.7	119.4 ± 8.1
5e	118.6 ± 7.9	119.3 ± 8.7	118.2 ± 8.4	119.1 ± 8.6	120.2 ± 8.8
5g	119.4 ± 8.2	120.9 ± 9.0	121.0 ± 9.2	119.8 ± 8.7	118.7 ± 8.5
5h	119.9 ± 8.7	118.3 ± 7.9	118.9 ± 8.0	118.5 ± 8.1	118.7 ± 8.3
5i	117.3 ± 7.6	118.4 ± 8.6	119.2 ± 8.3	117.2 ± 8.0	118.9 ± 8.5
5j	118.2 ± 7.8	118.9 ± 7.6	119.2 ± 8.0	117.4 ± 8.5	118.3 ± 8.6
5′a	118.8 ± 7.9	119.3 ± 8.1	119.2 ± 8.5	119.7 ± 8.8	119.6 ± 8.0
5′b	119.1 ± 8.2	118.5 ± 8.0	119.2 ± 8.7	119.5 ± 8.9	117.8 ± 8.2
5′c	118.2 ± 8.0	119.0 ± 8.3	118.9 ± 8.5	118.7 ± 8.2	119.6 ± 8.7
5′d	120.3 ± 8.4	119.5 ± 8.7	117.7 ± 8.2	118.6 ± 8.0	118.9 ± 8.5
5′e	119.5 ± 8.3	118.8 ± 8.4	118.0 ± 7.7	118.4 ± 8.2	118.7 ± 8.6
5′f	117.5 ± 7.4	118.7 ± 8.2	118.9 ± 7.8	118.6 ± 8.2	118.9 ± 8.4
5′g	119.1 ± 8.7	120.4 ± 8.5	118.5 ± 8.2	118.9 ± 8.6	117.7 ± 7.6
5′h	118.8 ± 8.0	119.6 ± 8.7	118.6 ± 8.3	118.7 ± 8.2	118.6 ± 8.5
5′i	116.9 ± 7.6	117.8 ± 8.0	118.1 ± 8.1	118.8 ± 8.3	119.0 ± 8.7
5′j	118.0 ± 8.0	118.9 ± 8.3	118.2 ± 8.1	119.5 ± 8.6	118.2 ± 8.1
6a	117.2 ± 7.8	118.5 ± 8.0	119.3 ± 8.5	117.8 ± 8.0	118.9 ± 7.9
6b	119.5 ± 7.9	117.2 ± 7.8	118.6 ± 8.2	118.4 ± 8.1	119.2 ± 8.2
6′a	116.6 ± 7.7	117.9 ± 8.0	119.2 ± 8.4	119.5 ± 8.3	118.4 ± 8.1
6′b	119.5 ± 8.3	120.7 ± 8.9	120.2 ± 9.2	117.6 ± 8.3	117.7 ± 7.9
7c	117.9 ± 8.0	117.7 ± 7.9	118.5 ± 8.1	119.2 ± 8.8	118.6 ± 8.6
7d	119.2 ± 8.5	118.5 ± 8.1	119.0 ± 8.4	118.6 ± 8.1	117.9 ± 7.9
7e	117.5 ± 7.7	117.9 ± 7.8	118.6 ± 8.1	119.0 ± 8.4	118.5 ± 8.7
7′c	118.7 ± 7.5	118.4 ± 7.6	119.6 ± 8.4	118.6 ± 8.4	119.0 ± 8.7
7′d	116.7 ± 7.5	117.8 ± 8.0	118.5 ± 7.9	117.9 ± 7.6	117.2 ± 7.8
7′e	120.5 ± 8.8	120.1 ± 8.5	118.7 ± 8.1	118.5 ± 8.3	118.6 ± 7.9
8c	117.3 ± 9.2	117.9 ± 8.5	118.7 ± 9.0	119.1 ± 8.5	118.3 ± 7.9
8d	117.7 ± 7.5	118.6 ± 7.9	120.3 ± 8.4	118.5 ± 9.1	117.7 ± 8.2
8e	116.9 ± 7.6	117.8 ± 8.2	117.9 ± 8.1	121.5 ± 9.2	118.0 ± 8.3
8′c	118.2 ± 8.1	119.4 ± 8.5	117.5 ± 9.1	118.4 ± 8.2	117.8 ± 7.9
8′d	116.7 ± 7.9	118.6 ± 8.3	119.0 ± 9.1	118.4 ± 8.2	117.6 ± 7.4
8′e	117.7 ± 8.0	119.3 ± 9.4	118.2 ± 9.0	118.5 ± 8.5	119.3 ± 8.7

$N = 10$, dose = 200 mg/kg.

initiated by adding the test solution to the apical or basolateral side of the monolayer. The various newly synthesized compounds across Caco-2 cell monolayers were valuated ($n = 3$) in the apical to basolateral (A → B) and basolateral to apical directions (B → A). The influence of efflux carriers on the permeability of the different compounds was also examined by comparing the permeability ratio P_{ratio} of absorptive transport P_{app} (A → B) to the secretory one P_{app} (B → A). Permeability coefficients for the synthetic compounds are summarized in Table 4.

According to the previous study, the compound with permeability coefficients $P_{app} < 1 \times 10^{-6}$, $1–10 \times 10^{-6}$, and $>10 \times 10^{-6}$ cm/s was defined as poorly, moderately, and well absorbed, respectively.[31] The compounds **14** and **14′a**,**b** exhibited moderate permeation through the Caco-2 cell monolayer. In contrast, after removing the NH_2 protecting group, that is, when **14** and **14′a**,**b** was converted into **6** and **6′a**,**b** the permeation was increased, that is, **6** and **6′a**,**b** belong to the well-absorbed compounds. A similar tendency was observed between the compounds **5** and **5′h**,**i** and **13** and **13′h**,**i**.

Table 4. Apparent permeability coefficients of **5** and **5′h**,**i**, **6** and **6′a**,**b**, **13** and **13′h**,**i**, **14** and **14′a**,**b**

Compound	$P_{app} \times 10^{-6}$(cm/s)		
	A → B	B → A	A → B/B → A
5h	11.89	7.01	1.70
5i	12.99	6.96	1.87
5′h	11.72	6.88	1.70
5′i	12.86	6.90	1.86
6a	10.22	7.05	1.45
6b	10.25	7.17	1.43
6′a	10.18	7.07	1.44
6′b	10.31	7.06	1.46
13h	10.22	7.00	1.46
13i	10.40	6.98	1.49
13′h	10.15	6.86	1.48
13′i	10.32	6.93	1.49
14a	8.97	7.87	1.14
14b	9.01	7.51	1.20
14′a	8.86	7.32	1.21
14′b	8.80	7.15	1.23

The standard deviations were generally less than 10% ($n = 4$).
A → B, from apical side to basolateral side.
B → A, from basolateral side to apical side.

Table 5. Composition of standard solution used for protracting standard curve

Tube number	1	2	3	4	5	6	7	8	9	10
Reactive solution 1(μl) [a]	200	200	200	200	200	200	200	200	200	200
Reactive solution 2(μl) [b]	200	200	200	200	200	200	200	200	200	200
Reactive solution 3(μl)	40	36	32	28	24	20	16	12	8	4
Buffer C (μl)	80	84	88	92	96	100	104	108	112	116
ATP (nmol)	20	18	16	14	12	10	8	6	4	2

[a] For reactive tube.
[b] For control tube.

Table 6. Inhibition effect of 1,3-dioxacycloalkanes on PKC

Agents	IC_{50} (mM)	Agents	IC_{50} (mM)	Agents	IC_{50} (mM)	Agents	IC_{50} (mM)
5a	1.62	**5′a**	1.64	**10h**	1.88	**10′h**	1.94
5b	1.72	**5′b**	1.76	**10i**	1.65	**10′i**	1.67
5c	1.22	**5′c**	1.30	**10j**	1.78	**10′j**	1.92
5d	1.87	**5′d**	190	**11a**	2.64	**11′a**	2.66
5e	1.44	**5′e**	1.48	**11b**	2.57	**11′b**	2.77
5f	1.48	**5′f**	1.70	**12a**	2.62	**12′a**	2.48
5g	1.54	**5′g**	1.66	**12b**	2.80	**12′b**	2.70
5h	1.10	**5′h**	1.14	**12c**	1.99	**12′c**	1.96
5i	0.89	**5′i**	0.93	**12d**	2.68	**12′d**	2.92
5j	1.23	**5′j**	1.35	**12e**	2.61	**12′e**	2.70
6a	1.67	**6′a**	1.68	**12f**	2.71	**12′f**	2.76
6b	1.62	**6′b**	1.70	**12g**	2.64	**12′g**	2.71
7c	2.49	**7′c**	2.55	**12h**	1.94	**12′h**	2.01
7d	2.68	**7′d**	2.73	**12i**	1.71	**12′i**	1.72
7e	2.66	**7′e**	2.72	**12j**	1.82	**12′j**	1.98
8c	2.71	**8′c**	2.74	**13c**	2.69	**13′c**	2.71
8d	2.76	**8′d**	2.79	**13d**	2.77	**13′d**	2.85
8e	2.81	**8′e**	2.85	**13e**	2.81	**13′e**	2.90
9a	2.59	**9′a**	2.63	**14c**	2.73	**14′c**	2.86
9b	2.42	**9′b**	2.67	**14d**	2.87	**14′d**	2.91
10a	2.43	**10′a**	2.52	**14e**	2.94	**14′e**	2.98
10b	2.58	**10′b**	2.62	**15c**	2.92	**15′c**	2.95
10c	1.83	**10′c**	1.91	**15d**	2.86	**15′d**	2.98
10d	2.78	**10′d**	2.80	**15e**	2.93	**15′e**	3.01
10e	2.48	**10′e**	2.56	**16c**	3.01	**16′c**	3.10
10f	2.59	**10′f**	2.64	**16d**	2.89	**16′d**	3.13
10g	2.48	**10′g**	2.55	**16e**	3.00	**16′e**	3.20

1-(5-Isoquinolylsulfonyl)-2-methylpiperazine was used as the positive control and its IC_{50} = 23 μM.

The in vivo anti-inflammatory effect of 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes most likely occurs via the inhibition of certain key enzymes involved in inflammation and/or cell signaling pathways. 1,3-Dioxane derivatives have been reported recently to possess PKC inhibitory activity and exert anti-inflammatory, anti-cancer, and reperfusion injury protection effects through their anti-proliferative and anti-inflammatory activities in human neutrophils and tumor cells. Therefore, we attempted to investigate the acting mechanism of 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes in PKC inhibition assay.[34,35] However, the preliminary results indicated that, most of these newly synthesized compounds displayed weak PKC inhibitor activities (Table 6), suggesting PKC might not be the target protein. Experiments are currently underway to ascertain the protein target(s) of the dioxacycloalkane compounds, in which other possible protein targets may also include cyclooxygenase and lipoxygenase, and phosphoinositide 3-kinase. Identification and understanding of the mechanism underlying the enzyme inhibition processes should prove beneficial for future development of chemoprevention agents.

3. Conclusion

We have identified 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes as a new series of potent anti-inflammatory agents. Twenty-four of the newly synthesized compounds provided the most interesting compounds in terms of in vivo anti-inflammatory activity. In addition, these compounds did not prolong tail bleeding time even at high doses (200 mg/kg). In conclusion, these newly synthesized compounds display a favorable pharmacological profile relative to the reference drug. Considering their efficacy and safety profiles, these newly synthesized compounds are worth to work further to detail the possible link of the inflammatory inhibition and cancer prevention.

4. Experimental

All reactions were carried out under nitrogen (1 bar). Melting points are uncorrected. Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere (1 bar). The agents used in this work were purchased from Sigma Chemical Co. (USA). Chromatography was performed on Qingdao silica gel H (Qingdao of China). The purities of the intermediates and the products were confirmed by TLC (Merck silica gel plates of type 60 F_{254}, 0.25 mm layer thickness, Germany) and HPLC (Waters, C18 column 4.6 × 150 mm, USA). NMR spectra were recorded at 300 MHz on a VXR-300 instrument or at 500 MHz on a Bruker Am-500 instrument in $CDCl_3$ with tetramethylsilane as internal standard. EI-MS was determined by Trace MS System (Thermo Finnigan, USA). Optical rotations were determined with a Schmidt + Haensch Polartromic D instrument (Germany). The statistical analysis of all the biological data was carried out by use of ANOVA test, $p < 0.05$ is considered significant.

4.1. HCl·L-Ser-OCH_3 (2a)

At 0 °C to 50 ml of anhydrous methanol, 3.75 ml (47.5 mmol) of thinly chloride was added dropwise. The solution was stirred at 0 °C for 30 min and then 5.0 g (47.6 mmol) of L-Ser was added. The reaction mixture was stirred at room temperature for 24 h and TLC(CH_3Cl/CH_3OH, 9:1) indicated complete disappear-

ance of L-Ser. The reaction mixture was evaporated under reduced pressure and the residue was triturated with petroleum ether repeatedly to provide 7304 mg (99%) of HCl·L-Ser-OCH_3 as a colorless powder (mp 161–162 °C) which was directly used for the next reaction.

4.2. HCl·L-Thr-OCH_3 (2b)

With the same procedure as that used for the preparation of **2a** from 4.998 g (47.5 mmol) of L-Thr 7867 mg (98%) of HCl·L-Thr-OCH_3 was obtained as a colorless powder (mp 140–142 °C).

4.3. HCl·L-Asp- $(OCH_3)_2$ (2c)

With the same procedure as that used for the preparation of **2a** from 6.332 g (47.5 mmol) of L-Asp 7.495 mg (98%) of HCl·L-Asp-$(OCH_3)_2$ was obtained as a colorless powder (mp 149–151 °C).

4.4. L-Glu-$(OCH_3)_2$ (2d)

With the same procedure as that used for the preparation of **2a** from 6.983 g (47.5 mmol) of L-Glu 8146 mg (98%) of HCl·L-Glu-$(OCH_3)_2$ was obtained as a colorless powder (mp 136–138 °C).

4.5. *N*-Phenyl-L-Ser-OCH_3 (2′a)

At 0 °C, to a suspension of 1.0 g (6.4 mmol) of HCl·L-Ser-OCH_3 in 30 ml of THF, 25 ml of saturated aqueous sodium carbonate was added. Under stirring to the solution 1.106 ml (8.3 mmol) of phenylacetyl chloride was added dropwise and the pH of the reaction mixture was adjusted to 8–9 by adding saturated aqueous sodium carbonate. The reaction mixture was stirred at room temperature for 3 h and TLC (CH_3Cl/CH_3OH, 19:1) indicated complete disappearance of L-Ser-OCH_3. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in 50 ml of ethyl acetate and then washed with saturated aqueous sodium bicarbonate (3× 10 ml), saturated aqueous of $KHSO_4$ (3× 10 ml), and saturated aqueous of NaCl (3× 10 ml) successively. The solution was evaporated under reduced pressure and the residue was purified by chromatography (petroleum ether/ethyl acetate, 5:1) to provide 1140 mg (75%) of $C_6H_5CH_2CO$-L-Ser-OCH_3 as a colorless powder (mp 77–79 °C).

4.6. *N*-Phenyl-L-Thr-OCH_3 (2′b)

With the same procedure as that used for the preparation of **2′a** from 1010 mg (6.0 mmol) of HCl·L-Thr-OCH_3 1171 mg (77%) of *N*-phenyl-L-Thr-OCH_3 was obtained as a colorless powder (mp 90–92 °C).

4.7. *N*-Phenyl-L-Asp-(OCH_3) (2′c)

With the same procedure as that used for the preparation of **2′a** from 1182 mg (6.0 mmol) of HCl·L-Asp-$(OCH_3)_2$ 1345 mg (80%) of *N*-phenyl-L-Asp-$(OCH_3)_2$ was obtained as a colorless powder (mp 90–92 °C).

4.8. *N*-Phenyl-L-Glu-(OCH_3) (2′d)

With the same procedure as that used for the preparation of **2′a** from 1274 mg (6.0 mmol) of HCl·L-Glu-$(OCH_3)_2$ 1433 mg (81%) of *N*-phenyl-L-Glu-$(OCH_3)_2$ was obtained as a colorless powder (mp 111–113 °C).

4.9. General procedure for the preparation of aminodiols 3a–e and 3′a–e

To the suspension of 200 mg (5.6 mmol) of KBH_4 in 10 ml of THF the solution of 1.0 mmol of HCl·L-AA-OCH_3 in 20 ml of THF was added. The reaction mixture was stirred at room temperature for 24 h and TLC (C_2H_5OH/H_2O, 7:1) indicated complete disappearance of HCl·L-AA-OCH_3. At 0 °C the reaction mixture was adjusted to pH 10 with hydrochloric acid (2 mol/L) and evaporated under reduced pressure. The residue was purified by chromatography (CH_3Cl/CH_3OH, 19:1).

4.9.1. 2-Aminopropane-1,3-diol hydrochloride (3a). Yield: 62%.

4.9.2. (2*R*,3*R*)-2-Aminobutane-1,3-diol hydrochloride (3b). Yield: 58%.

4.9.3. (2*S*)-2-Aminobutane-1,4-diol hydrochloride (3c). Yield: 39%.

4.9.4. (2*S*)-2-Aminoheptane-1,5-diol hydrochloride (3d). Yield: 42%. IR(film): ν/cm^{-1} = 3335, 3339, 3348, 1634, 1275, 1050. ^1H NMR (DMSO-d_6): δ/ppm = 7.623 (s, 2H), 4.228 (d, J = 6.21 Hz, 2H), 3.651 (m, J = 5.31 Hz, 1H), 3.526 (t, J = 5.44 Hz, 2H), 2.004 (s, 2H), 1.992 (m, J = 5.42 Hz, 2H), 1.562 (m, J = 5.42 Hz, 2H). ^{13}C NMR (DMSO-d_6): δ/ppm = 68.404, 63.027, 51.898, 28.587, 27.292. FAB-MS (*m*/*e*) 121 $[M+H]^+$. $[\alpha]_{20}^{D}$ −11.2 (*c* 4.0, H_2O). Anal. Calcd for $C_5H_{15}ClNO_2$: C, 49.97; H, 11.74; N 11.66. Found: C, 50.11; H, 11.89; N, 11.76.

4.9.5. *N*-[2-Hydroxy-1-(hydroxymethyl)ethyl]phenylacetamide (3″b). Yield: 91%.

4.9.6. (1*S*)-*N*-[3-Hydroxy-1-(hydroxymethyl)propyl]phenylacetamide (3′c). Yield: 93%.

4.9.7. (1*S*)-*N*-[4-Hydroxy-1-(hydroxymethyl)butyl]phenylacetamide (3′d). Yield: 93%.

4.10. General procedure for the preparation of 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes

A mixture of 2.0 mmol of aminodiol, 2.6 mmol of substitutedbenzaldehyde, 30 mg of tolylsulfonylic acid, 100 mg of anhydrous $NaSO_4$, 50 ml of chloroform, and 4 ml of THF was stirred at rt overnight until TLC($CHCl_3$/CH_3OH, 20:1) indicated complete disappearance of aminodiol. The reaction mixture was then adjusted to pH 7 with sodium carbonate and then filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by chromatography on silica gel.

4.11. General procedure for the preparation of 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes via enzymatic hydrolysis

A suspension of 1.0 mmol of 5-phenylacetylamino-2-substitutedphenyl-1,3-dioxacycloalkanes, 500 mg of the powder of penicillin acylase, and 20 ml of methanol/water was adjusted to pH 6.0 with hydrochloric acid and then shaken (37 °C, 50 times/1 min) for 4 h. TLC ($CHCl_3$/CH_3OH, 10:1) indicated the complete disappearance of 5-phenylacetylamino-2-substitutedphenyl-1,3-dioxacycloalkanes. The reaction mixture was filterted and the filtrate was adjusted to pH 8–9 with the aqueous sodium bicarbonate solution (5%). The solution was purified on the column of Sephadex G10 to remove the NaCl and then evaporated under reduced pressure to give 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes.

4.11.1. (*cis*)-(2-Phenyl-1,3-dioxan-5-yl)amine (5a). Yield: 40%. Mp 52–54 °C. IR(KBr): ν/cm^{-1} = 3245, 3188, 1647, 1639, 1601, 1579, 1502, 1459, 1051, 743, 692. ^1H NMR ($CDCl_3$): δ/ppm = 7.478 (d, J = 7.04 Hz, 2H), 7.363 (t, J = 7.56 Hz, 1H), 7.332 (t, J = 7.56 Hz, 2H), 5.510 (s, 1H), 4.141 (d, J = 11.71 Hz, 2H), 4.017 (d, J = 10.51 Hz, 2H), 2.787 (m, J = 10.32 Hz, 1H), 1.934 (s, 2H). ^{13}C NMR (DMSO-d_6): δ/ppm = 138.136, 128.964, 128.271, 125.865, 101.791, 73.300, 45.772. FAB-MS (*m*/*e*) 180 $[M+H]^+$. Anal. Calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 67.12; H, 7.23; N, 7.94.

4.11.2. (*cis*)-[2-(4′-Methylphenyl)-1,3-dioxan-5-yl]amine (5b). Yield: 35%. Mp 55–56 °C. IR(KBr): ν/cm^{-1} = 3247, 3189, 1645, 1635, 1602, 1580, 1503, 1461, 1382, 1048, 822. ^1H NMR ($CDCl_3$): δ/ppm = 7.363 (d, J = 7.82 Hz, 2H), 7.163 (d, J = 7.82 Hz, 2H), 5.470 (s, 1H), 4.134 (d, J = 4.41 Hz, 2H), 4.008 (d, J = 4.30 Hz, 2H), 2.786 (m, J = 4.41 Hz, 1H), 2.326 (s, 3H), 1.894 (s, 2H). ^{13}C NMR (DMSO-d_6): δ/ppm = 138.755, 135.367, 128.939, 125.766, 101.890, 73.316, 45.838, 21.237. FAB-MS (*m*/*e*) 194 $[M+H]^+$. Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.44; H, 7.71; N, 7.36.

4.11.3. (*cis*)-[2-(4′-Chlorophenyl)-1,3-dioxan-5-yl]amine (5c). Yield: 36%. Mp 76–78 °C. IR(KBr): ν/cm^{-1} = 3252, 3189, 1645, 1637, 1600, 1582, 1503, 1456, 1051, 821. ^1H NMR ($CDCl_3$): δ/ppm = 7.405 (d, J = 7.12 Hz, 2H), 7.288 (d, J = 7.12 Hz, 2H), 5.483 (s, 1H), 4.122 (d, J = 3.72 Hz, 2H), 4.029 (d, J = 6.41 Hz, 2H), 2.795 (s, 1H), 1.765 (s, 2H). ^{13}C NMR (DMSO-d_6): δ/ppm = 136.719, 134.749, 128.453, 127.389, 100.991, 73.407, 45.756. FAB-MS (*m*/*e*) 214 $[M+H]^+$. Anal. Calcd for $C_{10}H_{12}ClNO_2$: C, 56.21; H, 5.66; N, 6.56. Found: C, 56.14; H, 5.74; N, 6.64.

4.11.4. (*cis*)-2-[4′-Nitrophenyl]-1,3-dioxan-5-yl]amine (5d). Yield: 43%. Mp 112.5–113.5 °C. IR(KBr): ν/cm^{-1} = 3249, 3186, 1645, 1637, 1602, 1581, 1522, 1503, 1457, 1352, 1048, 823. ^1H NMR ($CDCl_3$): δ/ppm = 8.216 (d, J = 8.74 Hz, 2H), 7.661 (d, J = 8.74 Hz, 2H), 5.573 (s, 1H), 4.179 (d, J = 4.51 Hz, 2H), 4.052 (d, J = 4.32 Hz, 2H), 2.839 (m, J = 4.28 Hz, 1H), 1.733 (s, 2H). ^{13}C NMR (DMSO-d_6): δ/ppm = 148.191, 144.614, 127.084, 123.450, 100.077, 73.522, 45.707. FAB-MS (*m*/*e*) 225 $[M+H]^+$. Anal. Calcd for $C_{10}H_{12}N_2O_2$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.44; H, 5.34; N, 12.60.

4.11.5. (*cis*)-2-[3′-Nitrophenyl]-1,3-dioxan-5-yl]amine (5e). Yield: 44%. Mp 50–52 °C. IR(KBr): ν/cm^{-1} = 3247, 3186, 1645, 1637, 1600, 1581, 1522, 1502, 1458, 1351, 1048, 861, 773, 701. ^1H NMR ($CDCl_3$): δ/ppm = 8.367 (s, 1H), 8.194 (d, J = 8.22 Hz, 1H), 7.816 (d, J = 8.00 Hz, 1H), 7.534 (t, J = 8.00 Hz, 1H), 5.574 (s, 1H), 4.181 (d, J = 12.61 Hz, 2H), 4.082 (d, J = 11.40 Hz, 2H), 2.882 (m, J = 11.00 Hz, 1H), 2.126 (s, 2H). ^{13}C NMR (DMSO-d_6): δ/ppm = 146.117, 140.048, 132.169, 129.260, 123.780, 121.406, 99.969, 73.209, 45.649. FAB-MS (*m*/*e*) 225 $[M+H]^+$. Anal. Calcd for $C_{10}H_{12}N_2O_2$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.46; H, 5.44; N, 12.38.

4.11.6. (*S*,4*S*,5*R*)-(2-Phenyl-4-methyl-1,3-dioxan-5-yl)-amine (5f). Yield: 40%. Mp 48–50 °C. IR(KBr): ν/cm^{-1} = 3247, 3185, 1645, 1637, 1601, 1582, 1500, 1455, 1382, 1048, 742, 694. ^1H NMR ($CDCl_3$): δ/ppm = 7.472 (d, J = 6.72 Hz, 2H), 7.330 (t, J = 6.68 Hz, 1H), 7.330 (t, J = 6.67 Hz, 2H), 5.490 (s, 1H), 4.081 (m, J = 6.61 Hz, 1H), 4.020 (d, J = 4.52 Hz, 2H), 2.499 (t, J = 4.52 Hz, 1H), 1.671 (s, 2H), 1.237 (d, J = 4.50 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ/ppm = 136.153, 128.757, 128.123, 125.865, 101.560, 75.698, 73.868, 48.764, 17.611. FAB-MS (*m*/*e*) 194 $[M+H]^+$. $[\alpha]_{20}^{D}$ −8.1 (*c* 1.00, $CHCl_3$). Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.50; H, 7.90; N, 7.14.

4.11.7. (2*S*,4*S*,5*R*)-2-[(4′-Methylphenyl)-4-methyl-1,3- dioxan-5-yl]amine (5g). Yield: 45%. Mp 63–64 °C. IR(KBr): ν/cm^{-1} = 3257, 3190, 1649, 1635, 1602, 1583, 1500, 1457, 1382, 1049, 822. ^1H NMR ($CDCl_3$): δ/ppm = 7.362 (d, J = 8.41 Hz, 2H), 7.154 (d, J = 8.12 Hz, 2H), 5.487 (s, 1H), 4.119 (d, J = 4.55 Hz, 1H), 4.084 (d, J = 4.22 Hz, 1H), 4.040 (m, J = 6.10 Hz, 1H), 2.538 (m, J = 4.20 Hz, 1H), 2.318 (s, 3H), 1.649 (s, 2H), 1.257 (d, J = 6.60 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ/ppm = 138.656, 135.466, 128.914, 125.865, 101.783, 75.788, 73.967, 48.978, 21.212, 17.759. FAB-MS (*m*/*e*) 208 $[M+H]^+$. $[\alpha]_{20}^{D}$ −8.0 (*c* 1.00, $CHCl_3$). Anal. Calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.47; H, 8.22; N, 6.62.

4.11.8. (2*S*,4*S*,5*R*)-2-[(4′-Chlorophenyl)-4-methyl-1,3- dioxan-5-yl]amine (5h). Yield: 45%. Mp 45–46 °C. IR(KBr): ν/cm^{-1} = 3259, 3189, 1649, 1636, 1601, 1582, 1501, 1381, 1457, 1049, 822. ^1H NMR ($CDCl_3$): δ/ppm = 7.407 (t, J = 8.01 Hz, 2H), 7.326 (t, J = 8.01 Hz, 2H), 5.493 (s, 1H), 4.119 (m, J = 6.91 Hz, 1H), 4.077 (d, J = 5.70 Hz, 2H), 2.575 (m, J = 4.52 Hz, 1H), 1.668 (s, 2H), 1.267 (d, J = 6.60 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ/ppm = 136.768, 134.683, 128.436, 127.488, 100.909, 75.920, 74.000, 48.880, 17.734. FAB-MS (*m*/*e*) 228 $[M+H]^+$. $[\alpha]_{20}^{D}$ −8.0 (*c* 1.00, $CHCl_3$). Anal. Calcd for $C_{11}H_{14}ClNO_2$: C, 58.03; H, 6.20; N, 6.15. Found: C, 58.12; H, 6.29; N, 6.27.

4.11.9. (2*S*,4*S*,5*R*)-2-[(4′-Nitrophenyl)-4-methyl-1,3-dioxan-5-yl]amine (5i). Yield; 48%. Mp 80–82 °C. IR(KBr): ν/cm^{-1} = 3248, 3187, 1648, 1638, 1601, 1582, 1525, 1504, 1457, 1381, 1351, 1049, 822. ^1H NMR ($CDCl_3$): δ/ppm = 8.198 (d, J = 9.01 Hz, 2H), 7.659 (d, J = 9.01 Hz, 2H), 5.589 (s, 1H), 4.145 (m, J = 6.22 Hz, 1H),

4.109 (d, J = 5.71 Hz, 2H), 2.594 (m, J = 6.22 Hz, 1H), 1.593 (s, 2H), 1.289 (d, J = 6.32 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ/ppm = 148.101, 144.697, 127.142, 123.425, 99.945, 76.184, 74.165, 48.830, 17.668. FAB-MS (m/e) 239 $[M+H]^+$. $[\alpha]_{20}^{D}$ −8.3 (c 1.00, $CHCl_3$). Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 55.46; H, 5.92; N, 11.76. Found: C, 55.37; H, 5.84; N, 11.58.

4.11.10. (2*S*,4*S*,5*R*)-2-[(3′-Nitrophenyl)-4-methyl-1,3-dioxan-5-yl]amine (5j). Yield: 41%. Mp 50–52 °C. IR(KBr): ν/cm^{-1} = 3249, 3186, 1645, 1637, 1601, 1582, 1522, 1502, 1458, 1381, 1351, 1048, 743, 699. ^{1}H NMR ($CDCl_3$): δ/ppm = 8.361 (s, 1H), 8.191 (d, J = 8.42 Hz, 1H), 7.820 (d, J = 7.83 Hz, 1H), 7.527 (t, J = 8.02 Hz, 1H), 5.598 (s, 1H), 4.181 (m, J = 4.30 Hz, 1H), 4.111(d, J = 2.74 Hz, 2H), 2.612 (m, J = 3.05 Hz, 1H), 1.695 (s, 2H), 1.298 (d, J = 4.65 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ/ppm = 148.117, 140.180, 132.277, 129.252, 123.722, 121.480, 99.887, 76.135, 74.082, 48.830, 17.685. FAB-MS (m/e) 239 $[M+H]^+$. $[\alpha]_{20}^{D}$ −8.1 (c 1.00, $CHCl_3$). Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 55.46; H, 5.92; N, 11.76. Found: C, 55.60; H, 5.86; N, 11.86.

4.11.11. (*trans*)-(2-Phenyl-1,3-dioxan-5-yl)amine (5′a). Mp 58–60 °C. IR(KBr): ν/cm^{-1} = 3283, 3196, 2925, 2860, 1651, 1620, 1601, 1588, 1521, 1452, 1352, 1190, 1052, 741, 699. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.620 (d, J = 7.00 Hz, 2H), 7.502 (t, J = 7.50 Hz, 1H), 7.460 (t, J = 7.50 Hz, 2H), 5.614 (s, 1H), 4.273 (d, J = 11.40 Hz, 2H), 4.135 (d, J = 10.00 Hz, 2H), 2.941 (m, J = 10.01 Hz, 1H), 1.969 (s, 2H). ^{13}C NMR (DMSO-d_6): δ/ppm = 138.635, 129.443, 128.714, 126.694, 102.179, 73.871, 46.262. FAB-MS (m/e) 180 $[M+H]^+$. Anal. Calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 67.08; H, 7.26; N, 7.92.

4.11.12. (*trans*)-[2-(4′-Methylphenyl)-1,3-dioxan-5-yl]-amine (5′b). Yield: 94%. Mp 70–72 °C. IR(KBr): ν/cm^{-1} = 3249, 3186, 1643, 1633, 1601, 1582, 1501, 1451, 1381, 1049, 820. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.536 (d, J = 7.80 Hz, 2H), 7.365 (d, J = 7.80 Hz, 2H), 5.740 (s, 1H), 4.432 (d, J = 4.40 Hz, 2H), 4.265 (d, J = 4.32 Hz, 2H), 2.968 (m, J = 4.40 Hz, 1H), 2.634 (s, 3H), 1.997 (s, 2H). ^{13}C NMR (DMSO-d_6): δ/ppm = 139.145, 135.738, 129.193, 126.142, 102.189, 73.625, 46.383, 21.727. FAB-MS (m/e) 194 $[M+H]^+$. Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.48; H, 7.91; N, 7.13.

4.11.13. (*trans*)-[2-(4′-Chlorophenyl)-1,3-dioxan-5-yl]-amine (5′c). Yield: 92%. Mp 70–72 °C. IR(KBr): ν/cm^{-1} = 3254, 3187, 1642, 1635, 1602, 1580, 1501, 1454, 1050, 823. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.764 (d, J = 7.10 Hz, 2H), 7.513 (d, J = 7.10 Hz, 2H), 5.725 (s, 1H), 4.356 (d, J = 3.74 Hz, 2H), 4.219 (d, J = 6.32 Hz, 2H), 2.975 (s, 1H), 1.868 (s, 2H). ^{13}C NMR (DMSO-d_6): δ/ppm = 137.179, 135.194, 128.741, 127.610, 101.296, 73.744, 46.165. FAB-MS (m/e) 214 $[M+H]^+$. Anal. Calcd for $C_{10}H_{12}ClNO_2$: C, 56.21; H, 5.66; N, 6.56. Found: C, 56.31; H, 5.77; N, 6.44.

4.11.14. (*trans*)-2-[4′-Nitrophenyl]-1,3-dioxan-5-yl]amine (5′d). Yield: 93%. Mp 104–106 °C. IR(KBr): ν/cm^{-1} = 3251, 3188, 1644, 1636, 1601, 1580, 1525, 1501, 1456, 1350, 1049, 821. ^{1}H NMR ($CDCl_3$): δ/ppm = 8.330 (d, J = 8.44 Hz, 2H), 7.855 (d, J = 8.44 Hz, 2H), 5.768 (s, 1H), 4.394 (d, J = 4.54 Hz, 2H), 4.354 (d, J = 4.36 Hz, 2H), 2.985 (m, J = 4.42 Hz, 1H), 1.788 (s, 2H). ^{13}C NMR (DMSO-d_6): δ/ppm = 148.642, 145.041, 127.493, 123.745, 100.987, 74.152, 46.187. FAB-MS (m/e) 225 $[M+H]^+$. Anal. Calcd for $C_{10}H_{12}N_2O_2$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.66; H, 5.50; N, 12.30.

4.11.15. (*trans*)-2-[3′-Nitrophenyl]-1,3-dioxan-5-yl]amine (5′e). Yield: 95%. Mp 56–58 °C. IR(KBr): ν/cm^{-1} = 3249, 3187, 1646, 1635, 1602, 1580, 1525, 1501, 1456, 1350, 1049, 862, 770, 700. ^{1}H NMR ($CDCl_3$): δ/ppm = 8.416 (s, 1H), 8.285 (d, J = 8.20 Hz, 1H), 7.955 (d, J = 8.01 Hz, 1H), 7.841 (t, J = 8.01 Hz, 1H), 5.740 (s, 1H), 4.399 (d, J = 12.01 Hz, 2H), 4.275 (d, J = 11.00 Hz, 2H), 2.996 (m, J = 10.81 Hz, 1H), 2.234 (s, 2H). ^{13}C NMR (DMSO-d_6): δ/ppm = 146.548, 140.582, 132.687, 130.126, 124.178, 121.690, 100.314, 73.557, 46.164. FAB-MS (m/e) 225 $[M+H]^+$. Anal. Calcd for $C_{10}H_{12}N_2O_2$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.66; H, 5.51; N, 12.60.

4.11.16. (2*R*,4*S*,5*R*)-(2-Phenyl-4-methyl-1,3-dioxan-5-yl)-amine (5′f). Yield: 90%. Mp 58–60 °C. IR(KBr): ν/cm^{-1} = 3255, 3189, 1646, 1635, 1602, 1581, 1502, 1456, 1380, 1049, 741, 695. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.730 (d, J = 6.74 Hz, 2H), 7.516 (t, J = 6.62 Hz, 1H), 7.512 (t, J = 6.65 Hz, 2H), 5.687 (s, 1H), 4.318 (m, J = 6.63 Hz, 1H), 4.290 (d, J = 4.54 Hz, 2H), 2.701 (t, J = 4.54 Hz, 1H), 1.851 (s, 2H), 1.315 (d, J = 4.52 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ/ppm = 136.655, 129.175, 128.530, 126.157, 102.165, 76.202, 74.223, 49.320, 18.261. FAB-MS (m/e) 194 $[M+H]^+$. $[\alpha]_{20}^{D}$ 9.6 (c 1.00, $CHCl_3$). Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.28; H, 7.70; N, 7.36.

4.11.17. (2*R*,4*S*,5*R*)-[2-(4′-Methylphenyl)-4-methyl-1,3-dioxan-5-yl]amine (5′g). Yield: 92%. Mp 68–70 °C. IR(KBr): ν/cm^{-1} = 3259, 3191, 1647, 1633, 1601, 1581, 1502, 1455, 1381, 1051, 821. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.515 (d, J = 8.21 Hz, 2H), 7.336 (d, J = 8.10 Hz, 2H), 5.713 (s, 1H), 4.356 (d, J = 4.58 Hz, 1H), 4.298 (d, J = 4.26 Hz, 1H), 4.240 (m, J = 6.00 Hz, 1H), 2.834 (m, J = 4.25 Hz, 1H), 2.420 (s, 3H), 1.946 (s, 2H), 1.302 (d, J = 6.55 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ/ppm = 138.968, 135.970, 129.294, 126.275, 102.301, 76.278, 74.175, 49.285, 21.920, 18.275. FAB-MS (m/e) 208 $[M+H]^+$. $[\alpha]_{20}^{D}$ 9.7 (c 1.00, $CHCl_3$). Anal. Calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.68; H, 8.40; N, 6.85.

4.11.18. (2*R*,4*S*,5*R*)-2-[(4′-Chlorophenyl)-4-methyl-1,3-dioxan-5-yl]amine (5′h). Yield: 94%. Mp 51–53 °C. IR(KBr): ν/cm^{-1} = 3258, 3186, 1645, 1633, 1600, 1580, 1502, 1382, 1455, 1051, 821. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.672 (t, J = 8.00 Hz, 2H), 7.624 (t, J = 8.00 Hz, 2H), 5.700 (s, 1H), 4.350 (m, J = 6.94 Hz, 1H), 4.310 (d, J = 5.72 Hz, 2H), 2.835 (m, J = 4.56 Hz, 1H), 1.845 (s, 2H), 1.380 (d, J = 6.62 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ/ppm = 137.186, 135.090, 128.763, 127.983, 101.292, 76.284, 74.623, 49.307, 18.341. FAB-MS (m/e) 228 $[M+H]^+$. $[\alpha]_{20}^{D}$ 9.6 (c 1.00, $CHCl_3$). Anal.

Calcd for $C_{11}H_{14}ClNO_2$: C, 58.03; H, 6.20; N, 6.15. Found: C, 57.92; H, 6.09; N, 6.08.

4.11.19. (2*R*,4*S*,5*R*)-2-[(4′-Nitrophenyl)-4-methyl-1,3-dioxan-5-yl]amine (5′i). Yield: 95%. Mp 85–87 °C. IR(KBr): ν/cm^{-1} = 3255, 3189, 1647, 1636, 1602, 1580, 1523, 1502, 1455, 1382, 1350, 1048, 823. ^{1}H NMR ($CDCl_3$): δ/ppm = 8.316 (d, J = 9.00 Hz, 2H), 7.915 (d, J = 9.00 Hz, 2H), 5.810 (s, 1H), 4.328 (m, J = 6.24 Hz, 1H), 4.387 (d, J = 5.74 Hz, 2H), 2.816 (m, J = 6.24 Hz, 1H), 1.738 (s, 2H), 1.334 (d, J = 6.34 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ/ppm = 148.665, 144.984, 127.723, 123.752, 100.155, 76.726, 74.650, 49.216, 18.166. FAB-MS (*m*/*e*) 239 [M+H]$^+$. $[\alpha]_{20}^{D}$ 10.8 (*c* 1.00, $CHCl_3$). Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 55.46; H, 5.92; N, 11.76. Found: C, 55.58; H, 6.04; N, 11.82.

4.11.20. (2*R*,4*S*,5*R*)-2-[(3′-Nitrophenyl)-4-methyl-1,3-dioxan-5-yl]amine (5′j). Yield: 95%. Mp 57–59 °C. IR(KBr): ν/cm^{-1} = 3252, 3189, 1647, 1636, 1602, 1580, 1524, 1500, 1456, 1382, 1350, 1049, 741, 698. ^{1}H NMR ($CDCl_3$): δ/ppm = 8.398 (s, 1H), 8.224 (d, J = 8.40 Hz, 1H), 8.012 (d, J = 7.80 Hz, 1H), 7.711 (t, J = 8.00 Hz, 1H), 5.725 (s, 1H), 4.317 (m, J = 4.32 Hz, 1H), 4.297 (d, J = 2.76 Hz, 2H), 2.656 (m, J = 3.24 Hz, 1H), 1.733 (s, 2H), 1.328 (d, J = 4.66 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ/ppm = 148.627, 140.597, 132.588, 129.563, 124.222, 122.009, 100.387, 76.552, 74.385, 49.083, 18.159. FAB-MS (*m*/*e*) 239 [M+H]$^+$. $[\alpha]_{20}^{D}$ 13.2 (*c* 1.00, $CHCl_3$). Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 55.46; H, 5.92; N, 11.76. Found: C, 55.36; H, 6.03; N, 11.65.

4.11.21. (*cis*)-[2-(*E*-Phenylvinyl)-1,3-dioxan-5-yl]amine (6a). Mp 65–67 °C. IR(KBr): ν/cm^{-1} = 3249, 3188, 3030, 1673, 1645, 1635, 1601, 1581, 1500, 1457, 1048, 740, 696. ^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 7.881 (s, 2H), 7.278 (q, J = 7.12 Hz, 2H), 7.263 (t, J = 7.16 Hz, 2H), 7.151 (t, J = 7.16 Hz, 1H), 6.653 (d, J = 13.10 Hz, 1H), 6.257(d, J = 13.10 Hz, 1H), 5.404 (d, J = 7.32 Hz, 1H), 4.443 (d, J = 11.71 Hz, 4H). ^{13}C NMR ($CDCl_3$): δ/ppm = 135.030, 128.323, 127.656, 126.867, 126.112, 123.967, 105.882, 73.287, 52.537. FAB-MS (*m*/*e*) 206 [M+H]$^+$. Anal. Calcd for $C_{12}H_{16}ClNO_2$: C, 59.63; H, 6.67; N, 5.79. Found: C, 59.74; H, 6.81; N, 5.91.

4.11.22. (2*S*,4*S*,5*R*)-[2-(*E*-Phenylvinyl)-4-methyl-1,3-dioxan-5-yl]amine (6b). Mp 77–79 °C. IR(KBr): ν/cm^{-1} = 3250, 3190, 3031, 1675, 1644, 1636, 1602, 1581, 1503, 1455, 1380, 1049, 740, 696. ^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 7.815 (s, 2H), 7.279 (d, J = 7.21 Hz, 2H), 7.246 (t, J = 7.22 Hz, 2H), 7.169 (t, J = 7.21 Hz, 1H), 6.565 (d, J = 11.87 Hz, 1H), 6.266 (d, J = 11.87 Hz 1H), 5.364 (d, J = 6.04 Hz, 1H), 4.597 (m, J = 4.71 Hz, 1H), 4.386 (d, J = 4.74 Hz, 2H), 3.816 (m, J = 4.84 Hz, 1H), 1.211 (d, J = 4.84 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ/ppm = 135.320, 128.401, 127.720, 127.072, 126.240, 126.187, 105.543, 72.805, 72.684, 19.285. FAB-MS (*m*/*e*) 220 [M+H]$^+$. $[\alpha]_{20}^{D}$ −12.0 (*c* 1.0, H_2O). Anal. Calcd for $C_{13}H_8ClNO_2$: C, 61.05; H, 7.09; N, 5.48. Found: C, 61.12; H, 7.17; N, 5.53.

4.11.23. (*trans*)-[2-(*E*-Phenylvinyl)-1,3-dioxan-5-yl]amine (6′a). Yield: 90%. Mp 70–72 °C. IR(KBr): ν/cm^{-1} = 3252, 3187, 3032, 1675, 1646, 1636, 1602, 1580, 1501, 1455, 1049, 741, 698. ^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 8.003 (s, 2H), 7.548 (q, J = 7.10 Hz, 2H), 7.550 (t, J = 7.14 Hz, 2H), 7.302 (t, J = 7.14 Hz, 1H), 6.802 (d, J = 13.00 Hz, 1H), 6.526 (d, J = 13.00 Hz, 1H), 5.634 (d, J = 7.34 Hz, 1H), 4.638 (d, J = 11.51 Hz, 4H). ^{13}C NMR ($CDCl_3$): δ/ppm = 135.344, 128.612, 128.230, 127.174, 126.503, 124.326, 106.232, 73.603, 52.876. FAB-MS (*m*/*e*) 206 [MH]$^+$. Anal. Calcd for $C_{12}H_{16}ClNO_2$: C, 59.63; H, 6.67; N, 5.79. Found: C, 59.49; H, 6.58; N, 5.67.

4.11.24. (2*R*,4*S*,5*R*)-[2-(*E*-Phenylvinyl)-4-methyl-1,3-dioxan-5-yl]amine (6′b). Yield: 90%. Mp 82–84 °C. IR(KBr): ν/cm^{-1} = 3252, 3188, 3030, 1672, 1645, 1635, 1601, 1580, 1501, 1454, 1381, 1051, 741, 698. ^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 7.993 (s, 2H), 7.596 (d, J = 7.20 Hz, 2H), 7.560 (t, J = 7.20 Hz, 2H), 7.435 (t, J = 7.20 Hz, 1H), 6.763 (d, J = 11.25 Hz, 1H), 6.538 (d , J = 11.22 Hz 1H), 5.559 (d, J = 6.00 Hz, 1H), 4.799 (m, J = 4.74 Hz, 1H), 4.579 (d, J = 4.76 Hz, 2H), 3.993 (m, J = 4.86 Hz, 1H), 1.387 (d, J = 4.86 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ/ppm = 135.713, 128.714, 128.112, 127.394, 126.587, 126.692, 105.835, 73.217, 72.946, 19.665. FAB-MS (*m*/*e*) 220 [M+H]$^+$. $[\alpha]_{20}^{D}$ 14.5 (*c* 1.0, H_2O). Anal. Calcd for $C_{13}H_8ClNO_2$: C, 61.05; H, 7.09; N, 5.48. Found: C, 61.16; H, 7.01; N, 5.36.

4.11.25. (2*S*,5*S*)-2-(4′-Chlorophenyl-1,3-dioxaheptan-5-yl)-amine (7c). Colorless powder, mp 48–49 °C. IR(KBr): ν/cm^{-1} = 3345, 3044, 2960, 2915, 2843, 1644, 1605, 1552, 1464, 1370, 1176, 1052, 821, 742, 691. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.415 (d, J = 8.51 Hz, 2H), 7.326 (d, J = 8.51 Hz, 2H), 5.713 (s, 1H), 3.821 (d, J = 4.38 Hz, 2H), 3.543 (t, J = 6.01 Hz, 2H), 3.089 (s, 1H), 1.889 (m, J = 3.72 Hz, 2H), 1.457 (s, 2H). ^{13}C NMR ($CDCl_3$): δ/ppm = 147.886, 146.407, 127.465, 123.383, 99.276, 69.684, 61.269, 49.879, 38.688. FAB-MS (*m*/*e*) 229 [M+H]$^+$. $[\alpha]_{20}^{D}$ 8.0 (*c* 1.00, $CHCl_3$). Anal. Calcd for $C_{11}H_{12}ClNO_2$: C, 58.03; H, 6.20; N, 6.15. Found: C, 58.17; H, 6.09; N, 6.22.

4.11.26. (2*R*,5*S*)-2-(4′-Chlorophenyl-1,3-dioxaheptan-5-yl)amine (7′c). Colorless powder, mp 56–57 °C. IR(KBr): ν/cm^{-1} = 3342, 3041, 2966, 2917, 2840, 1641, 1602, 1542, 1450, 1376, 1169, 1053, 822, 742, 694. ^{1}H NMR ($CDCl_3$): δ/ppm = 7.431 (d, J = 8.01 Hz, 2H), 7.334 (d, J = 8.07 Hz, 2H), 5.695 (s, 1H), 3.894 (t, J = 3.22 Hz, 2H), 3.724 (d, J = 4.01 Hz, 2H), 3.134 (s, 1H), 2.023 (m, J = 2.43 Hz, 2H), 1.602 (m, J = 3.52 Hz, 2H), 1.469 (s, 2H). ^{13}C NMR ($CDCl_3$): δ/ppm = 147.886, 146.695, 127.705, 123.651, 99.652, 70.574, 62.040, 50.462, 38.987. FAB-MS (*m*/*e*) 229 [M+H]$^+$. $[\alpha]_{20}^{D}$ −6.0 (*c* 1.00, $CHCl_3$). Anal. Calcd for $C_{11}H_{12}ClNO_2$: C, 58.03; H, 6.20; N, 6.15. Found: C, 58.12; H, 6.19; N, 6.27.

4.11.27. (2*S*,5*S*)-2-(4′-Nitrophenyl-1,3-dioxaheptan-5-yl)-amine (7d). Colorless powder, mp 76–78 °C. IR(KBr): ν/cm^{-1} = 3345, 3042, 2970, 2919, 2851, 1642, 1600, 1561, 1521, 1462, 1372, 1352, 1171, 1050, 821, 742, 693. ^{1}H NMR ($CDCl_3$): δ/ppm = 8.216 (d, J = 9.04 Hz, 2H), 7.665 (d, J = 8.52 Hz, 2H), 5.793 (s, 1H), 3.862 (d, J = 4.31 Hz, 2H), 3.610 (t, J = 6.04 Hz, 2H), 3.153 (s, 1H), 1.821 (s, 2H), 1.789 (t, J = 3.95 Hz, 2H). ^{13}C NMR

($CDCl_3$): δ/ppm = 147.886, 146.407, 127.465, 123.383, 99.276, 69.684, 61.269, 49.879, 38.688. $[\alpha]_{20}^{D}$ 8.0 (*c* 1.00, $CHCl_3$). FAB-MS (*m*/*e*) 239 $[M+H]^+$. Anal. Calcd for $C_{11}H_{14}N_2O_4$: C, 55.46; H, 5.92; N, 11.76. Found: C, 55.36; H, 5.88; N, 11.82.

4.11.28. (2*R*,5*S*)-2-(4′-Nitrophenyl-1,3-dioxaheptan-5-yl)-amine (7′d). Colorless powder, mp 61–63 °C. IR(KBr): *v*/cm^{-1} = 3330, 3031, 2964, 2916, 2848, 1644, 1604, 1521, 1453, 1374, 1355, 1168, 1049, 821, 741, 693. ^1H NMR ($CDCl_3$): δ/ppm = 8.221 (d, *J* = 9.04 Hz, 2H), 7.679 (d, *J* = 8.04 Hz, 2H), 5.764 (s, 1H), 3.912 (m, *J* = 3.45 Hz, 2H), 3.702 (d, *J* = 5.85 Hz, 2H), 3.160 (s, 1H), 1.832 (m, *J* = 3.52 Hz, 2H), 1.401 (s, 2H). ^{13}C NMR ($CDCl_3$): δ/ppm = 147.886, 146.695, 127.705, 123.651, 99.652, 70.574, 62.040, 50.462, 38.987. $[\alpha]_{20}^{D}$ −16.0 (*c* 1.00, $CHCl_3$). FAB-MS (*m*/*e*) 239 $[M+H]^+$. Anal. Calcd for $C_{11}H_{14}N_2O_4$: C, 55.46; H, 5.92; N, 11.76. Found: C, 55.60; H, 6.02; N, 11.57.

4.11.29. (2*S*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaheptan-5-yl)-amine (7e). Colorless powder, mp 68–70 °C. IR(KBr): *v*/cm^{-1} = 3322, 3041, 2962, 2920, 2850, 1642, 1614, 1556, 1521, 1462, 1364, 1352, 1160, 1052, 861, 773, 742, 698. ^1H NMR ($CDCl_3$): δ/ppm = 8.374 (s, 1H). 8.194 (d, *J* = 7.82 Hz, 1H), 7.822 (d, *J* = 7.52 Hz, 1H), 7.546 (t, *J* = 8.01Hz, 1H), 5.796 (s, 1H), 3.855 (d, *J* = 4.34 Hz, 2H), 3.604 (t, *J* = 6.22 Hz, 2H), 3.142 (s, 1H), 1.976 (m, *J* = 4.84 Hz, 2H), 1.674 (s, 2H). ^{13}C NMR ($CDCl_3$): δ/ppm = 148.191, 141.730, 132.639, 129.194, 123.318, 121.719, 99.071, 69.525, 61.160, 49.844, 38.627. $[\alpha]_{20}^{D}$ 12.0 (*c* 1.00, $CHCl_3$). FAB-MS (*m*/*e*) 239 $[M+H]^+$. Anal. Calcd for $C_{11}H_{14}N_2O_4$: C, 55.46; H, 5.92; N, 11.76. Found: C, 55.33; H, 5.78; N, 11.92.

4.11.30. (2*R*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaheptan-5-yl)-amine (7′e). Colorless powder, mp 65–67 °C. IR(KBr): *v*/cm^{-1} = 3324, 3036, 2963, 2918, 2846, 1645, 1609, 1558, 1522, 1459, 1371, 1351, 1162, 1051, 862, 774, 739, 699. ^1H NMR ($CDCl_3$): δ/ppm = 8.330 (s, 1H), 8.145 (d, *J* = 8.42 Hz, 1H), 7.789 (t, *J* = 7.31 Hz, 1H), 7.505 (d, *J* = 7.34 Hz, 1H), 5.739 (s, 1H), 3.757 (d, *J* = 5.25 Hz, 2H), 3.475 (t, *J* = 5.24 Hz, 2H), 3.096 (m, *J* = 5.28 Hz,1H), 1.775 (m, *J* = 5.16 Hz, 2H), 1.456 (s, 2H). ^{13}C NMR ($CDCl_3$): δ/ppm = 147.638, 141.985, 132.953, 129.863, 123.142, 120.800, 98.593, 69.870, 62.631, 50.287, 38.096. $[\alpha]_{20}^{D}$ −6.0 (*c* 1.00, $CHCl_3$). FAB-MS (*m*/*e*) 239 $[M+H]^+$. Anal. Calcd for $C_{11}H_{14}N_2O_4$: C, 55.46; H, 5.92; N, 11.76. Found: C, 55.54; H, 5.81; N, 11.90.

4.11.31. (2*S*,5*S*)-2-(4′-Chlorophenyl-1,3-dioxaoctan-5-yl)-amine (8c). Colorless powder, mp 56–58 °C. IR(KBr): *v*/cm^{-1} = 3282, 3194, 2964, 2918, 2848, 1600, 1557, 1524, 1461, 1373, 1171, 1049, 820. ^1H NMR ($CDCl_3$): δ/ppm = 7.213 (d, *J* = 7.51 Hz, 2H), 7.150 (d, *J* = 7.51 Hz, 2H), 5.467 (s, 1H), 3.562 (d, *J* = 4.41 Hz, 2H), 3.334 (t, *J* = 5.14 Hz, 2H), 2.895 (m, *J* = 4.41 Hz, 1H), 2.231 (s, 2H), 1.506 (t, *J* = 5.14 Hz, 2H), 1.457 (t, *J* = 5.14 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ/ppm = 135.286, 132.878, 129.013, 128.891, 104.505, 72.343, 63.524, 51.727, 31.305, 25.878. FAB-MS (*m*/*e*) 242 $[M+H]^+$. $[\alpha]_{20}^{D}$ 9.3 (*c* 1.00, $CHCl_3$). Anal. Calcd for $C_{12}H_{16}ClNO_2$: C, 59.63; H, 6.67; N, 5.79. Found: C, 59.49; H, 6.59; N, 5.92.

4.11.32. (2*R*,5*S*)-2-(4′-Chlorophenyl-1,3-dioxaoctan-5-yl)-amine (8′c). Colorless powder, mp 62–64 °C. IR(KBr): *v*/cm^{-1} = 3284, 3197, 2962, 2913, 2845, 1604, 1547, 1455, 1371, 1167, 1048, 822. ^1H NMR ($CDCl_3$): δ/ppm = 7.401 (d, *J* = 7.50 Hz, 2H), 7.243 (d, *J* = 7.50 Hz, 2H), 5.773 (s, 1H), 3.726 (d, *J* = 4.40 Hz, 2H), 3.546 (t, *J* = 5.12 Hz, 2H), 3.004 (m, *J* = 4.40 Hz, 1H), 2.301 (s, 2H), 1.576 (t, *J* = 5.10 Hz, 2H), 1.751 (t, *J* = 5.10 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ/ppm = 135.973, 133.168, 129.725, 129.132, 105.009, 72.761, 64.421, 52.357, 31.684, 26.059. FAB-MS (*m*/*e*) 242 $[M+H]^+$. $[\alpha]_{20}^{D}$ −7.4 (*c* 1.00, $CHCl_3$). Anal. Calcd for $C_{12}H_{16}ClNO_2$: C, 59.63; H, 6.67; N, 5.79.Found: C, 59.80; H, 6.79; N, 5.66.

4.11.33. (2*S*,5*S*)-2-(4′-Nitrophenyl-1,3-dioxaoctan-5-yl)-amine (8d). Colorless powder, mp 81–83 °C. IR(KBr): *v*/cm^{-1} = 3284, 3197, 2965, 2913, 2846, 1605, 1564, 1520, 1464, 1368, 1351, 1176, 1052, 821. ^1H NMR ($CDCl_3$): δ/ppm = 8.154 (d, *J* = 8.92 Hz, 2H), 7.473 (d, *J* = 8.92 Hz, 2H), 5.468 (s, 1H), 3.581 (d, *J* = 4.34 Hz, 2H), 3.368 (t, *J* = 5.92 Hz, 2H), 2.931 (m, *J* = 4.36 Hz, 1H), 2.022 (s, 2H), 1.503 (m, *J* = 3.38 Hz, 2H), 1.457 (m, *J* = 3.38 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ/ppm = 147.482, 143.341, 128.470, 123.503, 104.561, 72.412, 63.493, 51.897, 31.369, 25.874. $[\alpha]_{20}^{D}$ 9.2 (*c* 1.00, $CHCl_3$). FAB-MS (*m*/*e*) 253 $[M+H]^+$. Anal. Calcd for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.10. Found: C, 57.24; H, 6.44; N, 11.21.

4.11.34. (2*R*,5*S*)-2-(4′-Nitrophenyl-1,3-dioxaoctan-5-yl)-amine (8′d). Colorless powder, mp 66–68 °C. IR(KBr): *v*/cm^{-1} = 3286, 3195, 2962, 2911, 2844, 1601, 1556, 1458, 1370, 1349, 1165, 1051, 820. ^1H NMR ($CDCl_3$): δ/ppm = 8.243 (d, *J* = 8.90 Hz, 2H), 7.768 (d, *J* = 8.90 Hz, 2H), 5.845 (s, 1H), 3.764 (d, *J* = 4.36 Hz, 2H), 3.683 (t, *J* = 5.90 Hz, 2H), 2.989 (m, *J* = 4.35 Hz, 1H), 2.041 (s, 2H), 1.576 (m, *J* = 3.39 Hz, 2H), 1.497 (m, *J* = 3.39 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ/ppm = 147.876, 143.901, 129.047, 124.012, 105.165, 73.011, 63.937, 52.314, 31.693, 26.176. $[\alpha]_{20}^{D}$ −11.7 (*c* 1.00, $CHCl_3$). FAB-MS (*m*/*e*) 253 $[M+H]^+$. Anal. Calcd for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.10. Found: C, 57.24; H, 6.34; N, 11.22.

4.11.35. (2*S*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaoctan-5-yl)-amine (8e). Colorless powder, mp 72–74 °C. IR(KBr): *v*/cm^{-1} = 3287, 3193, 2958, 2925, 2856, 1640, 1619, 1559, 1526, 1464, 1369, 1349, 1166, 1048, 861, 771, 702. ^1H NMR ($CDCl_3$): δ/ppm = 8.126 (s, 1H), 8.121 (d, *J* = 8.51 Hz, 1H), 7.576 (d, *J* = 8.52 Hz, 1H), 7.449 (t, *J* = 8.51Hz, 1H), 5.469 (s, 1H), 3.586 (d, *J* = 4.35 Hz, 2H), 3.376 (t, *J* = 4.28 Hz, 2H), 2.936 (d, *J* = 4.38 Hz, 2H), 2.142 (s, 2H), 1.509 (d, *J* = 4.33 Hz, 2H), 1.486 (m, *J* = 4.30 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ/ppm = 148.289, 138.037, 133.690, 129.319, 122.781, 122.171, 103.483, 63.586, 51.874, 31.337, 25.768. $[\alpha]_{20}^{D}$ 8.7 (*c* 1.00, $CHCl_3$). FAB-MS (*m*/*e*) 253 $[M+H]^+$. Anal. Calcd for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.10. Found: C, 57.01; H, 6.25; N, 11.22.

4.11.36. (2*R*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaoctan-5-yl)-amine (8′e). Colorless powder, mp 74–76 °C. IR(KBr): ν/cm^{-1} = 3287, 3194, 2955, 2922, 2853, 1614, 1554, 1522, 1462, 1366, 1351, 1164, 1050, 861, 773, 701. ^1H NMR ($CDCl_3$): δ/ppm = 8.217 (s, 1H), 8.153 (d, J = 8.50 Hz, 1H), 7.775 (d, J = 8.50 Hz, 1H), 7.598 (t, J = 8.50Hz, 1H), 5.865 (s, 1H), 3.687 (d, J = 4.36 Hz, 2H), 3.546 (t, J = 4.26 Hz, 2H), 2.978 (d, J = 4.35 Hz, 2H), 2.147 (s, 2H), 1.584 (d, J = 4.35 Hz, 2H), 1.502 (m, J = 4.32 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ/ppm = 148.930, 138.742, 134.025, 129.913, 123.017, 122.706, 103.845, 64.007, 52.162, 31.741, 26.140. $[\alpha]_{20}^{D}$ −7.4 (*c* 1.00, $CHCl_3$). FAB-MS (*m*/*e*) 253 $[M+H]^+$. Anal. Calcd for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.10. Found: C, 57.24; H, 6.40; N, 11.22.

4.12. General procedure for the synthesis of 5-benzoyl-amino-2-substitutedphenyl-1,3-dioxacycloalkanes[12]

At 0 °C, to the solution of 0.48 mmol of 5-amino-2-substitutedphenyl-1,3-dioxacycloalkanes, 49 mg (0.48 mmol) of triethylamine, and 3 ml chloroform, 67 mg (0.48 mmol) of benzoyl chloride was added drop-wise. The reaction mixture was stirred at room temperature for 3 h and TLC ($CHCl_3$/MeOH, 20:1) indicated complete disappearance of starting material. The reaction mixture was evaporated and the residue was dissolved in 3 ml ether. The solution was stirred at room temperature for 1 h and then filtered. The filtrate was evaporated and the residue was purified by silica gel chromatography ($CHCl_3$/MeOH, 15:1) to provide the title compound as a colorless powder.

4.12.1. (*cis*)-(2-Phenyl-1,3-dioxan-5-yl)benzamide (9a). Colorless powder, mp 133–135 °C. In a NOE experiment, a positive NOE between the NH at the 5-position and the proton of the phenyl at the 2-position was observed.

4.12.2. (*trans*)-(2-Phenyl-1,3-dioxan-5-yl)benzamide (9′a). Colorless powder, mp 146–148 °C. In a NOESY experiment, no NOE was observed.

4.12.3. (*cis*)-2-[4′-Methylphenyl]-1,3-dioxan-5-yl]benzamide (9b). Colorless powder, mp 134–136 °C. In a NOE experiment, a positive NOE between the NH at the 5-position and the proton of the phenyl at the 2-position was observed.

4.12.4. (*trans*)-2-[4′-Methylphenyl]-1,3-dioxan-5-yl]benzamide (9′b). Colorless powder, mp 146–148 °C. In a NOESY experiment, no NOE was observed.

4.12.5. (*cis*)-2-[4′-Chlorophenyl]-1,3-dioxan-5-yl]benzamide (9c). Colorless powder, mp 168–170 °C. In a NOE experiment, a positive NOE between the NH at the 5-position and the proton of the phenyl at the 2-position was observed.

4.12.6. (*trans*)-2-[4′-Chlorophenyl]-1,3-dioxan-5-yl]benzamide (9′c). Colorless powder, mp 133–135 °C. In a NOESY experiment, no NOE was observed.

4.12.7. (*cis*)-2-[4′-Nitrophenyl]-1,3-dioxan-5-yl]benzamide (9d). Colorless powder, mp 220–224 °C. In a NOE experiment, a positive NOE between the NH at the 5-position and the proton of the phenyl at the 2-position was observed.

4.12.8. (*trans*)-2-[4′-Nitrophenyl]-1,3-dioxan-5-yl]benzamide (9′d). Colorless powder, mp 120–122 °C. In a NOESY experiment, no NOE was observed.

4.12.9. (*cis*)-2-[3′-Nitrophenyl]-1,3-dioxan-5-yl]benzamide (9e). Mp 144–146 °C. In a NOE experiment, a positive NOE between the NH at the 5-position and the proton of the phenyl at the 2-position was observed.

4.12.10. (*trans*)-2-[3′-Nitrophenyl]-1,3-dioxan-5-yl]benzamide (9′e). Mp 130–132 °C. In a NOESY experiment, no NOE was observed. In a NOESY experiment, no NOE was observed.

4.12.11. (2*S*,4*S*,5*R*)-(2-Phenyl-4-methyl-1,3-dioxan-5-yl)-benzamide (9f). Colorless powder, mp 125–127 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.12. (2*R*,4*S*,5*R*)-(2-Phenyl-4-methyl-1,3-dioxan-5-yl)-benzamide (9′f). Colorless powder, mp 133–135 °C. In a NOESY experiment, no NOE was observed.

4.12.13. (2*S*,4*S*,5*R*)-[2-(4′-Methylphenyl)-4-methyl-1,3-dioxan-5-yl]benzamide (9g). Colorless powder, mp 152–154 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.14. (2*R*,4*S*,5*R*)-[2-(4′-Methylphenyl)-4-methyl-1,3-dioxan-5-yl]benzamide (9′g). Colorless powder, mp 144–146 °C. In a NOESY experiment, no NOE was observed.

4.12.15. (2*S*,4*S*,5*R*)-(2-(4′-Chlorophenyl)-4-methyl-1,3-dioxan-5-yl)benzamide (9h). Colorless powder, mp 125–127 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.16. (2*R*,4*S*,5*R*)-(2-(4′-Chlorophenyl)-4-methyl-1,3-dioxan-5-yl)benzamide (9′h). Colorless powder, mp 120–122 °C. In a NOESY experiment, no NOE was observed.

4.12.17. (2*S*,4*S*,5*R*)-(2-(4′-Nitrophenyl)-4-methyl-1,3-dioxan-5-yl)benzamide (9i). Colorless powder, mp 90–92 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.18. (2*R*,4*S*,5*R*)-(2-(4′-Nitrophenyl)-4-methyl-1,3-dioxan-5-yl)benzamide (9′i). Colorless powder, mp 112–114 °C. In a NOESY experiment, no NOE was observed.

4.12.19. (2*S*,4*S*,5*R*)-[2-(3′-Nitrophenyl)-4-methyl-1,3-dioxan-5-yl]benzamide (9j). Colorless powder, mp 140–142 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.20. (2*R*,4*S*,5*R*)-[2-(3′-Nitrophenyl)-4-methyl-1,3-dioxan-5-yl]benzamide (9′j). Colorless powder, mp 130–132 °C. In a NOESY experiment, no NOE was observed.

4.12.21. (*cis*)-[2-(*E*-Phenylvinyl)-1,3-dioxan-5-yl]benzamide (10a). Colorless powder, mp 119–121 °C. In a NOE experiment, a positive NOE between the NH at the 5-position and the proton of the phenyl at the 2-position was observed.

4.12.22. (*trans*)-[2-(*E*-Phenylvinyl)-1,3-dioxan-5-yl]benzamide (10′a). Colorless powder, mp 115–117 °C. In a NOESY experiment, no NOE was observed.

4.12.23. (2*S*,4*S*,5*R*)-[2-(*E*-Phenylvinyl)-4-methyl-1,3-dioxan-5-yl]benzamide (10b). Colorless powder, mp 123–125 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.24. (2*R*,4*S*,5*R*)-[2-(*E*-Phenylvinyl)-4-methyl-1,3-dioxan-5-yl]benzamide (10′b). Colorless powder, mp 116–118 °C. In a NOESY experiment, no NOE was observed.

4.12.25. (2*S*,5*S*)-2-(4-Chlorophenyl-1,3-dioxaheptan-5-yl)-benzamide (11c). Colorless powder, mp 129–131 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.26. (2*R*,5*S*)-2-(4-Chlorophenyl-1,3-dioxaheptan-5-yl)-benzamide (11′c). Colorless powder, mp 134–136 °C. In a NOESY experiment, no NOE was observed.

4.12.27. (2*S*,5*S*)-2-(4-Nitrophenyl-1,3-dioxaheptan-5-yl)benzamide (11d). Colorless powder, mp 121–123 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.28. (2*R*,5*S*)-2-(4-Nitrophenyl-1,3-dioxaheptan-5-yl)-benzamide (11′d). Colorless powder, mp 124–126 °C. In a NOESY experiment, no NOE was observed.

4.12.29. (2*S*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaheptan-5-yl)-benzamide (11e). Colorless powder, mp 114–116 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.30. (2*R*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaheptan-5-yl)-benzamide (11′e). Colorless powder, mp 117–119 °C. In a NOESY experiment, no NOE was observed.

4.12.31. (2*S*,5*S*)-2-(4′-Chlorophenyl-1,3-dioxaoctan-5-yl)-benzamide (12c). Colorless powder, mp 113–115 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.32. (2*R*,5*S*)-2-(4′-Chlorophenyl-1,3-dioxaoctan-5-yl)-benzamide (12′c). Colorless powder, mp 118–120 °C. In a NOESY experiment, no NOE was observed.

4.12.33. (2*S*,5*S*)-2-(4′-Nitrophenyl-1,3-dioxaoctan-5-yl)-benzamide (12d). Colorless powder, mp 133–135 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.34. (2*R*,5*S*)-2-(4′-Nitrophenyl-1,3-dioxaoctan-5-yl)-benzamide (12′d). Colorless powder, mp 116–118 °C. In a NOESY experiment, no NOE was observed.

4.12.35. (2*S*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaoctan-5-yl)-benzamide (12e). Colorless powder, mp 124–126 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.36. (2*R*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaoctan-5-yl)-benzamide (12′e). Colorless powder, mp 127–129 °C. In a NOESY experiment, no NOE was observed.

4.12.37. (*cis*)-(2-Phenyl-1,3-dioxan-5-yl)phenylacetamide (13a). Colorless powder, mp 150–152 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.38. (*trans*)-(2-Phenyl-1,3-dioxan-5-yl)phenylacetamide (13′a). Colorless powder, mp 194–195 °C. In a NOESY experiment, no NOE was observed.

4.12.39. (*cis*)-[2-(4′-Methylphenyl)-1,3-dioxan-5-yl]phenylacetamide (13b). Colorless powder, mp 185–187 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.40. (*trans*)-[2-(4′-Methylphenyl)-1,3-dioxan-5-yl]phenylacetamide (13′b). Colorless powder, mp 181–183 °C. In a NOESY experiment, no NOE was observed.

4.12.41. (*cis*)-[2-(4′-Chlorophenyl)-1,3-dioxan-5-yl]phenylacetamide (13c). Colorless powder, mp 170–172 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.42. (*trans*)-[2-(4′-Chlorophenyl)-1,3-dioxan-5-yl]phenylacetamide (13′c). Colorless powder, mp 256–258 °C. In a NOESY experiment, no NOE was observed.

4.12.43. (*cis*)-2-[4′-Nitrophenyl]-1,3-dioxan-5-yl]phenylacetamide (13d). Colorless powder, mp 201–203 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.44. (*trans*)-2-[4′-Nitrophenyl]-1,3-dioxan-5-yl]phenylacetamide (13′d). Colorless powder, mp 107–109 °C. In a NOESY experiment, no NOE was observed.

4.12.45. (*cis*)-2-[3′-Nitrophenyl]-1,3-dioxan-5-yl]phenylacetamide (13e). Mp 128–132 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.46. (*trans*)-2-[3′-Nitrophenyl]-1,3-dioxan-5-yl]phenylacetamide (13′e). Mp 141–143 °C. In a NOESY experiment, no NOE was observed.

4.12.47. (2*S*,4*S*,5*R*)-(2-Phenyl-4-methyl-1,3-dioxan-5-yl)-phenylacetamide (13f). Colorless powder, mp 144–146 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.48. (2*R*,4*S*,5*R*)-(2-Phenyl-4-methyl-1,3-dioxan-5-yl)-phenylacetamide (13′f). Colorless powder, mp 156–158 °C. In a NOESY experiment, no NOE was observed.

4.12.49. (2*S*,4*S*,5*R*)-2-(4′-Methylphenyl)-4-methyl-1,3-dioxan-5-yl]phenylacetamide (13g). Colorless powder, mp 159–161 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.50. (2*R*,4*S*,5*R*)-[2-(4′-Methylphenyl)-4-methyl-1,3-dioxan-5-yl]phenylacetamide (13′g). Colorless powder, mp 125–127 °C. In a NOESY experiment, no NOE was observed.

4.12.51. (2*S*,4*S*,5*R*)-(2-(4′-Chlorophenyl)-4-methyl-1,3-dioxan-5-yl)phenylacetamide (13h). Colorless powder, mp 137–139 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.52. (2*R*,4*S*,5*R*)-(2-(4′-Chlorophenyl)-4-methyl-1,3-dioxan-5-yl)phenylacetamide (13′h). Colorless powder, mp 129–131 °C. In a NOESY experiment, no NOE was observed.

4.12.53. (2*S*,4*S*,5*R*)-[2-(4′-Nitrophenyl)-4-methyl-1,3-dioxan-5-yl]phenylacetamide (13i). Colorless powder, mp 127–129 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.54. (2*R*,4*S*,5*R*)-[2-(4′-Nitrophenyl)-4-methyl-1,3-dioxan- 5-yl]phenylacetamide (13′i). Colorless powder, mp 120–122 °C. In a NOESY experiment, no NOE was observed.

4.12.55. (2*R*,4*S*,5*R*)-2-(3′-Nitrophenyl)-4-methyl-1,3-dioxan-5-yl]phenylacetamide (13j). Colorless powder, mp 147–149 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.56. (2*R*,4*S*,5*R*)-[2-(3′-Nitrophenyl)-4-methyl-1,3-dioxan-5-yl]phenylacetamide 13′j. Colorless powder, mp 140–142 °C. In a NOESY experiment, no NOE was observed.

4.12.57. (*cis*)-[2-(*E*-Phenylvinyl)-1,3-dioxan-5-yl]phenylacetamide (14a). Colorless powder, mp 135–136 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.58. (*trans*)-[2-(*E*-Phenylvinyl)-1,3-dioxan-5-yl]phenylacetamide (14′a). Colorless powder, mp 150–152 °C. In a NOESY experiment, no NOE was observed.

4.12.59. (2*S*,4*S*,5*R*)-[2-(*E*-phenylvinyl)-4-methyl-1,3-dioxan-5-yl]phenylacetyl amide (14b). Colorless powder, mp 125–127 °C. In NOESY experiment, NOEs between the CH_3 at the 4-position and the proton of phenyl at the 2-position, and between the CH_3 at the 4-position and the NH at the 5-position were observed.

4.12.60. (2*R*,4*S*,5*R*)-[2-(*E*-phenylvinyl)-4-methyl-1,3-dioxan-5-yl] phenylacetyl amide (14′b). Colorless powder, mp 141–143 °C. In a NOESY experiment, no NOE was observed.

4.12.61. (2*S*,5*S*)-2-(4′-Chlorophenyl-1,3-dioxaheptan-5-yl)phenylacetamide (15c). Colorless powder, mp 132–134 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.62. (2*R*,5*S*)-2-(4′-Chlorophenyl-1,3-dioxaheptan-5-yl)-phenylacetamide (15′c). Colorless powder, mp 139–141 °C. In a NOESY experiment, no NOE was observed. ^{13}C NMR ($CDCl_3$): δ/ppm = 169.957, 135.998, 135.634, 133.017, 129.986, 129.367, 129.048, 128.967, 127.891, 105.034, 69.591, 58.720, 45.565, 33.472. EI-MS (*m*/*e*) 345 $[M]^+$. $[\alpha]_{20}^{D}$ −15.0 (*c* 0.02, $CHCl_3$). Anal. Calcd for $C_{19}H_{20}ClNO_3$: C, 65.99; H, 5.83; N, 4.05. Found: C, 65.89; H, 5.79; N, 4.02.

4.12.63. (2*S*,5*S*)-2-(4′-Nitrophenyl-1,3-dioxaheptan-5-yl)-phenylacetamide (15d). Colorless powder, mp 125–127 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.64. (2*R*,5*S*)-2-(4′-Nitrophenyl-1,3-dioxaheptan-5-yl)-phenylacetamide (15′d). Colorless powder, mp 129–131 °C. In a NOESY experiment, no NOE was observed.

4.12.65. (2*S*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaheptan-5-yl)-phenylacetamide (15e). Colorless powder, mp 127–129 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.66. (2*R*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaheptan-5-yl)-phenylacetamide (15′e). Colorless powder, mp 135–137 °C. In a NOESY experiment, no NOE was observed.

4.12.67. (2*S*,5*S*)-2-(4′-Chlorophenyl-1,3-dioxaoctan-5-yl)-phenylacetamide (16c). Colorless powder, mp 120–122 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.68. (2*R*,5*S*)-2-(4′-Chlorophenyl-1,3-dioxaoctan-5-yl)-phenylacetamide (16′c). Colorless powder, mp 130–132 °C. In a NOESY experiment, no NOE was observed.

4.12.69. (2*S*,5*S*)-2-(4′-Nitrophenyl-1,3-dioxaoctan-5-yl)-phenylacetamide (16d). Colorless powder, mp 136–138 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.70. (2*R*,5*S*)-2-(4′-Nitrophenyl-1,3-dioxaoctan-5-yl)-phenylacetamide (16′d). Colorless powder, mp 150–152 °C. In a NOESY experiment, no NOE was observed.

4.12.71. (2*S*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaoctan-5-yl)-phenylacetamide (16e). Colorless powder, mp 131–133 °C. In NOESY experiment, NOE between the NH at the 5-position and the proton of phenyl at the 2-position was observed.

4.12.72. (2*R*,5*S*)-2-(3′-Nitrophenyl-1,3-dioxaoctan-5-yl)-phenylacetamide (16′e). Colorless powder, mp 138–140 °C. In a NOESY experiment, no NOE was observed.

4.13. In vivo anti-inflammatory assay

4.13.1. Animals. Male Kunming mice (about 25 g) were inbred and grown in the animal room at the college of Pharmacy, Peking University. The animal room was maintained at 23 ± 2 °C with a 12 h light/dark cycle. Food and tap water were supplied ad libitum. The ethical guidelines described in the NIH guide for care and use of Laboratory Animals were followed throughout the experiments.

4.13.2. Xylene-induced ear edema.[11,12] Male Kunming mice were randomly divided into three groups of 12 mice, namely the test group, vehicle control group, and positive control group. The mice in vehicle control group were administerted orally with a suspension of Aspirin in CMC at a dosage of 20 mg/kg, while the mice in the test group were administerted orally a suspension of the tested compounds in CMC at dosages of 20, 10, and 5 mg/kg. Thirty minutes later, 0.03 ml of xylene was applied to both the anterior and posterior surfaces of the right ear. The left ear was considered as control. Two hours after xylene application, mice were sacrificed and both ears were removed. Using a cork borer with a diameter of 7 mm, several circular sections were taken and weighed. The increase in weight caused by the irritant was measured through subtracting the weight of the untreated left ear section from that of the treated right ear section. The statistical analysis of the data was carried out by use of ANOVA test, $p < 0.05$ is considered significant.

4.14. Tail bleeding time measurement

The new anti-inflammatory agents were orally administered to male mice (body weight 18–22 g). After 30, 45, 60, and 90 min of administration, a mouse was placed in a tube holder with its tail protruding, and a 2-mm cut was made on the tail. Flowing blood until it stopped was gently wiped away with a tissue every 30 s until bleeding ceased and the time recorded.

4.15. PKC inhibition assay

4.15.1. Preparation of PKC. At 4 °C, 500 g of fresh bovine brains was excised, immersed and rinsed in 1 L of ice-cold buffer A (20 mM Tris–HCl, pH 7.5, 3 mM EDTA, 50 mM 2-mercaptoethanol, 1 mM $PhMeSO_2F$, and 10 mg/L aprotinin). The tissue was homogenized in 2.5 L of buffer A for 2 min and centrifuged at 9000*g* for 20 min. To the supernatant ammonium sulfate (21%, w/v) was slowly added. The mixture was centrifuged at 9000*g* for 20 min to remove the formed precipitates. To the supernatant additional ammonium sulfate was added to a final concentration of 45% (w/v). After centrifugation at 9000*g* for 20 min, the recovered precipitates were dissolved in 150 ml buffer B (20 mM Tris–HCl, pH 7.5, 2 mM EDTA, 50 mM 2-mercaptoethanol). The solution was dialyzed in 20-fold volume of buffer B for 3 days and then centrifuged at 30,000*g* for 30 min. The supernatant was applied to a 1-L DEAE–cellulose column equilibrated with buffer B. The column was washed with 2.5 L of buffer B at a flow rate of 125 ml/h. The enzyme was eluted from the column by a linear gradient of 0–0.3 M NaCl in buffer B at a flow rate of 60 ml/h. The fractions of 50 ml each were collected and were assayed for PKC activity. The fractions with high PKC activity were pooled and concentrated to 112 ml by ultrafiltration, adjusted to 30% glycerol, and stored at −85 °C.

4.15.2. Determination of PKC activity.[34,35]

4.15.2.1. Basic theory of method. After activation by phosphatidylserine (PS), diacylglycerol (DG), and Ca^+ PKC catalyzes the phosphorylation of Histone (III-S) by consuming ATP and produces ADP or AMP. Thus PKC specific activity may be defined as ATP's consumption rate as the result of per milligram enzymes catalyzing the phosphorylation of Histone (III-S). Consequently PKC activity may be determined via ATP's consumption by ion-pair reversed phase HPLC. The unit of PKC activity may be represented as nmol/min/mg.

4.15.2.2. Buffer and reactive solution. Buffer C: Tris–HCl 20 mM, pH 7.4; reactive solution 1: Tris–HCl 20 mM, PS 100 μg/ml, DG 10 μg/ml, $MgCl_2$ 10 mM, $CaCl_2$ 1 mM, Histone(III-S) 1 mg/ml, DTT 1 mM, pH 7.4; reactive solution 2: Tris–HCl 20 mM, $MgCl_2$ 10 mM, Histone(III-S) 1 mg/ml, DTT 1 mM, EGTA 0.1 mg/ml, pH 7.4; reactive solution 3: ATP 0.5 mM; enzymes: contained protein 5 mg/ml.

4.15.2.3. HPLC conditions. Reversed-phase Rainbow C18 column (Kromasil 4.6 × 150 mm), mobile phase included tetrabutyl ammonium hydroxide 5 mmol/L, methanol, and KH_2PO_4 (20:80, v/v, pH 7.0), flow rate: 1.0 ml/min, detected at 259 nm.

4.15.2.4. Protracting standard curve. According to Table 5 various solutions were added to control or reactive tube (1.5 ml plastic centrifugal tube). The tubes were kept at 30 °C for 10 min, and then at 100 °C for 1 min. After cooling, 300 μl of chloroform was added. The solution was surged for 1 min and centrifuged at 2000*g* for 5 min. To the supernatant 300 μl of chloroform was added. The solution was surged for 1 min and then centrifuged at 2000*g* for 5 min. Onto the HPLC column 20 μl of supernatant was injected, detected at 259 nm. The standard curve was protracted with ATP content as *X*-coordinate, peak area as *Y*-coordinate.

4.15.2.5. Solution of 1,3-dioxacycloalkanes. Each of the synthesized 1,3-dioxacycloalkanes was dissolved in buffer C to make 1,3-dioxacycloalkane containing solution for determination of PKC activity.

4.15.2.6. Determination of PKC activity. With the same procedure as used for protracting standard curve in the reactive tube the solution of tested compound was added. To the reactive tube 200 μl of reactive solution 1, 40 μl of reactive solution 3, 20 μl of PKC, and 60 μl of 1,3-dioxacycloalkane containing buffer C were added. The final concentration of 1,3-dioxacycloalkane was 0.01, 0.10, 1.00, 5.00, and 10.00 μmol/ml. To the controlled tube 200 μl of reactive solution 2, 40 μl of reactive solution 3, 20 μl of PKC, and 60 μl of buffer C were added. The tubes were kept at 30 °C for 10 min, and then at 100 °C for 1 min. After cooling 300 μl of chloroform was added. The solution was surged for 1 min and centrifuged at 2000*g* for 5 min. To the supernatant 300 μl of chloroform was added. The solution was surged for 1 min and then centrifuged at 2000*g* for 5 min. 20 μl of supernatant was injected to the HPLC column, detected at 259 nm. PKC specific activity was calculated according to standard curve. Their IC_{50} values were calculated by software of statistics.

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