Bioorganic & Medicinal Chemistry Letters 23 (2013) 2022–2026

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A new series of N2-substituted-5-(*p*-toluenesulfonylamino)phthalimide analogues as α-glucosidase inhibitors

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ARTICLE INFO

Article history:
Received 29 December 2012
Revised 25 January 2013
Accepted 1 February 2013
Available online 13 February 2013

Keywords:
5-(*p*-Toluenesulfonylamino)phthalimide
α-Glucosidase inhibitors
Postprandial hyperglycemia
Diabetes

ABSTRACT

Several members of a new family of non-sugar-type α-glycosidase inhibitors, bearing a 5-(*p*-toluenesulfonylamino)phthalimide moiety and various substituent at the N2 position, were synthesized and their activities were investigated. The newly synthesized compounds displayed different inhibition profile towards yeast α-glycosidase and rat intestinal α-glycosidase. Almost all the compounds had strong inhibitory activities against yeast α-glycosidase. Regarding rat intestinal α-glycosidase, only analogs with N2-aromatic substituents displayed varying degrees of inhibitory activities on rat intestinal maltase and lactase and nearly all compounds showed no inhibition against rat intestinal α-amylase. Structure–activity relationship studies indicated that 5-(*p*-toluenesulfonylamino)phthalimide moiety is a favorable scaffold to exert the α-glucosidase inhibitory activity and substituents at the N2 position have considerable influence on the efficacy of the inhibition activities.

Diabetes mellitus type 2 (T2DM), comprising 90% of all cases of diabetes mellitus is increasing dramatically and affecting about 5% of the world population. The burden of diabetes is driven by vascular complications such as cardiovascular disease, stroke, nephropathy, retinopathy, renal failure, and amputations of the extremities. With the admission of the fact that 'diabetes is a cardiovascular disease' (CVD), rise in CVD is being cautioned due to the worldwide increase in the prevalence of diabetes.[1,2] It is now generally accepted that these cardiovascular complications are related to prevailing hyperglycemia, particularly postprandial hyperglycemia (PPHG). Multiple works have convincingly demonstrated that blood glucose variability is more deleterious than chronic sustained hyperglycemia as a key player in contributing to diabetic micro- and macro-vascular complications.[3–5] In recent years, importance of PPHG has been recognized as an important cardiovascular risk factor. A growing body of evidence suggests that there is a strong association between PPHG and cardiovascular risk and other diabetic complications. PPHG is also identified as one of the earliest detectable abnormalities expressed in diabetes, better predictor of progression of diabetes and has been implicated in inducing oxidative stress that is recognized as a major pathophysiological link between CVD and diabetes.[6–8]

Therefore, agents that hold capacity of slowing down postprandial hyperglycemic excursion (PPHGE) and resultant oxidative stress may become therapeutics of colossal importance. Food starches contribute to major postprandial blood glucose. Slowing down of digestion and absorption of dietary starches either by means of dietary manipulation with low glycemic-index food or by inhibition of starch digesting enzyme α-glucosidase present at the intestinal brush borders, have shown promise in reducing PPHGE, hyperinsulinemia, burden of oxidative stress, and CVD.[9–11] α-Glucosidase inhibitors (AGIs) delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise.[12,13]

Several AGIs, including acarbose, voglibose, and miglitol, are clinically used in the effective treatment of type 2 diabetes mellitus. AGIs appear better therapeutic in controlling PPHGE in Asian people presumably because of their specific food habits. Only a few AGIs are available commercially and they are all sugar derivatives and require tedious multi-steps during preparation. Gastrointestinal side-effects, such as flatulence and diarrhea, are also problems for α-glucosidase inhibitors. Intense efforts have been directed towards the development of effective non-saccharide AGIs.[14–19] For instance, Kavitha et al. explored the binding mode of interaction of several sugar/non-sugar AGIs at the active site of α-glucosidase and developed a pharmacophore model which would represent the critical features responsible for α-glucosidase inhibitory activity, as illustrated in Figure 1a.[15]

These results encouraged us to explore a series of 5-(*p*-toluenesulfonylamino)phthalimide derivatives as a novel class of non-sugar-type AGIs based on the pharmacophore model. In this series, 5-(*p*-toluenesulfonylamino)phthalimide moiety was designed as a new scaffold possessing the similar structural characters as AGIs pharmacophore model described, and a variety of alkyl or aromatic

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http://dx.doi.org/10.1016/j.bmcl.2013.02.011

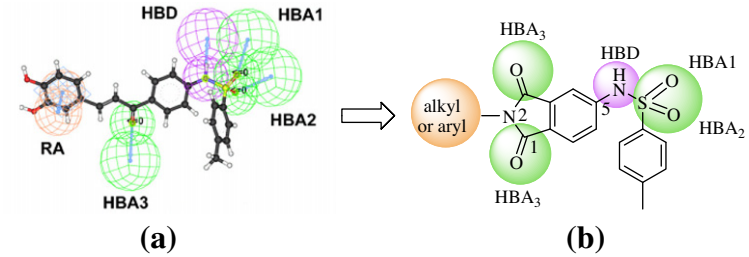


Figure 1. (a) Mapping of pharmacophore model for α-glucosidase inhibitory compounds.[15] (b) The general structure of target compounds.

moieties were connected to N2 position of phthalimide, as illustrated in Figure 1b. In the present study, a series of N2-substituted-5-(*p*-toluenesulfonylamino)phthalimide analogues (Table 1) was synthesized and their α-glucosidase inhibitory activities were evaluated against yeast α-glucosidase and rat intestinal α-glucosidase (maltase, lactase and amylase) and their structure–activity relationships were discussed.

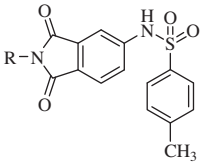
A generalised synthetic approach for the proposed compounds was shown in Schemes 1–4. Synthesis of the title compounds **4**–**15** was completed as depicted in Scheme 1. 5-Nitro-phthalimide potassium salt **3**, which was conveniently prepared according to the procedures described in literature,[20] was reacted with corresponding halogenated hydrocarbon to obtain the N-substituted-5-nitro-phthalimide intermediates **4a**–**14a**, respectively. These intermediates **4a**–**14a** were reduced with formamide and Pd/C to obtain N2-substituted-5-amino-phthalimide intermediates **4b**–**14b**, respectively, which underwent sulfonylation upon treatment with *para*-toluenesulfonic chloride in the presence of triethylamine gave the target compounds **4**–**14**. The intermediate **14a** was hydrolyzed to give intermediate **15a**, which carried the reduction and sulfonylation procedure under the same condition as described above to obtain compound **15**.

Compounds **17**–**18** were prepared via the synthetic route shown in Scheme 2. 5-Nitro-phthalimide **2** was reacted with formaldehyde to form *N*-hydroxymethyl-5-nitro-phthalimide **16**, followed by coupling with the corresponding phenol compound to provide N-substituted-5-nitro-phthalimide intermediates **17a**–**18a**, respectively. The intermediates **17a**–**18a** were submitted to the reduction and sulfonylation procedures after the phenolic hydroxy group was protected by acetyl to give **17c**–**18c**. Then the intermediates **17c**–**18c** underwent deprotection in presence of chlorhydric acid to obtain the target compounds **17**–**18**.

The intermediate **16** was treated with hydrochloric acid to gave *N*-chloromethyl-5-nitro-phthalimide **19** (Scheme 3), which underwent amination with morpholine or piperazine to obtain the N-substituted-5-nitro-phthalimide intermediates **20a**–**21a**, respectively. The intermediates **20a**–**21a** were submitted to reduction and sulfonylation procedures gave the target compounds **20**–**21**.

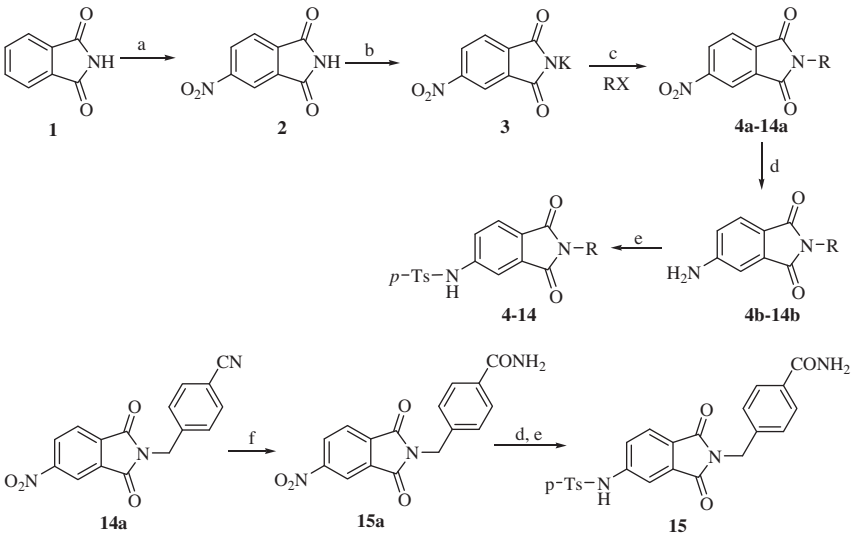
The following strategy was adopted to prepare final compounds **22**–**25** (Scheme 4). The intermediate **22a** was obtained by reaction of *N*-(2-bromoethyl)-5-nitro-phthalimide **5a** with 4-amino-benzene-1,2-di-ol diacetate and the intermediates **23a**–**25a** were prepared by reaction of 5-nitro-phthalimide potassium salt **3** with appropriate 2-bromoethyl phenyl ether. All the intermediates

Table 1
IC_{50} values (μM) for the inhibition of α-glucosidase by the synthesized compounds[a]

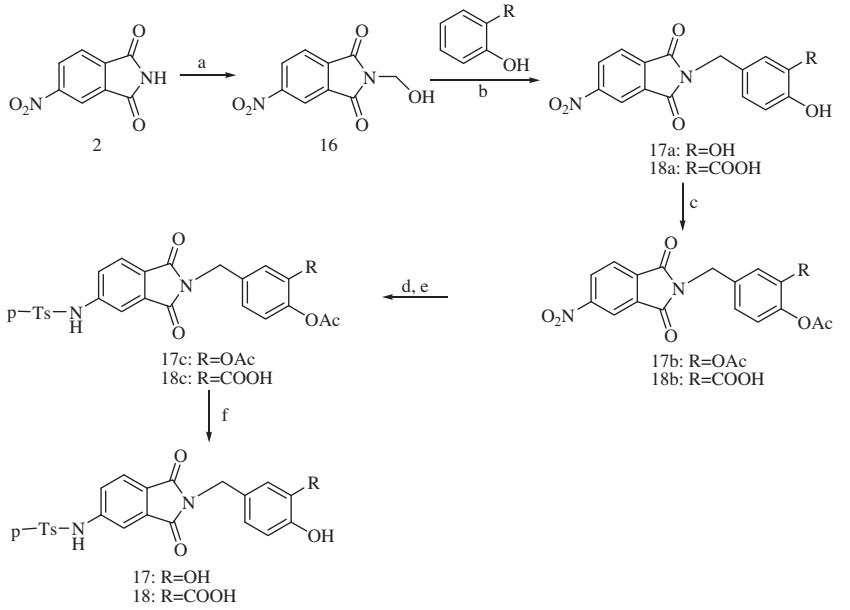


Compd	R	Yeast α-glucosidase	Rat intestinal α-glucosidase		
			Maltase	Lactase	α-Amylase
4	CH_3–	>200	>200	>200	>200
5	Br-CH_2-CH_2–	94.22 ± 4.36	>200	>200	>200
6	Br-CH_2-CH_2-CH_2–	88.43 ± 2.87	>200	>200	>200
7	Br-CH_2-$(CH_2)_2$-CH_2–	69.71 ± 3.65	>200	>200	>200
8	C_6H_5-CH_2–	54.16 ± 2.91	>200	>200	>200
9	4-F-C_6H_4-CH_2–	42.70 ± 0.86	194.02 ± 13.62	172.13 ± 10.39	>200
10	2-F-C_6H_4-CH_2–	40.77 ± 1.32	194.61 ± 10.76	180.47 ± 8.06	>200
11	4-Cl-C_6H_4-CH_2–	32.63 ± 0.67	188.2 ± 9.65	155.32 ± 9.699	>200
12	3,4-Cl_2-C_6H_4-CH_2–	28.74 ± 0.45	122.2 ± 4.98	100.94 ± 4.98	>200
13	C_6H_5-NH-CH_2CH_2–	58.45 ± 1.52	99.56 ± 4.02	176.53 ± 6.81	>200
14	4-NC-C_6H_4-CH_2–	66.50 ± 2.48	>200	199.02 ± 10.33	>200
15	4-H_2NCO-C_6H_4-CH_2–	60.32 ± 2.38	61.37 ± 1.88	84.15 ± 3.25	>200
17	3,4-$(OH)_2$-C_6H_3-CH_2–	72.62 ± 2.21	154.40 ± 6.12	164.44 ± 5.78	>200
18	3-HOOC-4-OH-C_6H_3-CH_2–	79.46 ± 2.09	101.15 ± 3.69	44.73 ± 1.38	188.01 ± 8.69
20	4-Morpholinyl	52.13 ± 1.52	>200	>200	>200
21	Piperazin-1-yl	46.57 ± 1.35	>200	>200	>200
22	3,4-$(OH)_2C_6H_3$-NH-CH_2CH_2–	44.48 ± 0.75	28.82 ± 1.02	66.64 ± 2.08	>200
23	3-OH-C_6H_4-O-CH_2CH_2–	28.72 ± 0.71	55.60 ± 1.51	110.53 ± 4.21	>200
24	4-OH-C_6H_4-O-CH_2CH_2–	25.53 ± 0.39	47.70 ± 1.85	100.46 ± 2.42	>200
25	C_6H_5-O-CH_2CH_2–	47.26 ± 1.08	51.13 ± 1.52	170.20 ± 3.62	>200
Acar		235 ± 3.89	25.52 ± 0.78	166.85 ± 6.65	8.65 ± 0.98

[a] The results summarized are the mean values of $n = 3$ for IC_{50} values.



Scheme 1. Synthetic pathway to compounds **4**–**15**. Reagents and conditions: (a) HNO_3, H_2SO_4, 5 °C to rt, 16 h, 75%; (b) KOH–MeOH, C_2H_5OH, rt, 3 h, 98%; (c) RX, K_2CO_3, DMF, reflux 1 h, overnight, 80–88%; (d) Pd/C, ammonium formate, THF, rt, 12 h, 80–95%; (e) *p*-TsCl, Et_3N, DMF, rt, 3 h, 62–75%; (f) Na_2CO_3, H_2O–MeOH–H_2O_2, Na_2MoO_4, rt, 4 h, 78%.

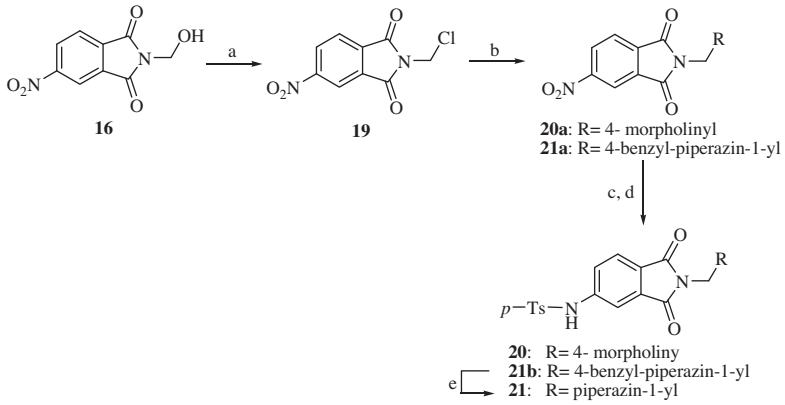


Scheme 2. Synthetic pathway to compounds **17**–**18**. Reagents and conditions: (a) 38% formaldehyde–H_2O, reflux 3 h, 77%; (b) HNO_3/H_2SO_4 = 5:1, rt, 7 h, 70–76%; (c) Ac_2O, reflux 3 h, 88%; (d) Pd/C, ammonium formate, THF, rt, 12 h, 93–95%; (e) *p*-TsCl, Et_3N, DMF, rt, 6 h, 65–69%; (f) 10% HCl, acetone, rt, 1 h, 85%.

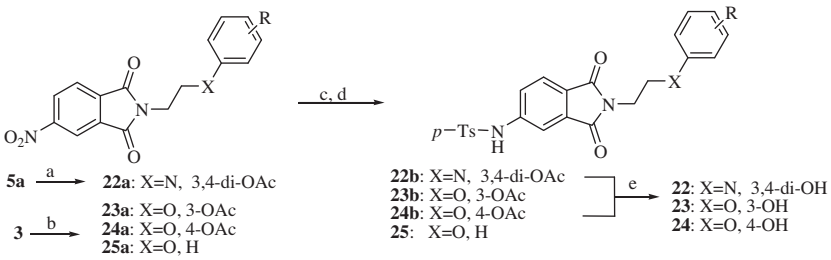
22a–**25a** underwent the similar procedure of reduction, sulfonylation and deprotection to give the target compounds **22**–**25**.

The structures of the target compounds were verified by ^1H NMR and mass spectrometry, while their purities were estimated by HPLC analysis.

α-Glucosidase of divers source has been used for screening and evaluation of α-glucosidase inhibitors. Yeast α-glucosidase has been mostly used because of its simple and convenient method.[21] In the present study, the α-glucosidase inhibitory activity of the newly synthesized compounds (Table 1) was firstly evaluated with yeast α-glucosidase. The inhibitory activity was determined as described in the literature,[19,22] and was expressed as the inhibitor concentration required for 50% inhibition of the α-glucosidase activity (IC_{50}) which is reported in Table 1. The results document that the synthesized N2-substituted-5-(*p*-toluene sulfonylamino)phthalimide derivatives exhibited obvious inhibitory activity against yeast α-glucosidase with IC_{50} values <100 μM, except the compound **4**. The efficacy of the inhibition activity was influenced by the variation of N2-substituents. Regarding structures of N2-substituents, the target compounds include three series. ① *N*2-Alkyl analogues, the compounds **4**–**7**, having small alkyl or bromoalkyl at the N2 position. ② N2-saturated heterocycles derivatives, the compounds **20** and **21**, having morpholine or piperazine ring in N2-substituent moiety. ③ N2-aromatic substituted analogues, including *N*2-benzyl analogues **8**–**12**, **14**, **15**, **17**, **18** and compounds **13**, **22**–**25** having an aryl moiety connected to N2 through a chain of three atoms. The IC_{50} values in Table 1 indicate that most of the N2-aromatic substituted analogues were more potent than *N*2-alkyl derivatives. Among *N*2-alkyl derivatives **4**–**7**, compound **4** had no activity while the inhibition potency of the other compounds increased with the length of the alkyl chain. Concerning the N2-aromatic substituted compounds, their



Scheme 3. Synthetic pathway to compounds **20**–**21**. Reagents and conditions: (a) concd hydrochloric acid, toluene, 65 °C, 48 h, 71%; (b) morpholine or 1-benzylpiperazine, K_2CO_3, acetone, 40 °C, 4.5 h, 91% and 89%; (c) Pd/C, ammonium formate, THF, rt, 2 h, 94–95%; (d) *p*-TsCl, Et_3N, DMF, rt, 3 h, 55–60%; (e) Pd/C, HCOOH, THF, rt, 8 h, 81%.



Scheme 4. Synthetic pathway to compounds **22**–**25**. Reagents and conditions: (a) 4-amino-benzene-1,2-di-ol diacetate, K_2CO_3, acetone, rt, 24 h; (b) corresponding 2-Br-ethyl-phenolic ether, DMF, 60 °C, 3 h, 55%; (c) Pd/C, H_2, 40 °C, 4 h, 98%; (d) *p*-TsCl, Et_3N, DMF, rt, 4 h, 65%; (d)10% HCl, acetone, rt, 1 h, 88%.

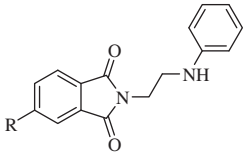
inhibitory potential changed with substituents on phenyl of N2 moieties. Among the *N*2-benzyl analogues, inhibitory effect increased with introduction of halogen atom at the benzyl moiety as compare with **8** and the strongest inhibition was found for compound **12** which bearing a 3,4-dichloro benzyl with an IC_{50} = 28.74 μM. In contrast with the expectation, introduction of hydroxy or other polar group to benzyl (**14**–**15**, **17**–**18**) decreased the activity. These may indicate that the hydrophobic group in this region may be favorable for the binding interaction. As the connection chain between *N*2-aryl and phthalimide scaffold increased to three atoms (**13**, **22**–**25**), introduction of a hydroxy group intends to increase the inhibitory potencies. Compound **24** displayed the strongest inhibition in all of the synthesized compounds with an IC_{50} = 25.53 μM. In N2-saturated heterocycles series, compounds **20**, **21** resemble aromatic substituted compounds in inhibitory potential. These results may suggest that 5-(*p*-toluenesulfonylamino)phthalimide is a favorable scaffold and the substitutes at the N2 position may be various structures to exert yeast α-glucosidase inhibitory activity.

The inhibition potencies and selectivity of the target compounds for several selected sugar hydrolases (maltase, lactase and α-amylase) were further evaluated with rat intestinal α-glucosidase.[23–26] Results are summarized in Table 1. The IC_{50} values data indicates that the inhibition profile of the target compounds towards rat intestinal α-glucosidase are different from that towards yeast α-glucosidase. No compound of the *N*2-alkyl derivatives and N2-saturated heterocycles derivatives had activity against rat intestinal α-glucosidase. No compounds displayed activity towards rat intestinal α-amylase except compounds **18** which showed only weaker inhibition (IC_{50} = 188.01 μM). Compounds having activities on rat intestinal α-glucosidase (maltase and/or lactase) generally have aromatic moieties in the N2-substituent regions, suggesting that aromatic structure may be favorable for rat intestinal α-glucosidase inhibitory activity. This observation is in agreement with the pharmacophore model described in Figure 1. Among the active compounds, **22** emerged as the strongest rat intestinal α-glucosidase inhibitor with IC_{50} values 28.82 and 66.64 μM for maltase and lactase, respectively. Other analogues **23**–**25** also showed obvious inhibitory activity against rat intestinal maltase with IC_{50} values within 47.70–51.13 μM and moderate activity against rat intestinal lactase with IC_{50} values within 100.46–170.20 μM. Only one of the nine *N*2-benzyl analogues, compound **15**, displayed strong activity against rat intestinal maltase and lactase (IC_{50} = 61.37 and 84.15 μM, respectively) and other analogues behaved as moderate inhibitors against rat intestinal maltase and lactase with the IC_{50} values more than 100 μM. It appears therefore that 3-atom connection chain between *N*2-aryl and phthalimide scaffold has added advantage in improving rat intestinal α-glucosidase inhibitory activity than *N*2-benzyl analogues. Introduction of hydroxy groups to *N*2-aryl of compound **13** yielding compound **22** significantly enhanced inhibitory potency. Introduction of one hydroxy substitution on *N*2-aryl, as in compound **23** and **24**, displayed no advantage when compared to compound **25**. These reflect that catechol structure of *N*2-aryl moiety may be important for improving the inhibitory potency.

The IC_{50} data (Table 1) also demonstrates that the target compounds behaved more selective inhibition of yeast α-glucosidase, since the IC_{50} values against yeast α-glucosidase are generally lower than the corresponding IC_{50} values against rat intestinal α-glucosidas.

In order to clarify the important role of *p*-toluenesulfonylamino group at the C5 position of phthalimide scaffold, several intermediates of compound **13** were tested and evaluated likewise. These intermediates, compounds **13a**, **13b** and **26**, have the same

Table 2
IC_{50} values (μM) for the inhibition of α-glucosidase by compounds **13**, **13a**, **13b** and **26**[a]



Compd	R	Yeast α-glucosidase	Rat intestinal α-glucosidase		
			Maltase	Lactase	α-Amylase
26	–H	>200	184.14 ± 7.16	>200	>200
13a	$–NO_2$	68.91 ± 1.29	111.44 ± 5.81	>200	>200
13b	$–NH_2$	137.93 ± 3.68	144.27 ± 8.72	>200	>200
13	*p*-Ts-NH_2–	58.45 ± 1.52	99.56 ± 4.22	176.53 ± 11.34	>200

[a] The results summarized are the mean values of $n = 3$ for IC_{50} values.

*N*2-(2-anilinoethyl) substituent as compound **13** but different substitutes at the C5 position, their structures and α-glucosidase inhibitory strength are presented in Table 2. Replacement of the *p*-toluenesulfonylamino group into $–NO_2$ group or $–NH_2$ group considerably reduced the inhibitory potencies towards both rat intestinal and yeast α-glucosidase, as evidenced by the IC_{50} values of **13a** and **13b**. Absence of substition at the C5 position, compound **26**, lead to nearly disappearance of activity. This may be due to favourable interactions of the *p*-toluenesulfonylamino group which could interact with the binding subsite through H-bonds by providing H-bond donors and H-bond receptors. But $–NO_2$ group could only provide H-bond receptors and $–NH_2$ group could mainly provide H-bond donors. From these results, it is fair to conclude that both of H-bond donors and H-bond receptors are essential structural features for the inhibitory activity. In our research, we also found that replacement of the tolylene of *p*-toluenesulfonylamino moiety to methyl also decreases the inhibition strength (data is not given here). This indicates the useful role of the aromatic ring of *p*-toluenesulfonylamin moiety which may interact with the binding subsite through hydrophobic interaction or π–π/π-cation interaction. These results verify the decisive role of *p*-toluenesulfonylamino group for the inhibitory activity that attributes to both sulfonylamino and aromatic moieties.

In conclusion, we have described the synthesis and evaluation of a series of N2-substituted-5-*p*-toluenesulfonylamino phthalimide derivatives as novel α-glucosidase inhibitors. Almost all compounds appeared to be strong inhibitors towards yeast α-glucosidase but only analogues with N2-aromatic substituents displayed varying degrees of inhibitory effects against rat intestinal maltase and/or lactase and nearly no compounds showed inhibitory activity against rat intestinal α-amylase. The toluenesulfonylamino group was demonstrated to be an essential structural moiety for this series and substituents at the N2 position had significant influence on inhibitory potencies. These results produce evidences in support of the rational design of the target compounds based on the pharmacophore model.[15] Although it seems that the present series derivatives being more selective for yeast α-glucosidase over rat intestinal α-glucosidase, compound **22**–**25** proved to be promising inhibitors for rat intestinal maltase and lactase. Molecular recognition in the target binding site in yeast α-glucosidase and rat intestinal α-glucosidase may be the reason for different inhibition profile of these compounds.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (Grant Nos. 21172177 and 81272448).

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24. Inhibitory activities assay with yeast α-glucosidase (Canada Bio Basic Inc.) was performed at 37 °C by using 4-nitro-phenyl-α-D-glucopyranoside as substrate in pH 7.28 buffer containing 10 mM sodium phsophate. Eight inhibitor concentrations around the IC_{50} values, approximately estimated in previous experiments, were employed. In each set of experiments the assay was performed in triplicate and acarbose (acar) was used as reference drug. The increased absorbance at 400 nm was compared with that of the control containing DMSO in the place of the test solution. IC_{50} values were measured graphically by a plot of percent activity versus log of the test compound concentration.
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26. Brush border membranes prepared from rat small intestine according to the method of Kessler et al.[23] were used as the enzyme source of rat intestinal maltase, lactase and α-amylase. Inhibitory activities of testes compounds against rat intestinal glucosidases were determined using an appropriate disaccharide as substrate and acarbose was used as reference drug. The released D-glucose was determined colorimetrically using Glucose-B Test Kit (Wako Pure Chem. Co., Osaka, Japan).