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Synthesis of novel β-amino ketones containing a *p*-aminobenzoic acid moiety and evaluation of their antidiabetic activities

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The synthesis of two series of β-amino ketones containing a *p*-aminobenzoic acid moiety (**TM-1** and **TM-2**) using a modified protocol of the Mannich reaction is reported. The molecular structures of a total of tweenty three new target compounds were characterized by ^{1}H NMR, ^{13}C NMR, ESI-MS and HR-MS. Subsequently, their antidiabetic activities were screened *in vitro*. The α-glucodase inhibition (α-GI) activity of compound **1e** reached a remarkable level of 66.50%. The peroxisome proliferator-activated receptor (PPAR) relative activation activities of six compounds are above 80%, and in particular **2i** displays an unprecedentedly high PPAR of 130.91%. The structure-activity relationships of the compounds were established. **2i** is also subject to further in-depth investigation.

diabetes mellitus, α-glucosidase, peroxisome proliferator-activated receptor, β-amino ketone, Mannich reaction

1 Introduction

Diabetes mellitus (DM) is a common metabolic disorder ever growing into epidemic proportions following the improvement in quality of life for an increasingly greater population in recent years [1], and the condition is now ranked the 3rd most fatal non-infectious chronic disease after cardiovascular disease and cancer. For the most widespread type 2 diabetes (T2DM), clinical drugs may be classified into insulin secretagogues (glinides and sulfonylureas), insulin sensitizers (biguanides, thiazolidinediones (TZDs)), aldose reductase inhibitors (e.g. epalrestat and tolrestat), glucose absorption effectors (e.g. *a*-glucosidase inhibitors such as acarbose, miglitol and voglibose). Also falling in the same category are the newly introduced dipeptidyl peptidase IV (DPP IV) inhibitors, for example alogliptin (approved in Japan in 2010) [2] and linagliptin (approved by the US FDA in 2011) [3]. Although somewhat useful in the treatment of T2DM, the long-term effects of these antidiabetic agents have not been fully evaluated and may lead to a variety of adverse consequences. For instance, insulin may cause weight gain and hypoglycaemia, and TZD insulin sensitizers may lead to multiple adverse effects including weight gain, edema, hepatotoxicity and elevated level of low density lipoprotein cholesterol (LDL-C) [4]. Thus the demand remains urgent for the development of new antidiabetic drugs with higher efficacy and lower toxicity for long term treatment.

Many of the commercially available T2DM drugs, despite being structurally diverse, share a few key features that are deemed critical to their functionalities. For example, 3-phenylpropanoic acid based nateglinide [5] (Novartis, 1999), an oral formulation of insulin secretion promoter

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effective in reducing blood glucose, and benzoic acid based repaglinide [6] (Novo Nordisk, 1998) and L-tyrosine derivatives [7] both contain carboxyl and amino functional groups. In addition, drugs like rosiglitazone (GSK, 1999), pioglitazone (Takeda, 1999) and epalrestat (Pharmaceutical, 1992), plus drug candidates muraglitazar (BMS 298585), DRF2189 [8], MCC555 [9] and GW1929, PTP1B inhibitors B [10] and C [11] (Chart 1), contain some sort of acidic part(s) or other functional group(s) with or without amino groups. Additionally, the majority of these molecules consist of two aromatic ring modules separated by a linker. These structural factors have to be taken into consideration in the development of new T2DM drugs.

The search for effective antidiabetic target molecules needs to start from examining the structural features of existing drugs, followed by designing a novel class of specific compounds and adopting facile synthetic methods. An important way is to obtain the desired bioactive molecules from domino or multicomponent reactions (MCRs). The Mannich reaction, one of the most important MCRs in organic synthesis [12, 13, 14], is commonly used for the preparation of β-amino ketones, including those potent for analgesic [15], tubercle [16, 17], inflammation [18], cancer [19] and bacteria [20]. Our group has recently identified a large group of β-amino ketones exhibiting androgen receptor modulating activity [21] and antidiabetic activities [22], It is apparent that many potent β-amino ketones contain a *p*-aminobenzoic acid moiety. These compounds often have the modular composition as outlined in Figure 1, including a hydrophilic portion A and a hydrophobic portion C connected by a linker portion B, and closely resemble the structural characteristics of TZDs and some L-tyrosine derivatives [7].

We are interested in the development of non-thiazolidinedione peroxisome proliferator-activated receptor (PPARγ) agonists in an attempt to surmount the pitfalls associated with the known TZDs [7]. Based on our previous research results, we decided to modify discrete parts of β-amino ketone lead compounds (Figure 1). As a well accepted scaffold in medicinal chemistry, *p*-aminobenzoic acid has been in-

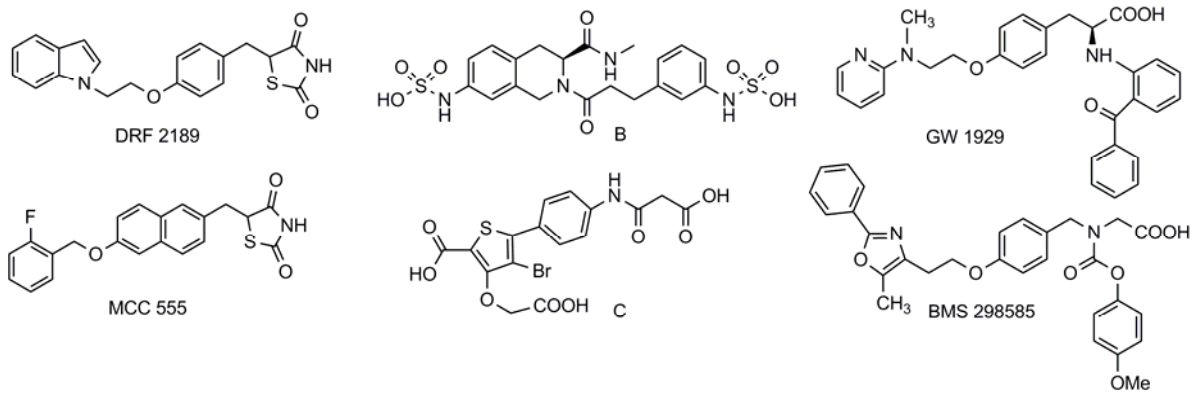


Chart 1 Representative candidates in clinical trials or active compounds reported.

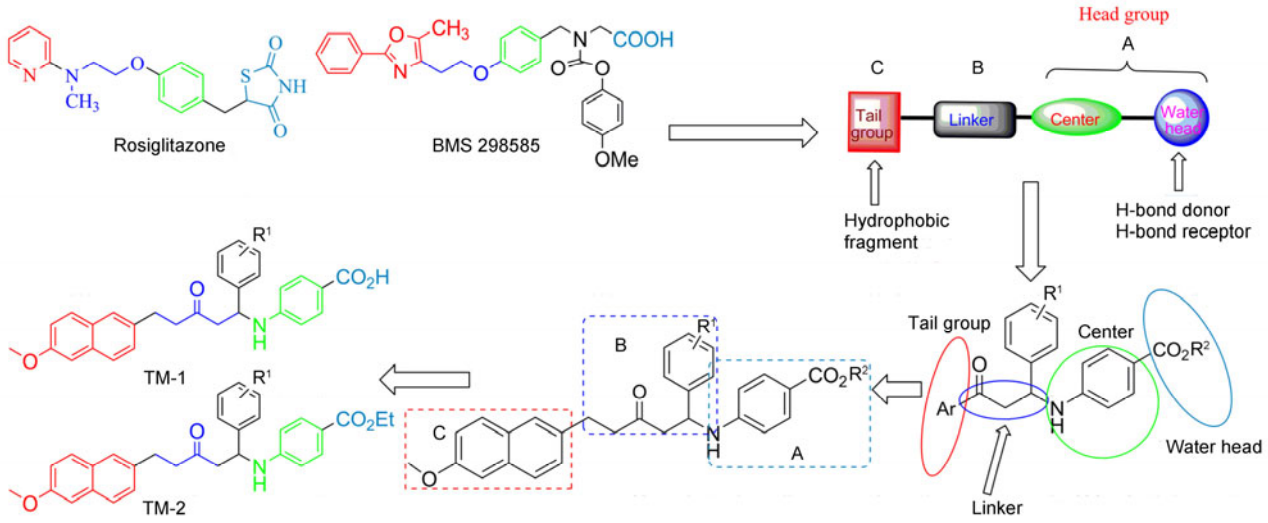


Figure 1 Engineering strategies for target molecules.

corporated in compounds displaying a variety of pharmacological effects, including anti-tumor [23], anticancer [24], anti-hepatitis B virus [25], anti-HIV integrase [26], antidiabetic [22, 27] and antibacterial activities [28, 29]. The *p*-aminobenzoic acid moiety is conserved as an essential pharmacophore which, similar to glinide, also acts as the indispensable amino component in the Mannich reaction. Alternatively, ethyl *p*-aminobenzoate, commonly present in toponarcosis, analgesic and antipruritic drugs, may be used as the hydrophilic portion A since it can be converted into *p*-aminobenzoic acid via *in vivo* metabolism.

It was reported [30] that the presence of a naphthalene ring led to more pronounced PPAR activities than analogues with a simple benzene ring. Presumably, the introduction of hydrocarbon chain between the carbonyl group and the aromatic ring in an aryl alkyl ketone alters the flexibility of the backbone which, in turn, changes its solubility and bioactivity. As such, inserting a nabumetone building block into β-amino ketones in place of portion C and part of portion B is desirable by virtue of its two methylene groups between the carbonyl group and the naphthalene ring and the two reactive sites for the Mannich reaction, which should lead to more backbone flexibility and, in turn, enhanced bioactivity. Currently, nabumetone is present in common non-steroidal anti-inflammatory drugs (NSAID) for rheumatic and inflammatory conditions [31]. The two reactive sites allow ready construction but may result in two kinds of products, which result in difficulties in obtaining high purity target molecules. Finally, an aromatic aldehyde with different substituent groups was selected as part of linker B. Based on our previous work and fresh considerations as highlighted above, we designed two series of β-amino ketones containing target molecules **TM-1** and **TM-2** as outlined in Figure 1. By synthesizing the two series of β-amino ketones through the Mannich reaction with the modified "one-pot, two-step and three-component" protocol and screening their PPAR activation and α-glucosidase inhibition, we were able to identify new lead molecules for antidiabetic drug and establish the all important structure-function relationship.

2 Results and discussion

2.1 Chemistry

The Mannich reaction is a classical tool for the synthesis of β-amino ketones, which may be achieved via a number of protocols. These include either a direct approach involving all-three starting materials aldehydes, ketones and amines [32, 33], exchange of amines [34], Michael addition of aromatic amines with α, β-unsaturated ketones [35], forming amine hydrochlorides [36], exchange of ketones [37], or condensation of Schiff bases with aromatic ketones [38] (Figure 2).

The Mannich reaction is one of the most important C–C and C–N bond forming reactions in organic synthesis. Prior to 1980, the conventional starting materials were formaldehyde, fatty amines and ketones, and to a lesser extent aromatic aldehydes and fatty amines [39]. It was not until 1991 when aromatic aldehydes, arylamines and ketones were first used as the starting materials [40]. Subsequently, the use of aromatic aldehydes and arylamines as starting materials was further explored [41, 42, 43]. We adopted a "one-pot, two-step and three-component" protocol to yield β-amino ketones (Scheme 1). Many target molecules showing various levels of potent antidiabetic activities have since been obtained [22, 44, 45, 46].

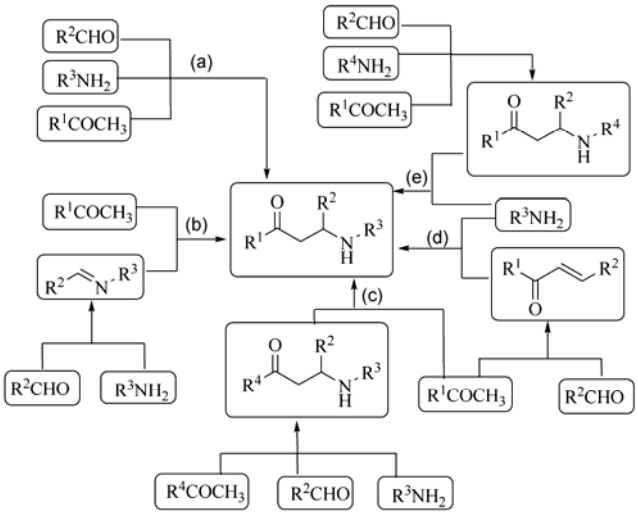
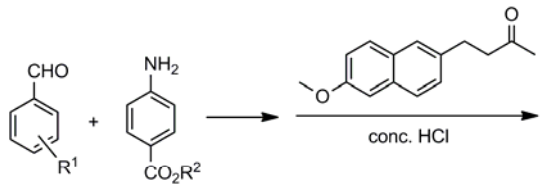
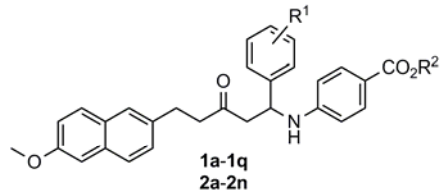


Figure 2 Current synthetic methodologies for Mannich bases. (a) Three-component one-pot reaction; (b) condensation of Schiff base with ketone; (c) exchange of ketone; (d) Michael addition; (e) exchange of amine.

A model reaction system involving nabumetone, *p*-chlorobenzaldehyde and *p*-aminobenzoic acid as starting materials was tested at different temperatures, namely 25, 30 and 40 °C; in different solvents including ethanol, methanol, benzene, chloroform or an ethanol-chloroform binary mixture; using different catalysts including concentrated hydrochloric acid, glacial acetic acid, nitric acid, phosphoric acid, iron(III) chloride, aluminium trichloride, or a solution of hydrogen chloride in ethyl acetate. The optimal reaction conditions were found to be an ethanol-chloroform mixture solution (*V*:*V* = 3:1), catalyzed by concentrated hydrochloric acid (0.1–0.2 mL) in the temperature range of 25–30 °C. In general, these parameters are not too dissimilar to previous findings for other *β*-amino ketone systems [22, 45].

As shown in Table 1, there appears to be a general trend for both **TM-1** and **TM-2** in that the yield of the final product β-amino ketone is closely dependent on the substituent group(s) on the benzene ring of the aromatic aldehyde. More specifically, aromatic aldehydes with electron-donating groups react more readily than those with electron-withdrawing groups, following the order **2l**(4-O-Bu^n) >**2j**(3-





1a-1q
2a-2n

1a: R^1 = 3-NO_2, R^2 = H; **2a**: R^1 = 3-NO_2, R^2 = Et;
1b: R^1 = 3, 4-diCl, R^2 = H; **2b**: R^1 = 3, 4-diCl, R^2 = Et;
1c: R^1 = 3-F, R^2 = H; **2c**: R^1 = 3-F, R^2 = Et;
1d: R^1 = 3-Cl, R^2 = H; **2d**: R^1 = 3-Cl, R^2 = Et;
1e: R^1 = 4-Cl, R^2 = H; **2e**: R^1 = 4-Cl, R^2 = Et;
1f: R^1 = 4-Br, R^2 = H; **2f**: R^1 = 4-Br, R^2 = Et;
1g: R^1 = H, R^2 = H; **2g**: R^1 = H, R^2 = Et;
1h: R^1 = 3-CH_3, R^2 = H; **2h**: R^1 = 3-CH_3, R^2 = Et;
1i: R^1 = 4-CH_3, R^2 = H; **2i**: R^1 = 4-CH_3, R^2 = Et;
1j: R^1 = 3-CH_3O, R^2 = H; **2j**: R^1 = 3-CH_3O, R^2 = Et;
1k: R^1 = 4-CH_3O, R^2 = H; **2k**: R^1 = 4-CH_3O, R^2 = Et;
1l: R^1 = 4-O-Bu^n, R^2 = H; **2l**: R^1 = 4-O-Bu^n, R^2 = Et;
1m: R^1 = 3, 4-OCH_2O, R^2 = H; **2m**: R^1 = 3, 4-OCH_2O, R^2 = Et;
1n: R^1 = 4-OH, R^2 = H; **2n**: R^1 = 4-OH, R^2 = Et;
1o: R^1 = 2, 4-diCl, R^2 = H;
1p: R^1 = 3-OH, R^2 = H;
1q:R^1 = 2-Cl, R^2 = H;

Scheme 1 Synthesis of target β-amino ketones.

CH_3O) > **2i**(4-CH_3) > **2c**(3-F) > **2e**(4-Cl). In particular, the substituent 4-O-Bu^n leads to the highest yield of over 90%. This can be attributed to its fairly low solubility in water, which is favorable for its separation from the reaction mixture, hence promoting the reaction toward completion and the subsequent recrystallization. However, aromatic aldehyde bearing stronger electron-withdrawing groups led to lower yield, following the order **2a**(3-NO_2) < **2d**(3-Cl) < **2e**(4-Cl) < **2c**(3-F) < **2i**(4-CH_3) < **2j**(3-CH_3O). This is probably correlated to the steric hindrance effect of the substituent and the resultant electronic density distribution of the molecule. Based on the results shown in Table 1, it becomes apparent that the nature, and location, of the substituent on the aromatic aldehyde have significant impact on the yield of the target molecules. Thus aromatic aldehydes with the strongest electron-withdrawing group (4-NO_2) or electron-donating group (4-OH) lead to the lowest yield, or even worse, do not undergo the reaction at all. In addition, aromatic aldehydes with moderately strong electron-withdrawing groups (e.g. 4-Br), or electron-donating groups (e.g. 4-Me, 4-MeO) produce reasonable yields in the range of 70–80%. The yields follow the order **1m**(3,4- OCH_2O) > **1l**(4-O-Bu^n) > **1k**(4-CH_3O) > **1j**(3-CH_3O) > **1h**(3-CH_3) > **1i**(4-CH_3); **1f**(4-Br) > **1b**(3,4-diCl) > **1o**(2,4- diCl) > **1d**(3-Cl) in **TM-1**, and **2l**(4-O-Bu^n) > **2j**(3-CH_3O) > **2i**(4-CH_3); **2f**(4-Br) > **2e**(4-Cl) > **2d**(3-Cl) in **TM-2**. It is also generally valid that aromatic aldehydes with *para*-substituents react faster than those with *ortho*- or *meta*-substituents. For instance, **1k**(4-CH_3O) > **1j**(3-CH_3O), **1e**(4-Cl) > **1q**(2-Cl) > **1d**(3-Cl), **1b**(3,4-diCl) > **1o**(2,4-diCl). All of these are correlated to the nature, and location, of the substitutent.

It is worth noting that aromatic aldehydes substituted by either 2,4-diCl, 3-OH or 2-Cl groups reacted readily with *p*-aminobenzoic acid to yield **1o**, **1p** and **1q**, respectively, but the expected analogous reaction with ethyl *p*-aminobenzoate did not proceed. The root cause remains unclear and is under continuing investigation. Fortunately, aromatic aldehydes substituted by a 4-OH group produced the corresponding β-amino ketones in moderate yields. Again, to our surprise, aromatic aldehydes substituted by 4-NO_2, 4-N(CH_3)$_2$, or furfuraldehyde, did not produce any of the corresponding β-amino ketones under the established conditions.

2.2 Biological activity

Positive controls for the assay of α-glucosidase inhibition and PPAR activation are Acarbose (100 μg mL^{-1}, 74%) and Rosiglitazone(10 μg mL^{-1}, 100%), respectively. The bioassay results of all target compounds are shown in Table 1.

2.2.1 α-Glucosidase inhibition

α-Glucosidase plays an important role in the digestion of carbohydrates and biosynthesis of viral envelope glycoproteins. α-Glucosidase inhibitors are promising candidates for antidiabetics [47], anti-AIDS [48], anti-viral hepatitis [49] and anticancer drugs [50]. In order to evaluate the antidiabetic activity of the target β-amino ketones, we adopted the assay of α-GI activity. From Table 1 and Figure 3, it can be found that the α-GI activities were weak for the majority of the β-amino ketones containing *p*-aminobenzoic acid (**TM-1**) or ethyl *p*-aminobenzoate moiety (**TM-2**) at a concentration of 10.0 μg mL^{-1}. A few compounds previously reported by our group are also listed in Table 1 for the purpose of investigating the structure-activity relationship (SAR) [22, 44]. As shown in Figure 3, the two series of compounds exhibit fairly consistent performances in their biological activities, except for the case of 3-CH_3. The products with the strong electron-withdrawing group 3-NO_2 and the strong electron-donating groups 3-OCH_3, 4-CH_3O or 3, 4-OCH_2O show very weak α-GI activities. However, those with the weak electron-withdrawing groups 4-Cl or 3,4-diCl are significantly better. For instance, the α-GI activity of **1e** (4-Cl) reached 66.50% at 10 μg mL^{-1}, very close to the positive control of acrobose at 10-fold higher concentration (74.12%, 100 μg mL^{-1}). In addition, the α-GI activity of **1b** (3,4-diCl) reached 48.50%. Furthermore, **1n** and **2b** gave values of 41.10% and 40.72%, respectively. These represent approximately 55% of the positive control activity of acrobose at a 10-fold higher concentration level.

Table 1 Yield, α-Glucosidase inhibition and PPAR relative activation of *β*-amino ketones *in vitro*

TM	R^1	Yield (%)	$C^{1\,a)}$ (nmol mL^{-1})	α-glucosidase inhibition (%)	PPAR relative activation (%) [b]
					PPAR (at conc. C^1)
1a	3-NO_2	60.1	20.06	6.40	8.75
1b	3,4-diCl	62.1	19.14	48.50	73.66
1c	3-F	75.6	21.22	10.00	28.65
1d	3-Cl	45.0	20.49	21.80	8.49
1e	4-Cl	84.4	20.49	66.50	3.34
1f	4-Br	75.1	18.78	33.19	17.88
1g	H	72.3	22.05	10.21	41.55
1h	3-CH_3	81.2	21.38	6.60	11.76
1i	4-CH_3	80.3	21.38	11.17	24.15
1j	3-CH_3O	75.3	20.68	9.35	12.59
1k	4-CH_3O	81.5	20.68	13.29	22.87
1l	4-O-Bu-n	82.5	19.02	12.21	12.66
1m	3,4-OCH_2O	89.0	20.10	7.93	13.33
1n	4-OH	57.8	21.31	41.10	39.79
1o	2,4-diCl	51.2	19.14	8.26	33.89
1p	3-HO	78.0	21.31	1.56	24.10
1q	2-Cl	50.2	20.49	6.39	29.40
2a	3-NO_2	30.7	18.99	10.69	4.58
2b	3,4-diCl	76.8	18.17	40.72	1.71
2c	3-F	73.7	20.02	13.84	25.68
2d	3-Cl	64.2	19.38	8.71	1.26
2e	4-Cl	71.1	19.38	24.18	3.63
2f	4-Br	83.2	17.84	29.24	20.31
2g	H	82.5	20.76	13.32	1.15
2h	3-CH_3	76.4	20.18	25.45	5.56
2i	4-CH_3	82.9	20.18	13.89	15.66
2j	3-CH_3O	86.5	19.55	10.34	29.32
2k	4-CH_3O	84.8	19.55	29.26	4.49
2l	4-O-Bu-n	93.6	18.06	10.37	14.26
2m	3,4-OCH_2O	73.0	19.03	1.75	11.56
2n	4-OH	56.4	20.10	34.38	3.23
Positive control				74	100

a) The concentration of each test compound is 10 μg mL^{-1} (the corresponding concentration in nmol mL^{-1} is shown in the Table); b) the value of PPAR is an average value of four measurements.

Table 2 PPAR relative activation of some β-amino ketones *in vitro*

Compound	**1c**	**1f**	**1i**	**1j**	**1k**	**1l**	**1o**	**1p**	**1q**	**2c**	**2f**	**2i**	**2j**	**2l**
$C^{2\,a)}$	10.61	9.39	10.69	10.34	10.34	9.51	9.57	10.66	10.24	10.01	8.92	10.09	9.78	9.03
PPAR[b]	35.91	85.93	93.47	72.31	103.32	68.53	53.20	23.11	64.92	63.70	88.48	130.91	80.66	40.41

a) The concentration of each test compound is 5.0 μg mL^{-1} (the corresponding concentration in nmol mL^{-1} is shown in the Table); b) the value of PPAR is an average value of four measurements. The positive control is rosiglitazone, with an activation of 100% (10 μg mL^{-1}).

The biological activity of the target molecules in the **TM-1** series is closely correlated to the intrinsic property of the substitutent group R^1 on the aromatic aldehyde. The α-GI activities of β-amino ketones with the electron-donating groups 3-CH_3, 4-CH_3, 3-CH_3O, 4-CH_3O, 4-C_4H_9O or 3,4-OCH_2O on the benzene ring of the aromatic aldehyde are all at the 10% level with no significant difference between them, with the exception of **1n**(4-OH) whose activity is about 41%. It is probable that the compound with comparatively larger electronic density variation across the aromatic aldehyde moiety has lower solubility which, in turn, weakens the competitive binding of α-glucosidases at the hypso-concentration. This inevitably leads to limited α-GI activity. In contrast, **1n**(4-OH) showed good solubility due to smaller 4-OH substituent. Therefore it is more likely to bind effectively to the protein substrate, an effect supplemented by the extended hydrogen bonding network and 3-D matching. This naturally leads to considerably higher bioactivities. These results indicate that the electronic density distribution is an important, but not the only, factor that

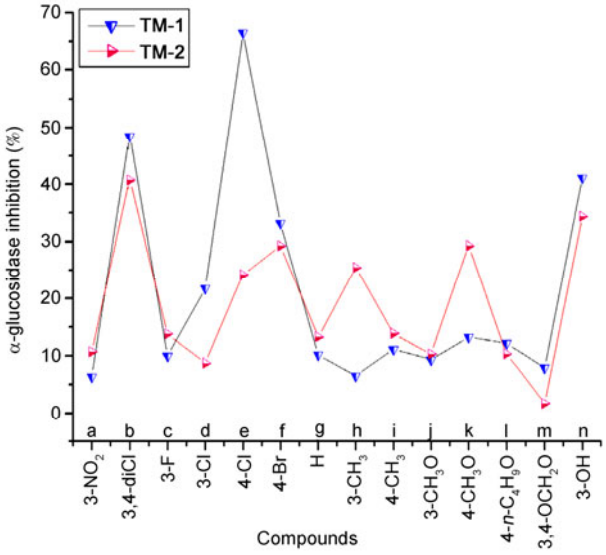


Figure 3 Comparison of the α-glucosidase inhibition of **TM-1** and **TM-2**.

dictates the bioactivities. Overall, the α-GI bioactivities of β-amino ketones with electron-withdrawing groups have considerable disparity. For chloro-substituted aromatic aldehydes, such as with 4-Cl, 3-Cl or 2-Cl substitutuents, the α-GI activities of the corresponding **TM-1** are 66.50%, 21.80% and 6.39%, respectively, showing additional trend beyond the electronic density distribution. This is most probably related to the 3-D conformation, steric hindrance and molecular rigidity—in short, the compatibility between the target molecule and the enzyme. The higher α-GI activity (48.50%) of 3,4-diCl (**1b**), as compared to 2,4-diCl (**1o**, 8.26%) and 2-Cl (**1q**, 6.39%) analogues, probably originates from the steric hindrance.

Further investigation into the SAR of the α-GI activity reveals that the compounds of both series **1** and **2** with electron-withdrawing groups on the aromatic aldehyde show a similar trend. However, in the presence of elecron-donating groups, **TM-2** outperforms **TM-1** in biological assay, showing the overall molecular structure dictates its bioactivities. Generally, for **TM-2** originating from *para*-substituted aromatic aldehydes, the α-GI activities are stronger than those with a *meta*-substituent. The α-GI activities vary as follows: **2n**(4-OH) > **2l** (4-C_4H_9O) > **2m**(3, 4-OCH_2O). This is intimately associated with molecular electronegativity as an exposed –OH group serves to enhance the hydrogen bonding network and solubility. This in turn improves the probability of colliding with the target species, and ultimately leads to higher bioactivities, exactly as in the case of **2n**(>40%). Based on these results, we propose that the –OH substituent in the product improves water-solubility and results in stronger binding to α-glucosidase. The α-GI activities of certain products with electron-withdrawing groups (3-NO_2, 3-F, 4-Br) appear to be inversely proportional to the electron-withdrawing strength of the substituents.

2.2.2 *PPAR activation*

The target molecules were evaluated *in vitro* for PPAR agonist activity at a concentration level of 10 μg mL^{-1}, showing mainly moderate activities. However, **1b** (R^1 = 3,4-diCl) exhibits an outstanding PPAR relative activation activity of 73.66%, outperforming the rest of the group by a significant margin. Examination of the SAR for PPAR agonist activity demonstrates that **TM-1** with a *p*-aminobenzoic acid moiety is more active than its **TM-2** counterpart (with an ethyl *p*-aminobenzoate moiety). This is primarily a reflection of the SAR: the built-in molecular pattern requires the presence of an acidic terminal. This is again probably related to the disparity of their solubilities. Moreover, enhancement in solubility not only increases the availability of molecules in the solution phase, it also improves the binding strength to PPAR receptor. It also probably affects the kinetic rate of hydrolysis of carboxylic acid esters which, in turn, modulates the biological activity. As shown in Figure 4, the changes in PPAR of **1** and **2** follow an almost identical trend with the notable exceptions of **b**(R^1 = 3, 4-diCl), **g**(R^1 = H) and **n**(R^1 = 4-OH) where some divergence is observed. In addition, **j**(R^1 = 3-CH_3O) and **k**(R^1 = 4-CH_3O) exhibit divergence on a much smaller scale. As for chloro-substituted target molecules, the difference in activity for the dichloro-substituted compound (**1b**) is greater than that for monochloro-substituted compound, especially in the **TM-1** series.

In **TM-1**, the β-amino ketones with electron-donating alkoxy or hydroxy groups, including 3-CH_3O, 4-CH_3O, 4-C_4H_9O, 3,4-OCH_2O, 3-OH and 4-OH, show similar PPAR relative activation activities under the 40% mark, except for 4-OH whose PPAR relative activation activity is over 70%.

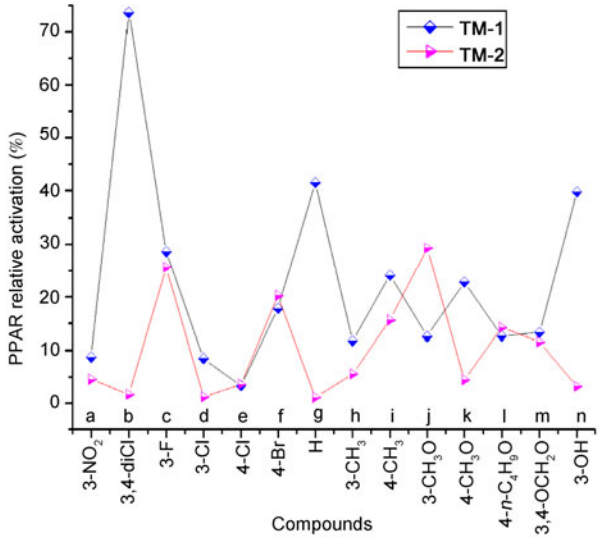


Figure 4 Comparison of PPAR relative activation of **1** and **2**.

This is likely attributed to its hydrophilic feature and more effective binding to its protein receptor. However, the product with a 3-OH substituent leads to a weaker PPAR than that with a 4-OH. We propose that a 3-OH substituent has a larger steric hindrance than a 4-OH substituent due to different localization and space volume. This is also the origin of the trend **1g**(H) > **1i**(4-CH_3) > **1h**(3-CH_3). As for chloro-substituted **TM-1**, the activity of the 3,4-diCl substituted compound is the strongest and the *ortho*-chlorosubstituted compound had better activity than the *meta*-chloro- or *para*-chloro-substituted compound. The root cause of this phenomenon remains unclear and is under continuing investigation. The trend in PPAR for **TM-2** is analogous to that for **TM-1** but with a somewhat smaller disparity.

It is surprising to note that both **TM-1** and **TM-2** exhibit greater PPAR relative activation at a 5.0 μg mL^{-1} concentration level than at a higher concentration of 10 μg mL^{-1} (Table 2 and Figure 5). **2i** and **1k** show the highest activities of 130.91% and 103.32%, respectively, indicating more potent performance than the deployed positive control of rosiglitazone (10 μg mL^{-1}, 100%). **1i**, **2f**, **1f** and **2j** also show remarkably high PPAR relative activation activities of 93.47%, 88.48%, 85.93% and 80.66%, respectively. The change in **2i** is most dramatic from a moderate 15.66% at 10 μg mL^{-1} to 130.91% at 5.0 μg mL^{-1}. The fact that higher concentrations of these compounds in Table 2 lead to reduced activation (Figure 5) is an important observation. Although its root cause remains unclear up to this point, further research is underway. We suppose that such disparities in PPAR may be related to the solubility, stability and conformation of the compounds, and may also reflect the activation effect at lower concentrations and the inhibition effect at higher concentrations.

TM-1 exhibits similar trend in the change of α-GI and PPAR relative activation (Figure 6(a)) with only one apparent departures (**1e**(4-Cl)), indicating that most of the **TM-1** series possess a dual-target property, while **TM-1e** is a single-target molecule. However, the correlation of the two bioactivities in the **TM-2** series is significantly weaker (Figure 6(b)), and indeed **TM-2b**, **TM-2j**, **TM-2k**, and **TM-2n** show completely the opposite trend. Such differences for **TM-2b** and **TM-2n** are particularly great, and characteristic of single-target binding.

It is interesting to observe, as shown in Figure 7, that the lipo-hydro distribution coefficients for **TM-1** and **TM-2** as a function of the individual substituent show a very similar trend, which once again conforms the critical role played by the substituent. For a given substituent, the magnitude of the

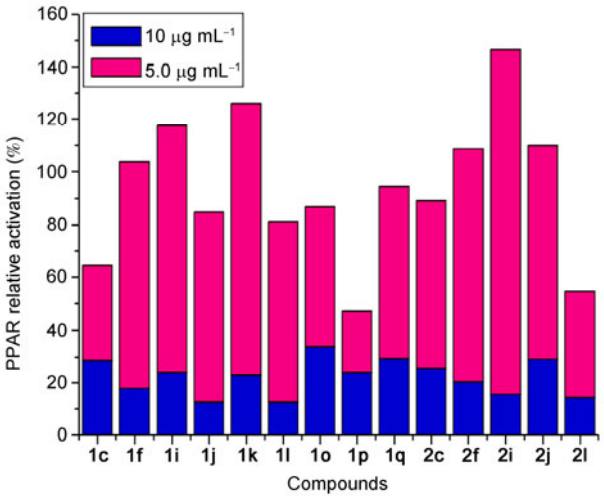


Figure 5 Comparison of PPAR relative activation at different concentrations for selected compounds.

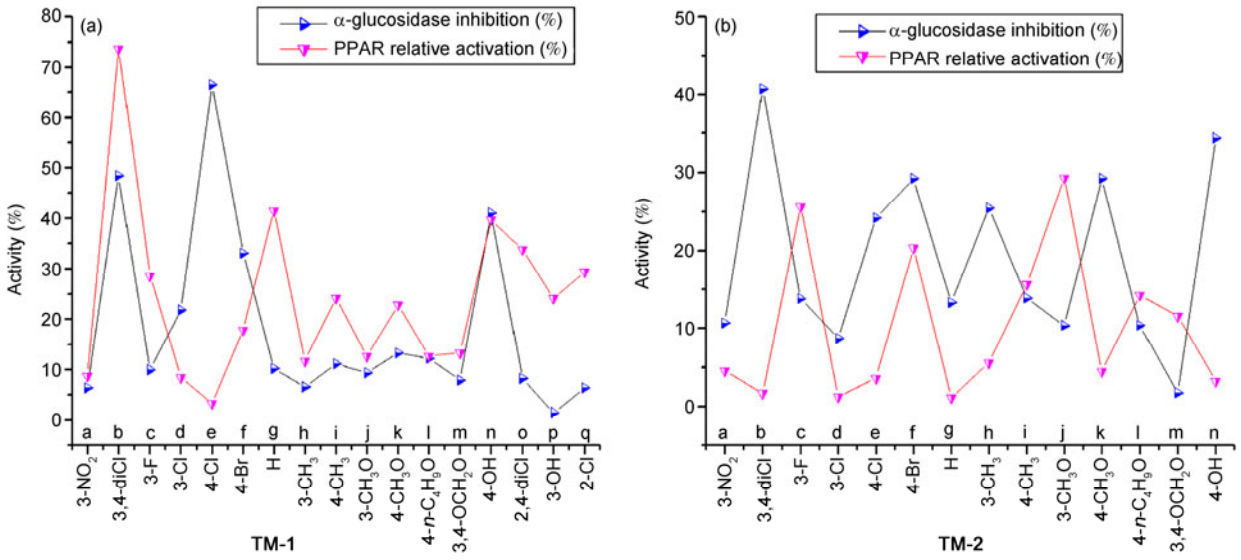


Figure 6 Comparison of α-GI and PPAR relative activation.

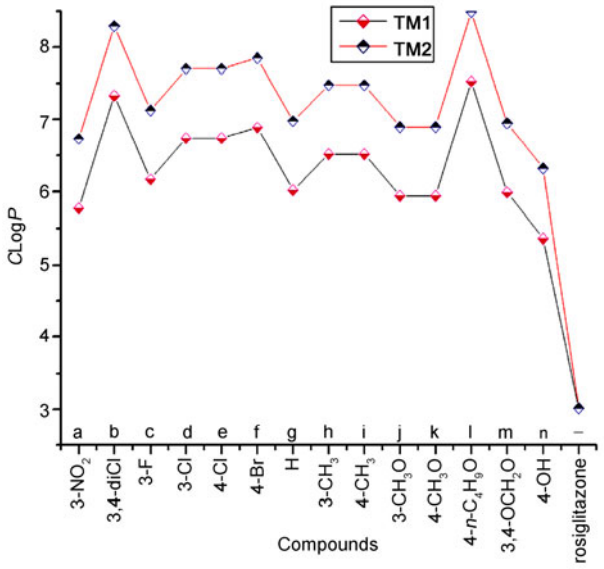


Figure 7 Relationship of *C*Log*P*-substituent for selected compounds.

lipo-hydro distribution coefficient for **TM-1** is smaller than that for **TM-2**. Furthermore, the coincidence of the trends of lipo-hydro distribution coefficient and biological activities for different substituents shows the close correlation between the two parameters. Therefore the impact of the lipo-hydro distribution coefficient should be taken into consideration in further optimization of the target molecules.

Examination of the 3-D structures (Figure 8) of a group of highly active species including **2i**, **1k**, **1j**, **2f**, **1f** and **2j** shows the absence of a U-shaped geometry, which has been suggested as an indispensible prerequisite for effective binding [30]. Instead, these potent molecules have a rectangular geometry, which is apparently consolidated by an R^1-bearing aromatic ring. Although the naphthalene ring and the other two benzene rings are non-coplanar, the structural module composed of naphthalene and 4-aminobenzoic acid (or ethyl 4-aminobenzoate) closely resembles rosiglitazone in the 3-D conformations. For those species with lower bioactivity such as **2b**, **2d** and **2g**, the naphthalene and ethyl 4-aminobenzoate rings are nearly coplanar, in contrast to the case for their high activity counterparts and Rosiglitazone. It has been reported [30] that the target molecules are bound in the cavity of a PPAR receptor in a U-shaped conformation. However, the high profile drug rosiglitazone does not conform to this general trend since it does not show any U-shaped conformation at low internal energy levels. It is hence plausible that fairly flexible molecules including rosiglitazone are able to accommodate the target molecules in their rectangular conformation before entering the receptor cavity for binding. Those molecules with sufficient flexibility show higher bioactivity. It transpires that, in addition to its own structural features, the target molecule should possess a high level of temporary spatial compatibility with the receptor at the very moment of binding [30, 47]. The target molecules **2i**, **1k**, **1j**, **2f**, **1f** and **2j** probably owe their high levels of bioactivity to such temporary structural compatibility with the receptor. It can be observed from Figure 9 that the PPAR activation and other parameters exhibit an irregular trend. Hence, taking into consideration the multiple factors governing the bioactivities—such as electronic density distribution, hydrogen bonding network, space volume and molecular flexibility—the trend in bioactivity as shown by the large group of high performance target molecules presented in this article clearly merits further investigation.

3 Experimental

3.1 General methods

Melting points were determined using an Electrothermal X-6 apparatus. ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were recorded on a Bruker AV 300 spectrometer using tetramethylsilane (TMS) as an internal

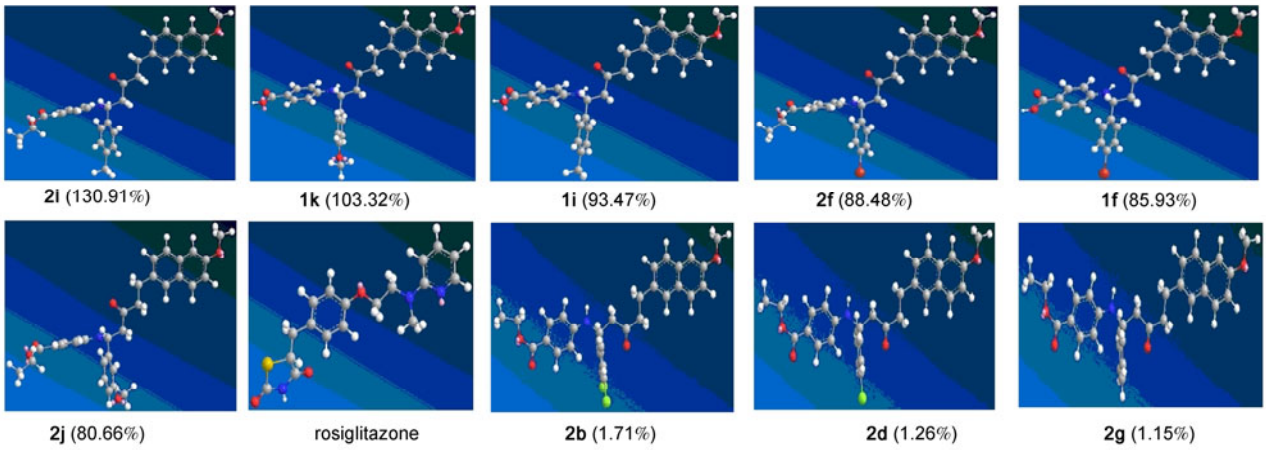


Figure 8 Three-dimensional structures of selected target molecules (PPAR).

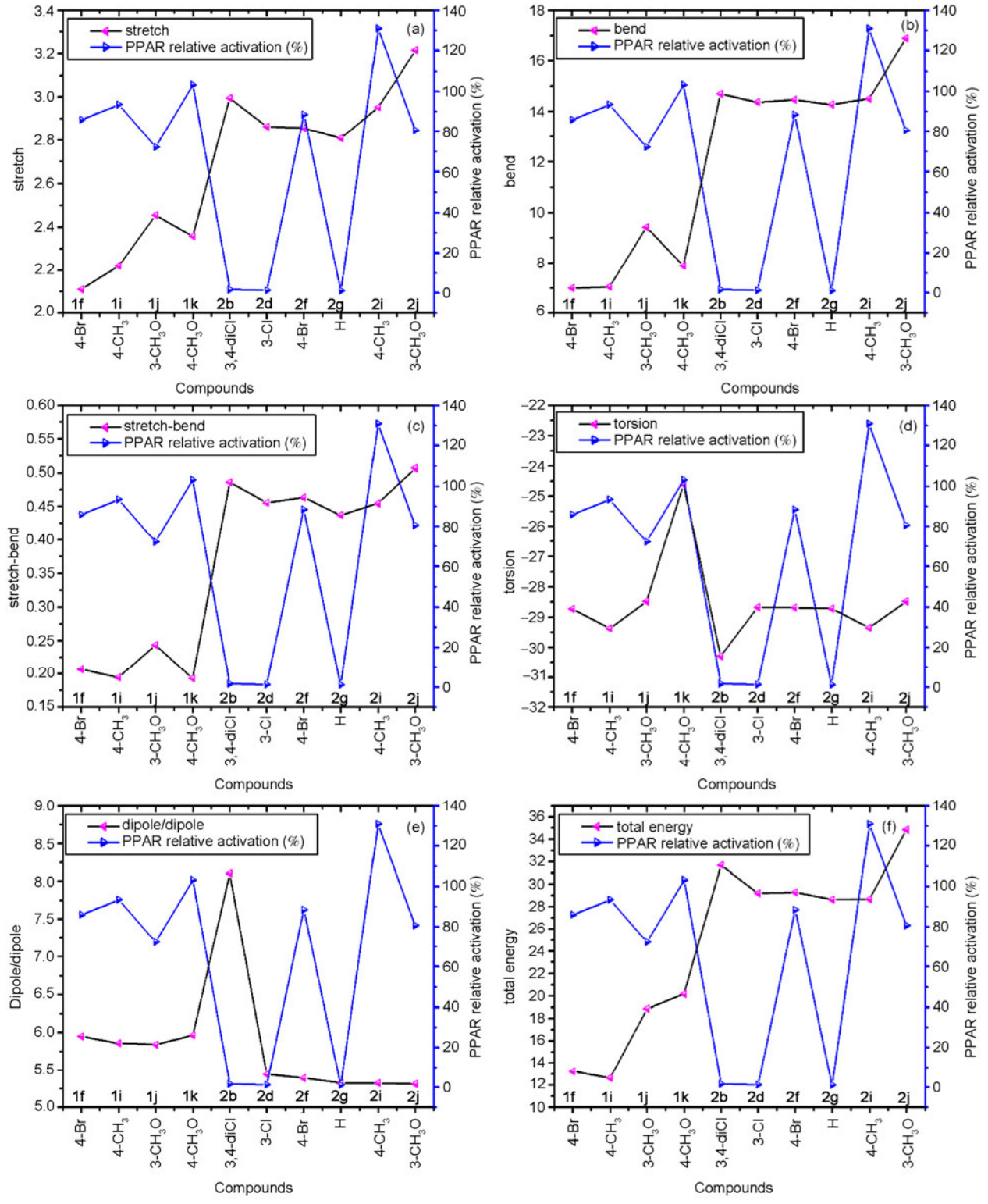


Figure 9 Diagrams of a set of additional characteristics for selected compounds and the corresponding PPAR. (a) PPAR-Stretch diagram; (b) PPAR-bend diagram; (c) PPAR-Stretch/bend diagram; (d) PPAR-Torsion diagram; (e) PPAR-Dipole/dipole diagram; (f) PPAR-Total energy diagram.

standard. The chemical shifts are reported in parts per million (ppm), the coupling constants (*J*) are expressed in hertz (Hz) and signals are described as singlet (s), doublet (d), triplet (t), quartet (q), as well as multiplet (m). Electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1946B instrument. High resolution mass spectra (HR

MS) analyses are conducted on a Daltonics Data Analysis 3.2 (Bruker) using the ESI technique. The progress of reactions and the purity of the compounds were monitored by thin layer chromatography (TLC) using solvent systems of different polarities. All chemical reagents and solvents were commercially available, and were used without further purification.

3.1.1 General procedure for the synthesis of β-amino ketones

To an alcoholic solution of aromatic aldehyde (2 mmol, 3–5 mL) in a round-bottom flask was added arylamine (2 mmol). The solution was stirred continuously at ambient temperature. When a great quantity of precipitate appeared, nabumetone (2 mmol) in chloroform (1–3 mL) and concentrated hydrochloric acid (0.1–0.2 mL) were added sequentially. The mixture was stirred vigorously at controlled temperature. The progress of the reaction was monitored by TLC. On completion of the reaction, the mixture was frozen in a refrigerator. A few hours later, the solid was subsequently collected by filtration. The filter cake was washed with water (2 × 3 mL) and 95% ethanol (2 × 3 mL), and then recrystallized from 95% ethanol. The yields vary from 30–97%. Melting points were determined, and the structures were confirmed by ^{1}H NMR, ^{13}C NMR, ESIMS and HRMS techniques.

3.1.2 Characterization of new target compounds

4-(1-(3-Fluorophenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxo pentylamino)benzoic acid (**1c**). Yield 75.6%, Mp 188–189 °C. ^{1}H NMR(DMSO-d_6, 300 MHz): δ 2.58–3.05 (6H, m), 3.90 (3H, s), 4.89–4.93 (1H, m), 5.87 (1H, s), 6.48 (2H, d, J = 8.6 Hz), 6.90 (1H, d, J = 6.9 Hz), 7.03–7.11 (3H, m), 7.20–7.25 (2H, m), 7.47 (1H, s), 7.52 (1H, s), 7.60–7.63 (2H, m), 7.73 (2H, d, J = 8.6 Hz), 11.98 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 206.4, 167.4, 156.7, 151.3, 139.9, 136.0, 132.8, 131.8, 131.0(2C), 129.5, 128.9, 129.0, 128.6, 127.9, 127.6, 127.5, 126.7, 125.9, 118.5, 117.9, 111.6(2C), 105.6, 55.2, 49.1, 47.9, 43.5, 28.8; ESI-MS (*m/z*): 472 $(M+1)^+$; HRMS: calcd for $C_{29}H_{26}FNNaO_4$ $[M+Na]^+$ 494.1738; found, 494.1728.

4-(1-(4-Bromophenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoic acid (**1f**). Yield 75.1%, Mp 205–207 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.81 (1H, dd, J_1 = 4.6, J_2 = 16.5 Hz), 2.85-2.90 (4H, m), 3.03 (1H, dd, J_1 = 8.8, J_2 = 16.2 Hz), 3.85 (3H, s), 4.95 (1H, dd, J_1 = 7.7, J_2 = 12.4 Hz), 6.54 (2H, d, J = 8.5 Hz), 6.97 (1H, d, J = 8.6 Hz), 7.11 (1H, d, J = 8.9 Hz), 7.25–7.30 (2H, m), 7.34 (2H, d, J = 8.3 Hz), 7.47 (2H, d, J = 8.3 Hz), 7.55 (1H, s), 7.60 (2H, d, J = 8.4 Hz), 7.67 (1H, d, J = 6.8 Hz), 7.70 (1H, d, J = 6.3 Hz), 12.04 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 206.9, 167.4, 156.8, 151.3, 142.7, 136.2, 132.8, 131.3, 131.0(2C), 129.6, 128.8(2C), 128.6 (2C), 127.7, 126.7, 125.9, 120.0, 118.5, 117.7, 111.8(2C), 105.7, 55.1, 51.7, 49.9, 43.5, 28.8; ESI-MS (*m/z*): 523 $(M+1)^+$; HRMS: calcd for $C_{29}H_{26}BrNNaO_4$, $[M+Na]^+$ 554.0937; found, 554.0915.

4-(5-(6-Methoxynaphthalen-2-yl)-3-oxo-1-*p*-tolylpentyla mino)benzoic acid (**1i**). Yield 80.3 %, Mp 190–192 ℃. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.23 (3H, s), 2.78 (1H, dd, J_1 = 4.6, J_2 = 6.5 Hz), 2.80-2.86 (4H, m), 3.00 (1H, dd, J_1 = 8.5, J_2 = 16.3 Hz), 3.85 (3H, s), 4.90 (1H, dd, J_1 = 7.8, J_2 = 12.5Hz), 6.53 (2H, d, J = 8.5 Hz), 6.92 (1H, d, J = 7.7 Hz), 7.08 (2H, d, J = 7.6 Hz), 7.12 (1H, d, J = 2.2 Hz), 7.25 (2H, d, J = 7.6 Hz), 7.25–7.29 (2H, m), 7.55 (1H, s), 7.58 (2H, d, J = 8.5 Hz), 7.67 (1H, d, J = 7.6 Hz), 7.69 (1H, d, J = 7.7 Hz), 11.98 (1H, s); ^{13}C NMR (DMSO-d_6, 75MHz): δ 207.1, 167.4, 156.8, 151.6, 140.1, 136.2, 136.0, 132.7, 130.9(2C), 129.0, 128.8, 128.5, 127.7(2C), 126.7, 126.3, 125.9(2C), 118.5, 117.4, 111.8(2C), 105.7, 55.1, 52.1, 50.2, 43.6, 28.8, 20.6; ESI-MS (*m/z*) 468 $(M+1)^+$; HRMS: calcd for $C_{30}H_{29}NNaO_4$, $[M+Na]^+$ 490.1989; found, 490.1969.

4-(5-(6-Methoxynaphthalen-2-yl)-1-(3-methoxyphenyl)-3-oxopentylamino)benzoic acid (**1j**). Yield 75.3%, Mp 145–147 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.79 (1H, dd, J_1 = 4.4, J_2 = 16.2 Hz), 2.84–2.91 (4H, m), 3.01 (1H, dd, J_1 = 9.2, J_2 = 16.3 Hz), 3.70 (3H, s), 3.85 (3H, s), 4.93 (1H, dd, J_1 = 7.6, J_2 = 12.3 Hz), 6.55 (2H, d, J = 8.6 Hz), 6.76 (1H, d, J = 7.8 Hz), 6.93 (1H, d, J = 6.1 Hz), 6.91–6.98 (2H, m), 7.10 (1H, dd, J_1 = 2.3, J_2 = 8.9 Hz), 7.19–7.23 (1H, m), 7.25 (1H, d, J = 2.3 Hz), 7.29 (1H, d, J = 8.4 Hz), 7.56 (1H, s), 7.59 (2H, d, J = 8.5 Hz), 7.67 (1H, d, J = 7.0 Hz), 7.70 (1H, d, J = 7.1 Hz), 12.01 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz,): δ 207.0, 167.4, 159.4, 156.8, 151.4, 145.0, 136.2, 132.7, 131.0(2C), 129.5, 128.8, 128.5, 127.7, 126.7, 125.8, 118.6, 118.5, 117.5, 112.3, 112.0(2C), 111.8, 105.7, 55.1, 54.9, 52.3, 50.2, 43.6, 28.8. HRMS: calcd for $C_{30}H_{29}NNaO_5$, $[M+Na]^+$ 506.1938; found, 506.1932.

4-(5-(6-Methoxynaphthalen-2-yl)-1-(4-methoxyphenyl)-3-oxopentylamino)benzoic acid (**1k**). Yield 81.5%, Mp 181–183 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.77 (1H, dd, J_1 = 3.8, J_2 = 16.2 Hz), 2.82–2.89 (4H, m), 3.01 (1H, dd, J_1 = 9.1, J_2 = 16.1 Hz), 3.69 (3H, s), 3.85 (3H, s), 4.90 (1H, dd, J_1 = 7.7, J_2 = 12.6 Hz), 6.54 (2H, d, J = 8.0 Hz), 6.84 (2H, d, J = 7.7 Hz), 6.92 (1H, d, J = 6.9 Hz), 7.11 (1H, d, J = 8.6 Hz), 7.29 (4H, m), 7.54 (1H, s), 7.59 (2H, d, J = 7.9 Hz), 7.67 (1H, d, J = 7.9 Hz), 7.69 (1H, d, J = 6.9 Hz), 12.02 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 207.3, 167.5, 158.2, 156.8, 151.6, 136.2, 135.0, 132.8, 131.0(2C), 128.8(2C), 128.6, 127.7, 127.6, 126.7(2C), 125.9, 118.5, 117.3, 113.8, 111.8(2C), 105.7, 55.1, 55.0, 51.7, 50.3, 43.6, 28.8; HRMS: calcd for $C_{30}H_{29}NNaO_5$, $[M+Na]^+$ 506.1938; found, 506.1933.

4-(1-(4-Butoxyphenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoic acid (**1l**). Yield 82.5%, Mp 196–198 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.91 (3H, t, J = 7.3 Hz), 1.39–1.44 (2H, m), 1.63–1.67 (2H, m), 2.77 (1H, dd, J_1 = 3.8, J_2 = 16.2 Hz), 2.82–2.89 (4H, m), 3.01 (1H, dd, J_1 = 8.4, J_2 = 16.3 Hz), 3.85 (3H, s), 3.89 (2H, t, J

= 6.3 Hz), 4.91 (1H, dd, J_1 = 7.8, J_2 = 12.5 Hz), 6.54 (2H, d, J = 8.5 Hz), 6.82 (2H, d, J = 8.3 Hz), 6.90 (1H, d, J = 7.7 Hz), 7.10 (1H, d, J = 6.9 Hz), 7.26 (2H, d, J = 8.2 Hz), 7.28 (2H, d, J = 6.5 Hz), 7.54 (1H, s), 7.59 (2H, d, J = 8.5 Hz), 7.67 (1H, d, J = 8.2 Hz), 7.69 (1H, d, J = 6.9 Hz), 12.00 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 207.2, 167.4, 157.6, 156.8, 151.6, 136.2, 134.8, 132.7, 130.9(2C), 128.8(2C), 128.5, 127.7, 127.5, 126.7, 125.8(2C), 118.5, 117.3, 114.3, 111.8(2C), 105.7, 67.0, 55.1, 51.8, 50.3, 43.6, 30.8, 28.8, 18.8, 13.7; ESI-MS (*m/z*): 564 $(M+K)^+$; HRMS: calcd for $C_{33}H_{35}NNaO_5$, $[M+Na]^+$ 548.2407; found, 548.2413.

4-(1-(2,4-Dichlorophenyl)-5-(6-methoxynaphthalen-2-yl) -3-oxopentylamino)benzoic acid (**1o**). Yield 51.2%, Mp 159–162 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.82 (1H, dd, J_1 = 4.4, J_2 = 16.2 Hz), 2.89–2.94 (4H, m), 3.02 (1H, dd, J_1 = 10.0, J_2 = 17.0 Hz), 3.85 (3H, s), 5.22 (1H, dd, J_1 = 7.6, J_2 = 12.3 Hz), 6.44 (2H, d, J = 8.4 Hz), 7.11 (2H, d, J = 8.6 Hz), 7.26 (1H, s), 7.32 (1H, d, J = 7.9 Hz), 7.40 (1H, s), 7.43 (1H, s), 7.59 (1H, s), 7.63 (2H, d, J = 8.6 Hz), 7.69 (1H, d, J = 6.4 Hz), 7.71 (1H, d, J = 5.8 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 206.3, 167.3, 156.8, 150.9, 139.2, 136.1, 132.8, 132.7, 132.4, 131.1(2C), 129.0, 128.9, 128.8, 128.6, 127.9, 127.5, 126.7, 125.9, 118.5, 118.1, 111.6(2C), 105.8, 55.1, 49.1, 47.7, 43.7, 28.8; ESI-MS (*m/z*): 522 $(M+1)^+$; HRMS: calcd for $C_{29}H_{25}Cl_2NNaO_4$, $[M+Na]^+$ 544.1053; found, 546.1043.

4-(1-(3-Hydroxyphenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoic acid (**1p**). Yield 78.0%, Mp 187–189 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.75–2.80 (1H, dd, J_1 = 4.4, J_2 = 16.4 Hz), 2.83–2.94 (4H, m), 2.96–3.04 (1H, dd, J_1 = 9.1, J_2 = 16.3 Hz), 3.85 (3H, s), 4.84–4.88 (1H, dd, J_1 = 8.0, J_2 = 11.6 Hz), 6.53 (2H, d, J = 8.6 Hz), 6.60 (1H, d, J = 8.1 Hz), 6.79 (2H, d, J = 8.4 Hz), 6.91 (1H, d, J = 7.4 Hz), 7.05–7.13 (2H, m), 7.25 (1H, s), 7.29 (1H, d, J = 8.4 Hz), 7.56 (1H, s), 7.60 (2H, d, J = 8.6 Hz), 7.67 (1H, d, J = 6.0 Hz), 7.70 (1H, d, J = 5.4 Hz), 9.35 (1H, s), 12.00 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 207.1, 167.5, 157.5, 156.8, 151.6, 144.8, 136.2, 132.8, 131.0(2C), 129.5, 128.8, 128.6, 127.7, 126.7, 125.9, 118.5, 117.4, 117.1, 113.9, 113.1, 111.7(2C), 105.7, 55.1, 52.3, 50.2, 43.6, 28.8; ESI-MS (*m/z*): 470 $(M+1)^+$; HRMS: calcd for $C_{29}H_{27}NNaO_5$, $[M+Na]^+$ 492.1781; found, 492.1767.

4-(1-(2-Chlorophenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoic acid (**1q**). Yield 50.2%, Mp 162–164 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.74-3.06 (6H, m), 3.85 (3H, s), 5.22–5.28 (1H, m), 6.44 (2H, d, J = 8.5 Hz), 7.05 (1H, d, J = 7.3 Hz), 7.11 (1H, d, J = 8.9 Hz), 7.26-7.33 (4H, m), 7.44 (2H, d, J = 6.7 Hz), 7.59 (1H, s), 7.62 (2H, d, J = 8.6 Hz), 7.68 (1H, d, J = 6.7 Hz), 7.71 (1H, d, J = 6.4 Hz), 12.06 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 206.5, 167.4, 156.8, 151.1, 139.9, 136.2, 132.8, 131.8, 131.1(2C), 129.5, 128.9, 128.8, 128.6, 127.8, 127.7, 127.5, 126.7, 125.9, 118.5, 117.9, 111.5(2C), 105.7, 55.1, 49.3, 48.0, 43.7, 28.8; ESI-MS (*m/z*): 488 $(M+1)^+$; HRMS: calcd for $C_{29}H_{26}ClNNaO_4$, $[M+Na]^+$ 510.1443; found, 510.1425.

Ethyl 4-(5-(6-methoxynaphthalen-2-yl)-1-(3-nitrophenyl)-3-oxopentylamino)benzoate (**2a**). Yield 30.7%, Mp 106–108 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.35 (3H, t, J = 7.1 Hz), 2.77–3.00 (6H, m), 3.91 (3H, s), 4.28 (2H, q, J = 7.1 Hz), 4.93 (1H, t, J = 5.8 Hz), 4.95 (1H, s), 6.40 (2H, d, J = 8.5 Hz), 7.08–7.14 (2H, m), 7.18 (1H, d, J = 8.2 Hz), 7.37–7.44 (2H, m), 7.58–7.61 (3H, m), 7.77 (2H, d, J = 8.5 Hz), 8.02 (1H, d, J = 8.5 Hz), 8.14 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 207.5, 166.7, 157.5, 150.0, 148.7, 144.3, 135.4, 133.2, 132.7, 131.8, 129.8(2C), 129.0, 128.1, 127.4, 127.2, 126.4, 122.6, 121.2, 120.0, 119.0, 112.6(2C), 105.7, 60.4, 55.4, 53.0, 49.8, 45.0, 29.5, 14.5; ESI-MS (*m/z*): 549 $(M+Na)^+$; HRMS: calcd for $C_{31}H_{30}N_2NaO_6$, $[M+Na]^+$ 549.1996; found 549.2003; calcd for $C_{31}H_{30}N_2KO_6$, $[M+K]^+$ 565.1741; found, 565.1748.

Ethyl 4-(1-(3,4-dichlorophenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoate (**2b**). Yield 76.8%, Mp 142–145 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.30 (3H, t, J = 7.1 Hz), 2.73 (2H, t, J = 7.2 Hz), 2.83 (2H, d, J = 5.9 Hz), 2.94 (2H, t, J = 7.2 Hz), 3.91 (3H, s), 4.25 (2H, q, J = 7.1 Hz), 4.75 (1H, t, J = 5.9 Hz), 4.91 (1H, s), 6.36 (2H, d, J = 8.5 Hz), 7.05–7.16 (4H, m), 7.24 (1H, d, J = 8.4 Hz), 7.33 (1H, s), 7.42 (1H, s), 7.58 (2H, d, J = 8.4 Hz), 7.74 (2H, d, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 207.8, 166.7, 157.5, 150.0, 142.3, 135.5, 133.3, 133.1, 131.7, 131.6, 131.5, 130.9(2C), 129.0, 128.3, 127.4, 127.2, 126.4, 125.7, 119.9, 119.1, 112.6(2C), 105.8, 60.4, 55.4, 52.9, 50.0, 45.2, 29.6, 14.5; ESI-MS (*m/z*): 572 $(M+Na)^+$; HRMS: calcd for $C_{31}H_{29}Cl_2NNaO_4$, $[M+Na]^+$ 572.1366; found, 572.1313.

Ethyl 4-(1-(3-fluorophenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoate (**2c**). Yield 73.7%, Mp 129–132 ℃. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.24 (3H, t, J = 6.9 Hz), 2.73–3.09 (6H, m), 3.85 (3H, s), 4.17 (2H, q, J = 6.9 Hz), 5.0 (1H, s), 6.57 (2H, d, J = 7.9 Hz), 6.98–7.02 (2H, m), 7.10 (1H, d, J = 7.6 Hz), 7.24–7.34 (5H, m), 7.56 (1H, s), 7.61–7.71 (4H, m); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 206.8, 165.8, 163.9, 160.7, 156.8, 151.6, 146.4, 136.2, 132.7, 130.8, 130.4, 130.3(2C), 128.8, 128.5, 127.6, 126.7, 125.8, 122.6, 118.5, 116.9, 113.0(2C), 105.7, 59.6, 55.1, 51.8, 49.8, 43.5, 28.8, 14.3; ESI-MS (*m/z*): 522 $(M+Na)^+$; HRMS: calcd for $C_{31}H_{30}FNNaO_4$, $[M+Na]^+$ 522.2138; found 522.2049, calcd for $C_{31}H_{30}FNKO_4$ $[M+K]^+$ 538.1883; found, 538.1796.

Ethyl 4-(1-(3-chlorophenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoate (**2d**). Yield 64.2%, Mp 132–134 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.33 (3H, t, J = 7.1 Hz), 2.69–2.83 (2H, m), 2.88 (2H, t, J = 5.9 Hz), 2.97 (2H, t, J = 7.2 Hz), 3.91 (3H, s), 4.28 (2H, q, J = 7.1 Hz), 4.81 (1H, t, J = 5.9 Hz), 4.93 (1H, s), 6.41 (2H, d, J = 8.6 Hz), 7.09–7.20 (7H, m), 7.45 (1H, s), 7.62 (2H, d, J = 8.6 Hz), 7.77 (2H, d, J = 8.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 207.9, 166.7, 157.5, 150.2, 144.0, 135.6, 134.9, 133.2,

131.4, 130.3(2C), 129.0, 128.6, 127.9, 127.7, 127.2, 126.4, 125.9, 124.4, 119.7, 119.0, 112.6(2C), 105.7, 60.3, 55.4, 53.5, 50.0, 45.3, 29.5, 14.5; ESI-MS (*m/z*): 538 $(M+Na)^+$; HRMS: calcd for $C_{31}H_{30}ClNNaO_4$, $[M+Na]^+$ 538.1028; found 538.1021, calcd for $C_{31}H_{30}ClNKO_4$, $[M+K]^+$ 554.0773; found, 554.0732.

Ethyl 4-(1-(4-chlorophenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoate (**2e**). Yield 71.1%, Mp 146–147 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.33 (3H, t, J = 7.1 Hz), 2.72 (2H, t, J = 7.2 Hz), 2.89 (2H, t, J = 5.3 Hz), 2.96 (2H, t, J = 7.2 Hz), 3.91 (3H, s), 4.28 (2H, q, J = 7.1 Hz), 4.82 (1H, t, J = 5.3 Hz), 6.42 (2H, d, J = 8.5 Hz), 7.04–7.09 (3H, m), 7.16–7.23 (4H, m), 7.45 (1H, s), 7.62 (2H, d, J = 8.5 Hz), 7.77 (2H, d, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.5, 166.1, 156.2, 151.7, 139.8, 133.6, 132.9, 132.3, 131.0, 130.1(2C), 129.4, 128.8(2C), 128.4(2C), 128.0, 127.2, 126.4, 119.2, 118.8, 114.0(2C), 105.9, 59.9, 53.3, 51.8, 50.6, 46.0, 29.1, 14.6; ESI-MS (*m/z*): 538 $(M+Na)^+$; HRMS: calcd for $C_{31}H_{30}ClNNaO_4$, $[M+Na]^+$ 538.1028; found 538.1017, calcd for $C_{31}H_{30}ClNKO_4$, $[M+K]^+$ 554.0773; found, 554.0737.

Ethyl 4-(1-(4-bromophenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoate (**2f**). Yield 83.2%, Mp 137–140 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.24 (3H, t, J = 6.9 Hz), 2.78–3.05 (6H, m), 3.85 (3H, s), 4.18 (2H, q, J = 6.9 Hz), 4.88–4.95 (1H, m), 6.56 (2H, d, J = 8.6 Hz), 7.04 (1H, d, J = 7.6 Hz), 7.04 (1H, d, J = 8.3 Hz), 7.25–7.35 (3H, m), 7.47 (2H, d, J = 7.5 Hz), 7.55 (1H, s), 7.62 (2H, d, J = 8.4 Hz), 7.66–7.71 (2H, m); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 206.8, 165.8, 156.8, 151.6, 142.6, 136.1, 132.7, 131.3, 130.8(2C), 128.9, 128.8, 128.5, 127.6, 126.7, 125.9, 120.0, 118.5, 116.8, 111.9(2C), 105.7, 59.6, 55.1, 51.7, 49.8, 43.5, 28.8, 14.3; ESI-MS (*m/z*): 582 $(M+Na)^+$, 560 $(M+1)^+$; HRMS: calcd for $C_{31}H_{30}BrNNaO_4$, $[M+Na]^+$ 582.1250; found 584.1259, calcd for $C_{31}H_{30}BrNKO_4$, $[M+K]^+$ 598.0995; found, 600.0987.

Ethyl 4-(5-(6-methoxynaphthalen-2-yl)-3-oxo-1-phenylpentylamino)benzoate (**2g**). Yield 82.5%, Mp 150–152 ℃. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.32 (3H, t, J = 7.1 Hz), 2.62-2.96 (6H, m), 3.90 (3H, s), 4.27 (2H, q, J = 7.1 Hz), 4.86 (1H, t, J = 6.1 Hz), 5.05 (1H, s), 6.44 (2H, d, J = 8.6 Hz), 7.04–7.23 (7H, m), 7.44 (1H, s), 7.61 (2H, d, J = 8.6 Hz), 7.76 (2H, d, J = 8.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.5, 166.8, 157.4, 150.5, 141.6, 135.8, 133.2, 131.5, 131.4(2C), 129.0(2C), 127.8, 127.7, 127.5, 127.1, 126.4, 126.2(2C), 119.3, 119.0, 112.5(2C), 105.7, 60.3, 55.3, 53.9, 50.1, 45.4, 29.4, 14.5; ESI-MS (*m/z*): 504 $(M+Na)^+$; HRMS: calcd for $C_{31}H_{31}NNaO_4$, $[M+Na]^+$ 504.2145; found 504.2157; calcd for $C_{31}H_{31}NKO_4$, $[M+K]^+$ 520.1890; found, 520.1894.

Ethyl 4-(5-(6-methoxynaphthalen-2-yl)-3-oxo-1-*m*-tolylpentylamino)benzoate (**2h**). Yield 76.4 %, Mp 136–138 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.33 (3H, t, J = 7.1 Hz), 2.30 (3H, s), 2.73 (2H, t, J = 7.3 Hz), 2.89 (2H, d, J = 6.2 Hz), 2.95 (2H, t, J = 7.3 Hz), 3.91 (3H, s), 4.29 (2H, q, J = 7.1 Hz), 4.83 (1H, t, J = 6.2 Hz), 4.95 (1H, s), 6.45 (2H, d, J = 8.7 Hz), 7.03–7.13 (4H, m), 7.14-7.19 (3H, m), 7.45 (1H, s), 7.62 (2H, d, J = 8.6 Hz), 7.77 (2H, d, J = 8.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.5, 166.9, 157.4, 150.6, 141.6, 138.6, 135.8, 133.2, 131.4(2C), 129.0, 128.9, 128.7, 128.5, 127.4, 127.1, 126.9, 126.3, 123.2, 119.2, 119.0, 112.5(2C), 105.7, 60.2, 55.4, 53.9, 50.2, 45.3, 29.4, 21.6, 14.5; ESI-MS (*m/z*): 518 $(M+Na)^+$; HRMS: calcd for $C_{32}H_{33}NNaO_4$, $[M+Na]^+$ 518.2302; found 518.2292, calcd for $C_{32}H_{33}NKO_4$, $[M+K]^+$ 534.2047; found, 534.2033.

Ethyl 4-(5-(6-methoxynaphthalen-2-yl)-3-oxo-1-*p*-tolylpentylamino)benzoate (**2i**). Yield 82.9%, Mp 135– 137 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.23 (3H, t, J = 6.9 Hz), 2.23 (3H, s), 2.75–3.06 (6H, m), 3.85 (3H, s), 4.18 (2H, q, J = 6.9 Hz), 4.89–4.94 (1H, m), 6.55 (2H, d, J = 8.4 Hz), 7.00 (1H, d, J = 7.6 Hz), 7.07–7.13 (3H, m), 7.24–7.30 (4H, m), 7.54 (1H, s), 7.60 (2H, d, J = 8.4 Hz), 7.66–7.71 (2H, m); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 207.1, 165.8, 156.8, 151.8, 140.0, 136.2, 136.0, 132.8, 130.7(2C), 129.0, 128.8, 128.6, 127.6(2C), 126.7, 126.3, 125.8(2C), 118.4, 116.6, 111.9(2C), 105.8, 59.5, 55.1, 52.1, 50.2, 43.6, 28.8, 20.6, 14.3; ESI-MS (*m/z*): 518 $(M+Na)^+$; HRMS: calcd for $C_{32}H_{33}NNaO_4$, $[M+Na]^+$ 518.2302; found 518.2302, calcd for $C_{32}H_{33}NKO_4$, $[M+K]^+$ 534.2047; found, 534.2051.

Ethyl 4-(5-(6-methoxynaphthalen-2-yl)-1-(3-methoxyphenyl)-3-oxopentylamino)benzoate (**2j**). Yield 86.5%, Mp 136–140 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.24 (3H, t, J = 7.0 Hz), 2.68–3.04 (6H, m), 3.70 (3H, s), 3.91 (3H, s), 4.18 (2H, q, J = 7.0 Hz), 4.87–4.94 (1H, m), 6.57 (2H, d, J = 8.5 Hz), 6.78 (1H, d, J = 7.6 Hz), 6.93–7.02 (3H, m), 7.13 (1H, m), 7.20–7.30 (3H, m), 7.56 (1H, s), 7.62 (2H, d, J = 8.5 Hz), 7.66–7.71 (2H, m); ^{13}C NMR (DMSO-d_6, 7 5 MHz): δ 207.0, 165.8, 159.4, 156.8, 151.8, 144.9, 136.2, 132.8, 130.8(2C), 129.5, 128.8, 128.6, 127.6, 126.7, 125.8, 118.6, 118.5, 116.6, 112.3, 112.1, 111.9(2C), 105.7, 59.6, 55.1, 54.9, 52.2, 50.1, 43.6, 28.8, 14.3; ESI-MS (*m/z*): 534 $(M+Na)^+$, 512 $(M+1)^+$; HRMS: calcd for $C_{32}H_{33}NNaO_5$, $[M+Na]^+$ 534.2251; found, 534.2249, calcd for $C_{32}H_{33}NKO_5$, $[M+K]^+$ 550.1996; found, 550.1995.

Ethyl 4-(5-(6-methoxynaphthalen-2-yl)-1-(4-methoxyphenyl)-3-oxopentylamino)benzoate (**2k**). Yield 84.8%, Mp 158–160 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.32 (3H, t, J = 7.1 Hz), 2.70 (2H, t, J = 7.3 Hz), 2.87 (2H, d, J = 6.0 Hz), 2.94 (2H, t, J = 7.3 Hz), 3.74 (3H, s), 3.90 (3H, s), 4.27 (2H, q, J = 7.1 Hz), 4.81 (1H, t, J = 6.0 Hz), 4.95 (1H, s), 6.44 (2H, d, J = 8.7 Hz), 6.79 (2H, d, J = 8.6 Hz), 7.11 (1H, dd, J_1 = 2.0, J_2 = 8.9 Hz), 7.16 (2H, d, J = 8.5 Hz), 7.16 (2H, d, J = 8.6 Hz), 7.43 (1H, s), 7.61 (2H, d, J = 8.4 Hz), 7.77 (2H, d, J = 8.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.6, 166.9, 159.0, 157.4, 150.6, 135.8, 133.5, 133.2, 131.4(2C), 129.0(2C), 127.6, 127.5, 127.3, 127.1, 126.4(2C), 119.2, 119.0, 114.4, 112.5(2C), 105.7, 60.3, 55.4, 55.3, 53.4, 50.1, 45.5, 29.4, 14.5; ESI- MS (*m/z*): 534 $(M+Na)^+$; HRMS:

calcd for $C_{32}H_{33}NNaO_5$, $[M+Na]^+$ 534.2251; found, 534.2249, calcd for $C_{32}H_{33}NKO_5$, $[M+K]^+$ 550.1996; found, 550.1995.

Ethyl 4-(1-(4-butoxyphenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoate (**2l**). Yield 93.6%, Mp 142−144 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.90 (3H, t, J = 7.3 Hz), 1.24 (3H, t, J = 7.0 Hz), 1.36−1.43 (2H, m), 1.62−1.67 (2H, m), 2.62−3.02 (6H, m), 3.85 (3H, s), 3.86−3.90 (2H, m), 4.18 (2H, q, J = 7.0 Hz), 4.85−4.91 (1H, m), 6.56 (2H, d, J = 8.5 Hz), 6.82 (2H, d, J = 8.3 Hz), 6.97 (1H, d, J = 7.7 Hz), 7.11 (1H, d), 7.25−7.29 (4H, m), 7.54 (1H, s), 7.61 (2H, d, J = 8.5 Hz), 7.66−7.71 (2H, m); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 207.2, 165.8, 157.7, 156.8, 151.8, 136.2, 134.7, 132.8, 130.7(2C), 128.8, 128.6(2C), 127.6, 127.5, 126.7, 125.8(2C), 118.5, 116.5, 114.3, 111.9(2C), 105.7, 67.0, 59.5, 55.1, 51.8, 50.2, 43.6, 30.8, 28.8, 18.8, 14.3, 13.7; ESI-MS (*m/z*): 576 $(M+Na)^+$, 554 $(M+1)^+$; HRMS: calcd for $C_{35}H_{39}NNaO_5$, $[M+Na]^+$ 576.2720; found, 576.2739, calcd for $C_{35}H_{39}NKO_5$, $[M+K]^+$ 592.2465; found, 592.2468.

Ethyl 4-(1-(benzo[d][1,3]dioxol-5-yl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoate (**2m**). Yield 73.0%, Mp 131−132 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.33 (3H, t, J = 7.1 Hz), 2.73−2.75 (2H, m), 2.93−2.98 (4H, m), 3.91 (3H, s), 4.28 (2H, q, J = 7.1 Hz), 4.75 (1H, t, J = 5.8 Hz), 5.91 (2H, s), 6.55 (2H, d, J = 8.4 Hz), 6.70 (2H, dd, J_1 = 7.9, J_2 = 16.9 Hz), 6.78 (1H, s), 7.08−7.20 (3H, m), 7.46 (1H, s), 7.62 (2H, d, J = 8.6 Hz), 7.78 (2H, d, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 208.4, 166.9, 155.7, 151.3, 148.3, 145.5, 137.7, 135.6, 133.2, 129.9(2C), 129.1, 127.5, 127.2, 126.4, 126.0, 125.4, 118.8, 117.4, 112.5(2C), 107.1, 107.0, 105.7, 60.3, 55.4, 55.1,53.2, 49.8, 45.3, 29.5, 14.6; ESI-MS (*m/z*): 548 $(M+Na)^+$, 526 $(M+1)^+$; HRMS: calcd for $C_{32}H_{31}NNaO_6$, $[M+Na]^+$ 548.2044; found, 548.2045, calcd for $C_{32}H_{31}NKO_6$, $[M+K]^+$ 564.1789; found, 564.1800.

Ethyl 4-(1-(4-hydroxyphenyl)-5-(6-methoxynaphthalen-2-yl)-3-oxopentylamino)benzoate (**2n**). Yield 56.4%, Mp 159−161 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24 (3H, t, J = 7.0 Hz), 2.72−3.04 (6H, m), 3.85 (3H, s), 4.18 (2H, q, J = 7.0 Hz), 4.84 (1H, dd, J_1 = 5.0, J_2 = 8.3 Hz), 6.55 (2H, d, J = 8.6 Hz), 6.68 (2H, d, J = 8.3 Hz), 7.10 (1H, dd, J = 2.0, 8.9 Hz), 7.17 (2H, d, J = 8.3 Hz), 7.28 (2H, d, J = 12.7 Hz), 7.54 (1H, s), 7.61 (2H, d, J = 8.6 Hz), 7.69 (2H, d, J = 8.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 207.6, 166.0, 156.9, 156.3, 151.9, 136.3, 133.3, 132.9, 130.9(2C), 129.0(2C), 128.7, 127.8, 127.7, 126.9, 126.0(2C), 118.6, 116.6, 115.2, 112.0(2C), 105.9, 59.8, 55.3, 51.9, 50.4, 43.8, 28.9, 14.5; ESI-MS (*m/z*): 520 $(M+Na)^+$, HRMS: calcd for $C_{31}H_{31}NNaO_5$, $[M+Na]^+$ 520.2094; found, 520.2070, calcd for $C_{31}H_{31}NKO_5$, $[M+K]^+$ 536.1839; found, 536.1815.

The characterization of other target compounds, including **1a**, **1b**, **1d**, **1e**, **1g**, **1h**, **1m**, **1n** has been reported by the authors' group previously [22].

3.2 Detection of α-glucosidase inhibition

α-Glucosidase inhibitory activity was determined in a 100 μL reaction mixer containing optimal rat-α-glucosidase (extracted from the rat small intestine of rat), 67 mmol L^{-1} pH 6.8 sodium phosphate buffer and different samples. Blank control (without enzyme and samples) or negative control (without sample) was set as above. After incubation at 37 °C for 10 min, 0.1 mol L^{-1} maltose was added and incubated for another 10 min at room temperature. The reaction was stopped with 200 μL of glucose and the optical density (OD) values at 490 nm recorded. The inhibition ratio was calculated according to the following equation: $I\% = [1-(OD_{Sample}-OD_{Blank}) / (OD_{Negative}-OD_{Blank})] \times 100\%$. Based on the inhibition value, IC_{50} was calculated using the 4 Parameter Logistic Model in Xlfit.

3.3 Determination of PPAR activation

HepG2 cells were cultured in low glucose Dulbecco's Modified Eagle's medium (DMEM) supplemented with 100 U mL^{-1} streptomycin and penicillin. One day prior to transfection, the cells were plated in 96-well plates with 1.5×10^4 cells per well. When the cells grew at a confluence of 70%, plasmid pPPRE-Luc with firefly luciferase reporter gene and the control plasmid phRL-TK with Renilla luciferase reporter gene were transfected into the cells. 24 hours after the transfection, the medium was replaced with fresh medium containing either different samples, rosiglitazone (positive control) or without sample (negative control). The cells without transfection were used as a blank control. After incubation for a further 24 h, the expression of luciferases was measured with a Dual-Luciferase Reporter Gene Assay Kit (Promega). The times the samples became activated (T%) were calculated as the following equation:

$$T = [(L_{1\ Sample} - L_{1\ Blank}) / (L_{1\ Negative} - L_{1\ Blank})] / [(L_{2\ Sample} - L_{2\ Blank}) / (L_{2\ Negative} - L_{2\ Blank})] \times 100\%$$

Here, L_1 represents the values for firefly luciferase and L_2 represents the values for Renilla luciferase.

4 Conclusion

A group of 31 β-amino ketones containing nabumetone, aromatic aldehyde and *p*-aminobenzoic acid moieties have been synthesized through direct Mannich reaction under optimized synthetic conditions. At a lower concentration level of 5.0 μg mL^{-1}, compounds **1k** and **2i** exert the highest PPAR relative activation activities 130.91% and 103.32%, respectively, exceeding the positive control value for rosiglitazone (10 μg mL^{-1}, 100%). Both are proven highly active target molecules with exceptional potential to become

commercially viable. They serve as concrete examples that β-amino ketones containing the 4-aminobenzoic acid unit represent a group of highly valuable lead molecules in the search for novel antidiabetic drugs.

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