European Journal of Medicinal Chemistry 83 (2014) 74–83

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

4,5-Di-substituted benzyl-imidazol-2-substituted amines as the structure template for the design and synthesis of reversal agents against P-gp-mediated multidrug resistance breast cancer cells

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ARTICLE INFO

Article history:
Received 29 January 2014
Received in revised form
17 May 2014
Accepted 9 June 2014
Available online 10 June 2014

Keywords:
Naamidine analogues
Multidrug resistance
Structure–activity relationship
P-glycoprotein
Modulator
Marine alkaloid

ABSTRACT

Over-expression of P-glycoprotein (P-gp), a primary multidrug transporter which is located in plasma membranes, plays a major role in the multidrug resistance (MDR) of cytotoxic chemotherapy. Naamidines are a class of marine imidazole alkaloids isolated from *Leucetta* and *Clathrina* sponges, possessing a Y-shaped scaffold. Based on the results previously obtained from the third-generation MDR modulator ONT-093 and other modulators developed in our group, we designed and synthesized a series of novel 4,5-di-substituted benzyl-1-methyl-1*H*-imidazol-2-substituted amines using the Naamidine scaffold as the structure template. Subsequently, their reversing activity for Taxol resistance has been evaluated in P-gp-mediated multidrug resistance breast cancer cell line MDA435/LCC6MDR. Compounds **12c** with a Y-shaped scaffold, and compound **17c** which is 'X-shaped' scaffold and possesses a 4-diethylamino group at aryl ring **B**, turned out to be the most potent P-gp modulators. It appears that compounds **12c** and **17c** at 1 μM concentration can sensitize LCC6MDR cells toward Taxol by 26.4 and 24.5 folds, with an EC_{50} 212.5 and 210.5 nM, respectively. These two compounds are about 5–6 folds more potent than verapamil (RF = 4.5). Moreover, compounds **12c** and **17c** did not exhibit obvious cytotoxicity in either cancer cell lines or normal mouse fibroblast cell lines. This study has demonstrated that the synthetic Naamidine analogues can be potentially employed as effective, safe modulators for the P-gp-mediated drug resistance cancer cells.

1. Introduction

One of the major obstacles for the treatment of cancers with chemotherapeutic agents is multidrug resistance (MDR) [1], which could be correlated with a number of mechanisms such as drug metabolism, p53 mutations, DNA repair capacity, hostile tumor microenvironment and the modified apoptotic pathways [2]. Primarily, the resistance to therapy is due to the over-expression of ATP-binding cassette (ABC) transporters, which often result in the increased efflux of chemotherapeutic drugs from cancer cells. Consequently, the intracellular concentration of drugs is lowered and their toxic effects towards malignant cells are ultimately alleviated [3]. Among the ABC transporters involved in MDR, the membrane permeability glycoprotein, termed P-glycoprotein (P-gp), plays the most critical role. Therefore, one would expect that the inhibition of P-gp over-expression could effectively reverse the multidrug resistance of tumor cells and restore their sensitivity to drugs, eventually improving the outcome of chemotherapy.

Over the past two decades, three generations of P-gp inhibitors have been developed, aiming to reverse the MDR in cancer cells. Particularly, the first and the second generation of inhibitors, such as Verapamil [4], Cyclosporine A [5], VX-710 [6] and PSC833 [7], mainly act by competitively binding to the P-gp substrate sites against the chemotherapy drugs, lacking sufficient affinity and specificity. In addition, most of these compounds are P450 enzyme inhibitors, which give rise to undesirable drug interactions; because they are essentially competing against combination therapy drugs, these compounds have exhibited inferior in vivo activity, high toxicity and unpredictable pharmacokinetic interactions [1,8].

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http://dx.doi.org/10.1016/j.ejmech.2014.06.016
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Notably, the third-generation of P-gp modulators such as Tariquidar (XR9576), Zosuquidar (LY335979), Elacridar (GF120918) and ONT-093 (OC144-093), not only exhibited little competitive effect against combination therapy drugs [9–12], but also showed less impact on their pharmacokinetic properties, indicating broader clinical applications. However, so far, none of the compounds reported has been approved by regulatory agencies, thus the discovery of novel MDR modulators with high activity and low toxicity still remains a huge challenge to pharmaceutical chemists.

Recently, our group has disclosed a series of effective MDR reversal agents, such as methylated Quercetin [13], permethyl Ningalin B analogues [14,15] and (*E*,*E*)-4,6-bis(styryl)-2-*O*-glucopyranosyl-pyrimidine [16], exhibiting remarkable modulating activity to ABC drug efflux pumps with low toxicity (Fig. 1). Specifically, it was found that Ningalin B derivatives **1a**, **1b**, **1c** and **1d** have displayed the best P-gp modulating activity, and they can sensitize LCC6MDR cells toward Taxol at 1 μM concentration by 18.2, 9.9, 42.7 and 42.7 folds, respectively. Even though a common "pharmacophore" has not been identified in the inhibitors or substrates of P-gp [6], for instance, the well-known third-generation MDR modulator ONT-093 (OC144-093) [12], it appears that these compounds do possess a Y-shaped scaffold composed of aromatic rings A, B and C (Fig. 1). Therefore, we envisioned that this common Y-shaped structure would be an important "pharmacophore" scaffold for P-gp inhibiting activity. On the other hand, it was found that the methoxy-substituted analogues, instead of the hydroxy-substituted ones, could increase the MDR reversal activity and reduce cytotoxicity [13,14,16]. Based on the results obtained from structure–activity relationship studies, we expect that another class of Y-shaped marine natural alkaloids, Naamidines, could be modified and developed into new P-gp modulators.

Naamidines (Fig. 1) are a class of marine imidazole alkaloids, which have been successively isolated from *Leucetta* and *Clathrina* sponges in the past thirty years [17,18]. It was found that these compounds exhibited interesting biological activities, such as moderate antitumor and antibacterial activities [17,19–24]. Comparing with the Y-shaped structure of ONT-093 (OC144-093), the linkers between the benzene and imidazole rings in the Naamidine structure are longer and more flexible, which should provide more conformation options for the binding with P-gp. As reported in the references [25–27], the introduction of lipophilic groups and hydrogen bond acceptors seems to favor the binding with P-gp, and the methylation of hydroxy groups, or removing hydantoin group at the 2-amino position could effectively reduce their cytotoxic activity [21]. Therefore, our design of the target molecules is based on the principles described as follows: the C4 and C5 positions of the central 2-aminoimidazole ring would be replaced with benzyl groups substituted with methoxy or diethylamino groups; by acylation reactions, the 2-amino group can be linked with moieties containing aryl rings which have been substituted with multiple methoxy groups. These novel Naamidine analogues have been examined for their MDR-reversing activity in the P-gp over-expressing cancer cell line MDA435/LCC6MDR.

2. Results and discussion

2.1. Chemistry

A series of Naamidine analogues have been prepared, in which the 2,4,5-positions of 1-methyl-1*H*-imidazole rings were substituted with different functional groups. Initially, three key intermediates **9a**–**c** containing multiple methoxy and diethylamino groups were prepared, as illustrated in Scheme 1. The synthesis of 4,5-bis(4-methoxybenzyl)-1-methyl-1*H*-imidazol-2-amine (**9a**) has been reported previously [28]. 4,5-Bis(3,4-dimethoxybenzyl)-1-methyl-1*H*-imidazol-2-amine (**9b**) and 4-(3,4-dimethoxybenzyl)-5-(4-(diethylamino)benzyl)-1-methyl-1*H*-imidazol-2-amine (**9c**) were prepared by a strategy similar to the reported one [28], employing 4,5-diiodo-1-methyl-1*H*-imidazole (**2**) as the starting material. At first, 4,5-diiodo-1-methyl-1*H*-imidazole (**2**) was treated with EtMgBr in anhydrous THF to furnish the desired 5-imidazolyl Grignard reagent, which was allowed to react

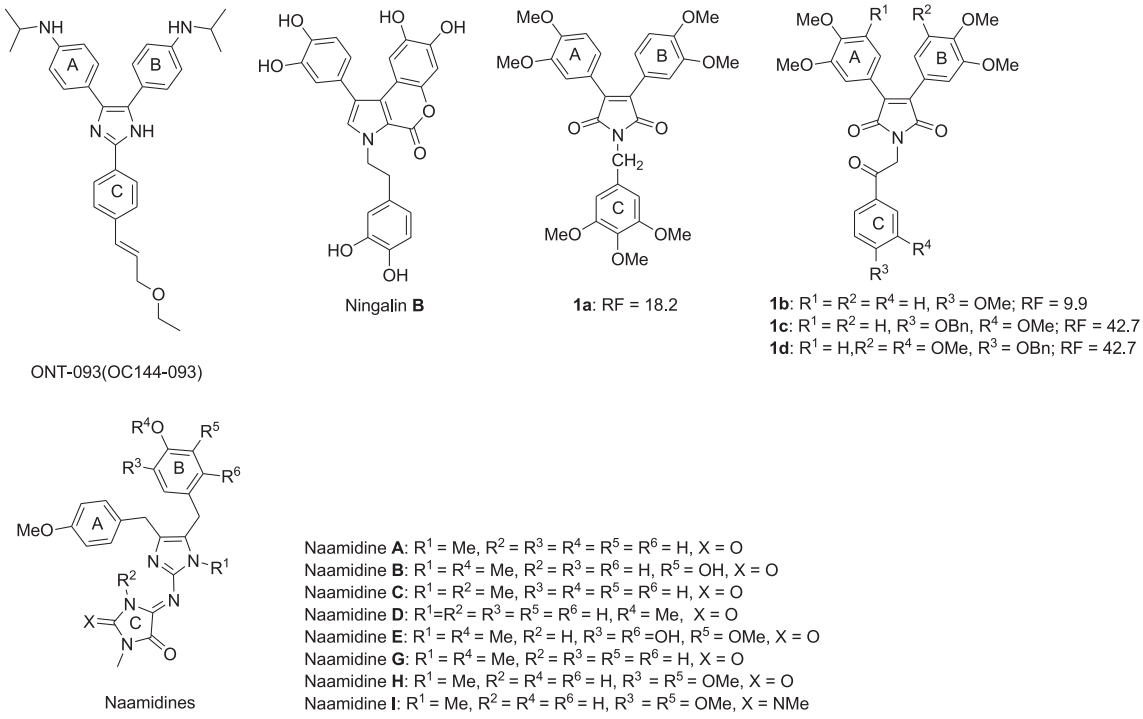
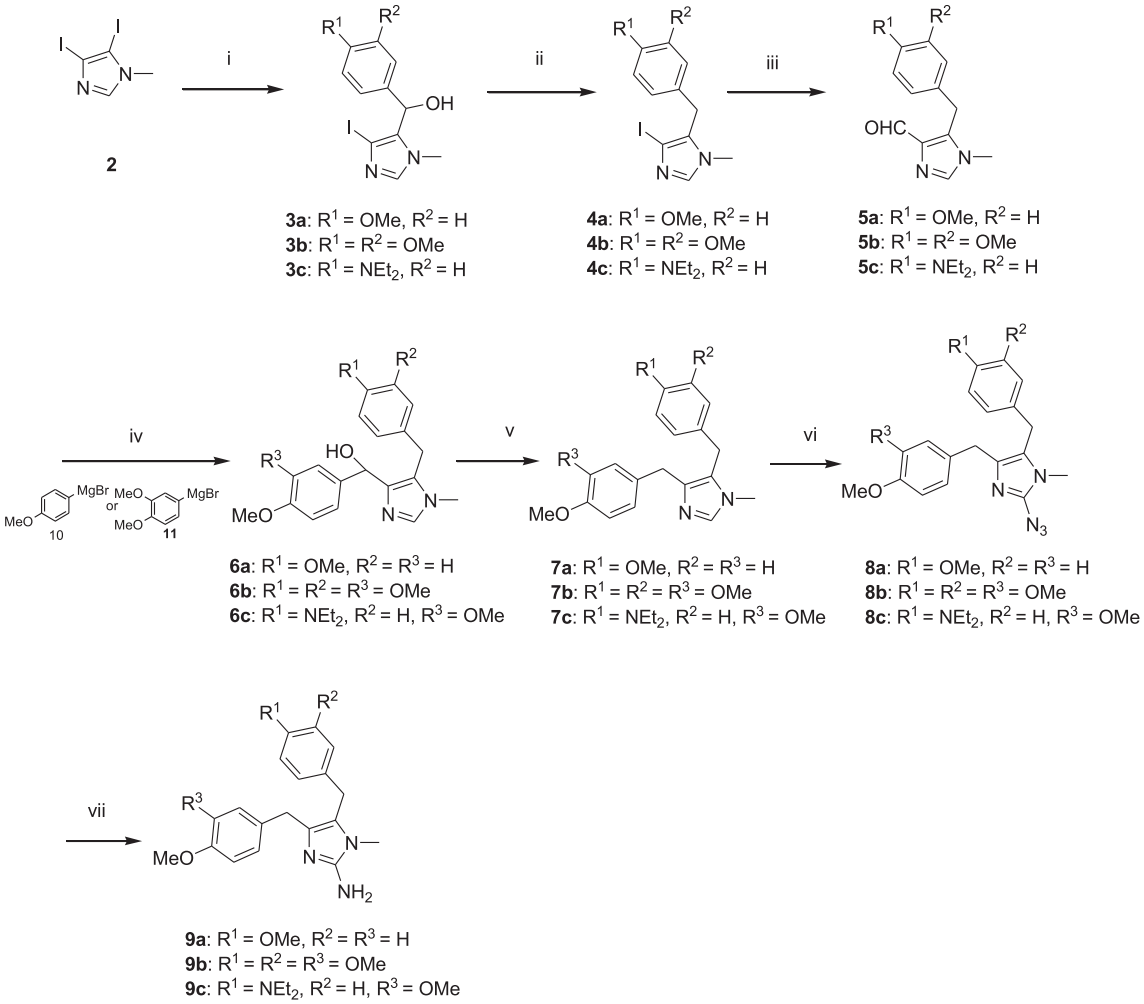


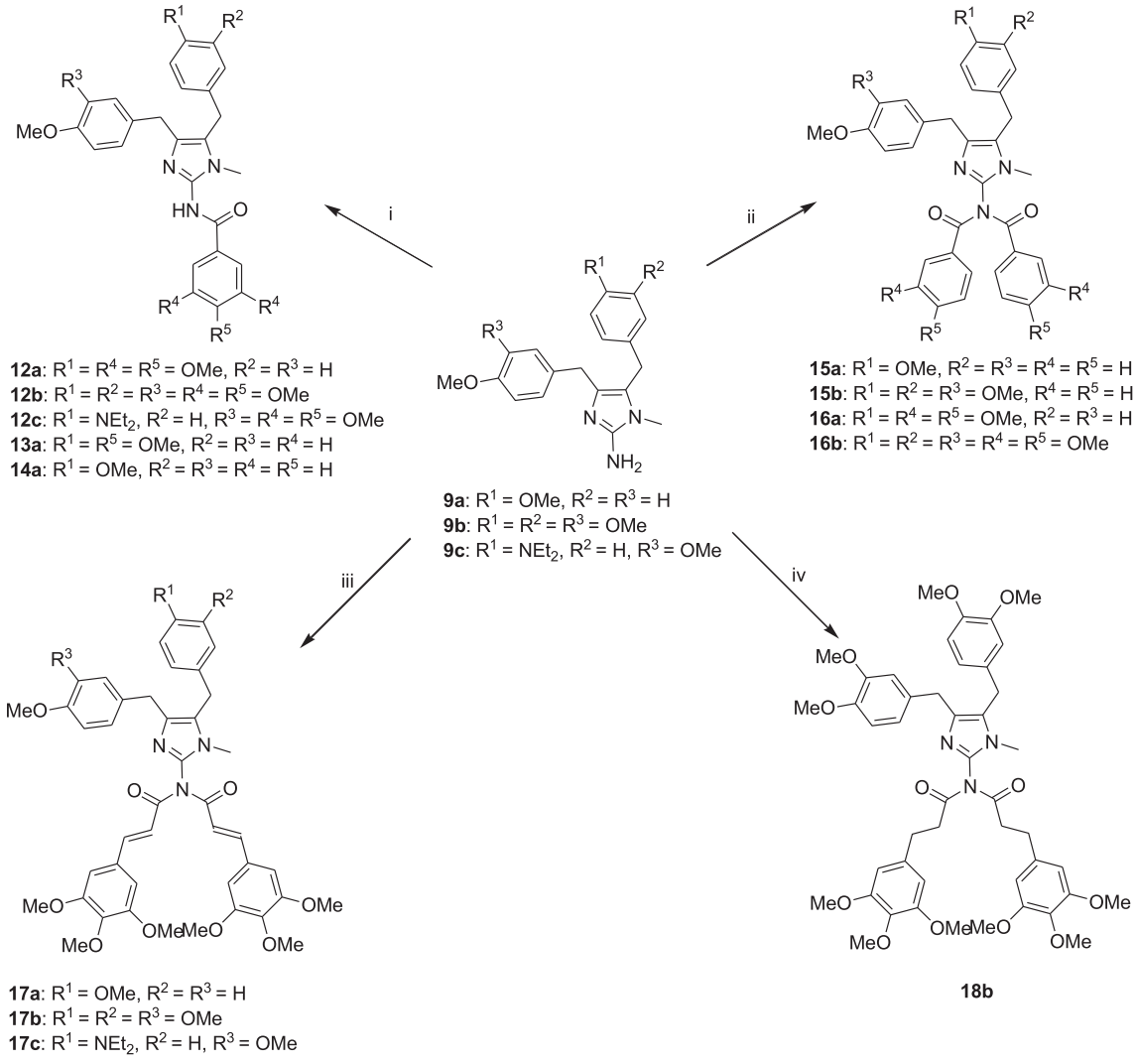
Fig. 1. Structures of compounds with Y-shaped scaffold.



Scheme 1. Synthesis of intermediates **9a**–**c**. (Reagents and conditions: (i) EtMgBr, THF, 25 °C, 0.5 h, then 4-methoxybenzaldehyde, 24 h (**3a**); EtMgBr, THF, 25 °C, 0.5 h, then 3,4-dimethoxybenzaldehyde, 24 h (**3b**); EtMgBr, THF, 25 °C, 0.5 h, then 4-(diethylamino)benzaldehyde, 24 h (**3c**); (ii) Et_3SiH, $BF_3{\cdot}Et_2O$, THF, 0 °C–60 °C, 24 h; (iii) EtMgBr, THF, 25 °C, 0.5 h, then *N*-methylformanilide, 12 h; (iv) **10**, THF, 25 °C, 12 h (**6a**); **11**, THF, 25 °C, 12 h (**6b** and **6c**); (v) Pd/C, H_2, MeOH, acetic acid, 40 atm, 50 °C, 24 h; (vi) *n*-BuLi, THF, −78 °C, 0.5 h, then TsN_3, 25 °C, 1 h; (vii) $NaBH_4$, MeOH, 0 °C–25 °C, 12 h.)

with 4-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde and 4-(diethylamino)benzaldehyde to afford the corresponding alcohols **3a**–**c**. Subsequent ionic reduction of the benzylic hydroxyl group with Et_3SiH and $BF_3{\cdot}Et_2O$ has provided the desired intermediates **4a**–**c**. These compounds were treated with EtMgBr in anhydrous THF to afford 4-imidazolyl Grignard reagents, which were allowed to react with *N*-methylformanilide to generate the corresponding aldehydes **5a**–**c**. At this stage, the resulting aldehydes were treated with *p*-methoxyphenylmagnesium bromide (**10**), or 1,2-dimethoxyphenylmagnesium bromide (**11**), affording alcohols **6a**–**c**. As previously reported [29] in the synthesis of Naamine C, treating these alcohols with Et_3SiH and $BF_3{\cdot}Et_2O$ would lead to the intramolecular Friedel–Crafts cyclization reaction, instead of the reduction of benzylic hydroxyl groups. Even though we found that $NaBH_4$ and $NiCl_2$ were possible reducing agents, difficult post-reaction processing due to the excess amount of nickel mud has hampered their suitability in this transformation. In order to improve this step, this reaction was attempted using Pd/C as the catalyst under hydrogen pressure (40 atm), and it appeared that the hydroxyl group has been eliminated completely, affording the desired products **7a**–**c** in an 81%, 72% and 77% yield, respectively. Next, compound **7a** was lithiated at the 2-position with stoichiometric amount of *n*-BuLi, and the resulting intermediate was treated with TsN_3 to afford azide product **8a**. Surprisingly, when this method was applied to substrate **7b**, the desired product **8b** was not obtained and most of the starting material was recovered. In Shunsaku Ohta's [30] study, this result has been attributed to the fact that lithiation takes place at the benzyl methylene group of the 5-position rather than the 2-position, which would be difficult to further react with TsN_3 due to the steric hindrance. Therefore, in his Naamine A synthesis, the 2-position of the imidazole ring was first brominated with NBS, and the resulting bromide was lithiated with *t*-BuLi, followed by TsN_3 treatment, ultimately affording the corresponding azide in a 32.8% total yield. In our study, **7b** and **7c** were treated with more than two equivalents of *n*-BuLi, thus the lithiation reaction takes place at both the 2-position of the imidazole ring and the benzyl methylene position. Subsequently, the resulting di-lithiated intermediate was treated with TsN_3 to afford the 2-substituted azide products **8b** and **8c** in a 51% and 42% yield, respectively. With these materials in hand, key intermediates **9a**–**c** can be readily prepared by $NaBH_4$ reduction of **8a**–**c** in MeOH.

As shown in Scheme 2, the target products can be synthesized by the acylation reaction of compounds **9a**–**c** with a series of acyl chlorides. Indeed, after treated with one equivalent of *n*-BuLi, compounds **9a**–**c** readily reacted with 3,4,5-trimethoxybenzoyl chloride to afford the corresponding products **12a**–**c**. Compound



Scheme 2. Synthesis of target products. (Reagents and conditions: (i) *n*-BuLi, THF, −78 °C, 0.5 h, then acyl chlorides, 25 °C, 2 h; (ii) benzoyl chlorides, DIPEA, THF, 0 °C–60 °C, 24 h; (iii) (*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl chloride, DIPEA, THF, 0 °C–60 °C, 24 h; (iv) 3-(3,4,5-trimethoxyphenyl)propanoyl chloride, DIPEA, THF, 0 °C–60 °C, 24 h.)

13a and **14a** were also obtained by the same approach. Interestingly, when excess DIPEA and benzoyl chloride were employed to react with **9a**–**c**, the *N*,*N*-benzoylbenzamide products **15a**–**b** have been obtained. Using the same synthetic strategy, compounds **16a**–**b**, **17a**–**c** and **18b** were also prepared, containing more benzene rings or methoxy groups.

2.2. Biological studies of Naamidine analogues

As demonstrated in the literature [14,31], the reversal effect of Naamidine analogues on the Taxol resistance can be determined using the MDA435/LCC6MDR cell line. The MDA435/LCC6MDR cell line is an ABCB1-transfected derivative of MDA435/LCC6 cell line, and the latter is an estrogen-independent breast cancer cell line. Notably, it was found that LCC6MDR cells are about 57.4 folds more resistant to Taxol treatment (IC_{50} = 149.3 ± 11.3 nM) than their parental LCC6 cells (IC_{50} = 2.6 ± 0.5 nM), which presumably should be due to the over-expression of P-gp. In order to measure the MDR reversal activity of Naamidine analogues, relative fold (RF) was used, which essentially is the ratio of the IC_{50} obtained without modulator to the one with modulator (1 μM) (Table 1). Verapamil, a well-known P-gp modulator, has been employed as the positive control. In order to study the correlation between the compound lipophilicity and MDR reversal activity, CLogPs of the Naamidine analogues have been calculated by software Sybyl 2.0, as illustrated in Table 1.

Notably, it appears that none of the intermediates **9a**–**c** has exhibited pronounced reversal activity (RF ≈ 1), in which the 2-amino group was not substituted. One of the most experimentally supported [33,34] mechanism for P-gp action is the "hydrophobic vacuum cleaner" model [32], which suggests that enhancing the lipophilicity of compounds should be essential for the improvement of P-gp modulating activity [26,27]. Therefore, it would be difficult for the Naamidine analogues **9a**–**c** to stay in the bilayer to interact with P-gp, because of their poor lipophilicity.

As expected, the introduction of multi-methoxy-substituted aryl groups to the 2-amino group of the imidazole ring has substantially increased compound lipophilicity, which possibly could improve the P-gp modulating activity of Naamidine analogues. Specifically, compounds **12a** (RF = 4.7, CLogP = 4.38), **12b** (RF = 4.0, CLogP = 3.85), **16a** (RF = 3.7, CLogP = 6.31) and **17a** (RF = 5.0, CLogP = 7.08) have exhibited similar modulating activity, using Verapamil (RF = 4.5, CLogP = 4.47) as the positive control. In addition, the modulating activity of **12a** (RF = 4.7) is at least 2 folds

Table 1
P-gp modulating activity of Naamidine analogues.

Compounds (1 μM)	CLogP[a]	Mean IC_{50} of taxol (nM)[b]	RF[c]
LCC6MDR[d]	–	149.3 ± 11.3	1.0
LCC6[e]	–	2.6 ± 0.5	–
Verapamil	4.47	32.9 ± 3.5	4.5
9a	3.39	120.1 ± 9.4	1.2
9b	2.87	164.1 ± 11.4	0.9
9c	4.44	147.3 ± 3.3	1.0
12a	4.38	30.1 ± 4.6	4.7
12b	3.85	37.9 ± 2.7	4.0
12c	5.42	5.8 ± 0.6	26.4
13a	5.14	100.7 ± 16.3	1.5
14a	5.05	66.4 ± 1.1	2.3
15a	6.77	58.7 ± 4.0	2.6
15b	6.25	118.0 ± 10.9	1.3
16a	6.31	40.9 ± 2.6	3.7
16b	5.79	89.8 ± 9.4	1.7
17a	7.08	30.3 ± 0.0	5.0
17b	6.56	92.1 ± 2.5	1.6
17c	8.14	6.2 ± 1.5	24.5
18b	5.20	138.8 ± 7.8	1.1

[a] CLogP values were generated using software Sybyl 2.0.
[b] IC_{50} values were determined using LCC6MDR cells after they were treated with different concentrations of Taxol in the presence of 1 μM Naamidine analogues or Verapamil. Each experiment has been repeated two times and the data were presented as the mean ± standard error of mean.
[c] RF = (IC_{50} without modulator)/(IC_{50} with modulator).
[d] No modulator was used in LCC6MDR cells.
[e] LCC6 cells were used without adding modulator.

higher than **13a** (RF = 1.5) and **14a** (RF = 2.3); compounds **16a** (RF = 3.7) and **16b** (RF = 1.7) also exhibited higher RF values than **15a** (RF = 2.6) and **15b** (RF = 1.3) respectively. These results suggest that increasing the number of methoxy groups on the phenyl ring at the 2-position of the imidazole ring is beneficial to the reversal activity, in accordance with the results obtained from our previous studies. Moreover, as disclosed recently [25,35], multiple methoxy substituents usually provide additional hydrogen bond acceptors, which should promote the binding to P-gp proteins.

Interestingly, we found that altering the substituents on the benzyl groups at the 4,5-positions of the imidazole ring could also significantly impact the reversal activity. Compared with the 4,5-bis-4-methoxybenzyl Naamidine analogues, the 4,5-bis-3,4-dimethoxybenzyl Naamidine analogues contain more methoxy groups but possess similar lipophilicity. However, none of them has exhibited decent reversal activity except compound **12b** (RF = 4.0). Specifically, compounds **15b** (RF = 1.3, CLogP = 6.25), **16b** (RF = 1.7, CLogP = 5.79) and **17b** (RF = 1.6, CLogP = 6.56) have displayed much weaker modulating activity than compounds **15a** (RF = 2.6, CLogP = 6.77), **16a** (RF = 3.7, CLogP = 6.31) and **17a** (RF = 5.0, CLogP = 7.08). To our delight, when the 5-position of imidazole ring was substituted with a 4-(diethylamino)benzyl group, the reversal activity has been greatly improved. It appear that compounds **12c** (RF = 26.4) and **17c** (RF = 24.5) have exhibited the best reversal activity, about 5–6 folds more potent than Verapamil (RF = 4.5). These results clearly indicated that modifying the benzyl groups at the 4,5-positions of the imidazole ring could be the most viable way to improve the binding affinity.

We next examined the EC_{50} and in vitro cytotoxicity of compounds **12c** and **17c**, in an effort to quantitatively evaluate their reversal potency and safety profile, as shown in Table 2. The EC_{50} is defined as the concentration of modulators required to reduce the IC_{50} by half. Each experiment has been repeated two or three times, and the data were presented as the mean ± standard error of mean. It was found that both **12c** (EC_{50} = 212.5 ± 22.6 nM) and **17c** (EC_{50} = 210.5 ± 15.0 nM) have produced a pronounced effect on reversing the Taxol cytotoxicity in the MDA435/LCC6 MDR cells. In

Table 2
EC_{50} and in vitro cytotoxicity of **12c** and **17c**.

Compounds	EC_{50} (nM)[a]	Cytotoxicity (IC_{50}, μM)[b]		
		LCC6	LCC6MDR	L929[c]
12c	212.5 ± 22.6	45.2 ± 4.65	38.3 ± 10.75	47.4 ± 6.75
17c	210.5 ± 15.0	13.7 ± 2.10	9.1 ± 1.85	18.2 ± 6.90

[a] EC_{50} was defined as the concentration of modulators required to reduce the IC_{50} by half.
[b] IC_{50} values were determined for LCC6, LCC6MDR and L929 cells after they were treated with a series of concentrations of **12c** and **17c**. Each experiment has been repeated two to three times, and the data were presented as the mean ± standard error of mean.
[c] L929 is a mouse connective tissue fibroblast cell line.

the in vitro cytotoxicity tests described as follows, the IC_{50} values of **12c** and **17c** were determined using cancer cell lines LCC6 and LCC6MDR as well as normal mouse fibroblast cell line L929. Specifically, compound **17c** (IC_{50} ≈ 10 μM) has exhibited moderate cytotoxic activity for three cell lines, whereas compound **12c** (IC_{50} ≈ 40 μM) showed weaker cytotoxic activity, which clearly suggests that **12c** should be a safer MDR modulator.

3. Conclusions

In this study, a series of novel 4,5-di-substituted benzyl-1-methyl-1*H*-imidazol-2-substituted amines have been designed, synthesized and evaluated for their P-gp-mediated MDR reversal activity. These synthetic compounds have exhibited moderate to excellent P-gp modulating activity against P-gp over-expressing breast cancer cell line LCC6MDR. Results obtained from preliminary SAR study suggest that compound lipophilicity is a critical factor for the P-gp modulating activity. Notably, increasing the number of methoxy groups on the phenyl groups presiding at the 2-position of the imidazole ring seems to be beneficial. On the other hand, it appears that altering the substituents of the benzyl groups located at the 4,5-position of the imidazole ring can significantly impact the reversal activity as well. To our delight, compounds **12c** and **17c**, which possess 4-(diethylamino) benzyl group at the 5-position of imidazole, have displayed the best P-gp modulating activity at a 1 μM concentration. As a matter of fact, they can re-sensitized LCC6MDR cells towards Taxol by 26.4 and 24.5 folds, with a EC_{50} 212.5 and 210.5 nM, respectively. Importantly, we found that compound **12c** displayed little in vitro cytotoxicity to either cancer cell lines or normal mouse fibroblast cell lines, exhibiting a better safety profile. In summary, this work has successfully demonstrated that the synthetic Naamidine analogues can be employed as effective, safe modulators for the P-gp-mediated MDR in cancer cells.

4. Experimental section

4.1. General

All moisture sensitive reactions were conducted under a nitrogen atmosphere in anhydrous, freshly distilled solvents. Anhydrous THF was distilled under nitrogen from Na. Starting materials and reagents, unless otherwise stated, were commercial grades and used without further purification. *p*-methoxyphenylmagnesium bromide (**10**) and 1,2-dimethoxyphenylmagnesium bromide (**11**) were prepared by literature procedures [36]. Acyl chlorides were prepared by corresponding acids and oxalyl chloride [37].

All reactions were monitored by thin-layer chromatography (TLC), on aluminum sheets (Silica gel 60-F254, E. Merck). Compounds were visualized by UV light. Flash chromatography was

carried out using silica-gel 60 (200–300 mesh). Silicagel chromatography solvents were analytical grade. Melting points were determined with an X-4 digital micro melting point tester (Taike Ltd., Beijing, China) and were uncorrected. ^{1}H NMR (600 or 500 MHz) and ^{13}C NMR (150 or 126 MHz) spectra were measured with TMS as an internal standard when $CDCl_3$ or DMSO-d_6 were used as a solvent. Chemical shifts are expressed in d (ppm) and coupling constants in *J* (Hz). High resolution (ESI) MS spectra were recorded using a QTOF-2 Micromass spectrometer.

4.1.1. 4,5-Bis(4-methoxybenzyl)-1-methyl-1H-imidazol-2-amine (**9a**)

9a was prepared by literature procedures [28] as a white solid; mp 162–163 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.12 (d, J = 8.6 Hz, 2H), 6.95 (d, J = 8.6 Hz, 2H), 6.81 (dd, J = 6.8, 4.8 Hz, 2H), 6.79 (d, J = 8.6 Hz, 2H), 3.78 (s, 5H), 3.75 (s, 3H), 3.74 (s, 2H), 3.17 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.60, 158.38, 147.04, 130.66, 129.57, 129.10, 128.96, 126.58, 121.28, 114.33, 114.15, 55.43, 55.31, 30.62, 29.95, 28.16; HRMS calcd for $(C_{20}H_{23}N_3O_2 + H)^+$ 338.1863, found 338.1857.

4.1.2. (4-Iodo-1-methyl-1H-imidazol-5-yl)(3,4-dimethoxyphenyl) methanol (**3b**)

EtMgBr (2.0 M solution in THF, 55 ml, 110 mmol) was added to a solution of 4,5-diiodo-1-methyl-1*H*-imidazole (35.1 g, 105 mmol) in anhydrous THF (500 ml) at 25 °C under N_2 atmosphere. The resulting mixture was stirred for 30 min and 3,4-dimethoxybenzaldehyde (17.4 g, 105 mmol) was added to this solution. After stirred at 25 °C for 24 h, sat. NH_4Cl (50 ml) was added to quench the reaction and the THF was removed under reduced pressure. Then the resulting suspension was filtered, washed by H_2O (300 ml) and Et_2O (50 ml), and dried in vacuo to give **3b** (35.3 g, 90%) as a white solid; mp 193–195 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.59 (s, 1H), 6.95 (d, J = 1.4 Hz, 1H), 6.91 (d, J = 8.3 Hz, 1H), 6.71–6.66 (m, 1H), 6.24–6.21 (m, 1H), 5.80 (d, J = 3.9 Hz, 1H), 3.72 (s, 3H), 3.72 (s, 3H), 3.41 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 149.00, 148.18, 141.75, 135.21, 134.77, 117.68, 112.08, 109.57, 85.56, 66.49, 55.94, 55.83, 33.11; HRMS calcd for $(C_{13}H_{15}N_2O_3I + H)^+$ 375.0200, found 375.0196.

4.1.3. (4-(Diethylamino)phenyl)(4-iodo-1-methyl-1H-imidazol-5-yl)methanol (**3c**)

3c was prepared as described for the synthesis of **3b** using EtMgBr (2.0 M solution in THF, 55 ml, 110 mmol), 4,5-diiodo-1-methyl-1*H*-imidazole (35.1 g, 105 mmol), 4-(diethylamino)benzaldehyde (18.6 g, 105 mmol) and anhydrous THF (500 ml). **3c** (35.9 g, 89%) was obtained as a white solid; mp 176–178 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.56 (s, 1H), 7.03 (d, J = 8.6 Hz, 2H), 6.62 (d, J = 8.8 Hz, 2H), 6.00 (d, J = 4.0 Hz, 1H), 5.75 (d, J = 3.9 Hz, 1H), 3.42 (s, 3H), 3.29 (q, J = 7.0 Hz, 4H), 1.05 (t, J = 7.0 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 146.89, 141.57, 135.57, 128.48, 126.64, 111.69, 85.17, 66.62, 44.04, 33.12, 12.85; HRMS calcd for $(C_{15}H_{20}N_3OI + H)^+$ 386.0724, found 386.0717.

4.1.4. 5-(3,4-Dimethoxybenzyl)-4-iodo-1-methyl-1H-imidazole (**4b**)

Et_3SiH (32 ml, 200 mmol) and $BF_3{\cdot}Et_2O$ (25.9 ml, 200 mmol) were slowly added to a solution of **3b** (33.7 g, 90 mmol) in anhydrous THF (500 ml) at 0 °C under N_2 atmosphere. Then the resulting mixture was heated to 60 °C. After stirred for 24 h, the reaction was cooled to room temperature and quenched by the addition of saturated aqueous solution of $NaHCO_3$. Then the THF was removed under reduced pressure and the resulting mixture was extracted with CH_2Cl_2 (100 ml) three times. The organic layer was dried by anhydrous Na_2SO_4 and concentrated to give **4b** (29.6 g, 92%) as a white solid; mp 96–98 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (s, 1H), 6.78 (d, J = 8.2 Hz, 1H), 6.68 (d, J = 1.6 Hz, 1H), 6.64 (d, J = 8.1 Hz, 1H), 3.92 (s, 2H), 3.85 (s, 3H), 3.83 (s, 3H), 3.45 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.25, 147.90, 139.38, 133.27, 129.69, 119.83, 111.26, 111.17, 84.75, 55.91, 55.86, 32.54, 30.26; HRMS calcd for $(C_{13}H_{15}N_2O_2I + H)^+$ 359.0251, found 359.0245.

4.1.5. N,N-diethyl-4-((4-iodo-1-methyl-1H-imidazol-5-yl)methyl) benzenamine (**4c**)

4c was prepared as described for the synthesis of **4b** using Et_3SiH (32 ml, 200 mmol), $BF_3{\cdot}Et_2O$ (25.9 ml, 200 mmol), **3c** (34.6 g, 90 mmol) and anhydrous THF (500 ml). **4c** (28.5 g, 86%) was obtained as a white solid; mp 115–117 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (s, 1H), 6.95 (d, J = 8.6 Hz, 2H), 6.60 (d, J = 8.7 Hz, 2H), 3.85 (s, 2H), 3.44 (s, 3H), 3.31 (q, J = 7.1 Hz, 4H), 1.13 (t, J = 7.1 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 146.66, 139.16, 133.87, 128.86, 123.57, 112.07, 84.38, 44.34, 32.55, 29.69, 12.53; HRMS calcd for $(C_{15}H_{20}N_3I + H)^+$ 370.0775, found 370.0774.

4.1.6. 5-(3,4-Dimethoxybenzyl)-1-methyl-1H-imidazole-4-carbaldehyde (**5b**)

EtMgBr (2.0 M solution in THF, 40 ml, 80 mmol) was added to a solution of **4b** (26.8 g, 75 mmol) in anhydrous THF (300 ml) at 25 °C under N_2 atmosphere. The resulting mixture was stirred for 30 min and *N*-methylformanilide (10.2 ml, 75 mmol) was added to this solution. After stirred at 25 °C for 12 h, sat. NH_4Cl (30 ml) was added to quench the reaction. Then the THF was removed under reduced pressure and the resulting mixture was extracted with CH_2Cl_2 (100 ml) three times. The organic layer was dried by anhydrous Na_2SO_4 and concentrated to give a yellow oil. The residue was purified by chromatography (acetoacetate/MeOH = 30/1, v/v) to give **5b** (16.9 g, 87%) as a light yellow solid; mp 90–92 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.01 (s, 1H), 7.44 (s, 1H), 6.76 (d, J = 8.1 Hz, 1H), 6.72 (s, 1H), 6.65 (d, J = 8.1 Hz, 1H), 4.34 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 3.49 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 187.61, 149.31, 148.00, 138.74, 138.20, 137.51, 128.93, 120.09, 111.49, 111.27, 55.88, 55.83, 31.52, 28.81; HRMS calcd for $(C_{14}H_{16}N_2O_3 + H)^+$ 261.1234, found 261.1229.

4.1.7. 5-(4-(Diethylamino)benzyl)-1-methyl-1H-imidazole-4-carbaldehyde (**5c**)

5c was prepared as described for the synthesis of **5b** using EtMgBr (2.0 M solution in THF, 40 ml, 80 mmol), **4c** (27.6 g, 75 mmol), *N*-methylformanilide (10.2 ml, 75 mmol) and anhydrous THF (300 ml). **5c** (17.7 g, 86%) was obtained as a white solid; mp 76–78 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.99 (s, 1H), 7.39 (s, 1H), 6.96 (d, J = 8.6 Hz, 2H), 6.57 (d, J = 8.7 Hz, 2H), 4.26 (s, 2H), 3.47 (s, 3H), 3.30 (q, J = 7.1 Hz, 4H), 1.12 (t, J = 7.0 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 187.20, 146.71, 138.71, 138.63, 138.07, 129.13, 122.64, 112.12, 44.32, 31.56, 28.18, 12.49; HRMS calcd for $(C_{14}H_{16}N_2O_3 + H)^+$ 272.1757, found 272.1756.

4.1.8. (5-(3,4-Dimethoxybenzyl)-1-methyl-1H-imidazol-4-yl)(3,4-dimethoxyphenyl)methanol (**6b**)

10 (2.0 M solution in THF, 35 ml, 70 mmol) was added to a solution of **5b** (16.9 g, 65 mmol) in anhydrous THF (200 ml). After stirred at 25 °C for 12 h under N_2 atmosphere, sat. NH_4Cl (20 ml) was added to quench the reaction. Then the THF was removed under reduced pressure and the resulting mixture was extracted with CH_2Cl_2 (100 ml) three times. The organic layer was dried by anhydrous Na_2SO_4 and concentrated to give a brown semi solid. The residue was purified by chromatography (acetoacetate/MeOH = 10/1, v/v) to give **6b** (24.1 g, 93%) as a white solid; mp 45–47 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.06 (s, 1H), 6.93 (d, J = 8.0 Hz, 1H), 6.75 (d, J = 8.2 Hz, 1H), 6.71 (d, J = 8.2 Hz, 1H),

6.52 (d, $J = 8.0$ Hz, 1H), 6.45 (s, 1H), 5.77 (s, 1H), 3.84 (s, 2H), 3.83 (s, 6H), 3.79 (s, 3H), 3.66 (s, 3H), 3.35 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.17, 148.89, 148.19, 147.69, 141.05, 136.84, 136.53, 130.31, 125.76, 119.87, 118.80, 111.23, 111.13, 110.76, 109.98, 69.74, 55.89, 55.87, 55.76, 55.70, 31.62, 28.56; HRMS calcd for $(C_{22}H_{26}N_2O_5 + H)^+$ 399.1914, found 399.1908.

4.1.9. (5-(4-(Diethylamino)benzyl)-1-methyl-1H-imidazol-4-yl)(3,4-dimethoxyphenyl)methanol (**6c**)

6c was prepared as described for the synthesis of **6b** using **11** (2.0 M solution in THF, 35 ml, 70 mmol) and **5c** (17.6 g, 65 mmol). **6c** (22.9 g, 86%) was obtained as a white solid; mp 117–119 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (s, 1H), 7.00 (s, 1H), 6.94 (d, $J = 7.7$ Hz, 1H), 6.80 (d, $J = 8.2$ Hz, 2H), 6.77 (d, $J = 8.2$ Hz, 1H), 6.54 (d, $J = 8.5$ Hz, 2H), 5.75 (s, 1H), 3.83 (s, 3H), 3.78 (s, 4H), 3.36 (s, 3H), 3.29 (q, $J = 7.0$ Hz, 4H), 1.12 (t, $J = 7.0$ Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.81, 148.09, 146.49, 136.69, 128.82, 126.35, 124.22, 118.75, 112.07, 110.80, 109.92, 69.71, 55.87, 55.72, 44.31, 31.64, 27.85, 12.51; HRMS calcd for $(C_{24}H_{31}N_3O_3 + H)^+$ 410.2438, found 410.2433.

4.1.10. 4,5-Bis(3,4-dimethoxybenzyl)-1-methyl-1H-imidazole (**7b**)

6b (11.9 g, 30 mmol) was dissolved in MeOH (200 ml) and acetic acid (20 ml). This reaction mixture was stirred under a hydrogen atmosphere (40 atm) in the presence of 10% Pd/C (200 mg) at 50 °C for 24 h. The catalyst was filtered and the filtrate was concentrated. Then the residue was neutralized by sat. Na_2CO_3 and extracted with CH_2Cl_2 (100 ml) three times. The organic layer was dried by anhydrous Na_2SO_4 and concentrated to give a brown oil. The residue was purified by chromatography (acetoacetate/MeOH = 10/1, v/v) to give **7b** (8.25 g, 72%) as a white solid; mp 73–76 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (s, 1H), 6.84 (d, $J = 1.5$ Hz, 1H), 6.80 (dd, $J = 8.2$, 1.6 Hz, 1H), 6.75 (d, $J = 4.2$ Hz, 1H), 6.73 (d, $J = 4.2$ Hz, 1H), 6.55 (dd, $J = 8.1$, 1.2 Hz, 1H), 6.44 (d, $J = 1.6$ Hz, 1H), 3.90 (s, 2H), 3.88 (s, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.66 (s, 3H), 3.34 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.16, 148.83, 147.64, 147.21, 138.81, 136.66, 133.72, 130.81, 125.53, 120.32, 119.85, 111.98, 111.16, 111.12, 55.90, 55.75, 55.67, 33.45, 31.68, 28.70; HRMS calcd for $(C_{22}H_{26}N_2O_4 + H)^+$ 383.1965, found 383.1959.

4.1.11. 4-((4-(3,4-Dimethoxybenzyl)-1-methyl-1H-imidazol-5-yl)methyl)-N,N-diethylbenzenamine (**7c**)

7c was prepared as described for the synthesis of **7b** using **6c** (12.3 g, 30 mmol) and 10% Pd/C (200 mg). **7c** (9.1 g, 77%) was obtained as a white solid; mp 108–120 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (s, 1H), 6.84 (d, $J = 8.7$ Hz, 2H), 6.82–6.79 (m, 2H), 6.75 (d, $J = 7.9$ Hz, 1H), 6.56 (d, $J = 8.7$ Hz, 2H), 3.87 (s, 2H), 3.85 (s, 2H), 3.82 (s, 3H), 3.78 (s, 3H), 3.36 (s, 3H), 3.30 (q, $J = 7.1$ Hz, 4H), 1.12 (t, $J = 7.1$ Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.78, 147.14, 146.46, 138.39, 136.41, 133.79, 128.78, 126.19, 124.75, 120.44, 112.09, 111.98, 111.20, 55.91, 55.70, 44.33, 33.44, 31.72, 27.96, 12.52; HRMS calcd for $(C_{24}H_{31}N_3O_2 + H)^+$ 394.2489, found 394.2483.

4.1.12. 4,5-Bis(3,4-dimethoxybenzyl)-2-azido-1-methyl-1H-imidazole (**8b**)

n-Butyl lithium (2.5 M solution in hexanes, 13.2 ml, 33 mmol) was added dropwise to a stirred solution of **7b** (5.73 g, 15 mmol) in anhydrous THF (100 ml) at −78 °C. Then the reaction was stirred for 0.5 h at the 25 °C. Then the reaction was cooled to −78 °C again and TsN_3 (10.2 g, 52 mmol) was added. After stirring for 1 h at 25 °C, the reaction mixture was quenched by the addition of sat. NH_4Cl (20 ml). Then the THF was removed under reduced pressure and the resulting mixture was extracted with CH_2Cl_2 (50 ml) three times. The organic layer was dried by anhydrous Na_2SO_4 and concentrated to give a pale brown oil, which was purified by chromatography (ligroin/acetoacetate = 2/1, v/v) to give **8b** (3.23 g, 51%) as a light brown solid; mp 71–74 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (d, $J = 1.6$ Hz, 1H), 6.79 (dd, $J = 8.2$, 1.5 Hz, 1H), 6.76–6.71 (m, 2H), 6.56 (dd, $J = 8.2$, 1.4 Hz, 1H), 6.45 (d, $J = 1.6$ Hz, 1H), 3.85 (s, 2H), 3.83 (s, 3H), 3.83 (s, 3H), 3.82 (s, 2H), 3.81 (s, 3H), 3.67 (s, 3H), 3.09 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 149.19, 148.83, 147.71, 147.32, 139.07, 136.09, 133.28, 130.54, 124.76, 120.27, 119.83, 112.01, 111.16, 111.10, 111.06, 55.90, 55.89, 55.78, 55.70, 33.14, 29.46, 29.11; HRMS calcd for $(C_{22}H_{25}N_5O_4 + H)^+$ 424.1979, found 424.1973.

4.1.13. 4-((4-(3,4-Bimethoxybenzyl)-2-azido-1-methyl-1H-imidazol-5-yl)methyl)-N,N-diethylbenzenamine (**8c**)

8c was prepared as described for the synthesis of **8b** using **7c** (5.89 g, 15 mmol), *n*-Butyl lithium (2.5 M solution in hexanes, 13.2 ml, 33 mmol) and TsN_3 (10.2 g, 52 mmol). **8c** (2.72 g, 42%) was obtained as a white solid; mp 101–103 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.85 (s, 1H), 6.83 (s, 2H), 6.80 (dd, $J = 8.2$, 1.8 Hz, 1H), 6.76 (d, $J = 8.2$ Hz, 1H), 6.56 (d, $J = 8.7$ Hz, 2H), 3.83 (s, 5H), 3.79 (s, 3H), 3.77 (s, 2H), 3.30 (q, $J = 7.0$ Hz, 4H), 3.11 (s, 3H), 1.12 (t, $J = 7.0$ Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.77, 147.23, 146.50, 138.75, 135.72, 133.37, 128.73, 125.45, 124.45, 120.36, 112.08, 112.00, 111.11, 55.88, 55.73, 44.32, 33.14, 29.49, 28.36, 12.50.

4.1.14. 4,5-Bis(3,4-dimethoxybenzyl)-1-methyl-1H-imidazol-2-amine (**9b**)

To a stirred solution of **8b** (2.12 g, 5 mmol) in MeOH (100 ml), $NaBH_4$ (1.89 g, 50 mmol) was slowly added at 0 °C. Then the reaction mixture was heated to 25 °C. After stirred at 25 °C for 12 h, the reaction mixture was quenched by the addition of sat. NH_4Cl (20 ml). Then the MeOH was removed under reduced pressure and the resulting mixture was extracted with CH_2Cl_2 (30 ml) three times. The organic layer was dried by anhydrous Na_2SO_4 and concentrated to give **9b** (1.75 g, 88%) as a white solid; mp 160–164 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.80 (s, 1H), 6.76 (d, $J = 8.2$ Hz, 1H), 6.74 (s, 2H), 6.55 (d, $J = 8.1$ Hz, 1H), 6.51 (d, $J = 1.8$ Hz, 1H), 3.84 (s, 3H), 3.81 (s, 3H), 3.80 (s, 5H), 3.75 (s, 2H), 3.73 (s, 3H), 3.28 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 149.53, 149.33, 148.30, 148.10, 146.92, 129.89, 128.62, 123.19, 121.14, 120.50, 119.94, 111.99, 111.59, 111.13, 56.15, 56.06, 56.01, 55.95, 30.23, 30.12, 28.32; HRMS calcd for $(C_{22}H_{27}N_3O_4 + H)^+$ 398.2074, found 398.2068.

4.1.15. 4-(3,4-Dimethoxybenzyl)-5-(4-(diethylamino)benzyl)-1-methyl-1H-imidazol-2-amine (**9c**)

9c was prepared as described for the synthesis of **9b** using **8c** (2.17 g, 5 mmol) and $NaBH_4$ (0.95 g, 25 mmol). **9c** (1.69 g, 83%) was obtained as a white solid; mp 113–117 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.23 (s, 2H), 6.86 (d, $J = 8.7$ Hz, 2H), 6.77 (s, 3H), 6.58 (d, $J = 8.7$ Hz, 2H), 3.82 (s, 3H), 3.82 (s, 3H), 3.74 (s, 4H), 3.32 (q, $J = 7.1$ Hz, 4H), 3.29 (s, 3H), 1.14 (t, $J = 7.1$ Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 149.28, 148.05, 146.96, 146.84, 129.71, 128.79, 122.29, 122.08, 121.77, 120.61, 112.26, 111.80, 111.52, 56.08, 55.99, 44.43, 30.26, 30.02, 27.62, 12.59; HRMS calcd for $(C_{24}H_{32}N_4O_2 + H)^+$ 409.2598, found 409.2595.

4.1.16. N-(4,5-bis(4-methoxybenzyl)-1-methyl-1H-imidazol-2-yl)-3,4,5-trimethoxybenzamide (**12a**)

n-Butyl lithium (2.5 M solution in hexanes, 0.4 ml, 1.0 mmol) was added dropwise to a stirred solution of **9a** (0.337 g, 1 mmol) in anhydrous THF (20 ml) at −78 °C under N_2 atmosphere and the reaction was stirred for 0.5 h at rt. Then the reaction mixture was cooled to −78 °C again and 3,4,5-Trimethoxybenzoyl chloride (0.23 g, 1 mmol) dissolved in anhydrous THF (5 ml) was added. After stirring for 2 h at 25 °C, the reaction mixture was quenched by the addition of sat. NH_4Cl (20 ml). Then the THF was removed under reduced pressure and the resulting mixture was extracted with

CH_2Cl_2 (30 ml) three times. The organic layer was dried by anhydrous Na_2SO_4 and concentrated. The residue was purified by chromatography (ligroin/EtOAc = 1/1, v/v) to give **12a** (0.351 g, 66%) as a white solid; mp 117–120 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 (s, 2H), 7.10 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.6 Hz, 2H), 6.86–6.82 (m, 4H), 3.92 (s, 2H), 3.91 (s, 6H), 3.88 (s, 3H), 3.84 (s, 2H), 3.79 (s, 3H), 3.79 (s, 3H), 3.37 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.76, 158.72, 152.66, 140.39, 134.06, 129.39, 129.10, 128.95, 128.77, 120.89, 114.53, 114.42, 105.85, 60.94, 56.15, 55.42, 55.40, 55.38, 29.78, 28.91, 28.07; HRMS calcd for $(C_{30}H_{33}N_3O_6 + H)^+$ 532.2442, found 532.2430.

4.1.17. N-(4,5-bis(3,4-dimethoxybenzyl)-1-methyl-1H-imidazol-2-yl)-3,4,5-trimethoxybenzamide (**12b**)

12b was prepared as described for the synthesis of **12a** using **9b** (0.198 g, 0.5 mmol), *n*-Butyl lithium (2.5 M solution in hexanes, 0.2 ml, 0.5 mmol) and 3,4,5-Trimethoxybenzoyl chloride (0.12 g, 0.5 mmol). **12b** (0.213 g, 72%) was obtained as a white solid; mp 120–122 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 (s, 2H), 6.79 (s, 1H), 6.78 (d, J = 1.6 Hz, 1H), 6.75 (dd, J = 8.2, 1.9 Hz, 1H), 6.65 (d, J = 1.9 Hz, 1H), 6.63 (d, J = 8.2 Hz, 1H), 6.60 (d, J = 1.8 Hz, 1H), 3.94 (s, 2H), 3.91 (s, 6H), 3.88 (s, 3H), 3.86 (s, 3H), 3.85 (s, 5H), 3.80 (s, 3H), 3.78 (s, 3H), 3.40 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.69, 149.43, 148.24, 140.47, 129.65, 129.28, 120.40, 119.89, 111.66, 111.49, 111.13, 105.85, 60.95, 56.16, 56.06, 55.98, 30.31, 29.09, 28.54; HRMS calcd for $(C_{32}H_{37}N_3O_8 + H)^+$ 592.2653, found 592.2643.

4.1.18. N-(4-(3,4-dimethoxybenzyl)-5-(4-(diethylamino)benzyl)-1-methyl-1H-imidazol-2-yl)-3,4,5-trimethoxybenzamide (**12c**)

12c was prepared as described for the synthesis of **12a** using **9c** (0.204 g, 0.5 mmol), *n*-Butyl lithium (2.5 M solution in hexanes, 0.2 ml, 0.5 mmol) and 3,4,5-Trimethoxybenzoyl chloride (0.12 g, 0.5 mmol). **12c** (0.138 g, 46%) was obtained as a white solid; mp 132–135 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (s, 2H), 6.94 (d, J = 8.3 Hz, 2H), 6.80 (d, J = 8.1 Hz, 1H), 6.76 (d, J = 8.2 Hz, 1H), 6.63 (s, 1H), 6.60 (d, J = 8.4 Hz, 2H), 3.91 (s, 6H), 3.88 (s, 5H), 3.85 (s, 3H), 3.84 (s, 2H), 3.78 (s, 3H), 3.42 (s, 3H), 3.32 (q, J = 7.0 Hz, 4H), 1.14 (t, J = 7.0 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.87, 152.54, 149.28, 148.08, 146.78, 140.26, 134.00, 129.66, 128.68, 122.95, 121.43, 120.38, 112.15, 111.55, 111.46, 105.74, 60.82, 56.04, 55.93, 55.81, 44.35, 30.18, 29.01, 27.72, 12.46; HRMS calcd for $(C_{34}H_{42}N_4O_6 + H)^+$ 603.3177, found 603.3169.

4.1.19. N-(4,5-bis(4-methoxybenzyl)-1-methyl-1H-imidazol-2-yl)-4-methoxybenzamide (**13a**)

13a was prepared as described for the synthesis of **12a** using **9a** (0.169 g, 0.5 mmol), *n*-Butyl lithium (2.5 M solution in hexanes, 0.2 ml, 0.5 mmol) and 4-methoxybenzoyl chloride (0.08 g, 0.5 mmol). **13a** (0.092 g, 39%) was obtained as a white solid; mp 134–136 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.19 (d, J = 8.6 Hz, 2H), 7.10 (d, J = 8.5 Hz, 2H), 7.02 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 3.2 Hz, 2H), 6.83 (d, J = 3.2 Hz, 2H), 3.91 (s, 2H), 3.84 (s, 5H), 3.79 (s, 3H), 3.78 (s, 3H), 3.35 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.77, 158.70, 158.68, 130.51, 129.38, 129.28, 128.94, 128.90, 120.68, 114.49, 114.40, 113.11, 55.43, 55.41, 55.38, 29.80, 28.89, 28.07; HRMS calcd for $(C_{28}H_{29}N_3O_4 + H)^+$ 472.2231, found 472.2220.

4.1.20. N-(4,5-bis(4-methoxybenzyl)-1-methyl-1H-imidazol-2-yl) benzamide (**14a**)

14a was prepared as described for the synthesis of **12a** using **9a** (0.169 g, 0.5 mmol), *n*-Butyl lithium (2.5 M solution in hexanes, 0.2 ml, 0.5 mmol) and benzoyl chloride (0.07 g, 0.5 mmol). **14a** (0.077 g, 35%) was obtained as a white solid; mp 103–106 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.24–8.21 (m, 2H), 7.43–7.36 (m, 3H), 7.10 (d, J = 8.5 Hz, 2H), 7.02 (d, J = 8.5 Hz, 2H), 6.85 (t, J = 1.9 Hz, 2H), 6.84 (t, J = 1.8 Hz, 2H), 5.30 (s, 1H), 3.92 (s, 2H), 3.84 (s, 2H), 3.79 (s, 3H), 3.79 (s, 3H), 3.37 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.55, 158.63, 158.60, 138.42, 130.49, 129.27, 129.08, 128.83, 128.71, 128.62, 127.77, 120.73, 114.41, 114.31, 55.31, 55.29, 29.69, 28.91, 27.98; HRMS calcd for $(C_{27}H_{27}N_3O_3 + H)^+$ 442.2125, found 442.2125.

4.1.21. N-(4,5-bis(4-methoxybenzyl)-1-methyl-1H-imidazol-2-yl)-N-benzoylbenzamide (**15a**)

Benzoyl chloride (0.16 g, 1.1 mmol) dissolved in anhydrous THF (5 ml) and DIPEA (1 ml) was added to a solution of **9a** (0.169 g, 0.5 mmol) in anhydrous THF (10 ml) at 25 °C under N_2 atmosphere. After stirred for 24 h under N_2 atmosphere, the reaction was quenched by the addition of sat.$NaHCO_3$ (10 ml). Then the THF was removed under reduced pressure and the resulting mixture was extracted with CH_2Cl_2 (30 ml) three times. The organic layer was dried by anhydrous Na_2SO_4 and concentrated. The residue was purified by chromatography (ligroin/EtOAc = 1/1, v/v) to give **15a** (0.179 g, 66%) as a white solid; mp 135–137 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, J = 7.4 Hz, 4H), 7.53 (t, J = 7.4 Hz, 2H), 7.37 (t, J = 7.8 Hz, 4H), 6.89 (d, J = 8.5 Hz, 2H), 6.69 (d, J = 8.5 Hz, 2H), 6.65 (d, J = 8.6 Hz, 2H), 6.54 (d, J = 8.6 Hz, 2H), 3.81 (s, 2H), 3.78 (s, 2H), 3.77 (s, 3H), 3.76 (s, 3H), 3.10 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.81, 158.18, 157.76, 138.07, 137.57, 133.62, 132.86, 132.80, 129.57, 129.31, 129.30, 128.62, 128.60, 126.80, 114.03, 113.64, 55.37, 55.34, 32.47, 30.26, 28.58; HRMS calcd for $(C_{34}H_{31}N_3O_4 + H)^+$ 546.2387, found 546.2380.

4.1.22. N-(4,5-bis(3,4-dimethoxybenzyl)-1-methyl-1H-imidazol-2-yl)-N-benzoylbenzamide (**15b**)

15b was prepared as described for the synthesis of **15a** using **9b** (0.199 g, 0.5 mmol) and benzoyl chloride (0.16 g, 1.1 mmol). **15b** (0.180 g, 61%) was obtained as a white solid; mp 192–194 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82–7.77 (m, 4H), 7.50 (t, J = 7.5 Hz, 2H), 7.35 (t, J = 7.8 Hz, 4H), 6.72 (d, J = 1.8 Hz, 1H), 6.65 (d, J = 8.1 Hz, 1H), 6.61 (d, J = 8.2 Hz, 1H), 6.56 (dd, J = 8.1, 1.7 Hz, 1H), 6.26 (d, J = 1.8 Hz, 1H), 6.24–6.22 (m, 1H), 3.83 (s, 10H), 3.72 (s, 3H), 3.55 (s, 3H), 3.15 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.80, 149.24, 148.83, 147.67, 147.26, 138.08, 137.54, 133.52, 132.91, 130.16, 129.36, 128.62, 126.75, 120.22, 119.54, 112.01, 111.07, 55.93, 55.76, 33.04, 30.27, 29.16; HRMS calcd for $(C_{36}H_{35}N_3O_6 + H)^+$ 606.2599, found 606.2594.

4.1.23. N-(4,5-bis(4-methoxybenzyl)-1-methyl-1H-imidazol-2-yl)-N-3,4-dimethoxybenzoyl-3,4-dimethoxybenzamide (**16a**)

16a was prepared as described for the synthesis of **15a** using **9a** (0.169 g, 0.5 mmol) and 3,4-dimethoxybenzoyl chloride (0.200 g, 1 mmol). **16a** (0.146 g, 44%) was obtained as a white solid; mp 171–173 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (dd, J = 8.4, 2.0 Hz, 2H), 7.40 (d, J = 2.0 Hz, 2H), 6.97 (d, J = 8.6 Hz, 2H), 6.76 (d, J = 8.5 Hz, 2H), 6.71 (d, J = 8.6 Hz, 2H), 6.65 (d, J = 8.7 Hz, 2H), 6.58 (d, J = 8.6 Hz, 2H), 3.92 (s, 6H), 3.83 (s, 2H), 3.81 (s, 6H), 3.80 (s, 2H), 3.76 (s, 3H), 3.75 (s, 3H), 3.07 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.37, 158.24, 157.85, 153.05, 148.94, 138.81, 137.42, 132.88, 129.60, 129.43, 128.62, 126.56, 125.84, 123.80, 113.94, 113.62, 112.15, 110.24, 56.09, 56.04, 55.27, 42.08, 32.61, 30.23, 28.59; HRMS calcd for $(C_{38}H_{39}N_3O_8 + H)^+$ 666.2810, found 666.2814.

4.1.24. N-(4,5-bis(3,4-dimethoxybenzyl)-1-methyl-1H-imidazol-2-yl)-N-3,4-dimethoxybenzoyl-3,4-dimethoxybenzamide (**16b**)

16b was prepared as described for the synthesis of **15a** using **9b** (0.199 g, 0.5 mmol) and 3,4-dimethoxybenzoyl chloride (0.200 g, 1 mmol). **16b** (0.188 g, 52%) was obtained as a white solid; mp 138–142 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49 (dd, J = 8.4, 2.0 Hz, 2H), 7.42 (d, J = 2.0 Hz, 2H), 6.75 (d, J = 8.5 Hz, 2H), 6.73 (d, J = 1.8 Hz, 1H), 6.66 (d, J = 8.2 Hz, 1H), 6.60–6.57 (m, 2H), 6.35 (d,

J = 1.7 Hz, 1H), 6.12 (d, J = 8.2 Hz, 1H), 3.91 (s, 6H), 3.85 (s, 2H), 3.84 (s, 2H), 3.82 (s, 6H), 3.81 (s, 6H), 3.70 (s, 3H), 3.60 (s, 3H), 3.12 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.37, 153.10, 149.19, 148.95, 148.80, 147.69, 147.29, 138.83, 137.41, 133.47, 130.12, 126.43, 125.77, 123.88, 120.31, 119.46, 112.17, 110.99, 110.26, 56.09, 56.01, 33.10, 30.25, 29.08; HRMS calcd for $(C_{40}H_{43}N_3O_{10} + H)^+$ 726.3021, found 726.3031.

4.1.25. (2E)-N-(4,5-bis(4-methoxybenzyl)-1-methyl-1H-imidazol-2-yl)-3-(3,4,5-trimethoxyphenyl)-N-((E)-3-(3,4,5-trimethoxyphenyl)acryloyl)acrylamide (**17a**)

17a was prepared as described for the synthesis of **15a** using 8a (0.169 g, 0.5 mmol) and (*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl chloride (0.256 g, 1 mmol). **17a** (0.210 g, 54%) was obtained as a white solid; mp 77–80 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.71 (d, J = 15.5 Hz, 2H), 7.18 (d, J = 8.6 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 6.76 (d, J = 15.5 Hz, 2H), 6.73–6.69 (m, 6H), 6.59 (d, J = 8.7 Hz, 2H), 3.94 (s, 2H), 3.93 (s, 2H), 3.89 (s, 6H), 3.82 (s, 12H), 3.73 (s, 3H), 3.67 (s, 3H), 3.14 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.83, 158.29, 157.86, 153.40, 146.12, 140.70, 137.64, 137.17, 132.54, 129.82, 129.48, 129.29, 128.49, 127.15, 118.13, 114.06, 113.77, 105.83, 60.97, 56.20, 55.13, 32.68, 30.13, 28.68; HRMS calcd for $(C_{44}H_{47}N_3O_{10} + H)^+$ 778.3334, found 778.3334.

4.1.26. (2E)-N-(4,5-bis(3,4-dimethoxybenzyl)-1-methyl-1H-imidazol-2-yl)-3-(3,4,5-trimethoxyphenyl)-N-((E)-3-(3,4,5-trimethoxyphenyl)acryloyl)acrylamide (**17b**)

17b was prepared as described for the synthesis of **15a** using 8b (0.199 g, 0.5 mmol) and (*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl chloride (0.256 g, 1 mmol). **17b** (0.183 g, 43%) was obtained as a white solid; mp 89–93 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.72 (s, 1H), 6.88 (d, J = 1.9 Hz, 1H), 6.82 (s, 1H), 6.80 (s, 1H), 6.78–6.75 (m, 1H), 6.72 (s, 4H), 6.66 (d, J = 8.2 Hz, 1H), 6.53 (d, J = 1.8 Hz, 1H), 6.46 (d, J = 8.2 Hz, 1H), 6.38 (dd, J = 8.2, 1.8 Hz, 1H), 3.97 (s, 2H), 3.94 (s, 2H), 3.89 (s, 6H), 3.83 (s, 12H), 3.81 (s, 3H), 3.75 (s, 3H), 3.73 (s, 3H), 3.68 (s, 3H), 3.16 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.90, 153.52, 149.25, 148.89, 147.78, 147.34, 146.30, 140.75, 137.68, 137.35, 133.22, 130.05, 129.93, 127.05, 120.13, 119.18, 118.24, 111.87, 111.07, 105.85, 61.08, 56.26, 55.76, 33.24, 30.27, 29.16; HRMS calcd for $(C_{46}H_{51}N_3O_{12} + H)^+$ 838.3546, found 838.3548.

4.1.27. (2E)-N-(4-(3,4-dimethoxybenzyl)-5-(4-(diethylamino) benzyl)-1-methyl-1H-imidazol-2-yl)-3-(3,4,5-trimethoxyphenyl)-N-((E)-3-(3,4,5-trimethoxyphenyl)acryloyl)acrylamide (**17c**)

17c was prepared as described for the synthesis of **15a** using **9c** (0.204 g, 0.5 mmol) and (*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl chloride (0.256 g, 1 mmol). **17c** (0.212 g, 50%) was obtained as a white solid; mp 99–101 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 (d, J = 15.5 Hz, 2H), 6.88 (d, J = 1.9 Hz, 1H), 6.81 (dd, J = 8.1, 1.9 Hz, 1H), 6.78 (s, 2H), 6.76 (d, J = 6.8 Hz, 2H), 6.71 (s, 4H), 6.67 (d, J = 8.2 Hz, 1H), 6.35 (d, J = 8.7 Hz, 2H), 3.93 (s, 4H), 3.89 (s, 6H), 3.82 (s, 12H), 3.81 (s, 3H), 3.71 (s, 3H), 3.20 (q, J = 7.0 Hz, 4H), 3.16 (s, 3H), 1.05 (t, J = 7.0 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.97, 153.49, 148.83, 147.25, 146.57, 146.21, 140.69, 137.48, 133.44, 129.91, 128.46, 127.68, 123.77, 120.22, 118.19, 111.97, 111.04, 105.78, 100.00, 61.07, 56.23, 55.77, 44.29, 33.24, 30.26, 28.54, 12.49; HRMS calcd for $(C_{48}H_{56}N_4O_{10} + H)^+$ 849.4069, found 849.4079.

4.1.28. N-(3-(3,4,5-trimethoxyphenyl)propanoyl)-N-(4,5-bis(3,4-dimethoxybenzyl)-1-methyl-1H-imidazol-2-yl)-3-(3,4,5-trimethoxyphenyl)propanamide (**18b**)

18b was prepared as described for the synthesis of **15a** using **9b** (0.199 g, 0.5 mmol) and 3-(3,4,5-trimethoxyphenyl)propanoyl chloride (0.258 g, 1 mmol). **18b** (0.155 g, 37%) was obtained as a white solid; mp 103–105 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.76 (d, J = 1.7 Hz, 1H), 6.67 (dt, J = 16.5, 5.0 Hz, 3H), 6.50 (dd, J = 8.2, 1.7 Hz, 1H), 6.36 (d, J = 3.9 Hz, 5H), 3.89 (s, 4H), 3.82 (s, 3H), 3.81 (s, 3H), 3.80 (s, 6H), 3.78 (s, 12H), 3.73 (s, 3H), 3.60 (s, 3H), 3.11–3.04 (m, 2H), 2.90 (s, 3H), 2.90–2.83 (m, 4H), 2.73–2.65 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.38, 153.27, 149.33, 148.92, 147.80, 147.40, 137.29, 137.24, 136.50, 136.22, 133.08, 129.92, 127.06, 120.12, 119.60, 111.76, 111.26, 111.15, 110.71, 105.45, 100.00, 60.92, 60.90, 56.15, 55.98, 39.71, 33.12, 30.92, 29.85, 29.14; HRMS calcd for $(C_{46}H_{55}N_3O_{12} + H)^+$ 842.3859, found 842.3854.

4.2. Material for biological studies

DMSO and taxol were purchased from Sigma–Aldrich. Dulbecco's modified Eagle's medium (DMEM), trypsin–ethylenediaminetetraacetic acid (EDTA), and penicillin/streptomycin were from Gibco BRL. Fetal bovine serum (FBS) was from Hyclone Laboratories. 2-(4, 5-Dimethylthiazol-2-yl)-5-[3-(carboxymethoxy)phenyl]-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) and phenazine methosulfate (PMS) were purchased from Promega. Human breast cancer cell lines LCC6 and LCC6MDR were kindly provided by Dr. Robert Clarke (Georgetown University, Washington, DC). L929, a mouse connective tissue fibroblast, was purchased from ATCC [14].

4.3. Cell culture

LCC6, LCC6MDR and L929 cells were maintained in DMEM media supplemented with 10% (v/v) heat-inactivated FBS and 100 units/ml penicillin and 100 μg/mL streptomycin. Cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2. When the cells reached confluence, they were detached by 0.05% trypsin-EDTA [14,38].

4.4. P-gp modulating activity

Six-thousand cells of LCC6MDR and anticancer drug taxol were mixed with or without modulators to a final volume of 200 μL in each well of 96-well plates. The plates were then incubated for 5 days at 37 °C. The Cell Titer 96 aqueous assay was used to measure the cell proliferation according to the manufacturer's instructions. MTS (2 mg/mL) and PMS (0.92 mg/mL) were mixed in a ratio of 20:1. An aliquot (10 μL) of the freshly prepared MTS/PMS mixture was added to each well and the plate was incubated for 2 h at 37 °C. Optical absorbance at 490 nm was then recorded with a microplate absorbance reader (Bio-Rad). IC_{50} values were calculated from the doseresponse curves of MTS assays (Prism 4.0). All experiments were performed in triplicate and repeated at least thrice, and the results were represented as mean ± standard error of mean [14,39].

4.5. Cytotoxicity assay

Ten-thousand cells of LCC6-MDR or LCC6 or L929 were mixed with a series of concentrations of naamidine analogues to a final volume of 100 μL in each well of 96-well plates. The plates were then incubated for 3 days at 37 °C. Then 50% inhibitory concentration (IC_{50}) of naamidine analogues was determined using MTS proliferation assay as described previously [14].

Acknowledgment

This work was supported by grants from National Natural Science Foundation of China (NSFC) (81373322), NSFC-Shandong Joint Fund (U1406402) and a Special Fund for Marine Scientific Research in the Public Interest of China (201005024).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.06.016.

References

[1] R. Krishna, L.D. Mayer, Multidrug resistance (MDR) in cancer. Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs, Eur. J. Pharm. Sci. 11 (2000) 265–283.

[2] E. Teodori, S. Dei, S. Scapecchi, F. Gualtieri, The medicinal chemistry of multidrug resistance (MDR) reversing drugs, Farmaco 57 (2002) 385–415.

[3] C.H. Choi, ABC transporters as multidrug resistance mechanisms and the development of chemosensitizers for their reversal, Cancer Cell. Int. 5 (2005) 30.

[4] R.F. Ozols, R.E. Cunnion, R.W. Klecker Jr., T.C. Hamilton, Y. Ostchega, J.E. Parrillo, R.C. Young, Verapamil and adriamycin in the treatment of drug-resistant ovarian cancer patients, J. Clin. Oncol. 5 (1987) 641–647.

[5] J.S. Kuhl, B.I. Sikic, K.G. Blume, N.J. Chao, Use of etoposide in combination with cyclosporin for purging multidrug-resistant leukemic cells from bone marrow in a mouse model, Exp. Hematol. 20 (1992) 1048–1054.

[6] F.J. Sharom, The P-glycoprotein efflux pump: how does it transport drugs? J. Membr. Biol. 160 (1997) 161–175.

[7] P.R. Twentyman, N.M. Bleehen, Resistance modification by PSC-833, a novel non-immunosuppressive cyclosporin [corrected], Eur. J. Cancer 27 (1991) 1639–1642.

[8] M. Cvetkovic, B. Leake, M.F. Fromm, G.R. Wilkinson, R.B. Kim, OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine, Drug Metab. Dispos. 27 (1999) 866–871.

[9] S. Nobili, I. Landini, B. Giglioni, E. Mini, Pharmacological strategies for overcoming multidrug resistance, Curr. Drug Targets 7 (2006) 861–879.

[10] P. Ruff, D.A. Vorobiof, J.P. Jordaan, G.S. Demetriou, S.D. Moodley, A.L. Nosworthy, I.D. Werner, J. Raats, L.J. Burgess, A randomized, placebo-controlled, double-blind phase 2 study of docetaxel compared to docetaxel plus zosuquidar (LY335979) in women with metastatic or locally recurrent breast cancer who have received one prior chemotherapy regimen, Cancer Chemother. Pharmacol. 64 (2009) 763–768.

[11] C.M. Kruijtzer, J.H. Beijnen, H. Rosing, W.W. ten Bokkel Huinink, M. Schot, R.C. Jewell, E.M. Paul, J.H. Schellens, Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and P-glycoprotein inhibitor GF120918, J. Clin. Oncol. 20 (2002) 2943–2950.

[12] I.E. Kuppens, T.M. Bosch, M.J. van Maanen, H. Rosing, A. Fitzpatrick, J.H. Beijnen, J.H. Schellens, Oral bioavailability of docetaxel in combination with OC144-093 (ONT-093), Cancer Chemother. Pharmacol. 55 (2005) 72–78.

[13] J. Yuan, I.L. Wong, T. Jiang, S.W. Wang, T. Liu, B.J. Wen, L.M. Chow, B. Wan Sheng, Synthesis of methylated quercetin derivatives and their reversal activities on P-gp- and BCRP-mediated multidrug resistance tumour cells, Eur. J. Med. Chem. 54 (2012) 413–422.

[14] P.Y. Zhang, I.L. Wong, C.S. Yan, X.Y. Zhang, T. Jiang, L.M. Chow, S.B. Wan, Design and syntheses of permethyl ningalin B analogues: potent multidrug resistance (MDR) reversal agents of cancer cells, J. Med. Chem. 53 (2010) 5108–5120.

[15] J.W. Bin, I.L. Wong, X. Hu, Z.X. Yu, L.F. Xing, T. Jiang, L.M. Chow, W.S. Biao, Structure-activity relationship study of permethyl ningalin B analogues as p-glycoprotein chemosensitizers, J. Med. Chem. 56 (2013) 9057–9070.

[16] L. Gao, Q. Liu, S.M. Ren, S.B. Wan, T. Jiang, I.L.K. Wong, L.M.C. Chow, S.X. Wang, Synthesis of a novel series of (E,E)-4,6-bis(styryl)-2-O-glucopyranosyl-pyrimidines and their potent multidrug resistance (MDR) reversal activity against cancer cells, J. Carbohydr. Chem. 31 (2012) 620–633.

[17] R.A. Nkansah, J.D. Sullivan, R.L. Giles, R.E. Looper, Synthetic design and biological activity of 2-aminoimidazole marine natural products, Abstr. Pap. Am. Chem. Soc. 241 (2011).

[18] P.B. Koswatta, C.J. Lovely, Structure and synthesis of 2-aminoimidazole alkaloids from *Leucetta* and *Clathrina* sponges, Nat. Prod. Rep. 28 (2011) 511–528.

[19] D.C. Dunbar, J.M. Rimoldi, A.M. Clark, M. Kelly, M.T. Hamann, Anti-cryptococcal and nitric oxide synthase inhibitory imidazole alkaloids from the calcareous sponge *Leucetta* cf *chagosensis*, Tetrahedron 56 (2000) 8795–8798.

[20] I. Mancini, G. Guella, C. Debitus, F. Pietra, Novel naamidine-type alkaloids and mixed-ligand zinc(II) complexes from a calcareous sponge, *Leucetta* sp., of the Coral sea, Helvetica Chim. Acta 78 (1995) 1178–1184.

[21] N. Aberle, J. Catimel, E.C. Nice, K.G. Watson, Synthesis and biological evaluation of analogues of the anti-tumor alkaloid naamidine A, Bioorg. Med. Chem. Lett. 17 (2007) 3741–3744.

[22] B.R. Copp, C.R. Fairchild, L. Cornell, A.M. Casazza, S. Robinson, C.M. Ireland, Naamidine A is an antagonist of the epidermal growth factor receptor and an in vivo active antitumor agent, J. Med. Chem. 41 (1998) 3909–3911.

[23] R.D. James, D.A. Jones, W. Aalbersberg, C.M. Ireland, Naamidine A intensifies the phosphotransferase activity of extracellular signal-regulated kinases causing A-431 cells to arrest in G1, Mol. Cancer Ther. 2 (2003) 747–751.

[24] S. Tsukamoto, T. Kawabata, H. Kato, T. Ohta, H. Rotinsulu, R.E. Mangindaan, R.W. van Soest, K. Ukai, H. Kobayashi, M. Namikoshi, Naamidines H and I, cytotoxic imidazole alkaloids from the Indonesian marine sponge *Leucetta chagosensis*, J. Nat. Prod. 70 (2007) 1658–1660.

[25] A. Seelig, X.L. Blatter, F. Wohnsland, Substrate recognition by P-glycoprotein and the multidrug resistance-associated protein MRP1: a comparison, Int. J. Clin. Pharmacol. Ther. 38 (2000) 111–121.

[26] M. Schmidt, J. Ungvari, J. Glode, B. Dobner, A. Langner, New 1,3-dioxolane and 1,3-dioxane derivatives as effective modulators to overcome multidrug resistance, Bioorg. Med. Chem. 15 (2007) 2283–2297.

[27] D. Berger, R. Citarella, M. Dutia, L. Greenberger, W. Hallett, R. Paul, D. Powell, Novel multidrug resistance reversal agents, J. Med. Chem. 42 (1999) 2145–2161.

[28] P.B. Koswatta, C.J. Lovely, Total syntheses of naamidine G and 14-methoxynaamidine G, Tetrahedron Lett. 51 (2010) 164–166.

[29] S. Nakamura, I. Kawasaki, M. Kunimura, M. Matsui, Y. Noma, M. Yamashita, S. Ohta, Total synthesis of naamine C and pyronaamidine, antitumor marine imidazole alkaloids, J. Chem. Soc. Perk Trans. 1 (2002) 1061–1066.

[30] S. Ohta, N. Tsuno, S. Nakamura, N. Taguchi, M. Yamashita, I. Kawasaki, M. Fujieda, Total syntheses of naamine A and naamidine A, marine imidazole alkaloids, Heterocycles 53 (2000) 1939–1955.

[31] F. Leonessa, D. Green, T. Licht, A. Wright, K. Wingate-Legette, J. Lippman, M.M. Gottesman, R. Clarke, MDA435/LCC6 and MDA435/LCC6MDR1: ascites models of human breast cancer, Br. J. Cancer 73 (1996) 154–161.

[32] C.F. Higgins, M.M. Gottesman, Is the multidrug transporter a flippase? Trends Biochem. Sci. 17 (1992) 18–21.

[33] Y. Raviv, H.B. Pollard, E.P. Bruggemann, I. Pastan, M.M. Gottesman, Photosensitized labeling of a functional multidrug transporter in living drug-resistant tumor cells, J. Biol. Chem. 265 (1990) 3975–3980.

[34] L. Homolya, Z. Hollo, U.A. Germann, I. Pastan, M.M. Gottesman, B. Sarkadi, Fluorescent cellular indicators are extruded by the multidrug resistance protein, J. Biol. Chem. 268 (1993) 21493–21496.

[35] I.K. Pajeva, M. Wiese, Pharmacophore model of drugs involved in P-glycoprotein multidrug resistance: explanation of structural variety (hypothesis), J. Med. Chem. 45 (2002) 5671–5686.

[36] P.B. Koswatta, R. Sivappa, H.V. Dias, C.J. Lovely, Total synthesis of (+/−)-calcaridine A and (+/−)-epi-calcaridine A, Org. Lett. 10 (2008) 5055–5058.

[37] A. Ghinet, B. Rigo, J.P. Henichart, D. Le Broc-Ryckewaert, J. Pommery, N. Pommery, X. Thuru, B. Quesnel, P. Gautret, Synthesis and biological evaluation of phenstatin metabolites, Bioorg. Med. Chem. 19 (2011) 6042–6054.

[38] K.F. Chan, Y.Z. Zhao, B.A. Burkett, I.L.K. Wong, L.M.C. Chow, T.H. Chan, Flavonoid dimers as bivalent modulators for P-glycoprotein-based multidrug resistance: synthetic apigenin homodimers linked with defined-length poly(ethylene glycol) spacers increase drug retention and enhance chemosensitivity in resistant cancer cells, J. Med. Chem. 49 (2006) 6742–6759.

[39] I.L.K. Wong, K.F. Chan, K.H. Tsang, C.Y. Lam, Y. Zhao, T.H. Chan, L.M.C. Chow, Modulation of multidrug resistance protein 1 (MRP1/ABCC1)-mediated multidrug resistance by bivalent apigenin homodimers and their derivatives, J. Med. Chem. 52 (2009) 5311–5322.