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# Novel symmetrical ureas as modulators of protein arginine methyl transferases

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ABSTRACT

Methylation of histone arginine residues is an epigenetic mark related to gene expression that is implicated in a variety of biological processes and can be reversed by small-molecule modulators of protein arginine methyltransferases (PRMTs). A series of symmetrical ureas, designed as analogues of the known PRMT1 inhibitor AMI-1 have been synthesized using Pd-catalyzed Ar–N amide bond formation processes or carbonylation reactions as key steps. Their inhibitory profile has been characterized. The enzymatic assays showed a weak effect on PRMT1 and PRMT5 activity for most of the compounds. The acyclic urea that exhibited the strongest effect on the inhibition of the PRMT1 activity also showed the greatest effect on the expression of some androgen receptor target genes (TMPRSS2 and FKBP5), which may be related with its enzymatic activity. Surprisingly, AMI-1 behaved as an activator of PRMT5 activity, a result not reported so far.



## 1. Introduction

Methylation of lysine and arginine residues of histones by methyl transferases (KMTs and PRMTs, respectively) is related to gene expression.[1] Contrarily to histone acetylation, histone methylation is an epigenetic mark that does not alter the total charge of the histone tails, although it reduces the affinity for anionic molecules such as DNA due to the increase of basicity and hydrophobicity of the histone tail. A large number of methyltransferases of arginine and lysine residues has been identified,[2] and most use the methyl group donor *S*-adenosyl-L-methionine (*S*-AdoMet or SAM). The process can be viewed as a classical nucleophilic substitution reaction at the methyl group by the partially deprotonated terminal amino groups of histones lysine or arginine residues.[3] Whereas histone lysine methylation produces increasingly methylated amines, histone arginine methylation can generate both mono- and/or dimethylarginine derivatives, and the latter as symmetric or non-symmetric isomers.

At least eleven mammalian PRMT family members that share a highly conserved catalytic domain have been identified to date.[1c,4] They are classified in four groups according to their product selectivity. Type I PRMTs (PRMT1, 3, 4, 6 and 8) produce non-symmetric dimethylarginines, Type II PRMTs (PRMT5, 7 and 9) form the symmetric counterparts, and both types catalyze the formation of monomethylarginine, Type III produces monomethylarginine marks only (PRMT7 has shown this activity on certain substrates), and Type IV monomethylates the internal guanidinium nitrogen and has only been characterized in yeast. The activity of PRMT2, 10 and 11 has yet to be fully characterized.

Arginine methylation is implicated in a variety of biological processes that include euchromatin maintenance, RNA processing, signal transduction, transcriptional regulation and DNA repair.[1c,4b] PRMT4/CARM1 is a co-activator of androgen and estrogen receptors, and is found overexpressed in hormone-dependent prostate and breast tumors. Targeting PRMTs with small-molecule modulators is potentially a promising approach to treat different diseases, for instance cancer of the prostate and breast where this approach would allow indirect targeting of the steroid receptors via their associated cofactors.

Inhibitors of methyl-transferase enzymes that are based on the structure of the SAM cofactor or its product *S*-adenosyl-L-homocysteine (AdoHcy), such as AdOx, methylthioadenosine and sinefungin, are in general unselective.[5] A bisubstrate structure which incorporates an arginine end group at the position of the methyl sulfonium ion of SAM is however more selective for PRMT inhibition and discriminates between PRMT1 and CARM1 in favor of the former.[6]

Non-mechanism based inhibitors of arginine methylation (AMIs; for example, AMI-1, 1 and AMI-3, 2) were discovered by HTS of compound libraries (Fig. 1).[7] AMI-1 **1**, which contains a symmetrical sulfonated naphthylurea, was characterized as a selective and cell-permeable PRMT inhibitor and was proposed to dock to the AdoMet binding pocket, based on molecular modeling.[8]

Also shown in Figure 1 are selected PRMT inhibitors, including allantodapsone **3**,[9] stilbamidine **4**,[9] α-methylthioglycolic amide **5**

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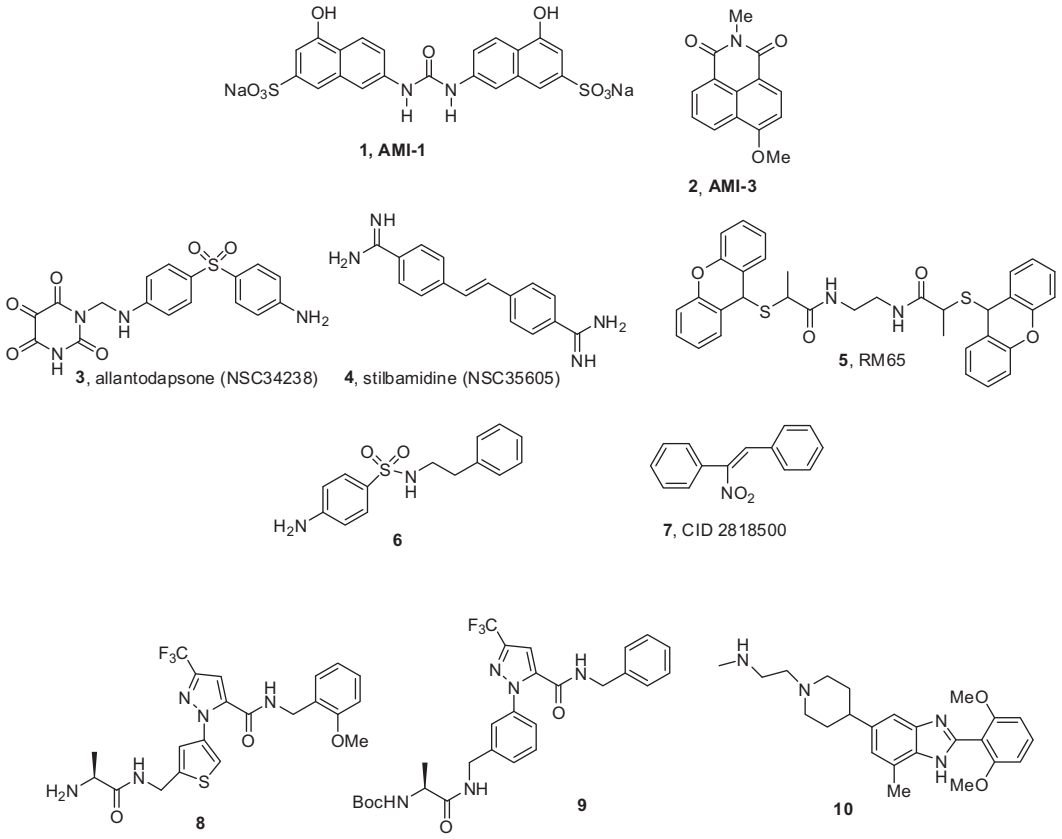



**Figure 1.** Selected PRMT modulators.

(RM-65),[10] benzo[*b*]imidazoles,[10b,11] sulfonamide **6**,[10b] nitroderivative **7**,[12] and pyrazoles **8**, **9** and **10**, which are nanomolar inhibitors of CARM1.[11,13]

Analogues of AMI-1 **1** where the sulfonic acid and urea groups are replaced by carboxylic acid and bis-amide functionalities, respectively, have recently been reported (Fig. 2),[14] with bis-carboxylic acid derivative **11** and its positional isomer as the most potent and selective PRMT inhibitors.[14] Diazocompounds related to AMI-1 that preserve the naphthosulfonic acid (**12**) have been found to directly target the substrate.[15] Acyl derivatives of *para*-aminosulfonamides and dapsone **13** also contain a urea but in non-symmetrical structures.[16] Recently, small-molecule enhancers of arginine methylation with aryl ureido acetamido indole carboxylate structures which function as CARM1 activators have been discovered.[17]

Based on the scaffolds discovered by Bedford et al.[7] we started a program aiming at developing inhibitors of PRMT1 and focused on the symmetrical structure of AMI-1 **1**. We designed analogues (Fig. 2) in which ureas are part of pyrimidin-2-ones and imidazolidin-2-ones heterocycles (Series I), and others with greater conformational flexibility around the termini by using unsaturated and saturated monocyclic and acyclic analogues of the naphthyl groups (Series II). We also considered a third group of analogues in which the carboxylic acid was replaced by a sulfonamide, which confers greater length to these uncharged molecules (Series III).

## 2. Chemistry

The synthesis of Series I includes an amidation of halobenzaldehydes with ureas[18] and a Horner–Wadsworth–Emmons reaction to construct the unsaturated chain of the cinnamates. For the first step Xantphos **17** was selected as ligand of Pd(0) or Pd(II) precatalysts in a 1:3 Pd/L ratio (to avoid aryl exchange processes in the phosphine),[19] with $Cs_2CO_3$ as base in dioxane,[20] conditions that

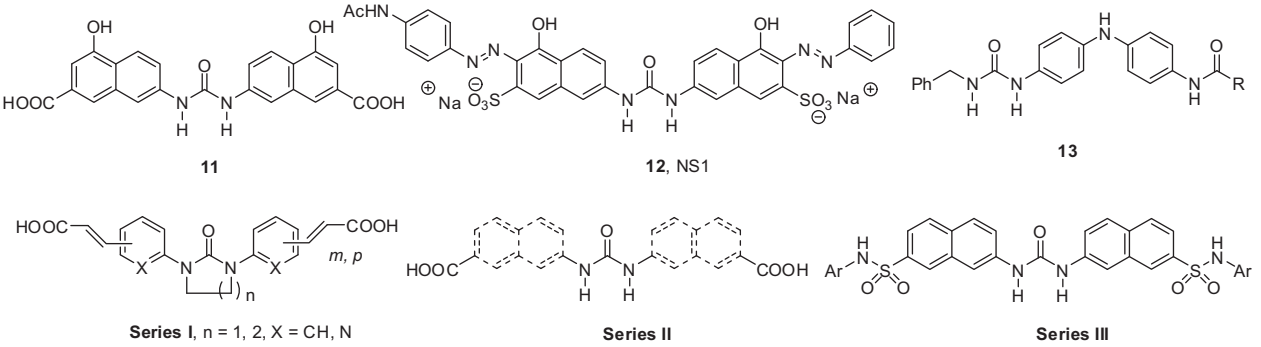



**Figure 2.** AMI-1-based PRMT inhibitors and novel structures reported in this work.

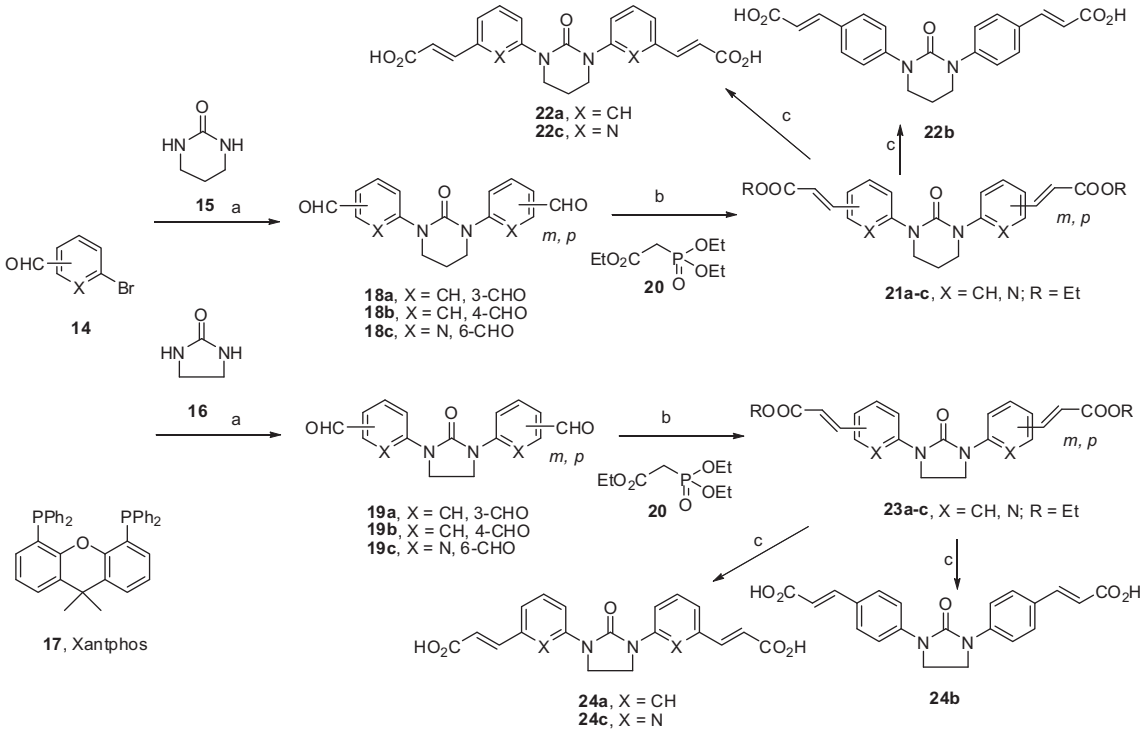
had proven successful for the preparation of both symmetrical and non-symmetrical ureas.[21] The reaction of bromobenzaldehydes with urea led to disappointing yields (4–7% symmetrical substituted ureas), which did not improve with changes in reaction conditions. Using ethyl *p*-bromobenzoate and a 1:3 Pd/L ratio the major products were the monosubstituted urea in dioxane (22% yield), and the symmetrical urea (62% yield) in DMF. This general procedure using DMF proved useful for the direct coupling of cyclic ureas to bromoarylaldehydes. The reaction of the bromobenzaldehyde isomers **14a–b** and the analogue 2-pyridinecarboxaldehyde **14c** with tetrahydropyrimidinin-2-(1*H*)-one **15** and imidazolidin-2-one **16** provided the corresponding adducts **18a–c** and **19a–c**. In general the coupling product was obtained in good to excellent yields with minor amounts of the monosubstituted derivative in selected cases (4% and 1% yield, respectively, in the case of **18c** and **19c**). Only the reaction of pyridinecarboxaldehyde **14c** with imidazolidin-2-one **16** was inefficient (28% yield). In addition, no reaction was obtained when *p*-bromobenzaldehyde was treated with other cyclic ureas, such as 1*H*-benzo[*d*]imidazol-2(3*H*)-one, with thiourea or with tetrahydropyrimidinin-2-(1*H*)-thione. In the case of *N*,*N*′-dimethylurea the reaction afforded instead a diarylamine, 4,4′-(methylazanediyl)dibenzaldehyde, the result of a rearrangement of the monosubstituted urea with release of methylisocyanate, as described previously.[22]

Chain extension was based on the HWE reaction[23] of the corresponding benzaldehydes with the anion of ethyl 2-(diethoxyphosphonyl)acetate **20** generated with *n*-BuLi in THF/DMPU (for **19c**, HNa in DMF was used due to solubility problems). Yields were good to excellent in all cases with the exception of the reaction of **19b**. The condensation took place with complete stereoselectivity and only the *E* unsaturated diesters **21a–c** and **23a–c** were obtained, as judged from the analysis of the vinyl signals on the $^1$H NMR spectra. Final saponification using aqueous NaOH in EtOH at 80 °C (25 °C for **22b**) afforded the desired symmetrical acrylates with heterocyclic ureas as connectors **22a–c** and **24a–c** in variable yields that were in general higher for the tetrahydropyrimidinin-2-(1*H*)-ones **22** (Scheme 1).

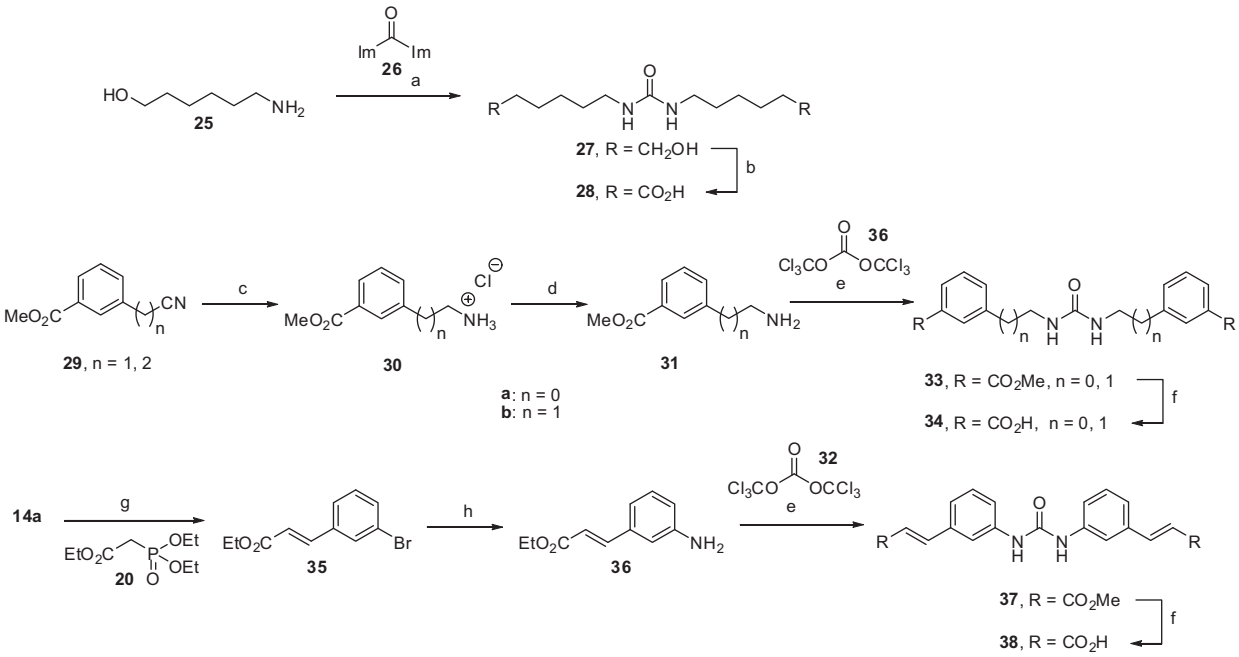
For Series II, which features alkenyl or alkylureas as substituents of benzoic acids, we selected instead the carbonylation of the corresponding amines (Scheme 2). The reaction of commercial methyl 6-aminohexanoate with carbonyldiimidazole **26**[24] or with $Co_2(CO)_8$ as carbonyl sources under microwave irradiation[25] led to the substituted urea in 65% and 44%, respectively. However, saponification of the ester was unsuccessful using a variety of bases (10% aqueous NaOH, $Ba(OH)_2$, LiOH, $KOSiMe_3$, $Me_3SnOH$). Alternatively, the carbonylation of 6-aminohexanol **25** with carbonyldiimidazole **26** (30%) followed by Jones oxidation (72%) of **27** provided the desired urea **28** (Scheme 2).

The synthesis of the ureas attached to benzoic acids via an alkyl chain involved the carbonylation of the amines obtained by reduction of the nitriles (Scheme 2). Commercial 3-cyanobenzoate **29a** and homologue **29b** (prepared by displacement of methyl 3-bromomethylbenzoate with NaCN in DMF in 85% yield) were hydrogenated in the presence of 10% Pd/C and HCl (83% and 63%, respectively) and the hydrochlorides **30** neutralized with $Et_3N$. Using triphosgene **32** as carbonyl source, the ureas **33a** and **33b** were acquired in 89% and 86% yield, respectively, and then were saponified by treatment with 10% LiOH in THF to afford carboxylic acids **34a** (82%) and **34b** (61%).

The carbonylation method was also successful for the preparation of **38** (Scheme 2). In this case, the steps were reversed, with a HWE to cinnamate **35** preceding the formation of the aniline **36** upon treatment of **35** with sodium azide in a DMSO/$H_2O$ mixture using a CuI/L-proline catalytic system at 110 °C.[26] Addition of triphosgene **32** to the aniline provided diester **37** and final hydrolysis led to dicarboxylic acid **38** in the yields shown in Scheme 2.




**Scheme 1.** Synthesis of analogues of Series I. Reagents and conditions: (a) **15** or **16**, $Pd_2dba_3{\cdot}CHCl_3$, Xantphos **17**, $Cs_2CO_3$, DMF, 100 °C (**18a**, 52%; **18b**, 81%; **18c**, 71%; **19a**, 91%; **19b**, 72%; **19c**, 28%); (b) *n*-BuLi, DMPU, THF, 0 to −78 °C, 2 h (**21a**, 90%; **21b**, 81%; **21c**, 90%; **23a**, 81%; **23b**, 12%; **23c**, 60%); (c) 10% aq NaOH, EtOH, 80 °C, 17 h (**22a**, 83%; **22b**, 80%; **22c**, 36%; **24a**, 48%; **24b**, 30%; **24c**, 86%).



**Scheme 2.** Synthesis of analogues of Series II. Reagents and conditions: (a) $Et_3N$, THF, 25 °C, 120 h, 30%; (b) $CrO_3$, $H_2SO_4$, acetone/$H_2O$, 25 °C, 2 h, 72%; (c) $H_2$, Pd/C 10%, HCl, 25 °C, 17 h (**30a**, 83%; **30b**, 63%); (d) $Et_3N$, $CH_2Cl_2$, 30 min; (e) (i) $Et_3N$, $C_6H_6$, 25 °C, 12 h, (ii) **31** or **36**, acetone, 25 °C, 2 h (**33a**, 89%; **33b**, 86%; **37**, 71%); (f) 10% aq LiOH, THF, 80 °C, 24 h (**34a**, 82%; **34b**, 61%; **38**, 86%); (g) *n*-BuLi, DMPU, THF, from −78 to 25 °C, 6 h, 96%; (h) $NaN_3$, CuI, L-proline, NaOH, DMSO, EtOH, 110 °C, 20 h, 56%.

Unfortunately, the formation of thioureas with Lawesson's reagent[27] was unsuccessful for models **33a** and **34a**, which were fully recovered after 17 h stirring at 70 °C.

Lastly, longer analogues of AMI-1, Series III, represented by sulfonamides **45**, were prepared from 7-amino-2-naphthalenesulphonic acid **39**.[28] The sequence (Scheme 3) comprises acetylation of the aniline ($Ac_2O$, pyridine, 84%), formation of the sodium sulphonate (MeONa, MeOH, 57%), activation as sulphonyl chloride ($POCl_3$, DMA, 44%) and addition of the corresponding anilines **41** in the presence of Hünig's base. No reaction took place with halogenated anilines (2,5-dibromoaniline, 4-chloro-3-trifluoromethylaniline and 4-ciano-3-trifluoromethylaniline). Reaction of **42** with 5 M NaOH and work-up afforded the ammonium salts, which were treated with $Et_3N$ and used in the next reaction. Their treatment with triphosgene **32** according to the above method provided **45a** and **45b** in low yields (23% and 7%), which might be due to solubility problems and difficulties in the purification process.

## 3. Results and discussion

### 3.1. Activity on PRMT enzymes

We first tested the activity of the synthesized compounds on recombinant PRMTs using AMI-1 **1** as standard for enzyme inhibition. The assays measured the modulation of human recombinant PRMT1 (expressed in *E. coli*) and PRMT5 (expressed in *Sf*9 cells) enzymes using histone H4 peptide as substrate precoated in a commercially available enzymatic assay kit.[29] PRMT1 and PRMT5 were chosen as representative members of the type I and type II enzymes and catalyze the non-symmetric and symmetric dimethylation of arginines, respectively. AMI-3 **2** ($IC_{50}$ of 16.3 μM for human PRMT1)[7] was also included in this study in order to have a broader comparison with other PRMT inhibitors.

The results show (Fig. 3A) that at 100 μM AMI-1 **1** was the most potent inhibitor of PRMT1 enzymatic activity (an $IC_{50}$ value of 8.8 μM was previously measured for AMI-1). Compound **28** proved to be the second most potent compound, followed by AMI-3 **2**, and both showed very potent inhibition of enzymatic activity. Other compounds like **22a**, **24a** and **22c** also inhibited PRMT1 activity but less potently than those mentioned before. The remaining compounds exhibited significantly weaker inhibition. The $IC_{50}$ values of the two most potent compounds, **28** and **24a** are 6.1 μM and 25.2 μM, respectively (see SI).

A similar assay performed to measure the PRMT5 enzymatic activity assay revealed that AMI-1 **1** is an activator of PRMT5, whereas **24c** and **34b** proved to be the most potent inhibitors (Fig. 3B).

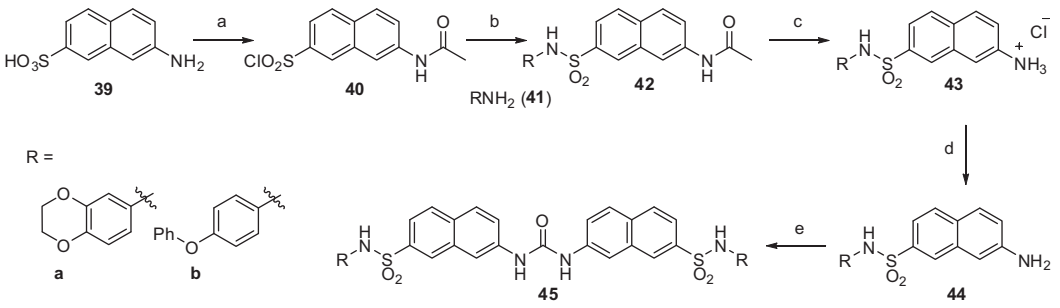
### 3.2. Cellular characterization of the AMI-1 analogues

#### 3.2.1. Effect on cell viability and proliferation

After completing the enzymatic PRMT assays, the effect of the compounds on the proliferation and viability of VCaP and LAPC-4 prostate cancer cell lines was tested. None of these compounds showed an effect on proliferation, except compounds **45a** and **45b**, which had inhibitory effects on the LAPC-4 cell line. The $IC_{50}$ values for **45a** and **45b** were 1.5 and 8.6 μM, respectively. The effect in the case of **45b** may be due to toxic (non-apoptotic) effects related to the high concentration used, since a dose-dependent effect on the proliferation was not observed.

#### 3.2.2. Effect on the expression of androgen receptor target genes

As mentioned before, several PRMTs have been described as cofactors/co-activators of the androgen and estrogen class of nuclear receptors, making them putative targets for the treatment of hormone-dependent cancers.[7,16,30] In order to determine the effects of the analogues on the expression of androgen-dependent genes in VCaP cells, we quantified the mRNA levels of selected genes after androgen stimulation in the absence or in the presence of the compounds (not shown). Among all the modulators tested, only **28** showed a significant effect. We therefore analyzed in detail the effects of this compound on a number of androgen-controlled genes in the VCaP cell line. Using different concentrations we could




**Scheme 3.** Synthesis of analogues of Series III. Reagents and conditions: (a) (i) $Ac_2O$, pyridine, 25 °C, 8 h, 84%, (ii) MeONa, MeOH, 25 °C, 12 h, 57%; (iii) $POCl_3$, DMA, 25 °C, 24 h, 44%; (b) R-$NH_2$ **41**, Hünig base, THF/$CHCl_3$, 25 °C, 12 h (**42a**, 44%; **42b**, 53%); (c) 5 M NaOH, MeOH, 60 °C, 120 h (**43a**, 88%; **43b**, 91%). (d) $Et_3N$, $CH_2Cl_2$, 30 min; (e) (i) triphosgene **32**, $Et_3N$, $C_6D_6$, 25 °C, 12 h, (ii) **44**, acetone 2 h (**45a**, 23%; **45b**, 7%).

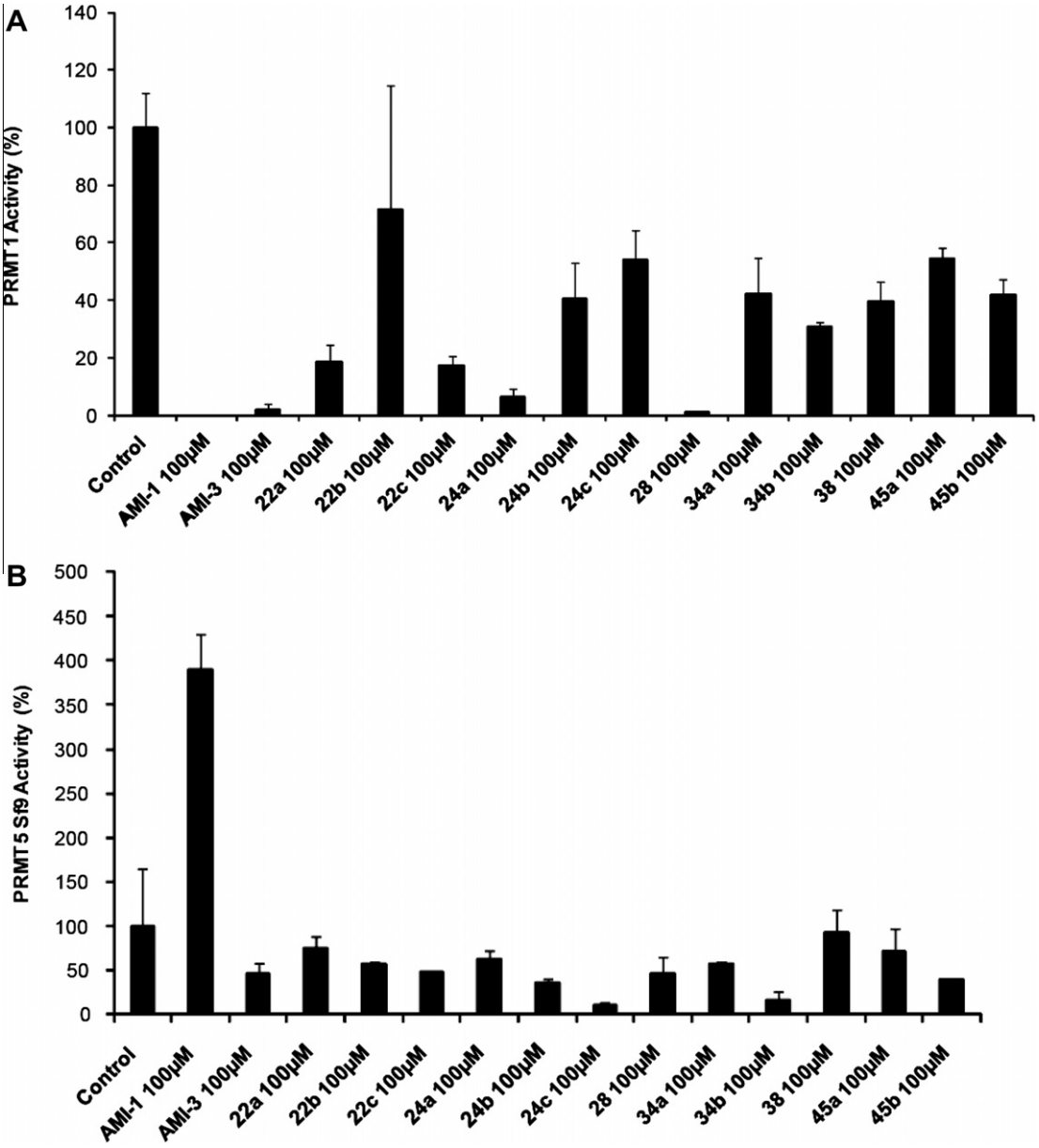



**Figure 3.** In vitro methylation assays to evaluate the effect of compounds on PRMT activity. (A) PRMT1 assay in the presence of AMI-1 **1**, AMI-3 **2** and compounds of Series I, II and III. (B) PRMT5 assay in the presence of the same compounds. The data represent the average value of independent duplicates; error bars represent standard deviation (SD) of biological duplicates. See Section 5 for a detailed experimental procedure.

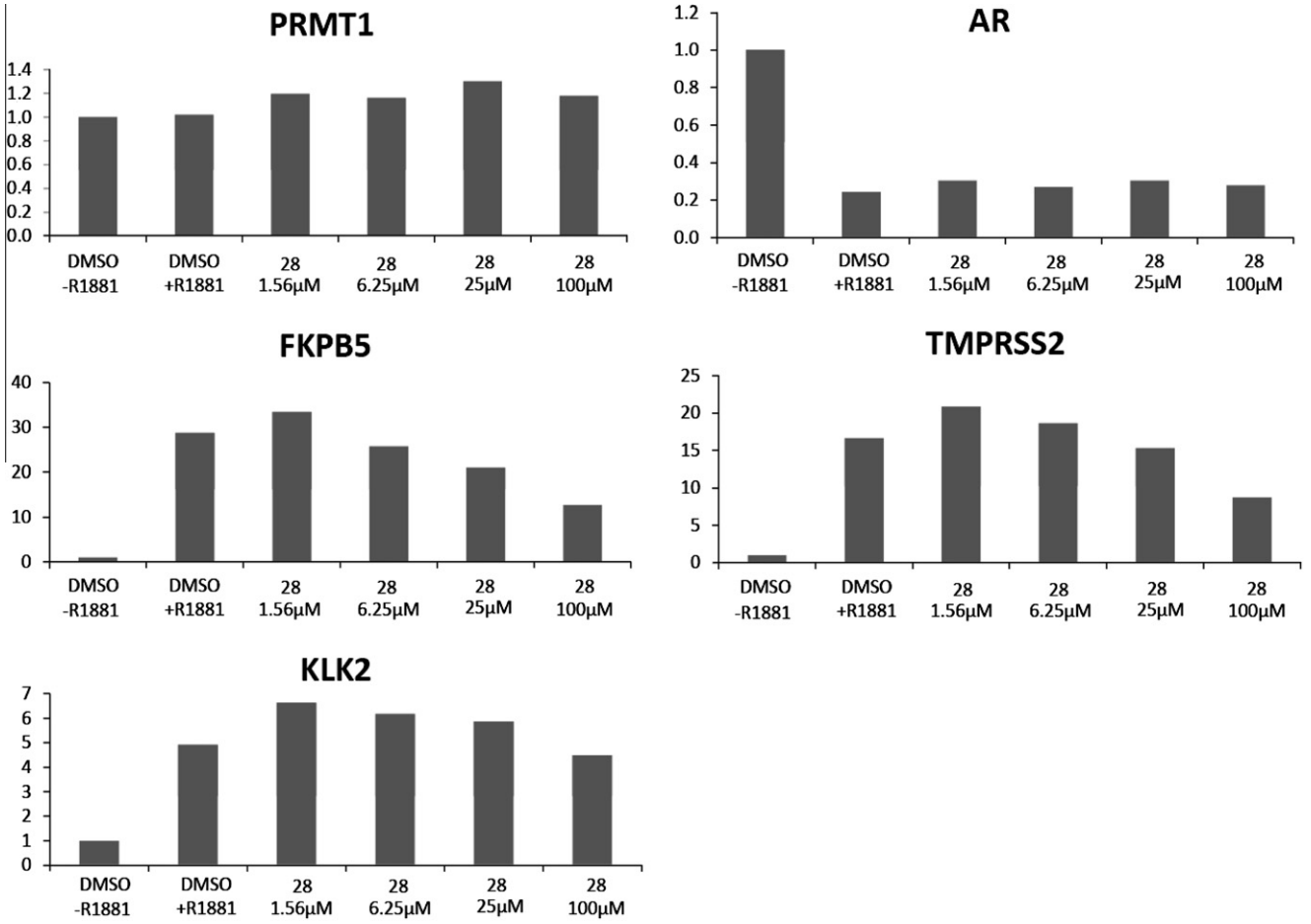



**Figure 4.** Transcriptional regulation of androgen-dependent genes by compound **28**. The first bar of the graphs represents the expression without androgen stimulation. The second bar shows the expression after treatment with R1881, a synthetic non-aromatizable androgen used for AR activation. The other bars correspond to the expression levels in presence of androgen and of increasing concentrations of compound **28**. The data represent the average value of independent triplicates.

demonstrate the specific, dose-dependent inhibitory effects of **28** on the expression of several genes that are stimulated by androgens such as TMPRSS2 and FKBP5. This was however not seen for all androgen-regulated genes since the levels of KLK2 remained unaltered. The expression of PRMT1, which is not androgen dependent, was not affected either. AR levels were reduced by androgen treatment of VCaP cells, as reported before,[31] but did not vary after application of **28** (Fig. 4).

## 4. Conclusions

Three series of analogues of the known PRMT1 inhibitor AMI-1 have been synthesized using as key steps Pd-catalyzed Ar–N amide bond formation processes for cyclic ureas or carbonylation reactions for amines/anilines for the parent and substituted ureas. All compounds share the symmetrical structure, composed of a central urea, which can be part of pyrimidin-2-one or imidazolidin-2-one heterocycles, and terminal moieties with carboxylic acid or sulphonamide functionalities. Differences are found in the length of the chain linking the central urea to the termini, in the relative position of these two functional groups as aryl ring substituents or in the presence of partially or totally saturated chains, which confers in the latter case greater conformational flexibility to the compounds.

The biological evaluation of the three series using in vitro enzymatic assays showed a low effect on the inhibition of PRMT1 and PRMT5 enzymes for most of the compounds. The strongest effect on the symmetric (PRMT1 assay) arginine dimethylation reactions was noticed with **28**, **22a**, **22c** and **24a**, whereas **24c** and **34b** were the most active inhibitors of PRMT5. In this regard, it is not straightforward to establish a correlation between the structure of these analogues and their activity. In addition, in this assay, AMI-1 behaved as an activator of PRMT5 activity, a result not reported so far. Acyclic urea **28** showed the greatest effect on the expression of some androgen receptor target genes, which may correlate with its PRMT1 enzymatic inhibitory activity. The absence of effects of **22a**, **22c** and **24a** may be due to less efficient cell penetration. Since the potency of the compounds is low, we can conclude that these symmetrical ureas inspired by AMI-1 are not apparent inhibitors of PRMT1 and 5. These scaffolds should be ruled out as starting points to develop more potent PRMT inhibitors.

## 5. Experimental section

### 5.1. Biochemistry methods

#### 5.1.1. In vitro methylation assay

The assay was performed with PRMT Direct Activity Assay kits for PRMT1 and PRMT5 purchased from BPS Bioscience (San Diego, CA, USA).[29]

*PRMT1 and PRMT5 assay kits*: PRMT kits are designed to measure PRMT1 or PRMT5 activity using purified PRMT1 or PRMT5 or extracts cells containing PRMT1 or PRMT5. These kits include a 96-well plate precoated with histone H4 peptide substrate, the primary antibody against methylated arginine-3 residue of histone

H4, the secondary HRP-labeled antibody, *S*-adenosylmethionine, methyltransferase assay buffer and purified human recombinant PRMT1 (expressed in *E. coli*) or PRMT5 (expressed in HEK293 cells) enzyme for 100 enzyme reactions. In addition to PRMT enzymes provided with both kits, PRMT1 and PRMT5 expressed in *Sf9* cells were also purchased to compare the results obtained with both enzymes and select the one with the highest activity. The highly specific primary antibody recognizes methylated R3 residue of histone H4 (asymmetric methylation in case of the antibody for PRMT1 and symmetric methylation for PRMT5). Detection of methyltransferase activity is done in three steps. First, *S*-adenosylmethionine is incubated with a sample containing assay buffer and methyltransferase enzyme for one hour. Next, a primary antibody is added followed by an incubation time of one hour. Finally, the plate is treated with a secondary HRP-labeled antibody, and the HRP substrate to produce chemiluminiscence that can be measured using a chemiluminiscence reader.

25 mM stock solutions of the compounds in DMSO were prepared. PRMTs assays were performed in the presence of the analogues at 100 μM (solutions in water) plus a reaction mixture composed of 20 μL of a solution of PRMT enzyme, 12.5 μL of TBST assay buffer provided with the assay kit (1 × TBS, pH 8.0, containing 0.05% Tween20), 2.5 μL of *S*-adenosylmethionine (400 μM stock solution) and 15 μL of the solution of the compounds in water. $IC_{50}$ values were calculated by measuring the enzymatic activity after treatment with 1.56, 6.25, 25 and 100 μM solutions of the compounds. The programme BioDataFit1.02 was used to fit the data to a sigmoidal function ($logEC_{50}$), which provided the desired values (see Supplementary data).

#### 5.1.2. Cell viability assay

The LAPC-4 and VCaP prostate cancer cell lines were obtained from ATCC (Manassas, VA, USA). LAPC-4 cells were grown in RPMI1640 without phenol red supplemented with 10% cFCS and 2 mM L-glutamine at a concentration of 4000 cells/well in a 96-well microtiter plate. One day later, the cells were treated with 1 nM R1881 and different compound concentrations. VCaP cells were grown in DMEM with phenol red supplemented with 10% cFCS at a concentration of 16,000 cells/well in a 96-well microtiter plate. One day later, the cells were treated with 0.1 nM R1881 and different compound concentrations. For both cell lines, the cell number was determined seven days later by Alamar Blue staining (Invitrogen, Life Technologies, Darmstadt, Germany) in a Victor3 luminometer (PerkinElmer, Rodgau, Germany).

#### 5.1.3. Effect on the expression of androgen receptor target genes

RNA was prepared from VCaP cells using the RNeasy extraction kit (Qiagen, Hilden, Germany) and reverse-transcribed with the SuperScript Reverse Transcriptase kit (Invitrogen). Gene expression was measured using specific fluorogenic probes and measured on a Fast Real-Time PCR system (Applied Biosystems, Life Technologies, Darmstadt, Germany). Human cyclophilin A levels were determined as internal control for normalization.

### 5.2. Chemistry methods

#### 5.2.1. General

Solvents were dried according to published methods and distilled before use. HPLC grade solvents were used for HPLC purification. All other reagents were commercial compounds of the highest purity available. All reactions were carried out under argon atmosphere, and those not involving aqueous reagents were carried out in oven-dried glassware. Analytical thin layer chromatography (TLC) was performed on aluminium plates with Merck Kieselgel 60F254 and visualized by UV irradiation (254 nm) or by staining with a solution of phosphomolibdic acid. Flash column chromatography was carried out using Merck Kieselgel 60 (230–400 mesh) under pressure. Infrared spectra were obtained on a JASCO FTIR 4200 spectrophotometer, from a thin film deposited onto a NaCl glass. $^1$H NMR spectra were recorded in $CDCl_3$, $CD_3OD$, DMSO-$d_6$ and $(CD_3)_2CO$ at ambient temperature on a Bruker AMX-400 spectrometer at 400 MHz with residual protic solvent as the internal reference ($CDCl_3$, $\delta_H$ = 7.26 ppm; $(CD_3)_2CO$, $\delta_H$ = 2.05 ppm; $CD_3OD$, $\delta_H$ = 3.31; DMSO-$d_6$, $\delta_H$ = 2.50); chemical shifts ($\delta$) are given in parts per million (ppm), and coupling constants ($J$) are given in Hertz (Hz). The proton spectra are reported as follows: $\delta$ (multiplicity, coupling constant $J$, number of protons, assignment). $^{13}$C NMR spectra were recorded in $CDCl_3$ $CD_3OD$, DMSO-$d_6$ and $(CD_3)_2CO$ at ambient temperature on the same spectrometer at 100 MHz, with the central peak of $CDCl_3$ ($\delta_C$ = 77.0 ppm) $CD_3OD$ ($\delta_C$ = 49.0 ppm), DMSO-$d_6$ ($\delta_C$ = 39.4 ppm) or $(CD_3)_2CO$ ($\delta_C$ = 30.8 ppm) as the internal reference. DEPT135 sequence was used to aid in the assignment of signals in the $^{13}$C NMR spectra. Melting points were determined on a Stuart SMP10 apparatus. Elemental analyses were determined on a Carlo Erba EA 1108 analyzer. Mass Spectrometry. Experiments were performed on an APEX III FT-ICR MS (Bruker Daltonics, Billerica, MA), equipped with a 7T actively shielded magnet. Ions were generated using an Apollo API electrospray ionization (ESI) source, with a voltage between 1800 and 2200 V (to optimize ionisation efficiency) applied to the needle, and a counter voltage of 450 V applied to the capillary. Samples were prepared by adding a spray solution of 70:29.9:0.1 (v/v/v) $CH_3OH$/water/formic acid to a solution of the sample at a v/v ratio of 1 to 5% to give the best signal-to-noise ratio.

Data acquisition and data processing were performed using the XMASS software, version 6.1.2 (Bruker Daltonics). FAB Experiments were performed on a VG AutoSpec instrument, using 3-nitrobenzylalcohol or glycerol as matrices.

The purity of the compounds was established in most cases by elemental analysis and, for those that did not gave suitable crystals, by HPLC, and found to be greater than 95%.

#### 5.2.2. (1,3)-Bis-(1-formyl-phen-3-yl)-tetrahydropyrimidin-2(1*H*)-one (18a)

**5.2.2.1. General procedure for the palladium-catalyzed amidation of arylbromides with ureas.** A solution of 3-bromobenzaldehyde **14a** (0.2 g, 0.125 mL, 1.08 mmol), tetrahydropyrimidin-2(1*H*)-one **15** (0.07 g, 0.7 mmol), $Pd_2dba_3.CHCl_3$ (0.0056 g, 0.0054 mmol), Xantphos (0.0094 g, 0.016 mmol) and dried $Cs_2CO_3$ (0.49 g, 1.51 mmol) in DMF (6 mL) was stirred at 100 °C for 4 h. The reaction mixture was cooled down to room temperature and filtered through a pad of Celite®. The solvent was evaporated to afford, after purification by column chromatography (silica gel, 97.5:2.5 $CH_2Cl_2$/MeOH), 0.087 g (52%) of a solid identified as (1,3)-bis-(1-formyl-phen-3-yl)-tetrahydropyrimidin-2(1*H*)-one **18a**. $^1$H NMR (400.13 MHz, $CDCl_3$): $\delta$ 9.99 (s, 2H, CHO), 7.87 (t, $J$ = 1.8 Hz, 2H, ArH), 7.71–7.66 (m, 4H, ArH), 7.51 (t, $J$ = 7.8 Hz, 2H, ArH), 3.91 (t, $J$ = 5.9 Hz, 4H, 2 × $CH_2$), 2.35 (quint., $J$ = 5.9 Hz, 2H, $CH_2$) ppm. $^{13}$C NMR (100.62 MHz, $CDCl_3$): $\delta$ 191.9 (d, 2×), 154.2 (s), 144.5 (s, 2×), 137.2 (s, 2×), 132.0 (d, 2×), 129.4 (d, 2×), 127.2 (d, 2×), 126.1 (d, 2×), 49.0 (t, 2×), 23.0 (t) ppm. MS (EI): $m/z$ (%) 309 ($[M+1]^+$, 7), 308 ($[M]^+$, 100), 307 (19), 133 (45), 132 (35), 105 (15), 77 (14). HMRS (EI): Calcd for $C_{18}H_{16}N_2O_3$, 308.1161; found, 308.1164. IR (NaCl): $\nu$ 2839 (w, C–H), 1694 (s, C═O), 1650 (s, C═O), 1590 (m), 1482 (s), 1426 (s), 1307 (s), 1203 (s) $cm^{-1}$. UV (MeOH): $\lambda_{max}$ 242 nm. Mp: 146–148 °C (EtOAc/hexane).

#### 5.2.3. (*E*,*E*)-(1,3)-Bis-[3-(1-ethoxycarbonyl-ethen-2-yl)-phen-1-yl]-tetrahydropyrimidin-2(1*H*)-one (21a)

**5.2.3.1. General procedure for the Horner–Wadsworth–Emmons reaction.** A cooled (0 °C) solution of ethyl 2-(diethoxyphosphoryl)acetate **20** (0.07 g, 0.06 mL, 0.31 mmol) in THF

(0.75 mL) was treated with *n*-BuLi (0.18 mL, 1.61 M en hexane, 0.29 mmol) and DMPU (1 mL) and the mixture was stirred for 30 min. The reaction was cooled down to −78 °C, a solution of (1,3)-bis-(1-formyl-phen-3-yl)-tetrahydropyrimidin-2(1*H*)-one **18a** (0.04 g, 0.13 mmol) in THF (0.75 mL) was added and the resulting mixture was stirred for 1.5 h at −78 °C and then was allowed to warm up to 25 °C for 30 min. $H_2O$ was added and the mixture was extracted with $Et_2O$ (3×). The combined organic layers were washed with water (3×), brine (3×) and dried ($Na_2SO_4$) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 97.5:2.5 $CH_2Cl_2$/MeOH) to afford 0.052 g (90%) of a solid identified as (*E*,*E*)-(1,3)-bis-[3-(1-ethoxycarbonyl-ethen-2-yl)-phen-1-yl]-tetrahydropyrimidin-2(1*H*)-one **21a**. $^1$H NMR (400.13 MHz, $CDCl_3$): $\delta$ 7.65 (d, $J$ = 16.0 Hz, 2H), 7.53 (s, 2H, ArH), 7.37–7.34 (m, 6H, ArH), 6.42 (d, $J$ = 16.0 Hz, 2H), 4.25 (q, $J$ = 7.1 Hz, 4H, $CO_2CH_2CH_3$), 3.85 (t, $J$ = 5.8 Hz, 4H, 2 × $CH_2$), 2.31 (quint., $J$ = 5.8 Hz, 2H, $CH_2$), 1.32 (t, $J$ = 7.1 Hz, 6H, $CO_2CH_2CH_3$) ppm. $^{13}$C NMR (100.62 MHz, $CDCl_3$): $\delta$ 167.0 (s, 2×), 154.3 (s), 144.4 (s, 2×), 144.2 (d, 2×), 135.3 (s, 2×), 129.3 (d, 2×), 127.6 (d, 2×), 125.4 (d, 2×), 125.3 (d, 2×), 118.9 (d, 2×), 60.6 (t, 2×), 49.2 (t, 2×), 23.2 (t), 14.4 (q, 2×) ppm. MS (EI): *m*/*z* (%) 449 ([M+1]$^+$, 13), 448 ([M]$^+$, 89), 419 (69), 403 (15), 374 (14), 373 (100), 184 (13), 172 (12), 158 (61), 130 (25), 102 (22). HMRS (EI): Calcd for $C_{26}H_{28}N_2O_5$ ([M]$^+$), 448.1998; found, 448.1995. IR (NaCl): $\nu$ 2980 (w, C–H), 1709 (s, C=O), 1645 (s, C=O), 1585 (w, C=C), 1482 (m), 1426 (m), 1303 (s), 1176 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 261 nm. Mp: 136–139 °C ($CH_2Cl_2$/MeOH).

### 5.2.4. (*E*,*E*)-3,3′-[(2-Oxodihydropyrimidine-1,3-diyl)-bis-(1,3-phenylene)]-diacrylic acid (22a)

**5.2.4.1. General procedure for the hydrolysis of esters with 10% NaOH.** To a solution of (*E*,*E*)-(1,3)-bis-[3-(ethoxycarbonyle-then-1-yl)-phenyl]-tetrahydropyrimidin-2(1*H*)-one **21a** (0.04 g, 0.09 mmol) in EtOH (2.7 mL) was added a 10% aqueous solution of NaOH (0.09 mL, 0.89 mmol) and the mixture was stirred at 80 °C for 17 h. The reaction was quenched with a 10% aqueous solution of HCl and the solid was filtered off to afford, 0.029 g (83%) of a solid identified of (*E*,*E*)-3,3′-[(2-oxodihydropyrimidin-1,3-diyl)-bis-(1,3-phenylene)]-diacrylic acid **22a**. $^1$H NMR (400.13 MHz, DMSO-$d_6$): $\delta$ 12.39 (br s, 2H, $CO_2H$), 7.68 (s, 2H, ArH), 7.58 (d, $J$ = 16.0 Hz, 2H), 7.47 (d, $J$ = 6.9 Hz, 2H, ArH), 7.41–7.35 (m, 4H, ArH, 2 × $CH_2$), 6.54 (d, $J$ = 16.0 Hz, 2H, $CH_2$), 3.81 (t, $J$ = 5.7 Hz, 4H, 2 × $CH_2$), 2.21 (quint., $J$ = 5.7 Hz, 2H, $CH_2$) ppm. $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 167.5 (s, 2×), 153.4 (s), 144.7 (s, 2×), 143.6 (d, 2×), 134.6 (s, 2×), 128.8 (d, 2×), 127.8 (d, 2×), 125.1 (d, 2×), 124.9 (d, 2×), 119.4 (d, 2×), 48.7 (t, 2×), 22.5 (t) ppm. MS (FAB$^+$): *m*/*z* (%) 393 ([M+H]$^+$, 100), 392 ([M]$^+$, 21), 157 (11), 154 (30). HMRS (FAB$^+$): Calcd for $C_{22}H_{21}N_2O_5$ ([M+H]$^+$), 393.1450; found, 393.1450. IR (neat): $\nu$ 3500–2500 (br, O–H), 1678 (s, C=O), 1642 (s, C=O), 1485 (s), 1424 (s), 1307 (s), 1201 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 259 nm. Mp: 292–295 °C (EtOH). Purity trace: RPHPLC-ESI (Sunfire® $C_{18}$ 5 μm, 250 × 46 mm, gradient from 95:5 to 0:100 $H_2O$/$CH_3CN$, 25 min, 1 mL/min, $t_R$ = 14.4 min; 98% purity).

### 5.2.5. 6,6′-Ureylen-di-hexanoic acid (28)

To a cooled (0 °C) solution of 1,3-bis-(6-hydroxyhex-1-yl)-urea **27** (0.05 g, 0.12 mmol) in acetone (0.65 mL) was added dropwise the Jones reagent (0.07 g $CrO_3$, 0.059 mL $H_2SO_4$, 0.69 mL $H_2O$) and the mixture was stirred for 2 h at 25 °C. The reaction mixture was quenched with water and the mixture was extracted with EtOAc (5×). The combined organic layers were washed with brine (3×), dried ($Na_2SO_4$) and the solvent was evaporated to afford 0.04 g (72%) of a white solid identified as 6,6′-ureylen-di-hexanoic acid **28**. $^1$H NMR (400.13 MHz, $CD_3OD$): $\delta$ 3.11 (t, $J$ = 6.9 Hz, 4H, 2 × $CH_2$), 2.30 (t, $J$ = 7.4 Hz, 4H, 2 × $CH_2$), 1.67–1.60 (m, 4H, 2 × $CH_2$), 1.54–1.46 (m, 4H, 2 × $CH_2$), 1.42–1.33 (m, 4H, 2 × $CH_2$) ppm. $^{13}$C NMR (100.62 MHz, $CD_3OD$): $\delta$ 177.6 (s, 2×), 161.3 (s), 40.8 (t, 2×), 34.9 (t, 2×), 31.0 (t, 2×), 27.5 (t, 2×), 25.8 (t, 2×) ppm. HMRS (ESI$^+$): Calcd for $C_{13}H_{24}N_2NaO_5$ ([M+Na]$^+$), 311.1572; found, 311.1577. IR (NaCl): $\nu$ 3330 (br, O–H/N–H), 2926 (m, C–H), 2859 (m, C–H), 1694 (s, C=O), 1610 (m, C=O), 1570 (s), 1261 (s), 1203 (m) cm$^{-1}$. Mp: 136–139 °C ($CH_2Cl_2$/MeOH). This compound has been previously described.[32]

### 5.2.6. Methyl 3-(cyanomethyl)benzoate (29b)

A suspension of methyl-3-(bromomethyl)benzoate (0.5 g, 2.18 mmol) and sodium cyanide (0.16 g, 3.27 mmol) in DMF (0.93 mL) and $H_2O$ (0.04 mL) was stirred at 75 °C for 5 h. The reaction was quenched with water and extracted with EtOAc. The combined organic layers were washed with $H_2O$ (3×), dried ($Na_2SO_4$) and the solvents were evaporated. The residue was purified by column chromatography (silica gel, from 80:20 hexane/EtOAc to 70:30 hexane/EtOAc) to afford 0.324 g (85%) of a colourless oil identified as methyl 3-(cyanomethyl)benzoate **29b**. $^1$H NMR (400.13 MHz, $CDCl_3$): $\delta$ 8.02–8.00 (m, 2H, ArH), 7.56–7.53 (m, 1H, ArH), 7.50–7.46 (m, 1H, ArH), 3.93 (s, 3H, $CH_3$), 3.81 (s, 2H, $CH_2$) ppm. $^{13}$C NMR (100.62 MHz, $CDCl_3$): $\delta$ 166.3 (s), 132.3 (d), 131.0 (s), 130.5 (s), 129.3 (d), 129.2 (d), 129.1 (d), 117.5 (s), 52.3 (q), 23.4 (t) ppm. HMRS (ESI$^+$): Calcd for $C_{10}H_{10}NO_2$ ([M+H]$^+$), 176.0706; found, 176.0703. IR (NaCl): $\nu$ 3003 (w, C–H), 2954 (w, C–H), 2252 (w, C≡N), 1722 (s, C=O), 1439 (m), 1287 (m) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 283, 229 nm.

### 5.2.7. (3-Methoxycarbonyl-phen-1-yl)-methanammonium chloride (30a)

A suspensión of methyl 3-cyanobenzoate **29a** (1.0 g, 6.20 mmol), 10% Pd/C (0.48 g) and concd HCl (0.66 mL, 6.20 mmol) in MeOH (60 mL) was stirred overnight under a hydrogen atmosphere. The mixture was filtered through a pad of Celite®, the solvent was evaporated and the solid was crystallized (EtOH/$Et_2O$) to afford 1.04 g (83%) of a solid identified as (3-methoxycarbonyl)-phen-1-yl)-methanamonium chloride **30a**. $^1$H NMR (400.13 MHz, $CD_3OD$) $\delta$ 8.14 (s, 1H, ArH), 8.06 (d, $J$ = 7.8 Hz, 1H, ArH), 7.69 (d, $J$ = 7.3 Hz, 1H, ArH), 7.57 (t, $J$ = 7.7 Hz, 1H, ArH), 4.18 (s, 2H, $CH_2$), 3.91 (s, 3H, $CH_3$) ppm. $^{13}$C NMR (100.62 MHz, $CD_3OD$): $\delta$ 167.8 (s), 135.1 (s), 134.8 (d), 132.2 (s), 131.1 (d, 2×), 130.5 (d), 52.8 (q), 43.9 (t) ppm. HMRS (ESI$^+$): Calcd for $C_9H_{12}NO_2$ ([M−Cl]$^+$), 166.0863, found 166.0858. IR (neat): $\nu$ 3200–2600 (br, N–H), 3158 (w, N–H), 2959 (w, C–H), 2807 (m, C–H), 1689 (s, C=O), 1607 (w), 1473 (w), 1450 (w), 1289 (s), 1213 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 282, 228 nm. Mp: 165–166 °C (EtOH/$Et_2O$).

### 5.2.8. 1-[3-(Methoxycarbonyl)-phen-1-yl]-ethan-1-ammonium chloride (30b)

Following the general procedure for reduction of nitriles, the reaction of methyl 3-(cyanomethyl)benzoate **29b** (0.22 g, 1.23 mmol), 10% Pd/C (0.095 g) and concd HCl (0.039 mL, 1.227 mmol) in MeOH (12.0 mL) afforded 0.166 g (63%) of a solid identified as 2-[3-(methoxycarbonyl)-phen-1-yl)-ethan-1-amonium chloride **30b**. $^1$H NMR (400.13 MHz, $CD_3OD$) $\delta$ 7.96 (s, 1H, ArH), 7.94–7.91 (m, 1H, ArH), 7.57 (d, $J$ = 7.7, 1H, ArH), 7.48 (t, $J$ = 7.7, 1H, ArH), 3.90 (s, 3H), 3.24–3.20 (m, 2H), 3.06 (t, $J$ = 7.8, 2H) ppm. $^{13}$C NMR (100.62 MHz, $CD_3OD$): $\delta$ 168.2 (s), 138.6 (s), 134.7 (d), 132.0 (s), 130.8 (d), 130.2 (d), 129.40 (d), 52.7 (q), 41.7 (t), 34.2 (t) ppm. HMRS (ESI$^+$): Calcd for $C_{10}H_{14}NO_2$ ([M−Cl]$^+$), 180.1019, found 180.1027. IR (neat): $\nu$ 3200–2800 (br, N–H), 2971 (m, C–H), 2893 (m, C–H), 2798 (w, C–H), 2732 (w, C–H), 1719 (s, C=O), 1595 (w), 1479 (m), 1437 (m), 1279 (s), 1213 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 285, 224 nm. Mp: 150–151 °C (EtOH/$Et_2O$).

**5.2.9. *N,N′*-bis-(3-Methoxycarbonyl-phen-1-yl-methyl)urea (33a)**

**5.2.9.1. General procedure for the carbonylation reaction using triphosgene.** A solution of methyl 3-(aminomethyl)benzoate **31a** (previously obtained from (3-methoxycarbonyl-phen-1-yl)-methanammonium chloride **30a** by treatment of $Et_3N$) (0.23 g, 1.53 mmol) and $Et_3N$ (0.48 mL, 0.35 g, 3.48 mmol) in benzene (22.2 mL) was added a solution of triphosgene **32** (0.23 g, 1.53 mmol) in benzene (22.2 mL). The mixture was stirred overnight at 25 °C and then the solvent was evaporated. The residue was dissolved in acetone (9.8 mL), another portion of 3-(aminomethyl)benzoate **31a** (0.23 g, 1.53 mmol) was added , and the mixture was stirred for 2 h at 25 °C. The residue was purified by crystallization (EtOH/$Et_2O$) to afford 0.242 g (89%) of a solid identified as *N,N′*-bis-(3-methoxycarbonyl-phen-1-yl-methyl)urea **33a**. $^1$H NMR (400.13 MHz, DMSO-$d_6$) $\delta$ 7.87 (s, 2H, NH), 7.82 (d, $J$ = 7.6 Hz, 2H, ArH), 7.53 (d, $J$ = 7.7 Hz, 2H, ArH), 7.46 (t, $J$ = 7.6 Hz, 2H, ArH), 6.64 (t, $J$ = 6.1 Hz, 2H, 2 × NH), 4.29 (d, $J$ = 6.1 Hz, 4H, 2 × $CH_2$), 3.84 (s, 6H, 2 × $CH_3$) ppm. $^{13}$C NMR (100.62 MHz, DMSO-$d_6$): $\delta$ 166.3 (s, 2×), 158.0 (s), 141.8 (s, 2×), 131.9 (d, 2×), 129.6 (s, 2×), 128.6 (d, 2×), 127.5 (d, 2×), 127.4 (d, 2×), 52.1 (q, 2×), 42.6 (t, 2×) ppm. HMRS (ESI$^+$): Calcd for $C_{19}H_{21}N_2O_5$ ([M+H]$^+$), 357.1445, found 357.1445. IR (neat): $\nu$ 3328 (w, N–H), 3009 (w, C–H), 2952 (w, C–H), 1714 (s, C=O), 1617 (s, C=O), 1564 (m), 1499 (m) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 287 nm. Mp: 180–182 °C (EtOH/$Et_2O$).

**5.2.10. *N,N′*-bis-2-[3-(Methoxycarbonyl)-phen-1-yl]-ethan-1-ylurea (33b)**

Following the general procedure for the carbonylation reaction with triphosgene, the reaction of methyl 3-(2-aminoethyl)benzoate **31b** (previously obtained from 2-[3-(methoxycarbonyl)-phen-1-yl]-ethan-1-ammonium chloride **30b** by treatment with $Et_3N$) (0.1 g, 0.61 mmol), $Et_3N$ (0.19 mL, 0.14 g, 1.38 mmol) and triphosgene **32** (0.09 g, 0.31 mmol) in benzene (15.7 mL) at 25 °C overnight, and the reaction of the residue with another portion of 3-(2-aminoethyl)benzoate **31b** (0.1 g, 0.61 mmol) in acetone (3.9 mL) afforded, after purification by column chromatography (silica gel, from 95:5 $CH_2Cl_2$/MeOH to 90:10 $CH_2Cl_2$/MeOH), 0.10 g (86%) of a solid identified as *N,N′*-bis-2-[3-(methoxycarbonyl)-phen-1-yl]-ethan-1-ylurea **33b**. $^1$H NMR (400.13 MHz, $CDCl_3$) $\delta$ 7.89–7.86 (m, 4H), 7.39–7.34 (m, 4H), 4.24 (t, $J$ = 5.5 Hz, 2H, NH), 3.90 (s, 6H, 2 × $CH_3$), 3.44 (dd, $J$ = 6.8, 5.5 Hz, 4H, 2 × $CH_2$), 2.85 (t, $J$ = 6.8 Hz, 4H, 2 × $CH_2$) ppm. $^{13}$C NMR (100.62 MHz, $CDCl_3$): $\delta$ 167.1 (s, 2×), 158.5 (s), 139.7 (s, 2×), 133.5 (d, 2×), 130.1 (s, 2×), 129.8 (d, 2×), 128.5 (d, 2×), 127.6 (d, 2×), 52.0 (q, 2×), 41.3 (t, 2×), 36.3 (t, 2×) ppm. HMRS (ESI$^+$): Calcd for $C_{21}H_{25}N_2O_5$ ([M+H]$^+$), 385.1758, found 385.1769. IR (neat): $\nu$ 3309 (m, N–H), 2956(w, C–H), 2873 (w, C–H), 1720 (s, C=O), 1583 (s), 1434 (m), 1282(s), 1200 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 283 nm. Mp: 182–184 °C (EtOH/$Et_2O$).

**5.2.11. 3,3′-(Ureylen-di-*N,N′*-methyl)-dibenzoic acid (34a)**

**5.2.11.1. General procedure for hydrolysis of esters using LiOH.** A 10% aqueous solution of LiOH (0.67 mL, 2.81 mmol) was added to *N,N′*-bis-(3-methoxycarbonyl-phen-1-yl-methyl)-urea **33a** (0.1 g, 0.28 mmol) in THF (8.6 mL) and the mixture was stirred at 80 °C for 22 h. The solvent was evaporated and the residue was purified by crystallization (MeOH/$Et_2O$) to afford 0.076 g (82%) of a solid identified as 3,3′-(ureylen-di-*N,N′*-methyl)-dibenzoic acid **34a**. $^1$H NMR (400.13 MHz, DMSO-$d_6$) $\delta$ 12.95 (s, 2H, OH), 7.86 (s, 2H, ArH), 7.80 (d, $J$ = 7.6 Hz, 2H, ArH), 7.50 (d, $J$ = 7.6 Hz, 2H, ArH), 7.43 (t, $J$ = 7.6 Hz, 2H, ArH), 6.66 (t, $J$ = 6.1 Hz, 2H, NH), 4.29 (d, $J$ = 6.1 Hz, 4H, 2 × $CH_2$) ppm. $^{13}$C NMR (100.62 MHz, DMSO-$d_6$) $\delta$ 167.4 (s, 2×), 158.1 (s), 141.6 (s, 2×), 131.5 (d, 2×), 130.7 (s, 2×), 128.5 (d, 2×), 127.8 (d, 2×), 127.5 (d, 2×), 42.7 (t, 2×) ppm. HMRS (ESI$^+$): Calcd for $C_{17}H_{17}N_2O_5$ ([M+H]$^+$), 329.1132, found, 329.1135. IR (neat): $\nu$ 3309 (w, N–H), 2877 (m, C–H), 1678 (m, C=O), 1571 (m), 1421 (m), 1286 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 283 nm. Mp: 260–261 °C (MeOH/$Et_2O$). Purity trace: RPHPLC-ESI (Sunfire® $C_{18}$ 5 μm, 250 × 46 mm, gradient from 95:5 to 0:100 $H_2O$/$CH_3CN$, 25 min, 1 mL/min, $t_R$ = 12.3 min; 100% purity).

**5.2.12. 3,3′-(Ureylen-di-*N,N′*-ethan-2-yl)-dibenzoic acid (34b)**

Following the general procedure for hydrolysis of esters with LiOH, the reaction of *N,N′*-bis-2-[3-(methoxycarbonyl)-phen-1-yl]-ethan-1-ylurea (0.07 g, 0.18 mmol), a 10% aqueous solution of LiOH (0.04 mL, 1.82 mmol) in THF (5.6 mL) at 80 °C for 24 h afforded, after purification by crystallization (EtOH/$Et_2O$), 0.4 g (62%) of a solid identified as 3,3′-(ureylen-di-*N,N′*-ethan-2-yl)dibenzoic acid **34b**. $^1$H NMR (400.13 MHz, DMSO-$d_6$) $\delta$ 7.78–7.76 (m, 4H, ArH), 7.44–7.39 (m, 4H, ArH), 3.22 (t, $J$ = 7.1 Hz, 4H, 2 × $CH_2$), 2.72 (t, $J$ = 7.1 Hz, 4H, 2 × $CH_2$) ppm. $^{13}$C NMR (100.62 MHz, DMSO-$d_6$) $\delta$ 167.4 (s, 2×), 158.0 (s), 140.2 (s, 2×), 133.3 (d, 2×), 130.8 (s, 2×), 129.6 (d, 2×), 128.6 (d, 2×), 127.1 (d, 2×), 40.8 (t, 2×), 35.9 (t, 2×) ppm. HMRS (ESI$^+$): Calcd for $C_{19}H_{21}N_2O_5$ ([M+H]$^+$), 357.1445, found, 357.1429. IR (neat): $\nu$ 3360 (w, N–H), 3164 (w, N–H), 2943 (w, C–H), 1683 (s, C=O), 1626 (m, C=O), 1584 (m), 1419 (m) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 318, 284, 226 nm. Mp: 213–215 °C (EtOH/$Et_2O$). Purity trace: RPHPLC-ESI (Sunfire® $C_{18}$ 5 μm, 250 × 46 mm, gradient from 95:5 to 0:100 $H_2O$/$CH_3CN$, 25 min, 1 mL/min, $t_R$ = 13.0 min; 94% purity).

**5.2.13. (*E*)-Ethyl 3-bromophenylacrylate (35)**

Following the general procedure for the Horner–Wadsworth–Emmons reaction, the reaction of 3-bromobenzaldehyde **14a** (1.0 g, 0.63 mL, 5.40 mmol), ethyl 2-(diethoxyphosphoryl)acetate **20** (2.18 g, 1.95 mL, 9.73 mmol), *n*-BuLi (7.07 mL, 1.30 M in hexane, 9.19 mmol), DMPU (1.87 g, 1.76 mL, 14.59 mmol) in THF (9.1 mL) afforded, after purification by column chromatography (silica gel, 95:5 hexane/EtOAc), 1.322 g (96%) of a solid identified as (*E*)-ethyl 3-bromophenylacrylate **35**. $^1$H NMR (400.13 MHz, $CDCl_3$): $\delta$ 7.66 (t, $J$ = 1.7 Hz, 1H, ArH), 7.59 (d, $J$ = 16.0 Hz, 1H, 1H), 7.49 (dt, $J$ = 7.9, 1.0 Hz, 1H, ArH), 7.42 (d, $J$ = 7.9 Hz, 1H, ArH), 7.24 (t, $J$ = 7.9 Hz, 1H, ArH), 6.42 (d, $J$ = 16.0 Hz, 1H), 4.26 (q, $J$ = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 1.33 (t, $J$ = 7.1 Hz, 3H, $CO_2CH_2CH_3$) ppm. $^{13}$C NMR (100.62 MHz, $CDCl_3$): $\delta$ 166.6 (s), 142.9 (d), 136.7 (s), 133.1 (d), 130.8 (d), 130.5 (d), 126.8 (d), 123.1 (s), 119.9 (d), 60.8 (t), 14.4 (q) ppm. MS (EI): *m/z* (%) 256 ([M]$^+$ $^{81}$[Br], 28), 254 ([M]$^+$ $^{79}$[Br], 30), 228 (26), 211 (82), 209 (86), 183 (26), 102 (100). HMRS (EI): Calcd for $C_{11}H_{11}O_2{}^{81}Br$ ([M]$^+$ $^{81}$[Br]), 255.9922; found, 255.9929, and Calcd for $C_{11}H_{11}O_2{}^{79}Br$ ([M]$^+$ $^{79}$[Br]), 253.9942; found, 253.9950. IR (NaCl): $\nu$ 2981 (w, C–H), 1712 (s, C=O), 1639 (m), 1561 (w), 1473 (w), 1309 (s), 1177 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 273, 223 nm. Mp: 34–36 °C (hexane/EtOAc).

**5.2.14. (*E*)-Ethyl 3-aminophenylacrylate (36)**

To a solution of (*E*)-ethyl 3-bromophenylacrylate **35** (0.55 g, 2.16 mmol) in DMSO/EtOH (31:16 mL) were added $NaN_3$ (1.81 g, 27.81 mmol), L-proline (0.22 g, 1.94 mmol), NaOH (0.08 g, 2.09 mmol) and CuI (0.37 g, 1.94 mmol) and the mixture was stirred at 110 °C for 20 h. The reaction was cooled down to 25 °C and a 1:1 mixture of EtOAc/$H_2O$ was added, and the undissolved solid was filtered off. The layers were separated, the organic layer was dried ($Na_2SO_4$), and the solvent was evaporated. The residue was purified by chromatography (silica gel, from $CH_2Cl_2$ to 95:5 $CH_2Cl_2$/MeOH) to afford 0.233 g (56%) of an oil identified as (*E*)-ethyl 3-aminophenylacrylate **36**. $^1$H NMR (400.13 MHz, $CD_3OD$): $\delta$ 7.56 (d, $J$ = 16.0 Hz, 1H), 7.11 (t, $J$ = 7.8 Hz, 1H, ArH), 6.92 (s, 1H, ArH), 6.87 (d, $J$ = 7.6 Hz, 1H, ArH), 6.75 (dd, $J$ = 7.9, 1.6 Hz, 1H, ArH), 6.40 (d, $J$ = 16.0 Hz, 1H), 4.22 (q, $J$ = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 1.31 (t, $J$ = 7.1 Hz, 3H, $CO_2CH_2CH_3$) ppm. $^{13}$C NMR (100.62 MHz,

$CD_3OD$): δ 168.8 (s), 149.5 (s), 146.8 (d), 136.4 (s), 130.6 (d), 119.2 (d), 118.6 (d), 118.2 (d), 115.2 (d), 61.6 (t), 14.6 (q) ppm. MS (EI): *m*/*z* (%) 192 ([M+1]$^+$, 12), 191 ([M]$^+$, 100), 162 (22), 147 (30), 146 (85), 119 (51), 118 (55), 117 (43), 91 (37). HMRS (EI): Cacld for $C_{11}H_{13}NO_2$, 191.0946; found, 191.0947. IR (NaCl): *ν* 3456 (w, N–H), 3367 (w, N–H), 2924 (m, C–H), 2853 (w, C–H), 1703 (s, C=O), 1633 (s), 1602 (m), 1305 (m), 1176 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 283, 251 nm.

#### 5.2.15. *N*,*N*′-bis-[3-(Ethoxycarbonyl-ethen-2-yl)-phen-1-yl]urea (37)

Following the general procedure for the carbonylation reaction using triphosgene, the reaction of (*E*)-ethyl 3-aminophenylacrylate **36** (0.17 g, 0.89 mmol), $Et_3N$ (0.20 g, 0.28 mL, 2.0 mmol) and triphosgene (0.13 g, 0.44 mmol) in benzene (23.4 mL) at 25 °C overnight, and then the reaction of the residue with another portion of (*E*)-ethyl 3-aminophenylacrylate **36** (0.17 g, 0.89 mmol) in acetone (5.7 mL) at 25 °C for 2 h afforded, after purification by chromatography (silica gel, from 80:20 hexane/EtOAc to 50:50 hexane/EtOAc) and crystallization (EtOH/$Et_2O$), 0.129 g (71%) of a solid identified as *N*,*N*′-bis-[3-(ethoxycarbonyl-ethen-2-yl)-phen-1-yl]urea **37**. $^1$H NMR (400.13 MHz, DMSO-$d_6$): δ 8.84 (s, 2H, NH), 7.76 (s, 2H, ArH), 7.62 (d, *J* = 16.0 Hz, 2H), 7.50 (d, *J* = 6.7 Hz, 2H, ArH), 7.37–7.32 (m, 4H, ArH), 6.54 (d, *J* = 16.0 Hz, 2H), 4.20 (q, *J* = 7.1 Hz, 4H, $CO_2CH_2CH_3$), 1.27 (t, *J* = 7.1 Hz, 6H, $CO_2CH_2CH_3$) ppm. $^{13}$C NMR (100.62 MHz, DMSO-$d_6$) δ 166.1 (s, 2×), 152.6 (s), 144.4 (d, 2×), 140.1 (s, 2×), 134.5 (s, 2×), 129.4 (d, 2×), 121.8 (d, 2×), 120.5 (d, 2×), 118.2 (d, 2×), 118.0 (d, 2×), 60.1 (t, 2×), 14.2 (q, 2×) ppm. HMRS (ESI$^+$): Calcd for $C_{23}H_{25}N_2O_5$ ([M+H]$^+$), 409.1758; found, 409.1743. IR (neat): *ν* 3291 (m, N–H), 2990 (w, C–H), 1714 (s, C=O), 1628 (w, C=O), 1559 (s), 1477 (m), 1314 (s), 1175 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 282 nm. Mp: 182–184 °C (EtOH/$Et_2O$).

#### 5.2.16. (*E*,*E*)-3,3′-(Ureylendi-*N*,*N*′-phen-3-yl)-diacrylic acid (38)

Following the general procedure for hydrolysis of esters, the reaction of *N*,*N*′-bis-[3-(ethoxycarbonyl-ethen-2-yl)-phen-1-yl]urea **37** (0.06 g, 0.15 mmol), a 10% aqueous solution of LiOH (0.04 mL, 1.5 mmol) in THF (4.6 mL) at 80 °C for 22 h afforded, after purification by crystallization (EtOH/$Et_2O$), 0.045 g (86%) of a solid identified as (*E*,*E*)-3,3′-(ureylendi-*N*,*N*′-phen-3-yl)-diacrylic acid **38**. $^1$H NMR (400.13 MHz, DMSO-$d_6$) δ 12.45 (s, 2H, $CO_2H$), 8.85 (s, 2H), 7.72 (s, 2H, ArH), 7.56 (d, *J* = 15.9 Hz, 2H), 7.50 (d, *J* = 6.4 Hz, 2H, ArH), 7.36–7.33 (m, 4H, ArH), 6.45 (d, *J* = 15.9 Hz, 2H) ppm. $^{13}$C NMR (100.62 MHz, DMSO-$d_6$) δ 167.4 (s, 2×), 152.6 (s), 144.0 (d, 2×), 140.1 (s, 2×), 134.7 (s, 2×), 129.4 (d, 2×), 121.8 (d, 2×), 120.3 (d, 2×), 119.2 (d, 2×), 117.7 (d, 2×) ppm. HMRS (ESI$^+$): Calcd for $C_{19}H_{17}N_2O_5$ ([M+H]$^+$), 353.1132; found, 353.1130. IR (neat): *ν* 3306 (w, N–H), 2957 (w, C–H), 2820 (w, C–H), 1684 (s, C=O), 1635 (s, C=O), 1588 (m), 1555 (m) 1438 (w) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 258 nm. Mp: >300 °C (EtOH/$Et_2O$, dec.). Purity trace: RPHPLC-ESI (Sunfire® $C_{18}$ 5 μm, 250 × 46 mm, gradient from 95:5 to 0:100 $H_2O$/$CH_3CN$, 25 min, 1 mL/min, $t_R$ = 14.4 min; 100% purity).

#### 5.2.17. 7-Acetamidonaphthalene-2-sulphonyl chloride (40)

**5.2.17.1. General procedure for the protection of anilines.** To a cooled (0 °C) solution of sodium 7-aminonaphthalene-2-sulphonate **39** (2.0 g, 8.16 mmol) in pyridine (10 mL) was added dropwise acetic anhydride (10 mL) and the reaction was allowed to warm to 25 °C and stirred for 8 h. The suspension was poured into a mixture of $Et_2O$ (35 mL) and THF (10 mL). The resulting solid was filtered off and washed with $H_2O$ to afford 1.81 g (84%) of a white solid identified as 7-acetamidonaphthalene-2-sulphonic acid, which was used in the next reaction without further purification.

**5.2.17.2. General procedure for the synthesis of chlorosulphonates.** To a solution of 7-acetamidonaphthalene-2-sulphonic acid (0.426 g, 1.613 mmol) in MeOH (4 mL) was added MeONa (0.09 g, 1.61 mmol) and the mixture was stirred at 25 °C overnight. The solid was filtered off and dried to afford 0.264 (57%) of a solid identified as sodium 7-acetamidonaphthalene-2-sulphonate, which was used in the next step without further purification.

To a solution of sodium 7-acetamidonaphthalene-2-sulphonate (0.27 g, 0.93 mmol) was added phosphorus oxychloride (4.94 g, 32,185 mmol, 3 mL), the resulting suspension was cooled down to 0 °C and *N*,*N*-dimethylacetamide (0.12 g, 1,40 mmol, 0.13 mL) was added dropwise. The mixture was warmed up to 25 °C and stirred for 24 h. Then the suspension was poured into ice-cold $H_2O$ (2 mL) and the mixture was stored for 3 days in the fridge. Then more $H_2O$ was added and the precipitate was filtered off and washed with $H_2O$ to obtain 0.115 (44%) of a white solid identified as 7-acetamidonaphthalene-2-sulphonyl chloride **40**. $^1$H NMR (400.13 MHz, DMSO-$d_6$) δ 10.25 (s, 1H, N–H), 8.23 (s, 1H, N–H), 8.00 (s, 1H, ArH), 7.83 (d, *J* = 8.8 Hz, 1H, ArH), 7.78 (d, *J* = 8.5 Hz, 1H, ArH), 7.69 (dd, *J* = 8.8, 1.8 Hz, 1H, ArH), 7.60 (dd, *J* = 8.5, 1.4 Hz, 1H, ArH), 2.10 (s, 3H, $CH_3$) ppm. $^{13}$C NMR (100.62 MHz, DMSO-$d_6$) δ 168.8 (s), 145.5 (s), 137.6 (s), 132.6 (s), 129.5 (s), 128.1 (d), 127.4 (d), 123.7 (d), 122.4 (d), 120.7 (d), 115.6 (d), 24.2 (q) ppm. HMRS (ESI$^+$): Calcd for $C_{12}H_{11}ClNO_3S$ ([M+H]$^+$), 284.0143, found, 284.0140. IR (neat): *ν* 3257 (m, N–H), 3081 (w, C–H), 1659 (s, C=O), 1556 (s, C=O), 1366 (s), 1167 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 258 nm. Mp: 144–146 (MeOH/$H_2O$).

#### 5.2.18. N-[7-(N′-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-sulphamoyl)-naphthalen-2-yl]-acetamide (42a)

**5.2.18.1. General procedure to the formation of sulphonamides.** To the solution of 2,3-dihydrobenzo[*b*][1,4]dioxin-6-amine **41a** (0.27 g, 1.77 mmol), *N*,*N*-diisopropylamine (0.3 g, 0.5 mL, 2.97 mmol) in $CHCl_3$ (2 mL) was added 7-acetamidonaphthalene-2-sulphonyl chloride **40** (0.2 g, 0.71 mmol) in a 1:1 THF/$CHCl_3$ mixture (4 mL). The reaction mixture was stirred overnight at 25 °C, then the solvent was evaporated and the residue was purified by crystallization ($H_2O$, adjusting to pH 3 with a 37% saturated aqueous solution of HCl) to afford 0.151 g (54%) of a solid identified as *N*-[7-(*N*′-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-sulphamoyl)-naphthalen-2-yl]-acetamide **42a**. $^1$H NMR (400.13 MHz, DMSO-$d_6$) δ 10.30 (s, 1H, N–H), 10.05 (s, 1H, N–H), 8.36 (s, 1H, ArH), 8.18 (s, 1H, ArH), 7.99 (d, *J* = 8.7 Hz, 1H, ArH), 7.94 (d, *J* = 8.9 Hz, 1H, ArH), 7.74 (dd, *J* = 8.9, 1.8 Hz, 1H, ArH), 7.61 (dd, *J* = 8.6, 1.6 Hz, 1H, ArH), 6.67 (d, *J* = 8.6 Hz, 1H, ArH), 6.59 (d, *J* = 2.4 Hz, 1H, ArH), 6.54 (dd, *J* = 8.6, 2.5 Hz, 1H, ArH), 4.11 (s, 4H, 2 × $CH_2$), 2.11 (s, 3H, $CH_3$) ppm. $^{13}$C NMR (100.62 MHz, DMSO-$d_6$) δ 169.0 (s), 143.2 (s), 140.6 (s), 138.5 (s), 137.0 (s), 132.2 (s), 130.9 (s), 130.8 (s), 129.1 (d), 128.6 (d), 127.0 (d), 122.6 (d), 120.7 (d), 117.3 (d), 115.7 (d), 114.3 (d), 110.2 (d), 64.1 (t), 63.8 (t), 24.2 (q) ppm. HMRS (ESI$^+$): Calcd for $C_{20}H_{19}N_2O_5S$ ([M+H]$^+$), 399.1009, found, 399.1021. IR (neat): *ν* 3263 (m, N–H), 3080 (w, C–H), 1666 (m, C=O), 1587 (m), 1558 (m), 1505 (m), 1317 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 254 nm. Mp: >300 °C ($H_2O$, dec.).

#### 5.2.19. *N*-[7-(*N*′-4-Phenoxyphen-1-yl-sulphamoyl)-naphthalen-2-yl]-acetamide (42b)

Following the general procedure to the formation of sulphonamides, the reaction of 7-acetamidonaphthalene-2-sulphonyl chloride **40** (0.1 g, 0.35 mmol) in a 1:1 THF/$CHCl_3$ mixture (2 mL), 4-phenoxyaniline **41b** (0.09 g, 0.76 mL) and *N*,*N*-diisopropylamine (0.13 g, 0.26 mL, 1.5 mmol) in $CHCl_3$ (1 mL) afforded 0.067 g (44%) of a white solid identified as *N*-[7-(*N*′-4-phenoxyphen-1-yl-sulphamoyl)-naphthalen-2-yl]-acetamide **42b**. $^1$H NMR (400.13 MHz, DMSO-$d_6$) δ 10.28 (s, 1H, N–H), 10.21 (s, 1H, N–H), 8.37 (s, 1H, ArH), 8.18 (s, 1H, ArH), 8.00 (d, *J* = 8.7 Hz, 1H, ArH), 7.94 (d,

*J* = 8.9 Hz, 1H, ArH), 7.74 (dd, *J* = 8.9, 2.0 Hz, 1H, ArH), 7.63 (dd, *J* = 8.6, 1.8 Hz, 1H, ArH), 7.4–7.3 (m, 2H, ArH), 7.2–7.0 (m, 3H, ArH), 6.9–6.8 (m, 4H, ArH), 2.11 (s, 3H, $CH_3$) ppm. $^{13}$C NMR (100.62 MHz, DMSO-$d_6$) $\delta$ 168.9 (s), 156.9 (s), 153.1 (s), 138.4 (s), 136.9 (s), 133.2 (s), 132.2 (s), 130.8 (s), 130.0 (d, 2×), 129.1 (d), 128.6 (d), 127.1 (d), 123.2 (d), 122.8 (d, 2×), 122.6 (d), 120.6 (d), 119.6 (d, 2×), 118.1 (d, 2×), 115.7 (d), 24.1 (q) ppm. HMRS (ESI$^+$): Calcd for $C_{24}H_{21}N_2O_4S$ ([M+H]$^+$), 433.1217, found, 433.1204. IR (neat): $\nu$ 3243 (m, N–H), 3100 (w, C–H), 1660 (m, C=O), 1585 (m), 1558 (m), 1497 (m), 1328 (m), 1154 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 247 nm. Mp: >300 ($H_2O$, dec.).

#### 5.2.20. 7-[*N*-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-sulphamoyl]-naphthalen-2-ammonium chloride (43a)

**5.2.20.1. General procedure for deprotection of amines.** To a solution of *N*-[7-(*N*′-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-sulphamoyl]-naphthalen-2-yl)acetamide **42a** (0.08 g, 0.19 mmol) in MeOH (1.2 mL) was added a 5 M aqueous solution of NaOH (0.38 g, 0.38 mL, 9.41 mmol) and the resulting solution was heated at 60 °C for 120 h. The mixture was cooled down and then acidified with a 6 M aqueous solution of HCl until pH 1. The resulting solid was filtered off and then the solvent was evaporated to afford, 0.067 g (91%) of a solid identified as 7-[*N*-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-sulphamoyl]-naphthalen-2-ammonium chloride **43a**. $^1$H NMR (400.13 MHz, DMSO-$d_6$) $\delta$ 10.06 (s, 1H, N–H), 8.04 (s, 1H), 7.87 (d, *J* = 8.6 Hz, 1H, ArH), 7.79 (d, *J* = 8.8 Hz, 1H, ArH), 7.45 (d, *J* = 8.6 Hz, 1H, ArH), 7.24 (d, *J* = 8.2 Hz, 2H, ArH), 6.66 (d, *J* = 8.6 Hz, 1H, ArH), 6.60 (d, *J* = 2.4 Hz, 1H, ArH), 6.55 (dd, *J* = 8.6, 2.5 Hz, 1H, ArH), 4.11 (s, 4H, 2 × $CH_2$) ppm. HMRS (ESI$^+$): Calcd for $C_{18}H_{17}N_2O_4S$ ([M−Cl]$^+$), 357.0904, found, 357.0911. IR (neat): $\nu$ 3400–2600 (br, N–H), 2869 (w, C–H), 1625 (w), 1595 (w), 1502 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 248 nm. Mp: >300 °C ($H_2O$, dec.).

#### 5.2.21. 7-[*N*-(4-Phenoxyphen-1-yl)-sulphamoyl]-naphthalen-2-ammonium chloride (43b)

Following the general procedure for the deprotection of amines, the reaction of *N*-[7-(*N*′-(4-phenoxyphenyl)-sulphamoyl)-naphthalen-2-yl]-acetamide (0.07 g, 0.15 mmol) and a 5 M aqueous solution of NaOH (0.43 g, 0.42 mL, 10.79 mmol) in MeOH (1 mL) at 60 °C for 120 h afforded 0.058 g (88%) of a solid identified as 7-[*N*-(4-phenoxyphen-1-yl)-sulphamoyl]-naphthalen-2-ammonium chloride **43b**. $^1$H NMR (400.13 MHz, DMSO-$d_6$) $\delta$ 10.29 (s, 1H, N–H), 8.11 (s, 1H, ArH), 7.93 (d, *J* = 8.6 Hz, 1H, ArH), 7.86 (d, *J* = 8.7 Hz, 1H, ArH), 7.53 (d, *J* = 8.5 Hz, 1H, ArH), 7.38 (s, 1H, ArH), 7.32 (t, *J* = 7.7 Hz, 3H, ArH), 7.11–7.05 (m, 3H, ArH), 6.86 (d, *J* = 8.8 Hz, 4H, ArH) ppm. HMRS (ESI$^+$): Calcd for $C_{22}H_{19}N_2O_3S$ ([M−Cl]$^+$), 391.1111, found, 391.1105. IR (neat): $\nu$ 3200–2600 (br, N–H), 2853 (w, C–H), 1590 (m), 1498 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 246 nm. Mp: >300 °C ($H_2O$, dec.).

#### 5.2.22. *N*,*N*′-bis-[7-(*N*-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-sulphamoyl)-naphthalene-2-yl]-urea (45a)

Following the general procedure for the carbonylation with triphosgene, the reaction of 7-amino-*N*-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-naphthalene-2-sulfonamide **44a** (previously obtained from the corresponding ammoniun chloride **43a** by treatment with $Et_3N$) (0.07 g, 0.19 mmol), $Et_3N$ (0.06 mL, 0.04 g, 0.42 mmol) and triphosgene **32** (0.03 g, 0.09 mmol) in benzene (4.9 mL) and then reaction of the residue with another portion of 7-amino-*N*-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)naphthalene-2-sulphonamide (0.07 g, 0.19 mmol) in acetone (1.4 mL) afforded, after purification by column chromatography (silica gel, 90:10 $CH_2Cl_2$/MeOH), 4.9 mg (7%) of a solid identified as *N*,*N*′-bis-[7-(*N*-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-sulphamoyl)-naphthalene-2-yl]-urea **45a**. $^1$H NMR (400.13 MHz, DMSO-$d_6$) $\delta$ 10.05 (s, 2H, 2 × NH), 9.22 (s, 2H, 2 × NH), 8.21 (s, 4H, ArH), 8.00 (d, *J* = 8.8 Hz, 2H, ArH), 7.96 (d, *J* = 9.0 Hz, 2H, ArH), 7.76 (dd, *J* = 8.8, 1.5 Hz, 2H, ArH), 7.61 (dd, *J* = 8.7, 1.3 Hz, 2H, ArH), 6.68 (d, *J* = 8.7 Hz, 2H, ArH), 6.61 (d, *J* = 2.4 Hz, 2H, ArH), 6.56 (dd, *J* = 8.6, 2.4 Hz, 2H, ArH), 4.12 (s, 8H, 4 × $CH_2$) ppm. $^{13}$C NMR (100.62 MHz, DMSO-$d_6$) $\delta$ 152.6 (s), 143.2 (s, 2×), 140.5 (s, 2×), 138.7 (s, 2×), 137.1 (s, 2×), 132.4 (s, 2×), 130.9 (s, 2×), 130.2 (s, 2×), 129.02 (d, 2×), 128.7 (d, 2×), 126.7 (d, 2×), 122.4 (d, 2×), 120.2 (s, 2×), 117.2 (d, 2×), 114.5 (d, 2×), 114.3 (d, 2×), 110.1 (d, 2×), 64.0 (t, 4×), 63.8 (t, 4×) ppm. HMRS (ESI$^+$): Calcd for $C_{37}H_{30}N_4NaO_9S_2$ ([M+Na]$^+$), 761.1346, found, 761.1354. IR (neat): $\nu$ 3410 (w, N–H), 3365 (w, N–H), 3254 (w, N–H), 2929 (w, N–H), 2878 (w, C–H), 1718 (w, C=O), 1541 (m), 1503 (m), 1147 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 263, 240 nm. Mp: 258–260 °C ($CH_2Cl_2$/MeOH).

#### 5.2.23. *N*,*N*′-bis-[7-(*N*-(4-Phenoxyphenyl)-sulphamoyl)-naphthalene-2-yl]-urea (45b)

Following the general procedure for the carbonylation with triphosgene, the reaction of 7-[amino-*N*-(4-phenoxyphenyl)]-naphthalene-2-sulphonamide **44b** (previously obtained from corresponding ammoniun chloride **43b** by treatment with $Et_3N$) (0.09 g, 0.22 mmol), $Et_3N$ (0.07 mL, 0.05 g, 0.5 mmol) and triphosgene **32** (0.03 g, 0.11 mmol) in benzene (5.7 mL) and then the reaction of the residue with another portion of 7-[amino-*N*-(4-phenoxyphenyl)]-naphthalene-2-sulphonamide **44b** (0.09 g, 0.22 mmol) in acetone (1.7 mL) afforded, after purification by column chromatography (silica gel, from 97.5:2.5 $CH_2Cl_2$/MeOH to 95:5 $CH_2Cl_2$/MeOH), 0.021 g (23%) of a solid identified as *N*,*N*′-bis-[7-(*N*-(4-phenoxyphenyl)-sulfamoyl)-naphthalene-2-yl]-urea **45b**, which still contained small amounts of triethylammonium chloride. $^1$H NMR (400.13 MHz, DMSO-$d_6$) $\delta$ 10.22 (s, 2H, 2 × NH), 10.01 (s, 2H, 2 × NH), 8.15 (d, *J* = 11.0 Hz, 4H, ArH), 7.98 (d, *J* = 8.6 Hz, 2H, ArH), 7.93 (d, *J* = 8.9 Hz, 2H, ArH), 7.69 (dd, *J* = 8.9, 2.0 Hz, 2H, ArH), 7.61 (dd, *J* = 8.6, 1.6 Hz, 2H, ArH), 7.31 (t, *J* = 7.9 Hz, 4H, ArH), 7.14–7.03 (m, 6H, ArH), 6.89–6.85 (m, 8H, ArH) ppm. $^{13}$C NMR (100.62 MHz, DMSO-$d_6$) $\delta$ 156.9 (s), 153.6 (s, 2×), 153.1 (s, 2×), 138.5 (s, 2×), 136.9 (s, 2×), 133.1 (s, 2×), 132.2 (s, 2×), 130.4 (s, 2×), 129.9 (d, 4×), 129.0 (d, 2×), 128.6 (d, 2×), 126.8 (d, 2×), 123.2 (d, 2×), 122.8 (d, 4×), 122.1 (d, 2×), 120.3 (d, 2×), 119.6 (d, 4×), 118.1 (d, 4×), 114.3 (d, 2×) ppm. IR (neat): $\nu$ 3248 (w, N–H), 3063 (w, C–H), 2978 (w, C–H), 1707 (m, C=O), 1590 (m), 1542 (m), 1496 (s), 1215 (s), 1153 (s) cm$^{-1}$. UV (MeOH): $\lambda_{max}$ 246 nm.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.01.017.

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