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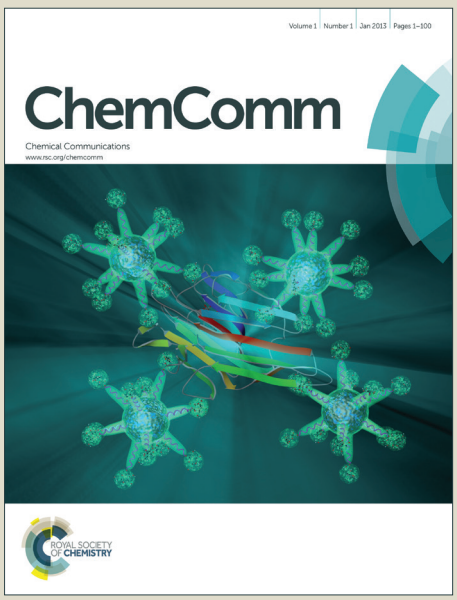

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Supramolecular pyridyl urea gels as soft matter with antibacterial properties against MRSA and/or *E. coli*

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The synthesis and characterisation of novel aryl-pyridyl ureas is described, which form self-assembly structures *via* extended hydrogen bonding and π–π interactions in the solid state, and in selected cases, forms supramolecular gels with antimicrobial properties against *Staphylococcus aureus* and/or *Escherichia coli*

Hydrogen bonding is a powerful structural interaction that is commonly used in synthetic self-assembly supramolecular structures.[1,2] Ureas and thioureas are particularly suited for such synthesis; providing two directional hydrogen bond donors and an acceptor.[1-4] Being easily accessed synthetically, they can adopt either *syn* and *anti* conformation[5,6] and are known to have high affinity for anions.[7-11] Urea/thiourea structures have also found to play a major role in human biology and physiology and they are also of industrial value; an example being polyurea, an elastomer that is often used for protective coating. It is also well known that hydrogen bonding structures[12] like ureas[13] and polyurea derivatives[14] can possess antibacterial properties against strains such as Gram(+) (*e.g*. *Bacillus subtilis* and *Staphylococcus aureus*) and Gram(-) (*e.g. Escherichia coli* and *Salmonella typhi*) bacteria.

Gels are highly versatile materials[1,2] with properties suited for use in variety of applications.[15a] This includes its use in drug delivery,[15b] but polymeric hydrogels have been shown to be suitable for coating implants with the view of preventing surface bacterial growth[15c]. In an extensive body of work, Steed *et al.*[11,15] have recently developed examples of pyridyl based ureas and studied their supramolecular properties in details. This has included the study of their ability to form soft matter such as supramolecular gels[1,2] and as ligands for the formation of metal organic frameworks (MOFs).[16] Similarly, Wu *et al.,*[17] Das *et al*.,[18] Hay *et al*.,[19] and Nangia *et al.*[4] have also studied the use of pyridyl ureas in such supramolecular applications. Inspired by this work, we set out to develop new families of simple pyridyl urea and thiosemicarbazide[20] derivatives, with the view of exploring their gelation properties and use for sensing and biomedical applications; an area that is fast growing within supramolecular chemistry.[21,22] Herein we present three families of novel aryl pyridyl ureas; some of which function as supramolecular gelators possessing antibacterial properties.

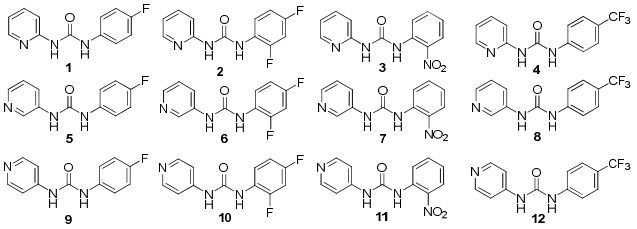

Fig. 1 The pyridyl ureas **1-12** developed in this study.

Compounds **1-12**, Fig. 1, were synthesised in a single step each from commercially available reagents, by reacting the relevant 2-, 3- or 4-pyridyl amine with 4-fluorophenyl-, 2,4-difluorophenyl-, 2-nitrophenyl- and 4-trifluoromethylphenylisocyanates under microwave conditions for 50 minutes at 100°C in CH_3CN. All products were fully characterised and for all, the ^{1}H NMR spectrum in DMSO-d_6 clearly showed the urea proton resonances as broad signals occurring between 8-11 ppm (see ESI).

Steed *et al*. have demonstrated that urea-pyridyl structures tend to crystallise to give urea NH…$N_{pyridyl}$ hydrogen-bonding interactions.[23] Such intermolecular networks would also be expected to dominate in the case of the 3- and 4-pyridyl ureas **5-12**, developed herein, while in the case of **1-4**, the formation of hydrogen bonding dimers would be foreseen, consisting of both intermolecular and intramolecular hydrogen bonding interactions.[2a,3]

Of the compounds developed herein, **1-3, 5-7** and **10-12** gave crystals suitable for X-ray diffraction analysis upon crystallisation from CH_3CN, or CH_3CN/MeOH (**9** was reported by Wu *et al*.[18]). The crystal structures of compounds **2**, **6**, **12** and **7**, and the intermolecular hydrogen bonding which takes place for these structures are shown in Fig. 2a-d, respectively (See ESI for remaining structures). All the structures of 2-pyridyl derivatives showed that the urea protons adopted an *anti* conformation and, as a result, a dimer is formed with both moieties oriented in opposite directions related by a centre of inversion. In the case of **1-3**, the expected dimer shows two intramolecular and two intermolecular hydrogen bonds. For **2**, Fig. 2a, the intermolecular hydrogen bonding arises between the NH urea proton adjacent to the pyridyl unit and oxygen atom of the urea carbonyl with the bond distance of 2.843(2)Å. These NH…O interactions result in the formation of

aforementioned dimeric structures in solid state.[23a] The intramolecular hydrogen bonding arises from the additional urea NH to the nitrogen atom of the pyridine. This is possible because the nitrogen atom of the pyridyl group is in an ideal position to accept a hydrogen bond from the NH urea group thus stabilising the *anti* conformation.[23a] Moreover, the molecules pack with multiple and extended face-to-face and edge-to-face π-stacking interactions giving rise to supramolecular polymeric network.

The crystal structure of **6**, Fig 2b, demonstrated that the molecules adopt a planar conformation in the solid state, where the urea protons are in *cis* conformation. These interact in an intermolecular fashion with the $N_{pyridyl}$ atom of an adjacent molecule through hydrogen-bonding, that has been referred to by Steed *et al.*[23a] as a 'urea pyridyl synthon II' geometry. Here, the classical urea α-tape motif was not observed, and each molecule is offset to the next. As in the case of **2**, the aryl fluorine atoms also play a significant role in the long-range interactions of the packing of **6**,[24] the overall packing being supported by face-to-face π-stacking (See ESI). Compound **7**, Figure 2d, also adopts a modified 'urea pyridyl synthon II' configuration, but different to that seen by **6** due to the presence of the nitro group. Here, a supramolecular hydrogen bonding polymer is also formed, which is extended into three dimensions through face-to-face and edge-to-face π–π interactions. However, unlike that in **6** and **12**, the $N_{pyridyl}$ moiety interacts only with the NH urea proton of the adjacent molecule; the (distal) NH-aryl proton is hydrogen bonded to the 2-nitro group in a six-membered ring system.

The ability of these structures to function as low molecular weight gelators was investigated in various solvents. The results show that for structures **1**, **6** and **7** all formed robust gels under a variety of solvent in 1% weight (See ESI) that were stable towards a tube inversion tests. The gel formation was reversible, as upon heating, the solution phase was obtained, which after sonication reformed the gel. Several others formed self-assembly structures such as aggregates or suspensions in a variety of solvents and solvent mixtures, including compounds **3**, **8** and **12**. These, at 1% weight, did not observe the tube inversion tests, and hence, cannot be classified as robust gel-like material. Fig. 3a shows scanning electron microscopy (SEM) images of the organogel formed upon sonication of **1** (1% by weight) in THF solution, demonstrating the formation of fibres or 'splinter' like morphology, as has been reported by Steed *et al.*[2b] Both **6** and **7** gave rise to organogel formation in THF, as well as gel formation in a mixture of 8:2 THF:H_2O solution. While **1** did not give rise to the formation of a gel in such THF:H_2O mixture, however the addition of $AgNO_3$ to a solution of **1** (1% by weight) in THF:H_2O generated a gel with similar morphology to that shown in Fig. 3a (See ESI), except possessing a more fibrous texture, Fig. 3c. The three hydrogels were also doped with cresol red, an acid sensitive pH indicator.[25] This resulted in the formation of red coloured hydrogels (See ESI). Upon exposure of these gels to HCl gas, the intermolecular hydrogen bonding interactions were disrupted, resulting in de-gelation, while upon exposure to ammonia, the gel remained intact, demonstrating no such hydrogen bonding disruption. These results also demonstrate that these gels are highly versatile, and can host other molecules within their fibres network, which is currently under investigation in our laboratory.

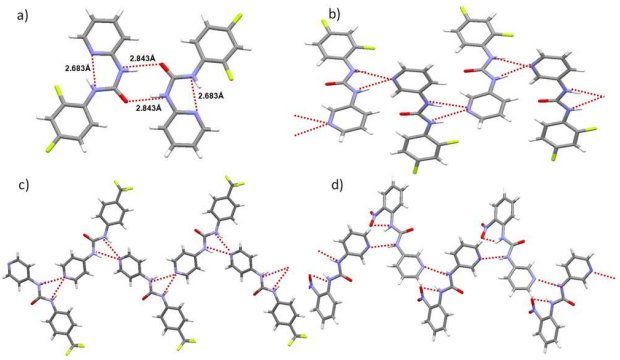

Fig. 2 The crystal structure of a) centrosymmetric dimeric structure of **2**; b) and c) The Steed 'urea pyridyl synthon II' seen for **6** and **12**, respectively; d) The modified Steed 'urea pyridyl synthon II' consisting of a single NH…$N_{pyridyl}$ hydrogen bonding in **7**.

It is reasonable to speculate that compounds **1-12** might possess antibacterial properties as hydrogen bonding plays a major role in the function of many antibacterial agents, such as Vancomycin,

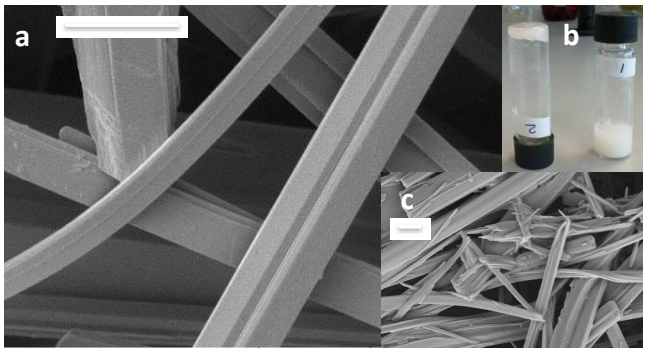

Fig. 3 a) SEM imaging (bar 10 μm) of organogel formed after 24 h using 1% by weight of **1** in THF. b) **1** upon gelation in: *1*) 1% by weight in MeOH; *2*) 1% by weight in a mixture of toluene:THF:$CHCl_3$ solution. c) SEM image (bar 2 μm) of $AgNO_3$ containing gel of **1** (1% by weight) in 8:2 THF:H_2O mixture.

which can inhibit bacterial cell wall synthesis by forming hydrogen bond interactions with terminal *D*-alanyl-*D*-alanine moieties of the NAM/NAG-peptides. With this in mind, compounds **1-12** and (the gels formed) were all tested for their antibacterial activities towards Gram(+) MRSA and Gram(-) *E.coli* strains using in the case of the free compounds a qualitative Kirby Bauer disk diffusion method or in the case of the gels, by using a liquid turbidity test. The areas of clearance were measured for compounds **1-12** (See ESI) to compare relative activities. Of these, **9** and **12** gave the most interesting and promising results, where **9** showed good activity against both strains, while **12** showed more activity towards MRSA after 18 hours of incubation at 37ºC, Fig. 4, at three different concentrations (denoted A→C in Fig. 4). The Kirby Bauer disk diffusion results for the 2-nitro substituted analogue **11** are also shown in Fig 4a.

Interestingly of the three-pyridyl families (*i.e.* **1-4**; **5-8** and **9-12**), compounds **1-4** were seen to have the least activity (area of clearance of 7 mm, 6 mm and 10 mm when 4.3 μmol, 3.9 μmol, and 3.6 μmol, were applied to the disc for **1**, **3** and **4** using MRSA); displaying a moderate selectivity in most cases for MRSA, while the 3-pyridine analogues **7** and **8** showed moderate activity towards *E.coli* both resulting in an area of clearance of 8 mm and 7 mm. However, and as demonstrated for **12** in Fig. 4a, the 4-pyridyl family gave the most potent activity [*e.g.* **12**, (are of clearance of 13 mm (1.8 μmol,) and 20 mm (3.6 μmol) for MRSA]. In addition to these experiments, we also carried out Alamar blue viability assays on these compounds using HeLa cells. These showed that the compounds were either not toxic or only slightly toxic. Hence, we were unable to determine EC50 values for these.

Having demonstrated that these structurally simple pyridyl ureas could function as antibacterial agents, we next investigated the abilities of the hydro- and organo gels for such activity using the aforementioned liquid turbidity test. The results demonstrated that in gel form, all were able to inhibit bacterial growth over 16-18 hours. Even compound **1**, which showed moderate activity (MIC = 4.3) prevented bacterial growth for both MRSA and *E.coli* as demonstrated in Fig. 4b, which also shows the bacterial growth in a solution (as cloudy solution) in a control experiment after 18 hours. These results clearly demonstrate that the supramolecular nature of

the systems, *i.e.* the formation of a self-assembly gel, changes the antibacterial properties of the pyridyl urea structures. While this

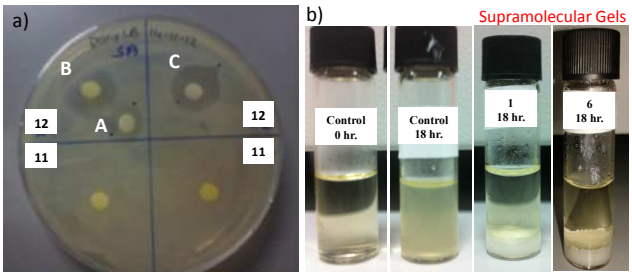

Fig. 4 a) Kirby-Bauer disk diffusion susceptibility test results for compounds **12** (top) and **11** (bottom, showing no clearance) using MRSA A) 0.72 µmol, B) 1.8 µmol and C) 3.6 µmol of **12**, respectively. b) Liquid turbidity test studies for gels **1** and **6** using *E.coli* demonstrating that bacterial growth is inhibited over 18 hours.

phenomenon is not well understood, it is possible that the overall supramolecular structure can give rise to multiple hydrogen bonding interactions that can interact with the bacterial cell wall and possibly disrupt it or prevent its formation. Such properties are highly desirable, particularly as the soft-matter is easily applicable and as such highly attractive for use in coating implants to prevent onset of bacterial infection.[15,26] Self-assembly soft matter are good candidates for application in therapeutic delivery[26] as they can be easily moulded, shaped and made to be responsive to external stimuli, such as pH[25]. Since the above urea gels were reversibly formed[1b] we anticipated that our systems could also 'naturally' degrade with time, for instance, upon interactions with biological anions, such as carboxylates,[2c] which could competitively interrupt the hydrogen bonding networks with in the gels. This we demonstrated by subjecting the gels formed from **6**, and the $AgNO_3$ based gel of **1**, with a solution of acetate. These showed that while the degradation was initially slow, the gels indeed 'dissolved' over a period of days. As these gels can host other organic substrates (*e.g.* cresol red), this results potentially also allows for their application as drug delivery systems, whereupon such degradation, substrates such as known antibacterial drugs can be released. We are currently investigating the formation of such dual functional gels and their antimicrobial properties in greater details.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Synthesis and characterisations; Figures 1-20]. See DOI: 10.1039/c000000x/

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Supramolecular pyridyl urea gels as soft matter with antibacterial properties against MRSA and/or *E. coli*

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The development of a family of twelve aryl pyridyl ureas, their crystallography and the ability of a number of these to form hydrogen bonding supramolecular gels with antimicrobial properties is described.