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Direct synthesis of 5- and 6-substituted 2-aminopyrimidines as potential non-natural nucleobase analogues†

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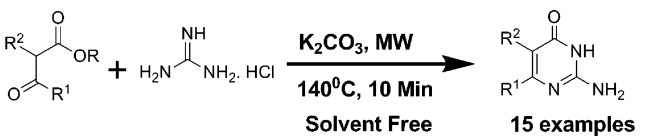
A series of 2-aminopyrimidine derivatives, substituted at 5- and 6-positions, were synthesized. The reaction was carried out in a single step by treatment of the corresponding β-ketoester or β-aldehydoester with guanidine hydrochloride in the presence of K_2CO_3, in a microwave-assisted method without the requirement of solvent. A unique 1 : 1 co-crystal structure was obtained which shows that a 6-phenyl-2-aminopyrimidinone forms a strong nucleobase-pair with cytosine, involving three hydrogen bonds. The base-pair was found to be as strong as that of natural guanine:cytosine (G:C), signifying the potential application of the synthesized derivatives. Additionally, we also report a second co-crystal involving 5-isopropyl-6-methyl-2-aminopyrimidinone and cytosine in a 1 : 1 ratio, which also shows strong base-pairing properties.

Heterocyclic compounds, such as pyrimidines, have found a wide range of applications in the pharmaceutical industry as anti-bacterial, anti-viral and anti-tumor agents, as well as their applications as artificial base-pairs.[1–5] Biological activities of such heterocycles are largely due to their structural resemblance to the nucleobases or coenzymes, enabling them to act as potential inhibitors.

A substantial number of 2-aminopyrimidine compounds were synthesized and many derivatives have been found to be clinically active molecules that exhibit cytotoxic, antibacterial and other kinds of inhibition properties.[6–9] Developing low-cost and efficient synthetic methodologies are important for the production of such compounds. Most commonly, 2-aminopyrimidine derivatives are synthesized following two procedures: (a) through reaction of substituted β-ketoester with guanidine.[10–12] The process requires long reflux and use of substantial amount of concentrated base as well as organic solvents; (b) three-component Biginelli reaction involving a β-ketoester, an aldehyde and guanidine, in presence of strong base and organic solvent.[13–15] In this article, we describe a one-pot synthesis of a series of 2-amino-4-pyrimidinones, a class of 2-aminopyrimidines or commonly known as isocytosines, in a microwave-directed method in solvent free condition. The reaction proceeds smoothly in presence of a mild base (K_2CO_3). The synthesized isocytosines vary in their substitutions at C-5 and C-6 positions (Scheme 1). Although microwave-assisted reactions have been applied to synthesize pyrimidines and uracil derivatives, to the best of our knowledge, it was never reported for the synthesis of 2-aminopyrimidine compounds.[16–20]

Isocytosine is an isomer of cytosine which has tendency to form reverse Watson–Crick base pair with guanine leading to formation of parallel-stranded DNA helix.[21–23] Sugiyama *et al.* have demonstrated the use of oligonucleotides containing isocytosine to selectively recognize guanine as well as isoguanine, a potential oxidative lesion in DNA.[24] Moreover, C-glycosidic isocytidine was employed as triplex forming oligonucleotides whereas N-glycosidic isocytosine was reported for diagnostic assay of branched DNA.[25–27] Isocytosine based self-assembled supramolecular polymers have also been used for the development of smart materials.[28] Apart from being used as a probe nucleobase, isocytosine and their derivatives were widely studied as inhibitors and pharmaceutically important molecules. Development of such isocytosine derivatives with varying stereo-electronic properties, through convenient methods and studying the crystal structures, therefore, will be highly relevant for potential biological as well as pharmaceutical applications.

Compounds **1–15** were synthesized in a closed vessel CEM Discover LabMate microwave reactor in absence of solvent, as



Scheme 1 Schematic presentation for the synthesis of 2-amino-4-pyrimidinones. R, R^1 and R^2 are given in Table 1.

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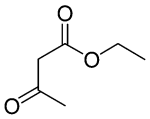
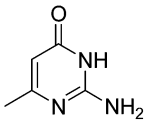
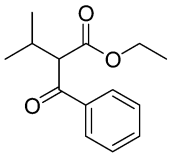
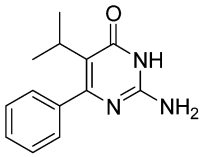
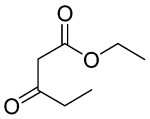
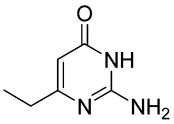
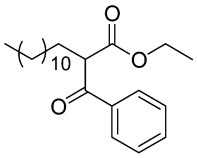
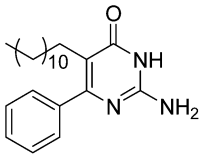
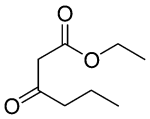
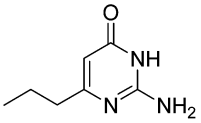
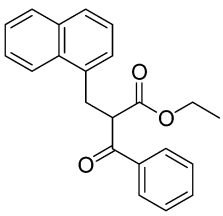
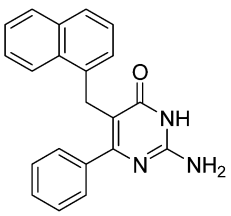
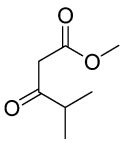
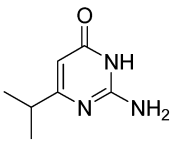
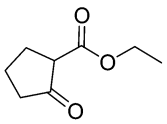
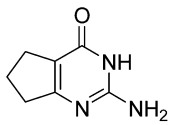
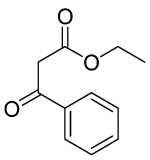
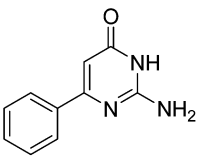
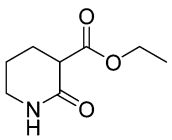
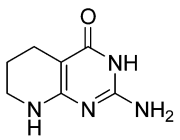
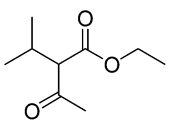
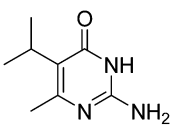
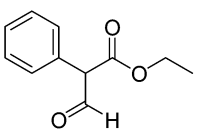
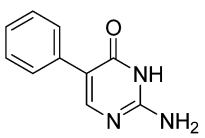
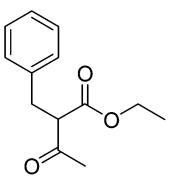
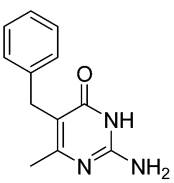
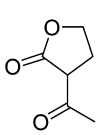
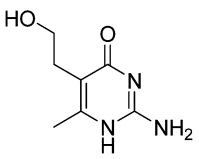
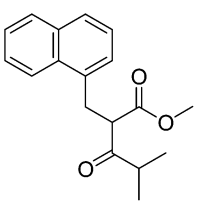
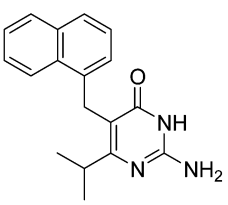
shown in Table 1. In general, 2 mmol of the substrate ester was taken with 4 mmol of guanidine hydrochloride along with 2 mmol of K_2CO_3 in a closed reaction vessel. The reaction attained completion upon irradiation for about 10 minutes and the temperature of the reaction vessel was kept at around 140 °C (see ESI†). In order to show diversity, a wide range of β-ketoesters (**1–12**), β-amidoester (**13**) and β-aldehydoester (**14**) were used as substrates, leading to formation of a variety of 2-amino-4-pyrimidinones, including a functionalized derivative (**15**), in a single step. The reaction

Table 1 Synthesis of various 2-amino-4-pyrimidinone derivatives (**1–15**) in a microwave-assisted method. All irradiations were performed at 140 °C for 10 min, in a closed vessel

S. no.	Substrate (**1a–8a**)	Product (**1–8**)	Yield%	S. no.	Substrate (**9a–15a**)	Product (**9–15**)	Yield%
1			85	9			72
2			82	10			60
3			84	11			61
4			79	12			86
5			82	13			70
6			76	14			80
7			73	15			70
8			67				

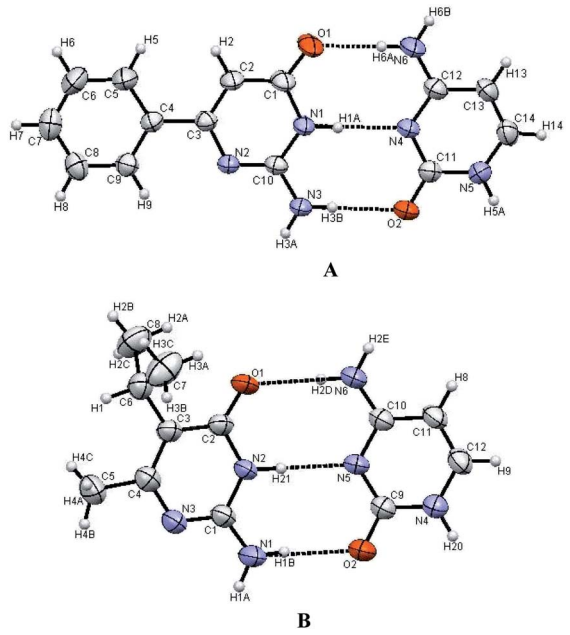


Fig. 1 ORTEP diagrams of the co-crystals. **1A** represents the ORTEP diagram for 6-phenylisocytosine (**5**):cytosine. H-bond distance: O1···N6 (2.81 Å), N1···N4 (2.87 Å), O3···N2 (2.90 Å). **1B** represents the ORTEP diagram for 5-isopropyl-6-methylisocytosine (**6**):cytosine. H-bond distance: O1···N6 (2.93 Å), N2···N5 (2.95 Å) and N1···O2 (2.92 Å).

occurs when the solid guanidine hydrochloride melts into the liquid substrate ester inside the microwave reactor. The reaction did not proceed in absence K_2CO_3, even at higher temperature. To our understanding, this mild base primarily acts as a scavenger of hydrochloric acid, present in the guanidine salt. The yields of the reactions were increased when two equivalents of guanidine hydrochloride was used instead of stoichiometric ratio. It is noteworthy from Table 1 that the reactivity of the substituted β-ketoesters **6–11** were found to be lowered, presumably due to increased steric crowding in the transition state. This can be evident from the mechanism proposed earlier by our group, where we had trapped the partially condensed intermediate.[19] Use of organic bases such as, DBU and triethylamine, in place of K_2CO_3, exhibited very poor yield.

Another important aspect of this article is to analyse the co-crystal structures of the synthesized isocytosine derivatives with free nucleobase cytosine. Evidence of such co-crystals involving modified nucleobases is rare.[29–31] We have obtained two co-crystals, 6-phenylisocytosine (**5**):cytosine and 5-isopropyl-6-methylisocytosine (**6**):cytosine, both in 1 : 1 ratio.[32] Fig. 1 depicts the ORTEP diagrams of the co-crystals obtained from methanol–water (2 : 1 v/v). It can be observed from the ORTEP diagram that the co-crystal of compound **5** with cytosine shows remarkably strong base-pair formation involving three hydrogen bonds, similar to that of natural G:C base pair. The strength of the H-bonds of the co-crystal were found to be as strong as that of Watson–Crick G:C base pair.[33] Compound **5**, therefore, could have potential applications as non-natural nucleotide and as oligonucleotide probe to selectively recognize cytosine in DNA. The co-crystal structure of compound **6** with cytosine, on the other hand, show relatively weaker H-bonds as compared to natural G:C base-pair.

The supramolecular self-assembly of the co-crystals were also studied. The 6-phenylisocytosine (**5**):cytosine co-crystal shows a helix-type molecular architecture, as demonstrated in Fig. 2. On the other hand, the co-crystal of 5-isopropyl-6-methylisocytosine (**6**):cytosine, presents a unique hexagonal self-assembled structure connected by water molecules, creating a rectangular void space. The beauty of such crystals is that, by varying the substitution at 5- and 6-position of 2-amino-4-pyrimidinones, shape of the molecular architecture could be controlled.

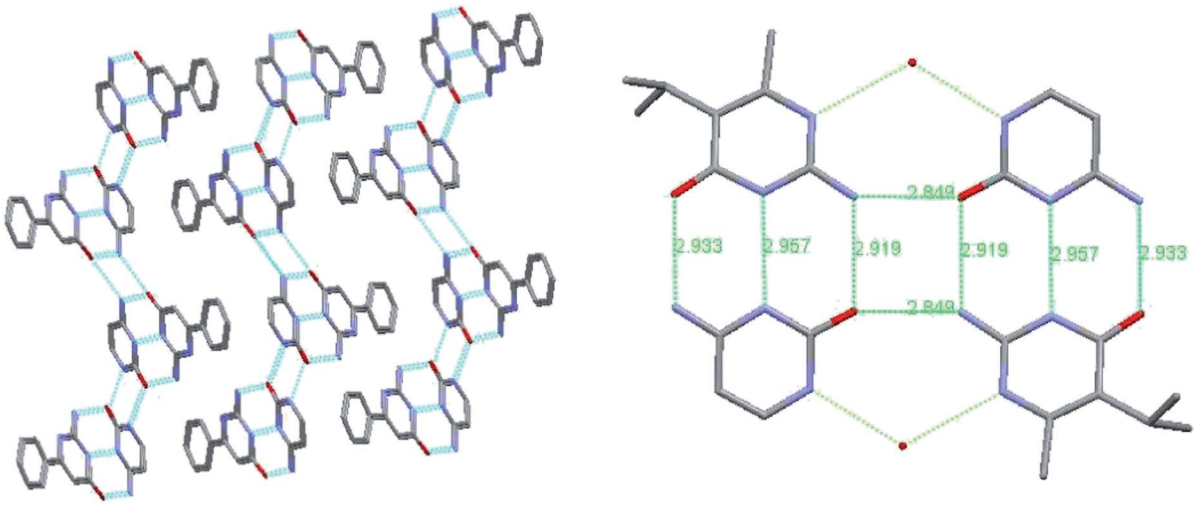


Fig. 2 Supramolecular architecture of the co-crystals. Left: co-crystal of compound **5** with cytosine. Right: co-crystal of compound **6** with cytosine. Red dots represent water molecules.

Conclusions

In this communication we have demonstrated a one-pot, microwave-directed methodology for the synthesis of biologically active 2-amino-4-pyrimidinones, a class of 2-aminopyrimidines. The high-yield reactions were performed in presence of mild base (K_2CO_3) without any solvent and were completed in a very short period of time (10 min). In order to study potential utilities of the synthesized compounds as artificial nucleobase-pairs, we have isolated co-crystals which show strong pairing properties with cytosine, as strong as that of natural G:C base-pair. Such modified nucleobase analogues could have potential applications for biomolecular recognition, apart from their pharmaceutical uses.

Acknowledgements

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