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ARTICLE TYPE

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

**K. Radhakrishnan, Namita Sharma and Lal Mohan Kundu***

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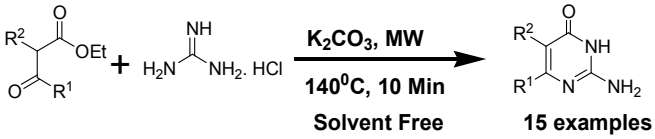
**A series of 2-aminopyrimidine derivatives, substituted at 5- and 6- positions, were synthesized. The reaction was carried out in a single step by treatment of corresponding β-ketoester or β-aldehydoester with guanidine hydrochloride in presence of $K_2CO_3$, in a microwave-assisted method without the requirement of solvent. A unique 1:1 co-crystal structure was obtained which shows that a 6-phenyl-2-aminopyrimidine 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-aminopyrimidine and cytosine in a 1:1 ratio, which also shows strong base-pairing properties.**

Heterocyclic compounds, such as pyrimidines, have found a long range of applications in pharmaceutical industry as anti-bacterial, anti-viral and anti-tumor agents, apart from their applications as artificial base-pairs.[1-5] Biological activities of such heterocycles are largely due to their structural resemblance with that of 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.

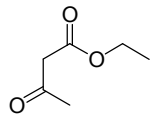
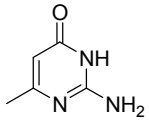
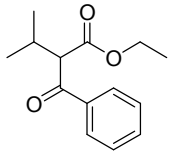
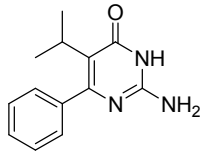
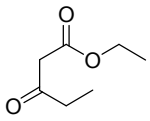
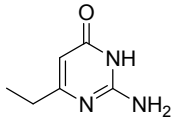
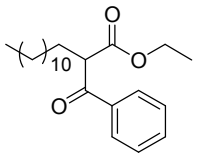
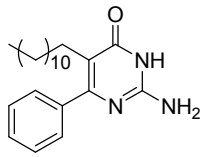
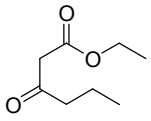
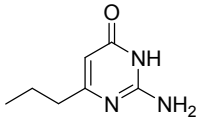
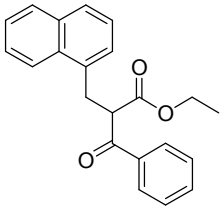
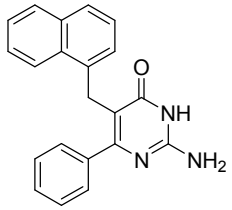
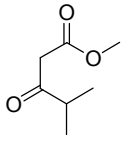
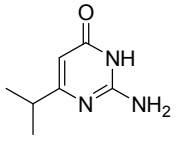
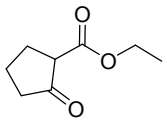
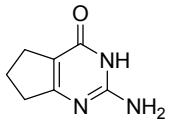
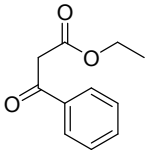
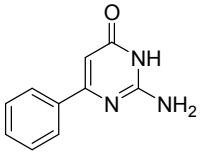
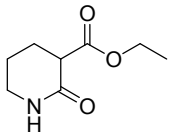
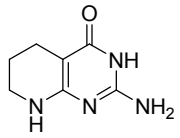
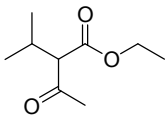
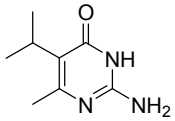
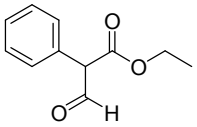
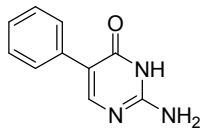
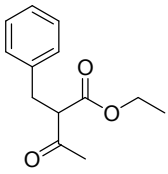
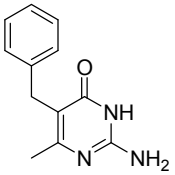
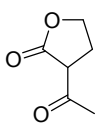
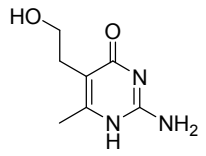
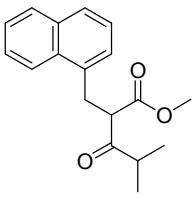
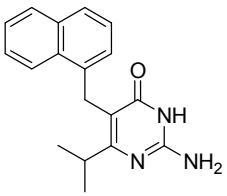



**Scheme- 1:** Schematic presentation for the synthesis of 2-amino-4-pyrimidinones. $R^1$ and $R^2$ are given in table-1.

Compounds **1-15** were synthesized in a closed vessel *CEM Discover LabMate* microwave reactor in absence of solvent, as 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 supporting information). 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 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.

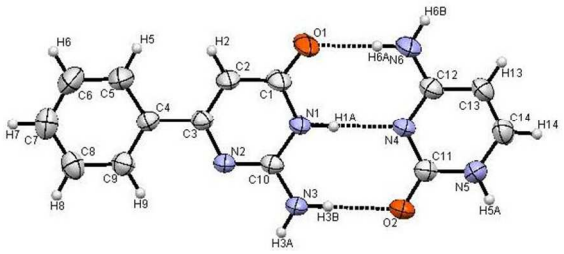
**Table 1**: Synthesis of various 2-amino-4-pyrimidinone derivatives **(1-15)** in a microwave-assisted method. All irradiations were performed at 140°C for 10 min, in a closed vessel.

| 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 | | | | |

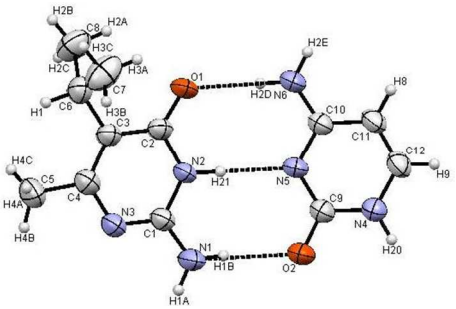
Another important aspect of this article is to analyse the co-crystal structures of the synthesized isocytosine derivatives with free nucleobase cytosine. Evidence of such co-crystals involving modified nucleobases is rare.[29-31] We have obtained

two co-crystals, 6-phenylisocytosine (**5**): cytosine and 5-isopropyl-6-methylisocytosine (**6**):cytosine, both in 1:1 ratio.[32] Figure-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 figure-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.




**1A**




**1B**

**Fig. 1: ORTEP** diagrams of the co-crysrals. **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).

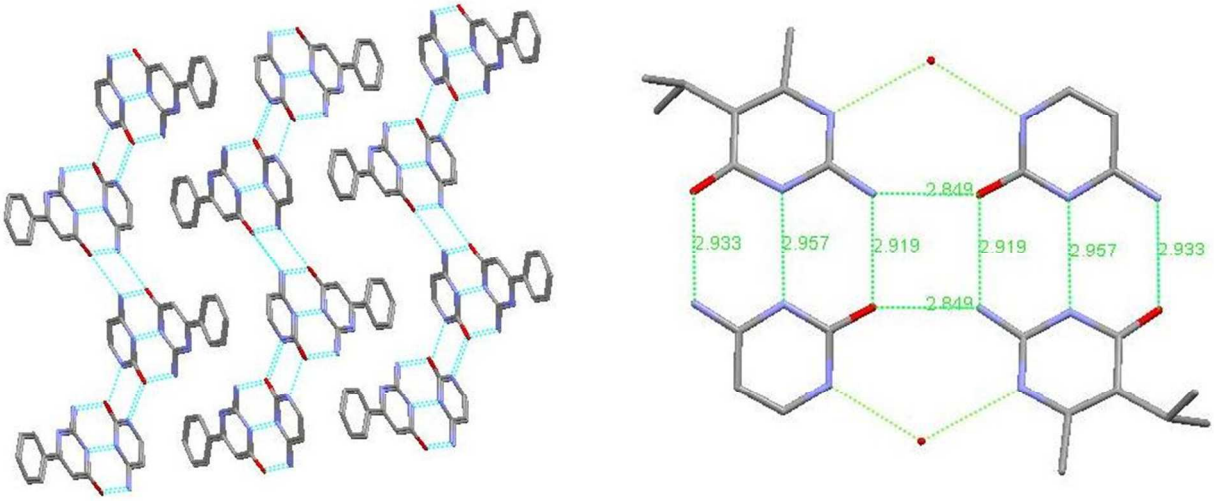



**Fig. 2:** Supramolecular architecture of the co-crystals. **Left:** co-crystal of compound **5** with cytosine. **Right:** co-crystal of compound **6** with cytosine. Red dots represent water molecules.

## 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.

## Acknowledgement

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## Notes and references

[*] *Dr. Lal Mohan Kundu, Department of Chemistry, Indian Institute of Technology Guwahati, North Guwahati, 781039, Assam, India. Fax: +91-3612582349; Tel: +91-3612582326; E-mail: lmkundu@iitg.ernet.in*

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Co-crystal data for compound **6**:cytosine complex: CCDC # 980516. Formula: $C_8H_{13}N_3O/\ C_4H_5N_3O.H_2O$. Temperature (K): 296 (2). Monoclinic, P21/n. a = 15.5249(9) Å, b = 5.5769(3) Å, c = 18.7323(11) Å. α = 90.00°, β = 112.714(3)°, γ = 90.00°. Unit cell volume = 1496.07(15) Å. Z = 4. μ = 0.098 $mm^{-1}$, ρ (calc) = 1.316 $gcm^{-3}$, Mo Kα radiation, R1 = 0.0405, $R1_{all\ data}$ = 0.0528, wR (F2) = 0.1109, wR (F2) $_{all\ data}$ = 0.1209
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