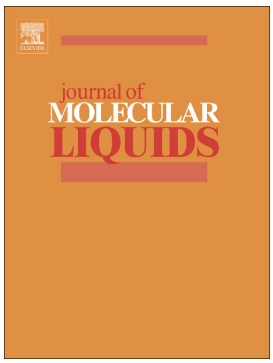

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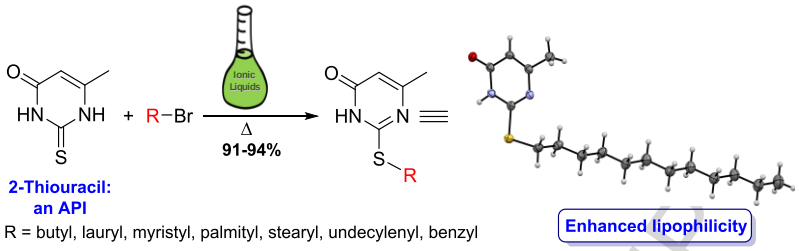

Studies on Solubility and *S*-alkylation of 2-Thiouracil in Ionic Liquids

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ABSTRACT

Ionic liquids have been exploited to assist dissolution of poorly soluble (and poorly bioavailable) drugs, enhancing permeation through physiological barriers to deliver drugs to target sites. Herein, the solubility of 6-methyl-2-thiouracil – a common antithyroid drug, with low solubility in water and common organic solvents – was studied by employing six different imidazolium-based ionic liquids with variable anions. We demonstrate facile, regiospecific *S*-alkylation of 2-thiouracil with various lipophilic side chains in high yields (91–94%) and in the absence of catalysts. The reaction yields are correlated with the H-bond formation ability between the ILs' anions and the solute, indicating that the hydrogen bond is perhaps responsible for the high solubility of 2-thiouracil in [C_2mim][OAc] and [C_2mim][Cl].

Keywords:
Ionic liquids
2-Thiouracil
APIs dissolution
Drug delivery

Drug delivery systems are critical elements in the efficacy of a clinically useful drug and must be designed to transport the drug to its target, at a desirable rate. However, many drug candidates exhibit poor solubility profiles, which often disqualifies them from further clinical testing. The extraordinary ability of ionic liquids to serve as an exotic class of room-temperature solvents to dissolve many poorly soluble active pharmaceutical ingredients (APIs) and further enhance these drugs' cell membrane permeability and therapeutic efficacy has been vastly studied [1-4]. Ionic liquids (ILs), are defined as salts which remain liquid below 100 °C, have attracted much interest as neoteric solvents in pharmaceutical dissolution and synthesis due to their unique physical and solvating properties. That is, unlike common organic solvents, ILs present outstanding properties due to their ionic nature such as low vapor pressure and desirable solvation capacity. Furthermore, ILs are considered as tunable materials with unprecedented properties, which can be modified by simply selecting appropriate cations and anions to design tailor-made properties for specific applications, ranging from synthesis and separation to energy and medicine [5-8].

The first synthesis of a pharmaceutical in a IL-based medium ([C_4mim][PF_6]) was reported by Seddon *et al.*, where Pravadoline – an anti-inflammatory and analgesic drug – was synthesized in high yield and both synthesis and separation steps were performed in the IL [9]. A benchmark contribution in this field was made by Salunkhe and coworkers who compared solubility of nucleosides, *i.e.*, adenosine, cytosine, and guanosine, in various IL systems.

They reported that the formation of H-bonds between the ILs' anion and the APIs improved the solubility of ribonucleosides when compared to common organic solvents [10]. Likewise, Moniruzzaman *et al.*, found that a hydrophilic IL, [C_2mim][OAc], showed high dissolving capability for acyclovir – an antiviral API used for treatment of herpes simplex virus [11]. Bogel-Lukasik *et al.*, reported that [N_{1122}(OH)][NTf_2], [BA][NO_3] and [DDA][NO_3] are great solvents for the dissolution of antituberculosis, *e.g.*, isoniazid and pyrazinecarboxamide [12]. Further, enhanced solubilities of paracetamol and ibuprofen in [$C_n$mim][PF_6] IL (n = 4 and 6) is reported by Leek *et al.*, [13]. Porter and coworkers demonstrated the conversion of poorly water-soluble drugs such as itraconazole, cinnarizine, and halofantrine into lipophilic ILs for introduction into lipid-based formulation. They reported that the formation of lipophilic ILs improves the drugs' solubility in lipid-based formulations, leading to promote the exposure of poorly water-soluble drugs after oral administration [14]. Bica *et al.*, employed [C_2mim][OAc] for extraction of shikimic acid (starting material for the important anti-influenza drug, Tamiflu), indicating that the hydrogen bonding of the IL anion to shikimic acid is responsible for the extraction outcome [15].

The mercapto analogs of pyrimidine bases (*e.g.* 2-thiouracils) are essential modified units of natural and synthetic nucleic acids. Their *S*-, *N*-, or *O*-substituted analogs have shown remarkable therapeutic properties. For example, darapladib and rilapladib (Figure 1) are potent lipoprotein-associated phospholipase A2 (Lp-PLA2) inhibitors – developed by GSK for the treatment of atherosclerosis, coronary artery disease, diabetic macular edema and Alzheimer. Phase II and III clinical trials of those drugs are now in progress [16]. In addition, 2-thiouracil derivatives, along with methimazole, are currently the most commonly employed medications for the treatment of hyperthyroidism. They contain thiourea pharmacophore, which can form stable electron donor-acceptor complexes with the Lewis acid diiodine [17]. Although widely used, 2-thiouracil derivatives are difficult to study because of their insolubility in water and organic solvents, and

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only very few reports addressed the solubility behavior of thiouracil derivatives in molecular solvents [18, 19].

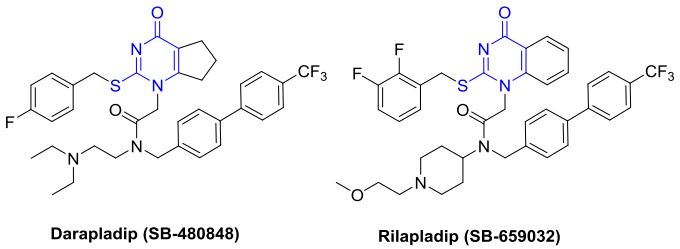
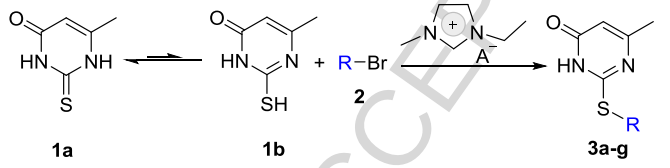


Figure 1. Structures of darapladib and rilapladip Lp-PLA2 inhibitors, featuring a 2-thiouracil ring system.

Thiouracil-based compounds exist in six tautomeric equilibria. Both theoretical and experimental studies indicate that the oxothione form (**1**) is the most stable in an inert matrix (Scheme 1). A thorough literature search revealed that the selective alkylation of N, O or S sites depend on various factors, *viz.*, reaction conditions and the nature of the alkyl halides [20]. Although impressive progress has been made in the development of new methodologies to induce regioselectivity for 2-thiouracil, as well as in the mechanistic understanding of these alkylation reactions [21], there are no reports of a general and straightforward method for selective *S*-alkylation of 2-thiouracil. Alkylation has been traditionally performed under basic conditions (DBU, KOH) and in organic solvents (CH_3CN, DMF) [22]. However, several disadvantages in the base-catalyzed alkylation process has hampered its scale-up. One such limitation is high catalyst loading, which leads to a tedious purification process to remove catalyst residue. Additionally, the strong basic nature of catalysts restrict the substrate scope of the process.

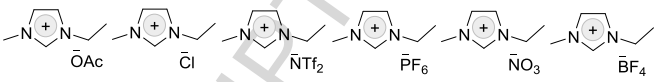
Impressed by the extraordinary solvating power of ILs to dissolve poorly soluble drugs, we have become attentive to new developments in the area. We decided to attempt exploiting IL [C_2mim][X] systems to incorporate various alkyl and alkenyl chains (C_4 and C_{18} carbon atoms) as well as benzyl on sulfur atom of 6-methyl-2-thiouracil in order to produce hydrophobic modified nucleosides, enhancing the cell barrier permeability to improve its pharmacokinetic properties (Scheme 1). This effort



Scheme 1. General reaction of regiospecific *S*-alkylation of 6-methyl-2-thiouracil in ionic liquids.

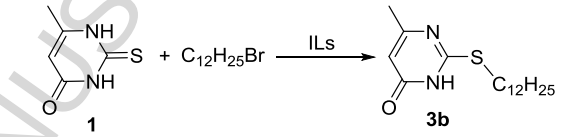
led to the development of the first successful example of catalyst-free approach for regiospecific synthesis of *S*-substituted thiopyrimidine rings. The ultimate goal is to enable incorporation into lipid-based formulations and integration into lipid absorption pathway. Here we report our results.

A set of ILs based on 1-ethyl-3-methylimidazolium ([C_2mim]) cation with different anions (Scheme 2) was selected for this study. To screen the dissolution efficiency of different ILs, we initially focused on the influence of anions on the yields of the alkylation reactions. Several theoretical and experimental investigations already addressed the solvation behavior of 1-ethyl-3-methylimidazolium-based ILs for various solutes [23, 24]. We decided to test different anions, varying in hydrophilicity, to identify optimum ILs for a model reaction (Table 1). As to be expected, we found that [C_2mim][OAc] and [C_2mim][Cl] showed the best results for dissolution of the solute at elevated temperatures (Table 1, Entries 2 and 6), which perhaps is correlated with the strength of intermolecular hydrogen bonding between the anions and the solute as well as dipolarity-polarizability of ILs and solute [25]. However, more investigations would be required to prove it. [C_2mim][OAc] is often considered as an excellent IL for biomass dissolution [15]. The moderate dissolution of **1** was observed for the hydrophobic IL [C_2mim][NTf_2] (Table 1, Entry 7). While promising results were obtained using [C_2mim][Cl], [C_2mim][BF_4] and [C_2mim][PF_6] proved to be the least efficient when they were used as solvents to dissolve 6-methyl-2-thiouracil, shown by their considerable low yields for the model reaction (Table 1, Entries 9, 10).



Scheme 2. 1-Ethyl-3-methylimidazolium ILs used in this study.

Table 1. Impact of reaction conditions and different ILs on the *S*-alkylation of 6-methyl-2-thiouracil.



Entry	ILs	Conditions	Conversion (%)[a]
1	[C_2mim][OAc]	rt, 8h	83
2	[C_2mim][OAc]	65 °C, 3h	94
3	[C_2mim][OAc]	80 °C, 3h	94
4	[C_2mim][Cl]	rt, 8h	79
5	[C_2mim][Cl]	65 °C, 3h	82
6	[C_2mim][Cl]	80 °C, 5h	87
7	[C_2mim][NTf_2]	65 °C, 3h	48
8	[C_2mim][NO_3]	65 °C, 3h	67
9	[C_2mim][BF_4]	65 °C, 3h	<30
10	[C_2mim][PF_6]	65 °C, 3h	<30

[a] Conversion measured by ^{1}H NMR.

We further evaluate the effect of pH over the solubility of the solute in [C_2mim][OAc] to address if the solute could be better dissolved in its neutral or charged form. The change of pH from 9.0 to 4.3 did not impact the solubility, meaning that electrostatic interactions between the charged solute and the IL does not have a major effect on the solubility of the solute in IL medium.

In order to have further insight into the reaction mechanism in ILs, DFT calculations were conducted using 6-methyl-2-thiouracil (**1**) and methyl bromide as a model reaction and showed as a two-step reaction in a gas phase (Figure 2). The first step is the S_N2 nucleophilic attack of the thioxo group at the methyl carbon of methyl bromide, followed by a proton abstraction at the N1 amine, which is favored proton donor to the proton at the N3 position.

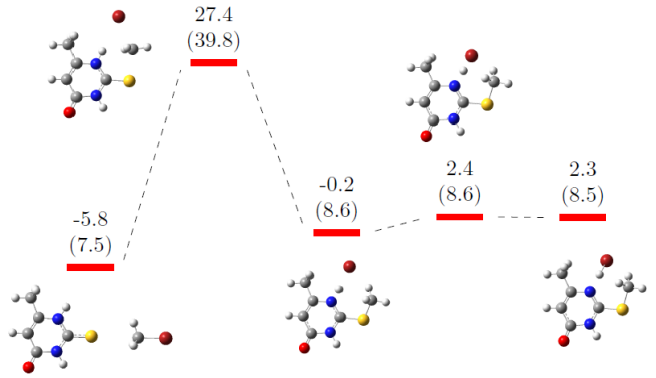


Figure 2. Gas phase reaction profile for reaction between 6-methyl-2-thiouracil **1** and CH_3Br computed at M06-2X [26]/6-311++G(d,p) level (see Supporting Information for more details and references). All energies are in kcal/mol and with respect to the separated 6-methyl-2-thiouracil and methyl bromide, and the numbers in parentheses are Gibbs free energies for the corresponding reaction step.

Encouraged by the initial results, the scope of 2-thiouracil substitution was investigated *via* forming enhanced lipophilic products with incorporation of long saturated and unsaturated chains, identical to natural fatty acids (Scheme 1). The substitution reaction of **1a** with alkyl, alkenyl and benzyl bromides (**2**) afforded exclusively *S*-substituted 2-thiouracil compounds (**3**) in excellent yields (91–94%), characterized by NMR, IR and ESI mass spectroscopy. Empirical data confirmed that hydrophilic tails of thiopyrimidine cores fluidize the cell membrane to create pathways for the diffusion of molecules and enhance drug permeation [27]. In addition, it was reported that increasing the chain length bonded to the 2-thiouracil motif showed significant rate enhancement, supporting the idea that the sulfur group was located in lipophilic environment [28]. Except **3a** and **3g**, the other products (**3b**–**f**) contain long alkyl and alkenyl appendages identical to that in natural fatty acids. Compounds **3a**–**e** feature fully saturated side chains. Compound **3f** bears C_{11} side chains with a terminal *ene* group, identical to undecylenic acid.

The experimental procedure was very simple, in that, 6-methyl-2-thiouracil was added in excess amount to the ILs, the mixture was then stirred under constant agitation (1000 rpm), at room temperature for 30 min. After reaching the equilibrium (preliminary tests on the time required to achieve the equilibrium were carried out), the alkyl/alkenyl/benzyl bromides (**2**) were added and stirred at 65 °C for 3 hours. Results given are based on three independent experiments. The products (**3**) precipitated out when ice water was added to the reaction mixture. No chromatographic separation required, and products were simply purified by crystallizing in DMF. All the new products were isolated as white crystalline solids, and were characterized by ^1H and ^{13}C NMR as well as IR spectra all of which fully comport with their proposed structures and formulations (see Supplementary Information).

The exclusive formation of *S*-alkylated products was proven by the X-ray crystal structure of **3b**, which was obtained from the slow evaporation of a solution of the compound in CH_3CN/DMF [29]. Not surprisingly, **3b** showed higher solubility in molecular solvents, compared to **1**, due to the presence of the hydrophobic side chains. The asymmetric unit is shown in Figure 3. **3b** crystallizes with the monoclinic space group, $P2_1/c$, with four formula units per unit cell, each of which has its *all-trans* linear C_{12} appendages. The crystals of **3b** self-organize *via* various noncovalent interactions (*i.e.* H-bonding and van der Waals) and form an interdigitated bilayer-structure with separate polar and nonpolar domains (see SI, Figure S1). The nonpolar regions of the bilayer are held together through van der Waals forces between the alkyl chains. Adjacent 2-thiouracil rings form a strong two-point hydrogen bonded dimer (N–H···O, d_H = 2.751Å) in the polar region of the structure (see SI, Figure S2).

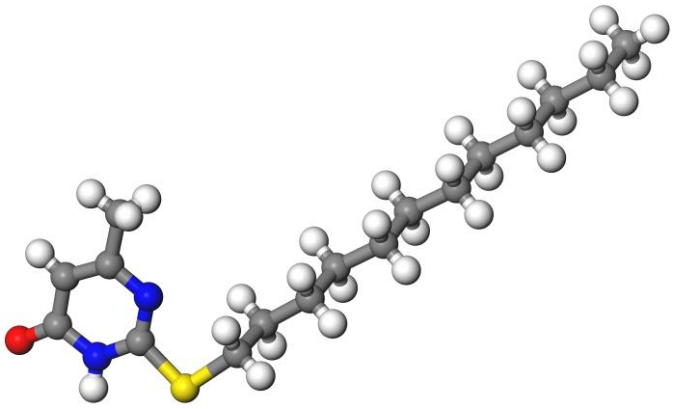


Figure 3. Crystal structure of **3b** (180 K), showing the *S*-alkylated product [29].

Overall, the present results indicate that 1-ethyl-3-methylimidazolium-based ionic liquids not only offers considerable promise for dissolution of a pharmaceutical compound, *e.g.*, 6-methyl-2-thiouracil, but also mild, catalyst-free and regiospecific *S*-substitution could be carried out on 2-thiouracil motif in ILs. For the first time, the pharmaceutically active ingredient was not only effortlessly dissolved in ionic liquids, but efficiently transferred to its lipophilic derivatives as important modified nucleosides intermediates. In this study, 6-methyl-2-thiouracil is converted into its lipophilic analogs to facilitate incorporation into lipid-based formulations and demonstrated potential for integration into lipid absorption pathways. The reactions proceed quickly and cleanly and high isolated yields (91−94%) were obtained for all seven examples evaluated, as determined by single-crystal X-ray diffraction, NMR, and IR spectroscopy techniques. The single-crystal XRD data conclusively confirms the formation of *S*-substituted products (versus *N*- or *O*- substitution). In particular, our methodology allows for the use of [C_2mim][OAc] ionic liquids as the green(er) reaction solvents, enhancing the solubility of 2-thiouracil in comparison to common organic solvents. The yields of the reactions in various ionic liquids depend on the structure of the anions – the ability of some anions to form strong hydrogen bonds likely led to the higher yields.

Acknowledgments

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[29] Crystallographic data for **3b**: $C_{17}H_{30}N_2OS$, M_r = 310.49, 0.40 x 0.05 x 0.05 mm, Monoclinic, $P2_1/c$, a = 24.8512(19) Å, b = 4.6661(3) Å, c = 16.1664(14) Å, β = 106.729(9)º, V = 1795.3(2) Å^3, Z = 4, ρ_{calcd} = 1.149 gcm^{-3}, μ = 0.182 mm^{-1}, Mo-$K\alpha$ radiation, λ = 0.71073Å, T = 180 K, $2\theta_{max}$ = 50.04º ($-19 \le h \le 29$, $-3 \le k \le 5$, $-19 \le l \le 13$), $F(000)$ = 680, 6760 measured reflections, $R1$ = 0.0660 for 1839 reflections ($I>2\sigma(I)$), $wR2$ = 0.1442 for 3172 independent reflections (all data) and 196 parameters, S = 1.023. CCDC 1828246 contains the supplementary crystallographic data.

Highlights:

- Solubility of 2-thiouracil is tested in imidazolium-based ionic liquids.
- The role of the various anions for the solubility of 2-thiouracil is studied.
- 2-thiouracil is an antithyroid drug with low solubility in H_2O & organic solvents.
- Regiospecific *S*-alkylation of 2-thiouracil with lipophilic tails is reported.