Green Chemistry

Cite this: *Green Chem.*, 2011, **13**, 2344

www.rsc.org/greenchem

COMMUNICATION

Toxic on purpose: ionic liquid fungicides as combinatorial crop protecting agents†

Katharina Bica,[a,b] Louise R. Cooke,[c] Patrick Nugent,[c] Christiaan Rijksen[b] and Robin D. Rogers*[b,d]

Received 14th February 2011, Accepted 31st May 2011
DOI: 10.1039/c1gc15170c

We present an ionic liquid strategy for novel hydrophobic fungicide forms of the actives thiabendazole and imazalil with increased rain persistence and activity against potato tuber diseases.

Only two fungicides are currently approved in the UK for use against potato tuber diseases such as dry rot (*Fusarium* spp.), gangrene (*Phoma* spp.), silver scurf (*Helminthosporium solani*), and skin spot (*Polyscytalum pustulans*): the benzimidazole derivative thiabendazole **1** and the imidazole-derivative imazalil **2**.[1] Thiabendazole has been widely used on potatoes since the mid-1970s both pre-planting and post-harvest.[2] However, problems with its use include resistance conferred by specific mutations (single base changes) in the β-tubulin gene, which have affected its activity in controlling silver scurf, skin spot, and dry rot caused by *F. sambucinum*, and its limited penetration into the potato tissue, since most of the active stays on the skin.[3] Imazalil was introduced for potato tuber disease control in the early 1980s[4] and is now available both alone and formulated with thiabendazole, but, like thiabendazole, it suffers from limited penetration into the tuber.

Within the last decade, ionic liquids (ILs, currently defined as salts melting below 100 °C) have been studied for an increasing number of applications where low or no toxicity is needed, despite the biological activity of many of the ILs.[5,6] Subsequently, applications have arisen taking advantage of known biological activity of several ions that could be made into ILs leading to new applications. For example, the antimicrobial properties inherent to some ILs have been recognized as valuable tools for biocide applications, including the development of bioactive coatings against MRSA or other pathogens, antiseptics or antifouling agents, or anti-cancer agents.[7–9]

While these examples mainly deal with traditionally studied IL ions such as 1-alkyl-3-methyl-imidazolium salts, ILs can also be directly prepared from pharmaceutically active ingredients.[10,11] We have recently shown that an IL form of a formerly solid drug cannot only preclude polymorphism, but also influences important properties such as solubility, bioavailablity, and stability.[12] Furthermore, IL forms may provide novel delivery options with improved membrane penetration or iontophoresis.[13]

Another field where the combination of biological activity and IL form might bring operational benefits is in agrochemistry.[14,15] For example, a hydrophobic IL form of an agrochemical could improve rain-fastness, reduce drift and the amount of chemicals which need to be applied, and also enhance movement within the plant to achieve greater uniformity of protection. Improved persistence might be achieved by movement into plant tissue or into the cuticle/epicuticular wax followed by gradual re-distribution.

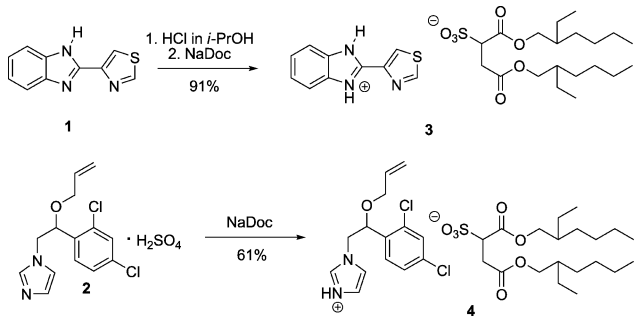
Enhanced curative activity against plant pathogens might result from re-distribution into plant tissue, since in the absence of translaminar or systemic movement, a fungicide can only act on the target pathogen before it penetrates plant tissue. The downside of increasing penetration would be the impact on residues; however, this would not matter if a pre-planting seed tuber treatment was used for seed-borne diseases. Additionally, a dual functional approach combining active cations and anion in one IL formulation might lead to synergistic or antagonistic effects (as observed for pharmaceutically active ILs) which might even be used to overcome resistance.[16]

We sought to study the utility of an IL approach to a major problem affecting the agricultural economy in Northern Ireland, diseases of potato tubers. We present herein novel active ILs composed of the fungicides thiabendazole **1** and imazalil **2** and discuss their activity against potato tuber pathogenic fungi (Scheme 1).

Thiabendazole and imazalil have an inherent basicity due to free nitrogen atoms that can easily be protonated. If paired with an appropriate acid, the acid/base equilibrium lies far enough to the right to obtain a low-melting, protic IL. For our initial studies, we chose docusate (bis(2-ethylhexyl) sulfosuccinate) as a suitable counterion for its ability to form stable, hydrophobic

[a]*Institute of Applied Synthetic Chemistry, Vienna University of Technology, 1060, Vienna, Austria*
[b]*The Queen's University of Belfast, QUILL, School of Chemistry and Chemical Engineering, Belfast, Northern Ireland, BT9 5AG*
[c]*Applied Plant Science and Biometrics Division, Agri-Food and Biosciences Institute, Newforge Lane, Belfast, UK BT9 5PX*
[d]*The University of Alabama, Department of Chemistry and Center for Green Manufacturing, Tuscaloosa, AL, 35487, USA.*
E-mail: rdrogers@as.ua.edu

† Electronic supplementary information (ESI) available: Synthetical procedures and analytical data of compound **3** and **4**, origin of isolates, and detailed information on *in vivo* assays. See DOI: 10.1039/c1gc15170c



Scheme 1 Synthesis of IL fungicide formulations of thiabendazole **1** and imazalil **2** (shown as its sulfate salt).

ILs, as well as its known emollient and skin penetration features.[17–19]

As a general strategy, the neutral fungicide was first converted to the chloride salt by treatment with aqueous hydrochloric acid (Scheme 1). Subsequent anion metathesis was achieved by a straightforward metathesis reaction with one equivalent sodium docusate in a mixture of acetone and water. The newly formed ILs were then extracted with dichloromethane and, after extractive removal of inorganic impurities with H_2O, isolated by evaporation in good yields. These suggested techniques can typically reduce the metal and halide contamination of the final IL to ≤ 0.05%. Although ILs based on alkylated thiazolium cations have been previously described,[20] here protonation was obtained selectively on the benzimidazole heterocyclic system, thus giving rise to thiabendazolium docusate **3** in 91% yield. Based on the commercial availability of imazalil as its sulfate salt, this protocol was slightly modified for the synthesis of imazalilium docusate **4**. Anion metathesis with sodium docusate was performed in anhydrous chloroform and gave imazalilium docusate **4** in a similar manner in 61% yield.

As is typical for hydrophobic ILs, these novel fungicide formulations were insoluble in water, in polar-aprotic solvents such as hexane or benzene, and also in ethyl acetate or diethyl ether. On the other hand, good solubility was observed in short-chain alcohols (MeOH, EtOH), in halogenated solvents, and in polar-aprotic solvents such as acetone, DMF or DMSO.

Both thiabendazolium and imazalilium docusate **3** and **4** were obtained as viscous liquids with glass transition temperatures at −16.3 °C and −31.0 °C, respectively. However, after some time both IL formulations solidified into crystalline solids melting at 46.9 or 64.0 °C, thus still fitting the current definition of ILs. When investigating the thermal stability using thermogravimetrical analysis (TGA), we observed single-step decompositions and excellent stabilities of >200 °C for both IL fungicides, indicating that the formation of a low-melting or liquid salt formulation is not related to limited stability.

We have carried out *in vitro* tests of the IL thiabendazolium docusate **3** in comparison to the neutral fungicide using selected isolates of *Fusarium* spp., the cause of potato dry rot. *F. coeruleum* and *F. culmorum* are sensitive to thiabendazole whereas isolates of *F. sambucinum* are frequently resistant. Isolates of *Phoma* spp., the cause of gangrene, and *Phytophthora erythroseptica*, the cause of pink rot, were also tested.

Thiabendazolium docusate **3** was prepared as a solution in EtOH and studied at concentrations of 250 and 25 μM (equivalent to 50 and 5 mg thiabendazole **1** per liter) in agar (malt agar for *Phoma* spp. and potato dextrose agar for the other organisms) to determine *in vitro* activity against mycelial growth of the test organisms. The final concentration of EtOH in agar was 1% v/v, which gave 0–10% inhibition of mycelial growth; results were calculated using a 1% ethanol control.

We were pleasantly surprised to see that the activity of thiabendazole **1** against the *Fusarium* and *Phoma* spp. was retained for the hydrophobic IL formulation (Fig. 1, Table 1). Furthermore, while thiabendazole **1** is not considered active against the Oomycete pathogen, *P. erythroseptica*, there is some evidence that the IL **3** is more active. It is interesting to note that a simple co-formulation of the neutral active thiabendazole with

Table 1 Sensitivity of potato tuber pathogens to derivatives of thiabendazole

		Inhibition (%)		
Isolate	Concentration (μM)	**1**	**3**	**1**-Stearic acid
F. sambucinum ex SASA	250	89	96	89
	25	22	19	24
F. sambucinum L'gall 53	250	93	68	82
	25	19	11	20
F. sambucinum L'gall 11	250	76	83	82
	25	8	19	10
F. coeruleum ex B7/06 T8	250	99	99	99
	25	99	99	99
F. coeruleum ex B16/08 P31	250	100	100	100
	25	100	100	100
F. culmorum P13	250	100	100	100
	25	100	100	100
Phoma exigua 9.1	250	100	99	99
	25	95	94	95
Phoma foveata BL2 05 T3	250	100	99	99
	25	98	95	97
Phytophthora erythroseptica BL2/08 P31	250	51	97	54
	25	4	33	10
Phytophthora erythroseptica Rooster Eire	250	31	91	34
	25	8	11	11

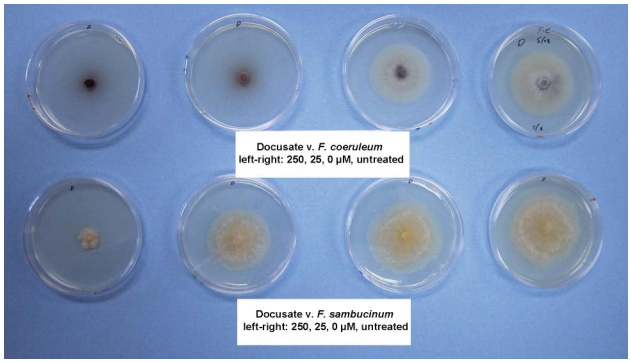


Fig. 1 *In vitro* activity of thiabendazolium docusate **3** against *F. coeruleum* and *F. sambucinum*.

Table 2 Sensitivity of potato tuber pathogens to derivatives of imazalil

Isolate	EC_{50}[a] µM	
	2	**4**
F. sambucinum ex SASA	0.7	1.0
F. sambucinum L'gall 11	1.8	2.6
F. coeruleum ex B7/06 T8	12.9	19.2
F. culmorum P13	0.4	0.4
Phoma exigua 9.1	5.6	7.3
Phoma foveata BL2/5 T3	2.7	4.6
P. erythroseptica BL2/08 P31	>127	38.4
P. erythroseptica Rooster Eire	>127	100.4

[a] Concentration required to reduce mycelial growth *in vitro* by 50%.

a second hydrophobic molecule such as stearic acid – that did not lead to protonation and IL formation[21] – did not enhance the activity, as was observed for **3**, but gave values similar to those of thiabendazole alone. This behaviour might be explained by the tendency of hydrophobic ILs to form ion pairs in solution, which makes them inherently different from a simple co-formulation of the active compounds with a second hydrophobic neutral.[22]

In a similar manner, imazalilium docusate **4** was tested at 127, 25, 12.7, 2.5 and 1.27 µM (equivalent to 0.5, 1, 5, 10 and 50 mg imazalil **2** per liter) against some of the same pathogen isolates. Again, we observed that activity was retained with EC_{50} values similar to those obtained with neutral imazalil (Table 2). Imazalil, an inhibitor of fungal ergosterol synthesis, is not active against Oomycete pathogens, but the hydrophobic IL imazalilium docusate **4** showed some limited activity against *P. erythroseptica*.

An IL strategy can be used for novel fungicide formulations with increased hydrophobicity against potato tuber pathogens. The basicity inherent in many fungicides, particularly in imidazoles or benzimidazoles allows simple transformation into a hydrophobic IL form in straightforward metathesis reactions with hydrophobic anions such as the common emmolient docusate. In addition to the potential for increased persistence of these novel hydrophobic fungicide formulations, biological testing showed that fungitoxicity against a range of potato tuber pathogens was not only retained but enhanced. These early results might not only lead to a dramatic reduction of future fungicide applications, but should kick-start ionic liquid research in crop protection.

Acknowledgements

Mark Wilson is thanked for technical assistance with the *in vitro* sensitivity testing.

Notes and references

1 J. C. Peters, A. K. Lees, D. W. Cullen, L. Sullivan, G. P. Stroud and A. C. Cunnington, *Plant Pathol.*, 2008, **57**, 262–271.
2 G. A. Hide, S. M. Hall and K. J. Boorer, *Plant Pathol.*, 1988, **37**, 377–380.
3 G. J. McKay and L. R. Cooke, *FEMS Microbiol. Lett.*, 1997, **152**, 371–378.
4 G. Cayley, G. A. Hide, P. J. Read and Y. Dunne, *Potato Res.*, 1983, **26**, 163–173.
5 *Ionic Liquids in Synthesis*, ed. P. Wasserscheid and T. Welton, Wiley-VCH, Weinheim, 2nd edn, 2008.
6 J. Ranke, S. Stolte, R. Stoermann, J. Arning and B. Jastorff, *Chem. Rev.*, 2007, **107**, 2183–2206.
7 L. Carson, P. K. W. Chau, M. J. Earle, M. A. Gilea, B. F. Gilmore, S. P. Gorman, M. T. McCann and K. R. Seddon, *Green Chem.*, 2009, **11**, 492–497.
8 J. Pernak, K. Sobaszkiewicz and I. Mirska, *Green Chem.*, 2003, **5**, 52–56.
9 D. Demberelnyamba, K.-S. Kim, S. Choi, S.-Y. Park, H. Lee, C.-J. Kim and I.-D. Yoo, *Bioorg. Med. Chem.*, 2004, **12**, 853–857.
10 J. Stoimenovski, D. R. MacFarlane, K. Bica and R. D. Rogers, *Pharm. Res.*, 2010, **27**, 521–526.
11 W. L. Hough and R. D. Rogers, *Bull. Chem. Soc. Jpn.*, 2007, **80**, 2262–2269.
12 (*a*) K. Bica, C. Rijksen, M. Nieuwenheuzen and R. D. Rogers, *Phys. Chem. Chem. Phys.*, 2010, **12**, 2011–2017; (*b*) K. Bica and R. D. Rogers, *Chem. Commun.*, 2010, **46**, 1215–1217.
13 H. Hamamoto and Y. Miwa, *PCT. Int. Appl. 2009075094A1*, 2009. Etodolac Patch (MRX-7EAT) became available for Phase I clinical trial US. http://www.medrx.co.jp/english/newsrelease.html. (last accessed 15/12/2009).
14 V. J. Kramer, D. G. Ouse, N. R. Pearson, H. Tank and M. W. Zetler, *U.S. Pat. Appl. 20080207452 A1*, 2008.
15 R. D. Rogers, D. T. Daly, R. P. Swatloski, W. L. Hough, J. H. Davis, M. Smiglak, J. Pernak and S. K. Spear, *PCT Int. Appl. 2007044693*, 2007.
16 W. L. Hough, M. Smiglak, H. Rodriguez, R. P. Swatloski, S. K. Spear, D. T. Daly, J. Pernak, J. E. Grisel, R. D. Carliss, M. D. Soutullo, J. H. Davis Jr. and R. D. Rogers, *New J. Chem.*, 2007, **31**, 1429–1436.
17 J. H. Davis, Jr and P. A. Fox, *Chem. Commun.*, 2003, 1209–1212.
18 G. Liversidge, W. M. Eickhoff, K. J. Illig, P. Sarpotdar and S. B. Ruddy, *PCT Int. Appl. 9620735*, 1996.
19 I. Legen, M. Salobir and J. Kerc, *Int. J. Pharm.*, 2005, **291**, 183–188.
20 J. H. Davis and K. J Forrester, *Tetrahedron Lett.*, 1999, **40**, 1621–1622.
21 In contrast to **3** and **4**, analytical data for the co-formulation of thiabendazolium and stearic acid did not show any sign of IL or salt formation. See: K. Bica, J. Shamshina, W. Hough, D. MacFarlane and R. D. Rogers, *Chem. Commun.*, 2011, **47**, 2267–2269.
22 (*a*) K. J. Fraser, E. I. Izgorodina, M. Forsyth, J. L. Scott and D. R. MacFarlane, *Chem. Commun.*, 2007, 3817–3819; (*b*) D. F. Kennedy and C. J. Drummond, *J. Phys. Chem.*, 2009, **113**, 5690–5693.

