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Bioorganic & Medicinal Chemistry Letters 17 (2007) 5222–5226
Bioorganic & Medicinal Chemistry Letters

Synthesis and SAR studies of novel antifungal 1,2,3-triazines

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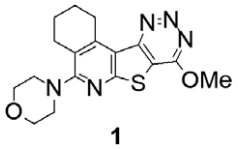
Received 25 May 2007; revised 25 June 2007; accepted 27 June 2007
Available online 30 June 2007

Abstract—A novel series of pyridothieno-1,2,3-triazines with potent antifungal activity against *Erysiphe graminis f. sp. tritici* has been discovered. Two complementary synthetic routes to compounds of this type have been developed and used to efficiently explore the structure–activity relationships around the lead compound. The incorporation of oxygen atoms into the side chains of the molecules has allowed the solubility of the compounds to be increased 10-fold whilst retaining biological activity.

As part of our ongoing search for novel compounds possessing antifungal properties, we purchased the unusual polycyclic 1,2,3-triazine **1**. Initial tests showed this compound to have promising activity. Further, more detailed, biological testing confirmed that **1** possessed potent protectant activity against wheat powdery mildew (*Erysiphe graminis f. sp. tritici*).



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Although the chemistry of 1,2,3-triazines is less well explored than that of other triazine isomers there are reports of pyridothieno-1,2,3-triazines possessing pharmacological effects, notably anti-allergic activity.[1–4] There are isolated reports of compounds of this type showing antiprotozoal[5] or antimicrobial activity,[6] although in the latter case the presence of a reactive chlorine on the triazine ring is required for activity. A recent report, published following the completion of our work, discloses compounds similar to those described here as possessing moderate antitumour activity.[7] However, there are only two references in the literature to compounds having the tetracyclic ring system of **1** and no reports of compounds of this structural type possessing potent antifungal effects of the type that we observed.[7,8]

The promising antifungal activity of **1** prompted us to further explore the potential of this area. A number of analogues of the lead structure were purchased and tested. Results confirmed the biological signal and provided some initial indication of the structure–activity relationships in this chemical class.

In order to explore the scope for activity in more detail we needed to devise synthetic routes that would facilitate the efficient variation of key parts of the lead molecule. In particular we focussed on keeping the pyridothienotriazine core of the molecule constant and exploring variation of the peripheral methoxy and morpholine groups.

Cyclohexanone was converted into thiopyranthione **2**[9] and the morpholine installed by rearrangement of **2**.[10] The thiophene was constructed by S-alkylation and cyclisation.[11] Diazotisation of the amine and acid-mediated cyclisation onto the nitrile generated chlorotriazine **3**.[3,12,13] Finally, the methoxy group was inserted by displacement of chlorine[3,4] to provide **1** in 16% overall yield (Scheme 1). This route allowed an efficient variation of the methoxy group by the use of different nucleophiles in the final step; we employed a range of alcohols, thiols and amines successfully. By altering the starting ketone we were also able to prepare analogues with variation in the fused cyclohexyl ring.

In order to explore variation of the morpholine group efficiently we devised a second synthetic route which proceeded via hydroxythiopyridone **4**.[14] The thiophene and triazine rings were constructed and the methoxy group added as before. Conversion of pyridone **5** to the corresponding *O*-triflate allowed the morpholine to be installed to provide **1** in 15% overall yield (Scheme 2).[15]

Keywords: Antifungal; 1,2,3-Triazine.
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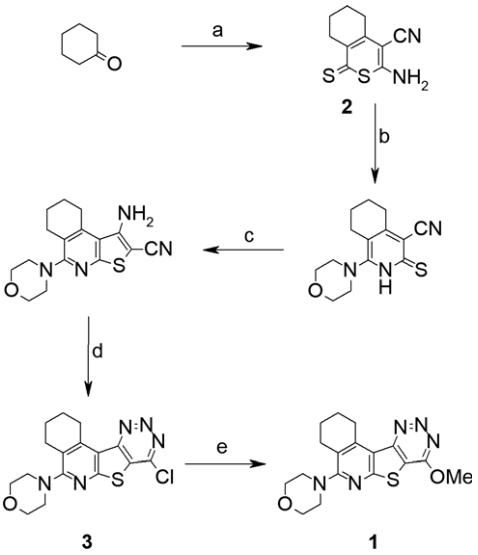
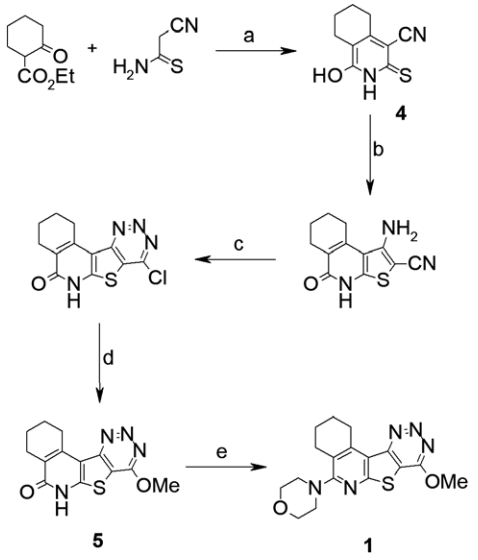
0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2007.06.076



Scheme 1. Reagents and conditions: (a) $CH_2(CN)_2$, CS_2, Et_3N, MeOH, 83%; (b) morpholine, EtOH, reflux, 47%; (c) i—ICH_2CN, KOH, DMF; ii—KOH, DMF, 92%; (d) $NaNO_2$, concd HCl, H_2O, 94%; (e) NaOMe, MeOH, reflux, 48%.



Scheme 2. Reagents and conditions: (a) KOH, MeOH, reflux, 70%; (b) $ClCH_2CN$, KOH, DMF, 79%; (c) $NaNO_2$, concd HCl, H_2O, 77%; (d) NaOMe, MeOH, reflux, 99%; (e) i—NaH, DMF; ii—$PhNTf_2$; iii—morpholine, 37%.

We used this route to replace the morpholine group with a range of amines and alkoxy groups.

Both of these routes proved amenable to robotic and multiple parallel synthesis methods and a wide range of analogues of the original lead were prepared in a rapid and efficient manner.

Using the route outlined in Scheme 1 we conducted a limited exploration of the tetracyclic core of the lead (Fig. 1).

The cyclopentyl analogue **6** maintained activity, but compounds containing either an oxygen (**7**) or sulfur

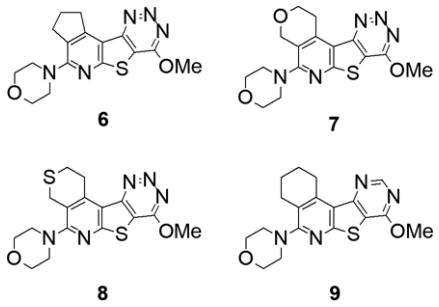
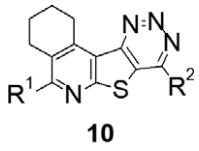


Figure 1. Variations of the core ring system.

(**8**) atom in this ring were inactive. Replacement of the 1,2,3-triazine ring with a pyrimidine (**9**) resulted in the complete loss of antifungal activity.

Given the apparent lack of scope to vary the core ring system the majority of our efforts were directed towards compounds of general structure **10** in which the ring system was kept constant and the peripheral groups were altered (Table 1).



There was limited scope for variation of the group (R^2) on the 1,2,3-triazine. A small increase in size of the alkoxy group was tolerated (**11** and **12**). However, the introduction of branching (**13** and **14**), or of significantly larger groups (**15** and **16**), was not permitted. Compounds containing thioethers in this position were active. These followed the trends for the alkoxy series, with small groups giving good activity (**17** and **18**) and larger ones resulting in inactive compounds (**19**). The unsubstituted thiol (triazinethione) **20** was also inactive, as was the chlorotriazine **3**. All compounds examined with nitrogen-containing groups in this position, including secondary (**21** and **22**) and tertiary (**23** and **24**) amines and hydrazines (**25** and **26**), were completely inactive.[16] A noteworthy feature of the SAR at this position of the molecule was an apparently very steep cut-off in activity, with compounds showing either excellent (>90% disease control) or no activity.

There was greater scope to vary the morpholine group in **1** and maintain activity. Compounds with tertiary amines in this position possessed good antifungal activity (**27** and **28**). A single large substituent on nitrogen was well tolerated (**29**), although the activity appeared to decrease with groups much larger than morpholine itself (**30**). There is some indication that for large groups the presence of a heteroatom is beneficial (compare **31** and **32**). Secondary amines were less active (compare **33** with **32**). It is notable that alkoxy groups were tolerated at this position (**34**) suggesting that it was the presence of the N–H group of the secondary amine that was unfavourable. Consistent with this is the inactivity of the

Table 1. Structure and antifungal activity of key compounds

Compound	Structure (**10**)		Antifungal activity[a]
	R^1	R^2	
1	Morpholino	MeO	100
3	Morpholino	Cl	0
5	HO	MeO	0
11	Morpholino	EtO	100
12	Morpholino	^{n}PrO	94
13	Morpholino	^{i}PrO	0
14	Morpholino	CyclopentylO	0
15	Morpholino	$Me_2CHCH_2CH_2O$	0
16	Morpholino	2-FurylCH_2O	0
17	Morpholino	MeS	100
18	Morpholino	EtS	100
19	Morpholino	$PhCH_2S$	0
20	Morpholino	HS	0
21	Morpholino	^{n}BuNH	0
22	Morpholino	$MeOCH_2CH_2NH$	0
23	Morpholino	Et_2N	0
24	Morpholino	Morpholino	0
25	Morpholino	H_2NNH	0
26	Morpholino	PhHNNH	0
27	Et_2N	MeO	100
28	Pyrrolidino	MeO	92
29	$PhCH_2(Me)N$	MeO	100
30	2,6-di(Me)Morpholino	MeO	72
31	^{n}Bu(Me)N	MeO	83
32	$MeOCH_2CH_2(Me)N$	MeO	100
33	$MeOCH_2CH_2NH$	MeO	33
34	^{n}PrO	MeO	100
35	CF_3SO_2O	MeO	40
36	Morpholino	$MeOCH_2CH_2O$	100
37	$(MeOCH_2CH_2)_2N$	MeO	67
38	$MeOCH_2CH_2(Me)N$	$MeOCH_2CH_2O$	100
Fenpropimorph[b]			97

[a] Percent control of *Erysiphe graminis f. sp. tritici* following application of the compound at 100 μg/mL in acetone/water (1:9) in a one day protectant test (for a detailed procedure see the Supplementary data).
[b] Commercial standard powdery mildewicide for comparison.

pyridone **5**, compared with weak activity seen for the derived triflate **35**.

These initial studies clearly established that significant scope existed to vary the lead structure and maintain a high level of antifungal activity. They also indicated some structural limits beyond which the antifungal activity declined or was lost completely.

Although it showed excellent antifungal activity in glasshouse tests,[17] compound **1** may not possess the physical properties required to fully express this activity in a more demanding field situation. The measured log *P* octanol[18] value of 4.25 is in the preferred range of 1.5–5, defined from an analysis of commercial fungicides.[19] However, **1** had a high melting point and very low aqueous solubility (Table 2). This level of solubility is such that it could severely compromise the antifungal activity of the compound in a field situation; a minimum aqueous solubility of about 2 μg/mL is reported for highly active commercial fungicides.[19] We therefore turned our attention to the design and synthesis of analogues that, whilst fulfilling the structural requirements for antifungal activity established by our initial studies, would also be more water soluble.

Table 2. Physical properties of selected compounds

Compound	C log *P*[a]	CHI log *P*[b]	Melting point (°C)	Solubility[c] (μg/mL)
1	2.33	4.06	209–211	0.08
12	3.39	—	163–165	—
15	4.32	—	139–141	—
27	3.73	5.32	—	0.08
30	3.37	4.72	227–229	<0.14
32	2.56	4.31	112–114	0.32
33	2.32	3.90	225–227	0.14
34	3.80	—	200–202	—
36	2.08	3.82	153–155	0.21
37	2.44	4.09	123–125	0.79
38	2.31	4.12	90–92	0.48

[a] Predicted log *P* (Ref. 22).
[b] Chromatographic hydrophobicity index derived log *P* (Ref. 21).
[c] Aqueous solubility measured at pH 7.

High crystallinity is a common feature of these compounds due to the planar tetracyclic core of the structure. Rather than modifying this core, which our preliminary studies had shown to be important for maintaining activity, we sought to decrease melting point and increase solubility in water by a suitable

choice of peripheral groups that were also compatible with achieving high levels of antifungal activity.

Simply increasing the bulk of the group (R^1) on the pyridine ring, with the aim of reducing the ability of the molecules to stack, and hence their crystallinity, proved unsuccessful due to increased lipophilicity. Thus dimethylmorpholine **30** had a high melting point and low aqueous solubility and showed reduced antifungal activity compared to the lead **1**. Replacement of the morpholine group with diethylamine (**27**) also failed to increase solubility. Changing the amine to an ether (**34**) made little difference to the melting point.

Increasing the size of the alkoxy group on the triazine ring initially seemed more promising, with a significant (46 °C) reduction in melting point observed on moving from methoxy (**1**) to *n*-propoxy (**12**). This trend continued, with the isopentyloxy analogue **15** having a melting point a further 24 °C lower. However, as discussed above, this compound was inactive, and both compounds were much more lipophilic than the lead **1**, more than offsetting any benefit from reduced melting point with respect to solubility in water.

We therefore decided to explore the insertion of heteroatoms, especially oxygen, into the alkyl side chains of the molecule. This is an approach that we successfully developed in previous projects,[20] which resulted in a significant increase in solubility of the molecules without exerting a detrimental effect on lipophilicity or biological activity. We began by exploring the alkoxy group on the triazine, and found that replacing the *n*-propoxy group in **12** with methoxyethoxy gave a compound (**36**) with a slightly reduced melting point, but significantly greater solubility than **1**. Encouragingly **36** also maintained good biological activity. Applying the same approach to the substituent on the pyridine ring also resulted in increased solubility, without altering lipophilicity or melting point (compare methoxyethylamine **33** with the morpholine **1**). Replacing the N–H in **33** with N–Me to give **32** dramatically reduced the melting point and significantly increased the water solubility. Extending this approach to the bis(methoxyethyl)amine **37** resulted in an additional increase in solubility. However, as expected from the SAR discussed above, this compound was less active due to the large size of the amine substituent on the pyridine ring. Combining oxygen-containing side chains of the preferred size at each end of the molecule resulted in compound **38** which had very similar log *P* octanol to the original lead **1**, but a melting point 120 °C lower and sixfold higher aqueous solubility. Compound **38** also showed good antifungal activity.

A common feature of the compounds made in this series was that established prediction methods failed to correctly estimate their physical properties. Thus, for the lead compound **1** the direct measurement of log *P* octanol[18] gave a value of 4.25 which was in good agreement with the value of 4.06 determined indirectly from measurements of chromatographic hydrophobicity index (CHI).[21] However, a structure-based prediction of log *P* octanol using Clog *P*[22] was 1.7 log units too low compared to the CHI derived value. This discrepancy proved to be reasonably consistent across a range of analogues (Table 2). Similarly the aqueous solubility of **1** was about an order of magnitude lower than the value of 0.9 μg/mL estimated from measured log *P* and melting point using Yalkowsky's general solubility equation.[23] This unexpectedly low solubility appears to be a feature of this class of chemistry. Of the 600 compounds in this series examined approximately 50% had estimated log *P* octanol values (obtained by applying a +1.7 log unit correction factor to the value calculated using Clog *P*) in the preferred 1.5–5 range. However, only about 5% were predicted to have aqueous solubility greater than 1 μg/mL and those that did failed to satisfy the SAR requirements for antifungal activity.

In conclusion, a novel area of chemistry with promising antifungal activity, particularly against wheat powdery mildew, has been discovered. The development of two complementary synthetic routes allowed an efficient exploration of structure–activity relationships around the pyridothieno-1,2,3-triazine **1**. By careful selection of substituents on the core ring system we were able to design and prepare compounds with significantly reduced melting point and increased water solubility. However, we were unable to design compounds which both satisfied the structural requirements for good activity and possessed physical properties that could allow the expression of this activity in a field environment.

Acknowledgments

We thank Paul Stanley and Susan Crosland for analytical support; and Beth Shirley for determining CHI log *P*.

Supplementary data

Supplementary data, including typical synthetic experimental procedures and details of the biological test method used, are available in the online version of this article. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.06.076.

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