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Pyrrolo[1,2-*a*][1,4]benzodiazepine: A novel class of non-azole anti-dermatophyte anti-fungal agents

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Abstract—Broad screening revealed compound **1a** to be a novel anti-fungal agent with high specificity towards dermatophytes. The anti-fungal structure–activity relationship of this novel class of 5,6-dihydro-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepines is described together with its mode of action that appeared to be the inhibition of squalene epoxidase. Preliminary in vivo results of the most active compounds are also reported.

In view of the increasing resistance to existing azole anti-fungals,[1] and the lack of sufficient chemical diversity in the existing classes of anti-fungals, the need for new anti-fungals remains high.[2] Although potent systemic broad-spectrum anti-fungals are preferred, there is ample room for compounds with a more focused application. In fact, the largest branch of the anti-fungal market is dermatology. It includes conditions such as onychomycosis, athlete's foot, tinea corporis and the like, that can be treated by both topical and oral regimes. Two major classes are dominating this market: the azole anti-fungals (14-α-demethylase inhibitors) such as ketoconazole,[3] itraconazole[4] and fluconazole,[5] and squalene epoxidase inhibitors such as thiocarbamates (e.g., tolnaftate)[6], naftifine,[7] terbinafine[8] and butenafine[9] (Scheme 1).

As well as target directed projects, general in vitro screening of the corporate compound library on yeast and mould species was conducted, revealing compound **1a** to be a novel molecule with specific activity against dermatophyte species, *Aspergillus fumigatus* and *Candida parapsilosis*. In this communication, we would like to report on the preliminary structure–activity relationship of this novel class of anti-fungals.

Compound **1a** has a 5,6-dihydro-4-(4-ethylphenyl)-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepine structure, which is scarcely described in the literature,[10] and the potential anti-infective activity of this heterocyclic class has not been reported. The synthesis of this novel template is easily achieved via a four-step synthesis depicted in Scheme 2. Substituted 2-amino-benzonitriles are commercially accessible or easily accessible via synthetic routes described in the literature.[11] The amine was cyclized in good yield via a modified Hantzsch pyrrole synthesis using 2,5-diethoxytetrahydrofurane in refluxing acetic acid. Then the nitrile was reduced using lithium aluminium hydride in dry tetrahydrofuran. Finally, the corresponding 2-*N*-pyrrolobenzyl amine was treated with the selected aldehyde to give the corresponding imine. This immediately gave an intramolecular Mannich reaction upon treatment with dry acid,

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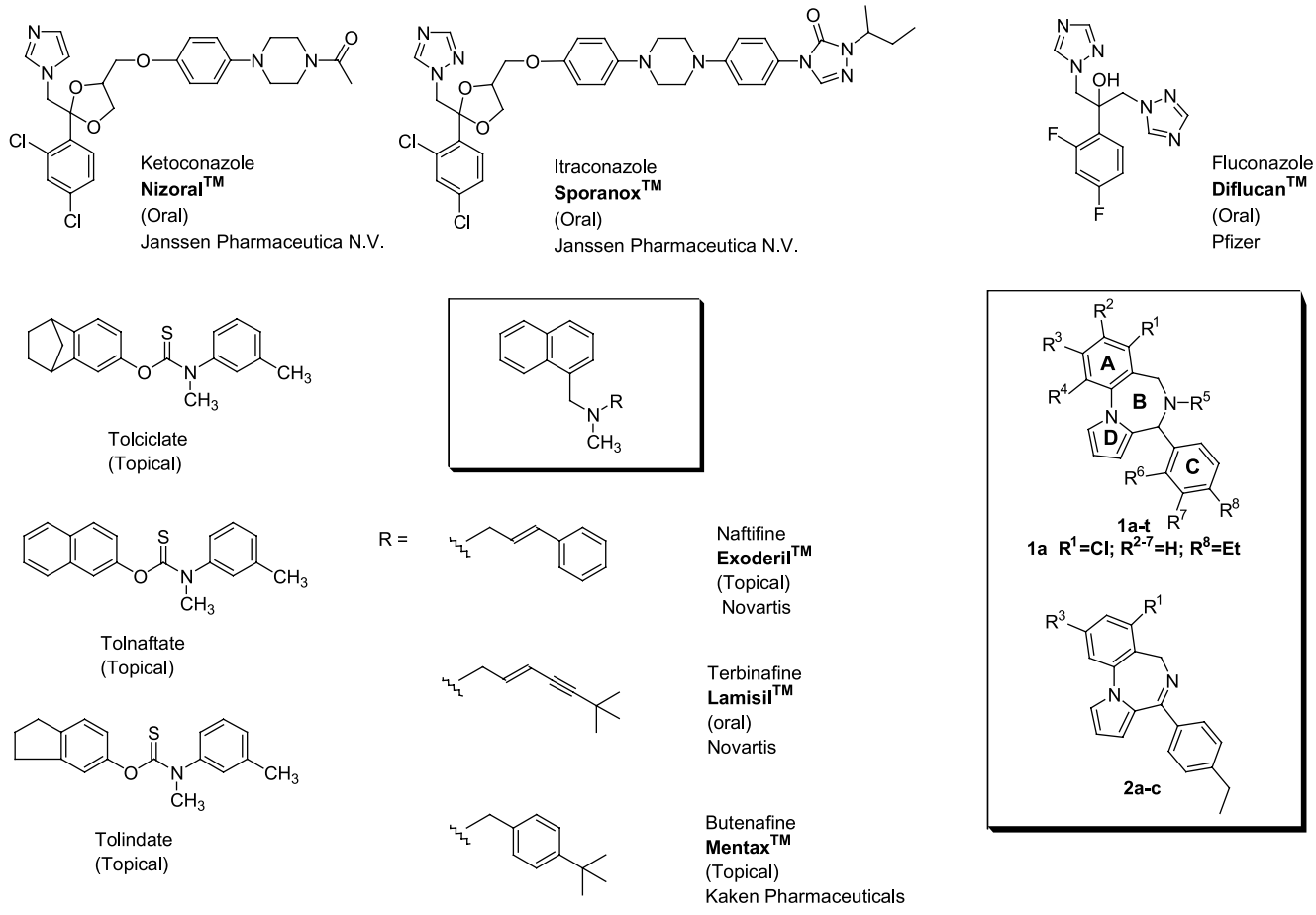
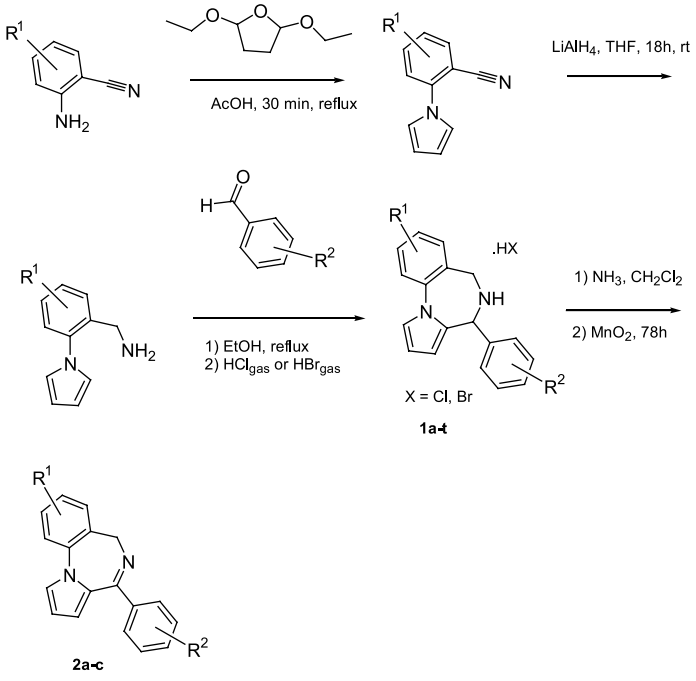


Scheme 1.



Scheme 2.

Table 1. Structure–activity relationship of substituted 5,6-dihydro-4-phenyl-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepine **1a–t**

Compound ID	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	*A. fumigatus*[a] B42928	*C. parapsilosis* B66126	*Cr. neoformans* B66663	*M. canis* B68128	*S. schenkii* B64284	*T. mentagrophytes* B70554	*T. rubrum* B68183
Terbinafine									0.32	0.32	1	0.03	0.32	0.01	0.003
1a	Cl	H	H	H	H	H	H	Et	3.2	1	100	0.32	100	0.1	0.03
1b (+)-*S*	Cl	H	H	H	H	H	H	Et	55	100	100	3.2	100	1	0.32
1c (−)-*R*	Cl	H	H	H	H	H	H	Et	1	0.32	100	0.32	100	0.01	0.03
1d	H	H	H	H	H	H	H	Et	100	100	100	100	100	3.2	1
1e	F	H	H	H	H	H	H	Et	10	10	100	3.2	100	1	0.32
1f	H	Cl	H	H	H	H	H	Et	100	100	100	100	100	51.6	3.2
1g	H	H	Cl	H	H	H	H	Et	100	100	100	1	100	1	0.1
1h	H	H	H	Cl	H	H	H	Et	100	100	100	3.2	100	3.2	1
1I	Me	H	H	H	H	H	H	Et	100	55	100	2.1	100	1	0.21
1j	SMe	H	H	H	H	H	H	Et	100	100	100	3.2	100	3.2	0.32
1k	OMe	H	H	H	H	H	H	Et	100	100	100	10	100	10	3.2
1l	Cl	H	H	H	Me	H	H	Et	100	100	100	100	100	55	51.6
1m	Cl	H	H	H	H	H	H	H	100	100	100	100	100	100	0.32
1n	Cl	H	H	H	H	H	H	Me	100	100	100	2.1	100	2.1	0.1
1o	Cl	H	H	H	H	H	H	nPr	100	6.6	100	0.66	100	0.66	0.32
1p	Cl	H	H	H	H	H	H	tBu	100	55	100	0.66	55	1	1
1q	Cl	H	H	H	H	Et	H	H	100	100	100	100	100	100	10
1r	Cl	H	H	H	H	H	Et	H	10	10	100	1	100	1	0.1
1s	Cl	H	H	H	H	H	H	Br	10	100	100	0.32	100	0.21	0.21
1t	Cl	H	H	H	H	H	H	Cl	51.6	100	100	0.66	100	0.66	0.32

[a] MIC in μM: microdilution test method in dilute casein hydrolysate-yeast extract-glucose medium (CYG), evaluation by OD measurement.[15,16] Reduction of test growth to <35% of control growth is interpreted as an indicator of inhibitory activity.

such as hydrochloric or hydrobromic acid in an appropriate solvent, usually diethyl ether or alcohol, to give the target compounds **1a–t**. Consequently, most of the compounds tested were hydrochloric or hydrobromic acid salts. Compound **1a** was separated in its enantiomers **1b** and **1c** using chiral HPLC.[12] Vibrational circular dichroism (VCD)–FTIR measurement and cancellation revealed the absolute configuration for compounds **1b** and **1c** to be the *S*- and *R*-configuration, respectively.[13] The corresponding 6-(4-ethylphenyl)-6*H*-pyrrolo[1,2-*a*][1,4]benzodiazepines **2a–c**[14] were elaborated by oxidation with manganese dioxide starting from the free base forms of **1a**, **1g** and **1d**, respectively.

The substitution pattern of rings A, B and C was explored intensively (see Table 1). Compound **1a** showed superior activity towards its regioisomers bearing a chlorine atom at positions -8 (R^2), -9 (R^3) and -10 (R^4). Contrary to **1a** (R^1 = Cl), other substituents in position-7 such as hydrogen (**1d**), 7-fluorine (**1e**), 7-methyl (**1i**), 7-thiomethyl (**1j**) and 7-methoxy (**1k**) were no longer active against *A. fumigatus* and *C. parapsilosis*, and were at least ten times less active against dermatophytes.

The 4-ethylphenyl substituent of compound **1a** appeared to be superior to its regioisomers (**1q** and **1r**), homologues (**1n–p**) and the unsubstituted phenyl substituent (**1m**). *A. fumigatus* and *C. parapsilosis*, in particular, seemed to be very sensitive towards both changes in substitution pattern on ring-A and -C, and the stereochemistry of the 5,6-dihydro-4-(4-ethylphenyl)-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepine (see Table 1). Compound **1c**, one of the enantiomers of **1a**, clearly inhibited *A. fumigatus* and *C. parapsilosis* more potently than the other enantiomer **1b** (see Table 1).

Quite surprisingly, apart from the activity against *C. parapsilosis*, none of these compounds showed any activity against other *Candida* species such as: *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis* and *Candida kefyr*. Inspired by the resemblance of the breadth of anti-fungal spectrum with that of terbinafine, compound **1a**'s inhibition of sterol biosynthesis was studied. After treatment with a 300 nM solution of **1a** (Fig. 1), a strong accumulation of squalene was revealed, suggesting that squalene epoxidase is the biochemical target. Moreover, this experiment together with the MIC values of **1a** (Table 1) revealed an overall 10-fold difference in potency between **1a** and terbinafine.[8] Nevertheless, closer analogues to butenafine, another squalene epoxidase inhibitor,[9] such as 4-*tert*-butyl **1p** and the *N*-methyl analogue **1l** did not result in a better activity.

Surprisingly, oxidation of the 5,6-dihydro-4*H*-pyrrolo-[1,2-*a*][1,4]benzodiazepines core towards a benzodiazepine (**2a**) resulted in superior activity compared to that of **1a** (see Table 2). **2a**'s anti-fungal spectrum was comparable to that of the enantiomer **1c**, but its activity against *A. fumigatus* was three times higher. In terms of the breadth of spectrum, the imines **2b** and **2c** revealed a promising tendency to shift to an even broader spectrum: their anti-fungal spectrum included both *Sporothrix schenkii* and *Cryptococcus neoformans*. Compound **2b** in particular showed a tremendous improvement in activity against *Cr. neoformans* and *S. schenkii*.

Compound **1a** was tested in two in vivo models previously described. *Trichophyton quinckeanum* infected mice[16] treated either intraperitoneally or orally with 40 mg/kg of compound **1a** started at the same day of infection and continued for six consecutive days did not result in a reduction in mean cutaneous lesion severity scores when compared with the placebo at day 6. On the other hand, topical application of 1 g/day of a 2% **1a** w/w carbowax[19] cream on the skin of guinea pigs with cutaneous *Microsporum canis* infection[17,18] resulted in an almost complete cure after 2 weeks of treatment, starting 3 days after infection. Unfortunately, no positive results were obtained with compound **1a**, its enantiomer **1c** or oxidized form **2a** when administered 40 mg/kg orally to *M. canis* infected guinea pigs.[16] Intravenous administration at 2 mg/kg and oral administration at 10 mg/kg of **1c** in guinea pigs resulted in very

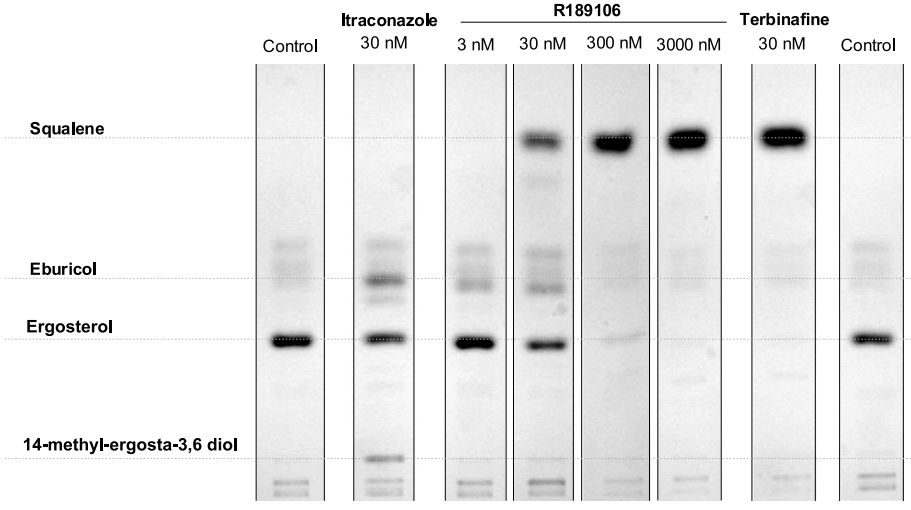


Figure 1. *Trichophyton rubrum* B68183 24 h sterol analysis: R189106 (**1a**) mode of action.

Table 2. Structure–activity relationship of 6-(4-ethylphenyl)-6*H*-pyrrolo[1,2-*a*][1,4]benzodiazepines **2a–c**

Compound ID	R_1	R_3	*A. fumigatus*[a] B42928	*C. parapsilosis* B66126	*Cr. Neoformans* B66663	*M. canis* B68128	*S. schenkii* B64284	*T. mentagrophytes* B70554	*T. rubrum* B68183
1c (–) **R**	Cl	H	1	0.32	100	0.32	100	0.01	0.03
2a	Cl	H	0.32	0.32	100	0.03	100	0.03	0.01
2b	H	Cl	0.32	1	1	0.32	6.6	0.1	0.065
2c	H	H	1	3.2	100	0.32	10	0.32	0.065

[a] MIC in μM: microdilution test method in dilute casein hydrolysate-yeast extract-glucose medium (CYG), evaluation by OD measurement.[15,16] Reduction of test growth to <35% of control growth is interpreted as an indicator of inhibitory activity.

low plasma concentrations after oral administration, and a rapid decrease of the plasma concentration in both cases. This poor bio-availability (<5%) might explain the lack of in vivo activity in guinea pigs.

In conclusion, compounds **1a**, **1c** and **2a** have been identified as a new class of pyrrolo[1,2-*a*][1,4]benzodiazepine anti-fungal agents with an anti-dermatophyte spectrum similar to that of terbinafine. Biochemical assessment of the mode of action of **1a** pointed to squalene epoxidase inhibition, a clinically validated target with the fungicidal potential. Overall, the potency gap between this new class and terbinafine is rather small.

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12. Chiral separation of **1a** on a Chiralpak™ AD HPLC column with 100% ethanol as eluent gave optical pure **1b** as the first fraction and **1c** as the second fraction. Compound **1c**: mp 250–252 °C (*i*-PrOH/HCl); $[\alpha]_D^{20}$ – 196.05 (*c* 0.516 in MeOH); ^{1}H NMR (360 MHz, DMSO-d_6) δ ppm 1.21 (t, J = 7.6 Hz, 3H) 2.66 (q, J = 7.5 Hz, 2H) 3.84 (d, J = 13.9 Hz, 1H) 4.53 (d, J = 13.9 Hz, 1H) 5.17 (s, 1H) 5.84 (ddd, J = 3.6, 1.6, 0.8 Hz, 1H) 6.28 (dd, J = 3.6, 2.9 Hz, 1H) 7.32 (d, J = 8.2 Hz, 2H) 7.40 (dd, J = 2.9, 1.6 Hz, 1H) 7.63 (m, 2H) 7.69 (m, 1H) 7.72 (d, J = 8.4 Hz, 2H) 10.61 (br s, 2H).
13. IR and VCD spectra were recorded at 6 cm^{-1} resolution on an FTIR Equinox spectrometer equipped with the VCD module PMA 37 (Bruker, Germany). A low-pass filter (<1800 cm^{-1}), BaF2 polarizer, ZnSe modulator (Hinds instruments) oscillating at a frequency of 50 Hz and MCT (InfraRed Associates) detector were used. Samples were dissolved in CD_2Cl_2 and placed in a demountable KBr cell with a 0.09 mm Teflon spacer (1 h collection time). OPUS software (Bruker, Germany) was used for spectral processing. A thorough conformational search is performed at the molecular mechanics level. A MM3 stochastic search is performed in combination with a systematic MMFF search with a dihedral grid of 30°. The located minima were optimized using Gaussian03RB5 at the B3LYP/6-31G* level. All conformations within 5 kcal/mol interval were used to simulate VCD and IR spectrum. Dipole and rotational strengths were calculated at the same B3LYP/6-31G* level, using the standard Gaussian (75,302)p grid.
14. Compound **2a**: ^{1}H NMR (360 MHz, $CDCl_3$) δ ppm 1.23 (t, J = 7.6 Hz, 3H) 2.66 (q, J = 7.6 Hz, 2H) 4.15 (br s, 1H) 5.48 (br s, 1H) 6.43 (dd, J = 3.8, 2.84 Hz, 1H) 6.51 (dd, J = 3.8, 1.7 Hz, 1H) 7.18 (d, J = 8.2 Hz, 2H) 7.27 (m, 2H) 7.32 (dd, J = 2.9, 1.7 Hz, 1H) 7.37 (dd, J = 6.8, 2.5 Hz, 1H) 7.63 (d, J = 8.2 Hz, 2H).
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