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Synthesis and antifungal activity of novel oxazolidin-2-one-linked 1,2,3-triazole derivatives†

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Novel oxazolidin-2-one-linked 1,2,3-triazole derivatives (**4a–k**) were synthesized by straightforward and versatile azide–enolate (3 + 2) cycloaddition. The series of compounds was screened for antifungal activity against four filamentous fungi as well as six yeast species of *Candida* spp. According to their efficiency and breadth of scope, they can be ordered as **4k** > **4d** > **4h** > **4a**, especially in relation to the activity displayed against *Candida glabrata* ATCC-34138, *Trichosporon cutaneum* ATCC-28592 and *Mucor hiemalis* ATCC-8690, *i.e.* compounds **4d**, **4h** and **4k** showed excellent activity against *C. glabrata* (MIC 0.12, 0.25 and 0.12 μg mL^{-1}, respectively), better than that of itraconazole (MIC 1 μg ml^{-1}). The activity of compound **4d** (MIC = 2 μg mL^{-1}) was higher than that observed for the standard antifungal drug (MIC = 8 μg mL^{-1}) against *Trichosporon cutaneum*, while compound **4k** displayed an excellent antimycotic activity against *Mucor hiemalis* (MIC = 2 μg mL^{-1} *vs.* 4 μg mL^{-1} for itraconazole). In addition, we describe herein a novel mild and eco-friendly synthetic protocol for obtaining β-ketosulfones (adducts to afford compounds **4a–k**) from α-brominated carbonyls in an aqueous nanomicellar medium at room temperature.

1. Introduction

Both oxazolidin-2-one[1] and 1,2,3-triazole[2] cores are very well-recognized pharmacophores in the literature. They have become extremely versatile in medicinal chemistry, featuring a number of clinically used drugs (*e.g.* Linezolid **1** or Tazobactam **2**, Fig. 1). Consequently, several antimicrobials based on one or the other scaffold have been reported in the literature.[3] On the other hand, the study of *both cores in the same molecule* (**3**) as an antibacterial agent has been reported[4] and patented (*e.g.* **3a** (ref. 5) and **3b** (ref. 6)). This year – 2017 – Melinta Therapeutics Inc. announced the acceptance of FDA of the Investigational New Drug (IND) application for topical Radezolid **3c**, a second-generation oxazolidinone/triazole compound discovered by Melinta scientists as a novel antibiotic to treat serious bacterial infections.[7] Curiously, antifungal activity has not been demonstrated for these kinds of compounds to the best of our knowledge.

Even though the mechanism of action of oxazolidinone[8] and triazole[9] cores has been described, the inhibition of monoamine oxidase A (MAO-A)[4a] as well as the RNA-binding process[4g] has been proposed as the mode of action for oxazolidin-2-one-linked 1,2,3-triazole.

Due to the strong resistance of fungi to current drugs, there is a continuous search for antifungal agents. As part of our ongoing research, we herein describe the synthesis and biological evaluation of novel oxazolidin-2-one-linked 1,2,3-triazole derivatives (**4**).

2. Chemistry

Our initial study began by obtaining the starting materials, azide/oxazolidin-2-one **8** and ketones **11** (Scheme 1). Firstly, chlorine derivative **7** was synthesized (61%) by coupling between 2-chloroethyl isocyanate **6** and benzoin **5**.[10] Subsequently, the nucleophilic substitution of **7** by the azide ion furnished compound **8** (58%).

Ketones **11a–11g** were synthesized since, unlike **11i–11j**, they are not commercially available (ketone **11h** was kindly donated by Syntex-La Roche). For these purposes, we have developed a novel organic solvent-free synthesis of

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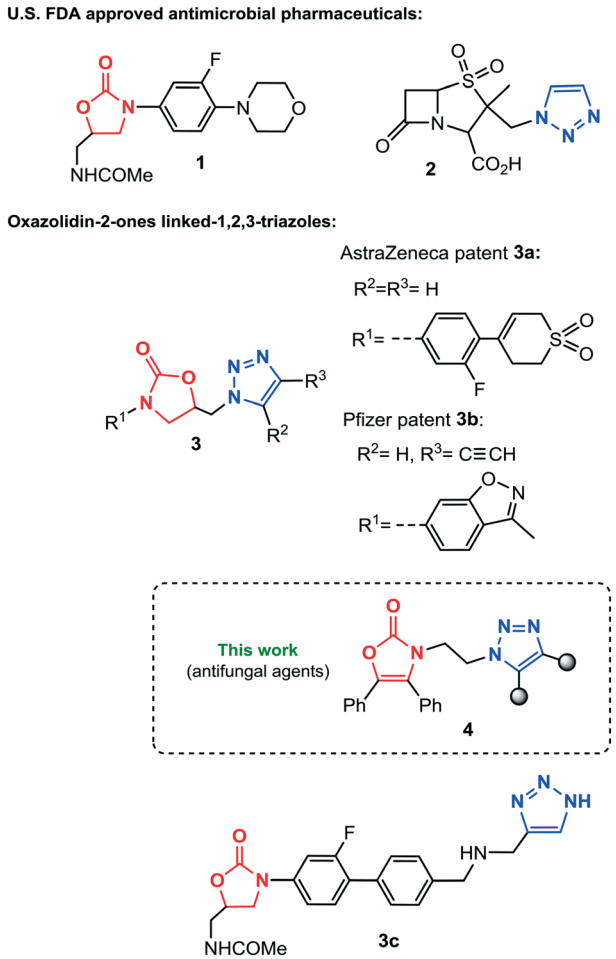


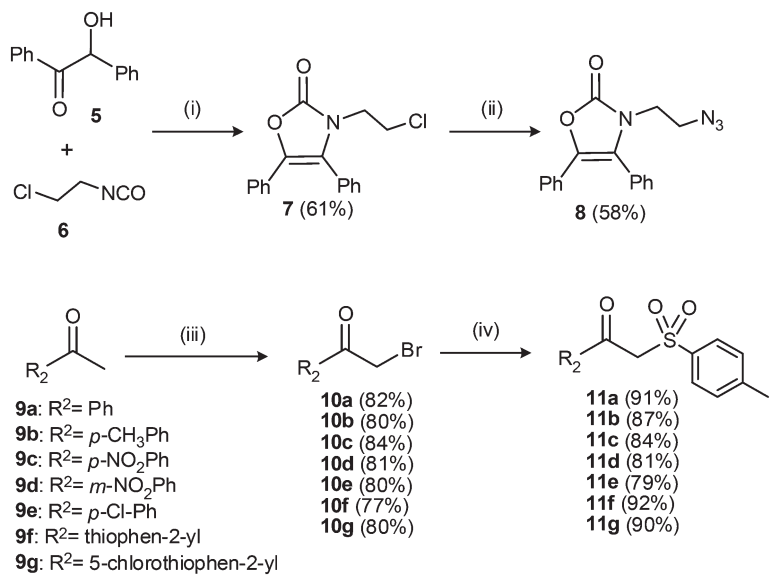
Fig. 1 The novel series of compounds **4** involves the extremely important oxazolidin-2-one and 1,2,3-triazole pharmacophoric cores. A similar feature is found in antimicrobial oxazolidin-2-one-linked 1,2,3-triazole **3**, including lead compounds **1** and **2**.

β-ketosulfones in an aqueous nanomicellar medium by the concomitant use of the surfactant reagent SPGS-550-M or 'Nok' (now available at Sigma-Aldrich[11]) and sodium *p*-toluenesulfinate from α-bromoketones. The surfactant nature of NOK (a third generation surfactant) allows it to act as an efficient nanoreactor in catalytic amounts. The study and scope of this novel methodology are described in the ESI† in greater detail. Therefore, we decided to apply such a methodology in the synthesis of compound **11** from **10**, which in turn was accomplished by reported protocols[12] with *N*-bromosuccinimide (NBS) as the bromine source.

Although Cu-catalyzed azide–alkyne cycloaddition (CuAAC) is the conventional method for obtaining 1,2,3-triazole moieties,[13] other strategies have emerged as alternatives for such a purpose. We previously reported[14] a novel synthetic protocol to achieve the efficient assembly of 1,4,5-trisubstituted 1,2,3-triazole cores through azide–enolate cycloaddition. In consequence, oxazolidin-2-one-linked 1,2,3-triazole derivatives (**4**) were synthesized by the efficient 1,3-dipolar cycloaddition of azide/oxazolidin-2-one in the presence of enolates prepared *in situ* from ketones **11** activated by DBU as the base. Table 1 summarizes these outcomes.

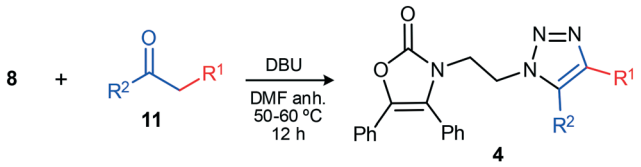
3. Microbiology

Compounds **4a–k** were tested for their *in vitro* activity against four filamentous fungi: *Aspergillus fumigatus* ATCC-16907, *Trichosporon cutaneum* ATCC-28592, *Rhizopus oryzae* ATCC-10329 and *Mucor hiemalis* ATCC-8690. These compounds were also evaluated, employing standardized microbiological methods developed by the CLSI, against six yeast specimens: *Candida albicans* ATCC-10231, *Candida utilis* ATCC-9226, *Candida tropicalis* ATCC-13803, *Candida parapsilopsis* ATCC-22019,



Scheme 1 Reagents and conditions: (i) N_2, 180 °C, 24 h. (ii) NaN_3 (1.1 eq.), DMF anh., 60 °C, 12 h, N_2. (iii) NBS (1.1 eq.), $TsOH{\cdot}H_2O$ (1.0 eq.), MeCN, 60 °C, 4 h. (iv) SPGS-550-M aq. (2% w/w), *p*-Tol-SO_2Na (1.5 eq.), r.t., 12 h.

Table 1 Synthesis of oxazolidin-2-one-linked 1,2,3-triazoles **4a–k** from azide **8** by coupling with active ketones **11**



Entry[a]	Ketone	Triazole[b] (yield%)[c]
1	**11a:** R^2 = Ph, R^1 = SO_2-*p*-Tol	**4a** (68)
2	**11b:** R^2 = *p*-CH_3Ph, R^1 = SO_2-*p*-Tol	**4b** (61)
3	**11c:** R^2 = *p*-NO_2-Ph, R^1 = SO_2-*p*-Tol	**4c** (74)
4	**11d:** R^2 = *m*-NO_2Ph, R^1 = SO_2-*p*-Tol	**4d** (70)
5	**11e:** R^2 = *p*-Cl-Ph, R^1 = SO_2-*p*-Tol	**4e** (72)
6	**11f:** R^2 = thiophen-2-yl, R^1 = SO_2-*p*-Tol	**4f** (68)
7	**11g:** R^2 = 5-chlorothiophen-2-yl, R^1 = SO_2-*p*-Tol	**4g** (64)
8	**11h:** R^2 = pentyl, R^1 = SO_2Ph	**4h** (71)
9	**11i:** R^2 = CH_3, R^1 = $COCH_3$	**4i** (73)
10	**11j:** R^2 = Ph, R^1 = COPh	**4j** (71)
11	**11k:** R^2 = Ph, R^1 = CN	**4k** (67)

[a] Reaction conditions: To a solution of compound **8** (1.0 eq.) and **11** (1.0 eq.) in DMF anh., DBU (2.0 eq.) was added. The reaction mixture was stirred at 50–60 °C for 12–24 h. [b] Confirmed by ^{1}H-NMR, ^{13}C-NMR and MS. [c] Yields refer to chromatographically pure isolated compounds.

Candida glabrata ATCC-34138, and *Candida krusei* ATCC-14243. Then, the sensitivity of the filamentous microorganisms was determined by the microdilution M38-A method,[15] and that of the yeast fungi with the M27-A3 method.[16]

Such antifungal activity was compared to that of itraconazole as the standard antifungal drug. The minimum inhibitory concentration (MIC) values of the standard and compounds **4a–k**, expressed in micrograms per milliliter, were determined in 96-well plates using MOPS (3-[*N*-morpholino]propanesulfonic acid buffered RPMI-1640 medium, Sigma-Aldrich).

4. Results and discussion

The antifungal activity of the test compounds is summarized in Table 2. Compounds **4d**, **4h** and **4k** showed excellent activity against *C. glabrata* (MIC 0.12, 0.25 and 0.12 μg mL^{-1}, respectively), better than that of itraconazole (MIC 1 μg ml^{-1}). The activity of compound **4d** (MIC = 2 μg mL^{-1}) was higher than that observed for the standard antifungal drug (MIC = 8 μg mL^{-1}) against *Trichosporon cutaneum*, while compound **4k** displayed an excellent antimycotic activity against *Mucor hiemalis* (MIC = 2 μg mL^{-1} *vs.* 4 μg mL^{-1} for itraconazole). Compounds **4a**, **4c** and **4e** proved to be moderate antifungal agents against *Aspergillus fumigatus* strains. The current results suggest that the presence of either the *a*-CN or *p*-NO_2Ph group at position 5 of the triazole cores increases the biological activity of these compounds in both yeast and filamentous fungi.

These outcomes can also be described by the 'sensitivity' parameters of yeasts, according to the breakpoints described in the M27-A3 document (Table 3). In general, *C. glabrata*, *C. krusei* and *C. parapsilosis* showed some susceptibility to the test compounds, whereas *C. albicans*, *C. tropicalis* and *C. utilis* were resistant to all of them.

Table 2 *In vitro* antifungal activities of the synthetized compounds (MIC, μg mL^{-1})

Compound	*C. alb*	*C. trop*	*C. uti*	*C. kru*	*C. gla*	*C. par*	*M. hie*	*A. fum*	*T. cut*	*R. ory*
4a	8	8	8	0.5	1	8	4	2	8	16
4b	1	8	4	8	2	0.25	16	8	8	16
4c	4	8	4	0.25	4	8	16	2	8	16
4d	8	8	8	8	0.12	0.5	16	16	2	16
4e	8	8	8	8	4	8	16	4	8	16
4f	8	8	8	8	1	8	16	8	8	16
4g	8	8	8	2	2	4	16	16	8	16
4h	8	8	8	8	0.25	8	16	16	8	16
4i	8	8	8	8	2	4	16	16	8	8
4j	8	8	8	8	2	8	16	16	8	16
4k	8	8	8	0.5	0.12	8	2	16	8	16
Standard[a]	0.03	0.06	0.25	0.25	1	0.06	4	1	8	1

Abbreviations: *C. alb.*, *Candida albicans*; *C. trop.*, *Candida tropicalis*; *C. uti.*, *Candida utilis*; *C. kru.*, *Candida krusei*; *C.gla.*, *Candida glabrata*, *C. par.*, *Candida parapsilosis*; *M. hie.*, *Mucor hiemalis*; *A. fum.*, *Aspergillus fumigatus*; *T. cut.*, *Trichosporon cutaneum*; *R. ory.*, *Rhizopus oryzae*.[a] Itraconazole.

Table 3 Determination of the sensitivity of yeast (according to document M27-A3): Susceptible (S), dose-dependent sensitive (SDD) and resistant (R)

Compound	*C. alb*	*C. trop*	*C. uti*	*C. kru*	*C. gla*	*C. par*
4a	R	R	R	SDD	R	R
4b	R	R	R	R	R	SDD
4c	R	R	R	SDD	R	R
4d	R	R	R	R	S	SDD
4e	R	R	R	R	R	R
4f	R	R	R	R	R	R
4g	R	R	R	R	R	R
4h	R	R	R	R	SDD	R
4i	R	R	R	R	R	R
4j	R	R	R	R	R	R
4k	R	R	R	SDD	S	R
Standard[a]	S	S	SDD	SDD	R	S

Abbreviations: *C. alb.*, *Candida albicans*; *C. trop.*, *Candida tropicalis*; *C. uti.*, *Candida utilis*; *C. kru.*, *Candida krusei*; *C.gla.*, *Candida glabrata*, *C. par.*, *Candida parapsilosis*. [a] Itraconazole. Interpretive criteria: breakpoints (MIC, μg mL^{-1}) = 0.12 [S], 0.25–0.5 [SDD], 1 [R].

5. Conclusion

In summary, eleven oxazolidin-2-one-linked 1,2,3-triazole derivatives (**4a–k**) were synthesized in good yields based on azide–enolate 1,3-dipolar cycloaddition. *In vitro* assays demonstrated that compound **4k** is the most efficient antimicrobial agent, since it was either better than or comparable to itraconazole against three species (*C. glabrata, M. hiemalis* and *T. cutaneum*). The second best antimicrobial activity was exhibited by compound **4d**, which was much better than the reference drug against two species (*C. glabrata* and *T. cutaneum*). In consequence, these compounds can be considered as drug candidates for future complementary biological studies. In addition, we have developed a novel organic solvent-free synthesis of β-ketosulfones in an aqueous nanomicellar medium. The surfactant nature of SPGS-550-M or 'NOK' (a third generation surfactant) allows it to act as an efficient nanoreactor in catalytic amounts. The notable advantages of this methodology over those previously reported include its simplicity of handling, mild conditions, high yields, cheap reagents and great tolerance of functional groups.

Conflicts of interest

The authors declare no competing interest.

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