Synthesis and antifungal activity of polycyclic pyridone derivatives with anti-hyphal and biofilm formation activity against *Candida albicans*

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KEYWORDS

Pyridone, hyphal formation, biofilm formation, *Candida albicans*, XTT reduction assay

ABSTRACT

Thirty-five pyridone derivatives were synthesized, with derivatization conducted on polycyclic pyridone scaffolds, including *cis*- or *trans*-oxydecalin and other cyclic structures, by domino－Knoevenagel－electrocyclic reactions. The anti-fungal activities of the synthesized compounds were tested against *Candida albicans*. Ten compounds inhibited hyphal formation without inhibiting growth. Pyridones with anti-hyphal formation activity (**4c**, **6d**, **12a** and **12c**) were tested for their ability to inhibit biofilm formation. Compound **6d** showed both anti-hyphal and biofilm inhibition activity.

Candida albicans is a dimorphic fungus that transforms from a yeast to a hyphal form. The capacity of *C. albicans* to switch from yeast to hypha is associated with antifungal drug resistance, virulence, invasion and colonization, and the development of spatially organized architectures of highly structured mature biofilms.[1] Biofilm formation by fungi undergoing the yeast-to-hypha transition is thus a target of antifungal drugs. Several small-molecule inhibitors of hyphal formation have been discovered but the number of lead compounds are poor compared to typically used antifungal agents (e.g., azole and polyene type antibiotics)[2] and thus anti-hyphal and anti-biofilm formation pharmacophores are required.

Natural pyridone derivatives isolated from marine-derived fungi are attractive lead compounds for anti-hyphal formation agents. For example, didymellamide A was isolated from *Stagonosporopsis cucurbitacearum* and showed antifungal activity against azole-resistant *C. albicans*.[3] Trichodin A was isolated from *Trichoderma* sp. and showed antifungal activity against *C. albicans*.[4] These compounds both contain the pyridone moiety and other cyclic structures (Fig. 1).

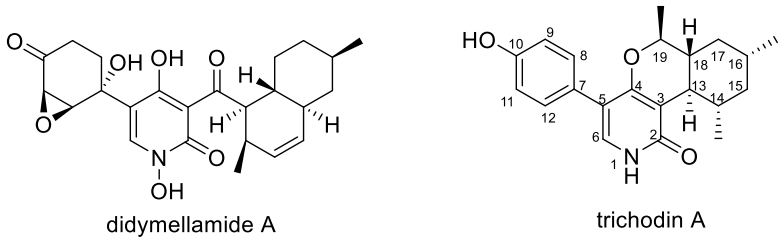


Fig. 1 Structures of polycyclic pyridones isolated from natural resources.

The skeletal diversity of a small molecule library is important for discovering bioactive leads. Diversity-oriented synthesis (DOS) and scaffold diversity synthesis (SDS) have been used to increase structural diversity.[5] A derivatized lead compound with a skeletal scaffold could fill the

three-dimensional (3D) surfaces of chemical space and interact with biological macromolecules in a selective manner. Skeletal diversification has previously been achieved by intramolecular cyclization reactions initiated by reagent- and substrate-controlled site-selective activation of different pairs of functional groups strategically placed around a linear template.

Starting from this interesting pharmacological profile, we have designed pyridone derivatives with cyclic scaffolds following the approach of combining in a single molecule two different pharmacophores. C-5 substituted pyridone with pyran structure is selected as the common scaffold with trichodin A. Targets of derivatization were A) substituent at C-5, B) *cis* or *trans* oxydecalin unit, C) presence of prenyl unit at C-21 and D) presence of chlorine at C-6 (Fig. 2).

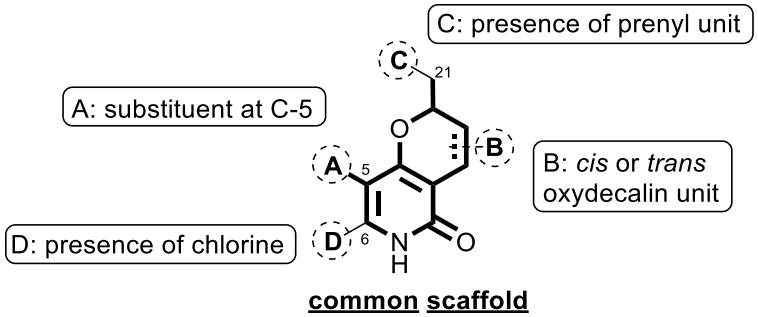


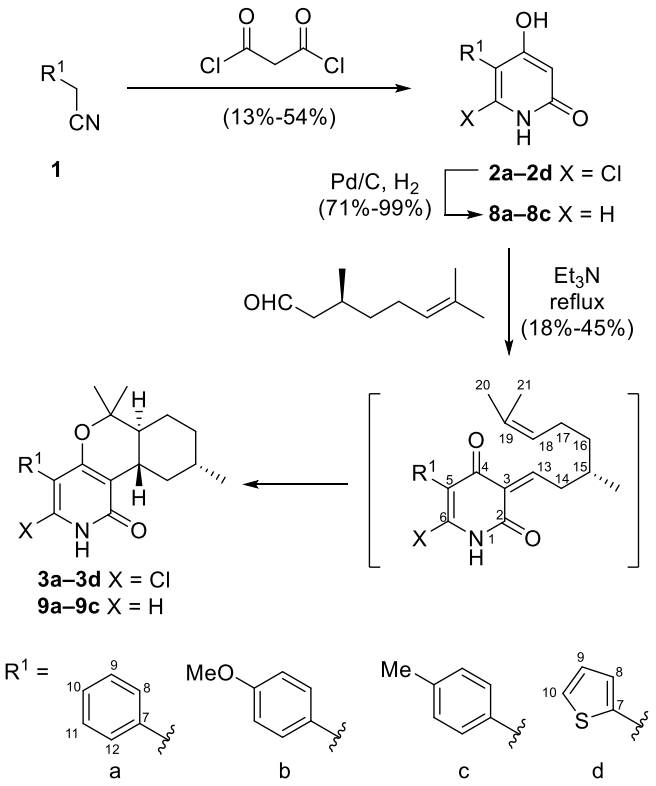
Fig. 2 Molecular design of pyridone derivatives.

These pyridone derivatives with cyclic scaffolds were synthesized by intermolecular electrocyclic reactions and their antifungal activities towards hyphal formation and biofilm formation were tested using *C. albicans*.

Polycyclic pyridone derivatives were synthesized from 4-hydroxy-2-pyridone *via* domino－Knoevenagel－electrocyclic reactions.[6] C-5-substituted 4-hydroxy-2-pyridones (**2a**-**2d**) were prepared from acetonitrile derivatives (**1a**-**1d**) and malonyl chloride according to the literature.[7] Knoevenagel condensation of pyridone and aldehyde gave intermediates. An additional

electrocyclic reaction between the heterodiene at C-4 and C-3 to C-13 and the dienophile at C-18 to C-19 in the intermediate afforded the polycyclic pyridone skeleton. This electrocyclic reaction yielded an oxydecalin ring with an altered ring juncture corresponding to the aldehyde and base.[8] Functional group diversity (C-3, prenyl group, chlorine) was introduced by using the corresponding acetonitrile, aldehyde, and by dechlorination.

(−)-Citronellal was used for this reaction to yield tricyclic pyridone derivatives with *trans*-annulation of pyran derivatives (**3a-3d**) through a hetero Diels–Alder reaction. (Scheme 1) The stereochemistry **3b** was determined from the coupling constants of ^{1}H-NMR and NOESY correlations. The large coupling constants between H-13 and H-18 (11.5 Hz) indicated that the ring juncture was *trans*. The NOESY correlations between H-13 and H-15, H-14α and H-18 determined their relative configuration (Fig. 3). The other tricyclic pyridone derivatives had the same relative configuration because they were generated using the same synthetic process.



Scheme 1 Synthesis of tricyclic pyridone derivatives.

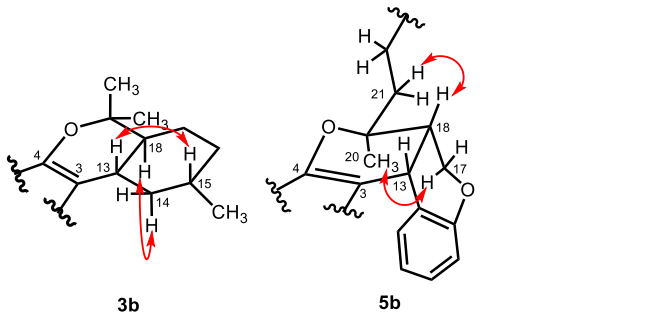
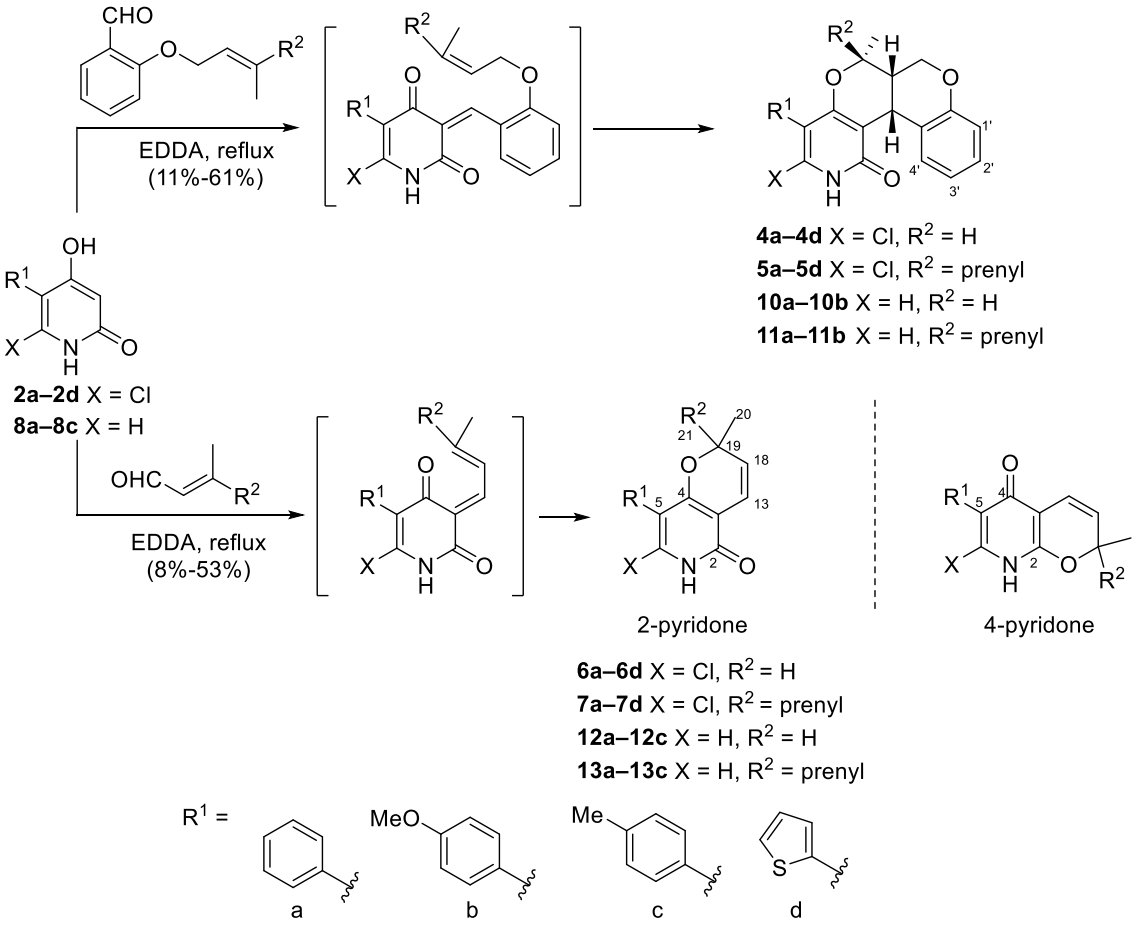


Fig. 3 NOESY correlations (red arrows) of **3b** and **5b**.

The *cis*-annulations of oxydecalin derivatives (**4a-4d**, **5a-5d**) were generated using 2-prenyloxy or 2-geranyloxybenzaldehyde (Scheme 2). The hetero Diels-Alder reaction did not proceed in the presence of triethylamine as base and an intermediate was obtained, whereas the use of ethylene diammonium diacetate (EDDA) as a base under reflux generated the oxydecalin skeleton. The

coupling constant between H-13 and H-18 of **5b** (6.0 Hz) indicated that the stereochemistry of the ring juncture was *cis*. The NOESY correlations between H-17α and H-20, H-18 and H-21 provided the relative configuration of **5b** (Fig. 3). The other tetracyclic pyridone derivatives (**4a-4d** and **5a-5d**) had the same relative configuration because they were generated using the same synthetic process.



Scheme 2 Synthesis of the tetra- and bicyclic pyridone derivatives.

Use of the aldehyde with an *E*-alkene at C-2' and C-3' (citral and 3-methyl-2-butenal) generated bicyclic pyridone derivatives (**6a-6d** and **7a-7d**) by oxa-electrocyclization (Scheme 2).

Manikandan reported that electrocyclization using EDDA at reflux or microwave condition

provided 2- and 4-pyridone derivatives.[8] ^{13}C-NMR spectroscopic data allowed determination of whether these compounds were 2- or 4-pyridone. The δ_C value for C-5 of the 3-phenyl-2-pyridone derivative was around 110 ppm, whereas that of the 3-phenyl-4-pyridone derivative was around 120 ppm.[9] Since δ_C in C-5 of all synthesized polycyclic pyridones was around 110 ppm, these compounds were likely 2-pyridone derivatives. **6a** was crystallized from *n*-hexane-EtOAc to give colorless needles. X-ray structure of **6a** suggested synthesized bicyclic pyridones were 2-pyridones (deposited at the Cambridge Crystallographic Data Centre, reference number CCDC 2056814). 2-Pyridone has been regarded as the prototype for the lactam−lactim tautomerization. **6a** was existed as lactim form in the crystal structure (Fig. 4).

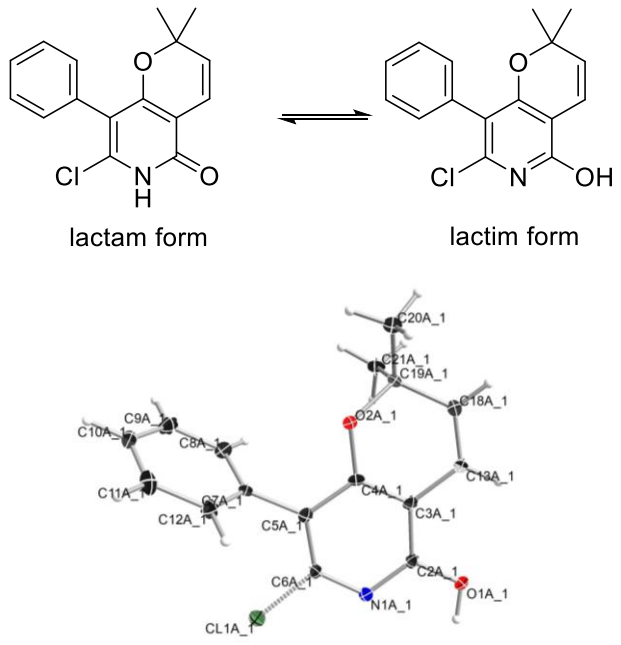


Fig. 4 Lactam−lactim tautomerization and X-ray structure of **6a**.

Polycyclic 6-hydropyridone derivatives (**9a-9c**, **10a-10c**, **11a-11c**, **12a-12c** and **13a-13c**) were also synthesized as the same pathway from 6-hydropyridone intermediates (**8a-8c**) prepared by

dechlorination by using Pd/C (Schemes 1 and 2)[7]. Dechlorination of thienylpyridone (**2d**) didn't progress due to the presence of sulfur atom. Thirty-five pyridones derivatized by substitution at C-5 (phenyl, 4-methylphenyl, 4-methoxyphenyl, thienyl), by the presence of chlorine at C-6, and by alteration of the scaffold of the pericyclic pyridone skeleton, were prepared (Table 1).

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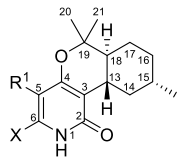
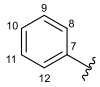
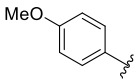
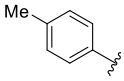
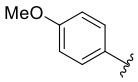
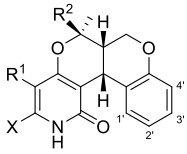
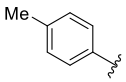
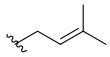
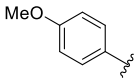
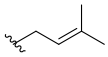
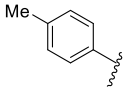
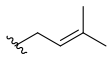
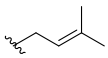
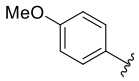
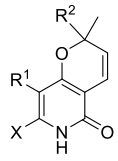
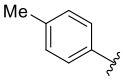
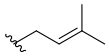
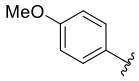
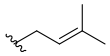
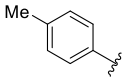
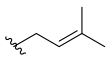
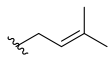
	**3a** Cl **9a** H		–	**3b** Cl **9b** H		–
	3c Cl **9c** H		–	**3d** Cl		–
	4a Cl **10a** H		CH_3	**4b** Cl **10b** H		CH_3
	**4c** Cl **10c** H		CH_3	**4d** Cl		CH_3
	5a Cl **11a** H			**5b** Cl **11b** H		
	5c Cl **11c** H			**5d** Cl		
	6a Cl **12a** H		CH_3	**6b** Cl **12b** H		CH_3
	**6c** Cl **12c** H		CH_3	**6d** Cl		CH_3
	7a Cl **13a** H			**7b** Cl **13b** H		
	7c Cl **13c** H			**7d** Cl		

Table 1 Synthesized pyridone derivatives.

The anti-hyphal formation activities of these compounds were tested using *C. albicans* (SC-5314). Hyphal formation was induced by growth in spider medium (nutrient broth, mannitol and K_2PO_4). Farnesol, a quorum-sensing molecule in *C. albicans*, inhibits hyphal formation and was used as a positive control.[10] Colonies were visually observed at the bottom of wells of a 96 well microplate in the presence of all samples, showing that these compounds did not inhibit the growth of *C. albicans*.

The results are shown in Fig. 5. Four compounds (**4c**, **6d**, **12a** and **12c**) at 25 μg/mL inhibited hyphal growth by over 50%. Six compounds (**3d**, **4d**, **5c**, **6a**, **6c** and 7**c**) showed minor activity (30-50% inhibition) whereas the other derivatives showed no inhibitory activity. The four active derivatives showed dose dependency in the range 3.12 to 25 μg/mL (Fig. 6).

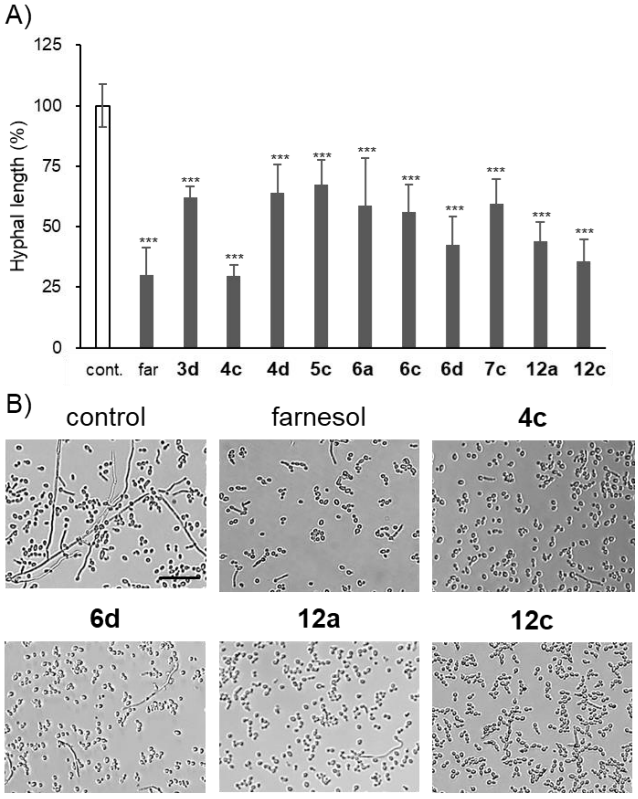


Fig. 5 Hyphal growth of *C. albicans* (SC5314) in spider medium. (A) Measured hyphal length

in the presence of the active pyridones and farnesol at 25 μg/mL. (B) The inhibitory effect of the active pyridones at 25 μg/mL on *C. albicans* SC5314. Farnesol (far) was used as a positive control. ***: $P< 0.001$ vs. control. Scale bars represent 50 μm.

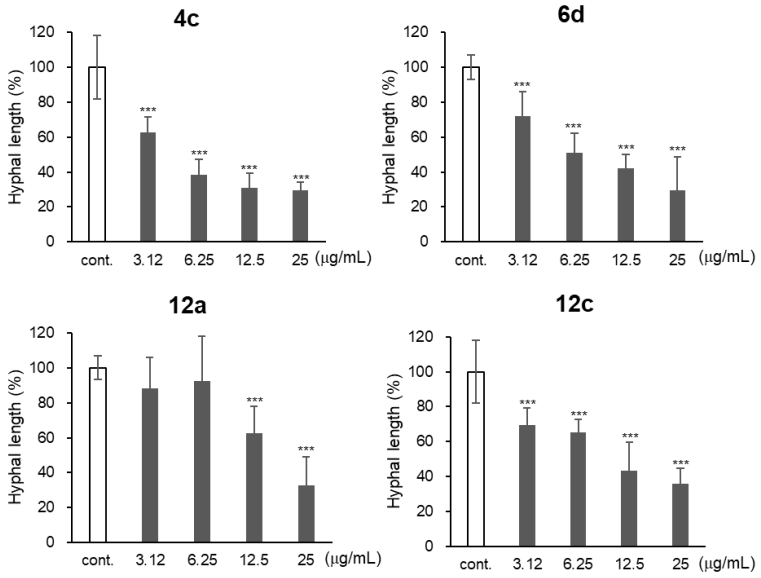


Fig. 6 Inhibition of *C. albicans* hyphal formation using **4c**, **6d**, **12a** and **12c**. ***: $P< 0.001$ vs. control.

Structure activity-relationships based on these results suggest that five of the seven bicyclic pyridones without prenyl sidechains (**6a-6d** and **12a-12c**) showed inhibitory activity. This hit rate was superior to that obtained using other polycyclic pyridone derivatives. Moreover, 6-hydro pyridone exhibited enhanced inhibitory activity compared with **6a** and **6c** and with **12a** and **12c**.

The four active compounds (**4c**, **6d**, **12a** and **12c**) were investigated in biofilm formation tests. Biofilms were grown at 37°C using RPMI 1640 medium and evaluated by the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) reduction assay.[11] Minocycline was used as the positive control. Compound **6d** reduced biofilm formation between 6.25 to 25 μg/mL (Fig. 7) whereas **12a** and **12c** showed mild inhibitory activity (respective inhibitory

activities of 18.7 and 17.9% at 25 μg/mL) and **4c** did not inhibit biofilm formation.

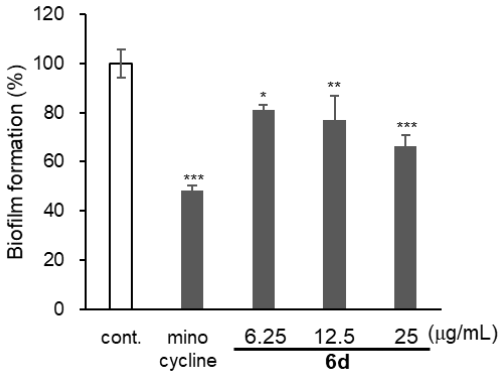


Fig. 7 Inhibition of biofilm formation by *C. albicans* using **6d**. *, **, ***: P<0.05, 0.01, 0.001 vs. control. Minocycline was tested in 10 μg/mL and used as positive control.

In conclusion, polycyclic pyridones were synthesized with the structural features of trichodin A. Four derivatives (**4c**, **6d**, **12a** and **12c**) inhibited hyphal formation by *C. albicans*, and **6d** also inhibited biofilm formation. These results suggest that **6d** may inhibit biofilm formation by modulating the hyphal formation and reducing cellular adhesion. Our findings illustrate the potential of pyridones as antifungal agents.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data to this article can be found online at

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