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Antifungal quinazolinones from marine-derived *Bacillus cereus* and their preparation

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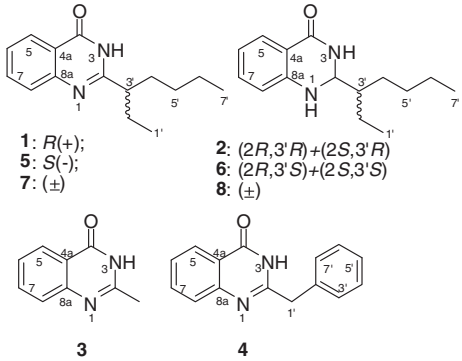
ABSTRACT

Two new quinazolinones alkaloids, *R*(+)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**1**) and (2*R*,3′*R*)+(2*S*,3′*R*)-2-(heptan-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (**2**) (a pair of epimers), as well as seven known analogues, 2-methylquinazolin-4(3*H*)-one (**3**), 2-benzylquinazolin-4(3H)-one (**4**), *cyclo*-(Pro–Ile), *cyclo*-(Pro–Leu), *cyclo*-(Pro–Val), *cyclo*-(Pro–Phe), and *cyclo*-(Tyr–Pro) were isolated from the *n*-butyl alcohol extract of the marine-derived bacterium *Bacillus cereus* 041381. The new compounds were identified by spectroscopic analysis and chemical synthesis. Four optical isomers **5**–**8** were also synthesized. Compounds **1**–**8** all showed moderate antifungal activity against Candida albicans with MIC values of 1.3–15.6 μM. Compound **5** exhibits the most powerful antifungal activity, which may reveal that S-configuration and 2,3-double bond were necessary for antifungal activity, and the racemization at C-2 and C-3′ reduced the antifungal activity.

Fungi are important opportunistic pathogens of humans, which could cause superficial or invasive infections. The majority of fungal pathogens include *Cryptococcus neoformans* and species of *Candida* and *Aspergillus*, with *Candida albicans* the most notable example of an opportunistic pathogen, causing both superficial infections and invasive fungal disease in immunocompromised individuals.[1] Unfortunately, the emergence of drug resistance in pathogenic fungi is all but inevitable.[2] Therefore, it is necessary to find new and efficacious drugs to solve these problems. In our continuing research on seeking microbial secondary metabolites with anti-*C. albicans* activities, a bacteria strain 041381 identified as *Bacillus cereus*, was isolated from a sea mud collected in Baimajin, Danzhou, Hainan, China. The *n*-BuOH extract of *B. cereus* 041381 was found to be active against *C. albicans*. Chemical investigation on the secondary metabolites afforded two new quinazolinones alkaloids, *R*(+)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**1**) and a mixture of (2*R*,3′*R*)- and (2*S*,3′*R*)-2-(heptan-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (**2**), along with seven known compounds, 2-methylquinazolin-4(3*H*)-one (**3**),[3] 2-benzylquinazolin-4(3*H*)-one (**4**),[3] *cyclo*-(Pro–Ile),[4] *cyclo*-(Pro–Leu),[5] *cyclo*-(Pro–Val),[6] *cyclo*-(Pro–Phe),[6] and *cyclo*-(Tyr–Pro).[7] Their structures including absolute configurations were elucidated by spectroscopic analysis and chemical synthesis. Meanwhile, four optical isomers, *S*(−)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**5**), (2*R*,3′*S*)+(2*S*,3′*S*)-2-(heptan-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (**6**), (±)-2-(heptan-3-yl) quinazolin-4(3*H*)-one (**7**), and (±)-2-(heptan-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (**8**), were prepared. These eight quinazolinones (**1**–**8**) all exhibited antifungal activity against *C. albicans* with MIC values of 2.5, 2.5, 15.6, 10.6, 1.3, 5.0, 10.2, 10.1 μM (ketoconazole as positive control, MIC 0.2 μM), respectively.



B. cereus 041381 was cultured under static conditions in a seawater based culture medium (4% amidulin and 0.1% yeast powder) at 28 °C for four days. The *n*-BuOH extract of the whole fermentation broth was subjected to extensive chromatography to give the new compounds **1** and **2** (Supplementary data).

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Table 1
^{1}H and ^{13}C NMR Data for **1** and **2** (500, 125 MHz, $CDCl_3$, TMS, δ ppm)

No	**1**		**2** [(2*R*,3′*R*)+(2*S*,3′*R*)]			
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1				4.09, br s		4.09, br s
2	159.0, C		67.3, C	4.96, dd ('t' like, 3.7)	67.3, C	4.96, dd ('t' like, 3.7)
3		10.3, br s		5.87, br s		5.87, br s
4	163.2, C		165.5, C		165.4, C	
4a	120.8, C		115.7, C		115.7, C	
5	126.3, CH	8.28, d (8.0)	128.6, CH	7.88, d (6.7)	128.6, CH	7.88, d (6.7)
6	126.3, CH	7.48, t (7.3)	119.1, CH	6.84, t (6.8)	119.1, CH	6.84, t (6.8)
7	134.7, CH	7.77, t (6.9)	133.8, CH	7.29, t (7.4)	133.8, CH	7.29, t (7.4)
8	127.4, CH	7.73, d (7.6)	114.6, CH	6.67, d (8.0)	114.6, CH	6.67, d (8.0)
8a	149.0, C		147.8, C		147.8, C	
1′	12.0, CH_3	0.9, t (7.3)	11.9, CH_3	0.99, t (7.8)	11.8, CH_3	0.97, t (7.5)
2′	26.9, CH_2	1.80, m	22.0, CH_2	1.40, m; 1.58, m	21.9, CH_2	1.40, m; 1.58, m
3′	48.5, CH	2.60, m	44.1, CH	1.50, m	44.1, CH_2	1.50, m
4′	33.2, CH_2	1.79, m	28.51, CH_2	1.34, m; 1.55, m	28.47, CH_2	1.34, m; 1.55, m
5′	29.6, CH_2	1.25, m	29.7, CH_2	1.34, m	29.6, CH_2	1.34, m
6′	22.6, CH_2	1.32, m	23.01, CH_2	1.33, m	22.99, CH_2	1.33, m
7′	13.9, CH_3	0.85, t (6.8)	14.0, CH_3	0.92, t (6.8)	14.0, CH_3	0.90, t (6.9)

Compound **1** was isolated as a white crystal with $[\alpha]_D^{20}$ +2.1 (*c* 0.1, acetone).[8] Its molecular formula was determined as $C_{15}H_{20}N_2O$ according to the HRESIMS at *m*/*z* 245.1655 $[M+H]^+$ (calcd 245.1654) and NMR data (Table 1). Four aromatic protons at δ 8.28 (1H, d, *J* = 8.0 Hz, H-5), 7.48 (1H, 't' like, *J* = 7.3, 7.7 Hz, H-6), 7.77 (1H, 't' like, *J* = 7.7, 7.0 Hz, H-7) and 7.73 (1H, d, *J* = 7.0 Hz, H-8) revealed the presence of an *o*-disubstituted benzene nucleus that was further supported by ^{1}H–^{1}H COSY connections from H-5 to H-8 through H-6 and H-7 (Fig. 1). Besides, the existence of an amide carbonyl group (δ 163.2) and a sp^2 quaternary carbon (δ 159.0) established the nucleus of **1** as quinazolin-4(3*H*)-one (Fig. 1).[3] The rest atoms were linked as 3-heptanyl that was deduced by analysis of the upfield ^{1}H and ^{13}C signals and ^{1}H–^{1}H COSY spectra (Fig. 1). The key long-range connections between H-2′ (δ 1.80) and H-4′ (δ 1.79) with the imino carbon (δ 159.0) indicated that 3-heptanyl was connected to C-2 of quinazolin-4(3*H*)-one nucleus. Thus, constitution of **1** was elucidated as 2-(heptan-3-yl)quinazolin-4(3*H*)-one.

Mixture **2** was obtained as a white crystal with the molecular formula $C_{15}H_{22}N_2O$ based on the HRESIMS at *m*/*z* 247.1819 $[M+H]^+$ (calcd 247.1810), two hydrogen atoms more than that of compound **1**.[8] Accordingly, the ^{1}H NMR and ^{13}C NMR spectra of **2** showed resemblance to **1**, except that the imino carbon signal at δ 159.0 (C-2) in **1** was replaced by a sp^3 methine signal at δ 67.3. In addition, a new NH signal was emerged at δ 4.09 and the original NH signal shifted to upfield (δ 5.87). These data supported **2** as the hydrogenation derivative at C=N double band of **1** that was further confirmed by the key HMBC correlations from H-1 (δ 4.09) to C-4a (δ 115.7) and C-8 (δ 114.6), and also by ^{1}H–^{1}H COSY connection between H-2 (δ 4.96) and H-3′ (δ 1.50). Thus, the constitution of **2** was determined to be 2-(heptan-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one. A pair of ^{13}C NMR signals in 3-heptanyl moiety ($C_{1'} \sim C_{7'}$) and C-4 (Table 1) suggested **2** as a pair of epimers at C-2 that could not be isolated.

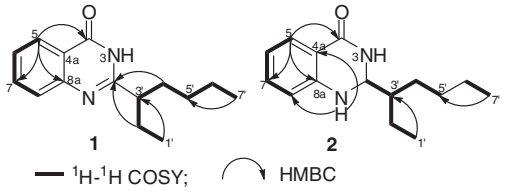
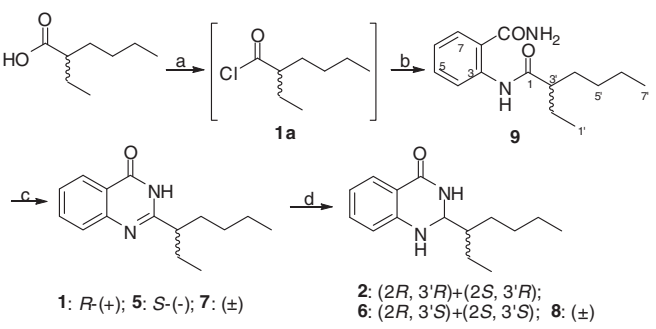


Figure 1. Key ^{1}H–^{1}H COSY and HMBC correlations of **1** and **2**.

The absolute configuration of C-3′ in **1** was determined by total synthesis from *R*(+)- and *S*(−)-2-ethylhexanoic acid and the comparison of the specific rotation between artificial and natural products. The synthesis work was initiated from the chiral resolution of (±)-2-ethylhexanoic acid by forming diastereomeric salt with *R*(+)-1-phenylethanamine.[9] *R*(+)-1-Phenylethanaminium (+)-2-ethylhexanoate was crystallized firstly from petroleum ether, while *R*(+)-1-phenylethanaminium (−)-2-ethylhexanoate was left in the mother liquor. After acidification by hydrochloric acid, respectively, *S*(+)-2-ethylhexanoic acid ($[\alpha]_D^{20}$ +10.2 (*c* 2.0, acetone)) and *R*(−)-2-ethylhexanoic acid ($[\alpha]_D^{20}$ −4.4 (*c* 2.0, acetone)) were recovered with 38% yield (91% ee) and 11% yield (42% ee),[10] respectively.

The chemical synthesis of **1** and **2** was outlined in Scheme 1. The key intermediate, 2-(2-ethylhexanamido)benzamide (**9**),[11] was prepared from the amidation of *o*-aminobenzamide with 2-ethylhexanoyl chloride (**1a**) produced by chloration of 2-ethylhexanoic acid with oxalyl chloride. Intramolecular condensation of **9** yielded the target product by reacting with HMDS and I_2 in CH_2Cl_2.[12] Using above method, *S*(−)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**5**) ($[\alpha]_D^{20}$ −1.1 (*c* 1.0, acetone)) and *R*(+)-2-(heptan-3-yl)quinazolin-4(3*H*)-one ($[\alpha]_D^{20}$ +0.4 (*c* 1.0, acetone)) were synthesized from *S*(+)-2-ethylhexanoic acid and *R*(−)-2-ethylhexanoic acid, respectively. NMR and specific rotation comparison between artificial and natural products revealed that the absolute configuration of **1** was *R*-. Therefore, the structure of compound **1** was determined as *R*(+)-2-(heptan-3-yl)quinazolin-4(3*H*)-one.[13,14]

Reduction of *R*(+)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**1**) and *S*(−)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**5**) with $NaBH_4$ in HOAc afforded two pairs of unisolable epimers, (2*R*,3′*R*)+(2*S*,3′*R*)-2-



Scheme 1. Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, rt; (b) *o*-aminobenzamide, Et_3N, CH_2Cl_2, rt; (c) HMDS/I_2, CH_2Cl_2; (d) $NaBH_4$, AcOH, $-10 \sim 50$ °C.

(heptan-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one and (2*R*,3′*S*)+(2*S*, 3′*S*)-2-(heptan-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (**6**), respectively. The ^{13}C NMR signals of these two pairs of artificial products both displayed doublets in 3-heptanyl moiety ($C_{1'}$–$C_{7'}$) and C-4 (Table 1). The NMR comparison revealed that the proton signals of **2** were nearly the same to those of synthetic (2*R*,3′*R*)+(2*S*,3′*R*)-2-(heptan-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one and different from those of **6**. Therefore, structure of **2** was elucidated as (2*R*,3′*R*)+(2*S*,3′*R*)-2-(heptan-3-yl)-2,3-dihydro-quinazolin-4(1*H*)-one.[15,16]

To discuss structure–activity relationship of quinazolinones and dihydroquinazolinones, two racemic products (±)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**7**) and (±)-2-(heptan-3-yl)-2, 3-dihydroquinazolin-4(1*H*)-one (**8**) were also prepared from (±)-2-ethylhexanoic acid by the same procedure.[17,18]

The antifungal activity of compounds **1**–**8** were evaluated by an agar dilution method.[19] Compounds **1**–**8** all displayed moderate growth inhibition on *C. albicans*, with MIC values of 2.5, 2.5, 15.6, 10.6, 1.3, 5.0, 10.2, and 10.1 μM, respectively (positive control, ketoconazole, MIC 0.2 μM).[20] Compound **5** exhibits most powerful antifungal activity, which may reveal that S-configuration and 2,3-double bond were necessary for antifungal activity, and the racemization at C-2 and C-3′ reduced the antifungal activity.

Acknowledgments

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Supplementary data

Supplementary data (^1H and ^{13}C NMR, DEPT spectra of compounds **1**, **2**, **5**, **6**, **9**, and 16S rRNA sequence of *B. cereus* 041381, and the isolation procedures of the compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.002.

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8. *R*(+)-2-(Heptan-3-yl)quinazolin-4(3*H*)-one (**1**): white crystal (MeOH); mp 123–125 °C; $[\alpha]_D^{20}$ +2.1 (*c* 0.1, acetone); UV (MeOH) λ_{max} (log ε) 202 (4.17), 224 (4.23), 265 (3.73), 304 (3.44), 316 (3.35) nm; IR (KBr) ν_{max} 3177, 3034, 2959, 2906, 1674, 1609, 1494, 1465, 1241, 1196, 890, 768 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) see Table 1. (2*R*,3′*R*)+(2*S*,3′*R*)-2-(Heptan-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (**2**): white crystal (MeOH); $[\alpha]_D^{20}$ +3.9 (*c* 0.1, acetone); UV (MeOH) λ_{max} (log ε) 221 (4.17), 252 (3.42), 349 (3.18) nm; IR (KBr) ν_{max} 3290, 3063, 2955, 2928, 2867, 1646, 1517, 1489, 1460, 1395, 1312, 1262, 1151, 750 cm^{-1}, ^1H (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) see Table 1.
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11. Preparation of *R*(−)-2-(2-ethylhexanamido)benzamide (**9**). To a stirred solution of *R*-(−)-2-ethylhexanoic acid (80 mg, 0.56 mmol) in CH_2Cl_2 (10 mL) at rt was added dropwise oxalyl chloride (142 mg, 1.12 mmol). After stirring the mixture for 5 h at rt, the solvent was removed in vacuo. The residue was redissolved in CH_2Cl_2 (10 mL) and added dropwise to a stirred solution of *o*-aminobenzamide (76 mg, 0.56 mmol) and Et_3N (62 mg, 0.62 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for another 1 h at rt and concentrated in vacuo. The obtained residue was dissolved in EtOAc (20 mL) and washed with H_2O, 5% $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. The concentration was purified by CC on SiO_2 eluting with 4:1 petroleum ether–EtOAc (v/v, 4:1) to give the desired **9** as a white crystal (125 mg, 85% yield). mp 152–153 °C; $[\alpha]_D^{20}$ −1.3 (*c* 1.0, acetone); ^1H NMR ($CDCl_3$, 500 MHz) δ: 11.2 (1H, s, H-2), 8.68 (1H, d, *J* = 8.4 Hz, H-7), 7.54 (1H, d, *J* = 8.0 Hz, H-4), 7.49 (1H, t, *J* = 8.0 Hz, H-5), 7.06 (1H, t, *J* = 7.7 Hz, H-6), 6.31 (1H, br s, –NH_2a), 5.75 (1H, br s, –NH_2b), 2.18 (1H, m, H-3′), 1.71 (2H, m, H-2′), 1.55∼1.59 (4H, m, H-4′, H-5′), 1.32 (2H, m, H-6′), 0.94 (3H, t, *J* = 7.5 Hz, H-1′), 0.85 (3H, t, *J* = 6.8 Hz, H-7′); ESI-MS *m*/*z* 263.2 $[M+H]^+$, 285.1 [M+Na].
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13. Preparation of *R*(+)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**1**). To a stirred solution of **9** (60 mg, 0.23 mmol) and I_2 (175 mg, 0.69 mmol) in CH_2Cl_2 (10 mL) was added dropwise HMDS (148 mg, 0.92 mmol) at rt and the mixture was stirred for 12 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with 5% $Na_2S_2O_3$ solution, water and brine, and dried over anhydrous Na_2SO_4. The concentration was purified by CC on SiO_2 eluted with 6:1 petroleum ether–EtOAc to give the synthetic **1** as a white crystal (48 mg, 86% yield). $[\alpha]_D^{20}$ +0.4 (*c* 1, acetone); NMR data were consistent to the natural.
14. Preparation of *S*(−)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**5**). The procedure for preparation of **1** was followed using *S*(+)-2-ethylhexanoate (80 mg, 0.56 mmol), oxalyl chloride (142 mg, 1.12 mmol), *o*-aminobenzamide (76 mg, 0.56 mmol) and Et_3N (62 mg, 0.62 mmol) to give *S*(+)-2-(2-ethylhexanamido)benzamide (115 mg, 80% yiled). mp 148–149 °C; $[\alpha]_D^{20}$ +1.6 (*c* 1.0, acetone); NMR data was consisitent to **9**. *S*(+)-2-(2-Ethylhexanamido) benzamide (60 mg, 0.23 mmol) reacted with I_2 (175 mg, 0.69 mmol) and HMDS (148 mg, 0.92 mmol) in CH_2Cl_2 to afford compound **5** as a white crystal (47 mg, 84% yield). mp 93–94 °C; $[\alpha]_D^{20}$ −1.1 (*c* 1.0, acetone); NMR data was consistent t.
15. Preparation of (2*R*,3′*R*)+(2*S*,3′*R*)-2-(heptan-3-yl)-2,3-dihydro- quinazolin-4(1*H*)-one (**2**). To a stirred mixture of *R*(+)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**1**) (15 mg, 0.06 mmol) and $NaBH_4$ (23 mg, 0.60 mmol) was added 4 mL HOAc at −10 °C and heated to 50 °C for 48 h. The reaction mixture was quenched with saturated $NaHCO_3$, extracted with EtOAc, dried over Na_2SO_4. The concentration was purified by HPLC eluted with MeOH–H_2O (v/v 75:25) to give the synthetic **2** as a white crystal (7.4 mg, 50%). $[\alpha]_D^{20}$ +1.3 (*c* 1, acetone); ^1H NMR ($CDCl_3$, 600 MHz) δ: 7.88 (2H, d, *J* = 7.7 Hz, H-5), 7.30 (2H, t, *J* = 7.3 Hz, H-7), 6.84 (2H, t, *J* = 7.3 Hz, H-6), 6.66 (2H, d, *J* = 7.7 Hz, H-8), 5.71 (2H, br s, H-3), 4.96 (2H, 't' like, *J* = 3.7 Hz, H-2), 4.06 (2H, br s, H-1), 1.58 (2H, m, H-2′a), 1.55 (2H, m, H-4′a), 1.50 (2H, m, H-3′), 1.40 (2H, m, H-2′b), 1.34 (6H, m, H-4′b/H-5′), 1.33 (4H, m, H-6′), 0.98 (3H, t, *J* = 7.8 Hz, H-1′)/0.96 (3H, t, *J* = 7.8 Hz, H-1′), 0.92 (3H, t, *J* = 6.9 Hz, H-7′)/0.90 (3H, t, *J* = 6.9 Hz, H-7′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 165.70/165.66 (C, C-4), 147.9 × 2 (C, C-8a), 133.9 × 2 (CH, C-7), 128.6 × 2 (CH, C-5), 119.2 × 2 (CH, C-6), 115.8 × 2 (C, C-4a), 114.7 × 2 (CH, C-8), 67.4 × 2 (CH, C-2), 44.2 × 2 (CH, C-3′), 29.8/29.7 (CH_2, C-5′), 28.6 × 2 (CH_2, C-4′), 23.1 × 2 (CH_2, C-6′), 22.0 × 2 (CH_2, C-2′), 14.1 × 2 (CH_3, C-7′), 12.0/11.9 (CH_3, C-1′); ESI-MS *m*/*z* 247.2 $[M+H]^+$.
16. Preparation of (2*R*,3′*S*)+(2*S*,3′*S*)-2-(heptan-3-yl)-2,3-dihydro- quinazolin-4(1*H*)-one (**6**). The procedure for preparation of **2** was followed using *S*(−)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (20 mg, 0.082 mmol) and $NaBH_4$ (32 mg, 0.82 mmol) to give **6** as a white crystal (10.4 mg, 52% yield). $[\alpha]_D^{20}$ +1.3 (*c* 1.0, acetone); ^1H NMR ($CDCl_3$, 600 MHz) δ: 7.87 (2H, d, *J* = 7.7 Hz, H-5), 7.28 (2H, t, *J* = 7.7 Hz, H-7), 6.82 (2H, t, *J* = 7.7 Hz, H-6), 6.67 (2H, d, *J* = 7.7 Hz, H-8), 6.39 (2H, br s, H-3), 4.94 (2H, dd, *J* = 3.3, 6.6 Hz, H-2), 4.19 (2H, br s, H-1), 1.62 (2H, m, H-2′a), 1.56 (2H, m, H-4′a), 1.52 (2H, m, H-2′b), 1.45 (2H, m, H-3′), 1.39 (2H, m, H-4′b), 1.32 (4H, m, H-5′), 1.30 (4H, m, H-6′), 0.97 (3H, t, *J* = 6.6 Hz, H-1′)/0.95 (3H, t, *J* = 6.6 Hz, H-1′), 0.89 (3H, t, *J* = 6.6 Hz, H-7′)/0.88 (3H, t, *J* = 6.6 Hz, H-7′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 165.73/165.70 (C, C-4), 147.9 × 2 (C, C-8a), 133.8 × 2 (CH, C-7), 128.6 × 2 (CH, C-5), 119.1 × 2 (CH, C-6), 115.8 × 2 (C, C-4a), 114.7 × 2 (CH, C-8), 67.4 × 2 (CH, C-2), 44.2 × 2 (CH, C-3′), 29.8/29.7 (CH_2, C-5′), 28.6 × 2 (CH_2, C-4′), 23.13/23.09 (CH_2, C-6′), 22.0 × 2 (CH_2, C-2′), 14.1 × 2 (CH_3, C-7′), 12.0/11.9 (CH_3, C-1′); ESI-MS *m*/*z* 247.2 $[M+H]^+$.
17. Preparation of (±)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**7**). The procedure for preparation of **1** was followed using (±)-2-ethylhexanoate (108 mg, 0.75 mmol), oxalyl chloride (190 mg, 1.5 mmol), *o*-aminobenzamide (102 mg, 0.75 mmol) and Et_3N (83 mg, 0.83 mmol) to give (±)-2-(2-ethylhexanamido)benzamide (169 mg, 86% yield). (±)-2-(2-Ethylhexanamido)benzamide (120 mg, 0.46 mmol) reacted with I_2 (350 mg, 1.38 mmol) and HMDS (296 mg, 1.84 mmol) in CH_2Cl_2 to give **7** as a white crystal (95 mg, 85% yield). NMR data was consistent to **1**.
18. Preparation of (±)-2-(heptan-3-yl)-2,3-dihydroquinazolin-4(1*H*)-one (**8**). The procedure for preparation of **2** was followed using (±)-2-(heptan-3-yl)quinazolin-4(3*H*)-one (**7**) (20 mg, 0.082 mmol) and $NaBH_4$ (32 mg, 0.82 mmol) to give **8** as a white powder (9.6 mg, 48% yield).
19. Zaika, L. L. *J. Food Safety* **1988**, *9*, 97.
20. The antifungal activities against *Candida albicans* were evaluated by an agar dilution method.[19] The tested strain, *C. albicans*, was cultivated in in YPD agar plates at 37 °C. Compounds **1**–**8** and ketoconazole (positive control) were dissolved in methanol at different concentrations from 10 to 0.078 μg/mL by the continuous two-fold dilution methods. A 10 μL quantity of test solution was absorbed by a paper disc (5 mm diameter) and placed on the assay plates. After 12 h incubation, zones of inhibition (mm in diameter) were recorded. The minimum inhibitory concentrations (MICs) were defined as the lowest concentration at which no microbial growth could be observed. The MIC values of compounds **1**–**8** were 2.5, 2.5, 15.6, 10.6, 1.3, 5.0, 10.2, and 10.1 μM, respectively (ketoconazole with MIC value of 0.2 μM).