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Retro-aza-Michael reaction of an *o*-aminophenol adduct in protic solvents inspired by natural products

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ABSTRACT

α,β-Unsaturated carbonyls are reactive group often found in bioactive small molecules. Their non-specific reaction with biomolecules can be the cause of the low efficacy and unexpected side-effects of the molecule. Accordingly, unprotected α,β-unsaturated carbonyls are not often found in drugs. Here, we report that *o*-aminophenol is a new masking group of α,β-unsaturated ketone, which is inspired by natural products saccharothriolides. *o*-Aminophenol adduct of α,β-unsaturated ketone, but not those of α,β-unsaturated amide or ester, undergoes a retro-Michael reaction to yield *o*-aminophenol and the Michael acceptor. This reaction was observed only in protic solvents, such as MeOH and aqueous MeOH. In contrast, *o*-anisidine was not eliminated from its Michael adduct. *o*-Aminophenol may be a promising masking tool of highly-reactive bioactive α,β-unsaturated carbonyl compounds.

1. Introduction

Michael acceptors in bioactive small molecules can form covalent bonds with nucleophiles in target proteins.[1–4] They are often found in natural products and have potent biological activities.[5,6] Recently, Michael acceptors were embedded in synthetic anticancer drugs, such as afatinib and ibrutinib. Both compounds have an α,β-unsaturated amide moiety and show irreversible binding with their target proteins.[7,8] In general, however, the high chemical reactivity of the Michael acceptors can lead to the risk of side effects by binding to off-target molecules.[1] To overcome this problem, chemical protection of the Michael acceptor or substitution with alternative functionalities have been investigated.[9–13] For example, installing sulfoxides at the β position of the α,β-unsaturated ester in brefeldin A furnished a prodrug of brefeldin A.[9] 3-Aminopropanamide, which is stable *in vitro*, was found to be converted to acrylamide in cells.[10] Very recently, α-chlorofluoroacetamide with weak intrinsic reactivity was reported as an effective warhead in cells.[13]

Saccharothriolides (STLs) are 10-membered macrolides, isolated from the culture broth of an actinomycete *Saccharothrix* sp. by our group (Figure 1a, S1).[14–17] The variety of the substitution at C-7 is derived from the Michael reaction between the precursor molecule, presaccharothriolide X (preSTL-X, **2**), and nucleophiles (Figure 1a). The structure–activity relationship study using STL congeners revealed that STL-B (**1**) and preSTL-X (**2**) were most potent cytotoxic STL congeners against human fibrosarcoma HT1080 cells. PreSTL-X (**2**) seems to be the active substance since STL-B (**1**) released *o*-aminophenol and thereby generated preSTL-X (**2**) in aqueous conditions (Figure 1a).[17] This retro-Michael reaction was not observed in the case of STL-A, -G, or -K, which had anthranilic acid, *o*-anisidine, or *m*-aminophenol at the C-7 position, respectively (Figure S1). However, the mechanism and the scope of this elimination was not known.

We have investigated the potential of *o*-aminophenol as a protective group for Michael acceptors using simple model compounds. Among the Michael adducts tested, the *o*-aminophenol adduct of an α,β-unsaturated ketone showed retro-Michael reactivity. This reaction was not observed when *o*-anisidine was used. In contrast, α,β-unsaturated amides and esters were not tolerated by this reversible protection by *o*-aminophenol. Herein we report that the transient protection of α,β-unsaturated ketones by *o*-aminophenol is a general protecting group strategy and not limited to STL-B.

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2. Results and discussion

2.1. Preference of Michael acceptors

The retro-Michael reaction of STL-B (**1**) proceeded spontaneously in aqueous solvents to yield preSTL-X (**2**) and *o*-aminophenol (Figure 1a).[17] To investigate the scope of this reaction, we examined three Michael adducts (**3**, **4** and **5**) which were synthesized from α,β-unsaturated ketone (**6**), ester (**7**), and amide (**8**), respectively (Figure 1b & 1c). *o*-Aminophenol adducts **3**, **4**, and **5** were left in aqueous MeOH at 37 °C for two days and then analyzed by HPLC (Fig 2, S2). We found that amount of keto-type adduct **3** decreased while *o*-aminophenol and Michael acceptor **6** appeared. Conversely, *o*-aminophenol and the corresponding Michael acceptors (**7** or **8**) were not detected when the ester or amide-type adducts (**4** and **5**, respectively) were incubated. These results revealed that the spontaneous retro-Michael reaction of the *o*-aminophenol adduct is not limited to STL-B. Instead, this reaction was specifically observed for the *o*-aminophenol adduct of α,β-unsaturated ketones. The acidity of the α-proton of the carbonyl is likely important for this reaction. Amides and esters reduce the acidity of the α-protons and thus hamper the spontaneous retro-Michael reaction.

2.2. Effect of solvent on the retro-Michael reaction

In the case of STL-B (**1**), the retro-Michael reaction proceeded in aqueous MeOH, but not in neat MeOH.[17] We examined the effect of solvents on the reactivity of compound **3** in an NMR tube. When **3** was dissolved in CD_3CN or DMSO-d_6 and incubated at 37 °C for two days, the ^1H NMR spectra showed no change (Figure S3). In contrast, the signals corresponding to the Michael acceptor **6** appeared when **3** was incubated in CD_3OD or CD_3OD/D_2O (4:1) (Figure 3). These results suggested that protic solvents promoted this retro-Michael reaction.

When we found that STL-B (**1**) released *o*-aminophenol in an aqueous MeOH, but not in neat MeOH, we could not explain the molecular mechanism.[17] The simple model compound **3** underwent the retro-Michael reaction both in neat MeOH and aqueous MeOH but not in MeCN or DMSO, which suggested that the elimination reaction requires a protic solvent.

The activity in the presence of H_2O in this reaction seems to be higher than that of MeOH. In the ^1H NMR spectra, deuterium exchange in Michael acceptor **6** and adduct **3** was observed both in CD_3OD/D_2O (4:1) and in CD_3OD, but the degree of the exchange was higher in the

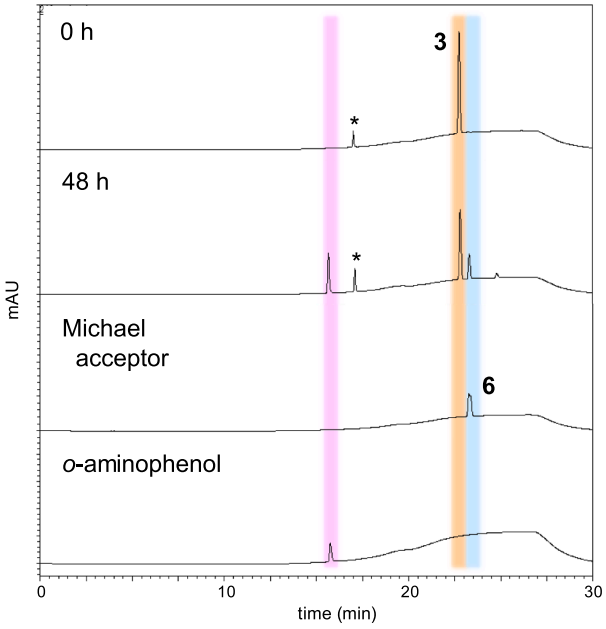

Fig. 2. Retro-Michael reaction of the *o*-aminophenol adducts (**3**). Compound **3** was dissolved in MeOH/H_2O (4:1), incubated at 37 °C, and analyzed by HPLC. Top panel: the sample just after preparation (0 h); second from the top: the sample after 48 h incubation. Chromatograms for the Michael acceptor (**6**) and *o*-aminophenol are also shown. The asterisks indicate caffeine, which was used as an internal standard. See the experimental section for details.

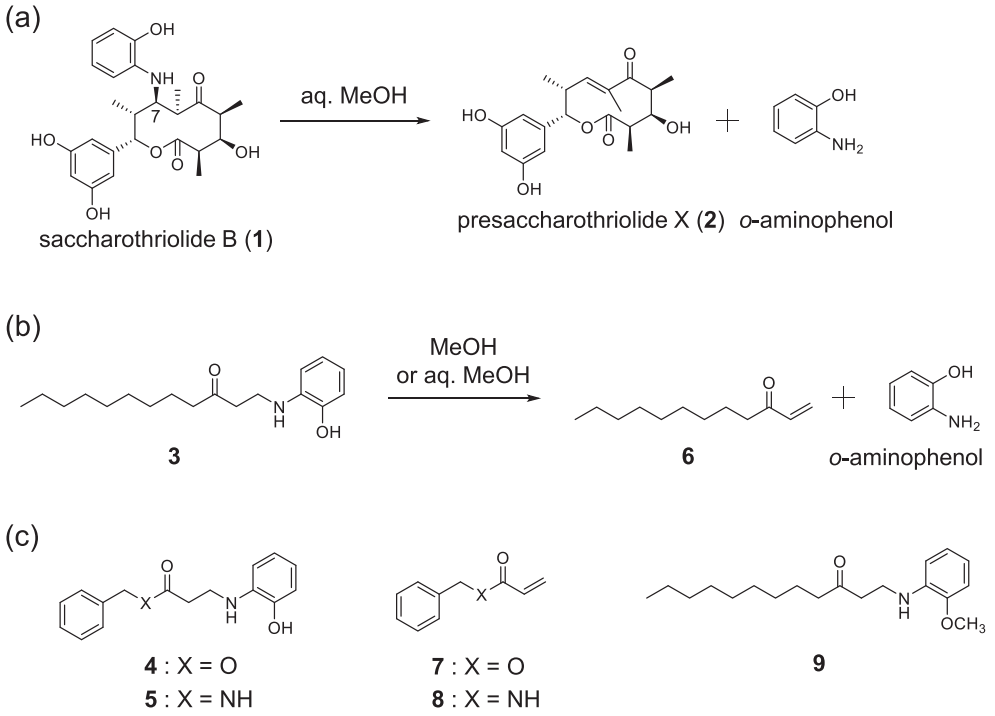

Fig. 1. Chemical structures of STLs and model Michael acceptors and adducts. (a) STL-B (**1**) releases *o*-aminophenol to generate preSTL-X (**2**). (b, c) Chemical structures of model compounds: keto-type (**3**, **6**, **9**), ester-type (**4**, **7**), and amide-type (**5**, **8**).

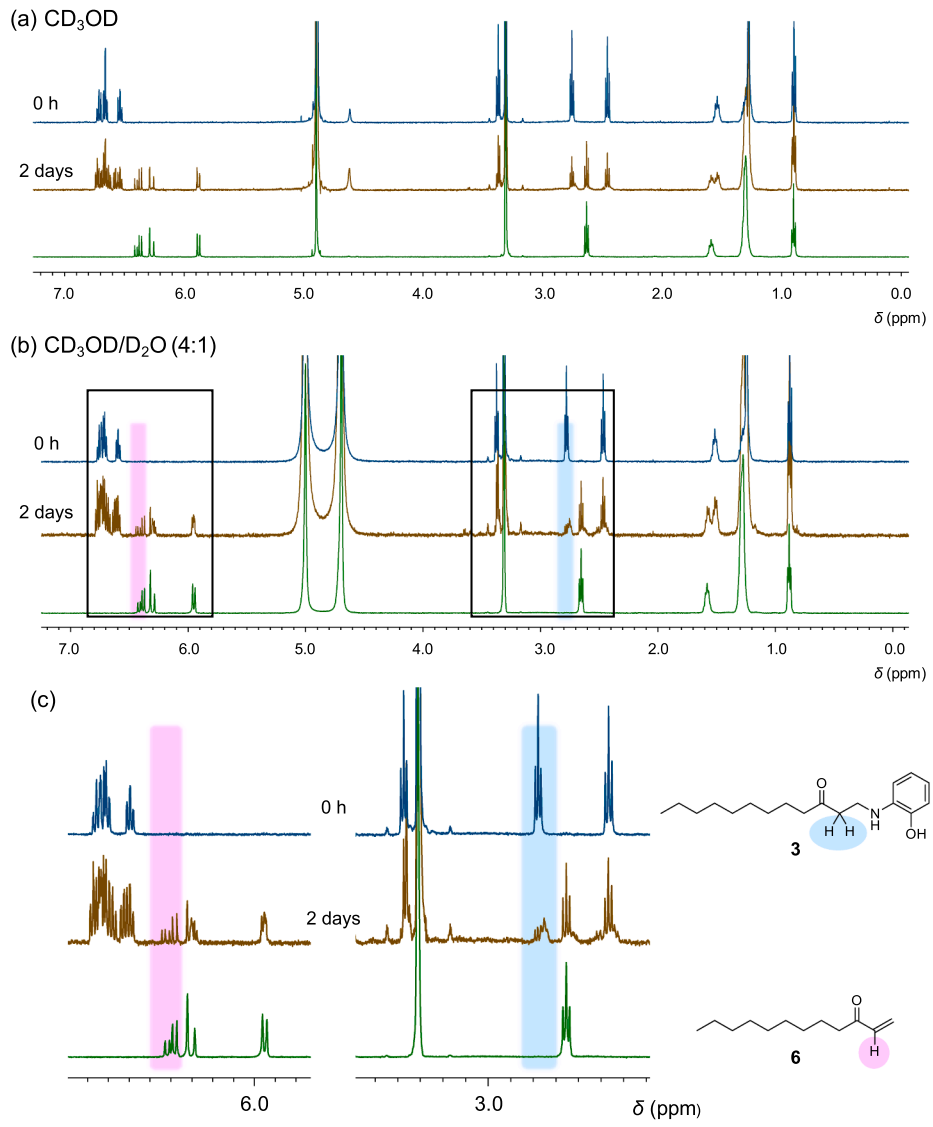

Fig. 3. Effect of solvents on the retro-Michael reaction of compound **3** (500 MHz ^{1}H NMR). *o*-Aminophenol adduct **3** was dissolved in CD_3OD (a) or CD_3OD/D_2O (4:1) (b). Top blue: compound **3** just after being dissolved in the solvent; middle brown; compound **3** after two days at 37 °C; bottom green: Michael acceptor **6**. (c) Enlarged view of the ^{1}H NMR spectra shown in (b). The proton signals highlighted in blue and pink correspond to the α-protons in compounds **3** and **6**, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

presence of D_2O (Figure 3c, S4). In CD_3OD, the peak area of the α methylene protons was 1.27H, indicating more than 35% of the protons were replaced by deuterium. Conversely, in the presence of D_2O, the peak area was 0.67H, which indicated more than 65% of the protons were replaced by deuterium (Figure S4c). These deuterium exchanges seem to couple with the retro-Michael reaction, as this exchange was not observed when the *o*-anisidine adduct was examined (Figure S6, see below).

2.3. Quantitative analyses of elimination of *o*-aminophenol in protic environments

The NMR analyses of compound **3** indicated that aqueous MeOH has higher catalytic activity in the retro-Michael reaction than that of neat MeOH. To investigate the solvent effect in a quantitative manner, we conducted time-dependent analyses of the retro-Michael reaction of compound **3** in MeOH or MeOH/water (4:1) (Figure 4). In MeOH, the

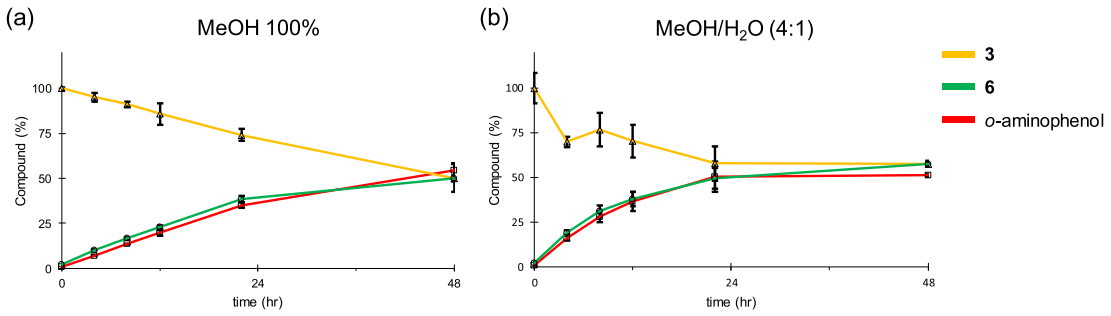

Fig. 4. Quantitative analyses of the elimination of *o*-aminophenol from compound **3**. Reaction was monitored in MeOH (a) or MeOH/H_2O (4:1) (b). Compound **3** (1 mM) was incubated at 37 °C for 48 h. Data represent the mean ± SD of three independent experiments. See the experimental section for details.

amount of compound **3** linearly decreased, whereas the amounts of *o*-aminophenol and the Michael acceptor **6** increased at a similar speed. After 48 h incubation, the remaining amount of compound **3** was estimated to be 50 (±7.7) %. The amount of *o*-aminophenol and compound **6** were 54 (±3.9) and 50 (±1.7) % relative to starting material **3**, respectively. In the presence of water, the reaction was faster than that in neat MeOH, and it took 22 h to reach the equilibrium. At that time, the amount of compound **3**, *o*-aminophenol, and compound **6** were 58 (±9.8), 50 (±8.3), and 50 (±5.8) %, respectively. These results unveiled that aqueous MeOH is a more effective solvent for the retro-Michael reaction of the *o*-aminophenol Michael adduct than neat MeOH. Although the promotion of Michael addition with anilines by polar protic solvents, without any promoting agent, has been reported,[18] this is the first report of a retro-Michael reaction promoted by protic solvents and no additional reagents. Additionally, the effects of pH for this reaction were tested. The reactions were monitored in MeOH/NaOAc aq. (4:1) and MeOH/Tris-HCl aq. (4:1) as models of acidic (pH 4.0) and basic (pH 8.8) conditions. The results were not different from that of neutral condition, they took *ca.* 24 h to reach the equilibrium (Figure S5). It is noted that compound **3** gave no detectable byproducts in these conditions.

2.4. Inert Michael adduct with o-anisidine

STL-G, an STL derivative that has *o*-anisidine at the C-7 position, did not show any retro-Michael reactivity (Figure S1).[17] We finally examined the requirement of the phenolic hydroxy group on *o*-aminophenol. To examine this phenomenon using a model compound, compound **9** was synthesized, and the reactivity was monitored by NMR and HPLC (Figure 1c, S6). As expected, compound **9** showed no change in the ^{1}H NMR spectrum in CD_3OD/D_2O (4:1) (Figure S6a). Quantitative analyses by HPLC confirmed that compound **9** is too stable to yield Michael acceptor **6** both in MeOH and MeOH/H_2O (4:1) (Figure S6b).

2.5. Plausible mechanism of retro-Michael reaction

We found that compound **9** neither released the Michael acceptor nor showed deuterium exchange in protic solvents. This indicated that the phenolic alcohol is required not only for the keto-enol equilibrium but also for the elimination reaction. Enol formation can be accomplished by forming the zwitterion, as shown in Figure 5. The zwitterion is more stable in protic solvents than in non-protic solvents, which is consistent with the retro-Michael reaction occurring protic solvents. The enol form of compound **3** undergoes deuteration or elimination. The proposed elimination mechanism of *o*-aminophenol is similar to that of the E1cB reaction by which a Mannich base can be converted to an enone.[19] In this case, however, alkylation of the amine to form a quaternary amine and a base to start the deamination reaction are required. Alternatively, protic solvents can generate a hydrogen-bonding network to form the enol, followed by deuteration or elimination (Figure S7). However, the difference between neat MeOH and aqueous MeOH remains to be explained.

3. Conclusion

In summary, we demonstrated that the retro-Michael reaction of the *o*-aminophenol Michael adduct is observed not only in STL-B (**1**) but also in the simple model compound **3**. α,β-Unsaturated ketones were compatible with this reaction, which was promoted in protic solvents, especially aqueous methanol. It is noted that this elimination reaction can be controlled by protecting the phenolic alcohol. This phenomenon may be applied as a drug-delivery system consisting of labile bioactive compounds possessing an α,β-unsaturated ketone. Protection of the phenolic alcohol will be crucial for tissue- or microenvironment-selective release of the bioactive compounds, which is currently being investigated in our group.

4. Experimental section

4.1. General procedures

All commercially available reagents were purchased from Nacalai, Fujifilm-Wako or Tokyo-Kasei unless described. Silica gel column was performed using Kanto Silica Gel 60 N (63–210 mm) (Kanto Chemical). TLC was performed using glass-backed silica gel 60F254 (Merck). NMR spectra were recorded on a JOEL 500 MHz instrument (JEOL). ^{1}H and ^{13}C chemical shifts are shown relative to the residual solvent: δ_H 7.26 and δ_C 77.16 for $CDCl_3$; δ_H 2.50 for $(CD_3)_2SO$; δ_H 1.94 for CD_3CN; δ_H 3.31 and δ_C 49.00 for CD_3OD. Chemical shifts (δ) are shown in parts per million (ppm) and coupling constants (J) are in hertz (Hz). High-resolution mass spectra were recorded on an ESI-ITTOF-MS (Shimadzu) or a FAB-MS JEOL JMS-700 double-focusing mass spectrometer (JEOL).

4.2. Synthesis of the compounds

4.2.1. Dodec-1-en-3-one (6)

To a stirred solution of decanal (0.83 g, 5.3 mmol) in THF (10.6 mL) was added vinylmagnesium bromide (1.0 M in THF, 6.4 mL, 6.4 mmol) at 0 °C. After being stirred for 1 h, the mixture was warmed up to room temperature and stirred for an additional five days. The reaction was quenched with 3 M HCl aqueous solution and the layers were separated. The aqueous layer was extracted six times with Et_2O, and the combined organic layers were washed with sat aq $NaHCO_3$, dried over Na_2SO_4, and evaporated. The residue was subjected to silica gel column chromatography (*n*-hexane/EtOAc) to afford a fraction containing 1-dodecen-3-ol

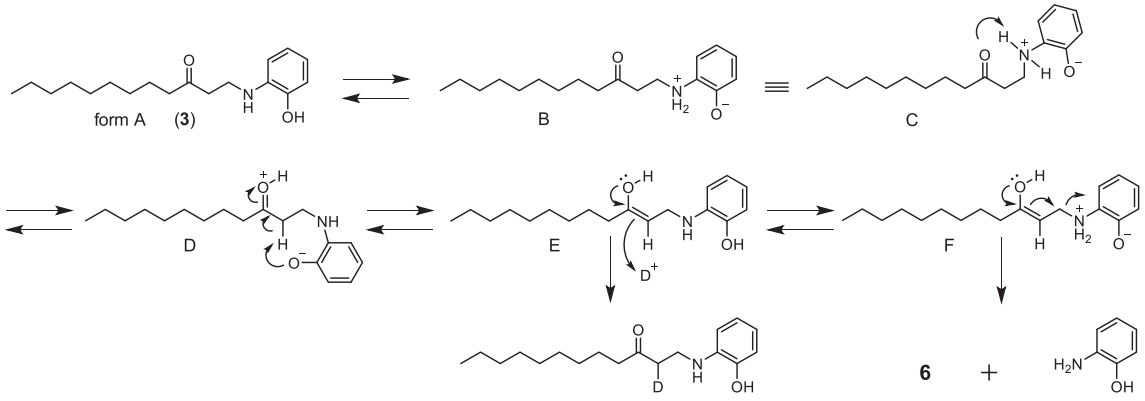

Fig. 5. Proposed mechanism of the retro-Michael reaction. Compound **3** (form A) exists as a zwitterion (form B), which may promote enol formation (form C to E and F). Generation of form E and F can be followed by deuteration or elimination of aminophenol.

(1.17 g). To a stirred solution of oxalyl chloride (0.75 mL, 9.5 mmol) in CH_2Cl_2 (34 mL) was added DMSO (0.90 mL, 12.7 mmol) at −78 °C. The resulting solution was stirred at −78 °C for 30 min, and then a solution containing dodec-1-en-3-ol (1.17 g in 2.5 mL CH_2Cl_2) was added. After being stirred at −78 °C for 45 min, triethylamine (4.4 mL, 31.8 mmol) was added to the mixture. After 10 min the reaction mixture was warmed up to room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel open column chromatography (*n*-hexane/EtOAc) to afford compound **6** as a colorless oil (557.9 mg, 58% over two steps): ^{1}H NMR (CD_3OD, 500 MHz) δ 6.38 (dd, J = 17.8, 10.3 Hz, 1H), 6.27 (dd, J = 17.8, 1.3 Hz, 1H), 5.88 (dd, J = 10.3, 1.3 Hz, 1H), 2.63 (t, J = 7.5 Hz, 2H), 1.54–1.64 (m, 2H), 1.36–1.24 (overlapped, 12H), 0.90 (t, J = 7.0 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 203.4, 137.7, 129.2, 40.3, 33.1, 30.61, 30,59, 30.4, 30.3, 25.2, 23.7, 14.4; HR-MS (FAB) $[M + H]^+$ *m/z* 183.1746 (calcd. for $C_{12}H_{23}O$, 183.1749).

4.2.2. 1-((2-Hydroxyphenyl) amino) dodecan-3-one (3)

Compound **6** (10.4 mg, 0.057 mmol) and *o*-aminophenol (31.1 mg, 0.28 mmol) were dissolved in CH_3CN (57.0 μL) and stirred at room temperature for 17 h. The mixture was subjected to silica gel column chromatography (*n*-hexane/EtOAc) to afford compound **3** as an orange solid (11.2 mg, 67%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.87–6.80 (m, 1H), 6.78–6.66 (overlapped, 3H), 3.39 (t, J = 6.0 Hz, 2H), 2.73 (t, J = 6.0 Hz, 2H), 2.42 (t, J = 7.5 Hz, 2H), 1.62–1.52 (m, 2H), 1.34–1.18 (overlapped, 12H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 211.1, 145.3, 136.2, 121.4, 119.3, 114.8, 114.2, 43.5, 42.0, 39.8, 32.0, 29.6, 29.5, 29.4, 29.3, 23.9, 22.8, 14.3; HR-MS (ESI) $[M + H]^+$ *m/z* 292.2278 (calcd. for $C_{18}H_{30}NO_2$, 292.2271).

4.2.3. Benzyl 3-((2-hydroxyphenyl) amino) propanoate (4)

Benzyl acrylate (**7**) (210.8 mg, 1.3 mmol), *o*-aminophenol (709.3 mg, 6.5 mmol) and silica gel (700.8 mg) were dissolved in CH_3CN (1.3 mL) and stirred at 86 °C for 15.5 h. The mixture was subjected to silica gel column chromatography (*n*-hexane/EtOAc) to afford compound **4** as a dark brown oil (202.5 mg, 57%): ^{1}H NMR (CD_3OD, 500 MHz) δ 7.37–7.28 (overlapped, 5H), 6.73–6.62 (overlapped, 3H), 6.57–6.51 (m, 1H), 5.14 (s, 2H), 3.44 (t, J = 6.5 Hz, 2H), 2.67 (t, J = 6.3 Hz, 2H); ^{13}C NMR (CD_3OD, 125 MHz) δ 173.9, 146.4, 137.8, 137.5, 129.5 (2C), 129.18 (2C), 129.16, 121.2, 118.9, 114.8, 112.7, 67.4, 40.8, 35.0; HR-MS (ESI) $[M + H]^+$ *m/z* 272.1283 (calcd. for $C_{16}H_{18}NO_3$, 272.1281).

4.2.4. N-benzylacrylamide (8)

To a stirred solution of benzylamine (0.22 mL, 2.0 mmol) in CH_2Cl_2 (5 mL) were added Et_3N (0.31 mL, 2.2 mmol) and acryloyl chloride (0.18 mL, 2.2 mmol) at 0 °C. After being stirred at room temperature for 1 h, the reaction was quenched with sat. $NaHCO_3$ and extracted with CH_2Cl_2 three times. The combined organic layers were dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc) to afford *N*-benzylacrylamide (**8**) as a white solid (221.1 mg, 69%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.26 (overlapped, 5H), 6.33 (dd, J = 17.0, 1.5 Hz, 1H), 6.11 (dd, J = 17.0, 10.5 Hz, 1H), 5.88 (br. s, 1H), 5.67 (dd, J = 10.3, 1.8 Hz, 1H), 4.52 (d, J = 6.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.5, 138.1, 130.7, 128.9 (2C), 128.1 (2C), 127.8, 127.0, 43.9 ; HR-MS (ESI) $[M + H]^+$ *m/z* 162.0916 (calcd. for $C_{10}H_{12}NO$, 162.0913).

4.2.5. N-Benzyl-3-((2-hydroxyphenyl) amino) propanamide (5)

N-benzylacrylamide (**8**) (209.6 mg, 1.3 mmol), *o*-aminophenol (709.3 mg, 6.5 mmol) and silica gel (700.8 mg) were dissolved in CH_3CN (1.3 mL) and stirred at 85 °C for 36 h. The mixture was purified by silica gel column chromatography (*n*-hexane/EtOAc) to afford compound **5** as a dark brown oil (46.5 mg, 13%): ^{1}H NMR (CD_3OD, 500 MHz) δ 7.31–7.24 (overlapped, 4H), 7.24–7.19 (m, 1H), 6.74–6.66 (overlapped, 3H), 6.56–6.50 (m, 1H), 4.37 (s, 2H), 3.45 (t, J = 6.5 Hz, 2H), 2.55 (t, J = 6.8 Hz, 2H); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.4, 146.3, 139.8, 138.0, 129.5 (2C), 128.5 (2C), 128.1, 121.2, 118.7, 114.7, 112.6, 44.1, 41.5, 36.7 ; HR-MS (ESI) $[M + H]^+$ *m/z* 271.1440 (calcd. for $C_{16}H_{19}N_2O_2$, 271.1441).

4.2.6. 1-((2-Methoxyphenyl) amino) dodecan-3-one (9)

Compound **6** (10.4 mg, 0.057 mmol) and *o*-anisidine (35.0 mg, 0.28 mmol) were dissolved in CH_3CN (57.0 μL) and stirred at room temperature overnight. The mixture was purified by silica gel column chromatography (*n*-hexane/EtOAc) to afford compound **9** as a white solid (10.0 mg, 57%): ^{1}H NMR (CD_3OD, 500 MHz) δ 6.84–6.79 (m, 2H), 6.69–6.63 (m, 2H), 3.81 (s, 3H), 3.38 (t, J = 6.8 Hz, 2H), 2.76 (t, J = 6.3 Hz, 2H), 2.45 (t, J = 7.3 Hz, 2H), 1.57–1.50 (m, 2H), 1.35–1.21 (overlapped, 12H), 0.90 (t, J = 7.0 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 212.9, 149.2, 138.3, 122.3, 119.1, 112.5, 111.0, 56.0, 43.8, 42.3, 40.0, 33.1, 30.60, 30.58, 30.4, 30.2, 24.8, 23.7, 14.5; HR-MS (ESI) $[M + H]^+$ *m/z* 306.2435 (calcd. for $C_{19}H_{32}NO_2$, 306.2428).

4.2.7. Monitoring of the conversion of o-aminophenol Michael adduct

Compounds (**3**, **4**, **5**, and **9**) (1.0 mM) and caffeine (100 μM) were dissolved in MeOH, MeOH/milliQ water (4:1), MeOH/0.1 M NaOAc aq. (4:1) or MeOH/50 mM Tris-HCl aq. (4:1) and incubated at 37 °C. A portion of the reaction mixture and the authentic samples were analyzed by HPLC. HPLC conditions for compounds **3** and **9**: Cosmosil MS II Φ 4.6 × 250 mm, 0.8 mL/min, 5–100% aq MeOH; HPLC conditions for compounds **4** and **5**: Cosmosil PBr Φ 4.6 × 250 mm, 0.8 mL/min, 5–100% aq MeOH. Chromatograms observed at 210 nm (**3**–**9**, *o*-aminophenol, *o*-anisidine, and caffeine) were used for measuring the amount of the compounds.

4.2.8. ^{1}H NMR-based monitoring

Compounds **3** or **9** (3.7–4.6 mM) were dissolved into CD_3OD or CD_3OD/D_2O (4:1). The mixtures in NMR tubes were incubated at 37 °C sealing with parafilm. The chemical shifts of ^{1}H NMR were recorded at 1 day and 2 days after the incubation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2021.116059.

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