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Base-Mediated Oxidative Degradation of Secondary Amides Derived from *p*-Amino Phenol to Primary Amides in Drug Molecules

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ABSTRACT

One of the most common functional groups encountered in drug molecules is the amide, and the most common degradation pathway for amides is base-mediated hydrolysis to its constituent amine and carboxylic acid. Herein, we report for the first time, a base-mediated oxidative degradation pathway of secondary amides to primary amides. This transformation also represents a novel synthetic methodology, reported for the first time in this work, in transforming secondary amides to primary amides without using any oxidative reagents. The introduction of this mechanism into the pharmaceutical literature is important given that the mechanism and required reactants are present to carry out the chemistry in dosage forms.

Introduction

Degradation of the active pharmaceutical ingredient (API) in drug products (DP) impacts the safety and efficacy of drugs, which prompts pharmaceutical scientists to study and understand degradation pathways. The most common degradation pathways operating on API in DP are hydrolytic and oxidative.[1] Mechanistically, oxidative degradation pathways are often more complex than hydrolytic pathways. The commonly recognized reactive species in oxidative degradation are alkoxy, hydroxy or hydroperoxy radicals, peroxides, metal ions and singlet oxygen.[1,2] Oxidation involving a radical species follows a one-electron pathway, whereas peroxides invoke a two-electron oxidation mechanism. Typically, the role of metal ions in oxidative degradation is to catalyze the oxidation of peroxides to peroxy/hydroxy/alkoxy radicals, which in turn oxidize the API.[1,2] It is also known that some oxidative degradation pathways involve electron transfer from transition metal ions.[3,4] Recently we have shown a novel oxidative pathway involving iron(III), where certain class of drug molecules can be oxidized in the absence of peroxides.[5]

In a recent drug development effort in our laboratories, a drug substance containing an amide functionality showed an unusual degradation product under accelerated stress in the solid state as well as in solution forced stress studies under basic (NaOH) conditions. The expected degradation products from an amide are typically the corresponding carboxylic acid and amine, which are formed under acid- or base-catalyzed hydrolysis. But, in this case, the observed degradation product was a primary amide (Scheme 1).

To the best of our knowledge, this degradation pathway for amides has not been reported in the drug degradation literature. Also, to the best of our knowledge, conversion of a secondary amide to a primary amide, effected under basic conditions without any added oxidizing agent, has not been reported in the organic chemistry literature. In this paper, we report the preliminary findings elucidating the mechanism of this unique degradation pathway.

Materials

Reagents and solvents, used for the synthesis and reactivity studies, are reagent grade and purchased from commercial sources. Solvents and trifluoracetic acid, used in chromatography and mass spectrometry, are HPLC grade and purchased from commercial sources.

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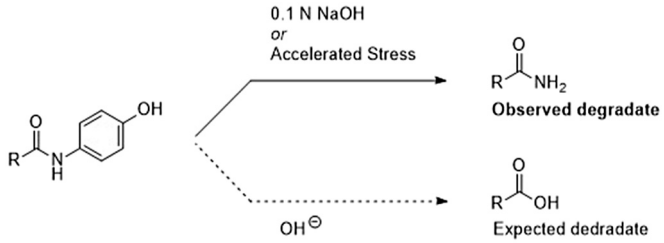


Scheme 1. Unusual degradation of a secondary amide to primary amide.

Experiment and Analysis

The solution state forced stress experiments were carried out by adding one part 1 N aqueous NaOH to nine part (by volume) substrate solution (~0.1 mg/mL) in 1:1H_2O—MeCN, and heating the resulting solution at 60 °C. Oxidative forced stress experiments with azobisisobutyronitrile (AIBN) and hydrogen peroxide (H_2O_2) were performed as described elsewhere.[6]

Reversed-phase chromatography for the analysis of the forced stress experiments were carried out on an Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA) equipped with a quaternary pump, heated column compartment, diode array detector, and autosampler. The chromatographic conditions included a Supelco Ascentis Express C18 column of dimensions 100 mm × 3 mm and 2.7 μm particle size held at 45 °C; flow rate was maintained at 1 mL/min; injection volume was 5 μL; tray temperature was ambient; detector wavelength was 254 nm; mobile phase A consisted of a mixture of 0.05% trifluoroacetic acid (TFA) in water; mobile phase B consisted of 0.05% TFA in acetonitrile. The mobile phase gradient is shown in Table 1.

For the LC/MS analysis of the forced stress experiments, an Agilent 1100 series HPLC with a quaternary pump, diode array detector and autosampler was used to separate the components of the reaction mixture prior to MS analysis. The chromatographic conditions used in the experiment included: Supelco Ascentis Express C18 column of dimensions 100 mm × 3 mm and 2.7 μm particle size held at 45 °C; flow rate maintained at 0.9 mL/min; injection volume of 5 μL; tray temperature at 25 °C; detection wavelength at 254 nm with spectrum scan from 190 to 400 nm. Mobile Phase A consisting of 0.05% TFA in water; and Mobile Phase B consisting of 0.05% TFA in MeCN. The mobile phase gradient is shown in Table 2. A Thermo Scientific LCQ Fleet MS was used in series with the Agilent 1100 HPLC for mass detections. The LCQ Fleet MS used ESI operated in positive ion mode. Capillary temperature was set to 250 °C, the source voltage set to 4.5 kV and source current set to 100 μA.

For the LC/MS analysis of synthetic reactions and isolated synthetic products, samples were injected (0.5 μL) onto a Waters Acquity UPLC BEH C18 column (1 cm × 500 mm, 1.7 μm) from Waters Corp. (Milford, MA) and eluted with a linear gradient delivered at a flow rate of 300 μL/min by an Acquity Ultra-Performance Liquid Chromatography system (Waters Corp., Milford, MA). Mobile phases consisted of water/acetonitrile/TFA at a ratio of 99.95%, 0%, and 0.05% (v:v:v) for solvent A and a ratio of 0%, 99.95%, and 0.05% (v:v:v) for solvent B. The following elution conditions were set: 5% of solvent B for 0.1 min, followed by a linear increase of B from 5 to 99% within 2 min. Column temperature was maintained at 50 °C. An SQD detector (Waters Corp., Milford, MA) was used coupled to the UPLC system. The mass spectrometer was equipped with an electrospray ion source. The capillary was set to 3.0 kV. The cone and extraction voltages were 20.0 eV and 3.0 eV, respectively. Source and desolvation temperatures were 120 °C and 400 °C, respectively.

Synthetic compounds were purified by normal phase flash silica chromatography using an automated purification system (ISCO) using Redisep® disposable flash cartridges with peak detection at 254 nm. Alternatively, compounds were purified by preparative reversed-phase HPLC using a Gilson 215 liquid handler equipped with a Waters Sunfire® C18 column (150 × 30 mm I.D.) with a linear 40 mL/min gradient over 20 min (90:10 to 5:95H2O containing 0.1% trifluoroacetic acid:MeCN) and detection at 215 nm.

NMR spectra were recorded at 500 MHz for ^{1}H on a Varian spectrometer in the stated solvent. The chemical shifts are given in ppm, referenced to the deuterated solvent signal or tetramethylsilane.

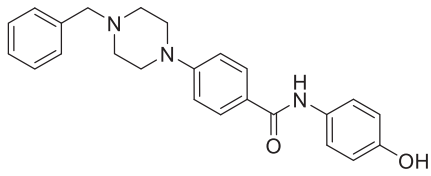
Table 1
Mobile Phase Gradient Used in the Reversed-Phase Chromatography Analyzing Forced Stress Experiments.

Time (Minute)	%A (0.05% TFA in H_2O)	%B (0.05% TFA in MeCN)
0	95	5
5	0	100
7	0	100
7.1	95	5
10	95	5

Table 2
Mobile Phase Gradient Used in the LC/MS Analysis of Forced Stress Experiments.

Time (Minute)	%A (0.05% TFA in H_2O)	%B (0.05% TFA in MeCN)
0	95	5
3.5	0	100
4	0	100
4.01	95	5
5	95	5

Synthesis



4-(4-benzylpiperazin-1-yl)-*N*-(4-hydroxyphenyl)benzamide (**1**)

tert-*Butyl 4-(4-((4-hydroxyphenyl)carbamoyl)phenyl)piperazine-1-carboxylate (1A)*

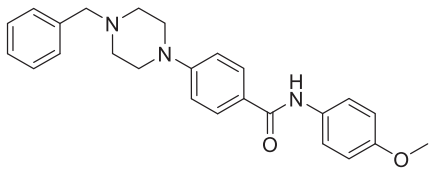
A mixture of 4-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)benzoic acid (250 mg, 0.816 mmol), 4-aminophenol (223 mg, 2.04 mmol), diisopropylethylamine (428 μL, 2.45 mmol), and HATU (388 mg, 1.02 mmol) in DMF (4 mL) was stirred at rt. After 16 h, the mixture was added to H_2O and extracted with 2× EtOAc. The combined organic layers were washed with water and saturated aqueous NaCl, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford crude **1A**.

N-(4-hydroxyphenyl)-4-(piperazin-1-yl)benzamide dihydrochloride (1B)

HCl gas was bubbled through a mixture of *tert*-butyl 4-(4-((4-hydroxyphenyl)carbamoyl)phenyl)piperazine-1-carboxylate (**1A**, 209 mg, 0.526 mmol) in diethyl ether at 0 °C for 20 s. The mixture was allowed to warm to room temperature while stirring for 16h, and then concentrated to afford crude **1B**.

4-(4-Benzylpiperazin-1-yl)-N-(4-hydroxyphenyl)benzamide (1)

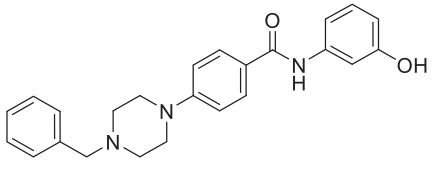
To a mixture of *N*-(4-hydroxyphenyl)-4-(piperazin-1-yl)benzamide dihydrochloride (**1B**, 190 mg, 0.513 mmol) and benzaldehyde (1 mL, 9.87 mmol) in DMF (1 mL) was added sodium triacetoxyborohydride (218 mg, 1.026 mmol) and the resulting mixture was stirred at rt. After 16 h, a saturated solution of aqueous sodium bicarbonate was added. The resulting mixture was extracted with 2× EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by preparative reversed-phase HPLC to afford the title compound as white solid. ESI MS *m/z*: 388.4 ($[M + H]^+$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.70 (s, 1H), 9.17 (s, 1H), 7.83 (d, J = 8.9 Hz, 2H), 7.50 (d, J = 8.9 Hz, 2H), 7.34 (d, J = 4.6 Hz, 4H), 7.31–7.23 (m, 1H), 6.98 (d, J = 9.0 Hz, 2H), 6.71 (d, J = 8.9 Hz, 2H), 3.54 (s, 2H), 3.31–3.26 (m, 4H), 3.17 (d, J = 5.3 Hz, 2H).



4-(4-benzylpiperazin-1-yl)-*N*-(4-methoxyphenyl)benzamide (**2**)

4-(4-Benzylpiperazin-1-yl)-N-(4-methoxyphenyl)benzamide (2)

Compound **2** was prepared according to the procedure from 4-(4-benzylpiperazin-1-yl)-*N*-(4-hydroxyphenyl)benzamide (**1**) using 4-methoyxaniline. ESI MS *m/z*: 402.5 ($[M + H]^+$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.80 (s, 1H), 7.85 (d, J = 9.0 Hz, 2H), 7.65 (d, J = 9.1 Hz, 2H), 7.35 (d, J = 4.6 Hz, 4H), 7.27 (q, J = 4.7 Hz, 1H), 6.99 (d, J = 9.0 Hz, 2H), 6.90 (d, J = 9.1 Hz, 2H), 3.74 (s, 3H), 3.54 (s, 2H), 3.31–3.26 (m, 4H), 2.52 (m, 4H).



4-(4-benzylpiperazin-1-yl)-*N*-(3-hydroxyphenyl)benzamide (**3**)

Ethyl 4-(4-benzylpiperazin-1-yl)benzoate (3A)

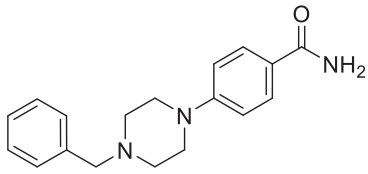
To a mixture of ethyl 4-(piperazin-1-yl)benzoate (750 mg, 3.20 mmol) and benzaldehyde (1.62 mL, 16.0 mmol) in DMF (10 mL) was added sodium triacetoxyborohydride (1.36 g, 6.40 mmol) and the resulting mixture was stirred at rt. After 16 h, a saturated solution of aqueous sodium bicarbonate was added. The resulting mixture was extracted with 2× EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using 0–100% ethyl acetate gradient in hexanes to afford **3A**.

4-(4-Benzylpiperazin-1-yl)benzoic Acid (3B)

To a mixture of ethyl 4-(4-benzylpiperazin-1-yl)benzoate (**3A**), 1.04 g, 3.20 mmol) in 1:1 MeOH:THF (32 mL) was added 1 M aqueous sodium hydroxide (6.40 mL, 6.40 mmol) and the resulting mixture was stirred at rt. After 16 h, 1 M aqueous HCl (6.40 mL, 6.40 mmol) was added and the resulting mixture was concentrated under reduced pressure to afford **3B** along with 2 equivalents of sodium chloride.

4-(4-Benzylpiperazin-1-yl)-N-(3-hydroxyphenyl)benzamide (3)

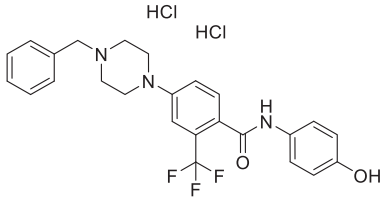
A mixture of 4-(4-benzylpiperazin-1-yl)benzoic acid disodium chloride (**3B**, 209 mg, 0.506 mmol), 3-aminophenol (138 mg, 1.26 mmol), diisopropylethylamine (442 μL, 2.53 mmol), and HATU (240 mg, 0.632 mmol) in DMF (1.7 mL) was stirred at rt. After 16 h, the mixture was purified by preparative reversed-phase HPLC to afford **3** as white solid. ESI MS *m/z*: 388.5 ($[M + H]^+$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.78 (s, 1H), 9.32 (s, 1H), 7.84 (d, J = 8.9 Hz, 2H), 7.35 (d, J = 4.5 Hz, 5H), 7.30–7.24 (m, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.08 (t, J = 8.0 Hz, 1H), 6.99 (d, J = 8.9 Hz, 2H), 6.46 (d, J = 7.9 Hz, 1H), 3.54 (s, 2H), 3.29 (m, 4H), 2.53 (m, 4H).



4-(4-benzylpiperazin-1-yl)benzamide (**4**)

4-(4-Benzylpiperazin-1-yl)benzamide (4)

A mixture of 4-(4-benzylpiperazin-1-yl)benzoic acid disodium chloride (**3B**, 106 mg, 0.257 mmol), ammonium bicarbonate (41 mg, 0.513 mmol), pyridine (42 μL, 0.513 mmol), and BOC-anhydride (119 μL, 0.513 mmol) in dioxane (0.64 mL) was stirred at rt. After 16 h, the mixture was concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using 0–10% MeOH gradient in CH_2Cl_2 to afford **4** as solid. ESI MS *m/z*: 296.3 ($[M + H]^+$). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.73 (d, J = 8.9 Hz, 2H), 7.40–7.33 (m, 4H), 7.30 (dd, J = 5.8, 2.7 Hz, 1H), 6.89 (d, J = 8.9 Hz, 2H), 3.59 (s, 2H), 3.37–3.30 (m, 4H), 2.66–2.58 (m, 4H).



4-(4-benzylpiperazin-1-yl)-*N*-(4-hydroxyphenyl)-2-(trifluoromethyl)benzamide dihydrochloride (**5**)

tert-*Butyl 4-(4-(methoxycarbonyl)-3-(trifluoromethyl)phenyl) piperazine-1-carboxylate (5A)*

A mixture of methyl 4-bromo-2-(trifluoromethyl) benzoate (300 mg, 1.06 mmol), *tert*-butyl piperazine-1-carboxylate, XPhos Pd GII (167 mg, 0.212 mmol), and cesium carbonate (863 mg, 2.65 mmol) in toluene (5.3 mL) was stirred at 120 °C. After 16 h, The mixture was concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using 0–100% ethyl acetate gradient in hexanes to afford **5A** as white solid.

4-(4-(tert-Butoxycarbonyl)piperazin-1-yl)-2-(trifluoromethyl) benzoic Acid (5B)

To a mixture of *tert*-butyl 4-(4-(methoxycarbonyl)-3-(trifluoromethyl)phenyl)piperazine-1-carboxylate (**5A**, 227 mg, 0.584 mmol) in 1:1 MeOH:THF (5.8 mL) was added 1 M aqueous sodium hydroxide (1.169 mL, 1.17 mmol) and the resulting mixture was stirred at 70 °C. After 7 h, 1 M aqueous HCl (1.17 mL, 1.17 mmol) was added and the resulting mixture was concentrated under reduced pressure to afford **5B** along with 2 equivalents of sodium chloride.

tert-*Butyl 4-(4-((4-hydroxyphenyl)carbamoyl)-3-(trifluoromethyl) phenyl)piperazine-1-carboxylate (5C)*

A mixture of 4-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-(trifluoromethyl)benzoic acid disodium chloride (**5B**, 287 mg, 0.584 mmol), 4-aminophenol (127 mg, 1.17 mmol), diisopropylethylamine (306 μL, 1.75 mmol), and HATU (278 mg, 0.730 mmol) in DMF (2.92 mL) was stirred at rt. After 16 h, the mixture was poured into water, and the resulting mixture was extracted with 2× EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using 0–100% ethyl acetate gradient in hexanes to afford **5C**.

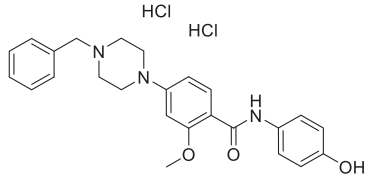
N-*(4-hydroxyphenyl)-4-(piperazin-1-yl)-2-(trifluoromethyl) benzamide dihydrochloride (5D)*

A mixture of *tert*-butyl 4-(4-((4-hydroxyphenyl)carbamoyl)-3-(trifluoromethyl)phenyl)piperazine-1-carboxylate (**5C**, 272 mg, 0.584 mmol) and TFA (450 μL, 5.84 mmol) in CH_2Cl_2 (5.8 mL) was stirred at rt. After 4 h, conc HCl (96 μL, 1.17 mmol) was added and the resulting mixture was concentrated under reduced pressure to afford **5D**.

4-(4-Benzylpiperazin-1-yl)-N-*(4-hydroxyphenyl)-2-(trifluoromethyl)benzamide dihydrochloride (5)*

To a mixture of *N*-(4-hydroxyphenyl)-4-(piperazin-1-yl)-2-(trifluoromethyl)benzamide dihydrochloride (**5D**, 149 mg, 0.340 mmol) and benzaldehyde (0.726 mL, 7.16 mmol) in DMF (0.7 mL) was added sodium triacetoxyborohydride (144 mg, 0.680 mmol) and the resulting mixture was stirred at rt. After 16 h, a saturated solution of aqueous sodium bicarbonate was added. The resulting mixture was extracted with 2× EtOAc. The combined organic layers were washed with saturated aqueous NaCl, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using 0–10% MeOH gradient in CH_2Cl_2 containing 0.1% NH_4OH to afford white solid. The solid was dissolved in 1:1 CH_2Cl_2:MeOH and conc. HCl (84 μL, 5.84 mmol) was added. The resulting mixture was concentrated under reduced pressure to afford **5** as white solid. ESI MS *m/z*: 456.3 ($[M + H]^+$). ^1H NMR (500 MHz, DMSO-d_6) δ 11.23 (s, 1H), 10.09 (s, 1H), 7.64 (dd, $J = 6.4, 2.8$ Hz, 2H), 7.54 (d, $J = 8.5$ Hz, 1H), 7.51–7.47 (m, 3H), 7.45 (d, $J = 8.9$ Hz, 2H), 7.33–7.24 (m, 2H), 6.72 (d, $J = 8.8$ Hz, 2H), 4.39 (d, $J = 4.9$ Hz, 2H), 4.03 (d, $J = 13.4$ Hz, 2H), 3.38 (d, $J = 11.7$ Hz, 2H), 3.30 (t, $J = 12.5$ Hz, 2H), 3.23–3.07 (m, 2H).

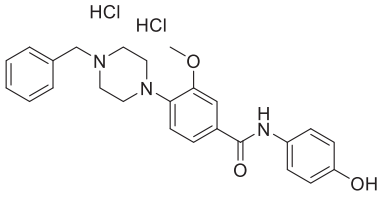
4-(4-Benzylpiperazin-1-yl)-N-*(4-hydroxyphenyl)-2-*



4-(4-benzylpiperazin-1-yl)-*N*-(4-hydroxyphenyl)-2-methoxybenzamide dihydrochloride (**6**)

methoxybenzamide dihydrochloride (6)

Compound **6** was prepared according to the procedure for 4-(4-benzylpiperazin-1-yl)-*N*-(4-hydroxyphenyl)-2-(trifluoromethyl) benzamide dihydrochloride (**5**) using methyl 4-bromo-2-methoxybenzoate. ESI MS *m/z*: 418.4 ($[M + H]^+$). ^1H NMR (500 MHz, DMSO-d_6) δ 11.00 (s, 1H), 9.64 (s, 1H), 7.75 (d, $J = 9.3$ Hz, 1H), 7.63 (dd, $J = 6.5, 2.8$ Hz, 2H), 7.53–7.45 (m, 5H), 6.72 (d, $J = 8.8$ Hz, 2H), 6.66 (d, $J = 5.7$ Hz, 2H), 4.40 (d, $J = 5.0$ Hz, 2H), 4.04 (d, $J = 13.4$ Hz, 2H), 3.97 (s, 3H), 3.38 (d, $J = 11.9$ Hz, 2H), 3.28 (t, $J = 12.1$ Hz, 2H), 3.19–3.07 (m, 2H).



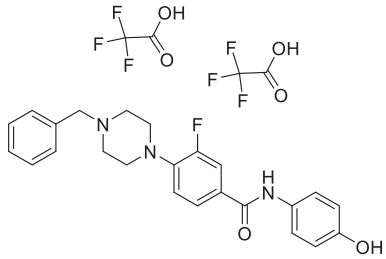
4-(4-benzylpiperazin-1-yl)-*N*-(4-hydroxyphenyl)-3-methoxybenzamide dihydrochloride (**7**)

*4-(4-Benzylpiperazin-1-yl)-*N*-(4-hydroxyphenyl)-3-methoxybenzamide dihydrochloride (7)*

Compound **7** was prepared according to the procedure for 4-(4-benzylpiperazin-1-yl)-*N*-(4-hydroxyphenyl)-2-(trifluoromethyl) benzamide dihydrochloride (**5**) using methyl 4-iodo-3-methoxybenzoate. ESI MS *m/z*: 418.4 ($[M + H]^+$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.17 (s, 1H), 9.90 (s, 1H), 7.71–7.63 (m, 2H), 7.57 (d, J = 8.3 Hz, 1H), 7.54–7.42 (m, 6H), 7.00 (d, J = 8.3 Hz, 1H), 6.74 (d, J = 8.8 Hz, 2H), 4.48–4.34 (m, 2H), 3.88 (s, 3H), 3.62 (d, J = 11.9 Hz, 2H), 3.38 (d, J = 11.1 Hz, 2H), 3.29–3.06 (m, 4H).

4-(4-Benzylpiperazin-1-yl)-3-fluorobenzoic Acid Hydrochloride (8A)

To a mixture of 3-fluoro-4-(piperazin-1-yl)benzoic acid hydrochloride (231 mg, 0.886 mmol) and benzaldehyde (0.449 mL, 4.43 mmol) in DMF (4.4 mL) was added sodium triacetoxyborohydride (376 mg, 1.77 mmol) and the resulting mixture was stirred at rt. After 16 h, the mixture was purified by preparative reversed-phase HPLC to afford the product as white solid.



4-(4-benzylpiperazin-1-yl)-3-fluoro-*N*-(4-hydroxyphenyl)benzamide bis(2,2,2-trifluoroacetate) (**8**)

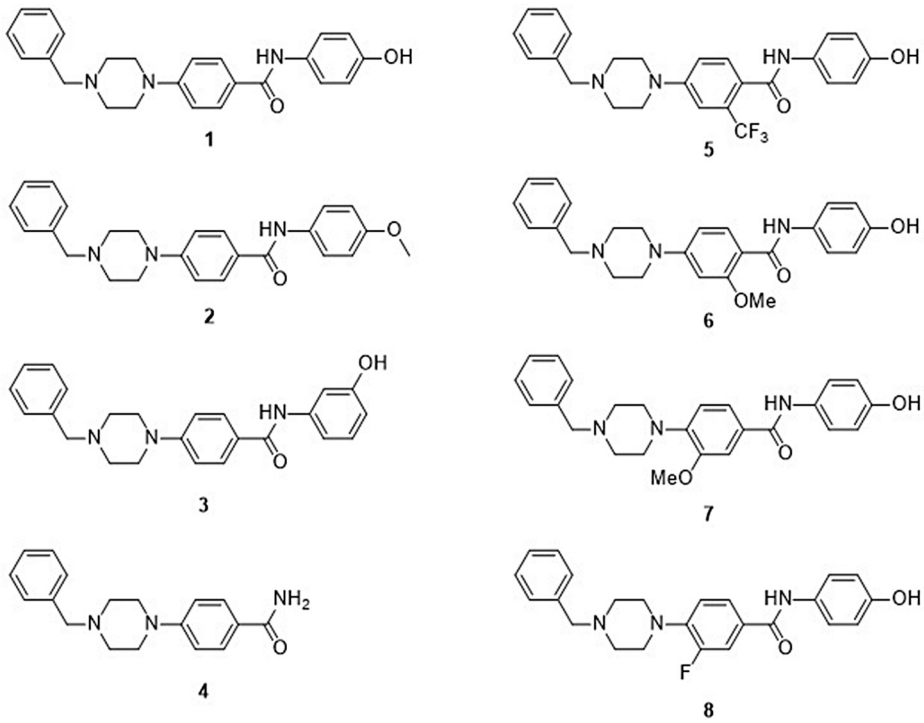


Fig. 1. Structures of molecules synthesized.

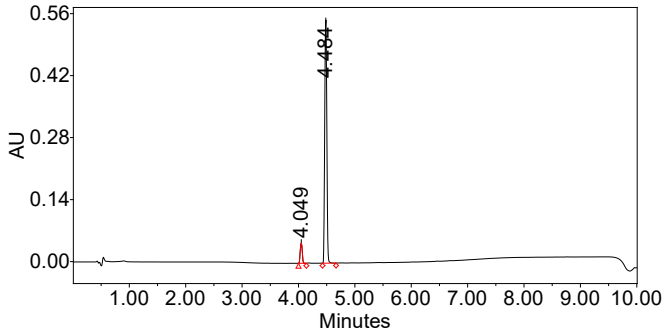


Fig. 2. Chromatogram from basic forced stress experiment, run in 0.1 N NaOH at 60 °C for 4 h, of **5**.

*4-(4-Benzylpiperazin-1-yl)-3-fluoro-*N*-(4-hydroxyphenyl) benzamide bis(2,2,2-trifluoroacetate) (8)*

A mixture of 4-(4-benzylpiperazin-1-yl)-3-fluorobenzoic acid hydrochloride (**8A**, 75 mg, 0.214 mmol), 4-aminophenol (70 mg, 0.641 mmol), diisopropylethylamine (187 μL, 1.07 mmol), and HATU (102 mg, 0.267 mmol) in DMF (1 mL) was stirred at rt. After 16 h, the mixture was purified by preparative reversed-phase HPLC to afford the title compound as off-white solid. ESI MS *m/z*: 406.4 ($[M + H]^+$). ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.74 (dd, J = 23.2, 11.0 Hz, 2H), 7.60–7.51 (m, 5H), 7.43 (d, J = 8.8 Hz, 2H), 7.17 (t, J = 8.5 Hz, 1H), 6.79 (d, J = 8.9 Hz, 2H), 4.40 (s, 2H), 3.78–3.35 (m, 5H), 3.31–3.01 (m, 3H).

Structures of molecules synthesized are shown collectively in Fig. 1.

Results

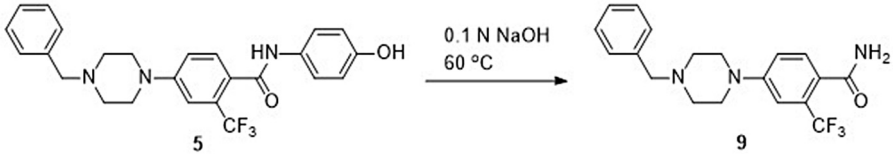
A typical forced degradation experiment run with **5** in 0.1 N NaOH at 60 °C for 4 h shows the formation of the unexpected degradation product **9** (Fig. 2 and Scheme 2). LC/MS of the peaks at 4.049 min and 4.484 min corresponds to the degradation product **9** and parent **5,** respectively, as shown in Scheme 2.

Fig. 3 plots the base-mediated degradation of compounds **1**, **5**, **6**, **7** and **8** over 24 h (time points: 4, 8, 12 and 24 h). Note that compounds **1**, **7** and **8** degrade completely between 12 h and 24 h time points. The effect of varying concentration of NaOH on the degradation of **1** is shown in Fig. 4. The amount of degradation product formed increases linearly with increase in the concentration of NaOH (0–0.1 N).

Identity of degradation products was established using the observed mass from LC/MS analysis of the basic forced stress experiments confirming the amide and not the carboxylic acid product. In one case, an authentic sample of **4** was synthesized and its UV spectrum, mass and retention time from chromatography were compared with those from the degradation product formed in the basic forced stress of **1** (Scheme 3). UV spectrum, mass and chromatographic retention time of the degradation product in Scheme 3 are found to be identical to those of **4**. Fig. 5 shows the overlay of chromatograms of the basic forced stress experiment of **1**, compound **4**, and the spiking of the basic forced stress experiment of **1** with **4**.

Discussion

One of the most common structural motifs in drug molecules is the amide functional group, and the hydrolytic cleavage is a



Scheme 2. Basic forced stress reaction of **5**.

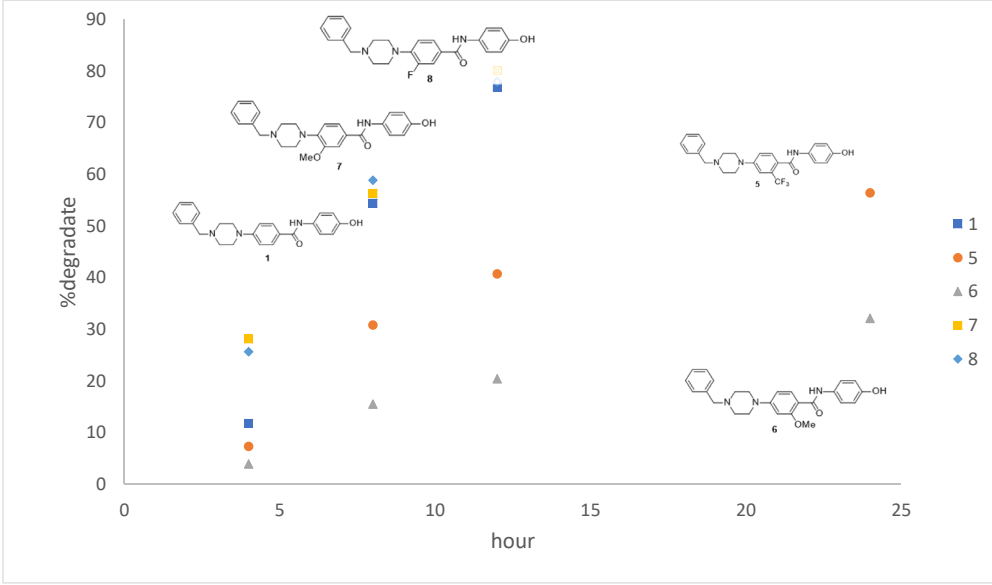


Fig. 3. Base-mediated degradation in 0.1 N NaOH at 60 °C.

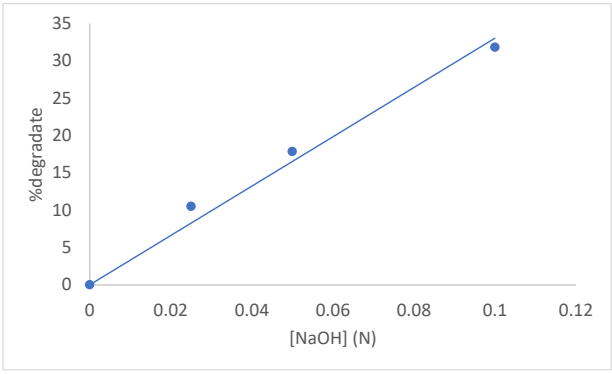
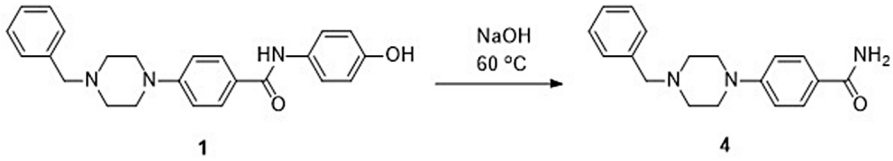


Fig. 4. Effect of the concentration of NaOH on the rate of degradation of **1**. Datapoints collected at 5.5 h.

humidity studies. In the process of evaluating the stability of a development candidate containing a secondary amide group, in our laboratories, we encountered unexpected results. Under both basic solution forced stress studies on the API and accelerated stress solid state studies on the formulated API, a primary amide degradation product was formed, instead of the corresponding carboxylic acid (Scheme 1). To study this unusual degradation pathway further, we synthesized molecules with similarities to the development compound (secondary amide) but utilized commonly available synthons to build a variety of structures to probe the mechanism as shown in Fig. 1.

The degradation profile resulting from basic forced stress of **1** is quite different from **2** and **3** – while compound **1** degrades to yield the degradate **4, 2** and **3** do not degrade at all under the same condition (Scheme 5). Furthermore, compound **1** does not degrade



Scheme 3. Basic forced stress reaction of **1**.

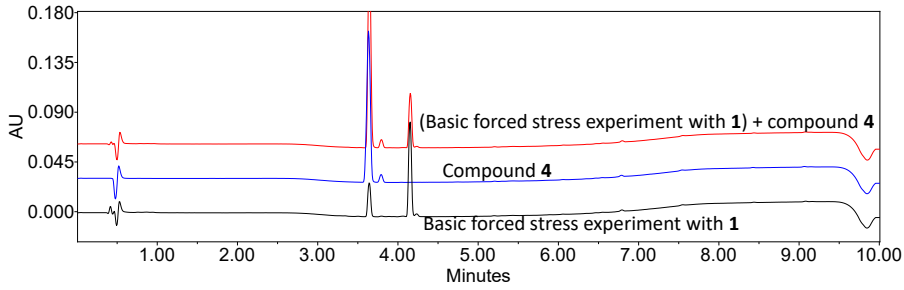
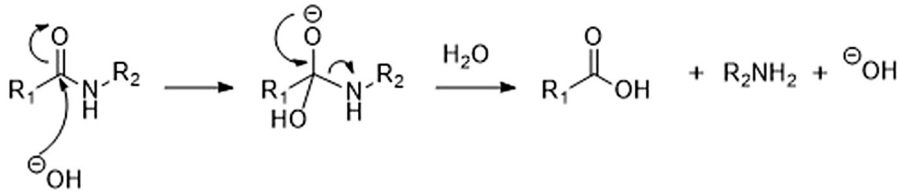


Fig. 5. Overlay of chromatograms from the basic forced stress experiment of **1**, compound **4** and spiked forced stress experiment of **1** with **4**.

prevalent degradation pathway for amides in drug molecules. Hydrolysis of the amide functional group results in the constituent carboxylic acid and amine, and the hydrolysis is usually mediated by base, as shown in Scheme 4 for a secondary amide.

under acidic forced stress conditions (with 0.1 N HCl).

This differential reactivity of **1** from **2** and **3** indicates that a free phenolic hydroxy group at the *p*-position (to the amide functionality) on the phenyl ring is essential for this degradation pathway to



Scheme 4. Base-mediated hydrolysis of secondary amides.

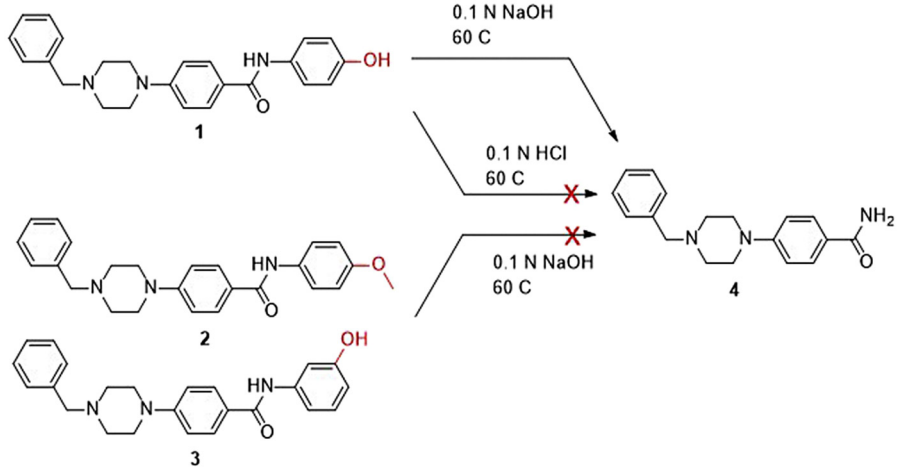
Susceptibility to hydrolytic degradation of drug molecules is determined by basic/acidic forced degradation experiments[1] and also by accelerated stress solid state elevated temperature and

operate – methylation of the phenolic hydroxy group in **2** or moving the phenolic hydroxy group from the *p*-to *m*-position in **3** shuts down the degradation pathway.

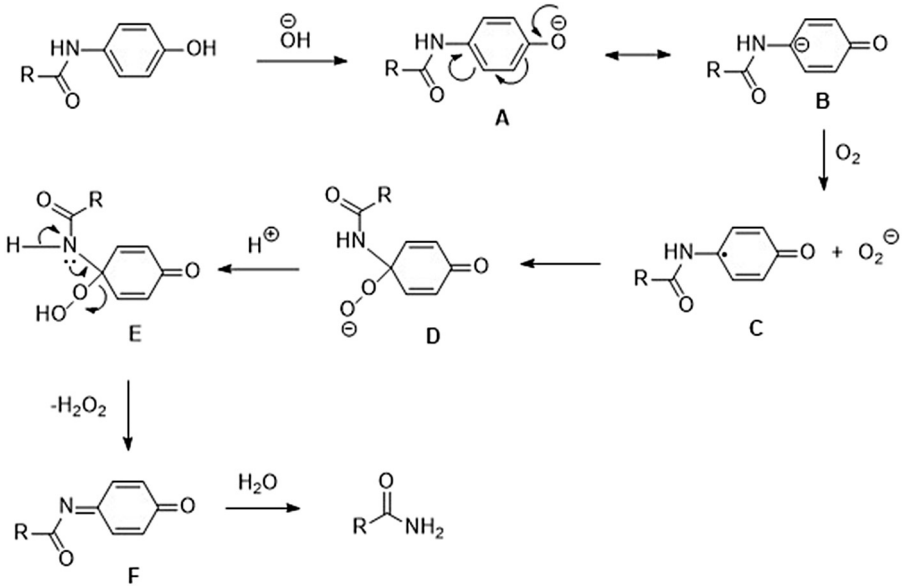
Running the basic forced stress experiment on **1** under N_2-atmosphere almost completely suppresses the formation of the degradate **4**, indicating participation of molecular oxygen in the degradation pathway. Moreover, under oxidative forced stress conditions, using AIBN or H_2O_2, **1** does not degrade to **4**.

Based on the above observations, we propose a degradation mechanism as shown in Scheme 6. The first step in this mechanistic pathway is the base-mediated deprotonation of the *p*-hydroxy group on the phenyl ring. One of the resonance forms of phenolate **A** is the carbanion **B**, which then transfers an electron to dissolved molecular oxygen (triplet electronic state, $^3O_2$) to form the *C*-centered radical, **C** and superoxide anion.[7] Spin inversion of the superoxide anion, followed by combination with **C** forms the peroxy anion **D**.[8,7] Protonation of the peroxy anion **D** gives the hydroperoxide **E**. Elimination of H_2O_2 from **E**, followed by hydrolysis, gives the primary amide.

In an effort to understand the electronic effects on the rate of degradation, compounds **5**–**8** were synthesized, where the central phenyl ring has been substituted with the electron-donating -OMe group or the electron-withdrawing $-CF_3$ or $-F$ groups. Assuming first order kinetics, the rate of degradation has been determined as shown in Fig. 6, and the results are tabulated in Table 3. The rates of degradation have been found to be **6** < **5** < **1** = **7** = **8**. All three compounds, **1**, **7** and **8**, are seemingly unaffected by the electronic effects of substituents on the central phenyl ring – **1** is unsubstituted, **7** is substituted with an electron-donating -OMe, and **8** has an electron-withdrawing $-F$ substituent. Interestingly, **5** and **6**, showing different rates of degradation from each other and lower



Scheme 5. Reactivities of **1** under basic and acidic forced stress, and **2** and **3** under basic forced stress.



Scheme 6. Proposed mechanism for the oxidative degradation of secondary amides to primary amides under basic conditions.

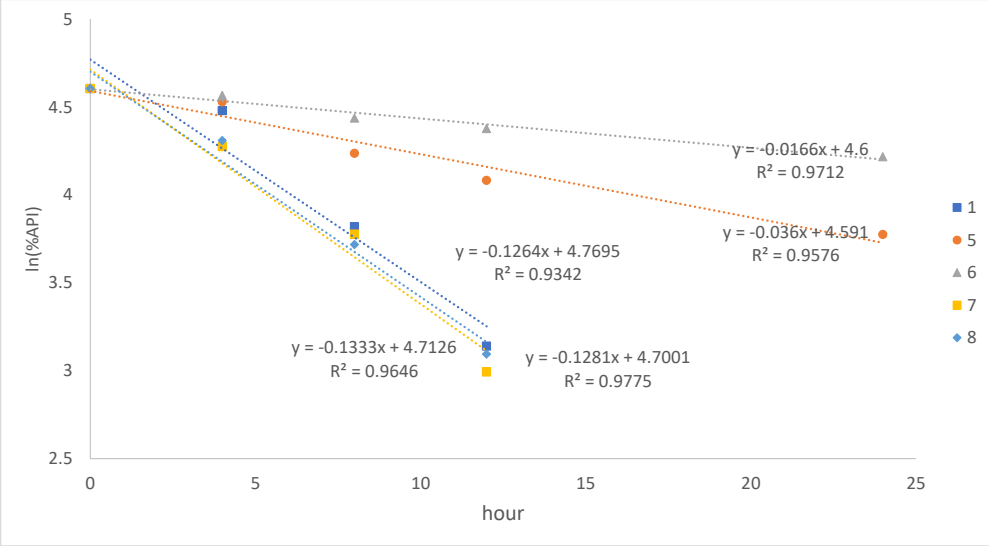


Fig. 6. First order rate plot for degradation of Compounds **1**, **5**, **6**, **7**, and **8**.

rates of degradation than **1**, **7** and **8**, contain the electron-withdrawing $-CF_3$ group and electron-donating -OMe group, respectively. These data do not point towards a trend where the rate of degradation is dependent on the electronics of the central phenyl ring. However, from a spatial point of view (*i.e.*, the position of the substituents), electronics of the substituents proximal to the amide group (electron-withdrawing $-CF_3$ in **5** and electron-donating -OMe in **6**) appear to influence the rate of degradation, whereas electronics of the substituents distal to the amide group (neutral –H in **1**, electron-donating -OMe in **7,** and electron-withdrawing –F in **8**) do not affect the rate. More work will be required to better understand this effect.

Amides are present in many drug molecules[9] and are useful synthons in organic transformations.[10–13]Thus, a new methodology involving easy conversion of secondary or tertiary amides to their corresponding primary amides has important implication in pharmaceutical degradation chemistry and synthetic organic chemistry. In published literature, these transformations have been accomplished with the use of oxidizing reagents.[14–17] A recent publication[18] reports the transformation of tertiary amides to primary amides using $(NH_4)_2CO_3$. However, as of the time of publication, we are unaware of any report in published literature detailing the transformation of a secondary amide to the corresponding primary amide without using any oxidative reagents. The novel degradation pathway reported in this paper can be used as a new methodology for the synthesis of primary amides under mild basic conditions. Furthermore, this transformation establishes the utility of 4-hydroxyphenyl as an easily removable protecting group for primary amides in organic synthesis.

Table 3
Rate of Degradation in 0.1 N NaOH at 60 °C.

Compound	Rate Constant (h^{-1})
1	1.3×10^{-1}
5	3.6×10^{-2}
6	1.6×10^{-2}
7	1.3×10^{-1}
8	1.3×10^{-1}

Conclusions

In this work, we showed a novel oxidative degradation pathway for amide functional groups in drug molecules, under basic conditions in solution state and under accelerated stress on solid state formulated API. A series of molecules have been synthesized to probe the degradation mechanism. Although data from this work indicate effect of electronic factors on the rate of degradation, more work is needed to arrive at a better understanding of such factors. This work also presents a novel methodology in organic synthesis, whereby secondary amides can be transformed into synthetically valuable primary amides without the use of oxidizing reagents.

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