Synthesis of a Pilot-Scale Library of 4-amino-2-(diethylaminomethyl)phenol (ADOC) Analogues for Testing of Organophosphate-Inhibited Acetylcholinesterase Reactivation Ability

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Herein we present a short synthetic method for the generation of 4-amino-2-(diethylaminomethyl)phenol (ADOC) analogs 4a-c and 6a-c from commercially available and inexpensive acetaminophen (2). The method was used to prepare and make available a pilot-scale library of analogs for in vitro biological evaluation in the USAMRICD’s multi-agent screen for nerve agent-inhibited acetylcholinesterase reactivation. Our hope is that this method will facilitate SAR studies directed toward the development ADOC-based, non-oxime therapies for treating nerve agent intoxication.
Background and Introduction

4-amino-2-(diethylaminomethyl)phenol (ADOC) was disclosed as a reactivator of nerve agent inhibited acetylcholinesterase (Figure 1). Previous structure activity relationship (SAR) studies of this molecule helped to elucidate the role of the hydroxyl, diethylamino and NH₂ amino groups in the molecule’s reactivation activity. The hydroxyl group appears essential for reactivation, as methylation of the hydroxyl abolishes reactivation activity. The diethylamino group seems to function in both reactivation and molecular recognition for binding in the active site. Reduction or increase in steric bulk around the benzylic nitrogen serves to reduce activation potential. Similarly, the NH₂ amino group appears to be involved in active site binding and recognition where addition of sterically bulky groups attenuates reactivation.

![Figure 1. Structure of ADOC (1) and brief summary of SAR](image)

In order to support additional SAR studies of ADOC (1) we developed a synthetic method to generate structural analogs, beginning with commercially available and inexpensive acetaminophen (Tylenol) (2) (Scheme 1A). Because of the apparent importance of the hydroxyl group, our efforts focused on developing a route to modify the NH₂ amino and benzyl amino groups in order to further investigate the effect of structural modifications at these locations on reactivation of nerve agent-inhibited acetylcholinesterase (Scheme 1B and 1C). Alkylated analogs 4a-c were accessed in 5 steps from 2. The tert-butylcarbamate group (“boc”) was chosen to facilitate isolation and purification of synthetic intermediates and the final compounds. Alkylation of the NH₂ amino group was readily achieved by a one-pot reductive alkylation of p-aminophenol (8, Scheme 3). The requisite benzyl amine was next installed by an efficient electrophilic aromatic substitution reaction between phenols 3a-c, N,N-diethylamine, and formaldehyde (the Betti reaction). Finally, the boc group was readily cleaved under acidic conditions to furnish the desired analogs. We initially sought to generate benzyl amino analogs 6a-c through a short 2-step synthetic sequence from a Betti reaction on 2 with the requisite secondary amine, followed by a deacetylation reaction. The Betti reaction proceeded smoothly; however, deacetylation under acidic conditions worked poorly (discussed below). We were happy to find that boc-protected phenol 5 provided access to the title compounds 6a-c.
Scheme 1: Synthesis of ADOC (1) and analogs from Tylenol (2).

**Results and Discussion**

**Preparation of N-alky analogs 4a-c**

Our initial efforts focused on a 3-step route to generate analogs 4a-c from acetaminophen (2), whereby the desired compounds would be achieved directly by an alkylation of ADOC (1) (Scheme 2). Although 1 was readily obtained from 2 by a Betti reaction with \(N,N\)-diethylamine, followed by deacetylation, we found that direct alkylation of 1 with propionaldehyde produced a mixture of undesired compounds, confirmed by \(^1\)HNMR, which prompted us to pursue an alternative method for generating 4a-c.

**Scheme 2.** Initial efforts to synthesize 4b.

Hypothesizing that the basic benzylamine group of 1 may be interfering with the proposed imine intermediate produced in the alkylation step, we next attempted alkylation first, followed by installation of the \(N,N\)-diethylamino group second (Scheme 3). Deacetylation of 2 provided \(p\)-aminophenol (8) which smoothly underwent alkylation to furnish anilines 9a-c. Next, since the Betti reaction proceeds through addition of the starting phenol to an iminum ion, we chose to install a tert-butylcarbamate (“boc”) group to avoid any potential unwanted reactivity from the nitrogen of 9a-c. In addition, installation of the boc group would likely facilitate any purification steps and could be easily removed as a final step. We were delighted to find that 9a-c provided penultimate intermediates 10a-c. Attempts were next made to remove the boc group under acidic conditions with trifluoroacetic acid (TFA). Although TFA did indeed remove the boc
group, it also generated a side product which we were unable to separate from the desired analogs 4a-c (not shown). Ultimately we found that the boc group could be removed under mildly acidic conditions with HCl to furnish 4a-c.


Preparation of benzylamino analogs 6a-c

Our initial efforts focused on the synthesis of 11a-c as they would have provided the most direct route to analogs 6a-c (Scheme 4). Intermediates 11a-c were readily obtained; however, we were unable to completely remove the acetyl group under acidic conditions and in every instance observed a mixture of unreacted starting material, degraded starting material, and some desired product. Based on our success with the synthesis of 4a-c (Scheme 3), we opted for a route involving boc-protected intermediate 5, which we were happy to discover was easily elaborated to furnish the desired compounds.

Scheme 4. Synthesis of benzylamino analogs 6a-c.
Conclusion

Herein we presented a short synthetic method for the generation of ADOC analogs 4a-c and 6a-c from commercially available and inexpensive acetaminophen (2). The method was used to prepare and make available a pilot-scale library of analogs for in vitro biological evaluation in the USAMRICD’s multi-agent screen for nerve agent-inhibited acetylcholinesterase reactivation. Our hope is that this method will facilitate SAR studies directed toward the development ADOC-based, non-oxime therapies for treating nerve agent intoxication.
Experiments

**Materials:** Unless otherwise specified, all commercially available reagents were used as received. All reactions using dried solvents were carried out under an atmosphere of room air with stirring.

**Instrumentation:** $^1$H NMR spectra were obtained on a 400 MHz Bruker NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS (s, δ 0.0 ppm). Multiplicities are given as: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), m (multiplet), bs (broad singlet). Chromatographic purifications were performed by flash chromatography with Teledyne Isco 4 gram or 12 gram RediSepRf silica gel normal phase columns on a Teledyne Isco Combiblack EZ Prep purification system. Compounds were detected by UV absorbance at 254 nm and 280 nm, as well as evaporative light scattering detection. The eluting solvent for the purification of each compound was determined by thin layer chromatography (TLC) on Millipore aluminum backed silica gel-coated plates (Silica Gel 60, F254) and visualized by UV light at 254 nm.

The following abbreviations are used throughout: room temperature (rt), ethyl acetate (EtOAc), methanol (MeOH), ethanol (EtOH), tert-butyloxycarbonyl (boc), dichloromethane (DCM), and triethylamine (TEA), tetrahydrofuran (THF), isopropanol (iPrOH)

\[
\text{(8) } p\text{-aminophenol}
\]

Acetaminophen (2) (3.0 g, mmol) was dissolved in 3M HCl in water (15 mL) and refluxed for 4 hours. The reaction was then allowed to cool to room temperature, and the pH was adjusted to ~8 with saturated NaHCO$_3$ and transferred to a separatory funnel. The aqueous layer was extracted 3x with EtOAc. The combined organic fractions were dried of Na$_2$SO$_4$, filtered, and concentrated to provide the title compound as a brown solid (1.6 g, 73%). $^1$H NMR (500 MHz, d-DMSO) δ 8.38 (s, 1H), 6.48 (d, J = 8.7 Hz, 2H), 6.42 (d, J = 8.7 Hz, 2H).

**General Experiment A:** Preparation of 5 and 3a-c

$p$-aminophenol (8) (1 equiv.), di-tert-butyloxycarbonyl (1 equiv), TEA (2 equiv), and DCM or THF (0.25 M) were combined, and the mixture was allowed to stir at rt for 24 hours. The reaction was then concentrated in vacuo, and the title compounds were either used without further purification or isolated by flash column chromatography.
The title compound was prepared from \( p \)-aminophenol (8) (0.138 g, 1.3 mmol) according to general experiment A. Reaction progress was monitored by TLC using DCM with 5% MeOH and 1% TEA and was complete after 24 hours. The reaction was then concentrated, dried under vacuum to provide a brown solid, and used in the subsequent step without further purification. \(^1\)HNMR (500 MHz, \( d-\)Acetone) \( \delta \) 8.13 (bs, 1H), 7.36 (m, 2H), 6.79 (d, \( J = 8.8 \) Hz, 2H), 1.31 (s, 9H).

### General Experiment B – Preparation of 9a-c

Ammonia (7N in MeOH, 2.62 mL, 18.3 mmol, 10 equiv) and formic acid (0.690 mL, 18.3 mmol, 10 equiv) were combined in iPrOH (15 mL) at rt and stirred for about 5 minutes. The resultant ammonium formate was then dissolved in a minimum amount of water (~1.5 mL). To this solution was next added 10% Pd/C (0.06 g, 30% by weight with respect to \( p \)-aminophenol (8)). The mixture was allowed to stir ~5 minutes after which time \( p \)-aminophenol (2) (0.20 g, 1.83 mmol, 1.0 equiv) was added followed by the respective aldehyde (acetaldehyde, propionaldehyde, isobutyraldehyde, 1.83 mmol, 1.0 equiv). The reaction was monitored by TLC and was complete by 1 hour. iPrOH was removed in vacuo, the reaction contents were taken up in EtOAc and water and the layers were separated. The organic layer was collected, and the aqueous layer was extracted twice more with EtOAc. Combined organic fractions were dried over Na₂SO₄ and concentrated in vacuo to provide the title compounds 9a-c as thin yellow oils which were used in subsequent reactions without further purification.
The title compound was prepared from \( p \)-aminophenol (8) (0.20 g, 1.83 mmol) according to general experiment B. \(^1\)HNMR (500 MHz, CDCl\(_3\)) \( \delta \) 6.73 (d, \( J = 8.2 \) Hz, 2H), 6.57 (d, \( J = 8.3 \) Hz, 2H), 3.12 (q, \( J = 6.8 \) Hz, 2), 1.25 (t, \( J = 7.2 \) Hz, 3H).

**General Experiment C – Preparation of 10a-c, 11a, and 12a-c**

Aminophenol 2, 5, or 3a-c (1 equiv), formaldehyde (1.1 equiv), primary or secondary amine (1 equiv), and EtOH (0.5 M) were combined in a round bottom flask, and the mixture was refluxed using a Findenser Super Air Condenser for 24 hours with stirring. The reaction was then allowed to cool, and products were visualized using TLC in a solvent system of DCM with 5% MeOH, 1% TEA. The reaction mixture was then adjusted to pH \( \approx 8.0 \) using saturated sodium bicarbonate followed by the addition of EtOAc. Three extractions were performed on the aqueous layer, and the combined organic layers were collected, dried over Na\(_2\)SO\(_4\), concentrated and dried in vacuo. The crude reaction mixture was then purified on a 12 g silica flash column at a flow rate of 30 mL/min with a solvent system of DCM with 1% TEA with a gradient of MeOH increasing from 0% to 10% from 0 minutes to 17 minutes followed by a ramp up to 20% MeOH from 17 minutes to 20 minutes. Collections containing product were then concentrated in vacuo to provide the target compounds.

The title compound was prepared from aminophenol 3a (0.117g, 0.493 mmol) according to general experiment C. Flash column chromatography provided 10a as an oil (0.100g, 63%). \(^1\)HNMR (500 MHz, CDCl\(_3\)) \( \delta \) 6.95 (bs, 1H), 6.77 (m, 2H), 3.76 (s, 2H), 3.61 (q, \( J = 7.1 \) Hz, 2H), 2.64 (q, \( J = 7.2 \) Hz, 4H), 1.43 (s, 9H), 1.13 (m, 9H).
The title compound was prepared from acetaminophen (2) (1.0g, 1.62 mmol) according to general experiment C. Flash column chromatography provided the title compound as an off white solid. $^1$HNMR (500 MHz, $d$-Acetone) $\delta$ 8.89 (bs, 1H), 7.39 (d, $J = 2.6$ Hz, 1H), 7.30 (dd, $J = 8.6$, $J = 2.6$ Hz, 1H), 6.62 (d, $J = 8.6$ Hz, 1H), 3.79 (s, 2H), 2.60 (m, 4H), 2.03 (s, 3H), 1.86 (m, 4H).

The title compound was prepared from tert-butyl (4-hydroxyphenyl)carbamate 5 (0.056g, 0.267 mmol) according to general experiment C. Flash column chromatography provided 12a as an oil (0.066g, 85%). $^1$HNMR (500 MHz, $d$-Acetone) $\delta$ 8.10 (bs, 1H), 7.30 (s, 1H), 7.21 (m, 1H), 6.64 (d, $J = 8.6$ Hz, 1H), 3.81 (s, 2H), 2.64 (m, 4H), 1.86 (m, 4H), 1.48 (s, 9H).

**General Experiment D – Boc cleavage**

Boc-protected phenol 10a-c or 12a-c (0.02-0.05g) was dissolved in 1M HCl in MeOH (3 mL) and allowed to reflux at 85°C for 2 hours with stirring. The reaction mixture was allowed to cool to room temperature and was then adjusted to pH ~8.0 using saturated sodium bicarbonate, and EtOAc was added. Three extractions were performed on the aqueous layer, and the combined organic layers were collected, dried over Na$_2$SO$_4$, concentrated and dried in vacuo. The crude reaction mixture was then purified on a 12 g silica flash column at a flow rate of 30 mL/min with a solvent system of DCM with 1% TEA with a gradient of MeOH increasing from 0% to 10% from 0 minutes to 17 minutes followed by a ramp up to 20% MeOH from 17 minutes to 20 minutes. The success of the purification was verified by TLC, and fractions containing product were then concentrated under vacuum.

(4a) 2-((diethylamino)methyl)-4-(ethylamino)phenol
The title compound was prepared from boc-protected phenol 10a (0.021g, 0.065 mmol) according to general experiment D. Work-up and concentration in vacuo provided ADOC analog 4a as a brown oil (0.013g, 89%). No purification was required. $^1$HNMR (500 MHz, CDCl$_3$) $\delta$ 6.70 (d, J = 8.6 Hz, 1H), 6.51 (dd, J = 8.5, J = 2.8 Hz, 1H), 6.34 (d, J = 2.7 Hz, 1H), 3.42 (s, 2H), 3.11 (q, J = 7.1 Hz, 2H), 2.63 (q, J = 7.2 Hz, 4H), 1.25 (t, J = 7.2 Hz, 3H), 1.12 (t, J = 7.2 Hz, 6H); $^{13}$CNMR (125 MHz, d-Acetone) 149.5, 142.1, 122.7, 116.0, 113.3, 112.7, 56.8, 46.1, 38.8, 14.2, 10.7; MS (ESI) m/z calculated for C$_{11}$H$_{23}$N$_2$O (M+H) 223.3, found 223.2.

(4b) 2-((diethylamino)methyl)-4-(propylamino)phenol

The title compound was prepared from boc-protected phenol 10b (0.021g, 0.062 mmol) according to general experiment D. Work-up and concentration in vacuo provided ADOC analog 4b as a brown oil (0.012g, 80%). No purification was required. $^1$HNMR (500 MHz, CDCl$_3$) $\delta$ 6.69 (d, J = 8.5 Hz, 1H), 6.50 (dd, J = 8.6, J = 2.7 Hz, 1H), 6.33 (d, J = 2.6 Hz, 1H), 3.71 (s, 2H), 3.03 (t, J = 7.1 Hz, 2H), 2.63 (q, J = 7.2 Hz, 4H), 1.63 (m, 2H), 1.12 (t, J = 7.2 Hz, 6H), 1.05 (t, J = 7.4 Hz, 3H); $^{13}$CNMR (125 MHz, CDCl$_3$) 150.1, 141.3, 122.8, 116.6, 113.7, 113.5, 57.12, 47.1, 46.3, 22.9, 11.7, 11.2; MS (ESI) m/z calculated for C$_{14}$H$_{25}$N$_2$O (M+H) 237.4, found 237.2.

(4c) 2-((diethylamino)methyl)-4-(isobutylamino)phenol

The title compound was prepared from boc-protected phenol 10c (0.025g, 0.071 mmol) according to general experiment D. Work-up and concentration in vacuo provided ADOC analog 4c as a brown oil (0.014g, 78%). No purification was required. $^1$HNMR (500 MHz, CDCl$_3$) $\delta$ 6.69 (d, J = 8.6 Hz, 1H), 6.50 (dd, J = 8.6, J = 2.6 Hz, 1H), 6.32 (d, J = 2.6 Hz, 1H), 3.71 (s, 2H), 2.87 (d, J = 6.8 Hz, 2H), 2.63 (q, J = 7.2 Hz, 4H), 1.88 (m, 1H), 1.12 (t, J = 7.2 Hz, 6H), 1.05 (d, J = 6.7 Hz, 6H); $^{13}$CNMR (125 MHz, d-Acetone) 149.9, 141.5, 122.7, 116.8, 113.7, 113.5, 57.0, 53.1, 46.3, 28.1, 20.6, 11.1; MS (ESI) m/z calculated for C$_{15}$H$_{27}$N$_2$O (M+H) 251.4, found 251.4.

(6a) 4-amino-2-(pyrrolidin-1-ylmethyl)phenol
The title compound was prepared from boc-protected phenol 12a (0.030g, 0.103 mmol) according to general experiment D. Flash column chromatography provided ADOC analog 6a as a brown oil (0.008g, 41%). $^1$HNMR (500 MHz, $d$-Acetone) $\delta$ 6.47 (s, 2H), 6.39 (s, 1H), 3.68 (s, 2H), 2.85 (bs, 2H), 2.58 (m, 4H), 1.80 (m, 4H); $^{13}$CNMR (125 MHz, $d$-Acetone) 149.8, 140.2, 123.1, 119.6, 115.8, 114.9, 58.6, 53.2, 23.3; MS (ESI) $m/z$ calculated for $C_{11}H_{17}N_2O$ (M+H) 193.3, found 193.3.

(6b) 4-amino-2-(piperidin-1-ylmethyl)phenol

The title compound was prepared from boc-protected phenol 12b (0.025g, 0.082 mmol) according to general experiment D. Flash column chromatography provided ADOC analog 6b as a brown oil (0.006g, 35%). $^1$HNMR (500 MHz, $d$-Acetone) $\delta$ 6.48 (s, 2H), 6.37 (s, 1H), 3.54 (s, 2H), 2.86 (bs, 4H), 1.61 (m, 4H), 1.50 (m, 2H); $^{13}$CNMR (125 MHz, $d$-Acetone) 149.6, 146.6, 122.0, 119.4, 115.2, 114.6, 61.9, 53.7, 26.1, 23.9; MS (ESI) $m/z$ calculated for $C_{12}H_{19}N_2O$ (M+H) 207.3, found 207.2.

(6c) 4-amino-2-(morpholinomethyl)phenol

The title compound was prepared from boc-protected phenol 12c (0.050g, 0.162 mmol) according to general experiment D. Flash column chromatography provided ADOC analog 6c as a brown oil (0.019g, 56%). $^1$HNMR (500 MHz, $d$-Acetone) $\delta$ 6.51 (s, 2H), 6.42 (s, 1H), 3.69 (bs, 4H), 3.59 (s, 2H), 2.52 (bs, 4H); $^{13}$CNMR (125 MHz, $d$-Acetone) 153.4, 143.7, 121.3, 120.4, 119.8, 115.7, 66.4, 61.05, 52.8; MS (ESI) $m/z$ calculated for $C_{11}H_{17}N_2O_2$ (M+H) 209.3, found 209.1.
References

