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Quinolinium salt as a potent inhibitor of lymphocyte apoptosis

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Abstract—The synthesis of several quinolinium salts and related compounds and their ability to inhibit glucocorticoid-induced apoptosis in murine thymocytes are described. Interestingly, 1-[2-methoxyimino-2-(4-pyrrolidin-1-yl-phenyl)ethyl]quinolinium bromide (**11**) showed a potent protective effect with an EC_{50} of 0.013 μM, which was at least 300-fold more potent than the reference compound pifithrin-α.

1. Introduction

Chemoprevention of cell death is a critical goal for pharmacologic intervention in a variety of clinical settings including ischemia and side effects of chemotherapy. Recently, a small molecule, 2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)-1-*p*-tolyl-ethanone hydrobromide,[1] referred to as pifithrin-α (PFTα, **1**, Fig. 1), was originally identified from a broad screen of 10,000 compounds to inhibit γ radiation-induced mitochondrial cell death.[2] In addition, this compound was shown to suppress the heat shock and glucocorticoid signaling pathways.[3] When glucocorticoid death-inducing signals reach the mitochondria, a rapid series of events ensues that afford a reliable and reproducible assay system for testing chemoprevention of cell death. There is a loss of the inner mitochondrial membrane potential and cells can no longer retain the dye $DiOC_6$ as an indicator of apoptosis. Once cells lose the integrity of the outer cell membrane, the dye propidium iodide (PI) is then no longer excluded from these cells.[4] Thus, in our studies the cytoprotective effect of each compound was determined by comparing the percentage of viable glucocorticoid treated murine thymocytes, which retained $DiOC_6$ and excluded PI, when treated with graded doses of compound.

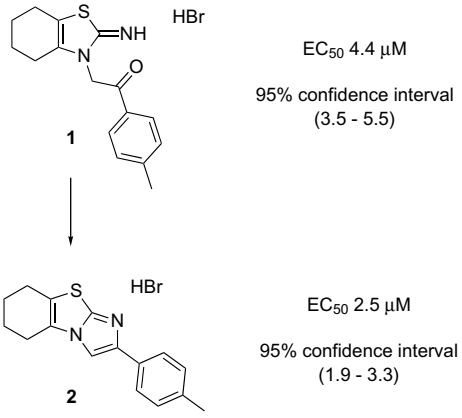

Figure 1. EC_{50} of **1** and **2**.

2. Synthesis and Biological Evaluation

Using **1** as a lead compound we aimed to optimize the cytoprotective effect by (1) exploring the activity of other ring system analogs isosteric with benzothiazoles and (2) stabilizing the open structure by removing or replacing the two imino group from the heterocyclic ring. As we noted early on in our studies of **1** and derivatives thereof, these 2-imino compounds, upon alkylation with α-haloacetophenones, show a strong tendency to cyclize to form the corresponding imidazo[2,1-*b*]benzothiazole (**2**), particularly in protic solvents.[5] Thus, while preparing isosteric ring system analogs, we synthesized a

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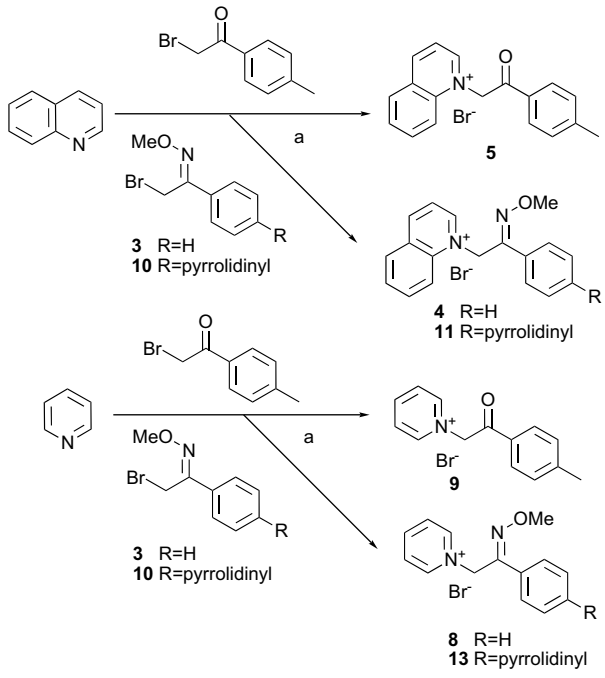
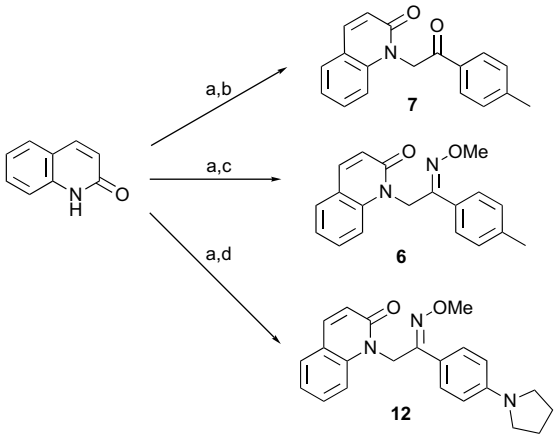
quinoline intermediate that displayed surprising activity in our thymocyte protection assay. This intermediate was formed by the simple alkylation of quinoline with the *O*-methyl oxime of an α-haloacetophenone. This report details the preparation and evaluation of this quinolinium salt and a variety of structurally related compounds.

Quinoline was treated with 2-bromo-1-*p*-tolylethanone *O*-methyl oxime (**3**)[6] in refluxing acetone to provide 1-(2-methoxyimino-2-*p*-tolylethyl)quinolinium bromide (**4**) (Scheme 1).[7] Proton NMR experiments, including NOE, indicated that **4** has the *E*-configuration, consistent with results of earlier studies using a *p*-bromo-substituted *O*-methyl oxime for alkylation of several quinolines and pyridines.[8] No other isomer was isolated or detected in the reaction. A comparison of the cytoprotective activity of **4** with **1** following in vitro dexamethasone treatment of mouse thymocytes showed **4** to be significantly more protective than **1** with EC_{50}s of 0.35 and 4.4 μM, respectively.

It was of interest to determine whether the oxime group was important for the activity observed for compound **4** relative to the usual ketone function as is present in compound **1**. Therefore, quinoline was alkylated with α-bromo-4′-methylacetophenone to provide the corresponding quinolinium salt (**5**).[9] This ketone was found to be devoid of significant cytoprotective activity, suggesting that the *O*-methyl oxime function is essential for bioactivity. Next, it was of interest to determine the importance of the quaternized quinoline moiety for bioactivity. A reasonable replacement for unsubstituted quinoline is 2-hydroxyquinoline, which would alkylate at the ring nitrogen producing a nonquaternized 2-quinolone derivative. Thus, 2-hydroxyquinoline was treated with sodium hydride in DMF followed by addition of oxime **3** to provide 1-(2-methoxyimino-2-*p*-tolylethyl)-1*H*-quinolin-2-one (**6**) (Scheme 2).[10] This oxime was found to be devoid of cytoprotective activity. The 2-hydroxyquinoline was also treated with the corresponding α-halo ketone under these same conditions to obtain the *N*-alkylated quinolone **7**[11], which also was not cytoprotective in the assay. To further explore the possible involvement of the quaternized system toward bioactivity, we prepared a 'ring-truncated' form of compound **4** by treatment of pyridine with oxime **3** to yield the pyridinium analog **8**.[12] This quaternized compound was weakly active in the cytoprotection assays, suggesting that both the oxime-containing alkyl and heterocyclic moieties are essential for potent activity. Indeed, alkylation of pyridine with the α-halo ketone gave compound **9**,[13] which was also found to be devoid of activity. A modification of the oxime-containing alkyl group was then made incorporating a pyrrolidinyl group at the *para* position of the phenyl ring, as had been done in studies of a similar series of compounds prepared recently in our laboratories.[5] In those studies, this modification to the usual ketone, α-bromo-4′-methylacetophenone, was found to provide significantly greater potency relative to **1** itself in the benzothiazole system. Thus, we prepared 2-bromo-1-*p*-(pyrrolidin-1-yl)ethanone *O*-methyl oxime (**10**)[14] and *N*-alkylated both quinoline and 2-hydroxyquinoline to provide compounds **11**[15] and **12**,[16] respectively. The quinolinium salt **11** was found to have potent cytoprotective activity while **12** was not active (Table 1). Indeed, **11** was greater than 25-fold more potent than **4** and 300-fold more potent than **1**. Finally, oxime **10** was then used to alkylate pyridine to provide the corresponding pyridinium salt (**13**),[17] which was found to be more active than the corresponding tolyl *O*-methyl oxime **8**, but less active than the quinolinium analog **11** and comparable to **4** in the cytoprotection assay.



Scheme 1. Reagent and conditions: (a) acetone, reflux, 2 h to 5 days.

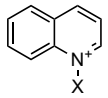
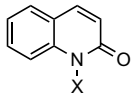
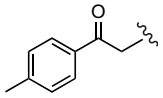
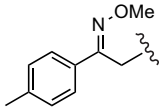
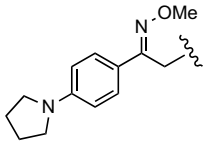


Scheme 2. Reagents and conditions: (a) NaH/DMF; (b) 2-bromo-4′-methyl-acetophenone/DMF; (c) **3**/DMF; (d) **10**/DMF.

Thus, this simple *para* substituent modification did improve the potency of the compounds in both quaternary salt series to protect murine thymocytes from glucocorticoid-induced cell death.

The reference compound **1** protects thymocytes from dexamethasone-induced cell death as reported by others

Table 1. In vitro cytoprotection activity of quaternary salts and related compounds

X =			
	**5** EC_{50} > 10 μM	**9** EC_{50} > 10 μM	**7** EC_{50} > 10 μM
	**4** EC_{50} = 0.35 μM (0.27–0.44)[a]	**8** EC_{50} = 5.1 μM (3.9–6.6)[a]	**6** EC_{50} > 10 μM
	**11** EC_{50} = 0.013 μM (0.0075–0.024)[a]	**13** EC_{50} = 0.28 μM (0.17–0.46)[a]	**12** EC_{50} > 10 μM

[a] 95% Confidence interval.

and confirmed here (Table 1 and Fig. 1).[3,18] The quinoline ring analog of **1** (compound **5**) demonstrated minimal if any protective effect. Thus, two modifications appear useful to enhance the potency of the quaternary salts: (1) a conversion of the ketone to an *O*-methyl oxime and (2) a pyrrolidinyl substituent on the phenyl moiety of the ketone.

3. Summary

We found that certain quinolinium and pyridinium salts bearing an oxime-containing alkyl group were able to potently prevent dexamethasone-induced cell death in a thymocyte protection assay. Single modifications deleting either the oxime function or the quaternized heterocycle completely abrogated the protective effect and only compounds bearing both of these groups in combination were significantly active. The biological basis of this observed structure–activity relationship is being actively investigated.

Acknowledgements

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6. 2-Bromo-1-*p*-tolylethanone *O*-methyl oxime (**3**). Two isomers in 87% total yield: *Z*-isomer ^{1}H NMR 500 MHz (DMSO-d_6): δ 2.33 (s, 3H), 3.83 (s, 3H), 4.62 (s, 2H), 7.26 (dd, 2H, *J* = 1.8, 8.2 Hz), 7.51 (d, 2H, *J* = 7.6 Hz); FABHRMS: found M^+, 242.0177 (calcd for $C_{10}H_{13}ONBr$: M^+, 242.0175). *E*-Isomer ^{1}H NMR 500 MHz (DMSO-d_6): δ 2.33 (s, 3H), 3.99 (s, 3H), 4.68 (s, 2H), 7.25 (d, 2H, *J* = 8.2 Hz), 7.61 (d, 2H, *J* = 7.3 Hz); FABHRMS: found M^+, 242.0175 (calcd for $C_{10}H_{13}ONBr$: M^+, 242.0175).
7. 1-(2-Methoxyimino-2-*p*-tolyl-ethyl)-quinolinium bromide (**4**). Yield 11%: mp 285–288 °C; ^{1}H NMR 500 MHz (DMSO-d_6): δ 2.19 (s, 3H), 4.06 (s, 3H), 6.46 (s, 2H), 7.08 (d, 2H, *J* = 7.9 Hz), 7.37 (d, 2H, *J* = 8.2 Hz), 8.04 (t, 1H, *J* = 7.3 Hz), 8.17 (m, 1H), 8.22 (d, 1H, *J* = 9.2 Hz), 8.32 (m, 1H), 8.44 (d, 1H, *J* = 7.9 Hz), 9.68 (d, 1H, *J* = 8.2 Hz), 9.28 (d, 1H, *J* = 5.8 Hz); Elem. Anal. found: C, 61.17; H, 5.18; N, 7.50 (calcd for $C_{19}H_{19}BrN_2O$: C, 61.47; H, 5.16; N, 7.55).
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9. 1-(2-Oxo-2-*p*-tolyl-ethyl)-quinolinium bromide (**5**). Yield 46%: mp 230–233 °C; ^{1}H NMR 500 MHz (DMSO-d_6): δ 2.45 (s, 3H), 7.02 (s, 2H), 7.49 (d, 2H, *J* = 7.9 Hz), 8.05 (d, 3H, *J* = 7.9 Hz), 8.21 (m, 1H), 8.32 (dd, 1H, *J* = 6.1, 7.9 Hz), 8.41 (d, 1H, *J* = 9.2 Hz), 8.55 (d, 1H, *J* = 8.2 Hz), 9.45 (d, 1H, *J* = 8.2 Hz), 9.56 (d, 1H, *J* = 5.5 Hz); Elem. Anal. found: C, 63.00; H, 4.77; N, 4.09 (calcd for $C_{18}H_{16}BrNO$: C, 63.17; H, 4.71; N, 4.09).
10. 1-(2-Methoxyimino-2-*p*-tolyl-ethyl)-1*H*-quinolin-2-one (**6**). Yield 28%: mp 129–132 °C; ^{1}H NMR 400 MHz (DMSO-d_6): δ 2.15 (s, 3H), 4.07 (s, 3H), 5.59 (s, 2H), 6.58 (d, 1H, *J* = 9.2 Hz), 6.98 (d, 2H, *J* = 7.0 Hz), 7.20 (m, 4H), 7.60 (s, 2H), 7.80 (d, 1H, *J* = 8.8 Hz); Elem. Anal. found: C, 74.12;

H, 5.85; N, 9.00 (calcd for $C_{19}H_{18}N_2O_2$: C, 74.49; H, 5.92; N, 9.14); MS (ESI) *m*/*z* 329.00 (MNa^+).

11. 1-(2-Oxo-2-*p*-tolyl-ethyl)-1*H*-quinolin-2-one (**7**). Yield 39%: mp 173–175 °C; ^{1}H NMR 400 MHz (DMSO-d_6): δ 2.43 (s, 3H), 5.87 (s, 2H), 6.67 (d, 1H, J = 9.3 Hz), 7.26 (t, 1H, J = 7.3 Hz), 7.33 (d, 1H, J = 8.3 Hz), 7.42 (d, 2H, J = 7.8 Hz), 7.52 (t, 1H, J = 7.8 Hz), 7.76 (d, 1H, J = 7.3 Hz), 8.00 (d, 1H, J = 9.8 Hz), 8.04 (d, 2H, J = 7.8 Hz); Elem. Anal. found: C, 77.70; H, 5.82; N, 5.28 (calcd for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05).
12. 1-(2-Methoxyimino-2-*p*-tolyl-ethyl)-pyridinium bromide (**8**). Yield 84%: mp 106–109 °C; ^{1}H NMR 400 MHz (DMSO-d_6): δ 3.30 (s, 3H), 3.99 (s, 3H), 6.10 (s, 2H), 7.24 (d, 2H, J = 5.6 Hz), 7.65 (d, 2H, J = 5.9 Hz), 8.13 (m, 2H), 8.60 (m, 1H), 9.07 (s, 2H); FABHRMS: found M^+, 241.1340 (calcd for $C_{15}H_{17}ON_2$: M^+, 241.1335).
13. 1-(2-Oxo-2-*p*-tolyl-ethyl)-pyridinium bromide (**9**). Yield 100%: mp 213–214 °C; ^{1}H NMR 400 MHz (DMSO-d_6): δ 2.45 (s, 3H), 6.50 (s, 2H), 7.48 (d, 2H, J = 7.3 Hz), 7.97 (d, 2H, J = 7.8 Hz), 8.28 (m, 2H), 8.74 (t, 1H, J = 7.3 Hz), 9.03 (d, 2H, J = 5.9 Hz); Elem. Anal. found: C, 55.46; H, 5.40; N, 4.85 (calcd for $C_{14}H_{14}BrNO{\cdot}1/2H_2O$: C, 55.83; H, 5.02; N, 4.65).
14. 2-Bromo-1-(4-pyrrolidin-1-yl-phenyl)-ethanone *O*-methyl-oxime (**10**). Yield 60%: mp 121–124 °C; ^{1}H NMR 400 MHz (DMSO-d_6): δ 1.95 (s, 4H), 3.25 (s, 4H), 3.93 (s, 3H), 4.61 (s, 2H), 6.53 (s, 2H), 7.52 (s, 2H); FABHRMS: found M^+, 296.0522 (calcd for $C_{13}H_{17}ON_2Br$: M^+, 296.0519).
15. 1-(2-Methoxyimino-2-(4-pyrrolidin-1-yl-phenyl)-ethyl]-quinolinium bromide (**11**). Yield 61%: mp 178–180 °C; ^{1}H NMR 400 MHz (DMSO-d_6): δ 1.90 (s, 4H), 3.15 (s, 4H), 3.99 (s, 3H), 6.39 (m, 4H), 7.36 (d, 2H, J = 7.3 Hz), 8.05 (t, 1H, J = 7.0 Hz), 8.18 (m, 1H), 8.30 (m, 2H), 8.46 (d, 1H, J = 8.1 Hz), 9.29 (d, 1H, J = 8.1 Hz), 9.65 (d, 1H, J = 5.1 Hz); FABHRMS: found M^+, 346.1920 (calcd for $C_{22}H_{24}ON_3$: M^+, 346.1914).
16. 1-(2-Methoxyimino-2-(4-pyrrolidin-1-yl-phenyl)-ethyl]-1*H*-quinolin-2-one (**12**). Yield 39%: mp 169–171 °C; ^{1}H NMR 400 MHz (DMSO-d_6): δ 1.94 (s, 4H), 3.22 (s, 4H), 3.93 (s, 3H), 5.51 (s, 2H), 6.51 (d, 2H, J = 8.1 Hz), 6.96 (d, 1H, J = 8.8 Hz), 7.46 (dd, 1H, J = 7.3, 7.7 Hz), 7.50 (d, 2H, J = 8.1 Hz), 7.69 (dd, 1H, J = 7.3, 7.7 Hz), 7.81 (d, 1H, J = 8.4 Hz), 7.89 (d, 1H, J = 8.1 Hz), 8.23 (d, 1H, J = 8.8 Hz); FABHRMS: found M^+, 361.1792 (calcd for $C_{22}H_{23}O_2N_3$: M^+, 361.1785).
17. 1-(2-Methoxyimino-2-(4-pyrrolidin-1-yl-phenyl)-ethyl]-pyridinium bromide (**13**). Yield 60%: mp 194–196 °C; ^{1}H NMR 400 MHz (DMSO-d_6): δ 1.95 (s, 4H), 3.24 (s, 4H), 3.92 (s, 3H), 5.96 (s, 2H), 6.52 (d, 2H, J = 8.8 Hz), 7.58 (d, 2H, J = 8.3 Hz), 8.12 (m, 2H), 8.59 (dd, 1H, J = 7.3, 7.8 Hz), 8.07 (d, 1H, J = 5.9 Hz); FABHRMS: found M^+, 296.1762 (calcd for $C_{18}H_{22}ON_3$: M^+, 296.1757).
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