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Quinol fatty alcohols as promoters of axonal growth

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Abstract—The synthesis of three series of quinol fatty alcohols (QFAs) and their biological activities on the promotion of axonal growth are described. Interestingly, the 15-(2,5-dimethoxyphenyl)pentadecan-1-ol, the QFA bearing 15 carbon atoms on the side chain ($n = 15$), shows the most potent promotion of axonal growth in the presence of both permissive and non-permissive naturally occurring substrates such as Sema3A and myelin proteins.

The adult CNS has limited capacity for regeneration. After a lesion has occurred, the site concerned undergoes many cellular and chemical changes. The site of lesion is invaded by a multitude of nerve cells such as astrocytes, microglial cells, oligodendrocytes and progenitor cells. This constitutes a mechanical barrier, called the 'glial scar',[1] that axon extensions are unable to cross. Moreover, these cells over-express many protein factors like myelin associated proteins (NOGO, MAG and OMgp),[2] Sema3A,[3] chondroitin sulfate proteoglycans all possessing strong inhibitory activity towards axon extension.[4,5] One therapeutic strategy emerging from the above considerations is the use of natural neurotrophic factors in order to improve neuron survival, sprouting and axon extension. Nonetheless, the major problems in applying neurotrophic factors are their poor system penetration, relative instability and wide range of local and systemic biological activities.[6] To address these limitations, innovative therapeutics[7] should consider the use of synthetic small molecules able to mimic the biological effects of natural neurotrophic factors while avoiding all the side effects.[8]

Initial screening of Chinese medicinal plants showed that an ω-alkanol, *n*-hexacosanol, possesses some neurotrophic effects.[9] The combination of this ω-alkanol with a retinol moiety gave tCFA15 (3-(15-hydroxypentadecyl)-2,4,4-trimethylcyclohexen-2-one). This compound is able to promote neuron survival and axon-specific growth on inhibitory substrates such as Sema3A or myelin associated proteins.[10,11]

Despite the mechanical and chemical inhibitory properties of the glial scar, the local microglial activation results in an excessive cellular respiration that generates an accumulation of reactive oxygen species[1] (ROSs) as well as nitric oxide.[12] ROSs attack cellular membranes through a process called 'lipid peroxidation' which results in apoptotic cell death. Compounds that are able to scavenge these oxidizing agents would be of particular interest to therapy. Synergistic effects have been observed in drug combination studies of trophic factors with antioxidants.[13] As a result, various synthetic compounds comprising an ω-alkanol chain have proven to be active towards nerve cells while maintaining a strong antioxidant activity.[14,15]

Ubiquinol, the antioxidant form of ubiquinone, is a well-known antioxidant[16,17] and its biological activity within the CNS is significant.[18,19] Since the ubiquinone moiety is structurally closely related to the antioxidant moiety of tCFA15, we designed a new series of neurotrophic compounds combining both the trophic effect of the ω-alkanol chain and the radical scavenging capacity of the hydroquinone ring.

In this study, we analyze the effects of quinol fatty alcohols (QFAs) as free radical scavengers and as promoters of axonal growth. To our great surprise, we found that

Keywords: Ubiquinol; Quinol fatty alcohols; Brain lesion; Glial scar; Axonal growth; Myelin proteins; Sema3A.
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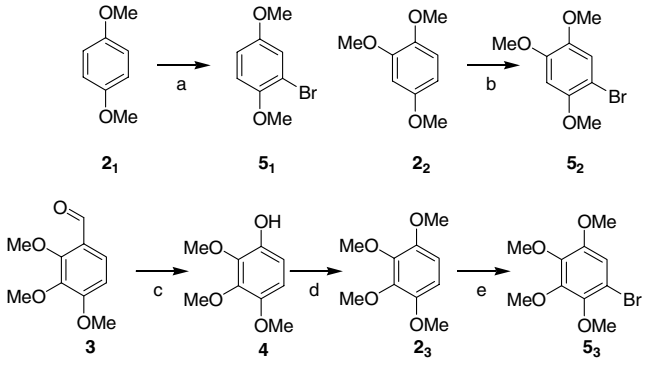
QFAs were able to counteract the inhibitory effects of proteic factors present within the glial scar. Such effects could lead to innovative therapeutic strategies for the treatment of CNS injuries.

Brominated methoxybenzenes $\mathbf{5_{1-3}}$ were the first intermediates needed to prepare the quinol fatty alcohols by means of a Sonogashira palladium catalyzed cross-coupling reaction. Compounds $\mathbf{5_1}$ and $\mathbf{5_2}$ were obtained, as described in the literature, in one step by the bromination of 1,4-dimethoxybenzene $\mathbf{2_1}$ and 1,2,4-trimethoxybenzene $\mathbf{2_2}$ by either the use of a *N*-bromosuccinimide[20] or bromine[21] in dichloromethane, respectively. Compound $\mathbf{5_3}$ was obtained in three steps from 2,3,4-trimethoxybenzene carboxaldehyde **3** which was oxidized to 2,3,4-trimethoxyphenol **4** through a Baeyer–Villiger reaction.[22] It was subsequently methylated with dimethylsulfate[23] in acetone and then brominated with NBS/TFA[24,25] (Scheme 1).

The terminal alkynes **9a–e** were prepared from O-protected bromoalcohols **8a–e** (Scheme 2). All bromoalcohols **7a–e** were obtained by monobromination of their respective diols **6a–e** in a mixture of HBr–cyclohexane.[26]

The C-10 and C-12 diols were commercially available. The C-13 and C-14 diols were prepared by reduction of the corresponding diacids with lithium aluminium hydride. The C-16 diol was obtained by reduction of the corresponding lactone, hexadecanolide.[26]



Scheme 1. Reagents and conditions: (a) NBS, CH_2Cl_2, reflux, 6 h, 80%; (b) Br_2, CH_2Cl_2, 0 °C, Quant; (c) i—*m*CPBA, CH_2Cl_2, 0 °C to rt; ii—NaOH 10%, MeOH, 95%; (d) Me_2SO_4, acetone, reflux, 4 h, 95%; (e) NBS, TFA, −20 °C to rt, 55%.

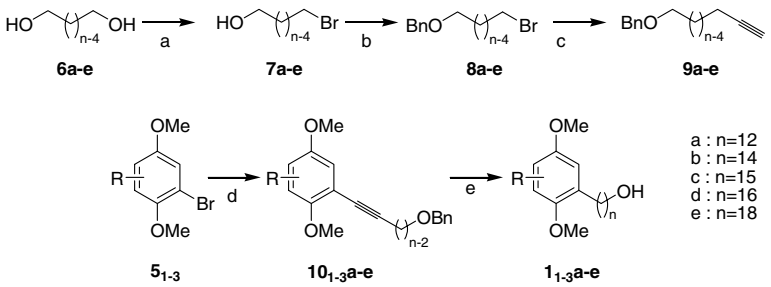
The synthesis was pursued by a Sonogashira-like palladium catalyzed cross-coupling reaction between the terminal alkynes **9a–e**[27,28] and the different arylbromides $\mathbf{5_{1-3}}$. Catalytic hydrogenation of $\mathbf{10_{1-3}a–e}$ with palladium on charcoal led to the quinol fatty alcohols $\mathbf{1_{1-3}a–e}$.

In an attempt to study the biological activity of QFAs, we analyzed the ability of each compound to promote axonal growth on mouse cortical neurons (Table 1). The experimental protocol has been described in a prior publication.[11] Briefly, mice embryos at the 15th day of gestation were dissected and cortical extracts were dissociated with trypsine. The neurons obtained were plated in 6-well plates and cultured on poly-L-lysine coverslips for 24 h at 37 °C, 5% CO_2. On day 2, neurons were incubated with the test compound and grown for 24 h at 37 °C, 5% CO_2. On day 3, neurons were fixed and immunostained with a primary anti-phosphoneurofilament antibody (SMi312, Sternberger) and a secondary Alexa488-coupled antibody. Coverslips were then mounted on plates with aqua-polymount. In a first series of experiments we determined which compounds exerted the strongest biological effect. An initial screening of all compounds at 10^{-9} M showed that compounds with a 15 carbon side chain were the most effective.

In a parallel experiment, we evaluated the ability of QFAs to scavenge hydroxyl radicals present in an ethanolic medium. To do so, we used the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) competition assay.[29] In the presence of hydroxyl radicals, ABTS is oxidized to the stable ABTS cation radical ($ABTS^{\cdot+}$) observed by its absorbance at 405 nm.

This method measures the relative ability of QFAs to scavenge hydroxyl radicals and thus inhibits the formation of $ABTS^{\cdot+}$ as measured by the decrease of its absorbance at 405 nm. Results are shown in Table 1.

Examination of Table 1 suggested that compounds bearing 15 carbon atoms on the side chain are the most active in each family,[30] with $\mathbf{1_1c}$, Q_2FA15, being the most active compound on the biological model tested even though its antioxidant activity is low. No special



Scheme 2. Reagents and conditions: (a) HBr, cyclohexane, reflux, 6 h, 75–89%; (b) i—NaH, THF, rt, 30 min; ii—BnBr, THF, reflux, 24 h, 72–88%; (c) lithium acetylide (ethylene diamine complex), DMSO, 0 °C to rt, overnight, 83–90%; (d) **8a–e**, piperidine, $Pd(PPh_3)_4$, 80 °C, 24 h, 71–90%; (e) Pd/C 5%, H_2, EtOH, rt, overnight, 80–92%.

Table 1. Compounds synthesized and tested

Compound number	Compound name	Molecular structure	HRMS (M+H$^+$)		Antioxidant activity	Axonal growth
			Calculated	Found	ABTS assay IC_{50} (μM)	%/control (1 nM)
—	Ethanol	—	—	—	—	100
—	Trolox®	—	—	—	600	—
—	tCFA15[a]	$C_{24}H_{44}O_2$	—	—	>10,000	150
1_1a	Q_2FA12	$C_{20}H_{34}O_3$	323.2581	323.2575	>10,000	109
1_1b	Q_2FA14	$C_{22}H_{38}O_3$	351.2880	351.2884	>10,000	110
1_1c	Q_2FA15	$C_{23}H_{40}O_3$	365.3050	365.3056	>10,000	181
1_1d	Q_2FA16	$C_{24}H_{42}O_3$	379.3207	379.3205	>10,000	120
1_1e	Q_2FA18	$C_{26}H_{46}O_3$	407.3520	407.3522	>10,000	109
1_2a	Q_3FA12	$C_{21}H_{35}O_4$	352.2614	352.2626	720	108
1_2b	Q_3FA14	$C_{23}H_{39}O_4$	380.2927	380.2928	720	109
1_2c	Q_3FA15	$C_{24}H_{41}O_4$	394.3083	394.3090	720	152
1_2d	Q_3FA16	$C_{25}H_{43}O_4$	408.3240	408.3243	720	135
1_2e	Q_3FA18	$C_{27}H_{47}O_4$	436.3553	436.3560	720	106
1_3a	Q_4FA12	$C_{22}H_{38}O_5$	383.2972	383.2788	***	100
1_3b	Q_4FA14	$C_{24}H_{42}O_5$	411.3105	411.3108	***	108
1_3c	Q_4FA15	$C_{25}H_{44}O_5$	425.3262	425.3265	***	158
1_3d	Q_4FA16	$C_{26}H_{46}O_5$	439.3418	439.3433	***	127
1_3e	Q_4FA18	$C_{28}H_{50}O_5$	467.3731	467.3735	***	103

Each compound is tested on the ABTS competition assay. The IC_{50} values were determined as the concentration of the compound required for a 50% diminution of the $ABTS^{\bullet+}$. *** stands for an antioxidant activity that did not reach the IC_{50}. Axonal growth was measured with UTHSCSA Image Tool 3.0 and is presented as the mean value of three different experiments. Variation was generally ±5%.

[a] Biological activity of tCFA15 is expressed at 10^{-9} M to be consistent with the remaining biological results presented.

correlation between the antioxidant and neurotrophic activity was found.

After the initial screening of our library of QFAs, a more triggered study was conducted. Figure 1 shows the dose-dependent effect of Q_2FA15 on the model of axonal growth. Q_2FA15 exerts its highest activity at 10–9 M. To be consistent with our model of a CNS lesion, we tested the activity of Q_2FA15 in the presence of naturally occurring inhibitory factors present within the glial scar. Table 2 shows that the growth promoting effect of Q_2FA15 is maintained in the presence of both myelin proteins (1 and 10 μg/mL) and Sema3 A (100 ng/mL), suggesting that our compound is able to counteract the inhibitory substrates of the glial scar.

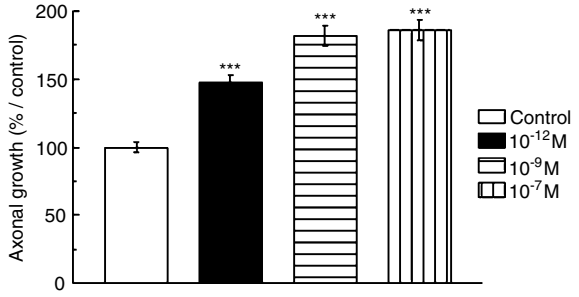


Figure 1. Dose-dependent promoting effect of Q_2FA15 on a model assay of axonal growth. Data are shown as means ± SEM. ***$p < 0.001$.

Lesioned axons within the CNS have little or no hope of regeneration. Previous studies in our laboratories have shown that tCFA15 was a potent promoter of axonal growth, acting at 10^{-7} M.[10,11] Herein, we have designed a similar compound that exerts a promoting effect 100 times stronger than tCFA15s.

Though no synergistic activity is noted between the antioxidant and the neurotrophic moieties, Q_2FA15 still bears a methylated quinol moiety which is highly antioxidant in its demethylated form[31] (IC_{50} = 6 μM on the ABTS assay). Multiple studies have shown that intracellular demethylases, based on cytochrome P450 activity, have the ability to demethylate such aromatic compounds within cells.[32]

In our case, methylation not only confers higher stability to Q_2FA15 but also allows easier CNS penetration. Demethylated compound within the cells should exert

Table 2. Promoting effect of Q_2FA15 as compared to tCFA15 upon naturally occurring inhibitory factors present in the glial scar

	No inhibitory substrate	Myelin proteins		Sema3A
		1 μg/mL	10 μg/mL	100 ng/mL
No treatment	0	−19.8	−37.6	−30.2
tCFA15 (10^{-7} M)	+80.0	+28.8	+5.0	+10.2
Q_2FA15 (10^{-9} M)	+81.0	+18.4	−5.3	+12.4

Data are shown in % of axonal growth as variations from control conditions.

its antioxidant activity as required by the biological system.

Finally, preliminary pharmacological studies have shown that Q_2FA15 requires cyclic nucleotides (cAMP/cGMP) to exert its biological effect. Recent findings have shown that the inhibitory effect of Sema3A can be circumvented by intracellular increases of cGMP,[33] whereas myelin-triggered inhibition can be circumvented by cAMP.[34] Moreover, one of the many targets of intracellular cGMP is cAMP-dependent phosphodiesterases resulting in an increase of intracellular cAMP.[35] In addition, cAMP-triggered axonal growth is known to result in over-expression of specific α1- and βIII-tubulin genes. CNS neurons express three subtypes of α-tubulin (α1, α2 and α4) and four subtypes of β-tubulin (βI, βII, βIII and βIV) genes. Microtubules consisting of α1- and βIII-tubulin are found in post-mitotic neurons and exert increased stability.[36]

All these data suggest that Q_2FA15 (**1_1c**) is a strong promoter of axonal growth that can circumvent the inhibitory properties of the glial scar. Its very strong biological activity makes it a very promising candidate for future therapies the aim of which is the promotion of functional recovery from CNS lesions.

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