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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3917–3920

Solid-phase synthesis of quinol fatty alcohols, design of N/O-substituted quinol fatty alcohols and comparative activities on axonal growth

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Received 1 April 2006; revised 10 May 2006; accepted 11 May 2006
Available online 30 May 2006

Abstract—Following the promising activity of Q_2FA15 on axonal growth, two new series of N/O-substituted QFAs were synthesized, based on a S_N2-type reaction. O-alkylated QFA bearing 14 carbon atoms on the side chain ($n = 14$) shows a very potent activity on axonal growth though lowered when compared to Q_2FA15. While O-alkylation allows good retention of the biological activity, N-alkylation abolishes it nonetheless. A solid-phase-supported synthesis of Q_2FA15 allowing the conception of new hybrid compounds is also described.

Regeneration of the CNS after injury is very limited due to the presence in the lesion site of many inhibitory factors such as myelin-associated proteins (MAG, OMGp and NOGO),[1] Sema3A[2] or chondroitin sulfate proteoglycan.[3,4] In a prior publication, we have shown that quinol fatty alcohols have the ability to promote axonal growth especially on inhibitory substrates present within the CNS.[5] Specifically, Q_2FA15 is able to promote axonal growth at 181% relative to control conditions, while allowing proper axonal growth on myelin proteins and Sema3A substrates.

Ω-Alkanol derivatives have proven in recent years to be very active towards various targets in the CNS.[6–8] The need of a synthetic strategy applicable to differently substituted ω-alkanols is nowadays a crucial aspect of our research. To address that, a synthetic strategy using solid-phase organic synthesis was designed in order to obtain Q_2FA15, based on the Sonogashira cross-coupling reaction between an arylbromide and a resin-supported terminal alkyne derivative.

Though Q_2FA15 is a very active compound, its chemical synthesis is rather tiresome. Based on a convergent synthetic route, four steps are needed for the synthesis of the side terminal alkyne chain, while an additional three steps are required for obtaining the final compound. In addition, the coupling reaction between the quinol and the alkyne moieties is based on Sonogashira cross-coupling[9,10] therefore using palladium catalysts.[11]

Palladium is known to interact with organic compounds and common purification procedures are often not sufficient to eliminate the excess metal catalyst.[12] In order to circumvent that problem, pharmaceutical industries are using techniques such as distillation, adsorption,[13] crystallization[14] and extraction[15] thus reducing palladium levels to acceptable amounts.

In this paper, we thereby describe the design of two new series of O-alkylated and N-alkylated quinol fatty alcohols where the carbon–carbon bond between the quinol and the ω-alkanol moieties was replaced by a C–O and C–N bond, respectively. O/N-alkylated QFAs were tested on a cellular model of axonal growth in order to compare their activity to lead compound Q_2FA15, while their free radical scavenging activity was also evaluated.

Solid-phase Sonogashira cross-coupling reactions are usually accomplished between a supported arylhalide/

Keywords: Quinol fatty alcohols; Solid-phase organic synthesis; S_N2-type reactions; Axonal growth; Myelin proteins; Sema3A.
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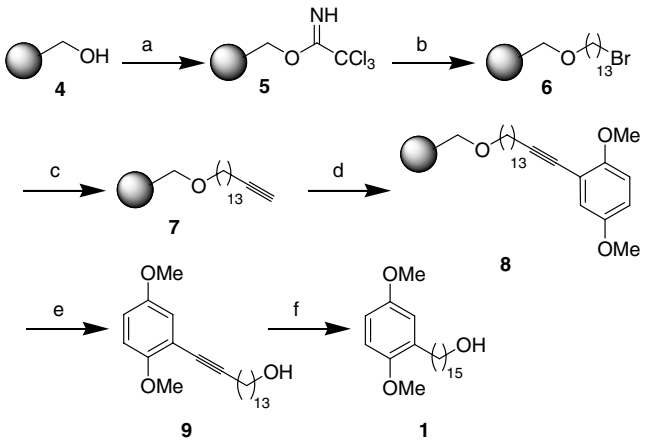
doi:10.1016/j.bmcl.2006.05.027

triflate and soluble terminal alkynes.[16] In our case, supported terminal alkynes were needed in order to be coupled to a variety of arylhalides/triflates. To do so, we used a trichloroacetamidate[17] Wang resin **5** which was subsequently substituted by 13-bromotridecan-1-ol in a mixture of methylene chloride and cyclohexane in acidic conditions to give resin **6**. Terminal alkyne **7** was obtained with lithium acetylide in DMSO.[18] The Sonogashira cross-coupling reaction was followed by a cleavage of our compound from the Wang resin with 10% TFA in methylene chloride and water. A final catalytic hydrogenation gave Q_2FA15 **1** (Scheme 1).

O-alkylated QFAs **2a–d** were obtained by a S_N2-type reaction between 2,5-dimethoxyphenol **14** and bromoalcohols **11a–d** with potassium carbonate in acetone.[19] 2,5-dimethoxyphenol **14** was obtained through a Baeyer–Villiger oxidation of 2,5-dimethoxycarboxaldehyde **13**.[20]



Scheme 1. Reagents and conditions: (a) CCl_3CN, DBU, 0 °C, 67%; (b) $BF_3 \cdot OEt_2$, 13-bromotridecan-1-ol, cyclohexane, CH_2Cl_2, 90%; (c) lithium acetylide (ethylene diamine complex), 0 °C to rt, 95%; (d) 1-bromo-2,5-dimethoxyphenol, $Pd(PPh_3)_4$, piperidine, 80 °C, 90%; (e) TFA 10%, CH_2Cl_2, H_2O, rt, 80%; (f) H_2, Pd/C 10%, EtOH, 90%.

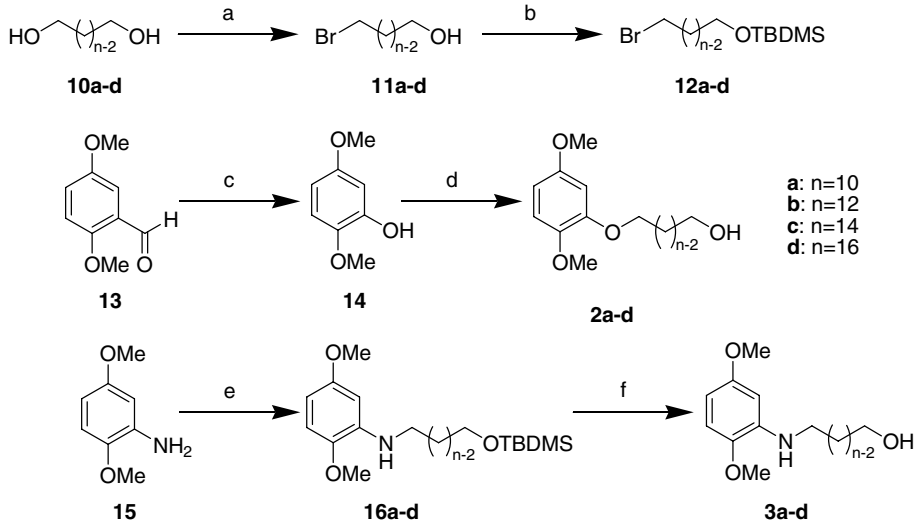
N-alkylated QFAs **3a–d** were obtained by a S_N2-type reaction between commercial 2,5-dimethoxyaniline **15** and protected bromoalcohols **12a–d** with *n*-BuLi in THF,[21] followed by deprotection with TBAF[22] (Scheme 2).

The C10 and C12 diols were commercially available. Diol C14 was obtained by reduction of the corresponding diacid with lithium aluminium hydride. Diol C16 is obtained by reduction of the corresponding lactone with the same reducing agent.[6] Bromoalcohols **11a–d** were obtained by monobromination of the corresponding diols in a mixture of HBr–cyclohexane.[6] The use of *tert*-butyldimethylsilyl chloride in methylene chloride[23] gave silylated bromoalkanols **12a–d**.

In an attempt to study the biological activity of our heteroatom-alkylated 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.[24] Briefly, mice embryos at the 15th day of gestation were dissected and cortical extracts were dissociated with trypsin. 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.

A complete screening of all compounds at 10^{-9} M showed that O-alkylated compound (*O*-QFA14) with a 14-carbon side chain was the most effective, while its N-alkylated homologue had no apparent biological activity (Fig. 1).



Scheme 2. Reagents and conditions: (a) HBr 47%, cyclohexane, reflux, 6 h, 79–89%; (b) TBDMS-Cl, Imid., CH_2Cl_2, rt, 4 h, 89–95%; (c) i—mCPBA, CH_2Cl_2, 0 °C to rt, ii—NaOH 10%, MeOH, 95%; (d) K_2CO_3, **11a–d**, acetone, reflux, 6 h, 62–70%; (e) i—*n*-BuLi, THF, 0 °C, ii—**12a–d**, THF, 60 °C, iii—dioxane, 60 °C, 14 h, 54–65%; (f) TBAF, THF, rt, 4 h, 93–95%.

Table 1. Compounds synthesized and tested

Compound	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	—
1	Q_2FA15	$C_{23}H_{40}O_3$	365.3050	365.3056	>10,000	181
2a	*O*-QFA10	$C_{18}H_{30}O_4$	311.2217	311.2211	***	122
2b	*O*-QFA12	$C_{20}H_{34}O_4$	339.2530	339.2526	***	145
2c	*O*-QFA14	$C_{22}H_{38}O_4$	367.2843	367.2848	***	165
2d	*O*-QFA16	$C_{24}H_{42}O_4$	395.3156	395.3159	***	109
3a	*N*-QFA10	$C_{18}H_{31}NO_3$	310.2377	310.2380	***	100
3b	*N*-QFA12	$C_{20}H_{35}NO_3$	338.2690	338.2701	***	102
3c	*N*-QFA14	$C_{22}H_{39}NO_3$	366.2847	366.2831	***	105
3d	*N*-QFA16	$C_{24}H_{43}NO_3$	394.3316	394.3322	***	104

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%.

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.[25] In the presence of hydroxyl radicals, ABTS is oxidized to the stable ABTS cation radical (ABTS$^{\bullet+}$) observed by its absorbance at 405 nm.

This method measures the relative ability of *C*/*O*/*N*-QFAs to scavenge hydroxyl radicals and thus inhibit the formation of ABTS$^{\bullet+}$ as measured by the decrease of its absorbance at 405 nm. Results are shown in Table 1.

Though antioxidant activity of *O*-QFA14[26] (**2c**) and *N*-QFA14 (**3c**) seems to be higher than that of Q_2FA15[27] (**1**), their biological activity towards regeneration of axon extension is affected. *O*-QFA14 seems to exert a biological activity that is comparatively similar to that of Q_2FA15 though 10% lower. However, *N*-QFA14 is totally inactive towards axonal growth. This lack of biological activity could be due to protonation of *N*-QFA14 in our aqueous cell-culture medium.

Ω-Alkanols are known to interact with the cell membrane and slightly destabilize it.[28] Diffusion through the cell membrane through a flip-flop process is a possible outcome. A protonated compound would bear an extra positive charge which would not allow proper compound–membrane interaction (Fig. 2).

Furthermore, the nitrogen of *N*-QFA14 could hydrogen bond to the methoxy moiety of the benzene ring,[29] hence blocking the compound in a certain configuration that does not allow proper interaction with the cell membrane.

In this paper, we describe a solid-phase synthesis of Q_2FA15. Though the overall yield is significantly lower than that of the previously described synthesis[5] (Table 2), this approach allows us to obtain our compound in a very straightforward manner using significantly less purification steps and less organic solvents. This synthesis should be easily applicable to the design of new potentially active hybrid compounds.

The syntheses of N/O-alkylated QFAs are on the other hand overly simple and require fewer steps to obtain the final compounds. Both syntheses are based on S_N2-type reactions therefore needing no transition metal catalysts. Though the activity of *N*-QFAs on axonal growth is nil, *O*-QFA14 appears to have the capacity to promote axonal growth to comparable extents as Q_2FA15.

Altogether, these data suggest that though C-alkylated QFAs, and other hybrid compounds, are biologically

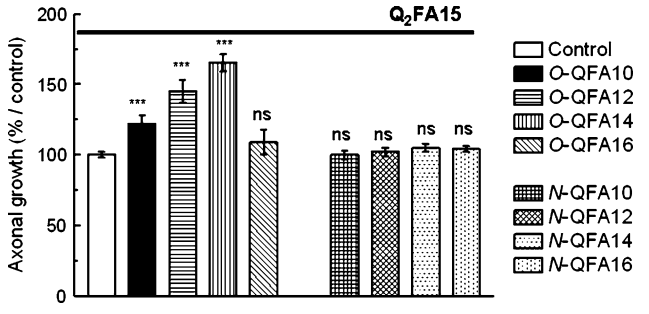


Figure 1. Promoting effect of *O*-QFAs and *N*-QFAs according to the side-chain length on a model assay of axonal growth. Compounds are tested at 10^{-9} M. Data are shown as means ± SEM. ***$p < 0.001$, ns, not significant.

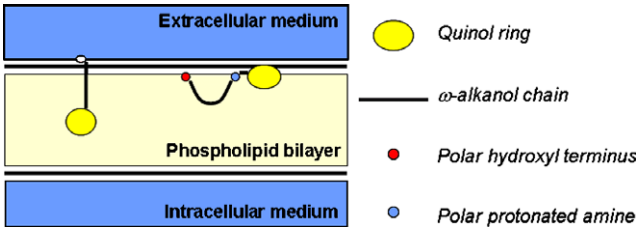


Figure 2. Possible configurations of *O*-QFA14 and protonated *N*-QFA14. The difference in membrane position is the most plausible explanation of why *N*-QFA14 is an inactive compound.

Table 2. Comparison of the synthetic strategies and biological activities of C-alkylated, O-alkylated and N-alkylated QFAs

	Number of steps	Coupling reaction	Overall yield (%)	Biological activity
Q_2FA15	3	Sonogashira	51	Active
spQ_2FA15	6	Sonogashira	37	Active
O-QFA14	2	S_N2	65	Active
N-QFA14	3	S_N2	54	Inactive

sp, solid phase.

active and their design should be pursued. Alternatives such as O-alkylation should be considered as a means of simplifying the synthetic routes followed, while allowing simpler and cleaner chemistry without significantly affecting the biological activity.

Acknowledgments

This research was partially supported by Meiji Dairies Corporation (B.L. and M.H.) and ACI Jeunes Chercheurs (D.B.).

References and notes

1. Filbin, M. T. *Nat. Rev. Neurosci.* **2003**, *4*, 703.
2. Pasterkamp, R. J.; Verhaagen, J. *Brain Res. Rev.* **2001**, *35*, 36.
3. Liu, Y.; Kim, D.; Himes, B. T.; Chow, S. Y.; Schallert, T., et al. *J. Neurosci.* **1999**, *19*, 4370.
4. Ramer, M. S.; Priestley, J. V.; McMahon, S. B. *Nature* **2000**, *403*, 312.
5. Hanbali, M.; Vela-Ruiz, M.; Bagnard, D.; Luu, B. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2637.
6. Girlanda-Junges, C.; Keyling-Bilger, F.; Schmitt, G.; Luu, B. *Tetrahedron* **1998**, *54*, 7735.
7. Muller, T.; Grandbarbe, L.; Morga, E.; Heuschling, P.; Luu, B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6023.
8. Coowar, D.; Bouissac, J.; Hanbali, M.; Paschaki, M.; Mohier, E.; Luu, B. *J. Med. Chem.* **2004**, *47*, 6270.
9. Hofman, S. *Synthesis* **1998**, 479.
10. Negishi, E. In *Handbook of Organopalladium Chemistry for Organic Synthesis*; Wiley, Eds.; 2002; Vol. 1, pp 493–529.
11. Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4442.
12. Garrett, C. E.; Prasad, K. *Adv. Synth. Catal.* **2004**, *346*, 889.
13. Rosso, V. W.; Lust, D. A.; Bernot, P. J.; Grosso, G. A.; Modi, S. P., et al. *Org. Process Res. Dev.* **1997**, *1*, 311.
14. Königsberger, K.; Chen, G.-P.; Wu, R.; Girgis, M. J.; Prasad, K., et al. *Org. Process Res. Dev.* **2003**, *7*, 731.
15. Villa, M.; Cannata, V.; Rosi, A.; Allegrini, P. WO Patent 98,516,46, 1998.
16. Bräse, S.; Kirchhoff, J. H.; Köbberling, J. *Tetrahedron* **2003**, *59*, 885.
17. Hanessian, S.; Xie, F. *Tetrahedron Lett.* **1998**, *39*, 733.
18. Jayasuriya, N.; Bosak, S.; Regen, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 5844.
19. Horie, T.; Tsukayama, M.; Kourai, H.; Yokoyama, C.; Furukawa, M., et al. *J. Med. Chem.* **1986**, *29*, 2256.
20. Tisdale, E. J.; Chowdhury, C.; Vong, B. G.; Li, H.; Theodorakis, E. A. *Org. Lett.* **2002**, *4*, 909.
21. Vitale, A. A.; Chiocconi, A. A. *J. Chem. Res., Synop.* **1996**, *7*, 336.
22. Nakayama, Y.; Kumar, B. J.; Kobayashi, Y. *J. Org. Chem.* **2000**, *65*, 707.
23. Wuts, P. G. M.; Bigelow, S. S. *J. Org. Chem.* **1988**, *53*, 5023.
24. Hanbali, M.; Bernard, F.; Berton, C.; Gatineau, G.; Luu, B., et al. *J. Neurochem.* **2004**, *90*, 1423.
25. Poeggeler, B.; Thuermann, S.; Dose, A.; Schoenke, M.; Burkhardt, S., et al. *J. Pineal Res.* **2002**, *33*, 20.
26. *O*-QFA14. Calcd: C, 72.09; H, 10.45. Found: C, 71.98; H, 10.15.
27. Q_2FA15. Calcd: C, 75.77; H, 11.06. Found: C, 75.63; H, 11.05.
28. Jover, E.; Gonzalez de Aguilar, J. L.; Luu, B.; Lutz-Bucher, B. *Eur. J. Pharmacol.* **2005**, *516*, 197.
29. Aguilar-Martinez, M.; Bautista-Martinez, J. A.; Macias-Ruvalcaba, N.; Gonzalez, I.; Tovar, E., et al. *J. Org. Chem.* **2001**, *66*, 8349.