Bioorganic & Medicinal Chemistry Letters 22 (2012) 3751–3753

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Wake promoting agents: Search for next generation modafinil, lessons learned: Part III

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ARTICLE INFO

Article history:
Received 13 February 2012
Revised 22 March 2012
Accepted 3 April 2012
Available online 13 April 2012

Keywords:
Wake-promoting agent
Modafinil
Transporters

ABSTRACT

In searching for a next generation molecule to the novel wake promoting agent modafinil (compound **1**), a series of fluorene-derived wakefulness enhancing agents were developed and evaluated in rat. Extensive pharmacokinetic studies of a potent member of the series (compound **15**) revealed that the wake promotion activity of the analog was likely due to an active metabolite (compound **3**).

Disorders of 'wakefulness' including states of impaired alertness, vigilance and attention affect millions of individuals. Relatively few pharmacotherapies are available to treat such symptoms. Modafinil (compound **1**, Fig. 1), a novel agent, pharmacologically distinct from classical stimulants (caffeine, amphetamine, and methylphenidate), improves wakefulness in a variety of species and is efficacious in humans with few peripheral or central side effects.[1] While the precise mode of action of modafinil has yet to be well-defined, due to its modest binding activity, mechanistic studies have suggested that dopamine (DAT) and norepinephrine transporters (NET) contribute to modafinil's wake-promoting pharmacology with a causal or indirect relationship.[2–4]

While modafinil continues to be evaluated for expanded clinical applications, a few aspects of its overall profile have served as the cornerstone for efforts to identify a follow-on molecule. Modafinil demonstrates modest inhibition of CYP2C19 (IC_{50} = 11 μM) but shows virtually no interaction with CYP3A4 and CYP2D6. Since clinical studies demonstrated that human plasma concentrations can reach levels greater than 30 μM at efficacious dose, the potential for drug–drug interactions is possible which will become important as modafinil is used as an adjunctive therapy in patients with psychiatric and/or neurological disorders. Furthermore, it was anticipated that a follow-on molecule from a different structural scaffold but having a modafinil-like (or better) profile, distinct from classical psychostimulants, might shed more light in elucidating the mechanism of action of modafinil. Also, a molecule of this class might help to elucidate wake promoting mechanisms in general, especially contributory roles played by various transporters. Previously, our laboratories disclosed biphenyl-derived wake promoting agents, exemplified by structure **2** (Fig. 1).[5] In this Letter, we report another aspect of our discovery effort disclosing a series of fluorene-based wake promoting agents (in rat) exemplified by generic structure **A** (Fig. 1).

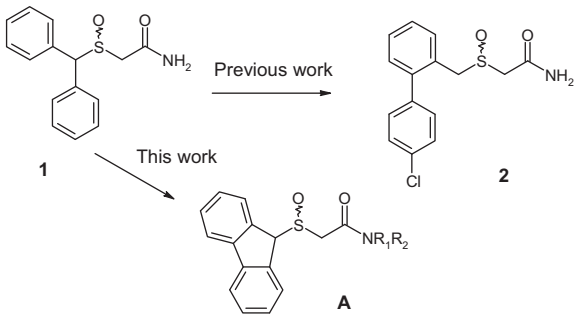

Figure 1. Chemical structures of compound **1**, compound **2**, and current series (**A**).

Scheme 1 depicts the general synthetic scheme that was utilized to generate the target compounds. Coupling of compounds **3a–b** and **4** in acidic media produced generic compound **5** that on basic hydrolysis followed by treatment with chloroacetic acid generated carboxylic acid **6**. Compound **6**, in turn, was converted to amide of generic structure **7**. Compound **7**, on controlled oxidation, generated target compounds **8–19**.

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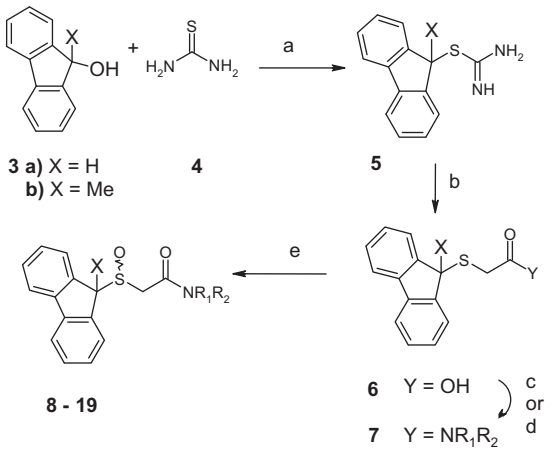
http://dx.doi.org/10.1016/j.bmcl.2012.04.031



Scheme 1. Reagents and conditions: (a) 48% HBr, H_2O, mixing at 60 °C followed by reflux, 0.5 h, 90%; (b) (i) 10 N NaOH, 80 °C, 1 h; (ii) $ClCH_2COOH$, reflux, 2 h, 80% over two steps; (c) for Y = NH_2: (i) $SOCl_2$, C_6H_6, reflux, 3 h; (ii) 28% NH_4OH, CH_2Cl_2, 0 °C – rt, 0.5 h, 70% over two steps; (d) for Y = NR_1R_2: HATU, HOAt, NMM, DMF, 0 °C to rt, overnight, 50–70%; (e) 30% H_2O_2, gl. acetic acid, rt, 2 h, 80–90%.

At the outset of our synthetic program, no well-defined molecular target(s)[4b,c] and no historical database of modafinil's wake promotion structure–activity relationships existed in the literature.[6] Thus the members of the new series were initially evaluated in a DAT binding assay (rat).[7] Subsequently cumulative wake-promoting activity in rats [i.e., total time (min) awake over a period of 3 h after dosing (3 h AUC) at 100 mg/kg ip] was utilized as the biological activity of the new analogs following our previously published procedure.[5] Compounds were also evaluated in a CYP2C19 enzyme inhibitory assay. Table 1 displays the biological data for modafinil, analogs **8–19** and compound **3** (vide infra).

As mentioned previously, at the outset of our synthetic program, no known molecular target describing modafinil-SAR existed in literature. Thus the exploration began with locking two phenyl rings of the parent molecule via a central five-member ring maintaining the distant sufinylacetamide moiety, thought to be unique for a CNS drug. As shown in Table 1, the initial analog **8** displayed similar wake promoting activity of parent compound **1**. However, compound **8** did not display any significant DAT inhibitory activity, bringing the contributory role of DAT in the wake promoting activity of this compound into question (vide infra). The corresponding methyl analog, compound **9** bearing a quaternary carbon center at the fluorene scaffold was comparatively less active in the wake promotion assay. This suggested a detrimental effect of steric congestion on activity from this site. While modification of the primary carboxamide moiety of compound **8** to a secondary methylamide, as in compound **10**, was slightly detrimental, extension of the methyl chain to an ethanol moiety (compound **11**) produced wake promoting activity raising the possibility of a beneficial interaction from a distant site. While conversion of the primary carboxamide of compound **8** to a linear tertiary carboxamide (compound **12**) was not beneficial, introduction of a cyclic amine moiety in the region was significantly productive (compounds **13**, **14** and **15**; note these compounds were screened at 30 mg/kg ip since residual waking activity beyond 3 h was observed at the higher dose). Hypothesizing additional steric bulk in the carboxamide area might be beneficial, the series was further extended by including amino acid residues for example compounds **16**, **17**, **18**, and **19**. Note the last three compounds were tested as a mixture of diastereomers. All four compounds displayed superior activity in the

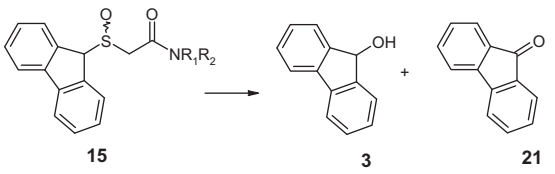
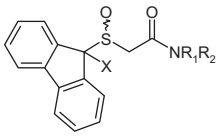


Figure 2. Metabolism of compound **15** in rat.

Table 1
Biological data of new analogs



Compound	X	NR_1R_2	DAT IC_{50} (μM)	Wake 3 h AUC (min)[a]	2C19 IC_{50} (μM) or %Inhib. at 10 μM
1[b]		Modafinil	3.70	117 ± 13*	11
8[b]	H	NH_2	>100	116 ± 8*	>100
9[b]	Me	NH_2	>100	71 ± 5	26%
10[b]	H	NHMe	>100	83 ± 6*	48%
11[b]	H	$NH(CH_2)_2OH$	>100	167 ± 5*	>100
12[b]	H	NEt_2	>100	99 ± 5*	>100
13[b,c]	H	*N*-Morpholinyl	>100	158 ± 10*	30%
14[b,c]	H	*N*-Piperazinyl	>100	119 ± 17	>100
15[b,c]	H	*N*-(4-Acetyl)-piperazinyl	>100	146 ± 12	>100
16[b]	H	*N*-Glycinamide	>100	140 ± 10*	>100
17[d]	H	(*S*)-*N*-Alaninylamide	>100	151 ± 14*	>100
18[d]	H	(*S*)-*N*-Serinylamide	>100	136 ± 14*	>100
19[d]	H	(*S*)-*N*-Prolinylamide	>100	169 ± 13*	>100
3	—	—	9	163 ± 3*	—
Vehicle[e]		—	—	65 ± 9	—

[a] Mean ± SEM; *P <0.05 versus within-experiment vehicle control.
[b] Tested as a mixture of enantiomers.
[c] Tested at a dose of 30 mg/kg.
[d] Tested as a mixture of diastereomers.
[e] Average of vehicle group: mean and SEM values (N = 11) for compounds shown.

Table 2
Pharmacokinetic parameters

Pharmacokinetic parameters	Plasma			Brain		
	Compound **15**	Compound **3**	Compound **21**	Compound **15**	Compound **3**	Compound **21**
C_{max}, ng/g	6286 ± 2973	263 ± 44	255 ± 70	416 ± 272	4384 ± 844	971 ± 309
t_{max}, h	0.8 ± 0.2	2 ± 0	1.2 ± 0.4	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
AUC_{0-t}, ng h/g	7501 ± 2657	1081 ± 149	939 ± 214	295 ± 252	11639 ± 2773	2349 ± 665
Mean ± SEM, n = 3	Dosed as compound **15** at 116 mg/kg ip					

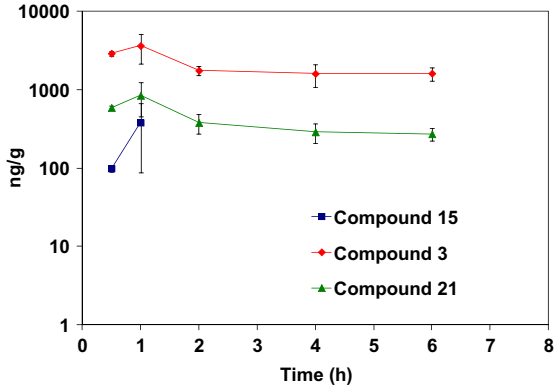


Figure 3. Brain levels in rats dosed with compound **15** 116 mg/kg ip, 0.5% methylcellulose, 0.2% tween 80.

wake promotion assay. As shown in Table 1, compounds **8**–**19** did not display any significant DAT inhibitory activity, compared to modafinil's modest activity in the same assay. Similarly, most of them (except **9**, **10** and **13**) did not offer any unwarranted 2C19-liability.

From this series, representative compound **15** was advanced for additional profiling as well as measuring its wake promotion activity in a higher species. However, the compound displayed a lack of wake promoting activity in dogs. Subsequent detailed pharmacokinetic work in rats revealed that compound **15** underwent metabolism generating compound **3** (major metabolite) and compound **21** (minor metabolite) in rats (Fig. 2, NR_1R_2 = N-(4-Acetyl)-piperazinyl).

After ip dosing of compound **15** in rats, plasma levels of the two metabolites were ~4% of the level of the parent compound as measured by C_{max} and 13% as represented by the AUC. However, in brain the parent was extensively converted to compound **3** and to a lesser extent to compound **21** (Table 2 and Fig. 3). C_{max} concentrations of compound **3** in brain were over 10-fold that of the parent compound with an AUC ca 40 times that of compound **15**. To a lesser extent, compound **21** was also formed in the brain following administration of compound **15**.

As shown in Table 1, subsequent screening in wake promoting assay in rats, compound **3** (100 mg/kg ip) was found to be active. Thus, it is likely that the wake promoting activity of compound **15** in rats might have arisen from the activity of compound **3**. On the other hand, such metabolism might be minimal in dogs. It should be noted that compound **3** maintains some DAT binding activity, though less active than modafinil, raising the possibility of involvement of this transporter in its wake promoting activity in rats. However, no additional study was carried out with compound **3**, as a more efficacious molecule (compound **2**, Fig. 1), active both in rat and dog wake promotion assays, had already been identified.

In this Letter, we disclosed a series of fluorene-derived wakefulness promoting agents (in rat) in search of a next generation molecule to modafinil. From this work, representative compound **15** was advanced for additional profiling. However, compound **15** lacked wake promoting activity in dogs. Investigative work revealed that the wake promoting activity of compound **15** in rats might have been due to the reactive metabolite **3**.

Acknowledgments

Authors wish to acknowledge the scientific support and encouragement of Drs. Donna Bozyczko-Coyne, John P. Mallamo and Jeffry L. Vaught during the course of this research. Authors sincerely thank the reviewers for their constructive criticism and valuable suggestions to improve the quality of the manuscript.

References and Notes

1. Modafinil had been introduced in the market by Cephalon, Inc., Frazer, PA under the trade name Provigil®. Detailed information on the drug could be found in the website www.provigil.com (accessed on February 12, 2012).
2. Andersen, M. L.; Kessler, E.; Murnane, K. S.; McClung, J. C.; Tufik, S.; Howell, L. L. *Psychopharmacology* **2010**, *210*, 439; (b) Wisor, J. P.; Nishino, S.; Sora, I.; Uhl, G. L.; Mignot, E.; Edgar, D. M. *J. Neurosci.* **2001**, *21*, 1787.
3. Volkow, N. D.; Fowler, J. S.; Logan, J.; Alexoff, D.; Zhu, W.; Telang, F.; Wang, G. J.; Jayne, M.; Hooker, J. M.; Wong, C.; Hubbard, B.; Carter, P.; Warner, D.; King, P.; Shea, C.; Xu, Y.; Muench, L.; Apelskog-Torres, K. *JAMA* **2009**, *301*, 1148.
4. (a) Madras, B. K.; Xie, Z.; Lin, Z.; Jassen, A.; Panas, H.; Lynch, L.; Johnson, R.; Livni, E.; Spencer, T. J.; Bonab, A. A.; Miller, G. M.; Fischman, A. J. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 561; (b) Nishino, S.; Mao, S.; Sampathkumaran, R.; Shelton, J. *Sleep Res. Online* **1998**, *1*, 49; (c) Mignot, E.; Nishino, S.; Guilleminault, C.; Demont, W. C. *Sleep* **1994**, *17*, 436.
5. Dunn, D.; Hostetler, G.; Iqbal, M.; Messina-McLaughlin, P.; Reiboldt, A.; Lin, Y. G.; Gruner, J.; Bacon, E. R.; Ator, M. A.; Chatterjee, S. *Bioorg. Med. Chem. Lett.*, in press.
6. After our work was completed, following publication describing several modafinil analogs appeared in the literature: Cao, J.; Prisinzano, T. E.; Okunola, O. M.; Kopajtic, T.; Shook, M.; Katz, J. L.; Newman, A. M. *ACS Med. Chem. Lett.* **2011**, *2*, 48.
7. *Protocol for DAT binding assay:* Frozen striata (Pel-Freez) were homogenized in 10 volumes of buffer (10 mM $NaPO_4$ pH 7.4; 140 mM NaCl) and centrifuged at 10,000 *g* for 10 min (4 °C). The pellet was suspended and centrifuged again with the remaining pellet suspended to a final concentration of 1 mg tissue/ml. 3H-WIN-35428 (Perkin–Elmer) was added to a final concentration of 4 nM and incubated for 120 min at room temperature. Reactions were terminated by filtration. Nomifensine (20 μM) was used to determine nonspecific binding.