



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

Derek Dunn, Greg Hostetler, Mohamed Iqbal, Val R. Marcy, Yin Guo Lin, Bruce Jones, Lisa D. Aimone, John Gruner, Mark A. Ator, Edward R. Bacon, Sankar Chatterjee\*

Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380-4245, USA

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### 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**).

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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.<sup>1</sup> 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.<sup>2–4</sup>

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 ( $IC_{50} = 11 \mu M$ ) but shows virtually no interaction with CYP3A4 and CYP2D6. Since clinical studies demonstrated that human plasma concentrations can reach levels greater than  $30 \mu M$  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

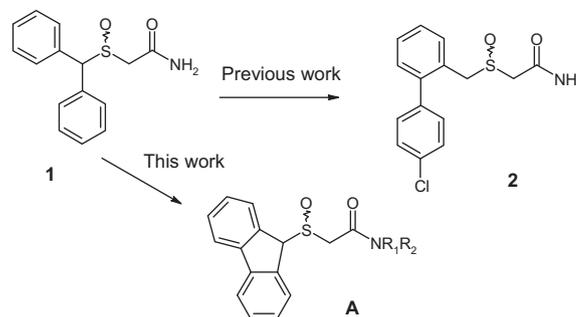


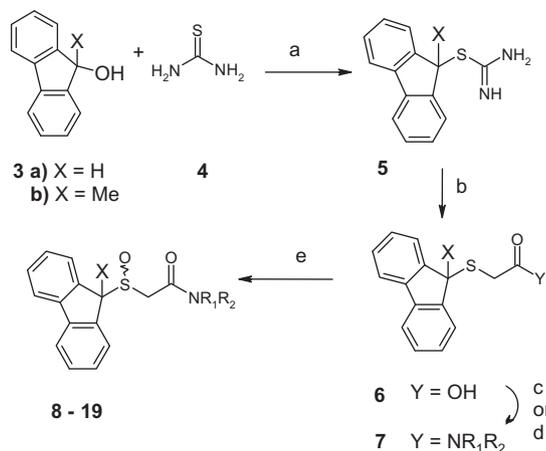
Figure 1. Chemical structures of compound **1**, compound **2**, and current series (**A**).

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).<sup>5</sup> 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).

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**.

\* Corresponding author.

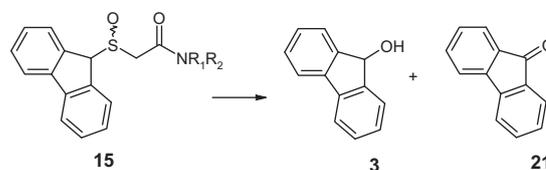
E-mail address: Sankar.Chatterjee24@gmail.com (S. Chatterjee).



**Scheme 1.** Reagents and conditions: (a) 48% HBr, H<sub>2</sub>O, mixing at 60 °C followed by reflux, 0.5 h, 90%; (b) (i) 10 N NaOH, 80 °C, 1 h; (ii) ClCH<sub>2</sub>COOH, reflux, 2 h, 80% over two steps; (c) for Y = NH<sub>2</sub>: (i) SOCl<sub>2</sub>, C<sub>6</sub>H<sub>6</sub>, reflux, 3 h; (ii) 28% NH<sub>4</sub>OH, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C – rt, 0.5 h, 70% over two steps; (d) for Y = NR<sub>1</sub>R<sub>2</sub>: HATU, HOAt, NMM, DMF, 0 °C to rt, overnight, 50–70%; (e) 30% H<sub>2</sub>O<sub>2</sub>, gl. acetic acid, rt, 2 h, 80–90%.

At the outset of our synthetic program, no well-defined molecular target(s)<sup>4b,c</sup> and no historical database of modafinil's wake promotion structure–activity relationships existed in the literature.<sup>6</sup> Thus the members of the new series were initially evaluated in a DAT binding assay (rat).<sup>7</sup> 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.<sup>5</sup> 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).

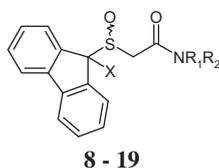
As mentioned previously, at the outset of our synthetic program, no known molecular target describing modafinil-SAR existed



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

in literature. Thus the exploration began with locking two phenyl rings of the parent molecule via a central five-member ring maintaining the distant sulfinylacetamide 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

**Table 1**  
Biological data of new analogs



Compound	X	NR <sub>1</sub> R <sub>2</sub>	DAT IC <sub>50</sub> (μM)	Wake 3 h AUC (min) <sup>a</sup>	2C19 IC <sub>50</sub> (μM) or %Inhib. at 10 μM
1 <sup>b</sup>	H	Modafinil	3.70	117 ± 13*	11
8 <sup>b</sup>	H	NH <sub>2</sub>	>100	116 ± 8*	>100
9 <sup>b</sup>	Me	NH <sub>2</sub>	>100	71 ± 5	26%
10 <sup>b</sup>	H	NHMe	>100	83 ± 6*	48%
11 <sup>b</sup>	H	NH(CH <sub>2</sub> ) <sub>2</sub> OH	>100	167 ± 5*	>100
12 <sup>b</sup>	H	NEt <sub>2</sub>	>100	99 ± 5*	>100
13 <sup>b,c</sup>	H	N-Morpholinyl	>100	158 ± 10*	30%
14 <sup>b,c</sup>	H	N-Piperazinyl	>100	119 ± 17	>100
15 <sup>b,c</sup>	H	N-(4-Acetyl)-piperazinyl	>100	146 ± 12	>100
16 <sup>b</sup>	H	N-Glycinamide	>100	140 ± 10*	>100
17 <sup>d</sup>	H	(S)-N-Alaninylamide	>100	151 ± 14*	>100
18 <sup>d</sup>	H	(S)-N-Serinylamide	>100	136 ± 14*	>100
19 <sup>d</sup>	H	(S)-N-Prolinylamide	>100	169 ± 13*	>100
3	—	—	9	163 ± 3*	—
Vehicle <sup>e</sup>	—	—	—	65 ± 9	—

<sup>a</sup> Mean ± SEM; \*P < 0.05 versus within-experiment vehicle control.

<sup>b</sup> Tested as a mixture of enantiomers.

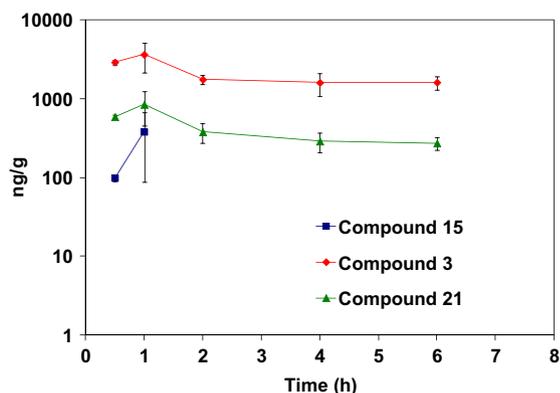
<sup>c</sup> Tested at a dose of 30 mg/kg.

<sup>d</sup> Tested as a mixture of diastereomers.

<sup>e</sup> 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 <sub>0-t</sub> , 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-ility.

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<sub>1</sub>R<sub>2</sub> = 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.

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