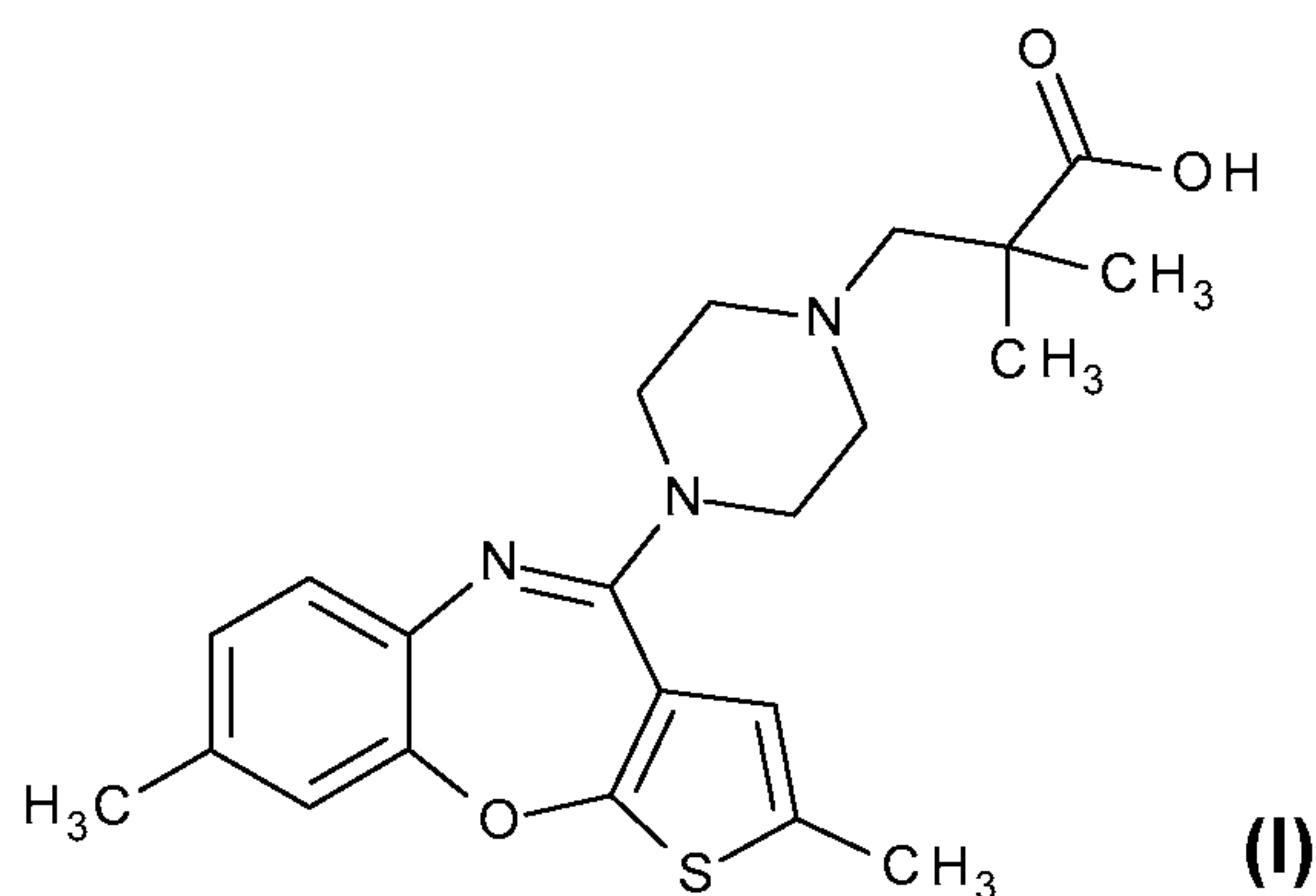




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(54) **Titre :** COMPOSES (THIENO[2,3-B][1,5]BENZOXAZEPIN-4-YL)PIPERAZIN-1-YLES EN TANT QUE COMPOSES A DOUBLE
ACTIVITE AGONISTES INVERSES H1/ANTAGONISTES 5-HT_{2A}
(54) **Title:** (THIENO[2,3-B][1,5]BENZOXAZEPIN-4-YL)PIPERAZIN-1-YL COMPOUNDS AS DUAL ACTIVITY H1 INVERSE
AGONISTS/5-HT_{2A} ANTAGONISTS



(57) **Abrégé/Abstract:**

A dual H1 inverse agonist/5-HT_{2A} receptor antagonist of the formula (I), its uses, and methods for its preparation are described.



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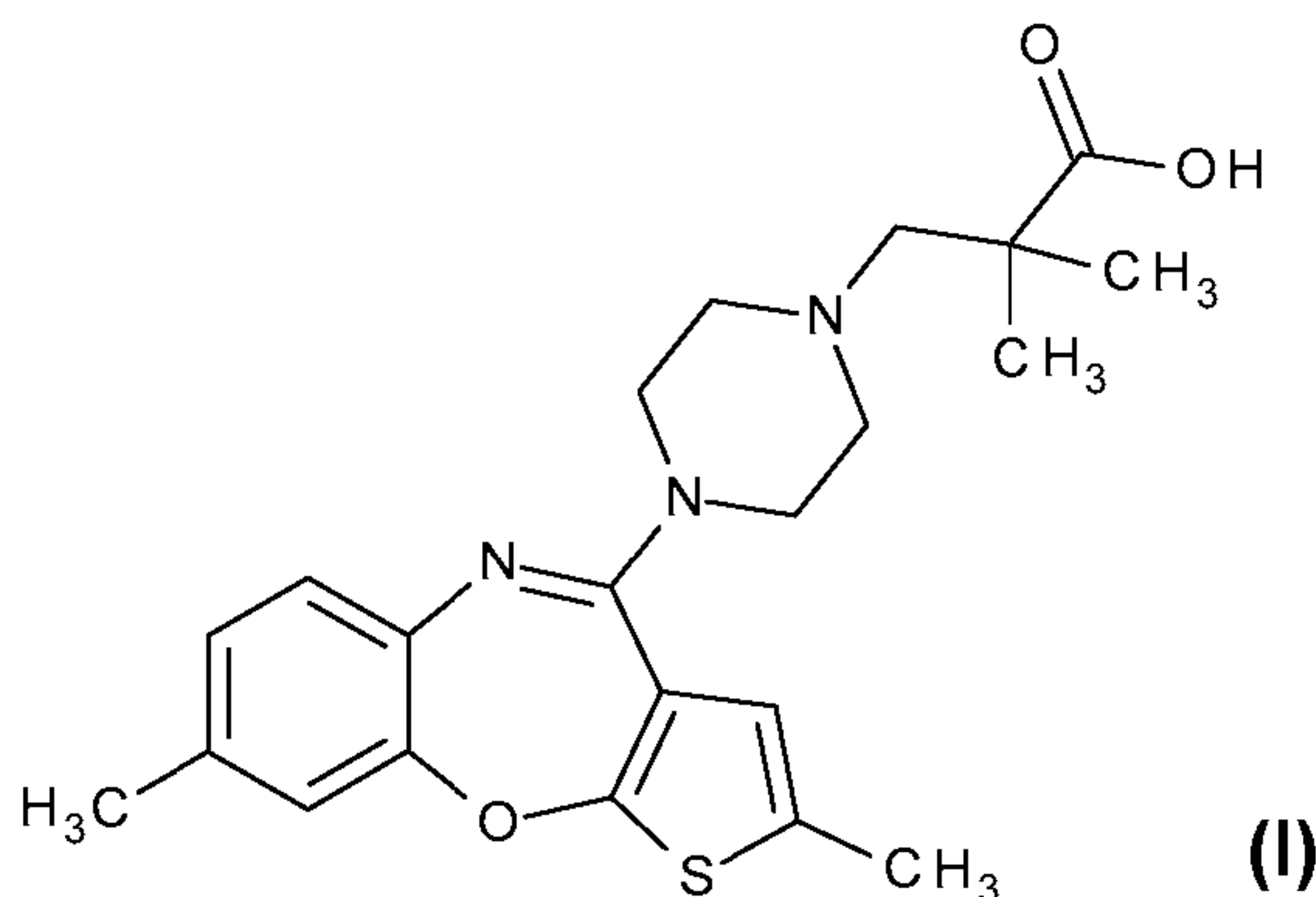
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(54) **Title:** (THIENO[2,3-b][1,5]BENZOXAZEPIN-4-YL)PIPERAZIN-1-YL COMPOUNDS AS DUAL ACTIVITY H1 INVERSE AGONISTS/5-HT_{2A} ANTAGONISTS



(I)

(57) **Abstract:** A dual H1 inverse agonist/5-HT_{2A} receptor antagonist of the formula (I), its uses, and methods for its preparation are described.

-1-

**(THIENO[2,3-b][1,5]BENZOXAZEPIN-4-YL)PIPERAZIN-1-YL COMPOUNDS
AS DUAL ACTIVITY H1 INVERSE AGONISTS/5-HT_{2A} ANTAGONISTS**

Histamine plays an important role in a variety of physiological processes through
5 its interaction with at least four different G-protein coupled receptors, the H1-H4
receptors. In the CNS, H1 receptors play a key role in the sleep regulation cycle and H1
antagonists/inverse agonists are known to induce somnolence.

Likewise, serotonin plays important roles in a variety of physiological processes
through its interaction with at least fourteen different G-protein coupled receptors.
10 Modulation of 5-HT_{2A} receptors in the CNS plays a key role in the sleep regulation cycle
and 5-HT_{2A} antagonists have been shown to improve slow wave sleep and sleep
maintenance in patients with insomnia.

Compounds having H1 or 5-HT_{2A} inverse agonist or antagonist activity have been
used in the treatment of insomnia (e.g. doxepin and trazodone, respectively) and have
15 exhibited significant pharmacological effects in animal sleep studies. However, no
selective dual activity H1/5-HT_{2A} inverse agonists/antagonists are currently commercially
available.

WO 2007/022068 describes certain substituted (thieno[2,3-b][1,5]benzodiazepine-
4-yl)piperazin-1-yl and (thieno[2,3-b][1,5]benzoxazepine-4-yl)piperazin-1-yl compounds
20 for treating sleep disorders.

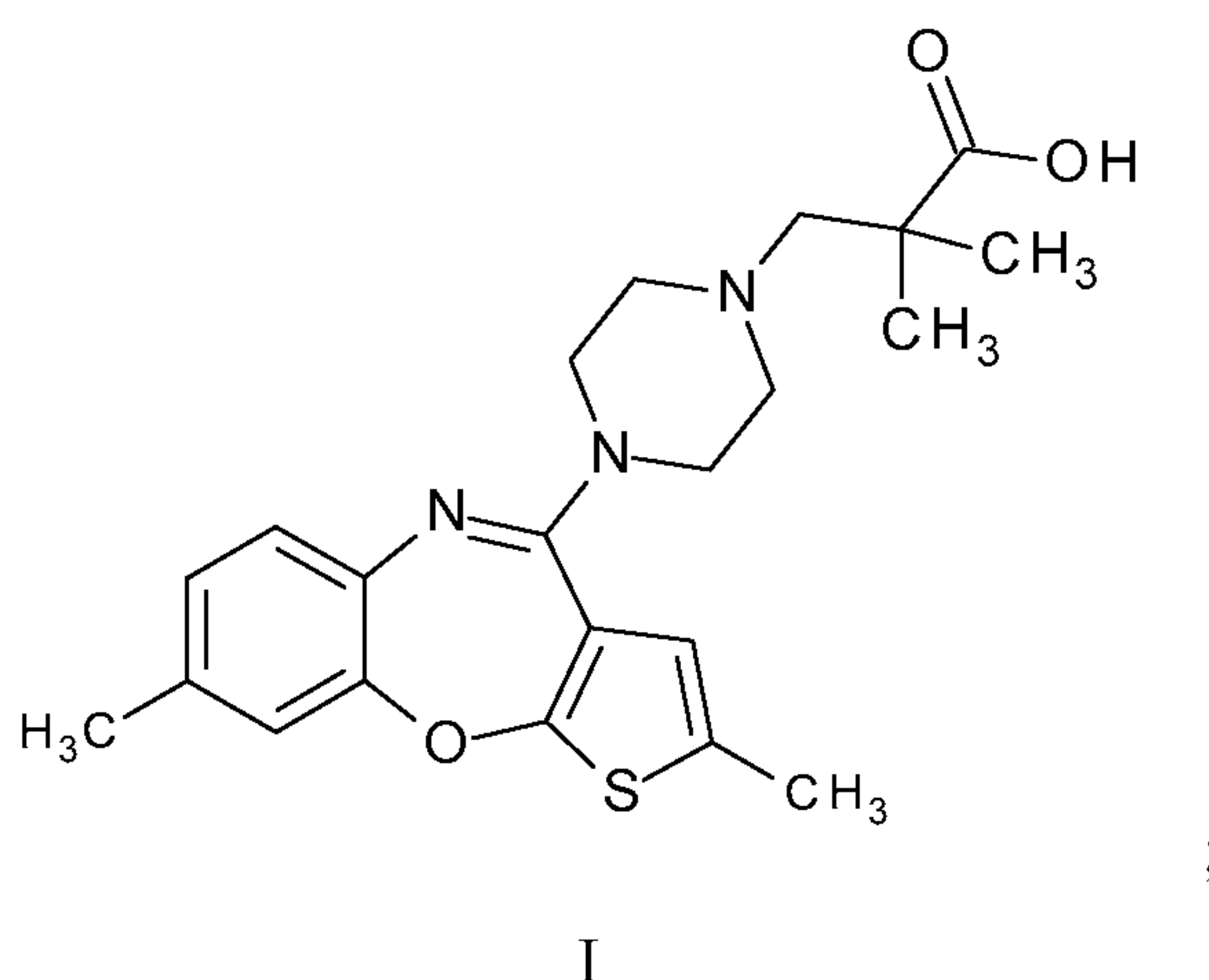
The present invention provides 3-[4-(2,8-dimethyl-thieno[2,3-
b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethyl-propanoic acid and
pharmaceutically acceptable salts thereof, having high inverse agonist potency for the H1
receptor, high antagonist potency for the 5-HT_{2A} receptor, and good selectivity for these
25 receptors, particularly as against other histamine receptors, serotonin receptors and other
physiologically relevant receptors, particularly as against the 5-HT_{2C} receptor, GABA_A
receptor, muscarinic receptors, dopaminergic receptors, adrenergic receptors, and the
hERG channel. These compounds also demonstrate through animal models that they may
be useful for the treatment of sleep disorders characterized by poor sleep maintenance.
30 As such, the compounds are believed to be useful for the treatment of sleep disorders
characterized by poor sleep latency or poor sleep maintenance or both, such as the
treatment of insomnia, as for example chronic or transient primary insomnia, or chronic

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or transient secondary insomnia, or both. Examples of secondary insomnia include, but are not limited to insomnia associated with depressive disorders (e.g. major depressive disorder, dysthymia, and/or cyclothymia), insomnia associated with anxiety disorders (e.g. generalized anxiety disorder and/or social phobia), insomnia associated with pain
 5 (e.g. fibromyalgia, chronic bone or joint pain, such as associated with inflammatory arthritis or osteoarthritis, or diabetic neuropathic pain), insomnia associated with allergic reactions (e.g. allergic asthma, pruritus, rhinitis, congestion, etc.), insomnia associated lung or airway disorders (e.g. with obstructive sleep apnea, reactive airway disease, etc.), insomnia associated with psychiatric disorders, dementia, and/or neurodegenerative
 10 diseases, and/or insomnia associated with circadian rhythm sleep disorders (e.g. shift work sleep disorder, jet lag disorder, delayed sleep phase disorder, advanced phase sleep disorder, and non-24 hr. sleep-wake syndrome, etc.).

Further, the compounds of the present invention demonstrate potentiation of their effects on non-rapid eye movement sleep (NREM sleep) when coadministered with
 15 selective serotonin reuptake inhibitors.

The present invention provides a compound of Formula I



or a pharmaceutically acceptable salt thereof. That is to say 3-[4-(2,8-dimethylthieno[2,3-
 20 b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethylpropanoic acid or a pharmaceutically acceptable salt thereof.

In another aspect of the invention there is provided a pharmaceutical composition comprising a compound of Formula I or a pharmaceutically acceptable salt thereof, in

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combination with at least one pharmaceutically acceptable carrier, diluent, or excipient. Furthermore, this aspect of the invention provides a pharmaceutical composition for treating insomnia, as for example insomnia characterized by prolonged sleep latency or poor sleep maintenance or both, as for example primary insomnia, jet lag, shift work
5 sleep disorder, delayed sleep phase disorder, advanced phase sleep disorder, and/or non-24 hr. sleep-wake disorders, comprising a compound of Formula I or a pharmaceutically acceptable salt thereof, in combination with one or more pharmaceutically acceptable excipients, carriers, or diluents.

A further embodiment of this aspect of the invention provides a pharmaceutical
10 composition comprising a compound according to Formula I, or pharmaceutically acceptable salt thereof, in combination with at least one pharmaceutically acceptable carrier, excipient or diluent, and optionally other therapeutic ingredients. In a yet further embodiment of this aspect of the invention, the pharmaceutical composition further comprises a second therapeutic agent which is a serotonin reuptake inhibitor, as for
15 example citalopram, paroxetine, fluoxetine and/or fluvoxetine.

The present invention also provides a method of treating insomnia, as for example insomnia characterized by prolonged sleep latency or poor sleep maintenance or both, as for example primary insomnia, jet lag, shift work sleep disorder, delayed sleep phase disorder, advanced phase sleep disorder, and/or non-24 hr. sleep-wake disorders, in a
20 mammal comprising administering to a mammal in need of such treatment an effective amount of a compound of Formula I or a pharmaceutically acceptable salt thereof. In another embodiment of this aspect of the invention, the method further comprises administering in simultaneous, separate or sequential combination, a second therapeutic agent which is a serotonin reuptake inhibitor, as for example citalopram, paroxetine,
25 fluoxetine and/or fluvoxetine. In one particular embodiment of these methods of treatment, the mammal is a human.

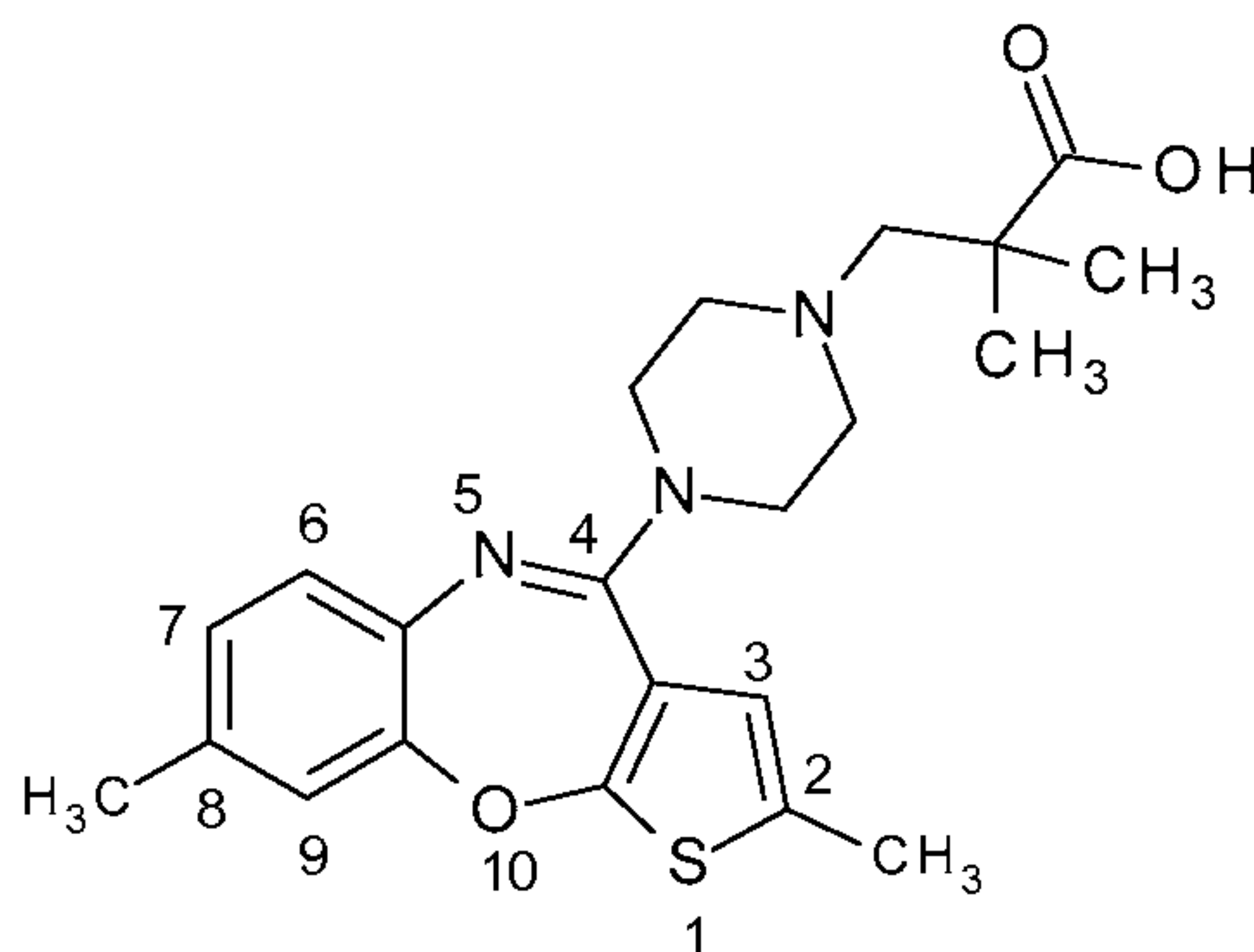
This invention also provides a compound of Formula I or a pharmaceutically acceptable salt thereof for use in therapy. Within this aspect, the invention provides a compound of Formula I, or a pharmaceutically acceptable salt thereof, for use in the
30 treatment of insomnia. In further embodiments, the insomnia is characterized by prolonged sleep latency or poor sleep maintenance or both, as for example primary insomnia, jet lag, shift work sleep disorder, delayed sleep phase disorder, advanced phase

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sleep disorder, and/or non-24 hr. sleep-wake disorders. In another embodiment of this aspect, the invention provides a compound according to Formula I, or a pharmaceutically acceptable salt thereof, for use in simultaneous, separate or sequential combination with a serotonin reuptake inhibitor, as for example citalopram, paroxetine, fluoxetine and/or
5 fluoxetine, in the treatment of insomnia. One particular embodiment of this aspect of the inventions, the uses are in mammals, particular humans.

Another aspect of this invention provides the use of a compound of Formula I, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of insomnia, as for example primary insomnia characterized by prolonged sleep
10 latency or poor sleep maintenance or both, as for example primary insomnia, jet lag, shift work sleep disorder, delayed sleep phase disorder, advanced phase sleep disorder, and/or non-24 hr. sleep-wake disorders. Another embodiment of this aspect of the invention provides the use of a compound of Formula I, or a pharmaceutically acceptable salt thereof, and a second therapeutic agent which is a serotonin reuptake inhibitor, as for
15 example citalopram, paroxetine, fluoxetine and/or fluoxetine, in the manufacture of a medicament for the treatment of insomnia, as for example, insomnia characterized by prolonged sleep latency and/or poor sleep maintenance, as for example primary insomnia, jet lag, shift work sleep disorder, delayed sleep phase disorder, advanced phase sleep disorder, and/or non-24 hr. sleep-wake disorders.

20 For clarity, the following numbering of the tricyclic ring structure will be used throughout the application:



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The compound of this invention has basic and acidic moieties, and accordingly reacts with a number of organic and inorganic acids and bases to form pharmaceutically acceptable salts. Pharmaceutically acceptable salts of the compound of the present invention are contemplated within the scope of the present application. The term

5 "pharmaceutically acceptable salt" as used herein, refers to any salt of a compound of the invention that is substantially non-toxic to living organisms. Such salts include those listed in Journal of Pharmaceutical Science, **66**, 2-19 (1977), which are known to the skilled artisan.

10 Abbreviations used herein are defined as follows:

"Brine" means saturated aqueous NaCl solution.

"DMEM" means Dulbecco's Minimum Eagle's Medium.

"DMSO" means dimethyl sulfoxide.

"EDTA" means ethylenediaminetetraacetic acid.

15 "Equiv" means equivalent(s).

"FBS" means fetal bovine serum.

"HEPES" means 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid.

"HPLC" means high pressure liquid chromatography.

"hr." means hour or hours.

20 "IC₅₀" means the concentration at which 50% of the maximum inhibition is achieved.

"LC-MS" means HPLC-mass spectrography.

"MeOH" means methanol.

"min." means minute or minutes.

25 "MS" means mass spectroscopy.

"MS (ES+)" means mass spectroscopy using electrospray ionization.

"NMR" means nuclear magnetic resonance.

"RO" means receptor occupancy.

"THF" means tetrahydrofuran.

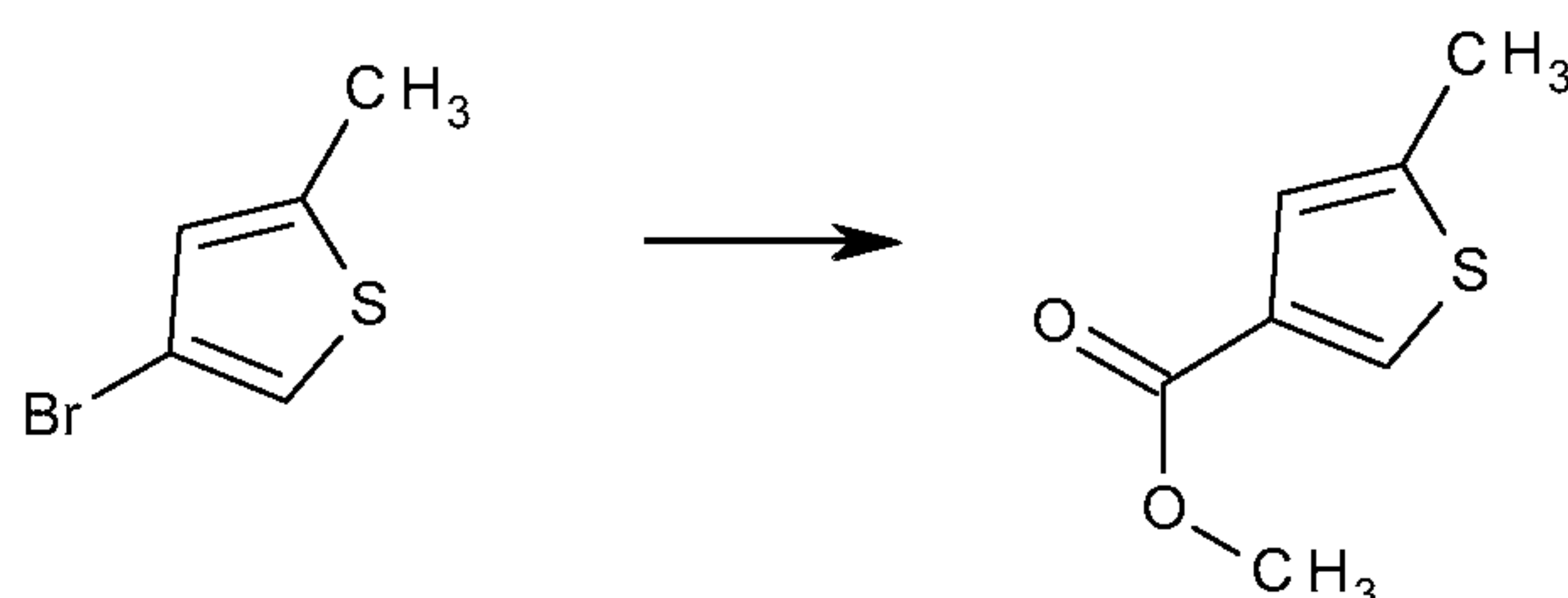
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General Chemistry

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The compound of the present invention can be prepared according to the following synthetic examples.

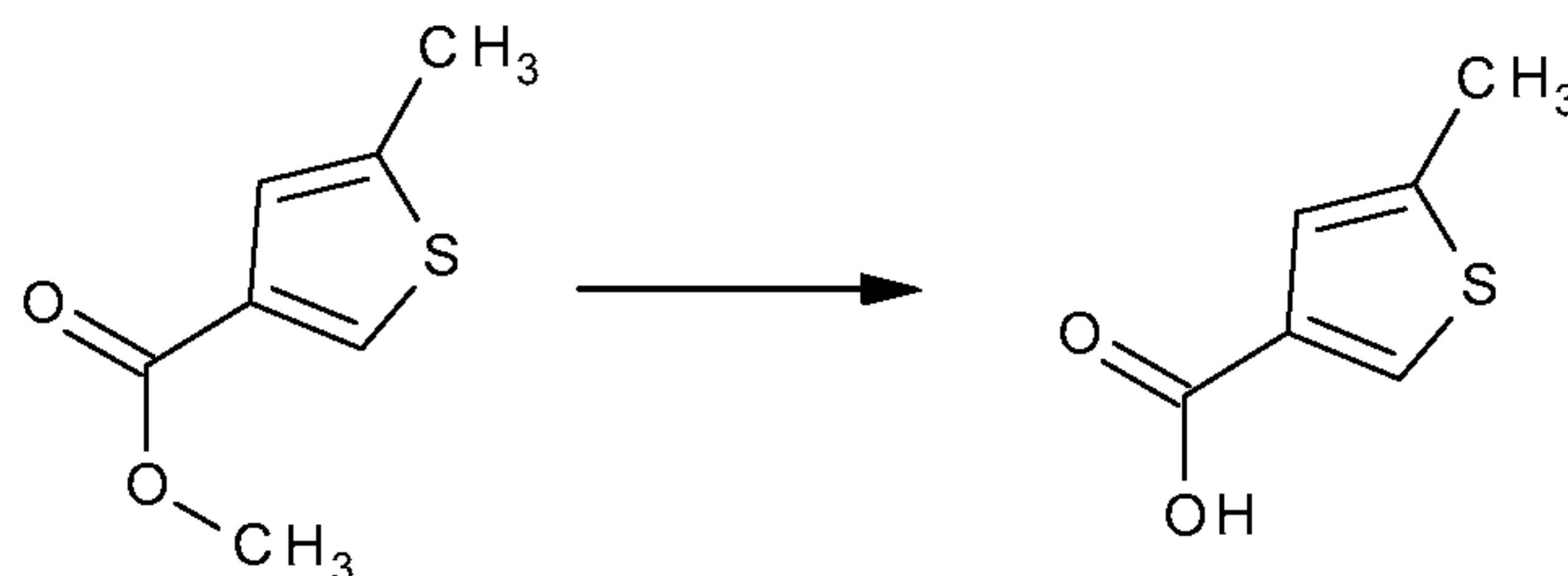
Preparation 1: Methyl 5-methylthiophene-3-carboxylate



5

Combine 4-bromo-2-methyl-thiophene (24.5 g, 138.37 mmol), bis-(1,3-diphenylphosphino)propane (2.35 g, 5.53 mmol), palladium acetate (1.24 g, 5.53 mmol), triethylamine (38.57 mL, 276.73 mmol), methanol (40 mL) and dimethyl sulphoxide (80 mL) and purge the reaction vessel with carbon monoxide. Pressurize to 30 PSI with carbon monoxide and heat to 70°C, with stirring overnight under carbon monoxide. Evaporate the volatiles under reduced pressure to give a dark orange solution. Add water (300 mL) and extract with ethyl acetate (3 x 150 mL) then wash the combined organic extracts with water (2 x 100 mL). Dry over sodium sulphate, filter and evaporate under reduced pressure. Add 10% diethyl ether in iso-hexane (500 mL) and remove the resulting dark yellow precipitate by filtration. Evaporate the filtrate under reduced pressure to give methyl 5-methylthiophene-3-carboxylate (18.894 g, 87.42% yield) as a liquid. ¹H NMR (400.13 MHz, CDCl₃): δ 7.84 (d, *J* = 1.5, 1H), 7.16 (t, *J* = 1.5, 1H), 3.83 (s, 3H) and 2.46 (d, *J* = 1.5, 3H).

20 Preparation 2. 5-Methylthiophene-3-carboxylic acid



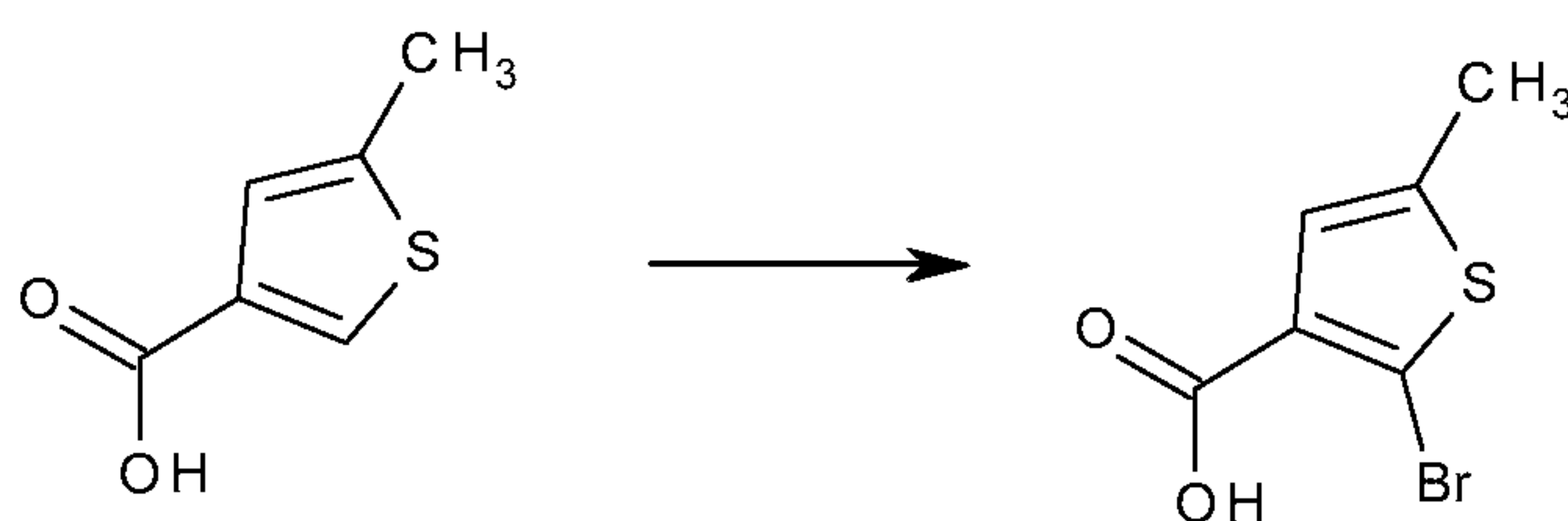
To a 250 mL flask add methyl 5-methylthiophene-3-carboxylate (16.4 g, 104.99 mmoles, 1.00 equiv) and dissolve in methanol (57.40 mL). Add NaOH (12.60 g,

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314.97 mmol, 3 equiv) and water (19.68 mL). Heat the suspension at reflux (65°C) for 45 min., until the reaction is complete by LC-MS. Cool the reaction mixture to room temperature, then add 5M aqueous HCl (85 mL, 5vol), followed by ethyl acetate (130 mL, 7.5vol). Stir for 15 min., until all solids are dissolved. Separate the layers and
5 extract the aqueous layer with ethyl acetate (2 x 130 mL). Wash the combined organics with water (3 x 130 mL), then brine (85 mL). Dry over sodium sulfate, filter and concentrate to give a solid of 5-methylthiophene-3-carboxylic acid (13.93 g, 93.32% yield). MS (m/z): 141.0 (M-H). ¹H NMR (400.13 MHz, CDCl₃): 7.99 (d, *J* = 1.5 Hz, 1H), 7.20 (d, *t* = 1.5 Hz, 1H), 2.50 (d, *J* = 1.5, 3H).

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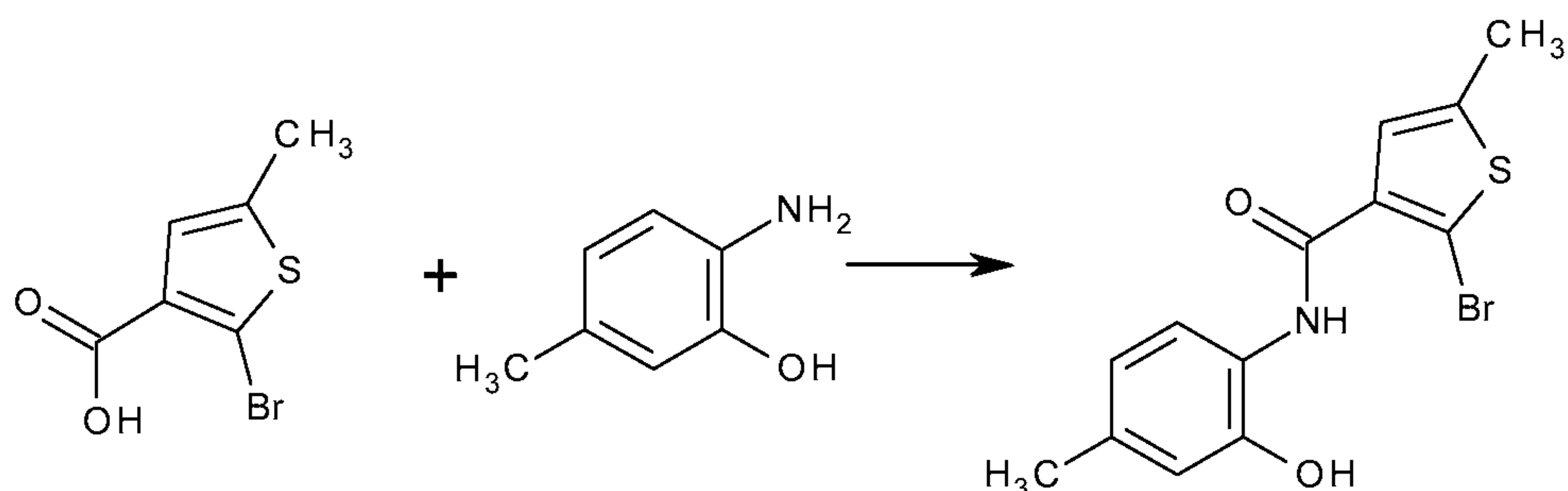
Preparation 3. 2-Bromo-5-methyl-thiophene-3-carboxylic acid



To a solution of 5-methylthiophene-3-carboxylic acid (13.93 g, 97.98 mmol, 1.00 equiv) in acetic acid (119.80 mL), add bromine (5.03 mL, 97.98 mmol, 1.00 equiv)
15 dropwise as a solution in acetic acid (59.90 mL) over 20 min. Stir the reaction mixture at room temperature for 1 hr., until the reaction is complete by LC-MS. Pour the reaction mixture slowly into water (696.50 mL) at room temperature, and collect the resulting precipitate by filtration. Wash with water (100 mL) and dry under vacuum and nitrogen on a pad for 16 hr. to give a white solid of 2-bromo-5-methylthiophene-3-carboxylic acid
20 (20.55 g, 94.88% yield). MS (m/z): 218.89, 220.79 (M-H). ¹H NMR (400.13 MHz, CDCl₃): 7.08 (d, *J* = 1.5 Hz, 1H), 2.42 (s, 3H).

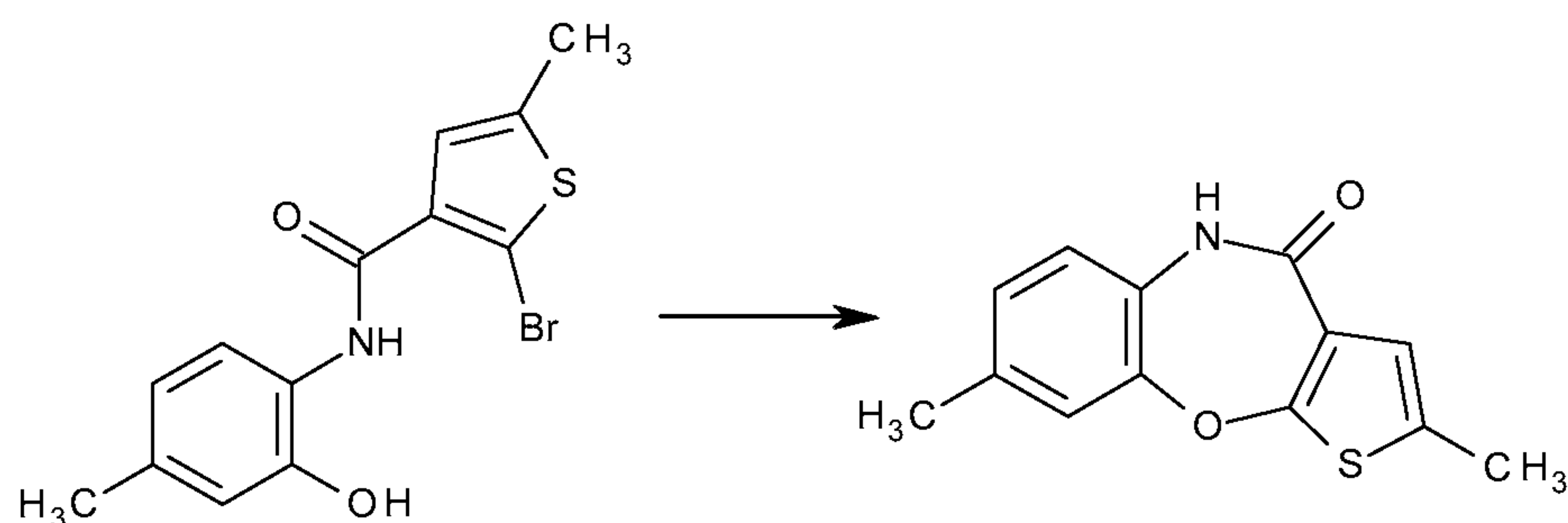
Preparation 4. 2-Bromo-N-(2-hydroxy-4-methyl-phenyl)-5-methylthiophene-3-carboxamide

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To a suspension of 2-bromo-5-methyl-thiophene-3-carboxylic acid (45.2 g, 204.46 mmols, 1.00 equiv) in dichloromethane (316.40 mL) in a 1 L flask, add dimethylformamide (500 μ L), followed by 2M oxalyl chloride in dichloromethane (112.45 mL, 224.90 mmols, 1.1 equiv) dropwise (Caution gas evolution). Stir the reaction mixture at room temperature for 2 hr., until the reaction mixture becomes a clear, dark solution and the reaction is complete by TLC (eluent EtOAc 30% in iso-hexane). Evaporate the solution to dryness and then redissolve the resulting solid in anhydrous tetrahydrofuran (678 mL). Add this solution to a flask containing 6-amino-m-cresol (33.40 g, 265.79 mmols 1.3 equiv), pyridine (82.67 mL, 1.02 moles, 5 equiv) and anhydrous tetrahydrofuran (678 mL). Stir at room temperature for 2 hr. until the reaction is complete by LC-MS. Pour the reaction mixture into 1M aqueous HCl (2.71 L, 60 vol) with stirring and collect the resulting precipitate by filtration. Wash with 1M aqueous HCl (250 mL), then water (250 mL), and dry under vacuum on a filter pad overnight to give a red solid 2-bromo-N-(2-hydroxy-4-methyl-phenyl)-5-methyl-thiophene-3-carboxamide (62.34 g; 93.47% yield). If necessary, the solid can be purified further by washing with methanol. MS (m/z): 325.98, 327.93 (M+H).

Preparation 5. 2,8-Dimethyl-5H-thieno[2,3-b][1,5]benzoxazepin-4-one

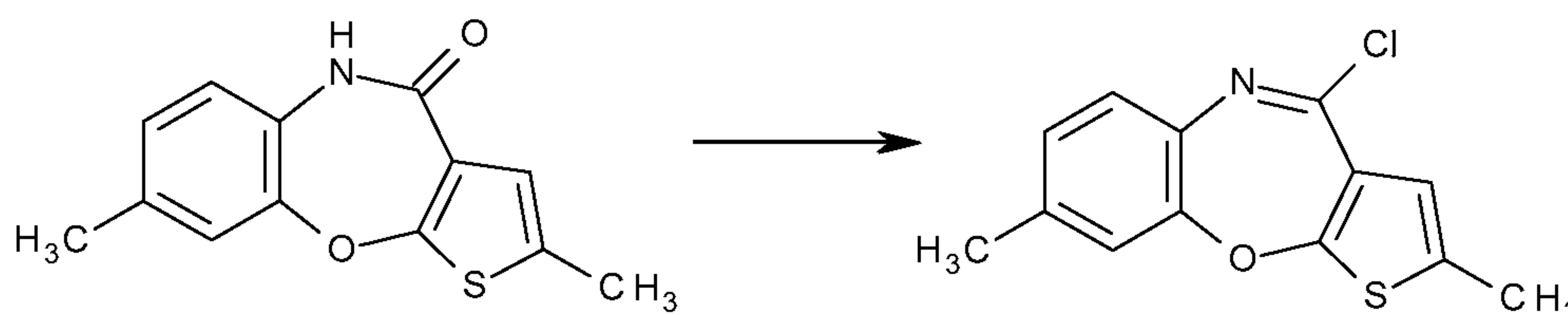


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In a 500 mL flask dissolve 2-bromo-N-(2-hydroxy-4-methyl-phenyl)-5-methyl-thiophene-3-carboxamide (62.34 g, 191.10 mmol, 1.00 equiv) in dimethyl sulfoxide (1.56 L) and add potassium carbonate (79.23 g, 573.30 mmol, 3 equiv). Heat the reaction mixture to 140°C with stirring for 4 hr., until the reaction is complete by LC-MS.

- 5 Cool the reaction mixture to room temperature and then pour into water (2.5 L) controlling the exotherm with an ice bath. Acidify with 2M aqueous HCl (450 mL), extract with dichloromethane (4 x 800 mL) and wash the organic phase with saturated aqueous sodium bicarbonate (1.2 L), then water (2 x 2 L). Dry, filter and concentrate. Purify by flash chromatography on silica gel (750 g) eluting with 0% to 20% ethyl acetate
 10 in dichloromethane to give a solid of 2,8-dimethyl-5H-thieno[2,3-b][1,5]benzoxazepin-4-one (28.1 g; 59.94%). MS (m/z): 246.09 (M+H).

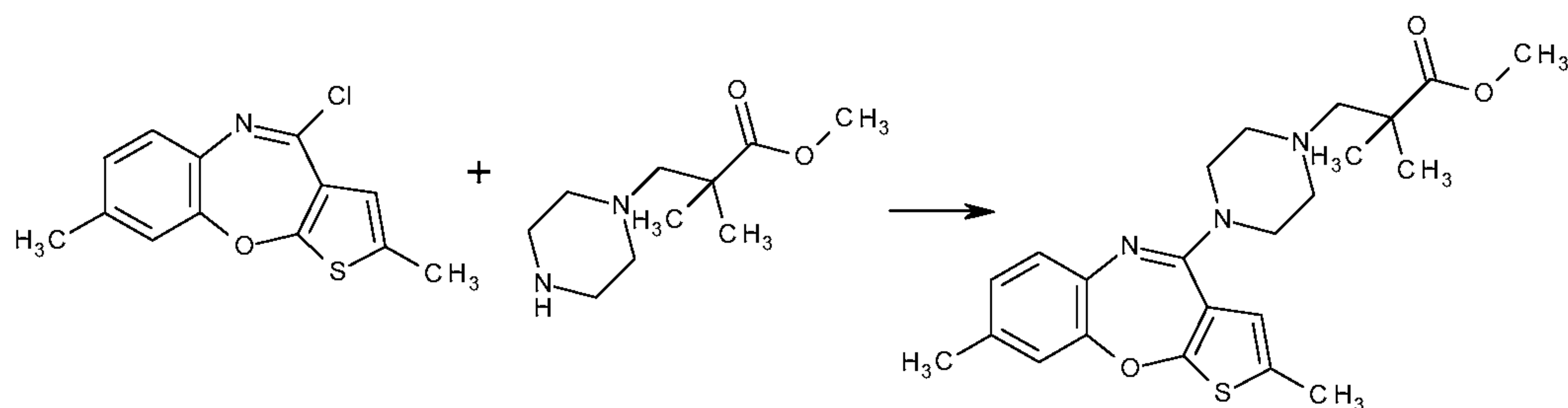
Preparation 6. 4-Chloro-2,8-dimethyl-thieno[2,3-b][1,5]benzoxazepine



- 15 Heat a suspension of 2,8-dimethyl-5H-thieno[2,3-b][1,5]benzoxazepin-4-one (26.84 g, 109.42 mmol, 1.00 equiv) in methoxybenzene (107.36 mL) in a 500 mL flask to about 60°C. Add N,N-dimethylaniline (38.89 mL, 306.37 mmol, 2.8 equiv) in one portion, followed by phosphoryl chloride (23.39 mL, 251.66 mmol, 2.3 equiv) over 15 min. Heat the resulting solution at 100°C for 2 hr., until the reaction is complete by LC-
 20 MS. Cool the reaction mixture slightly and evaporate to give a dark oil of 4-chloro-2,8-dimethyl-thieno[2,3-b][1,5]benzoxazepine (28.85g, assumed quantitative). MS (m/z): 264.0 (M+H).

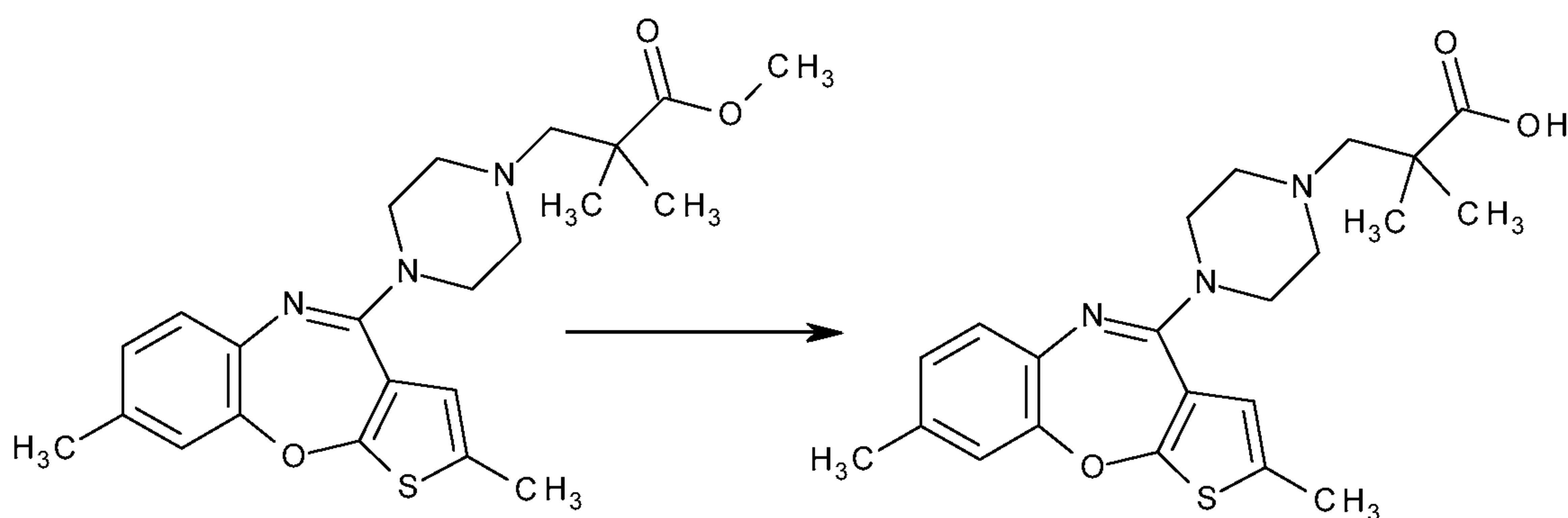
- Preparation 7. Methyl 3-[4-(2,8-dimethylthieno[2,3-b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethyl-propanoate
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To a solution of 4-chloro-2,8-dimethylthieno[2,3-b][1,5]benzoxazepine (28.85 g, 109.39 mmol, 1.00 equiv) in acetonitrile (288.50 mL), add methyl 2,2-dimethyl-3-piperazin-1-ylpropanoate (59.77 g, 218.77 mmol, 2 equiv) and potassium carbonate (136.06 g, 984.47 mmol, 9 equiv). Heat the mixture at a gentle reflux (82°C) for 3 hr., until the reaction is complete by LC-MS. Cool the reaction and evaporate. Then partition between water (300 mL) and ethyl acetate (3 x 300 mL). Wash the combined organic extracts with water (2 x 300 mL), then brine. Dry over sodium sulfate, filter and evaporate. Purify by flash chromatography on silica gel (800 g) eluting with 0% to 30% ethyl acetate in iso-hexane to give a thick oil of methyl 3-[4-(2,8-dimethylthieno[2,3-b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethylpropanoate (37.95 g; 81.14% yield). MS (m/z): 428.1 (M+H).

Example 1. 3-[4-(2,8-Dimethylthieno[2,3-b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethylpropanoic acid

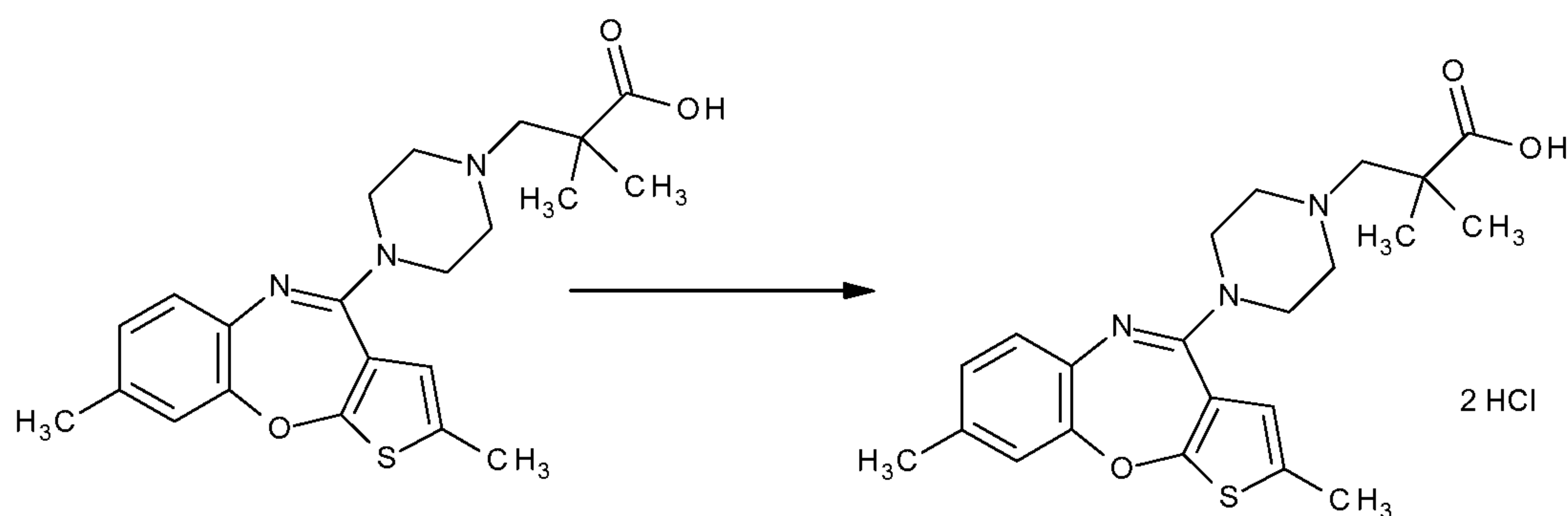


To a slurry of methyl 3-[4-(2,8-dimethylthieno[2,3-b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethylpropanoate (37.95 g, 88.76 mmol, 1.00 equiv) in

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isopropyl alcohol (227.70 mL) and water (227.70 mL), add sodium hydroxide (10.65 g, 266.27 mmol, 3 equiv) and heat the mixture to gentle reflux (85°C) for 1.5 hr., until complete dissolution occurs and the reaction is complete by LC-MS. Cool to room temperature and neutralize to around pH 6.0 - 6.5 using 2M aqueous HCl (approx. 120 mL), to give a precipitated solid. Remove the organic solvent in vacuo to give a suspension. Check the pH of the suspension and if necessary further adjust to pH 6.0-6.5 with 2M aqueous HCl (approx. 15 mL). Filter the suspension and wash with water, then dry overnight under vacuum. Slurry the crude solid material in acetonitrile (100 mL, 2.5vol), sonicate, and then gently heat (40°C) with stirring for 15 min. Filter, wash with acetonitrile (3 x 20 mL), then dry the product under vacuum to give a solid of 3-[4-(2,8-dimethylthieno[2,3-b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethyl-propanoic acid (29.5 g, 80.37% yield). MS (m/z): 414.1 (M+H). ¹H NMR (400.13 MHz, CDCl₃): 6.99 (d, J = 7.8 Hz, 1H), 6.90 (dd, J = 7.8 and 1.5 Hz, 1H), 6.83 (d, J = 1.5 Hz, 1H), 6.28 (s, 1H), 3.67 (br s, 4H), 2.86 (br s, 4H), 2.59 (s, 2H), 2.34 (s, 3H), 2.28 (s, 3H) 1.26 (s, 6H)

Example 2. 3-[4-(2,8-Dimethylthieno[2,3-b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethyl-propanoic acid dihydrochloride



20

To a suspension of 3-[4-(2,8-dimethylthieno[2,3-b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethyl-propanoic acid (300 mg, 725.44 μmol, 1.00 equiv) in isopropyl alcohol (4.50 mL) at 50°C, add 4M HCl in 1,4-dioxane (453.40 μL, 1.81 mmol, 2.5 equiv). Heat the resulting solution to 60°C for 30 min., then cool to room temperature. Evaporate under vacuum, slurry the resulting solid in diethyl ether (4.50

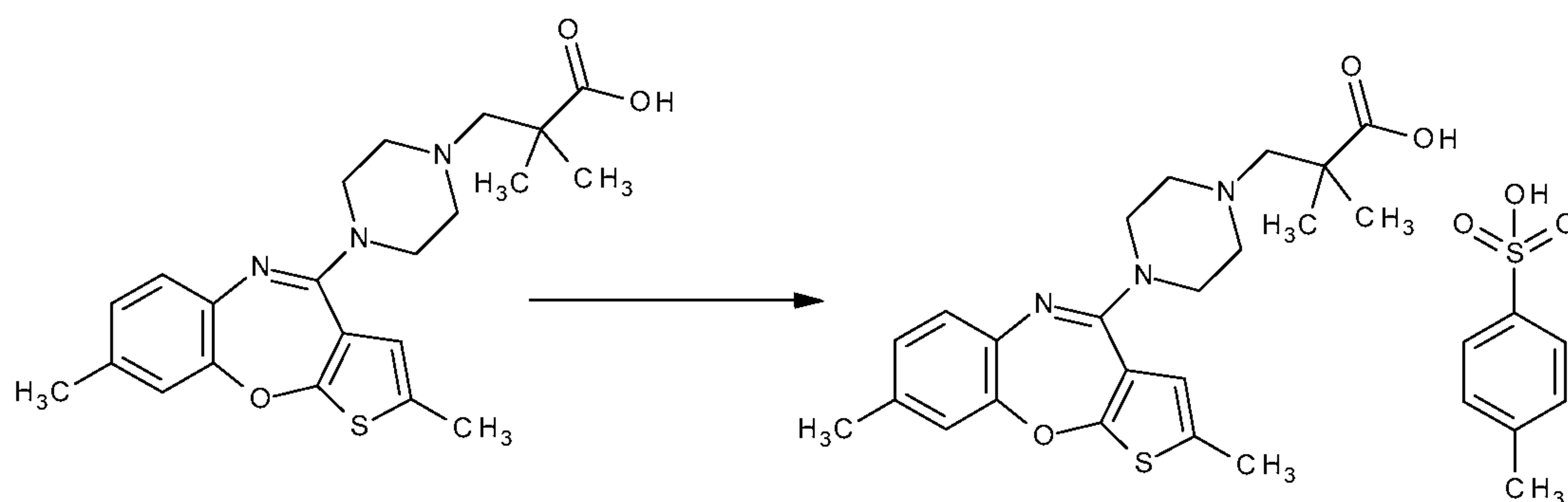
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mL) for 15 min., then collect by filtration, and dry on the pad. Further dry in vacuum oven for 1 hr. to give a crystalline solid of 3-[4-(2,8-dimethylthieno[2,3-b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethyl-propanoic acid dihydrochloride (346 mg, 98.0%). MS (m/z): 414.0 (M+H).

5

Example 3. 3-[4-(2,8-Dimethylthieno[2,3-b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethyl-propanoic acid; 4-methylbenzenesulfonic acid



To a suspension of 3-[4-(2,8-dimethylthieno[2,3-b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethyl-propanoic acid (300 mg, 725.44 μ moles, 1.00 equiv) in ethanol (6 mL) at 50°C, add p-toluenesulfonic acid (124.92 mg, 725.44 μ moles, 1 equiv) and stir the resulting solution at 50°C for 30 min. Cool to room temperature and evaporate under vacuum to give a solid. Slurry the solid in diethyl ether (4.50 mL) for 15 min., then collect by filtration, and dry on the pad. Further dry in vacuum oven for 1 hr. to give a crystalline solid of 3-[4-(2,8-dimethylthieno[2,3-b][1,5]benzoxazepin-4-yl)piperazin-1-yl]-2,2-dimethyl-propanoic acid; 4-methylbenzenesulfonic acid (413 mg, 97%). MS (m/z): 414.0 (M+H).

Literature data (Morairty SR, Hedley L, Flores J, Martin R, Kilduff TS. (2008) *Selective 5-HT_{2A} and 5-HT₆ receptor antagonists promote sleep in rats. Sleep* **31**, 34-44.; and Barbier, A.J., and Bradbury, M.J., *Histaminergic Control of Sleep-Wake Cycles: Recent Therapeutic Advances for Sleep and Wake Disorders*, CNS & Neurological Disorders – Drug Targets, vol 6, pg. 31-43 (2007)) and data generated in non-clinical animal studies support a role for dual activity H1 inverse agonists / 5-HT_{2A} antagonists in

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the treatment of insomnia and in the symptomatic treatment of insomnia associated with other disorders such as depressive disorders, anxiety disorders, pain, allergies, lung or airway disorders, psychiatric disorders, dementia, and/or neurodegenerative diseases, and/or circadian rhythm sleep disorders. Specifically it is found that certain dual activity
5 H1 inverse agonists / 5-HT_{2A} antagonists are effective in increasing total sleep time using EEG monitored rodents without disproportionate or clinically relevant hypoactivity, decrease in REM sleep, or hypersomnolence.

To further demonstrate the characteristics of the present compounds, they may be run in the following *in vitro* and *in vivo* assays:

10

In vitro binding and activity assays:

H1 competition binding assay

[³H]-Pyrilamine binding experiments are carried out in SPA (scintillation
15 proximity assay) 96-well format. Membranes used in this assay are prepared from HEK-293 cells stably expressing recombinant H1 receptor (human). The incubation is initiated by the addition of a mixture of WGA PVT SPA beads (1mg/well, Perkin Elmer (MA, USA) RPNQ0001) and 3 µg membranes to assay buffer (67 mM Tris; pH 7.6) containing 3.5 nM [³H]-Pyrilamine and varying concentrations of the test compound (10 point
20 concentration response curves). Non-specific binding is determined in the presence of 10 µM Triprolidine. Samples are incubated for 4 hr. at room temperature (22° C) and then read in a Microbeta Trilux.

5-HT_{2A} competition binding assay

[³H]-Ketanserin binding experiments are carried out in SPA 96-well format.
25 Membranes used in this assay are prepared from AV-12 cells stably expressing recombinant 5-HT_{2A} receptor (human). The incubation is initiated by the addition of a mixture of WGA YSi SPA beads (1mg/well, Perkin Elmer (MA, USA), RPNQ0011) and 2 µg membranes to assay buffer (67 mM Tris, 0.5 mM EDTA; pH 7.6) containing 3.1 nM
30 [³H]-Ketanserin and varying concentrations of the test compound (10 point concentration response curves). Non-specific binding is determined in the presence of 20 µM 1-(1-

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1-Naphthyl) piperazine. Samples are incubated for 4 hr. at room temperature (22° C) and then read in a Microbeta Trilux.

5-HT_{2C} competition binding assay

5 [¹²⁵I]-(±)DOI binding experiments are carried out in SPA 96-well format. Membranes used in this assay are prepared from AV-12 cells stably expressing recombinant 5-HT_{2C} receptor (human). The incubation is initiated by the addition of a mixture of WGA PVT SPA beads (0.5 mg/well, Perkin Elmer (MA, USA), RPNQ0001) and 2.5 µg membranes to assay buffer (50 mM Tris-HCl, 10 mM MgCl₂, 0.5 mM EDTA, 10 10 µM pargyline, 0.1% ascorbic acid, pH7.4) containing 0.2 nM [[¹²⁵I]-(±)DOI and varying concentrations of the test compound (10 point concentration response curves). Non-specific binding is determined in the presence of 20 µM 1-(1-Naphthyl) piperazine. Samples are incubated for 4 hr. at room temperature (22° C) and then read in a Microbeta Trilux.

15

Binding data analysis

Curves are evaluated using a 4-parameter logistic nonlinear equation to obtain the concentration of competitor causing 50% inhibition of radioligand binding (IC₅₀). Equilibrium dissociation constants (K_i) are calculated according to the equation K_i = 20 IC₅₀/(1+L/K_d), where L equals the concentration of radioligand used in the experiment and K_d equals the equilibrium dissociation constant of the radioligand for the receptor, determined from standard saturation analysis or homologous competition experiments. Reported values for K_i, where n values are indicated, are shown as geometric mean ± the standard error of the mean (SEM), with the number of replicate determinations indicated 25 by n. Geometric means are calculated by the equation $\text{GeoMean} = 10^{(\text{Average}(\log K_i 1 + \log K_i 2 + \dots \log K_i n)/\text{sqrt } n)}$.

GABA_A antagonism using native receptors in primary neuronal cultures

Activity of compounds on native GABA_A receptors is evaluated by monitoring 30 calcium fluxes using a 96 well format FLIPR® system (Fluorometric Imaging Plate Reader (FLIPR®, Molecular Devices). Briefly, cortical embryonic neurons are dissociated from E18 rat embryos and plated at optimum density into black-walled,

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transparent bottom poly-D-lysine coated 96-well FLIPR® plates. After loading the cells with a calcium sensitive dye (Fluo4-AM, Molecular Devices), the cells are bathed in a solution containing low chloride (chloride replaced by gluconate). Under these conditions activation of GABA_A receptors causes an efflux of chloride ions (in the direction of the chemical gradient), which results in membrane depolarization and consequently activation of voltage gated calcium channels (VGCCs). Calcium influx through VGCCs is recorded and analysed offline using the FLIPR® system. For a pharmacological validation of the assay, concentration response curves (CRC) are recorded for the standard agonist (GABA) and standard antagonist (Gabazine). Any effects are determined in CRC mode against a fixed concentration of agonist GABA at 10 µM (equivalent to an EC₉₀ GABA response).

Methods:

The antagonist effects of compounds are quantified using 10-point dose response curves by comparing the peak fluorescent responses to the agonist GABA in the presence and absence of compound. The assay window is defined as the maximal response obtained by GABA at its predetermined EC₉₀ concentration minus the response obtained by a fully inhibiting concentration of gabazine (50 µM). Antagonist effects are calculated as a percent of the assay window. All data are calculated as relative IC₅₀ values using a four-parameter logistic curve fitting program (Prism Graphpad® 3.01). Antagonist potencies for all compounds are compared to gabazine with three replicates in each assay run.

Further, the compounds of the invention may be tested in binding assays and functional activity assays by well known methods for other physiologically important receptors such as, but not limited to, the hERG channel, other serotonin receptors (specifically 5-HT_{1B}, 5-HT_{1D}, receptors, lack of agonist activity at 5-HT_{2B} receptors, 5-HT_{2C}, 5-HT₅, 5-HT₆, and 5-HT₇ receptors), dopaminergic receptors (specifically D1, D2, and D3), GABA_A receptors, adrenergic receptors and monoamine transporters.

The compound of example 2 is tested essentially as described above and is found to have activity profiles as shown in Table 1.

Table 1. Selectivity data

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	Example 2
H1 K _i (nM)	131
5-HT _{2A} K _i (nM)	11.3
5-HT _{2B} K _i (nM)	95.8
5-HT _{2B} Agonist EC ₅₀ (nM)	>10,000
5-HT _{2B} Antagonist K _b (nM)	45
5-HT _{2C} K _i (nM)	883
GABA _A IC ₅₀ (μM)	98
hERG Channel (μM)	>100
Dopamine D ₁ K _i (nM)	2,350
Dopamine D ₂ K _i (nM)	2,480
Dopamine D ₃ K _i (nM)	>5,660
5-HT _{1B} K _i (nM)	ND
5-HT _{1D} K _i (nM)	>3,980
5-HT ₅ K _i (nM)	>8,830
5-HT ₇ K _i (nM)	>2,060
Adrenergic alpha _{1A} K _i (nM)	>10,500
Adrenergic alpha _{1B} K _i (nM)	>16,200
Adrenergic alpha _{2A} K _i (nM)	>9,020
Adrenergic alpha _{2B} K _i (nM)	3,710
Adrenergic alpha _{2C} K _i (nM)	>5,550
Serotonin Transporter K _i (nM)	>651
Norepinephrine Transport K _i (nM)	>674
Dopamine Transporter K _i (nM)	>877

Therefore, physiologically relevant doses of the compounds of the invention are expected to provide substantial inhibition of H1 and 5-HT_{2A} receptors in vivo, while not substantially interacting with other physiologically relevant receptors, and thus are

5 expected to provide the desired pharmacology while avoiding undesired effects associated

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with off-target activity. Such undesired effects include, but are not limited to the following: 5-HT_{2C} antagonist activity associated with treatment emergent weight gain, 5-HT_{2B} agonist activity associated with valvulopathy, hERG channel modulation associated with QT prolongation, and GABA_A antagonist activity associated with seizure activity. Furthermore, interference with sleep/wake physiology is avoided by the selectivity over dopamine receptors, other serotonin receptors, adrenergic receptors, and monoamine transporters.

5-HT_{2A} Receptor Occupancy: Receptor occupancy (RO) is assayed to demonstrate antagonist/inverse agonist activity at the 5-HT_{2A} Receptor *in vivo*. Briefly, male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing approximately 230-280 grams are given ad lib access to food and water until the beginning of the 3-hr. experimental protocol. 1 mg/kg ketanserin (non-selective 5-HT_{2A} antagonist) is used as a positive control to establish assay validity. Test compounds or control are administered by oral gavage in a vehicle comprised of 20% hydroxypropyl beta-cyclodextrin. MDL 100907 ((R)-(+)- α -(2,3-Dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol), a selective 5-HT_{2A} antagonist, is used as a tracer. MDL 100907 is suspended in water with 5 μ l dilute lactic acid (1 mg/ml), diluted to 6 μ g/ml with saline, and administered in a volume of 1 mL/kg intravenously via the lateral tail vein to yield a tracer dose of 3 μ g/kg. Rats are administered test compound, ketanserin, or vehicle (N = 4), followed 1 hr. later with an intravenous, 3 μ g/kg tracer dose of MDL 100907. It is at the time of tracer administration that RO is considered to be measured. Fifteen min. after tracer administration, rats are sacrificed by cervical dislocation. Plasma samples are collected and samples of the frontal cortex and cerebellum are removed. The level of MDL 100907 tracer is measured in each cortical and cerebellar sample. RO is calculated using the well-established ratio method which employs a region of high receptor density representative of total binding (frontal cortex) normalized by an area without or with very low levels of receptor (cerebellum). This region, referred to as the null region, represents nonspecific binding of the ligand probe. Vehicle ratio of the tracer levels in cortex relative to cerebellum represents 0% occupancy. A ratio of 1 represents 100% occupancy and is achieved when all specific binding to the 5-HT_{2A} receptor of the MDL 100907 tracer is blocked. The intermediate ratios of cortical to cerebellar tracer from the test

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compound pretreated group are interpolated linearly between the ratio of tracer levels in the vehicle-treated animals (0% occupancy) and a ratio of 1 (100% occupancy) in order to determine the percent 5-HT_{2A} RO.

5 MDL 100907 Analysis: Cortex and cerebellar samples are weighed and placed in conical centrifuge tubes on ice. Four volumes (w/v) of acetonitrile containing 0.1% formic acid is added to each tube. The samples are then homogenized and centrifuged at 14,000 RPM (21,920 x g) for 16 min. Supernatant is diluted by adding 100 - 900 µL sterile water in HPLC injection vials for LC/MS/MS analysis. Analysis of MDL 100907 is carried out
10 using an Agilent model 1200 HPLC (Agilent Technologies, Palo Alto, CA) and an API 4000 mass spectrometer. The chromatographic separation is on a 2.1 X 50 mm C18 column (Agilent part number 971700-907) with a mobile phase consisting of 60% acetonitrile in water with an overall 0.1% formic acid content. Detection of MDL 100907 is accomplished by monitoring the precursor to product ion transition with a mass to
15 charge ratio (m/z) of 374.2 to 123.0. Standards are prepared by adding known quantities of analyte to brain tissue samples from non-treated rats and processing as described above.

Statistical Methods: Curves for each study are fitted to a 4 parameter logistic function
20 with the bottom fixed at 0% using JMP® version 8.0 (SAS Institute Inc, Cary NC) and the absolute ED₅₀ is calculated by the software. Values are given as means, standard errors and 95% confidence intervals. The compound of Example 2 is tested essentially as described and is found to achieve high 5-HT_{2A} receptor occupancy with an ED₅₀ of 0.09 mg/kg.

25

Inhibition of DOI Induced Headshake Activity: The *in vivo* 5-HT_{2A} receptor antagonist activity of the compound of the present invention is further demonstrated by its ability to block head shaking activity induced by the 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI). (see for example Bartoszyk GD, van Amsterdam C, Böttcher
30 H, Seyfried CA. EMD 281014, a new selective serotonin 5-HT_{2A} receptor antagonist. *Eur J Pharmacol.* 2003 **473**: 229-230.) Briefly, male C57BL/6J mice (20-25 g, Charles

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River) are housed in standard housing conditions (32 mice in a large IVC cage, 07.00 to 19.00 light phase, constant temperature (19-23°C) and humidity (50% +/-10), ad lib food and water). Mice received either vehicle (0.25% Methyl cellulose), DOI (3 mg/kg in saline) or test compound at 10mg/kg PO plus DOI (3 mg/kg in saline). Test compounds are individually evaluated in groups of four per experiment with n=4 for each compound, together with vehicle and DOI+vehicle (n=8). After a test compound pre-treatment time of 60 min. the mice receive either vehicle (saline) or 3 mg/kg DOI dosed subcutaneously, and are then placed into clear perspex observation chambers. Five min. after DOI or vehicle administration the number of visually scored head shakes exhibited by each individual mouse is counted for 15 min. The data is analyzed using an ANOVA and post-hoc Dunnet's Test. The compound of example 2 is tested essentially as described and is found to inhibit the DOI induced headshake response at 100% at 10 mg/kg.

Sleep and behavioral monitoring in rats: The compound of the present invention is tested in rats for its ability to increase the amount of sleep or decrease sleep interruption or both without undesired effects such as inhibition of REM sleep, waking motor impairment, and/or rebound insomnia. Test animals are continuously monitored by electroencephalograms (EEG), electromyograms (EMG), and motion to measure cumulative nonREM sleep, cumulative total sleep, average sleep bout duration, longest sleep bout duration, rebound insomnia, REM sleep inhibition and locomotor activity intensity during wakefulness. Methods for such studies are known in the art (see for example methods described in Edgar DM, Seidel WF. Modafinil induces wakefulness without intensifying motor activity or subsequent rebound hypersomnolence in the rat. *J Pharmacology & Experimental Therapeutics* 1997; **283**: 757-769; van Gelder RN, Edgar DM, Dement WC. Real-time automated sleep scoring: validation of a microcomputer-based system for mice. *Sleep* 1991, **14**: 48-55; and Gross BA, Walsh CM, Turakhia AA, Booth V, Mashour GA, Poe GR. Open-source logic-based automated sleep scoring software using electrophysiological recordings in rats. *J Neurosci Methods*. 2009; **184(1)**:10-8.) Studies are conducted as follows:

30

Animal preparation. Adult, male Wistar rats (approximately 270-300 g at time of surgery) are surgically fitted for chronic recording of EEG, EMG, and motion as follows:

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Rats are surgically prepared with a cranial implant consisting of four stainless steel screws for EEG recording (two frontal [3.9 mm anterior from bregma, and ± 2.0 mm mediolaterally] and two occipital [6.4 mm posterior from bregma, ± 5.5 mm mediolaterally]), and with two Teflon-coated stainless steel wires for EMG recording (positioned under the nuchal trapezoid muscles). All leads are soldered to a miniature connector (Microtech, Boothwyn, PA) prior to surgery. The implant assembly is affixed to the skull by the combination of the stainless steel EEG recording screws, cyanoacrylate applied between the implant connector and skull, and dental acrylic. Locomotor activity is monitored via a miniature transmitter (Minimitter PDT4000G, Philips Respironics, Bend, OR) surgically placed into the abdomen. At least 3 weeks are allowed for recovery.

Recording environment. Each rat is housed individually within a microisolator cage modified with an inserted polycarbonate filter-top riser to allow more vertical headroom. A flexible cable that minimally restricts movement is connected at one end to a commutator affixed to the cage top and at the other end to the animal's cranial implant. Each cage is located within separate, ventilated compartments of a stainless steel sleep-wake recording chamber. Food and water are available *ad libitum* and the ambient temperature is maintained at about $23 \pm 1^\circ\text{C}$. A 24-hr light-dark cycle (LD 12:12) using fluorescent light is maintained throughout the study. Relative humidity averages approximately 50%. Animals are undisturbed for at least 30 hrs before and after each treatment.

Study design and dosing. The vehicle (placebo, methylcellulose 15 centipoise 0.25% in water) or one of the test compound dose levels is administered orally at 1 mL/kg pseudo-randomly such that no rat receives the same treatment twice, and no rat receives more than two of the 8 treatments in any one study. Each rat is removed from its cage for about a minute to be weighed and treated. At least 6 days "washout" period precede and follow each treatment.

30

Data collection. Sleep and wakefulness discrimination may be automated (e.g., Van Gelder et al. 1991 (above); Edgar et al. 1997 (above); Winrow CJ, et al.,

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Neuropharmacology 2010; **58(1)**:185-94.; and Gross et al., 2009 (above). EEG is amplified and filtered (X10,000, bandpass 1-30 Hz), EMG is amplified and integrated (bandpass 10-100 Hz, RMS integration), and non-specific locomotor activity (LMA) is monitored simultaneously. Arousal states are classified in 10 second epochs as non-REM sleep, REM sleep, wakefulness, or theta-dominated wakefulness. Locomotor activity (LMA) is recorded as counts per minute and is detected by commercially available telemetry receivers (ER4000, Minimitter, Bend, OR).

Statistical Analysis. All animals having at least one outcome are included in the summary results (for example, we include appropriate data from an animal treatment for which telemetry data is usable but EEG data is not). The post-treatment observation period is divided into post-dosing intervals appropriate to each Outcome, where the time of dosing is defined as the start of Hour = 0, and outcomes are summarized in the observation period by computing either the mean hourly or the cumulative value across each period (see legend of Table 2 for precise definition of each Outcome). Sleep bouts are analyzed on the log scale to stabilize the variation, all other variates are analyzed on the linear scale. Each outcome in each period is analyzed by analysis of covariance using treatment group and treatment date as factors and the corresponding pre-treatment interval, 24 hrs earlier, as the covariate. Adjusted means and the change from vehicle means and their corresponding standard errors are summarized for each treatment group. Outcomes analyzed on the log scale are back-transformed to report geometric means and mean ratio-to-vehicle results.

The compound of Example 2 is tested essentially as described. The compound of Example 2 is found to significantly increase cumulative NREM sleep time and cumulative total sleep time without significant rebound insomnia, or inhibition of locomotor intensity (LMI) at 3 mg/kg. REM sleep inhibition, however, cannot be ruled out by these data. (See sleep profile and locomotor activity intensity in Table 2.)

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Table 2. Compound of Example 3.

Dose (mg/kg PO)	<u>Efficacy variables</u>			<u>Undesired effect variables</u>		
	<u>Cumulative NREM sleep</u>			<u>Rebound Insomnia</u>		
	<i>N</i>	Adj.Mean	SE	<i>N</i>	Adj.Mean	LCL
10	4	36.7	8.8	4	0.4	-9.0
3	8	29.2	6.9	8	-1.3	-8.5
1	10	31.9	6.5	10	-3.8	-10.6
0.5	9	21.7	6.8	9	-0.3	-7.4
0.25	4	34.3	9.1	4	1.0	-8.2
0.025	4	1.3	8.9	4	0.3	-9.0

Dose (mg/kg PO)	<u>Cumulative Total sleep</u>			<u>REM inhibition</u>		
	<i>N</i>	Adj.Mean	SE	<i>N</i>	Adj.Mean	LCL
	<i>N</i>	Adj.Mean	SE	<i>N</i>	Adj.Mean	LCL
10	4	32.8	9.9	4	-12.1	-20.9
3	8	26.9	7.8	8	-4.2	-11.2
1	10	30.8	7.3	10	0.1	-6.4
0.5	9	21.7	7.9	9	0.7	-6.1
0.25	4	33.5	10.3	4	-1.5	-10.4
0.025	4	-2.3	10.1	4	-2.7	-11.6

Dose (mg/kg PO)	<u>Average Sleep Bout</u>			<u>Locomotor Activity Intensity</u>		
	<i>N</i>	Adj.Mean	SE	<i>N</i>	Adj.Mean	LCL
	<i>N</i>	Adj.Mean	SE	<i>N</i>	Adj.Mean	LCL
10	4	1.7	0.3	4	-3.7	-6.5
3	8	1.5	0.2	8	-2.6	-4.8
1	10	1.7	0.2	10	-2.5	-4.6
0.5	9	1.6	0.2	9	0.0	-2.2
0.25	4	1.6	0.2	4	-1.6	-4.4
0.025	4	1.0	0.1	4	-0.1	-2.9

Dose (mg/kg PO)	<u>Longest Sleep Bout</u>					
	<i>N</i>	Adj.Mean	SE			
	<i>N</i>	Adj.Mean	SE			
10	4	1.9	0.4			
3	8	1.9	0.3			
1	10	2.2	0.3			
0.5	9	2.1	0.3			
0.25	4	1.6	0.3			
0.025	4	1.0	0.2			

Table 2. Outcome statistics: Abbreviations: *N* = sample size; Adj.Mean = adjusted group mean value relative to vehicle controls; SE = standard error of

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the mean; LCL = lower 95% confidence limit, NREM = non-REM, *i.e.*, all sleep other than REM sleep. The parallel reference vehicle group sample size was $N=27$.

Definitions and units — means are adjusted differences from vehicle controls:

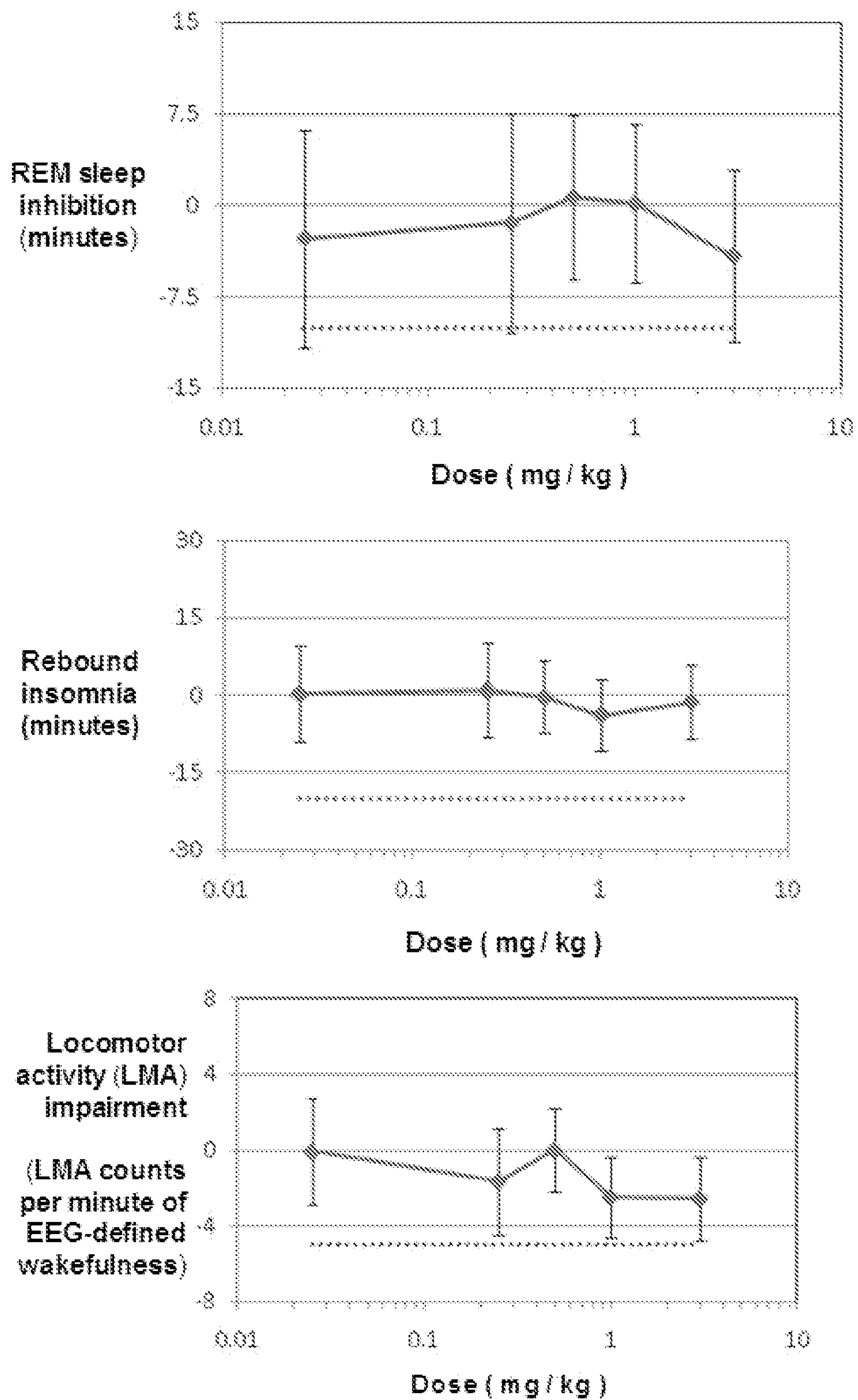
- 5 • Cumulative sleep: across the first 6 hr. post-treatment, in minutes ('Total sleep' denotes NREM sleep + REM sleep).
- Average sleep bout: average of hourly-averaged sleep bouts, across the first 6 hr. post-treatment, expressed as n -fold increase over vehicle controls.
- 10 • Longest sleep bout: the longest sleep bout in the first 6 hr. post-treatment, expressed as n -fold increase over vehicle controls.
- Rebound insomnia: cumulative minutes of NREM+REM sleep during the first 3 hr. of the lights on period, *i.e.*, 7th, 8th and 9th hours post-treatment.
- REM inhibition: cumulative minutes of REM sleep during the first 12 hr. post-treatment.
- 15 • Locomotor Activity (LMA) Intensity: expressed as LMA counts per minute of EEG-defined wakefulness, averaged across the first 6 hr. post-treatment.

Determining efficacy. The threshold efficacy for each of the four efficacy variables is calculated by plotting the increase in each variable relative to vehicle controls during the
 20 6 hr. period after treatment against log(dose). The threshold efficacy for each variable is that dose, estimated by 4 parameter logistic nonlinear regression, which gives the defined efficacy threshold value; +30 min. of additional accumulated non-REM sleep, +25 min. of additional accumulated total sleep, 1.75x increase in average sleep bout duration, and 1.5x increase in longest sleep bout duration. The compound of example 2 is found to
 25 have threshold efficacious doses as shown in Table 3.

Table 3	Estimated efficacy dose (mg/kg)	95% confidence interval (mg/kg)
NREM accumulation = 30 min.	0.05	0.04-n.e.*
total sleep accumulation = 25 min.	0.07	0.03-0.09
longest sleep bout (1.75-fold increase)	0.26	0.07-0.20
average sleep bout (1.5-fold increase)	0.21	0.03-0.06

*n.e. = not estimable, statistically.

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Determining undesired effects. Each 'undesired effect' outcome variable (see Table 2

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legend for definitions), is plotted against log(dose). The threshold value for REM sleep inhibition is defined as a cumulative reduction of REM sleep of -10 min. The threshold value for rebound insomnia is defined as -20 min. The threshold value for reduced LMI is defined as -5 locomotor activity counts per minute of EEG-defined wakefulness. A
5 significant undesired effect is defined to occur when the lower confidence limit goes below the threshold value at any dose at or below 10 times the average efficacious dose, *and* a dose response trend is evident for doses above the threshold efficacy dose. For the compound of Example 2, REM sleep inhibition exceeded the threshold value at 3 mg/kg, but this dose is more than 10 times the most conservative efficacy dose of 0.26
10 mg/kg (Table 3); apparent REM sleep inhibition at 0.25 mg/kg and 0.025 mg/kg are not part of a dose related trend and are not statistically significant at those doses, and may therefore be considered an artifact of the small sample size at those doses; nevertheless, REM sleep inhibition cannot be ruled out by these data. It is concluded that no undesired occurrences of REM rebound insomnia, or reduction in LMI are observed within 10 times
15 the most conservative efficacy dose, but REM sleep inhibition cannot be ruled out. Negative values indicate REM inhibition, rebound insomnia and reduced LMI, respectively.

In further studies, the compounds of the present invention may be coadministered with selective serotonin reuptake inhibitors to demonstrate a potentiation of their effect on
20 non-rapid eye movement sleep (NREM sleep) and sleep maintenance. The compound of Example 2 is coadministered with citalopram in rat sleep studies essentially as described above for the compound alone, and is found to significantly increase NREM sleep at significantly lower doses.

25 Plasma Clearance: It is important in a compound useful for treating sleep disorders such as insomnia that it be adequately cleared from the body with a favorable rate of clearance to avoid unwanted effects such as prolonged somnolence beyond the desired sleep period, daytime sleepiness, impaired cognition after waking, etc. The present invention provides compounds with improved rates of clearance. Rate of clearance can be assayed
30 essentially as described below.

Male Sprague Dawley rats (body weight range 250-320 g) with indwelling femoral arterial cannulae are obtained from Charles River, Wilmington, MA 01887, USA.

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Test compound is administered intravenously in solution (1 mL/kg) in 20% Captisol[®] in 22.5 mM phosphate buffer, pH 2, at a final drug concentration of 1.0 mg/mL (free base equivalents). Blood samples are obtained using the indwelling cannula over 24 hr. Samples of plasma are obtained by centrifugation and stored frozen (-20°C), or on dry ice, prior to analysis.

Male Beagle dogs (body weight range 10-12 kg) are obtained from Marshall Bioresources, USA. Test compound is administered intravenously in solution (1 mL/kg) in 20% Captisol[®] in 22.5 mM phosphate buffer, pH 2, at a final drug concentration of 1.0 mg/mL (free base equivalents). Blood samples are obtained from the jugular vein over 24 hr. Samples of plasma are obtained by centrifugation and stored frozen (-20°C) prior to analysis.

Frozen plasma samples are thawed to room temperature for bioanalysis of concentrations of test compound. A related internal standard compound in acetonitrile/methanol (1:1, v/v) is added to all samples of plasma (1:1, v/v). The samples are centrifuged to remove precipitated protein prior to analysis. The supernatants are analysed by injection and rapid gradient elution on a Javelin Betasil C18 column (20 x 2.1 mm cartridge, Mobile phase A: Water/ 1 M NH₄HCO₃ , 2000:10 v/v, Mobile Phase B: MeOH/ 1 M NH₄HCO₃ , 2000:10 v/v). The eluted analytes are detected by LC-MS-MS analysis using a Sciex API 4000 triple quadrupole mass spectrometer. Concentrations of compound are determined from standards prepared and analysed under identical conditions. Clearance is calculated using non-compartmental analysis in Watson 7.4, Thermo Fisher Scientific, Inc.

$$\text{Clearance} = \frac{\text{Dose}}{\text{Area under curve for plasma concentration / time}}$$

The compound of Example 2 is run essentially as described and is found to have a favorable clearance profile:

	Example	Clearance (mL/min./Kg)	
		Rat	Dog
30	2	27 (+/- 7.3, n=3)	2.8 (+/- 1.2, n=3)

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While it is possible to administer the compounds as employed in the methods of this invention directly without any formulation, the compounds are usually administered in the form of pharmaceutical compositions comprising the compound, or a pharmaceutically acceptable salt thereof, as an active ingredient and at least one pharmaceutically acceptable carrier, diluent and/or excipient. These compositions can be administered by a variety of routes including oral, sublingual, nasal, subcutaneous, intravenous, and intramuscular. Such pharmaceutical compositions and processes for preparing them are well known in the art. See, e.g., Remington: The Science and Practice of Pharmacy (University of the Sciences in Philadelphia, ed., 21st ed., Lippincott Williams & Wilkins Co., 2005).

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.1 to about 60 mg, more usually about 0.5 to about 30 mg, as for example between about 1 and about 10 mg of the active ingredient. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with at least one suitable pharmaceutically acceptable carrier, diluent and/or excipient.

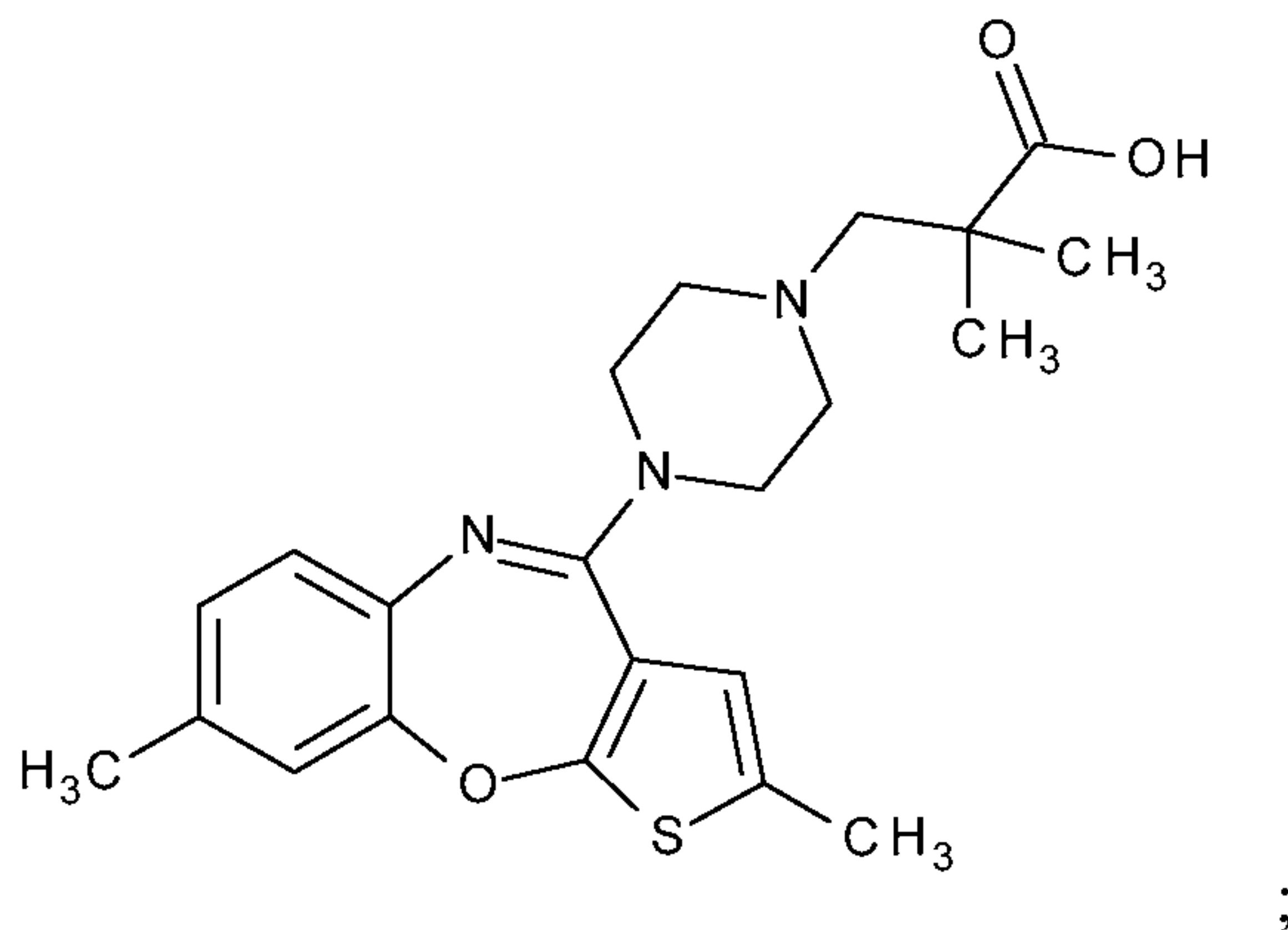
The compounds of Formula I are generally effective over a wide dosage range. For example, dosages per day normally fall within the range of about 0.002 to about 1.0 mg/kg, more usually from about 0.008 to 0.5 mg/kg, and as for example between 0.015 and 0.15 mg/kg of body weight. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. It will be understood that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound or compounds administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms.

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We Claim:

1. A compound of the formula



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or a pharmaceutically acceptable salt thereof.

2. A compound according to Claim 1 which is an HCl salt.

10 3. A compound according to Claim 1 which is a tosylate salt.

4. A pharmaceutical composition comprising a compound according to Claim 1, or a pharmaceutically acceptable salt thereof, in combination with at least one pharmaceutically acceptable carrier, diluent, or excipient.

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5. A method of treating insomnia in a mammal comprising administering to a mammal in need of such treatment an effective amount of a compound according to Claim 1, or a pharmaceutically acceptable salt thereof.

20 6. The method of Claim 5 where the mammal is a human.

7. The method of Claim 5 where the insomnia is characterized by difficulties in sleep onset or sleep maintenance or both.

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8. The method of Claim 7 where the mammal is a human.

9. A compound according to Claim 1, or a pharmaceutically acceptable salt thereof, for use in therapy.

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10. A compound according to Claim 1, or a pharmaceutically acceptable salt thereof, for use in the treatment of insomnia.

11. The compound for use according to Claim 10, or a pharmaceutically acceptable salt thereof, where the insomnia is characterized by difficulties in sleep onset or sleep maintenance or both.

12. The compound for use according to either Claim 10 or 11 in a human.

13. The use of a compound according to Claim 1, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of insomnia.

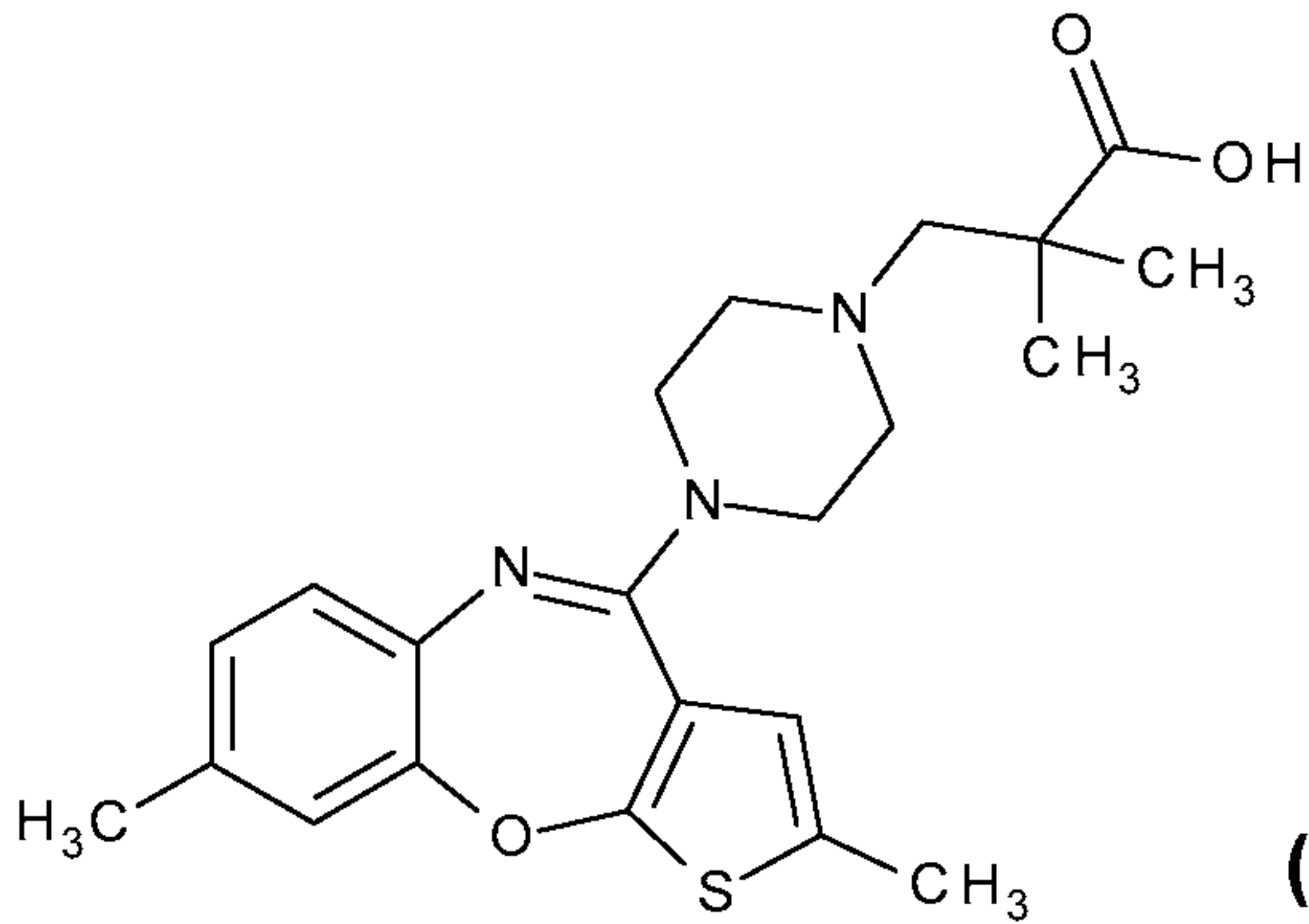
14. The use according to Claim 13, where the insomnia is characterized by difficulties in sleep onset or sleep maintenance or both in a mammal.

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15. A pharmaceutical composition comprising a compound according to claim 1, or a pharmaceutically acceptable salt thereof, in combination with at least one pharmaceutically acceptable carrier, excipient or diluent, and optionally other therapeutic ingredients.

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16. The pharmaceutical composition of Claim 15 where the other therapeutic ingredient comprises a selective serotonin reuptake inhibitor.



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