Title: TREATMENT OF COGNITIVE IMPAIRMENT WITH COMBINATION THERAPY

Abstract: Treatment of varying degrees of cognitive impairment associated with Alzheimer's disease with a combination of a phosphodiesterase 4 inhibitor and an acetylcholinesterase inhibitor, including roilumilast and donepezil hydrochloride.
Treatment of Cognitive Impairment with Combination Therapy

Field of the Invention

The present invention is directed to therapies for the treatment of cognitive impairment. More particularly, the present invention is directed to the treatment of cognitive impairment associated with Alzheimer's disease with a combination of (1) a phosphodiesterase 4 inhibitor selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide, and (2) an acetylcholinesterase inhibitor selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

Background of the Invention

Decline in cognitive function is a common occurrence in the aging population. Cognitive impairment has a negative impact on daily activities and quality of life. (Mattson MP et al; Physiol Rev Vol. 82, 2000, pp 637-672). The loss of cognitive function is pronounced and severe in patients suffering from pathological conditions such as Alzheimer's disease or other types of dementia. Further, prominent cognitive deficits are also present in depressed and schizophrenic patients (Blaney PH; Psychol Bull Vol 99, 1986, pp 229-246. Frith C; BR Med Bull, Vol 52, 1996, pp 618-626). Cognitive impairment has a significant impact on the quality of life of these patients. Hence, it is of critical importance that strategies and therapeutics to counteract cognitive decline are developed.

Phosphodiesterases have recently gained increased attention as potential new targets for cognition enhancement. Phosphodiesterases are enzymes that hydrolyze cyclic AMP (cAMP) and/or cyclic GMP (cGMP) in various cell types, including the brain. Evidence is accumulating that second messenger molecules, cGMP and cAMP, are important in memory processes in general and long-term potentiation in particular.

Several acetylcholinesterase inhibitors have been approved between 1997 and 2001 for the treatment of dementia in patients with mild, moderate or severe Alzheimer disease.

In the International patent applications WO2002074726, WO2005061 458, WO20061 10588 and WO2006044528 combinations of phosphodiesterase 4 inhibitors with inter alia phosphodiesterase 10 inhibitors, calcium channel blockers, adenosine receptor modulators, NMDA receptor modulators, acetylcholinesterase inhibitors, phosphatidylserine, melatonin, vitamin B6, Vitamin B12, vitamin C or vitamin E are mentioned as allegedly being useful for the treatment of cognitive impairment.

Side effects noted with the administration of acetylcholinesterase inhibitors include diarrhea, nausea and vomiting. The incidence and the severity of these side effects increases with the dose amount and are in general more pronounced at the initiation of the treatment or after dose increases. Side effects noted in connection with the administration of phosphodiesterase 4 inhibitors mainly include diarrhea, nausea and headache. As with the administration of acetylcholinesterase inhibitors, the incidence and the severity of the side effects following phosphodiesterase 4 inhibitor administration is correlated with the dose strength and occur more pronounced at the initiation of the treatment.

It is an object of the present invention to increase the efficacy in the treatment of cognitive impairment while at the same time keeping the side effects on a minimal level by using a combination of an acetylcholinesterase inhibitor with a particular low dose of a phosphodiesterase 4 inhibitor for the treatment of cognitive impairment.

Definitions

As used herein, the phrase "therapeutically effective amount" refers to the amounts of active compounds that elicit the biological or medicinal response that is being sought in a tissue, system, animal, individual or human by a researcher, veterinarian, medical doctor or other clinician, which includes one or more of the following:

(1) inhibiting the disease and its progression; for example, inhibiting a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., arresting further development of the pathology and/or symptomatology) such as in the case of cognitive impairment, arresting or delaying a) the decline in memory (long term and/or short term), b) the decline in decision making, c) the decline in executive functions (e.g., reasoning, problem-solving, planning), d) the decline in language skills (e.g., naming, fluency, expressive speech, and comprehension), e) the decline in visuospatial skills, and f) stop the decline in attentional control (e.g., simple and divided attention), and

(2) ameliorating the disease; for example, ameliorating a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., reversing the pathology and/or symptomatology) such as in the case of cognitive impairment, a) improvement in memory, b) improvement in decision making, c) improvement in executive functions (e.g., reasoning, problem-solving, planning), d) improvement in language skills
(e.g., naming, fluency, expressive speech, and comprehension), e) improvement in visuospatial skills, and f) improvement in attentional control (e.g., simple and divided attention).

As used herein, the term "mammal" has its ordinary meaning in the art and includes, e.g. humans, mice, rats, rabbits, dogs, cats, bovines, horses, swine, and monkey, with preference given to humans.

As used herein, the phrase "cognitive impairment" refers to any decline in one or more of memory functions, decision making, executive functions, language skills, visuospatial skills, or attentional control.

As used herein, the phrases "treatment of cognitive impairment" or "treating cognitive impairment" refer to one of

(a) treating mild cognitive impairment;
(b) delaying the progression from mild cognitive impairment to cognitive impairment associated with Alzheimer's disease; and
(c) treating cognitive impairment associated with Alzheimer's disease.

Sharp demarcations between normal cognition and mild cognitive impairment and between mild cognitive impairment and cognitive impairment associated with Alzheimer's disease are difficult. Clinical judgement must be used to make these distinctions. As used herein, the phrase "mild cognitive impairment" refers to the symptomatic predementia phase of Alzheimer's disease. Criteria that should be met in order to diagnose a person with "mild cognitive impairment" include the following (Albert M S et al; Alzheimer's & Dementia 2011 Vol 7, pp 270-279):

- there should be evidence of concern about a change in cognition, in comparison with the person's previous level
- there should be evidence of lower performance in one or more cognitive domains that is greater than expected for the patient's age, and educational background; this lower performance can occur in a variety of cognitive domains, including memory, executive function, attention, language, and visuospatial skills
- Persons with mild cognitive impairment commonly have mild problems performing complex functional tasks which they used to perform previously, such as paying bills, preparing a meal, or shopping; they may take more time, be less efficient, and make more errors at performing such activities than in the past
- The cognitive changes are sufficiently mild that there is no evidence of a significant impairment in social or occupational life
- Scores on cognitive tests for individuals with mild cognitive impairment are typically 1 to 1.5 standard deviations below the mean for their age and education matched peers on culturally appropriate normative data (i.e., for the impaired domain(s)); age and educational norms are available for some tests, as for example Verbal Learning Tests such as California Verbal Learning Test (CVLT) or Free and Cued Selective Reminding Test (FCSRT).
In general, “pharmaceutically acceptable salts” refers to salts with inorganic bases, salts with organic bases, salts with inorganic acids, salts with organic acids, and salts with basic or acidic amino acids.

Examples of salts with inorganic bases may include salts with alkali metals such as sodium and potassium, salts with alkaline earth metals such as calcium and magnesium, and salts with aluminum.

Examples of salts with organic bases may include salts with trimethylamine, triethylamine, pyridine, picoline, ethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, and N,N-dibenzylethlenediamine.

Examples of salts with inorganic acids may include salts with hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid, and phosphoric acid.

Examples of salts with organic acids may include salts with formic acid, acetic acid, trifluoroacetic acid, fumaric acid, oxalic acid, tartaric acid, maleic acid, citric acid, succinic acid, malic acid, methanesulfonic acid, benzenesulfonic acid, and p-toluenesulfonic acid.

Examples of salts with basic amino acids may include salts with arginine, lysine, ornithine, etc; examples of salts with acidic amino acids may include salts with aspartic acid, and glutamic acid.

"Concurrent administration" (also including "concomitant administration"), as used herein, means that both the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor (a) are administered to the mammal in need of the treatment in a single dosage form for simultaneous, concomitant administration or (b) are administered to the mammal in need of the treatment in two separate dosage forms, and the two separate dosage forms are administered immediately one after the other. In this context, the two separate dosage forms are administered immediately one after the other, if the dosages are administered within between 0 and 15 minutes of each other; or more preferably within between 0 and 5 minutes of each other; or most preferably within between 0 and 1 minute of each other.

"Sequential administration" (also including "administering sequentially"), as used herein, means that the phosphodiesterase 4 inhibitor is administered to the mammal in need of the treatment in one dosage form and the acetylcholinesterase 4 inhibitor is administered to the mammal in need of the treatment in another separate dosage form, wherein the second dosage form is administered to the mammal in need of the treatment while the first dosage form still has an effect on the mammal being treated. In a preferred embodiment of the invention, the first and the second dosage form are administered within such a time interval that the effect of the combined treatment on the mammal being treated is synergistic. In this context, the two separate dosage forms are considered to be administered sequentially, if the two dosage forms are administered at least 15 but no more than 240 minutes apart, preferably between 15 and 120 minutes apart, and more preferable between 15 and 60 minutes apart.
"Unit dosage forms", as used herein, 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 a suitable pharmaceutical excipient. Such dosage units can refer to volume or weight of the therapeutic.

Summary of the Invention

The present invention provides the following:

1. A method of treating cognitive impairment in a mammal in need of such treatment, comprising administering to a mammal suffering from cognitive impairment a therapeutically effective amount of a combination of
   (1) a phosphodiesterase 4 inhibitor; and
   (2) an acetylcholinesterase inhibitor;

   wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide, and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil, a pharmaceutical acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine, and a pharmaceutically acceptable salt of rivastigmine.

2. The method according to above-mentioned 1, wherein said treating cognitive impairment is selected from the group consisting of
   (a) treating mild cognitive impairment;
   (b) delaying progression from mild cognitive impairment to cognitive impairment associated with Alzheimer’s disease; and
   (c) treating cognitive impairment associated with Alzheimer’s disease.

3. The method according to above mentioned 1, wherein said treating cognitive impairment is treating mild cognitive impairment.

4. The method according to above mentioned 1, wherein said treating cognitive impairment is delaying progression from mild cognitive impairment to cognitive impairment associated with Alzheimer’s disease.

5. The method according to above mentioned 1, wherein said treating cognitive impairment is treating cognitive impairment associated with Alzheimer’s disease.

6. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast and a pharmaceutically acceptable salt of roflumilast.
7. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide.

8. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast.

9. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide.

10. The method according to any one of above-mentioned 1 to 7, wherein the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

11. The method according to any one of above-mentioned 1 to 7, wherein the acetylcholinesterase inhibitor is donepezil hydrochloride.

12. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

13. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is donepezil hydrochloride.

14. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

15. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is donepezil hydrochloride.

16. The method according to any one of above-mentioned 1 to 7, wherein the acetylcholinesterase inhibitor is selected from the group of galantamine and a pharmaceutically acceptable salt of galantamine.

17. The method according to any one of above-mentioned 1 to 7, wherein the acetylcholinesterase inhibitor is galantamine hydrobromide.
18. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of galantamine and a pharmaceutically acceptable salt of galantamine.

19. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is galantamine hydrobromide.

20. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of galantamine and a pharmaceutically acceptable salt of galantamine.

21. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is galantamine hydrobromide.

22. The method according to any one of above-mentioned 1 to 7, wherein the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

23. The method according to any one of above-mentioned 1 to 7, wherein the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate.

24. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

25. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate.

26. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

27. The method according to any one of above-mentioned 1 to 5, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate.

28. The method according to any one of above-mentioned 10, 11, 16, 17, 22 and 23, wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of between 50 and 300 meg.
29. The method according to any one of above-mentioned 10, 11, 16, 17, 22 and 23, wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of between 50 and 150 meg.

30. The method according to any one of above-mentioned 12, 13, 18, 19, 24 and 25, wherein the phosphodiesterase 4 inhibitor is roflumilast and wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of between 50 and 300 meg.

31. The method according to any one of above-mentioned 12, 13, 18, 19, 24 and 25, wherein the phosphodiesterase 4 inhibitor is roflumilast and wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of between 50 and 150 meg.

32. The method according to any one of above-mentioned 14, 15, 20, 21, 26 and 27, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of between 50 and 300 meg.

33. The method according to any one of above-mentioned 14, 15, 20, 21, 26 and 27, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of between 50 and 150 meg.

34. The method according to any one of the above-mentioned 29, 31 and 33, wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of 50 meg.

35. The method according to any one of the above-mentioned 29, 31 and 33, wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of 62.5 meg.

36. The method according to any one of the above-mentioned 29, 31 and 33, wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of 75 meg.

37. The method according to any one of the above-mentioned 29, 31 and 33, wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of 100 meg.

38. The method according to any one of the above-mentioned 29, 31 and 33, wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of 125 meg.

39. The method according to any one of the above-mentioned 29, 31 and 33, wherein the phosphodiesterase 4 inhibitor is administered at a daily dose of 150 meg.

40. The method according to above-mentioned 13, wherein roflumilast is administered at a daily dose of between 50 and 150 meg and donepezil hydrochloride is administered at a daily dose of between 5 and 23 mg.
41. The method according to above-mentioned 13, wherein roflumilast is administered at a daily dose selected from 50, 75, 100 or 125 mg and donepezil hydrochloride is administered at a daily dose selected from 5 or 10 mg.

42. The method according to above-mentioned 19, wherein roflumilast is administered at a daily dose of between 50 and 150 mg and galantamine hydrobromide is administered at a daily dose corresponding to between 4 and 12 mg of galantamine.

43. The method according to above-mentioned 19, wherein roflumilast is administered at a daily dose selected from 50, 75, 100 or 125 mg and galantamine hydrobromide is administered at a daily dose corresponding to 4 or 8 mg of galantamine.

44. The method according to above-mentioned 25, wherein roflumilast is administered at a daily dose of between 50 and 150 mg and rivastigmine hydrogen (2R,3R) tartrate is administered at a daily dose corresponding to between 3 and 12 mg of rivastigmine.

45. The method according to above-mentioned 25, wherein roflumilast is administered at a daily dose selected from 50, 75, 100 or 125 mg and rivastigmine hydrogen (2R,3R) tartrate is administered at a daily dose corresponding to 3 or 6 mg of rivastigmine.

46. The method according to any one of above-mentioned 1 to 45, wherein the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are administered in one single dosage form.

47. The method according to any one of above-mentioned 1 to 45, wherein the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are administered concurrently or sequentially in two separate dosage forms.

48. A pharmaceutical composition, comprising:
   a. a phosphodiesterase 4 inhibitor in combination with
   b. an acetylcholinesterase inhibitor, and
   c. a pharmaceutically acceptable carrier,

wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide, the acetylcholinesterase inhibitor is selected from the group consisting of donepezil, a pharmaceutical acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine, and a pharmaceutically acceptable salt of rivastigmine.
49. The pharmaceutical composition according to above-mentioned 48, wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast and a pharmaceutically acceptable salt of roflumilast.

50. The pharmaceutical composition according to above-mentioned 48, wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide.

51. The pharmaceutical composition according to above-mentioned 48, wherein the phosphodiesterase 4 inhibitor is roflumilast.

52. The pharmaceutical composition according to above-mentioned 48, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide.

53. The pharmaceutical composition according to any one of above-mentioned 48 to 50, wherein the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

54. The pharmaceutical composition according to any one of above-mentioned 48 to 50, wherein the acetylcholinesterase inhibitor is donepezil hydrochloride.

55. The pharmaceutical composition according to above-mentioned 48, wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

56. The pharmaceutical composition according to above-mentioned 48, wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is donepezil hydrochloride.

57. The pharmaceutical composition according to above-mentioned 48, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

58. The pharmaceutical composition according to above-mentioned 48, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is donepezil hydrochloride.

59. The pharmaceutical composition according to any one of above-mentioned 48 to 50, wherein the acetylcholinesterase inhibitor is selected from the group consisting of galantamine and a pharmaceutically acceptable salt of galantamine.
60. The pharmaceutical composition according to any one of above-mentioned 48 to 50, wherein
the acetylcholinesterase inhibitor is galantamine hydrobromide.

61. The pharmaceutical composition according to above-mentioned 48, wherein the
phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected
from the group consisting of galantamine and a pharmaceutically acceptable salt of
galantamine.

62. The pharmaceutical composition according to above-mentioned 48, wherein the
phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is
galantamine hydrobromide.

63. The pharmaceutical composition according to above-mentioned 48, wherein the
phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is
selected from the group consisting of galantamine and a pharmaceutically acceptable salt of
galantamine.

64. The pharmaceutical composition according to above-mentioned 48, wherein the
phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is
galantamine hydrobromide.

65. The pharmaceutical composition according to any one of above-mentioned 48 to 50, wherein
the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a
pharmaceutically acceptable salt of rivastigmine.

66. The pharmaceutical composition according to any one of above-mentioned 48 to 50, wherein
the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate.

67. The pharmaceutical composition according to above-mentioned 48, wherein the
phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected
from the group consisting of rivastigmine and a pharmaceutically acceptable salt of
rivastigmine.

68. The pharmaceutical composition according to above-mentioned 48, wherein the
phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is
rivastigmine hydrogen (2R,3R) tartrate.

69. The pharmaceutical composition according to above-mentioned 48, wherein the
phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is
selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of
rivastigmine.
70. The pharmaceutical composition according to above-mentioned 48, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is rivastigmine tartrate.

71. The pharmaceutical composition according to any one of the above-mentioned 53, 54, 59, 60, 65 and 66, wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.

72. The pharmaceutical composition according to any one of the above-mentioned 53, 54, 59, 60, 65 and 66, wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 meg.

73. The pharmaceutical composition according to any one of above-mentioned 55, 56, 61, 62, 67 and 68, wherein the phosphodiesterase 4 inhibitor is roflumilast and wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.

74. The pharmaceutical composition according to any one of above-mentioned 55, 56, 61, 62, 67 and 68, wherein the phosphodiesterase 4 inhibitor is roflumilast and wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 meg.

75. The pharmaceutical composition according to any one of above-mentioned 57, 58, 63, 64, 69 and 70, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.

76. The pharmaceutical composition according to any one of above-mentioned 57, 58, 63, 64, 69 and 70, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 meg.

77. The pharmaceutical composition according to any one of above-mentioned 72, 74 and 76, wherein the phosphodiesterase 4 inhibitor is present in an amount of 50 meg.

78. The pharmaceutical composition according to any one of above-mentioned 72, 74 and 76, wherein the phosphodiesterase 4 inhibitor is present in an amount of 62.5 meg.

79. The pharmaceutical composition according to any one of above-mentioned 72, 74 and 76, wherein the phosphodiesterase 4 inhibitor is present in an amount of 75 meg.

80. The pharmaceutical composition according to any one of above-mentioned 72, 74 and 76, wherein the phosphodiesterase 4 inhibitor is present in an amount of 100 meg.
81. The pharmaceutical composition according to any one of above-mentioned 72, 74 and 76, wherein the phosphodiesterase 4 inhibitor is present in an amount of 125 meq.

82. The pharmaceutical composition according to any one of above-mentioned 72, 74 and 76, wherein the phosphodiesterase 4 inhibitor is present in an amount of 150 meq.

83. The pharmaceutical composition according to above-mentioned 56, wherein roflumilast is present in an amount of between 50 and 150 meq and donepezil hydrochloride is present in an amount of between 5 and 23 mg.

84. The pharmaceutical composition according to above-mentioned 56, wherein roflumilast is present in an amount selected from 50, 75, 100 or 125 meq and donepezil hydrochloride is present in an amount selected from 5 or 10 mg.

85. The pharmaceutical composition according to above-mentioned 62, wherein roflumilast is present in an amount of between 50 and 150 meq and galantamine hydrobromide is present in an amount corresponding to between 4 and 12 mg of galantamine.

86. The pharmaceutical composition according to above-mentioned 62, wherein roflumilast is present in an amount selected from 50, 75, 100 or 125 meq and galantamine hydrobromide is present in an amount corresponding to 4 or 8 mg of galantamine.

87. The pharmaceutical composition according to above-mentioned 68, wherein roflumilast is present in an amount of between 50 and 150 meq and rivastigmine hydrogen (2R,3R) tartrate is present in an amount corresponding to between 3 and 12 mg of rivastigmine.

88. The pharmaceutical composition according to above-mentioned 68, wherein roflumilast is present in an amount selected from 50, 75, 100 or 125 meq and rivastigmine hydrogen (2R,3R) tartrate is present in an amount corresponding to 3 or 6 mg of rivastigmine.

89. The pharmaceutical composition according to one of above-mentioned 48 to 88, wherein the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are to be administered concurrently in one single dosage form.

90. The pharmaceutical composition according to one of above-mentioned 48 to 88, wherein the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are to be administered concurrently or sequentially in two separate dosage forms.

**Brief Description of the Figures**

40 Figure 1 depicts the dose response effects of rolipram and of roflumilast on the discrimination index in the object location task test following a 24-hour retention interval in mice.
Figure 2 is a graph illustrating the number of words correctly remembered by healthy adult subjects during the Verbal Learning Task (VLT) following administration of various doses of roflumilast (1st, 2nd, and 3rd recall, 45 min delayed and 24 h delayed).

Figure 3 illustrates quantitative bar graph analysis of the results of the electroencephalography data depicting the effect of roflumilast on Event-Related Potentials during the VLT (3rd trial only).

Figure 4 is a graph illustrating the number of words correctly remembered by 60 to 80 year old subjects during the Verbal Learning Task (VLT) following administration of various doses of roflumilast (1st, 2nd, 3rd recall, 45 min delayed and 24 h delayed).

Figure 5 illustrates the effects of different roflumilast doses on a scopolamine induced memory deficit in the Object recognition task in male Wistar rats.

Figure 6 illustrates the combined effects of sub-efficacious doses of roflumilast and donepezil on a scopolamine induced memory deficit in the Object recognition task in male Wistar rats.

Detailed Description of the Invention

The present invention provides a combination of a phosphodiesterase 4 inhibitor with an acetylcholinesterase inhibitor for the treatment of cognitive impairment. More particularly, the combination of the present invention can be used to treat mild cognitive impairment, to delay the progression from mild cognitive impairment to cognitive impairment associated with Alzheimer’s disease, as well as to treat cognitive impairment associated with Alzheimer’s disease.

The phosphodiesterase 4 inhibitor used in the present invention is selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide.

Roflumilast is the only phosphodiesterase 4 inhibitor that has been approved for the treatment of severe chronic obstructive pulmonary disease (COPD). The US label states that roflumilast is indicated as a treatment to reduce the risk of COPD exacerbations in patients with severe COPD associated with chronic bronchitis and a history of exacerbations. The recommended dosage for patients with COPD is one 500 meg tablet per day.

A major metabolite of roflumilast in humans and several animal species is roflumilast-N-oxide, which is by itself a potent phosphodiesterase 4 inhibitor. It is believed that in humans roflumilast-N-oxide accounts for more than 90% of overall phosphodiesterase 4 inhibition, and therefore roflumilast-N-oxide largely governs the pharmacological effects observed in humans after the administration of roflumilast.
Mouse studies have shown a pronounced effect on spatial memory measured by the object location task test following a single subcutaneous administration of 0.03 mg/kg roflumilast, while nearly no effect was detected after a single subcutaneous administration of 0.01 mg/kg roflumilast and completely no effect was noted following a single subcutaneous administration of 0.1 mg/kg roflumilast.

Based on the results obtained in the object location task test in mice, a clinical trial involving healthy 18 to 35 year old adults was performed using a single oral administration of a capsulated formulation containing 100 meg, 300 meg or 1000 meg roflumilast.

In this clinical trial the group of healthy adults receiving a single oral dose of 100 meg of roflumilast showed a considerable improvement with respect to the number of correct words recalled (an average 2.5 words improvement after the third trial) in the verbal learning task (VLT). EEG measurements performed simultaneously with VLT testing revealed that Event-Related Potential (ERP), P600 demonstrated the strongest increase of amplitude, also in the group of healthy adults receiving a single oral dose of 100 meg of roflumilast.

In the above-indicated clinical trial roflumilast was administered once in a single oral dose of 100 meg, 300 meg or 1000 meg. Due to the pharmacokinetics of roflumilast and its metabolite roflumilast-N-oxide and the median plasma half life of these compounds the steady state plasma concentration levels in a once a day (24 h) repeated dosing regimen of roflumilast/roflumilast-N-oxide is about two-fold compared to the plasma concentration levels following a once a day single dosing. Thus, the administration of a single oral dose of 100 meg, 300 meg or 1000 meg roflumilast leads to comparable plasma concentration levels as 50 meg, 150 meg and 500 meg roflumilast in the steady state once a day (24 h) repeated dosing regimen.

In rat studies the data obtained in the mouse studies have been confirmed. A single intraperitoneally administered dose of 0.003 mg/kg roflumilast was able to fully restore spatial memory function (measured by the object recognition task) in rats treated with scopolamine to induce memory deficit. Single intraperitoneally administered doses of 0.01 mg/kg, 0.03 mg/kg, 0.001 mg/kg and 0.0003 mg/kg roflumilast showed increasingly less efficacy on restoring spatial memory function. No effect was noted following a single intraperitoneally administration of 0.0001 mg/kg roflumilast.

In order (a) to confirm the results seen in the clinical trial with the healthy 18 to 35 year old adults and (b) to detect whether perhaps even more substantial improvement might be observed in aged adults with a certain degree of cognitive impairment, a clinical study is conducted with forty 60 to 80 year old subjects with one group having a more pronounced cognitive decline (impaired group: 1 to 2 standard deviations below the mean for their age and education matched peers on culturally appropriate normative data assessed with Verbal Learning Test) as well as with an aged matched control group (control group: -0.5 - +0.5 standard deviations below and above the mean for their age and education...
matched peers on culturally appropriate normative data assessed with Verbal Learning Test). The two patients groups are tested for cognitive battery (Verbal Learning Task, Spatial Memory Task and Stroop Task) and EEG battery (ERP's, sensory gating and novelty oddball task) tests. The data obtained from an interim analysis of that clinical trial based on 9 subjects of the impaired group and 4 subjects of the control group appears to confirm the effects seen in the Verbal Learning Task in the earlier trial with healthy adults after administration of 100 meg of roflumilast.

All these data indicate that roflumilast administered in doses considerably lower than the approved once a day dose for the treatment of severe COPD (500 meg), is effective in improving cognitive impairment.

In addition, it has been shown that the combination of the non-effective dose of 0.0001 mg/kg of roflumilast with the sub-efficacious dose of 0.01 mg/kg donepezil hydrochloride acts synergistically by fully restoring spatial memory function (measured by the object recognition task) in rats treated with scopolamine to induce memory deficit.

These rat data taken together with the above-mentioned data for mono treatment with low dose roflumilast indicate that low dose roflumilast not only in form of mono treatment but also in form of a combination treatment with an acetylcholinesterase inhibitor, such as donepezil, is able to improve cognitive impairment.

The acetylcholinesterase inhibitor used in the present invention is selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

Side effects noted in connection with the administration of acetylcholinesterase inhibitors include diarrhea, nausea and vomiting. The incidence and the severity of these side effects increases with the dose amount and are more pronounced at the initiation of the treatment or after dose increases.

Side effects noted in connection with the administration of phosphodiesterase 4 inhibitors mainly include diarrhea, nausea and headache. As with the administration of acetylcholinesterase inhibitors, the incidence and the severity of the side effects following roflumilast administration is correlated with the dose strength and occur more pronounced at the initiation of the treatment; incidence and severity are more pronounced at the 4-8 week interval of treatment.

Based on the above-indicated discovery that the phosphodiesterase 4 inhibitor roflumilast has an effect on cognitive impairment in humans at a dose considerably below the approved dose for the treatment of severe COPD, cognitive impairment may be treated by the use of a combination treatment of low dose roflumilast with an acetylcholinesterase inhibitor. This allows greater synergistic and combined therapeutic effects of the phosphodiesterase 4 inhibitor roflumilast and the
acetylcholinesterase inhibitor, while keeping the potential side effects minimal due to the low dose of roflumilast.

In a first aspect, the present invention is directed to a method of treating cognitive impairment in a mammal in need of such treatment, comprising administering to a mammal suffering from cognitive impairment a therapeutically effective amount of a combination of

(1) a phosphodiesterase 4 inhibitor; and
(2) an acetylcholinesterase inhibitor;

wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide, and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil, a pharmaceutical acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine, and a pharmaceutically acceptable salt of rivastigmine.

Cognitive impairment refers to any decline in one or more of memory functions, decision making, executive functions, language skills, visuospatial skills, or attentional control.

In a second aspect, the present invention is directed to a method of treating cognitive impairment in a mammal in need of such treatment, including administering to a mammal suffering from cognitive impairment a therapeutically effective amount of a combination of

(1) a phosphodiesterase 4 inhibitor; and
(2) an acetylcholinesterase inhibitor;

wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil, a pharmaceutical acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine, and a pharmaceutically acceptable salt of rivastigmine; and wherein the treatment of cognitive impairment can mean any one of:

(a) treating mild cognitive impairment;
(b) delaying the progression from mild cognitive impairment to cognitive impairment associated with Alzheimer's disease; and
(c) treating cognitive impairment associated with Alzheimer's disease.

In one embodiment of the second aspect of the invention, treating cognitive impairment means treating mild cognitive impairment.

In a further embodiment of the second aspect of the invention, treating cognitive impairment means delaying the progression of mild cognitive impairment to cognitive impairment associated with Alzheimer's disease.
In a further embodiment of the second aspect of the invention, treating cognitive impairment means treating cognitive impairment associated with Alzheimer’s disease.

The combination of the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are co-administered to the mammal (patient) in need of treatment in form of a pharmaceutical composition.

In a third aspect, the present invention is therefore directed to a pharmaceutical composition, including:

a. a phosphodiesterase 4 inhibitor in combination with
b. an acetylcholinesterase inhibitor, and
c. a pharmaceutically acceptable carrier,

wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide, the acetylcholinesterase inhibitor is selected from the group consisting of donepezil, a pharmaceutical acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine, and a pharmaceutically acceptable salt of rivastigmine.

In a preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast and a pharmaceutically acceptable salt of roflumilast.

In another preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide.

In another preferred embodiment of the first, second and third aspect of the invention, the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

In another preferred embodiment of the first, second and third aspect of the invention, the acetylcholinesterase inhibitor is selected from the group consisting of galantamine and a pharmaceutically acceptable salt of galantamine.

In another preferred embodiment of the first, second and third aspect of the invention, the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

In another preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast and a
pharmaceutically acceptable salt of roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

In another preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

In another preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast and a pharmaceutically acceptable salt of roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of galantamine and a pharmaceutically acceptable salt of galantamine.

In another preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of galantamine and a pharmaceutically acceptable salt of galantamine.

In another preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast and a pharmaceutically acceptable salt of roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

In another preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

In a particularly preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast.

In another particularly preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast-N-oxide.

In another particularly preferred embodiment of the first, second and third aspect of the invention, the acetylcholinesterase inhibitor is donepezil hydrochloride.
In another particularly preferred embodiment of the first, second and third aspect of the invention, the acetylcholinesterase inhibitor is galantamine hydrobromide.

In another particularly preferred embodiment of the first, second and third aspect of the invention, the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate.

In another particularly preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is donepezil hydrochloride.

In another particularly preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is galantamine hydrobromide.

In another particularly preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is donepezil hydrochloride.

In another particularly preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is galantamine hydrobromide.

In another particularly preferred embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate.

In the present invention, roflumilast or roflumilast-N-oxide may be co-administered with an acetylcholinesterase inhibitor at a daily dose of about 50 mg to about 300 mg, such as a dose of 50, 62.5, 75, 100, 125, 150, 175, 200, 250 or 300 mg, preferably at a daily dose of 50, 62.5, 75, 100, 125 or 150 mg, more preferably at a daily dose of 50, 75, 100 or 125 mg.

In the present invention, donepezil may be co-administered with the phosphodiesterase 4 inhibitor at a daily dose of about 2.281 mg to about 20.984 mg, such as a dose of 2.281, 4.562, 6.843, 9.123, 13.685, 18.247 or 20.984 mg (corresponding to a daily dose of 2.5, 5, 7.5, 10, 15, 20 or 23 mg of donepezil hydrochloride); preferably, at a daily dose of 2.281, 4.562, 6.843 or 9.123 mg (corresponding to a daily dose of 2.5, 5, 7.5 and 10 mg of donepezil hydrobromide).
As preferred daily dose combinations of the phosphodiesterase 4 inhibitor roflumilast and the acetylcholinesterase inhibitor donepezil hydrochloride may be mentioned:

<table>
<thead>
<tr>
<th>roflumilast</th>
<th>donepezil hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 mcg</td>
<td>10 mg</td>
</tr>
<tr>
<td>125 mcg</td>
<td>5 mg</td>
</tr>
<tr>
<td>100 mcg</td>
<td>10 mg</td>
</tr>
<tr>
<td>100 mcg</td>
<td>5 mg</td>
</tr>
<tr>
<td>75 mcg</td>
<td>10 mg</td>
</tr>
<tr>
<td>75 mcg</td>
<td>5 mg</td>
</tr>
<tr>
<td>50 mcg</td>
<td>10 mg</td>
</tr>
<tr>
<td>50 mcg</td>
<td>5 mg</td>
</tr>
</tbody>
</table>

In the present invention, galantamine may be co-administered with the phosphodiesterase 4 inhibitor at a daily dose of about 2 to about 12 mg, such as 2, 4, 6, 8, 10 or 12 mg (corresponding to 2.563, 5.126, 7.689, 10.253, 12.815 or 15.379 mg of galantamine hydrobromide); preferably at a daily dose of 2, 4, 6 or 8 mg corresponding to 2.563, 5.126, 7.689 or 10.253 mg of galantamine hydrobromide).

As preferred daily dose combinations of the phosphodiesterase 4 inhibitor roflumilast and the acetylcholinesterase inhibitor galantamine hydrobromide may be mentioned:

<table>
<thead>
<tr>
<th>roflumilast</th>
<th>galantamine hydrobromide</th>
<th>corresponding to galantamine (free base)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 meg</td>
<td>10.253 mg</td>
<td>8 mg</td>
</tr>
<tr>
<td>125 meg</td>
<td>5.126 mg</td>
<td>4 mg</td>
</tr>
<tr>
<td>100 meg</td>
<td>10.253 mg</td>
<td>8 mg</td>
</tr>
<tr>
<td>100 meg</td>
<td>5.126 mg</td>
<td>4 mg</td>
</tr>
<tr>
<td>75 meg</td>
<td>10.253 mg</td>
<td>8 mg</td>
</tr>
<tr>
<td>75 meg</td>
<td>5.126 mg</td>
<td>4 mg</td>
</tr>
<tr>
<td>50 meg</td>
<td>10.253 mg</td>
<td>8 mg</td>
</tr>
<tr>
<td>50 meg</td>
<td>5.126 mg</td>
<td>4 mg</td>
</tr>
</tbody>
</table>
In the present invention, rivastigmine may be co-administered with a phosphodiesterase 4 inhibitor at a daily dose about 2 to about 12 mg, such as 2, 3, 6, 9 or 12 mg (corresponding to twice a day 1.600, 2.399, 4.799, 7.198 or 9.597 mg of rivastigmine hydrogen (2R,3R) tartrate); preferably at a daily dose of 2, 3, 6 or 9 mg (corresponding to twice a day 1.600, 2.399, 4.799 or 7.198 of rivastigmine hydrogen (2R,3R) tartrate).

As preferred daily dose combinations of the phosphodiesterase 4 inhibitor roflumilast and the acetylcholinesterase inhibitor rivastigmine hydrogen (2R,3R) tartrate may be mentioned:

<table>
<thead>
<tr>
<th>roflumilast</th>
<th>rivastigmine hydrogen (2R,3R) tartrate</th>
<th>Corresponding to rivastigmine (free base)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 mcg</td>
<td>2 x 4.799 mg</td>
<td>2 x 3 mg</td>
</tr>
<tr>
<td>125 mcg</td>
<td>2 x 2.399 mg</td>
<td>2 x 1.5 mg</td>
</tr>
<tr>
<td>100 mcg</td>
<td>2 x 4.799 mg</td>
<td>2 x 3 mg</td>
</tr>
<tr>
<td>100 mcg</td>
<td>2 x 2.399 mg</td>
<td>2 x 1.5 mg</td>
</tr>
<tr>
<td>75 mcg</td>
<td>2 x 4.799 mg</td>
<td>2 x 3 mg</td>
</tr>
<tr>
<td>75 mcg</td>
<td>2 x 2.399 mg</td>
<td>2 x 1.5 mg</td>
</tr>
<tr>
<td>50 mcg</td>
<td>2 x 4.799 mg</td>
<td>2 x 3 mg</td>
</tr>
<tr>
<td>50 mcg</td>
<td>2 x 2.399 mg</td>
<td>2 x 1.5 mg</td>
</tr>
</tbody>
</table>

In the table above, the dosage of roflumilast is given as a single dose per day. The rivastigmine hydrogen (2R, 3R) tartrate is given in the amount stated, twice per day, to account for the short half life of this drug (for example, “2 x 2.399 mg” means that during a treatment day, for example in the morning one 2.399 mg rivastigmine hydrogen (2R,3R) tartrate tablet is taken and another one in the evening).

In one embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is administered at a daily dose of 300 mg.

In a further embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of 300 mg.

In another embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is administered at a daily dose of 250 mg.

In another embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of 250 mg.
In another embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is administered at a daily dose of 200 meg.

In another embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of 200 meg.

In another embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is administered at a daily dose of 175 meg.

In another embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of 175 meg.

In a preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is administered at a daily dose of between 50 and 150 meg.

In a particularly preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of between 50 and 150 meg.

In another preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is administered at a daily dose of 150 meg.

In another particularly preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of 150 meg.

In another preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is administered at a daily dose of 125 meg.

In another particularly preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of 125 meg.

In another preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is administered at a daily dose of 100 meg.

In another particularly preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of 100 meg.

In another preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is administered at a daily dose of 75 meg.
In another particularly preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of 75 mg.

In another preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is administered at a daily dose of 62.5 mg.

In another particularly preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of 62.5 mg.

In another preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is administered at a daily dose of 50 mg.

In another particularly preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of 50 mg.

In a further preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of between 50 and 150 mg and the acetylcholinesterase inhibitor is donepezil hydrochloride and is administered at a daily dose of between 5 and 23 mg.

In a further preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose selected from 50, 75, 100 or 125 mg and the acetylcholinesterase inhibitor is donepezil hydrochloride and is administered at a daily dose selected from 5 or 10 mg.

In a further preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of between 50 and 150 mg and the acetylcholinesterase inhibitor is galantamine hydrobromide and is administered at a daily dose corresponding to between 4 and 12 mg of galantamine.

In a further preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose selected from 50, 75, 100 or 125 mg and the acetylcholinesterase inhibitor is galantamine hydrobromide and is administered at a daily dose corresponding to 4 or 8 mg of galantamine.

In a further preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of between 50 and 150 mg and the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate and is administered at a daily dose corresponding to between 3 and 12 mg of rivastigmine.
In a further preferred embodiment of the first and second aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is administered at a daily dose of between 50 and 125 meg and the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate and is administered at a daily dose corresponding to 3 or 6 mg rivastigmine.

In one embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 300 meg.

In a further embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of 300 meg.

In another embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 250 meg.

In another embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of 250 meg.

In another embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 200 meg.

In another embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of 200 meg.

In another embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 175 meg.

In another embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of 175 meg.

In a preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of between 50 and 150 meg.

In a particularly preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of between 50 and 150 meg.

In another preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 150 meg.
In another particularly preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of 150 meg.

In another preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 125 meg.

In another particularly preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of 125 meg.

In another preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 100 meg.

In another particularly preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of 100 meg.

In another preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 75 meg.

In another particularly preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of 75 meg.

In another preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 62.5 meg.

In another particularly preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of 62.5 meg.

In another preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 50 meg.

In another particularly preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of 50 meg.
In a further preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of between 50 and 150 mg and the acetylcholinesterase inhibitor is donepezil hydrochloride and is present in the pharmaceutical composition in an amount of between 5 and 23 mg.

In a further particularly preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount selected from 50, 75, 100 or 125 mg and the acetylcholinesterase inhibitor is donepezil hydrochloride and is present in the pharmaceutical composition in an amount selected from 5 or 10 mg.

In a further preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of between 50 and 150 mg and the acetylcholinesterase inhibitor is galantamine hydrobromide and is present in the pharmaceutical composition in an amount corresponding to between 4 and 12 mg of galantamine.

In a further particularly preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount selected from 50, 75, 100 or 125 mg and the acetylcholinesterase inhibitor is galantamine hydrobromide and is present in the pharmaceutical composition in an amount corresponding to 4 or 8 mg of galantamine.

In a further preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount of between 50 and 150 mg and the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate and is present in the pharmaceutical composition in an amount corresponding to between 3 and 12 mg of rivastigmine.

In a further particularly preferred embodiment of the third aspect of the invention, the phosphodiesterase 4 inhibitor is roflumilast and is present in the pharmaceutical composition in an amount selected from 50, 75, 100 or 125 mg and the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate and is present in the pharmaceutical composition in an amount corresponding to 3 or 6 mg of rivastigmine.

The phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor may be co-administered either in one single dosage form which contains both the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor or in two separated dosage forms in which one contains the phosphodiesterase 4 inhibitor and the other contains the acetylcholinesterase inhibitor.
In a further embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are administered in one single dosage form.

In another further embodiment of the first, second and third aspect of the invention, the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are administered concurrently or sequentially in two separate dosage forms.

In another preferred embodiment of the invention the concurrent or sequential administration of a phosphodiesterase 4 inhibitor according to the invention and an acetylcholinesterase inhibitor according to the invention leads to a synergistic effect (a) with regard to the efficacy in the treatment of cognitive impairment (i.e. the effect on one or more of the above mentioned pathologies or sympatomatologies of cognitive impairment of the combination will be greater than the sum of the effects on one or more of the above mentioned pathologies or sympatomatologies of cognitive impairment of the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor when administered alone) or (b) with regard to the minimization of side effects typically seen in connection with the administration of phosphodiesterase 4 inhibitors and acetylcholinesterase inhibitors (i.e. the number of occurrences of side effects and/or their severity will be lower for the combination than the sum of the number of occurrences of side effects and/or their severity for the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor when administered alone) or (c) with regard to both (a) and (b).

In another preferred embodiment of the invention the phosphodiesterase 4 inhibitor according to the invention and the acetylcholinesterase inhibitor according to the invention are present in the pharmaceutical composition according to the invention in amounts that lead to a synergistic effect (a) with regard to the efficacy in the treatment of cognitive impairment (i.e. the effect on one or more of the above mentioned pathologies or sympatomatologies of cognitive impairment of the combination will be greater than the sum of the effects on one or more of the above mentioned pathologies or sympatomatologies of cognitive impairment of the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor when administered alone) or (b) with regard to the minimization of side effects typically seen in connection with the administration of phosphodiesterase 4 inhibitors and acetylcholinesterase inhibitors (i.e. the number of occurrences of side effects and/or their severity will be lower for the combination than the sum of the number of occurrences of side effects and/or their severity for the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor when administered alone) or (c) with regard to both (a) and (b).

*Phosphodiesterase 4 inhibitor Roflumilast*

The chemical name of roflumilast is N-(3,5-dichloropyridin-4-yl)-3-cyclopropylmethoxy-4difluoromethoxybenzamide [or alternatively: 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyridin-4-yl)benzamide].
The structural formula of roflumilast is:

![Structural formula of roflumilast]

The phosphodiesterase 4 inhibitor roflumilast is disclosed in U.S. Patent 5,712,298 (hereby incorporated by reference in its entirety).

Pharmaceutically acceptable salts of roflumilast may include the sodium and the potassium salt of roflumilast. Roflumilast is preferably used in its free form rather than in the form of a pharmaceutically acceptable salt thereof.

The chemical name of roflumilast-N-oxide is 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloro-1-oxypyrid-4-yl)benzamide. Roflumilast-N-oxide (also referred to as the pyridyl N-oxide of roflumilast), is the major active metabolite of roflumilast in humans, and is a potent phosphodiesterase 4 inhibitor.

Pharmaceutically acceptable salts of roflumilast-N-oxide include the sodium and the potassium salt of roflumilast-N-oxide. Roflumilast-N-oxide is preferably used in its free form rather than in the form of a pharmaceutically acceptable salt thereof.

Roflumilast may be synthesized as disclosed in U.S. Patents 5,712,298 and 7,470,791. Each of these U.S. patents is hereby incorporated by reference in its entirety.

Roflumilast may be formulated in a variety of dosage forms for administration by several routes of administration. Roflumilast tablets may be prepared as disclosed in U.S. Patent 7,951,397, which is hereby incorporated by reference in its entirety. Taste masking formulations for oral dosage forms are disclosed in WO2006/097456 (U.S. patent application 2008/0193544) which is hereby incorporated by reference in its entirety.

Transdermal dosage forms for roflumilast are disclosed in WO2003/099334 (U.S. patent application 2006/0084684) which is hereby incorporated by reference in its entirety) as are other formulations for topical administration, e.g., creams, ointments, gels and pastes. Preparations of roflumilast solutions for injection are disclosed in WO2006/032675 (U.S. patent application 2007/0259009 which is hereby incorporated by reference in its entirety).
Acetylcholinesterase Inhibitors

1. Donepezil

The chemical name of donepezil is \((\pm)-2,3\text{-dihydro-5,6-dimethoxy-2-[1-(phenylmethyl)-4-piperidinyl]methyl}-1\text{-H-inden}-1\text{-one. Its empirical formula is } C_{24}H_{29}NO_3 \text{ and it has a molecular weight of 379.5.}

Donepezil is used in medicaments mainly in the form of its hydrochloride salt. Donepezil, respectively, donepezil hydrochloride, is a reversible inhibitor of the enzyme acetylcholinesterase.

The structural formula of donepezil hydrochloride is:

![Structural formula of donepezil hydrochloride](image)

Donepezil and a method for its synthesis are disclosed in U.S. patent 4,895,841, which is hereby incorporated by reference in its entirety.

As pharmaceutically acceptable salts of donepezil may be mentioned donepezil hydrochloride, donepezil hydrobromide, donepezil fumarate, donepezil tartrate, donepezil oxalate and donepezil benzoate. A particularly preferred pharmaceutically acceptable salt of donepezil is donepezil hydrochloride.

Additional processes for the preparation of donepezil are described in U.S. patents 5,606,064, 6,252,081, 7,148,354 and in the international patent application WO9722584. Polymorphs of donepezil hydrochloride are described in U.S. patents 5,985,864 and 6,140,321. Polymorphic crystals of donepezil are described in U.S. patent 6,245,911. In U.S. patent publication US2008/234485 a method for the preparation of donepezil hydrobromide and crystals thereof is described. Each of these patents and published U.S. patent applications, respectively, is hereby incorporated by reference, in its entirety.

Donepezil may be formulated in a variety of dosage forms for administration by several routes of administration. Donepezil hydrochloride is, for example, formulated in tablet form in dosages of 5 mg, 10 mg and 23 mg (amount refers to donepezil hydrochloride), as well as in orally disintegrating tablet form in dosages of 5 mg and 10 mg. Orally disintegrating tablets comprising donepezil hydrochloride
are described in U.S. patent 7,727,548. Taste masked pharmaceutical compositions comprising
donepezil hydrochloride are described in U.S. patent 7,727,552. Each of these patents is hereby
incorporated by reference, in its entirety.

2. Galantamine

The chemical name of galantamine is (4aS,6R,8aS)-4a,5,9,10,11,12-hexahydro-3-methoxy-1-
1-methyl-6H-benzofuro[3a,2-ef][2]benzazepin-6-ol. Its empirical formula is C_{17}H_{21}NO_{3} and it has
a molecular weight of 287.35.

Galantamine is used in medicaments mainly in the form of its hydrobromide salt. Like donepezil
hydrochloride, galantamine, and its respective hydrobromide salt galantamine hydrobromide, is a
reversible inhibitor of the enzyme acetylcholinesterase.

The structural formula of galantamine hydrobromide is:

![Structural formula of galantamine hydrobromide]

The use of galantamine for the treatment of Alzheimer's disease is described in U.S. patent
4,663,318, which is hereby incorporated by reference in its entirety.

The preferred pharmaceutically acceptable salt of galantamine is galantamine hydrobromide.

Galantamine may be formulated in a variety of dosage forms for administration by several routes of
administration. Galantamine hydrobromide is, for example, formulated as fast-dissolving oral tablet
form in dosages of 4 mg, 8 mg and 12 mg (base equivalent) or 5.126 mg, 10.253 mg, and 15.379 mg
of galantamine hydrobromide. Fast-dissolving tablets comprising galantamine hydrobromide are
described for example in U.S. patents 6,099,863 and 6,358,527. Galantamine hydrobromide is also
formulated in extended release oral tablet form in dosages of 8 mg, 16 mg and 24 mg (base
equivalent). Extended release galantamine compositions are described in U.S. patent 7,160,559.

Each of these patents is hereby incorporated by reference, in its entirety. Galantamine hydrobromide
can be also formulated as an oral solution containing galantamine hydrobromide, equivalent to, for
example, 4 mg/mL of galantamine base.
3. Rivastigmine

The chemical name of rivastigmine is (S)-N-Ethyl-N-methyl-3-[1-(dimethylamino)ethyl]-phenyl carbamate. Its empirical formula is C_{14}H_{22}N_{2}O_{2} and it has a molecular weight of 250.34.

Rivastigmine is used in medicaments mainly in the form of its tartrate salt. Like donepezil hydrochloride, rivastigmine, and its respective tartrate salt rivastigmine hydrogen (2R, 3R) tartrate, is a reversible inhibitor of the enzyme acetylcholinesterase.

The structural formula of rivastigmine hydrogen tartrate is:

```
O
N
CH(CH_3)CH(CH_3)
H
CH(CH_3)

C_4H_6O_6
```

N-Ethyl-N-methyl-3-[1-(dimethylamino)ethyl]-phenyl carbamate and its use in the treatment in senile dementia or Alzheimer's disease is described in U.S. patent 4,948,807. (S)-N-Ethyl-N-methyl-3-[1-(dimethylamino)ethyl]-phenyl carbamate is described in U.S. patent 5,602,176. Each of these patents is hereby incorporated by reference, in its entirety.

The preferred pharmaceutically acceptable salt of rivastigmine is rivastigmine hydrogen (2R, 3R) tartrate.

Rivastigmine may be formulated in a variety of dosage forms for administration by several routes of administration. Rivastigmine hydrogen (2R, 3R) tartrate is, for example, formulated as hard gelatine capsules containing rivastigmine hydrogen (2R, 3R) tartrate equivalent to 1.5, 3, 4.5 and 6 mg of rivastigmine base. Typically, these hard gelatin capsules are taken two times a day. Extended release rivastigmine compositions which allow a once a day administration of rivastigmine are described in U.S. patent 6,565,883, which is hereby incorporated by reference, in its entirety. Rivastigmine hydrogen (2R, 3R) tartrate can be also formulated in form of an oral solution containing rivastigmine hydrogen (2R, 3R) tartrate, equivalent to, for example, 2 mg/mL of rivastigmine base.

Combination Therapy

The phosphodiesterase 4 inhibitor selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide, is co-administered with the acetylcholinesterase inhibitor selected from the
group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine, concurrently or sequentially, and by the same or different route(s) of administration.

The phosphodiesterase 4 inhibitor is co-administered with the acetylcholinesterase inhibitor, in the same or different formulations, including, but not limited to:

a) a single oral dosage form containing both the phosphodiesterase 4 inhibitor, and the acetylcholinesterase inhibitor;

b) two separate oral dosage forms wherein one oral dosage form contains the phosphodiesterase 4 inhibitor, and the other oral dosage form contains the acetylcholinesterase inhibitor;

c) a single transdermal dosage form containing both (1) the phosphodiesterase 4 inhibitor, and (2) the acetylcholinesterase inhibitor;

d) two separate transdermal dosage forms wherein one transdermal dosage form contains the phosphodiesterase 4 inhibitor, and the other transdermal dosage form contains the acetylcholinesterase inhibitor;

e) a single intravenous dosage form containing both (1) the phosphodiesterase 4 inhibitor, and (2) the acetylcholinesterase inhibitor;

f) two separate intravenous dosage forms wherein one intravenous dosage form contains the phosphodiesterase 4 inhibitor, and the other intravenous dosage form contains the acetylcholinesterase inhibitor;

g) two separate dosage forms wherein the first dosage form contains the phosphodiesterase 4 inhibitor, and the second dosage form contains the acetylcholinesterase inhibitor and wherein the first and the second dosage form are administered by different routes of administration.

The preferred dosage form is a single oral dosage form providing administration of (1) a phosphodiesterase 4 inhibitor selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and (2) an acetylcholinesterase inhibitor selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine.
Suitable oral dosage forms include tablets, capsules, powders, pills, solutions, suspensions, emulsions, pastes and granules. The most preferred oral dosage forms include tablets, each tablet containing both (1) a PDE4 inhibitor selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and (2) an acetylcholinesterase inhibitor selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

If the PDE4 inhibitor selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and the acetylcholinesterase inhibitor selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine are not administered in the same dosage form, each active ingredient may be administered before or after the other. Such administration may be made sequentially.

Dosage information for combination treatment:

The combination of (1) a PDE4 inhibitor selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and (2) an acetylcholinesterase inhibitor selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine may be co-administered once daily, or twice, three or four times a day.

For the combination treatment of (1) a PDE4 inhibitor selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and (2) an acetylcholinesterase inhibitor selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine, once daily co-administration is particularly preferred.

The oral dosage forms for once daily co-administration of a combination of (1) a PDE4 inhibitor selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and (2) an acetylcholinesterase inhibitor selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine, may be either in the form of
a) a single oral dosage form, which contains both (1) a PDE4 inhibitor selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and (2) an acetylcholinesterase inhibitor selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine;

or in the form of

b) two separated oral dosage forms, in which one dosage form contains the PDE4 inhibitor selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and the other dosage form contains the acetylcholinesterase inhibitor selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine;

Roflumilast may be co-administered with the acetylcholinesterase inhibitor at a daily dose of about 50 to about 300 meg, such as 50, 62.5, 75, 100, 125, 150, 175, 200, 250 or 300 meg; preferably at a daily dose of 50, 62.5, 75, 100, 125 or 150 meg, more preferably at a daily dose of 50, 75, 100 or 125 meg. If roflumilast is used in the form of a pharmaceutically acceptable salt, the dose of such salt is calculated so that the dose of roflumilast fits with the above given numbers.

Roflumilast-N-oxide may be co-administered with the acetylcholinesterase inhibitor at a daily dose of about 50 to about 300 meg, such as 50, 62.5, 75, 100, 125, 150, 175, 200, 250 or 300 meg; preferably at a daily dose of 50, 62.5, 75, 100, 125 or 150 meg, more preferably at a daily dose of 50, 75, 100 or 125 meg. If roflumilast-N-oxide is used in the form of a pharmaceutically acceptable salt, the dose of such salt is calculated so that the dose of roflumilast-N-oxide fits with the above given numbers.

Donepezil may be co-administered with the phosphodiesterase 4 inhibitor at a daily dose of about 2.281 mg to about 20.984 mg, such as a dose of 2.281, 4.562, 6.843, 9.123, 13.685, 18.247 or 20.984 mg (corresponding to a daily dose of 2.5, 5, 7.5, 10, 15, 20 or 23 mg of donepezil hydrochloride); preferably, at a daily dose of 2.281, 4.562, 6.843 or 9.123 mg (corresponding to a daily dose of 2.5, 5, 7.5 or 10 mg of donepezil hydrochloride).

Most preferably, the amount of roflumilast and donepezil hydrochloride, respectively, in the oral dosage form for once daily co-administration is selected from 50, 75, 100 or 125 meg for roflumilast and 5 or 10 mg for donepezil hydrochloride.
Galantamine may be co-administered with the phosphodiesterase 4 inhibitor at a daily dose of about 2 to about 12 mg, such as 2, 4, 6, 8, 10 or 12 mg (corresponding to 2.563, 5.126, 7.689, 10.253, 12.815 or 15.379 mg of galantamine hydrobromide); preferably at a daily dose of 2, 4, 6 or 8 mg (corresponding to 2.563, 5.126, 7.689 or 10.253 mg of galantamine hydrobromide).

Most preferably, the amount of roflumilast and galantamine hydrobromide, respectively, in the oral dosage form for once daily co-administration is selected from 50, 75, 100 or 125 mg for roflumilast and 5.126 or 10.253 mg for galantamine hydrobromide (corresponding to 2.563, 5.126, 7.689 or 10.253 mg of galantamine hydrobromide).

Rivastigmine may be co-administered with a phosphodiesterase 4 inhibitor at a daily dose of about 2 to about 12 mg, such as 2, 3, 6, 9 or 12 mg (corresponding to twice a day 1.600, 2.399, 4.799, 7.198 and 9.597 mg of rivastigmine hydrogen (2R,3R) tartrate); preferably at a daily dose of 2, 3, 6 or 9 mg (corresponding to twice a day 1.600, 2.399, 4.799 or 7.198 of rivastigmine hydrogen (2R,3R) tartrate).

Most preferably, the amount of roflumilast and rivastigmine hydrogen (2R,3R) tartrate, respectively, in the oral dosage form for co-administration is selected from 50, 75, 100 or 125 mg for roflumilast and 4.799 or 9.597 mg for rivastigmine hydrogen (2R,3R) tartrate (corresponding to 3 or 6 mg of rivastigmine).

Examples of roflumilast tablet formulations:

Example A: 250 mg roflumilast tablet

| Roflumilast | 0.250mg |
| Lactose monohydrate | 49.660mg |
| Corn starch | 13.390mg |
| Polyvidone K90 | 1.300mg |
| Magnesium stearate | 0.650mg |

Example B: 125 mg roflumilast tablet

| Roflumilast | 0.125mg |
| Lactose monohydrate | 49.660mg |
| Corn starch | 13.390mg |
| Polyvidone K90 | 1.300mg |
| Magnesium stearate | 0.650mg |

Example C: 100 mg roflumilast tablet

| Roflumilast | 0.100mg |
| Lactose monohydrate | 49.660mg |
| Corn starch | 13.390mg |
Polyvidone K90 1.300mg
Magnesium stearate 0.650mg

Example D: 75 mg roflumilast tablet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roflumilast</td>
<td>0.075mg</td>
</tr>
<tr>
<td>Lactose monohydrate</td>
<td>49.660mg</td>
</tr>
<tr>
<td>Corn starch</td>
<td>13.390mg</td>
</tr>
<tr>
<td>Polyvidone K90</td>
<td>1.300mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.650mg</td>
</tr>
</tbody>
</table>

Example E: 50 mcg roflumilast

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roflumilast</td>
<td>0.050mg</td>
</tr>
<tr>
<td>Lactose monohydrate</td>
<td>49.660mg</td>
</tr>
<tr>
<td>Corn starch</td>
<td>13.390mg</td>
</tr>
<tr>
<td>Polyvidone K90</td>
<td>1.300mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.650mg</td>
</tr>
</tbody>
</table>

Examples F: 10 mg Oral disintegrating tablet donepezil hydrochloride

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donepezil hydrochloride</td>
<td>10 mg</td>
</tr>
<tr>
<td>Colloidal silicon dioxide</td>
<td>2.5 mg</td>
</tr>
<tr>
<td>Mannitol</td>
<td>258 mg</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>7.5 mg</td>
</tr>
<tr>
<td>Polyvinyl alcohol</td>
<td>2 mg</td>
</tr>
</tbody>
</table>

Example G: 4 mg Galantamine (corresponding to 5.126 mg galantamine hydrobromide) tablet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galantamine hydrobromide</td>
<td>5.126 mg</td>
</tr>
<tr>
<td>Spray dried mixture lactose monohydrate and microcrystalline cellulose (75:25)</td>
<td>51.454 mg</td>
</tr>
<tr>
<td>Crospolyvidone</td>
<td>3 mg</td>
</tr>
<tr>
<td>Colloidal anhydrous silica</td>
<td>0.12 mg</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>0.3 mg</td>
</tr>
<tr>
<td>Film Coat</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Hypromellose</td>
<td>2.5 mg</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>0.603 µl</td>
</tr>
<tr>
<td>Talc</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Titanium Dioxide</td>
<td>0.75 mg</td>
</tr>
<tr>
<td>Colorants</td>
<td>0.0032 mg</td>
</tr>
</tbody>
</table>

Example H: 8 mg Galantamine (corresponding to 10.253 mg galantamine hydrobromide) tablet

<table>
<thead>
<tr>
<th>Galantamine hydrobromide</th>
<th>10.253 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray dried mixture</td>
<td>102.907 mg</td>
</tr>
<tr>
<td>lactose monohydrate and</td>
<td></td>
</tr>
<tr>
<td>microcrystalline cellulose</td>
<td></td>
</tr>
<tr>
<td>(75:25)</td>
<td></td>
</tr>
<tr>
<td>Crospolyvidone</td>
<td>6 mg</td>
</tr>
<tr>
<td>Colloidal anhydrous silica</td>
<td>0.24 mg</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>0.6 mg</td>
</tr>
<tr>
<td>Film Coat</td>
<td></td>
</tr>
<tr>
<td>Hypromellose</td>
<td>4 mg</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>0.965 µl</td>
</tr>
<tr>
<td>Talc</td>
<td>0.8 mg</td>
</tr>
<tr>
<td>Titanium Dioxide</td>
<td>1.2 mg</td>
</tr>
<tr>
<td>Colorants</td>
<td>0.013 mg</td>
</tr>
</tbody>
</table>

Example I: 12 mg Galantamine (corresponding to 15.379 mg galantamine hydrobromide) tablet

<table>
<thead>
<tr>
<th>Galantamine hydrobromide</th>
<th>15.379 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray dried mixture</td>
<td>154.361 mg</td>
</tr>
<tr>
<td>lactose monohydrate and</td>
<td></td>
</tr>
<tr>
<td>microcrystalline cellulose</td>
<td></td>
</tr>
<tr>
<td>(75:25)</td>
<td></td>
</tr>
<tr>
<td>Crospolyvidone</td>
<td>9 mg</td>
</tr>
<tr>
<td>Colloidal anhydrous silica</td>
<td>0.36 mg</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>0.9 mg</td>
</tr>
<tr>
<td>Film Coat</td>
<td></td>
</tr>
<tr>
<td>Hypromellose</td>
<td>5 mg</td>
</tr>
</tbody>
</table>
Example J: 16 mg Galantamine (corresponding to 20.506 mg galantamine hydrobromide) tablet

<table>
<thead>
<tr>
<th>Galantamine hydrobromide</th>
<th>20.506 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray dried mixture lactose monohydrate and microcrystalline cellulose (75:25)</td>
<td>205.814 mg</td>
</tr>
<tr>
<td>Crospolyvidone</td>
<td>12 mg</td>
</tr>
<tr>
<td>Collodial anhydrous silica</td>
<td>0.48 mg</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>1.2 mg</td>
</tr>
<tr>
<td>Film Coat</td>
<td></td>
</tr>
<tr>
<td>Hypromellose</td>
<td>6 mg</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>1.448 µl</td>
</tr>
<tr>
<td>Talc</td>
<td>1.2 mg</td>
</tr>
<tr>
<td>Titanium Dioxide</td>
<td>1.8 mg</td>
</tr>
<tr>
<td>Colorants</td>
<td>0.130 mg</td>
</tr>
</tbody>
</table>

Example K: Different oral pharmaceutical compositions, including, inter alia, extended release compositions for rivastigmine hydrogen (2R,3R) tartrate) are disclosed in international patent application WO00/19985, corresponding to US 6,565,883, the disclosure of which is hereby incorporated by reference in its entirety.

Pharmaceutical Formulations and Dosage Forms

When employed as pharmaceuticals, the compounds of the invention (the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are collectively referred to as 'the compounds of the invention' in the present specification) can be administered in the form of pharmaceutical compositions. These compositions can be prepared in a manner well known in the pharmaceutical art, and can be administered by a variety of routes. Administration can be pulmonary (e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, subcutaneous, intraperitoneal intramuscular or injection or infusion. Parenteral administration can be in the form of a single bolus dose, or can be, for example, by a continuous perfusion pump.
compositions and formulations for topical administration can include transdermal patches, conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or both of (1) a phosphodiesterase 4 inhibitor selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide, and (2) an acetylcholinesterase inhibitor selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine, in combination with one or more pharmaceutically acceptable carriers. As the pharmaceutically acceptable carriers, those known in the art can be employed. In making the compositions of the invention, the active ingredients are typically mixed with an excipient, diluted by an excipient or enclosed within such a carrier in the form of, for example, a capsule, sachet, paper, or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

The compositions can be formulated in a unit dosage form, each dosage containing an amount of each active ingredient as described above.

The active compounds can be effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It will be understood, however, that the amount of the compound actually administered will usually be determined by a physician, according to the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredients are mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of the active ingredients. When referring to these preformulation compositions as homogeneous, the active ingredients are typically dispersed evenly throughout the composition so that the composition can be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above.

The liquid forms in which the compounds and compositions of the present invention can be incorporated for administration orally or by injection include aqueous solutions, suitably flavored
syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.
EXAMPLES

The specific examples below are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent.

5

Example 1: Analysis of Cognitive Improvement Effects of Roflumilast on Spatial Memory in Mice

The objective of this study was to evaluate the cognitive improvement effects of Roflumilast on spatial memory employing Object Location Task (OLT) in male C57BL/6NCrl mice. Roflumilast was compared with data of the already established PDE4 inhibitor Rolipram in the same model.

METHODS

Maintenance of Animals Twenty-four 7 month-old male C57BL/6NCrl mice (Charles River, L'Arbresle, France) were used (average body weights: 27.6 g). The animals were kept under a 12/12-hour light/dark cycle (lights on from 07.00 pm to 07.00 am) with free access to food and water. All testing was done between 09.00 am and maximally 06.00 pm.

Preparation of Compounds for Administration Both rolipram (Sigma-Aldrich St. Louis, USA; MW 275.34) and roflumilast (Biocrea, Radebeul, Germany; MW 403.21) were dissolved in dimethylsulfoxide (DMSO) and kept at 4 °C; this stock solution was used for further dilutions in 0.5% methylcellulose. Each of rolipram and roflumilast is used in form of its free base. All injected solutions consisted of 0.5% methylcellulose with a fixed DMSO percentages (1.2%) (vehicle).

Object Location Task Studies in Mice For the Object Location Task (OLT), doses of 0.01 mg/kg, 0.03 mg/kg and 0.1 mg/kg of Rolipram or Roflumilast or vehicle were administered subcutaneously (s.c.). Based on previous findings, PDE4 inhibitor single administration was performed 3 hours after the first trial as this has an optimum effect on object memory performance. The injection volume was 5 µl/kg.

The OLT apparatus consisted of a circular arena, 40 cm in diameter. Half of the 40 cm transparent polyvinyl chloride wall was covered from the outside with white paper. Two objects were placed symmetrically about 10 cm away from the wall on the separation line, between the transparent and covered side of the arena. Four different sets of objects were available: (1) a cone made of brass (maximal diameter 6 cm and total height 3.8 cm), (2) a transparent glass bottle (diameter 2.7 cm, height 8.5 cm) filled with sand and water, (3) a massive metal cube (2.5 cm x 5 cm x 7.5 cm) with two holes (diameter 1.5 cm), and (4) a massive aluminum cube with a tapering top (4.5 cm x 4.5 cm x 8.5 cm).

A testing session comprised two trials of 4 minutes. Before each trial, mice were placed in an empty Makrolon cage (incubation cage) for the same amount of time as the trial (4 min). During the first trial (T1), two identical objects were placed symmetrically about 10 cm away from the wall on the separation line between the transparent and covered side of the arena. After the first exploration period of 4 min, the mouse was put back in its home cage. Mice then received treatment at 3 hours post T1. Subsequently, after a predetermined delay interval (24h), the mouse was placed in the
apparatus for the second trial of 4 min (T2). Two identical objects as in T1 were used; one object was placed in the previously used position, whereas the other was placed in a novel position. The novel position of the object could either be a fixed distance towards the front or a fixed distance towards the back of the arena for both objects. The times spent exploring each object during T1 and T2 were recorded manually using a personal computer. All objects and locations were used in a balanced manner to exclude possible object and/or location preferences. To avoid olfactory cues, the objects were thoroughly cleaned with 70% ethanol after each trial. The testing order of conditions was determined randomly.

Statistical Data Analysis The measurements reflected the time spent by the mice in exploring each object during T1 and T2. The time spent in exploring the two identical samples in T1 were represented by 'a1' and 'a2', respectively. The time spent in exploring the sample and the new object in T2 were represented by 'a' and 'b', respectively. From these exploration times the following variables were calculated: e1, e2, and d2 (Table 1). The d2 index is a relative measure of discrimination corrected for exploratory activity. The d2 index can range from -1 to 1, with -1 or 1 indicating complete preference for the familiar or novel object, respectively, and 0 signifying no preference for either object.

<table>
<thead>
<tr>
<th>Trial number</th>
<th>Exploration time (sec)</th>
<th>Discrimination index</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>e1 = a1 + a2</td>
<td>-</td>
</tr>
<tr>
<td>T2</td>
<td>e2 = a + b</td>
<td>d2 = (b - a) / e2</td>
</tr>
</tbody>
</table>

One-sample t-statistics were performed in order to assess whether the d2 index for each treatment group differed significantly from zero. However, comparison of the value of d2 with the value zero with no variance may not be the most suitable way of analyzing object recognition since there was an increased chance of making a type I error. Treatment groups were therefore also compared using one-way ANOVAs. When the overall ANOVA was significant, a post-hoc analysis with Bonferroni t-tests (all pairwise comparisons) was performed. A n a level of 0.05 was considered significant.

RESULTS

The results of the exploration times (e1 and e2) and the discrimination measures (d2) for each of the different groups are summarized in Table 2. There were no differences in exploration time between treatment conditions for both T1 (e1: F(6.113) = 1.27, n.s.) and T2 (e2: F(6.113) = 1.66, n.s.). One mouse was excluded from the analysis in the rolipram 0.1 mg/kg and roflumilast 0.01 mg/kg condition due to insufficient exploration times (< 7.5 seconds). Number of animals used in the study was: vehicle 23; rolipram 0.01 mg/kg: 16; rolipram 0.03 mg/kg: 16; rolipram 0.1 mg/kg: 15; roflumilast 0.01 mg/kg: 15; roflumilast 0.03 mg/kg: 16; roflumilast 0.1 mg/kg: 16.
Table 2: Means (± SEM) for the Derived Measures in the OLT

A: Rolipram

<table>
<thead>
<tr>
<th>Group number</th>
<th>Dose level Rolipram (mg/kg, s.c.)</th>
<th>e1 (s)</th>
<th>e2 (s)</th>
<th>d2 index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>13.87 (0.73)</td>
<td>13.36 (0.71)</td>
<td>-0.05 (-0.04)</td>
</tr>
<tr>
<td>2</td>
<td>0.01</td>
<td>15.58 (0.95)</td>
<td>12.99 (0.84)</td>
<td>0.08 (0.05)</td>
</tr>
<tr>
<td>3</td>
<td>0.03</td>
<td>15.45 (1.35)</td>
<td>13.56 (0.95)</td>
<td>0.13 (0.05)  #</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>15.82 (1.03)</td>
<td>12.29 (0.08)</td>
<td>0.07 (0.05)</td>
</tr>
</tbody>
</table>

e1, total exploration time during T1
e2, total exploration time during T2
d2 index, discrimination index between the new and familiar objects for T2

The d2 index differed from zero by one-sample f-tests: #: p<0.05.

B: Roflumilast

<table>
<thead>
<tr>
<th>Group number</th>
<th>Dose level Roflumilast (mg/kg, s.c.)</th>
<th>e1 (s)</th>
<th>e2 (s)</th>
<th>d2 index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>13.87 (0.73)</td>
<td>13.36 (0.71)</td>
<td>-0.05 (-0.04)</td>
</tr>
<tr>
<td>2</td>
<td>0.01</td>
<td>15.95 (1.11)</td>
<td>14.13 (1.22)</td>
<td>0.04 (0.05)</td>
</tr>
<tr>
<td>3</td>
<td>0.03</td>
<td>15.62 (1.37)</td>
<td>16.13 (1.18)</td>
<td>0.34 (0.03)  ###</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>17.51 (1.36)</td>
<td>13.73 (1.11)</td>
<td>0.00 (0.06)</td>
</tr>
</tbody>
</table>

e1, total exploration time during T1
e2, total exploration time during T2
d2 index, discrimination index between the new and familiar objects for T2

The d2 index differed from zero by one-sample f-tests: ###: p < 0.001.

One-sample t-tests showed that the d2 indices of the rolipram 0.03 mg/kg and roflumilast 0.03 mg/kg conditions significantly differed from zero, indicating that mice discriminated between locations after twenty-four hours (Table 2 and Figure 1). Between group comparisons showed significant differences between rolipram conditions (F(3.68) = 3.99, p < 0.05). Post-hoc analysis revealed that the d2 index in the rolipram 0.03 mg/kg condition differed significantly from the vehicle condition (Figure 1). Between group comparisons of the roflumilast conditions also showed significant differences (F(3.68) = 15.71, p < 0.001). Post-hoc analysis revealed that the d2 index of the roflumilast 0.03 mg/kg condition differed significantly from the vehicle condition (Figure 1).

In the OLT, roflumilast and rolipram were effective at the same dose of 0.03 mg/kg in improving spatial memory. Interestingly, the discrimination index (d2) for the Roﬂumilast treatment had a higher absolute value compared with the rolipram treatment, indicating that Roﬂumilast may have a stronger impact on spatial memory performance.
Since emesis is a typical side-effect of PDE4 inhibitors the emetic potential of roflumilast and rolipram was investigated in parallel using the xylazine/ketamine induced α2-adrenergic receptor-mediated anesthesia test. The results confirmed that the two phosphodiesterase 4 inhibitors have different effects on emesis. Rolipram showed a strong emetic potential already with a dose of 0.3 mg/kg. In contrast, roflumilast only showed a tendency towards emetic potential at a dose of 3.0 mg/kg.

The present data show that roflumilast is a better alternative for memory enhancement than rolipram since its effect on memory is more potent while its emetic potential is much lower (i.e., wider therapeutic window in human) than that of rolipram.

Example 2: Analysis of Effects of Roflumilast on Cognition in Healthy Adults.

The objective of this proof-of-concept study was to validate Roflumilast as cognitive enhancer using a translational behavior (i.e., cognitive testing) - EEG (i.e., brain electrical activity) approach. The study was intended to demonstrate whether memory, as well as attention, information processing, and executive function improved upon administration of Roflumilast in healthy adults. This single center, randomized, double blind, efficacy study had a four-period crossover design and used single administration in healthy adults (n=20; 18 to 35 years; both males and females) of Roflumilast (capsulated formulation of 100 mg, 300 mg, and 1000 mg) and of placebo with each period being ten to twenty-one days apart.

METHOD

Verbal Learning Task Analysis: The study utilized Verbal Learning Task (VLT) to analyze the increased number of words remembered following roflumilast administration. The VLT consisted of displaying 30 monosyllable words on a computer screen for a period of 60 seconds. Immediately after the presentation of the words on the computer screen subjects were asked to report as many words as they could recall by memory. This process (presentation and recall) was repeated further two times (VLT, immediate recall, 3 trials). In addition, 45 min and 24 h after the last presentation, subjects were again asked to report as many words as they could recall by memory (VLT, delayed recall 45 mh and 24 h). The set of three recall trials was conducted 60 minutes following administration of the roflumilast therapy.

Event Related Potential Analysis: An electroencephalogram (EEG) cap was used to place a set of 32 EEG electrodes according to the international 10-20 system on the subjects. Event Related Potentials (ERPs) were extracted by averaging the responses within an epoch of 100 ms before and 1000 ms after stimulus onset covering P300, N400, and P600. Separate averages were made for correct and incorrect responses within a task and for different trial types. EEG measurements were done simultaneously with VLT testing. ERPs were calculated from the words that were called during
immediate recall (encoding), and from the words that were recognized and from those that were not during the recognition condition at 45 minutes. The ERP components of P300, N400, and P600 were compared to examine whether the initial stimulus processing during the learning trials differs from word to word. Finally, ERPs to the old and new items during the recognition task were measured.

In addition to the VLT (immediate recall and delayed recall), the subjects were also tested in the Spatial Memory Task, the Stroop task and the Continuous Performance task (a description of these additional cognitive battery tests can be found in Example 3).

Statistical Data Analyses: Human data was analyzed using IBM SPSS Statistics version 20. General Linear Models for repeated measures were applied with the placebo condition included as contrast. Statistical outcomes for Tests of Within-Subjects Effects and Tests of Within-Subjects Contrasts were regarded for immediate and delayed free recall scores and for the summed immediate recall score (i.e. immediate 1 + immediate 2 + immediate 3). The factor Treatment (4 levels; placebo, roflumilast 100 meg, roflumilast 300 meg and roflumilast 1000 meg) was included as a within subjects factor. For the analysis of the EEG data, the factor Channel (5 levels; Fz, FCz, Cz, CPz, and Pz) was included as a second within subjects factor. Peak and latency values of three memory related ERP's were analysed; i.e. P300, N400 and P600. In case of significant findings (p < 0.05) post-hoc t-tests were performed to reveal which of the five midline electrodes contributed to the effect.

RESULTS

Low dose roflumilast (i.e., 100 meg) but not higher doses (i.e., 300 and 1000 meg) showed significant increase in the number of correct words recalled only after the 3rd trial of VLT (Figure 2).

In parallel, the corresponding EEG measurements revealed that ERP, P600 demonstrated increased amplitude with low dose Roflumilast (i.e., 100 meg) but not with higher doses (i.e., 300 and 1000 meg) (Figure 3).

Figure 2 and 3 do show some effect also for the 300 meg and 1000 meg dose. Analysing the roflumilast/roflumilast-N-oxide blood plasma levels of the treated healthy volunteers, it was revealed that some individuals in the 300 meg as well as in the 1000 meg dose group exhibited plasma concentration levels comparable to the plasma concentration level of the individuals from the 100 meg dose group. If the response is dependent on a specific targeted plasma concentration level range, then individuals with similar plasma concentration levels would be expected to show similar responses. Most likely, the reformulated roflumilast capsules used in the trial lead to some variability in the absorption profile of roflumilast in the 20 healthy adults. Therefore the effect of the 300 meg and the 1000 meg dose actually is probably to a certain extent lower than shown in Figure 2 and 3.

In the VLT, Roflumilast was effective at the single dose of 100 meg in improving number of correct words recalled (average 2.5 words). This is a meaningful effect considering the age group and the
education levels of the participants (i.e., young adult college students). Based on these results, one can expect to see larger effect with Roflumilast in elderly subjects with naturally occurring cognitive decline. Further, during the recall analyses, increased brain activity was observed at P600 only with low dose, 100 meg Roflumilast. This finding further supports the fact that the improvement observed on behavioural outcome (i.e., recalling more correct words) is a reflection of enhancement in brain activity captured and measured by EEG/ERPs.

No statistically significant differences were found between placebo and drug treatment in the Spatial Memory Task, the Stroop task and the Continuous Performance task.

The results of this study, taken together with the rodent data, indicate that low doses but not high doses (such as the approved once daily dose for the treatment of severe COPD; 500 meg) of Roflumilast are effective in improving cognitive functioning (e.g., memory deficits). Low dose Roflumilast, with better side effect and tolerability profile offers more suitable treatment for Mild Cognitive Impairment (MCI) and cognitive impairment associated with Alzheimer’s disease.

Example 3: Analysis of cognitive effects of roflumilast on age-related memory impairment

The primary objective of this study is to examine any improvement, following roflumilast administration, in memory of elderly subjects having (a) normal age-related memory impairment or (b) having enhanced age-related memory impairment by means of behavioral tasks.

The secondary objective of this study is to assess the effects of roflumilast on the electrophysiological correlates of memory and cognition.

METHOD

The study is conducted according to a double-blind, placebo-controlled, four-period cross-over design. Forty healthy subjects, both male and female within an age range of 60 to 80 years are planned to be included in the study in 2 groups: 1) 20 subjects with a memory performance between 1-2 Standard Deviation below the average for their age, gender, and educated level (Impaired Elderly) and 2) 20 subjects with age (± 3 years), gender, and educational level matched (in order to speed up the study matching was stopped after the interim analysis) with an average memory performance between 0.5 Standard Deviation below and 0.5 Standard Deviation above from normative values (Healthy Elderly). Classification in terms of impaired or healthy elderly will be determined by a one-off testing of memory performance using the Rey Verbal Learning Task (Rey A; L'examen psychologique dans les cas d'encephalopathy traumatique 1958 Paris; Presses Universitaire de France; or Van der Elst et al; J Int Neuropsychol Soc 2005, 11 (3), pp 290-302) according to fully standardized procedures as applied in The Maastricht Aging Study (Jolles et al; Maastricht Aging Study; determinants of cognitive aging; Maastricht, The Netherlands, Neuropsych Publishers 1995). Normative data for each individual subject will be derived also from the Maastricht Aging Study using

All subjects within their corresponding groups (either Healthy Elderly n=20 or Impaired Elderly n=20) will be randomized in a double blind fashion to 1 of 4 treatment sequences, each sequence consisting of the following periods: A) Placebo + placebo; B) Roflumilast 100 meg + placebo; C) Roflumilast 250 meg + placebo and D) Roflumilast 1000 meg (500 + 500 meg) according to a computer-generated allocation schedule in a cross-over design. Between each of the four treatment sequences there will be a 12 days washout period.

Cognitive status will be quantified using computerized cognitive battery, a validated tool for measuring the cognitive impairment in humans. The battery will consist of: VLT, Spatial Memory Task (SMT), Stroop Task, and Bond-Lader Visual Analogue Scales (BL-VAS).

Brain electrical activity changes will be quantified with EEG battery tests. The EEG battery tests will be administered to all subjects during VLT, SMT, Stroop as well as for sensory gating and Novelty oddball task.

Overview of testing day for each Treatment Period (Day 1 and 2) is given below:

<table>
<thead>
<tr>
<th>Time (min); Relative to dosing</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>-5</td>
<td>BL-VAS</td>
</tr>
<tr>
<td>0</td>
<td>Dosing (A, B, C or D)</td>
</tr>
<tr>
<td>55</td>
<td>Baseline EEG recording (5 min; eyes closed)</td>
</tr>
<tr>
<td>60</td>
<td>VLT Immediate recall, 3 trials (10 min)</td>
</tr>
<tr>
<td>70</td>
<td>Pharmacokinetic (PK) blood sampling</td>
</tr>
<tr>
<td>75</td>
<td>SMT immediate recall (10 min)</td>
</tr>
<tr>
<td>85</td>
<td>Stroop task (10 min)</td>
</tr>
<tr>
<td>95</td>
<td>Sensory gating (10 min)</td>
</tr>
<tr>
<td>105</td>
<td>BL-VAS (5 min)</td>
</tr>
<tr>
<td>110</td>
<td>VLT Delayed recall (3 min) and recognition (3 min)</td>
</tr>
<tr>
<td>120</td>
<td>SMT delayed recall (5 min)</td>
</tr>
<tr>
<td>125</td>
<td>Novelty Oddball task (10 min)</td>
</tr>
<tr>
<td>135</td>
<td>EEG recording, resting state (5 min, eyes closed)</td>
</tr>
<tr>
<td>140</td>
<td>PK blood sampling</td>
</tr>
<tr>
<td>145</td>
<td>Participants return home</td>
</tr>
<tr>
<td>Next Day</td>
<td></td>
</tr>
<tr>
<td>1430</td>
<td>Participants arrive</td>
</tr>
<tr>
<td>1435</td>
<td>BL-VAS (5 min)</td>
</tr>
<tr>
<td>1440 (24 h)</td>
<td>VLT Delayed recall (3 min) and recognition (3 min)</td>
</tr>
</tbody>
</table>
1450 SMT picture recognition and delayed recall (10 min)
1460 Stroop task (10 min)
1470 PK Blood sampling

5 Verbal learning task (VLT): The Rey VLT as modified by Riedel and colleagues (Riedel, Klaasen et al, Psychopharmacology (Berlin) 1999 Vol 141(4) pp 362-369) is used. This modified VLT maximizes the possibility of measuring enhancement rather than only impairment, by means of prolonging the list. The test consists of a list of 30 monosyllabic words (18 nouns and 12 adjectives). The words are shown on a computer screen for 1 second. Three trials with the same item sequence are presented. Each trial ends with a free recall of the words (immediate recall). Forty-five minutes after the first exposure, the subject is asked to recall as many words as possible (delayed recall). Subsequently, a recognition test is presented, consisting of 15 former words and 15 new but comparable words (distracters). The words are shown on a computer screen for 2 seconds and subjects are asked to rate whether they were presented in the learning trial by a "yes/no" response. The inter-word interval is 2 seconds. 24 Hours after the immediate recall, subjects will return to the lab for a second delayed recall and recognition. The remaining 15 old words and 15 new words will be presented during recognition. EEG will be recorded during the immediate recall and the first recognition test on the test day. No EEG recording will be performed during the first delayed recall and recognition at the 24 h measurement. The number of words correctly recalled will be collected during the three immediate learning trials (first, second, third and total) delayed, and recognition periods. The number of words correctly recalled in the learning trials is summed to yield the total immediate free recall score.

20 Spatial memory task (SMT): The spatial memory task assesses spatial memory and is based on the object relocation task by Postma and colleagues (Kessels, Postma et al, Behav Res Methods Instrum Comput. 1999, Vol 31(3) pp 423-428). It consists of one immediate and two delayed conditions. In the immediate condition, a set of 10 pictures will be presented one by one on different locations within a white square on a computer screen. All pictures are everyday, easy-to-name objects, presented in gray scale (± 3.5 x 5 cm). Each picture will be presented for 2000 msec with an interstimulus interval of 1000 msec. This will be followed by a "relocation" part, which consists of the presentation of a picture in the middle of the screen, followed by a "1" and a "2" being presented on two different locations. The participants' task is to decide where the picture was originally presented, in location "1" or location "2". The "1" and "2" will remain on the screen until the participant responds. After relocation, which is accomplished by a button press, the next picture will be presented followed by a "1/2" choice option. This continues until all 10 pictures have been relocated. Thereafter, the next set of 10 pictures will be presented. A total of 6 sets of 10 pictures are displayed. Forty-five minutes later, subjects will perform the first delayed version. The original locations are not presented again. Subjects immediately start with the relocation part of the task.

35 Twenty-four hours after the immediate condition, subjects will return to the lab and perform the task again. This time, the SMT will include a recognition phase. They are shown 60 old pictures (i.e. from
the SMT task) and 60 new pictures (i.e. not seen before in the SMT task), in 6 blocks of 20 pictures each (each block contains 10 old and 10 new pictures). The subjects have to rate within 2 seconds whether they were presented with these pictures in the learning trials by a "yes/no" response. If the subject indicates that they have seen a picture before, they are again presented with a "1" and a "2" on two different locations (regardless of the correctness of their response). Once more, they have to decide where the picture was originally presented in location "1" or location "2". The "1" and "2" will remain on the screen until the subject responds. If the subject indicates that the picture presented is new, no reply with regard to the original location has to be made. The space bar can be pressed instead, and the next picture will appear after a brief interval requiring the next "yes/no" response. As with the other tests, the EEG will be recorded during this task and this will later be analysed. No EEG will be recorded during the 24h-measurement. The number of correctly localized items will be collected during the immediate and the two delayed periods.

Stroop task:   The Stroop task is well known for its ability to induce interference, and assesses response inhibition and focused attention. In this task, colour names are printed in coloured ink; in the congruent category, the colour name and the colour of the ink are the same, in the incongruent category they are not. The subjects have to name the colour of the ink, not the words themselves. However, because of the urge to read the printed words (even if one is asked to ignore them) interference occurs. Since the printed words and ink colour differ in the incongruent category, interference is larger in this category than in the congruent category; this is called the "Stroop effect" and is known to remain even after extended practices (Gazzaniga, Ivry et al 2002, Cognitive neuroscience: The biology of the mind, W.W. Norton & Company, Inc). The colours used in this task are blue, red, green and yellow. The colour of the ink has to be named by pressing one out of four buttons, which each represent one of the colours. The main performance measures are the reaction time (RT) and the number of errors. The Stroop task will also be presented at the 24h measurement. EEG will be recorded during the first but not the second presentation of the task (i.e. no EEG at the 24h measurement) and it will be analysed similarly to the EEG recorded during VLT.

Sensory gating: Subjects will be presented with one type of auditory stimuli, a click with a duration of 3 ms, constructed from a 1000 Hz tone. Clicks will be presented in pairs with an interval of 500 ms between the first (S1) and the second (S2) click. The interval between click pairs will be random between 6 and 10 s and the intensity of the click is around 60 dB. The subjects will be asked to sit quietly and listen to the tones. EEG will be recorded during this task, of which ERPs will be calculated offline. The most important ERP component is the P50, which is usually reduced in amplitude to the second as compared to the first click. By calculating the ratio (S2/S1), an indication of the amount of gating can be obtained.

Novelty oddball task:   The novelty oddball task assesses involuntary attention processes. It is a passive paradigm, in which three types of auditory stimuli are presented while the subject watches a silent movie/cartoon and ignores the stimulation. The stimuli consist of frequent standard, infrequent deviant and infrequent novel stimuli. The standard and deviant stimuli will be 500 Hz and 750 Hz
tones with two upper harmonic components (1000 and 1500, 1500 and 2250 Hz, respectively). The intensity of the first and second harmonic components is decreased compared to the fundamental by 3 and 6 dB, respectively. The use of those stimuli will be counterbalanced between subjects, but will remain constant for the different measurements within subjects. Novel stimuli consist of three stimulus categories of 20 different sounds, namely animal, human, and mechanical sounds. The deviant and novel stimuli will each be presented in 12.5% of the trials. All sounds have duration of 300 ms with 10 ms rise and fall times and will be presented with a 1000 ms stimulus onset asynchrony and equal intensities to both ears using a headphone. No behavioral measures will be recorded. ERPs will be recorded, from which the N100 will be analysed. Furthermore, the response to the standard will be subtracted from the deviant and novel stimuli, which enables the visualization of the mismatch negativity and P3a components, the latter being a novelty response. The amplitudes and latencies of these components will be compared between the deviant-standard and novel standard responses. The P3a component measures the involuntary switch to novel stimuli, whereas the mismatch negativity is a measure of sensory memory.

Bond-Lader Visual Analogue Scales (BL-VAS): The BL-VAS (Bond A and Lader M, 1974; Br J Med Psychol Vol 47, pp. 211-218) will be used in order to assess alertness, calmness, and contentedness. BL-VAS consists of 16 100 mm visual analogue scales anchored by antonyms (eg. Alert-Drowsy, Lethargic-Energetic; etc) and be applied on testing Day 1 and 2 of each treatment period.

For the EEG measurements, an EEG cap will be used to place a set of 32 EEG electrodes according to the international 10-20 system. A reference and a ground will be placed at the linked mastoids and at the forehead, respectively. Eye movements will be detected by horizontal and vertical electro-oculogram (EOG) recordings. Before electrode attachment, the positions will be slightly scrubbed with a gel in order to provide a good measurement. Both EEG and EOG will be filtered between 0.01 and 100 Hz and sampled at 500 Hz. Offline, the EEG will be checked for EOG activity and other artifacts. The EEG that contains artifacts will be excluded from analysis. ERPs will be extracted by averaging the responses within an epoch of 100 ms before and 1000 ms after stimulus onset. Separate averages will be made for correct and incorrect responses within a task and for different trial types. With regard to the VLT, the following measures will be used. ERPs will be calculated from the thirty words presented during each of the three immediate recall trials separately. Additionally, ERP’s for both old and new word, as presented during the recognition paradigm at 45 min will be calculated. Primarily, the P300, N400, and P600 components will be analysed for both tasks.

Expected outcome: It is expected that in this elderly study population, in particular in the group of subjects with an enhanced age-associated memory impairment, the signals seen in the young healthy subjects in the study of example 2, will be confirmed and will at the same time be more pronounced, i.e. (1) efficacy of low dose roflumilast and (2) inefficacy of the single administration dose (1000 meg) correlating with the repeated administration dose of roflumilast approved for the treatment of severe chronic obstructive pulmonary disease (500 meg) will be confirmed.
Interim Analysis

An interim analysis has been performed based on the data of 9 subjects of the Impaired Elderly group and 4 subjects of the Healthy Elderly group. For this purpose, cognitive battery test scores (VLT, SMT, Stroop task and BL-VAS) and concentrations of roflumilast and roflumilast-N-oxide in plasma have been analysed and summarized by dose over each scheduled sampling time using descriptive statistics.

Results of Interim Analysis

The results are based on a pooled interim analysis from 9 subjects of the Impaired Elderly group and 4 subjects of the Healthy Elderly group who have already completed the study.

In the present study, subjects of the Healthy Elderly group remembered 10 words after 3 learning trials on a 30 words list task, whereas subjects of the Impaired Elderly group remembered 7.9 words after 3 learning trials on the 30 word list task.

Though the interim analysis population was small, there were effects indicative of activity. Compared to placebo, low dose roflumilast (i.e., 100 meg) but not higher doses (i.e., 250 and 1000 meg) showed a statistically significant (p<0.05) increase in the number of correct words immediately recalled (i.e., 1.5 words) after the 3rd trial of VLT (Figure 4). Compared to placebo, low and high dose roflumilast (i.e., 100 and 1000 meg, respectively) but not middle dose (i.e., 250 meg) showed a statistically significant (p<0.05) increase in the number of correct words (i.e., 1.7 and 1.6 words, respectively) recalled after 45 min delayed recall trial of VLT (Figure 4). Also, the subjects reported significantly higher (subjective) alertness in comparison with placebo only at 45 min after the low dose, based on BL-VAS scores.

No statistically significant differences were detected between placebo and drug treatment in the Spatial Memory Task, and Stroop task.

The exposure to roflumilast and roflumilast N-oxide were consistent with those previously reported.

Following a single low dose of roflumilast (100 meg), an improvement in the number of correct words immediately recalled (i.e., 1.5 words) was observed. As with immediate recall, a similar improvement seen in delayed recall appears to confirm its clinical meaning. The placebo-corrected fold-improvement was 1.16 in the present interim data of the elderly study. This was consistent with the fold-improvement of 1.12 in the healthy volunteer study, though the absolute word improvement on the 3rd trial from 8.81 to 10.28 words in elderly subjects was smaller than that of 21.25 to 23.7 words on the 3rd trial in young volunteers in the previous study (Example 2). Taken together, the interim data analysis of the elderly subjects supports the finding that low dose roflumilast enhances episodic
memory performance observed in young volunteers, thereby adding considerable weight to its potential usefulness in prodromal dementia (Mild cognitive impairment (MCI)).

The results of this study, taken together with the results in healthy adults and the rodent data, indicate that low doses of roflumilast may be effective in improving cognitive functioning (e.g., memory deficits). Low dose roflumilast, with better side effect and tolerability profile compared to the approved once daily dose for the treatment of severe COPD (500 meg) offers more suitable treatment for the cognitive impairment associated with aging [dementia, Mild Cognitive Impairment (MCI) and Alzheimer’s disease] as well as with Schizophrenia.

Example 4: Analysis of Cognitive Improvement Effects of Roflumilast alone and in combination with Donepezil on memory performance in rats

The objective of this study is to evaluate the efficacious dose range of roflumilast as well as possible synergistic effects of a combination of roflumilast and the acetylcholinesterase inhibitor donepezil with regard to cognitive improvement effects by using scopolamine induced memory deficit on object memory performance in male Wistar rats. Memory acquisition processes are investigated using the object recognition test (ORT).

Methods

Maitenance of Animals: Twenty four 3-4 month old male Wistar rats (Charles River, Sulzfeld, Germany) were used for the study. All animals were housed individually in standard green line Tecniplast IVC cages on Stardust bedding. The animals were housed on a reversed 12/12 h light/dark cycle (lights on from 07:00 pm to 07:00 am) and had free access to food and water. The rats were housed and tested in the same room. A radio, playing softly, provided background noise in the room. All testing was performed between 09:00 am and 06:00 pm.

Preparation of Compounds: Drugs were prepared daily, except as indicated otherwise. Roflumilast (Takeda, Konstanz, Germany, MW 403.21) was dissolved in 98% methylcellulose solution (0.5% methylcellulose) and 2% Tween 80. Roflumilast in doses of 0 (vehicle), 0.0001, 0.0003, 0.001, 0.003, 0.01 and 0.03 mg/kg was administered intraperitonally (ip, injection volume 1 ml/kg). Scopolamine (in form of scopolamine hydrobromide) and donepezil (in form of donepezil hydrochloride) were both dissolved in saline (0.9% NaCl). Scopolamine hydrobromide was as well administered intraperitonally at a dose of 0.1 mg/kg (ip, injection volume 1ml/kg). Donepezil hydrochloride was dissolved in saline (Prckaerts et al; 2012, Neuropharmacology Vol 62, 1099-1110). Donepezil hydrochloride was administered p.o. (po, injection volume 1ml/kg) at a dose of 0.1 mg/kg. All compounds were administered 30 min before T1.
Object Recognition Task in Rats: The testing order of conditions was determined randomly. The experimenter was blind of testing protocol. A within design was used and all treatment conditions were balanced over rats. The ORT was performed as described elsewhere (Ennaceur and Delacour, 1988, Behav Brain Res, Vol 31, pp 47-59; Akkerman et al; 2012, Behav Brain Res, Vol 232, pp 335-347). The apparatus consisted of a circular arena, 83 cm in diameter. The back half of the 40 cm high wall was made of grey PVC and the front was made of transparent PVC. Fluorescent red tubes and a light bulb provided a constant illumination of about 20 lux on the floor of the apparatus. The light intensity was equal in the different parts of the apparatus.

Two objects were placed in symmetrical positions at the mid-line between the gray and transparent halves of the arena, about ten centimeters away from wall. Four different sets of objects were available: 1) a standard 1 L brown transparent glass bottle (diameter 10 cm, height 22 cm) filled with water, 2) a metal cube (10.0 x 5.0 x 7.5 cm) with two holes (diameter 1.9 cm), 3) a cone consisting of a gray PVC base (maximal diameter 18 cm) with a collar on top made of brass (total height 16 cm), and 4) an aluminium cube with a tapering top (13.0 x 8.0 x 8.0 cm). Objects were presented to the animals in a balanced manner to avoid object or place biases. Rats were unable to displace the objects.

A test session comprised two trials, each with durations of 3 min. During the learning trial (T1) the apparatus contains two identical objects (object a1 and a2). Rats were always introduced into the apparatus with their nose towards the transparent wall segment (i.e. facing outwards to the front of the arena). Subsequently, rats were put back in its home cage for a 1 h interval. After the retention interval, rats were put back into the arena for the learning trial (T2). In T2, the two objects from T1 were replaced by one identical copy (a3) and a different novel object (b). The times spent in exploring each object during T1 and T2 were recorded manually on a personal computer.

Exploration was defined in the following manner: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. Sitting on the object was not considered as exploratory behavior. In order to avoid the presence of olfactory cues, the objects were thoroughly cleaned with a 70% ethanol solution before each trial.

Experimental procedure:

Prior to compound testing, the animals were handled for 5 min on 2 consecutive days and allowed to explore the ORT arena, also for 5 min. Subsequently, the animals were accustomed to the complete ORT testing procedure without receiving any injection. As soon as the animals showed good discrimination performance at a 1 h interval, the testing for the roflumilast dose response study was started. First, the saline/vehicle and scopolamine (0.1 mg/kg)/vehicle conditions were tested to verify that the cholinergic deficit model effectively impaired object memory. Next, a dose-response curve was created by testing several doses (0.0001, 0.0003, 0.001, 0.003, 0.01 and 0.03 mg/kg) of
roflumilast in combination with scopolamine. Table 3 below shows a schematic overview of the names and details of the different conditions in the dose-response study.

Table 3 - Experimental conditions of the roflumilast dose-response study

<table>
<thead>
<tr>
<th>Condition Name</th>
<th>Dose Scopolamine (mg/kg)</th>
<th>Dose Roflumilast (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>0 (vehicle)</td>
<td>0 (vehicle)</td>
</tr>
<tr>
<td>scopolamine</td>
<td>0.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>0.0001 mg/kg</td>
<td>0.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>0.0003 mg/kg</td>
<td>0.1</td>
<td>0.001</td>
</tr>
<tr>
<td>0.001 mg/kg</td>
<td>0.1</td>
<td>0.003</td>
</tr>
<tr>
<td>0.003 mg/kg</td>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>0.01 mg/kg</td>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>0.03 mg/kg</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

Scopolamine and roflumilast were administered 30 min before T1. The retention time between T1 and T2 was 1 h.

The study to evaluate the potential synergy of a combined administration of roflumilast and donepezil was conducted by using the least efficacious roflumilast dose from the dose-response experiment. Thus, a sub-efficacious dose (0.0001 mg/kg) of roflumilast was tested in combination with a sub-efficacious dose (0.1 mg/kg; no effect on object memory performance; Prickaerts et al 2005, Psychopharmacology Vol 177, pp 381-390) of donepezil to investigate whether the two compounds could synergistically improve memory performance. Table 4 below shows schematically the treatment conditions of the synergy study.

Table 4 - Experimental conditions of the roflumilast-donepezil synergy study

<table>
<thead>
<tr>
<th>Condition Name</th>
<th>Dose Scopolamine (mg/kg)</th>
<th>Dose Roflumilast (mg/kg)</th>
<th>Dose Donepezil (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>veh/veh/veh</td>
<td>0 (vehicle)</td>
<td>0 (vehicle)</td>
<td>0 (vehicle)</td>
</tr>
<tr>
<td>scop/veh/veh</td>
<td>0.1</td>
<td>0 (vehicle)</td>
<td>0 (vehicle)</td>
</tr>
<tr>
<td>scop/veh/dpz</td>
<td>0.1</td>
<td>0 (vehicle)</td>
<td>0.1</td>
</tr>
<tr>
<td>scop/rof/veh</td>
<td>0.1</td>
<td>0.0001</td>
<td>0 (vehicle)</td>
</tr>
<tr>
<td>scop/rof/dpz</td>
<td>0.1</td>
<td>0.0001</td>
<td>0.1</td>
</tr>
</tbody>
</table>

All compounds were administered 30 min before T1. The retention interval between T1 and T2 were 1 h. (veh: vehicle; scop: scopolamine; dpz: donepezil; rof: roflumilast)
Statistical Analysis:
The readout parameters of the object recognition task are the times that rats spent on exploring each object during T1 and T2. The exploration time (in seconds) of each object during T1 are presented as "a1" and "a2". The time spent in exploring the familiar and the new object in T2 are presented as "a" and "b", respectively. Using this information, the following variables are calculated: $e_1, e_2$ and $d_2$ (see Table 5 below). The $d_2$ index is a relative measure of discrimination corrected for exploratory activity and has been shown not to be correlated with $e_1$ and $e_2$ (Akkerman et al, 2012, Behav Brain Res Vol 232, pp 317-322). The $d_2$ index can range from -1 to 1. A significant difference from zero indicates that rats remembered the objects from T1 and a difference from the vehicle condition signifies an actual memory improvement. Of note, rats require a minimum amount of exploration in order to show reliable memory performance (Akkerman et al, 2012, Behav Brain Res Vol 232, pp 335-347). Therefore, animals are removed from the analysis if they spend less than 6 sec or 9 sec exploring the objects during T1 and T2, respectively. In the present study, none of the animals had to be excluded.

Table 5 - Object recognition task (Read-out Measures and their calculations)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Exploration time (sec)</th>
<th>Discrimination Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>$e_1 = a_1 + a_2$</td>
<td>$d_2 = (b - a) / e_2$</td>
</tr>
<tr>
<td>T2</td>
<td>$e_2 = a + b$</td>
<td></td>
</tr>
</tbody>
</table>

One sample t-statistics were performed to assess whether the $d_2$ index for each treatment condition was significantly different from zero (chance level). However, comparison of the value of $d_2$ with the value zero with no variance is not the most suitable way of analyzing object recognition since there is an increased chance of making a type I error. For this reason, treatment conditions were also compared using one-way ANOVA. In case of significant differences between treatment conditions, post-hoc analyses were performed using Bonferroni t-tests to compare each treatment to the vehicle condition. All statistical analyses were performed using an a of 0.05.

Results

a) The effects of roflumilast on a scopolamine induced memory deficit in the ORT

The results of the dose-response experiment with roflumilast in the scopolamine induced memory deficit are summarized in Table 6. One way ANOVA revealed no significant differences between treatment conditions in the level of exploration in T1 ($e_1$: $F(7, 120)=1.09$, n.s.) and T2 ($e_2$: $F(7, 120)=0.82$, n.s.)

Table 6 - Mean values (± SEM) of the different ORT measures in the roflumilast dose response study

<table>
<thead>
<tr>
<th>Condition</th>
<th>$e_1$ (sec)</th>
<th>$e_2$ (sec)</th>
<th>$d_2$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>23.88 (2.31)</td>
<td>25.31 (1.84)</td>
<td>0.43 (0.05)</td>
<td>16</td>
</tr>
</tbody>
</table>
One sample t-tests were performed on the d2 measures, a significant difference from zero (indicated by hash-signs; #: p < 0.05; ###: p < 0.001) indicates that the animals remembered the object from T1.

One sample t-tests were used to compare the d2 of the different treatment to zero. It was found that the d2 values of all conditions were significantly higher than zero, except the d2 values of the scopolamine and 0.0001 mg/kg conditions (see Table 6). One-way ANOVA revealed significant differences in d2 between treatment conditions (F(3,81)=6.67, p<0.001). Post-hoc analyses with Bonferroni t-tests showed that the vehicle, 0.003 mg/kg, 0.01 mg/kg and 0.03 mg/kg conditions has a significantly higher d2 value compared to the scopolamine condition. On the other hand, only the scopolamine and 0.0001 mg/kg conditions were significantly lower compared to the vehicle condition.

Taken together these data indicate that roflumilast was able to fully restore memory function at doses of 0.003 mg/kg and higher whereas animals treated with 0.0003 mg/kg and 0.001 mg/kg only showed intermediate memory improvement, i.e. were only different from zero. The dose of 0.0001 mg/kg roflumilast had no effect on memory performance.

The effects of the different doses of roflumilast on object memory performance are graphically presented in Figure 5.

b) The combined effects of sub-efficacious doses of roflumilast and donepezil on a scopolamine induced memory deficit in the ORT

The results of the roflumilast-donepezil study are summarized in Table 7. One way ANOVA revealed no significant differences between treatment conditions in the level of exploration in T1 (e1: F(4,115)=0.44, n.s.) and T2 (e2: F(4,115)=1.46, n.s.).

<table>
<thead>
<tr>
<th>Condition</th>
<th>e1 (s)</th>
<th>e2 (s)</th>
<th>d2</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>veh/veh/veh</td>
<td>26.85 (2.07)</td>
<td>29.07 (1.72)</td>
<td>0.31 (0.07) ***</td>
<td>24</td>
</tr>
<tr>
<td>scop/veh/veh</td>
<td>24.77 (1.86)</td>
<td>30.76 (2.14)</td>
<td>-0.04 (0.05)</td>
<td>24</td>
</tr>
</tbody>
</table>
One sample t-tests were used to compare the d2 of the different treatments to zero. It was found that only the d2 value of the veh/veh/veh and scop/rof/dpz conditions were significantly higher than zero.

One-way ANOVA revealed significant d2 differences between treatment conditions (F(4, 115)=1.378, p<0.001). Post-hoc analyses with Bonferroni t-tests showed that the veh/veh/veh and scop/rof/dpz conditions had a significantly higher d2 value compared to the scop/veh/veh condition. On the other hand, the scop/veh/veh, scop/veh/dpz and scop/rof/veh conditions were all significantly lower compared to the veh/veh/veh condition. No difference was found between the veh/veh/veh condition and the scop/rof/dpz condition. Taken together, these data show that the combined sub-efficacious doses of roflumilast and donepezil were able to fully restore memory function. The effects of the different conditions on object memory performance are graphically presented in Figure 6.
Further aspects of the invention

a) A pharmaceutical composition for use in the treatment of cognitive impairment, comprising
   I) a phosphodiesterase 4 inhibitor in combination with
   II) an acetylcholinesterase inhibitor, and
   III) a pharmaceutically acceptable carrier,

   wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

b) The pharmaceutical composition according to above-mentioned a), wherein treatment of cognitive impairment means any one of
   (a) treatment of mild cognitive impairment;
   (b) delay of progression from mild cognitive impairment to cognitive impairment associated with Alzheimer's disease; and
   (c) treatment of cognitive impairment associated with Alzheimer's disease.

c) The pharmaceutical composition according to above-mentioned a), wherein treatment of cognitive impairment is treatment of mild cognitive impairment.

d) The pharmaceutical composition according to above-mentioned a), wherein treatment of cognitive impairment is delay of progression from mild cognitive impairment to cognitive impairment associated with Alzheimer's disease.

e) The pharmaceutical composition according to above-mentioned a), wherein treatment of cognitive impairment is treatment of cognitive impairment associated with Alzheimer's disease.

f) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is selected from roflumilast or a pharmaceutically acceptable salt of roflumilast.

g) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is selected from roflumilast-N-oxide or a pharmaceutically acceptable salt of roflumilast-N-oxide.

h) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast.

i) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide.
j) The pharmaceutical composition according to any one of above-mentioned a) to g), wherein the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

k) The pharmaceutical composition according to any one of above-mentioned a) to g), wherein the acetylcholinesterase inhibitor is donepezil hydrochloride.

l) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

m) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is donepezil hydrochloride.

n) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

o) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is donepezil hydrochloride.

p) The pharmaceutical composition according to any one of above-mentioned a) to g), wherein the acetylcholinesterase inhibitor is selected from the group consisting of galantamine and a pharmaceutically acceptable salt of galantamine.

q) Pharmaceutical composition according to any one of a) to g), wherein the acetylcholinesterase inhibitor is galantamine hydrobromide.

r) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of galantamine and a pharmaceutically acceptable salt of hydrobromide.

s) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is galantamine hydrobromide.

t) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of galantamine and a pharmaceutically acceptable salt of galantamine.
u) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is galantamine hydrobromide.

v) The pharmaceutical composition according to any one of above-mentioned a) to g), wherein the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

w) The pharmaceutical composition according to any one of a) to g), wherein the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate.

x) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

y) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate.

z) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

aa) The pharmaceutical composition according to any one of above-mentioned a) to e), wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate.

bb) The pharmaceutical composition according to any one of above-mentioned j), k), p), q), v) or w), wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.

c) The pharmaceutical composition according to any one of above-mentioned j), k), p), q), v) or w), wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 meg.

dd) The pharmaceutical composition according to any one of above-mentioned i), m), r), s), x) or y), wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.

ee) The pharmaceutical composition according to any one of above-mentioned l), m), r), s), x) or y), wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 meg.

ff) The pharmaceutical composition according to any one of above-mentioned n), o), t), u), z) or aa), wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.
gg) The pharmaceutical composition according to any one of above-mentioned n), o), t), u), z) or aa), wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 mg.

hh) The pharmaceutical composition according to any one of above-mentioned cc), ee) or gg), wherein the phosphodiesterase 4 inhibitor is present in an amount of 50 mg.

ii) The pharmaceutical composition according to any one of above-mentioned cc), ee) or gg), wherein the phosphodiesterase 4 inhibitor is present in an amount of 62.5 mg.

jj) The pharmaceutical composition according to any one of above-mentioned cc), ee) or gg), wherein the phosphodiesterase 4 inhibitor is present in an amount of 75 mg.

kk) The pharmaceutical composition according to any one of above-mentioned cc), ee) or gg), wherein the phosphodiesterase 4 inhibitor is present in an amount of 100 mg.

ll) The pharmaceutical composition according to any one of above-mentioned cc), ee) or gg), wherein the phosphodiesterase 4 inhibitor is present in an amount of 125 mg.

mm) The pharmaceutical composition according to any one of above-mentioned cc), ee) or gg), wherein the phosphodiesterase 4 inhibitor is present in an amount of 150 mg.

nn) The pharmaceutical composition according to above-mentioned m), wherein roflumilast is present in an amount of between 50 and 150 mg and donepezil hydrochloride and is present in an amount of between 5 and 23 mg.

oo) The pharmaceutical composition according to above-mentioned m), wherein roflumilast is present in an amount selected from 50, 75, 100 or 125 mg and donepezil hydrochloride is present in an amount selected from 5 or 10 mg.

pp) The pharmaceutical composition according to above-mentioned s), wherein roflumilast is present in an amount of between 50 and 150 mg and galantamine hydrobromide is present in an amount corresponding to between 4 and 12 mg of galantamine.

qq) The pharmaceutical composition according to above-mentioned s), wherein roflumilast is present in an amount selected from 50, 75, 100 or 125 mg and galantamine hydrobromide is present in an amount corresponding to 4 or 8 mg of galantamine.

rr) The pharmaceutical composition according to above-mentioned y), wherein roflumilast is present in an amount of between 50 and 150 mg and and rivastigmine hydrogen (2R,3R) tartrate is present in an amount corresponding to between 3 and 12 mg of rivastigmine.
ss) The pharmaceutical composition according to above-mentioned y), wherein roflumilast is present in an amount selected from 50, 75, 100 or 125 mg and rivastigmine hydrogen (2R,3R) tartrate is present in an amount corresponding to 3 or 6 mg of rivastigmine.

5 tt) The pharmaceutical composition according to any one of above-mentioned a) to ss), wherein the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are administered in one single dosage form.

uu) The pharmaceutical composition according to any one of above-mentioned a) to ss), wherein the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are administered concurrently or sequentially in two separate dosage forms.

vv) Use of a phosphodiesterase 4 inhibitor in combination with an acetylcholinesterase inhibitor for the manufacture of a pharmaceutical composition for the treatment of cognitive impairment, wherein the phosphodiesterase 4 inhibitor is selected from the group of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil, a pharmaceutically acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

ww) Use according to above-mentioned vv), wherein treatment of cognitive impairment means any of a) Treatment of mild cognitive impairment; b) Delay of progression from mild cognitive impairment to cognitive impairment associated with Alzheimer's disease; and c) Treatment of cognitive impairment associated with Alzheimer's disease.

xx) Use according to above-mentioned vv), wherein treatment of cognitive impairment is treatment of mild cognitive impairment.

yy) Use according to above-mentioned vv), wherein treatment of cognitive impairment is delay of progression from mild cognitive impairment to cognitive impairment associated with Alzheimer's disease.

zz) Use according to above-mentioned vv), wherein treatment of cognitive impairment is treatment of cognitive impairment associated with Alzheimer's disease

aaa) Use according to any one of above-mentioned vv) to zz), wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast and a pharmaceutically acceptable salt of roflumilast.
Use according to any one of above-mentioned vv) to zz), wherein the phosphodiesterase inhibitor is selected from the group consisting of roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide.

Use according to any one of above-mentioned vv) to zz), wherein the phosphodiesterase inhibitor is roflumilast.

Use according to any one of above-mentioned vv) to zz), wherein the phosphodiesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

Use according to any one of above-mentioned vv) to bbb), wherein the acetylcholinesterase inhibitor is donepezil hydrochloride.

Use according to any one of above-mentioned vv) to bbb), wherein the acetylcholinesterase inhibitor is donepezil hydrochloride.

Use according to any one of above-mentioned vv) to bbb), wherein the acetylcholinesterase inhibitor is selected from the group consisting of galantamine and a pharmaceutically acceptable salt of galantamine.

Use according to any one of above-mentioned vv) to bbb), wherein the acetylcholinesterase inhibitor is galantamine hydrobromide.
Use according to any one of above-mentioned v) to zz), wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is galantamine hydrobromide.

Use according to any one of above-mentioned v) to zz), wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of galantamine and a pharmaceutically acceptable salt of galantamine.

Use according to any one of above-mentioned v) to zz), wherein the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

Use according to any one of above-mentioned v) to zz), wherein the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate.

Use according to any one of above-mentioned v) to zz), wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of rivastigmine and a pharmaceutically acceptable salt of rivastigmine.

Use according to any one of above-mentioned v) to zz), wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is rivastigmine hydrogen (2R,3R) tartrate.

Use according to any one of above-mentioned eee), fff), kkk), iii), qqq) and rrr), wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of between 50 and 300 meg.

Use according to any one of above-mentioned eee), fff), kkk), iii), qqq) and rrr), wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of between 50 and 150 meg.
yyy) Use according to any of above-mentioned ggg), hhh), mmm), nnn), sss) and ttt), wherein the phosphodiesterase 4 inhibitor is roflumilast and wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of between 50 and 300 mg.

zzz) Use according to any of above-mentioned ggg), hhh), mmm), nnn), sss) and ttt), wherein the phosphodiesterase 4 inhibitor is roflumilast and wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of between 50 and 150 mg.

aaaa) Use according to any of above-mentioned iii), jjj), ooo), pp), uuu) and vvv), wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of between 50 and 300 mg.

bbbb) Use according to any of above-mentioned iii), jjj), ooo), pp), uuu) and vvv), wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 50 and 150 mg.

cccc) Use according to any of above-mentioned xxx), zzz) and bbbb), wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 50 mg.

dddd) Use according to any of above-mentioned xxx) , zzz) and bbbb), wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 62.5 mg.

eeee) Use according to any of above-mentioned xxx), zzz) and bbbb), wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 75 mg.

ffff) Use according to any of above-mentioned xxx), zzz) and bbbb), wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 100 mg.

9999) Use according to any of above-mentioned xxx), zzz) and bbbb), wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 125 mg.

hhhh) Use according to any of above-mentioned xxx), zzz) and bbbb), wherein the phosphodiesterase 4 inhibitor is present in the pharmaceutical composition in an amount of 150 mg.

iii) Use according to above-mentioned hhh), wherein roflumilast is present in the pharmaceutical composition in an amount of between 50 and 150 meg and donepezil hydrochloride and is present in the pharmaceutical composition in an amount of between 5 and 23 mg.
jjjj) Use according to above-mentioned hhh), wherein roflumilast is present in the pharmaceutical composition in an amount selected from 50, 75, 100 or 125 mg and donepezil hydrochloride is present in the pharmaceutical composition in an amount of 5 or 10 mg.

5 kkkk) Use according to above-mentioned nnn), wherein roflumilast is present in the pharmaceutical composition in an amount of between 50 and 150 mg and galantamine hydrobromide is present in the pharmaceutical composition in an amount corresponding to between 4 and 12 mg of galantamine.

10 mmmm) Use according to above-mentioned ttt), wherein roflumilast is present in the pharmaceutical composition in an amount of between 50 and 150 mg and rivastigmine hydrogen (2R,3R) tartrate is present in the pharmaceutical composition in an amount corresponding to between 3 and 12 mg of rivastigmine.

15 nnnn) Use according to above-mentioned ttt), wherein roflumilast is present in the pharmaceutical composition in an amount selected from 50, 75, 100 or 125 mg and rivastigmine hydrogen (2R,3R) tartrate is present in the pharmaceutical composition in an amount corresponding to 3 or 6 mg of rivastigmine.

20 oooo) Use according to any one of above-mentioned vv) to nnnn), wherein the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are to be administered in one single dosage form.

30 pppp) Use according to any one of above-mentioned vv) to nnnn), wherein the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are to be administered concurrently or sequentially in two separate dosage forms.
1. A pharmaceutical composition, comprising:
   a. a phosphodiesterase 4 inhibitor in combination with
   b. an acetylcholinesterase inhibitor, and
   c. a pharmaceutically acceptable carrier,

   wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil, a pharmaceutical acceptable salt of donepezil, galantamine, a pharmaceutically acceptable salt of galantamine, rivastigmine, and a pharmaceutically acceptable salt of rivastigmine.

2. The pharmaceutical composition according to claim 1, wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast and a pharmaceutically acceptable salt of roflumilast.

3. The pharmaceutical composition according to claim 1, wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide.

4. The pharmaceutical composition according to claim 1, wherein the phosphodiesterase 4 inhibitor is roflumilast.

5. The pharmaceutical composition according to claim 1, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide.

6. The pharmaceutical composition according to any one of claims 1 to 3, wherein the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

7. The pharmaceutical composition according to any one of claims 1 to 3, wherein the acetylcholinesterase inhibitor is donepezil hydrochloride.

8. The pharmaceutical composition according to claim 1, wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

9. The pharmaceutical composition according to claim 1, wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is donepezil hydrochloride.
10. The pharmaceutical composition according to claim 1, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

11. The pharmaceutical composition according to claim 1, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is donepezil hydrochloride.

12. The pharmaceutical composition according to any one of claims 6 to 7, wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.

13. The pharmaceutical composition according to any one of claims 6 to 7, wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 meg.

14. The pharmaceutical composition according to any one of claims 8 to 9, wherein the phosphodiesterase 4 inhibitor is roflumilast and wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.

15. The pharmaceutical composition according to any one of claims 8 to 9, wherein the phosphodiesterase 4 inhibitor is roflumilast and wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 meg.

16. The pharmaceutical composition according to any one of claims 10 to 11, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.

17. The pharmaceutical composition according to any one of claims 10 to 11, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 meg.

18. The pharmaceutical composition according to any one of claims 13, 15 and 17, wherein the phosphodiesterase 4 inhibitor is present in an amount of 50 meg.

19. The pharmaceutical composition according to any one of claims 13, 15 and 17, wherein the phosphodiesterase 4 inhibitor is present in an amount of 62.5 meg.

20. The pharmaceutical composition according to any one of claims 13, 15 and 17, wherein the phosphodiesterase 4 inhibitor is present in an amount of 75 meg.
21. The pharmaceutical composition according to any one of claims 13, 15 and 17, wherein the phosphodiesterase 4 inhibitor is present in an amount of 100 mg.

22. The pharmaceutical composition according to any one of claims 13, 15 and 17, wherein the phosphodiesterase 4 inhibitor is present in an amount of 125 mg.

23. The pharmaceutical composition according to any one of claims 13, 15 and 17, wherein the phosphodiesterase 4 inhibitor is present in an amount of 150 mg.

24. The pharmaceutical composition according to claim 9, wherein roflumilast is present in an amount of between 50 and 150 mg and donepezil hydrochloride is present in an amount of between 5 and 23 mg.

25. The pharmaceutical composition according to claim 9, wherein roflumilast is present in an amount selected from 50, 75, 100 or 125 mg and donepezil hydrochloride is present in an amount selected from 5 or 10 mg.

26. The pharmaceutical composition according to any one of claims 1 to 25, wherein the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are to be administered concurrently in one single dosage form.

27. The pharmaceutical composition according to any one of claims 1 to 25, wherein the phosphodiesterase 4 inhibitor and the acetylcholinesterase inhibitor are to be administered concurrently or sequentially in two separate dosage forms.

28. A pharmaceutical composition for use in the treatment of cognitive impairment, comprising
   I) a phosphodiesterase 4 inhibitor in combination with
   II) an acetylcholinesterase inhibitor, and
   III) a pharmaceutically acceptable carrier,

   wherein the phosphodiesterase 4 inhibitor is selected from the group consisting of roflumilast, a pharmaceutically acceptable salt of roflumilast, roflumilast-N-oxide and a pharmaceutically acceptable salt of roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

29. The pharmaceutical composition according to claim 28, wherein treatment of cognitive impairment means any one of
   (a) treatment of mild cognitive impairment;
   (b) delay of progression from mild cognitive impairment to cognitive impairment associated with Alzheimer's disease; and
   (c) treatment of cognitive impairment associated with Alzheimer's disease.
30. The pharmaceutical composition according to claim 28, wherein treatment of cognitive impairment is treatment of mild cognitive impairment.

31. The pharmaceutical composition according to claim 28, wherein treatment of cognitive impairment is delay of progression from mild cognitive impairment to cognitive impairment associated with Alzheimer's disease.

32. The pharmaceutical composition according to claim 28, wherein treatment of cognitive impairment is treatment of cognitive impairment associated with Alzheimer's disease.

33. The pharmaceutical composition according to any one of claims 28 to 32, wherein the phosphodiesterase 4 inhibitor is selected from roflumilast or a pharmaceutically acceptable salt of roflumilast.

34. The pharmaceutical composition according to any one of claims 28 to 32, wherein the phosphodiesterase 4 inhibitor is selected from roflumilast-N-oxide or a pharmaceutically acceptable salt of roflumilast-N-oxide.

35. The pharmaceutical composition according to any one of claims 28 to 32, wherein the phosphodiesterase 4 inhibitor is roflumilast.

36. The pharmaceutical composition according to any one of claims 28 to 32, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide.

37. The pharmaceutical composition according to any one of claims 28 to 34, wherein the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

38. The pharmaceutical composition according to any one of claims 28 to 34, wherein the acetylcholinesterase inhibitor is donepezil hydrochloride.

39. The pharmaceutical composition according to any one of claims 28 to 32, wherein the phosphodiesterase 4 inhibitor is roflumilast and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.

40. The pharmaceutical composition according to any one of claims 28 to 32, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is donepezil hydrochloride.

41. The pharmaceutical composition according to any one of claims 28 to 32, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is selected from the group consisting of donepezil and a pharmaceutically acceptable salt of donepezil.
42. The pharmaceutical composition according to any one of claims 28 to 32, wherein the phosphodiesterase 4 inhibitor is roflumilast-N-oxide and the acetylcholinesterase inhibitor is donepezil hydrochloride.

43. The pharmaceutical composition according to any one of claims 37 to 38, wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.

44. The pharmaceutical composition according to any one of claims 37 to 38, wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 meg.

45. The pharmaceutical composition according to any one of claims 39 to 40, wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.

46. The pharmaceutical composition according to any one of claims 39 to 40, wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 meg.

47. The pharmaceutical composition according to any one of claims 41 to 42, wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 300 meg.

48. The pharmaceutical composition according to any one of claims 41 to 42, wherein the phosphodiesterase 4 inhibitor is present in an amount of between 50 and 150 meg.

49. The pharmaceutical composition according to any one of claims 44, 46 and 48, wherein the phosphodiesterase 4 inhibitor is present in an amount of 50 meg.

50. The pharmaceutical composition according to any one of claims 44, 46 and 48, wherein the phosphodiesterase 4 inhibitor is present in an amount of 62.5 meg.

51. The pharmaceutical composition according to any one of claims 44, 46 and 48, wherein the phosphodiesterase 4 inhibitor is present in an amount of 75 meg.

52. The pharmaceutical composition according to any one of claims 44, 46 and 48, wherein the phosphodiesterase 4 inhibitor is present in an amount of 100 meg.

53. The pharmaceutical composition according to any one of claims 44, 46 and 48, wherein the phosphodiesterase 4 inhibitor is present in an amount of 125 meg.

54. The pharmaceutical composition according to any one of claims 44, 46 and 48, wherein the phosphodiesterase 4 inhibitor is present in an amount of 150 meg.
Figure 1: Dose-response effects of rolipram (s.c) and roflumilast (s.c.) on the discrimination index (d2) in the object location task after a twenty-four hour retention interval in 7 months old C57BL/6NCrl mice (means + SEM)

When compared with vehicle treatment, both rolipram and roflumilast 0.03 mg/kg conditions had a significantly higher d2 index. A significant difference from the vehicle condition is depicted with asterisks (Bonferroni comparison t-test: *: p < 0.05, **: p < 0.001). A difference from zero is depicted with hashes (one-sample t-tests: #: p < 0.05, ###: p < 0.001).
Figure 2: The effect of Roflumilast after the 3rd recall trial in VLT for number of correct words

![Free recall - correct items](image)

VLT analyses using a General Linear Model for repeated measures, with the placebo condition included as contrast demonstrated that roflumilast caused increase in number of correct words reached to statistically significant levels after only 100 mcg dose and observed only at 3rd recall (tests of within subjects contrasts: 100 mcg vs. placebo: **p = 0.004; 300 mcg vs. placebo: p = 0.624; 1000 mcg vs. placebo: p = 0.137).
Figure 3: The effect of Roflumilast during the 3rd recall trial of VLT on ERPs

P600 after word presentation during 3rd recall trial

μV

placebo  100 mcg  300 mcg  1000 mcg

**

EEG co-measurements using a General Linear Model for repeated measures, with the placebo condition included as contrast during VLT 3rd recall demonstrated that roflumilast 100 mcg caused statistically significant induction in mean peak values at the Pz electrode (tests of Within Subjects Contrast, factor Treatment: placebo – 100 mcg: p = 0.048; placebo – 300 mcg: p = 0.109; placebo – 1000 mcg: p = 0.440). Post-hoc paired sample t-tests were performed to specify the effects for the 5 midline electrodes in the 100 mcg condition as compared to placebo: Fz: p = 0.465; FCz: p = 0.315; Cz: p = 0.015; CPz: p = 0.025; Pz: p = 0.002. The values in the figure refer to the mean peak values measured at the Pz electrode, i.e. **p = 0.002.
Figure 4: The effect of Roflumilast on VLT parameter of number of correct words

VLT analyses using a General Linear Model for repeated measures, with the placebo condition included as contrast demonstrated that roflumilast caused increase in number of correct words reached to statistically significant levels for 3rd recall at 100 mcg and delayed (45min) recall at 100 and 1000 mcg

Tests of within subjects contrasts:

3rd recall:
- 100 mcg vs. placebo: *p = 0.024;
- 250 mcg vs. placebo: p= 0.245;
- 1000 mcg vs. placebo: p = 0.527

Delayed (45 min) recall:
- 100 mcg vs. placebo: *p = 0.015;
- 250 mcg vs. placebo: p= 0.351;
- 1000 mcg vs. placebo: *p = 0.021
Figure 5: The effect of different doses of roflumilast on a scopolamine induced memory deficit in the object location task in male Wistar rats.

Figure 5 shows the average d2 value and SEM of each treatment condition in the roflumilast dose response study. The discrimination index (d2) is indicated on the y-axis and the different treatment conditions are shown on the x-axis. Hash signs indicate a difference from zero (#: p<0.05; ###: p<0.001), a difference from the vehicle condition is indicated with dollar signs ($$: p<0.01; $$$: p<0.001) and a difference from the scopolamine condition is indicated with asterisk (*: p<0.05; **: p<0.01; ***: p<0.001).
Figure 6: The combined effects of sub-efficacious doses of roflumilast and donepezil on a scopolamine induced memory deficit in the object location task in male Wistar rats.

Figure 6 shows the average d2 value and SEM of each treatment condition in the roflumilast-donepezil study. The discrimination index (d2) is indicated on the y-axis and the different treatment conditions are shown on the x-axis. Hash signs indicate a difference from zero (###: p<0.001), a difference from the veh/veh/veh condition is indicated with dollar signs ($$: p<0.01; $$$: p<0.001) and a difference from the scop/veh/veh condition is indicated with asterisk (**: p<0.001).
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

ADD.

According to International Patent Classification (IPC) and/or both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>wo 01/87281 A2 (SMITHKLINE BEECHAM PLC [GB] ; HAGAN JAMES [GB]) 22 November 2001 (2001-11-22) claims 1, 2 ----- 1-54</td>
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Further documents are listed in the continuation of Box C.

X See patent family annex.

* Special categories of cited documents:
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  E* earlier application or patent but published on or after the international filing date
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  O* document referring to an oral disclosure, use, exhibition or other means
  P* document published prior to the international filing date but later than the priority date claimed
  T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  X* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search

21 October 2014

Date of mailing of the international search report

27/10/2014

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Authorized officer

Terenzi, Carl a
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<td>A</td>
<td>US 2006/183776 A9 (PRATT RAYMOND [US]) 17 August 2006 (2006-08-17) page 1, paragraphs 5, 6, 10</td>
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<td>A</td>
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