ISOINDOLINE DERIVATIVES FOR USE AS AMPK ACTIVATORS

DESCRIPTION

Field of the Invention

The present invention relates in general to the pharmaceutical field, and more precisely it refers to isoindoline derivatives of formula (I) reported in the following, that are activators of an enzymatic complex, the protein kinase activated by adenosine monophosphate (abbreviated in the following as AMPK, acronym for the whole English term 5' Adenosine MonoPhosphate-activated protein Kinase). They are useful for the prophylaxis and treatment of metabolic disorders, such as diabetes and obesity, and of immune-mediated inflammatory diseases and cancer. The present invention also relates to a process for the preparation of these compounds.

State of the Art

AMPK is a heterotrimeric kinase of the serine / threonine kinase family, known to be involved in the regulation of metabolic pathways of the energy demand / consumption, and plays a key role in maintaining adequate levels of ATP in the cells in conditions that deplete these levels, such as exercise, hunger, hypoxia and rapid cell growth.

Once activated by phosphorylation of a critical threonine residue (Thr172), the AMPK kinase activates the pathways that generate ATP, such as glucose transport in muscles, fatty acid oxidation, and autophagy, while inhibiting the pathways consuming ATP, such as the synthesis of cholesterol and fatty acids, and the protein synthesis. In addition to regulating metabolic enzymes and other proteins by direct phosphorylation, the AMPK enzyme complex is also known to regulate co-activators and transcription factors such as PGC1a, FOXO proteins, HDAC, p300 and CREB. It moreover regulates the activity of another key metabolic element, mTOR, with which it allows cells to respond appropriately to metabolic stress and to regulate cell growth and differentiation, as well as autophagy.

Thanks to the central role played by AMP in energy homeostasis in cells and in the whole body, this enzymatic complex represents a very attractive target for the treatment of various metabolic diseases. In particular, the discovery that physical activity activates AMPK in skeletal muscles leading to increased glucose uptake indicates a therapeutic potential of compounds that are AMPK activators to treat type 2 diabetes. Furthermore, in obese insulin-resistant patients, a reduced AMPK activity in adipose tissue was observed, together with an increased expression of genes associated with inflammation compared to what was observed in control subjects, suggesting also in this case that a reduced activity of AMPK could play a role in the causes of the disease.

Activation of AMPK in hepatic and adipose tissue modulates different biochemical pathways that balance lipogenesis and lipolysis to maintain ATP. The enzyme complex AMPK inhibits the lipid synthesis through the phosphorylation and inhibition of ACC1 and HMG-CoA reductase, together with the transcriptional regulation of lipogenic transcription factors involved in the synthesis of fatty acids and cholesterol, SREBP-1C and SREBP-2, respectively.

Furthermore, this enzyme complex critically contributes to the modulation of immune / inflammatory cell functions (i.e. macrophages, neutrophils, lymphocytes, dendritic cells), such as the production of cytokines, chemotaxis, cytotoxicity, apoptosis and proliferation.

Alterations in the expression of AMPK and / or in its activity have proved to be important in the pathophysiology of immune-mediated inflammatory diseases characterized by abnormal functions of immune cells, such as psoriasis, inflammatory bowel disease, rheumatoid arthritis, atherosclerosis and some neurodegenerative diseases (i.e. of Huntington, Alzheimer's syndrome and Parkinson's disease), thus confirming the relevance of this protein in the modulation of immune / inflammatory responses.

In addition to the regulation of the activity of immune cells, the AMPK enzyme complex is also involved in blocking carcinogenesis, by counteracting most of the metabolic changes that occur in rapidly proliferating cells by acting on their metabolic state.

Based on this experimental evidence, AMPK can rightly be considered a relevant molecular target for the treatment of metabolic diseases, including type 2 diabetes and obesity, as well as of immune-mediated inflammatory diseases and cancer, thus making strongly felt the need to provide powerful and effective AMPK activator compounds.

Up to now, several classes of compounds have been developed that are considered AMPK activators. The main class is that of the so-called "direct activators", which are small molecules able to bind to the enzyme complex and to activate it, triggering a change in its conformation that allows further activation by phosphorylation of the Thr172 key residue in one of the subunits of the complex. A first example of compound considered as a direct AMPK activator is represented by the adenosine analogue 5-aminoimidazole-4-carboxamide riboside, known as AICAR. This molecule, which is an AMP-mimetic and is thus able to regulate AMP-dependent enzymes too, cannot be considered a specific AMPK activator. Furthermore, despite having received the marketing authorization, AICAR is a product characterized by a very short half-life.

Further direct AMPK activators have also been described, the thienopyridone derivative A769662 developed by Abbott Laboratories, and the benzoimidazole derivative 991 developed by Merck Sharp and Dohme. They both bind to the allosteric binding site for drug and metabolite (ADaM) of AMPK, therefore they do not work in the presence of mutations in the enzyme AMPK complex that stabilize

the ADaM site. Furthermore, both these compounds are characterized by a modest oral bioavailability.

A further product developed by Metabasis Pharmaceutics as a direct activator of AMPK is the isoxazolyl-furan-2-phosphonic acid C2 that promotes the activity of AMPK mimicking AMP. Even this product, however, does not meet the requirements for the development of an effective drug: because of its high hydrophilicity, the cell walls are in fact impermeable to C2, which has already been set aside in favour of one of its prodrugs, the corresponding isopropyl phosphoester C13, which exhibits better bioavailability.

Various other compounds have been described in the literature as AMPK activators and many of them are currently in clinical trials; none of them, however, has for the moment still obtained the marketing authorization as a drug.

The need to identify novel compounds that are powerful and efficient AMPK activators without presenting the drawbacks mentioned above for the known compounds, therefore remains very much felt.

Summary of the Invention

Now the Applicant has found that derivatives having a isoindoline-1 ,3-dionic nucleus, suitably functionalized at nitrogen atom in position 2 and substituted at position 4 or 5 of the benzo-fused ring, are able to significantly stimulate the AMPK enzymatic complex activity, involved in particular, as illustrated above, in the regulation of metabolic pathways for the production and consumption of cellular energy.

It is therefore a subject of the invention the comounds of general formula (I) wherein:

A is selected from the group consisting of C1-C6 alkyl, cyano C1-C4 alkyl, aryl, heteroaryl, heterocycle, aryl C1-C4 alkyl, heteroaryl C1-C4 alkyl, and heterocycle C1-C4 alkyl, wherein each aryl, heteroaryl or heterocycle group is optionally substituted with one or more substituents, equal or different from each other and selected from the group consisting of halogen, cyano, nitro, trifluoromethyl, methoxy, hydroxy, methyl-thio, mercapto, amino, carboxy, formyl, carbamoyl, alkylcarbonyl, arylcarbonyl, sulphamoyl, alkylamido, arylamido, alkylureido, arylureido, alkylsulphonamido, arylsulphonamido, aryl, heteroaryl, and heterocycle;

B is absent or is selected from among O, S, SO, S02, NH, and N(H)CO;

R is selected from the group consisting of aryl, heteroaryl, heterocycle, wherein each aryl, heteroaryl or heterocycle group is optionally substituted by one or more substituents equal or different between each other and selected from the group consisting of halogen, cyano, nitro, trifluoromethyl, methoxy, hydroxy, methyl-thio, mercapto, amino, carboxy, formyl, carbamoyl, alkylcarbonyl, arylcarbonyl, sulphamoyl, alkylamido, arylamido, alkylureido, arylureido, alkylsulphonamido, arylsulphonamido,

aryl, heteroaryl, and heterocycle, and pharmaceutically acceptable salts thereof, for use in the prophylaxis and treatment of diseases or disorders that benefit from activation of the AMPK enzymatic complex, for example metabolic disorders, such as diabetes and obesity, immune-mediated inflammatory pathologies and cancer.

The compounds of general formula (I) defined above wherein A is cyanomethyl, B is N(H)CO and R is phenyl optionally substituted, a pharmaceutical composition comprising at least one of these compounds in admixture with one or more pharmaceutically acceptable excipients and/or diluents and/or vehicles, and the process for their preparation, are further subjects of the invention.

Further important characteristics of the compounds of formula (I), of the process for preparing them, of the pharmaceutical compositions comprising them and of the related medical use according to the invention are reported in the following detailed description.

Brief Description of the Drawings

The Figures 1 and 2 here attached show the most significant results obtained in the experimental studies described in detail in the following. In particular:

-Figure 1 illustrates the level of activation of AMPK in the C2C12 cell line after 30 minutes of exposure to the isoindoline-1 ,3-dionic compound 1 obtained as disclosed in Example 2 below and to berberine (BBR), reference compound, both tested at concentration 10 microM;

-Figure 2 illustrates the level of AMPK activation in the C2C12 cell line after 30 minutes of exposure to isoindoline-1 ,3-dionic compounds 1 and 25 described below, tested at concentration 20 microM, and to acadesine (ACA), reference compound, tested at concentration 200 microM.

Detailed Description of the Invention

In the present invention, the term "halogen" refers to fluorine, chloro, bromo or iodo, if not defined otherwise.

The term "alkyl" refers to a monovalent saturated hydrocarbon radical bearing a linear or branched residue, if not indicated otherwise. The "alkyl" group in the present invention, when consisting of 2 or more carbon atoms, may comprise double or triple carbon-carbon bonds or, when consisting of 3 or more carbon atoms, may form cyclic residues.

The term "aryl" refers to a cyclic or bicyclic aromatic group, consisting of a minimum of 6 to a maximum of 10 carbon atoms, for example phenyl or naphthyl, except differently defined.

The terms "heteroaryl" and "heterocycle" refer respectively to heteroaromatic compounds and to non-aromatic heterocyclic compounds, formed by a minimum of 5 to a maximum of 12 members and containing from 1 to 3 heteroatoms, selected from the group consisting of N, O, S, SO and S02.

In the present invention, the term "pharmaceutically acceptable salt" refers to derivatives of the isoindoline-1 ,3-dionic compounds of general formula (I) wherein the compound has been suitably

modified by conversion of any basic or acid group, if present, into the corresponding addition salt with any acid or base conventionally considered as acceptable for pharmaceutical uses.

According to a preferred embodiment of the present invention the isoindoline-1 ,3-dionic derivatives for use have general formula (I) wherein A is cyanomethyl, B is N(H)CO and R is phenyl optionally substituted with one or more substituents selected from hydroxy or methoxy.

Non-limitative examples of isoindoline-1,3-dionic derivatives of general formula (I) for the use according to the present invention are selected from the following:

1 : N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

2: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2-methoxy-benzamide;

3: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3-methoxy-benzamide;

4: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-4-methoxy-benzamide;

5: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3,4-dimethoxy-benzamide;

6: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3,5-dimethoxy-benzamide;

7: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2,6-dimethoxy-benzamide;

8: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2-hydroxy-benzamide;

9: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3-hydroxy-benzamide;

10: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-4-hydroxy-benzamide;

11 : N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3,4-dihydroxy-benzamide;

12: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3,5-dihydroxy-benzamide;

13: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2,6-dihydroxy-benzamide;

14: 2-amino-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

15: 3-amino-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

16: 4-amino-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

17: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2-nitro-benzamide;

18: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3-nitro-benzamide;

19: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-4-nitro-benzamide;

20: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2-fluoro-benzamide;

21 : N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3-fluoro-benzamide;

22: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-4-fluoro-benzamide;

23: 2-chloro-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

24: 3-chloro-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

25: 4-chloro-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

26: 2-bromo-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

27: 3-bromo-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

28: 4-bromo-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

29: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2-(trifluoromethyl)benzamide;

30: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3-(trifluoromethyl)benzamide;

31 : N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-4-(trifluoromethyl)benzamide;

32: 4-cyano-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

33: 4-cyano-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

34: 4-cyano-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)benzamide;

35: N-(2-(cyanomethyl)-1 ,3-dioxoisoindolin-5-yl)-[1 , 1 '-biphenyl]-4-carboxamide;

36: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2'-methoxy-[1 , T-biphenyl]-4-carboxamide;

37: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3'-methoxy- [1,1'-biphenyl]-4-carboxamide;

38: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-4'-methoxy- [1,1'-biphenyl]-4-carboxamide;

39: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3',4'-dimethoxy- [1,1'-biphenyl]-4-carboxamide;

40: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3',5'-dimethoxy- [1,1'-biphenyl]-4-carboxamide;

41 : N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2',6'-dimethoxy- [1,1'-biphenyl]-4-carboxamide;

42: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2'-hydroxy- [1,1'-biphenyl]-4-carboxamide;

43: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3'-hydroxy- [1,1'-biphenyl]-4-carboxamide;

44: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-4'-hydroxy- [1,1'-biphenyl]-4-carboxamide;

45: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3',4'-dihydroxy- [1,1'-biphenyl]-4-carboxamide;

46: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3',5'-dihydroxy- [1,1'-biphenyl]-4-carboxamide;

47: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2',6'-dihydroxy- [1,1'-biphenyl]-4-carboxamide;

48: 2'-amino-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)- [1,1'-biphenyl]-4-carboxamide;

49: 3'-amino-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)- [1,1'-biphenyl]-4-carboxamide;

50: 4'-amino-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)- [1,1'-biphenyl]-4-carboxamide;

51 : N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2'-nitro-[1,1'-biphenyl]-4-carboxamide;

52: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3'-nitro-[1,1'-biphenyl]-4-carboxamide;

53: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-4'-nitro-[1,1'-biphenyl]-4-carboxamide;

54: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2'-fluoro-[1,1'-biphenyl]-4-carboxamide;

55: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3'-fluoro-[1,1'-biphenyl]-4-carboxamide;

56: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-4'-fluoro-[1,1'-biphenyl]-4-carboxamide;

57: 2'-chloro-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-[1,1'-biphenyl]-4-carboxamide;

58: 3'-chloro-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-[1,1'-biphenyl]-4-carboxamide;

59: 4'-chloro-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-[1,1'-biphenyl]-4-carboxamide;

60: 2'-bromo-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-[1,1'-biphenyl]-4-carboxamide;

61: 3'-bromo-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-[1,1'-biphenyl]-4-carboxamide;

62: 4'-bromo-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-[1,1’-biphenyl]-4-carboxamide;

63: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-2'-(trifluoromethyl)-[1,1'-biphenyl]-4-carboxamide;

64: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-3'-(trifluoromethyl)-[1,1’-biphenyl]-4-carboxamide;

65: N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-4'-(trifluoromethyl)-[1,1’-biphenyl]-4-carboxamide;

66: 2'-cyano-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-[1,1’-biphenyl]-4-carboxamide;

67: 3'-cyano-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-[1,1’-biphenyl]-4-carboxamide;

68: 4'-cyano-N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-[1,1’-biphenyl]-4-carboxamide;

69: N- (2 -benzyl-1,3-dioxoisoindolin-5-yl)benzamide;

70: N- (2- (2-methoxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide;

71 : N- (2- (3-methoxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide;

72: N- (2- (4-methoxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide;

73: N- (2- (3,4-dimethoxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide

74: N- (2- (3,5-dimethoxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide

75: N- (2- (2,6-dimethoxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide

76: N- (2- (2-hydroxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide;

77: N- (2- (3-hydroxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide;

78: N- (2- (4-hydroxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide;

79: N- (2- (3,4-dihydroxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide;

80: N- (2- (3,5-dihydroxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide;

81 : N- (2- (2,6-dihydroxybenzyl)-1,3-dioxoisoindolin-5-yl)benzamide;

82: N- (2- benzyl-1,3-dioxoisoindolin-5-yl)-2-methoxybenzamide;

83: N- (2- benzyl-1,3-dioxoisoindolin-5-yl)-3-methoxybenzamide;

84: N- (2 -benzyl-1,3-dioxoisoindolin-5-yl)-4-methoxybenzamide;

85: N- (2 -benzyl-1,3-dioxoisoindolin-5-yl)-3,4-dimethoxybenzamide;

86: N- (2 -benzyl-1,3-dioxoisoindolin-5-yl)-3,5-dimethoxybenzamide;

87: N- (2 -benzyl-1,3-dioxoisoindolin-5-yl)-2,6-dimethoxybenzamide;

88: N- (2 -benzyl-1,3-dioxoisoindolin-5-yl)-2-hydroxybenzamide;

89: N- (2 -benzyl-1,3-dioxoisoindolin-5-yl)-3-hydroxybenzamide;

90: N- (2- benzyl-1,3-dioxoisoindolin-5-yl)-4-hydroxybenzamide;

91 : N- (2- benzyl-1,3-dioxoisoindolin-5-yl)-3,4-dihydroxybenzamide;

92: N- (2- benzyl-1,3-dioxoisoindolin-5-yl)-3,5-dihydroxybenzamide;

93: N- (2- benzyl-1,3-dioxoisoindolin-5-yl)-2,6-dihydroxybenzamide.

With reference to the Scheme 1 reported in the following, the present description provides moreover a process for the preparation of the isoindoline-1 ,3-dionic derivatives of general formula (I) wherein B is selected from O, S, SO, SO2, NH and N(H)CO and A and R are defined as above, and pharmaceutically acceptable salts thereof, comprising the steps of:

i) suitable functionalization or conversion of an amino group present on an isoindoline-1 ,3-dione at position 4 or 5 of the benzo-fused ring to yield the desired -B-R substituent;

ii) suitable functionalization at the isoindoline nitrogen on position 2 of the derivative coming from step i) to introduce the substituent A depending on the desired compound of formula (I).

Depending on the desired compound of general formula (I) wherein B is selected from O, S, SO, $S0_2$, NH and N(H)CO and wherein A and R are defined as above, different functionalization with different reagents will be carried out in the step i) of the above said process, according to procedures and with reagents that can be easily identified by any person with ordinary skills in the art. For example, if a compound of general formula (I) is wanted wherein B is NH, the amino group on the benzo-fused ring in step i) is converted by reaction with alkyl halides. If a compound of general formula (I) is indeed desired wherein B is N(H)CO, the same amino group as above is converted by reaction with acyl halides. If it is indeed desired a compound of general formula (I) wherein B is O, the amino group on the benzo-fused ring is converted in step i) into a hydroxyl group then reacted with alkyl halides. Finally, if a compound of general formula (I) wherein B is S, or SO or SO2 is desired, the amino group on the benzo-fused ring is converted in step i) of the above said process into a sulfhydryl group, which is subsequently reacted with alkyl halides to obtain the compounds of formula (I) wherein B is S, or further oxidized to yield the compounds of formula (I) wherein B is SO or SO2.

The following Scheme 1 illustrates a non-limitative scheme of the above said process of the invention, particularly useful to obtain the compounds of general formula (I) wherein B, at position 5 of the benzo-fused ring, is selected from O, S, SO, SO2, NH and N(H)CO and A and R are defined as above.

In the first step i), the starting reagent 5-aminoisoindoline-1 ,3-dione of formula (II) is converted into the desired compounds of general formula (I) according to the different alternative procedures of step i) illustrated in the Scheme 1 below as a), b), c) and d) wherein the amino group on the benzo- fused ring can be functionalized or converted into hydroxyl or sulfhydryl, as explained above, depending on the kind of spacer B and of substituent R that is wanted in compounds (I), before alkylation at the isoindoline nitrogen on position 2 of the ring.

Scheme 1

wherein A and R are as defined above.

The compounds of general formula (I) reported above wherein B is absent and A and R are as defined above can be prepared in general by a process of preparation comprising the steps of:

i'') reaction of substitution of a leaving group on an isoindoline-1 ,3-dione bearing the leaving group in position 4 or 5 of the benzo-fused ring to introduce on it the desired R substituent;

ii'') functionalization at the isoindoline nitrogen in position 2 of the derivative coming from step i'') to introduce the substituent A depending on the desired compound of formula (I).

In the following Scheme 2 the process above defined is schematically illustrated, with reference to the preparation of compounds of general formula (I) wherein B is absent and R is aryl, with R in position 5 on the benzo-fused ring.

Scheme 2

The compounds of general formula (I) according to the invention, wherein B is absent and R is different from aryl, can be prepared with procedures analogues to that illustrated above in Scheme 2, or by conversion of the so obtained compound (I) wherein R is aryl, according to modes and with reagents and conditions easily identifiable by any person with ordinary skills in the art.

The compounds of general formula (I) above wherein A is cyanomethyl, B is N(H)CO and R is phenyl optionally substituted, can be prepared by a process comprising the following steps:

i') first step of alkylation of an amino group on an isoindoline-1 ,3-dione nucleus to yield the corresponding benzamidic derivative,

ii') second step of alkylation at the isoindoline nitrogen, wherein the benzamidic derivative obtained in step i') is converted into the compound of general formula

(I) wherein A is cyanomethyl.

In the following Scheme 3 the process defined above is schematically illustrated with reference to the preparation of compounds of general formula (I) wherein A is cyanomethyl, B is N(H)CO and R is phenyl, with -B-R in position 5 on the benzo-fused ring:

Scheme 3

With reference to the Scheme 3 above, in the first step, the 5-aminoisoindoline-1 ,3-dione of formula (II), a commercial product, is reacted preferably with an amount of benzoylchloride in the presence of a base, such as trimethylamine in the solvent toluene, to form the N-(1 ,3-dioxoisoindolin-5-yl)benzamide of formula (IV), following a procedure previously disclosed in the literature (Zhou, W. et al. MedChemComm, 2016, 7(2), 292-296).

Always with reference to the Scheme 3, in the second step, the N-(1 ,3-dioxoisoindolin-5-yl)benzamide of formula (IV) is converted into the activator of AMPK having formula (I) by reaction with an amount of chloroacetonitrile in the presence of a base, such as potassium carbonate in solvent DMF, to form N-(2-(cyanomethyl)-1 ,3-dioxoisoindolin-5-yl)benzamide.

The compounds of general formula (I) defined above according to the invention are useful in the prophylaxis and/or treatment of diseases or disorders that benefit from the activation of the enzymatic complex AMPK, in particular metabolic diseases, immuno-mediated inflammatory diseases and cancer. They can be used, alone or in combination of two or more compounds, in pharmaceutical compositions with pharmaceutically acceptable vehicles, excipients and/or diluents, and possible further active principles having known activity, such as antidiabetic agents, anti-inflammatory agents, and anticancer agents, chemotherapeutic or not-chemotherapeutic agents, in order to increase their therapeutic efficacy. The present compounds can be present in the compositions as such or in the form of pharmaceutically acceptable salts.

The present pharmaceutical compositions can be formulated in various pharmaceutical forms, for different administration routes, for example as oral, topical or injectable compositions, according to the conventional methods, in the form of tablets, granules, powder, capsules, syrup, aqueous solution, aqueous suspension, oily solution, oily suspension, emulsion or microemulsion, to be used for the oral, intramuscular, intravenous, subcutaneous or topical administration.

EXAMPLES

In the following examples, the synthetic procedures described refer to the following experimental conditions, where not indicated otherwise:

- Temperatures are expressed in Celsius degrees (°C);
- Organic solutions have been dried on anhydrous magnesium sulphate; evaporation of the solvent was carried out by using a rotary evaporator and working at reduced pressure;
- Thin Layer Chromatography (TLC) has been carried out on Merck 60 F-254 plates; the column chromatography has been carried out using silica gel as the stationary phase and the chromatographic system Biotage;
- Time indicated in each procedure, required for obtainment of the desired product, was determined by TLC on reaction mixture;
- The final product of each reaction was characterized by chemical-physical and spectroscopic data;
- The yield indicated for each product is indicative and it does not necessarily correspond to that obtainable by a reaction carried out in an optimal way;
- The melting points have been measured by a Reichert-Kofler equipment and have not been corrected;
- The proton nuclear magnetic resonance (1 H-NMR) spectra were recorded with a Bruker Ultrashield spectrometer operating at 400 MHz, using dimethyl sulfoxide (DMSO-d6) as solvent. The following abbreviations were used: s for singlet; d for doublet, t for triplet, q for quartet, m for multiplet, exc for protons exchangeable with D2O.

Example 1. Preparation of N-(1,3-dioxoisoindolin-5-yl)benzamide

1.00 mmol of 5-aminoisoindoline-1 ,3-dione, commercial product, was solubilized in 1.0 mL of toluene and added with 1.20 mmol of commercial benzoylchloride and 1.20 mmol of triethylamine, then heated under reflux for 6 hours. After cooling, the solid that separates was collected by filtration, then dried under vacuum and purified by crystallization in ethanol. 250.3 mg of the desired product of the title were so obtained (yield 94%). It was characterized by chemical-physical and spectroscopical data.

M.p. (°C): >300. 1 H-NMR (δ, DMSO, d6): 7.649 (t, 1 H, Ar, J=7.25), 7.825 (t, 2H, Ar, J=7.69), 7.825 (d, 2H, Ar, J=8.32), 7.993 (d, 1 H, Ar, J=7.04), 8.145 (dd, 1 H, Ar, J=7.76, J=1.722), 8.331 (s, 1 H, Ar), 10.792 (s, 1 H, NH, exc), 11.269 (s, 1 H, NH, exc).

Example 2. Preparation of N-(2-(cyanomethyl)-1 ,3-dioxoisoindolin-5-yl)benzamide (Compound 1)

1.00 mmol of N-(1 ,3-dioxoisoindolin-5-yl)benzamide was solubilized in 1.0 ml_ of DMF and added with 1.20 mmol of commercial 2-chloroacetonitrile and 1.20 mmol of potassium carbonate, then heated at reflux for 2 hours. After cooling, the solvent was evaporated to dryness under reduced pressure and the so obtained residue was added with water. The solid that separates was collected by filtration, dried under vacuum and purified by crystallization in methanol. 174.0 mg of the desired product of the title were so obtained (yield 57%). It was characterized by chemical-physical and spectroscopical data.

M.p. (°C): 248-250. 1 H-NMR (δ, DMSO, d6): 4.741 (s, 2H, CH), 7.585 (t, 2H, Ar, J=7.68), 7.659 (t, 1 H, Ar, J=7.28), 7.961 (d, 2H, Ar, J=8.25), 8.003 (d, 1 H, Ar, J=7.92, J=8.81), 8.199 (dd, 1 H, Ar, J=8.25, J=1.88), 10.874 (s, 1 H, NH, exc).

The experimental procedures described in Examples 1 and 2 have been then repeated and the isoindoline-1 ,3-dionic derivatives 2-13 of the present invention listed above have been prepared.

Example 3. Preparation of 4-chloro-N-(1,3-dioxoisoindolin-5-yl)benzamide 1.00 mmol of 5-aminoisoindoline-1 ,3-dione, commercial product, was solubilized in 1.0 ml_ of toluene and added with 1.20 mmol of commercial 4-chlorobenzoylchloride and 1.20 mmol of triethylamine, then heated at reflux for 10 hours. After cooling, the solid that separates was collected by filtration, dried under vacuum and purified by crystallization in methanol. 285.5 mg of the desired product of the title were so obtained (yield 95%). It was characterized by chemical-physical and spectroscopical data.

M.p. (°C): >300. 1 H-NMR (δ, DMSO, d6): 8.318 (d, 1 H, Ar, J=1.64), 8.131 (dd, 1 H, Ar, J=8.24, J=1.84), 8.024 (d, 2H, Ar, J=8.60), 7.838 (d, 1 H, Ar, J=8.16), 7.659 (d, 2H, Ar, J=8.61), 10.844 (s, 1 H, NH, exc), 1 1.272 (s, 1 H, NH, exc).

Example 4. Preparation of N-(2-(cyanomethyl)-1,3-dioxoisoindolin-5-yl)-4-chloro-benzamide (Compound 25)

1.00 mmol of 4-chloro-N-(1,3-dioxoisoindolin-5-yl)benzamide prepared as described above in Example 3 was solubilized in 1.0 ml_ of DMF and added with 1.20 mmol of commercial 2-chloroacetonitrile and 1.20 mmol of potassium carbonate, then heated at reflux for 1 hour. After cooling, the solvent was evaporated to dryness at reduced pressure and the residue obtained was added with water. The solid that separates was collected by filtration, dried under vacuum and purified by crystallization in methanol. 186.7 mg of the desired product of the title were so obtained (yield 55%). It was characterized by chemical-physical and spectroscopical data.

M.p. (°C): 233-235. 1 H-NMR (δ, DMSO, d6): 4.739 (s, 2H, CH), 7.667 (d, 2H, Ar, J=8.32), 7.963 (d, 1 H, Ar, J=8.16), 8.033 (d, 2H, Ar, J=8.37), 8.180 (d, 1 H, Ar, J=6.84), 8.420 (s, 1 H, Ar), 1 1.272 (s, 1 H, NH, exc).

Example 5. In vitro assays

The functional efficacy of the present isoindoline-1,3-dionic derivatives of general formula (I) was verified by in vitro assays, carried out on cell lines of C2C12 murine myoblasts. The cells were treated with the isoindoline-1,3-dionic derivatives under examination, used at concentration of 10 microM. After 30 minutes, the level of phosphorylation of the AMPK protein at the Thr172 residue was determined, using the Western Blot method. The results obtained, expressed in terms of ratio between the phosphorylated protein and the native protein, were compared with the analogous data acquired in the presence of berberine (BBR), an AMPK activator of natural origin used as reference product. Figure 1 illustrates the level of activation of AMPK in the C2C12 cell line after 30 minutes of exposure to the isoindoline-1,3-dionic compound 1 obtained as described above in Example 2 and to berberine, reference product, both tested at concentration of 10 microM. In analogous way the functional efficacy of the isoindoline- 1,3-dionic derivatives 2-13 of the present invention, prepared as described above, was studied and verified.

Figure 2 illustrates the level of AMPK activation in the C2C12 cell line after 30 minutes of exposure to the isoindoline-1 ,3-dionic derivatives 1 and 25 obtained as described above in Examples 2 and 4, tested at concentration of 20 microM, and by comparison to acadesine (ACA), only AMPK agonist product put on the market until today, tested at concentration of 0.2 milliM. The tested isoindoline-1 ,3-dionic derivatives 1 and 25 significantly activate the target protein with respect to the vehicle group and they do not show a significantly different efficacy with respect to the reference product ACA, which was however used at a concentration 10 times higher than the derivatives tested.

The values indicated in figures are expressed as mean ± DS (n=2,3), * $p < 0.05$,

** $p < 0.01$ and *** $p < 0.001$, with respect to vehicle group.