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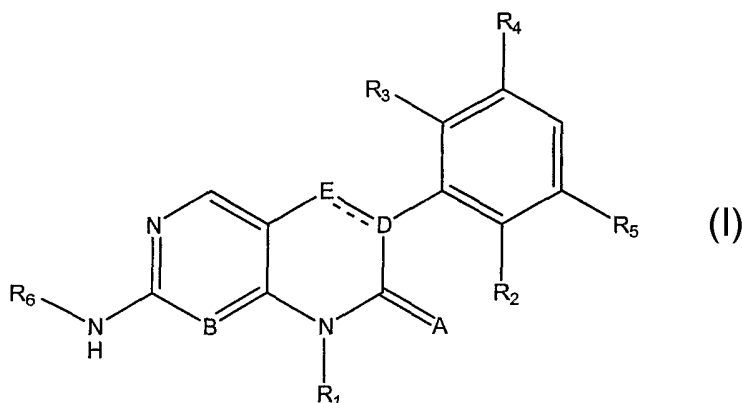
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(54) **Title:** USE OF KINASE INHIBITORS TO PROMOTE NEOCHONDROGENESIS



therefor in the specification.

(57) **Abstract:** This invention provides methods for utilizing compounds that inhibit cyclin-dependent kinase and tyrosine kinase enzymes in the promotion of neochondrogenesis and the enhancement, protection and repair of cartilage. In certain embodiments the invention relates to methods of using compounds of formula (I); and pharmaceutically acceptable salts thereof, to promote neochondrogenesis, wherein, R₁, R₂, R₃, R₄, R₅, R₆, A, B, D, and E have any of the values defined

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USE OF KINASE INHIBITORS TO PROMOTE NEOCHONDROGENESIS

FIELD OF THE INVENTION

This invention relates to the use of kinase inhibiting compounds, including tyrosine kinase inhibiting compounds, in the promotion of neochondrogenesis and the enhancement, protection and repair of cartilage. The methods particularly use substituted pyrimidopyrimidines, dihydropyrimidopyrimidines, pyridopyrimidines, naphthyridines and pyridopyrazines that inhibit cyclin-dependent kinase and tyrosine kinase enzymes.

BACKGROUND OF THE INVENTION

Tyrosine kinases are an integral part of growth factor receptors and are essential for the propagation of growth factor signal transduction leading to cellular proliferation, differentiation, and migration. Growth factor receptors are also known as receptor tyrosine kinases (RTKs). The aberrant regulation of growth factors or their cognate receptors are reputed to play a critical role in the progression of proliferative diseases. Fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) have been implicated as important mediators of tumor promoted angiogenesis. Solid tumors are dependent upon the formation of new blood vessels from preexisting vessels (angiogenesis) to nourish their growth and to provide a conduit for metastases. Accordingly, inhibitors of the FGF and VEGF RTKs, as well as other tyrosine kinases, have been disclosed as useful agents for the prevention and treatment of proliferative diseases dependent on these enzymes.

WO 00/24744 (Harris et al.), WO 96/13262 (Thompson et al.), WO 01/64679 (Adams et al.), WO 01/29041 and WO 01/29042 (both to Dunn et al.) disclose pyrimidopyrimidinone compounds useful in treating proliferative diseases.

SUMMARY OF THE INVENTION

This invention provides a method of promoting localized neochondrogenesis in a mammal comprising administering locally to a cartilage in the mammal a pharmaceutically effective amount of a tyrosine kinase inhibitor. Neochondrogenesis is the process of forming new cartilage, including the proliferation and differentiation of mesenchymal stem cells and formation of cartilage matrix.

This invention comprises methods of inhibiting, ameliorating, repairing or preventing damage or degradation of, or of inducing growth and repair of, cartilage in a recipient, the methods comprising applying or administering directly to the recipient's cartilage a pharmaceutically effective amount of a tyrosine kinase inhibitor. Direct application or administration of the tyrosine kinase inhibitor as used herein refers to a non-systemic administration of the inhibitor in a site-specific manner to or near the cartilage(s) in question. The methods herein include parenteral application of the compound into or onto the cartilage or local or topical application of the inhibitor such that a pharmaceutically effective amount of the inhibitor will reach the cartilage and promote

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localized chondrocyte proliferation without significant systemic distribution of the inhibitor. One in vivo measure of desired distribution of a pharmaceutically effective amount of the tyrosine kinase inhibitor in question is the delivery of an amount of inhibitor sufficient to enhance chondrocyte growth at or near the locus of administration, without measurably increasing chondrocyte proliferation at other sites in the body.

The methods herein include the application or administration directly or topically on cartilage tissue, including articular or hyaline cartilage, fibrocartilage and elastic cartilage, that is experiencing or subject to degradation, including the damage associated with trauma and degenerative disorders, such as cartilage damage associated with Osteoarthritis, rheumatoid arthritis and rheumatoid variants. One method of this invention comprises administering a pharmaceutically effective amount of a tyrosine kinase inhibitor intra-articularly to a cartilaginous tissue experiencing or subject to degradation or damage. In another embodiment, the pharmaceutically effective amount of a tyrosine kinase inhibitor is administered in a formulation including one or more pharmaceutically acceptable carriers or excipients.

The methods of this invention may be used in the protection and repair of cartilage tissues and used to foster rehabilitation and quality of life in a recipient. The methods herein may be utilized for repair or recovery of cartilaginous tissues resulting from osteoarthritis, trauma, including traumatic mechanical destruction or progressive mechanical degradation or destruction (wear and tear), or infections, including bacterial, viral and fungal infections, sometimes referred to as septic or infectious arthritis, suppurative arthritis, reactive arthritis or Gonococcal arthritis. They may further assist in cartilage damage, degradation or atrophy resulting from maladies including chronic inflammatory synovitis, forms of chondritis, such as nasal, bilateral auricular or respiratory tract chondritis, initial or relapsing polychondritis, chronic atrophic polychondritis, or generalized or systemic chondromalacia, or cartilaginous neoplasias. The repair may also be utilized to treat cartilage damage associated with connective or bone tissue diseases including osteomyelitis, gout, chondromalacia, juvenile rheumatoid arthritis, psoriatic arthritis, hemophilic arthritis, Reiters Syndrome, Scleroderma, Systemic Lupus Erythematosus, Ankylosing Spondylitis, herniated vertebral discs, etc.

The methods, formulation and regimens herein may also be utilized to repair, strengthen or rebuild cartilage tissues following surgical procedures requiring reduction, building up or manipulation of cartilage tissues, including reconstructive or cosmetic reductions or manipulations of cartilage. Also included herein is a method of increasing the strength, thickness or mass of an existing cartilage tissue. Increasing existing cartilage in the absence of specific trauma or significant degradation may be useful in the prophylactic strengthening or supporting of cartilaginous tissue where the existing tissue may have potential for failure due to age, use or exceptional physical demands.

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Increasing the thickness, mass or strength of cartilaginous tissues may also be desired at sites wherein the tissue will encounter prosthetic devices.

The methods of this invention are useful in treating or enhancing recovery of cartilage damage or degradation brought about due to the trauma associated with repetition or sports injuries, including the degradation, tearing, compression and dislocation of cartilaginous tissues. Non-limiting examples of repetition- or sports-related cartilage injuries and maladies that may be improved with the methods of this invention include chondromalacia, or tearing or damage to articular, meniscal cartilage, wrist, ankle, acromioclavicular, or vertebral cartilage.

The methods of this invention may also be characterized as methods of initiating, enhancing or promoting neochondrogenesis in a cartilage tissue, the method comprising administering locally or directly to the cartilage tissue a neochondrogenesis enhancing amount of a tyrosine kinase inhibitor, as described herein.

Tyrosine kinase inhibitors may be administered within the scope of this invention in conjunction with medical procedures designed to clean, repair or evaluate cartilaginous tissues, such as arthroscopic lavage (arthroscopic washout) and debridement procedures.

The methods herein include veterinary methods of treatment, amelioration, inhibition or prevention of cartilage damage or diminishment associated with veterinary maladies, including osteochondritis, joint dysplasias or spondylosis, degenerative joint disease, Osteoarthritis, Rheumatoid Arthritis, and the maladies and methods described herein that are equally relevant to non-human mammals.

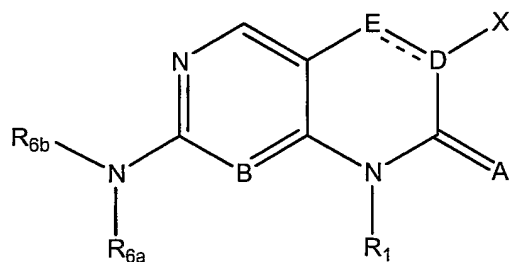
Another use of the compounds of this invention comprises utilizing an effective amount of a tyrosine kinase inhibitor to promote and enhance the growth of chondrocytes in vitro. Cartilage cells grown in vitro may then be utilized in physiologically acceptable solutions, formulations and matrices to enhance cartilage repair or growth in damaged, degraded or otherwise insufficiently developed cartilage in vivo. These methods may be further understood as comprising the addition of a cartilage enhancing amount of a tyrosine kinase inhibitor to an in vitro culture of chondrocytes. Such in vitro cultures include those known in the art for the growth of chondrocytes and may include cartilage enhancing growth media and components including bone morphogenetic protein (BMP)-6, dexamethasone, transforming growth factor (TGF)-beta3, etc.

Tyrosine kinase inhibitor compounds may also be used to foster cartilage growth following cartilage cells or tissues allografted or autografted into a recipient. Fostering greater chondrocyte growth using the modes of administration described herein may be used to hasten the integration of new cells or tissues and strengthen the resulting cartilaginous presence in the concerned area.

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DETAILED DESCRIPTION OF THE INVENTION

One embodiment of the methods of this invention comprises administering to an animal in need thereof, particularly to a mammal in need thereof, a pharmaceutically effective amount of a compound of formula:



wherein:

A is O, NH₂, NH(C₁-C₆ alkyl), N(C₁-C₆ alkyl)₂, or -NHC(O)-NHR₁₂;

10 R₁₂ is C₁-C₆ straight or branched chain alkyl, preferably C₁-C₄ straight or branched chain alkyl, or -(CH₂)_n-C₃-C₈ cycloalkyl ring, preferably -(CH₂)_n-C₃-C₇ cycloalkyl, wherein n is an integer of from 1 to 3;

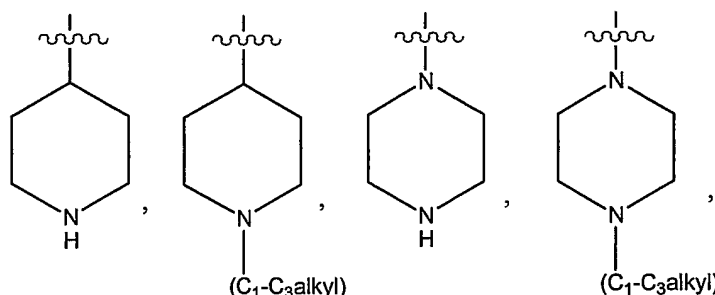
B and D and E are independently selected from CH or N;

E is from CH (when the dashed line indicates a double bond), CH₂ or N, with the proviso that E and D are not both N;

15 R₁ is selected from the group of C₁-C₆ straight or branched chain alkyl, preferably C₁-C₄ straight or branched chain alkyl, optionally substituted by -COOH, or;

20 a) a phenyl, benzyl or C₃-C₈ cycloalkyl group, preferably C₃-C₇ cycloalkyl, or -CH₂-C₃-C₈ cycloalkyl group, preferably -CH₂-C₃-C₇ cycloalkyl, with the rings of the phenyl, benzyl or cycloalkyl groups being optionally substituted by 1 or 2 COOH or -CH₂-COOH groups; or

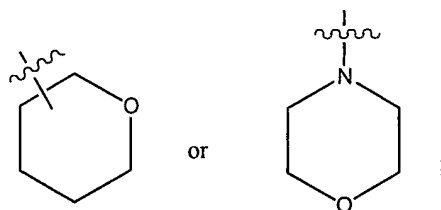
b) a piperidinyl or piperazinyl moiety selected from group of:



the rings of the piperidinyl or piperazinyl moieties being optionally substituted by 1 or 2 COOH or -CH₂-COOH groups; or

25 c) a tetrahydropyranyl or morpholinyl moiety of the formulae:

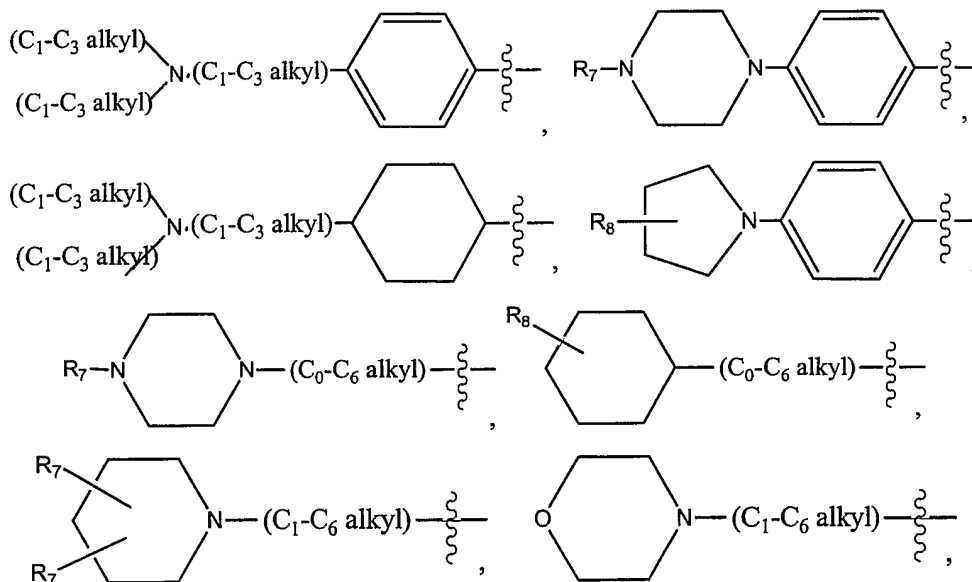
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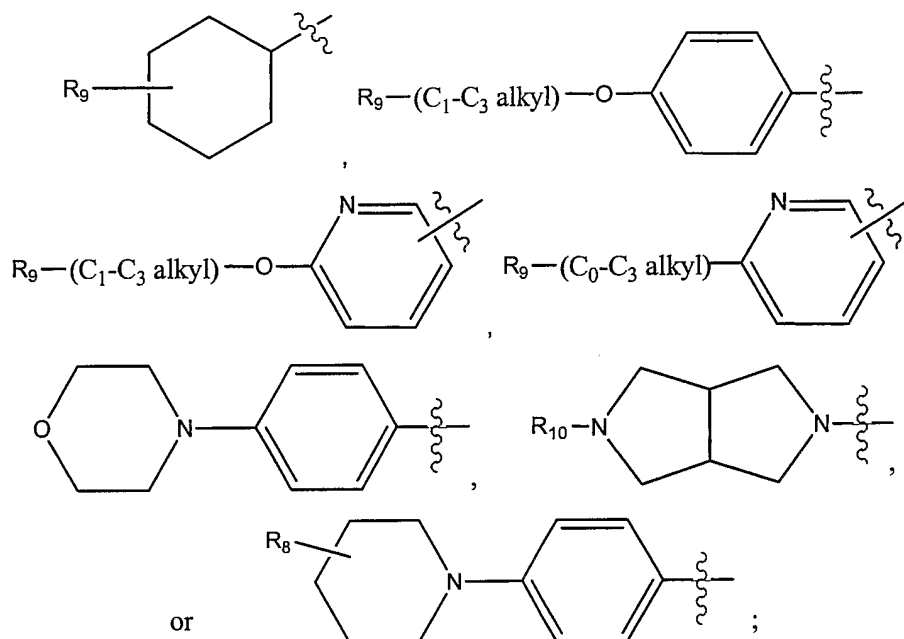
X is C₁-C₆ alkyl, or optionally substituted phenyl, benzyl, C₃-C₇ cycloalkyl, -CH₂-C₃-C₇ cycloalkyl, or 6-membered or 7-membered heterocyclic or heteroaromatic moieties containing one or two heteroatoms selected from N, O or S; with each of the phenyl, benzyl, cycloalkyl, heterocyclic or heteroaromatic moieties being optionally substituted by from 1 to 4 substituents selected from C₁-C₆ alkyl, preferably C₁-C₃ alkyl, C₁-C₆ alkoxy, preferably C₁-C₃ alkoxy, halogen, -NH₂, NO₂, OH, C₁-C₆ haloalkyl, preferably C₁-C₃ haloalkyl, C₁-C₆ haloalkoxy, preferably C₁-C₃ haloalkoxy;

R_{6a} is selected from H or C₁-C₆ alkyl, preferably C₁-C₃ alkyl;

R_{6b} is selected from the group of H, -(C₁-C₅ alkyl)-NH₂, -(C₁-C₅ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₅ alkyl)-N-(C₁-C₃ alkyl-R₁₁)₂, -O-(C₁-C₅ alkyl)-NH₂, -O-(C₁-C₅ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -O-(C₁-C₅ alkyl)-N-(C₁-C₃ alkyl-R₁₁)₂, -CH(CH₂OH)₂, -(C₁-C₃ alkyl)-(CH₂OH)₂, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-NH₂, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-N(C₁-C₃ alkyl-R₁₁)₂, phenyl substituted by 1 or 2 groups selected from NH₂, -N(C₁-C₃ alkyl), -N(C₁-C₃ alkyl)₂, CN or -(C₁-C₃ alkyl)-tetrazole, or C₁-C₆ alkyl,



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5 with each of the alkyl chains of any group in this R_4 definition being optionally substituted by from 1 to 4 OH groups;

R_7 in each instance is independently selected from H, $-NH_2$, $NH(C_1-C_3 \text{ alkyl})$, $N(C_1-C_3 \text{ alkyl})_2$, or $C_1-C_3 \text{ alkyl}$;

R_8 is H, OH or $C_1-C_3 \text{ alkyl}$;

10 R_9 is H, OH, $-NH_2$, $NH(C_1-C_3 \text{ alkyl})$, or $N(C_1-C_3 \text{ alkyl})_2$;

R_{10} is H or $C_1-C_3 \text{ alkyl}$;

R_{11} is H, CN, OH, NH_2 , F, or CF_3 ;

or a pharmaceutically acceptable salt thereof.

15 In certain embodiments, the compound of formula I, or a pharmaceutically acceptable salt thereof, is selected from the group of:

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

20 (S,S)-1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[(5-hydroxymethyl-2-phenyl-[1,3]dioxolan-4-ylmethyl)-amino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

(S,S)-1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3,4-trihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

25 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

7-(3-Amino-2-hydroxy-propylamino)-1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

5 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; and

10 7-(4-Diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one.

In certain embodiments, the compound of formula I, or a pharmaceutically acceptable salt thereof, is selected from the group consisting of:

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

15 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

20 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

7-(4-Amino-2,3-dihydroxy-butylamino)-1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-([S,S]-2,3,4-trihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

25 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

7-(4-Amino-2,3-dihydroxy-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

30 7-{3-[Bis-(2-hydroxy-ethyl)-amino]-propylamino}-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; and

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one.

In certain embodiments, the compound of formula I, or a pharmaceutically acceptable salt thereof, is selected from the group consisting of:

35 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-butylamino)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

Ethyl-4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylate;

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4-[3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;

7-Amino-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

5 1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

10 (S)-1-Cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

4-[3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid;

15 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

20 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(3-ethoxy-2,6-difluoro-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

(R)-1-Cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; and

25 1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(trans-4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one.

In certain embodiments, the methods of the present invention comprise treating a mammal suffering from osteoarthritis.

30 In certain embodiments, the methods of the present invention comprise treating a mammal suffering from cartilage degradation.

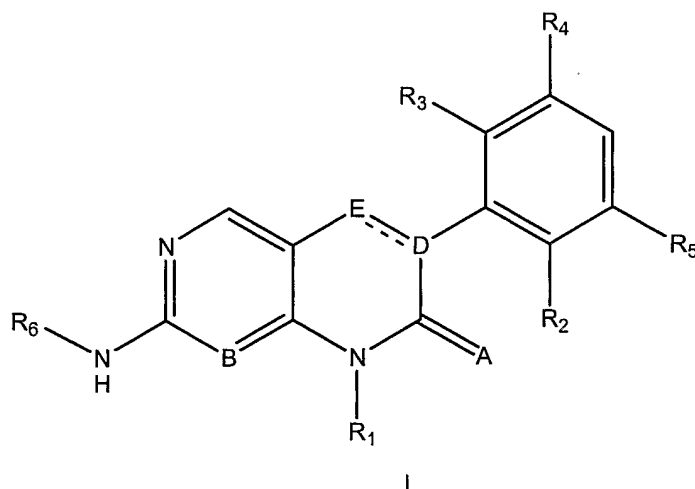
In certain embodiments, the methods of the present invention comprise administered intra-articularly a compound of formula I, or a pharmaceutically acceptable salt thereof.

35 In certain embodiments, the methods of the present invention comprise administered intra-articularly to a knee joint a compound of formula I, or a pharmaceutically acceptable salt thereof.

In certain embodiments, the methods of the present invention comprise administered intra-articularly to a knee joint a compound of formula I, or a pharmaceutically acceptable salt thereof, at a dose from 0.001 to 0.1 mg/kg.

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In certain embodiments, the present invention relates to the use of a compound, or a pharmaceutically acceptable salt thereof, of formula I:



5

wherein:

A is O, NH₂, NH(C₁-C₆ alkyl), N(C₁-C₆ alkyl)₂, or -NHC(O)-NHR₁₂;

R₁₂ is C₁-C₆ straight or branched chain alkyl, or -(CH₂)_n-C₃-C₈ cycloalkyl ring; n is an integer of from 1 to 3;

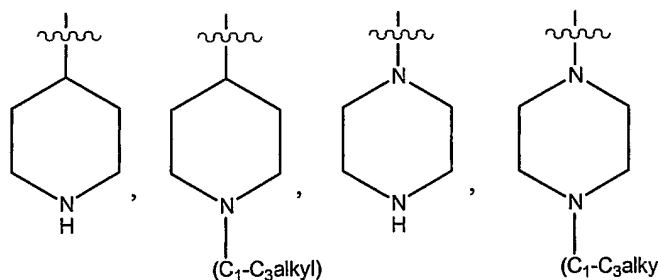
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B, D and E are independently selected from CH or N, with the proviso that E and D are not both N;

R₁ is selected from the group of C₁-C₆ straight or branched chain alkyl, optionally substituted by -COOH, or;

15

- a) a phenyl, benzyl or C₃-C₈ cycloalkyl ring, or -CH₂-C₃-C₈ cycloalkyl ring, with the phenyl, benzyl or cycloalkyl rings being optionally substituted by 1 or 2 COOH or -CH₂-COOH groups; or
- b) a piperidiny or piperazinyl moiety selected from group of:

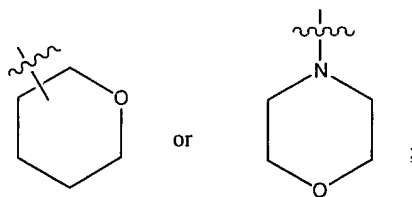


the rings of the piperidiny or piperazinyl moieties being optionally substituted by 1 or 2 COOH or -CH₂-COOH groups; or

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- c) a tetrahydropyranyl or morpholinyl moiety of the formulae:

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R_2 is H, Cl or F;

R_3 is H, Cl or F, with the proviso that at least one of R_2 or R_3 is F;

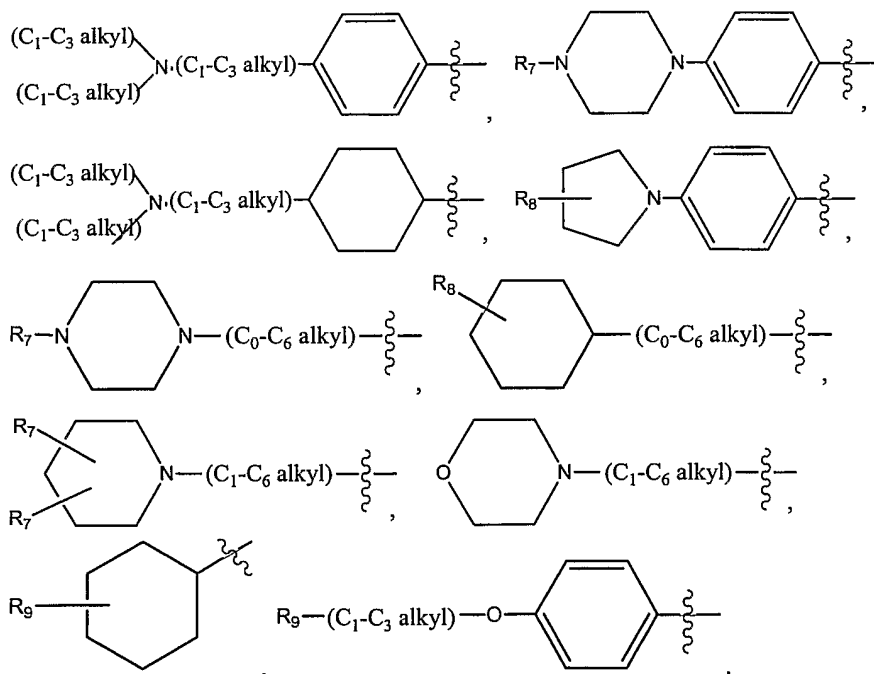
R_4 is H, OH, -OCH₃, or -OCH₂CH₃, with the proviso that, if R_4 is H, R_2

5 and R_3 are not H;

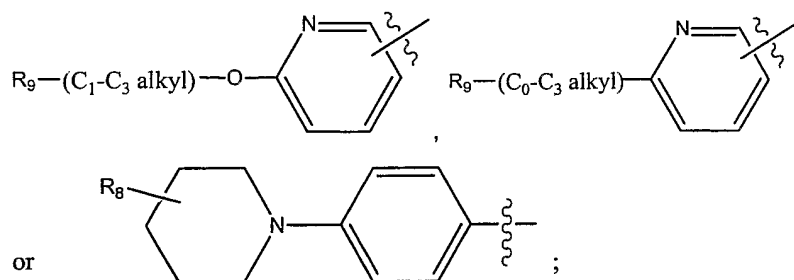
R_5 is -OCH₃, or -OCH₂CH₃;

R_6 is selected from the group of H, -(C₁-C₅ alkyl)-NH₂, -(C₁-C₅ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₅ alkyl)-N-(C₁-C₃ alkyl)-R₁₁, -O-(C₁-C₅ alkyl)-NH₂, -O-(C₁-C₅ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -O-(C₁-C₅ alkyl)-N-(C₁-C₃ alkyl)-R₁₁, -CH(CH₂OH)₂, -(C₁-C₃ alkyl)-(CH₂OH)₂, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-NH₂, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-N(C₁-C₃ alkyl)-R₁₁, phenyl substituted by one or two groups selected from NH₂, -N(C₁-C₃ alkyl), -N(C₁-C₃ alkyl)₂, CN or -(C₁-C₃ alkyl)-tetrazole, or C₁-C₆ alkyl,

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-11-



with each of the alkyl chains of any group in this R_4 definition being optionally substituted by from 1 to 4 OH groups;

5 R_7 in each instance is independently selected from H, $-NH_2$, $NH(C_1-C_3 \text{ alkyl})$, $N(C_1-C_3 \text{ alkyl})_2$, or $C_1-C_3 \text{ alkyl}$;

R_8 is H, OH or $C_1-C_3 \text{ alkyl}$;

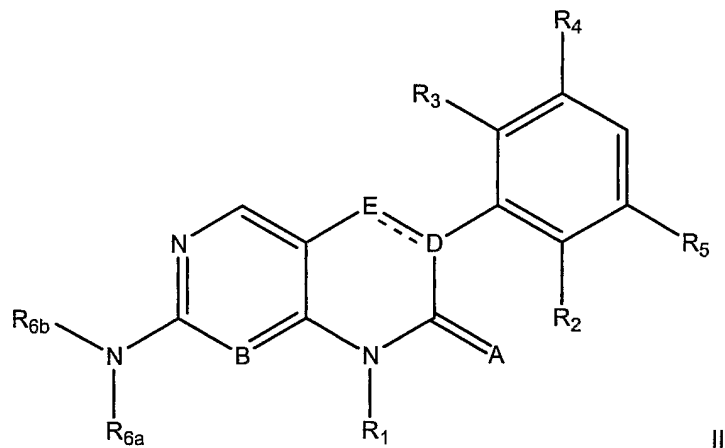
9 H, OH, $-NH_2$, $NH(C_1-C_3 \text{ alkyl})$, or $N(C_1-C_3 \text{ alkyl})_2$;

R_{10} is H or $C_1-C_3 \text{ alkyl}$;

10 R_{11} is H, CN, OH, NH_2 , F, or CF_3 ;

in the manufacture of a medicament for promoting neochondrogenesis in a mammal in need thereof.

In one embodiment, the methods of this invention comprise administration to an animal in need thereof, particularly to a mammal in need thereof, compounds of formula II:



wherein:

A is O, NH_2 , $NH(C_1-C_6 \text{ alkyl})$, $N(C_1-C_6 \text{ alkyl})_2$, or $-NHC(O)-NHR_{12}$;

20 R_{12} is C_1-C_6 straight or branched chain alkyl, preferably C_1-C_4 straight or branched chain alkyl, or $-(CH_2)_n-C_3-C_8$ cycloalkyl ring, preferably $-(CH_2)_n-C_3-C_7$ cycloalkyl, wherein n is an integer of from 1 to 3;

B and D and E are independently selected from CH or N;

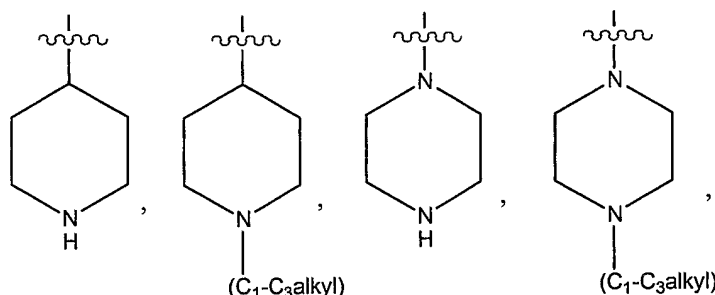
-12-

E is from CH (when the dashed line indicates a double bond), CH₂ or N, with the proviso that E and D are not both N;

R₁ is selected from the group of C₁-C₆ straight or branched chain alkyl, preferably C₁-C₄ straight or branched chain alkyl, optionally substituted by -COOH, or;

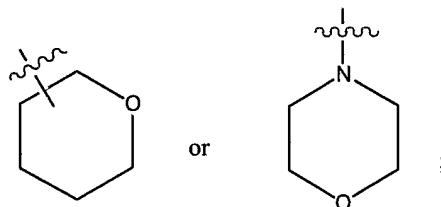
- 5 a) a phenyl, benzyl or C₃-C₈ cycloalkyl group, preferably C₃-C₇ cycloalkyl, or -CH₂-C₃-C₈ cycloalkyl group, preferably -CH₂-C₃-C₇ cycloalkyl, with the rings of the phenyl, benzyl or cycloalkyl groups being optionally substituted by 1 or 2 COOH or -CH₂-COOH groups; or

- b) a piperidiny or piperaziny moiety selected from group of:



the rings of the piperidiny or piperaziny moieties being optionally substituted by 1 or 2 COOH or -CH₂-COOH groups; or

- c) a tetrahydropyranyl or morpholinyl moiety of the formulae:



R₂ is H, Cl or F;

R₃ is H, Cl or F, with the proviso that at least one of R₂ or R₃ is F;

R₄ is H, OH, -OCH₃, or -OCH₂CH₃, with the proviso that, if R₄ is H, R₂ and R₃ are not H;

R₅ is -OCH₃, or -OCH₂CH₃;

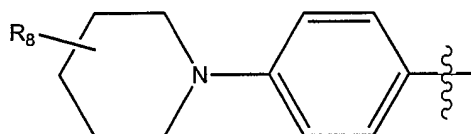
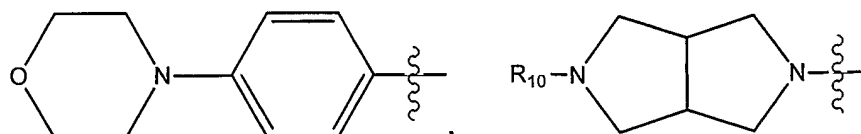
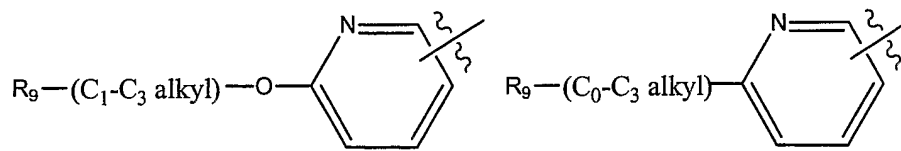
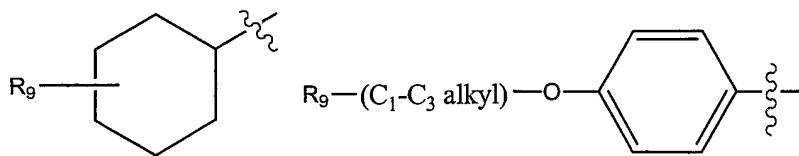
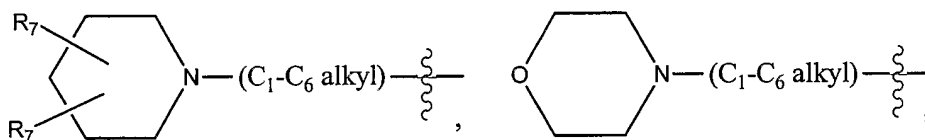
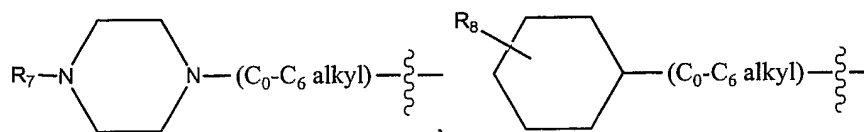
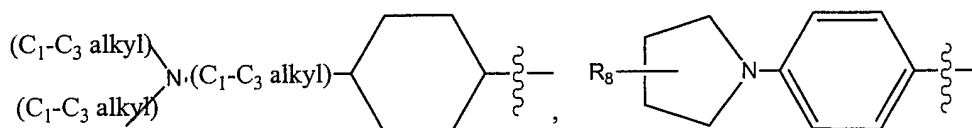
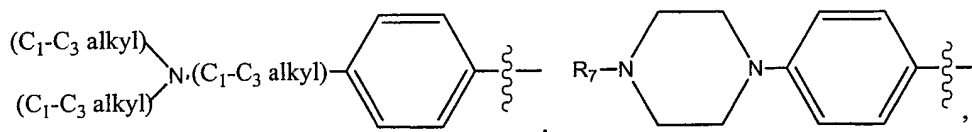
- 20 R_{6a} is selected from H or C₁-C₆ alkyl, preferably C₁-C₃ alkyl;

R_{6b} is selected from the group of H, -(C₁-C₅ alkyl)-NH₂, -(C₁-C₅ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₅ alkyl)-N-(C₁-C₃ alkyl-R₁₁)₂, -O-(C₁-C₅ alkyl)-NH₂, -O-(C₁-C₅ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -O-(C₁-C₅ alkyl)-N-(C₁-C₃ alkyl-R₁₁)₂, -CH(CH₂OH)₂, -(C₁-C₃ alkyl)-(CH₂OH)₂, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-NH₂, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₃ alkyl)-O-(C₁-

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C₃ alkyl)-N(C₁-C₃ alkyl-R₁₁)₂, phenyl substituted by 1 or 2 groups selected from NH₂, -N(C₁-C₃ alkyl), -N(C₁-C₃ alkyl)₂, CN or -(C₁-C₃ alkyl)-tetrazole, or C₁-C₆ alkyl,



or

with each of the alkyl chains of any group in this R₄ definition being optionally substituted by from 1 to 4 OH groups;

R₇ in each instance is independently selected from H, -NH₂, NH(C₁-C₃ alkyl), N(C₁-C₃ alkyl)₂, or C₁-C₃ alkyl;

R₈ is H, OH or C₁-C₃ alkyl;

R₉ is H, OH, -NH₂, NH(C₁-C₃ alkyl), or N(C₁-C₃ alkyl)₂;

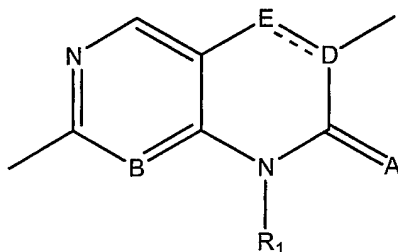
-14-

R_{10} is H or C_1 - C_3 alkyl;

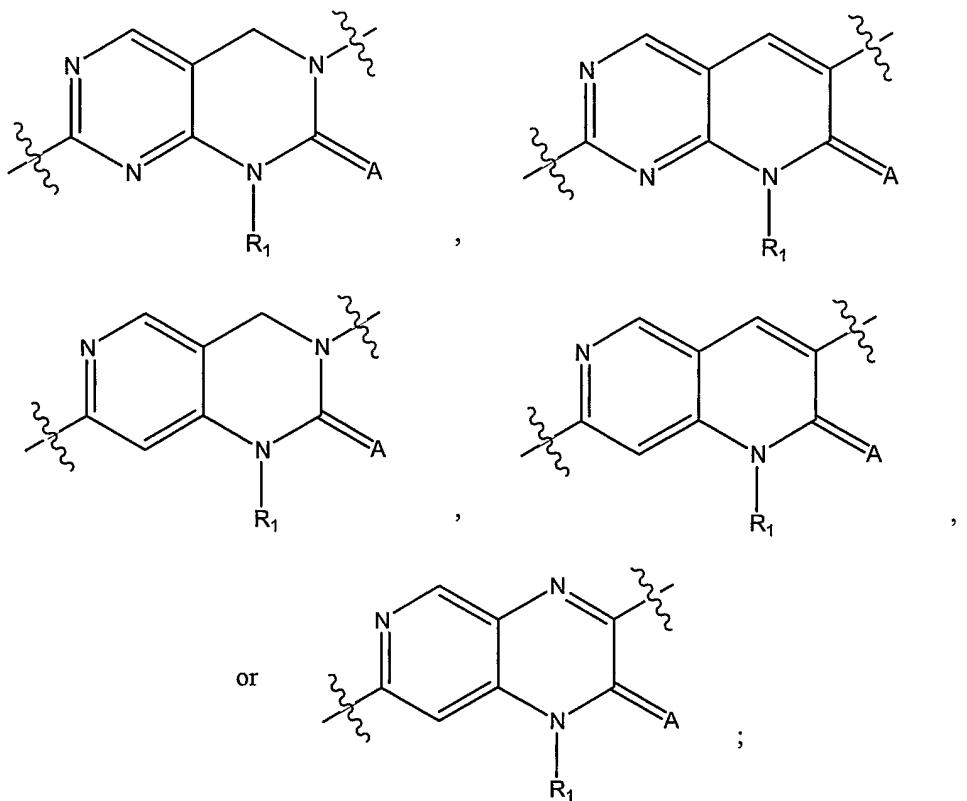
R_{11} is H, CN, OH, NH_2 , F, or CF_3 ;

or a pharmaceutically acceptable salt thereof.

This invention also includes subgroups of the compounds described above in
 5 which the core structure designated:



is selected independently in each subgroup from the moieties:



10

wherein, in each case, the variables A, R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , R_{11} ,
 and R_{12} are as defined above. The dashed lines (----) in the structures above indicate an
 optional double bond at the position indicated.

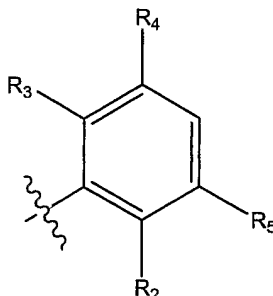
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Within each of the groups and subgroups described herein is another subgroup of
 compounds of this invention wherein the variable A is O. Another subgroup within each
 group or subgroup of compounds comprises those wherein A is selected from NH_2 ,

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NH(C₁-C₆ alkyl) or N(C₁-C₆ alkyl)₂. A further subgroup therein comprises those compounds wherein A is selected from NH₂, NH(C₁-C₃ alkyl) or N(C₁-C₃ alkyl)₂.

In each description of compounds herein it is understood the moiety designated by the structure:



5

includes, but is not limited to, the representative groups 2,6-difluoro-3,5-dimethoxy-phenyl; 2,6-difluoro-3,5-diethoxy-phenyl; 3-ethoxy-2,6-difluoro-5-methoxy-phenyl; 2-fluoro-3,5-dimethoxy-phenyl; 2-fluoro-3,5-diethoxy-phenyl; 3-ethoxy-2-fluoro-5-methoxy-phenyl; 5-ethoxy-2-fluoro-3-methoxy-phenyl; 5-methoxy-2,6-difluoro-3-hydroxy-phenyl; 2-fluoro-3-hydroxy-5-methoxy-phenyl; 2-fluoro-3-methoxy-5-hydroxy-phenyl; 2-fluoro-3-hydroxy-5-ethoxy-phenyl; 2-fluoro-3-ethoxy-5-hydroxy-phenyl; 2,6-difluoro-3-methoxy-phenyl, 2,6-difluoro-3-ethoxy-phenyl, 2,6-difluoro-3-ethoxy-phenyl, 2-chloro-6-fluoro-3,5-dimethoxy-phenyl, 2-chloro-5-ethoxy-6-fluoro-3-methoxy-phenyl, 2-chloro-3-ethoxy-6-fluoro-5-methoxy-phenyl, 2-chloro-3-ethoxy-6-fluoro-5-hydroxy-phenyl, 2-chloro-3-methoxy-6-fluoro-5-hydroxy-phenyl, 2-chloro-6-fluoro-3-methoxy-phenyl, or 6-chloro-3-ethoxy-2-fluoro-phenyl. Within each of the groups of compounds described herein is a subgroup wherein one of R₂ and R₃ is H and the other is F, and R₄ and R₅ are as defined above. In another subgroup within each group or subgroup described herein, R₂ and R₃ are both F and R₄ and R₅ are as defined above.

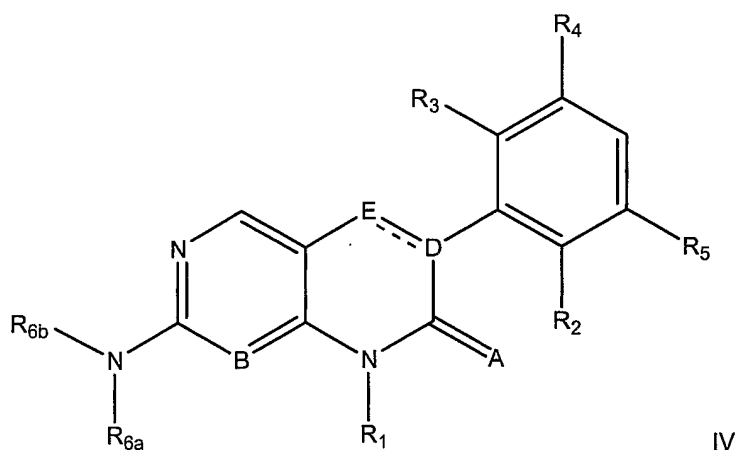
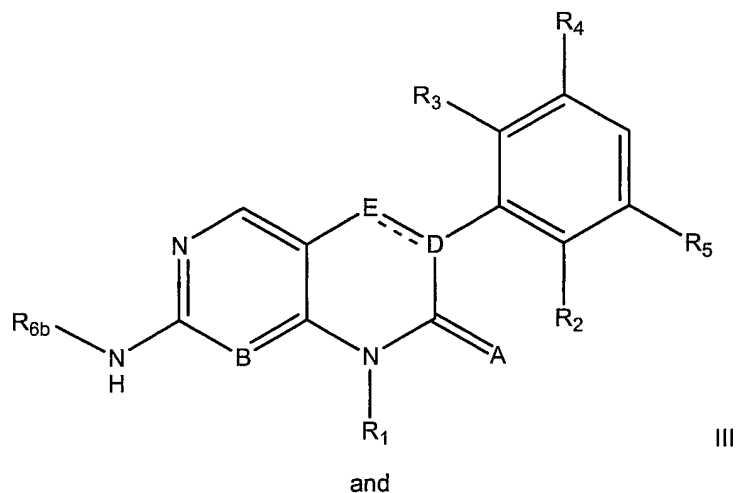
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Within each compound description herein also exists a further subgroup in which R₁ is selected from C₁-C₆ straight or branched chain alkyl, preferably C₁-C₄ straight or branched chain alkyl, optionally substituted by -COOH, or a benzyl or C₃-C₈ cycloalkyl ring, preferably C₃-C₇ cycloalkyl, with the benzyl or cycloalkyl rings being optionally substituted by 1 or 2 COOH groups.

25

Two groups of compounds of this invention comprises those of the formulae III and IV:

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wherein:

5 R₁ is C₁-C₆ straight or branched chain alkyl, preferably C₁-C₄ straight or branched chain alkyl, optionally substituted by -COOH, or; a phenyl, benzyl, or C₃-C₈ cycloalkyl ring, preferably C₃-C₇ cycloalkyl, or -CH₂-C₃-C₈ cycloalkyl ring, preferably -CH₂-C₃-C₇ cycloalkyl, with the phenyl, benzyl or cycloalkyl rings being optionally substituted by 1 or 2 COOH or -CH₂-COOH groups;

10 R₂ is H or F; and A, B, D, E, R₃, R₄, R₅, R_{6a} (when present), R_{6b}, R₇, R₈, R₉, R₁₀, R₁₁ and R₁₂ are as defined above; or a pharmaceutically acceptable salt thereof.

It will also be understood that the definition of C₃-C₈ cycloalkyl ring includes fully saturated rings, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl groups, partially saturated carbocyclic rings having from 3 to 8 ring carbon atoms, such as cyclohexene, and bridged cycloalkyl groups, such as bicyclo[2.2.1]heptane.

It will also be understood that additional kinase inhibiting compounds known in the art may be used in the methods of treatment, combination therapies and drug

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regimens of this invention. These include, but are not limited to, the kinase inhibiting compounds disclosed in U.S. Patent No. 5,620,981, U.S. Patent No. 5,733,914, WO 02/12238, WO 2004/011465, U.S. Pat. No. 6,150,373 (Harris et al), U.S. Pat. No. 6,605,617 (Renhowe et al.), U.S. Pat. No. 6,602,863 (Bridges et al.), U.S. Pat. No. 6,596,746 (Das et al.), U.S. Pat. Appln. 20030158224A1 (Renhow et al.), U.S. Pat. Appln. 20030100567 (Bilodeau et al.), U.S. Pat. Appln. 20030087881 (Bridges et al.), U.S. Pat. Appln. 20030065180 (Tsou et al.), U.S. Pat. Appln. 20030064996 (Bilodeau et al.), U.S. Pat. No. 6,562,818 (Bridges), U.S. Pat. No. 6,521,618 (Boschelli et al.), U.S. Pat. No. 6,479,512 (Fraley et al.), U.S. Pat. No. 6,455,534 (Bridges et al.), U.S. Pat. No. 6,420,382 (Fraley et al.), U.S. Pat. Appln. 20020137755 (Bilodeau et al.), U.S. Pat. Appln. 20020107392 A1 (Renhowe et al.), U.S. Pat. Appln. 20020103230 (Renhowe et al.), U.S. Pat. No. 6,344,459 (Bridges et al.), U.S. Pat. No. 6,344,455 (Bridges et al.), U.S. Pat. No. 6,313,138 (Fraley et al.), U.S. Pat. No. 6,306,874 (Fraley et al.), and U.S. Pat. No. 6,153,617 (Fraley et al.), U.S. Pat. No. 6,150,359 (Bawarian et al.), WO 99/52936 (Yalamoori et al.), WO 00/47583 (Hudkins et al.), WO 01/09121 (Moset et al), U.S. Pat. No. 6,455,525 (Singh et al.), U.S. Pat. Appln. No. 2003158199 (Stieber et al.), U.S. Pat. No. 5,990,146 (Boschelli et al.), U.S. Pat. No. 6,100,254 (Budde et al.), U.S. Pat. No. 6,218,388 (Boschelli et al.), U.S. Pat. No. 6,316,479 (McMahon et al.), U.S. Pat. No. 6,649,635 (McMahon et al.), U.S. Pat. Appln. No. 20040102360 (Barnett et al.) and others.

In embodiments, the present invention relates to methods of promoting the growth of mammalian chondrocytes in vitro comprising administering to the chondrocytes in vitro a pharmaceutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

The compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms, including hydrated forms, are equivalent to unsolvated forms and are intended to be encompassed within the scope of the present invention.

The compounds of Formula I are capable of further forming both pharmaceutically acceptable formulations comprising salts, including but not limited to acid addition and/or base salts, solvates and N-oxides. This invention also provides pharmaceutical formulations comprising a compound of Formula I together with a pharmaceutically acceptable carrier, diluent, or excipient therefor. All of these forms are within the present invention.

Pharmaceutically acceptable acid addition salts of the compounds of Formula I include salts derived from inorganic acids such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydriodic, phosphorus, and the like, as well as the salts derived from organic acids, such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanolic acids, hydroxy alkanolic acids, alkanedioic acids, aromatic acids, aliphatic and

aromatic sulfonic acids, etc. Such salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, toluenesulfonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like. Also contemplated are the salts of amino acids such as arginate, gluconate, galacturonate, and the like; see, for example, Berge, et al., "Pharmaceutical Salts," *J. of Pharmaceutical Science*, 1977;66:1-19.

The acid addition salts of the basic compounds are prepared by contacting the free base form with a sufficient amount of the desired acid to produce the salt in the conventional manner. The free base form may be regenerated by contacting the salt form with a base and isolating the free base in the conventional manner. The free base forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free base for purposes of the present invention.

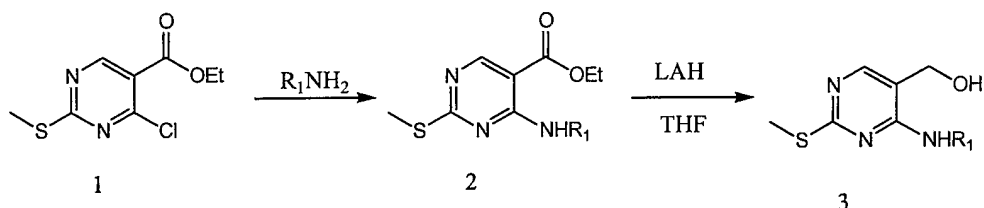
Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metal hydroxides, or of organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine, and procaine; see, for example, Berge, supra, 1977.

The base addition salts of acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in a conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention.

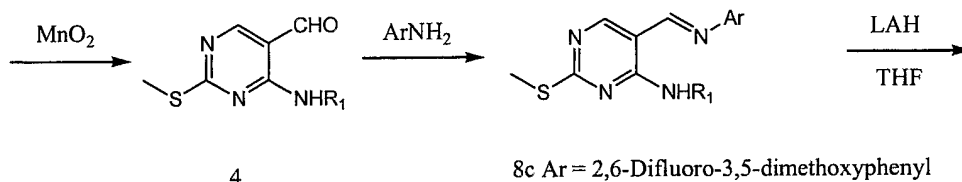
3-Phenyl-pyrimido[4,5-d]pyrimidin-2-one compounds of this invention may be prepared by methods known in the art, including those described in WO 99/61444 (Dobrusin et al.), and WO 01/29042 (Dunn et al.). An example of useful synthetic routes includes that seen in Scheme 1. Treatment of 4-Chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester (1) with an R₁-substituted amine, wherein R₁ is as defined herein, including alkylamines and aryl amines, forms the corresponding 4-R₁-amino-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester (2a-2h). This treatment may be carried out in an art recognized solvent, such as tetrahydrofuran, or dichloromethane. Reduction of the ester (2a-2h) to the corresponding alcohol (3a-3h) can be carried out in art recognized methods, such as using lithium aluminum hydride in tetrahydrofuran.

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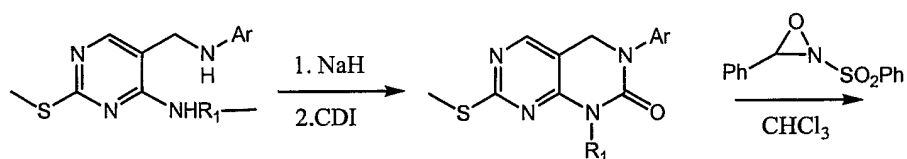
Scheme 1



Subsequent oxidation of the alcohol (3) yields the corresponding aldehyde (4), which may be treated with an aryl amine, $ArNH_2$, wherein Ar is as defined for its optionally substituted groups at the desired position, to provide the corresponding 2-methylsulfanyl-4- R_1 -amino-5-arylimino-pyrimidine (8c).

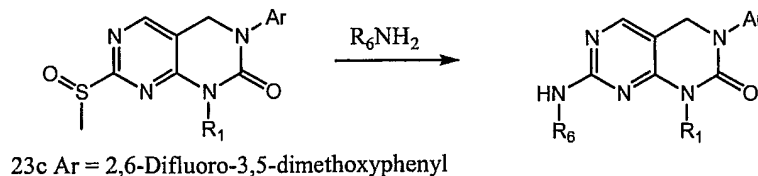


The 5-arylamino compound (8c) may then be reduced to the corresponding 5-aryl-amine (13c) using conventional reducing agents, such as sodium borohydride, lithium aluminum hydride or sodium triacetoxyborohydride under art recognized conditions. Cyclization of the amine (13c) to provide the 1- R_1 -7-methylsulfanyl-3-aryl-1H-pyrimido[4,5-d]pyrimidin-2-one (18c) may be accomplished using sodium hydride and carbonyldiimidazole (CDI). Oxaziridine-mediated oxidation of the 7-position sulfanyl compounds (18c) provides the corresponding sulfinyl compounds (23c), which may then be reacted with an R_6 -substituted amine, wherein the R_6 positional groups are as defined herein, to provide the desired 7- R_6 -amino-3-Phenyl-pyrimidin-2-one compounds.



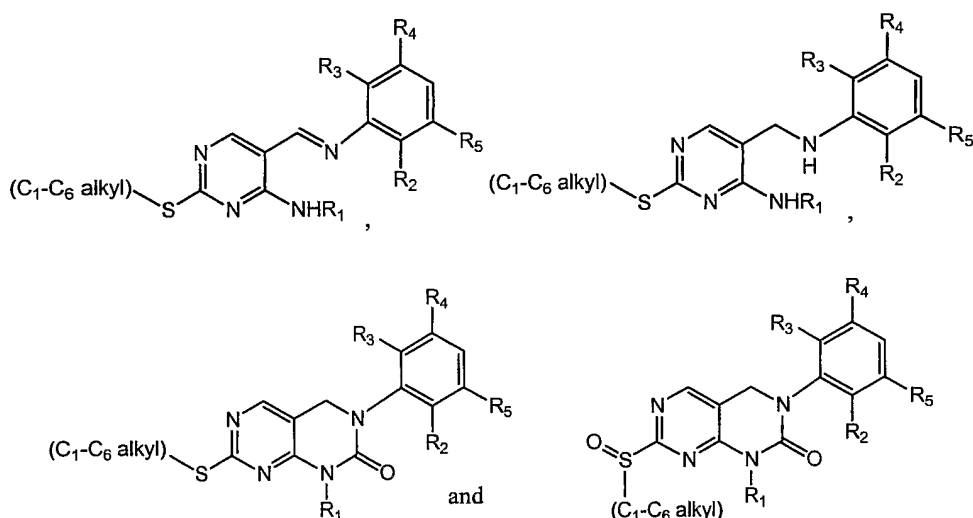
13c Ar = 2,6-Difluoro-3,5-dimethoxyphenyl

18c Ar = 2,6-Difluoro-3,5-dimethoxyphenyl



As depicted in Scheme 1, above, this application also comprises compounds useful in preparation of the pharmaceutically useful compounds described herein, the compounds having the formulae:

-20-

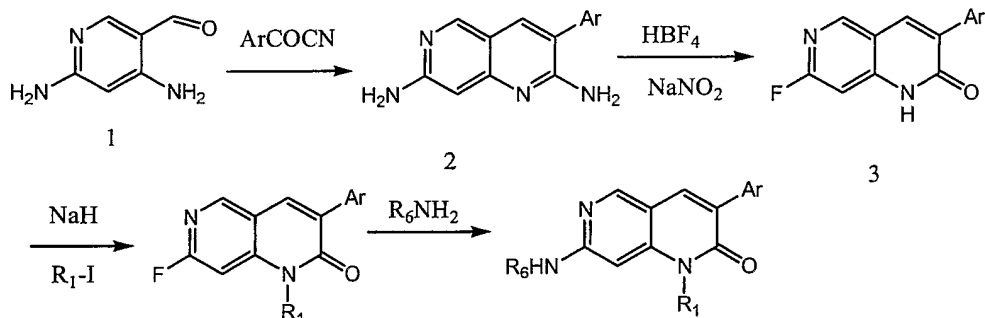


wherein R_1 , R_2 , R_3 , R_4 , and R_5 may be as defined in each instance herein.

Preparation of naphthyridone compounds useful in the methods of this invention
 5 can also be accomplished by methods known in the art, such as those described in
 WO 99/09030 (Barvian et al.). As seen in Scheme 2, 4,6-Diamino-
 3-pyridylcarboxaldehyde (1) can be reacted with an appropriate aryl acetonitrile
 (ArCOCN) to provide the corresponding 3-aryl-[1,6]naphthyridine-2,7-diamine (2), also
 described in U.S. Patent No. 5,620,981, after the manner reported by Hawes et al., *J.*
 10 *Heterocycl. Chem.*, 1972:9,703. The 3-aryl-[1,6]naphthyridine-2,7-diamine (2) may be
 converted to 3-aryl-7-fluoro-[1,6]naphthyridine-2-one (3) by a diazotization reaction in 50%
 aqueous fluoroboric acid in a large excess (up to 8 equivalents) of solid sodium nitrite at
 low temperature (at or below -5°C) for several days, after the manner previously
 described by Rewcastle et al., *J. Med. Chem.*, 1996;39,1823. The corresponding R_1 -
 15 substituted compounds (4) of this invention, wherein R_1 is as defined herein, may be
 produced by treatment of the 3-aryl-7-fluoro-[1,6]naphthyridine-2-one (3) with an
 appropriate R_1 -iodide, such as an appropriate alkyl iodide, in the presence of a base in a
 suitable dry, unreactive solvent, such as dimethylformamide, at from about 0°C to about
 20 20°C . A small amount of the corresponding 2-position $-\text{OR}_1$ compound may result from
 competing O-alkylation and can be removed by convention chromatography techniques.
 The resulting 7-Fluoro-1- R_1 -3-aryl-1H[1,6]naphthyridin-2-one compounds (4) may be
 treated with an appropriate aromatic or aliphatic amine ($R_6\text{NH}_2$) to yield the desired
 naphthyridone compounds of this invention. In the reaction Schemes 1-6, the listings of
 25 $R_6\text{NH}_2$ reactants and $-\text{NHR}_6$ substituents refer equally to all of the options of $\text{NHR}_{6a}\text{R}_{6b}$
 reactants and $-\text{NR}_{6a}\text{R}_{6b}$ substituents described herein.

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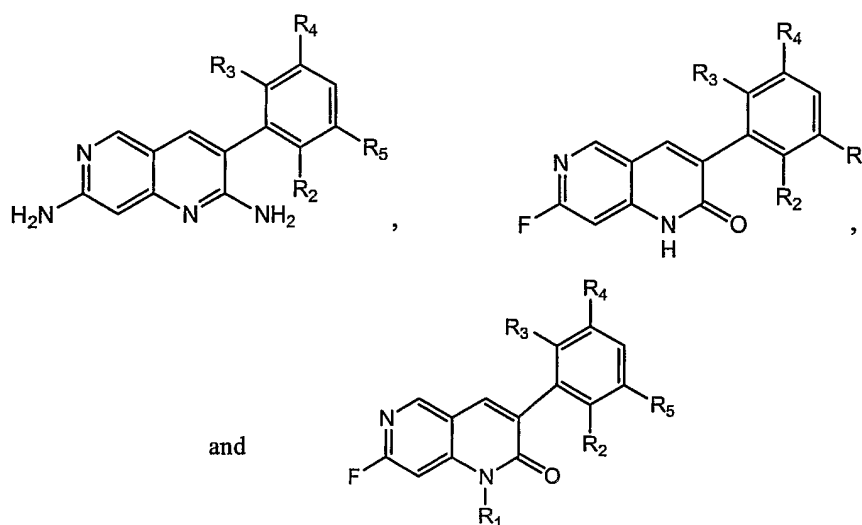
Scheme 2



Pteridinone compounds of this invention may be prepared by methods known in the art, including those described in WO 01/19825 (Denny et al.). An example of these methods is the reaction of 2,4-Dichloro-5-nitro-pyrimidine (1) with an appropriate R_1 -amine, preferably at reduced temperatures, to form 2-Chloro-5-nitro-pyrimidin-4-yl)- R_1 -amine (2), wherein R_1 is as defined for the corresponding positional groups herein.

Additional treatment with R_6 -primary amine, preferably at ambient temperatures, provides the corresponding 5-nitro-2,4-diaminopyrimidine compound (3), wherein R_6 is as defined for the corresponding positional groups herein. The nitro compound (3) can then be reduced by conventional methods to the corresponding 2,4,5-triaminopyrimidine (4) through conventional means, such as catalytic hydrogenation. Treatment of the amine (4) with an appropriately substituted oxophenylacetic acid ester, preferably at elevated temperatures of from about 50°C to about 150°C , produces the desired cyclized 2-substituted amino-6-phenyl-8H-pteridin-7-one (5).

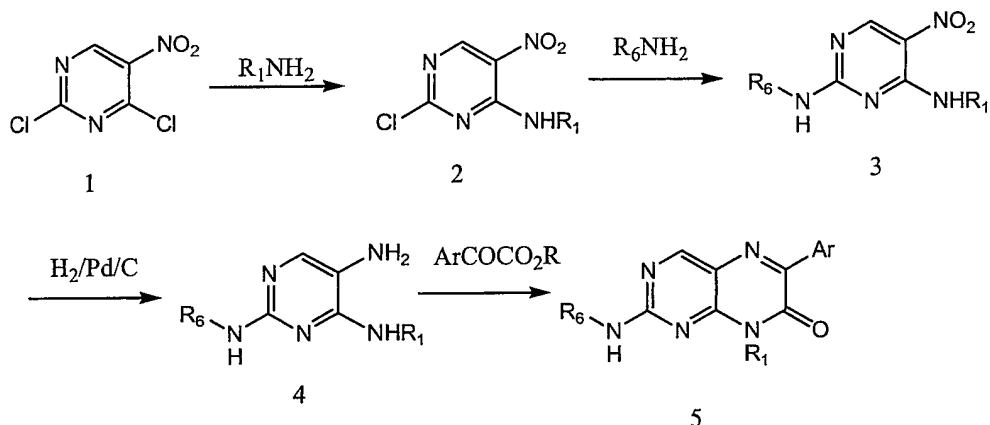
The methods and regimens of this invention may utilize compounds of the formulae:



wherein R_1 , R_2 , R_3 , R_4 , and R_5 may be as defined in each instance herein.

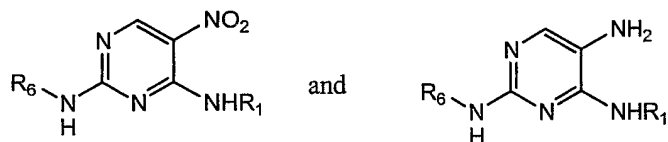
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Scheme 3



Synthetic routes to prepare pteridinone ureas (5), as defined herein, include the techniques described in WO 01/19825 (Denny et al.). As seen in Scheme 4, 2,4-Dichloro-5-nitro-pyrimidine (1) may be treated with ammonia to provide the corresponding 2-Chloro-5-nitro-pyrimidin-4-yl-amine (2). Reduction of the nitro group, for instance by reaction with hydrogen in the presence of a catalyst such as palladium on carbon, and treatment with an R_6 -substituted amine yields the N- R_6 -pyrimidine-2,4,5-triamine (3). Cyclization to pteridine (4) is accomplished by reaction of the triamine (3) with an agent such as an aryl ketonitrile ($ArCOCN$). Subsequent treatment of the pteridine (4) with an appropriate isocyanate, wherein R_7 is as defined herein, in the presence of NaH provides the desired urea (5).

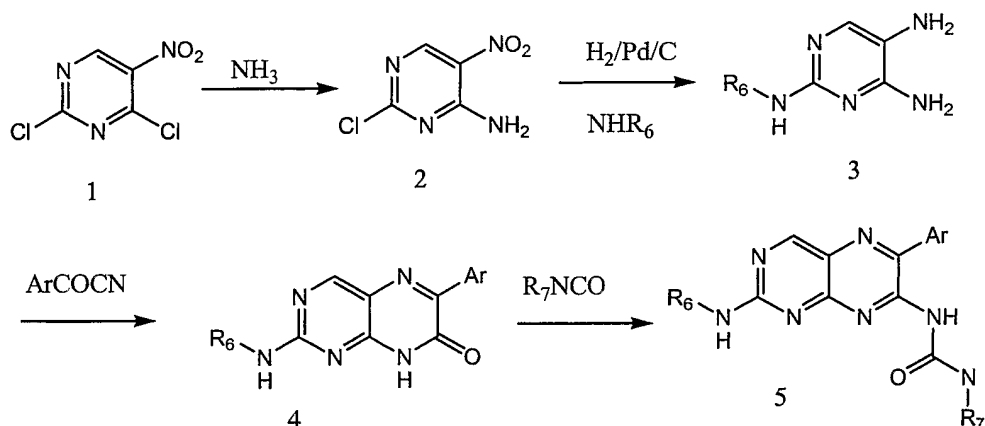
The methods of this invention may further utilize compounds of the formulae:



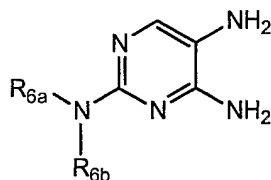
wherein R_1 and R_6 are as defined herein, which may be used in the synthesis of the pharmaceutically useful pteridinone compounds of this invention.

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Scheme 4



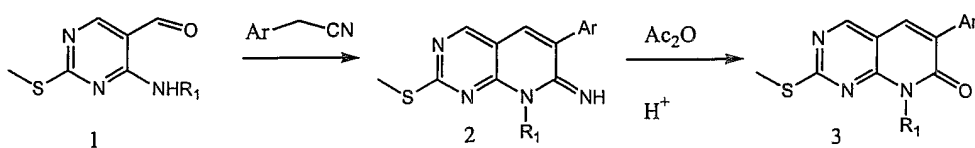
As depicted in Scheme 4, above, compounds useful in the methods herein may be synthesized utilizing triaminopyrimidine compounds of the formula:



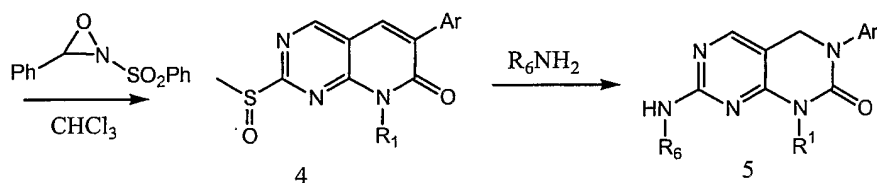
wherein R_{6a} and R_{6b} are as defined herein.

Pyridopyrimidine compounds useful in the methods of this invention can also be prepared by methods known in the art. Examples of references describing their synthesis include U.S. Pat. No. 5,733,913 (Blankley et al.), U.S. Pat. No. 5,733,914 (Blankley et al.), WO 02/12237 (Beylin et al.), and WO 9833798 (Boschelli et al.). Scheme 5, below, demonstrates a procedure for preparation of pyridopyrimidine compounds of this invention. As depicted, an appropriate 4-(substituted amino)-2-methylsulfanyl-pyrimidine-5-carboxaldehyde (1) (*J. Med. Chem.*, 1998;41(22):4365-4377 or *J. Med. Chem.*, 1998;41(17):3276-3292) can be treated with acetonitrile agent in the presence of a base and suitable solvent to afford the condensed 6-(aryl)-8-(substituted)-2-methylsulfanyl-8H-pyrido[2,3-d]pyrimidin-7-ylideneamine product (2). Product (2) can be acylated, followed by catalyzed hydrolysis, to the corresponding 7-keto compound (3). The methylthio group can then be oxidized to the respective sulfoxide (4), which is displaced to form the 4-aminopyridine or substituted 4-aminopyridine product (5). Additional reagents, conditions and solvents for these steps may be seen in WO 01/12238 (Hamby et al.).

Scheme 5

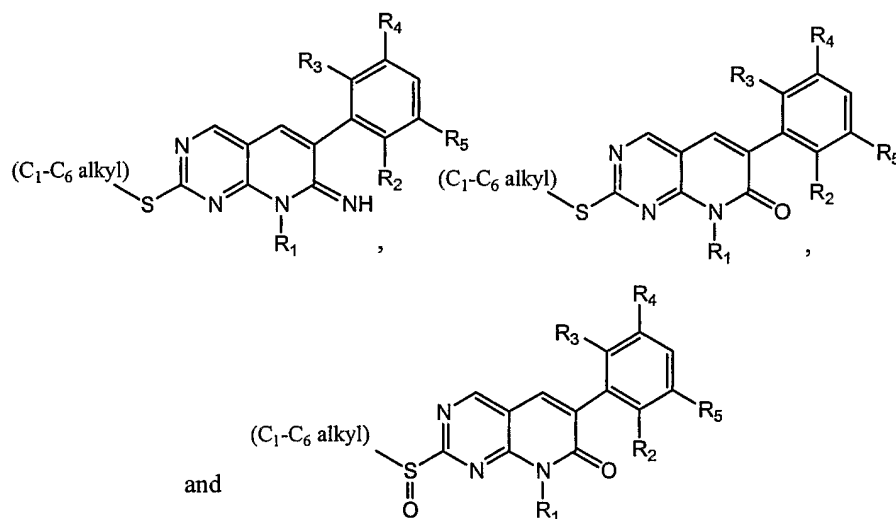


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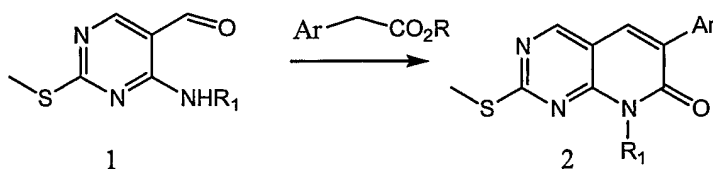
A further synthesis of pyridopyrimidone compounds of this invention can be accomplished by the alternate condensation route demonstrated in Scheme 6. In this method, the initial preparation of the 4-thioalkyl-7-keto compound (2) is accomplished by condensation of the 4-(substituted amino)-2-methylsulfanyl-pyrimidine-5-carboxaldehyde (1) with a substituted aryl acetic acid ester, such as a phenyl acetic acid alkyl ester, in the presence of a suitable base, such as 1,8-diazabicyclo[5.4.0]undec-7-ene. The reaction can be accomplished neat or in a solvent such as dimethylformamide or dimethyl sulfoxide.

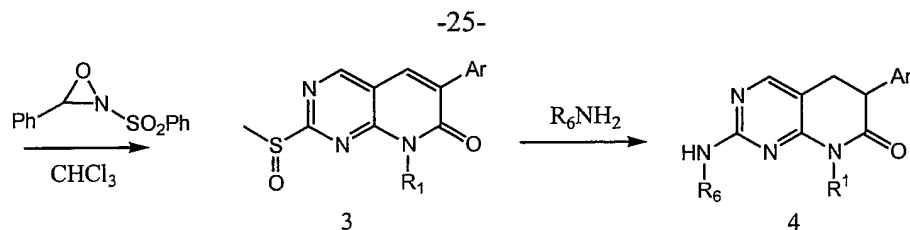
This invention also comprises methods of treatment, inhibition or prevention of cartilage damage or degradation utilizing compounds of the formulae:



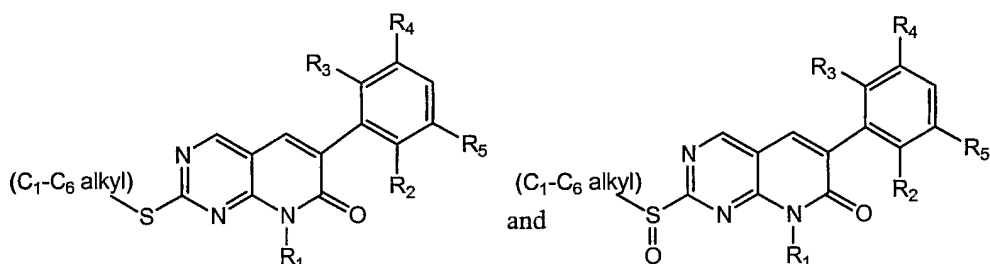
wherein the variables R_1 , R_2 , R_3 , R_4 , and R_5 may be as defined in each instance herein.

Scheme 6





As noted in Scheme 6, this invention also comprises compounds of the formulae:



wherein the variables R₁, R₂, R₃, R₄, and R₅ may be as defined in each instance herein.

5 The following non-limiting descriptions also demonstrate methods for synthesis of compounds useful in the methods herein.

1) Preparation of Anilines

Route A for preparation of 2,6-Difluoro-3,5-dimethoxyaniline

2,6-Difluoro-3,5-dimethoxyaniline can be prepared as follows:

10

Methyl 4-(benzylamino)-2,3,5,6-tetrafluorobenzoate

Methyl pentafluorobenzoate (41.4 g, 183 mmol) was treated with N-methylpyrrolidinone (36.0 g) and the solution stirred and cooled to 5°C under a N₂ atmosphere. Benzylamine (19.8 g, 185 mmol) was added dropwise over 27 min. at 5°C-20°C. The resulting thick yellow slurry was cooled to 2°C over 25 min. and N,N-diisopropyl ethylamine (27.0 g, 209 mmol) was added over 30 min. at 3°C-10°C. The mixture was allowed to warm to 18°C and stirred for 2 hrs. It was then heated to 60°C and stirred another 2 hs at 58°C-67°C. The resulting solution was cooled to room temperature and stirred overnight. The mixture was treated with toluene (200 mL) and methyl *tert*-butyl ether (120 mL) and extracted with water (150 mL) followed by 10% aqueous acetic acid (2 × 50 mL). The organic layer was concentrated to a solid, which was recrystallized from toluene (45 mL) and heptane (50 mL). After cooling to -20°C, the crystals were collected, washed with heptane (2 × 25 mL) and vacuum dried at 35°C to give methyl 4-(benzylamino)-2,3,5,6-tetrafluorobenzoate (49.3 g, 157 mmol): mp 96-97°C.

25

Methyl 4-(benzylamino)-3,5-difluoro-2,6-dimethoxybenzoate

Methyl 4-(benzylamino)-2,3,5,6-tetrafluorobenzoate (24.0 g, 76.6 mmol) was cooled to 5°C-10°C under a nitrogen atmosphere and a 25 weight % solution of sodium

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methoxide in methanol (53.5 mL, 234 mmol) was added dropwise over 5 minutes with continued cooling. The resulting mixture was stirred with ice bath cooling for another 10 minutes, the ice bath was removed and the mixture allowed to warm to 35°C-40°C. It was recooled to room temperature and held for 4.5 hours before heating to 65°C-70°C where it was held for 3.5 hours. The mixture was cooled to room temperature and held overnight. Acetic acid (7.5 g) was added followed by methyl *tert*-butyl ether (250 mL) and water (100 mL). The lower aqueous layer was separated and the organic layer extracted with saturated, aqueous sodium bicarbonate solution (2 × 50 mL) followed by water (25 mL). The organic solution was concentrated under vacuum to give methyl 4-(benzylamino)-3,5-difluoro-2,6-dimethoxybenzoate as a yellow oil (24.5 g).

4-(Benzylamino)-3,5-difluoro-2,6-dimethoxybenzoic acid

Methyl 4-(benzylamino)-3,5-difluoro-2,6-dimethoxybenzoate (24.0 g) was dissolved in ethanol (75 mL) and the solution stirred and cooled to 10°C under a nitrogen atmosphere. Sodium hydroxide, 50% aqueous solution (12.5 g, 156 mmol) was added dropwise over 10 minutes at 10°C-15°C followed by ethanol (10 mL). The solution was stirred at 15°C-25°C. A thick precipitate formed. Stirring was continued for a total of 2 hours at 15°C-25°C. The mixture was heated to 50°C-55°C where it was held for 1.5 hours. The mixture was allowed to cool to room temperature and held overnight. Water (100 mL) was added and the resulting solution cooled in an ice bath and acidified with 37% HCl (13.5 mL) to pH 2. The mixture was extracted with toluene (100 mL) and methyl *tert*-butyl ether (100 mL) and the organic extract washed with water (10 mL) and concentrated to an oil. This was triturated with toluene (20 mL) to give crystals which were collected and vacuum dried at 45°C to give 4-(benzylamino)-3,5-difluoro-2,6-dimethoxybenzoic acid (19.4 g, 60 mmol): mp: 85°C-86°C (decomposition with gas evolution).

N-Benzyl-2,6-difluoro-3,5-dimethoxyaniline

4-(Benzylamino)-3,5-difluoro-2,6-dimethoxybenzoic acid (9.1 g, 28.1 mmol) was treated with chlorobenzene (45 mL) and the solution heated to reflux under nitrogen where it was maintained for 16 hours. The solution was cooled to room temperature and extracted with saturated, aqueous sodium bicarbonate solution (2 × 5 mL) followed by water. The organic layer was concentrated under reduced pressure to an oil that crystallized on standing. This was recrystallized from heptane (10 mL) and toluene (5 mL), the mixture cooled to -10°C, filtered and the crystals washed with heptane (10 mL) and vacuum dried at 35°C to give N-benzyl-2,6-difluoro-3,5-dimethoxyaniline (7.4 g, 26.5 mmol). mp: 65°C-66°C.

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2,6-Difluoro-3,5-dimethoxyaniline

N-Benzyl-2,6-difluoro-3,5-dimethoxyaniline (6.0 g, 21.5 mmol) was dissolved in THF (40 mL) and the solution hydrogenated over 20% palladium hydroxide on carbon catalyst (0.6 g) at 20°C-25°C 48-50 psi hydrogen pressure for 16 hours. The mixture was filtered, the catalyst washed with THF (2 × 15 mL) and the combined filtrates concentrated to a solid. This was recrystallized from heptane (10 mL) and toluene (4.5 mL). The mixture was cooled to -10°C before filtering. The crystals were washed with heptane (2 × 10 mL) and vacuum dried at 35°C to give 2,6-difluoro-3,5-dimethoxyaniline as off-white needles (3.85 g, 20.4 mmol). mp: 78°C-79°C.

Route B for the preparation of 2,6-Difluoro-3,5-dimethoxyaniline**2,6-Difluoro-3,5-dimethoxy benzoic acid methyl ester**

A solution of 336 g (1.71 mol) of methyl 3,5-dimethoxy benzoate in 0.84 L of acetonitrile was cooled down to 0°C on an ice bath under nitrogen atmosphere. A suspension of 912 g (2.57 mol) of SelectFluor: 1-chloromethyl-4-fluoro-1,4-diazobicyclo[2.2.2] octane bis(tetrafluoroborate) in 16 L acetonitrile was added keeping the temperature close to 0°C. The reaction was stirred overnight warming up to room temperature. The next day, the reaction mixture was poured into 10 L of the sodium carbonate solution and extracted with 20 L of t-butylmethyl ether. The organic layer was washed with 3.75 L of brine, dried with sodium sulfate, filtered and evaporated. The crude mixture was separated by Biotage 150 column chromatography eluting with a gradient of heptane:EtOAc 30:1(36 L) to 25:1(36 L) to 20:1(54 L) to 15:1(54 L) to obtain 119.29 g (30%) of the title compound. MS (APCI) (m+1)/z 233.1.

2,6-Difluoro-3,5-dimethoxy benzoic acid

A suspension of 115 g (0.49 mol) of 2,6-difluoro-3,5-dimethoxy benzoic acid methyl ester and 42.9 g (1.07 mol) of solid sodium hydroxide in 1.4 L of anhydrous ethanol was refluxed for 24 hours. The ethanol was concentrated *in vacuo* and the solid residue was dissolved in water and extracted two times with ethyl ether. The aqueous layer was acidified with concentrated hydrochloric acid and a white precipitate was filtered, washed with cold water and dried *in vacuo* to give 87.9 g (81%) of the title compound. MS (APCI) (m+1)/z 219.1.

(2,6-Difluoro-3,5-dimethoxy-phenyl)-carbamic acid tert-butyl ester

To a stirred mixture of 47.7 g (0.22 mol) of 2,6-difluoro-3,5-dimethoxy benzoic acid, 32 mL (0.22 mol) of triethylamine and 16.2 g (0.22 mol) of t-butanol in 500 mL of toluene, 48.2 mL (0.22 mol) of diphenylphosphoryl azide was added in one portion and the mixture was heated to 60°C-70°C. After approximately 1 hour, the reaction was completed and the mixture was cooled down. The toluene was removed *in vacuo*. Then,

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ethyl acetate was added and the resulting solution was washed two times with saturated potassium hydrogen-phosphate solution, two times with saturated sodium bicarbonate solution, one time with brine, dried over sodium sulfate, filtered and evaporated. The crude product was purified by column chromatography: 1 kg of silica eluting with dichloromethane to give 45 g (71%) of the title compound.

2,6-Difluoro-3,5-dimethoxy-phenylamine

To 34.6 g (0.12 mol) of (2,6-difluoro-3,5-dimethoxy-phenyl)-carbamic acid tert-butyl ester, 120 mL of trifluoroacetic acid was added to give a homogeneous solution which was stirred at room temperature for 30 minutes. After 30 min., the reaction mixture was concentrated *in vacuo*. To the residue, a saturated solution of sodium bicarbonate was carefully added to pH 8. The resulting suspension was extracted twice with diethylether. Combined diethylether layers were washed once with brine, dried over sodium sulfate, filtered and evaporated to give 22 g (97%) of title compound. MS (APCI) (m+1)/z 190.1.

2-Fluoro-3,5-dimethoxy-benzoic acid methyl ester

A solution of 168 g (0.86 mol) of methyl 3,5-dimethoxy benzoate in 0.42 L of acetonitrile was cooled down to 0°C on an ice bath under nitrogen atmosphere. A suspension of 456 g (1.3 mol) of SelectFluor: 1-chloromethyl-4-fluoro-1,4-diazobicyclo[2.2.2] octane bis(tetrafluoroborate) in 8 L acetonitrile was added keeping the temperature close to 0°C. The reaction was stirred overnight warming up to room temperature. The next day, the reaction mixture was poured into 5 L of the sodium carbonate solution and extracted with 20 L of t-butylmethyl ether. The organic layer was washed with 1.875 L of brine, dried with sodium sulfate, filtered and evaporated. The crude mixture was separated by Biotage column chromatography eluting with a gradient of heptane:EtOAc 30:1(18 L) to 25:1(18 L) to 20:1(27 L) to 15:1(27 L) to obtain 73.6 g (40%) of the title compound. MS (APCI) (m+1)/z 215.1.

2-Fluoro-3,5-dimethoxy benzoic acid

A suspension of 66.7 g (0.27 mol) of 2-fluoro-3,5-dimethoxy-benzoic acid methyl ester and 27 g (0.675 mol) of solid sodium hydroxide in 815 mL of anhydrous ethanol was refluxed for 24 hours. The ethanol was concentrated *in vacuo* and the solid residue was dissolved in water and extracted two times with ethyl ether. The aqueous layer was acidified with concentrated hydrochloric acid and a white precipitate was filtered, washed with cold water and dried *in vacuo* to afford 53.9 g (86.5%) of the title compound. MS (APCI) (m+1)/z 201.0.

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(2-Fluoro-3,5-dimethoxy-phenyl)-carbamic acid tert-butyl ester

To a stirred mixture of 54.5 g (0.27 mol) of 2-fluoro-3,5-dimethoxy benzoic acid, 38 mL (0.27 mol) of triethylamine and 20.1 g (0.27 mol) of t-butanol in 500 mL of toluene, 59.9 mL (0.27 mol) of diphenylphosphoryl azide was added in one portion and the mixture was heated to 60°C-70°C. After approx. 1 hour, the reaction was completed and the mixture was cooled down. The toluene was removed *in vacuo*. Then, ethyl acetate was added and the resulting solution was washed two times with saturated potassium hydrogen-phosphate solution, two times with saturated sodium bicarbonate solution, one time with brine, dried over sodium sulfate, filtered and evaporated. The crude product was purified by column chromatography: 1.4 kg of silica eluting with dichloromethane to give 63.2 g (86%) of the title compound. MS (APCI) (m+1)/z 270.0.

2-Fluoro-3,5-dimethoxy-phenylamine

To 56.6 g (0.21 mol) of (2-fluoro-3,5-dimethoxy-phenyl)-carbamic acid *tert*-butyl ester, 184 mL of trifluoroacetic acid was added to give a homogeneous solution which was stirred at room temperature for 30 minutes. After 30 minutes, the reaction mixture was concentrated *in vacuo*. To the residue, a saturated solution of sodium bicarbonate was carefully added to pH 8. The resulting suspension was extracted two times with diethylether. The combined diethylether layers were washed one time with brine, dried over sodium sulfate, filtered and evaporated to give 34.1 g (95.5%) of title compound. MS (APCI) (m+1)/z 172.0.

1-Ethoxy-2,4-difluoro-benzene

Into a solution of 2,4-difluorophenol (69 g, 0.53 mol) and acetone (500 mL) was added 1.2 equivalents of potassium carbonate (89 g, 0.64 mol) followed by 1.3 equivalents of iodoethane (108 g, 0.69 mol). The reaction mixture is warmed to reflux for 4 hours then concentrated *in vacuo*. The residue was dissolved in ethyl acetate (500 mL), washed with a saturated aqueous solution of sodium chloride (3 × 200 mL), dried over Na₂SO₄, and concentrated *in vacuo* to give 75 g (90%) of the title compound as yellow oil, which was used without further purification in the next step.

3-Ethoxy-2,6-difluoro-benzoic acid

A solution of 1-ethoxy-2,4-difluoro-benzene (16 g, 0.10 mol) in dry THF (170 mL) was cooled to -78°C and lithium diisopropylamide (62 mL, 0.12 mol) was added dropwise. The reaction mixture was stirred at -78°C for 1.5 hours and then CO₂ (from dry ice, dried through H₂SO₄) was introduced. After 1 hour, the reaction mixture was warmed to 0°C and 6M HCl was added to adjust pH to 2-3. The solution was extracted with ethyl acetate (3 × 100 mL), extracted with 5% aq. NaOH (3 × 100 mL). The NaOH extracts are adjusted

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to pH 1 with HCl and the white solid separated is filtered, washed with water then dried in vacuum oven to give the title compound **3**, as white solid. Yield: 16.2 g (80%), mp: 157°C-158°C.

5 **(3-Ethoxy-2,6-difluoro-phenyl)-carbamic acid tert-butyl ester**

To a mixture of 3,6-difluoro-3-ethoxybenzoic acid (15.8 g, 78.2 mmol), triethyl amine (12.2 mL, 8.9 g, 86 mmol) and *tert*-butanol (8.3 mL, 6.4 g, 86 mmol) in toluene (250 mL) was added diphenylphosphoryl azide (18 mL, 82.1 mmol). The reaction was heated at 60°C-70°C for 2 hours. The reaction mixture was evaporated and the residue
10 was dissolved in ethyl acetate (250 mL), washed with KH₂PO₄ (3×), aq. satd. NaHCO₃ (3×), brine (2×), dried over Na₂SO₄, filtered and evaporated under vacuum to give the title compound as white solid. The above solid was used directly in the next step without purification. Yield: 20.5 g, 96%

3-Ethoxy-2,6-difluoro-phenylamine

15 A solution of (3-ethoxy-2,6-difluoro-phenyl)-carbamic acid tert-butyl ester (135 g, 0.525 mol) in a mixture of dichloromethane and 10 equivalents trifluoroacetic acid (598 g, 5.25 mol) was stirred at room temperature for 1 hour and concentrated *in vacuo*. The pH of the residue was adjusted to pH 8 with a saturated aqueous solution of sodium bicarbonate. The organic layer was separated and the aqueous layer is extracted with
20 ethyl acetate (3 × 250 mL). The organic extracts were combined, washed with a saturated aqueous solution of sodium chloride (2×), dried and concentrated *in vacuo*. Chromatography of the yellow residual liquid down silica gel using 25:1 hexane:ethyl acetate gave 65 g (66%) of the title compound as oil.

2) Preparation of Esters

25 **Procedure A**

General Procedure for the Parallel Synthesis of 4-Substituted-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl esters (2)

Into an Argonaut Technologies' Quest 205 100 mL reactor was added 8.0 g (34.4 mmol) of 4-chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester in 70 mL
30 of dry THF. To the reaction mixture was added 1.1 equivalents of triethylamine followed by a solution of 1.1 equivalents of amine (R₁NH₂) in 10 mL of dry THF. The reaction mixture was agitated overnight under a nitrogen atmosphere at room temperature. The THF solution was drained from the reaction vessel, and the residue was washed with THF. The THF solutions were combined, evaporated, and redissolved in 35 mL of ethyl
35 acetate. The ethyl acetate solution was added back into the reaction vessel containing the residue, followed by 40 mL of 0.5 M NaOH. The reaction was agitated and the aqueous layer removed. The ethyl acetate solution was washed twice with 0.5 M NaOH

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and once with a saturated solution of sodium chloride. The ethyl acetate layer was dried with magnesium sulfate and concentrated *in vacuo*. The residue was stirred with pentane and evaporated to give the desired product.

4-Cyclopropylamino-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester

Using procedure A, 4-chloro-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester and 2.16 g (37.8 mmol) of cyclopropylamine were reacted to give 7.80 g (90%) of the title compound as a solid: mp 41°C-42°C. MS (APCI) (m+1)/z 254.0. Analysis calculated for C₁₁H₁₅N₃SO₂: C, 52.16; H, 5.97; N, 16.59. Found: C, 52.26; H, 5.82; N, 16.53.

3) Preparation of alcohols

Procedure B

General Procedure for the Parallel Synthesis of 4-Substituted-2-methylamino-pyrimidin-5-yl)-methanols

(4-Cyclopropylamino-2-methylamino-pyrimidin-5-yl)-methanol

Into an Argonaut Technologies' Quest 205 100 mL reactor was added 1.5 equivalents of 1 M LAH in THF. To the LAH solution was added 3.90 g (15.4 mmol) of 4-cyclopropylamino-2-methylsulfanyl-pyrimidine-5-carboxylic acid ethyl ester prepared above. The reaction mixture was agitated overnight under a nitrogen atmosphere at room temperature. The reaction was quenched by sequential addition of 0.87 mL water, followed by 0.87 mL of 15% NaOH solution, and finally 2.61 mL of water. The reaction was filtered and the residue was washed with THF. The THF solutions were combined and concentrated *in vacuo*. The residue was stirred with pentane and evaporated to give 2.99 g (92%) of (4-cyclopropylamino-2-methylamino-pyrimidin-5-yl)-methanol as a solid: mp 139°C-140°C. MS (APCI) (m+1)/z 212.0. Analysis calculated for C₉H₁₁N₃SO·0.10 H₂O: C, 50.73; H, 6.24; N, 19.72. Found: C, 50.68; H, 6.09; N, 19.36.

4) Preparation of aldehydes

Procedure C

General Procedure for the Synthesis of 4-Substituted-2-methylsulfanyl-pyrimidine-5-carbaldehydes

To a solution of 4-substituted-2-methylamino-pyrimidin-5-yl)-methanol prepared above in 100 mL of CHCl₃ was added 9 equivalents of MnO₂. The reaction was stirred at room temperature overnight then filtered through Celite. The filter pad was washed with CHCl₃ and the filtrate was concentrated *in vacuo*. The residue was stirred with pentane and evaporated to give the desired product.

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4-Cyclopropylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde

Using Procedure C, 2.88 g (13.6 mmol) of (4-cyclopropylamino-2-methylamino-pyrimidin-5-yl)-methanol gave 2.65 g (93%) of the title compound as a white solid: mp 69.5°C-70°C. MS (APCI) (m+1)/z 210.1

5 Analysis calculated for $C_9H_{11}N_3SO$: C, 51.66; H, 5.30; N, 20.08. Found: C, 51.81; H, 5.21; N, 19.83.

5) Preparation of Imines**(5-[(2,6-Difluoro-3,5-dimethoxy-phenylimino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-ethyl-amine**

10 In a 500-mL round bottom flask, 3.00 g (15.86 mmol) of 2,6-difluoro-3,5-dimethoxy-phenylamine and 3.13 g (15.86 mmol) of 4-ethylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde in 150 mL of toluene with 1.11 g (4.76 mmol) of camphorsulfonic acid was fitted with a Dean Stark trap and heated at reflux. During the
15 day, the toluene was drained 3 times from the finger, replenished with 100 mL of the solvent and continued heating at reflux under nitrogen atmosphere overnight. The toluene was concentrated *in vacuo* and dried to give 7.19 g (quantitative) of the title compound. No further purification was necessary. MS (APCI) (m+1)/z 369.1.

Procedure D**20 General Procedure for the Condensation of Anilines with Aldehydes (4)
Cyclopropyl-(5-[(2,6-difluoro-3,5-dimethoxy-phenylimino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-amine**

To a solution of the 7.00 g (33.4 mmol) of 4-cyclopropylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde in 500 mL of toluene was added 6.33 g (33.4 mmol,
25 1 equivalent) of 2,6-difluoro-3,5-dimethoxy-phenylamine and 1.94 g (8.36 mmol, 0.25 equivalents) of camphorsulfonic acid. The reaction was warmed to reflux and the formed water was removed with the aid of a Dean-Stark trap. After reacting for 48 hours, the reaction was concentrated *in vacuo*. The residue was stirred with ether and filtered. The filter pad was dissolved in dichloromethane, stirred with silica gel, and concentrated
30 *in vacuo*. The residue was placed in the sample injection module of a Biotage FLASH 75 chromatographic apparatus. Chromatography of the crude material down a Biotage 500 g silica gel column with 2:1 hexane/ethyl acetate, then 1:1 hexane/ethyl acetate and finally 9:1 ethyl acetate/ethanol gave 8.92 g (70%) of title compound as a solid: mp 172°C-174°C. MS (APCI) (m+1)/z 381.0
35 Analysis calculated for $C_{17}H_{18}F_2N_4SO_2$: C, 53.67; H, 4.77; N, 14.73. Found: C, 53.54; H, 4.58; N, 14.61.

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Cyclopentyl-(5-[(2,6-difluoro-3,5-dimethoxy-phenylimino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-amine

In a 1-L round bottom flask, 10.00 g (0.0528 mol) of 2,6-difluoro-3,5-dimethoxy-phenylamine and 12.42 g (0.0523 mol) of 4-cyclopentylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde in 500 mL of toluene with 3.65 g (0.0157 mol) of camphorsulfonic acid was fitted with a Dean Stark trap and heated at reflux. During the day, the toluene was drained three times from the finger and continued heating at reflux under nitrogen atmosphere overnight. The toluene was concentrated *in vacuo* and dried to give 25.30 g (quantitative) of the title compound. No further purification was necessary. MS (APCI) (m+1)/z 409.2.

Cyclopentyl-(5-[(3-ethoxy-2,6-difluoro-phenylimino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-amine

To a solution of the 8.22 g (34.7 mmol) of 4-cyclopentylamino-2-methylsulfanyl-pyrimidine-5-carbaldehyde in 170 mL of toluene was added 6.00 g (34.7 mmol, 1 equivalent) of 3-ethoxy-2,6-difluoro-phenylamine and 2.01 g (8.66 mmol, 0.25 equivalents) of camphorsulfonic acid. The reaction was warmed to reflux and the formed water was removed with the aid of a Dean-Stark trap. After reacting for 48 hours, the reaction was concentrated *in vacuo*. The residue was dissolved in dichloromethane and neutralized with a saturated aqueous solution of sodium bicarbonate. The resulting emulsion was filtered through fiberglass filter paper. The methylene chloride layer was isolated from the filtrate, then washed first with a saturated aqueous solution of sodium bicarbonate, followed by a saturated aqueous solution of sodium chloride. The methylene chloride layer was dried with magnesium sulfate and concentrated *in vacuo*. The residue was dissolved in dichloromethane, stirred with silica gel, and concentrated *in vacuo*. The residue was placed in the sample injection module of a Biotage FLASH 75 chromatographic apparatus. Chromatography of the crude material on a Biotage 500 g silica gel column with 4:1 hexane/ethyl acetate gave 11.1 g (82%) of the title compound as a solid: mp 136°C-141°C. MS (APCI) (m+1)/z 393.2. Analysis calculated for C₁₉H₂₂F₂N₄SO: C, 58.15; H, 5.65; N, 14.28. Found: C, 58.22; H, 5.68; N, 14.26.

6) Preparation of Amines- (reduction of imines)**(5-[(2,6-Difluoro-3,5-dimethoxy-phenylamino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-ethyl-amine**

A solution of 7.19 g (assume 15.86 mmol) of (5-[(2,6-difluoro-3,5-dimethoxy-phenylimino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-ethyl-amine in 130 mL of anhydrous tetrahydrofuran was cooled to 0°C using an ice/acetone bath. The cooled mixture was treated dropwise via syringe with 23.79 mL (23.79 mol) of 1 M solution of lithium

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aluminum hydride in tetrahydrofuran which resulted initially with vigorous bubbling. The resulting mixture continued to stir at 0°C for 1 hour under nitrogen atmosphere. The ice bath was removed and the reaction mixture was allowed to warm up to room temperature and continued stirring at room temperature overnight. The reaction mixture was cooled to 0°C using an ice/acetone bath and quenched by the slow addition of 23.79 mL of water, 23.79 mL of 15% sodium hydroxide and 47.58 mL of water. The salts were filtered over a bed of Celite and removed the tetrahydrofuran *in vacuo*. The residue was redissolved in dichloromethane and filtered again. The layers were separated and dried the organic layer over sodium sulfate, filtered and evaporated. The crude product was purified using medium-pressure chromatography eluting with 2:1 hexanes/ethyl acetate to give 4.45 g (76%) of the title compound: mp 95°C-99°C. MS (APCI) (m+1)/z 371.1.

Procedure E

General Procedure for the Reduction of Imines

15 Cyclopropyl-(5-[(2,6-difluoro-3,5-dimethoxy-phenylamino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-amine

A solution of in 8.61 g (22.6 mmol) of cyclopropyl-(5-[(2,6-difluoro-3,5-dimethoxy-phenylimino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-amine in 400 mL of dry THF was cooled in an ice/water bath. To the cooled solution was added dropwise 1 equivalent (22.6 mL, 22.6 mmol) of a 1 M LAH solution in THF. The reaction was stirred cold for 2 hours and then quenched by sequential addition of 0.4 mL water, followed by 1.6 mL of 15% NaOH solution, and finally 0.88 mL of water. The reaction was filtered through Celite and the filter pad was washed well with THF. The THF filtrate was concentrated *in vacuo*. The residue was dissolved in dichloromethane, washed with a saturated solution of NaCl, and concentrated *in vacuo*. Stirring the residue with diethyl ether and concentrating the suspension *in vacuo* gave 8.38 g (97%) of the title compound as a solid: mp 48°C-50°C. MS (APCI) (m+1)/z 383.4 Analysis calculated for C₁₇H₂₀F₂N₄SO₂: C, 53.39; H, 5.27; N, 14.65. Found: C, 53.44; H, 5.29; N, 14.31.

30 Cyclopentyl-(5-[(2,6-difluoro-3,5-dimethoxy-phenylamino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-amine

A solution of 25.30 g (assume 0.0523 mol) of (5-[(2,6-difluoro-3,5-dimethoxy-phenylimino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-ethyl-amine in 425 mL of anhydrous tetrahydrofuran was cooled to 0°C using an ice/acetone bath. The cooled mixture was treated dropwise via syringe with 78.50 mL (0.0785 mol) 1 M solution of lithium aluminum hydride in tetrahydrofuran which resulted initially with vigorous bubbling. The resulting mixture continued to stir at 0°C for 1 hour under nitrogen atmosphere. The ice bath was removed and the reaction mixture was allowed to warm up to room temperature and

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continued stirring at room temperature overnight. The reaction mixture was cooled to 0°C using an ice/acetone bath and quenched by the slow addition of 78.50 mL of water, 78.50 mL of 15% sodium hydroxide and 157 mL of water. The salts were filtered over a bed of Celite and removed the tetrahydrofuran *in vacuo*. The residue was redissolved in dichloromethane and filtered again. The layers were separated and dried the organic layer over sodium sulfate, filtered and evaporated. The crude product was purified using medium-pressure chromatography eluting with a gradient of 2:1 to 1:1 hexanes/ethyl acetate to give 16.34 g (76%) of title compound. MS (APCI) (m+1)/z 411.1.

10 **Cyclopentyl-{5-[(3-ethoxy-2,6-difluoro-phenylamino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl}-amine**

A solution of 8.00 g (20.4 mmol) cyclopentyl-{5-[(3-ethoxy-2,6-difluoro-phenylimino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl}-amine prepared above, in 200 mL dry THF, was cooled in an ice / water bath. To the cooled solution was added dropwise 1 equivalent (20.4 m, 20.4 mmol) of a 1 M LAH solution in THF. The reaction was stirred cold for 4.5 hours and then warmed to 50°C for 3.5 hours. The reaction was cooled to room temperature, treated with another 0.5 equivalent (11.0 mL, 11.0 mmol) of 1 M LAH solution in THF, and stirred at room temperature overnight. The reaction was quenched by sequential addition of 1.3 mL water, followed by 5.2 mL of 15% NaOH solution, and finally 2.9 mL of water. The reaction was filtered through Celite and the filter pad was washed well with THF. The THF filtrate was concentrated *in vacuo*. The residue was dissolved in dichloromethane, washed with a saturated solution of NaCl, and concentrated *in vacuo* to give 8.03 g (100%) of the title compound as a waxy solid: MS (APCI) (m+1)/z 395.1. Analysis calculated for C₁₉H₂₄F₂N₄SO:

25 C, 57.85; H, 6.13; N, 14.20. Found: C, 57.92; H, 6.19; N, 14.01.

7) Formation of Pyrimidine pyrimidinones

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methylsulfanyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

30 A solution of 4.92 g (11.58 mmol) of (5-[(2,6-difluoro-3,5-dimethoxy-phenylamino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-ethyl-amine in 85 mL of anhydrous tetrahydrofuran was cooled to 0°C using an ice/acetone bath. The reaction mixture was treated with 1.16 g (28.95 mmol) of sodium hydride and continued stirring at 0°C for 30 minutes. After 30 minutes, 5.63 g (34.74 mmol) of 1,1'-carbonyldiimidazole was added and continued to stir at 0°C for an additional 30 minutes. After 1 hour, the ice bath was removed and the reaction mixture was allowed to warm up to room temperature. The reaction mixture heated at reflux overnight under nitrogen atmosphere. Evaporated the tetrahydrofuran and partitioned the residue between dichloromethane and water. The aqueous layer was extracted one time with 250 mL dichloromethane. The

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dichloromethane extracts were combined, dried over sodium sulfate, filtered and evaporated. The crude product was purified using medium-pressure chromatography eluting with 2:1 hexanes/ethyl acetate to give 4.23 g (92%) of the title compound: mp 165°C-168°C. MS (APCI) (m+1)/z 397.1.

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Procedure F**1-Cyclopropyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfanyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one**

10 A solution of 7.66 g (20.0 mmol) of cyclopropyl-(5-[(2,6-difluoro-3,5-dimethoxy-phenylamino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-amine in 150 mL of dry THF was cooled to 0°C. To this solution was added 2.00 g (50.1 mmol, 2.5 equivalents) of NaH as a 60% oil suspension. The reaction was stirred for 0.5 hour, the ice bath was removed, and the reaction was stirred for another 0.5 hour. To the reaction was added 9.74 g (60.1 mmol, 3.0 equivalents) of CDI. After 0.5 hour the reaction was warmed to reflux for 15 24 hours. The reaction was concentrated *in vacuo* and the residue was partitioned between dichloromethane and a saturated solution of ammonium chloride. The dichloromethane layer was washed again with ammonium chloride and then once with a saturated solution of NaCl, dried, and concentrated *in vacuo*. The residue was stirred with diethyl ether and the suspension filtered to give 7.73 g (93%) of the title compound as a solid: mp 173°C-176°C. MS (APCI) (m+1)/z 409.1
20 Analysis calculated for C₁₈H₁₈F₂N₄SO₃: C, 52.93; H, 4.44; N, 13.72.
Found: C, 52.74; H, 4.34; N, 13.80.

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfanyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

25 A solution of 16.33 g (0.0398 mol) of cyclopentyl-(5-[(2,6-difluoro-3,5-dimethoxy-phenylamino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl)-amine in 288 mL of anhydrous tetrahydrofuran was cooled to 0°C using an ice/acetone bath. The reaction mixture was treated with 3.98 g (0.0995 mol) of sodium hydride and continued stirring at 0°C for 30 30 minutes. After 30 minutes, 19.35 g (0.019 mol) 1,1'-carbonyldiimidazole was added and continued to stir at 0°C for an additional 30 minutes. After 1 hour, the ice bath was removed and the reaction mixture was allowed to warm up to room temperature. The reaction mixture heated at reflux overnight under nitrogen atmosphere. Evaporated the tetrahydrofuran and partitioned the residue between dichloromethane and water. The aqueous layer was extracted one time with 500 mL dichloromethane. The 35 dichloromethane extracts were combined, dried over sodium sulfate, filtered and evaporated. There was a portion of the crude material that did not dissolve in the eluent. Filtered the solid and dried in the housevac oven overnight to give the title compound,

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6.80 g of crop 1. The remaining crude material was purified using medium pressure-chromatography, eluting with a gradient of 2:1 hexanes/ethyl acetate to 1:1 hexanes/ethyl acetate to give the title compound as a white solid, 8.74 g of crop 2. Combined the crops to give a total of 15.54 g (90%) of the title compound. MS (APCI) (m+1)/z 437.1.

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1-Cyclopentyl-3-(3-ethoxy-2,6-difluoro-phenyl)-7-methylsulfanyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

A solution of 1.00 g (2.53 mmol) of cyclopentyl-{5-[(3-ethoxy-2,6-difluoro-phenylamino)-methyl]-2-methylsulfanyl-pyrimidin-4-yl}-amine in 20 mL of dry THF was cooled to 0°C. To this solution was added 0.25 g (6.33 mmol, 2.5 equivalents) of NaH as a 60% oil suspension. The reaction was stirred for 0.5 hour, the ice bath was removed, and the reaction was stirred for another 0.5 hour. To the reaction was added 1.24 g (7.60 mmol, 3.0 equivalents) of CDI. After 0.5 hour the reaction was warmed to reflux for 24 hours. The reaction was concentrated *in vacuo* and the residue was partitioned between dichloromethane and a saturated solution of ammonium chloride. The dichloromethane layer was washed again with ammonium chloride and then once with a saturated solution of NaCl, dried, and concentrated *in vacuo*. The crude material was eluted onto a Biotage 90 g silica gel column that had been pre-equilibrated with 3:1 hexane/ethyl acetate using the same solvent mixture. Fractions of pure product were combined and concentrated *in vacuo* to give 0.86 g (81%) of the title compound as a foam: mp 45°C dec. MS (APCI) (m+1)/z 421.2
Analysis calculated for C₂₀H₂₂F₂N₄SO₂: C, 57.13; H, 5.27; N, 13.32.
Found: C, 57.12; H, 5.08; N, 13.21.

25

8) Formation of Sulfoxides

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

A solution of 4.23 g (10.67 mmol) of 3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methylsulfanyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one in 90 mL of chloroform was treated with 3.35 g (12.80 mmol) of 3-phenyl-2-(phenylsulfonyl)-oxaziridine and stirred at room temp. under N₂ atmosphere overnight. Crude product was purified by medium-pressure chromatography eluting with straight ethyl acetate and a gradient of 1% to 3% MeOH in chloroform to give 4.45 g (quantitative) title compound. MS (APCI) (m+1)/z 413.1.

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Procedure H**General Procedure for the Synthesis of Sulfoxides****1-Cyclopropyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one**

5 0.64 g (1.57 mmol) of 1-cyclopropyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfanyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one in 20 mL of CHCl_3 was reacted with ($\pm$)-*trans*-2-benzenesulfonyl-3-phenyl-oxaziridine. The reaction was stirred overnight at room temperature and then concentrated *in vacuo*. The reaction was placed directly on a Biotage 90 g silica gel column. Chromatography, eluting with 9:1 ethyl acetate/ethanol, gave 0.66 g (99%) of the title compound as a solid: mp 198.5°C-199.5°C. MS (APCI) ($m+1$)/ z 425.0. Analysis calculated for $\text{C}_{18}\text{H}_{18}\text{F}_2\text{N}_4\text{SO}_4$: C, 50.94; H, 4.27; N, 13.20. Found: C, 51.13; H, 4.13; N, 12.98.

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

15 A solution of 15.53 g (0.0356 mol) of 1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfanyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one in 295 mL of chloroform was treated with 11.16 g (0.0427 mol) of 3-phenyl-2-(phenylsulfonyl)-oxaziridine and stirred at room temperature under nitrogen atmosphere overnight. The reaction mixture was evaporated *in vacuo* to approximately 30 mL of volume. The crude product was purified using medium-pressure chromatography eluting with a gradient of straight ethyl acetate to 9:0.5:0.25 ethyl acetate/methanol/triethylamine to give 15.54 g (96%) of the title compound: mp 167°C-169°C. MS (APCI) ($m+1$)/ z 453.1.

3-(3-Ethoxy-2,6-difluoro-phenyl)-1-(1-ethyl-propyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

25 Using procedure H, 0.82 g (1.95 mmol) of 1-Cyclopentyl-3-(3-ethoxy-2,6-difluoro-phenyl)-7-methylsulfanyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one in 15 mL of CHCl_3 was reacted with ($\pm$)-*trans*-2-benzenesulfonyl-3-phenyl-oxaziridine. In this instance the reaction was run for 6 hours and the crude residue was placed directly on a Biotage 90 g silica gel column. Chromatography, eluting with ethyl acetate, gave 0.79 g (94%) of the title compound as a solid: mp 57°C dec. MS (APCI) ($m+1$)/ z 437.1 Analysis calculated for $\text{C}_{20}\text{H}_{22}\text{F}_2\text{N}_4\text{SO}_3$: C, 55.04; H, 5.08; N, 12.84. Found: C, 54.91; H, 5.10; N, 12.54.

10) Synthesis of amine side chains**(4S, 5R)-2,2,5-Trimethyl-[1,3]dioxolane-4-carboxylic acid benzylamide**

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A flask was charged with 70 mL dry tetrahydrofuran, 3.17 g (17.7 mmol) (4S,5R)-2,2,5-trimethyl[1,3]dioxolane-4-carbonyl chloride, and the solution was cooled in a 0°C bath. To this solution, 3.48 mL (31.9 mmol) benzyl amine was slowly added, and a precipitate formed immediately. The reaction mixture was stirred for 1 hour at 0°C and 1 hour at room temperature. The reaction mixture was filtered through Celite to remove salts, and the tetrahydrofuran was removed under reduced pressure giving 4.41 g (100%) of the title compound as a yellow oil. No further purification was necessary. MS (APCI) (m+1)/z 250.1.

(2S,3R)-N-Benzyl-2,3-dihydroxy-butamide

A flask was charged with 4.23 g (17.0 mmol) (4S,5R)-2,2,5-trimethyl[1,3]dioxolane-4-carboxylic acid benzylamide, 30 mL acetonitrile, and 17 mL 0.5 M aqueous hydrochloric acid solution. This mixture was stirred overnight at room temperature. The solution was made neutral by the addition of 1 M aqueous sodium hydroxide solution. The solvent was removed under reduced pressure, and the crude residue was dried in a vacuum oven. The dry, crude material was purified by column chromatography (20:1 dichloromethane/methanol) to give 3.03 g (85%) of the title compound as a white solid. MS (APCI) (m+1)/z 210.1.

(2R,3R)-1-Benzylamino-butane-2,3-diol

A flask was charged with 3.03 g (14.5 mmol) (2S,3R)-N-benzyl-2,3-dihydroxy-butamide, 60 mL dry THF, and the mixture was cooled to 0°C under a nitrogen atmosphere. To this solution, 1.65 g (43.5 mmol) lithium aluminum hydride powder was added in small portions. The mixture was stirred at 0°C for 1 hour, allowed to warm to room temperature, and then heated at reflux overnight. The reaction mixture was then cooled to 0°C and quenched by the slow addition of 1.65 mL water, 1.65 mL 15% aqueous sodium hydroxide solution, and 4.95 mL water, sequentially. The solids were filtered off, and the solvent was removed under reduced pressure. The crude material was purified by column chromatography (1:1 dichloromethane/ methanol) to give 1.41 g (50%) of the title compound as a white solid. MS (APCI) (m+1)/z 196.1.

(2R,3R)-1-Amino-butane-2,3-diol

A pressure safe flask was charged with 1.41 g (7.22 mmol) (2R,3R)-1-benzylamino-butane-2,3-diol and 30 mL methanol and palladium on carbon catalyst. This solution was stirred overnight under an atmosphere of hydrogen. The catalyst was removed by filtration through Celite, and the solvent was removed under reduced pressure giving 0.726 g (96%) of the title compound as a yellow oil which crystallized on standing. MS (APCI) (m+1)/z 106.1.

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9) Formation of final products- displacement of sulfoxides and derivatives**Procedure J****General Procedure for Parallel Synthesis of 1-Substituted-7-[4-(2-diethylamino-ethoxy)-phenylamino]-3-(3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-ones**

Into an Argonaut Technologies' Quest 205 100 mL reactor was added the sulfoxide (20) prepared above. To the reaction was added 2 equivalents of 4-(2-diethylamino-ethoxy)-phenylamine dissolved in 30 mL of dry CH₃CN followed by 3 equivalents of trifluoroacetic acid. The reaction mixture was agitated at 65°C for 37 hours under a nitrogen atmosphere. The reaction was drained from the reaction vessel, evaporated, and redissolved in 50 mL of dichloromethane. The dichloromethane solution was added back into the reaction vessel followed by 30 mL of a saturated solution of sodium bicarbonate. The reaction was agitated and the dichloromethane removed. The dichloromethane solution was washed again with a saturated solution of sodium bicarbonate and then with a saturated solution of sodium chloride. The dichloromethane layer was dried with magnesium sulfate and concentrated *in vacuo*. The residue was suspended in 20 mL of diethyl ether. To the suspension was added a solution of 2 equivalents of triethylamine in 5 mL of diethyl ether followed by a solution of 1.1 equivalents of di-*tert*-butyl dicarbonate in 5 mL of diethyl ether. The reaction was agitated at room temperature for 4 hours. The reaction was cooled to 0°C and diluted with 30 mL of hexane. The suspension was filtered. The filter pad was washed with 1:1 diethyl ether/hexane, and dried at 45°C overnight *in vacuo* to give desired product.

Procedure K**General Procedure for the Reaction of Substituted Alkylamines with Sulfoxides**

To a sulfoxide prepared above was added a solution of 3 equivalents of amine in dry dioxane. The reaction was warmed to 75°C for 24 hours, then concentrated *in vacuo*. The residue was dissolved in dichloromethane. The dichloromethane solution was extracted twice with a saturated solution of sodium bicarbonate then once with a saturated solution of sodium chloride, dried with magnesium sulfate, and concentrated *in vacuo*. The crude product was chromatographed using a Biotage Quad 3 chromatographic apparatus with a 90 g silica gel column and the appropriate solvent systems. Pure fractions were combined and concentrated *in vacuo*. The residue was stirred with ether and concentrated *in vacuo* to give the desired product.

Procedure L**General Procedure for the Reaction of Substituted Alkylamines with Sulfoxides**

To a sulfoxide prepared above was added a solution of 3 equivalents of amine and 1.1 equivalents of camphorsulfonic acid in dry dioxane. The reaction was warmed to

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75°C for 24 hours, then concentrated *in vacuo*. The residue was dissolved in dichloromethane. The dichloromethane solution was extracted twice with a saturated solution of sodium bicarbonate then once with a saturated solution of sodium chloride, dried with magnesium sulfate, and concentrated *in vacuo*. The crude product was chromatographed using a Biotage Quad 3 chromatographic apparatus with a 90 g silica gel column and the appropriate solvent systems. Pure fractions were combined and concentrated *in vacuo*. The residue was stirred with ether and concentrated *in vacuo* to give the desired product.

This invention also provides pharmaceutical formulations comprising a compound of Formula I together with a pharmaceutically acceptable carrier, diluent, or excipient therefore.

Liquid form preparations include solutions, suspensions, and emulsions such as water or water/propylene glycol solutions. For parenteral injection into cartilaginous tissues, liquid preparations can be formulated in solution in aqueous polyethylene glycol solution, isotonic saline, 5% aqueous glucose, lactated Ringer's solution and the like. Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavors, and stabilizing and thickening agents as desired. Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water and mixing with a viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, or other well-known suspending agents.

The pharmaceutical preparations of the invention are preferably in unit dosage form. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation.

A pharmaceutically or therapeutically effective amount or dose of a compound of this invention for intraarticular administration to a joint will generally be from about 0.1 mg to about 10 mg per joint, such as a knee joint, shoulder joint, elbow joint, etc. In certain embodiments, the dose administered intra-articularly is from 0.001 to 0.1 mg/kg, or from 0.001 to 0.03 mg/kg, or from 0.01 to 0.03 mg/kg.

A pharmaceutically or therapeutically effective amount or dose of a compound of this invention is an amount that will provide inhibition of cartilage damage or degradation or stimulate new growth of damaged or degraded cartilage tissues. A pharmaceutically or therapeutically effective amount or dose of a compound of this invention will also be understood to be an amount sufficient to provide a preventative, inhibitory, ameliorating or diminishing effect on damage or degradation described herein, their symptoms or physiological origins.

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The kinase inhibiting compounds described herein can be administered neat to cartilage tissues or applied in formulations known in the art for direct or intraarticular administration to cartilaginous tissues. The compounds may also be administered into spaces or tissues adjacent the cartilage in question such that the compound will
5 encounter the cartilage in question without substantial relative systemic dispersal. Such administrations may include injection or other delivery of a pharmaceutically effective amount of a kinase inhibiting compound into the synovial fluid or bursa(e) associated with a joint.

The formulations may include pharmaceutically acceptable adjuvants, excipients
10 and carriers, including biodegradable polymers and co-polymers, buffers, solvents, surfactants, diluents, emulsifiers, emollients, hydrogels, hydrophilic agents, lipoproteins and other fatty acid derivatives, liposomes and other micelles, microporous membranes, mucoadhesives and biodegradable microspheres. For intraarticular injections, the compounds may be suspended or maintained in a solution of physiologically acceptable
15 aqueous media, such as buffered saline, Ringer's Injection, etc.

Useful formulations known in the art include the emulsion and micellar formulations described in U.S. Pat. Nos. 6,586,003 and 6,120,794 (both to Liu et al.), the polymeric systems described in U.S. Pat. Application 20020164374 (Jackson et al.), the porous drug matrices disclosed in U.S. Pat. Application 20020142050 and WO 0072827
20 (both to Straub et al.), and in soluble polymeric solutions, such as those described in U.S. Pat. No. 5,273,742 and WO 93/12802 (both to Gould et al.). It will be further understood that the methods and compounds herein may also be used in conjunction with other systemic or intraarticular relief or treatment methods used for related maladies. These include the intraarticular administration of morphine, dexamethasone, hyaluronic acid and
25 its salts and analogs, glucocorticoid and other corticosteroid treatments, injectable yttrium colloid suspension, etc.

The compounds of this invention may also be integrated into or onto physiologically acceptable matrices, polymers, gels, etc., for application to cartilage in need of repair or additional cartilaginous growth. Examples of such physiologically
30 acceptable carriers or materials known in the art include thermosensitive polysaccharide gels, such as chitosan-based polymer solutions, natural hydrogels, such as alginates, fibrin, collagen gels, etc., natural polymeric materials (such as devitalized cartilage matrix, collagen fibers, etc.), synthetic polymers (including permanent materials and those to be resorbed over time), biodegradable polymers, and polymers with adsorbed proteins or
35 immobilized functional groups.

Useful biodegradable polymers include polymethylmethacrylate, glycolic acid, polyglycolic acid (PGA), polylactic acid (PLA), poly-DL-lactic-co-glycolic acid (PLGA), copolymers of glycolic, polyglycolic and polylactic acids, polyhydroxyalkonates, poly(dioxanone), poly(trimethylene carbonate) copolymers, and poly(ϵ -caprolactone)

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homopolymers and copolymers, poly anhydrides and polyorthoesters. Combinations of physiologically acceptable polymers and natural materials may also be used, such as a combination of poly-DL-lactic-co-glycolic acid (PLGA) and collagen gel.

5 The physiologically acceptable materials described above may include extracellular matrix molecules of connective tissues, such as collagen, hyaluronan and glycosaminoglycans, or their physiologically acceptable synthetic equivalents.

10 Tyrosine kinase inhibiting agents may also be added to regiments and agents known in the art for promoting or assisting cartilage repair or replacement, including Carticel (autologous cultured chondrocytes), available from Genzyme Corporation, Cambridge, MA.

15 Compounds of this invention may be administered to a specified site over time as a dosed, regimented or continuous infusion using infusion pump technologies. Examples of useful infusion pumps known in the art include the 2000 Plus™ Ambulatory Infusion Pump, 4000 Plus™ Ambulatory Infusion Pump, and 4000 CMSTM (Clinical Management System) Multi-therapy Ambulatory Infusion System with Flexible Communication Capabilities devices manufactured by Curlin Medical, Huntington Beach, CA, the Rhythmic™ Volumetric continuous flow infusion pump and Rythmic™ Intermittent pump available from Micrel Medical Devices S.A., Athens, Greece, and the I-FlowVIP ambulatory infusion pump available from Progressive Motion, Santa Barbara, CA, the Baxter.

20 The compounds described herein may be administered through infusion in any biocompatible formulation or material compatible with the infusion technology in question. Examples of useful biocompatible materials include sterile physiologic saline, buffered Ringer's solution, etc.

25

Preparation of Parenteral Solution

30 In a solution of 700 mL of propylene glycol and 200 mL of water for injection is suspended 20.0 g of 1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(4-dimethylamino-butylamino)-1H-pyrimido[4,5-d]pyrimidin-2-one with stirring. After suspension is complete, the pH is adjusted to 5.5 with hydrochloric acid, and the volume is made up to 1000 mL with water for injection. The formulation is sterilized, filled into 5.0 mL ampoules, each containing 2.0 mL (representing 40 mg of invention compound) and sealed under nitrogen.

35 This invention will be further understood by the following non-limiting examples.

EXAMPLE 1

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

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To a solution of sodium ethylthiolate [generated from sodium hydride (200 mg, 8.32 mmol) and ethanethiol (616 μ L, 8.32 mmol) in dimethylformamide (20 mL)], 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one in dimethylformamide (8 mL) was added. The resulting solution was then stirred at 75°C for 90 minutes. The solvent was removed under reduced pressure to afford a light brown oil. Dichloromethane (100 mL) and water (20 mL) were added. The bilayer thus afforded was then stirred vigorously while 4 N hydrochloric acid was added. Enough hydrochloric acid was added until the aqueous layer reached pH = 5. The layers were separated; the aqueous layer was extracted with dichloromethane (2 $\times$ 80 mL). The organic layers were combined and set aside. Saturated aqueous sodium chloride (20 mL) was to the aqueous layers and was extracted with ethyl acetate (2 $\times$ 80 mL). The organic layers were combined, dried (magnesium sulfate), filtered, and concentrated under reduced pressure. To the yellow oil (794 mg) thus afforded, hexanes were added to remove non-polar impurities. The supernatant was removed, and the remaining residue was placed in vacuo. The oil was purified via reverse phase HPLC to give the title compound (289 mg, 54%) as a clear amber gum: MS (APCI) 519.2, 520.2.

EXAMPLE 2

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

In a 1-L round bottom flask, 8.00 g (17.78 mmol) of 1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one in 260 mL of dioxane was treated with 5.10 g (35.36 mmol) of 4-diethylamino-butylamine and heated at reflux for 10 hours under nitrogen atmosphere. The dioxane was evaporated *in vacuo*. The crude product was purified using medium-pressure chromatography eluting with a gradient of 9:0.5:0.25 to 9:1:0.5 ethyl acetate/methanol/triethylamine to give 8.14 g (86%) of the title compound as a pure partially crystalline solid. A 50 mg portion of the solid was recrystallized with 25% ethanol in hexanes to give 35 mg of title compound as pure crystalline solid: mp 132°C-133°C. MS (APCI) (m+1)/z 533.3.

EXAMPLE 3

(S,S)-1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[(5-hydroxymethyl-2-phenyl-[1,3]dioxolan-4-ylmethyl)-amino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

(S,S)-1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[(5-hydroxymethyl-2-phenyl-[1,3]dioxolan-4-ylmethyl)-amino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as in Example 3 using 0.5 g (1.1 mmol) 1-Cyclopentyl-3-(2-fluoro-3,5-

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dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.69 g (3.3 mmol) (S,S)-(5-Aminomethyl-2-phenyl [1,3]dioxolan-4-yl)-methanol. The product was purified by column chromatograph via 5:1 dichloromethane/methanol. This gave 0.59 g (89%) of (S,S)-1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[(5-hydroxymethyl-2-phenyl-[1,3]dioxolan-4-ylmethyl)-amino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one as a white solid: MS (APCI) m/z 598.2, 599.3.

EXAMPLE 4

(S,S)-1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3,4-trihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

(S,S)-1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[(5-hydroxymethyl-2-phenyl-[1,3]dioxolan-4-ylmethyl)-amino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one (0.57 g, 0.95 mmol) was dissolved in 90 mL methanol containing 12 mL 10% HCl acid. The mixture was stirred at room temperature for 9 hours. Adjusted pH = 12 by adding 1 M NaOH. Stripped the solvent. The residue was dissolved in methanol. Insoluble NaCl was removed by filtration. The filtrate was evaporated to dryness and the residue was purified by column chromatography (5:1 dichloromethane/methanol) to give 0.275 g (57%) (S,S)-1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3,4-trihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one as white solid: mp = 160°C (dec.); MS (APCI) m/z 510.1, 511.1.

EXAMPLE 5

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

To a solution of 0.511 g (1.13 mmol) of 1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was added 0.39 g (3.39 mmol) of *trans*-4-aminocyclohexanol. The resulting mixture was heated at 110°C oil bath temperature for 2 days under N₂ atmosphere. The dioxane was evaporated. The crude product was purified by medium-pressure chromatography eluting with 20:1 dichloromethane/methanol to give 0.50 g (88%) of title compound: mp 208°C-212°C (dec). MS (APCI) (m+1)/z 504.2.

EXAMPLE 6

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as described in Example 5 using 0.50 g (1.11 mmol) of 1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and

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0.30 g (3.32 mmol) of serinol. The crude product was purified using medium-pressure chromatography eluting with 20:1 dichloromethane/methanol to give 0.38 g (72%) of the title compound: mp 172°C-174°C. MS (APCI) (m+1)/z 480.1.

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EXAMPLE 7

7-(3-Amino-2-hydroxy-propylamino)-1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

To a solution of 0.50 g (1.11 mmol) of 1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was added 0.31 g (3.32 mmol) of 1,3-diamino-2-hydroxypropane. The resulting mixture was heated at an oil bath temperature of 95°C for 18 hours under nitrogen atmosphere. The dioxane was evaporated. The crude product was purified using medium-pressure chromatography eluting with 30:6:1 dichloromethane/ methanol/triethylamine to give 0.273 g (52%) of the title compound: mp 167°C (dec). MS (APCI) (m+1)/z 479.2.

EXAMPLE 8

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as described in Example 7 using 0.50 g (1.11 mmol) of 1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.33 mL (3.32 mmol) of 2-(2-aminoethoxy)ethanol. The crude product was purified using medium-pressure chromatography eluting with 20:1 dichloromethane/methanol to give 0.50 g (92%) of the title compound: mp 160°C-163°C. MS (APCI) (m+1)/z 494.3.

EXAMPLE 9

1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

Using Procedure K, 0.63 g (1.48 mmol) of 1-cyclopropyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 4-(diethylamino)butylamine in 20 mL of dioxane were reacted. Chromatography with 9:1:0.5 ethyl acetate/ethanol/triethylamine gave 0.62 g (83%) of the title compound as a solid: mp 139°C-141°C.

MS (APCI) (m+1)/z 505.2. Analysis calculated for C₂₅H₃₄N₆F₂O₃:
C, 59.51; H, 6.79; N, 16.66. Found: C, 59.56; H, 6.77; N, 16.49.

EXAMPLE 10

7-(4-Diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

7-(4-Diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as described in Example 3 using 0.46 g (1.11 mmol) of 3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methylsulfinyl-3,4-

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dihydro-1*H*-pyrimido[4,5-*d*]pyrimidin-2-one and 0.32 g (2.22 mmol) of 4-diethylamino-butylamine. The crude product was purified using a gradient of 9:1 ethyl acetate/methanol to 9:0.25:0.25 to 9:0.5:0.25 ethyl acetate/methanol/triethylamine to give 0.4833 g (83%) of the title compound: mp 140°C-141°C. MS (APCI) (*m*+1)/*z* 493.2.

5

EXAMPLE 11

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1*H*-pyrimido[4,5-*d*]pyrimidin-2-one

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1*H*-pyrimido[4,5-*d*]pyrimidin-2-one was prepared as described in Example 5 using 0.5 g (1.21 mmol) of 3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methylsulfinyl-3,4-dihydro-1*H*-pyrimido[4,5-*d*]pyrimidin-2-one and 0.33 g (3.64 mmol) of serinol. The crude product was purified using medium-pressure chromatography eluting with 20:1 dichloromethane/methanol to give 0.51 g (96%) of the title compound: mp 168°C-172°C. MS (APCI) (*m*+1)/*z* 440.1.

15

EXAMPLE 12

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1*H*-pyrimido[4,5-*d*]pyrimidin-2-one

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1*H*-pyrimido[4,5-*d*]pyrimidin-2-one was prepared as described in Example 5 using 0.5 g (1.21 mmol) of 3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methylsulfinyl-3,4-dihydro-1*H*-pyrimido[4,5-*d*]pyrimidin-2-one and 0.42 g (3.64 mmol) of *trans*-4-aminocyclohexanol. The crude product was purified using medium-pressure chromatography eluting with 20:1 dichloromethane/methanol to give 0.50 g (89%) of the title compound: mp 198°C-202°C. MS (APCI) (*m*+1)/*z* 464.1.

25

EXAMPLE 13

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-butylamino)-3,4-dihydro-1*H*-pyrimido[4,5-*d*]pyrimidin-2-one

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-butylamino)-3,4-dihydro-1*H*-pyrimido[4,5-*d*]pyrimidin-2-one was prepared as described in Example 7 using 0.49 g (1.08 mmol) of 1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-3,4-dihydro-1*H*-pyrimido[4,5-*d*]pyrimidin-2-one and 0.28 g (2.66 mmol) of (2*R*,3*R*)-1-amino-butane-2,3-diol. The crude product was purified using medium-pressure chromatography eluting with 20:1 dichloromethane/methanol to give 0.50 g (94%) of the title compound: mp 195°C-196°C (dec). MS (APCI) (*m*+1)/*z* 494.2.

30

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EXAMPLE 14

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

5 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as described in Example 7 using 0.50 g (1.11 mmol) of 1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.30 g (3.32 mmol) R-(+)-3-amino-1,2-propanediol. The crude product was purified using medium-pressure chromatography eluting with 9:1:0.5 ethyl
10 acetate/methanol/triethylamine to give 0.42 g (78%) of the title compound: mp 118°C-122°C. MS (APCI) (m+1)/z 480.2.

EXAMPLE 15

7-(4-Amino-2,3-dihydroxy-butylamino)-1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

15 7-(4-Amino-2,3-dihydroxy-butylamino)-1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as in Example 2 using 0.5 g (1.1 mmol) 1-cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.397 g (3.3 mmol) 1,4-Diamino-butane-2,3-diol. The final product was purified by column chromatography (40:6:1 dichloromethane/methanol/sat. aqueous ammonium hydroxide solution) giving
20 0.18 g (32%) of the final product: mp 158°C (dec.); MS (APCI) m/z 509.1, 510.1.

EXAMPLE 16

1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-([S,S]-2,3,4-trihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

25 1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-([S,S]-2,3,4-trihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as in Example 2 using 0.40 g (0.92 mmol) 1-cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.33 g (2.76 mmol) (S,S)-4-amino-1,2,3-butanetriol. The final product was purified by column chromatography (20:1 dichloromethane/methanol) to get 0.05 g (11%) as a white solid: mp 86°C-88°C; MS (APCI) m/z 492.2, 493.2.
30

EXAMPLE 17

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

35 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as described in

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Example 2 using 0.50 g (1.21 mmol) of 3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.36 mL (3.64 mmol) of 2-(2-aminoethoxy)ethanol. The crude product was purified using medium-pressure chromatography eluting with 20:1 dichloromethane/methanol to give 0.50 g (91%) of the title compound: mp 159°C-160°C. MS (APCI) (m+1)/z 454.1.

EXAMPLE 18

7-(4-Amino-2,3-dihydroxy-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

7-(4-Amino-2,3-dihydroxy-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as in Example 2 using 0.5 g (1.2 mmol) 3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.433 g (3.6 mmol) (S,S)-1,4-diaminobutane-2,3-diol. The final product was purified by column chromatography (40:6:1 dichloromethane/methanol/sat. aqueous ammonium hydroxide solution) which gave 0.24 g (43%) of the final product as a white solid: mp 115°C (dec.); MS (APCI) m/z 469.1, 470.2.

EXAMPLE 19

7-{3-[Bis-(2-hydroxy-ethyl)-amino]-propylamino}-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared using 0.50 g (1.21 mmol) of 3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.59 g (3.64 mmol) of N-(3-amino-propyl)diethanolamine. The crude product was purified using medium-pressure chromatography eluting with a gradient of 9:1 ethyl acetate/methanol to 9:1:0.25 to 9:1:0.5 ethyl acetate/methanol/triethylamine to give 0.43 g (69%) of the title compound: mp 128°C-129°C. MS (APCI) (m+1)/z 511.2.

EXAMPLE 20

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as described in Example 2 using 1.00 g (2.21 mmol) of 1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.60 g (6.63 mmol) of S-(-)-3-amino-1,2-propanediol. The crude product was purified using medium-pressure chromatography eluting with 9:1:0.5 ethyl

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acetate/methanol/triethylamine to give 0.98 g (92%) of the title compound: mp 179°C-182°C. MS (APCI) (m+1)/z 480.2.

EXAMPLE 21

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-butylamino)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-butylamino)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as described in Example 7 using 0.40 g (0.97 mmol) of 3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.245 g (2.33 mmol) of (2R,3R)-1-amino-butane-2,3-diol. The crude product was purified using medium-pressure chromatography eluting with 20:1 dichloromethane/methanol to give 0.40 g (91%) of title compound: mp 171°C-173°C. MS (APCI) (m+1)/z 454.2.

EXAMPLE 22

Ethyl-4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylate

The title compound was prepared as follows:

a) 5-[2,6-Difluoro-3,5-dimethoxy-phenylamino)-methyl]-2-methylsulfonyl-pyrimidin-4-ylamine

A mixture of 4-Amino-2-methylsulfonyl-pyrimidine-5-carbaldehyde (2.45 g, 14.5 mmol), 2,6-difluoro-3,5-dimethoxyaniline (2.75 g, 14.5 mmol), and camphorsulfonic acid (1.01 g, 4.35 mmol) in toluene (80 mL) was heated at reflux for 18 hours (note: the flask was equipped with a Dean-Stark trap). The solvent was removed under reduced pressure to afford a yellow solid. The crude product was sufficiently pure, so it was taken on to the next step. To a suspension of the crude imine (~4.93 g) in tetrahydrofuran (60 mL) at 0°C, lithium aluminum hydride (820 mg, 21.6 mmol) was added in several portions. Caution, gas evolution! The resulting yellow suspension was then stirred at 0°C for 1 hour, whereupon a green solution was afforded. Water (1 mL), 15% aqueous sodium hydroxide (1 mL), and water (3 mL) were added sequentially. Care is advised during this quenching protocol. The resulting mixture was then stirred at 0°C for 10 minutes. The suspension was then filtered over a pad of Celite, and the Celite was rinsed with dichloromethane. The combined filtrates were concentrated under reduced to give a thick oil. Recrystallization was accomplished with a solvent mixture of dichloromethane:hexanes:methanol (17:8.5:1, 312 mL) to give 1.85 g of the desired aniline. The mother liquor was concentrated and recrystallized with the same solvent mixture to give an additional 0.48 g of product. The second mother liquor was concentrated and recrystallized to afford 0.84 g. The precipitates were combined to give 3.17 g (64%, for 2 steps) of the title compound: MS (APCI) 343.1, 344.1.

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b) 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylsulfanyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

To a 0°C suspension of sodium hydride [861 mg (1.44 g of 60%), 35.9 mmol] in dimethylformamide (20 mL) was added 5-[2,6-Difluoro-3,5-dimethoxy-phenylamino)-methyl]-2-methylsulfanyl-pyrimidin-4-ylamine (3.07 g, 8.97 mmol) in dimethyl-formamide (24 mL). The resulting heterogeneous mixture was stirred at 0°C for 5 minutes and then at room temperature for 90 minutes. The mixture was cooled to 0°C and carbonyldiimidazole (5.09 g, 31.4 mmol) in dimethylformamide (15 mL) was added cautiously (gas evolution!). The resulting orange solution was stirred at room temperature for 3 days. Saturated aqueous ammonium chloride (10 mL) was added to the orange solution at 0°C, and the mixture was concentrated under reduced pressure. The suspension thus afforded was partitioned between dichloromethane (250 mL) and saturated ammonium chloride (50 mL). The layers were separated; the organic layer was washed with water (3 × 50 mL), dried (magnesium sulfate), filtered, and concentrated under reduced pressure to give a yellow solid. The solid was then triturated with a mixture of diethyl ether:dichloromethane (4:1, 50 mL) and then filtered to afford 2.35 g (71%) of title compound as an off-white solid: MS (APCI) 369.0, 370.1.

c) Ethyl-4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfanyl-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylate

To a 250-mL dry, round-bottomed flask was added 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylsulfanyl-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidine (1.5 g, 4.08 mmol), ethyl-4-hydroxycyclohexanecarboxylate (2.11 g, 12.23 mmol), triphenylphosphine (3.21 g, 12.23 mmol), and tetrahydrofuran (50 mL), and the solution was blanketed with a nitrogen atmosphere. The solution was then cooled to 0°C, and diisopropylazodicarboxylate (2.41 mL, 12.23 mmol) was added dropwise. The solution was then warmed to room temperature and stirred overnight. The crude mixture was then concentrated *in vacuo* and taken up in dichloromethane, washed with water, dried over sodium sulfate, and concentrated. The crude was purified by flash silica chromatography eluting with 3:2 hexane/ethyl acetate to yield the title compound (1.17 g, 55% yield): LRMS: 523.2 (M + H).

d) Ethyl-4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylate

To a solution of Ethyl-4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfanyl-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylate (1.17 g, 2.24 mmol) in chloroform (15 mL) was added *trans*-2-(phenylsulfonyl)-3-phenyloxaziridine (644 mg, 2.47 mmol). The solution was stirred at room temperature for 16 hrs, then concentrated *in vacuo*. The crude material was purified by flash silica chromatography

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eluting with 9:1 ethyl acetate/ethanol to give title compound (820 mg, 68% yield): LRMS: 539.1 (M + 1).

e) Ethyl-4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylate

5 To a 100-mL round-bottomed flask was added Ethyl-4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylate (820 mg, 1.52 mmol), dioxane (60 mL), then a 2 M solution of methylamine in tetrahydrofuran (7.6 mL, 15.2 mmol), and the solution was heated to 60°C under an atmosphere of nitrogen for 5 hours. The solution was then concentrated
10 *in vacuo*, and reacted further without purification: LRMS: 506.2 (M + H).

EXAMPLE 23

4-[3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid

15 To a 250-mL round-bottomed flask was added Ethyl-4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylate (crude 1.5 mmol), methanol (70 mL), water (20 mL), and sodium hydroxide (600 mg, 15 mmol), and the mixture was heated to 50°C for 3 days. The crude
20 reaction solution was then concentrated to less than 10 mL *in vacuo*, and then the volume was increased to 50 mL with water. The pH was then adjusted to 4 with 6 M hydrochloric acid, and the resulting white precipitate was collected by filtration and dried *in vacuo* overnight to deliver the title compound (460 mg, 63% yield for 2 steps) as a white solid: mp 148°C, LRMS: 478.2 (M + H).

EXAMPLE 24

25 **7-Amino-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; compound with trifluoroacetic acid**

The title compound was prepared as follows:

a) 4-[3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid *tert*-butyl ester

30 To a suspension of 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one (2.24 g, 6.08 mmol), *tert*-Butyl-4-hydroxy-1-piperidinylcarboxylate (3.66 g, 18.2 mmol), and triphenylphosphine (4.77 g, 18.2 mmol) in tetrahydrofuran (50 mL) at 0°C, diethyl azodicarboxylate (3.17 g, 18.2 mmol) was added dropwise. The resulting yellow suspension was then stirred at room temperature. Within
35 1 hour a yellow solution was afforded which was stirred at room temperature for 40 hours. The solvent was removed under reduced pressure to give a thick yellow oil (8.96 g).

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Attempts to purify the desired product from triphenylphosphine oxide and the reduced diethyl azodicarboxylate reagent failed, so the crude product was taken to the next step [note: by ^1H NMR the thick oil consisted of reduced diethyl azodicarboxylate:triphenylphosphine oxide:desired Mitsunobu product (3.2:2.58:1)]. The crude product (~2.73 g, based on ^1H NMR integration) was dissolved in chloroform (65 mL), and the solution thus afforded was cooled to 0°C , and Davis' oxaziridine (1.55 g, 5.94 mmol) was added in several portions. The solution was then stirred at room temperature for 20 hours; mass spec showed starting material, so an additional portion of the oxaziridine (430 mg) was added, and stirring of the reaction continued for 4 additional hours. The solution was concentrated under reduced pressure to a volume of ~15 mL. The sample was then purified using flash chromatography eluting with ethyl acetate and then dichloromethane/methanol (40:1). 2.93 g of a white solid were afforded. By ^1H NMR the solid was a mixture of desired product/reduced diethyl azodicarboxylate/triphenylphosphine oxide (14:2.9:1). The solid was repurified using flash chromatography method first eluting with ethyl acetate and then dichloromethane/methanol (50:1) to afford 2.02 g (59% for two steps) of the title compound as a white foam: MS (APCI) 468.2, 469.2.

b) 4-[7-Amino-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid *tert*-butyl ester

4-[3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylsulfinyl-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid *tert*-butyl ester (419 mg, 0.738 mmol) and liquid ammonia (20 mL) in dioxane (20 mL) were stirred in a sealed tube at room temperature for 24 hours. The solvent was removed under reduced pressure. The resulting residue was purified via flash chromatography eluting with a gradient of ethyl acetate:hexanes (2:1 to 5:1) to give 312 mg (81%) of the title compound: MS (APCI) 421.1, 465.2, 521.2.

c) 7-Amino-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; compound with trifluoroacetic acid

To a solution of 4-[7-Amino-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid *tert*-butyl ester (295 mg, 0.567 mmol) in dichloromethane (5 mL) at 0°C trifluoroacetic acid (647 μL) was added. The resulting solution was stirred at room temperature for 5 hours. The solution was then concentrated under reduced pressure. The resulting residue was concentrated with toluene (2×50 mL) to give a thick oil. Dichloromethane (2 mL) was added to the oil to produce a white precipitate. To accelerate precipitation diethyl ether (10 mL) was added. The mixture was then filtered to deliver 338 mg (92%) of the title compound as a white powder: mp 200°C - 220°C (decomposition); MS (APCI) 421.1, 422.2.

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EXAMPLE 25

1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

5 1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as in Example 2 using 0.4 g (0.92 mmol) 1-cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.25 g (2.76 mmol) serinol. The final product was purified by column chromatography (20:1 dichloromethane/methanol). This gave 0.29 g (68%) of 1-cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one as a white solid: mp 194°C-195°C; MS (APCI) m/z 462.2, 463.2.

EXAMPLE 26

1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

15 1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as in Example 2 using 0.4 g (1.01 mmol) 1-ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.27 g (3.03 mmol) 4-amino-butan-1-ol. The final product was purified by column chromatography (20:1 dichloromethane/methanol). This gave 0.375 g (88%) of 1-ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one as a white solid: mp 129°C-131°C; MS (APCI) m+1/z 420.

EXAMPLE 27

(S)-1-Cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

25 (S)-1-Cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as in Example 2 using 0.4 g (0.92 mmol) 1-cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.252 g (2.76 mmol) (S)-3-amino-1,2-propanediol. This gave 0.268 g (63%) of (S)-1-cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one as a white solid: mp 92°C-94°C; MS (APCI) m/z 462.2, 463.2.

EXAMPLE 28

1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

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A flask was charged with 0.312 g (0.79 mmol) of 1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one, 15 mL dioxane, and 0.273 g (2.37 mmol) of *trans*-4-Aminocyclohexanol, and the mixture was heated 48 hours at 95°C. The dioxane was removed under reduced pressure, and the crude residue was purified directly via column chromatography (20:1 dichloromethane/methanol). This procedure gave 0.215 g (66%) of 1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one as a white solid: mp = 198°C-204°C; MS (APCI) m+1/z 462.

EXAMPLE 29

4-[3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid *tert*-butyl ester

4-[3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid *tert*-butyl ester was synthesized in a same manner as 4-[3-(2,6-dichloro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid *tert*-butyl ester. However, 4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid *tert*-butyl ester (535 mg, 0.943 mmol) and 40% aqueous methylamine (407 μ L, 4.72 mmol) were used to afford the title compound (442 mg, 88%) as a white solid: MS (APCI) 435.1, 479.1, 535.2.

EXAMPLE 30

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; compound with trifluoroacetic acid

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; compound with trifluoroacetic acid was synthesized in the same manner as 3-(2,6-dichloro-3,5-dimethoxy-phenyl)-7-methylamino-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one, compound with trifluoroacetic acid; however, 4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid *tert*-butyl ester (411 mg, 0.187 mmol) and trifluoroacetic acid (878 μ L) were used to deliver the title compound (482 mg) as a white solid: HPLC = 88% pure; mp 135°C-140°C; MS (APCI) 435.1, 436.1.

EXAMPLE 31

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as described in Example 7 using 0.48 g (1.16 mmol) of 3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-methylsulfinyl-3,4-

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dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.32 g (3.49 mmol) of R-(+)-3-amino-1,2-propanediol. The crude product was purified by medium-pressure chromatography eluting with 20:1 dichloromethane/ methanol to give 0.346 g (68%) of title compound: mp 148°C-152°C. MS (APCI) (m+1)/z 440.1.

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EXAMPLE 32

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(3-ethoxy-2,6-difluoro-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

Using Procedure K, described above, 0.74 g (1.69 mmol) of 3-(3-ethoxy-2,6-difluoro-phenyl)-1-(1-ethyl-propyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 4-(diethylamino)butylamine in 20 mL of dioxane were reacted. Chromatography with 9:1:0.5 ethyl acetate/ethanol/ triethylamine gave 0.75 g (86%) of the title compound as a solid: mp 129°C-130°C. MS (APCI) (m+1)/z 512.47. Analysis calculated for C₂₇H₃₈N₆F₂O₂:

C, 62.77; H, 7.41; N, 16.27. Found: C, 62.79; H, 7.35; N, 16.10.

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EXAMPLE 33

(R)-1-Cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

R)-1-Cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as in Example 2 using 0.4 g (0.92 mmol) 1-cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.252 g (2.76 mmol) (R)-3-amino-1,2-propanediol. The final product was purified by column chromatography (20:1 dichloromethane/methanol). This gave 0.290 g (68%) of (R)-1-cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one as a white solid: mp 89°C-91°C; MS (APCI) m/z 462.2, 463.2.

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EXAMPLE 34

1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as in Example 2 using 0.350 g (0.887 mmol) 1-ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.267 mL (2.66 mmol) 2-(2-amino-ethoxy)-ethanol. The final product was purified by column chromatography (20:1 dichloromethane/methanol). This gave 0.331 g (86%) of 1-ethyl-3-(2-fluoro-3,5-

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dimethoxy-phenyl)-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one as a white solid: mp 143°C-146°C; MS (APCI) m+1/z 436.

EXAMPLE 35

1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(*trans*-4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one

1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(*trans*-4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one was prepared as in Example 2 using 0.5 g (1.15 mmol) 1-cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-methanesulfinyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one and 0.389 g (3.45 mmol) *trans*-4-hydroxy-cyclohexylamine. The final product was purified by column chromatography (20:1 dichloromethane/methanol). This gave 0.320 g (57%) of 1-cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(*trans*-4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one as a white solid: MP 108°C-110°C; MS (APCI) m/z 486.2, 487.2.

Additional non-limiting examples of pyrimido[4,5-d]pyrimidin-2-one compounds which may be utilized within the scope of this invention include:

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-
dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-
10 dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-
dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-
dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-
20 dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-hydroxy-5-methoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-methoxy-5-ethoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-
30 dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-
dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-
dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-hydroxy-5-methoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-diethylamino-butylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-
methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3,5-dimethoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-
dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-
dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-
dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-
dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-methoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-methoxy-5-ethoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopentyl-7-(4-hydroxy-cyclohexylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopropyl-7-(4-hydroxy-cyclohexylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Ethyl-7-(4-hydroxy-cyclohexylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-
methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3,5-dimethoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-methoxy-5-
ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-
ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-methoxy-
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-ethoxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-difluoro-3-hydroxy-
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-
methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3,5-dimethoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-methoxy-5-
ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-
ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-methoxy-
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-ethoxy-phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-difluoro-3-hydroxy-
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-methoxy
phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-
3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopentyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-dimethylamino-cyclohexylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopentyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-[5-(4-methyl-piperazin-1-yl)-pentylamino]-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 4-[-3-(2,6-Difluoro-3-hydroxy-5-methoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2,6-Difluoro-3,5-dimethoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 10 4-[-3-(2,6-Difluoro-3-methoxy-5-ethoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2,6-Difluoro-3-hydroxy-5-ethoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2,6-Difluoro-3-methoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 15 4-[-3-(2,6-Difluoro-3-ethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2,6-Difluoro-3-hydroxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2-Fluoro-3-hydroxy-5-methoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 20 4-[-3-(2-Fluoro-3,5-dimethoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2-Fluoro-3-methoxy-5-ethoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 25 4-[-3-(2-Fluoro-3-hydroxy-5-ethoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2-Fluoro-3-methoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2-Fluoro-3-ethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 30 4-[-3-(2-Difluoro-3-hydroxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2-Fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 35 4-[-3-(2-Fluoro-6-chloro-3,5-dimethoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2-Fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;

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- 4-[-3-(2-Fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2-Fluoro-6-chloro-3-methoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 5 4-[-3-(2-Fluoro-6-chloro-3-ethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 4-[-3-(2-Difluoro-6-chloro-3-hydroxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 10 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopentyl-7-(5-diisopropylamino-pentylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(5-diisopropylamino-pentylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Ethyl-7-(5-diisopropylamino-pentylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2,6-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-difluoro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopentyl-7-(4-amino-cyclohexylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopropyl-7-(4-amino-cyclohexylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3,5-dimethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-hydroxy-5-ethoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-methoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 30 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-fluoro-6-chloro-3-ethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; and
- 1-Ethyl-7-(4-amino-cyclohexylamino)-3-(2-difluoro-6-chloro-3-hydroxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 as well as the pharmaceutically acceptable salt and ester forms thereof.

Biological Examples

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Compound A is 6-(3,5-dimethoxy-phenyl)-8-ethyl-2-(pyridin-4-ylamino)-8H-pyrido[2,3-d]pyrimidine-7-one hydrochloride.

5 Compound B is 1-cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one.

Compound C is 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one trifluoroacetate.

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Biological Example 1

The following experiment demonstrates that intra-articular delivery of compounds of this invention, as represented by 6-(3,5-dimethoxy-phenyl)-8-ethyl-2-(pyridin-4-ylamino)-8H-pyrido[2,3-d]pyrimidine-7-one and 1-cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one, can be used for promoting repair and regeneration of the articular cartilage in the knee joint without inducing widespread mineralization of soft tissues.

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Materials and Methods

A rabbit model of chondral / subchondral defect (Baragi et al. (1997) *Osteoarthritis Cart.* 5 (4): 275-282) was used to evaluate the effects of intra-articular delivery of the FGFr antagonists 6-(3,5-dimethoxy-phenyl)-8-ethyl-2-(pyridin-4-ylamino)-8H-pyrido[2,3-d]pyrimidine-7-one (Compound A in Table 2) and 1-cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one (Compound B in Table 2).

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Surgery:

Using sterile technique, a medial parapatellar arthrotomy was performed, and the patella was luxated laterally. A sterilized steel drill bit and a dual torque low speed drill was used to create a 5 mm diameter defect in the articular cartilage of the trochlear groove. This defect was used to contain the test compound and the various delivery vehicles described below. The test compound-vehicle 'plug' was held in the defect by the patellar ligament. In some cases, periosteum removed from the medial aspect of tibia was used as graft patch. The patch was sutured on to the defect using 8-0 suture. The graft was then sealed with a fibrin-based glue (Tiseel VH Fibrin Sealant, available from Baxter Healthcare Corp.) to minimize leakage from the defect. The patella was repositioned to its original location within the trochlear groove. A three layer closure was be used to close the surgery site on the knee and tibia. After surgery, animals were returned to their cages and given food and water *ad libitum*. Throughout the study, animals appeared normal, with no detectable effects on appetite or movement.

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Drug Delivery:

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The test compounds were administered to the experimental animals either orally or intra-articularly (IA). Animals subjected to oral route of administration were gavaged daily with a uniform suspension of the various concentrations of the test compound in 0.5% carboxy methylcellulose aqueous suspension for the duration of the experiment.

5 Intraarticular delivery of test compounds was experimented using different delivery vehicles. In the first study, a collagen sponge the same diameter as the defect was soaked in a soluble collagen solution (Vitrogen[®] Collagen in Solution, available from Nutacon BV) containing the test compound and 'press fitted' into the articular cartilage defect. In the second study, animals had a collagen 'plug' made from thickened Vitrogen[®] Collagen in Solution and the test compound put into the defect. In the third study, 10 controlled-release formulations were tested for delivery of the test compounds. Compound B was formulated into a 7.8% solution containing 47% PLGA (Poly-lactide-co-glycolide copolymer). Compound A was formulated into a 5.8% solution containing 36% PLGA. The vehicle controls for these various groups used identically formulated solutions 15 without the compounds.

Plasma Drug Concentrations:

At sacrifice, animals were restrained and blood samples were taken for drug level quantification. Immediately afterwards, the animals were euthanized using Beuthanasia[®]-D Pentobarbital Sodium (Schering-Plough Animal Health, Kenilworth, NJ), and the 20 operated knee removed intact and placed into 10% NBF (Neutral, phosphate buffered 10% formalin, available from Anatech Ltd, Battle Creek, MI) in order to fix the tissues for histological analysis. The drug levels were measured using LC/MS/MS techniques.

Histology:

25 Tissue samples (stomach, heart, aorta and kidney) collected for assessing systemic toxicity of compounds were fixed in buffered neutral formalin and embedded in paraffin. Sections of tissue were cut and stained with H&E (hematoxylin & eosin) stain. Several soft tissue mineralization sections were also subjected to von Kossa staining method that is used for visualizing tissue mineralization.

Results:

30 IA administration of Compound A (see Table 1) reduced drug exposure and averted systemic toxicity that was observed in animals dosed orally. Moreover, IA delivery promoted chondrogenesis specifically in the joint of interest. More importantly, IA delivery, especially using the controlled-release formulations, was effective in inducing 35 chondrogenesis at significantly lower doses than those employed in the oral dosing paradigm (Table 2).

Conclusions:

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Based on the results of our studies, it can be concluded that kinase inhibitors when given intraarticularly can induce neochondrogenesis specifically in the cartilage tissues of a joint of interest without inducing systemic toxicity. Kinase inhibiting compounds may be used in promoting cartilage repair and regeneration at doses of less than 10 mg per localized administration. While the lowest effective pharmaceutically effective concentration may vary with the compound, it is recommended that for the purpose of cartilage repair and regeneration doses ranging between 0.1 to 10 mg per localized administration be given preferably using the controlled-release formulations such as the ones discussed herein. While the frequency and length of administration will depend on the formulations used and the extent of repair/regeneration needed, the frequency of IA drug application may be limited in some recipients to a maximum of one application every 4 weeks. Other delivery vehicles including use of osmotic pumps can also be employed. In order to avoid systemic toxicity, it is recommended that the use of these vehicles does not result in systemic kinase inhibitor blood levels of greater than 50 ng/ml.

Table 1

Dose	Route	Duration (weeks)	Exposure (ng/ml)	Toxicity
5	PO	2	50-100	+
5	IA	2	BLQ	-
40	PO	2	~3000	++
40	IA	2	~15	-
40	IA	4	~20	-

In Table 1, the single plus (+) in the Toxicity column represents evidence of increased chondrocyte proliferation in the adjacent growth plate, but no evidence of soft tissue mineralization. The double plus (++) indicates both evidence of growth plate chondrocyte proliferation and systemic soft tissue mineralization. The (-) sign in toxicity column indicates no apparent evidence of either soft tissue mineralization or growth plate chondrocyte proliferation. BLQ in Tables 1 and 2 indicates a concentration below a level of quantification, which was about 6.25 ng/ml.

Table 2. Effect of intraarticular administration of FGFr inhibitors on Chondrogenesis

Compound	Delivery vehicle	Dose (mg/kg)	Plasma level (ng/ml)	Chondrogenesis
A	PLGA	0.87	BLQ	++
A	Collagen sponge	5	BLQ	+
A	Collagen gel	40 (2 weeks)	15	++
A	Collagen gel	40 (4 weeks)	20	+++
B	PLGA	1.2	BLQ	++

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In Table 2, the single, double and triple plus indicators in the right column indicate relative amounts of chondrogenesis resulting from intraarticular administration of FGFr inhibitors. A single plus (+) represents evidence of increased chondrocyte proliferation at the site of administration, while double plus (++) and triple plus (+++) signs indicate clear and increasing evidence of neochondrogenesis following intraarticular administration of FGFr inhibitor.

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Biological Example 2

Protocol:

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The experiment began on day 0 using New Zealand Male Rabbits (2.5 kg) at doses of 5, 1, and 0.1 mg/kg/day delivered via pump intra-articularly into the knee joint. Alzet osmotic pumps (2ML1) were implanted subcutaneously, under sterile surgical conditions in the rabbit's left hindquarter. Compound B was dissolved in 1-methyl-2-pyrrolidinone (NMP) (25%) + propylene glycol (75%). The rabbits were sacrificed 7-days later. Plasma was collected in EDTA containing tubes (Vacutainer 7861, BD Biosciences) at Days 0, 3, 5 and 7 for drug level determination as well as determination of sodium and phosphorus levels. The knee joints were lavaged with saline (1.0ml) + Protease Inhibitor Cocktail (Complete, Roche Diagnostics) (1 tablet dissolved in 1 ml H₂O + 49 ml Saline). The rabbits' heart, stomach and kidneys were harvested for histology (H&E and von

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Kossa stained sections were examined by trained pathologist for signs of soft tissue mineralization).

Calcium and phosphorous were measured in rabbit serum using an Ortho Clinical Diagnostics Vitros 950 automated chemistry analyzer. Mean values for each dose group and time-point were compared to controls using a linear trend test within one factor analysis of variance at the 1% and 5% levels.

Compound B was extracted from plasma and knee lavage matrix via protein precipitation in a 96-well format. An eleven-point calibration curve was prepared using rabbit plasma or knee lavage in a range from 0.1 - 100 ng/mL. A 50 μ L aliquot of each sample was precipitated with 100 μ L of acetonitrile. Samples were vortexed for about 5 min, then centrifuged for 5 min at 4000 rpm. A 100 μ L aliquot of supernatant was transferred to a 96 well deep-well plate. A 2 μ L aliquot of each sample was injected onto the LC/MS/MS. Analysis was performed on a HTS PAL Leap auto sampler and Shimadzu LC-10 ADVP pump with an Applied Biosystems Sciex 4000 triple-quadrupole mass spectrometer using positive electrospray ionization in multiple reaction monitoring (MRM) mode. A MonoChrom 5 μ m CN, 2.0 x 50 cm analytical column was used for chromatographic separations provided by MetaChem.

Results:

Control (vehicle): animals exhibited a normal weight gain with no observable histological changes in any target tissue or knee joints.

5 mg/kg/day: animals experienced a 16% weight loss, along with tremors and a stiffened posture. Readily identifiable tissue mineralization was seen at necropsy in the heart, stomach and liver. Calcium levels were significantly decreased (7.1 ± 2.6 mg/dL) and Phosphorus levels were conversely increased (29.13 ± 7.87 mg/dL).

1 mg/kg/day: animals experienced a 19% weight loss, along with tremors and a stiffened posture and one animal was found dead on the 7th day. Readily identifiable tissue mineralization was seen at necropsy in the heart, stomach and liver. Calcium levels were significantly decreased (8.5 ± 2.5 mg/dL) and Phosphorus levels were conversely increased (18.7 ± 9.4 mg/dL).

0.1 mg/kg/day: animals experienced normal weight gain and no discernable clinical toxicity. Animals were devoid of tissue mineralization at the time of necropsy in the heart, stomach and liver. Calcium levels were in the normal range (5.0 ± 1.0 mg/dL) as were Phosphorus levels (13.7 ± 0.78 mg/dL).

The average blood levels of compound B are reported in Table 3:

Table 3

Blood levels of compound B (ng/ml)

Dose	0 hours	72 hours	120 hours	168 hours
0	Not measured	BLQ	Not measured	BLQ

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0.1	BLQ	BLQ	1.26 ± 1.26	BLQ
1	BLQ	4.47 ± 1.31	18.07 ± 3.29	15.6 ± 1.5
5	BLQ	4.33 ± 2.24	12.53 ± 0.83	55.63 ± 11.85

Biological Example 3

Protocol:

The study was begun on Day 0 using New Zealand Male Rabbits (2.5kg) at doses of 0.5, 0.3 and 0.1 mg/kg/day. Alzet osmotic pumps (2ML1) were implanted subcutaneously under sterile surgical conditions just above the rabbit's hindquarter. A PE 60 polypropylene catheter was sewn into the knee joint, run sub-dermal and attached to an Alzet (2ML1) osmotic pump. Compound B was dissolved in lactic acid (50mM), pH 4. Rabbits were sacrificed on Day 7 yielding experiment duration of 7-days. Plasma was collected at Days 0, 3, 5 and 7 for drug level determination as well as determination of sodium and phosphorus levels. The knee joints were lavaged with saline (1.0ml) + Protease Inhibitor Cocktail (Complete, Roche Diagnostics) (1 tablet dissolved in 1ml H₂O + 49 ml Saline).

The rabbits' heart, stomach and kidneys were harvested for histology (H&E and von Kossa stained sections were examined by trained pathologist for signs of soft tissue mineralization).

Calcium and phosphorous were measured in rabbit serum using an Ortho Clinical Diagnostics Vitros 950 automated chemistry analyzer. Mean values for each dose group and time-point were compared to controls using a linear trend test within one factor analysis of variance at the 1% and 5% levels.

Compound B was extracted from plasma and knee lavage matrix via protein precipitation in a 96-well format. An eleven-point calibration curve was prepared using rabbit plasma or knee lavage in a range from 0.1 - 100 ng/mL. A 50 μ L aliquot of each sample was precipitated with 100 μ L of acetonitrile. Samples were vortexed for about 5 min, then centrifuged for 5 min at 4000 rpm. A 100 μ L aliquot of supernatant was transferred to a 96 well deep-well plate. A 2 μ L aliquot of each sample was injected onto the LC/MS/MS. Analysis was performed on a HTS PAL Leap auto sampler and Shimadzu LC-10 ADVP pump with an Applied Biosystems Sciex 4000 triple-quadrupole mass spectrometer using positive electrospray ionization in multiple reaction monitoring (MRM) mode. A MonoChrom 5um CN, 2.0 x 50 cm analytical column was used for chromatographic separations provided by MetaChem.

Results:

Control (vehicle): animals exhibited a slight weight loss (3%) with no observable histological changes in any target tissue or knee joints. Calcium levels were 12.7 ± 1.0 mg/dL and Phosphorus 5.7 ± 0.15 mg/dL.

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0.5 mg/kg/day: animals experienced a 5% weight loss. Moderate tissue mineralization was seen on histology sections of the heart, stomach and liver. Calcium levels were 12.5 ± 0.06 mg/dL and Phosphorus 6.9 ± 0.03 mg/dL.

0.3 mg/kg/day: animals experienced a 3% weight loss. Tissue mineralization was minimal in histology sections of the heart liver and moderate in stomach. Calcium levels were significantly decreased (7.4 ± 0.12 mg/dL) and Phosphorus levels were conversely increased (12.5 ± 0.07 mg/dL).

0.1mg/kg/day: animals experienced 3% weight loss. Animals showed no signs of tissue mineralization on histology sections of the heart, stomach and liver. Calcium levels were in the normal range (5.9 ± 1.5 mg/dL) as were Phosphorus levels (12.6 ± 0.15 mg/dL).

The lavage levels of compound B in individual animals is reported in Table 4 for the animals' right knee (compound B) and left knee (control). The high levels of compound were detected in some of the samples from control samples (left knee samples). This was due to contamination of control samples with compound B that had adhered to the analytical column used for chromatographic separations of samples from drug treated animals. ALQ refers to above a level of quantitation.

Table 4
Lavage levels of compound B (ng/ml)

Dose	Right Knee	Left Knee
0	3.88	4.38
	3.22	6.43
0.1	14.0	109
	9.38	18200
0.3	17.1	31700,
	16.7	72400
	11.0	ALQ
0.5	18.0	160000
	4.86	246000
	7.45	120000

Plasma concentrations for Compound B for the 0.1 mg/kg, 0.3 mg/kg, and 0.5 mg/kg samples at every time-point were below quantitation levels (which was approximately below 0.1 ng/ml).

Biological Example 4

Purpose:

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Biological Example 2 demonstrated that compound B induced tissue mineralization even at lower doses over a period of 7 days. In the 0.5 mg/kg/day dose-group, mineralization was mild in the heart and moderate in the kidney and lung tissues. On the other hand, the mineralization in the 0.3 mg/kg day dose-group was minimal in the heart and kidney and mild in the stomach. Biological Example 4 was designed to determine the threshold at which mineralization is persists over a 4-week period of time. Biological Example 4 employed intra-articular administration of Compound B directly into the rabbit knee joint. In order to maintain the same rate of delivery as previous studies have employed, pumps were replaced after a period of two weeks.

Protocol:

Biological Example 4 was begun on Day 0 using New Zealand Male Rabbits (2.5 -2.7 kg) at doses of 0.2, 0.1, 0.05 mg/kg/day, given intra-articularly via the use of Alzet (2ML1) pumps. Compound B was dissolved in lactic acid (50 mM), pH 3. Using sterile conditions a PE 60 polypropylene catheter was sewn into the left knee joint and run under the skin and attached to an Alzet (2ML1) osmotic pump implanted just above the rabbit's hindquarter. Rabbits were sacrificed on Day 28. Plasma was collected at Days 0, 7, 14, 21 and 28 for drug level determination as well as determination of sodium and phosphorus levels. The knee joints were lavaged with saline (1.0ml) + Protease Inhibitor Cocktail (Complete, Roche Diagnostics) (1 Tablet dissolved in 1ml H₂O + 49 ml Saline).

The rabbits' heart, stomach and kidneys were harvested for histology (H&E and von Kossa stained sections were examined by trained pathologist for signs of soft tissue mineralization).

Calcium and phosphorous were measured in rabbit serum using an Ortho Clinical Diagnostics Vitros 950 automated chemistry analyzer. Mean values for each dose group and time-point were compared to controls using a linear trend test within one factor analysis of variance at the 1% and 5% levels.

Compound B was extracted from plasma and knee lavage matrix via protein precipitation in a 96-well format. An eleven-point calibration curve was prepared using rabbit plasma or knee lavage in a range from 0.1 - 100 ng/mL. A 50 µL aliquot of each sample was precipitated with 100 µL of acetonitrile. Samples were vortexed for about 5 min, then centrifuged for 5 min at 4000 rpm. A 100µL aliquot of supernatant was transferred to a 96 well deep-well plate. A 2 µL aliquot of each sample was injected onto the LC/MS/MS. Analysis was performed on a HTS PAL Leap auto sampler and Shimadzu LC-10 ADVP pump with an Applied Biosystems Sciex 4000 triple-quadrupole mass spectrometer using positive electrospray ionization in multiple reaction monitoring (MRM) mode. A MonoChrom 5µm CN, 2.0 x 50 cm analytical column was used for chromatographic separations provided by MetaChem.

Results:

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Control (vehicle): animals experienced a normal weight gain over the course of the study with no observable histological changes in any target tissue or knee joints.

Calcium levels were 13.07 ± 0.37 mg/dL and phosphorus 6.20 ± 0.25 mg/dL.

0.2 mg/kg/day: animals experienced a normal weight gain. Moderate tissue mineralization was seen on histology sections of the heart, stomach and liver. Calcium levels were 12.65 ± 0.15 mg/dL and phosphorus 7.6 ± 0.14 mg/dL. Histological changes observed in the joint treated with the compound included moderate necrosis of articular cartilage, mild cartilage hyperplasia and minimal changes in the growth plate.

0.1 mg/kg/day: animals experienced a normal weight gain. Minimal tissue mineralization was seen on histology section of the heart, stomach and liver. Calcium levels were in the normal range (6.9 ± 0.10 mg/dL) and phosphorus levels were also in the normal range (12.75 ± 0.45 mg/dL). Histological changes observed in the joint treated with the compound included moderate necrosis of articular cartilage, mild cartilage hyperplasia and minimal changes in the growth plate.

0.05 mg/kg/day: animals experienced normal weight gain. Animals showed no signs of tissue mineralization on histology sections of the heart, stomach and liver. Calcium levels were in the normal range (5.63 ± 0.34 mg/dL) as were phosphorus levels (13.03 ± 0.12 mg/dL). Minimal hyperplasia of cartilage was the only histological change observed in the joint treated with the compound.

Table 5

Blood levels of compound B (ng/ml)

Dose	0 hours	168 hours	336 hours	504 hours	672 hours
0	BLQ	BLQ	BLQ	BLQ	BLQ
0.05	BLQ	0.47 ± 0.1	0.54 ± 0.02	0.45 ± 0.03	0.39 ± 0.03
0.1	BLQ	0.79 ± 0.04	1.03 ± 0.08	0.83 ± 0.13	0.67 ± 0.18
0.2	BLQ	1.36 ± 0.19	2.58 ± 0.29	1.69 ± 0.11	2.05 ± 0.11

The lavage levels of compound B in individual animals are reported in Table 6 for the animals' right knee (compound B) and left knee (control). The high levels of compound were detected in some of the samples from control samples (left knee samples). This was due to contamination of control samples with compound B that had adhered to the analytical column used for chromatographic separations of samples from drug treated animals. ALQ refers to above a level of quantitation.

Table 6

Lavage levels of compound B (ng/ml)

Dose	Right Knee	Left Knee
0	BLQ	BLQ
	1.79	BLQ

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	6.01	BLQ
0.05	ALQ	17800
	14.6	11700
	10.6	69.8
0.1	1.9	11800
	0.98	613
0.2	3.12	BLQ
	5.6	2130
	2.6	4230

Results:

No relevant changes in phosphorous and calcium were observed relative to control in animals receiving 0.05 mg/kg for up to one month. In animals receiving 0.1 mg/kg, changes in phosphorous observed within the first week of dosing were either similar to those in controls or were small in magnitude and transient in nature. Small increases in phosphorous (13% versus control), however, were eventually observed 3 weeks after the start of dosing. Animals receiving 0.2 mg/kg had slightly larger increases in phosphorous levels (9 – 27%) relative to control but were considered significantly different between 5 and 7 days after the start of dosing. Mean calcium values for animals receiving 0.1 and 0.2 mg/kg were not considered significantly different from control.

Relative to controls, significant increases in mean phosphorous levels (14 – 42%) were observed in animals receiving 0.3 mg/kg (Days 3 and 7) and 0.5 mg/kg (Days 3, 5 and 7). Although the increases in phosphorous observed at these doses were smaller than those observed at higher doses, all individual animal values exceeded reference range on Day 3 and remained elevated through Day 7. No significant changes in calcium were observed at these doses.

Larger increases in mean phosphorous levels were observed on Days 5 and 7 in animals receiving 1 mg/kg (2.8 – 2.9-fold versus controls) and 5 mg/kg (3.5 – 5-fold versus controls), with higher values observed on Day 7 in both dose groups. The magnitudes of increase in phosphorous levels observed at these doses were at least twice those seen at 0.5 mg/kg and were accompanied by 25 – 48% decreases in mean calcium levels. The magnitudes of calcium decreases were inversely proportional to the magnitude of increase in phosphorous, and were sufficient to result in individual animal values that fell below reference range on Day 5 and / or Day 7 in all animals at 1 mg/kg and 2 of 3 animals at 5 mg/kg.

Conclusion:

Administration of Compound B resulted in dose and time-related increases in serum phosphorous levels at doses ≥ 0.1 mg/kg. The increases in phosphorous between doses of 0.1 and 0.5 mg/kg did not result in significant changes in calcium levels, while those increases in serum phosphorous observed at ≥ 1 mg/kg resulted in statistically

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significant decreases in serum calcium that fell below historical range in the majority of animals.

Biological Example 5

In Vitro Studies: Effect of Compound A, Compound B, and Compound C on Chondrogenesis

Purpose:

To examine the potential of the fibroblast growth factor receptor inhibitors Compound A, Compound B, and Compound C to enhance the matrix production of chondrogenic cells in vitro. Specifically, to examine the glycosaminoglycan (GAG) accumulation within the matrix produced from the chondrogenesis of bone marrow cells by an in vitro pellet culture system. (Johnstone et al. (1998) Exp Cell Res. 238(1):265-272.)

Methods:

Bone marrow was harvested from the femurs of 3-8 months old New Zealand White Rabbits. The bone marrow was diluted in sterile phosphate buffered saline and the mononuclear cells were obtained after centrifugation in Histsopaque 1077 (Sigma H8889; St. Louis, MO), a 1.077 g/mL solution of polysucrose and sodium diatrizoate. The mononuclear cells were expanded by subculture in tissue culture plates with Dulbecco's Modified Eagle Media (DMEM) (Gibco 11885; Grand Island, NY), antibiotic/antimycotic (Gibco 15240), and 10% fetal bovine serum (Gibco 10082).

After expansion, the cells were differentiated in pellet culture. 250,000 cells in 1 mL media were centrifuged at 500 x g for 5 minutes in 15 mL polypropylene tubes. The media for pellet culture included DMEM (Gibco 11995), antibiotic/antimycotic (Gibco 15240), 100 nM dexamethasone (Sigma D2915), 100 µM ascorbic acid – 2 phosphate (Sigma A8960), ITS+ Premix (BD Biosciences 354352; Bedford, MA) (6.25 ug/mL Insulin, 6.25 µg/mL transferrin, 6.25 ng/ml selenous acid, 1.25 mg/ml bovine serum albumin, 5.35 µg/ml linoleic acid), and 10 ng/ml transforming growth factor – beta3 (R&D Systems 243-B3-010/CF; Minneapolis, MN). The pellet culture media was also supplemented with basic Fibroblast Growth Factor (FGF-2) (R&D Systems 233-FB-025/CF), Compound A, Compound B, or Compound C to determine the effect of stimulation or inhibition of FGF receptors. Cells were cultured for 14 days with media changed twice a week. Conditioned media was saved and frozen at –80°C. After 14 days, cell pellets were digested in buffer overnight at 65°C and then stored at –80°C for later analysis. Digestion buffer was composed of 100 mM sodium phosphate (MCB Manufacturing Chemists SX0710-1; Cincinnati, OH), pH = 7.0, 5 mM L-cysteine (Sigma C7755), 5 mM EDTA (Gibco 15575), and 125 µg/ml papain (Sigma P3125).

GAG accumulation was measured by 1,9- dimethylmethylene blue assay. Chondroitin sulfate (Sigma C4384) standards measured as µg/ml, pellet digest, and

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media samples were mixed at 1:20 in dye measured by absorbance at 525 nm. The dye was composed of 16mg/L 1,9-dimethylmethylene blue powder (Polysciences 3610; Warrington, PA), 4 g/L sodium formate (Sigma 247596), 0.25% ethanol (Sigma E7023), and 0.4% formic acid (Mallinckrodt 2592; Paris, KY) (see Farndale et al. (1986) *Biochim Biophys Acta.* 883(2):173-7.

DNA content of the pellet digest was measured by the fluorochrome Hoechst 33258 (Rio Rad 1702480; Hercules, CA) and compared to a standard of calf thymus DNA measured as $\mu\text{g/ml}$ (see Kim et al. (1988) *Anal Biochem.* 1988 Oct;174(1):168-76).

Results:

Histology and immunohistochemistry of bone marrow cells cultured in a pellet culture system displayed a phenotype that included chondrocyte like morphology, positive staining for proteoglycan and collagen II, and minimal collagen I staining at the periphery.

Increased doses of FGF-2 in the media of the chondrogenic pellet culture system resulted in decreased GAG accumulation per DNA within the pellet. Results from exogenous FGF-2 doses ranging from 1 to 100 ng/mL are displayed in Table 7.

Table 7

FGF-2 decreases GAG accumulation in vitro

FGF-2 (ng/mL)	Pellet Digest GAG/DNA % of Control	Standard Deviation
Control	100.0	12.5
1	89.0	23.0
5	64.6	11.1
25	24.3	2.3
100	11.1	3.8

Compound A, Compound B, and Compound C were able to increase GAG/DNA accumulation within this system. Compound A, Compound B, or Compound C was added to the pellet culture system at 100 nM. The GAG/DNA accumulation is compared to control in Table 8.

Table 8

Compound (100 nM)	Pellet Digest GAG/DNA % of Control	Standard Deviation
Control	100.0	15.7
Compound A	101.2	4.9
Compound B	111.8	21.3
Compound C	125.2	2.0

Biological Example 6

In Vivo Studies: Effect of intra-articularly delivered Compound B in a rabbit ACL/meniscetomy model of osteoarthritis

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Procedure:

Forty skeletally mature (about 7 months old) male NZW rabbits were used. Animals had their anterior cruciate ligament transected along with removal of about 70% of the medial meniscus in the left knee to induce experimental osteoarthritis. After 2 weeks, a catheter was implanted intra-articularly with one end in the operated knee capsule and the other end attached to an osmotic pump, which was inserted subcutaneously dorsal to the knee. These pumps contained either vehicle (lactic acid buffer) or Compound B in the vehicle, at a dose of 0.1 mg/kg/day or 0.01 mg/kg/day. These pumps (Alzet model 2ML4) delivered a volume of 2.5 μ l/hr for 4 weeks in the 3 treatment groups (n=10).

After 4 weeks, animals were sacrificed and blood and urine collected. The knees were injected with 1 ml of saline and this was removed and collected. The operated knee joint was then removed, disarticulated, scored and imaged. It was then stained with India ink and imaged again. The knee joints, stomach, and kidney were then placed in 10% formalin for histological processing. Plasma and synovial lavage samples were sent for drug concentration determination.

Results:

Gross and histological data indicated that compound B at both 0.1 and 0.01 mg/kg/day doses promoted exuberant growth of extra-articular cartilage. However, the compound did not reduce or worsen the cartilage lesions resulting from surgical destabilization of the joint. Since the concentrations of the compound in the joint even at the low dose were high (> 1000 ng/ml), and joint architecture was altered due to exuberant growth of extra-articular cartilage, it was decided that additional studies with lower concentration of drug in the joint were needed to further assess the efficacy of compound.

Biological Example 7**In Vivo Studies: Effect of intra-articularly delivered Compound B in a rabbit articular defect model.**

Procedure: Thirty skeletally mature (about 10 months old) male NZW rabbits were used in this study. A full-thickness, cartilage defect was created in the left femoral condyle of these animals using the following surgical procedure. Using sterile technique, a medial parapatellar arthrotomy was performed, and the patella was luxated laterally. A sterilized steel drill bit and a dual torque low speed drill was used to create a 5 mm diameter defect in the articular cartilage of the trochlear groove. This defect was used to contain a formulation of 47% PLGA with or without compound B. While the vehicle group received only the formulation, treatment groups received the PLGA formulation containing either about 0.56 mg or about 0.062 mg of the compound / kg of weight of the animal.

After 4 weeks, animals were sacrificed and blood and urine collected. The knees were injected with 1 ml of saline and this was removed and collected. The operated knee

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joint was then removed and placed in 10% formalin for histological processing. Plasma and synovial lavage samples were sent for drug concentration determination.

Results:

5 Histological data indicated that the compound was effective in promoting cartilage repair. Both the doses of the compound, 0.062 and 0.56 mg / kg, were effective
The effects appeared to be dose-dependent.

10 Vehicle control animals had deep defects with fibro-osseous proliferation and few foci of chondrocyte formation. In the low-dose group, the cartilage defects were covered by proliferating chondrocytes. At the base of the defect, chondrocyte and fibro-myxomatous proliferation were noted. New bone formation within subchondral bone of epiphysis was observed. In the high dose group, the cartilage defects were filled with spindle cells and fibrin within myxomatous matrix. New bone formation was also observed in these animals.

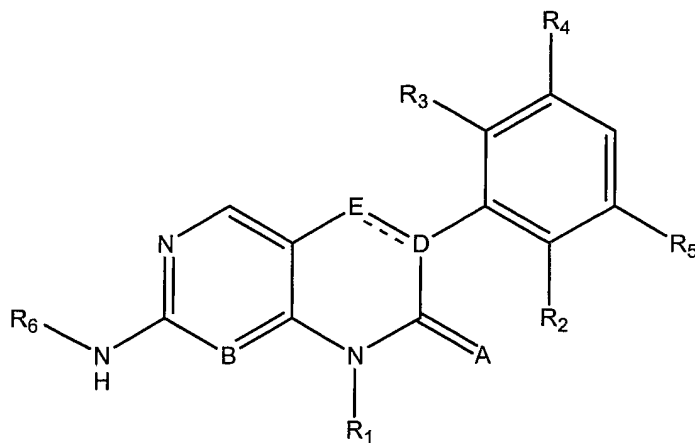
15 It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

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CLAIMS

What is claimed is:

1. A method of promoting neochondrogenesis in a cartilage in a mammal comprising administering locally to the cartilage in the mammal a pharmaceutically effective amount of a compound of formula I:



wherein:

A is O, NH₂, NH(C₁-C₆ alkyl), N(C₁-C₆ alkyl)₂, or -NHC(O)-NHR₁₂;

R₁₂ is C₁-C₆ straight or branched chain alkyl, or -(CH₂)_n-C₃-C₈

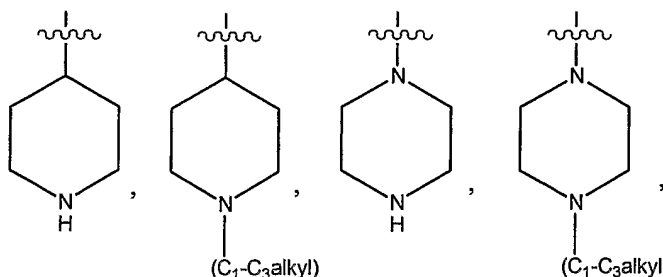
cycloalkyl ring; n is an integer of from 1 to 3;

B, D and E are independently selected from CH or N, with the proviso that E and D are not both N;

R₁ is selected from the group of C₁-C₆ straight or branched chain alkyl, optionally substituted by -COOH, or;

c) a phenyl, benzyl or C₃-C₈ cycloalkyl ring, or -CH₂-C₃-C₈ cycloalkyl ring, with the phenyl, benzyl or cycloalkyl rings being optionally substituted by 1 or 2 COOH or -CH₂-COOH groups; or

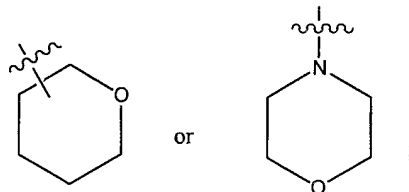
d) a piperidinyl or piperazinyl moiety selected from group of:



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the rings of the piperidinyl or piperazinyl moieties being optionally substituted by 1 or 2 COOH or -CH₂-COOH groups; or

c) a tetrahydropyranyl or morpholinyl moiety of the formulae:



5

R₂ is H, Cl or F;

R₃ is H, Cl or F, with the proviso that at least one of R₂ or R₃ is F;

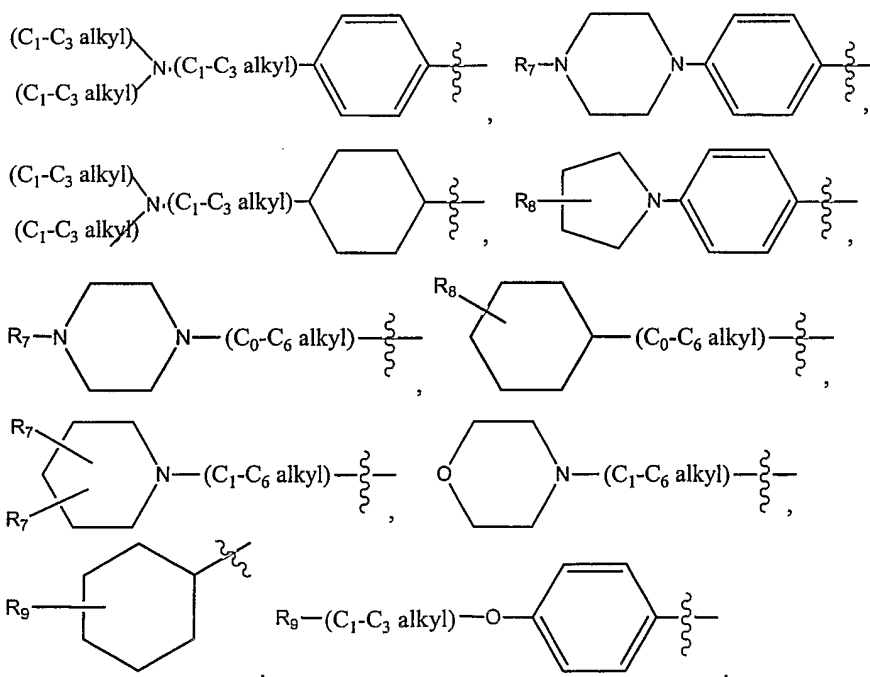
R₄ is H, OH, -OCH₃, or -OCH₂CH₃, with the proviso that, if R₄ is H, R₂ and R₃ are not H;

R₅ is -OCH₃, or -OCH₂CH₃;

10

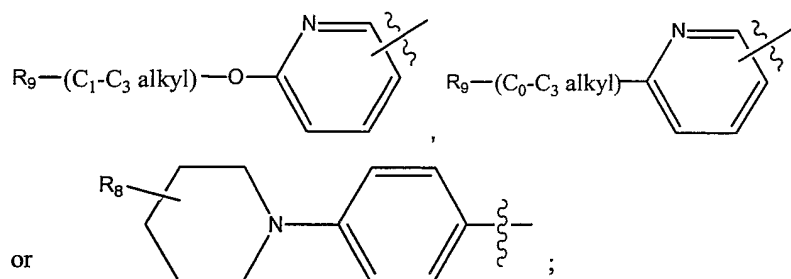
R₆ is selected from the group of H, -(C₁-C₅ alkyl)-NH₂, -(C₁-C₅ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₅ alkyl)-N-(C₁-C₃ alkyl)-R₁₁, -O-(C₁-C₅ alkyl)-NH₂, -O-(C₁-C₅ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -O-(C₁-C₅ alkyl)-N-(C₁-C₃ alkyl)-R₁₁, -CH(CH₂OH)₂, -(C₁-C₃ alkyl)-(CH₂OH)₂, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-NH₂, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-NH-(C₁-C₃ alkyl)-R₁₁, -(C₁-C₃ alkyl)-O-(C₁-C₃ alkyl)-N(C₁-C₃ alkyl)-R₁₁, phenyl substituted by one or two groups selected from NH₂, -N(C₁-C₃ alkyl), -N(C₁-C₃ alkyl)₂, CN or -(C₁-C₃ alkyl)-tetrazole, or C₁-C₆ alkyl,

15



20

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with each of the alkyl chains of any group in this R_4 definition being optionally substituted by from 1 to 4 OH groups;

5 R_7 in each instance is independently selected from H, $-NH_2$, $NH(C_1-C_3 \text{ alkyl})$, $N(C_1-C_3 \text{ alkyl})_2$, or $C_1-C_3 \text{ alkyl}$;

R_8 is H, OH or $C_1-C_3 \text{ alkyl}$;

9 H, OH, $-NH_2$, $NH(C_1-C_3 \text{ alkyl})$, or $N(C_1-C_3 \text{ alkyl})_2$;

R_{10} is H or $C_1-C_3 \text{ alkyl}$;

10 R_{11} is H, CN, OH, NH_2 , F, or CF_3 ;

or a pharmaceutically acceptable salt thereof.

2. The method of Claim 1 wherein the compound, or a pharmaceutically acceptable salt thereof, is selected from the group of:

15 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

20 (S,S)-1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[(5-hydroxymethyl-2-phenyl-[1,3]dioxolan-4-ylmethyl)-amino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

(S,S)-1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3,4-trihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

25 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

7-(3-Amino-2-hydroxy-propylamino)-1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

30 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; and

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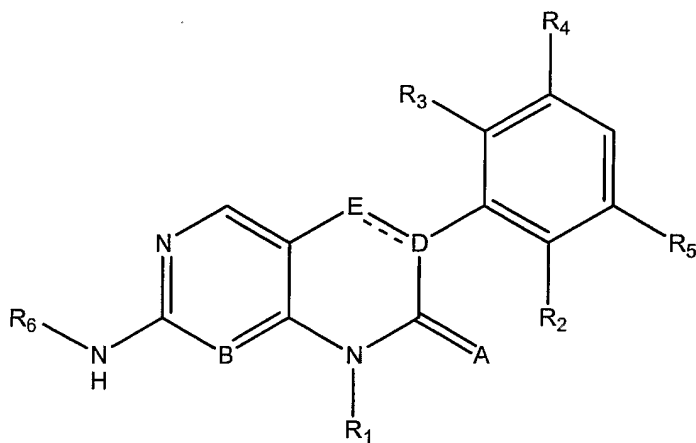
7-(4-Diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one.

3. The method of Claim 1 wherein the compound, or a pharmaceutically acceptable salt thereof, is selected from the group consisting of:
 - 5 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
 - 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
 - 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one
 - 10 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
 - 7-(4-Amino-2,3-dihydroxy-butylamino)-1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
 - 15 1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-([S,S]-2,3,4-trihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
 - 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
 - 7-(4-Amino-2,3-dihydroxy-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
 - 20 7-{3-[Bis-(2-hydroxy-ethyl)-amino]-propylamino}-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; and
 - 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one.
4. The method of Claim 1 wherein the compound, or a pharmaceutically acceptable salt thereof, is selected from the group consisting of:
 - 25 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-butylamino)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
 - Ethyl-4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylate;
 - 30 4-[3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
 - 7-Amino-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
 - 35 1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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- 1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- (S)-1-Cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 4-[3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid;
- 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(3-ethoxy-2,6-difluoro-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 (R)-1-Cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; and
- 1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(*trans*-4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one.
- 20
5. The method of claim 2, 3 or 4, wherein said mammal suffers from osteoarthritis.
6. The method of claim 2, 3, or 4, wherein said mammal suffers from cartilage degradation.
7. The method of claim 5, wherein said compound, or a pharmaceutically acceptable salt thereof, is administered intra-articularly.
- 25 8. The method of claim 7, wherein said compound, or a pharmaceutically acceptable salt thereof, is administered intra-articularly to a knee joint.
9. The method of claim 8, wherein said compound, or a pharmaceutically acceptable salt thereof, is administered at a dose from 0.001 to 0.1 mg/kg.
- 30 10. The use of a compound, or a pharmaceutically acceptable salt thereof, of formula I:

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wherein:

A is O, NH₂, NH(C₁-C₆ alkyl), N(C₁-C₆ alkyl)₂, or -NHC(O)-NHR₁₂;

R₁₂ is C₁-C₆ straight or branched chain alkyl, or -(CH₂)_n-C₃-C₈

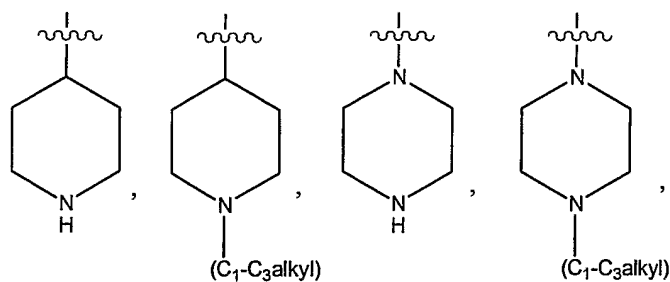
cycloalkyl ring; n is an integer of from 1 to 3;

B, D and E are independently selected from CH or N, with the proviso that E and D are not both N;

R₁ is selected from the group of C₁-C₆ straight or branched chain alkyl, optionally substituted by -COOH, or;

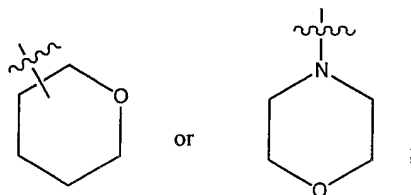
e) a phenyl, benzyl or C₃-C₈ cycloalkyl ring, or -CH₂-C₃-C₈ cycloalkyl ring, with the phenyl, benzyl or cycloalkyl rings being optionally substituted by 1 or 2 COOH or -CH₂-COOH groups; or

f) a piperidinyl or piperazinyl moiety selected from group of:



the rings of the piperidinyl or piperazinyl moieties being optionally substituted by 1 or 2 COOH or -CH₂-COOH groups; or

c) a tetrahydropyranyl or morpholinyl moiety of the formulae:



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 R_2 is H, Cl or F; R_3 is H, Cl or F, with the proviso that at least one of R_2 or R_3 is F; R_4 is H, OH, $-OCH_3$, or $-OCH_2CH_3$, with the proviso that, if R_4 is H, R_2 and R_3 are not H;

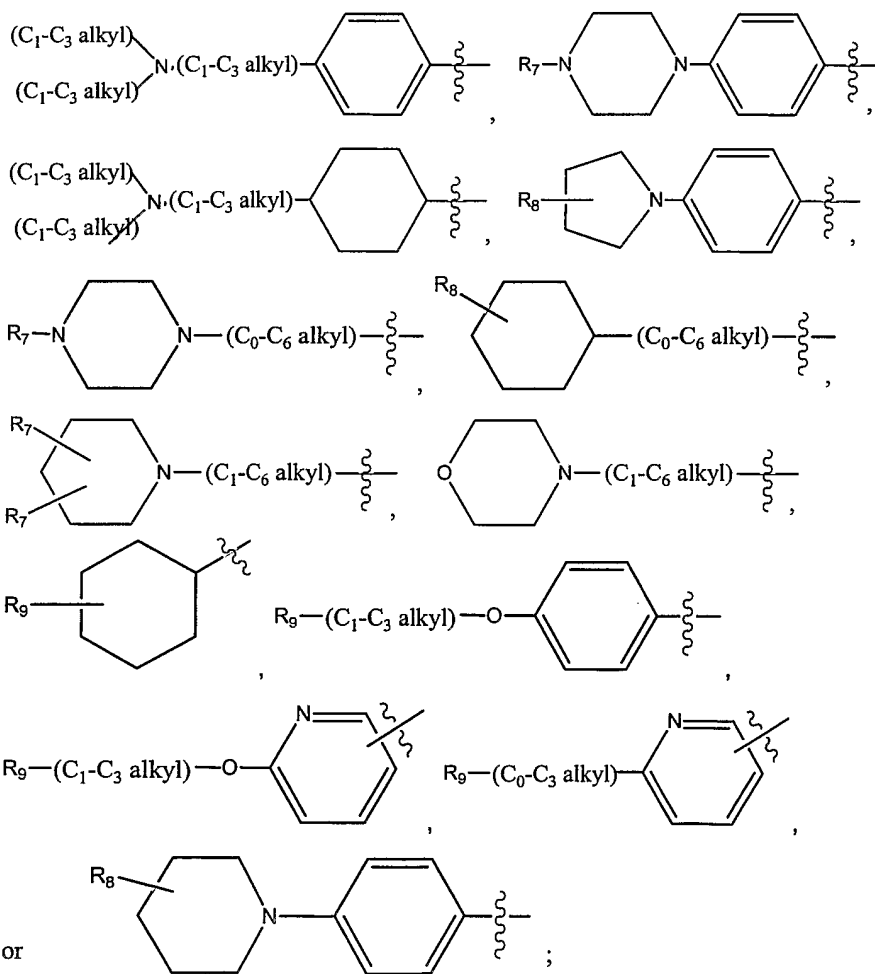
5

 R_5 is $-OCH_3$, or $-OCH_2CH_3$;

10

R_6 is selected from the group of H, $-(C_1-C_5 \text{ alkyl})-NH_2$, $-(C_1-C_5 \text{ alkyl})-NH-(C_1-C_3 \text{ alkyl})-R_{11}$, $-(C_1-C_5 \text{ alkyl})-N-(C_1-C_3 \text{ alkyl})-R_{11}^2$, $-O-(C_1-C_5 \text{ alkyl})-NH_2$, $-O-(C_1-C_5 \text{ alkyl})-NH-(C_1-C_3 \text{ alkyl})-R_{11}$, $-O-(C_1-C_5 \text{ alkyl})-N-(C_1-C_3 \text{ alkyl})-R_{11}^2$, $-CH(CH_2OH)_2$, $-(C_1-C_3 \text{ alkyl})-(CH_2OH)_2$, $-(C_1-C_3 \text{ alkyl})-O-(C_1-C_3 \text{ alkyl})-R_{11}$, $-(C_1-C_3 \text{ alkyl})-O-(C_1-C_3 \text{ alkyl})-NH_2$, $-(C_1-C_3 \text{ alkyl})-O-(C_1-C_3 \text{ alkyl})-NH-(C_1-C_3 \text{ alkyl})-R_{11}$, $-(C_1-C_3 \text{ alkyl})-O-(C_1-C_3 \text{ alkyl})-N(C_1-C_3 \text{ alkyl})-R_{11}^2$, phenyl substituted by one or two groups selected from NH_2 , $-N(C_1-C_3 \text{ alkyl})$, $-N(C_1-C_3 \text{ alkyl})^2$, CN or $-(C_1-C_3 \text{ alkyl})$ -tetrazole, or $C_1-C_6 \text{ alkyl}$,

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with each of the alkyl chains of any group in this R₄ definition being optionally substituted by from 1 to 4 OH groups;

R₇ in each instance is independently selected from H, -NH₂, NH(C₁-C₃ alkyl), N(C₁-C₃ alkyl)₂, or C₁-C₃ alkyl;

5 R₈ is H, OH or C₁-C₃ alkyl;

9 H, OH, -NH₂, NH(C₁-C₃ alkyl), or N(C₁-C₃ alkyl)₂;

R₁₀ is H or C₁-C₃ alkyl;

R₁₁ is H, CN, OH, NH₂, F, or CF₃;

10 in the manufacture of a medicament for promoting neochondrogenesis in a mammal in need thereof.

11. The use of claim 10, wherein said compound, or a pharmaceutically acceptable salt thereof, is administered intra-articularly.

12. The use of claim 11, wherein said compound, or a pharmaceutically acceptable salt thereof, is administered intra-articularly to a knee joint.

15 13. The use of claim 12, wherein said mammal suffers from osteoarthritis.

14. The use of claim 13, wherein said compound, or a pharmaceutically acceptable salt thereof, is administered at a dose from 0.001 to 0.1 mg/kg.

15. The use of claim 13, wherein said compound, or a pharmaceutically acceptable salt thereof, is selected from the group consisting of:

20 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3-hydroxy-5-methoxy phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

25 (S,S)-1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[(5-hydroxymethyl-2-phenyl-[1,3]dioxolan-4-ylmethyl)-amino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

(S,S)-1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3,4-trihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

30 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

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7-(3-Amino-2-hydroxy-propylamino)-1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

5 1-Cyclopropyl-7-(4-diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; and

7-(4-Diethylamino-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;

16. The use of Claim 13 wherein the compound, or a pharmaceutically acceptable salt thereof, is selected from the group consisting of:
- 10 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 15 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one
- 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 7-(4-Amino-2,3-dihydroxy-butylamino)-1-cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-([S,S]-2,3,4-trihydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-1-ethyl-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 25 7-(4-Amino-2,3-dihydroxy-butylamino)-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 7-[3-[Bis-(2-hydroxy-ethyl)-amino]-propylamino]-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; and
- 1-Cyclopentyl-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one.
- 30

17. The use of Claim 13 wherein the compound, or a pharmaceutically acceptable salt thereof, is selected from the group consisting of:
- 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-butylamino)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 35 Ethyl-4-[3-(2,6-difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylate;

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- 4-[3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-cyclohexanecarboxylic acid;
- 7-Amino-3-(2,6-difluoro-3,5-dimethoxy-phenyl)-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 5 1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(2-hydroxy-1-hydroxymethyl-ethylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-butylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 10 (S)-1-Cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 4-[3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-2-oxo-3,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-1-yl]-piperidinyl-1-carboxylic acid;
- 15 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-methylamino-1-piperidin-4-yl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 3-(2,6-Difluoro-3,5-dimethoxy-phenyl)-7-(2,3-dihydroxy-propylamino)-1-ethyl-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Cyclopentyl-7-(4-diethylamino-butylamino)-3-(3-ethoxy-2,6-difluoro-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 20 (R)-1-Cyclopentyl-7-(2,3-dihydroxy-propylamino)-3-(2-fluoro-3,5-dimethoxy-phenyl)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one;
- 1-Ethyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-[2-(2-hydroxy-ethoxy)-ethylamino]-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one; and
- 25 1-Cyclopentyl-3-(2-fluoro-3,5-dimethoxy-phenyl)-7-(*trans*-4-hydroxy-cyclohexylamino)-3,4-dihydro-1H-pyrimido[4,5-d]pyrimidin-2-one.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB2005/003058

A. CLASSIFICATION OF SUBJECT MATTER A61K31/519 A61K31/4985 A61P19/00				
According to International Patent Classification (IPC) or to 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 practical, search terms used) EPO-Internal, WPI Data				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
Y	US 2004/019210 A1 (CHIVIKAS CONNOLLY CLEO J ET AL) 29 January 2004 (2004-01-29) claims 1-9 paragraph '0692!	1-17		
Y	WO 02/12238 A (WARNER-LAMBERT COMPANY; HAMBY, JAMES, MARINO; KLUTCHKO, SYLVESTER; KRA) 14 February 2002 (2002-02-14) abstract claims 1-22	1-17		
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☒ Further documents are listed in the continuation of box C.				
☒ Patent family members are listed in annex.				
° Special categories of cited documents :				
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *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 </td> <td style="width: 50%; vertical-align: top;"> *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 *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family </td> </tr> </table>			*A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *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 *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family
A document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *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 *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family			
Date of the actual completion of the international search 14 December 2005		Date of mailing of the international search report 22/12/2005		
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Giacobbe, S		

INTERNATIONAL SEARCH REPORT

International Application No
PC 1, IB2005/003058

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HILL D J ET AL: "PEPTIDE GROWTH FACTORS AND THEIR INTERACTIONS DURING CHONDROGENESIS" PROGRESS IN GROWTH FACTOR RESEARCH, PERGAMON PRESS, GB, vol. 4, no. 1, 1992, pages 45-68, XP001105259 ISSN: 0955-2235 page 62, paragraph 1 -----	1-17
Y	WO 01/29042 A (F. HOFFMANN-LA ROCHE AG) 26 April 2001 (2001-04-26) cited in the application abstract -----	1-17
Y	DECHIARA THOMAS M ET AL: "Ror2, encoding a receptor-like tyrosine kinase, is required for cartilage and growth plate development" NATURE GENETICS, NATURE AMERICA, NEW YORK, US, vol. 24, no. 3, March 2000 (2000-03), pages 271-274, XP002177001 ISSN: 1061-4036 abstract -----	1-17
Y	NAWACHI K ET AL: "Tyrosine kinase-type receptor ErbB4 in chondrocytes: interaction with connective tissue growth factor and distribution in cartilage" FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 528, no. 1-3, 25 September 2002 (2002-09-25), pages 109-113, XP004383242 ISSN: 0014-5793 abstract -----	1-17
Y	KATO Y ET AL: "Fibroblast growth factor stimulates colony formation of differentiated chondrocytes in soft agar." JOURNAL OF CELLULAR PHYSIOLOGY. DEC 1987, vol. 133, no. 3, December 1987 (1987-12), pages 491-498, XP009058823 ISSN: 0021-9541 abstract -----	1-17

INTERNATIONAL SEARCH REPORT

national application No.
PCT/IB2005/003058

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-9 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB2005/003058

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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