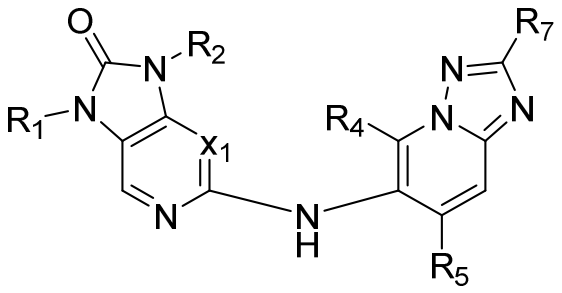
CLAIMS

1. A compound having the structure of Formula I:



(Formula I)

or a salt thereof,

wherein:

x_1 is C-R_3 or N;

R_1 is C_1-C_3 alkyl;

R_2 is cycloalkyl or heterocyclyl, and cycloalkyl and heterocyclyl are optionally substituted with one or more R_6;

R_3 is H or C_1-C_3 alkyl;

R_4 is H or C_1-C_3 alkyl;

R_5 is C_1-C_3 alkyl;

each R_6 is independently selected from hydroxy, halo, alkyl, alkoxy, cycloalkyl, amino, and cyano, or two R_6, taken together with the atom or atoms to which they are bonded, form a spirocyclic or fused ring; and

R_7 is H or C_1-C_3 alkyl,

provided that at least one of the following applies:

(a) x_1 is C-R_3;

(b) R_1 is C_2-C_3 alkyl;

(c) R_4 is C_1-C_3 alkyl;

(d) R_2 is substituted with one R_6, and R_6 is halo;

(e) R_2 is substituted with two R_6 that, taken together with the atom or atoms to which they are bonded, form a spirocyclic or fused ring; and

(f) R_2 is C_3-C_5 cycloalkyl optionally substituted with one or more R_6.

2. The compound of claim 1, wherein x_1 is C-R_3.

3. The compound of claim 2, wherein R_3 is H or methyl.

4. The compound of claim 1, wherein x_1 is N.

5. The compound of any one of the preceding claims, wherein R_1 is C_2-C_3 alkyl.

6. The compound of any one of claims 1-4, wherein R_1 is selected from methyl and ethyl.

7. The compound of any one of the preceding claims, wherein R_4 is C_1-C_3 alkyl.

8. The compound of any one of claims 1-6, wherein R_4 is H or methyl.

9. The compound of any one of the preceding claims, wherein R_2 is cycloalkyl.

10. The compound of any one of claims 1-8, wherein R_2 is heterocyclyl.

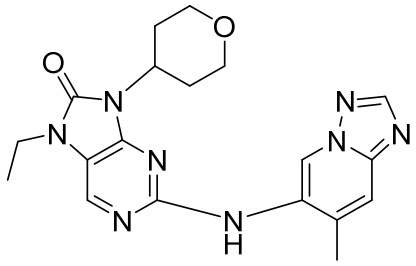
11. The compound of any one of the preceding claims, wherein R_2 is optionally substituted with one or more R_6 independently selected from hydroxy, halo, and cycloalkyl, or two R_6, taken together with the atom or atoms to which they are bonded, form a spirocyclic or fused ring.

12. The compound of any one of claims 1-10, wherein R_2 is optionally substituted with one or more R_6 independently selected from hydroxy, methoxy, and methyl.

13. The compound of any one of the preceding claims, wherein R_5 is methyl.

14. The compound of any one of the preceding claims, wherein R_7 is H or methyl.

15. A compound selected from: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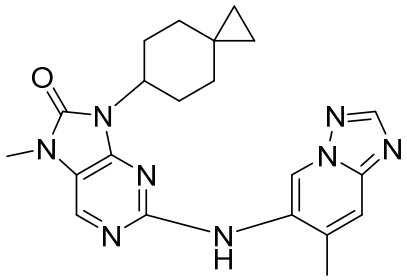
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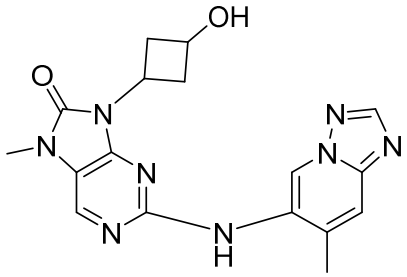
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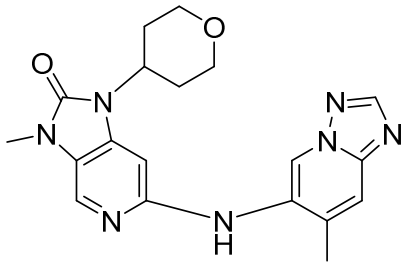
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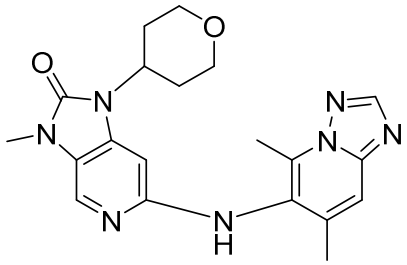
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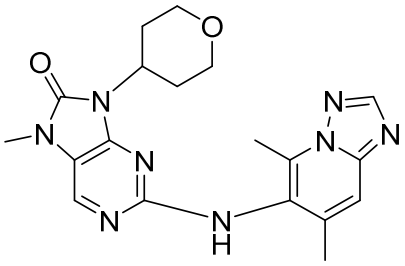

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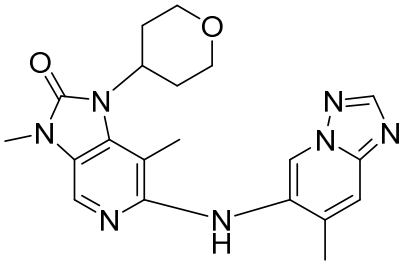


, or a salt thereof.

16. A composition comprising

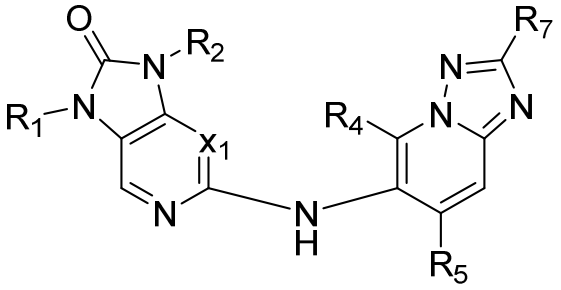
a) a DNA protein kinase inhibitor (DNA-PKI);

b) a DNA cutting agent;

c) optionally, a cell; and

d) optionally, a donor DNA;

wherein the DNA-PKI is a compound of Formula I



(Formula I)

or a salt thereof,

wherein:

x_1 is C-R_3 or N;

R_1 is C_1-C_3 alkyl;

R_2 is cycloalkyl or heterocyclyl, and cycloalkyl and heterocyclyl are optionally substituted with one or more R_6;

R_3 is H or C_1-C_3 alkyl;

R_4 is H or C_1-C_3 alkyl;

R_5 is C_1-C_3 alkyl;

each R_6 is independently selected from hydroxy, halo, alkyl, alkoxy, cycloalkyl, amino, and cyano, or two R_6, taken together with the atom or atoms to which they are bonded, form a spirocyclic or fused ring; and

R_7 is H or C_1-C_3 alkyl.

17. The composition of claim 16, wherein x_1 is N.

18. The composition of claim 16 or 17, wherein R_1 is methyl.

19. The composition of any one of claims 16-18, wherein R_4 is H.

20. The composition of any one of claims 16-19, wherein R_2 is cyclohexyl.

21. The composition of any one of claims 16-19, wherein R_2 is tetrahydropyranyl.

22. The composition of any one of claims 16-19, wherein R_2 is tetrahydrofuranyl.

23. The composition of any one of claims 16-22, wherein R_2 is optionally substituted with one or more R_6 independently selected from hydroxy, methoxy, and methyl.

24. The composition of any one of claims 16-23, wherein R_5 is methyl.

25. The composition of any one of claims 16-24, wherein R_7 is H or methyl.

26. The composition of claim 16, wherein the DNA-PKI is a compound of any one of claims 1-15.

27. A composition comprising

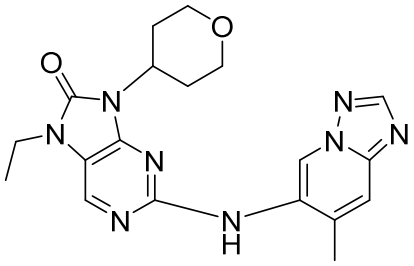
a) a DNA protein kinase inhibitor (DNA-PKI);

b) a DNA cutting agent;

c) optionally, a cell; and

d) optionally, a donor DNA;

wherein the DNA-PKI is selected from: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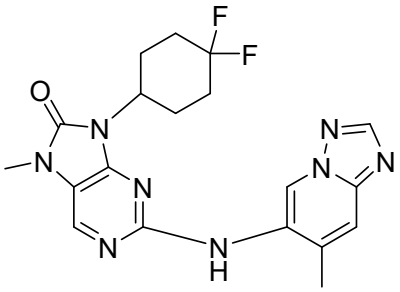
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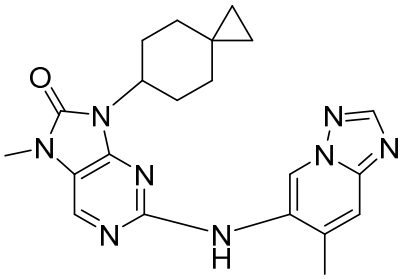
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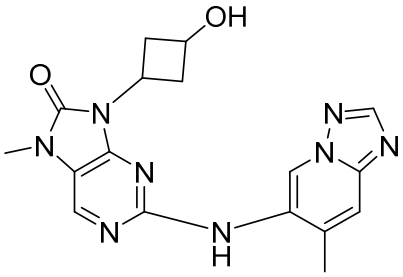
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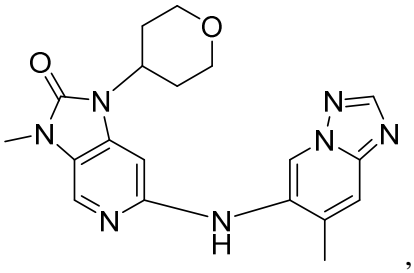
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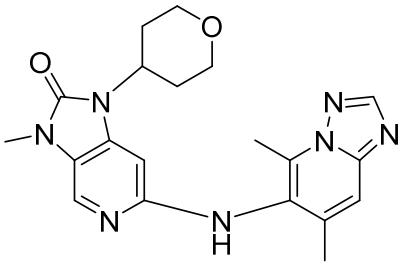
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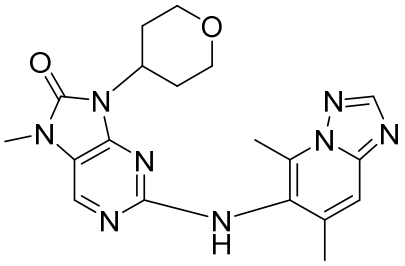
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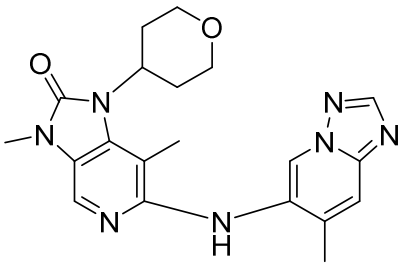

, or a salt thereof.

28. The composition of any one of claims 16-27, comprising a cell.

29. The composition of claim 28, wherein the cell is a eukaryotic cell, such as a liver cell.

30. The composition of claim 28, wherein the cell is useful in adoptive cell therapy (ACT).

31. The composition of claim 30, wherein the cell is a stem cell, such as a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC).

32. The composition of claim 30 or 31, wherein the cell is an immune cell.

33. The composition of claim 32, wherein the immune cell is a leukocyte or a lymphocyte.

34. The composition of claim 33, wherein the immune cell is a lymphocyte, such as a T cell, a B cell, or an NK cell.

35. The composition of claim 34, wherein the lymphocyte is a T cell, such as a primary T cell or a regulatory T cell.

36. The composition of any one of claims 28-35, wherein the cell is a human cell.

37. The composition of any one of claims 16-36, wherein the DNA cutting agent comprises a CRISPR/Cas nuclease component and optionally a guide RNA component.

38. The composition of claim 37, wherein the DNA cutting agent is selected from a zinc finger nuclease, a TALE effector domain nuclease (TALEN), a CRISPR/Cas nuclease component, and combinations thereof.

39. The composition of claim 38, wherein the CRISPR/Cas nuclease component comprises a Cas nuclease or an mRNA encoding the Cas nuclease.

40. The composition of claim 39, wherein the Cas nuclease is a Class 2 Cas nuclease, such as a Cas9 nuclease.

41. The composition of claim 40, wherein the Cas nuclease is a *S. pyogenes* Cas9 nuclease or a *N. meningitidis* Cas9 nuclease, such as Nme2Cas9.

42. The composition of claim 39, wherein the Cas nuclease is a Cas12a nuclease.

43. The composition of any one of claims 16-42, comprising a modified RNA.

44. The composition of any one of claims 37-43, wherein the guide RNA component is a guide RNA nucleic acid such as a modified or unmodified guide RNA.

45. The composition of claim 44, wherein the guide RNA nucleic acid is or encodes a dual-guide RNA (dgRNA) or is or encodes a single-guide (sgRNA).

46. The composition of claim 45, wherein the guide RNA nucleic acid is a modified gRNA; and the modified gRNA comprises a modification at one or more of the first five nucleotides at the 5' end or at the 3' end.

47. The composition of any one of claims 16-46, wherein the composition comprises a guide RNA nucleic acid and a Class 2 Cas nuclease mRNA; and the ratio of the mRNA to the guide RNA nucleic acid is from about 2:1 to 1:4 by weight.

48. The composition of any one of claims 16-47, comprising the donor DNA.

49. The composition of claim 48, wherein the donor DNA comprises a template comprising a sequence encoding a protein, a regulatory sequence, or a sequence encoding structural RNA.

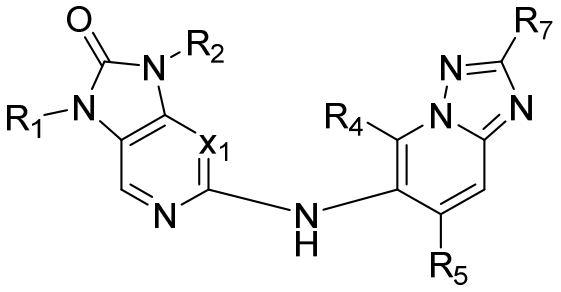
50. The composition of any one of claims 16-49, wherein the DNA cutting agent is present in a lipid nucleic acid assembly composition, such as a lipid nanoparticle (LNP) composition.

51. The composition of claim 50, wherein the lipid nucleic acid assembly composition comprises an ionizable lipid.

52. The composition of claim 28, wherein the cell is not a cancer cell.

53. An in vitro method for targeted genome editing in a cell, a method of repairing a double stranded DNA break in a cell, a method of inhibiting or suppressing repair of a DNA break in a cell via a non-homologous end joining (NHEJ) pathway, or a method of targeted insertion of a donor DNA into the genome of a cell, comprising contacting

the cell with a DNA cutting agent and a DNA-PKI, wherein the DNA-PKI is a compound of Formula I



(Formula I)

or a salt thereof,

wherein:

x_1 is C-R_3 or N;

R_1 is C_1-C_3 alkyl;

R_2 is cycloalkyl or heterocyclyl, and cycloalkyl and heterocyclyl are optionally substituted with one or more R_6;

R_3 is H or C_1-C_3 alkyl;

R_4 is H or C_1-C_3 alkyl;

R_5 is C_1-C_3 alkyl;

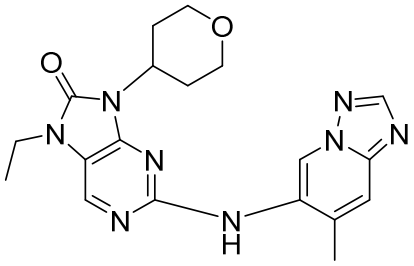
each R_6 is independently selected from hydroxy, halo, alkyl, alkoxy, cycloalkyl, amino, and cyano, or two R_6, taken together with the atom or atoms to which they are bonded, form a spirocyclic or fused ring; and

R_7 is H or C_1-C_3 alkyl.

54. The method of claim 53, wherein the DNA-PKI is a compound of any one of claims 1-15.

55. An in vitro method for targeted genome editing in a cell, a method of repairing a double stranded DNA break in a cell, a method of inhibiting or suppressing repair of a DNA break in a cell via a non-homologous end joining (NHEJ) pathway, or a method of targeted insertion of a donor DNA into the genome of a cell, comprising contacting

the cell with a DNA cutting agent and a DNA-PKI, wherein the DNA-PKI is selected from: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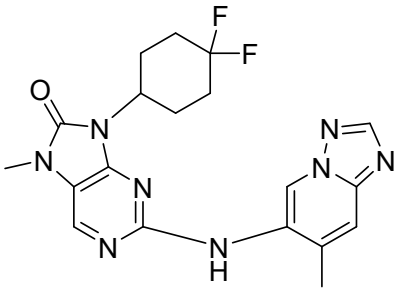
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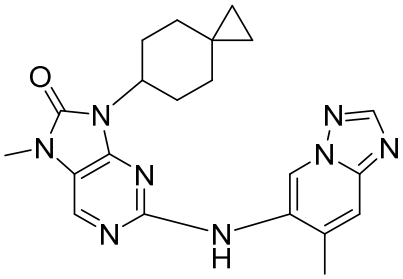
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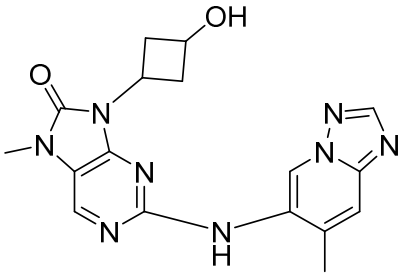
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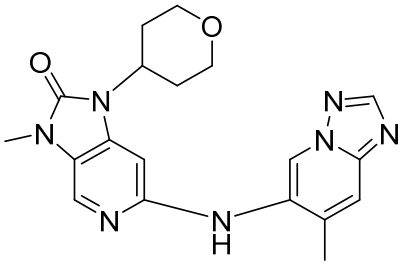
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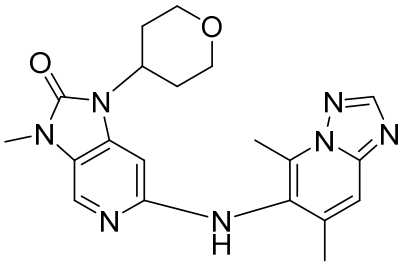
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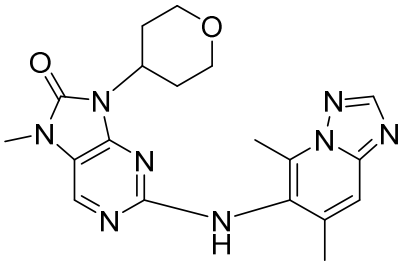
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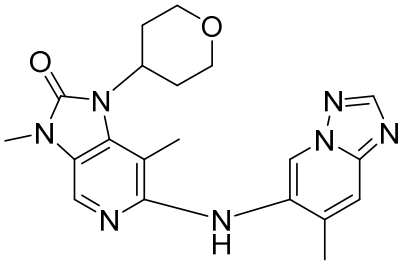
, or a salt thereof.

56. The method of any one of claims 53-55, wherein the cell is a eukaryotic cell, such as a liver cell.

57. The method of any one of claims 53-56, wherein the cell is useful in adoptive cell therapy (ACT).

58. The method of any one of claims 53-56, wherein the cell is a stem cell, such as a hematopoietic stem cell (HSC) or an induced pluripotent stem cell (iPSC).

59. The method of claim 57, wherein the cell is an immune cell.

60. The method of claim 59, wherein the immune cell is a lymphocyte, such as a T cell, a B cell, or an NK cell.

61. The method of any one of claims 53-60, wherein the DNA cutting agent is selected from a zinc finger nuclease, a TALE effector domain nuclease (TALEN), a CRISPR/Cas nuclease component, and combinations thereof.

62. The method of claim 61, wherein the CRISPR/Cas nuclease component comprises a Cas nuclease or an mRNA encoding the Cas nuclease.

63. The method of claim 62, wherein the Cas nuclease is a Class 2 Cas nuclease, such as a Cas9 nuclease.

64. The method of claim 63, wherein the Cas nuclease is a *S. pyogenes* Cas9 nuclease or a *N. meningitidis* Cas9 nuclease, such as Nme2Cas9.

65. The method of claim 63, wherein the Cas nuclease is a Cas12a nuclease.

66. The method of any one of claims 53-65, further comprising contacting the cell with a modified RNA.

67. The method of any one of claims 53-66, further comprising contacting the cell with a guide RNA nucleic acid.

68. The method of claim 67, wherein the guide RNA nucleic acid is a modified gRNA; and the modified gRNA comprises a modification at one or more of the first five nucleotides at the 5' end or at the 3' end.

69. The method of claim 67 or 68, wherein the DNA cutting agent is a Class 2 Cas nuclease mRNA; and the ratio of the mRNA to the guide RNA nucleic acid is from about 2:1 to 1:4 by weight.

70. The method of any one of claims 53-69, further comprising contacting the cell with a donor DNA.

71. The method of claim 70, wherein the donor DNA comprises a template comprising a sequence encoding a protein, a regulatory sequence, a sequence encoding structural RNA.

72. The method of any one of claims 53-71, comprising contacting the cell with a lipid nucleic acid assembly composition comprising the DNA cutting agent, such as a lipid nanoparticle (LNP) composition.

73. The method of claim 72, wherein the lipid nucleic acid assembly composition comprises an ionizable lipid.

74. The method of any one of claims 53-73, further comprising contacting the cell with a vector.