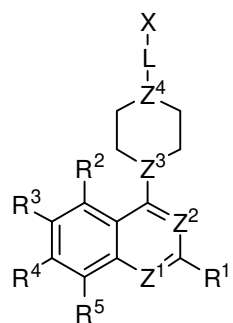


SUMMARY

[0005] Compounds, compositions and methods are provided for the inhibition of ENPP1. ENPP1 inhibitor compounds can act extracellularly to block the degradation of cGAMP. [AspectExamples](#) of the subject methods include contacting a sample with a ENPP1 inhibitor to inhibit the cGAMP hydrolysis activity of ENPP1. In some cases, the ENPP1 inhibitor is cell impermeable. Also provided are compositions and methods for treating cancer. [AspectExamples](#) of the methods include administering to a subject a therapeutically effective amount of an ENPP1 inhibitor to treat the subject for cancer. In certain cases the cancer is a solid tumor cancer. Also provided are methods of administering radiation therapy to a subject either before or after administering an ENPP1 inhibitor. The radiation therapy can be administered at a dosage and/or frequency effective to reduce radiation damage to the subject, but still instigate an immune response.

[0005a] [In one aspect, the present disclosure refers to an ENPP1 inhibitor of the formula:](#)



(VI)

[wherein,](#)

[X is a hydrophilic head group selected from phosphonic acid, phosphonate, phosphonate ester, phosphate, phosphate ester, thiophosphate, thiophosphate ester, phosphoramidate and thiophosphoramidate;](#)

[L is selected from -CH₂-, -\(CH₂\)₂-, -\(CH₂\)₃-, -\(CH₂\)₄-, -\(CH₂\)₅- and -\(CH₂\)₆-;](#)

[Z¹ and Z² are each independently selected from CR¹ and N;](#)

[Z³ and Z⁴ are each independently selected from CR and N, wherein R is H, alkyl or substituted alkyl;](#)

[each R¹ is independently selected from H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, heterocycle and substituted heterocycle;](#)

[R² and R⁵ are each independently selected from H, OH, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkoxy, substituted alkoxy, -OCF₃, halogen, amine, substituted amine, amide, heterocycle and substituted heterocycle;](#)

R³ and R⁴ are each independently selected from H, OH, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkoxy, substituted alkoxy, -OCF₃, halogen, amine, substituted amine, amide, heterocycle and substituted heterocycle; or R³ and R⁴ together with the carbon atoms to which they are attached form a fused ring selected from heterocycle, substituted heterocycle, cycloalkyl, substituted cycloalkyl, aryl and substituted aryl; or a pharmaceutically acceptable salt or a solvate thereof.

[0005b] In one aspect, the present disclosure refers to a pharmaceutical composition, comprising: an ENPP1 inhibitor as described herein; and a pharmaceutically acceptable excipient.

[0006] These and other advantages and features of the disclosure will become apparent to those persons skilled in the art upon reading the details of the compositions and methods of use, which are more fully described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The invention is best understood from the following detailed description when read in conjunction with the accompanying figures. The patent or application file contains at least one figure executed in color. It is emphasized that, according to common practice, the various features of the figures are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures. It is understood that the figures, described below, are for illustration purposes only. The figures are not intended to limit the scope of the present teachings in any way.

[0008] **FIG. 1A to FIG. 1C** shows data illustrating that an exemplary ENPP1 inhibitor can increase the amount of extracellular cGAMP present in a cell system.

[0009] **FIG. 2A to FIG. 2B** illustrates that an exemplary ENPP1 inhibitor can increase cGAMP-stimulated interferon transcription.

[0010] **FIG. 3A to FIG. 3B** shows data illustrating that an exemplary ENPP1 inhibitor can increase the number of tumor-associated dendritic cells in a mouse tumor model.

[0011] **FIG. 4A to FIG. 4C** illustrates that ENPP1 inhibition synergizes with IR treatment and anti-CTLA-4 to exert anti-tumor effects.

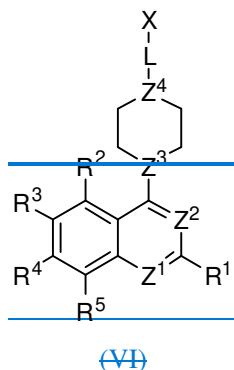
[0012] **FIG. 5** shows a schematic illustrating that ENPP1 is an innate immune checkpoint that regulates the immunotransmitter cGAMP.

DEFINITIONS

[0013] Before embodiments of the present disclosure are further described, it is to be understood that this disclosure is not limited to particular embodiments described, as such may, of course, vary. It

What is claimed is:

1. ~~_____~~ An ENPP1 inhibitor of the formula:



wherein,

~~X is a hydrophilic head group selected from phosphonic acid, phosphonate, phosphonate ester, phosphate, phosphate ester, thiophosphate, thiophosphate ester, phosphoramidate and thiophosphoramidate;~~

~~L is a linker;~~

~~Z¹ and Z² are each independently selected from CR¹ and N;~~

~~Z³ and Z⁴ are each independently selected from CR and N, wherein R is H, alkyl or substituted alkyl;~~

~~each R¹ is independently selected from H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, heterocycle and substituted heterocycle;~~

~~R² and R⁵ are each independently selected from H, OH, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkoxy, substituted alkoxy, OCF₃, halogen, amine, substituted amine, amide, heterocycle and substituted heterocycle;~~

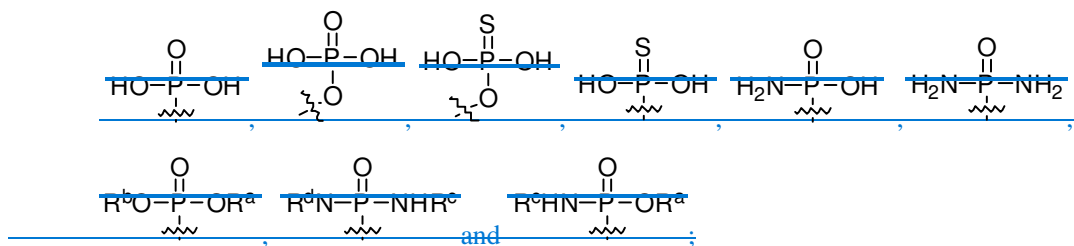
~~R³ and R⁴ are each independently selected from H, OH, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkoxy, substituted alkoxy, OCF₃, halogen, amine, substituted amine, amide, heterocycle and substituted heterocycle; or R³ and R⁴ together with the carbon atoms to which they are attached form a fused selected from heterocycle, substituted heterocycle, cycloalkyl, substituted cycloalkyl, aryl and substituted aryl;~~

~~or a pro drug, a pharmaceutically acceptable salt or a solvate thereof.~~

2. ~~_____~~ The ENPP1 inhibitor of claim 1, wherein:

~~L is selected from —CH₂—, —(CH₂)₂—, —(CH₂)₃—, —(CH₂)₄—, —(CH₂)₅— and —(CH₂)₆—;~~

~~X is selected from:~~



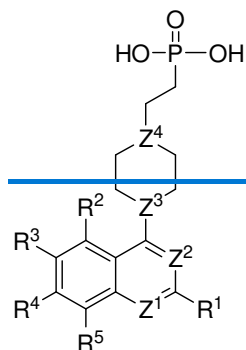
wherein:

R^a and R^b are each independently selected from aryl, alkyl, $\text{CH}_2\text{OC}(\text{O})\text{R}^e$, $\text{CH}_2\text{OC}(\text{O})\text{OR}^e$;

and

R^c and R^d are each independently selected from $\text{C}(\text{CH}_3)\text{C}(\text{O})\text{OR}^e$, alkyl and wherein R^e is alkyl.

3. The ENPP1 inhibitor of claim 2, wherein the inhibitor is of the formula:



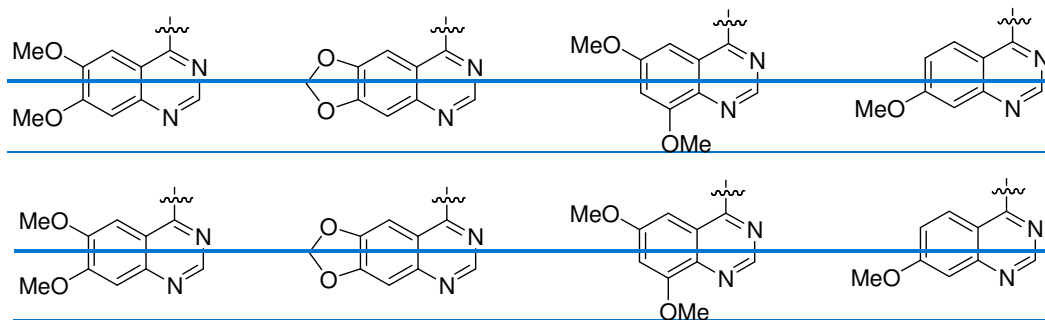
wherein,

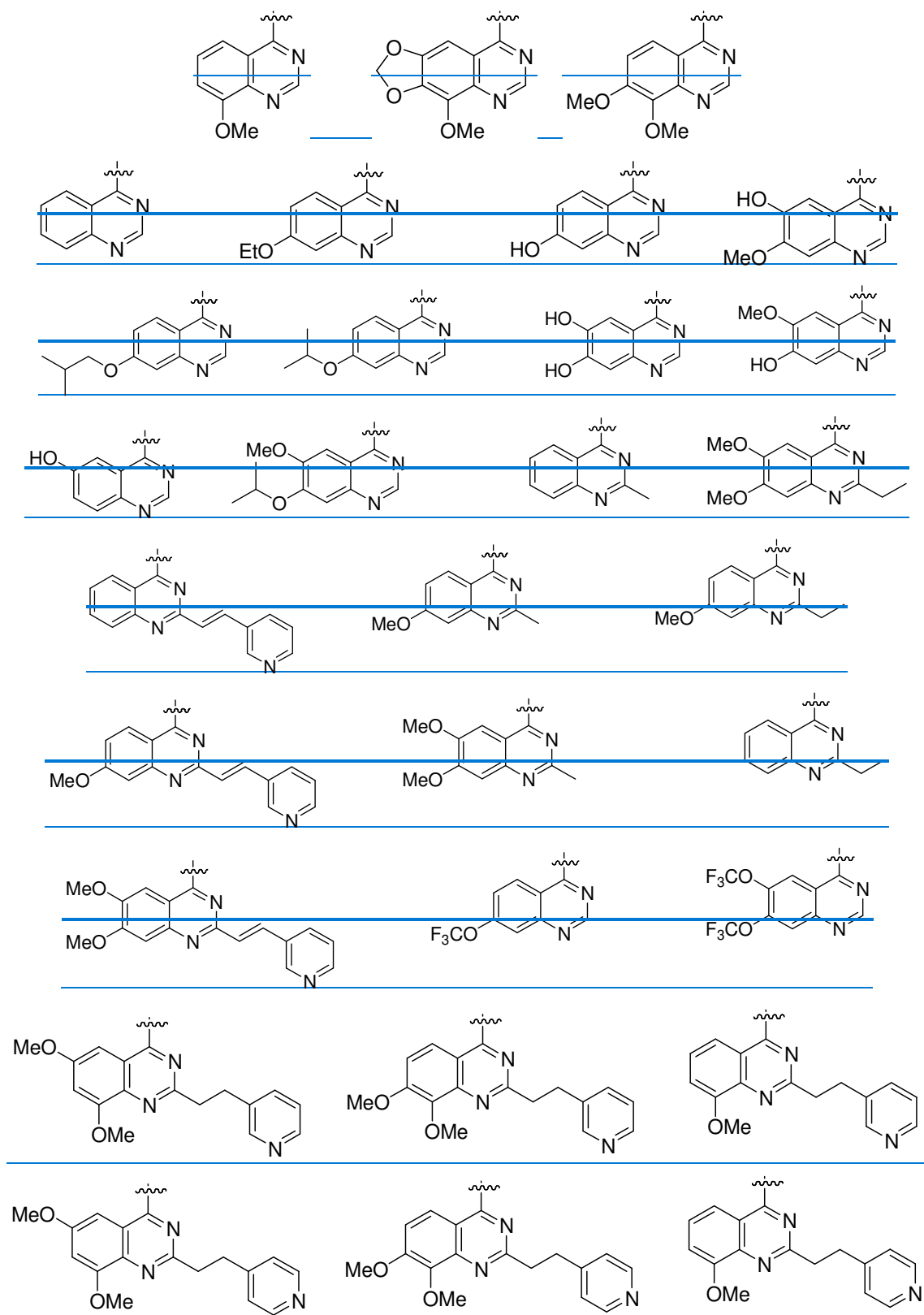
Z^1 and Z^2 are each N;

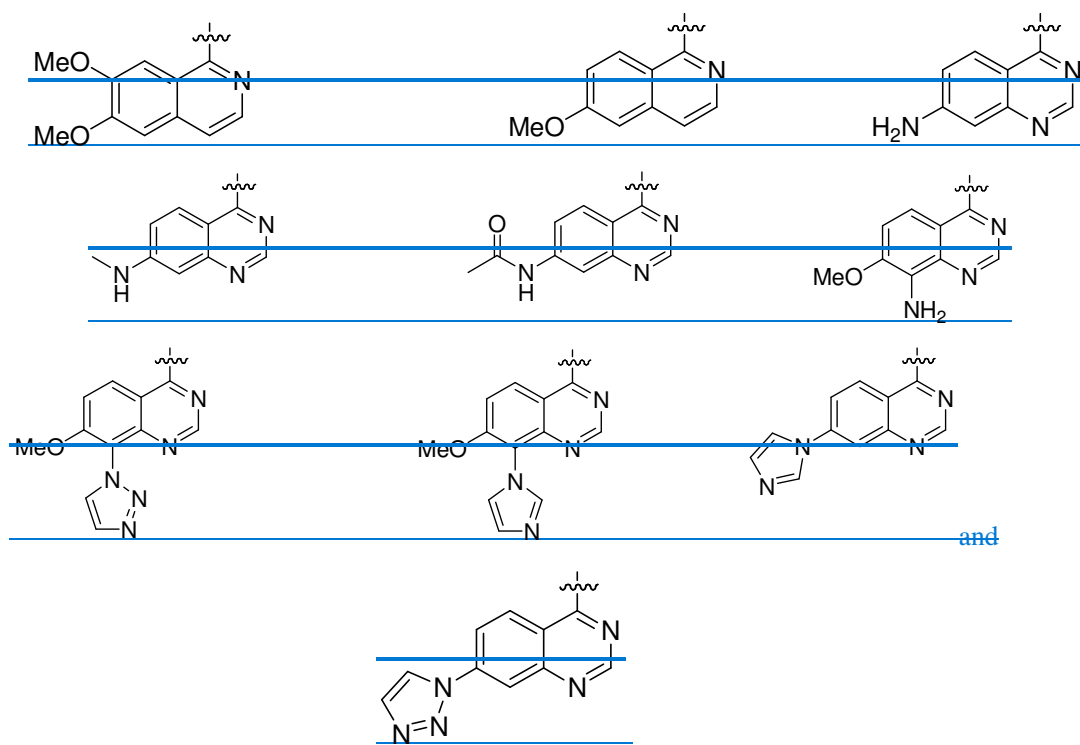
Z^3 is N; and

Z^4 is CH or N.

4. The ENPP1 inhibitor of any one of claims 1-3, wherein the inhibitor comprises a group selected from:







5. ~~The ENPP1 inhibitor of claim 1, wherein the inhibitor is a compound of Table 1 or Table 2.~~

6. ~~A method of inhibiting ENPP1, the method comprising:~~

~~contacting a sample comprising ENPP1 with an ENPP1 inhibitor to inhibit cGAMP hydrolysis activity of the ENPP1, wherein the ENPP1 inhibitor is of formula (I):~~



~~wherein:~~

~~Y is selected from aryl, substituted aryl, heteroaryl, substituted heteroaryl, carbocycle, substituted carbocycle, heterocycle and substituted heterocycle;~~

~~A is selected from carbocycle, substituted carbocycle, heterocycle and substituted heterocycle;~~

~~L is a covalent bond or a linker; and~~

~~X is a hydrophilic head group,~~

~~or a pro drug, a pharmaceutically acceptable salt or a solvate thereof.~~

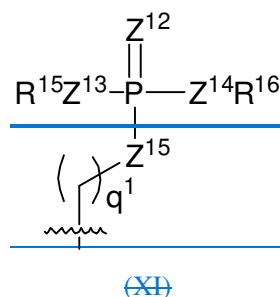
7. ~~The method of claim 6, wherein the ENPP1 inhibitor is cell impermeable.~~

8. ~~The method of claim 6 or 7, wherein the sample is a cellular sample.~~

9. ~~The method of claim 8, wherein the sample comprises cGAMP and cGAMP levels are elevated in the cellular sample relative to a control sample not contacted with the inhibitor.~~

10. ~~The method of claim 6, wherein the hydrophilic head group (X) is selected from phosphonic acid, phosphonate, phosphonate ester, phosphate, phosphate ester, thiophosphate, thiophosphate ester, phosphoramidate and thiophosphoramidate.~~

11. ~~The method of claim 10, wherein L-X comprises a group of formula (XI):~~



~~wherein:~~

~~Z¹² is selected from O and S;~~

~~Z¹³ and Z¹⁴ are each independently selected from O and NR' wherein R' is H, alkyl or substituted alkyl;~~

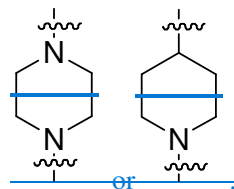
~~Z¹⁵ is selected from O and CH₂;~~

~~R¹⁵ and R¹⁶ are each independently selected from H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkoxy, substituted alkoxy, aryl, substituted aryl, an acyl group, heterocycle, substituted heterocycle cycloalkyl and substituted cycloalkyl; and~~

~~q¹ is an integer from 0 to 5.~~

12. ~~The method of any one of claims 6-11, wherein A is selected from piperidine, substituted piperidine, piperazine and substituted piperazine.~~

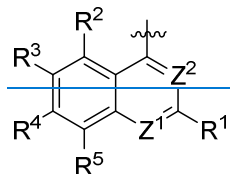
13. ~~The method of claim 12, wherein A is:~~



14. ~~The method of any one of claims 6-13, wherein L is (CH₂)_n, and n is 1-6.~~

15. — The method of any one of claims 6-14, wherein Y is selected from quinazoline, substituted quinazoline, quinoline, substituted quinoline, naphthalene, substituted naphthalene, isoquinoline, substituted isoquinoline, 7H-purine, substituted 7H-purine, pyrimidine, substituted pyrimidine.

16. — The method of claim 15, wherein Y is of the formula:



wherein:

Z^1 and Z^2 are each independently selected from CR^1 and N;

each R^1 is independently selected from H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, heterocycle and substituted heterocycle;

R^2 and R^5 are each independently selected from H, OH, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkoxy, substituted alkoxy, OCF_3 , halogen, amine, substituted amine, amide, heterocycle and substituted heterocycle;

R^3 and R^4 are each independently selected from H, OH, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkoxy, substituted alkoxy, OCF_3 , halogen, amine, substituted amine, amide, heterocycle and substituted heterocycle; or R^3 and R^4 together with the carbon atoms to which they are attached form a fused ring selected from heterocycle, substituted heterocycle, cycloalkyl, substituted cycloalkyl, aryl and substituted aryl.

17. — A pharmaceutical composition, comprising:

a ENPP1 inhibitor of any one of claims 1-5; and

a pharmaceutically acceptable excipient.

18. — A method of treating cancer, the method comprising:

administering to a subject with cancer a therapeutically effective amount of an ENPP1 inhibitor to treat the subject for cancer;

wherein the ENPP1 inhibitor is of formula (I):



wherein:

Y is selected from aryl, substituted aryl, heteroaryl, substituted heteroaryl, carbocycle, substituted carbocycle, heterocycle and substituted heterocycle;

~~A is selected from carbocycle, substituted carbocycle, heterocycle and substituted heterocycle;~~

~~L is a covalent bond or a linker; and~~

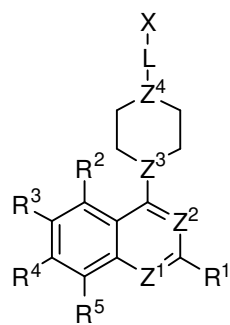
~~X is a hydrophilic head group;~~

~~or a pro drug, a pharmaceutically acceptable salt or a solvate thereof.~~

~~19. The method of claim 18, where the cancer is a solid tumor cancer.~~

~~20. The method of claim 18 or 19, wherein the cancer is breast cancer.~~

1. An ENPP1 inhibitor of the formula:



(VI)

wherein,

X is a hydrophilic head group selected from phosphonic acid, phosphonate, phosphonate ester, phosphate, phosphate ester, thiophosphate, thiophosphate ester, phosphoramidate and thiophosphoramidate;

L is selected from $-\text{CH}_2-$, $-(\text{CH}_2)_2-$, $-(\text{CH}_2)_3-$, $-(\text{CH}_2)_4-$, $-(\text{CH}_2)_5-$ and $-(\text{CH}_2)_6-$;

Z^1 and Z^2 are each independently selected from CR¹ and N;

Z^3 and Z^4 are each independently selected from CR and N, wherein R is H, alkyl or substituted alkyl;

each R¹ is independently selected from H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, heterocycle and substituted heterocycle;

R² and R⁵ are each independently selected from H, OH, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkoxy, substituted alkoxy, $-\text{OCF}_3$, halogen, amine, substituted amine, amide, heterocycle and substituted heterocycle;

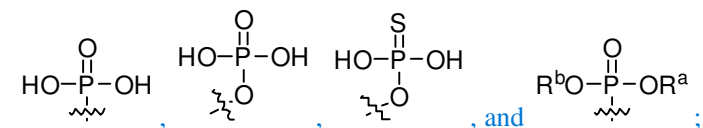
R³ and R⁴ are each independently selected from H, OH, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkoxy, substituted alkoxy, $-\text{OCF}_3$, halogen, amine, substituted amine, amide, heterocycle and substituted heterocycle; or R³ and R⁴ together with the carbon atoms to which they

are attached form a fused ring selected from heterocycle, substituted heterocycle, cycloalkyl, substituted cycloalkyl, aryl and substituted aryl;

or a pharmaceutically acceptable salt or a solvate thereof.

2. The ENPP1 inhibitor of claim 1, wherein:

X is selected from:

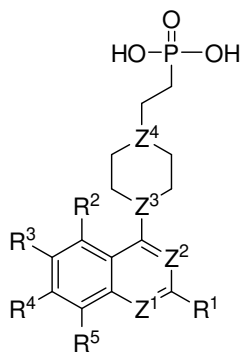


wherein:

R^a and R^b are each independently selected from aryl, alkyl, -CH₂OC(O)R^c, and -CH₂OC(O)OR^c; and

wherein R^c is alkyl.

3. The ENPP1 inhibitor of claim 2, wherein the inhibitor is of the formula:



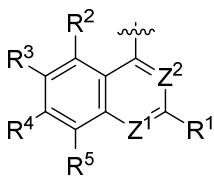
wherein,

Z¹ and Z² are each N;

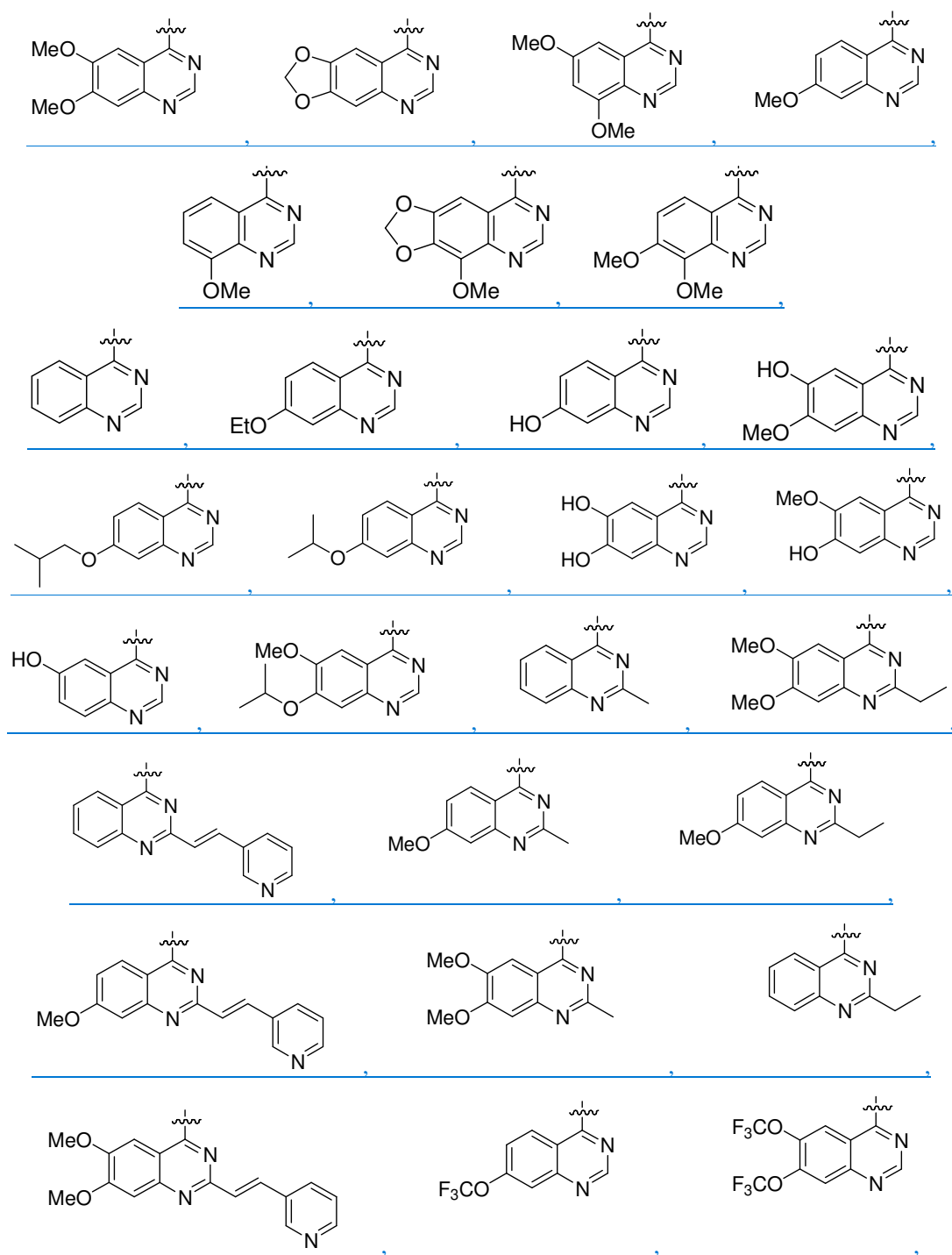
Z³ is N; and

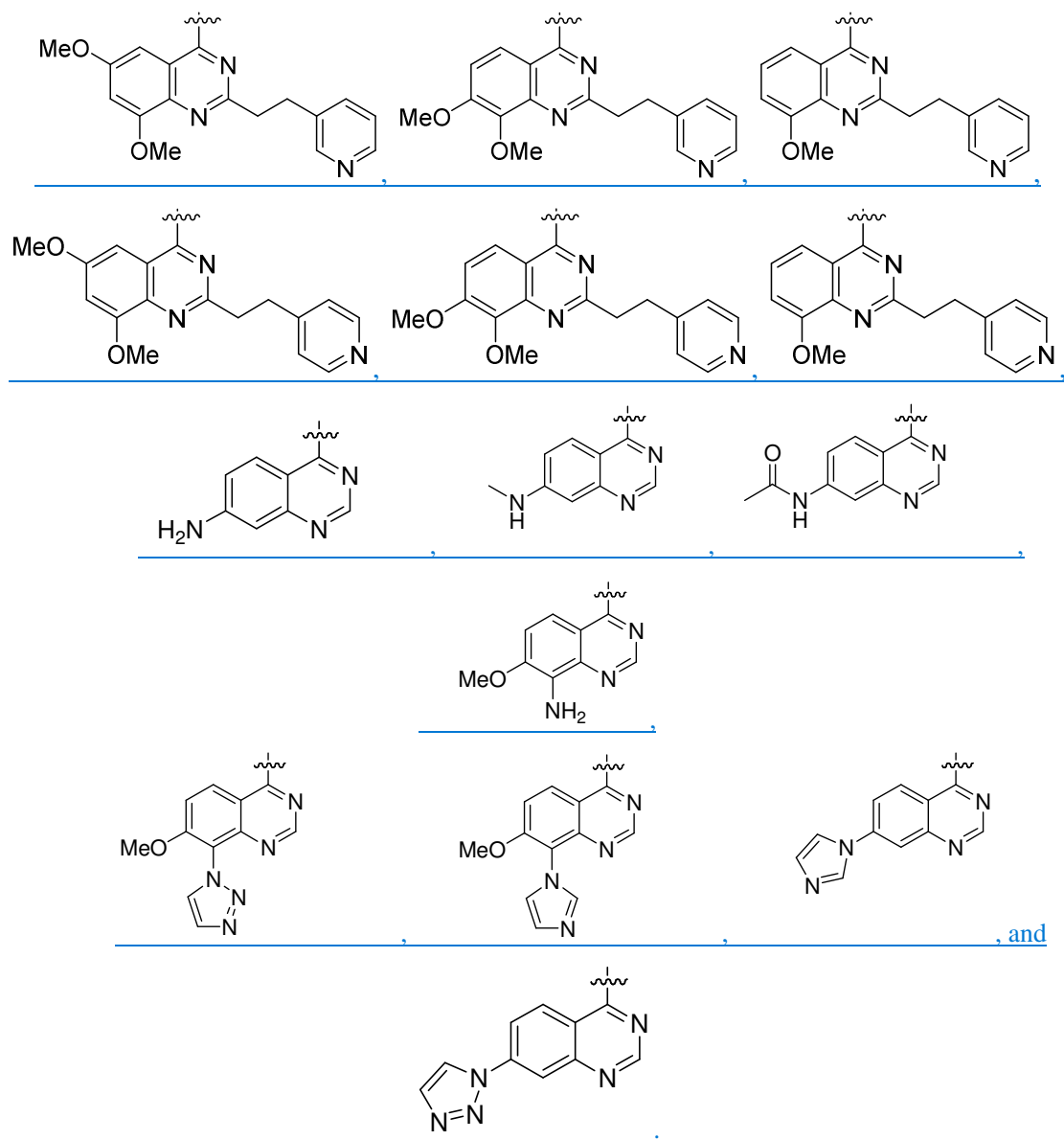
Z⁴ is CH or N.

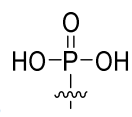
4. The ENPP1 inhibitor of any one of claims 1-3, wherein the portion of the molecule



represented by is selected from:





5. The ENPP1 inhibitor of claim 1 or 2, wherein X is .

6. The ENPP1 inhibitor of claim 1 or 2, wherein L is $-(CH_2)_2-$.

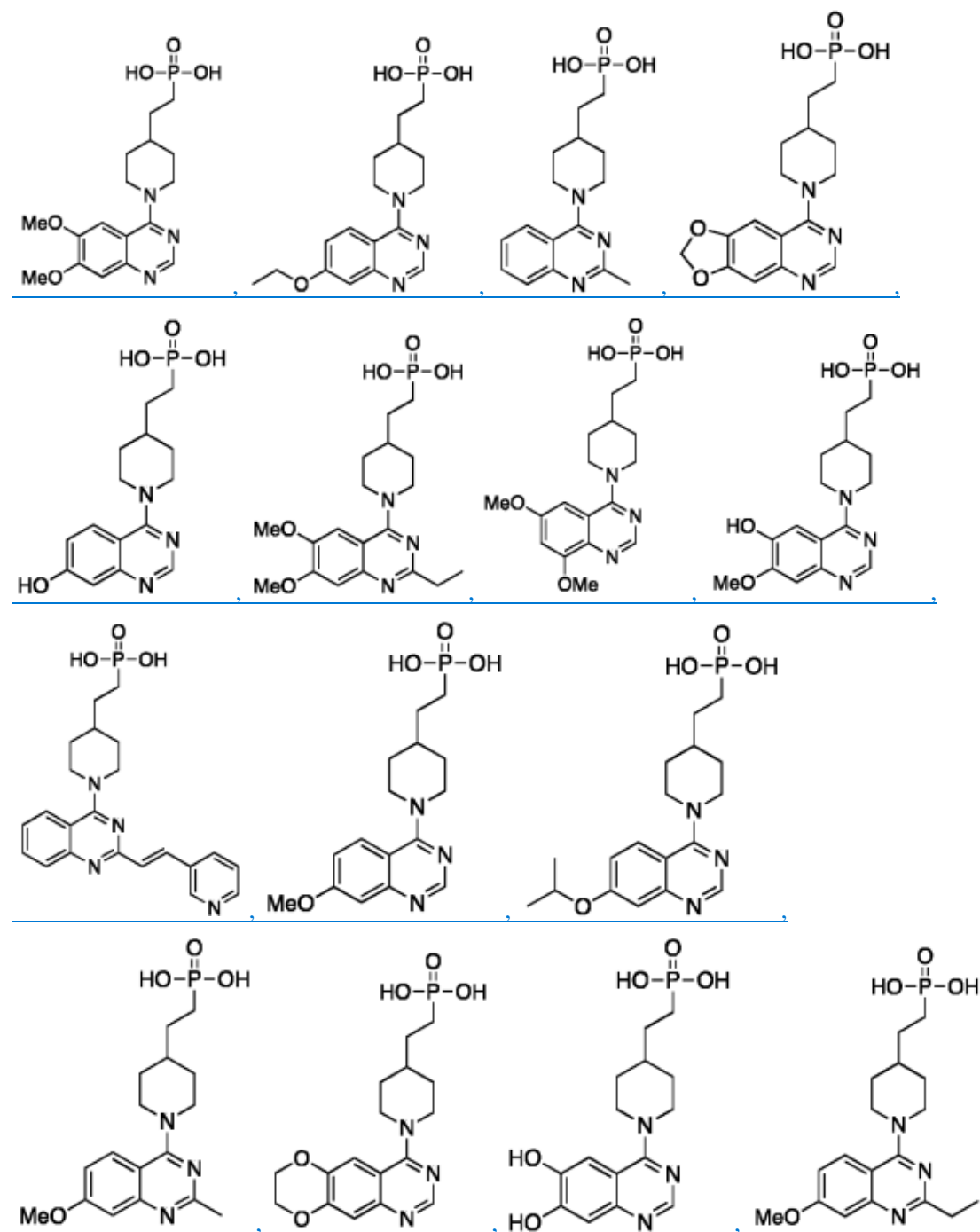
7. The ENPP1 inhibitor of claim 1 or 2, wherein Z^1 and Z^2 are each N.

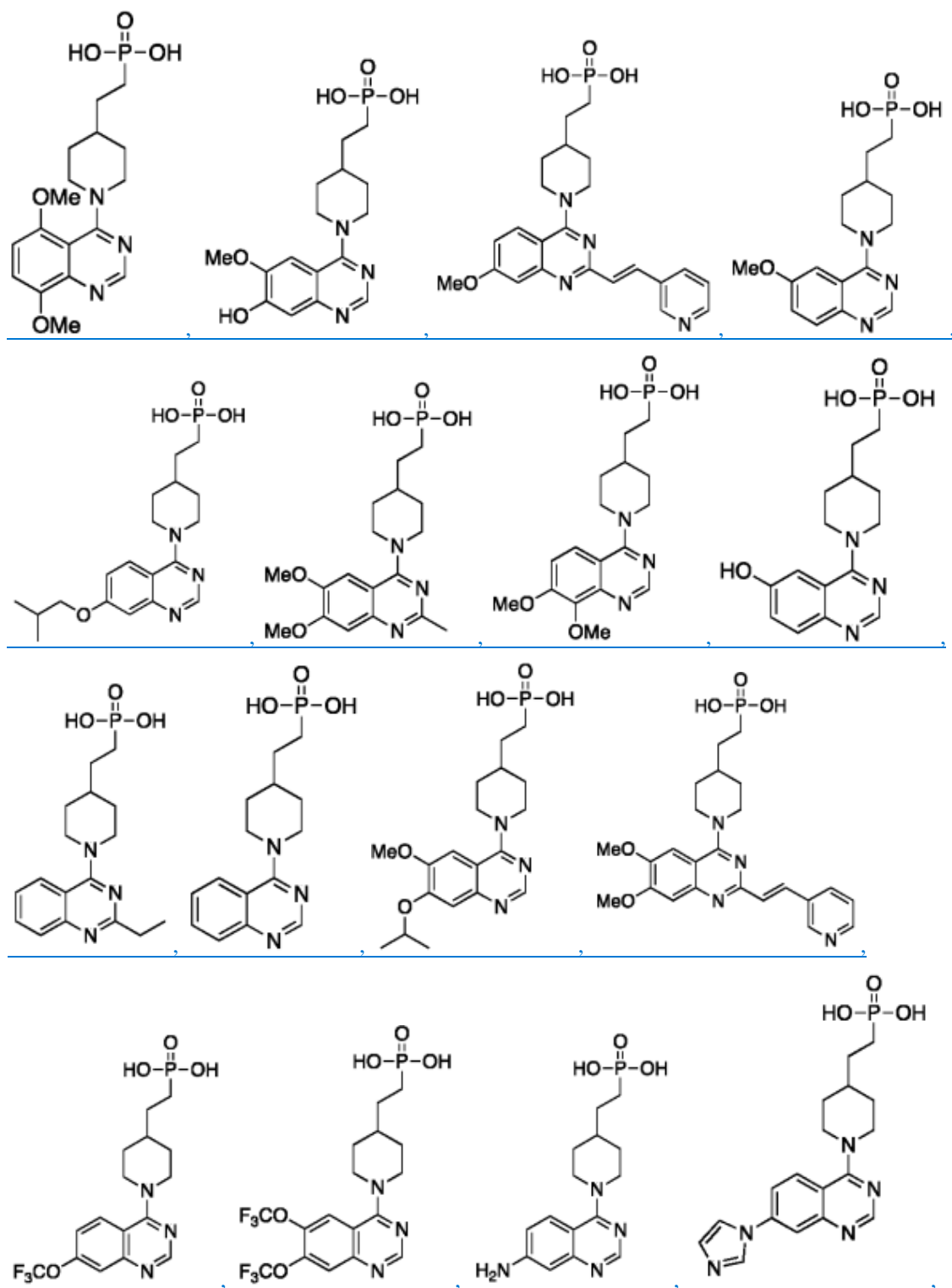
8. The ENPP1 inhibitor of any one of claims 1-3, wherein R^1 is hydrogen.

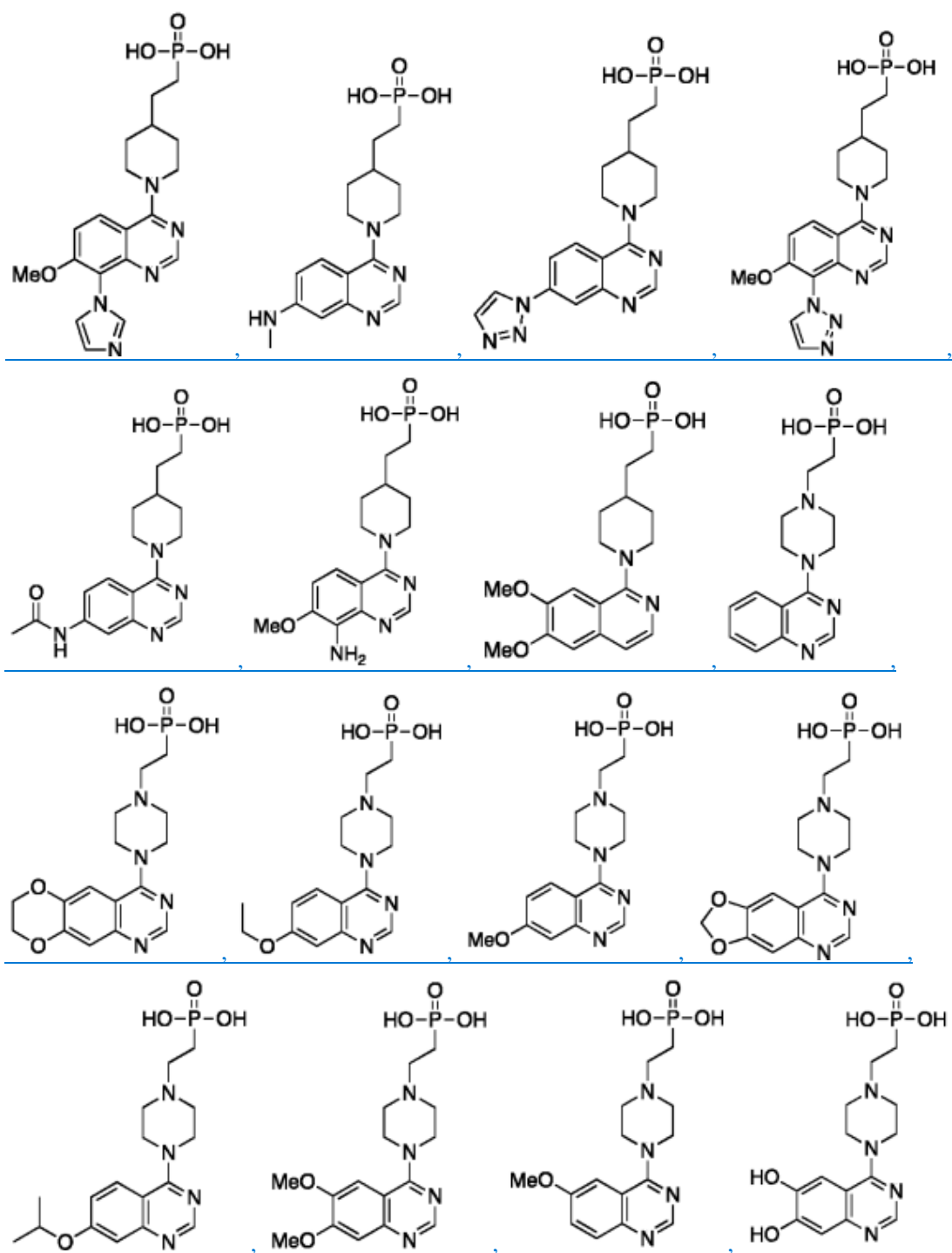
9. The ENPP1 inhibitor of any one of claims 1-3, wherein R⁵ is alkoxy.

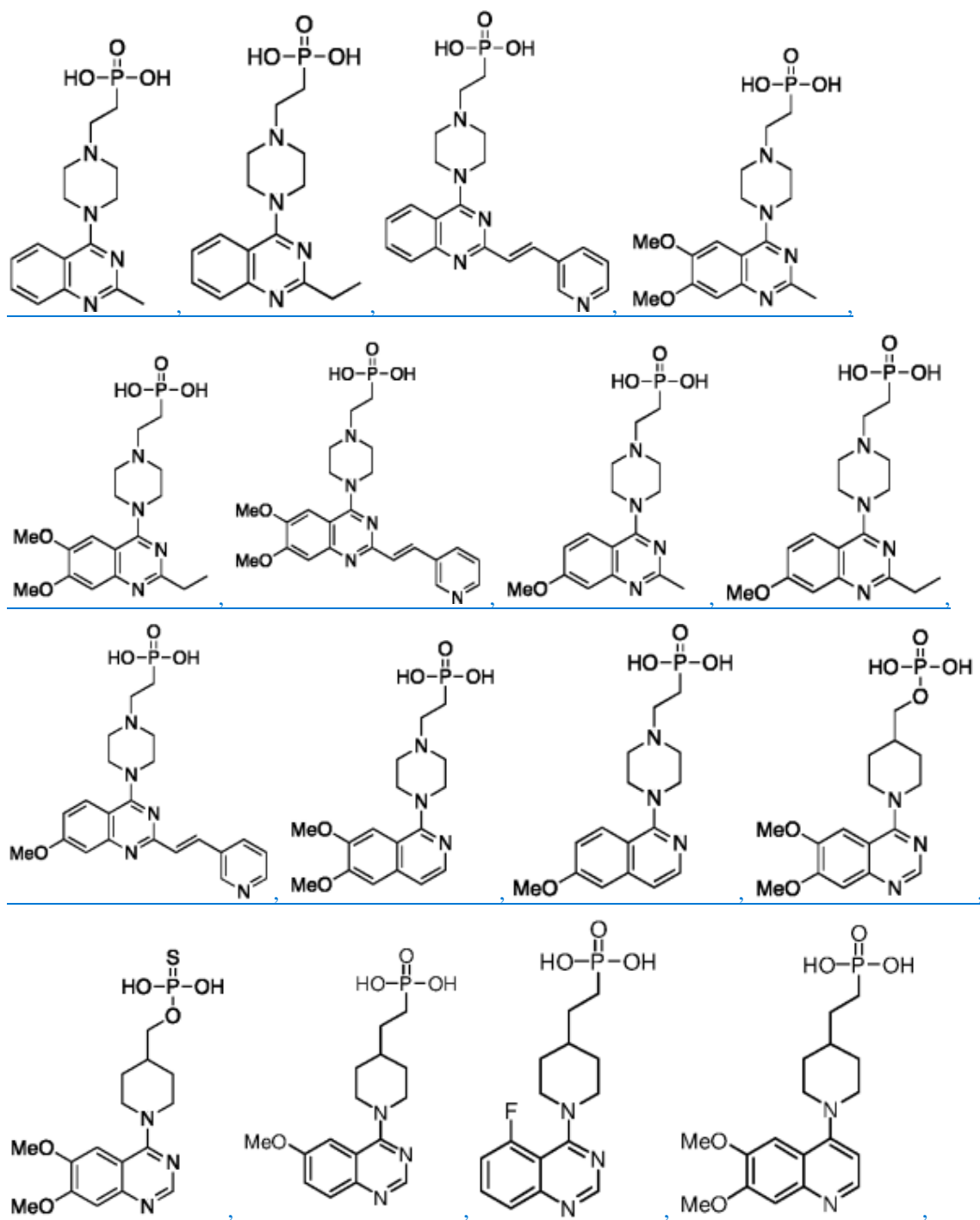
10. The ENPP1 inhibitor of claim 9, wherein R⁵ is methoxy.

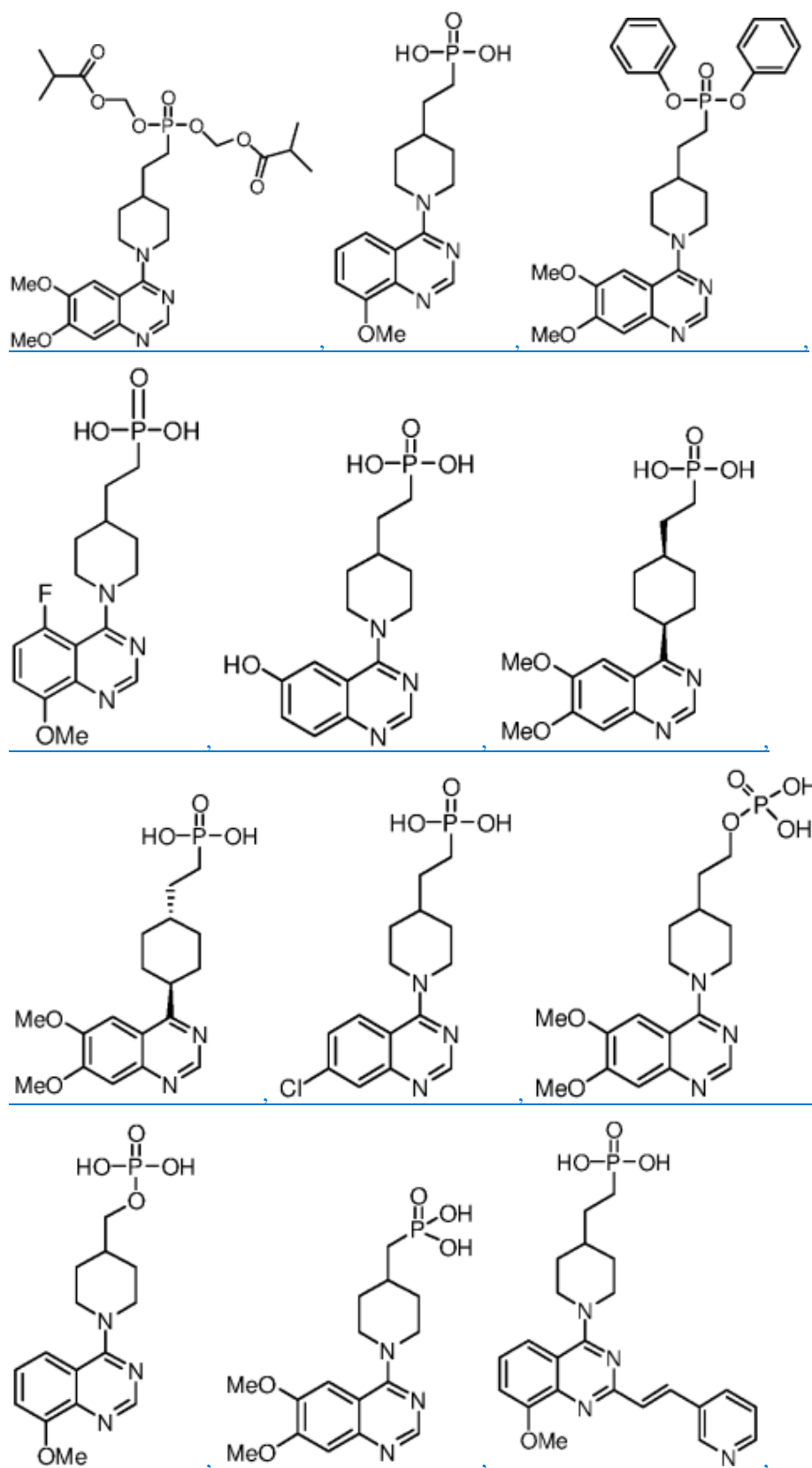
11. The ENPP1 inhibitor of claim 1, wherein the ENPP1 inhibitor is selected from:

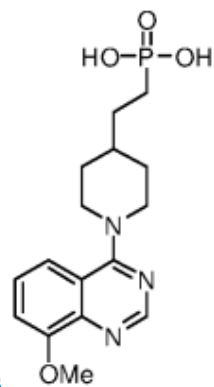
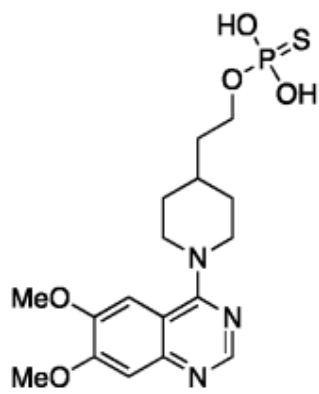
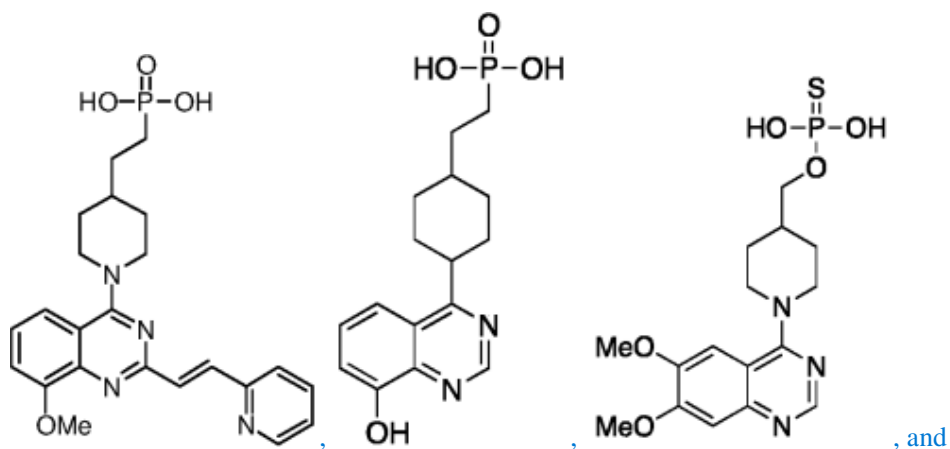


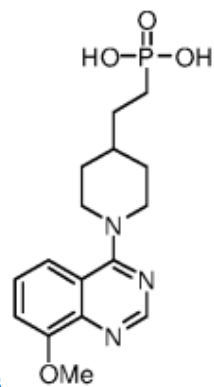


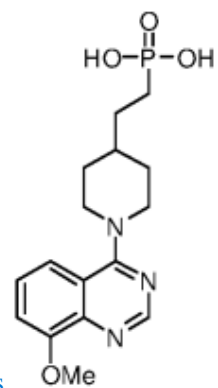








12. The ENPP1 inhibitor of claim 1, wherein the ENPP1 inhibitor is , or a pharmaceutically acceptable salt or solvate thereof.



13. The ENPP1 inhibitor of claim 1, wherein the ENPP1 inhibitor is

14. A pharmaceutical composition, comprising:
an ENPP1 inhibitor of any one of claims 1-13; and
a pharmaceutically acceptable excipient.