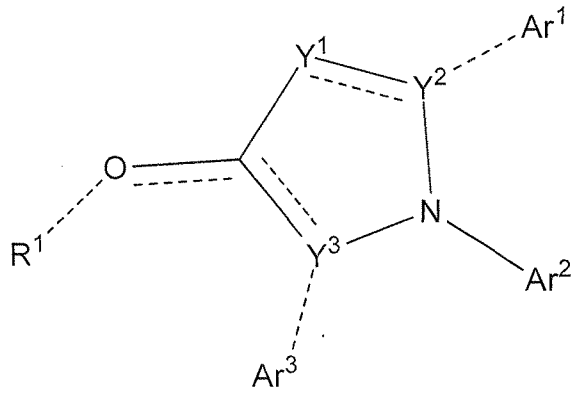
compound MI-2, an irreversible MALT1 protease inhibitor, was identified as a lead compound with nanomolar activity in cell-based assays and selective activity against ABC-DLBCLs. Importantly we show that MALT1 inhibitors kill ABC-DLBCLs *in vitro* and *in vivo*, are non-toxic to animals and also suppress primary human non GCB-DLBCL specimens. Hence we demonstrate that MALT1 is a bona fide therapeutic target, and provide a lead compound that forms the basis of a new class of therapeutic agents for B-cell lymphomas.

The ~~inventiondisclosure~~ provides, in ~~various embodimentsone aspect~~, a method of modulating MALT1, comprising contacting MALT1 with an effective amount or concentration of a compound of formula (I)



(I)

wherein

a dashed bond indicates that a bond can be present or absent;

when a double bond is present between Y^1 and Y^2, Y^1 is N or CR, Y^2 is C, and Ar^1 is present; when a single bond is present between Y^1 and Y^2, Y^1 is CR_2, Y^2 is O or S, and Ar^1 is absent, and each independently selected R is H or (C1-C6)alkyl;

R^1 is alkyl, alkoxyalkyl, or arylalkyl, wherein any alkyl, alkoxyalkyl, or arylalkyl, can be mono- or independently multi-substituted with halo or (C1-C6)alkoxy, provided that when a double bond is present between the oxygen atom and the ring comprising Y^3, R^1 is absent and Ar^3 is present, and when a single bond is present between the oxygen atom and the ring, R^1 is present, a double bond between Y^3 and the carbon atom bearing the oxygen atom is present, and Ar^3 is absent;

Ar^1 is phenyl substituted with 1-3 J^1 groups; J^1 is halo or (C1-C6)alkoxy;

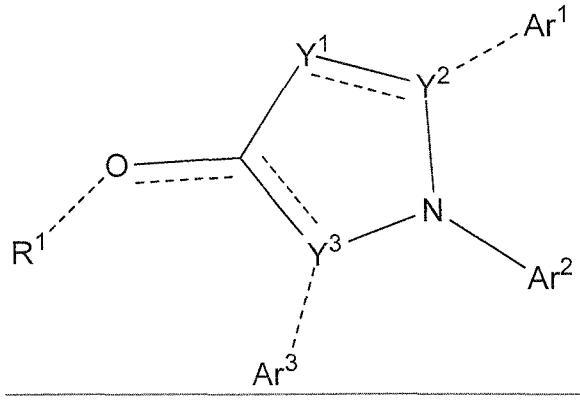
Ar^2 is phenyl substituted with 1-3 J^2 groups; J^2 is a group of formula $-N(R)C(O)-R^2$ and R^2 is alkyl, aryl, or arylamino, wherein any alkyl, aryl, or arylamino is substituted with 0-2 halo, nitro, or (C1-C6)alkoxy groups;

Ar^3 is phenyl substituted with 1-3 J^3 groups; J^3 is halo or (C1-C6)alkoxy;

or any salt, hydrate, tautomer, or stereoisomer thereof.

The ~~invention~~disclosure further provides, in various embodiments, a method of treating or preventing cancer comprising administering to a patient an effective dose of a compound of formula (I) as defined above. More specifically, the cancer can be a lymphoma, such as a diffuse large B-cell lymphoma (DLBCL).

In another aspect, there is provided a compound of formula (I) for use in treating or preventing cancer, wherein said compound of formula (I) is:



(I)

wherein

a dashed bond indicates that a bond can be present or absent;

when a double bond is present between Y^1 and Y^2, Y^1 is N or CR, Y^2 is C, and Ar^1 is present; when a single bond is present between Y^1 and Y^2, Y^1 is CR_2, Y^2 is O or S, and Ar^1 is absent, and each independently selected R is H or (C1-C6)alkyl;

R^1 is alkyl, alkoxyalkyl, or arylalkyl, wherein any alkyl, alkoxyalkyl, or arylalkyl, can be mono- or independently multi-substituted with halo or (C1-C6)alkoxy, provided that when a double bond is present between the oxygen atom and the ring comprising Y^3, R^1 is absent and Ar^3 is present, and when a single bond is present between the oxygen atom and the ring, R^1 is present, a double bond between Y^3 and the carbon atom bearing the oxygen atom is present, and Ar^3 is absent;

Ar^1 is phenyl substituted with 1-3 J^1 groups; J^1 is halo or (C1-C6)alkoxy;

Ar^2 is phenyl substituted with 1-3 J^2 groups; J^2 is a group of formula -N(R)C(O)-R^2 and R^2 is alkyl, aryl, or arylamino, wherein any alkyl, aryl, or arylamino is substituted with 0-2 halo, nitro, or (C1-C6)alkoxy groups;

Ar^3 is phenyl substituted with 1-3 J^3 groups; J^3 is halo or (C1-C6)alkoxy;

or any salt, hydrate, tautomer, or stereoisomer thereof.

The ~~invention~~disclosure further provides, in ~~various embodiments~~another aspect, a method of identifying a small molecule modulator of MALT1, comprising contacting a recombinant form of MALT1 (340-789) fused with a leucine zipper dimerization motif (LZ-MALT1) and a candidate modulator compound, using the MALT1 substrate peptide LRSR linked to the fluorogen AMC (7-amino-4-methylcoumarin), such that cleavage of the Ac-LRSR-AMC substrate by MALT1 results in release of AMC and a fluorescent signal, wherein a decrease in the cleavage of the Ac-LRSR-AMC substrate by the recombinant form of MALT1 in the presence of the candidate modulator indicates that the candidate modulator is a small molecule modulator of MALT1.

BRIEF DESCRIPTION OF THE FIGURES

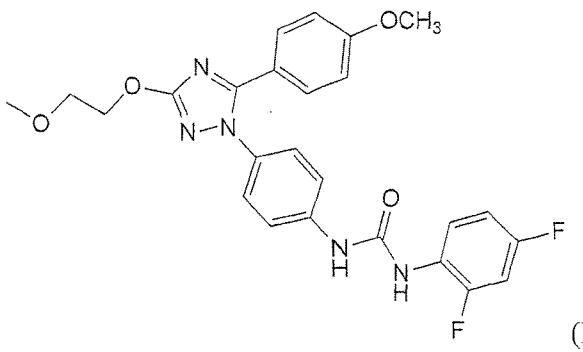
Figure 1A depicts two perspective views of the structure of a recombinant form of MALT1 (340-789) fused with a leucine zipper dimerization motif (LZ-MALT1), which promotes its dimerization and activation.

Figure 1B is a graphic representation of the results by which 324 candidate compounds were selected from a compound library for validation in a concentration response assay using LZ-MALT1

Figure 1C is a graphic representation of the results by which nineteen compounds were selected for further validation based on their biochemical activity (IC_{50} <20 μM).

Figure 1D shows the chemical structure of compound MI-2.

Figure 1E shows a photograph of a Western blot of gel electrophoresis results demonstrating that MI-2 caused a dose-dependent decrease in MALT1-mediated cleavage, noted by an increase in the uncleaved CYLD protein and a



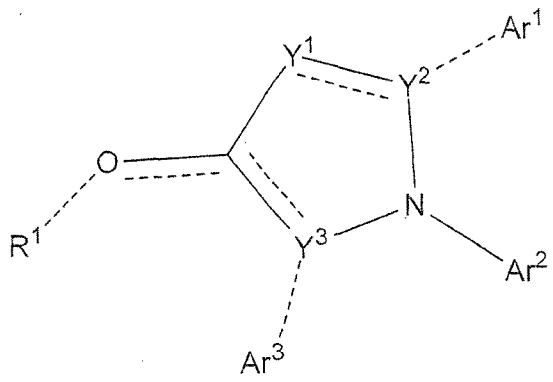
(MI-2A5),

or any salt, hydrate, tautomer, or stereoisomer thereof.

~~5. The method of claim 1 wherein the MALT1 is disposed within a living animal.~~

~~6. The method of claim 5 wherein the living animal is a human being afflicted with cancer.~~

~~7~~5. ~~A method of treating or preventing cancer comprising administering to a patient an effective dose of a~~A compound of formula (I) for use in treating or preventing cancer, wherein said compound of formula (I) is:



(I)

wherein

a dashed bond indicates that a bond can be present or absent;

when a double bond is present between Y^1 and Y^2, Y^1 is N or CR, Y^2 is C, and Ar^1 is present; when a single bond is present between Y^1 and Y^2, Y^1 is CR_2, Y^2 is O or S, and Ar^1 is absent, and each independently selected R is H or (C1-C6)alkyl;

R^1 is alkyl, alkoxyalkyl, or arylalkyl, wherein any alkyl, alkoxyalkyl, or arylalkyl, can be mono- or independently multi-substituted with halo or (C1-

C6)alkoxy, provided that when a double bond is present between the oxygen atom and the ring comprising Y^3, R^1 is absent and Ar^3 is present, and when a single bond is present between the oxygen atom and the ring, R^1 is present, a double bond between Y^3 and the carbon atom bearing the oxygen atom is present, and Ar^3 is absent;

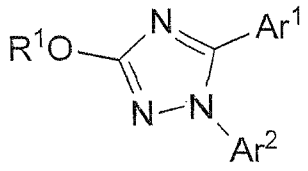
Ar^1 is phenyl substituted with 1-3 J^1 groups; J^1 is halo or (C1-C6)alkoxy;

Ar^2 is phenyl substituted with 1-3 J^2 groups; J^2 is a group of formula -N(R)C(O)-R^2 and R^2 is alkyl, aryl, or arylamino, wherein any alkyl, aryl, or arylamino is substituted with 0-2 halo, nitro, or (C1-C6)alkoxy groups;

Ar^3 is phenyl substituted with 1-3 J^3 groups; J^3 is halo or (C1-C6)alkoxy;

or any salt, hydrate, tautomer, or stereoisomer thereof.

~~86~~. The ~~method~~compound of claim ~~75~~, wherein the compound of formula (I) is a compound of formula (IA)

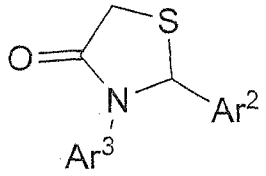


(IA)

wherein R^1, Ar^1, and Ar^2 are as defined for formula (I),

or any salt, hydrate, tautomer, or stereoisomer thereof.

~~97~~. The ~~method~~compound of claim ~~75~~, wherein the compound of formula (I) is a compound of formula IB)

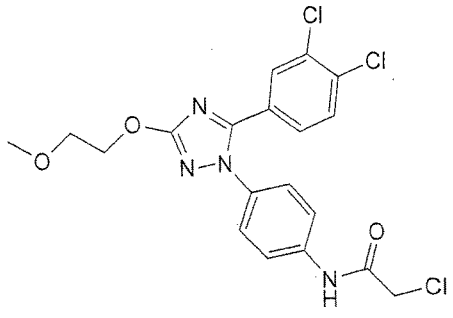


(IB),

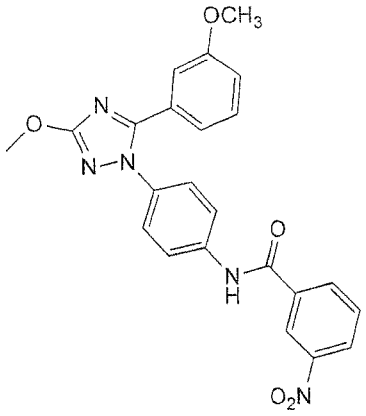
wherein Ar^2 and Ar^3 are as defined for formula (I),

or any salt, hydrate, tautomer, or stereoisomer thereof.

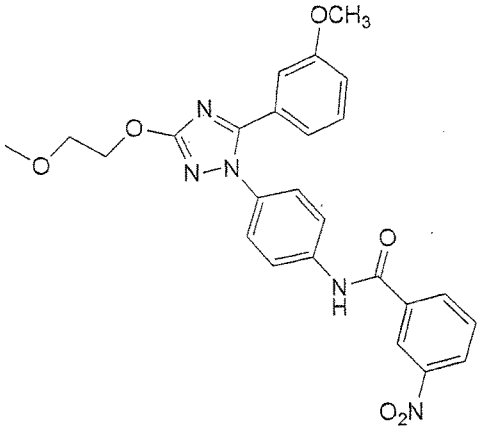
~~108~~. The ~~method~~compound of claim ~~75~~, wherein the compound is any of



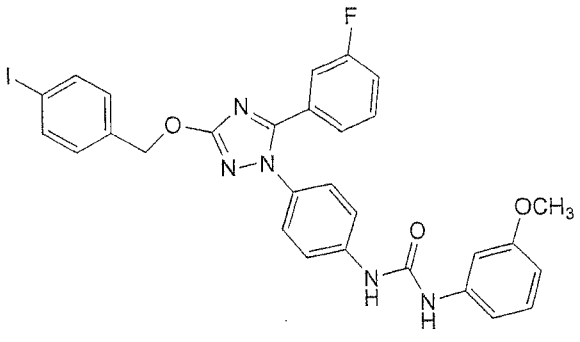
(MI-2)



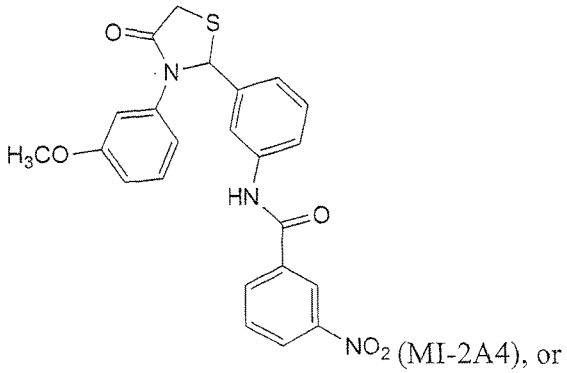
(MI-2A1)



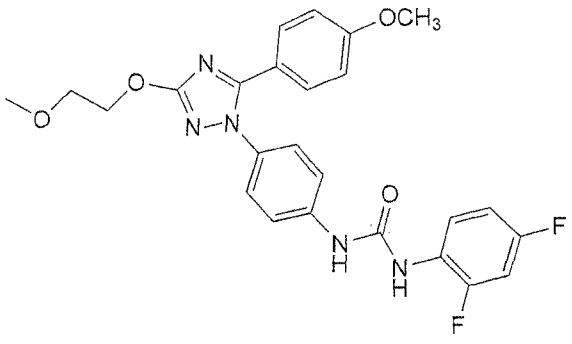
(MI-2A2)



(MI-2A3)



(MI-2A4), or



(MI-2A5),

or any salt, hydrate, tautomer, or stereoisomer thereof.

~~11~~9. The ~~method~~compound of claim ~~7~~5, wherein the cancer is a lymphoma.

~~12~~10. The ~~method~~compound of claim ~~11~~9, wherein the lymphoma is a diffuse large B-cell lymphoma.

~~13~~11. A method of identifying a small molecule modulator of MALT1, comprising contacting a recombinant form of MALT1 (340-789) fused with a leucine zipper dimerization motif (LZ-MALT1) and a candidate modulator compound, using the MALT1 substrate peptide LRSR linked to the fluorogen AMC (7-amino-4-methylcoumarin), such that cleavage of the Ac-LRSR-AMC substrate by MALT1 results in release of AMC and a fluorescent signal, wherein a decrease in the cleavage of the Ac-LRSR-AMC substrate by the recombinant form of MALT1 in the presence of the candidate modulator indicates that the candidate modulator is a small molecule modulator of MALT1.