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(56) Fremdragne publikationer:
WO-A1-2007/059613
WO-A1-2011/005790
WO-A2-2007/007207
ROMAGNOLI, R. ET AL.: 'Symmetrical alfa- bromoacryloylamido diaryldienone derivatives as a novel series of antiproliferative agents. Design, synthesis and biological evaluation' BIOORG. MED. CHEM. LETT. vol. 20, no. 9, 2010, pages 2733 - 2739, XP027012823
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ALEO, E. ET AL.: 'Idenfitication o new compounds that trigger apoptosome-independent caspase activation and apoptosis' CANCER RES. vol. 66, no. 18, 2006, pages 9235 - 9244, XP055149448

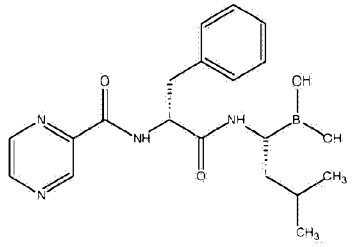
DESCRIPTION

FIELD OF THE INVENTION

[0001] The invention relates to the compounds and the compounds for use in treating cancer in a patient by inhibiting deubiquitinating activity. More particularly, the invention relates to compounds for use in a method of treating a cancer in a patient who has proved resistant to treatment by at least one anti-cancer medicine. Most particularly, the invention relates to a compound for use in the method and to a pharmaceutical composition comprising the compound.

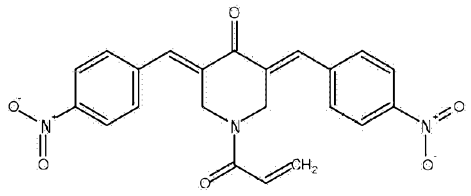
BACKGROUND OF THE INVENTION

[0002] Tumor cells display enhanced sensitivity to disruptions in the ubiquitin-proteasome system (UPS) making this an attractive target for the development of anti-cancer therapies (1). Ubiquitin-tagged substrates are degraded by the 26S proteasome, a multi-subunit complex comprising a proteolytic 20S core (20S CP) capped by 19S regulatory particles (19S RP) (2,3). The 20S CP has evolved as an important target for anti-cancer drug development, resulting in the approval of bortezomib (Velcade®) for treatment of myeloic leukemia (4).



bortezomib

[0003] The compound b-AP15 (NSC687852) is known to induce p53-independent and cathepsin-D-dependent apoptosis (5,6).



b-AP15 (NSC687852)

OBJECTS OF THE INVENTION

[0004] It is an object of the invention to provide a compound for use in a method of treating cancer in a patient by inhibiting deubiquitinating activity, in particular a cancer refractory to state-of-the-art chemotherapy.

[0005] WO 2007/007207 discloses compounds useful for treating a variety of cell proliferative disorders such as cancers. The compounds are based on a 6-carbon core having phenyl rings attached and acts by inhibiting tyrosine kinase activity. There is no discussion about inhibition of the proteasome by blocking ubiquitination at all.

[0006] Romagnoli et al., (Symmetrical alfa bromoacryloylamido diaryldieone derivatives as a novel serie of antiproliferative agents. Design, synthesis and biological evaluation. Bioorg. Med. Chem. Lett., Vol. 20, No. 9, 2010) describes 1,5 diaryl-3-oxo-1,4-pentadienyl-compounds (Ib) demonstrating anticancer activity. The author concludes that hybrid compounds containing the bromoalphabromoacryloyl moieties are potential anticancer drugs. Again, there is no discussion regarding proteasome ubiquitination inhibition ad no guidance to develop compounds of general formula I of the present application.

[0007] WO 2011/005790 discloses curcumin derivatives, having antioxidant properties and suggests using these for protecting the normal cells from associated oxidative da mage during chemotherapy, thereby increasing anticancer efficacy.

[0008] Aleo et al., (Identification of new compounds that trigger apoptosome-independent caspase activation and apoptosis, Cancer Res, Vol. 66, No. 18, 2006) and Berndtsson *et al.,* 2009 (6), Both disclose compounds having a 3,5-di(phenylmethylene)-piperidin-4-one that act against cancer via inhibition of the ubiquitin-proteasome system. The compounds inhibit the ubiquitin isopeptidase activity of the proteasome and induce apoptosis.
The chalcone derivatives of Aleo *et al.,* 2006, inhibits a broader range of DUBs, i. e., are less specific than the compounds of the present invention. Both articles are silent regarding subunit 19S RP of the proteasome as well of refractory cancers.

[0009] WO 2007/059613 discloses 4-piperidone derivatives having anticancer effect but do not disclose compounds of the present invention (7-14 membered ring) nor discuss action on the 19S-unit of the 26S-proteasome.

[0010] Another object of the invention is to provide a compound of the aforementioned kind, which has improved solubility at physiological pH in respect of functionally equivalent compounds known in the art.

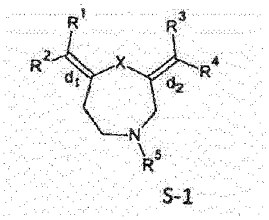
[0011] An additional object of the invention is to provide compounds for use in treating cancer.

[0012] A further object of the invention is to provide a pharmaceutical composition comprising the compound.

[0013] Still further objects of the invention will become evident by studying the following summary of the invention, a number of preferred embodiments thereof illustrated in a drawing, and the appended claims.

SUMMARY OF THE INVENTION

[0014] According to the present invention is disclosed a compound of the general structure S:



S-1

capable of abrogating the deubiquitinating (DUB) activity of the 19S RP DUBs.
The compound of the invention is recognized as pertaining to a novel class of proteasome inhibitors of which the known compound b-AP15 is a representative.

[0015] In particular, according to the present invention, the compound of the invention inhibits the activity of two 19S RP DUBs, UCHL5 and USP14 while not affecting non-proteasomal DUBs. More particularly, the compound of the invention has effect in the treatment of a cancer tumor refractory to state-of-the-art chemotherapy due to over-expression of the intrinsic apoptosis-inhibitor Bcl-2.

[0016] In another preferred
embodiment, the compound is effective in the treatment of a cancer refractory to any anti-cancer drug known in the art.

[0017] In this application, "refractory to treatment" signifies that treatment of a cancer with a single dose of an anti-cancer medicine does not substantially reduce the growth rate of the cancer observed immediately prior to the treatment, such as reducing the growth rate per month by not more than 25 per cent or 10 per cent or even 5 percent or less. An accepted measure of tumor growth is the change of volume of a non-disseminated cancer.

[0018] An example of a cancer amenable to treatment by the method of the invention is multiple myeloma. Other examples of cancers amenable to treatment comprise lung cancer, prostate cancer, colon cancer, ovary cancer, pancreas cancer, breast cancer, neck & head cancer.

[0019] In the compound of the invention of the general structure S-1,

R^1, R^2 at double bond d1 and R^3, R^4 at double bond d2 can, independent of each other, have a configuration opposite to that of formula S-1, X is CO or CS;

R^1 and R^3 are H;

R^2 and R^4 are, independent of each other, H; C_{1-6}-alkyl; C_{1-5}-alkylCO; phenyl or 6-memberedheteroaryl optionally substituted by 1-3 of: C_{1-6}-alkyl, C_{1-6}-alkoxy, CN,-$COOC_{1-6}$ alkyl, COOH, NO_2, F, Cl, CF_3, NH_2, NHC_{1-6}-alkyl, $N(C_{1-6}\text{-alkyl})_2$, $CONR^7R^8$, with the proviso that one or more of H in alkyl and alkoxy can be substituted by fluoro;

R^5 is H; C_{1-6}-alkyl; C_{2-6}-alkenyl; C_{1-3}-alkoxy-C_{2-6}-alkyl-; C_{1-3}-alkoxy-C_{2-6}-alkenyl-; aryl-C_{0-6}-alkyl-; heteroaryl-C_{0-6}-alkyl-; heterocyclyl-C_{0-6}-alkyl-; cycloalkyl-C_{0-6}-alkyl-; -C_{1-6}-alkyl-$COOC_{1-6}$-alkyl; -C_{2-6}-alkyl-aryloxy; COR^6;

R^6 is selected from: C_{1-6}-alkyl; C_{2-6}-alkenyl; C_{1-6}-alkoxy; C_{1-3}-alkoxy-C_{1-6}-alkyl-; C_{1-3}-alkoxy-C_{1-6}-alkenyl-; aryl-C_{0-6}-alkyl-; heteroaryl-C_{0-6}-alkyl-; heterocyclyl-C_{0-6}-alkyl-; cycloalkyl-

C_{0-6}-alkyl-; -C_{1-6}-alkyl-$COOC_{1-6}$-alkyl; NH_2; -NHC_{1-6}-alkyl; -$N(C_{1-6}$-alkyl$)_2$; -C_{0-6}-alkyl-aryloxy;

R^7, R^8 are, independent of each other, H or C_{1-3}-alkyl.

[0020] It is preferred for both of R^2 and R^4 to be H; C_{1-6}-alkyl; C_{1-5}-alkylCO; phenyl or 6-membered heteroaryl optionally substituted by 1-3 of: C_{1-6}-alkyl, C_{1-6}-alkoxy, CN, $COOC_{1-6}$-alkyl, COOH, NO_2, F, Cl, CF_3, NH_2, NHC_{1-6}-alkyl, $N(C_{1-6}$-alkyl$)_2$, $CONR^7R^8$, with the proviso that one or more of H in alkyl and alkoxy can be substituted by fluoro, and wherein substitution of phenyl is preferably at one or more of positions 3, 4, 5.

[0021] It is particularly preferred for both of R^2 and R^4 to be phenyl substituted at one or more of positions 3, 4, 5 by 1-3, preferably by 1 or 2, of: C_{1-6}-alkyl, C_{1-6}-alkoxy, CN, -$COOC_{1-6}$ alkyl, COOH, NO_2, F, Cl, CF_3, NH_2, NHC_{1-6}-alkyl, $N(C_{1-6}$-alkyl$)_2$, $CONR^7R^8$, with the proviso that one or more of H in alkyl and alkoxy can be substituted by fluoro. Most preferred are electron-withdrawing substituents, in particular F, Cl, trifluoromethyl, NO_2, CN.

[0022] It is preferred for R^5 to be selected from the group consisting of H, methyl, acetyl, $COCH=CH_2$, 2-acetoxyethyl.

[0023] According to a preferred aspect of the invention X = CO. According to another preferred aspect of the invention R^1 and R^3 are both H. According to a third preferred aspect of the invention, R^2 and R^4 are, independent of each other, phenyl or 6-membered heteroaryl optionally substituted by 1-3 of: C_{1-6}-alkyl, C_{1-6}-alkoxy, CN, -$COOC_{1-6}$-alkyl, COOH, NO_2, F, Cl, CF_3, NH_2, NHC_{1-6}-alkyl, $N(C_{1-6}$-alkyl$)_2$, $CONR^7R^8$, phenyl being preferred and substitution of phenyl, if any, being preferred in one or more of positions 3, 4, 5.

[0024] According to a preferred aspect of the invention R^1, R^2 at double bond d1 and R^3, R^4 at double bond d2 have the configuration of formula S-1; X is CO or CS; R^1 and R^3 are, independent of each other, H; R^2 and R^4 are, independent of each other, H; C_{1-6}-alkyl; C_{1-5}-alkylCO; phenyl or 6-membered heteroaryl
substituted with 1-3 of: CN, NO_2, F, Cl, NH_2, NHC_{1-6}-alkyl, $N(C_{1-6}$-alkyl$)_2$, COC_{1-6}-alkyl; R^5 is H, C_{1-6}-alkyl, C_{2-6}-alkenyl, C_{1-3}-alkoxy-C_{1-6}-alkyl, C_{1-3}alkoxy-C_{1-6}-alkenyl, aryl, heteroaryl, heterocyclyl, C_{1-6}-alkyl-heteroaryl, C_{1-6}-alkyl-heterocyclyl, C_{1-6}-alkyl-cycloalkyl, C_{1-6}-alkylaryl, CO-C_{1-6}-alkyl, CO-vinyl, CO-allyl, CO-aryl, CO-cycloalkyl. It is preferred, independent of each other, for X to be CO, for R^2 and R^4 to be substituted phenyl, for R^5 to be selected from COR^6, in particular from CO-C_{1-6}-alkyl, CO-cycloalkyl, CO-vinyl, CO-allyl.

[0025] "Aryl" refers to a monocyclic or bicyclic hydrocarbon of from 6 to 10 carbon atoms comprising at least one aromatic ring. "Aryloxy" refers to an aryl group bonded to an oxygen atom. "Heteroaryl" represents a monocyclic ring system having 5 or 6 ring atoms, of which one or more are selected independently from oxygen, nitrogen, sulphur. "Alkyl" denotes straight or branched

alkyl. "Alkenyl" denotes straight or branched alkenyl. "Alkoxy" denotes straight or branched alkoxy. "Cycloalkyl" refers to a saturated monocyclic hydrocarbon of from 3 to 7 carbon atoms.

[0026] Preferred compounds of the invention of the general structure S-1 are disclosed in Tables 1 and 2.

Table 1. Preferred compounds of the invention

X = CO, R^1 = R^3 = H, R^5 is H or alkyl					
#	R^2	R^4	R^3	HCT116, FMCA, IC50 (μM)	MeJuSo-UB, IncuCyte lowest effective conc. (μM)
1516	phenyl	phenyl	H		1.2
1517	4-methoxyphenyl	4-methoxyphenyl	H		
1518	4-chlorphenyl	4-chlorophenyl	H		1.6
1533	3-acetylphenyl	3-acetylphenyl	H		
1535	3-nitrophenyl	3-nitrophenyl	H	5.9	
1536	2-nitrophenyl	2-nitrophenyl	H	6.9	
1537	4-nitrophenyl	4-nitrophenyl	H	4.1	
1560	4-nitrophenyl*	H**	H	1.0	
1561	4-fluorophenyl	4-fluorophenyl	H	1.0	1
1562	4-fluoro-3-nitrophenyl	4-fluoro-3-nitrophenyl	H	0.5	0.25
1563	4-nitrophenyl	4-nitrophenyl	methyl	1.5	0.5
1564	4-fluorophenyl	4-fluorophenyl	methyl	0.9	16
1565	4-fluoro-3-nitrophenyl	4-fluoro-3-nitrophenyl	methyl	1.5	0.5
1566	3-nitrophenyl	3-nitrophenyl	methyl	2.3	0.5
1574	4-fluorophenyl	4-fluorophenyl	propyl	2.8	0.5
1575	4-nitrophenyl	4-methoxyphenyl	H	2.1	1
1576	4-fluorophenyl	4-methoxyphenyl	H	1.6	8
1577	4-fluorophenyl	4-methoxyphenyl	methyl	4.9	8
1582	4-fluorophenyl	4-chlorophenyl	H	1.7	2
1583	4-chlorophenyl	4-nitrophenyl	methyl	3.3	2
1584	4-chlorophenyl	4-nitrophenyl	H	1.8	2
1585	4-fluorophenyl	4-nitrophenyl	H	1.5	2
1586	4-chlorophenyl	4-fluorophenyl	H	1.1	0.5
1587	4-fluorophenyl	4-nitrophenyl	methyl	3.0	1
1588	4-nitrophenyl	4-methoxyphenyl	methyl	3.1	1
1589	4-chlorophenyl	4-fluorophenyl	methyl	2.5	1

X = CO, R^1 = R^3 = H, R^5 is H or alkyl					
#	R^2	R^4	R^3	HCT116, FMCA, IC50 (µM)	MeJuSo-UB, IncuCyte lowest effective conc. (µM)
1590	4-chlorophenyl	4-methoxyphenyl	methyl	2.0	1
1591	4-nitrophenyl	4-chlorophenyl	H	0.9	0.5
1592	4-chlorophenyl	4-nitrophenyl	H	12	4
1593	4-nitrophenyl	4-fluorophenyl	methyl	2.9	1
1594	4-nitrophenyl	4-chlorophenyl	methyl	2.6	1
1595	4-fluorophenyl	4-chlorophenyl	methyl	2.3	1
1596	4-nitrophenyl	4-fluorophenyl	H	2.6	2
1608	3-chloro-4-fluorophenyl	3-chloro-4-fluorophenyl	methyl	1.8	1
1609	4-fluoro-3-trifluoromethyl-phenyl	4-fluoro-3-trifluoromethyl-phenyl	methyl	1.4	1
1610	3,4-difluorophenyl	3,4-difluorophenyl	methyl	1.7	1
1611	3-fluoro-5-trifluoromethyl-phenyl	3-fluoro-5-trifluoromethyl-phenyl	H	1.1	0.5
1612	3-fluoro-5-trifluoromethyl-phenyl	3-fluoro-5-trifluoromethyl-phenyl	methyl	0.7	0.25
1613	4-nitrophenyl	4-nitrophenyl	H	25	32
1614	4-nitrophenyl	4-nitrophenyl	methyl	7.4	8
1615	4-chloro-3-trifluoromethylphenyl	4-chloro-3-trifluoromethyl phenyl	methyl	0.9	0.5
1616	3,4,5-trifluoromethylphenyl	3,4,5-trifluoromethylphenyl	methyl	1.4	0.5
1617	4-trifluoromethylphenyl	4-trifluorometylphenyl	methyl	1.6	1
1618	3-cyano-4-fluorophenyl	3-cyano-4-fluorophenyl	methyl	1.5	0.5
1619	3-carbonylaminophenyl	3-carbonylaminophenyl	H	30	32
1620	3-nitrophenyl	3-nitrophenyl	methyl	>32	no effect
1621	4-cyanophenyl	4-cyanophenyl	methyl	4.5	2
1622	4-fluoro-3-trifluoromethyl-phenyl	4-fluoro-3-trifluoromethyl-phenyl	H	0.8	0.5

X = CO, R^1 = R^3 = H, R^5 is H or alkyl					
#	R^2	R^4	R^3	HCT116, FMCA, IC50 (µM)	MeJuSo-UB, IncuCyte lowest effective conc. (µM)
1623	3-cyano-4-fluorophenyl	3-cyano-4-fluorophenyl	H	1.0	0.5
1624	3-fluoro-4-trifluoromethyl-phenyl	3-fluoro-4-trifluoromethyl-phenyl	methyl	1.8	1
1625	4-cyanophenyl	4-cyanophenyl	H	0.9	0.5
1626	3-fluoro-4-trifluoromethyl-phenyl	3-fluoro-4-trifluoromethyl-phenyl	H	0.8	8
* or H or a mixture of H and 4-nitrophenyl ** or 4-nitrophenyl or a mixture of H and 4-nitrophenyl					

[0027] Due to protonation of their amino group the solubility in aqueous media of azepanone compounds of the invention of which R^5 is not acyl as well as of correspondingly substituted piperidin-4-ones increases with decreasing pH. However, according to an important aspect azepanone compounds of the invention of which R^5 is not acyl (that is, not -COR^6) have superior solubility in aqueous media at physiological pH in comparison with correspondingly substituted piperidin-4-ones. While the solubility of these azepanones and piperidine-4-ones increases in going from a high pH to a low pH, the increase starts at higher pH values for the azepanones than for the corresponding piperidin-4-ones. In this application "physiological pH" is a pH of from about 6 to about 8, in particular from 7.0 to 7.5.

Table 2. Preferred compounds of the invention

X = CO, R^1 = R^3 = H, R^5 = COR^6					
#	R^2	R^4	R^6	HCT116, FMCA, IC50 (µM)	MeJuSo-UB, lowest effective conc. (µM)
1505	4-nitrophenyl	4-nitrophenyl	2-pyrrolidinyl	5.2	16
1507	4-nitrophenyl	4-nitrophenyl	2-(1-carboxyethyl-ethyl)	4.4	8
1520	phenyl	phenyl	vinyl		
1521	phenyl	phenyl	cyclobutyl		
1525	4-methoxyphenyl	4-methoxyphenyl	cyclobutyl		
1526	4-methoxyphenyl	4-methoxyphenyl	cyclopropyl		

X = CO, R^1 = R^3 = H, R^5 = COR^6					
#	R^2	R^4	R^6	HCT116, FMCA, IC50 (μM)	MeJuSo-UB, lowest effective conc. (μM)
1527	4-chlorophenyl	4-chlorophenyl	cyclobutyl		2
1546	4-nitrophenyl	4-nitrophenyl	vinyl	1.2	2
1567	4-nitrophenyl	4-nitrophenyl	methyl	0.6	0.5
1568	4-fluorophenyl	4-fluorophenyl	vinyl	1.5	2
1569	4-fluorophenyl	4-fluorophenyl	vinyl	2.0	4
1570	4-fluoro-3-nitrophenyl	4-fluoro-3-nitrophenyl	vinyl	0.5	0.25
1571	4-fluoro-3-nitrophenyl	4-fluoro-3-nitrophenyl	methyl	0.9	0.25
1572	3-nitrophenyl	3-nitrophenyl	vinyl	2.5	0.5
1578	4-fluorophenyl	4-methoxyphenyl	methyl	7.0	8
1579	4-fluorophenyl	4-methoxyphenyl	methyl	5.9	8
1580	4-nitrophenyl	4-methoxyphenyl	vinyl	1.5	8
1581	4-nitrophenyl	4-methoxyphenyl	methyl	7.2	8
1597	4-nitrophenyl	4-chlorophenyl	methyl	1.3	1
1627	4-trifluoromethylphenyl	4-trifluoromethylphenyl	methyl	0.7	1
1628	3,4-difluorophenyl	3,4-difluorophyenyl	methyl	2.3	1
1629	3,4,5-trifluorophenyl	3,4,5-trifluorophenyl	methyl	0.6	1
1630	4-chloro-3-fluorophenyl	4-chloro-3-fluorophenyl	methyl	0.9	0.5
1631	3-chloro-4-fluorophenyl	3-chloro-4-fluorophenyl	methyl	1.0	32
1633	4-chlorophenyl	4-chlorophenyl	2-acetoxyethyl	2.2	4
1635	4-chlorophenyl	4-chlorophenyl	benzyl	1.4	2
1636	4-chlorophenyl	4-chlorophenyl	1-(3-phenyl-2-propenyl)	2.0	1
1637	4-chlorophenyl	4-chlorophenyl	3-pyridyl	2.1	2
1638	4-chlorophenyl	4-chlorophenyl	2-thiophenyl	2.0	2
1639	4-chlorophenyl	4-chlorophenyl	4-hydroxy-3-ethoxybenzyl	1.2	1
1640	4-chlorophenyl	4-chlorophenyl	methyl-(2-methoxy-carboxyl)phenyl	1.9	1

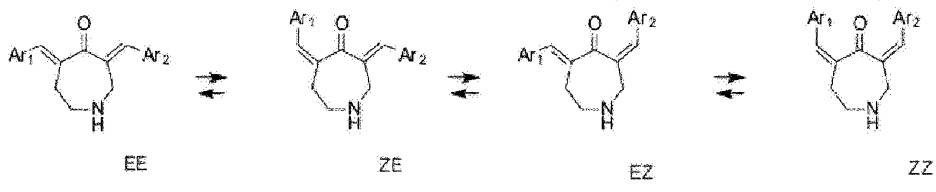
X = CO, R^1 = R^3 = H, R^5 = COR^6					
#	R^2	R^4	R^6	HCT116, FMCA, IC50 (µM)	MeJuSo-UB, lowest effective conc. (µM)
1641	4-trifluoromethylphenyl	4-trifluoromethylphenyl	methyl-3-pyridyl	2.7	2
1642	4-trifluoromethylphenyl	4-trifluoromethylphenyl	2-oxo-acetoxyethyl	1.2	1
1643	4-trifluoromethylphenyl	4-trifluoromethylphenyl	3-(3-oxo-propanoyloxy-methyl)	0.9	1
1644	4-trifluoromethylphenyl	4-trifluoromethylphenyl	1-oxo-2-(2-pyridyl)ethyl	1.4	1
1645	4-chloro-3-fluorophenyl	4-chloro-3-fluorophenyl	2-oxo-acetoxyethyl	2.5	2
1646	4-fluoro-3-nitrophenyl	4-fluoro-3-nitrophenyl	2-oxo-acetoxymethyl	1.1	1
1647	4-fluoro-3-nitrophenyl	4-fluoro-3-nitrophenyl	3-(3-oxo-propanoyloxy-methyl)	0.7	0.25
1648	4-fluoro-3-nitrophenyl	4-fluoro-3-nitrophenyl	1-oxo-2-(2-pyridyl)ethyl	0.8	0.5
1649	3,4,5-trifluorophenyl	3,4,5-trifluorophenyl	methyl-(2-methoxy-carboxyl)phenyl	0.9	0.5
1650	3,4,5-trifluorophenyl	3,4,5-trifluorophenyl	methyl-3-pyridyl	1.9	1
1651	3,4,5-trifluorophenyl	3,4,5-trifluorophenyl	2-oxo-acetoxyethyl	0.8	0.5
1652	3,4,5-trifluorophenyl	3,4,5-trifluorophenyl	3-(3-oxo-propanoyloxy-methyl	0.6	0.5
1653	3,4,5-trifluorophenyl	3,4,5-trifluorophenyl	1-oxo-2-(2-pyridyl)ethyl	0.71	1
1654	4-chloro-3-trifluoro methyl-phenyl	4-chloro-3-fluoromethyl-phenyl	methyl-(2-methoxy-carboxyl)phenyl	1.2	1
1655	4-chloro-3-trifluoro methyl-phenyl	4-chloro-3-trifluoro methyl-phenyl	methyl-3-pyridyl	1.0	1
1656	4-chloro-3-trifluoro methyl-phenyl	4-chloro-3-trifluoro methyl-phenyl	2-oxo-acetoxyethyl	0.7	0.5
1657	4-chloro-3-trifluoro methyl-phenyl	4-chloro-3-trifluoro methyl-phenyl	acetyl	0.6	0.5

X = CO, R^1 = R^3 = H, R^5 = COR^6					
#	R^2	R^4	R^6	HCT116, FMCA, IC50 (µM)	MeJuSo-UB, lowest effective conc. (µM)
1658	4-chloro-3-trifluoro methyl-phenyl	4-chloro-3-trifluoro methyl-phenyl	1-oxo-2-(2-pyridyl)ethyl	0.8	0.5
1659	4-trifluoromethylphenyl	4-trifluoromethylphenyl	2-acetoxyethyl	2.4	2
1660	4-chloro-3-fluorophenyl	4-chloro-3-fluorophenyl	2-acetoxyethyl	2.4	2
1661	4-chloro-3-fluorophenyl	4-chloro-3-fluorophenyl	methylcarboxyl	24	32
1662	4-fluoro-3-nitrophenyl	4-fluoro-3-nitrophenyl	2-acetoxyethyl	2.1	1
1663	4-fluoro-3-nitrophenyl	4-fluoro-3-nitrophenyl	methylcarboxyl	3.1	4
1664	3,4,5-trifluorophenyl	3,4,5-trifluorophenyl	2-acetoxylethyl	2.8	4
1665	3,4,5-trifluorophenyl	3,4,5-trifluorophenyl	methylcarboxyl	5.0	8
1666	4-chloro-3-trifluoromethylphenyl	4-chloro-3-trifluoromethylphenyl	2-acetoxyethyl	1.3	1

[0028] The solubility in aqueous media of compounds of the invention of which R^5 is acyl (that is, -COR^6) is substantially independent of pH.

[0029] Particularly preferred compounds of the invention are compounds nos. 1561, 1562, 1567, 1570, 1571, 1586, 1591, 1600, 1612, 1618, 1622, 1625, 1643, 1644, 1647, 1648, 1649, 1652, 1653, 1656, 1657, 1658, 1662. Most preferred compounds of the invention are compounds nos. 1570, 1571, 1625, 1662.

[0030] Since the compound of the invention comprises a 1,5-disubstituted 1,4-pentene-3-one moiety it can exist in four cis/trans isomers EE, ZE, ES, ZZ. In defining the compound of the invention this isomerism is defined in the foregoing as "R^1, R^2 at double bond d1 and R^3, R^4 at double bond d2 can, independent of each other, have a configuration opposite to that of formula S1". The compound of the invention comprises any such isomer and any mixture of such isomers.



[0031] In synthesis the compound of the invention is obtained as a mixture of isomer but sometimes also in form of the isomer with the lowest solubility in the particular solvent, from which it precipitates or crystallizes. While pure isomers thus can be obtained under controlled conditions, the pharmacological effect of the compound is exhibited by all isomers. The reason for this is their equilibration in the presence of water or other hydroxylic or sulfhydrylic solvent or agent, which is accelerated by acid and base catalysis.

[0032] Accordingly, the term "compound of the invention" as used herein comprises a pure isomer of the aforementioned kind as well as a mixture of two or more such isomers. The rate of equilibration of the compound of the invention in aqueous body fluid is sufficient to provide for substantial equilibration within a single treatment period.

[0033] The compound of the invention comprises an azepane moiety, preferably an azepane-4-one moiety. According to an important aspect of the invention, the compound of the invention exhibits a cytotoxic activity superior to that of a structurally corresponding compound comprising a piperidine moiety, such as a 4-piperidinone moiety.

[0034] According to another important aspect of the invention, the compound of the invention comprising an azepane moiety, in particular an azepan-4-one moiety exhibits a solubility in a liquid carrier suitable for administration to a patient, such as dimethyl sulfoxide, superior to that of a structurally corresponding compound comprising a piperidine moiety, such as a 4-piperidinone moiety.

[0035] A "single treatment period" is the period of time elapsing between administration and consumption of the compound of the invention, that is, the point in time at which the concentration of the compound of the invention at a site of action, such as in a tumor, has been reduced by 90 % or 95 % or 99 % and more. In a pharmaceutical composition, an isomer or a mixture of isomers of the compound of the invention is stabilized against isomerization by careful exclusion of moisture.

[0036] The use of the invention comprises administering to the patient in need a pharmacologically effective dose of the compound of the invention in a suitable pharmaceutical carrier, such as, for instance, dissolved or suspended in an aqueous carrier or in a carrier comprising dimethyl sulfoxide or N,N-dimethylacetamide. Administration can be by any suitable route, such as by intravenous, intramuscular, intraperitoneal or subcutaneous injection or infusion. Other methods of administration, in particular *per os,* are also contemplated, such as in form of tablets or hard or soft gelatin capsules.

[0037] The person skilled in the art knows how to determine a pharmacologically effective dose. Such a dose may be from 0.0001g/kg to 0.1 g/kg body weight, in particular from 0.001 g/kg to 0.01 g/kg body weight, consideration being given to whether the agent is administered systemically or locally.

[0038] Consistent with DUB inhibition, treatment with the compound of the invention causes the accumulation of polyubiquitinated proteins of higher molecular weight in comparison with bortezomib treatment, and results in a stronger unfolded protein response. According to the

invention, it has also been found that apoptosis induction by the compound of the invention differs from that of bortezomib by being insensitive to disruption of the p53 tumor suppressor and insensitive to overexpression of the apoptosis inhibitor Bcl-2.

[0039] According to the present invention use of the compounds for treatment inhibits tumor progression in human and mouse tumor *in vivo* models of breast, lung, colon, head & neck carcinoma, and inhibits infiltration in an acute myeloid leukaemia (AML) model. In consequence, inhibiting the DUB activity of the 19S RP by the compound of the invention is disclosed to be a viable option for the treatment of cancer in humans and animals.

[0040] According to a preferred aspect of the invention the 19S RP DUBs comprise UCHL5 and USP14. According to another preferred aspect of the invention the deubiquitinating (DUB) activity of non-proteasomal DUBs is not affected by the compound of the invention. The compound of the invention can be administered dissolved or suspended in a liquid carrier by any suitable route, such as by intravenous, intramuscular and subcutaneous administration. Alternatively or additionally, the compound of the invention can be administered perorally, such as in form of a tablet or capsule. A useful pharmacologically effective dose of the compound of the invention is from 0.0001g/kg to 0.1 g/kg body weight, in particular from 0.001 g/kg to 0.01 g/kg body weight, consideration being given to whether the compound is administered systemically or locally.

[0041] The compound of the invention blocks cellular proteasome function, as confirmed by use of a reporter cell line, which expresses ubiquitin tagged to yellow fluorescent protein (UbG76V-YFP) constitutively targeted for proteasomal degradation (12). Immunoblotting and flow cytometry revealed a dose dependent accumulation of the Ub-YFP reporter (IC50=0.8 μM) suggesting an impairment of proteasome function. Since inhibition of proteasome function is characterized by defects in ubiquitin turnover (13) colon carcinoma HCT116 cells were treated with the compound of the invention and the level of ubiqutin conjugation analyzed by immunoblotting. The treatment caused the rapid time dependent accumulation of polyubiquitinated proteins of a higher molecular weight in comparison with the 20S CP inhibitor bortezomib, suggesting that the compound of the invention inhibits an alternative branch of the UPS. The increase in polyubiquitin is associated with a strong proteotoxic response characterized by induction of HSPA6 (Hsp70B'), HSPA1B and DNAJB1 (Hsp40).

[0042] The turnover of many cell cycle regulatory proteins is controlled by the UPS including inhibitors of the cyclin-dependent kinase $p21^{Cip1}$, $p27^{Kip1}$ and the tumor suppressor p53 (4). Treatment with the compound of the invention increases their levels in a dose dependent manner without altering the levels of ornithine decarboxylase 1 (ODC1), an ubiquitin-independent proteasome substrate (8). The increase in cell cycle regulators was concomitant with growth arrest in the G2/M phase boundary and increased sub G1 DNA content. The cell cycle arrest observed is not associated with increased levels of DNA damage markers such as phosphorylated p53 (at Ser 15) (9) or H2AX (at Ser 139) (10), suggesting that b-AP15 is not a genotoxic agent.

[0043] The increase in sub G1 DNA, caspase-3 activation and cleavage of poly-ADP ribose polymerase (PARP) and cytokeratin is associated with an overall decrease in cell viability at drug concentrations that induce the accumulation of polyubiquitin connecting UPS inhibition and

apoptosis. Apoptosis induction by bortezomib is sensitive to the status of the p53 tumor suppressor and over-expression of the anti-apoptotic Bcl-2 oncoprotein (11, 12). By using isogenic clones of HCT116 colon cancer cells it was demonstrated that b-AP15 induced apoptosis is insensitive to over-expression of Bcl-2 and disruption of the apoptotic regulators p52, BAX or PUMA. Measurement of cytotoxic activity shows that the compound of the invention is more toxic to the colon carcinoma cell line HTC-116 than to immortalized retinal pigment epithelial cells (hTERT-RPE1) and peripheral blood mononuclear cells (PBMC). The compound of the invention exhibits a higher degree of cytotoxic activity towards the HTC-116 cells than towards normal cell types.

[0044] The observed reduction in cellular proteasome activity cannot be explained by inhibition of proteolytic activities of the β subunits of the 20S CP. *In vitro* experiments using activity-specific substrates do not show inhibition in any of the proteolytic activities of the 20S CP or 26S proteasome, disassociation of the 19S RP and 20S CP or inhibition of polyubiquitin binding to the proteasome.

[0045] The compound of the invention comprises an α-β dienone entity with two sterically accessible β carbons. A structurally similar pharmacophore has been earlier described to be comprised by a class of ubiquitin isopeptidase inhibitors (13). However, when cellular DUB activity was tested using ubiquitin 7-amido-4-methylcoumarin (Ub-AMC) on treated cells treated with the compound of the invention, no reduction in Ub-AMC cleavage was observed. This demonstrates that the compound of the invention is not a general DUB inhibitor. While not wishing to be bound by theory, the similarities in pharmacophore structure and the data showing that compound of the invention inhibits proteasome activity independent of the 20S CP indicate that the compound of the invention inhibits the proteasome by blocking the deubiquitinating activity of the 19S RP.

[0046] *In vitro* assays using Ub-AMC and purified 19S RP or 26S proteasomes confirmed that the compound of the invention inhibits the deubiquitinating activity of both the 19S RP and 26S proteasome. Recombinant ubiquitin-GFP is a substrate for 19S RP DUB activity (15). Treatment of 19S RP with b-AP15 efficiently inhibited the cleavage of Ub-GFP and ubiquitinated HDM2. The type of ubiquitin bonds present in the polyubiquitin chain determines the fate of an ubiquitin-modified substrate.

[0047] K48 linked polyubiquitin chains generally target conjoined proteins for degradation (14), whereas K63 linked chains are involved in non-proteolytic roles including DNA repair (15) and mitotic chromosome segregation (16). Ubiquitin chain disassembly reactions revealed that the compound of the invention inhibits 19S RP processing of both K48 and K63 linked ubiquitin tetramers. The inhibition of ubiquitin chain disassembly observed may account for the accumulation of high molecular weight ubiquitin conjugates in cells treated with the compound of the invention.

[0048] The deubiquitinating activity of the proteasome is attributed to the action of three DUBs, UCHL5, USP14 and POH1, all localized within the 19S RP (17-19). Both UCHL5 and USP14 are sensitive to N-ethylmaleimide (NEM), a general inhibitor of cysteine proteases, whereas POH1 is insensitive to inhibition by NEM but sensitive to metal chelators such as N,N,N,N-tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN) (20). Inhibition experiments showed that residual DUB

activity is present even after co-treatment of 19S RP with NEM and the compound of the invention. This residual DUB activity was abolished upon co-treatment of 19S RP with the compound of the invention and TPEN, suggesting that the compound of the invention primarily inhibits one or both of the NEM sensitive cysteine DUBs. The β-carbons of the compound of the invention may serve as Michael acceptor moieties, resulting in covalent binding to cysteine residues in target proteins. *In vitro* assays showed, however, that the compound of the invention is a reversible inhibitor and that glutathione does not preclude the inhibitory activity of the compound.

[0049] To identify specifically which DUBs were inhibited by treatment with the compound of the invention, competitive labelling experiments were performed using hemagglutinin tagged ubiquitin vinylsulphonone (HA-UbVS), an active site directed probe that irreversibly reacts with DUBs of the cysteine class (17). Incubation of 19S RP or 26S proteasomes with the compound of the invention abolished Ub-VS labelling of two DUBs of molecular weights corresponding to UCHL5 and USP14. A similar result was obtained using UbVs on lysates derived from drug-treated cells. Immunoblot analysis showed a downward shift in molecular weight of both USP14 and UCHL5 due to loss of activity and decreased UbVs labelling. This is consistent with affinity-purified proteasomes from the compound of the invention treated cells displaying reduced DUB activity confined to the proteasome and not evident in cell lysates. Additional *in vitro* assays showed minimal inhibition of the compound of the invention on recombinant non-proteasomal cysteine DUBs, consistent with the notion that inhibition is not due to general cysteine reactivity.
The compound of the invention does substantially decrease and even stop tumor growth *in vivo,* as shown by its administration to mice bearing either a human tumor or mouse xenografts. When the compound of the invention is administered daily to SCID mice bearing FaDu head and neck carcinoma xenografts, significant inhibition of FaDu tumor growth is observed following daily treatment with the compound of the invention (treated/control tumor volume, T/C=0.4, p=<0.001). Tumor cell death was analyzed by measuring xenograft derived cytokeratin (CK18) in circulation. Cytokeratin-18 is a biomarker for apoptosis (21, 22); a significant increase in plasma levels of total human CK18 was observed (p=0.01). Levels of caspase cleaved CK18 (CK18-Asp396) increased moderately compared with total levels, suggesting that the compound of the invention has activity against tumor cells *in vivo*. The compound of the invention was also shown to inhibit tumor onset of HCT-116^{Bcl2+} colon carcinoma xenografts in nude mice, as demonstrated by significant delay in tumor onset in comparison to vehicle treated controls. Similarly, the compound of the invention inhibits tumor growth in syngenic mice models using less frequent administration schedules.

[0050] Ubiquitin C-terminal hydrolases (UCH) and ubiquitin specific proteases (USP) are major subgroups of the approximately one hundred DUBs encoded by the human genome (23). The mechanism of specificity of the compound of the invention for UCHL5 and USP14 in the 19S RP may be related to unique conformations of these enzymes in the 19S RP or due to drug-induced alterations of the 19S RP structure. The present findings are consistent with reports in the art indicating that loss of both UCHL5 and USP14, unlike loss of either one alone, leads to the accumulation of polyubiquitinated proteins and inhibition of cellular protein degradation (24).

[0051] The observation that DUB inhibition is associated with high molecular weight ubiquitin-substrate complexes seems to be of particular relevance. Strong expression of chaperone genes was observed in cells treated with the compound of the invention, indicating induction of a

proteotoxic response. High-molecular weight ubiquitin-substrate complexes accumulating as a result of DUB inhibition by the compound of the invention seem to generate strong cytotoxicity.

[0052] In the following the invention will be described in greater detail by reference to preferred embodiments thereof illustrated by a drawing comprising a number of figures.

DESCRIPTION OF THE FIGURES

[0053]

Figs. 1a to 1o are diagrams illustrating induction of dose-dependent cytotoxicity after 72 hours of continuous compound exposure to the reporter cell line HCT-116 by embodiments of the compound of the invention, as measured FMCA (Fluorometric Microculture Cytotoxicity Assay), as well as absence of such induction by structurally related compounds not comprised by the invention. Treated cells were compared to untreated controls (survival index);

Figs. 2a to 2e are diagrams illustrating the superior solubility of compounds of the invention in an aqueous media at physiological pH;

Figs. 3a to 3f are diagrams illustrating, by the method of Figs. 1a to 1o, the superior cytotoxicity of azepanone compounds of the invention in relation to structurally corresponding piperidin-4-one compounds not comprised by the invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

Methods

[0054] *In vitro* proteasome activity assays are performed in black 96-well microtitier plates using human 20S proteasome (Boston Biochem) in reaction buffer (25 mM Hepes, 0.5 mM EDTA, 0.03 % SDS) with Suc-LLVY-AMC, Z-LLE-AMC or Boc-LRRAMC used as substrates for proteasome activity. De-ubiquitinase activity assays are performed with human 19S RP (Boston Biochem) with ubiquitin-AMC as substrate. For FaDu xenograft studies a 100-µl-cell suspension containing $1x10^6$ cells is injected subcutaneously into the flank of SCID. Upon tumor take mice are randomized into control or treatment groups and administered with 5 mg kg^{-1} compound of the invention or vehicle. *In vivo* levels of apoptosis and cell death are determined from the detection of caspase cleaved and total levels of cytokeratin-18 in plasma using M30 Apoptosense® and M65 ELISA®s assays (Peviva). The methods are described below in more detail.

[0055] *Reagents*. Reagents were obtained from the following sources: 20S proteasome (E-360), 26S proteasome (E-365), 19S proteasome (E-366), Suc-LLVY-AMC (S-280), Z-LLE-AMC (S-230), Boc-LRR-AMC (S-300), Ubiquitin-AMC (U-550), Tetra-ubiquitin K63 (UC-310), Tetra-ubiquitin K48

(UC-210), deconjugating enzyme set (KE10), HA-Ubiquitin Vinyl Sulfone (U-212) (Boston Biochem); anti-β-actin (AC-15), ODC-1 (HPA001536) (Sigma Aldrich); anti-LC-3 (2775), anti-GAPDH (2118), anti-p44/42 MAPK (4695), anti-Phospho-p44/42 MAPK (9101)(Cell Signaling); N-ethylmaleimide (34115) (EMD Chemicals); anti-Ubiquitin K48 (Apu2), anti-Ubiquitin (MAB1510) (Millipore); anti-p53 (DO1), anti-UCHL5 (H-110), Hdm2 (SMP14) (Santa Cruz); anti-PARP (C2-10), anti-p27 (G173-524), anti-active Caspase 3 (C92-605) (BD Biosciences); anti-USP14 (A300-919A) (Bethyl Laboratories); anti-HA (12CA5) (Roche). Bortezomib was obtained from the Department of Oncology, Karolinska Hospital, Sweden.

[0056] *Cell culture*. MCF7 cells are maintained in MEM/10% fetal calf serum. HCT-116 p53 +/+, p53 -/-, Bcl-2 +/+, PUMA -/- and BAX -/- cells are maintained in McCoy's 5A modified medium/10% fetal calf serum. The HCT-116 p53 +/+, p53 -/-, PUMA -/- and BAX -/- are generated as described (25). The HCT-116 Bcl-2 +/+ cell line was generated by transfecting parental HCT-116 p53 +/+ cells with pCEP4 Bcl-2 (Addgene plasmid 16461) (26) and isolating high expression clones. FaDu and LLC3 cells are maintained in DMEM high glucose medium supplemented with 10% fetal calf serum, Na pyruvate, Hepes and non-essential amino acids. 4T1.12B carcinoma cells are maintained in RPMI medium supplemented with 10% fetal calf serum. The proteasome reporter cell line MelJuSo Ub-YFP was generated as described (12). Cells were maintained in Dulbecco's Modified Eagle's Medium/10 % fetal calf serum. The retinal epithelial cell line was generated as described (12). All cells are maintained in Dulbecco's Modified Eagle Medium/10 % fetal calf serum. The retinal epithelial cell line was generated as described (28). All cells are maintained at 37 °C in 5 % CO_2.

[0057] *Proteasome and DUB inhibition assays*. In vitro proteasome activity assays using 20S CP (2nM) (Boston Biochem) are performed at 37 °C in 100-µl reaction buffer (25 mM Hepes, 0.5 mM EDTA, 0.03 % SDS). Samples are incubated for 10 min with indicated compound followed by addition of 10 µM Suc-LLVY-AMC, Z-LLE-AMC or Boc-LRR-AMC for the detection of chymotrypsin-like, caspase-like and trypsin-like activity respectively. For DUB inhibition assays 19S RP (5 nM), 26S (5 nM) UCH-L1 (5 nM), UCH-L3 (0.3 nM), USP2CD (5 nM) USP7CD (5 nM) USP8CD (5 nM) and BAP1 (5 nM) are incubated with the compound of the invention followed by addition of ubiquitin-AMC (1000 nM). Fluorescence is monitored using Wallac Multilabel counter or Tecan Infinite M1000 equipped with 360 nm excitation and 460 nm emission filters.

[0058] *Substrate overlay assays*. Native gel electrophoresis is performed as described (29). In brief 4 µg of purified 26S proteasome (Boston Biochem) is mixed with 10 or 50 µM of the compound of the invention and incubated at 37 °C for 10 min. Samples are resolved on 4% non-denaturing PAGE. Gels are submerged in assay buffer (20 mM Tris-HCL, 5 mM $MgCl_2$, 1 mM ATP, 0.1 mM Suc-LLVY-AMC) and proteasomes are visualized under UV illumination.

[0059] *Ubiquitin-cleavage assay*. The recombinant Ub-GFP plasmid pet19b Ub-M-GFP is generated as described (30). In brief recombinant Ub-GFP is purified from BL21 E.coli cells by His affinity purification. For cleavage assays 19S RP (25 nM) is incubated with 10 mM NEM, 250 µM TPEN or 50 µM of the compound of the invention for 10 min followed by the addition of recombinant Ub-GFP (200 nM). Ubiquitin chain disassembly reactions are performed essentially as above except K48- or K63-linked ubiquitin tetramers (50 ng) are substituted for Ub-GFP. The

level of Ub-GFP cleavage or ubiquitin disassembly is determined by immunoblotting with anti-ubiquitin antibodies. The ubiquitinated Hdm2 substrate is generated according to the Boston Biochem protocol (K-200). For the cleavage assay 19S RP (25 nM) is incubated with 50 µM of the compound of the invention or DMSO for 10 min followed by the addition of ubiquitinated Hdm2 substrate (100 nM). The cleavage of ubiquitinated Hdm2 substrate and ubiquitinated Hdm2 is determined by immunoblotting with anti-Hdm2 antibodies.

[0060] *Proteasome isolation:* HCT-116 cells are treated with bortezomib (100 nM) or the compound of the invention (1 µM) for 3 hours. After stimulation, the cells are lysed in 50 mM HEPES pH 7.4, 250 mM sucrose, 10 mM $MgCl_2$, 2 mM ATP, 1 mM DTT and 0.025 % digitonin. Samples are sonicated briefly and incubated for 15 min on ice. Proteasomes from these samples are isolated according to the manufacturer's protocol.

[0061] *UbVS labelling of DUBs.* For labelling of DUBs in cell lysates sub confluent cells are harvested by trypsinization, washed three times with PBS, and centrifuged at 1500 RPM for 5 min. Cell pellets are lysed with buffer (50 mM HEPES pH 7.4, 250 mM sucrose, 10 mM $MgCl_2$, 2 mM ATP, 1 mM DTT) on ice for 15 min. Debris is removed by centrifugation and 25 µg of protein is labelled with 1 µM HA-UbVS for 30 min at 37 °C. Samples are resolved by SDS-PAGE and analyzed by immunoblotting with indicated antibodies.

[0062] *Determination of cell apoptosis and viability.* For determination of apoptosis parental HCT-116 p53 +/+ cells are treated with the increasing doses of the compound of the invention for 24 h. Treatment doses are based on the drug concentration that resulted in maximal apoptosis over a 24 h period. HCT-116 cells are seeded in 96-well microtiter plates at 10,000 cells per well and incubated overnight. Cells are treated with indicated drug for 24 h. At the end of the incubation period, NP40 is added to the tissue culture medium to 0.1 % and 25 µl of the content of each well was assayed using the M30-Apoptosense® ELISA as previously described (31). Cell viability is determined by measuring acid phosphatase activity or using the FMCA method (32). For the acid phosphatase activity cells are seeded at 5000 cells per well in 96-well culture plates and incubated for 12 h at 37 °C. Compounds are added to the cells in growth media and incubated for 72 h at 37 °C. Cells are washed with 200 µl warm PBS. 100 µl of para-nitrophenyl phosphate (pNPP, 2mg/ml) in Na acetate buffer pH 5 (NaAc 0.1 M, 0.1% Triton-X-100) is added per well. Cells are incubated for 2 h after which reaction was stopped by addition of 1N NaOH. Absorbance is measured at 405 nm. The dose-dependent cytotoxicity of a number of embodiments of the compound of the invention is illustrated in Figs. 1a-1o.

[0063] For the FMCA assay cells are seeded in the drug-prepared 384-well plates using the pipetting robot Precision 2000 (Bio-Tek Instruments Inc., Winooski, VT). The plates are incubated for 72 h and then transferred to an integrated HTS SAIGAN Core System consisting of an ORCA robot (Beckman Coulter) with CO_2 incubator (Cytomat 2C, Kendro, Sollentuna, Sweden), dispenser module (Multidrop 384, Titertek, Huntsville, AL), washer module (ELx 405, Bio-Tek Instruments Inc), delidding station, plate hotels, barcode reader (Beckman Coulter), liquid handler (Biomek 2000, Beckman Coulter) and a multipurpose reader (FLUOstar Optima, BMG Labtech GmbH, Offenburg, Germany) for automated FMCA. Survival index (SI) is defined as the fluorescence of test wells in percentage of controls with blank values subtracted.

[0064] *Cell-cycle analysis*. For determination of cell cycle HCT-116 cells are treated with the compound of the invention or DMSO cells are harvested by trypsinisation, washed and fixed in 70% ice cold EtOH for 12 h. The cells are re-suspended in staining solution containing propidium iodide (50 µg/ml) and RNAse A (0.5 µg/ml) in PBS. Samples are run on BD FACScalibur. The percentage of cells in each phase of the cell cycle is determined using ModFit software.

EXAMPLE 1. ***Exemplary synthesis of preferred embodiments to the compound of the invention***

[0065] *General information*. All solvents used were of HPLC grade or better. When anhydrous conditions were required, an excess of 3 Å molecular sieves were added to the solvent at least 24 h before use to ensure dryness. ^{1}H NMR nuclear magnetic resonance (NMR) was recorded on a Bruker Advance DPX 400 spectrometer at 400.1 MHz. Low resolution electrospray ionization mass spectra were obtained using an Agilent mass spectrometer in positive ionization mode. Flash chromatography was performed on Merck silica gel 60 (230-400 mesh). Analytical LCMS data were obtained with an Agilent mass spectrometer; Agilent 1100 system; A: ACE C8 column (50x3.0 mm, 5µM); gradient: 10-97 % acetonitrile in water/0.1 % TFA, in 3 min 1.0 mL/min, or B: xBridge C18 column (3.5 µM. 50x3.0 mm), gradient 10 % to 97 % acetonitrile in 10 mM NH_4HCO_3 (pH 10) in 3 min, 1 mL/min). Names of chemical structures were determined using Marvin Scech 5.2.6, ChemAxon.

(3E, 5E)-3,5-Bis(phenylmethylidene)azepan-4-one (# 1516) and (3E, 5E)-3,5-bis(4-methoxyphenylmethylidene)-azepan-4-one (# 1517)

[0066] Hexahydro-4H-azepin-4-one (0.45 g, 3.0 mmol), together with either benzaldehyde (0.70 g, 7.0 mmol), 4-methoxybenzaldehyde (0.90 g, 7.0 mmol) or 4-chlorobenzaldehyde (0.92 g, 7.0 mmol) was dissolved in acetic acid (10 mL). Then sulfuric acid (conc. 1 mL) was added drop-wise and the reactions were stirred for 24 hours at rt. Water (30 mL) was added and the precipitate filtered and dried *in vacuo* over night. No further purification was performed. Compound # 1516 was obtained with 99% purity determined by LCMS (System A) MS ESI^{+} m/z 290 [M+H]$^{+}$. Compound # 1517 was also obtained in 99% purity determined by LCMS (System A), MS ESI^{+} m/z 350 [M+H]$^{+}$. Compound # 1518 was obtained in 91% purity; LCMS (System A). MS ESI^{+} m/z 358 [M]$^{+}$, 360 [M+2]$^{+}$.

(3E, 5E)-3,5-bis(phenylmethylidene)-1-(prop-2-enoyl)-azepan-4-one (#1520)

[0067] (3E, 5E)-3,5-Bix(phenylmethylidene)azepan-4-one (# 1516) (50.0 mg, 0.182 mmol) and acrylic acid (14.4 mg, 0.20 mmol), HBTU (58.4 mg, 0.182 mmol), triethylamine (36.7 mg, 0.364 mmol) were dissolved in DMF (2 mL) and stirred over night. Ethyl acetate and brine were added and the products were extracted. The combined organic layers were dried and evaporated. The

crude product was diluted with methanol and purified by preparative HPLC. Compound # 1520 was obtained in 96% purity, MS-ESI$^+$ m/z 344 $[M+H]^+$.

(2R)-[(3E, 5E)-3,5-Bis(4-nitrophenylmethylidene)-4-oxo-1-(pyrrolidin-2-yl-carbonyl)-azepan trifluoroacetate (# 1505)

[0068] N-Boc-azepanone (100 mg, 0.47 mmol) and 4-nitrobenzaldehyde (156 mg, 1.03 mmol) were dissolved in acetic acid (10 mL). Then sulfuric acid (conc. 1mL) was added dropwise and the reactions were stirred at room temperature for three days. Then more aldehyde and sulfuric acid were added and the reaction stirred another 24 hours, more acid was added twice 24 hours apart. The reaction was quenched by addition of water and the precipitated crude intermediates were filtered off and washed with water. After drying the product *in vacuo* over night 2 x 35 mg (0.09 mmol) of the crude intermediate was weighed into two flasks and dissolved together with monoethyl succinate (14.8 mg, 0.10 mmol) in DCM/DMF (2 mL, 4:1). Triethylamine (19.3 µL, 0.14 mmol) was added and the mixture stirred for 5 min before addition of HATU (38.6 mg, 0.10 mmol). After continuing stirring for 12 hours more triethylamine and HATU was added and the stirring continued for 4 hours. The solvents were evaporated and the residue purified by preparative HPLC. The residue was dissolved in dichloromethane/trifluroacetic acid (5 mL, 4:1), stirred for 40 min and concentrated again. Compound # 1505 was obtained in 93% purity by LCMS (System A). MS ESI$^+$ m/z 477 [M+H]+.

EXAMPLE 2. ***Further exemplary syntheses of preferred embodiments of the compound of the invention***

[0069] *(2R)-2-{[(3E, 5E)-3,5-bis[(4-nitrophenyl)methylidene]-4-oxoazepan-1-carbonyl}pyrrolidinium trifluoroacetate (compound # 1505)*. N-boc Azepan-4-one (0.10 g, 0.47 mmol) and 4-nitrobenzaldehyde (156 mg, 1.0 mmol) were dissolved in acetic acid (10 mL), conc. H_2SO_4 (1 mL) was added drop-wise and the reaction stirred at rt over the weekend. More aldehyde (156 mg) and H_2SO_4 (1 mL) were added and stirring continued at rt over night. Another mL conc. H_2SO_4 was added and reaction stirred over night again. Conc. H_2SO_4 was added once more and the reaction stirred until complete (for two weeks). Upon addition of water a brown precipitate was formed, filtered off, washed with water, and dried under vacuum to give 339.5 mg of brown solid Intermediate 1, which was used without further purification. Intermediate 1 (35 mg, 0.09 mmol) and N-boc proline (22 mg, 0.10 mmol) were dissolved in DCM/DMF (4:1, 2 mL). TEA (19 µL, 0.14 mmol) was added and the mixture stirred for 5 min, then HATU (38.6 mg, 0.10 mmol) was added and the reaction stirred at rt overnight. More TEA (19 µL, 0.14 mmol) and HATU (38.6 mg, 0.10 mmol) was added, and the reaction stirred for another 4 h. The reaction mixture was concentrated and then purified by preparative LC (40-70 % ACN in 0.1% TFA) to give the product as a yellow solid. The solid was dissolved in DCM/TFA (4:1, 5 mL) and the solution stirred at rt for 40 min to remove the boc protective group. The TFA salt of the product was recovered as a yellow solid of 93% purity. LCMS A: Rt 1.94/1.99, m/z $[M+H]^+$ 477.1, B: Rt 2.28.

[0070] *(3E,5E)-1-(4-ethoxy-4-oxobutanoyl)-3,5-bis[(4-nitrophenyl)methylidene]-4-oxoazepon-1-ium trifluoroacetate (compound # 1507)*. Intermediate 1 (35 mg, 0.09 mmol) and N-boc proline (22 mg, 0.10 mmol) were dissolved in DCM/DMF (4:1, 2 mL). TEA (19 µL, 0.14 mmol) was added and the mixture stirred for 5 min, then HATU (38.6 mg, 0.10 mmol) was added and the reaction stirred at rt overnight. More TEA (19 µL, 0.14 mmol) and HATU (38.6 mg, 0.10 mmol) were added and the reaction stirred for another 4 h. The reaction mixture was concentrated and then purified on preparative LC (40-70 % ACN in 0.1% TFA) to give the TFA salt of the product as a yellow solid of 95% purity. LCMS A: Rt 2.48/2.50 m/z $[M+H]^+$ 508.1. B: Rt 2.48/2.52.

[0071] *(3E, 5E)-3,5-bis[(4-chlorophenyl)methylidene]azepan-4-one (compound # 1518)*. Azepan-4-one hydrochloride (0.45 g, 3.0 mmol) and 4-chlorobenzaldehyde (0.92 g, 6.6 mmol) were dissolved in acetic acid (10 mL), conc. H_2SO_4 (1 mL) was added drop-wise and the reaction stirred at rt for 24 h. After addition of water (30 mL) a precipitate was formed, filtered off, and dried in vacuum to give the product in 91% purity as a yellow solid. LCMS A: Rt 2.04 m/z $[M]^+$ 358.1.

[0072] *(3E, 5E)-3,5-bis(phenylmethylidene)-1-(prop-2-enoyl)azepon-4-one (compound # 1520)*. Azepan-4-one hydrochloride (50 mg, 0.182 mmol), acrylic acid (14 µL, 0.20 mmol), TBTU (58 mg, 0.182 mmol) and TEA (37 mg, 0.364 mmol) were dissolved in DMF (2 mL) and stirred at rt overnight. Brine and ethyl acetate were added and the phases separated. The organic phase was dried and the solvents evaporated after filtration. The crude product was dissolved in acetic acid (2 mL) and H_2SO_4 (0.2 mL). Benzaldehyde (50 µL) was added and the reaction stirred for 24 hours. Methanol and water were added to the mixture, which was purified by preparative LC. The title compound was isolated in 96% purity as a yellow solid. LCMS A: Rt 2.68 m/z $[M+H]^+$ 344.1.

[0073] *(3E, 5E)-3,5-bis(phenylmethylidene)-1-cyclobutanecarbonylazepan-4-one (compound # 1521)*. Azepan-4-one hydrochloride (50 mg, 0.182 mmol), cyclobutyric acid (14 µL, 0.20 mmol), TBTU (58 mg, 0.182 mmol) and TEA (37 mg, 0.364 mmol) were dissolved in DMF (2 mL) and stirred at rt overnight. Brine and ethyl acetate were added and the phases separated. The organic phase was dried and the solvents evaporated after filtration. The crude product was dissolved in acetic acid (2 mL) and H_2SO_4 (0.2 mL). Benzaldehyde (50 µL) was added and the reaction stirred for 24 h. Methanol and water were added to the mixture, which was purified by preparative LC. The title compound was isolated in 96% purity as a yellow solid. LCMS A: Rt 2.68 m/z $[M+H]^+$ 372.1.

[0074] *(3E,5E)-1-(2-cyclopropylacetyl)-3,5-bis[(4-methoxyphenyl)methylidenelazepon-4-one (compound 1526)*. Azepan-4-one hydrochloride (0.45 g, 3.0 mmol) and 4-methoxybenzaldehyde (0.90 g, 6.6 mmol) were dissolved in acetic acid (10 mL), conc. H_2SO_4 (1 mL) was added drop-wise, and the reaction stirred at rt for 24 h. Water (30 mL) was added. The precipitate was filtered off and dried in vacuum over night. The crude material (30 mg, 0.107 mmol), cyclopropylacetic acid (12 mg, 0.12 mmol), TBTU (41 mg, 0.13 mmol) and TEA (26 mg, 0.26 mmol) were dissolved in DMF (2 mL) and stirred at rt over night. Methanol (1.5 mL) and water (0.5 mL) were added and the product was purified by preparative LC to yield the solid product in 95% purity. LCMS A: Rt 2.51 m/z $[M+H]^+$ 432.2.

[0075] *(3E,5E)-5-[(3-nitrophenyl)methylidene]-3-(phenylmethylidene)azepan-4-one (compound # 1560)*. N-boc-Azepan-4-one (0.10 g, 0.47 mmol) and 3-nitrobenzaldehyde (156 mg, 1.0 mmol) were dissolved in acetic acid (5 mL), concentrated H_2SO_4 (0.5 mL) was added drop-wise and the reaction stirred at rt for 4 days. Then more concentrated H_2SO_4 (0.5 mL) and aldehyde (156 mg, 1.0 mmol) were added and stirring continued at rt for three weeks. A mixture of the mono- and di-condensation products was obtained. The mixture was purified by column chromatography (DCM/methanol) to give the intermediate amine Intermediate 2 as a brown oil (19 mg). Intermediate 2 was dissolved in acetic acid (1.5 mL) together with benzaldehyde. Conc. H_2SO_4 (0.05 mL) was added and the reaction stirred at rt overnight. Then more H_2SO_4 was added and the stirring continued for a week. More aldehyde (156 mg, 1.0 mmol) and H_2SO_4 was added and stirring continued for an additional 4 days. The reaction mixture was concentrated and purified by preparative LC to give the TFA-salt of the product as yellow solid in 98% purity. LCMS System A: Rt 1.78 m/z $[M+H]^+$ 335.1, System B: Rt 2.43/2.28.

[0076] *(3E,5E)-1-methyl-3,5-bis[(4-nitrophenyl)methylidene]azepon-4-one (compound # 1563)*. N-methylazepan-4-one·HCl (50 mg, 0.30 mmol) and 4-nitrobenzaldehyde were dissolved in acetic acid (5 mL) and stirred for 10 min, then conc. H_2SO_4 (50 µL) was added slowly and the mixture was stirred at rt overnight. More concentrated H_2SO_4 (100 µL) was added and stirring was continued at rt for 6 h. Additional 500 µL of concentrated H_2SO_4 was added and the reaction stirred overnight. A further 350 µL of conc. H_2SO_4 was added and stirring continued for additional 5 h, during which period further H_2SO_4 was added in two portions (500 µL and 250 µL). Then water (3 x reaction volume) was added and the mixture was stirred until rt was reached. The reaction mixture was extracted with ethyl acetate (3 x reaction volume). The phases were separated and the organic phase concentrated to yield a dark yellow viscous oil. The crude product was purified by preparative HPLC, (XBridge column; eluents 50 mM ammonium carbonate buffer at pH 10 and methanol) giving the title product as a yellow solid (26.3 mg). LCMS System A: Rt 1.87 m/z $[M+H]^+$ 394.1, System B: Rt 2.57.

[0077] *(3E,5E)-3,5-bis[(4-fluorophenyl)methylidenel-1-propylazepan-4-one (compound # 1574)*. Azepan-4-one hydrochloride (0.25 g, 1.68 mmol) and 4-fluorobenzaldehyde (0.416 g, 3.36 mmol) were dissolved in acetic acid (20 mL) and the solution stirred for 10 min, then conc. H_2SO_4 (200 µL) was slowly added and the solution was stirred at rt overnight. More conc. H_2SO_4 (1 mL) was added and stirring continued at rt. Another mL of conc. H_2SO_4 was added after 6 h, and the reaction stirred again overnight. The next day further 800 µL of conc. H_2 SO_4 was added and stirring continued for a period of five days, during which two portions of H_2SO_4 (1 mL and 0.5 mL) were added to the reaction mixture. Then water (3 x reaction volume) was added and the mixture stirred until rt was reached. The reaction mixture was extracted with ethyl acetate (10 x reaction volume). The organic phase was concentrated by evaporation. Water was added to the residue. A precipitate was formed and filtered off. The solid was washed with water and dried in vacuum to give Intermediate 3 as a yellow solid. A portion (15 mg, 0.05 mmol) thereof was dissolved in DCE-Propanal (4 µL, 0.06 mmol) was added, and the mixture stirred for 15 min at rt. Then $NaBH(OAc)_3$ (15.7 mg, 0.07 mmol) and acetic acid (2.6 µL, 0.05 mmol) were added and the reaction stirred at rt over night. The reaction was concentrated and the crude product purified by preparative LC

giving the product (7.2 mg) in 90% purity. LCMS System A: Rt 2.02 m/z $[M+H]^+$ 368.1, System B: Rt 3.21.

[0078] *(3E,5E)-3-[(4-methoxyphenyl)methylidene]-5-[(4-nitrophenyl)methylidene]azepan-4-one (compound # 1575)*. Azepan-4-one hydrochloride (0.25 g, 1.68 mmol) and 4-nitrobenzaldehyde (253 mg, 1.68 mmol) were dissolved in acetic acid (20 mL) and stirred for 10 min, then conc. H_2SO_4 (1 mL) was slowly added and the mixture stirred at rt for 8 days. On days 1-3 one portion conc. H_2SO_4 per day was added (0.5 mL, 0.75 mL, and 0.5 mL). Water (2 x reaction volume) was added and the mixture extracted with ethyl acetate (2 x reaction volume). The organic phase was concentrated by evaporation and dried to yield crude Intermediate 4. A portion of Intermediate 4 (100 mg, 0.41 mmol) was dissolved in acetic acid (6 mL) and stirred for 10 min, then concentrated H_2SO_4 (0.6 mL) was added slowly and the reaction stirred at rt for 6 days. Upon addition of water the product precipitated as a yellow solid. The precipitate was filtered off, washed with water and dried in vacuum to give the title compound as a yellow solid in 98% purity. LCMS System A: Rt 1.82 m/z $[M+H]^+$ 365.1, System B: Rt 2.41. ^{1}H-NMR (400 MHz, $CDCl_3$) [ppm] = 2.97-2.99 (m, 2H), 3.41-3.44 (m, 2H), 3.83 (bs, 3H), 4.28 (s, 2H), 7.06-7.08 (d, 2H), 7.47 (s, 1H), 7.59-7.62 (d, 2H), 7.76 (s, 1H), 7.78-7.80 (d, 2H), 8.27-8.29 (d, 2H).

[0079] *(3E,5E)-5-[(4-fluorophenyl)methylidene]-3-[(4-methoxyphenyl)methylidene]-1-methylazepan-4-one (compound # 1577)*. N-methylazepan-4-one hydrochloride (75 mg, 0.46 mmol) and 4-fluorobenzaldehyde were dissolved in acetic acid (7 mL) and stirred for 10 min, then conc. H_2SO_4 (350 µL) was added slowly and the mixture was stirred at rt for 8 days. More conc. H_2SO_4 was added during days 2-4 (0.175 mL, 0.35 mL, 0.25 mL respectively). Water was added and the solution extracted with ethyl acetate (twice the volume of reaction mixture). The organic phase was concentrated to give Intermediate 5. A portion of this intermediate (35 mg, 0.15 mmol) and 4-methoxybenzaldehyde (17 µL, 0.15 mmol) were dissolved in acetic acid (2.5 mL) and stirred for 10 min, then conc. H_2SO_4 (0.20 mL) was added slowly and the reaction stirred for five days. Water (2 x reation volume) was added and the reaction mixture extracted with ethyl acetate (2 x reaction volume). The organic layer was concentrated and water was added. A precipitate was formed and filtered off to give the title product (11.2 mg) in 91% purity as a yellow solid. LCMS System A: Rt 1.86 m/z $[M+H]^+$ 352.1, System B: Rt 2.79.

[0080] *(3E,5E)-1-acetyl-5-[(4-fluorophenyl)methylidene]-3-[(4-ethoxyphenyl)methylidene]azepon-4-one (compound # 1579)*. Azepan-4-one hydrochloride (0.25 g, 1.68 mmol) and 4-fluorobenzaldehyde (179 µL, 1.68 mmol) were dissolved in acetic acid (20 mL) and stirred for 10 min, then conc. H_2SO_4 (1 mL) was slowly added and the mixture was stirred at rt for 8 days with addition of conc. H_2SO_4 during the first three days (0.5 mL, 0.75 mL and 0.5 mL respectively). Water (2 x reaction volume) was added and the mixture extracted with ethyl acetate (2 x mixture volume). The organic phase was concentrated and dried to give the crude Intermediate 6. A portion of this intermediate (100 mg, 0.46 mmol) was dissolved in acetic acid (6 mL) and stirred for 10 min, then concentrated H_2SO_4 (0.6 mL) was added slowly and the reaction stirred at rt for 7 days. Water was added (1 x volume) and the mixture was neutralized with saturated aqueous $NaHCO_3$. The formed precipitate was filtered off, washed with water and dried in vacuum to yield

Intermediate 7 (31.5 mg) as a yellow solid of 91% purity. LCMS System A: Rt 1.85 m/z $[M+H]^+$ 338. Intermediate 7 (10 mg) was dissolved in DCM (1 mL) and TEA (5.0 µL, 0.04 mmol) was added. The mixture was stirred for 10 min, then acetyl chloride (2.3 µL, 0.03 mmol) was added and the reaction stirred at rt for 30 min. The reaction was washed with water, saturated aqueous $NaHCO_3$ and brine. The organic phase was concentrated to give the title compound (6.4 mg) as a yellow solid of 90% purity. LCMS System A: Rt 2.35 m/z $[M+H]^+$ 380.1, System B: Rt 2.37. ^{1}H-NMR (400 MHz, $CDCl_3$): [ppm] = 1.70, 1.90, 1.98 and 1.99 (4 x s, 3H, CH_3CO-, signals from the two regioisomers and their acetate rotamers), 2.89-3.01 (m, 2H), 3.68-3.77 (m, 2H), 3.79, 3.79, 3.79, 3.08 (4 x s, 3H, -OMe, signals from the two regioisomers and their acetate rotamers), 4.65-4.68 (m, 2H), 7.0-7.04 and 7.098-7.103 (2 x m, 2H), 7.22-7.30 (m, 3H), 7.48-7.62 (m, 5H).

[0081] *(3E,5E)-5-[(4-chlorophenyl)methylidene]-3-[(4-nitrophenyl)methylidene]azepan-4-one (compound # 1583)*. N-methylazepan-4-one hydrochloride (75 mg, 0.46 mmol) and 4-chlorobenzaldehyde (64 mg, 0.46 mmol) were dissolved in acetic acid (7 mL) and stirred for 10 min, then conc. H_2SO_4 (350 µL) was added slowly and the mixture was stirred at rt for 8 days. More conc. H_2SO_4 was added during days 2-4 (0.175 mL, 0.35 mL, 0.25 mL respectively). Water (2 x reaction volume) was added and the solution extracted with ethyl acetate (2 x reaction volume). The organic phase was concentrated to give Intermediate 8. A portion of the intermediate (35 mg, 0.14 mmol) and 4-nitrobenzaldehyde (69.5 mg, 0.46 mmol) were dissolved in acetic acid (2.5 mL) and stirred for 10 min, then conc. H_2SO_4 (200 µL) was added slowly and the mixture was stirred at rt for 5 days. More conc. H_2SO_4 (0.2 mL) was added, and stirring continued for 5 more days. Water (2 x reaction volume) was added and the solution extracted with ethyl acetate (2 x reaction volume). The organic phase was concentrated and the residue purified by preparative LC to give the title compound (1.8 mg) as a yellow solid of 94% purity. LCMS System A: Rt 1.98/2.04 m/z $[M+H]^+$ 383.1, System B: Rt 2.82/2.98.

Abbreviations

[0082]

Boc
: tert-butyloxycarbonyl

ACN
: acetonitrile

DCM
: dichloromethane

TFA
: trifluoroacetic acid

DMF
: dimethylformamide

TEA
: triethylamine

Rt

retention time

TBTU

O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate)

rt

room temperature

LC

liquid chromatography

EDC

1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide

HATU

2-(1H-7-Azabenzotriazol-1-yl)--1,1,3,3-tetramethyl uronium hexafluorophosphate;

DCE

1,2-Dichloroethane

[0083] EXAMPLE 3. *Pharmaceutical composition A (aqueous suspension).* The compound of the invention (25 mg) is dissolved in 1 ml of dimethyl sulfoxide. The solution is added drop-wise to 10 ml of vigorously stirred saline. The formed suspension, which can be stabilized by adding 1 % by weight of PVP, can be used for intramuscular, intravenous or subcutaneous administration.

[0084] EXAMPLE 4. *Pharmaceutical composition B (tablet).* Tablets for oral administration are produced by blending 2.0 g of the compound of the invention (powder, <10 mμ, 90 %) with microcrystalline cellulose (1.30 g), corn starch (0.50) g, silica (0.20) g, Mg stearate (0.12 mg). The mixture is dry compressed to 400 mg tablets, which are sugar coated.

[0085] EXAMPLE 5. *Pharmaceutical composition C (solution).* The compound of the invention (10 mg) is dissolved in 0.5 ml of Cremophor EL (BASF Corp.) and absolute ethanol was added to 1.0 ml. The clear solution is filled into glass vials for injection.

[0086] EXAMPLE 6. *Pharmaceutical composition D (solution).* For intraperitoneal administration in animal studies an aqueous composition a stock solution was prepared by dissolving the compound of the invention to a concentration of 2 mg/ml in Chremaphor EL/polyethylene glycol 400 1:1. (v/v) at room temperature or by heating to up to about 80 °C assisted by ultrasonication. A aliquot of the stock solution was diluted 1:10 with 0.9 % saline and used immediately for IP injection.

[0087] EXAMPLE 7. *Pharmaceutical composition E (solution).* For intraperitoneal administration a 25 % by weight Kolliphor HS15 stock solution was prepared by melting an entire container of Kolliphor HS15 (Sigma 42966) by warming to 60 °C and diluting with deionized water to 25 % w/w. To compound # 1570 (18.0 mg) in a 10 mL sample tube was added 10.0 mL of the stock solution and the tube vortexed, treated with ultrasound at about 50 °C for about 2 h, and occasionally heated to about 83 °C. The clear solution obtained was sterile filtered through a 0.2 μm cellulose syringe filter prior to injection. By the same procedure solutions of compounds # 1546 and # 1571 were prepared; these compounds were however not fully dissolved. The non-dissolved residue was weighed, and the weight deducted from the starting weight of compound (18 mg). It was found that the prepared solutions (10 ml) contained 8.5 mg and 11.0 mg, respectively, of

compounds # 1546 and # 1571.

[0088] EXAMPLE 8. *Pharmaceutical composition F (solution)*. For intraperitoneal administration a stock solution of 2-hydroxypropyl-β-cyclodextrin (Aldrich 332593) was prepared by dissolving the cyclodextrin in deionized water to a concentration of 30% w/w. To compound # 1649 (15.0 mg) in a 10 mL sample tube was added 10.0 mL of the stock solution. The tube was vortexed, treated with ultrasound at about 50 °C for about 2 h, and occasionally heated to about 83 °C. The solution obtained was sterile filtered through a 0.2 µm cellulose syringe filter prior to injection. The weight of residual compound # 1659 not dissolved was determined and used for correcting the concentration of the filtered solution to 82.5 % of the attempted concentration. By the same procedure a solution of compound # 1546 was prepared.

[0089] EXAMPLE 9. *The compound of the invention induces proteasome inhibition*. The reporter cell line MelJuSo Ub-YFP, which is engineered to accumulate yellow fluorescent protein (YFP) upon proteasome inhibition (12), was used for compound evaluation. The accumulation of YFP was measured for 48 hours in an IncuCyte-FLR system (Essen Bioscience, Essen, UK), which is an automated fluorescence microscope. Numbers of positive cells per field were used as a measure of proteasome inhibition.

[0090] EXAMPLE 10. *Determination of solubility of compounds of the invention in aqueous media*. In the diagrams of Figs.2a-2e solubility is expressed as Log S (mmol/ml; software ACD/Labs Inc.) Solubility is determined in aqueous buffer at various pH values and predicted for pure water at 25 °C. The algorithm uses a set of >6,800 compounds as reference. The diagrams show that azepanones of the invention can have a substantially increased solubility, such as by a factor 2 or more, in aqueous media at physiological pH, such as at a pH from 6 to 8, in particular of from 7.0 to 7.5, in comparison with correspondingly substituted piperidin-4-ones.

[0091] EXAMPLE 11. *Azepanes/azepanones of the invention exhibit higher cytotoxicity than structurally corresponding piperidines/piperidin-4-ones*
Figs. 3b, 3d, 3f are diagrams illustrating the cytotoxicity of compounds of the invention nos. 1546, 1547, and 1570 with a 7-membered ring moiety in comparison to structurally corresponding compounds not comprised by the invention with a 6-membered ring moiety. Their induction of dose-dependent cytotoxicity was determined after 72 hours of continuous compound exposure to the reporter cell line HCT-116. Treated cells were compared with untreated controls. Cytotoxicity is visualized as survival index (SI) over the range of about 90 % SI to about 0 % SI in dependence on compound concentration. It appears from the Figures that the compounds of the invention are more cytotoxic that the reference compounds since they are producing the same level of cytotoxicity at lower concentration.

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[0092]

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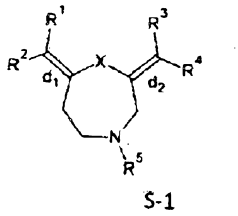
Patentkrav

1. Forbindelse med den almene struktur S-1



S-1

hvor

R^1, R^2 ved dobbeltbindingen d1 og R^3, R^4 ved dobbeltbindingen d2 uafhængigt af hinanden kan have en konfiguration, der er modsat konfigurationen af strukturen S-1

X er CO eller CS;

R^1 og R^3 er H;

R^2 og R^4 uafhængigt af hinanden er H; C_{1-6}-alkyl; C_{1-5}-alkyl-CO; phenyl eller 6-leddet heteroaryl, eventuelt substitueret med 1-3 af: C_{1-6}-alkyl, C_{1-6}-alkoxy, CN, -$COOC_{1-6}$-alkyl, COOH, NO_2, F, Cl, CF_3, NH_2, NHC_{1-6}-alkyl, $N(C_{1-6}\text{-alkyl})_2$, $CONR^7R^8$, med det forbehold, at et eller flere H i alkyl og alkoxy kan være erstattet af fluor;

R^5 er en hvilken som helst af H; C_{1-6}-alkyl; C_{2-6}-alkenyl; C_{1-3}-alkoxy-C_{2-6}-alkyl-; C_{1-3}-alkoxy-C_{2-6}-alkenyl-; aryl-C_{0-6}-alkyl-; heteroaryl-C_{0-6}-alkyl-; heterocyclyl-C_{0-6}-alkyl-; cycloalkyl-C_{0-6}-alkyl-; -C_{1-6}-alkyl-$COOC_{1-6}$-alkyl; -C_{2-6}-alkyl-aryloxy; COR^6;

R^6 er en hvilken som helst af C_{1-6}-alkyl; C_{2-6}-alkenyl; C_{1-6}-alkoxy; C_{1-3}-alkoxy-C_{1-6}-alkyl-; C_{1-3}-alkoxy-C_{1-6}-alkenyl-; aryl-C_{0-6}-alkyl-; heteroaryl-C_{0-6}-alkyl-; heterocyclyl-C_{0-6}-alkyl-; cycloalkyl-C_{0-6}-alkyl-; -C_{1-6}-alkyl-$COOC_{1-6}$-alkyl; NH_2; -NHC_{1-6}-alkyl; -$N(C_{1-6}\text{-alkyl})_2$; -$C_{0-6}$-alkyl-aryloxy;

R^7, R^8 uafhængigt af hinanden er H eller C_1-C_3-alkyl.

2. Forbindelse ifølge krav 1, hvor X = CO.

3. Forbindelse ifølge et hvilket som helst af kravene 1-2, hvor R^2 og R^4 er phenyl substitueret i en eller flere af positionerne 3, 4, 5.

4. Forbindelse ifølge et hvilket som helst af kravene 1 til 3, hvor R^5 er COR^6, og R^6 er C_{1-6}-alkyl eller C_{2-6}-alkenyl.

5. Forbindelse ifølge krav 4, hvor R^6 er C_{2-6}-alkenyl.

6. Forbindelse ifølge krav 4, hvor R^6 er C_{1-6}-alkyl.

7. Forbindelse ifølge krav 1, hvor R^1, R^2 ved dobbeltbindingen d^1 og R^3, R^4 ved dobbeltbindingen d^2 har konfigurationen af strukturen S-1,
X er CO eller CS;
R^1 og R^3 uafhængigt af hinanden er H;
R^2 og R^4 uafhængigt af hinanden er H; C_{1-6}-alkyl; C_{1-5}-alkylCO; phenyl eller 6-leddet heteroaryl substitueret med 1-3 af: CN, NO_2, F, Cl, NH_2, NHC_{1-6}-alkyl, $N(C_{1-6}$-alkyl$)_2$; COC_{1-6}-alkyl;
R^5 er H, C_{1-6}-alkyl, C_{2-6}-alkenyl, C_{1-3}alkoxy-C_{1-6}-alkyl, C_{1-3}-alkoxy-C_{1-6}-alkenyl, aryl, heteroaryl, heterocyclyl, C_{1-6}-alkyl-heteroaryl, C_{1-6}-alkyl-heterocyclyl, C_{1-6}-alkyl-cycloalkyl, C_{1-6}-alkyl-aryl, CO-C_{1-6}-alkyl, CO-vinyl, CO-allyl, CO-aryl, CO-cycloalkyl.

8. Forbindelse ifølge krav 7, hvor X er CO.

9. Forbindelse ifølge krav 7 eller 8, hvor R^2 og R^4 er substitueret phenyl.

10. Forbindelse ifølge et hvilket som helst af kravene 7 til 9, hvor R^5 er valgt blandt CO-C_{1-6}-alkyl, CO-cycloalkyl, CO-vinyl, CO-allyl.

11. Forbindelse ifølge krav 10, hvor R^5 er CO-vinyl.

12. Forbindelse ifølge krav 10, hvor R^5 er CO-C_{1-6}-alkyl.

13. Farmaceutisk sammensætning, der omfatter forbindelsen ifølge et hvilket som helst af kravene 1 til 12 og en farmaceutisk acceptabel bærer.

14. Farmaceutisk sammensætning ifølge krav 13 i form af en tablet eller kapsel eller et andet enkeltdosis-præparat til peroral administration.

15. Farmaceutisk sammensætning ifølge krav 13 i form af en opløsning eller suspension i en farmaceutisk acceptabel flydende bærer til injektion eller infusion.

16. Farmaceutisk sammensætning ifølge krav 15 til intravenøs, intramuskulær, intraperitoneal eller subkutan infusion eller injektion.

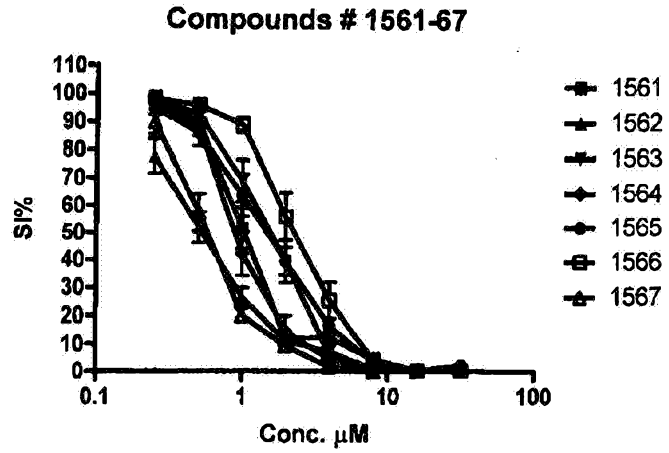
17. Forbindelse ifølge et hvilket som helst af kravene 1-12 eller farmaceutisk sammensætning ifølge et hvilket som helst af kravene 13-16 til anvendelse som lægemiddel.

18. Forbindelse ifølge et hvilket som helst af kravene 1-12 eller farmaceutisk sammensætning ifølge et hvilket som helst af kravene 13-16 til anvendelse ved behandling af cancer.

19. Forbindelse eller farmaceutisk sammensætning til anvendelse ifølge krav 18, hvor canceren er valgt blandt myelomatose, brystcancer, ovariecancer, lungecancer, coloncancer, prostatacancer og pancreascancer.

20. Forbindelse eller farmaceutisk sammensætning til anvendelse ifølge krav 19, hvor den farmakologisk effektive dosis er fra 0,0001 g/kg til 0,1 g/kg kropsvægt, især fra 0,001 g/kg til 0,01 g/kg kropsvægt, idet der tages højde for, om midlet administreres systemisk eller lokalt.

DRAWINGS



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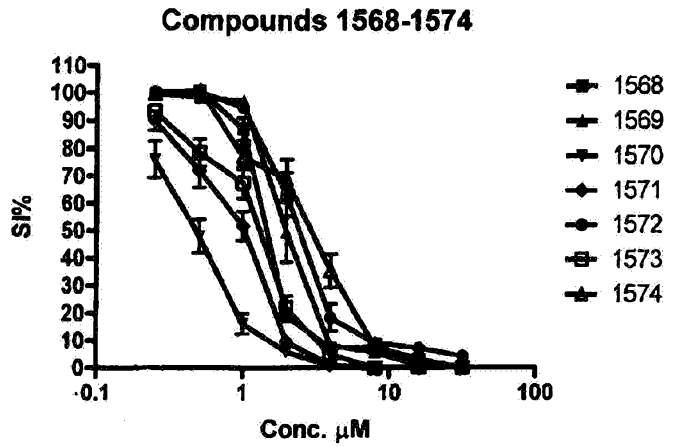

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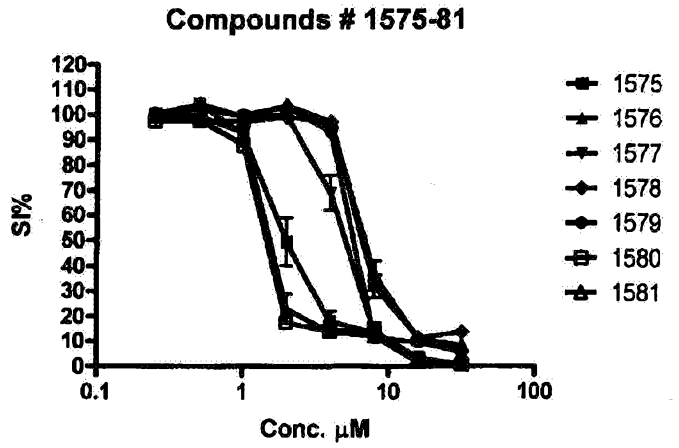

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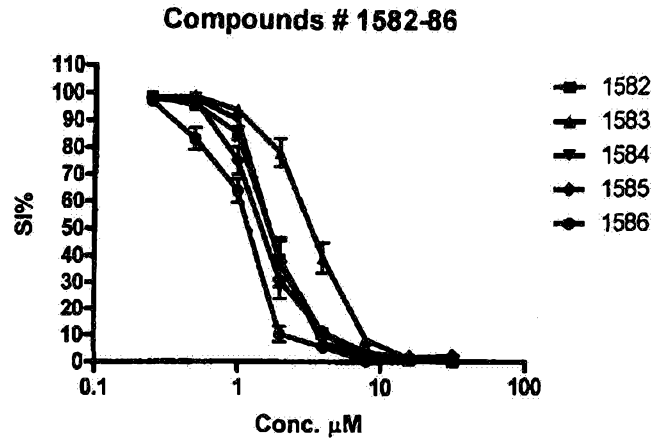

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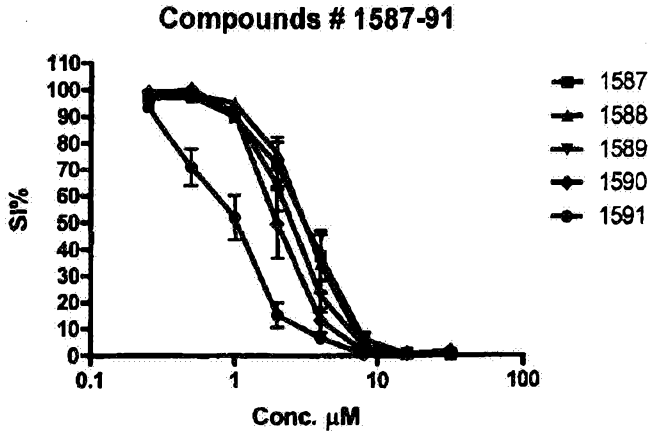
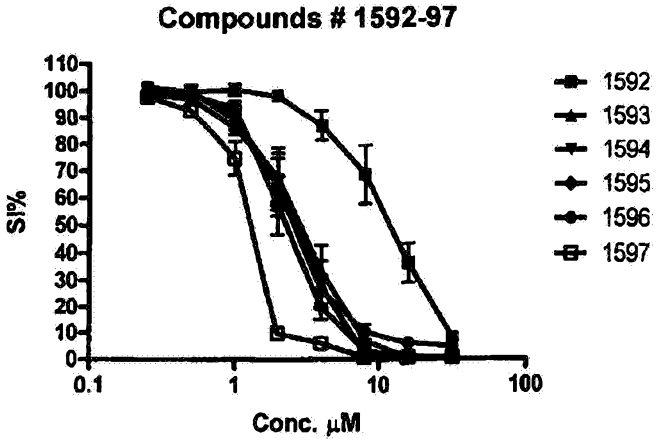


Fig. 1e



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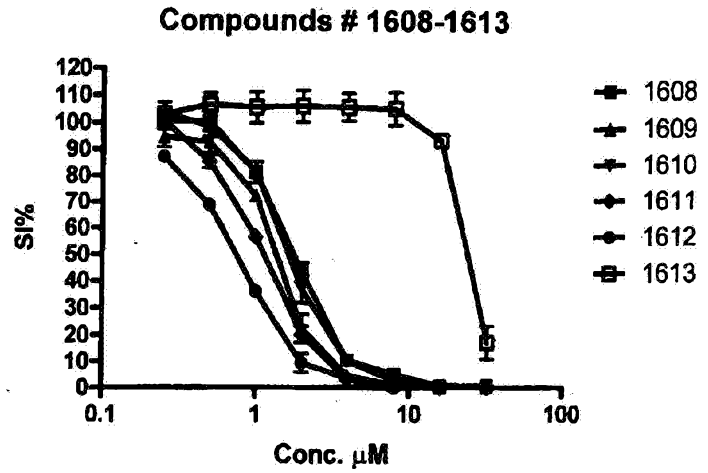

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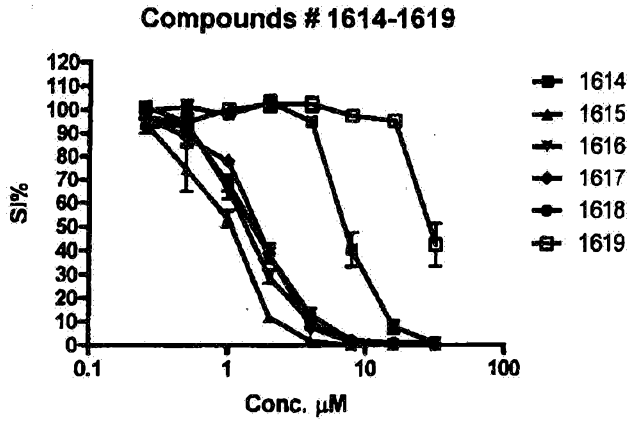
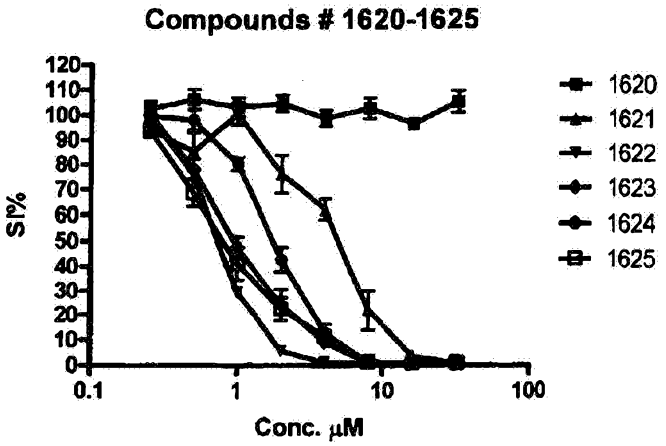


Fig. 1h



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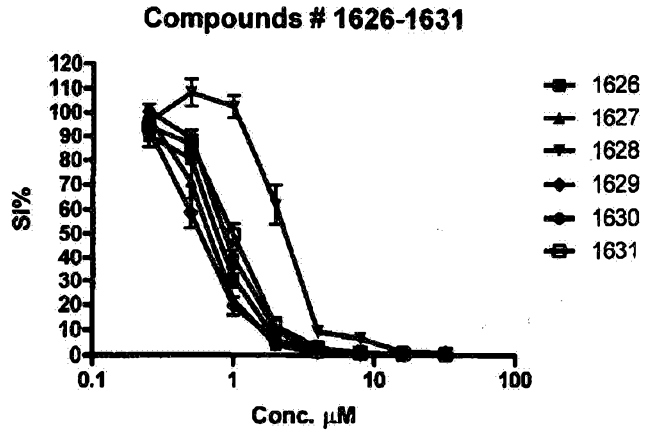

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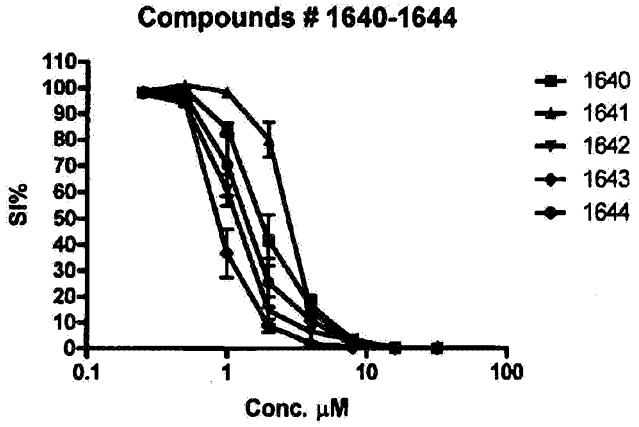

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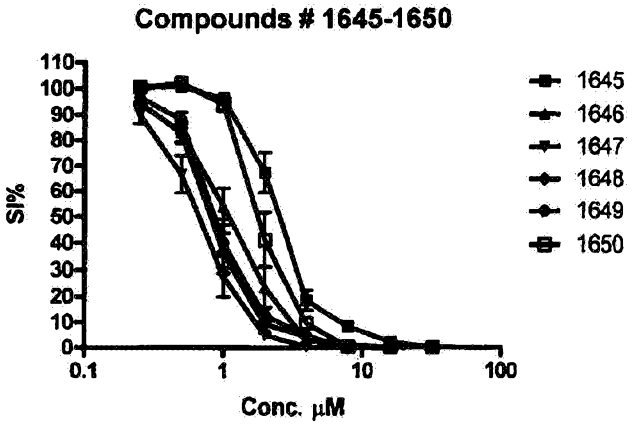

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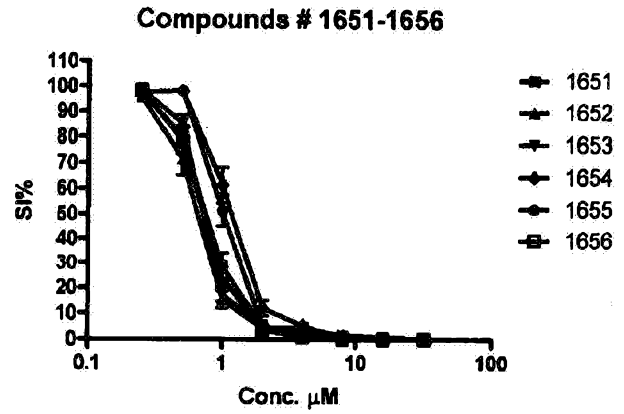

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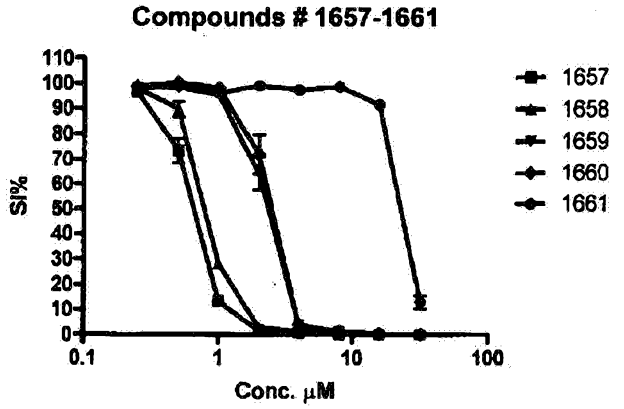

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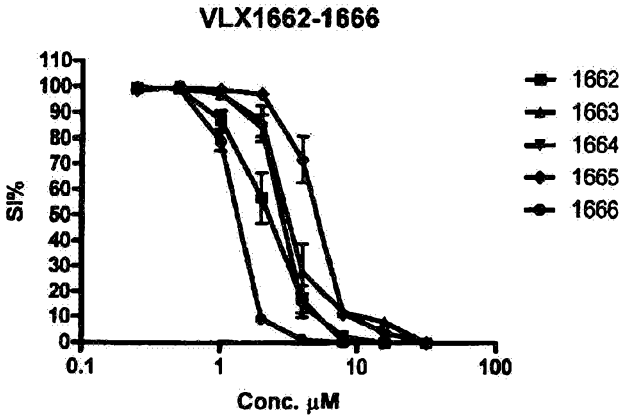

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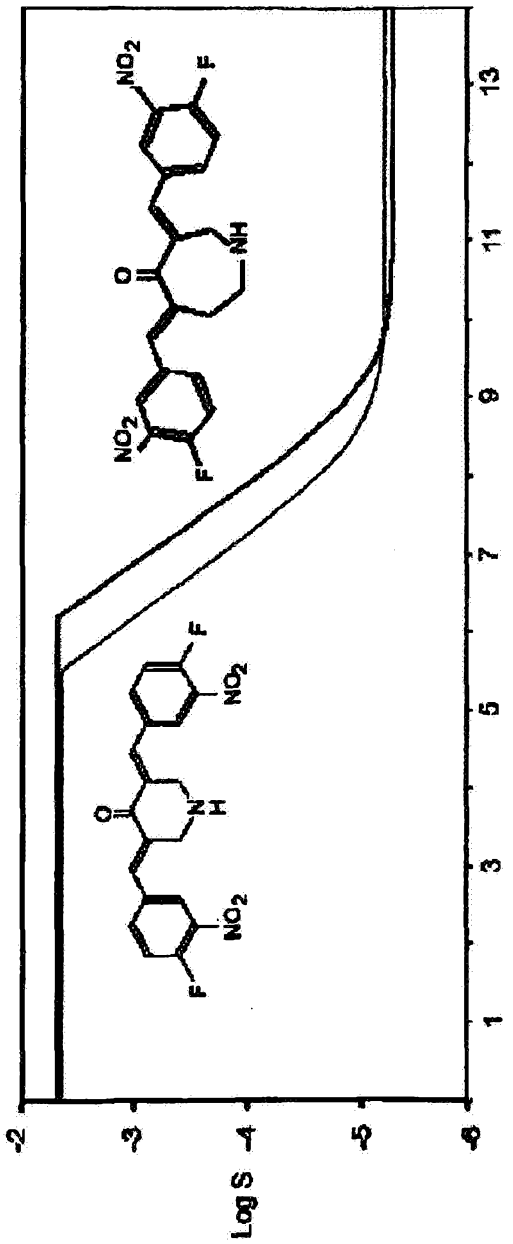

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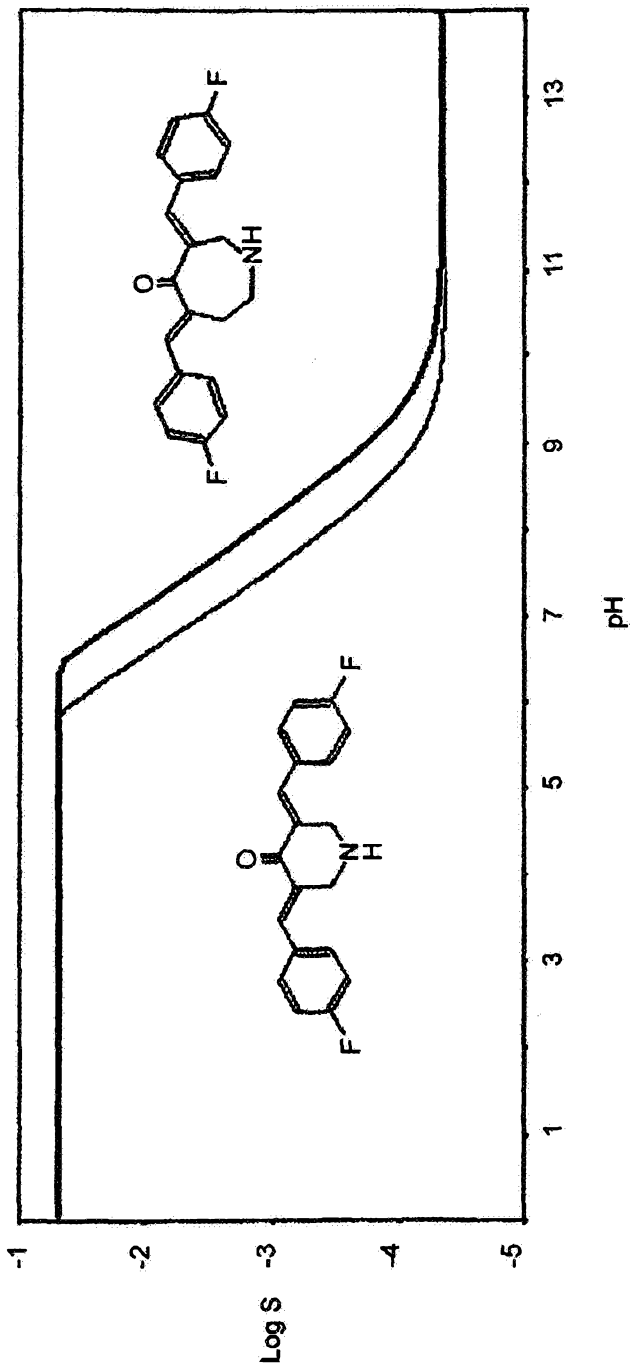

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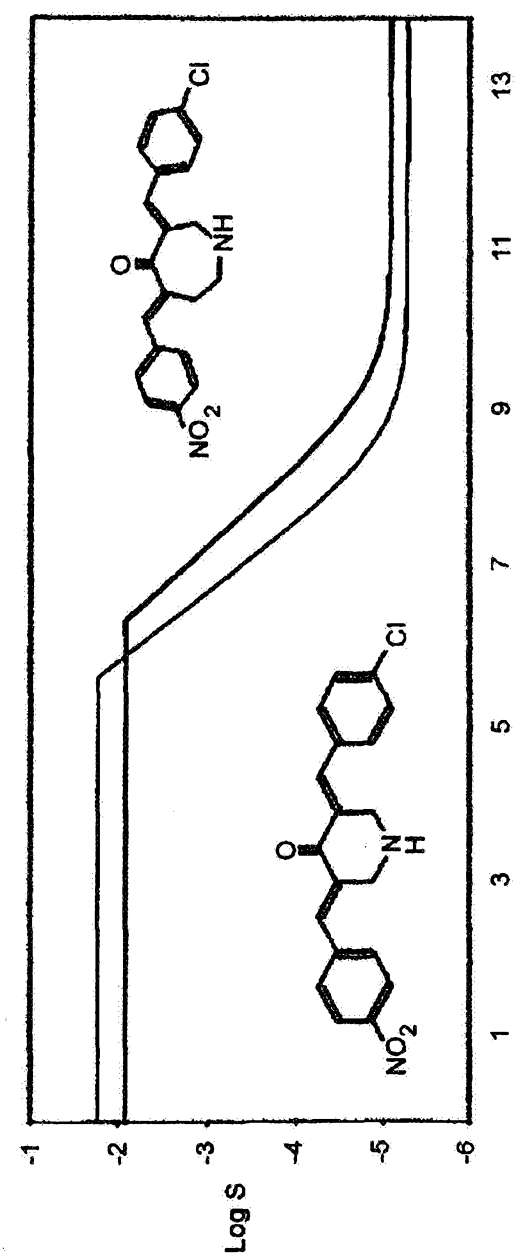

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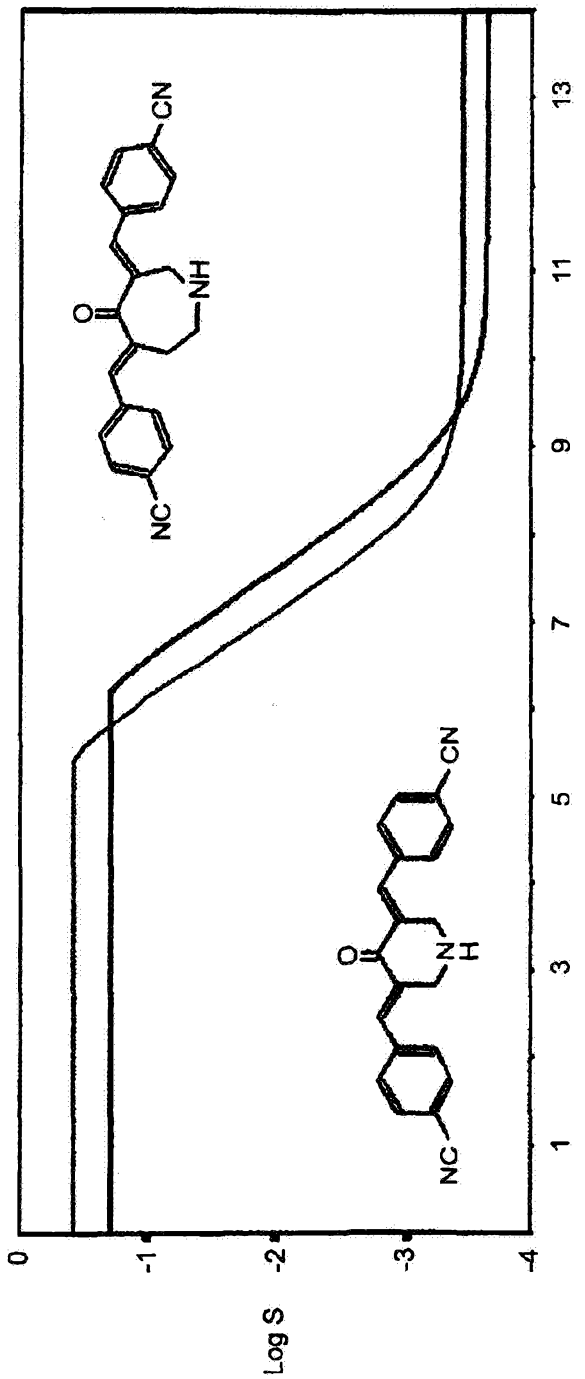

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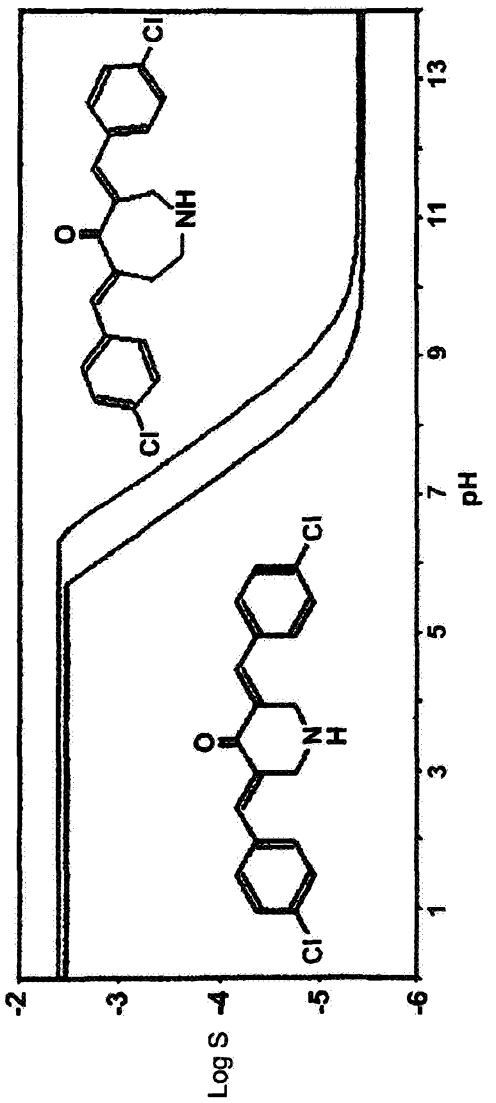

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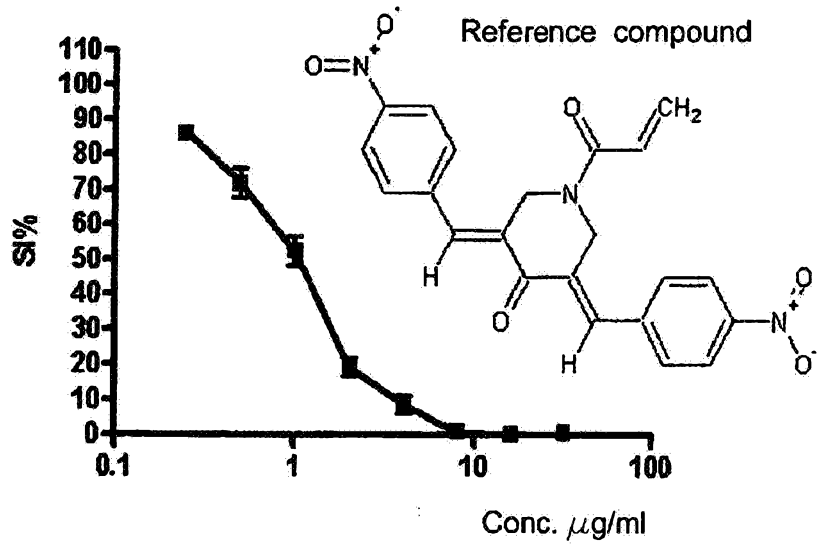
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Conc. μg/ml

Fig. 3a

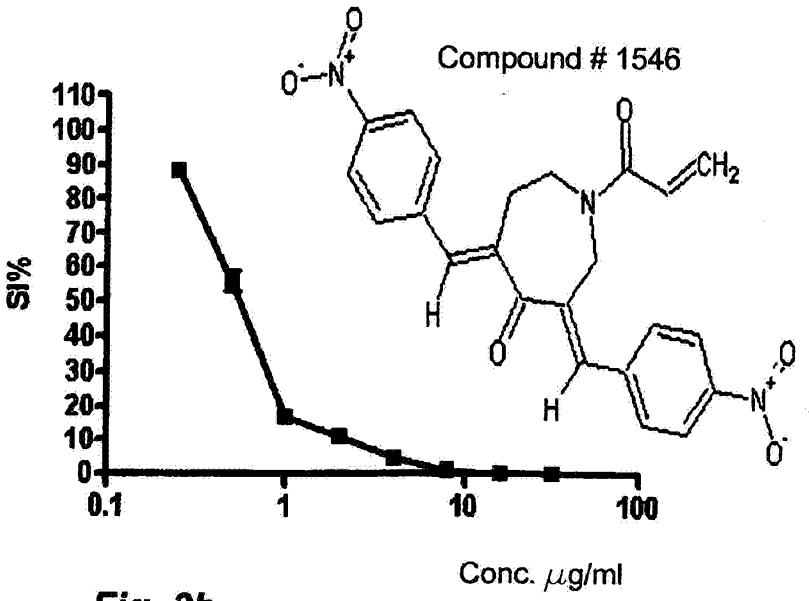
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Fig. 3b

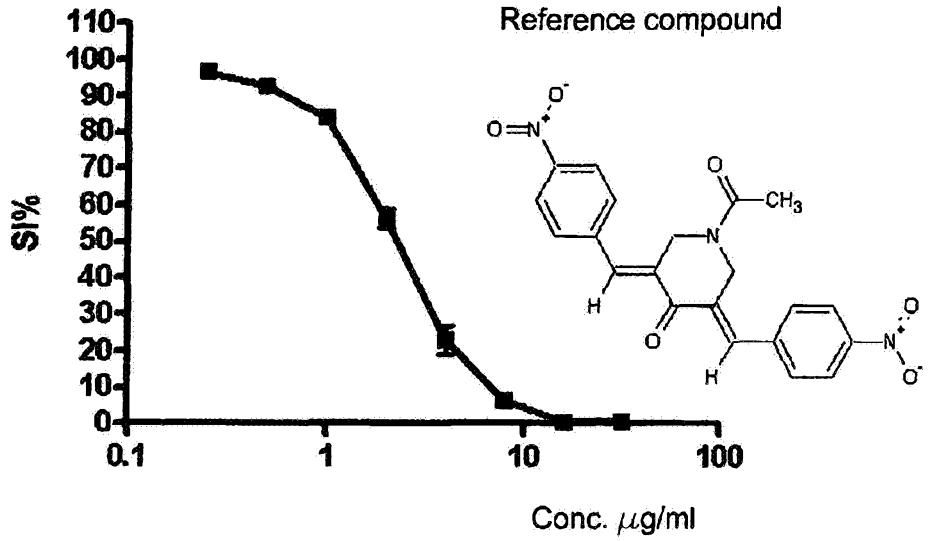
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Fig. 3c

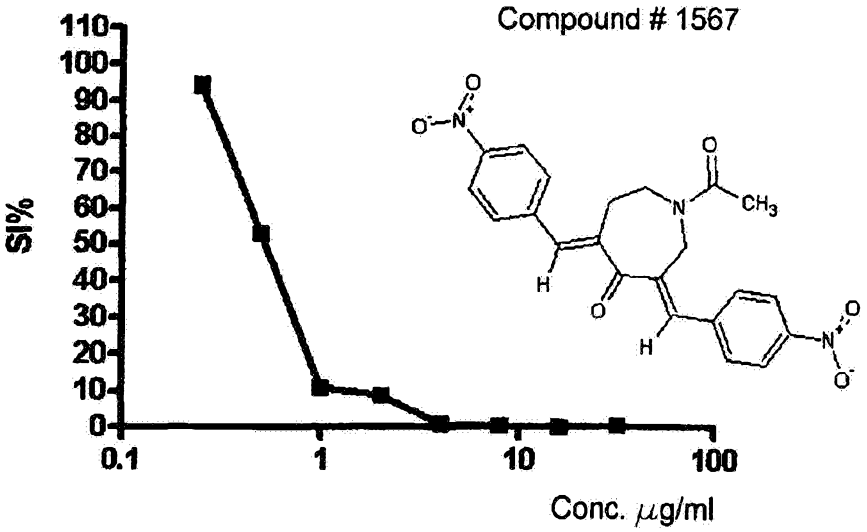
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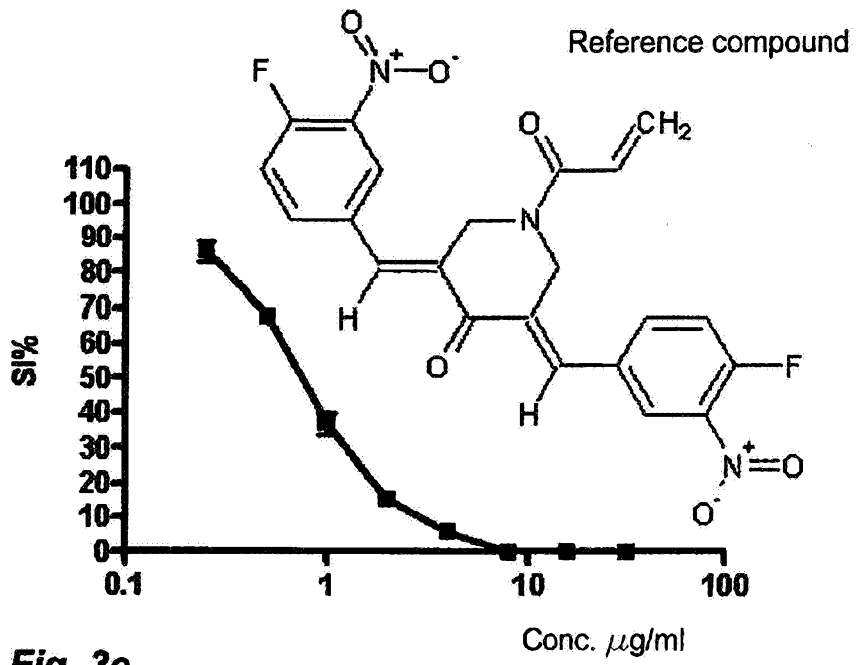
Fig. 3d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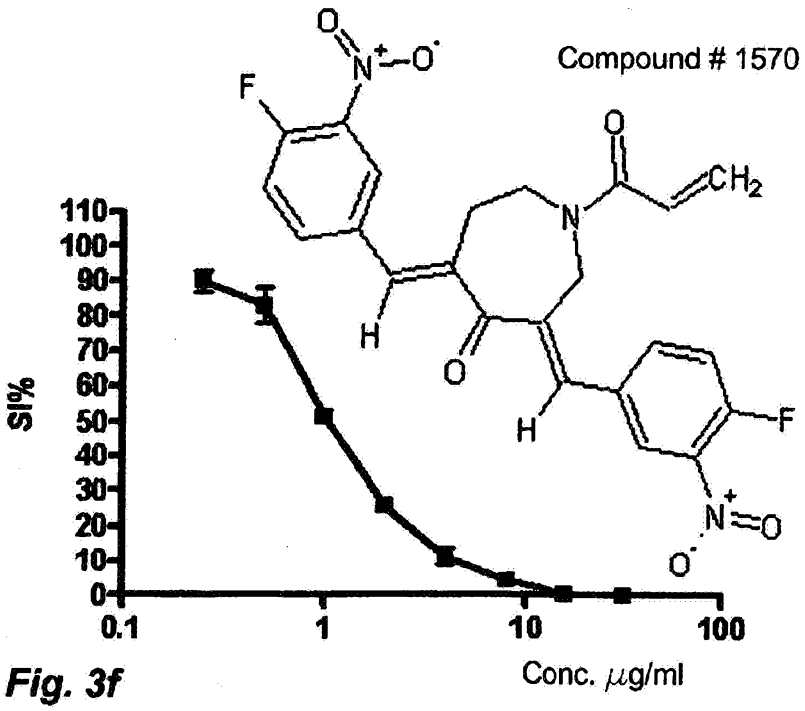
Conc. µg/ml

Fig. 3e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0.1
1
10
100
Conc. µg/ml

Fig. 3f