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Letter

Development of novel anti-cancer agents with a scaffold of tetrahydropyrido[4,3-d]pyrimidine-2,4-dione

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Development of novel anti-cancer agents with a scaffold of tetrahydropyrido[4,3-d]pyrimidine-2,4-dione

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KEYWORDS: TRAIL, AKT, ERK, Foxo3a, ATF4

ABSTRACT: ONC201 is a small molecular anti-cancer agent currently in multiple Phase II clinical trials. Based on the pharmacophore of ONC201, a series of small molecular compounds with a core structure of tetrahydropyrido[4,3-d]pyrimidine-2,4-dione were designed and synthesized. Preliminary mechanism studies of these compounds indicated that they can inhibit the phosphorylation of AKT and ERK, induce the dephosphorylation of Foxo3a, and promote the expression of TRAIL and the enhancement of activating transcription factor 4 (ATF4) in PC-3 cells. Structure-activity relationship (SAR) studies indicated that modifications of the substituted groups on the core structure can significantly improve the cellular activity of these compound. The most potent compounds are over 100 times more potent than ONC201 in inhibition of cell growth in a panel of different types of human cancer cell lines.

Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is tumor suppressor which induces apoptosis in a wide range of human cancer cell lines by binding to and activating pro-apoptotic death receptors 4 and 5 (DR4 and DR5). TRAIL can potently induce apoptosis in cancer cells while shows low toxicity to normal cells, therefore recombinant TRAIL and TRAIL-agonist antibodies targeting DR4 and DR5 have been evaluated in multiple clinical trials, however these macro-biomolecules didn't show sufficient effects in these trials.[1,2] The reasons for the failures of these trials are not clear, but one hypothesis is that their poor stability and bio-distribution may limit their usage as anti-cancer agents.[3] To overcome these drawbacks, Allen et al. initiated a campaign for searching small molecules which can up-regulate the expression of TRAIL gene and enhance the level of endogenous TRAIL by screening NCI chemical library. Their effort led to the identification of a small molecular compound ONC201 (also known as TIC10, **Figure 1**) as an inducer of the expression of TRAIL gene.[4,5] ONC201 can inhibit cell growth and induce robust apoptosis in a broad range of tumor cells, and the apoptosis induced by ONC201 is p53 independent.[5] Detail mechanism studies for ONC201 have been performed and the results indicated that ONC201 exerts its anti-cancer activity through two pathways, including both TRAIL-dependent and –independent pathways.[6-8] In the TRAIL-dependent pathway, ONC201 induces dual-inhibition of the phosphorylations of AKT and ERK, which leads to the dephosphorylation of Foxo3a. Dephosphorylated Foxo3a then translocates from the cytoplasm into the nucleus and binds to the promoter of TRAIL gene to up-regulate the transcription of TRAIL gene.[6] In the TRAIL-independent pathway, ONC201 can induce endoplasmic reticulum stress responses (ER) or integrated stress responses ISR) which culminate at the enhancement of activating transcription factor 4 (ATF4), that leads to the expression of many ATF4 targeted genes.[7,8] In solid tumors ONC201 can function through both of the two pathways, but in hematological malignancies it seems to act only through the TRAIL-independent mechanism.[6-8]

In preclinical studies ONC201 exhibited excellent drug properties, such as robust anti-proliferative and pro-apoptotic effects, benign toxicity profile, optimum aqueous solubility, favorable pharmacokinetic properties and oral bio-availability. It also shows broad synergistic effects with approved and investigational cancer therapies in many solid tumors and hematological malignancies in preclinical model.[9-11] Currently ONC201 is being evaluated in multiple Phase II clinical trials.[12]

Because of the promising anti-cancer activity of ONC201, we are interested in the design of novel anti-cancer agents with similar mechanism of functions as ONC201. In this paper, we reported our design, synthesis and preliminary mechanism studies of a class of tetrahydropyrido[4,3-d]pyrimidine-2,4-diones using ONC201 as a lead compound.

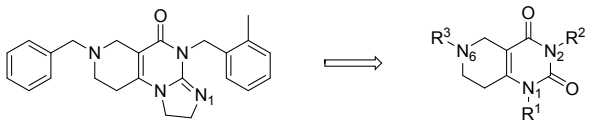
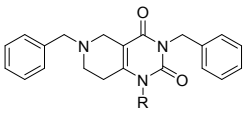


Figure 1. Design of analogues of ONC-201.

ONC201 has a unique tricyclic structure. Analyzing its structure, we think that N_1 in the five membered ring can function as a hydrogen bond acceptor, while the ethylenyl group in the five membered ring can provide hydrophobic interaction. Based upon this analysis, we designed a class of analogues of ONC201 with a core structure of tetrahydropyrido[4,3-d]pyrimidine-2,4-dione (**1**, **Figure 1**), in which N_1 in ONC201 (**Figure 1**) is replaced with an oxygen atom to maintain the ability as a hydrogen bond acceptor and a small hydrophobic substitute group R^1 is introduced to N_1 in **1**

to mimic the hydrophobic effect of the ethylenyl group in the five membered ring in ONC201. R^2 and R^3 are the groups mimicing the benzyl and 2-methylbenzyl groups respectively in ONC201 (**Figure 1**).

Table 1. Cell growth inhibition of compounds **2-6** and ONC201 in human breast cancer MDA-MB-231 cell.



Compound	R	MDA-MB-231 IC_{50} (μM)
ONC201		14.6±3.2
2	H	32.0±5.6
3	Me	1.9±0.8
4	Et	2.0±0.7
5	iPr	52.3±11.7
6	cyclopentyl	67.1±13.2

We at first designed and synthesized compounds **2-6** by introducing different substituted groups to N_1 of general structure **1**. In these five compounds the R^2 and R^3 groups in **1** were kept as benzyl groups. In our initial screen for the anti-cancer cellular activities of ONC201, we have found that ONC201 has modest anti-cancer cellular activity in MDA-MB-231 cell line, therefore **2-6** were tested together with ONC201 in this cell line for their abilities in inhibiting cell growth and the results are shown in **Table 1**. ONC201 shows an IC_{50} of 14.6 μM in this cell line, while compound **2** which doesn't have a substituted group on N_1 shows an IC_{50} of 32.0 μM and is about 2-times less potent than ONC201. Compounds **3** and **4** which contain a methyl or an ethyl group on N_1 show significantly improved cellular activity with IC_{50}s of 1.9 and 2.0 μM respectively and are about 7-times more potent than ONC201, suggesting that a small hydrophobic group at this position is critical to the cellular activity. However, compounds **5** and **6** which have an isopropyl or a cyclopentyl group on N_1 respectively are 4- to 5-times less potent than ONC201, indicating that the size of the substituted group on N_1 has critical influence to the cellular activity. To reach potent cellular activity, R^1 can only be a methyl or an ethyl group, a larger hydrophobic group on N_1 is detrimental.

In order to find whether these compounds have similar structure-activity relationship (SAR) in their anti-cancer cellular activities in other cancer cell lines, we selected a more potent compound **4** and a less potent compound **5** and screened their anti-cancer cellular activities together with ONC201 in a panel of different types of human cancer cell lines and the results are reported in **Table 2**. ONC201 shows IC_{50} values between 3-15 μM to most of the cancer cell lines we have tested, but is less sensitive to KYSE140 and RS4;11 cell lines with IC_{50} values of 58.1 and 31.4 μM respectively. It has been reported that ONC201 inhibits cell growth in most of sensitive human cancer cell lines with IC_{50}s between 1-10 μM, thus our results are consistent to the reported results. Similar to their activities to MDA-MB-231 cells, compound **5** is a few fold less potent than ONC201 to all of these cell lines, while compound **4** is 5-10 times more potent than ONC201. It is of note that the cells sensitive to ONC201 are also sensitive to compounds **4** and **5**, and the cells not sensitive to ONC201 are also not sensitive to compounds **4** and **5**, suggesting compounds **4** and **5** have similar mechanism of function as ONC201. Among all of the cell lines we have screened, PC3, MDA-MB-468 and H3122 are the most sensitive solid tumor cell lines, while MV4;11 is the most sensitive hematological tumor cell line to these compounds

Table 2. Cell growth inhibition of compounds **4**, **5** and **ONC201** in a panel of human cancer cell lines.

Cell lines	ONC201 IC_{50} (μM)	4 IC_{50} (μM)	5 IC_{50} (μM)
Colo205	15.1±3.3	2.9±1.1	48.1±16.3
KYSE140	58.1±15.9	13.8±4.3	65.9±21.6
PC3	8.9±3.2	1.7±0.3	22.9±8.3
MDA-MB-468	8.9±1.6	1.8±0.3	27.6±6.9
H3122	8.7±2.3	1.6±0.2	21.8±4.4
THP1	14.4±4.5	4.5±1.4	18.6±3.5
RS4;11	31.4±14.8	3.9±1.8	38.8±9.6
MV4;11	3.0±1.3	0.4±0.1	6.7±1.8
Molm13	7.5±1.9	0.9±0.2	28.9±7.4
Molm14	6.3±2.2	0.6±0.2	15.3±3.9

In order to explore the mechanism of function of our designed compounds, we performed preliminary mechanism studies for compounds **4** and **5** in comparison with ONC201. We at first evaluated these compounds for their ability in promoting TRAIL expression and inducing the production of SubG1 DNA in PC-3 and MDA-MB-468 cell lines, and the results indicated that all of these three compounds can dose dependently up-regulate TRAIL expression and enhance the production of subG1 DNA (**Figure 3**). Consistent to their cellular activities, compound **4** at 2.5 μM is more potent than 10 μM of ONC201 in promoting TRAIL expression and increasing the production of subG1 DNA, while compound **5** is less potent than ONC201.

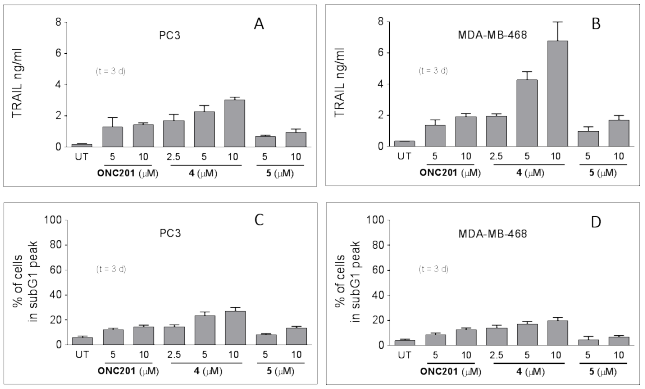


Figure 3. Compounds **4**, **5** and ONC201 promote the expression of TRAIL and the production of SubG1 DNA.

In TRAIL dependent pathway, ONC201 can inhibit the phosphorylation of AKT and ERK, which leads to the dephsophorylation of Foxo3a, therefore we treated PC3 cells with different concentrations of compounds **4**, **5** and ONC201,

and performed western blot analysis for these biomarkers (**Figure 4**). The results indicated that all of these three compounds can dose dependently inhibit the phosphorylation of ERK and AKT and reduce the phosphorylation of Foxo3a, and compound **4** is more potent than ONC201 while **5** is less potent than ONC201, therefore the ability of these compounds in inducing the changes of these biomarkers is also consistent to their cellular activity.

It has been reported that in TRAIL independent pathway, ONC201 can induce ER and ISR which culminate at the up-regulation of ATF4, therefore we also performed western blot analysis for ATF4, and the results indicated that all of these three compounds can dose dependently induce the up-regulation of ATF4, and again, compound **4** is more potent than ONC201, while compound **5** is less potent than ONC201 (**Figure 4**). Our biological studies thus suggest that our designed compounds have similar mechanism of functions as ONC201.

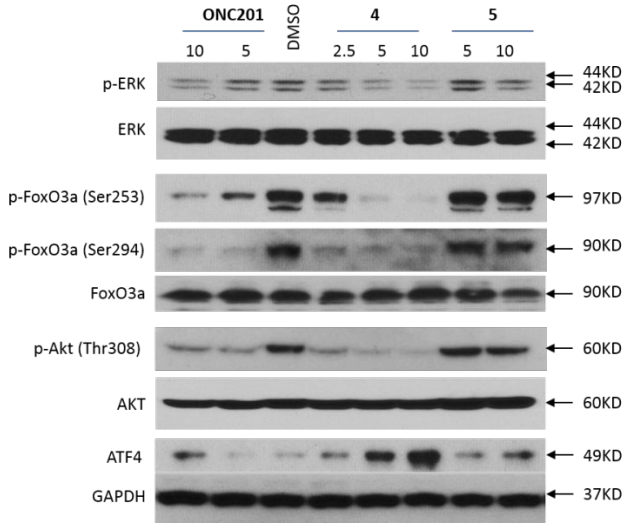
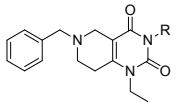


Figure 4. Western blot analysis for compounds **4** and **5** in comparison with ONC201 using human prostate cancer cell PC3. Cell was treated for 24 hours.

After confirming the mechanism of functions of our designed compounds, we then designed and synthesized compounds **7-25** (**Table 3**) to explore the substituted group on N_3 of the tetrahydropyrido[4,3-d]pyrimidine-2,4-dione core structure for its influence to the cellular activity using compound **4** as the lead compound. In these compounds the substituted groups on N_1 and N_6 were kept as ethyl and benzyl groups respectively. These compounds have been evaluated for their anti-cancer cellular activities in two sensitive cell lines H3122 and MV4;11 and the results are reported in **Table 3**. In these two cell lines, **4** shows IC_{50} values of 1.6 and 0.4 μM, respectively. Compounds **7** and **8**, in which a methyl or an ethyl group was used to replace the benzyl group on the N_3 of compound **4** respectively are 45 times less potent than **4** in inhibiting the cell growth of H3122 cell line and 25 times less potent than **4** in inhibiting the cell growth of MV4;11 cell line, suggesting that a larger hydrophobic group on N_3 is critical for the cellular activity. Replacement of the phenyl ring of the benzyl group with an aliphatic ring (compounds **9-11**) significantly decreases the cellular activity. In these three compounds, the anti-cancer cellular activity increases with the enlargement of the ring, but the most potent compound **11** in which a cyclohexyl group is used to replace the phenyl ring is still 7- to 9-times less potent than **4** in these two cell lines, indicating that the phenyl ring is important to the cellular activity.

Table 3. Cell growth inhibition of compounds **7-25** in H3122 and MV4;11 cell lines.



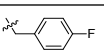
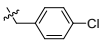
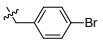
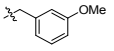
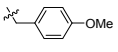
Compounds	R	H3122 IC_{50} (μM)	MV4;11 IC_{50} (μM)
ONC201		8.7±2.3	3.0±1.3
4	Bn	1.6±0.2	0.4±0.1
7	methyl	78.8±20.9	10.1±2.9
8	ethyl	72.9±18.7	9.8±2.3
9		69.2±15.4	8.9±3.1
10		51.9±16.2	5.9±2.2
11		14.8±3.3	2.7±0.9
12		4.7±1.6	0.9±0.2
13		3.9±1.9	0.6±0.2
14		0.8±0.2	0.2±0.1
15		3.9±1.3	0.6±0.2
16		2.6±0.9	0.3±0.1
17		0.4±0.1	0.07±0.02
18		2.7±0.8	0.6±0.2
19		1.6±0.4	0.2±0.1
20		0.6±0.2	0.08±0.02
21		1.9±0.5	0.5±0.1
22		0.7±0.2	0.09±0.03
23		1.9±0.3	0.4±0.1
24		0.9±0.3	0.2±0.1
25		0.6±0.2	0.09±0.03

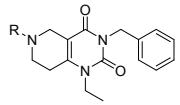
Introduction of a halo atom to the ortho or meta position of this phenyl ring doesn't have significant influence to the cellular activity. For example, compounds **12** and **13** which have a fluoro atom on the ortho or meta position of the phenyl ring respectively show comparable cellular activities in both of

the two cell lines and are only 2-times less potent than compound **4**. Compounds **18** and **19** which have a bromo atom on the ortho or meta position respectively are slightly more potent than **12** and **13**, suggesting that a larger halo atom on these two position is more favorable to the cellular activity. Different from the orhto and meta substitutions, introduction of a halo atom to the para position of this phenyl ring can improve the cellular activity by 2-5 fold (compounds **14**, **17** and **20** *vs* **4**). Compound **17** which has a chloro atom on the para positon of this phenyl ring shows IC_{50} values of 0.4 and 0.07 μM to H3122 and MV4;11 cell lines respectively and is the most potent compound in this series. We have also tried introduction of other hydrophobic groups, such as methoxyl, methyl and trifluoromethyl groups (**21**-**25**) to different positions of the phenyl ring and found the same trend. Interestingly, it seems that the position for introducing the substituted group but not the substituted group itself has more significant influence to the cellular activity. If these groups were introduced to the para position, the cellular activity can be improved, but if the groups were introduced to the ortho or meta position, the cellular activity was not dramatically influenced.

We then explored the substituted group on N_6 for its influence to the anti-cancer cellular activity by designing and synthesizing compounds **26**-**40**. In these compounds the substituted groups on N_1 and N_3 were kept as ethyl and benzyl groups respectively, while various hydrophobic groups were used to replace the benzyl group on N_6 of compound **4**. These compounds were also evaluated for their abilities in inhibiting cell growth in H3122 and MV4;11 cell lines and the results are reported in **Table 4**. Similar to the modifications of the substituted group on N_3, replacement of the benzyl group on N_6 of compound **4** with small hydrophobic groups, such as methyl, ethyl or cyclopropylmethyl significantly decreases the cellular activities (compounds **26**-**28** *vs* **4**) by more than 30 fold. Replacement of the benzyl group with a phenyethyl group (compound **29**) also decreases the cellular activities by more than 10 fold, indicating that this benzyl group is also critical for the cellular activity. We then tried introduction of a halo atom to different positions of the phenyl ring. For this phenyl ring, introduction of a F, Cl or Br atom to the ortho position dramatically decreases the cellular activity by 4-20 fold (**30**, **33** and **36** *vs* **4**), indicating that a substituted group on the ortho position is detrimental to the cellular activity. Introduction of a halo atom to the para position doesn't have significant influence to the cellular activity (**32**, **35** and **38** *vs* **4**), but compounds **31**, **34** and **37** which have a F, Cl and Br at the meta position of the phenyl ring have 3-20 times more potent cellular activities in these two cell lines than **4**. Compound **34** shows IC_{50} values of 0.3 and 0.02 μM in inhibiting the cell growth in these two cell lines and is the most potent compound in this series. We have also tried introduction of a methoxy group to the meta and para positions of the phenyl ring and found meta substituted compound **39** is slightly more potent than **4** while para substituted compound **40** is about 8 times less potent than **4**. These results also suggested that the meta substituted compound has more potent cellular activity, but **39** is 4 and 10 times less potent than **34** in inhibiting the cell growth of H3122 and MV4;11 cell lines respectively, indicating that a larger group at the meta position is not favorable.

Table 4. Cell growth inhibition of compounds **26**-**40** in H3122 and MV4;11 cell lines.

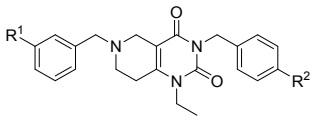
Compounds	R	H3122 IC_{50} (μM)	MV4;11 IC_{50} (μM)
ONC201		8.7±2.3	3.0±1.3
4	Bn	1.6±0.2	0.4±0.1
26	methyl	84.3±27.9	19.4±4.6
27	ethyl	79.6±24.6	18.8±3.9
28		54.7±18.8	13.2±3.3
29		22.5±5.9	3.8±0.9
30		17.3±4.6	1.6±0.3
31		0.4±0.1	0.04±0.01
32		3.1±0.8	0.5±0.2
33		21.9±2.7	3.3±0.9
34		0.3±0.1	0.02±0.01
35		4.1±0.9	0.6±0.2
36		32.5±11.7	3.9±1.1
37		0.6±0.1	0.07±0.02
38		3.0±0.9	0.3±0.1
39		1.2±0.3	0.2±0.1
40		11.9±4.7	1.6±0.3



Combining the SAR information obtained from optimizations of the substituted groups on the two phenyl rings we designed compounds **41**-**49** in which two halo atoms were introduced to the meta position of the phenyl ring in the benzyl group on N_6 and the para position of the phenyl ring in the benzyl group on N_3, respectively. These compounds have also been evaluated for their anti-cancer cellular activities in H3122 and MV4,11 cell lines and the results are reported in **Table 5**. All of these compounds show more potent or comparable cellular activities as the corresponding compounds in which only one of the two benzyl groups on N_3 and N_6 contains halo atom substitution at corresponding position. For example, compounds **41**-**43** which contain a para fluoro

substituted benzyl group on N_3 and a meta fluoro, chloro or bromo substituted benzyl group on N_6 respectively shows IC_{50} values of 24, 21 and 55 nM in inhibiting the cell growth of MV4,11 cell line and are 4-8 times more potent than **14** in which only the benzyl group on N_3 has a para fluoro substitution (**Table 3**). These three compounds are also slightly more potent than their corresponding compounds **31**, **34** and **37** which contain a benzyl group on N_3 and a fluoro, chloro or bromo substituted benzyl group on N_6 respectively. Compounds **43**, **46** and **49** which contain a meta bromo substituted benzyl group on N_3 show slightly less potent cellular activities than other compounds in this series, indicating that a larger substituted group at the meta position of this phenyl ring is not favorable to the cellular activity, consistent to the SAR found from the optimization of the benzyl group on N_3 (**Table 4**).

Table 5. Cell growth inhibition of compounds **41-49** in H3122 and MV4;11 cell lines.



Compounds	R^1	R^2	H3122 IC_{50} (nM)	MV4,11 IC_{50} (nM)
41	F	F	134±29	24±6
42	Cl	F	158±35	21±4
43	Br	F	326±49	55±7
44	F	Cl	217±31	33±7
45	Cl	Cl	147±32	23±6
46	Br	Cl	386±53	59±16
47	F	Br	223±59	39±13
48	Cl	Br	139±41	21±5
49	Br	Br	379±39	62±17

In summary, a series of tetrahydropyrido[4,3-d]pyrimidine-2,4-diones have been designed and synthesized as analogues of a clinical lead ONC201. Preliminary mechanism studies indicated that these compounds have similar mechanism of functions as ONC201. These compounds potently inhibit cell growth in a panel of human cancer cell lines and modifications of the substituted groups on the core structure can dramatically improve the cellular activities. Therefore, these compounds represent a class of novel anti-cancer agents worthy of further studies. More detailed SAR studies and investigations to identify the protein targets of these compounds is proceeding.

ASSOCIATED CONTENT

Supporting Information

The experimental details for the synthesis of the designed compounds, assays for cell growth inhibition, western blot analysis of the biomarkers, the detection of TRAIL concentration, the detection of SubG1 DNA

Supporting Information for analogues of ONC201.pdf

The Supporting Information is available free of charge on the ACS Publications website.

AUTHOR INFORMATION

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Author Contributions

All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Pan, G.; O'Rourke, K.; Chinnaiyan, A. M.; Gentz, R.; Ebner, R.; Ni, J.; Dixit, V. M. J. S. The Receptor for the Cytotoxic Ligand TRAIL. *Science*, **1997**, *276*, 111-113.

(2) Ashkenazi, A. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. *Nat. Rev. Cancer* **2002**, *2*, 420-430.

(3) Ashkenazi, A. Targeting the extrinsic apoptotic pathway in cancer: Lessons learned and future directions. *J. Clin. Invest.* **2015**, *125*, 487–489.

(4) Allen, J. E.; Krigsfeld, G.; Mayes, P. A.; Patel, L.; Dicker, D. T.; Patel, A. S.; Dolloff, N. G.; Messaris, E.; Scata, K. A.; Wang, W. Dual inactivation of Akt and ERK by TIC10 signals Foxo3a nuclear translocation, TRAIL gene induction, and potent antitumor effects. *Sci. Transl. Med.* **2013**, *5*, 171ra17.

(5) Allen, J. E.; Krigsfeld, G.; Patel, L.; Mayes, P. A.; Dicker, D. T.; Wu, G. S.; El-Deiry, W. S. Identification of TRAIL-inducing compounds highlights small molecule ONC201/TIC10 as a unique anti-cancer agent that activates the TRAIL pathway. *Mol. Cancer* **2015**, *14*, 99.

(6) Prabhu, V.V.; Allen, J. E.; Dicker, D. T; El-Deiry, W. S. Small-molecule ONC201/TIC10 targets chemotherapy-resistant colorectal cancer stem-like cells in an Akt/Foxo3a/ TRAIL-dependent manner. *Cancer Res.* **2015**, *75*, 1423-1432.

(7) Kline, C. L. B.; Heuvel, A. P. J. V. D.; Allen, J. E.; Prabhu, V. V.; Dicker, D. T.; Eldeiry, W. S. ONC201 kills solid tumor cells by triggering an integrated stress response dependent on ATF4 activation by specific eIF2α kinases. *Sci. Signal.* **2016**, *9*, ra18.

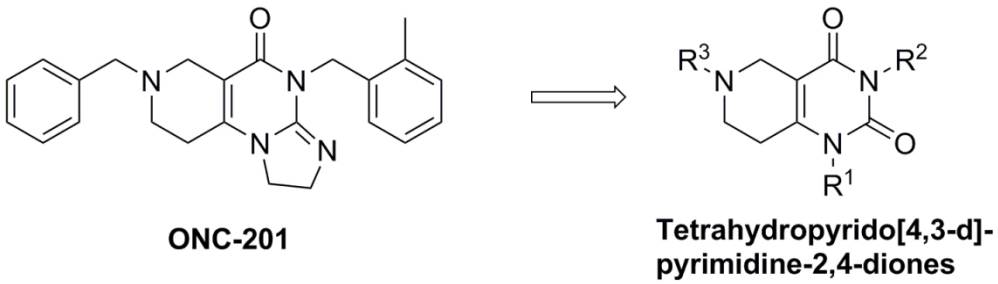
(8) Ishizawa, J.; Kojima, K.; Chachad, D.; Ruvolo, P.; Ruvolo, V.; Jacamo, R. O.; Borthakur, G.; Mu, H.; Zeng, Z.; Tabe, Y. ATF4 induction through an atypical integrated stress response to ONC201 triggers p53-independent apoptosis in hematological malignancies. *Sci. Signal.* **2016**, *9*, ra17.

(9) Talekar, M. K.; Allen, J. E.; Dicker, D. T.; El-Deiry, W. S. ONC201 induces cell death in pediatric non-Hodgkin's lymphoma cells. *Cell cycle* **2015**, *14*, 2422-2428.

(10) Allen, J. E.; Prabhu, V. V.; Talekar, M.; Ap, V. D. H.; Lim, B.; Dicker, D. T.; Fritz, J. L.; Beck, A.; Eldeiry, W. S. Genetic and Pharmacological Screens Converge in Identifying FLIP, BCL2, and IAP Proteins as Key Regulators of Sensitivity to the TRAIL-Inducing Anticancer Agent ONC201/TIC10. *Cancer Res.* **2015**, *75*, 1668.

(11) Jin, Z. Z.; Wang, W.; Fang, D. L.; Jin, Y. J. mTOR inhibition sensitizes ONC201-induced anti-colorectal cancer cell activity. *Biochem. Biophys. Res. Commun.* **2016**, *478*, 1515-1520.

(12) Allen, J. E.; Kline, C. L. B.; Prabhu, V. V.; Wagner, J.; Ishizawa, J.; Madhukar, N.; Lev, A.; Baumeister, M.; Zhou, L.; Lulla, A. J. O. Discovery and clinical introduction of first-in-class imipridone ONC201. *Oncotarget*, **2016**, *7*, 74380-74392.



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