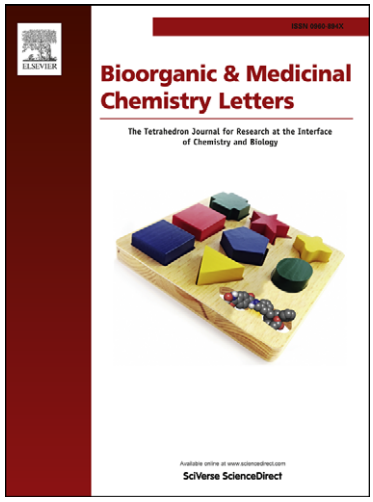


Design, synthesis and biological evaluation of novel imidazo[4,5-c]pyridine-carboxamide derivatives as PARP-1 inhibitors

Qihua Zhu, Xuyang Wang, Zhaoxing Chu, Guangwei He, Guangping Dong, Yungen Xu

PII: S0960-894X(13)00208-4
DOI: http://dx.doi.org/10.1016/j.bmcl.2013.02.032
Reference: BMCL 20150

To appear in: *Bioorganic & Medicinal Chemistry Letters*

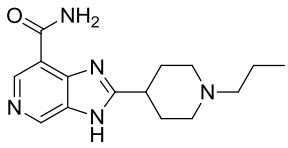
Received Date: 26 October 2012
Revised Date: 28 January 2013
Accepted Date: 6 February 2013

Please cite this article as: Zhu, Q., Wang, X., Chu, Z., He, G., Dong, G., Xu, Y., Design, synthesis and biological evaluation of novel imidazo[4,5-c]pyridinecarboxamide derivatives as PARP-1 inhibitors, *Bioorganic & Medicinal Chemistry Letters* (2013), doi: http://dx.doi.org/10.1016/j.bmcl.2013.02.032

Graphical Abstract

Design, synthesis and biological evaluation of novel imidazo[4,5-c]pyridinecarboxamide derivatives as PARP-1 inhibitors

Qihua Zhu[a], Xuyang Wang[a], Zhaoxing Chu[b], Guangwei He[b,*], Guangping Dong[a], Yungen Xu[a,*]



8d, IC_{50}=0.528μM

A series of novel imidazo[4,5-c]pyridinecarboxamide derivatives as PARP inhibitors is described.

Design, synthesis and biological evaluation of novel imidazo[4,5-c]pyridinecarboxamide derivatives as PARP-1 inhibitors

Qihua Zhu[a], Xuyang Wang[a], Zhaoxing Chu[b], Guangwei He[b,*], Guangping Dong[a], Yungen Xu[a,*]

[a]*Jiangsu Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, Nanjing 21009, China*

[b]*Hefei YiGong Pharmaceutical Co. Ltd., Hefei, 230088, China*

*Corresponding authors: YungenXu

Email: xu_yungen@hotmail.com

Tel.:86-25-83271244

Abstract

A series of novel cyclic amine-substituted imidazo[4,5-c]pyridinecarboxamide analogs were designed and synthesized. All the target compounds were evaluated for their PARP inhibition activity, and the result indicated that most of the compounds possessed inhibitory effect on PARP at the concentration of 1μM, among which compound **8d** (IC_{50}=0.528μM) was selected for evaluating the antitumor effect in vivo. The result showed the antitumor efficacy of the compound **8d** and cisplatin combination group in a mouse A549 model is similar with that of the ABT-888 and cisplatin combination group.

Keyword: Imidazo[4,5-c]pyridinecarboxamide; PARP-1; PARP Inhibitors; Antitumor

Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme with an important role in the process of genomic repair. To date, 18 members of the PARP family have been identified and characterized. Among the PARP family, PARP-1 is one of the most abundant proteins and has been believed the main isoform[1]. The PARP-1 enzyme has three structural regions: the DNA binding domain containing two zinc fingers, the automodification domain, and the catalytic domain[2]. The catalytic domain catalyze the transfer of ADP-ribose units from intracellular nicotinamide adenine dinucleotide (NAD^+) to nuclear acceptor proteins and is responsible for the formation of ADP-ribose polymers[3]. It is generally accepted that the catalytic activity of PARP-1 is stimulated by DNA damage caused by peroxidation, irradiation, and DNA-damaging chemicals, e.g., chemotherapeutic agents[4]. The major role of PARP-1 is important in number of cellular processes, thus it is regarded as a target for treating diseases related to ischemia-reperfusion injury and cancer. Indeed, PARP-1 inhibitors have been identified to be effective in animal models of ischemic stroke, traumatic brain injury, Parkinson's disease and cancer[5]. Moreover, recent studies have shown that cells deficient in BRCA1 and BRCA2, proteins involved in DNA double strand break repair by homologous recombination (HR) repair are highly sensitive to PARP-1 inhibitors[6].

Figure **1** is a schematic presentation of X-ray structure of NAD^+ binding to PARP-1, and formation of multiple hydrogen bond interactions between the carboxamide moiety and two critical amino acid residues in the PARP active site, Gly-863 and Ser-904[7]. In addition, Tyr907 forms a planar surface which has a π-π-interaction with the nicotinamide group and stabilizes the oxonuim ion. Early studies identified nicotinamide (IC_{50}=210μM) and 3-aminobenzamide (IC_{50}=30μM) as inhibitor of PARP-1[8]. However, these compounds show low potency and poor specificity. The tremendous academic and industrial efforts have been made to find new inhibitors with good potency against PARP-1. It is very pleasure that many groups have obtained good results. Over the past three decades, a variety of PARP-1 inhibitors have been reported[9]. It is clear that the majority of PARP inhibitors are based on the benzamide as pharmacophore, which mimics the nicotinamide moiety of NAD^+binding mode, by

conformational restriction of a primary amide or by transformation of an amide into a lactam.

Although a variety of PARP-1 inhibitors have been disclosed in the literature, many suffer from development problems such as toxicity, poor solubility, or poor pharmacokinetic profiles. The effort of pursuing new inhibitors with good potency against PARP-1 is still needed. The recent studies found that the benzimidazolecarboxamides core is one of the most potent scaffold structures for PARP-1 inhibitors, and many benzimidazole-4-carboxamide derivatives had been developed. For example, 2-[(R)-2-methyl-pyrrolidin-2-yl]-1H-benzimidazole-4-carboxamide (ABT-888, veliparib)[10] is currently on-going phase II clinical trials. In an effort to search novel PARP-1 inhibitors and explore alternative chemical templates, we took the pyridine ring instead of benzene ring in benzimidazole. And the recent studies[11] also found that incorporation of a basic amine into the 2-substituent of the benzimidazole ring system in many cases demonstrated good enzyme potency. On the basis of the above structure-activity relationship of PARP-1 inhibitors, we designed a series of cyclic amine-substituted imidazo [4,5-c]pyridinecarboxamide analogs as new PARP-1inhibtiors.

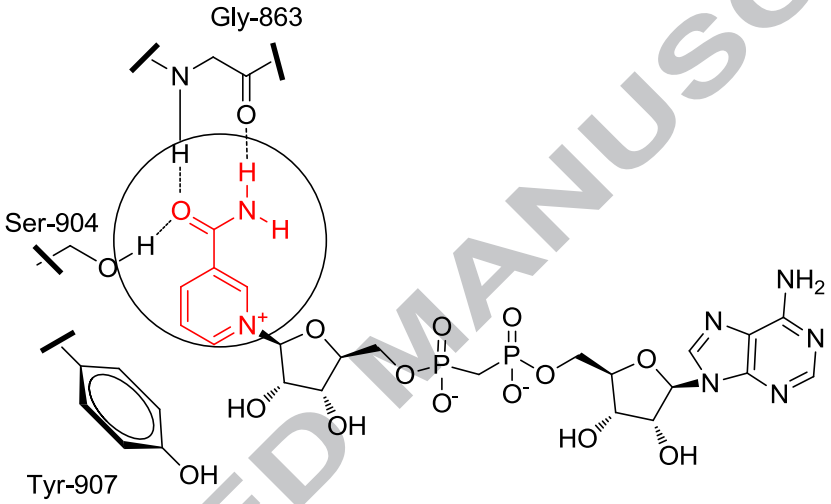


Figure 1. A schematic presentation of the X-ray structure of NAD^+ binding PARP-1. The cycled part of structure (red) showed critical hydrogen bond interactions of NAD^+ with PARP-1[7].

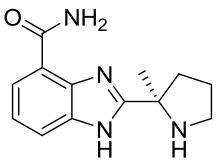


Figure 2. The structure of ABT-888

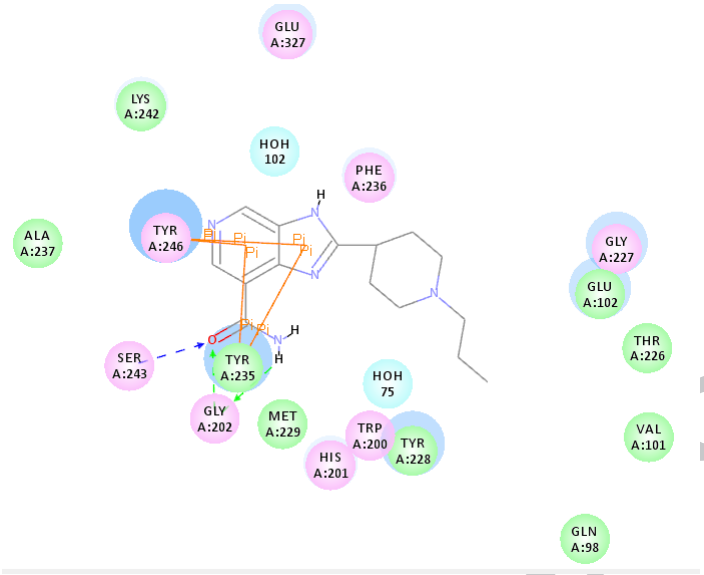
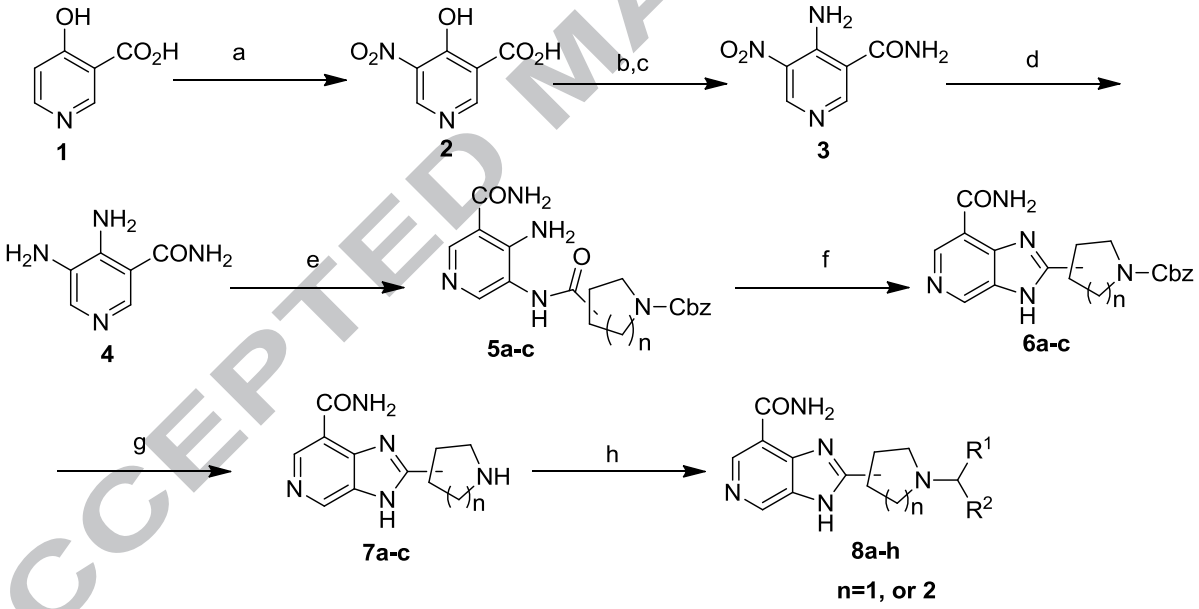


Figure 3. Docking of compound **8d** in the catalytic domain of human PARP-1 (PDB code: 2RCW)



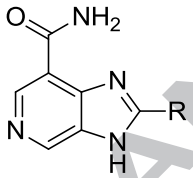
Scheme 1. Synthesis route for target compounds. Reactions and conditions: (a) conc H_2SO_4, fuming HNO_3; (b) $SOCl_2$; (c) $NH_3.H_2O$, CH_3CN, 0°C to r.t, 24h, 80%; (d) 10% Pd-C, H_2, MeOH, r.t., 12h, 90%; (e) i: 2-pyrrolidinecarboxylic acid, 4-pipecolinic acid, $SOCl_2$, Pyridine, methylene chloride, reflux, 2h; ii: PyBOP, 3-pipecolinic acid, N,N-diisopropylethylamine, DMF, r.t, 12h; (f) AcOH, 160°C, in an autoclave, 24h, 42%-50.3%; (g) 10% Pd-C, H_2, MeOH, r.t., 12h, 90%; (h) $R^1R^2C(O)$, $NaBH_3CN$, MeOH, r.t. 31%-65%;

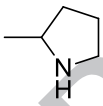
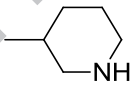
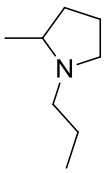
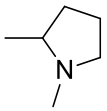
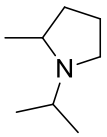
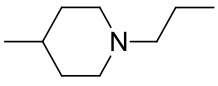
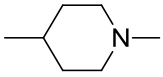
Herein, we describe the design and synthesis of a new series of imidazo[4,5-c]pyridinecarboxamide derivatives. Crystallographic structure of the human PARP-1 catalytic domain (PDB code: 2RCW) was obtained from the Protein Data Bank (PDB) database, and a docking study was performed to approach the development of potent inhibitors. Docking of compound **8d** in the catalytic domain of human PARP-1 is shown in **Figure 3**. The key interactions in the PARP-1 active site, consistent with previous literature reports, are highlighted. Both Ser-243 and

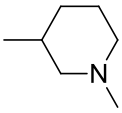
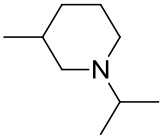
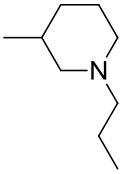
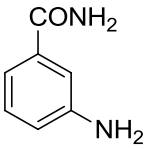
Gly-202 are involved in key hydrogen-bond interactions with the carboxamide group of **8d**. There is also a π-stacking interaction between the imidazo[4,5-c]pyridine ring and Tyr-246 and Tyr-235. These same interactions are also displayed in the binding of NAD^+ (**Fig. 1**) to the PARP-1 active site.This result led us to design its analogs.

To verify this docking study, imidazo[4,5-c]pyridine-7-carboxamide derivativeswere synthesized, as outlined in Scheme **1**. Nitration of **1** with mixed acid of fuming nitric acid and sulfuric acid afforded nitro compound **2**. Treatment of **2** with thionyl chloride smoothly produced the corresponding acid chloride, which was reacted with 12% aqueous ammonia at 0℃ for 30 min, then at room temperature overnight to give 4-amino-5-nitronicotinamide **3**. 4,5-Diaminonicotinamide **4**, obtained by catalytic hydrogenation of **3**, was coupling with saturated nitrogen-containing heterocyclic acid under PyBOP or $SOCl_2$ conditions to form intermediates **5a-c**, which were heated in acetic acid to give imidazo[4,5-c]pyridinecarboxamide derivatives **6a-c**. Deprotection of Cbz group using catalytic hydrogenation provide a secondary amines **7a-c**[12]. *N*-alkylation of **7a-c**withaldehydes or ketones under sodium cyanoborohydride reductive amination condition afforded tertiary amines **8a-h**[13].

Table1. The structures and PARP-1 enzyme inhibition activity of compounds **7a-c** and **8a-h**



Compd.	Substituents (R)	Inhibition (%)[a]	IC_{50} (μM)
7a		32.6	ND
7b		76.2	0.825
7c		60.3	0.636
8a		1.9	ND
8b		4.7	ND
8c		17.4	ND
8d		79.0	0.528
8e		56.6	1.452

8f		23.1	ND
8g		65.5	0.651
8h		67.1	0.938
3-AB		100[b]	ND
ABT-888		97	0.019

a: Inhibitory ratio % at 1μM; b: PARP-1 inhibition at the concentration of 100μM; ND, not determined.

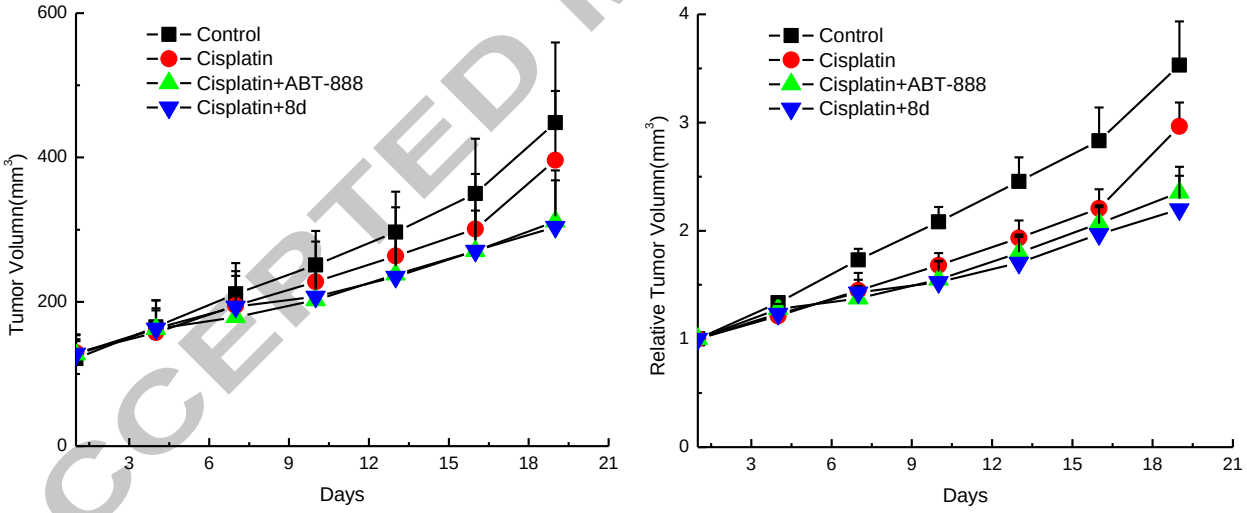


Figure 4. Antitumor efficacy of 8**d** in mice bearing tumors derived from A549 cells when dosed orally at 25 mg/kg for 19 days. For each group, n = 5.

All target compounds were evaluated in vitro for their PARP-1 enzyme inhibition activity (Table 1). The result showed that some target compounds possessed PARP-1 inhibition activities at the concentration of 1μM. Compounds with PARP-1inhibitory ratio >50% were selected to determine IC_{50} values, and the results were listed in table 1. Among compounds **7a-c**, compound**7a**, bearing a 2-pyrrolidinyl group at 2 position, showed low inhibitory activity, while the 3- or 4-piperidyl analogs **7b** and **7c** showed relatively good potency. Among the *N*-alkyl derivatives, compounds **8a-c**, deriving from **7a**, showed less active than **7a** in the enzyme assay. Compared to **7b**, its *N*-alkyl derivatives **8d** and **8e** showed similar inhibitory effect on PARP-1. The *N*-methyl derivatives **8f** exhibited less inhibition activity against PARP-1 than its parent compound **7c** and *N*-propyl or isopropyl analogues (**8g** or **8h**). Similar to **8f**, *N*-methyl derivatives **8b** and **8e** displayed weaker activity in the enzyme assay compared

to **7a** and **7b**.

Compound **8d** was selected for evaluating the in vivo antitumor effect, and the result showed the antitumor efficacy of the compound **8d** and cisplatin combination group in a mouse A549 model is similar with that of the ABT-888 and cisplatin combination group (**Figure 4**)[16]. The cisplatin/ABT-888 and cisplatin/8**d** combination group both showed significantly more effective than cisplatin alone in this model. Tumor regression was observed with both combination group, and both were well tolerated, with no mortality. Less than 10% body weight loss was seen during the experiment.

In summary, we reported the synthesis and biological evaluation of novel imidazo[4,5-c]pyridinecarboxamide derivatives as potent PARP-1 inhibitors. Compound **8d** (IC_{50}=0.528μM) was found to be the most potent one in vitro. In a mouse A549 model, the cisplatin/**8d** combination group is efficacious at well tolerated doses when administered orally. Further biological evaluation of this class of derivatives is ongoing and will be reported in the near future.

Acknowledgment

This work was supported by the enterprise innovation drug incubation base of the Ministry of Science and Technology, China (No.2012ZX09401-006) and the Graduate Students' Innovation Personnel Training Plan of China Pharmaceutical University (No. 2011IIBPY02).The authors are grateful to Institute of Pharmacology & Toxicology and Biochemical Pharmaceutics at Zhejiang University for their help in the biological test.

References

1. Ame, J. –C.; Spenlehauer, C.; de Murcia, G. BioEssays. 2004, 26, 1148.
2. (a) Rolli, V.; O'Farrell, M.; Menissier-de Muricia, J.; de Muricia, G. Biochemistry. 1997, 36, 12147; (b) de Murcia, G.; Schreiber, V.; Molinete, M.; saulier, B.; Poch, O.; Masson, M.; Niedergang, C.; Menissier de Murcia. Mol. Cell. Biochem. 1994, 138, 15.
3. Jeggo, P. A. Curr. Biol. 1998, 8, R49.
4. (a) Szabo, C.; Zingarelli, B.; O'Connor, M.; Salzman, A. L. Proc. Natl. Acad. Sci. U.S.A.1996, 93, 1753;(b)de Murica, J. M.; Niedergang, C.; Trucco, C.; Ricoul, M.; Dutrillaux, B.; Mark, M.; et. al. Proc. Natl. Acad. Sci. U.S.A.1997, 94, 7303; (c) Bowman, K. J.; White, A.; Golding, B. T.; Griffin, R. J.; Curtin, Br. J. Cancer 1998, 78, 1269.
5. (a) Zaremba, T.; Curtin, N. J. Anticancer Agents Med, Chem. 2007, 7, 515; (b)Graziani, G.; Battaini, F.; Zhang, J. Pharmacol. Res. 2005, 52. 1. 1.
6. (a) Bryant, H. E.; Schultz, N.; Thomas, H. D.; Parker, K. M.; Flower, D.; Lopez, E.; Kyle, S.; Meuth, M.; Curtin, N. J.; Helleday, t. Nature (London, U.K.) 2005, 434, 913; (b) MacCabe, N.; Tumer, N. C.; Lord, C. J.; Kluzek, K.; Bialkowska, A.; Swift, S.; Giavara, S.; O'Connor, M. J.; Tutt, A. N.; Zdzienicka, M. Z.; Smith, G. C. M.; Ashworth, A. Cancer Res. 2006, 66, 8109.
7. (a) Ame, J. –C.; Spenlehauer, C.; de Murcia, G. BioEssays 2004, 26, 882; (b) Virag, L.; Szabo, C.; Pharmacol. Rev. 2002,54, 375; (c) Jagtap, P.; Szabo, C. Nat. Rev. Drug. Discovery 2005, 4. 421.
8. (a) Purnell, M. R.; Whish, W. J. D. Biochem. J. 1980, 185, 775; (b) Durkacz, B. W.; Olusesan, O.; Gray, D. A.; Shall, S. Nature 1980, 283, 593; (c) Sestili, P.; Spadoni, G.; Balsamini, C.; Scovassi, I.; Cattabeni, F.; Duranti, E.; Cantoni, O.; Higgins, D.; Tomson, C. J. Cancer Res. Clin. Oncol. 1990, 116, 615.
9. (a) Park, C. H.; Chun, K.; Joe, B. Y.; Park, J. S.; Kim, Y. C.; Choi, J. S.; Ryu, D. K.; Koh. S. H.; Cho, G. W.; Kim, S. H.; Kim, M. H., Bioorg. Med. Chem. Lett. 2010, 20, 7, 2250; (b) Gandhi, V. B.; Luo, Y.; Liu, X.;

Shi, Y.; Klinghofer, V.; Johnson, E. F.; Park, C.; Giranda, V. L.; Penning, T. D.; Zhu, G. D. Bioorg. Med. Chem. Lett. 2010, 20, 3, 1023; (c) Pescatore, G.; Branca, D.; Fiore, F.;Kinzel, O.; Bufi, L. L.; Muraglia, E.; Orvieto, F.; Rowley, M.; Toniatti, C.; Torrisi, C.; Jones, P. Bioorg. Med. Chem. Lett. 2010, 20, 1094. (d) Menear, K. A.; Adcock, C.; Boulter, R.; Cockcroft, X. L.; Copsey, L.; Cranston, A.; Dillon, K. J.; Drzewiecki, J.; Garman, S.; Gomez, S.; Javaid, H.; Kerrigan, F.; Knights, C.; Lau, A.; Loh VM, J. R.; Matthews, I. T.; Moore, S.; O'Connor, M. J.; Smith, G. C.; Martin, N. M. J. Med. Chem. 2008, 51, 20, 6581. (e) Thomas, H. D.; Calabrese, C. R.; Batey, M. A.; Canan, S.; Hostomsky, Z.; Kyle, S.;Maegley, K. A.; Newell, D. R.; Skalitzky, D.; Wang, L. Z.; Webber, S. E.; Curtin, N.J. Mol. Cancer Ther. 2007, 6, 945.

10. (a) Donawho, C. K; Luo, Y.; Luo, Y.; Penning, T. D.; Bauch, J. L.; Bouska, J. J.; Bontcheva-Diaz, V. D.; Cox, B. F.; DeWeese, T. L.; Dillehay, L. E.; Ferguson, D. C.; Ghoreishi-Haack, N. S.; Grimm, D. R.; Guan, R.; Han, E. K.; Holley-Shanks, R. R.; Hristov, B.; Idler, K. B.; Jarvis, K.; Johnson, E. F.; Kleinberg, L. R.; Klinghofer, V.; Lasko, L. M.; Liu, X.; Marsh, K.C.; McGonigal, T. P.; Meulbroek, J. A.; Olson, A. M.; Palma, J. P.; Rodriguez, L. E.; Shi, Y.; Stavropoulos, J. A.; Tsurutani, A. C.; Zhu, G. D.; Rosenberg, S. H.; Giranda, V. L.; Frost, D. J. Clin. Cancer. Res. 2007, 13, 2728; (b) Penning, T. D.; Zhu, G. D.; Gandhi, V. B.; Gong, J.;Liu, X.; Shi, Y.; Klinghofer, V.; Johnson, E. F.; Donawho, C. K.; Frost, D. J.Bontcheva-Diaz, V.; Bouska, J. J.; Osterling, D. J.; Olson, A. M.; Marsh, K. C.; Luo, Y.; Giranda, V. L. J. Med. Chem. 2009, 52, 2, 514.

11. Penning, T. D.; Zhu, G. D.; Gandhi, V. B.; Gong, J.; Thomas, S.; Lubisch, W.; Grandel, R.; Wernet, W.; Park, C. H.; Fry, E. H.; Liu, X.; Shi, Y.; Klinghofer, V.; Johnson, E. F.; Donawho, C. K.; Frost, D. J.; Bontcheva-Diaz, V.; Bouska, J. J.; Olson, A. M.; Marsh. K, C.; Luo, Y.; Rosenberg, S. H.; Giranda, V. L. Bioorg. Med. Chem. 2008, 16, 14, 6965;

12. General Procedure for the synthesis of the target compounds 7a-7c: (**i**) A suspension of 2-pyrrolidinecarboxylic acid or 4-pipecolinic acid (10 mmol) in a mixture of dichloromethane (80 mL), thionyl chloride (3.5 mL) was heated to reflux overnight. The resulting solution was concentrated and acid chloride intermediate was used in the next step without additional purification. To a solution of 4,5-diaminonicotinamide **4** (6.5 mmol) in DMAc (20 mL) and pyridine (1.2 mL) was added the acid chloride in anhydrous THF (10 mL) at 0 °C. After stirring at room temperature overnight, the reaction mixture was concentrated under reduced pressure. The residue was poured into crushed ice. The precipitate of that formed was filtered off and dried to give the intermediate **5** which used in next step without purification. (**ii**) To a solution of 3-pipecolinic acid (10 mmol) and 5-diaminonicotinamide **4** (6.5 mmol) in DMF (10 mL) was treated with PyBOP (1.0mmol) and N,N-diisopropylethylamine (4.0 mmol). The reaction mixture was stirred at ambient temperature overnight. The solvent was removed using high vacuum. The residue was subjected to flash column chromatography using methylene chloride/methanol (15:1) to give the intermediate **5** as a white solid. The intermediate **5** was dissolved in glacial acetic acid (10 mL) in an autoclave and heated at 160 °C until the reaction was complete (monitoring by TLC). The solvent was removed and the solid residue was purified by column chromatography using methylene chloride/methanol (30:1) as eluentto give pure **6a-6c** in 42%-50.3% yield. A solution of **6a-6c** (2.7 mmol) in methanol (50 mL) was reduced with hydrogen in the presence of palladium on carbon (10%, 0.1 g). After stirring at room temperature for 12 h, the reaction mixture was filtered, and the filtrate was concentrated to give pure target compounds **7a-7c** in 90% yield.

13. General Procedure for the synthesis of the target compounds 8a-8h: A solution of 7a-7c in MeOH (10 mL) was stirred with formaldehyde, acetone or propionaldehyde at ambient temperature for 5 min.Sodium cyanoborohydride was added and the solution stirred at ambient temperature overnight. After

concentration, the residue was purified by column chromatography using methylene chloride/methanol (30:1 to 15:1) as eluent to give the target compounds **8a-8h** in 31-65% yield.

14. Analytical data for selected final compound **7b**: white solid. M.P.: 96-98°C. ^{1}H-NMR (300 MHz, DMSO-d6) δ: 8.92 (s, 1H), 8.89 (s, 1H), 8.76 (s, 1H), 7.73 (s, 1H), 3.12 (m, 3H), 2.68 (m, 2H), 2.02 (m, 2H), 1.80 (m, 2H). ^{13}C-NMR (DMSO, 75 MHz) δ: 166.9, 163.5, 144.1, 140.6, 138.6, 115.9, 44.8, 36.1, 30.7; ESI-MS (m/z): 246 $[M+1]^+$. HRMS (m/z) for $C_{12}H_{16}N_5O$: calcd 246.1349, found 246.1351.

15. Analytical data for selected final compound **8g**: yellow solid. M.P.: 122-124°C.^{1}H-NMR (300 MHz, CD_3OD) δ: 8.87(s, 1H), 8.82 (s, 1H), 3.31 (m, 1H), 3.22 (m, 1H), 2.91 (m, 2H), 2.78 (m, 1H), 2.47 (m, 1H), 2.14 (s, 1H), 1.80 (m, 3H), 1.14 (d, *J*=6.0 Hz, 6H); ^{13}C-NMR (CD_3OD, 75 MHz) δ: 168.9, 164.0, 145.3, 141.8, 139.5, 137.5, 117.4, 49.9, 56.5, 53.5, 37.9, 30.4, 25.2, 18.2, 18.0; ESI-MS (m/z): 288 $[M+1]^+$, 310 $[M+Na]^+$. HRMS (m/z) for $C_{15}H_{22}N_5O$: calcd 288.1819, found 288.1823.

16. The antitumor efficacy of target compounds was conducted in a mouse A549 model. For A549 syngeneic studies, 2×10^6 cells were injected subcutaneously in the flank of 5-week-old nude mice. When tumors reached volume of 100 to 300 mm^3, mice were randomized to form homogeneous groups and treatment started, dosing orally. Mice were dosed orally with cisplatin (2mg/kg) once every three days, while treated with ABT-888 (25mg/kg) or 8**d** (25mg/kg) twice a day respectively for 19 days, with tumor growth and body weight measurement done once every three days.