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Synthesis and structure–activity relationship studies of LLY-507 analogues as SMYD2 inhibitors

Bin Zhang[a,#], Liping Liao[b,c,#], Fan Wu[a,#], Fengcai Zhang[b,c], Zhongya Sun[b,c], Haijun Chen[a,*], Cheng Luo[b,c,*]

[a]*Key Laboratory of Molecule Synthesis and Function Discovery (Fujian Province University), College of Chemistry, Fuzhou University, Fuzhou, Fujian 350116, China*

[b]*State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai, 201203, China*

[c]*University of Chinese Academy of Sciences, 19 Yuquan Road, Beijing, 100049, China*

**E-mail addresses:*

chenhaij@gmail.com (H. Chen)

cluo@simm.ac.cn (C. Luo)

[#]These authors contribute equally to this work.

Abstract

SET and MYND domain-containing protein 2 (SMYD2), a lysine methyltransferase, is reported to catalyze the methylation of lysine residues on histone and non-histone proteins. As a potential target for cancer therapy, there are several SMYD2 inhibitors are reported, LLY-507 as a cell-active inhibitor exhibits submicromolar potency against SMYD2 in several cancer cell lines. To know which structural fragment of LLY-507 is suitable for chemical modification, three sites are chosen for structure–activity relationship studies (SARs). Among our focused library, compounds **43** and **44** with amide link on site C showed reasonably improved potency indicating that modification on this fragment is more flexible and introduction of electrophilic warheads in this position might provide lysine-targeting covalent inhibitors for SMYD2.

Keywords: SMYD2 inhibitors; Thermal shift assay; Structure-activity relationships.

Protein lysine methyltransferases play a critical role in epigenetic gene regulation.[1,2] The SMYD family, containing SET and MYND domains, is one special and important class of protein lysine methyltransferases.[3,4] Accumulating evidence has shown that SMYD2, one of the SMYD members, is overexpressed in various types of human cancers.[5-7] SMYD2 has been proposed as a potential oncogene that affects the proliferation, apoptosis, and metastasis of cancer cells via histone or non-histone methylation.[8-11] Therefore, there are intensive ongoing efforts to identify SMYD2 inhibitors as valuable chemical probes to elucidate the role of SMYD2 in cancer and other diseases.[12,13]

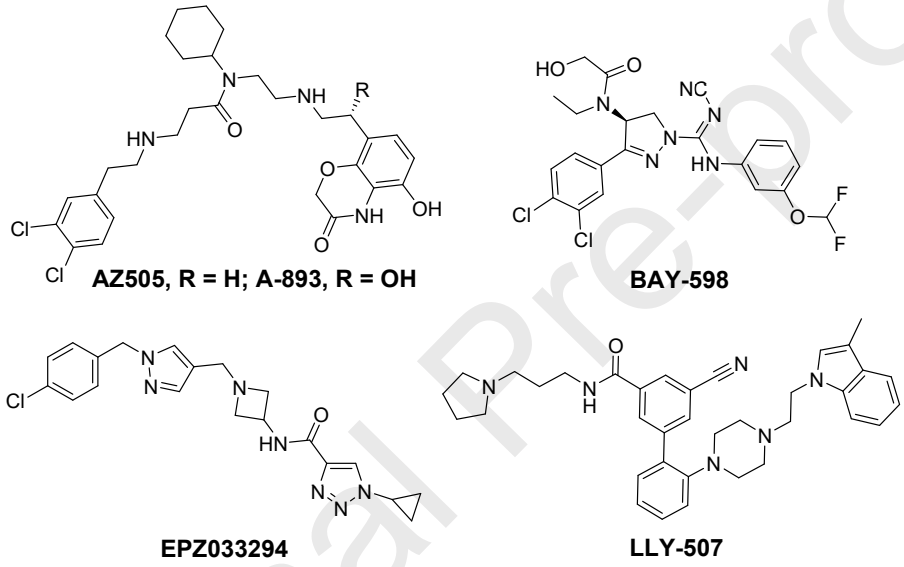


Fig. 1. Chemical structures of recently reported SMYD2 inhibitors.

The currently reported small-molecule inhibitors of SMYD2 are shown in **Fig. 1**.[14] AZ505 is the first reported inhibitor of SMYD2 with an inhibitory potency in the submicromolar range.[15,16] The crystal structures of SMYD2 in complex with these inhibitors show that except EPZ033294, they all bind in a similar fashion, occupying the peptide-binding groove of SMYD2.[15-17] Among them, LLY-507 is the first cell-active, selective small-molecule inhibitor of SMYD2 with an IC_{50} of 70 nM and inhibits the proliferation of various cancer cell lines.[18,19] Considering the large number of lysine residues in SMYD2 especially the area around the ligand binding sites, it is suitable to design covalent inhibitors by introduction of electrophilic warheads.[18,19] Previous findings show that replacement of the propyl pyrrolidine

moiety of LLY-507 with a variety of amines is unable to improve the potency.[20] In order to determine which site of LLY-507 is suitable for structural modification, we plan to choose other three chemical sites of LLY-507 through simple reactions for more information about SARs (**Fig. 2**): 1,1'-biphenyl region (site A), the key core of piperazine (site B), the side chain of 1-ethyl-3-methyl-1*H*-indole-methane (site C). Herein, we report the synthesis, pharmacological evaluation, and SARs of LLY-507 analogues as SMYD2 inhibitors.

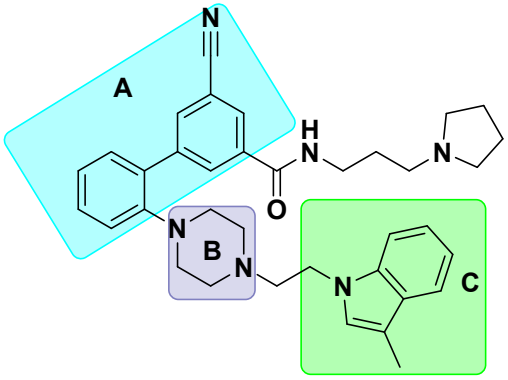
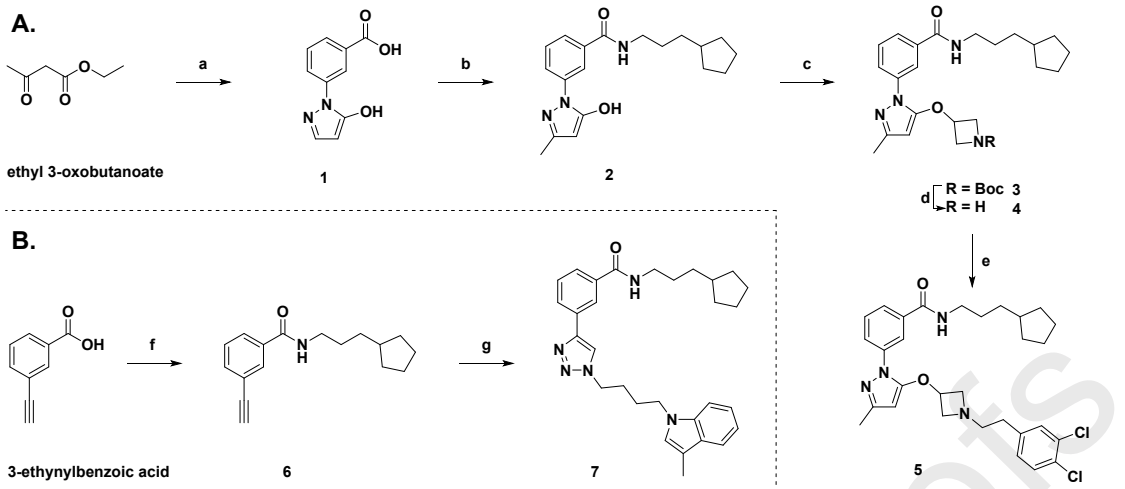


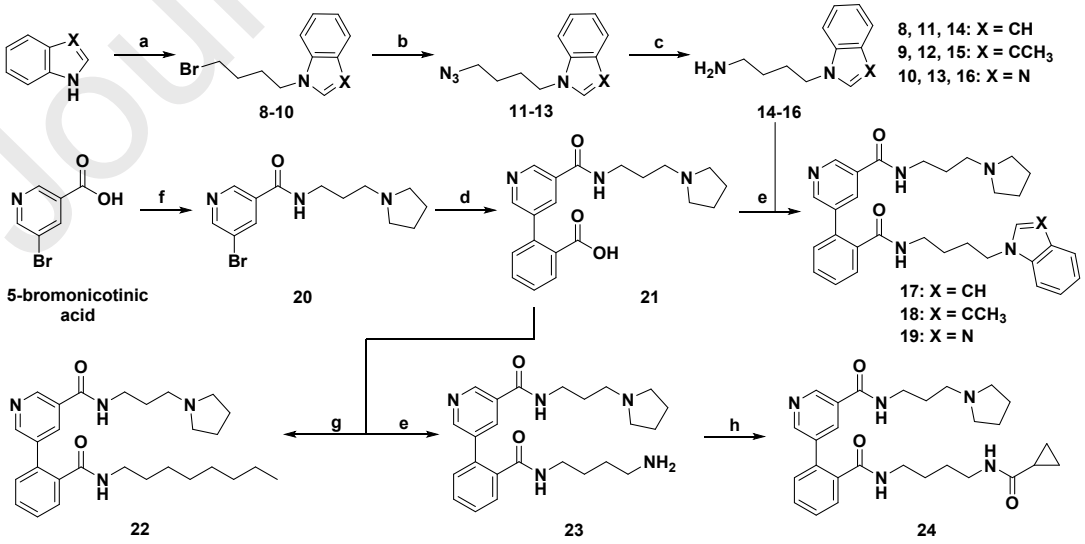
Fig. 2. Three structural modification sites based on the chemical structure of LLY-507: A. 1,1'-Biphenyl region; B. Other linkers; C. Side indole group.

The synthesis of LLY-507 analogues was outlined in **Schemes 1-4**. As shown in **Scheme 1**, coupling of ethyl acetoacetate with 3-hydrazineylbenzoic acid generated compound **1**. Amidation of compound **1** afforded amide **2**. Compound **2** was alkylated with *t*ert-butyl 3-iodoazetidine-1-carboxylate to give the intermediate **3,** followed by deprotection to furnish **4**. This intermediate was then treated with 3,4-dichlorophenethyl methanesulfonate to give compound **5**. Compound **7** was obtained via click reaction.[21]



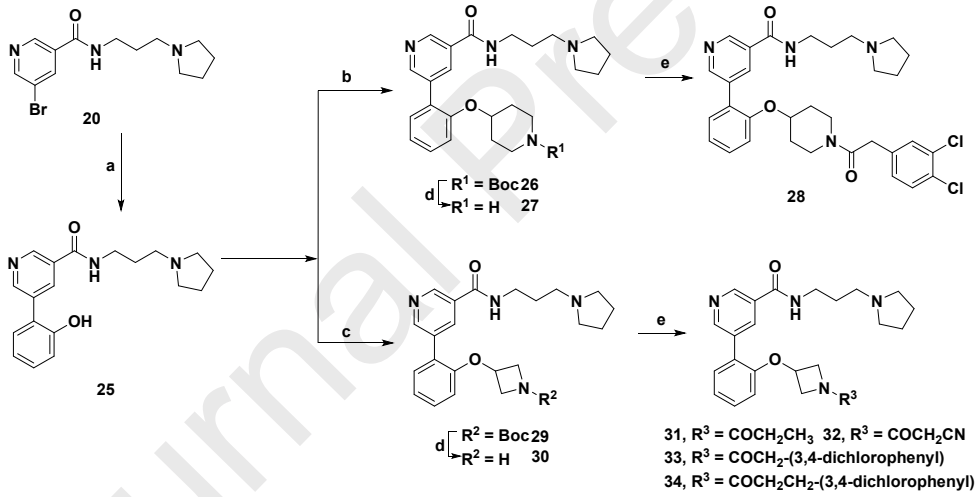
Scheme 1. Reagents and conditions: (a) 3-hydrazinylbenzoic acid, AcOH, 130 °C; (b) 3-(pyrrolidin-1-yl)propan-1-amine, HBTU, DIPEA, DMF, 25 °C; (c) *t*ert-butyl 3-iodoazetidine-1-carboxylate, Cs_2CO_3, DMF, 80 °C; (d) HCl in dioxane, MeOH, 25 °C; (e) 3,4-dichlorophenethyl methanesulfonate, K_2CO_3, CH_3CN, 70 °C; (f) 3-(pyrrolidin-1-yl)propan-1-amine, EDCI, HOBt, Et_3N, CH_2Cl_2, 25 °C; (g) **12**, $CuSO_4$, VcNa, THF/H_2O, 25 °C.

As shown in **Scheme 2**, the intermediates 14-16 were obtained by a simple three-step reaction. Treatment of 5-bromonicotinic acid with 3-pyrrolidin-1-ylpropan-1-amine provided **20**. Compound **21** was synthesized by Suzuki coupling reaction. The final products were obtained by employing a conventional amide coupling protocol.



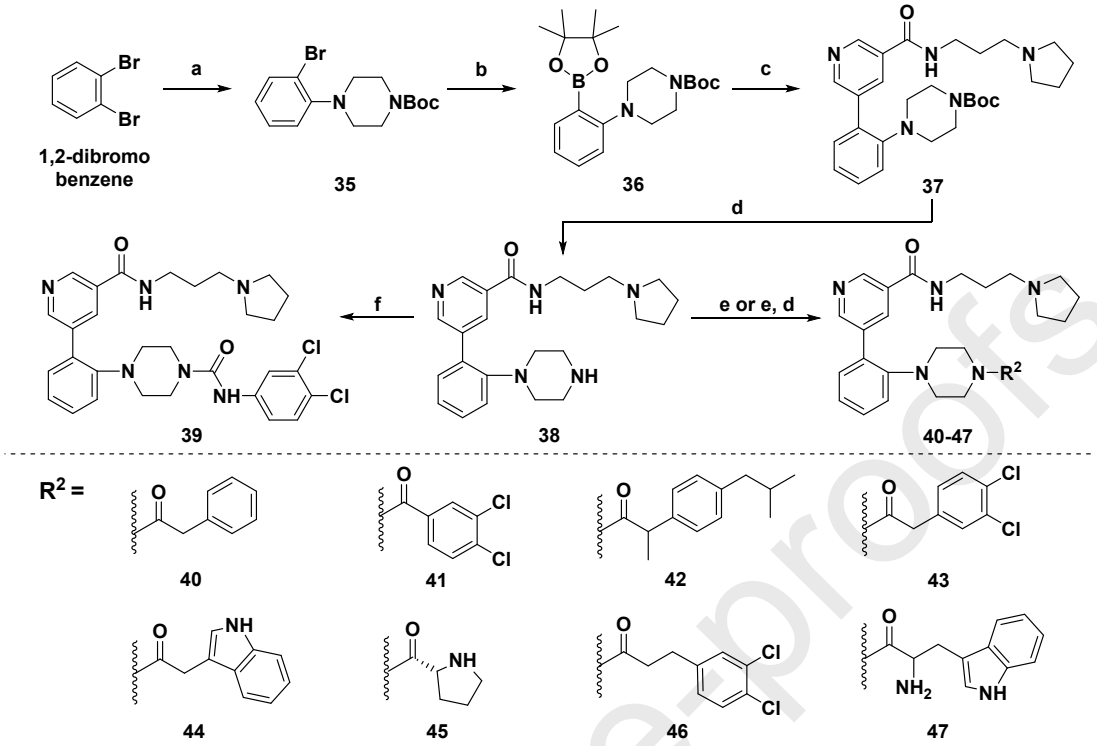
Scheme 2. Reagents and conditions: (a) 1,4-dibromobutane, NaH, dry THF, 25 °C; (b) NaN_3, DMSO, 80 °C; (c) PPh_3, THF/H_2O, 25 °C; (d) 2-carboxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, CH_3CN/H_2O, 90 °C; (e) HBTU, DIPEA, DMF, 25 °C; (f) 3-(pyrrolidin-1-yl)propan-1-amine, EDCI, HOBt, TEA, CH_2Cl_2, 25 °C; (g) octan-1-amine, HBTU, DIPEA, $THF/CHCl_3$, 25 °C; (h) cyclopropanecarboxylic acid, EDCI, $THF/CHCl_3$, 25 °C.

As shown in **Scheme 3**, compound **20** was subject to Suzuki coupling reaction with 2-hydroxyphenylboronic acid to afford **25**. Compound **28** was obtained by Mitsnobu reaction,[22] deprotection and coupling reaction with 3,4-dichlorophenylacetic acid. Compounds **31-34** were obtained by the similar reaction route with different organic acids.



Scheme 3. Reagents and conditions: (a) 2-hydroxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dioxane/H_2O, 80 °C; (b) *tert*-butyl 4-hydroxypiperidine-1-carboxylate, PPh_3, DIAD, THF, 25 °C; (c) *t*ert-butyl 3-iodoazetidine-1-carboxylate, Cs_2CO_3, DMF, 70 °C; (d) HCl in dioxane, MeOH, 25 °C; (e) RCOOH, EDCI, $CHCl_3$/THF, 25 °C.

As shown in **Scheme 4**, compound **36** was obtained via Buchwald-Hartwig amination and Miyaura-Boronization reaction. Subsequent reaction with **20** afforded **37**. Removal of Boc group provided piperazine **38**, which was suitable for derivatization under simple conditions to afford compounds **39-47**.



Scheme 4. Reagents and conditions: (a) 1-Boc-piperazine, $Pd_2(dba)_3$, BINAP, KO^tBu, toluene, 80 °C; (b) B_2Pin_2, $Pd(dppf)Cl_2$, KOAc, 1,4-dioxane, 80 °C; (c) **25**, $Pd(PPh_3)_4$, Na_2CO_3, dioxane/H_2O, 80 °C; (d) HCl in dioxane, MeOH, 25 °C; (e) R^2COOH, EDCI, $CHCl_3$/THF, 25 °C; (f) 1,2-dichloro-4-isocyanatobenzene, Et_3N, CH_2Cl_2, 25 °C.

To determine whether all the newly synthesized LLY-507 analogues bind to SMYD2, we utilized a fluorescence-based thermal shift assay to characterize the interaction between SMYD2 and these compounds.[23] The experimental results were presented as melting temperature (°C) and summarized in **Table 1**. Besides, all the newly synthesized LLY-507 analogues were assessed for their inhibitory activities against SMYD2 via in vitro methylation transfer assay. LLY-507 was employed as the positive control.[20] The IC_{50} values were illustrated in **Table 1**.

Table 1. SMYD2 inhibitory activity of the focused compound.

	PTS (°C) [a]	
	Compound :	
Compd.	protein	IC_{50} (nM)

	20:1	10:1	5:1	
5	NE[b]	NT[c]	NE	1699
7	NE	NT	NE	>5000
17	3.1	3.1	NT	75
18	2.7	NT	3	53
19	NE	NE	NT	1502
22	NE	NE	NT	>5000
23	NE	NE	NT	>5000
24	NE	NE	NT	>5000
28	1.2	NT	NE	81
31	NE[b]	NT[c]	NE	1914
32	NE	NT	NE	2415
33	2.2	NT	1.6	48
34	2.5	NT	NE	57
39	0	-1.0	NT	522
40	0.23	0.09	NT	1002
41	0.37	0.09	NT	891
42	-1.3	-0.61	NT	>2500
43	3.5	NT	3.6	17
44	3.7	2.8	NT	50
45	NE	NE	NT	1469
46	3.4	3.0	NT	24
47	1.4	1.0	NT	57
LLY-507	3.0	2.9	3.5	71

[a] PTS is a binding experiment between protein and compound. [b] NE: no effect. [c] NT: not tested.

As shown in **Table 1**, compounds **5** and **7** without 1, 1'-biphenyl group were inactive, suggesting the 1,1'-biphenyl group was critical for maintaining the SMYD2 inhibition. Hence, we synthesized LLY-507 analogues by replacement of 1,1'-biphenyl group with 2-(pyridin-3-yl)benzoic acid. Notably, compound **18** bearing a 4-(3-methyl-1*H*-indol-1-yl)butan-1-amine tail showed potent SMYD2 inhibitory activity with an IC_{50} value of 53 nM and a fluorescence-based thermal shift assay indicated that compound **18** dose-dependently shifted the melting temperature (T_m) of SMYD2 for 2.73 ^{o}C (**Fig. 3**). The inhibitory activity was also substantially maintained when the 4-(3-methyl-1*H*-indol-1-yl)butan-1-amine was replaced by 4-(1*H*-indol-1-yl)butan-1-amine. However, introducing other long-chain groups such

as 4-(1*H*-benzo[*d*]imidazol-1-yl)butan-1-amine (**19**), octan-1-amine (**22**), putrescine (**23**), or *N*-(4-aminobutyl)cyclopropanecarboxamide (**24**) would lead to a dramatical loss of SMYD2 inhibitory potency of compounds, indicating that the tolerance of this position was limited. Comparing of compounds **33**, **28** with **43,** it can be suggested that the piperazine group was critical for maintaining the SMYD2 inhibition. Hence, we examined how the replacement of the side indole group (site C) might affect SAR. We prepared analogues with various alternatives to the piperazine and observed that some of these modifications were tolerated. Notably, an attempt to introduce the 2-(3,4-dichlorophenyl)acetic acid led to compound **43** showing 4-fold improved potency with an IC_{50} value of 17 nM, whereas replacement with 3,4-dichlorobenzoic acid (**41**) was inactive. Furthermore, compound **39** with *N*-(3,4-dichlorophenyl)acetamide suffered a significant loss in potency. However, compound **46** with 3-(3,4-dichlorophenyl)propanoic acid substituted on the piperazine exhibited better inhibitory activity than LLY-507. Additionally, introduction of 2-phenylacetic acid (**40**) and ibuprofen (**42**) also led to significantly decreased in potency. We attributed this potency decrease to their unfavorable interaction with hydrophobic pocket in the binding site of the protein. The replacement of 1-ethyl-3-methyl-1*H*-indole with 2-(1*H*-indol-3-yl)acetic acid (**44**) or tryptophan (**47**) did not affect the potency, further indicating that this region would be combined with electrophilic warheads to provide Lysine-Targeting covalent inhibitors for SMYD2.[24]

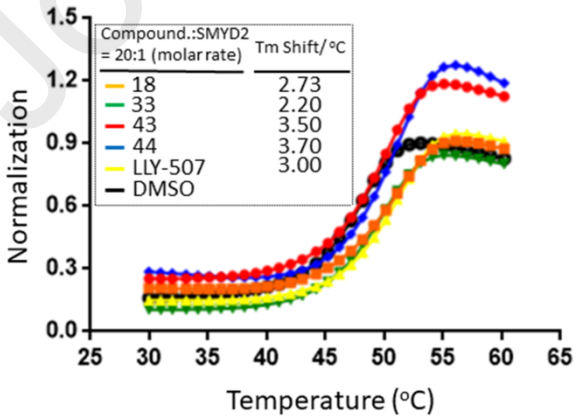


Fig. 3. The thermal shift assay displaying the stabilization of SMYD2 by compounds **18**, **33**, **43** and **44**.

Accumulating evidence supports that SMYD2 was overexpressed in gastric cancer cell lines, and inhibition of SMYD2 expression suppresses gastric cancer cell proliferation, survival, and tumor growth in vitro and in vivo.[25] Therefore, we further tested whether compounds **17-19**, **22**, **33**, **34**, **43**, **44**, and **46** could inhibit the cell proliferation rate of SMYD2 over-expressed gastric cancer cell lines AGS and NCI-87 (**Table 2**). Compounds **17-19** and **22** exhibited no inhibitory activity on AGS cells at low concentrations, which could arise from the reduced cell membrane permeability. As shown in **Table 2**, both compounds **43**, **44**, and **46**, the most potent SMYD2 inhibitors, exhibited significantly anti-proliferative activity against gastric cancer cell lines AGS (**Fig. 4**) and NCI-87. Among them, compound **44** (IC_{50} = 2.3 μM and 3 μM) displayed the best potency and efficacy in AGS and NCI-87 cell inhibition assays respectively, which was two-fold higher than LLY-507.

Table 2. In vitro anti-proliferative activity of newly synthesized compounds against human gastric cancer cell lines.

Compd.	IC_{50} (μM)		Compd	IC_{50} (μM)	
	AGS	NCI-N87		AGS	NCI-N87
17	50	50	**34**	17	NT
18	20	20	**43**	5.8	6.0
19	100	100	**44**	2.3	3.0
22	50	50	**46**	4.6	5.0
33	17.6	NT[a]	**LLY-507**	5.5	7.0

[a] NT: not tested.

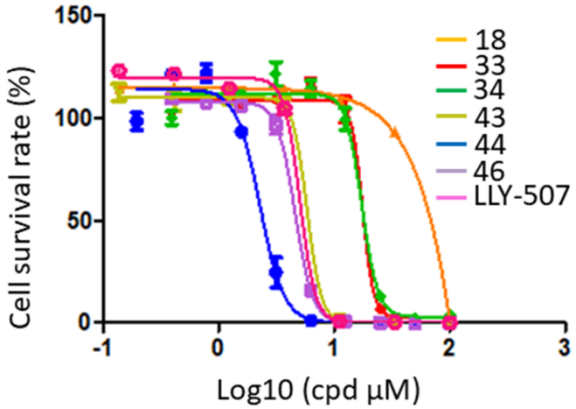


Fig. 4. The cell viability of the selected compounds in AGS cells.

The SARs of LLY-507 analogous as SMYD2 inhibitors were summarized in **Fig. 5**. Firstly, site A was critical for maintaining SMYD2 inhibition. When the piperazine (site B) group was replaced with 3-methoxyazetidine or 4-methoxypiperidine decreased activity was observed against SMYD2. Inhibitory activities also revealed that the substitution of the amino group leads to a significant loss in potency. An amino group (site C) was indispensable for exerting inhibitory activity. Another finding was that a bulkier terminal site C group resulted in reduced activity (**43** to **47**) from 17 nM to 57 nM. Activity gradually decreased when the site C group was larger than the ethyl group; in particular, **46** and **42**, with the largest group, showed no inhibitory activity toward SMYD2. Furthermore, comparing **41**, **45** with **43**, it could be concluded that the piperazine group linked by an ester group (n = 2) was essential for activity. LLY-507 analogues with the hydrophobic group on site C appeared to be more favorable in general and further indicating that this region was more flexible and could be replaced by many functionalized groups.

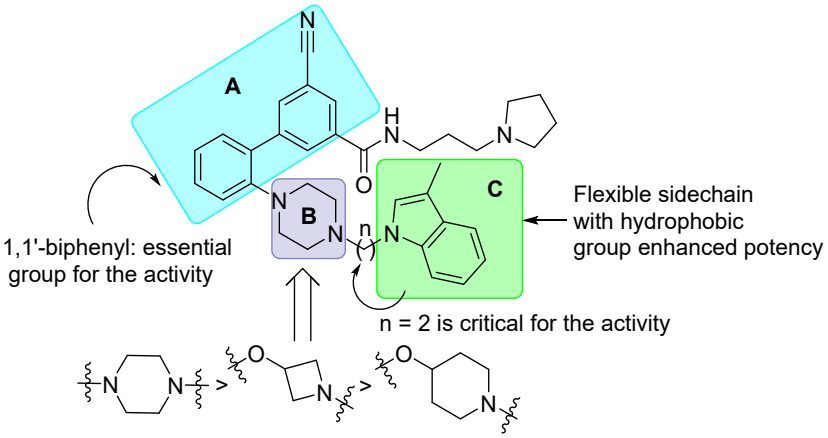


Fig. 5. SARs of LLY-507 analogous as SMYD2 inhibitors.

In summary, a series of novel LLY-507 analogs were synthesized and evaluated for their SMYD2 inhibitory activity. Among them, compounds **43** and **44** could inhibit the proliferation of gastric cancer cell lines AGS and NCI-87, which was equivalent to or better than LLY-507. Our results showed that the sites A and B are indispensable for exerting inhibitory activity, while site C was more flexible. Taken together, the site C could be combined with electrophilic warheads such as acrylamides and vinyl sulfones via amide reaction to provide targeting covalent inhibitors for SMYD2. This work would facilitate further development of new targeting covalent inhibitors for SMYD2.

Note

The authors declare no competing financial interest

Acknowledgments

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A supplementary date

Supplementary data associated with this article can be found, in the online

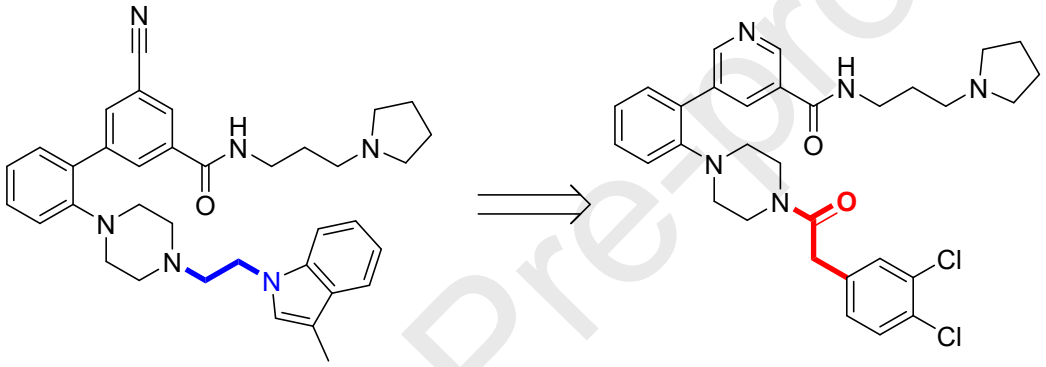
version, at https://doi.org/xx.

References

1. Bannister, A.J.; Kouzarides, T. *Nature* **2005**, *436*, 1103.
2. Kubicek, S.; Jenuwein, T. *Cell* **2004**, *119*, 903.
3. Al-Shar'i, N.A.; Alnabulsi, S.M. *J. Mol. Graph. Model.* **2016**, *68*, 147.
4. Hamamoto, R.; Saloura, V.; Nakamura, Y. *Nat. Rev. Cancer* **2015**, *15*, 110.
5. Reynoird, N.; Mazur, P.K.; Stellfeld, T.; Flores, N.M.; Lofgren, S.M.; Carlson, S.M.; Brambilla, E.; Hainaut, P.; Kaznowska, E.B.; Arrowsmith, C.H.; Khatri, P.; Stresemann, C.; Gozani, O.; Sage, J. *Genes Dev.* **2016**, *30*, 772.
6. Zhang, P.; Ruan, J.; Weng, W.; Tang, Y. *Leuk. Lymphoma* **2020**, *61*, 437.
7. Zuo, S.R.; Zuo, X.C.; He, Y.; Fang, W.J.; Wang, C.J.; Zou, H.; Chen, P.; Huang, L.F.; Huang, L.H.; Xiang, H.; Liu, S.K. *J. Cancer* **2018**, *9*, 321.
8. Abu-Farha, M.; Lambert, J.P.; Al-Madhoun, A.S.; Elisma, F.; Skerjanc, I.S.; Figeys, D. *Mol. Cell Proteomics* **2008**, *7*, 560.
9. Obermann, W.M.J. *J. Biol. Chem.* **2018**, *293*, 16479.
10. Tracy, C.; Warren, J.S.; Szulik, M.; Wang, L.; Garcia, J.; Makaju, A.; Russell, K.; Miller, M.; Franklin, S. *Curr. Opin. Physiol.* **2018**, *1*, 140.
11. Jiang, Y.; Trescott, L.; Holcomb, J.; Zhang, X.; Brunzelle, J.; Sirinupong, N.; Shi, X.; Yang, Z. *J. Mol. Biol.* **2014**, *426*, 3413.
12. Yi, X.; Jiang, X.J.; Fang, Z.M. *Clin. Epigenetics* **2019**, *11*, 112.
13. Singh, P.K. *Eur. J. Med. Chem.* **2019**, *166*, 351.
14. Fabini, E.; Manoni, E.; Ferroni, C.; Rio, A.D.; Bartolini, M. *Future Med. Chem.* **2019**, *11*, 901.
15. Thomenius, M.J.; Totman, J.; Harvey, D.; Mitchell, L.H.; Riera, T.V.; Cosmopoulos, K.; Grassian, A.R.; Klaus, C.; Foley, M.; Admirand, E.A.; Jahic, H.; Majer, C.; Wigle, T.; Jacques, S.L.; Gureasko, J.; Brach, D.; Lingaraj, T.; West, K.; Smith, S.; Rioux, N.; Waters, N.J.; Tang, C.; Raimondi, A.; Munchhof, M.; Mills, J.E.; Ribich, S.; Porter Scott, M.; Kuntz, K.W.; Janzen, W.P.; Moyer, M.; Smith, J.J.; Chesworth, R.; Copeland, R.A.; Boriack-Sjodin, P.A. *PLoS One* **2018**, *13*, e0197372.
16. Eggert, E.; Hillig, R.C.; Koehr, S.; Stockigt, D.; Weiske, J.; Barak, N.; Mowat, J.; Brumby, T.; Christ, C.D.; Ter Laak, A.; Lang, T.; Fernandez-Montalvan, A.E.; Badock, V.; Weinmann, H.; Hartung, I.V.; Barsyte-Lovejoy, D.; Szewczyk, M.; Kennedy, S.; Li, F.; Vedadi, M.; Brown, P.J.; Santhakumar, V.; Arrowsmith, C.H.; Stellfeld, T.; Stresemann, C. *J. Med. Chem.* **2016**, *59*, 4578.
17. Sweis, R.F.; Wang, Z.; Algire, M.; Arrowsmith, C.H.; Brown, P.J.; Chiang, G.G.; Guo, J.; Jakob, C.G.; Kennedy, S.; Li, F.; Maag, D.; Shaw, B.; Soni, N.B.; Vedadi, M.; Pappano, W.N. *ACS. Med. Chem. Lett.* **2015**, *6*, 695.
18. Lanouette, S.; Davey, J.A.; Elisma, F.; Ning, Z.; Figeys, D.; Chica, R.A.; Couture, J.F. *Structure* **2015**, *23*, 206.
19. Xu, S.; Zhong, C.; Zhang, T.; Ding, J. *J. Mol. Cell Biol.* **2011**, *3*, 293.
20. Cowen, S.D.; Russell, D.; Dakin, L.A.; Chen, H.; Larsen, N.A.; Godin, R.; Throner, S.; Zheng, X.; Molina, A.; Wu, J.; Cheung, T.; Howard, T.; Garcia-Arenas, R.; Keen, N.; Pendleton, C.S.; Pietenpol, J.A.; Ferguson, A.D. *J. Med. Chem.* **2016**, *59*, 11079.
21. Hein, C.D.; Liu, X.M.; Wang, D. *Pharm. Res.* **2008**, *25*, 2216.

22. Swamy, K.C.; Kumar, N.N.; Balaraman, E.; Kumar, K.V. *Chem. Rev.* **2009**, *109*, 2551.
23. Chen, B.; Ye, F.; Yu, L.; Jia, G.; Huang, X.; Zhang, X.; Peng, S.; Chen, K.; Wang, M.; Gong, S.; Zhang, R.; Yin, J.; Li, H.; Yang, Y.; Liu, H.; Zhang, J.; Zhang, H.; Zhang, A.; Jiang, H.; Luo, C.; Yang, C.G. *J. Am. Chem. Soc.* **2012**, *134*, 17963.
24. Pettinger, J.; Jones, K.; Cheeseman, M.D. *Angew. Chem. Int. Ed. Engl.* **2017**, *56*, 15200.
25. Komatsu, S.; Ichikawa, D.; Hirajima, S.; Nagata, H.; Nishimura, Y.; Kawaguchi, T.; Miyamae, M.; Okajima, W.; Ohashi, T.; Konishi, H.; Shiozaki, A.; Fujiwara, H.; Okamoto, K.; Tsuda, H.; Imoto, I.; Inazawa, J.; Otsuji, E. *Br. J. Cancer* **2015**, *112*, 357.

Graphical abstract



LLY-507
SMYD2: IC_{50} = 71 nM

Compound 43
SMYD2: IC_{50} = 17 nM

Highlights

- A series of LLY-507 analogues were designed and synthesized.
- A fluorescence-based thermal shift assay was utilized.
- Six analogues exhibited improved potency.
- This work would facilitate further development of covalent inhibitors for SMYD2.