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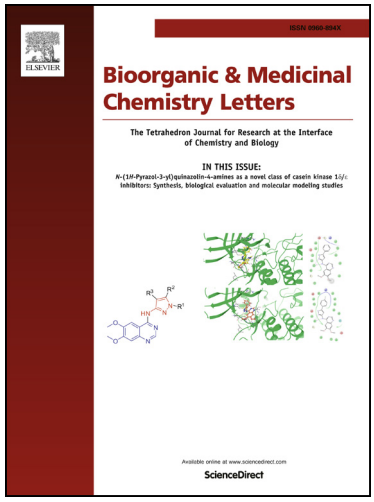

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Design, Synthesis and biological evaluation of indole derivatives as Vif inhibitors

Chunlan Pu [a, 1], Rong-Hua Luo [b, 1], Mengqi Zhang [a, 1], Xueyan Hou [a], Guoyi Yan [a], Jiang Luo [a], Yong-Tang Zheng [b,*], Rui Li [a,*]

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Abstract: The crystal structure of viral infectivity factor (Vif) was reported recently, which makes it possible to design new inhibitors against Vif by structure-based drug design. Through analysis of the protein surface of Vif, the C2 pocket located in the N-terminal was found, which is suit for developing small molecular inhibitors. Then, in our article, fragment-based virtual screening (FBVS) was conducted and a series of fragments was obtained, among which, **Zif-1** bearing indole scaffold and pyridine ring can form H-bonds with Tyr148 and Ile155. Subsequently, 19 derivatives of **Zif-1** were synthesized. Through the immune-fluorescence staining and western blot assays, **Zif-15** shows potent activity in inhibiting Vif-mediated A3G degradation. Further docking experiment shows that **Zif-15** form H-bond interactions with residues His139, Tyr148 and Ile155. Therefore, **Zif-15** is a promising lead compound against Vif that can be used to treat AIDS.

Keywords: APOBEC3G degradation; Vif inhibitor; FBVS; indole derivatives

Today, AIDS is one of the biggest public health problems in the world. In clinical, the existing 28 anti-human immunodeficiency virus (HIV) drugs can effectively delay AIDS progression, but cannot cure this disease and need lifelong medication.[1,2] Scientists are attempting to find a safe and effective HIV-1 vaccine that can prevent HIV-1 infection, but the result is disappointing.[3,4] Nowadays, considerable progress has been made in treating HIV-infected patients by using highly active antiretroviral therapy (HAART).[5] However, with the increasing incidence of drug resistant viruses and drug toxicity, it is urgent to find more anti-HIV drugs with new

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mechanisms.

Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (APOBEC3G, A3G) is a cytidine deaminase.[6] As one of the A3G family members, A3G is a major cellular host defense factor. It was believed that A3G is able to protect the host cell from retroviruses and severely weaken the infectivity of HIV-1.[7-9] The antiviral effect of A3G is not only through restricting DNA replication, but also via the deaminase-independent mechanisms.[10] However, A3G can be degradated by Vif through ubiquitin-mediated pathway.[11-15] Therefore, inhibition of Vif is an appealing way to treat HIV-1.[16,17] Several small molecule inhibitors of Vif inhibitor (**RN-18, MM-1**, **IMB-26** and benzimidazoles) were reported (Fig. 1).[18-22] In this study, we designed and synthesized a series of indole derivatives and found **Zif-15** as an effective Vif inhibitors, which may targeting the Vif–ElonginC interaction (Fig. 2).

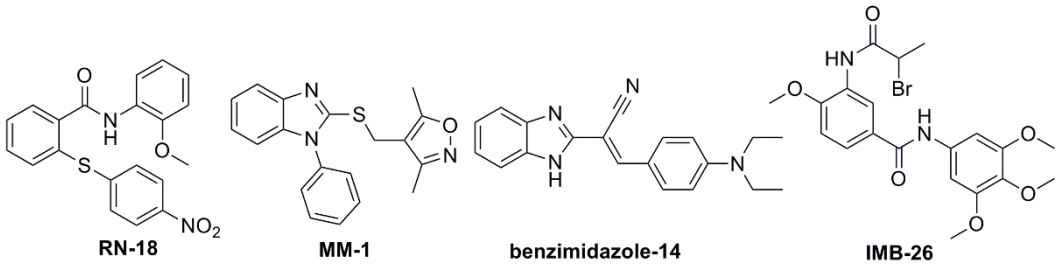

Fig. 1. *Structures of HIV-1 Vif inhibitors.*

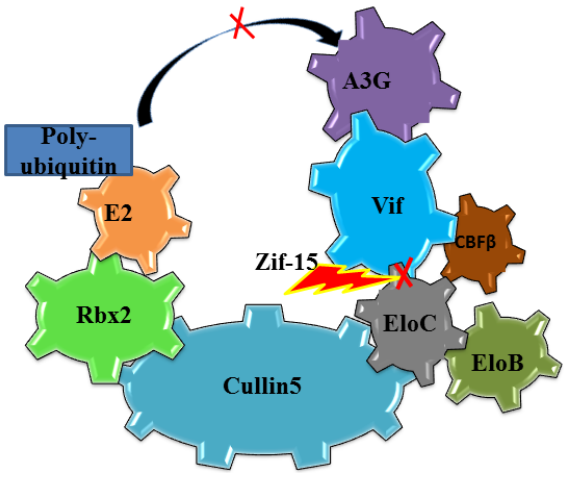

Fig. 2. *Schematic illustration of the mechanism of Vif inhibitor **Zif-15**. **Zif-15** binding to the C2 pocket of Vif, which might block the interaction of Vif with ElonginC (EloC), thereby inhibiting the formation of the E3 complex and thus impeding the degradation of A3G.*

The crystal structure of viral infectivity factor (Vif) was reported recently [23], which makes it

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possible to design new inhibitors against Vif by structure-based drug design. Firstly, to find the appropriate binding pocket, the crystal structure of Vif complex (PDB ID: 4N9F) was obtained from the Protein Data Bank (Fig. 3A). By using Discovery Studio 3.1 packages, five possible binding sites (C1~C5) were identified. As can be seen in Fig. 3B, C3~C5 are smaller in space that cannot accommodate small molecules (Table 1). Although the size of C1 was appropriate, ligands binding to this site may not be helpful in impeding the formation of E3 complex. C2 pocket, located in the N-terminal of Vif, is in vicinity of the ElonginC, hence, ligands binding to this pocket may inhibit the interaction of Vif with ElonginC, thus leading to the dissociation of E3 complex and prevention of A3G from degradation. Therefore, C2 pocket was used for the further FBVS campaign.

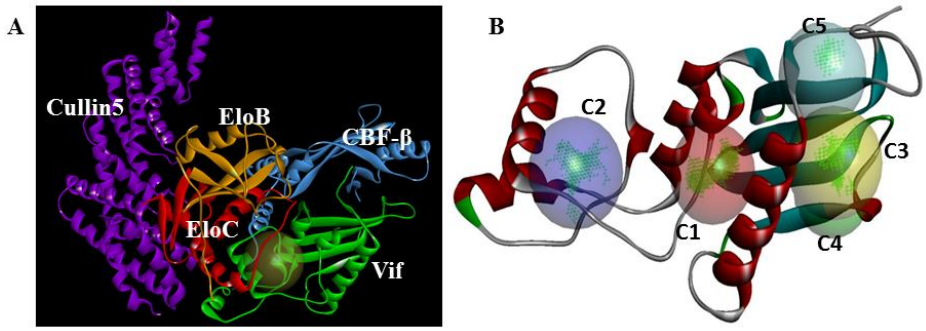

Fig. 3. *A. Crystal structure of the Vif-CBFbeta-Cullin5-EloB-EloC pentameric complex (the yellow transparent sphere represents the location of C2 pocket of Vif). B. Possible binding sites of Vif protein C1~C5.*

Table 1. *The location, size and function of the five binding sites.*

binding site	X	Y	Z	volume	color	function
C1	70.989	-87.494	-180.097	57.50	red	-
C2	72.881	-69.58	-181.951	43.25	blue	interact with ElonginC
C3	67.09	-100.832	-181.877	27.13	yellow	-
C4	55.443	-97.504	-187.186	14.50	green	-
C5	72.837	-100.88	-188.206	13.75	gray	interact with CBF-β

Based on the virtual screening of the ZINC fragment-based database, a series of fragments that can bind to the C2 pocket of Vif were discovered (Fig. 4A), and the structures of these fragments were provided in supporting information. Most of the fragments show attractive binding modes, among which **Zif-1** bearing the indole and pyridine ring can form hydrogen bonds with Tyr148 and Ile155 (Fig. 4B). Therefore, 19 novel derivatives of **Zif-1** were designed and

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synthesized.

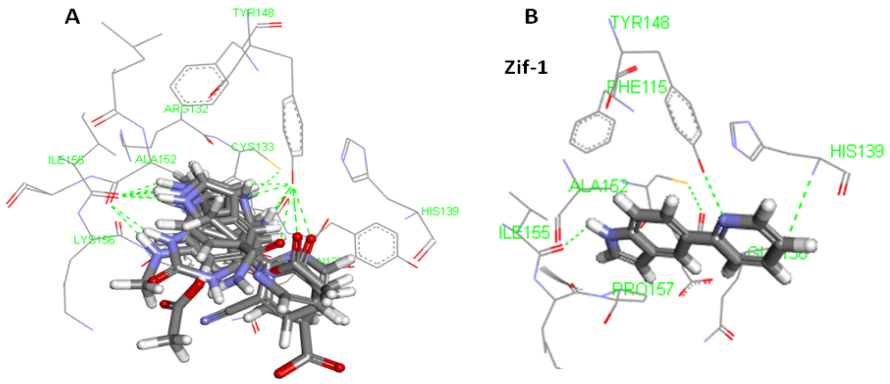
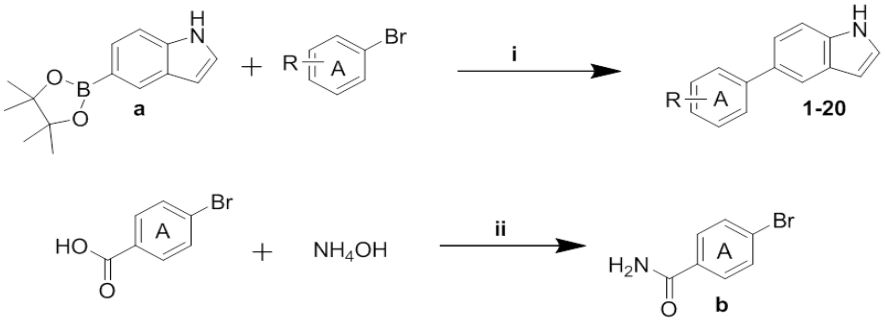


Fig. 4. A. *Overlapping image of the molecules obtained from the fragment-based virtual screening against Vif C2 pocket.* B. *Binding mode of Zif-1 in C2 pocket of Vif.*

The general preparation method of 5-substituted indole derivatives was outlined in Scheme 1. Briefly, 5-bromoindole was reacted with bis(pinacolato)diboron at 100 °C to prepare compound **a**. Compound **b** was obtained through the acylation of 6-bromonicotinic acid with 1,1'-carbonyldiimidazole (CDI) in THF.[24] The target compounds were generated by Suzuki cross-coupling reactions[25-27] of compound **a** with bromine substituted aromatic compounds. The structures of the all synthesized compounds were summarized in Table 2. The detailed reaction conditions, processing procedures, MS, NMR and yield data are provided in supporting information.



Scheme 1: *Reagents and conditions: (i) $PdCl_2(dppf)CH_2Cl_2$, K_2CO_3, 1,4-dioxane, ~60 °C, 4~5 h, 50~70%. (ii) CDI, THF, NH_4OH, rt, > 85%. (A represents phenyl or pyridyl moiety)*

Table 2. *The structures, biological activities and yields of the target compounds **1~20**.*

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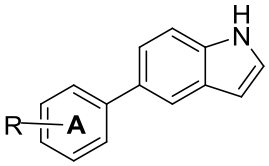

Compound	A[#]	R	MFI	A3G restoring level	Yields (%)
1	pyridin-2-yl	H	-	lowest	63
2	pyridin-2-yl	3-NH_2	++	medium	64
3	pyridin-2-yl	2-NH_2	+	low	52
4	pyridin-3-yl	2-NH_2	+	low	54
5	pyridin-3-yl	6-NH_2	++	medium	60
6	pyridin-2-yl	2-CHO	+	low	63
7	pyridin-2-yl	3-CHO	-	lowest	59
8	C_6H_5	4-CHO	-	lowest	57
9	C_6H_5	2-CHO	+	low	76
10	C_6H_5	3-CHO	+	low	56
11	C_6H_5	2-CN	-	lowest	55
12	C_6H_5	4-SO_2CH_3	++	medium	61
13	pyridin-3-yl	6-OCH_3	+	low	72
14	pyridin-2-yl	3-$CONH_2$	++	medium	24
15	pyridin-2-yl	3-COOH	+++	high	42
16	C_6H_5	4-COOH	++	medium	32
17	C_6H_5	4-CH_2COOH	-	lowest	59
18	pyridin-2-yl	4-COOH	++	medium	46
19	pyridin-2-yl	3-$CONH_2$	+	low	35
20	pyridin-3-yl	4-$CONH_2$	++	medium	42

[#] A represents benzene ring or pyridine ring.

To determinate their biological activities, the Tet-On system is used in regulating the expression of Vif in TREX-hvif-15 cell line.[28] The intensity of fluorescence was shown in Fig. 5A. Comparing with $A3G^+$, Vif^- group, the fluorescence of $A3G^+$, Vif^+ was dramatically decreased (Fig. 5B). Next, the 20 compounds were added to the $A3G^+$, Vif^+ system and the results show that the fluorescence intensity of **Zif-15** group is the strongest, similar to that of $A3G^+$, Vif^- group. Compounds **Zif-2**, **5**, **12**, **14**, **16**, **18** and **20** have the moderate fluorescence, while compounds **Zif-3**, **4**, **6**, **9**, **10**, **13** and **19** had relatively weak fluorescence. Besides, there is almost no fluorescence observed in compounds **Zif-1**, **7**, **8**, **11** and **17**.

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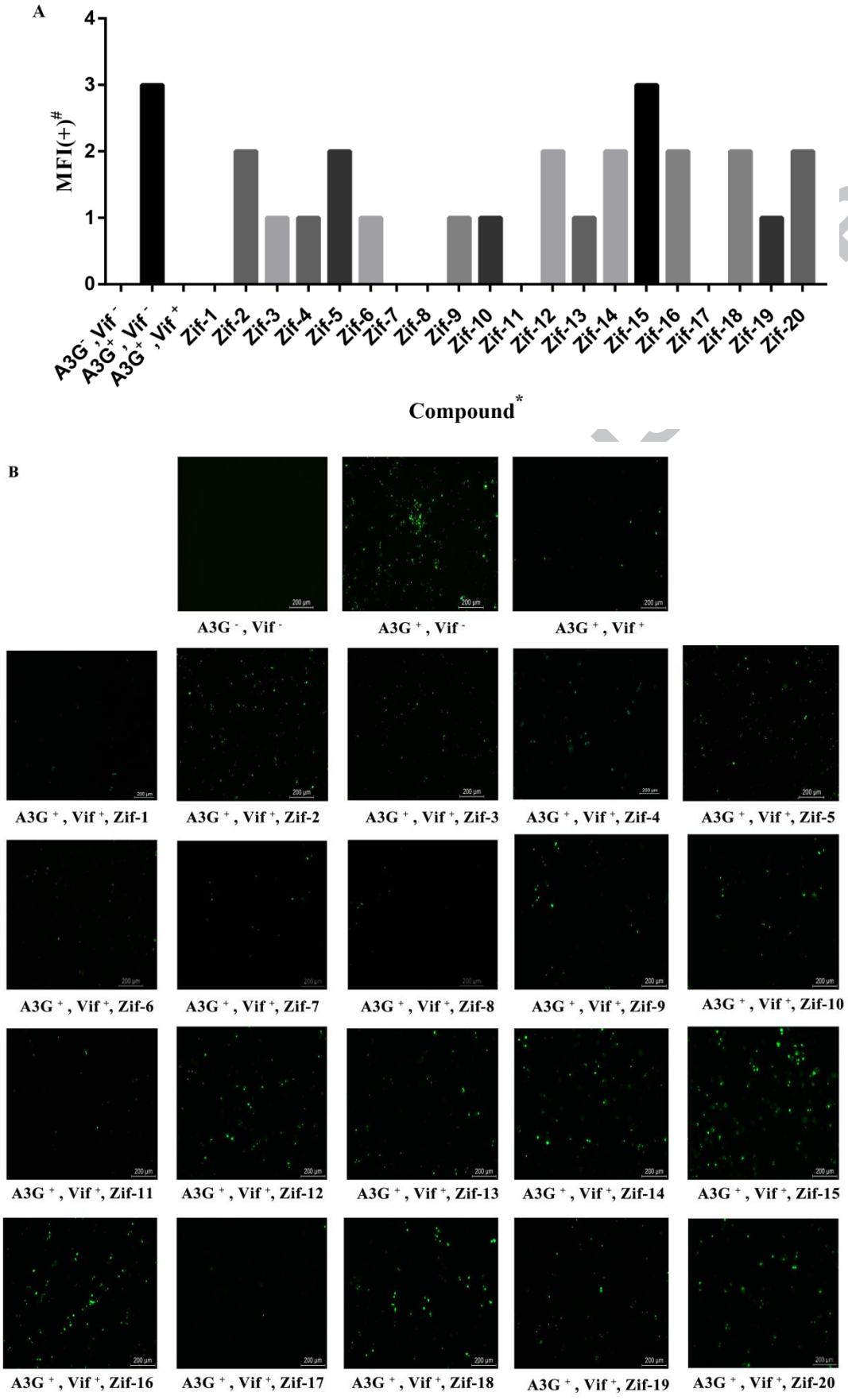

Fig. 5. *The mean fluorescent intensity (MFI) analysis of the effect of compound* ***1~20*** *on the*

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intensity of A3G. [#] *The fold change of A3G MFI. The intensity was described as following: -, no or less than 1 fold change; +, 1~2 fold change; ++, 2~3 fold change; +++, >3 fold change.* [*] *The concentration of compounds was 50 μM. A3G*$^{+}$*: TREX-hvif-15 cells were transfected with EYFP-N1-hA3G. Vif*$^{+}$*: the protein of Vif was induced by 1 μg/mL Dox.*

Next, to detect the variation of the amount of the Vif and A3G protein when treated by the 20 compounds, western blot experiments were done. The results are illustrated in Fig. 6. **Zif-15** restored the high level of expression of A3G. Compounds **Zif-2**, **5**, **12**, **14**, **16** and **18** showed the medium restoration level of expression of A3G, while other compounds **Zif-3**, **4**, **6**, **9**, **10**, **13** and **19** had a low A3G levels. **Zif-1**, **7**, **8**, **11** and **17** show no activity in restoring the A3G levels. The fluorescence intensity and the restoring level of A3G are shown in Table 2.

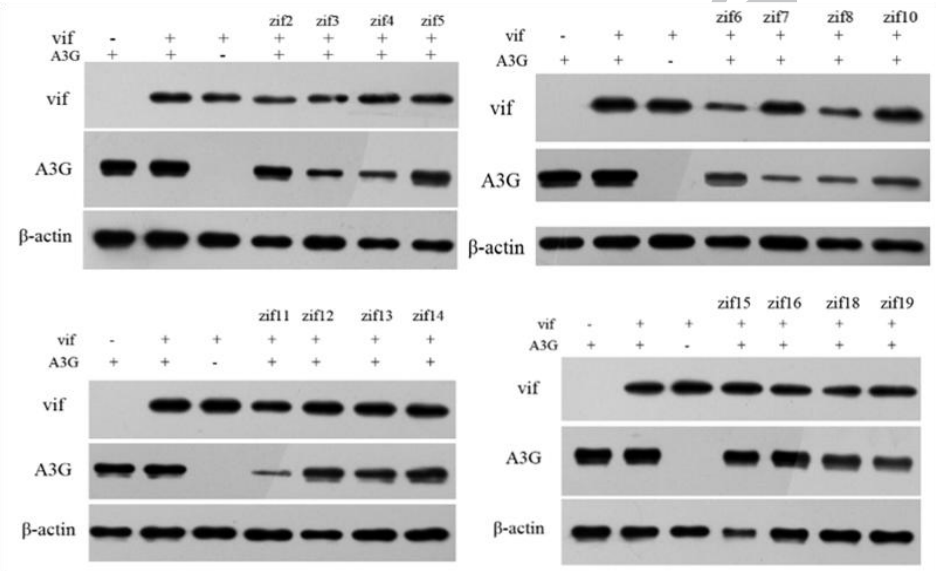

Fig. 6. *Effects of Compounds **1~20** on the expression of A3G and Vif. TREX-hvif-15 cells were transfected with EYFP-N1-hA3G, and Vif was induced by 0.1 μg/mL Dox. The cells were treated with or without compounds **1~20** in concentration of 20 μM, respectively.*

According to the structures and biological activities of compounds **1~20** (Table 2), it can be found that when the aromatic rings "A" is the pyridine-2-yl ring without any substituent group, **Zif-1** shows no activity. According to the interaction mode of **Zif-1** with C2 pocket of Vif (Fig. 4B), the substituent group with hydrogen donor might form the H-bond interaction with residue His139, thus increase the binding affinity. Hence, an amino group was introduced to C-3′ position of the pyridin-2-yl (**Zif-2**) and it increased the activity. When the amino group was introduced to the C-2′ position, the activity was dropped, which can be ascribed to the increased distance between amino group and His139. Next, changing the position of pyridine N atom to pyridin-3-yl (**Zif-4)** leads to the reduced activity, while further changing the amino position to para-position of the indole ring (**Zif-5**) leads to the partial restoration of the activity, which can be attributed to formation of H-bond with His139. According to the **Zif-1** binding mode, introduction of substituent group with hydrogen acceptor would not be helpful in binding. To validate our

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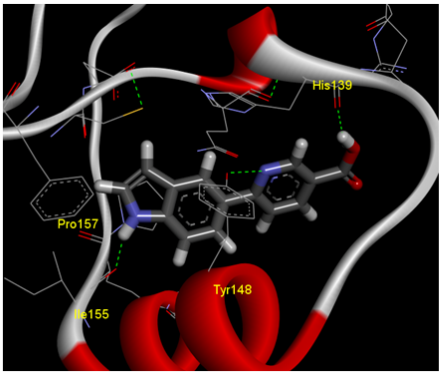
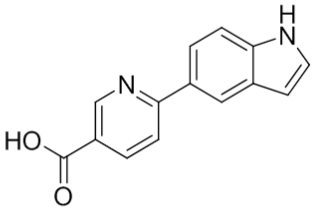
assumption, compounds substituted with aldehyde group (**Zif-6**~**Zif-10**), cyano group (**Zif-11**) and methoxy group (**Zif-13**) were synthesized, and they all show low to no activity, independent of the location of pyridine N atom and the substituent group. It should be noted that **Zif-12**, with methylsulfonyl substituted at the C-4′ position still retain some activity. Finally, compounds substituted by the groups with both hydrogen donor and acceptor, like acylamino group and carboxyl group, were synthesized (**Zif-14~16**, **Zif-18**, **Zif-20**) and their activity are all good, especially **Zif-15** shows the best activity. The binding mode of **Zif-15** with C2 pocket of Vif was provided in Fig.7. Specifically, the N atom of the pyridine and the H atom of -NH- on the indole ring of **Zif-15** form H-bonds with Tyr148 and Ile155, respectively. Apart from that, the H atom of the carboxyl group forms H-bond with His139. Besides, the indole core forms the hydrophobic interaction with Pro157. Due to loss of the H-bond of pyridine with Tyr148, the activity of **Zif-16** was decreased. When a methylene group was inserted to the carboxyl group, **Zif-17** shows almost no activity, which may be ascribed to the steric hindrance. As for the acylamino group, pyridine-2-yl (**Zif-14**, **20**) is superior to pyridine-3-yl (**Zif-19**) in activity, which is similar to the abovementioned **Zif-4**.





Zif-15

Fig. 7. *The binding mode of the ligand **Zif-15** in Vif C2 pocket.*

In summary, through analysis of the protein surface of Vif, the C2 pocket which is suit for developing small molecular inhibitors was found. Then FBVS was conducted to find a series of promising fragments. According to the binding mode of **Zif-1** with C2 pocket of Vif, 19 indole derivatives was designed and synthesized. Quantitative structure-activity relationship (QSAR) shows that the pyridine-2-yl and hydrogen donor group at the para-position is favorable, and indole core is indispensable for the potency. Among them, through fluorescence-based primary screening and western blot, **Zif-15** shows the best activity, and is deserved to be further optimized.

Acknowledgment

The Vif stably expressed cell line TREX-hvif-15 and APOBEC3G expressed plasmid

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Supporting Information

Experimental procedures and characterization data of compounds.

Conflicts of Interest

The authors declare no conflict of interest.

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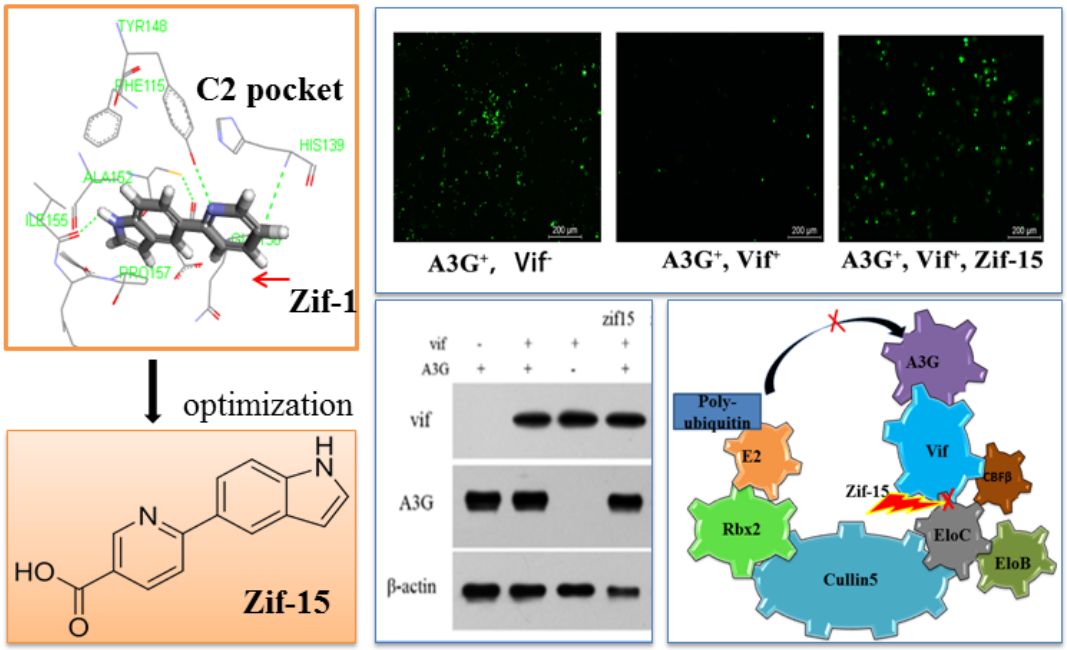
Graphical abstract:

FBVS was conducted against the C2 pocket of Vif, and **Zif-1** was selected for further optimization. Through the immune-fluorescence staining and western blot assays, **Zif-15** shows potent activity in inhibiting Vif-mediated A3G degradation.



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Highlights

1. C2 pocket of Vif was found which is suit for developing small molecular inhibitors.
2. FBVS was conducted against C2 pocket, and a series of fragments were obtained.

Zif-15 shows potent activity in inhibiting Vif-mediated A3G degradation.

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