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Synthesis and biological evaluation of novel quinoxalinone-based HIV-1 reverse transcriptase inhibitors

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Abstract: A series of novel N^4-substituted thiophen-3-ylsulfonylquinoxalinone derivatives were designed and synthesized by the variations of 2- and 5-position of thiophene ring. All target molecules were tested for their anti-HIV-1 replication activities and compounds **1b** and **1d** were found to be the most potent inhibitors with an IC_{50} value at 10^{-8} mol/L level. The preliminary structure-activity relationships were analyzed on the basis of the binding mode of compound **1b** predicted by CDOCKER.

Keywords: Quinoxalinone; HIV-1 reverse transcriptase inhibitor; Structure-activity relationship

1. Introduction

Since the human immunodeficiency virus 1 (HIV-1) was first confirmed as the causative agent of acquired immunodeficiency syndrome (AIDS), the tremendous progress has been achieved in the search of anti-HIV therapeutics.[1-3] The strategy of the highly active anti-retroviral therapy (HAART)[4,5] revolutionized the treatment of AIDS which is now considered as a manageable chronic disease. Non-nucleoside reverse-transcriptase inhibitors (NNRTIs) such as efavirenz which is one of the essential components in HAART. To date, in total five NNRTIs have been approved for the clinical treatment of HIV infection, where nevirapine, efavirenz and delavirdine are included as the first generation inhibitors and etravirine[6] and rilpivirine[7] as the second generation inhibitors. However, upon the pressure of anti-HIV agents, some of the key amino acid residues such as Tyr181Cys and Lys103Asn can readily mutate in the non-nucleoside inhibitor binding pocket of the reverse transcriptase. As a result, the therapeutic efficacy of the known NNRTIs was significantly decreased. Therefore, many efforts have been devoted to develop novel chemical entities possessing improved potency and resistant profiles.[8-14]

In our efforts to explore novel chemical entities of NNRTIs, 6-fluoro-N^4-(quinoline-8-sulfonyl)-3,4-dihydroquinoxalin-2(1*H*)-one **A** (Figure.1) was identified by screening our in-house library. The variation of substituents on quinoxaline scaffold resulted in the discovery of compound **1a** with an improved anti-HIV activity (IC_{50} = 0.2 μM).[15] With an aim to further

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improve the inhibitory activity, a series of novel 6-chloro-3-methyl-3,4-dihydroquinoxalin-2(1*H*)-ones bearing a 2,5-disubstituted thiophen-3-ylsulfonyl moiety were designed and synthesized. We herein wish to present the detailed synthesis, antiviral activities and preliminary structure-activity relationships (SAR) of these quinoxalines.

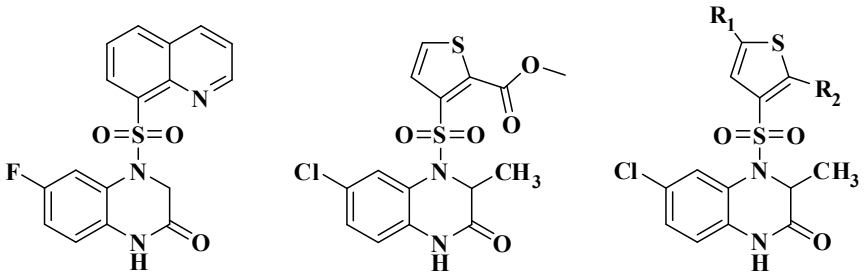
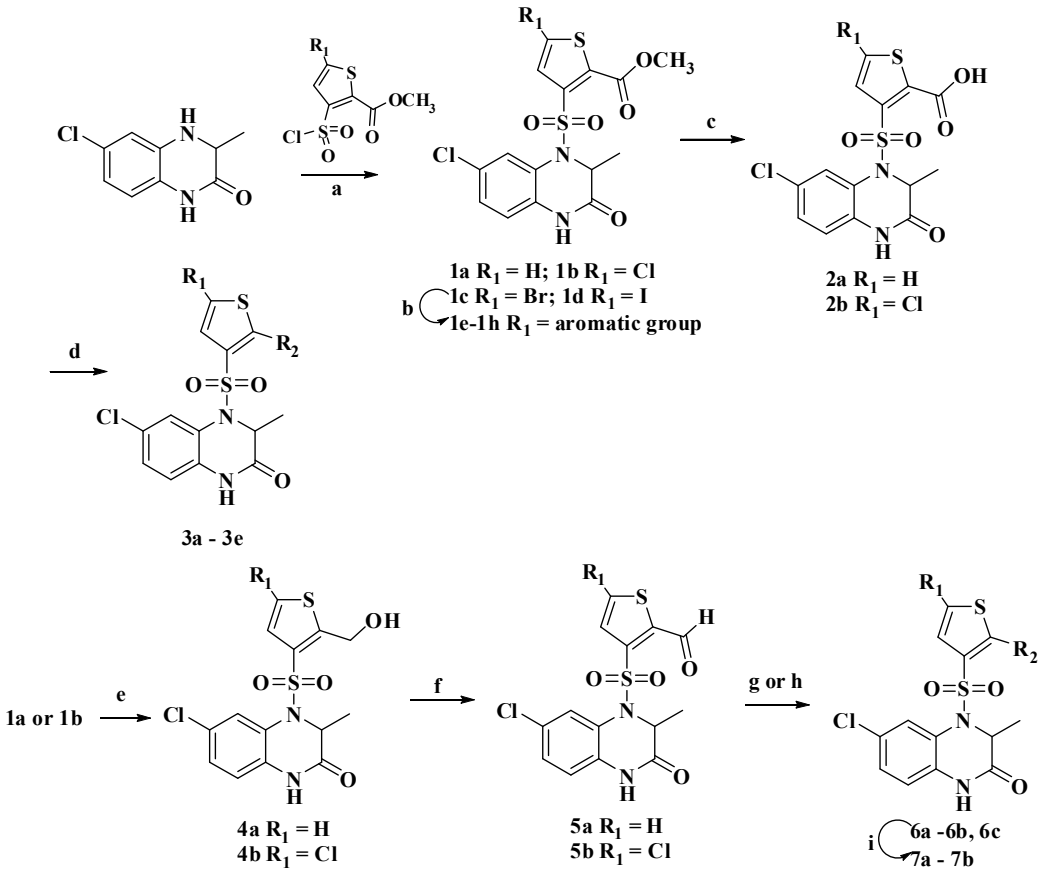


Figure1. Chemical structures of leads (**A** and **1a**) and designed quinoxalinone-based NNRTIs.

2. Chemistry

All tested compounds with variations at 2- and 5-position of thiophene ring were synthesized according to Scheme 1, and their chemical structures were shown in Table 1. The microwave irradiation of the mixture of 6-chloro-3-methyl-3,4- dihydroquinoxalin-2(1*H*)-one and methyl 5-halo-3-(chorosulfonyl)-thiophene-2-carboxylate in pyridine produced compounds **1b-1d** in the moderate yield. The Suzuki coupling of compound **1c** with various aromatic boronic acids were conducted under the reaction conditions of $Pd(OAc)_2$, Xantphos and triethylamine, and thus providing the corresponding biaryl compounds **1e-1h** in 25-62% yield. The diversification on 5-position of the thiophene ring was realized by the conversion of methoxycarbonyl group into various substituents. Hydrolysis of compounds **1a** and **1b** gave rise to carboxyl substituted compounds **2a** and **2b** in 89% and 97% yield, respectively. The formation of esters **3a-3c** and amides **3d** and **3e** were achieved in low to moderate yield by the reaction of compound **2a** or **2b** with alcohols, halides or amines, respectively. Compounds **1a** and **1b** were reduced with $LiAlH_4$ into compounds **4a** and **4b**, which were further oxidized by PDC into aldehydes **5a** and **5b** in 42% and 68% yield. Upon treatment with formic acid, the aldehydes **5a** and **5b** were condensed respectively with 2-aminothiazole to deliver the corresponding imines **6a** and **6b**, which were reduced with $NaHB(OAc)_3$ to lead to the formation of compounds **7a** and **7b** in 94% and 65% yield. Compound **6c** was achieved in 50% yield via the van Leusen reaction of compound **5b** with TosMIC reagent in the presence of K_2CO_3.

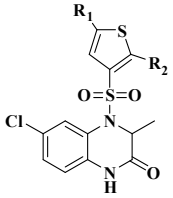


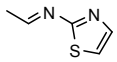
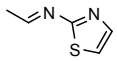
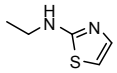
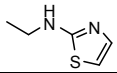
Scheme 1. Reagents and conditions: (a) sulfonyl chlorides, pyridine, microwave; (b) $Pd(OAc)_2$, Xantphos, Et_3N, $ArB(OH)_2$, toluene, 80℃; (c) LiOH/MeOH/H_2O/THF, r.t.; (d) *iso*-propanol, concentrated H_2SO_4; or 1-bromo-3-methylbut-2-ene/K_2CO_3/acetone; or *N*,*O*-dimethylhydroxyamine hydrochloride/EDC/DMAP; or *iso*-propylamine/BOP/Et_3N; (e) $LiAlH_4$/THF/0℃; (f) PDC/CH_2Cl_2/r.t.; (g) 2-aminothiazole, HCOOH/MeOH/reflux; (h) K_2CO_3, TosMIC, MeOH; (i) $NaHB(OAc)_3$/ CH_2Cl_2/r.t.

3. Biological Results and Discussion

The anti-HIV-1 activities of all synthesized compounds were evaluated by using a cell-based HIV-1 replication pharmacological model which was set up by HIV-1(pNL4-3) core packed with vesicular stomatitis virus glycoprotein as mentioned before.[15] The level of HIV-1 replication was presented by a reporter gene expression (i.e. luciferase activity) in infected cells. The results were expressed as IC_{50} values and summarized in Table 1. Nevirapine (NVP) was used as a reference molecule.

Table 1 The chemical structures and anti-HIV-1 replication activities of all tested compounds[a].



Compound	R_1	R_2	IC_{50} (μM)[b]
1a	H	$COOCH_3$	0.2
1b	Cl	$COOCH_3$	0.07
1c	Br	$COOCH_3$	0.13
1d	I	$COOCH_3$	0.075
1e	phenyl	$COOCH_3$	9.05
1f	*m*-methoxyphenyl	$COOCH_3$	9.68
1g	2-thienyl	$COOCH_3$	6.53
1h	2-furyl	$COOCH_3$	1.97
2a	H	COOH	NA[c]
2b	Cl	COOH	NA
3a	H	$COOCH(CH_3)_2$	1.09
3b	H	$COOCH_2CH=C(CH_3)_2$	0.77
3c	Cl	$COOCH(CH_3)_2$	5.55
3d	H	$CONCH_3(OCH_3)$	NA
3e	H	$CONHCH(CH_3)_2$	NA
4a	H	CH_2OH	NA
4b	Cl	CH_2OH	NA
5a	H	CHO	NA
5b	Cl	CHO	NA
6a	H		NA
6b	Cl		NA
6c	Cl		4.44
7a	H		NA
7b	Cl		NA

[a] Nevirapine was used as a control; IC_{50} for nevirapine was 39 nM.

[b] Compound dose (μM) required to inhibit the HIV replication activity by 50 %.

[c] NA: no activity

Our initial investigation was performed on the modification of the substituents on 5-position of the thiophene ring. As shown in Table 1, when a halogen was introduced onto 5-position (compounds **1b**-**1d**), an increased anti-HIV-1 replication activity was observed in comparison with lead compound **1a** (IC_{50} = 0.2 μM). Compound **1b** with a chloro substituent showed the most potent inhibitory activity with IC_{50} value of 0.07 μM., which was comparable to the inhibitory activity of compound **1d** with iodo substitution (IC_{50} = 0.075 μM). The replacement of a chloro group with a aromatic substituent (e.g., compounds **1e**-**1h**) led to reduction in anti-HIV-1 activity with IC_{50} values ranging from 1.97 μM to 9.68 μM. Therefore, it was suggested that the introduction of a bulky aromatic motif onto the thiophene ring was not favorable to the activity, probably due to the resulted steric hinderance. These results were consistent with the SARs on the known NNRTIs. It

has been demonstrated that chloro, cyano or methyl groups were the privileged fragments on the aromatic ring, which was located in the subpocket consisting of Tyr181, Tyr188 and Trp229.[8-12]

The effect of modification of 2-substituent on the thiophene ring was then explored while 5-substitution was maintained as a hydrogen or chloro group. The conversion of hydrophobic methoxylcarbonyl fragment into hydrophilic carboxylic (compounds **2a** and **2b**), hydroxymethyl (compounds **4a** and **4b**) or aldehyde groups (compound **5a** and **5b**) resulted in a complete loss of anti-HIV-1 replication activity, respectively. In comparison with compounds **1a** and **1b**, compounds **3a**-**3c** with a larger *iso*-propyl or 3-methyl-2-butenyl substituent linked to the thiophene ring via ester bond showed moderate activities (IC_{50}, 0.77-5.55 μM). Surprisingly, compounds **3d** and **3e,** where the hydrophobic alkyl group was linked to the thiophene ring via an amide bond, did not exhibit anti-HIV-1 activity at all. Compounds **6a**-**6b** and **7a**-**7b**, containing a thiazole fragment on 2-substituent, had no inhibitory activity on HIV-1 replication too. As an isostere of methyl ester, an oxazole was brought into 2-position of the thiophene ring and the resulted compound **6c** possessed moderate activity with an IC_{50} value of 4.44 μM. Taken together, it was suggested that substitution at 2-position of the thiophene ring with a hydrophobic substituent was beneficial to the inhibitory activity, while the large size of this bulky group was not allowed.

To investigate the binding mode of the synthesized N^4-substituted thiophen-3-ylsulfonylquinoxalinones, molecular modeling was performed using CDOCKER docking program (Accelrys Discovery Studio 2.5.5).[16] The coordinates of the RT-efavirenz complex (pdb code: 1FK9)[17] were employed. The binding poses of the most potent compound **1b** and the reference molecule efavirenz were shown in Figure 2. The position and orientation of compound **1b** were quite close to those of efavirenz. The quinoxalinone ring was located in a hydrophobic pocket formed by residues Leu100, Val106, Pro236 and Tyr318. The key hydrogen bonding interactions were observed between the amide moiety of quinoxalinone and the carbonyl oxygen and backbone nitrogen of Lys101, which were also frequently observed in some other typical NNRTIs bound to RT.[8,10,11] They were believed to be the crucial contributors to the binding affinity. The methyl substituent of compound **1b** adopted a similar orientation to that of trifluoromethyl group of efavirenz and projected into a small hydrophobic site constructed by the residues Val106, Val179 and Gly190. As expected, the 5-chloro-2-methoxylcarbonyl-3-sulfonylthiophene fragment was situated in a large hydrophobic subpocket consisting of Pro95, Tyr181, Tyr188 and Trp229, which was occupied by the cyclopropyl-propynyl of efavirenz in the complex. On the basis of the binding features of compound **1b**, several SAR results about this series of quinoxalinones could be elucidated further. The placement of a bulky aromatic group on 5-position of the thiophene ring led to a reduced activity probably due to its steric interaction with the residue Leu234. A significant increase in activity was observed when a hydrogen (compound **1a**) was displaced by a chloro substituent (compound **1b**) on 5-position of the thiophene ring. Presumably because the electron-withdrawing chloro substitution made a positive contribution to the π-π stacking interactions between the thiophene ring and the aromatic side chains of Tyr181, Tyr188 and Trp229. Therefore, the small electron-withdrawing substituents on 5-position of the thiophene ring would be favorable for enhancing the binding affinity. As illustrated in Figure 2, the 2-substituted methoxylcarbonyl group on the thiophene ring was surrounded by the

hydrophobic side chains of Leu100 and Glu138. In consistent with the above SAR investigation, the replacement of the methoxylcarbonyl substituent with a hydrophilic group led to a loss of activity.

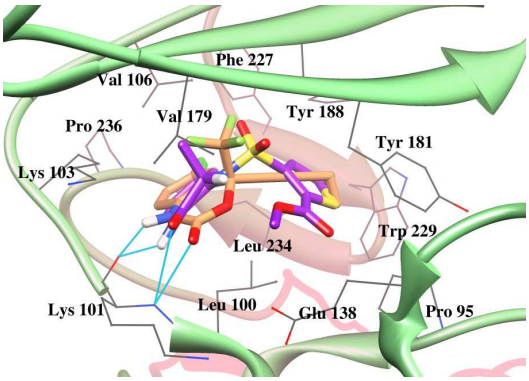


Figure 2. CDOCKER-modeled binding mode of compound **1b** (carbon atoms colored purple) in comparison with the crystal structure (1FK9 in PDB)[17] of efavirenz (carbon atoms colored orange). H-bonding interactions are presented with light blue line. Molecular image was generated with UCSF Chimera.[18]

The key amino acid mutations Y181C and K103N at the binding site of RT were commonly observed and rendered the virus resistant to many known NNRTIs. Therefore, seven title compounds (**1a-1d, 1h, 3a-3b**) were selected and tested for their inhibitory effect on the $HIV_{RT\text{-}K103N}$ and $HIV_{RT\text{-}Y181C}$ replication. Unfortunately, none of the compounds displayed effective inhibition against the two resistant strains (data shown in supporting information). It was suggested that the residues K103 and Y181 at the binding site played a key role in the interactions between the quinoxalinone derivatives and RT as well.

4. Conclusions

In summary, a range of novel quinoxalinones bearing 2,5-disubstituted-3-sulfonylthiophene motif were synthesized and their anti-HIV-1 activities were evaluated. Compounds **1b** and **1d**, which contained 5-chloro-2-methoxycarbonyl and 5-iodo-2-methoxycarbonyl thiophene fragment, were identified as the most potent NNRTIs with the IC_{50} values at 10^{-8} mol/L level. According to the molecular docking of the titled compounds, the SAR results were analyzed, and that will facilitate further development of novel potent quioxaline-based NNRTIs.

Acknowledgments

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References

1. R. J. Shattock, M. Warren, S. McCormack, C. A. Hankins, *Science* 2011, **333**, 42-43.

2. P. Zhan, X. W. Chen, D. Y. Li, Z. J. Fang, E. De Clercq, X. Y. Liu, *Med. Res. Rev.* 2013, **33(S1)**, E1-E72.
3. M. P. de Béthune, *Antiviral Res.* 2010, **85**, 75-90.
4. J. E. Gallant, E. DeJesus, J. R. Arribas, A. L. Pozniak, B. Gazzard, R. E. Campo, B. Lu, D. McColl, S. Chunk, J. Enejosa, J. J. Toole, A. K. Cheng, *N. Engl. J. Med.* 2006, **354**, 251-260.
5. S. Staszewski, J. Morates-Ramirez, K. T. Tashima, A. Rachlis, D. Skiest, J. Stanford, R. Stryker, P. Johnson, D. F. Labriola, D. Farina, D. J. Manion, N. M. Ruiz, *N. Engl. J. Med*. 1999, **341**, 1865-1873.
6. J. D. Croxtall, *Drugs* 2012, **72**, 847-869.
7. G. J. Zaharatos, M. A. Wainberg, *Ann. Med.* 2013, **45**, 236-241.
8. D. Y. Li, P. Zhan, E. De Clercq, X. Y. Liu, *J. Med. Chem.* 2012, **55**, 3595-3613.
9. P. Chong, P. Sebahar, M. Youngman, D. Garrido, H. C. Zhang, E. L. Stewart, R. T. Nolte, L. P. Wang, R. G. Ferris, M. Edelstein, K. Weaver, A. Mathis, A. Peat, *J. Med. Chem.* 2012, **55**, 10601-10609.
10. G. L. Regina, A. Coluccia, A. Brancale, F. Piscitelli, V. Famiglini, S. Cosconati, G. Maga, A. Samuele, E. Gonzalez, B. Clotet, D. Schols, J. A. Esté, E. Novellino, R. Silvestri, *J. Med. Chem* . 2012, **55**, 6634-6638.
11. L. Q. Sun, L. Zhu, K. D. Qian, B. J. Qin, L. Huang, C. H. Chen, K. H. Lee, L. Xie, *J. Med. Chem* . 2012, **55**, 7219-7229.
12. D. Y. Li, P. Zhan, H. Q. Liu, C. Pannecouque, J. Balzarini, E. De Clercq, X. Y. Liu, *Bioorg. Med. Chem.* 2013, **21**, 2128-2134.
13. A. Li, Y. Ouyang, Z. Y. Wang, Y. Y. Cao, X. Y. Liu, L. Ran, C. Li, L. Li, L. Zhang, K. Qiao, W. S. Xu, Y. Huang, Z. L. Zhang, C. Tian, Z. M. Liu, S. B. Jiang, Y. M. Shao, Y. S. Du, L. Y. Ma, X. W. Wang, J. Y. Liu, *J. Med. Chem* . 2013, **56**, 3593-3608.
14. M. Bollini, R. Gallardo-Macias, K. A. Spasov, J. Tirado-Rives, K. S. Anderson, W. L. Jorgensen, *Bioorg. Med. Chem. Lett.* 2013, **23**, 1110-1113.
15. B. L. Xu, Y. Sun, Y. Guo, Y. L. Cao, T. Yu, *Bioorg. Med. Chem.* 2009, **17**, 2767-2774.
16. G. S. Wu, D. H. Robertson, C. L. Brooks, V. Michal, *J. Comput. Chem.* 2003, **24**, 1549-1562.
17. J. Ren, J. Milton, K. L. Weaver, S. A. Short, D. I. Stuart, *Structure.* 2000, **8**, 1089-1094.
18. E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* 2004, **25**, 1605-1612.

Graphical Abstract:

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A series of novel quinoxalinone derivatives were identified as potent anti-HIV-1 agents with the IC_{50} values at 10^{-8} μmol/L level.