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SYNTHESIS AND ANTI-HIV ACTIVITY OF ARYLPIPERAZINYL FLUOROQUINOLONES: A NEW CLASS OF ANTI-HIV AGENTS

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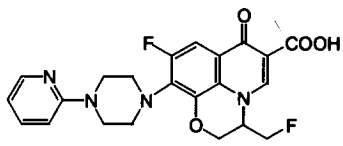
Abstract : Synthesis and anti-HIV activity of a series of novel arylpiperazinyl fluoroquinolones are reported. In the SAR study, the aryl substituents on the piperazine nitrogen were found to play an important role for the anti-HIV-1 activity. A few of the compounds exhibited potent anti-HIV activity: IC_{50}= 0.06μM in chronically infected cells.

Recent studies in HIV chemotherapy have produced stunning progress in a relatively short period of time.[1,2] Yet, as new resistant viral strains continue to evolve, there remains a need for the development of new classes of antiviral compounds to supplement the available technology. Our studies in this area began with the random screening of chemical libraries. Specifically, we have found that 9-fluoro-3-fluoromethyl-2,3-dihydro-10-{4-(2-pyridyl)-1-piperazinyl}-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid (R-71762), inhibits HIV-1 replication both in acutely and in chronically infected cells.[3]



R - 71762

In order to pinpoint the structural elements responsible for the anti-viral activity of R-71762 and to explore structural variations of this lead compound, we prepared a series of fluoroquinolone derivatives and tested their anti-HIV activities against HIV-1_{IIIB} chronically infected Molt-4 cells. Briefly, prewashed HIV-1_{IIIB} chronically infected Molt-4 cells (Molt-4/IIIB cells) were incubated with various concentrations of the compound at 37℃ under 5% CO_2 atmosphere. After 48h of incubation, the culture supernatants were harvested and the contents of p24 antigen were determined by ELISA (Cellular Product, USA). The percentages relative to the untreated control cultures were plotted on a graph and the 50% inhibitory

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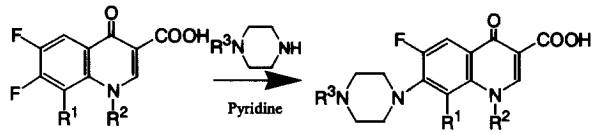
concentration (IC_{50}) was calculated. In order to distinguish anti-HIV-1 activities of the compounds from non-specific inhibition of cellular functions, their cytotoxic activities were also measured in the same cells. The cytotoxicities of the compounds were based on the viability of the cells remaining after harvest of the supernatants. Cell viability was determined by tetrazolium-based colorimetric assay.[4] The 50% cytotoxic concentration (CC_{50}) was calculated as above. Here we describe the results of our structure-activity relationship studies on arylpiperazinyl fluoroquinolones and report their potency against HIV-1.

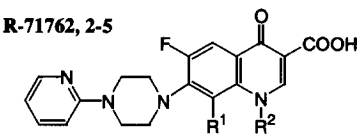
Scheme

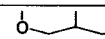
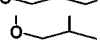


1 **2 - 30**

All compounds were prepared in a similar manner, according to the well-established method for the synthesis of antibacterial fluoroquinolones[5] (Scheme). Upon treatment with substituted piperazine, properly substituted 6,7-difluoroquinolone carboxylic acids **1** underwent aromatic ring amination through an aryl fluoride substitution reaction in pyridine to give the desired piperazinylfluoroquinolone derivatives **2 – 30**. Alternatively, for acids **1** wherein R^1 was not an electron-withdrawing group, the corresponding difluoroborate salts were used in order to promote the nucleophilic amination reaction.[6]

Table 1. Inhibitory activities of the compounds R-71762, **2-5**



Compound	R^1	R^2	IC_{50} (μM)*	CC_{50} (μM)*
R-71762			1.7±0.6	>60
2			3.7±1.4	39±5
3	F	cyclopropyl	50±4	>50
4	OCH_3	cyclopropyl	1.8±0.6	26±2
5	$OCHF_2$	cyclopropyl	0.25±0.04	15±2

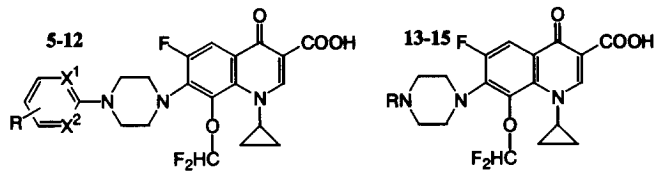
*: Mean ± SD of at least three independent experiments.

Structural modifications of the core skeleton of R-71762 were commenced at the bridge moiety between the 1 and 8 positions of the fluoroquinolone ring (R^1, R^2; Table 1). Compound **2** showed a 50% reduction in anti-HIV-1 activity compared with R-71762. This suggests a significant role of the bridge moiety in the lead compound, even though the cytotoxicity of compound 2 increased. To investigate the role of this component,

compounds **3-30** lacking the 1-8 ethyleneoxy bridge were tested. Following the precedence of antibacterial quinolone drugs,[7] we began with compounds possessing a cyclopropyl group as R^2. Compound **3**, possessing a fluorine atom as R^1, and a cyclopropyl group as R^2 had very weak anti-HIV activity. However, the anti-viral activity of the methoxy-substituted compound **4** was almost equal to that of the lead compound R-71762. Moreover, compound **4**'s potency could be further enhanced by substitution of the methoxy group's hydrogens with fluorine atoms. Finally, the difluoromethoxy-substituted compound **5** was found to be the most active in this series of compounds.

We next turned our attention towards the piperazine ring substituent. Results of the anti-HIV assays for a variety of substitution on the piperazine ring are shown in Table 2.

Table 2. Inhibitory activities of the compounds **5-15**



Compound	X^1	X^2	R	IC_{50} (μM)*	CC_{50} (μM)*
5	N	CH	H	0.25±0.04	15±2
6	N	N	H	0.97±0.25	>40
7	CH	CH	H	0.063±0.008	8.4±3.4
8	CH	CH	4–F	0.059±0.011	5.7±1.9
9	CH	CH	4–Cl	0.48±0.35	5.8±3.7
10	CH	CH	4–OMe	0.60±0.24	17±2
11	CH	CH	3–OMe	0.70±0.26	22±6
12	CH	CH	2–OMe	0.11±0.04	9.9±0.8
13	–	–	H	>60	>60
14	–	–	Me	>60	>60
15	–	–	$PhCH_2$	†	23±4

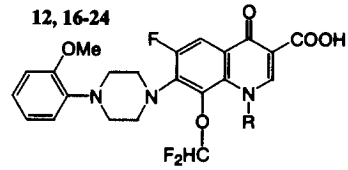
*: Mean ± SD of at least three independent experiments.
†: Not active at a non-cytotoxic concentration.

Clearly, the aryl substituents play an important role. For example, although the introduction of a pyrimidine ring (**6**) reduced anti-HIV potency notably, the phenyl-substituted compound **7** was 4 times more active than the parent compound **5**. Introduction of chlorine atom on the 4-position of the phenyl ring (compound **9**) resulted in a significant diminution of potency but fluorine atom case (compound **8**) did not. Similar tendencies were observed in the methoxy-substituted compound **10** and its regio-isomer **11**. This reduction of the inhibitory potency might be due to either the steric hindrance or electrostatic nature of the substituents or both. Quite interestingly, the ortho-substituted compound **12** was more potent than both its para-

and meta-substituted relatives, and its anti-HIV-1 activity was considerably high. Because of compound **12**'s favorable antiviral properties, we settled it as a lead compound for further derivatization (Table 3).

Alkylpiperazinyl fluoroquinolones, some of which are well known antibacterial agents, had no anti-HIV-1 activities at all (**13-15,** in Table 2). The precise reason for the loss of activity in these compounds is not clear. However, this observation once again highlights the general importance of the steric and electronic properties of the aromatic ring attached to the piperazinyl nitrogen.

Table 3. Inhibitory activities of the compounds **12, 16-24**



Compound	R	IC_{50} (μM)*	CC_{50} (μM)*
12	cyclopropyl	0.11±0.04	9.9±0.8
16	CH_3	0.14±0.04	13±1
17	CH_2CH_3	0.094±0.039	13±2
18	$CH(CH_3)_2$	0.10±0.05	8.9±0.8
19	CH_2CH_2F	0.27±0.08	22±8
20	CH_2CH_2OH	0.26±0.04	26±6
21	CH_2CH_2OAc	0.10±0.03	11±1
22	CH_2COOH	>55	>55
23	$CH_2CH_2N(CH_3)_2$	5.6±3.4	>55
24	$NHCH_3$	0.35±0.20	19±4

*: Mean ± SD of at least three independent experiments.

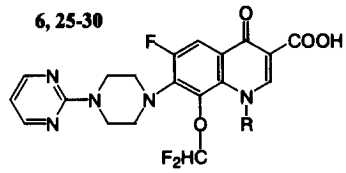
Further modification of **12** commenced at the N1 of the quinolone ring system (Table 3). A variety of substituents of moderate size maintained their original potency (**16-21**). However, a compound with an acidic sidechain at the 1 position had no detectable anti-HIV-1 activity (**22**). Modification with a basic sidechain at this position also resulted in a significant loss of potency (**23**), while the less basic diazine **24** retained the activity of the parent compound. In this group, compound **17**, with an ethyl group at the 1 position, was found to be the most potent (IC_{50}=0. 094±0.039 μM).

Another point of departure from the basic skeleton was compound **6** because of its remarkably low cytotoxicity (Table 4). The goal of this line of investigation was to discover where it would be possible to enhance the anti-HIV activity of the compound while retaining its low level of cytotoxicity. To this end we turned, once again, to the 1 position of the quinolone ring (Table 4). In contrast to the previous series **16-24**, the simple alkyl group derivatives at the 1 position substantially increased the potency (**25-27**). For example, **25** exhibited seven times higher inhibitory activity than **6**. In fact, the anti-HIV potency of **25** was similar to those of **12** and **17,** but at a significantly lower level of cytotoxicity. Compound **25** had the most favorable selectivity

index (CC_{50}/IC_{50}) among all of the compounds studied in this report. (Selectivity indexes of selected compounds are as follows; **7**: 133.3, **12**: 90.0, **17**: 138.3, **25**: 271.4). Other compounds with slightly polar groups at the 1 position (**28-30**) also showed low cytotoxicity but as with our experience with **22-24**, at the cost of reduced anti-HIV activity.

Table 4. Inhibitory activities of the compounds **6**, **25-30**

6, 25-30



Compound	R	IC_{50} (μM)*	CC_{50} (μM)*
6	cyclopropyl	0.97±0.25	>40
25	CH_3	0.14±0.01	38±2
26	CH_2CH_3	0.41±0.15	28±2
27	$CH(CH_3)_2$	0.23±0.10	31±6
28	CH_2CH_2F	0.93±0.06	>60
29	CH_2CH_2OH	0.79±0.04	>60
30	$NHCH_3$	0.50±0.06	>60

*: Mean ± SD of at least three independent experiments.

In summary, SAR studies based on the lead compound R-71762 using HIV-1 chronically infected cells have led to a number of interesting compounds with attractive anti-viral selectivity indexes. The bridge moiety connecting the 1 and 8 position of the fluoroquinolone ring is not necessary for the anti-HIV-1 activity, but the substituents at these positions play an important role in the anti-viral function. An aryl substituent on the piperazine ring seems necessary for anti-HIV-1 activity. Among the aryl groups examined, unsubstituted phenyl, o-methoxyphenyl and pyrimidine groups all seem suitable substituents in terms of their selectivity index. However, the o-methoxypheny-substituted compounds exhibited higher anti-HIV-1 potencies, while pyrimidine-substituted derivatives had lower cytotoxic characters. Interestingly, these compounds do not exhibit any inhibitory activity against the most common targets of current anti-HIV drugs, reverse transcriptase and HIV protease (data not shown). Compound **17** has been shown to inhibit HIV-1 transcription via an unknown mechanism.[8] In addition, **17** has been shown to inhibit replication of herpesviruses, including human cytomegalovirus, varicella-zoster virus and herpes simplex virus types 1 and 2, which are important opportunistic pathogens in AIDS patients.[9] Therefore, the fluoroquinolones reported here may prove to be a unique class of therapeutic agents against AIDS. Further research based on this SAR study is underway. More detailed results, including pharmacokinetic studies and the mechanism of anti-HIV-1 activity, will be described in future reports.

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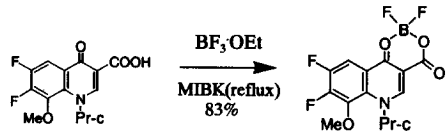

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