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# Evaluation of the *in vitro* antiretroviral potential of some Biginelli-type pyrimidines

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**Summary. –** Despite the success of highly active antiretroviral therapy, AIDS still remains as one of the most important world health problems. Toxicity of current available drugs and inevitable emergence of multi-drug resistant strains makes things worse. In the present study a series of novel Biginelli-type pyrimidine compounds were evaluated as potential anti-human immunodeficiency virus (HIV)-1 agents using green fluorescence protein (GFP) reporter single round HIV-1 infection assay. The rate of infected cells was monitored by flowcytometry. The effect of compounds on the cellular proliferation was considered as the cyotoxicity. The anti-HIV-1 active compounds were selected for HIV-1 replication and syncytium formation assays. The antiretroviral activity of compounds was measured against luciferase reporter A murine leukemia virus (AMLV) virions as the retrovirus control. Compounds 2, 5, 6, 8, 11, 12, 13, 17, 18, 20, and 21 were the most potent against HIV-1. Compound 8 had the 50% inhibitory concentration ($IC_{50}$) of 100 nmol/l for inhibiting HIV-1 replication and 50% cytotoxic concentration ($CC_{50}$) was up to 100 μmol/l (therapeutic index (TI) >1000). Results show that the active compounds were able to inhibit the retrovirus control as well. Analysis of structure of the studied compounds proved relationships with their anti-HIV-1 effects. Some of the studied compounds seem to be promising anti-HIV-1 drug candidates. Structural manipulation based on the well-defined structure-activity relationships might propose some new leads for anti-HIV-1 drug discovery programs.

**Keywords:** antivirals; HIV-1; Biginelli-type pyrimidines

## Introduction

Depletion of CD4 T lymphocytes is the main feature of AIDS, the infection caused by HIV-1 (Barre-Sinoussi *et al*., 1983; Fauci *et al*., 1984). This virus enters the host immune system cells through the interaction of ENVs with the target cell surface receptors: CD4 and one of the two chemokine receptors, CCR5 or CXCR4 (Alkhatib *et al*., 1996; Choe *et al*., 1996; Dragic *et al*., 1996). HIV-1 is an RNA virus, belonging to the retrovirus family. After target cell entry, virion transcribes its RNA genome to DNA and then integrates the proviral DNA to the host cell genome using reverse transcriptase (RT) and indegrase (IN) enzymes, respectively (Cook, 1993). Virus-producing cells have much shorter half-lives than latently infected ones in HIV-1-infected humans, suggesting a role for viral infection in the destruction of host cells. HIV-1 infection in tissue-culture conditions leads to syncytium formation and single cell lysis which are called cytopathic effects (Dedera and Ratner, 1991). There are reports of the cytostatic or cytotoxic effects of HIV-1 proteins

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**Abbreviations:** AMLV = A murine leukemia virus; DHPMs = 3,4-dihydropyrimidine-2(1*H*)-ones; ENV(s) = envelope glycoprotein(s); GFP = green fluorescence protein; HAART = highly active antiretroviral therapy; HIV = human immunodeficiency virus; IN = integrase; RT = reverse transcriptase; RTU = reverse transcriptase unit; SCR = single-cycle replicable; $IC_{50}$ = 50% inhibitory concentration; $CC_{50}$ = 50% cytotoxic concentration; SI = selectivity index ($CC_{25}$/$IC_{25}$); TI = therapeutic index ($CC_{50}$/$IC_{50}$); VSVG = vesicular stomatitis virus-glycoprotein

Tat, Vif, Vpr, Nef, and protease in cultured cells (Bartz and Emerman, 1999; Chen *et al*., 1999; Okada *et al*., 1997; Rasola *et al*., 2001). However, lysis of cultured T cells infected by HIV-1 and depletion of these cells in simian immunodeficiency virus infected monkeys has been observed without these viral proteins (Cao *et al*., 1996; McCloskey *et al*., 1997). Expression of the HIV-1 envelope glycoprotein (ENV) also results in cytopathic effects in cells expressing the appropriate receptors (LaBonte *et al*., 2003). HIV-1 ENVs can trigger the formation of lethal syncytia among infected cells due to cell-cell fusion (Sodroski *et al*., 1986).

Despite the success of highly active antiretroviral therapy, AIDS remains one of the most important world health problems. RT and IN which have critical roles during HIV-1 replication can be exploited as targets for anti-HIV-1 therapy and strongly show effectiveness particularly when employed in combination. Highly active antiretroviral therapy (HAART) dramatically decreases the number of AIDS cases, however, it is not able to eradicate HIV-1 infection from patients completely (Barreca *et al*., 2005; Imamichi, 2004; Wilkin *et al*., 2010). On the other hand, maintaining patients' adherence to antiretroviral therapy is difficult because of the toxicity of current available anti-HIV-1 substances (Siliciano *et al*., 2003; Tan *et al*., 2010). The inevitable emergence of drug-resistant mutants and multi-drug resistant strains makes things worse (Grant *et al*., 2002; Obiako *et al*., 2010). Therefore, there is increasing impetus for the discovery of new anti-HIV agents against current targeted or alternative sites in the viral life cycle.

In the beginning of 1890s the Italian chemist Pietro Biginelli reported a novel method for the one pot synthesis of 4-aryl-3,4-dihydropyrimidine-2(1*H*)-ones (DHPMs) which are known today as Biginelli-type pyrimidines (Biginelli, 1893). After nine decades of being ignored, the pharmacological properties of this interesting heterocyclic scaffold attracted worthwhile attention of medicinal chemists (Kappe, 2000). Since the early 1980s, a broad range of biological effects, including calcium channel modulation (Rovnyak *et al*., 1992), adrenoceptor blocking activity (Barrow *et al*., 2000), antitumor (Klein *et al*., 2007), antibacterial (Ashok *et al*., 2007), antioxidant (Stefani *et al*., 2006) and anti-inflammatory (Bahekar and Shinde, 2004) activities has been ascribed to this class of heterocycles. DHPMs also possess antiviral activity (Hurst and Hull, 1961). Nitracin, a nitrofuryl-substituted Biginelli-type pyrimidine has displayed good activity against the viruses of the trachoma group (Hurst, 1962). 3,4-Dihydropyrimidine-2(1*H*)-one moiety has been found in batzelladine A and B, natural marine alkaloids. These compounds are the first low molecular weight natural products reported as inhibitors of the binding of HIV gp-120 to CD4 cells (Patil *et al*., 1995).

In this study we report the anti-HIV-1 activity of forty-six 4-aryl-3,4-dihydropyrimidine-2(1*H*)-one (thione) derivatives. Single-round infection, replication and syncytium formation assays were the methods utilized for the evaluation of the anti-HIV-1 activity of the compounds. To prove the safety of the compounds, cytotoxicity was also determined.

## Materials and Methods

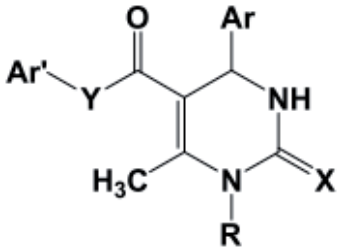
*Compounds.* General structure and structural details of the compounds used in this study are shown in Table 1. The preparation of compounds 1–16 was reported by our group previously (Soleymani and Memarian, 2010). Compounds 17–27, 34, 36, 38, 39, 41, 44, 45, and 46 were prepared according to literature (Besoluk *et al*., 2008; Jiang and You, 2007; Kumar *et al*., 2006; Lu *et al*., 2002; Russowsky *et al*., 2004; Zhan *et al*., 2008). The rest of the studied compounds were not reported yet. The compounds were dissolved in DMSO at different concentrations of 10 mmol/l to 10 μmol/l. These stocks were diluted 100 times in cell environment so that the final concentrations of compounds were 100 μmol/l to 10 nmol/l. Nevirapine (50 μmol/l) extracted from commercial tablets and DMSO (1% v/v) were used as positive and negative controls. All tests were performed in triplicate.

*Cells and viruses.* HEK293T, MT-2 and Cf2Th cells were used in this study. Cells were cultured in DMEM (293T and Cf2Th) and RPMI 1640 (MT-2) mediums (Chemicon, USA) supplemented with 15% heat inactivated FBS (Gibco, South America) plus appropriate concentrations of L-Glutamine (Gibco, South America) and penicillin-streptomycin (Bioidea, Iran). Transfections were done using polyfect reagent (Qiagen, USA) according to the instruction. GFP reporter and single-cycle replicable (SCR) HIV virions were produced as previously described (Sadat *et al*., 2011; Zabihollahi *et al*., 2011). pCL-ECO, pBABE-LUC and pMD2G (Addgene, USA) were used to produce luciferase reporter AMLV. Virus containing supernatants were collected and pooled 24, 48, and 72 hrs post transfection. Virus supernatants were clarified by filtering through 0.22 μm filters. Some virus stocks were concentrated by centrifuging at 50,000 × g for 2 hrs at 4°C. Stocks were analyzed for p24 content (HIV P24 ELISA, BIOMERIEUX) and infectious titer was determined before aliquoting and storing at -70°C (Cavrois *et al*., 2004).

*Single-round infection assay.* HIV-1 GFP and AMLV luciferase reporter virions were used for performing single-round infection assay. MT-2 cells ($4\times10^4$) were placed in each well of 96 well plates and infected by GFP reporter HIV-1. After 5.5 hrs, 200 μl of fresh medium was added to each well and cells were cultured for additional 3 days. Subsequently, the content of each well was transferred to the flowcytometry tubes containing 1 ml RPMI 1640. The fraction of GFP positive cells in each well was quantified using the flowcytometry (Partec, Germany) (Campbell *et al*., 2007). Concentration of compound which was able to inhibit the HIV-1 infection by 50% was considered as $IC_{50}$.

Cf2Th cells were infected with $10^4$ RT unit (RTU) luciferase reporter AMLV virions in each well of 96 well plates and cultured for 72 hrs. Cells were lysed and 100 μl of substrate (Promega, USA) was added to each well. The luminesence activity of plates was de-

**Table 1. Chemical structure of pyrimidine derivates used in this study for anti HIV-1 activity**




| Compound | Ar | Ar‘ | R | X | Y |
|---|---|---|---|---|---|
| 1 | 2-Chlorophenyl | 2-Chlorophenyl | - | S | NH |
| 2 | Phenyl | 4-Bromophenyl | - | O | NH |
| 3 | Phenyl | 4-Flourophenyl | - | O | NH |
| 4 | Phenyl | Cyclohexyl | - | O | NH |
| 5 | 2-Chlorophenyl | 2-Chlorophenyl | - | O | NH |
| 6 | 4-Chlorophenyl | 2-Chlorophenyl | - | O | NH |
| 7 | 2-Bromophenyl | 2-Chlorophenyl | - | O | NH |
| 8 | 3-Bromophenyl | 2-Chlorophenyl | - | O | NH |
| 9 | 4-Bromophenyl | 2-Chlorophenyl | - | O | NH |
| 10 | 4-Methylphenyl | 2-Chlorophenyl | - | O | NH |
| 11 | 3-Nitrophenyl | 2-Chlorophenyl | - | O | NH |
| 12 | 4-Nitrophenyl | 2-Chlorophenyl | - | O | NH |
| 13 | 3-Methoxyphenyl | 2-Chlorophenyl | - | O | NH |
| 14 | 4-Methoxyphenyl | 2-Chlorophenyl | - | O | NH |
| 15 | $CHCH_3$Phenyl | 2-Chlorophenyl | - | O | NH |
| 16 | Phenyl | Phenyl | - | O | NH |
| 17 | Thienyl | Methyl | - | S | O |
| 18 | Thienyl | Ethyl | - | S | O |
| 19 | Thienyl | *iso*-propyl | - | S | O |
| 20 | Thienyl | Isobutyl | - | S | O |
| 21 | Thienyl | *n*-Propyl | - | S | O |
| 22 | Thienyl | *tert*-Butyl | - | O | O |
| 23 | Thienyl | 2-Pyridyl | - | O | NH |
| 24 | Thienyl | 3-Pyridyl | - | O | NH |
| 25 | Thienyl | 2-Chlorophenyl | - | O | NH |
| 26 | Thienyl | 3-Chlorophenyl | - | O | NH |
| 27 | Thienyl | 4-Chlorophenyl | - | O | NH |
| 28 | $N_1$-Phenylamino-2-mercaptomethyl-5-imidazolyl | 2-Chlorophenyl | - | O | N |
| 29 | $N_1$-Phenylamino-2-mercaptomethyl-5-imidazolyl | 3-Chlorophenyl | - | O | NH |
| 30 | $N_1$-Phenylamino-2-mercaptomethyl-5-imidazolyl | 4-Chlorophenyl | - | O | NH |
| 31 | $N_1$-Benzyl-2-mercaptomethyl-5-imidazolyl | 2-Chlorophenyl | - | O | NH |
| 32 | $N_1$-Benzyl-2-mercaptomethyl-5-imidazolyl | 3-Chlorophenyl | - | O | NH |
| 33 | $N_1$-Benzyl-2-mercaptomethyl-5-imidazolyl | Ethyl | - | O | O |
| 34 | 4-Fluorophenyl | Ethyl | - | O | O |
| 35 | 4-Fluorophenyl | Ethyl | Methyl | O | O |
| 36 | 4-Fluorophenyl | Methyl | - | O | O |
| 37 | 4-Fluorophenyl | Methyl | Methyl | O | O |
| 38 | *n*-Propyl | Ethyl | - | O | O |
| 39 | 4-Bromophenyl | Ethyl | - | O | O |
| 40 | 4-Bromophenyl | Methyl | - | O | O |
| 41 | 4-Bromophenyl | Ethyl | - | S | O |
| 42 | 4-Bromophenyl | Methyl | - | S | O |
| 43 | 4-Bromophenyl | Ethyl | Methyl | O | O |
| 44 | Phenyl | Ethyl | - | O | O |
| 45 | Phenyl | Ethyl | Methyl | O | O |
| 46 | Phenyl | Phenyl | - | O | O |

**Table 2. Screening the anti-HIV-1 potential of the studied compounds**

| Compounds | Protection (μmol/l) | | Cytotoxicity (μmol/l) | | Therapeutic index | Selectivity index |
|---|---|---|---|---|---|---|
| | $IC_{50}$ | $IC_{25}$ | $CC_{50}$ | $CC_{25}$ | | |
| 1 | >100 | 0.5 | >100 | 21 | >1 | 42 |
| 2 | 33 | 0.1 | >100 | 91 | >3 | 182 |
| 3 | >100 | 0.3 | >100 | 17 | >1 | 56 |
| 4 | >100 | 0.1 | >100 | 10.3 | >1 | 103 |
| 5 | 82 | 0.09 | NT | NT | >1.2 | >1111 |
| 6 | 15 | 0.09 | NT | NT | >6.6 | >1111 |
| 7 | 75 | 20 | >100 | 89 | >1.3 | 4.4 |
| 8 | 87 | 1.1 | >100 | 64 | >1.17 | 58 |
| 9 | 24 | 0.08 | >100 | 73 | >4.1 | 912 |
| 10 | 9 | 0.07 | >100 | >100 | >11.1 | >1428 |
| 11 | 25 | 0.07 | NT | NT | >4 | >1428 |
| 12 | 96 | 0.09 | NT | NT | >1 | >1111 |
| 13 | 91 | 6 | >100 | 75 | >1 | 12.5 |
| 14 | 88 | 0.93 | >100 | 12 | >1.1 | 13 |
| 15 | >100 | 12 | 98 | 1.2 | >1 | 0.1 |
| 16 | >100 | 100 | NT | NT | >1 | >1 |
| 17 | >100 | 82 | NT | NT | >1 | >1.2 |
| 18 | 90.3 | 6.5 | 100 | 63 | 1.24 | 9.6 |
| 19 | 32.2 | 9.7 | 68.7 | 7.2 | 2.1 | 0.7 |
| 20 | 1.1 | 0.2 | 72.5 | 11 | 65.9 | 55 |
| 21 | 4.8 | 0.3 | 30.9 | 0.9 | 6.43 | 3 |
| 22 | NI | NI | 100 | 81 | <1 | <1 |
| 23 | >100 | 67 | >100 | >100 | >1 | >1.4 |
| 24 | NI | NI | >100 | >100 | <1 | <1 |
| 25 | 100 | 34 | >100 | 92 | >1 | 2.7 |
| 26 | NI | NI | >100 | 94 | <1 | <1 |
| 27 | 60 | 8 | >100 | 76 | 1.6 | 9.5 |
| 28 | NI | NI | >100 | 87 | <1 | <1 |
| 29 | 103 | 23 | 89 | 91 | 0.86 | 4 |
| 30 | >100 | 37 | >100 | 45 | >1 | 1.2 |
| 31 | NI | NI | >100 | >100 | <1 | <1 |
| 32 | 80 | 74 | >100 | 100 | >1.2 | 1.3 |
| 33 | NI | NI | NT | NT | 1 | 1 |
| 34 | NI | NI | NT | NT | 1 | 1 |
| 35 | NI | 95 | NT | NT | 1 | >1 |
| 36 | NI | NI | NT | NT | 1 | 1 |
| 37 | NI | NI | NT | NT | 1 | 1 |
| 38 | NI | NI | NT | NT | 1 | 1 |
| 39 | 89 | 13 | 100 | 53 | 1.1 | 4 |
| 40 | 1.1 | 0.6 | 99 | 11 | 90 | 18 |
| 41 | 82 | 66 | 45 | 15 | 0.5 | 0.22 |
| 42 | 71 | 52 | 87 | 43 | 1.22 | 0.8 |
| 43 | 85 | 19 | 81 | 32 | 0.95 | 2 |
| 44 | NI | 94 | NT | NT | 1 | >1 |
| 45 | 88 | 19 | NT | NT | >1.13 | >5.2 |
| 46 | 100 | 15 | 98 | NT | 1 | >6.6 |

NT = non toxic; NI = non inhibitory.

termined by using luminometer device (EG&G Berthold LB 96V Microplate Luminometer) (Madani *et al.,* 2007).

*Replication assay.* SCR virions are replicable no more than one cycle (Zabihollahi *et al.*, 2011). Vesicular stomatitis virus glycoprotein (VSVG)-SCR virions can infect 293 T cells and complete their replication cycle by assembling of inactive virions. HEK293T cells ($6\times10^4$) were seeded in each well of 24 well plates containing 250 µl of complete medium and infected with 400 ng P24 VSVG-SCR virions. Cells and virions were incubated together overnight and cells were then washed two times with pre-warmed 5% FBS supplemented DMEM. Complete medium (400 µl) was added into each well and cell supernatants were analyzed for p24 load after 48 hrs (HIV P24 ELISA, BIOMERIEUX).

*Syncytium formation assay.* MT-2 cells are sensitive to HIV induced syncytial formation. MT-2 cells ($2\times10^4$) were seeded in 96 well plates containing 80 µl of fresh media and 700 ng P24 VSVG-SCR virions. Complete fresh medium (200 µl) was added into each well after 16 hrs. Cells were incubated 48 hrs and the syncytiums were counted under a light microscope (Nikon TR300) in five fields of each well (Madani *et al.*, 2007).

*Cytotoxicity assay.* Toxicities of the compounds against MT-2 and 293T cells were quantified using XTT (Roche Molecular Biochemicals) cell proliferation assay (Lin *et al.*, 2003). Cells were cultured in 96 well plates ($3.5\times10^4$ cells/well) containing fresh phenol-red free medium and incubated for 72 hrs in a $CO_2$ incubator. Subsequently, 50 µl of XTT (sodium 3-[1 (phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid) was added into each well and incubated 4 hrs at 37°C. Plates were evaluated by using Bio-Tek ELx 800 ELISA reader at the test and reference OD of 450 and 630 nm, respectively. Concentration which reduces proliferation of 50% of cells was considered as $CC_{50}$.

## Results

### *Anti-HIV-1 potential of the compounds*

A primary screening of the studied compounds for anti-HIV-1 potential was performed using single-round infection and cytotoxicity assays. The anti-HIV-1 activity of the compounds was evaluated using single-round infection assay. The inhibitory and cytotoxicity of each compound is shown in Table 2. Compounds 5, 6, 10, 11, and 12 had considerable anti-HIV-1 activity with no remarkable toxicity for the cells. Since they were not toxic up to their maximal solubility concentration, selectivity index (SI) could not be determined mathematically. Some other compounds had not only good anti-HIV-1 activity but also toxicity on MT-2 cells (compounds 2, 4, 8, 9, 20, 21, 27, and 40). Compounds 19, 20, 21, 30, and 41 were the most toxic among the tested compounds. Compound 20 and 40 were the most active ones for inhibiting HIV infection ($IC_{50}$ of 1.1 µmol/l) but, they were highly toxic as well. Compound 10 inhibited viral infection with $IC_{50}$ and $IC_{25}$ of 9 and 0.07 µmol/l, respectively but was not toxic. The calculated SI for this compound was >1428 which shows its considerable anti-HIV-1 potential. Compounds 22, 24, 26, 28, and 31 did not show any inhibitory effect and compounds 33, 34, 35, 36, 37, 38, and 44 had neither of inhibitory and toxic effects up to the highest concentration.

### *Antiretroviral potential against AMLV and replication of HIV-1*

The anti-HIV active compounds were evaluated for inhibition of HIV-1 replication and AMLV infection. Compounds 2, 5, 6, 8, 9, 10, 17, 18, 19, 20, and 21 were the most potent compounds regarding the screening so their inhibitory activity was evaluated against HIV replication. The highly potent compounds showed similar anti-HIV activity in both, infection and replication assays (5, 6, 10, 11, and 12). The calculated TI values for compounds 18, 19, 20, and 21 were 333, 100, 97, and 101 respectively, which shows potent anti-HIV-1 activity. Compounds 6 and 10 did not show toxic effect for cells in their solubility range but compound 6 inhibited 50% of viral replication at concentration of 39 µmol/l. Compounds 20 and 21 had significant anti HIV replication activity but they were highly toxic as well. Compound 8 had $IC_{50}$ of 100 µmol/l and concentration of $CC_{50}$ was 100 µmol/l. The TI value of compound 8 was more than 1000 which shows considerable anti-HIV-1 potential of this agent. The anti-AMLV activity of all compounds was similar to their anti-HIV activity (Table 3). Compound 8 was not effective for inhibition of AMLV virions infection ($IC_{50}$ >100 µmol/l).

### *Inhibition of syncytium formation induced by HIV-1*

In this study, syncytium formation, as a factor for HIV-1 replication and anti-HIV-1 activity of some active compounds was measured. The results of syncytium formation assay which shows the inhibitory effect of compounds for HIV-1 replication is shown in Table 3. Compounds 19, 20, and 21 inhibit almost all the HIV-1 induced syncytium formation at concentration

**Table 3. The inhibitory activity against AMLV, HIV-1 replication, and syncytium formation**

| Compounds | $IC_{50}$[a] | | |
|---|---|---|---|
| | AMLV | HIV-1 | Syncytiums |
| 2 | 37 | 100 | 98 |
| 5 | 43 | 54 | 62 |
| 6 | 96 | 39 | 29 |
| 8 | >100 | 0.1 | 14 |
| 9 | 52 | 38 | 62 |
| 10 | 57 | 98 | 43 |
| 11 | 37 | 13 | 28 |
| 12 | 21 | 74 | 66 |
| 18 | 25 | 11 | 19 |
| 19 | 13 | 19 | 21 |
| 20 | 4 | 0.9 | 3 |
| 21 | 15 | 1.1 | 4 |

[a]$IC_{50}$ = 50% inhibitory concentration.

of 100 μmol/l. Compound 8 showed considerable anti-HIV-1 activity with lower toxicity among the studied compounds.

## Discussion

A series of Biginelli-type pyrimidines were evaluated as potential anti-HIV-1 agents. A screening for anti-HIV-1 activity using single round HIV-1 infection assay as well as cytotoxicity evaluation of the studied compounds were performed. Compounds with high anti-HIV-1 potential were selected for anti-HIV-1 replication assay. Replication assay shows the ability of compounds for inhibiting replication cycle of HIV-1 virions from entry to release from the host cells. In order to determine if the compounds had specific activity against HIV-1, the antiviral activity of the compounds against AMLV as a retrovirus control was measured. Syncytium formation assay was considered for detecting the possible activity of some active compounds against HIV-1 induced cytopathic effects. Almost all compounds with the highest activity in infection assay showed similar results in inhibiting HIV replication (2, 6, 9, 17, 18, 20, and 21). Compound 8 showed very high anti-HIV-1 activity in replication and syncytium formation assay, but on the other hand, it showed moderate effect in infection assay. This means, that it may inhibit the viral replication after insertion of provirus. All of the compounds which were evaluated for anti-AMLV activity showed similar results to anti-HIV-1 potential, leading us to conclusion, that this class of compounds is not affecting viral components.

Analysis of structure of studied compounds shows that all most active compounds (5, 6, 9, 10, 11, and 12) have a substituted phenyl group at C-4 position of the pyrimidine ring. Compounds with thienyl or substituted imidazole ring at this position do not show a remarkable anti-HIV-1effect (compare for example, the SI values of compounds 9–12 with the SI values of compounds 25 and 31). Compounds 11 and 12 with a nitrophenyl group at C-4 are the most active ones, but their selectivity indices were not possible to calculate mathematically since they were almost nontoxic to the MT-2 cells. All active compounds also have an aryl amide group at C-5 position of the pyrimidine ring. By comparing the SI value of compound 9 (912) with those for compounds 39 and 40 (4 and 18, respectively) we came to conclusion that the presence of an ester group at this position makes the compound less active. The last noteworthy point in the structure-activity relationships of the studied compounds can be concluded by comparing the selectivity indices of compounds 5 with 1, 39 with 41, and 40 with 42. As it can be seen, 2-thio carbonyl substitution at the C-2 position of the pyrimidine ring instead of 2-carbonyl group decreases the selectivity of the compound against the virion.

It should be emphasized that some of the most active compounds (5, 6, 8, 11, 12, and 13) were not so toxic to the target cell to disturb its normal activities and cause its death. Of course, the best scenario is that the drug doesn't affect the target cell at all. On the other hand, mentioned compounds showed high activity against HIV-1 infection and replication. These compounds seem to be promising candidates. Structural manipulation based on the well-defined structure-activity relationships might propose some new leads for anti-HIV-1 drug discovery programs.

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