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In vitro anti-HIV activity of diverse substituted nitrosopyrimidine analogues

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Abstract: A series of substituted nitrosopyrimidineanalogues were evaluated *in vitro* against the replication of HIV-1 and HIV-2 in MT-4 cells using MTT method. 4,6-Diamino-5-nitrosopyrimidine (**23**) showed an IC_{50} values of > 0.44 μM with SI <1. This means that **23**was cytotoxic to MT-4 cells at a CC_{50} value of > 0.44 μM.In addition, 6-amino-5-nitroso-2-phenylpyrimidine-4-one (**22**) and phenyl (2,6-diamino-5-nitrosopyrimidin-4-yl) benzoate (**36**) were cytotoxic to MT-4 cells at concentration of 6.92 and 6.85μM, respectively.

Keywords: Anti-HIV activity / Cytotoxicity / Nitrosopyrimidines

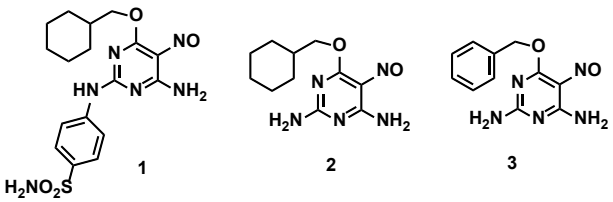
1. Introduction

Nitrosopyrimidines constitute a class of biologically relevant molecules, since exhibited activity as inhibitors of cyclin-dependent kinases (CDKs) (*e.g.*: **1** and **2**) [1] and of the DNA-repair protein *O*(6)-alkylguanine-DNA-alkyltransferase (AGT) **3**[2] leading to a renewed interest in the synthesis of these pyrimidine derivatives. Recently, Melguizo *et al.* [3-5] have synthesized several alkoxy-5-nitroso-pyrimidine analogues, aiming to evaluate their various biological activity, since they reported some nitropyrimidines as potential antifungal [6] and antibacterial agents [7]. In addition, pyrimidine and its derivatives demonstrated a diverse array of biological and pharmacological activities including antitumor [8-10], antimicrobial [11-14], and antihypertensive [15] as well as their cardiovascular [16] and diuretic [17] properties. Some pyrimidine analogues exhibited potent antiviral activity against a wide spectrum of unrelated viruses, such as poliovirus [18], and herpes virus[19] in addition to their activity as anti-HIV agents [20-22]. Two recent diarylpyrimidines (DAPY), rilpivirine [23] and etravirine [24, 25] have been classified as non-nucleoside

reverse transcriptase inhibitors (NNRTI's). Further, several pyrimidine derivatives exhibited significant antitumor activity as well *e.g.* imatinibmesylate (Gleevec) [26], a novel interesting agent for treatment of chronic Leukemia*via* the tyrosine kinase inhibition, contains a 2-amino-4-pyridylsubstituted pyrimidine moiety. 2,4-Diamino-N^4-6-diaryl-pyrimidines were reported as active agents in the proliferation of tumor cell lines *in vivo*, especiallyduodenum cancer [27]. Chauhan and Kumar [28] have reviewed extensively the biological significance of pyrazolo[3,4-*d*] pyrimidines.

Considering the significance of pyrimidines and in continuation of our ongoing work [29-33] on the synthesis and anti-HIV of various pyrimidine derivatives, we report here the anti-HIV activity of some substituted nitrosopyrimidinederivatives.

Some of the substituted nitrosopyrimidine derivatives presented in this study have been previously prepared in our laboratories meanwhile the other analogues were prepared by different groups [3-7, 34-36].

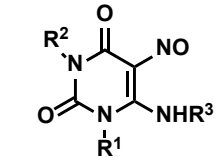


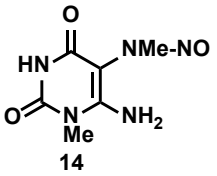
CDKs Inhibitors AGT Inhibitor

3. Results and discussion

2.1. Chemistry

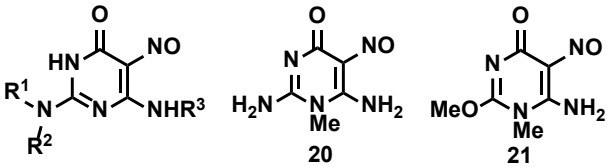
In the present study, the selected substituted 5-nitrosopyrimidine analogues **4-38** have been synthesized by different laboratories by various methods including the nitrosation of carbon 5 of substituted pyrimidines, aminolysis of the 2- or 4-alkoxy group to the corresponding 2- or 4-alkylamino-5-nitroso-pyrimidine derivatives, or alkylation of the free amino groups at carbons 2, 4 or 6 of 5-nitrosopyrimidine scaffold. Figures 1-5 represent different series of substituted 5-nitrosopyrimidine analogues, which have been evaluated for theiractivity against HIV. To the best of our knowledge, no study concerning the anti-HIV activity of nitrosopyrimidine analogues has been reported.





	R^1	R^2	R^3
4	H	H	H
5	Me	H	H
6	C_3H_7	H	H
7	CH_2-CH=CH_2	H	H
8	H	Me	H
9	Me	Me	H
10	C_3H_7	Me	H
11	C_3H_7	C_3H_7	H
12	Me	Me	Me
13	Me	Me	$CHMe_2$

Fig. 1.1,3-dialkylamino, or diamino-6-aminoor alkylamino-5-nitrosopyrimidine-2,4-dionederivatives (**4-13**)



	R^1	R^2	R^3
15	H	H	H
16	H	H	Me
17	Me	H	H
18	Me	Me	H
19	Me	H	Me

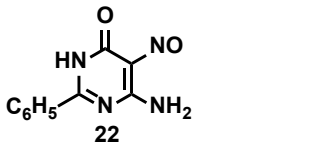
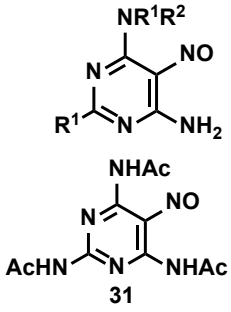
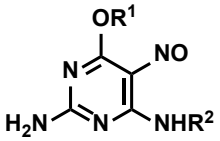


Fig. 2.2-(Dimethylamino) or 2-amino-6-(methylamino)or 6-amino-5-nitroso-pyrimidine-4-one derivatives (**15-19**), N^1-Me-(**20**), N^1-Me-2-methoxy- (**21**) and 2-phenyl-(**22**) analogues.



	R1	R2	R3
23	H	H	H
24	NHEt	H	H
25	$NHCH_2CH_2OH$	H	H
26	NH_2	H	Me
27	NH_2	Me	Me
28	SMe	H	H
29	SMe	Me	Me
30	C_6H_5	H	H

Fig. 3.N^6-Amino-N^4-(substituted amino)-2-(H, NH_2, alkyl, SMe or Ph)-5-nitrosopyrimidine derivatives (**23-30**) and 2,4,6-triacetamido analogue (**31**)



	R1	R2
32	Me	H
33	$CHMe_2$	Me
34	$CH_2C_6H_5$	H
35	C_5H_{11}	Me
36	COC_6H_5	H

Fig. 4.6-Alkoxy-N^4-methyl or N-alkyl, SMe or Ph)-5-nitrosopyrimidine derivatives(**32-36**).

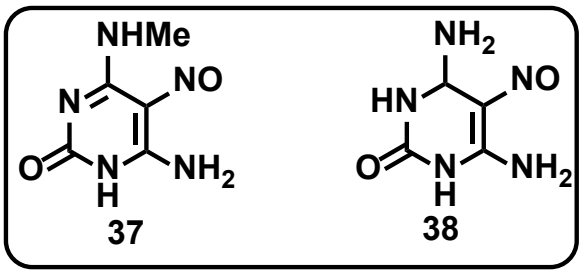


Fig. 5.6-Amino-(4-methylamino)-5-nitroso-pyrimidine-2-one (**37**) and 4,6-diamino analogue (**38**)

3. *In vitro* Anti-HIVAssay

Compounds **4-38** were tested for their *in vitro* anti-HIV-1 (strain III_B) and HIV-2 (strain ROD) activity in human T-lymphocyte (MT-4) cells, using MTT method [37]. The results are summarized in Table 1, in which the data for lamivudine (3TC) [38] and nevirapine [39] were included for comparison purposes. Compound **23** was found to be the only compound from the series inhibiting HIV-1 and HIV-2 replication (IC_{50} = >0.44 μM), but no selectivity can be witnessed selectivity index (SI<1). Compounds **22** and **36** showed moderate activity against HIV-1 and HIV-2 with IC_{50} values of >6.92 and >6.85 μM, respectively, with selectivity index (SI < 1). Such data means these compounds might be toxic at the therapeutic level. However, the potency of the compounds was much lower than the corresponding lead compounds, lamivudine (3TC) and nevirapine.

Appropriate reference compounds are always included in the experiments assessing the anti-HIV activity of new compounds. Our choice for 3TC and nevirapine as reference compounds is based on the fact that both nucleoside and nonnucleosides reverse transcriptase inhibitors (NRTIs, NNRTIs, respectively) are equipotent against HIV-1 and HIV-2.

With respect to SAR studies, the unsubstituted 5-nitropyrimidine analogue at carbon 2 (C_2-H), with two free amino groups at carbons 4 and 6 (compound **23**) showed generally triggers better activity, when compared to other analogues having substituents at C-2. Although compounds **22** and **36** having phenyl and amino groups at carbon 2 respectively, but revealed a moderate activity in comparison for those of the other analogues of the series. Such activity might be due to the tolerated of both compounds in the hydrophobic region of HIV RT. In conclusion, the unsubstituted carbon 2 of 5-nitrosopyrimidine analogue was found to be a potent agent against HIV and it might be considered a promising agent for further structural modifications andpharmacological evaluation.

Table 1. *In vitro* anti HIV-1 and HIV-2 activity and cytotoxicity of new nitrosopyrimidine

derivatives

Comp	HIV-1 (III_B) IC_{50} (μM)	HIV-2 (ROD) IC_{50} (μM)	CC_{50} (μM)	SI (III_B)	SI (ROD)
4	>58.13	>58.13	58.13	<1	<1
5	>86.63	>86.63	86.63	<1	<1
6	>98.25	>98.25	98.25	<1	<1
7	>106.50	>106.50	106.5	<1	<1
8	>92.50	>92.50	92.50	<1	<1
9	>06.90	>106.90	106.9	<1	<1
10	>125.00	>125.00	125.0	<1	<1
11	>59.50	>59.50	59.50	<1	<1
12	>104.20	>104.20	104.2	<1	<1
13	>108.50	>108.50	108.5	<1	<1
14	>111.00	>111.00	111.0	<1	<1
15	>97.70	>97.70	97.70	<1	<1
16	>91.50	>91.50	91.50	<1	<1
17	> 64.50	>64.50	64.50	<1	<1
18	>56.35	>56.35	56.35	<1	<1
19	>89.68	>89.68	89.68	<1	<1
20	>66.45	>66.45	66.45	<1	<1
21	>13.45	>13.45	13.45	<1	<1
22	>6.92	>6.92	6.92	<1	<1
23	>0.44	>0.44	0.44	<1	<1
24	>102.60	>102.60	102.6	<1	<1
25	>125.00	>125.00	125.0	<1	<1
26	> 57.45	> 57.45	57.45	<1	<1
27	> 69.03	>69.03	69.03	<1	<1
28	>11.43	>11.43	11.43	<1	<1
29	>65.45	>65.45	65.45	<1	<1
30	>67.18	>67.18	67.18	<1	<1
31	>12.98	>12.98	12.98	<1	<1
32	>25.03	>25.03	25.03	<1	<1
33	>44.68	>44.68	44.68	<1	<1
34	> 66.55	>66.55	66.55	<1	<1
35	>68.33	>66.33	66.33	<1	<1
36	>6.85	>6.85	6.85	<1	<1
37	> 96.63	> 96.63	96.63	<1	<1
38	>101.00	>101.00	101.0	<1	<1
3TC	>0.58	>2.27	>20	>34	> 9
Nevir-apine	0.075	>4.00	>4.00	>53	X1

Anti HIV-1 activity measured with strain III_B; anti HIV-2 activity measured with strain ROD; IC_{50} (μM): compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 and HIV-2 induced cytopathogenic effect; Average CC_{50} (μM) compound concentration that reduced the viability of mock-infected MT-4 cells by 50%; SI: selectivity index (CC_{50}/ IC_{50}). Data represent mean values (± S.D.) for two independent determinations.

4. Conclusion

In summary, a series of substituted nitrosopyrimidine analogues (**4-38**) has been evaluated against HIV activity. Compound **23** having 2,4-diamino groups with unsubstituted carbon 2 (C_2-H) is the most active analogue of the series, meanwhile **23** and **36** exhibited moderate activity against HIV. Due to the cytoxicity of these three analogues, they are in progress for antitumor evaluation.

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