European Journal of Chemistry 5 (4) (2014) 588-594

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Synthesis, anti-HIV activity and molecular modeling study of some new pyrimidine analogues

Yossra Abood Marich, Niran Jassim Al-Salihi and Najim Aboud Al-Masoudi *

Department of Chemistry, College of Science, University of Basrah, Basrah, 61001, Iraq

**Corresponding author at: Department of Chemistry, College of Science, University of Basrah, Basrah, 61001, Iraq.*
Tel.: +49.75.3134435. Fax: +49.75.3134435. E-mail address: najim.al-masoudi@gmx.de (N.A. Al-Masoudi).

ARTICLE INFORMATION

DOI: 10.5155/eurjchem.5.4.588-594.1109

Received: 15 June 2014
Received in revised form: 08 July 2014
Accepted: 08 July 2014
Online: 31 December 2014

KEYWORDS

Pyrimidines
Anti-HIV activity
Sodium hypochlorite
Molecular modeling study
Structure activity relationship
Non-nucleoside reverse transcriptase inhibitors

ABSTRACT

A new series of 2,6-diamino-5-arylazo-4-chloropyrimidine analogues (6-13) were synthesized from the pyrimidine scaffold 5, *via* diazotization with various amines. Nucleophilic displacement of compound 5 by ethanethiolate or arylthio nucleophiles, afforded the 4-alkylthio analogues (14-16). Treatment of compound 17 or 18 with thiourea under MWI gave the 4-thione derivatives 19 and 20, respectively. On treatment of compound 20 with 2-mercaptoacetic acid furnished the 4-thio analogue (21). Reaction of compound 19 or 20 with sodium hypochlorite followed by ammonium hydroxide afforded the 4-aminothio analogues 22 and 23, respectively. Oxidation of compound 23 with H_2O_2 led to the 4-sulphonamide derivative 24. All new compounds were evaluated for their *in vitro* antiviral activity against the replication of HIV-1 and HIV-2 in MT-4 cells. Compounds 14-16 and 21 showed an EC_{50} values of > 2.12, 1.99, 1.80 and 1.92 μg/mL, respectively. In addition, preliminary structure-activity relationship and molecular modeling of compound 15 has been studied.

1. Introduction

Pyrimidine and its derivatives demonstrated a diverse array of biological and pharmacological activities including antitumor [1-6], antimicrobial [7-10], and antihypertensive [11] in addition to their cardiovascular [12,13] and diuretic [14,15] properties. Some pyrimidine analogues exhibited potent antiviral activity against a wide spectrum of unrelated viruses, such as poliovirus [16] and herpes virus [17] and anti-HIV agents [18-20], whereas two recent diarylpyrimidines (DAPY), rilpivirine **1** [21] and etravirine **2** [22, 23] have been classified as non-nucleoside reverse transcriptase inhibitors (NNRTI's), meanwhile Chen *et al.* [24] have reported a new class of diarylpyrimidines (CHX-DAPYs) as potent NNRTI's. Further, several pyrimidine derivatives exhibited significant antitumor activity *e.g.* imatinib mesylate (Gleevec, **3**) [25], an interesting novel agent for the treatment of chronic Leukemia is the tyrosine kinase inhibitor which contains a 4-pyridyl-substituted pyrimidine-2-amine, in addition to a 2,4-diamino-N^4-6-diaryl-pyrimidines where the latter were identified to block the proliferation of tumor cell lines *in vivo*, especially duodenum cancer [26]. Monastrol **4** [27] is another model of pyrimidine derivative as inhibitor of kinesin Eg5 that interact with microtubuline and then causes mitotic arrest [28] (Figure 1).

Recently, Kim *et al.* [29] have reported some novel pyrimidine derivatives as potent acid pump antagonists (APAs). Jian *et al.* [30] have reviewed the biological and medicinal significance of pyrimidines extensively.

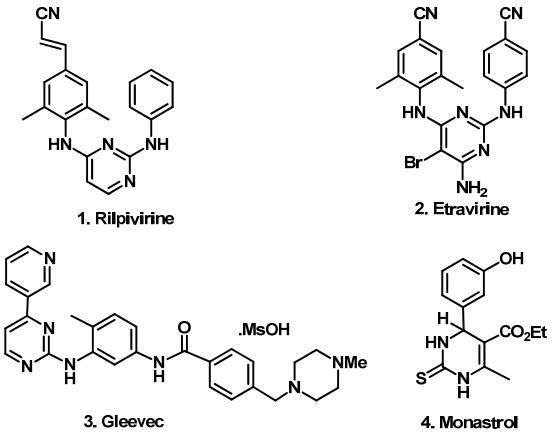
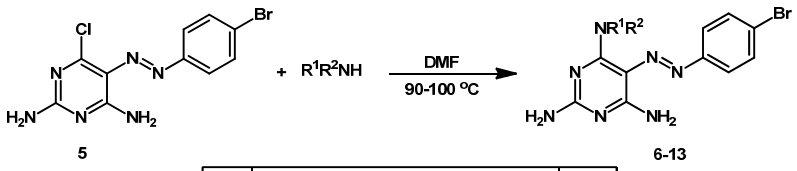


Figure 1. Pyrimidine analogues antitumor and anti-HIV drugs.

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.5.4.588-594.1109



Entry	R^1	R^2	R^1	R^2	Entry
6	H	$CH_2C_6H_5$	Ph	Ph	10
7	H	picolyl	piperidine		11
8	Me	Me	morpholine		12
9	Et	Et	piperazine		13

Scheme 1

In continuation of our ongoing work on the synthesis of new anti-HIV pyrimidine derivatives [31,32], we report here the synthesis of new of series of pyrimidines having substituted amino and thio groups with evaluation of their anti-HIV activity as well the SAR and molecular modeling study.

2. Experimental

2.1. Instrumentation

Melting points are uncorrected and were measured on a Büchi melting point apparatus B-545 (Büchi Labortechnik AG, Switzerland). NMR data were obtained on 400 and 600 MHz (^{1}H) and 150.91 MHz (^{13}C) spectrometers (Avance III, Bruker, Germany) with TMS as internal standard and on the δ scale in ppm. Heteronuclear assignments were verified by ^{1}H, ^{13}C HMBC and ^{1}H, ^{13}C HSQC NMR experiments. Microanalytical data were obtained with a Vario, Elemental analyzer (Shimadzu, Japan). Analytical silica gel TLC plates $60F_{254}$ were purchased from Merck. Microwave supported reaction was performed in a SmithSynthesizer (temperature control of irradiation power up to 800 W). All reagents were obtained from commercial suppliers and were used without further purification.

2.2. Synthesis

2.2.1. General procedure for the preparation of 2,6-diamino-4-alkylamino-5-((p-bromophenyl)diazenyl)pyrimidine derivatives **(6-13)**

A solution of compound **5** (164 mg, 0.50 mmol) in DMF (20 mL) and an appropriate amine (1.00 mmol) was heated in an oil bath at 90-100 °C for 4-5 h. Then water (25 mL) was added, the solution was cooled, and the yellow precipitate was collected, washed with water, and dried. Recrystallization from ethanol afforded the desired product (Scheme 1).

2,6-Diamino-4-benzylamino-5-p-bromophenylazopyrimidine (**6**): From benzylamine (107 mg). Yield: 110 mg (55%). M.p.: 160-167 °C. R_f = 0.75. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.84-8.14 (br. s., 2H, NH_2), 7.72 (d, 2H, J = 7.9 Hz, H-Ar), 7.54 (d, 2H, J = 7.9 Hz, H-Ar), 7.36-7.24 (m, 6H, H-Ar + CH_2NH), 6.62 (br s., 2H, NH_2), 4.72 (d, 2H, J = 6.5 Hz, CH_2). ^{13}C NMR (150.91 MHz, DMSO-d_6, δ, ppm): 163.3 ($C(2)_{pyrimid.}$), 161.0 ($C(4)_{pyrimid.}$), 152.0 ($C(6)_{pyrimid.}$), 139.0, 131.7, 128.3, 127.3, 126.9 ($C_{arom.}$), 122.7 (C-Br), 119.1 ($C_{arom.}$), 110.1 ($C(5)_{pyrimid.}$); 42.9 (CH_2). Anal. calcd. for $C_{17}H_{16}BrN_7$: C, 51.27; H, 4.05; N, 24.62. Found: C, 51.04; H, 3.92; N 24.41 %.

2,6-Diamino-5-((p-bromophenyl)diazenyl)-4-(2-picolyl amino)pyrimidine (**7**): From 2-picolylamine (108 mg). Yield: 64 mg (32%). M.p.: 193-196 °C. R_f = 0.54. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.50 (d, 2H, J = 4.8 Hz, NH_2), 8.36 (d, 1H, J = 8.2 Hz, $H_{picolyl}$-6'')-Ar), 7.78 (m, 3H, $H_{arom.}$-3' + $H_{arom.}$-5' + $H_{picolyl}$-4''), 7.55 (d, 2H, J = 8.2 Hz, $H_{picolyl}$-3'' + $H_{picolyl}$-5''), 7.31 (d, 2H, J = 8.0 Hz, $H_{arom.}$-2' + $H_{arom.}$-6'), 6.60 (br s., 2H, NH_2), 4.73 (d, 2H, $J_{NH,CH2}$ = 6.1 Hz, $HNCH_2$), 3.28 (d, 1H, $HNCH_2$). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 163.2 ($C(2)_{pyrimid.}$), 161.5 ($C(4)_{pyrimid.}$), 157.6 ($C(2')_{picolyl}$), 152.0 ($C(6)_{pyrimid.}$), 149.5 ($C(6')_{picolyl}$), 141.6 ($C(4')_{picolyl}$), 131.7, 122.9, 122.1 ($C_{arom.}$), 119.3 ($C(3')_{picolyl}$ + $C(5')_{picolyl}$), 110.1 ($C(5)_{pyrimid.}$), 40.0 (NCH_2). Anal. calcd. for $C_{16}H_{15}BrN_8$: C, 48.13; H, 3.79; N, 28.07. Found: C, 47.91; H, 3.68; N, 27.81 %.

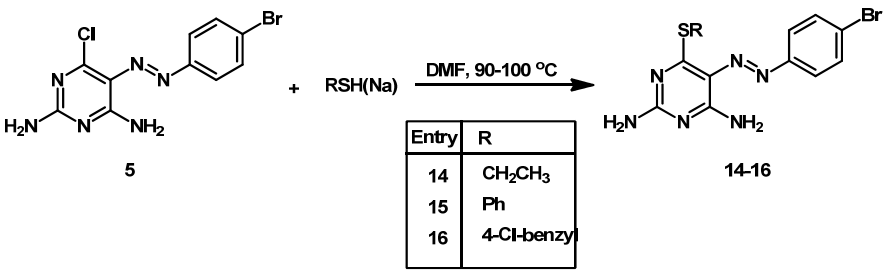
2,6-Diamino-5-((p-bromophenyl)diazenyl)-4-dimethylamino pyrimidine (**8**): From dimethylamine (45 mg). Yield: 124 mg (74%). M.p.: 204-207 °C (Dec.). R_f = 0.55. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.79 (br s., 2H, NH_2), 7.61 (d, 2H, J = 7.9 Hz, H-Ar), 7.54 (d, 2H, J = 7.9 Hz, H-Ar), 6.81 (br s., 2H, NH_2), 3.31 (s, 6H, NMe_2). ^{13}C NMR (DMSO-d_6, δ, ppm): 163.0 ($C(2)_{pyrimid.}$), 161.5 ($C(4)_{pyrimid.}$), 156.0 ($C(6)_{pyrimid.}$), 132.5, 130.2, 126.5 ($C_{arom.}$), 122.9 (C-Br), 104.1 ($C(5)_{pyrimid.}$), 39.1 (NMe_2). Anal. calcd. for $C_{12}H_{14}BrN_7$: C, 42.87; H, 4.20; N, 29.16. Found: C, 42.66; H, 4.08; N, 28.98 %.

2,6-Diamino-5-((p-bromophenyl)diazenyl)-4-diethylamino pyrimidine (**9**): From diethylamine (73 mg). Yield: 126 mg (69%). M.p.: 145-147 °C (Dec). R_f = 0.66. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.14 (br s., 2H, NH_2), 7.72 (d, 2H, J = 7.9 Hz, H-Ar), 7.68 (d, 2H, J = 7.9 Hz, H-Ar), 6.60 (br s., 2H, NH_2), 3.99 (q, 4H, J = 7.1 Hz, 2xCH_2CH_3), 1.23 (t, 6H, 2xCH_2CH_3). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 163.0 ($C(2)_{pyrimid.}$), 162.0 ($C(4)_{pyrimid.}$), 157.2 ($C(6)_{pyrimid.}$), 132.1, 130.6, 127.9 ($C_{arom.}$), 121.6 (C-Br), 100.0 ($C(5)_{pyrimid.}$), 41.6 (2xCH_2CH_3), 11.4 (2xCH_2CH_3). Anal. calcd. for $C_{14}H_{18}BrN_7$: C, 46.16; H, 4.98; N, 29.92. Found: C, 45.89; H, 4.88; N, 28.68 %.

2,6-Diamino-5-((p-bromophenyl)diazenyl)-4-diphenylamino pyrimidine (**10**): From diphenylamine (169 mg). Yield: 83 mg (36%). M.p.: 155-158 °C (Dec). R_f = 0.58. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.19 (br s., 2H, NH_2), 7.64-7.08 (m, 14H, H-Ar), 6.81 (br s., 2H, NH_2). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 163.1 ($C(2)_{pyrimid.}$), 161.3 ($C(4)_{pyrimid.}$), 156.0 ($C(6)_{pyrimid.}$), 140.8, 138.8, 132.6, 130.2, 129.6, 129.2, 127.3 ($C_{arom.}$), 123.2 (C-Br), 104.8 ($C(5)_{pyrimid.}$). Anal. calcd. for $C_{22}H_{18}BrN_7$: C, 57.40;H, 3.94; N, 21.30. Found: C, 57.18; H, 3.82; N, 21.06 %.

2,6-Diamino-4-piperidino-5-p-bromophenylazo-pyrimidine (**11**): From piperidine (85 mg). Yield: 167 mg (89%). M.p.: 190-197 °C. R_f = 0.90. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.96 (br s., 2H, NH_2), 7.78 (d, 2H, J = 8.0 Hz, $H_{arom.}$-3' + $H_{arom.}$-5'), 7.56 (d, 2H, J = 8.0 Hz, $H_{arom.}$-2' + $H_{arom.}$-6'), 6.69 (br s., 2H, NH_2), 3.71 (m, 4H, 2x$CH_{2(piperidin)}$), 1.65-1.50 (m, 6H, 3x$CH_{2(piperidin)}$). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 162.7 ($C(2)_{pyramid.}$), 160.8 ($C(4)_{pyrimid.}$), 157.0 ($C(6)_{pyrimid.}$), 132.7, 130.1, 128.6 ($C_{arom.}$), 123.3 (C-Br), 106.9 ($C(5)_{pyrimid.}$), 53.0 (2x$CH_{2(piperidin)}$), 25.9, 24.4 (3x$CH_{2(piperidin)}$). Anal. calcd. for $C_{15}H_{18}BrN_7$: C, 47.88; H, 4.82; N, 26.06. Found: C, 47.59; H, 4.68; N, 25.89 %.

2,6-Diamino-5-((p-bromophenyl)diazenyl)-4-morpholino-pyrimidine (**12**): From morpholine (87 mg). Yield: 166 mg (88%). M.p.: 238-242 °C. R_f = 0.58. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.79 (br s., 2H, NH_2), 7.62 (d, 2H, J = 7.9 Hz, $H_{arom.}$-3' +



Scheme 2

$H_{arom.}$-5'), 7.53 (d, 2H, *J* = 7.9 Hz, $H_{arom.}$-2' + $H_{arom.}$-6'), 6.84 (br s., 2H, NH_2), 3.88 (m, 4H 2x$CH_{2(morpholin)}$), 3.71 (m, 4H, 2x$CH_{2(morpholin)}$). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 163.3 ($C(2)_{pyrimid.}$), 161.3 ($C(4)_{pyrimid.}$), 156.8 ($C(6)_{pyrimid.}$), 132.6, 130.6, 127.5 ($C_{arom.}$), 122.9 (C-Br), 105.8 ($C(5)_{pyrimid.}$), 66.9 (2x$C_{morpholin}$), 49.6 (2x$C_{morpholin}$). Anal. calcd. for $C_{14}H_{16}BrN_7O$: C, 44.46; H, 4.26; N, 25.92. Found: C, 44.19; H, 4.08; N, 25.69 %.

2,6-Diamino-5-((p-bromophenyl)diazenyl)-4-piperazino-pyrimidine (**13**): From piperazine (86 mg). Yield: 153 mg (81%). M.p.: 238-242 °C. R_f = 0.11. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.07 (br s., 2H, NH_2), 7.62 (d, 2H, *J* = 7.7 Hz, $H_{arom.}$-3' + $H_{arom.}$-5'), 7.52 (d, 2H, *J* = 7.7 Hz, $H_{arom.}$-2' + $H_{arom.}$-6'), 6.66 (br s., 2H, NH_2), 3.30 (br s., 1H, NH), 3.09 (br s., 4H, 2x$CH_{2(piperazin)}$), 2.89 (br s., 4H, 2x$CH_{2(piperazin)}$). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 163.7 ($C(2)_{pyrimid.}$), 162.2 ($C(4)_{pyrimid.}$), 157.1 ($C(6)_{pyrimid.}$), 132.7, 130.4, 127.4, ($C_{arom.}$), 122.9 (C-Br), 105.8 ($C(5)_{pyrimid.}$), 49.6, 47.2 (4x$C_{piperazin}$). Anal. calcd. for $C_{14}H_{17}BrN_8$: C, 44.57; H, 4.54; N, 29.70. Found: C, 44.27; H, 4.40; N, 27.75 %.

2.2.2. General procedure for the preparation of 2,6-diamino-4-alkyl- and arylthio-5-((p-bromophenyl)diazenyl) pyrimidine derivatives (14-16)

A solution of compound **5** (100 mg, 0.31 mmol) in DMF (20 mL) and sodium ethanethiolate, sodium thiophenolate or *p*-chlorobenzenethiol (0.90 mmol) was heated in an oil bath at 90-100 °C for 4-6 h. After cooling, the solution was evaporated and the residue was co-evaporated with toluene (4×15 mL) to give a crude solid. Recrystallization from ethanol or ethanol:ether (1:3, *v*:*v*) afforded the desired product (Scheme 2).

5-((4-Bromophenyl)diazenyl)-6-(ethylthio)pyrimidine-2,4-diamine (**14**): From sodium ethanethiolate (76 mg). Yield: 81 mg (74%). M.p.: 236-238 °C. R_f = 0.63. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.26 (br s., 2H, NH_2), 8.19 (br s., 2H, NH_2), 7.72 (d, 2H, *J* = 7.9 Hz, $H_{arom.}$-3' + $H_{arom.}$-5'), 7.69 (d, 2H, *J* = 7.9 Hz, $H_{arom.}$-2' + $H_{arom.}$-6'), 3.99 (q, 2H, *J* = 7.1 Hz, CH_2CH_3), 1.23 (t, 3H, CH_2CH_3). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 163.0 ($C(4)_{pyrimid.}$), 162.0 ($C(2)_{pyrimid.}$), 157.2 ($C(6)_{pyrimid.}$), 132.1, 130.6, 127.9 ($C_{arom.}$), 121.6 (C-Br), 100.0 ($C(5)_{pyrimid.}$), 41.6 (SCH_2CH_3), 11.4 (SCH_2CH_3). MS ((+)-FAB): *m/z* = 352/354 $[M]^+$. Anal. calcd. for $C_{12}H_{13}BrN_6S$: C, 40.80; H, 3.71; N, 23.79. Found: C, 40.61; H, 3.60; N, 23.54 %.

2,4-Diamino-5-((p-bromophenyl)diazenyl)-6-(phenylthio) pyrimidine (**15**): From sodium benzenethiolate (117 mg). Yield: 85 mg (68%). M.p.: 224-226 °C (Dec.). R_f = 0.53. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.32 (br s., 2H, NH_2), 8.19 (br s., 2H, NH_2), 7.90-7.59 (m, 9H, $H_{arom.}$). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 181.4 ($C(4)_{pyrimid.}$), 164.6 ($C(2)_{pyrmid.}$), 156.0 ($C(6)_{pyrimid.}$), 133.5, 132.2, 132.1, 129.0, 127.8, 123.8 ($C_{arom.}$), 122.1 (C-Br), 118.1 ($C(5)_{pyrimid.}$). MS ((+)-FAB): *m/z* = 401/402 $[M]^+$. Anal. calcd. for $C_{16}H_{13}BrN_6S$: C, 47.89; H, 3.27; N, 20.94. Found: C, 47.77; H, 3.16; N, 20.76 %.

2,4-Diamino-5-((p-bromophenyl)diazenyl)-6-((4-chloro benzyl)thio)pyrimidine (**16**): From 4-chlorobenzylthiol (143 mg). Yield: 74 mg (53%). M.p.: 232-236 °C (Dec.). R_f = 0.55. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.26 (br s., 2H, NH_2), 8.19 (br s., 2H, NH_2), 7.75-7.29 (m, 8H, $H_{arom.}$), 4.36 (s, 2H, CH_2). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 164.6 ($C(4)_{pyrimid.}$), 161.1 ($C(2)_{pyrmid.}$), 155.9 ($C(6)_{pyrimid.}$), 132.2, 132.0, 131.1, 128.4, 123.2 ($C_{arom.}$), 122.1 (C-Br), 118.6 ($C(5)_{pyrimid.}$), 31.2 (CH_2). MS ((+)-FAB): *m/z* = 448/450 $[M]^+$. Anal. calcd. for $C_{17}H_{14}BrClN_6S$: C, 45.40; H, 3.14; N, 18.69. Found: C, 45.21; H, 3.02; N, 18.42 %.

2.2.3. Reaction of compound 17 and 18 with thiourea under MWI

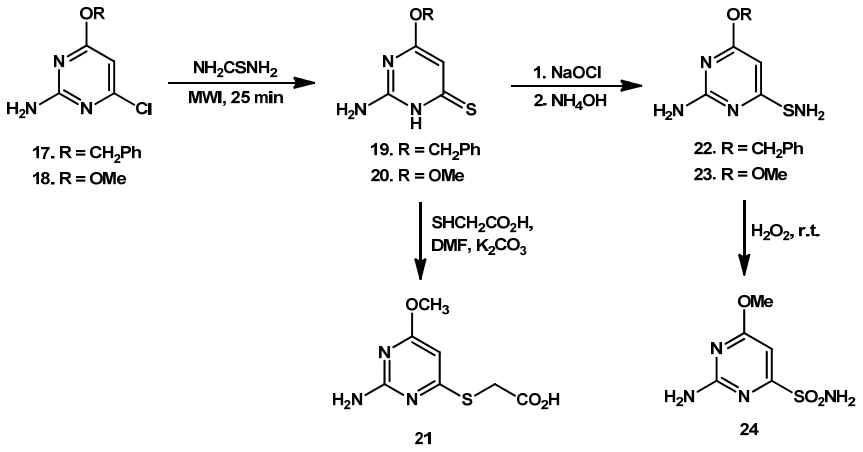
A crushed mixture of compound **17** (100 mg, 0.46 mmol) or **18** (100 mg, 0.71 mmol) and thiourea (10 mg, 1.31 mmol) was irradiated in a microwave oven (800 W) for 25 min. The mixture was poured into ice-cold water and the solid residue was washed with ether followed by a little amount of cold ethanol. Recrystallization from ethanol afforded the desired product (Scheme 3).

2-Amino-6-(benzyloxy)pyrimidine-4-thione (**19**): Yield: 76 mg (71%). M.p.: 136-138 °C. R_f = 0.57. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.42-7.33 (m, 5H, $H_{arom.}$), 7.07 (br s., 2H, NH_2), 6.15 (s, 1H, NH), 5.31 (s, 1H, $H_{pyrimid.}$-5), 5.13 (s, 2H, CH_2). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 184.3 (C6=S), 170.3 ($C(4)_{pyrimid.}$), 154.7 ($C(2)_{pyrimid.}$), 137.1 ($C(1')_{arom.}$), 128.9, 128.8, 128.4, 128.2 ($C_{arom.}$), 94.7 ($C(5)_{pyrimid.}$), 65.7 (CH_2). Anal. calcd. for $C_{11}H_{11}N_3OS$: C, 56.63; H, 4.75; N, 18.01. Found: C, 56.41; H, 4.65; N, 17.82 %.

2-Amino-6-methoxypyrimidine-4-thione (**20**): Yield: 76 mg (67%). M.p.: 289-292 °C (Dec.). R_f = 0.37. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.28 (br s., 2H, NH_2), 6.76 (s, 1H, NH), 5.35 (s, 1H, $H_{pyrimid.}$-5), 4.07 (s, 3H, OCH_3). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 184.9 (C=S), 171.8 ($C(4)_{pyrimid.}$), 153.8 ($C(2)_{pyrimid.}$), 94.1 ($C(5)_{pyrimid.}$), 57.0 (OMe). Anal. calcd. for $C_5H_7N_3OS$: C, 38.20; H, 4.49; N, 26.73. Found: C, 8.43; H, 4.37; N, 26.52 %.

2.2.4. Synthesis of 2-(2-amino-6-methoxypyrimidin-4-yl) mercapto acetic acid (21)

To a solution of compound **20** (55 mg, 0.34 mmol) in DMF (5 mL) containing potassium carbonate (30 mg) was added 2-mercaptoacetic acid (30 mg, 0.32 mmol) and stirred at room temperature for 16 h. After cooling, the mixture was filtered and the filtrated was evaporated to dryness and the residue was purified on a short column of SiO_2 (5 g). Elution with chloroform:methanol (95:5, *v*:*v*) afforded compound **21** (35 mg, 47%) (Scheme 3). M.p.: 142-154 °C (Dec.). R_f = 0.1. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.95 (s, 1H, OH), 7.23 (br s., 2H, NH_2), 6.33 (s, 1H, $H_{pyrimid.}$-5), 4.08 (s, 2H, CH_2), 3.88 (s, 3H, OMe). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 71.7 (CO_2H), 170.6 ($C(6)_{pyrimid.}$), 168.5 ($C4)_{pyrimid.}$), 155.9 ($C(2)_{pyrimid.}$), 94.6 ($C(5)_{pyrimid.}$), 51.9 (OMe), 31.0 (CH_2).



Scheme 3

Anal. calcd. for $C_7H_9N_3O_3S$: C, 39.06; H, 4.21; N, 19.52. Found: C, 38.82; H, 4.10; N, 19.32.

2.2.5. Reaction of compound 21 with sodium hypochlorite and ammonium hydroxide

Compound **21** (104 mg, 0.66 mmol) or **20** (54 mg, 0.34 mmol) was added to a solution of sodium hypochlorite (5 mL) and the reaction mixture was stirred at 60 °C for 4 h. Ammonium hydroxide (10 mL) was added to the reaction mixture, and stirred for an additional 1 h at room temperature, the precipitate was filtered and washed with water several times and dried, followed by washing with dry ether to give compound **22** or **23**, respectively (Scheme 3).

2-Amino-4-(aminothio)-6-(benzyloxy)pyrimidine (**22**): Yield: 111 mg (68%). M.p.: 286-289 °C. R_f = 0.32. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.43-7.33 (m, 5H, $H_{arom.}$), 7.33 (br s., 2H, NH_2), 6.14 (s, 1H, $H_{pyrimid.}$-5), 5.39 (br s., 2H, SNH_2), 5.31 (s, 2H, CH_2). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 189.4 ($CSNH_2$), 166.1 ($C(4)_{pyrimid.}$), 160.5 ($C2)_{pyrimid.}$), 129.0-126.9 ($C_{arom.}$), 100 ($C(5)_{pyrimid.}$). Anal. calcd. for $C_{11}H_{12}N_4OS$: C, 53.21; H, 4.87; N, 22.56. Found: C, 52.98; H, 4.70; N, 22.68 %.

2-Amino-4-(aminothio)-6-methoxypyrimidine (**23**): Yield: 36 mg (61%). M.p.: 288-291 °C. R_f = 0.43. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.41 (br s., 2H, NH_2), 5.99 (s, 1H, $H_{pyrimid.}$-5), 3.87 (s, 3H, OMe), 2.31 (s, 2H, SNH_2). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 189.0 ($C(6)_{pyrimid.}$), 167.8 ($C4)_{pyrimid.}$), 153.2 ($C(2)_{pyrimid.}$), 94.9 ($C(5_{pyrimid.}$), 57.0 (OMe). Anal. calcd. for $C_5H_8N_4OS$: C, 34.87; H, 4.68; N, 32.53. Found: C, 34.59; H, 4.55; N, 32.33 %.

2.2.6. Synthesis of 2-amino-6-methoxypyrimidine-4-sulfonamide (**24**)

A suspension of compound **23** (80 mg, 0.46 mmol) in H_2O_2 (5 mL) was stirred at room temperature for 4 h. The solution was evaporated to dryness and the residue was washed with water (3 x 10 mL), dried and then then with ether. The dried crude product was purified on a short SiO_2 column (5 g) using chloroform:methanol (4:1, *v*:*v*) as eluent, to give compound **24** (70 mg, 74%) (Scheme 3). M.p.: 156-158 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.32 (br s., 2H, NH_2), 6.51 (s, 1H, $H_{pyrimid.}$-5), 3.98 (s, 3H, OMe), 2.11 (br s., 2H, SO_2NH_2). ^{13}C NMR (150.91, DMSO-d_6, δ, ppm): 168.9 ($C(6)_{pyrimid.}$), 165.1 ($C4)_{pyrimid.}$), 154.7 ($C(2)_{pyrimid.}$), 96.2 ($C(5)_{pyrimid.}$), 57.2 (OMe). Anal. calcd. for $C_5H_8N_4O_3S$: C, 29.41; H, 3.95; N, 27.44. Found: C, 29.19; H, 3.86; N, 27.21 %.

3. Results and discussion

3.1. Chemistry

Recently, reported the synthesis of azopyrimidine **5** [33], and selected here as a starting material for the synthesis of new azopyrimidine analogues having alkyl and aryl amino groups at C-4. Thus, compound **5** was subjected to a nucleophilic displacement of chlorine group by treatment with various amines in DMF at 90-100 °C for 4-5 h, leading to the new derivatives **6**-**13** in good to moderate yields (55-89 %), except the products **7** and **10** were obtained in a lower yields 32 and 36%, respectively (Scheme 1), might due to the steric factor (Scheme 1).

The structures of **6**-**13** were established by ^{1}H, ^{13}C NMR and mass spectral data. In the ^{1}H NMR spectra, both amino groups at C-2 and C-6 of pyrimidine backbone appeared almost at the same regions as broad singlets at δ 6.84-6.38 ppm and δ 10.61-7.79 ppm, respectively, assigned by D_2O exchange. The amino groups at C-6 of the analogue **6** showed two broad singlets (δ 8.85 and 8.14 ppm) and not doublets as expected, might be due to the hydrogen bonding with the nitrogen of the azo group at C-5. The aromatic and the aliphatic protons were fully analyzed (*c.f.* Experimental section). In the ^{13}C NMR spectra of **6**-**13**, C-2 and C-4 of the pyrimidine scaffold resonated at the region δ 164.0-163.2 ppm and the region δ 162.3-160.0 ppm, respectively. C-5 and C-6 appeared at the regions δ 110.1-100.0 and 157.7-152.0 ppm, respectively. The aromatic carbon atoms C-4' carrying bromine residue were resonated the regions δ 123.8-118.9 ppm, while the aromatic carbon atoms C-1' - C-6' of the pyrimidine analogues **6**-**13** were resonated at the regions δ 140.8-122.6 ppm. The aliphatic carbon atoms were fully analyzed (*cf* Experimental section).

Next, the analogue **5** was used as a precursor for the synthesis of new 5-arylazo-4-ethyl(aryl)thio-pyrimidine derivatives to examine their antiviral activity in comparison to the azoaryl analogues **6**-**13**. Thus, treatment of compound **5** with sodium ethanethiolate, sodium thiophenolate, or *p*-chlorobenzylthiol in DMF afforded, *via* nucleophilic displacements of the chlorine group, **14**-**16** in 74, 68 and 53% yield, respectively (Scheme 2).

The structures of compounds **14**-**16** were assigned by the ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectra showed rather similar patterns for the phenyl and ethyl protons. The methylene and methyl protons of SEt group appeared as quartet and triplet at δ 3.99 and 1.36 ppm (J = 7.1 Hz), respectively, whereas the aromatic protons were fully analysed (*cf* Experimental section).

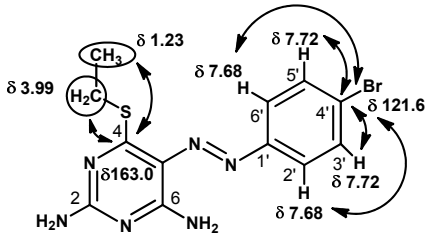


Figure 2. $J_{C,H}$ Correlations in the HMBC NMR spectrum of compound **14**.

In the ^{13}C NMR spectra of compounds **14-16**, C-4, C-5 and C-6 together with the aromatic carbon atoms were identified. However, compound **14** has been selected for further NMR experiment. In the gradient-selected HMBC spectrum [34] of compound **14**, C-4 of the pyrimidine ring at δ 163.0 ppm showed a $^2J_{C,H}$ coupling with methylene protons of $CH_3\boldsymbol{CH_2}S$ group at δ 3.99 ppm, in addition to a $^3J_{C,H}$ coupling with the methyl protons of the $\boldsymbol{CH_3}CH_2S$ group at δ 1.23 ppm. Further, the aromatic protons H-3' and H-5' at δ 7.72 ppm revealed two $^2J_{C,H}$ coupling with C-4' (C-Br) of the aromatic ring at δ 121.6 ppm, while the same carbon atom (C-Br) showed two $^3J_{C,H}$ couplings with H-2' and H-6' of the same ring at δ 7.68 ppm (Figure 2).

Our work was modified by conversion of the chloro residue in the pyrimidine scaffold to the thione, following Lawson and Tankler method [35]. Thus, compound **17** and **18** were treated with thiourea under microwave irradiation (MWI) in a free solvent condition for 15 min afforded, after purification, the pyrimidine-thione analogues **19** and **20** in 71 and 67 % yield, respectively. Treatment of compound **20** with 2-mercapto acetic acid in DMF in the presence of K_2CO_3 at room temperature for 16 h afforded regioselectively [36], after chromatographic purification, 4-thio-ethylacetic acid derivative **21** (47 %).

Next, treatment of compound **19** and **20** with sodium hypochlorite followed by ammonium hydroxide, following Rice *et al*. method [37], furnished after purification, the 4-aminothio-pyrimidine derivatives **22** and **23** in 61 and 68% yield, respectively. Oxidation of compound **23** with H_2O_2 at room temperature afforded, after chromatographic purify-cation, the sulfonamide **24** in 74% yield (Scheme 3).

The structures of compound **19-24** were elucidated from their ^1H and ^{13}C NMR spectra. In the ^1H NMR spectra of compounds **19** and **20**, H-5 of the pyrimidine ring were appeared at δ 5.31 and 5.39 ppm, respectively, whereas the protons of the amino groups at C-2 were resonated as broad singlet at δ 7.07 and 7.33 ppm, respectively. The SNH_2 protons of compound **22** resonated as a broad singlet at δ 5.39 ppm, while the singlet and broad singlet at δ 6.76 and 2.31 ppm were assigned to NH proton of the pyrimidine thione **20** and the SNH_2 protons at C-4 of the analogue **23**, respectively. The singlets at δ 5.35 and 5.99 ppm were attributed to H-5 of the pyrimidine ring, in addition to two singlets at δ 4.07 and 4.09 ppm, assigned to the methoxy group at C-6 of compound **20** and **23**, respectively. Compound **24** showed singlet and broad singlet at δ 6.51 and 2.11 ppm, identified as H-5 and SO_2NH_2 protons, respectively. In the ^{13}C NMR of compound **19-24**, the resonances at the lower field regions δ 189.4-168.9 ppm were assigned to C-6 (C-S) of the pyrimidine ring in comparison for those of the starting materials **17** and **18** at δ 164.1 and 162.7 ppm, respectively. C-2, C-4 and C-5 together with the substituents carbon atoms were fully identified (*cf* Experimental section). All the synthesized were confirmed also from their ^1H, ^{13}C HSQC [38] NMR spectra.

3.2. In vitro anti-HIV activity

Compounds **6-13**, **14-16** and **19-24** 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 based on an MTT assay [39]. The results are summarized in Table 1, in which the data for nevirapine (BOE/BIRG587) [40] and azidothymidine (DDN/AZT) [41] were included for comparison.

Table 1. *In-vitro* anti-HIV-1[a] and HIV-2 [b] of new pyrimidine analogues **4-16** and **19-27**.

Compound	Virus strain	EC_{50} (µg/mL) [c]	CC_{50} (µg/mL) [d]	SI [e]
6	III_B	> 45.23	45.23	< 1
	ROD	> 45.23	45.23	< 1
7	III_B	> 65.23	65.23	< 1
	ROD	> 65.23	65.23	< 1
8	III_B	> 32.60	32.60	< 1
	ROD	> 32.60	32.60	< 1
9	III_B	> 53.60	53.60	< 1
	ROD	> 53.60	53.60	< 1
10	III_B	> 35.10	35.10	< 1
	ROD	> 35.10	35.10	< 1
11	III_B	> 12.35	12.35	< 1
	ROD	> 12.35	12.35	< 1
12	III_B	> 28.05	28.05	< 1
	ROD	> 28.05	28.05	< 1
13	III_B	> 10.04	10.04	< 1
	ROD	> 10.04	10.04	< 1
14	III_B	> 2.12	2.12	< 1
	ROD	> 2.12	2.12	< 1
15	III_B	> 1.99	1.99	< 1
	ROD	> 1.99	1.99	< 1
16	III_B	> 1.80	1.80	< 1
	ROD	> 1.80	1.80	< 1
19	III_B	> 11.34	11.34	< 1
	ROD	> 11.34	11.34	< 1
20	III_B	> 5.67	5.67	< 1
	ROD	> 5.67	5.67	< 1
21	III_B	> 1.92	1.92	< 1
	ROD	> 1.92	1.92	< 1
22	III_B	> 3.38	3.38	< 1
	ROD	> 3.38	3.38	< 1
23	III_B	> 5.98	5.98	< 1
	ROD	> 5.98	5.98	< 1
24	III_B	> 10.18	10.18	< 1
	ROD	> 10.18	10.18	< 1
AZT	III_B	0.0022	> 25	> 11363
	ROD	> 25	> 25	> 26596
Nevirapine	III_B	0.050	0.050	> 80
	ROD	> 4.00	> 4.00	< 1

[a] Anti-HIV-1 activity measured with strain III_B.
[b] Anti-HIV-2 activity measured with strain ROD.
[c] Compound concentration required to achieve 50 % protection of MT-4 cells from the HIV-1 and 2-induced cytopathogenic effect.
[d] Compound concentration that reduces the viability of mock-infected MT-4 cells by 50%.
[e] SI: selectivity index (CC_{50}/EC_{50}).

Compounds-induced cytotoxicity was also measured in MT-4 cells parallel with the antiviral activity. All compounds are inactive except compounds **14-16** and **21** which showed EC_{50} values of > 2.12, 1.99, 1.80 and 1.92 µg/mL, respectively, but no selectivity was observed (SI < 1).

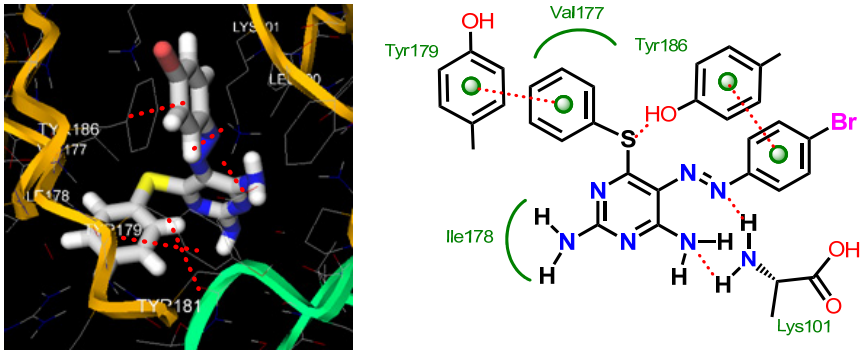


Figure 3. Docked conformation of compound **15** showing three hydrogen bonding: Lys101 with NH_2 at C-6 and N=N groups of the pyrimidine ring, in addition to Tyr186 with sulfur atom of the phenylthio group. It exhibited also hydrophobic interactions between phenyl moieties and Tyr179, Tyr186 of reverse transcriptase (RT) enzyme residues.

From the SAR analysis, we found that the alkyl- or arylthio substituents at C-4 of the pyrimidine ring, *e.g.* compounds **14-16** or thioalkyl acetic acid moiety at C-6 of the same ring, *e.g.*: compound **21** were well tolerated in the hydrophobic region of HIV RT and then showed higher activity than those of the alkyl- or arylamino substituents at C-4 of the same ring; *e. g.*: compounds **6-13**, and resulted in loss of activity. This means that the thio groups targeting the hydrophobic binding pocket of HIV-1 RT.

3.3. Molecular modeling analysis

The molecular docking was performed using SYBYL-X 1.1 and the docking results were shown by PyMOL [42]. Our molecular docking analysis of the new analogues based on the modeling study, which was performed to understand the binding mode of these analogues with the HIV-RT binding pocket (NNIBP) (PDB code: 3DLG, [43]).

Compound **15** has been selected for the docking modeling study, since its binding energy score -8.01, indicating a selectivity and potency profiles of substituted aryl-azopyrimidine to bind the active site of HIV-RT pocket (Figure 3). As shown in figure 3, the aromatic rings of compound **15** fitted into an aromatic rich subpocket surrounded by the aromatic side chains of Tyr179 and Tyr186. The pyrimidine backbone was located in the middle of the binding pocket, anchoring the phenylthio substituent at C-4 in a favourable position for hydrogen bonding with the OH group of Tyr186 and other two hydrogen bonding of amino group at C-6 and azo group (N=N) at C-5 with Lys103 of the RT enzyme. Overall, the combination of hydrophobic interaction and π-stacking appears to govern the binding of compound **15** with HIV RT.

4. Conclusion

In conclusion, synthesis of new 2,6-diamino-4-alkylamino-5-p-bromophenylazopyrimidine derivatives, **6-13**, the corresponding 4-ethyl- and arylthio analogues, **14-16**, and the 4-thio derivatives **19-25** has been described. All the new synthesized compounds have been evaluated for their activity against HIV-1 and 2. Compounds **14-16** and **21** exhibited potential activity against HIV-1, whereas the others analogues shown moderate to poor activity. Compound **15** have been selected for the molecular modeling study showing its binding to the reverse transcriptase enzyme pocket through three hydrogen bonding and two hydrophobic interactions.

Acknowledgement

We thank Prof. Christophe Pannecouque of Rega Institute for Medical Research, Katholieke Universiteit, Leuven, Belgium for the anti-HIV screening. Mr. Ulrich Haunz and Miss Anka Friemel of chemistry department, University of Konstanz, Germany are highly acknowledged for the NMR experiments.

References

[1]. Cocco, M. T.; Congiu, C.; Lilliu, V. *Bioorg. Med. Chem.* **2006**, *14*, 366-372.
[2]. Heidelberger, C.; Chaudhuri, N. K.; Danneberg, P.; Mooren, D.; Griesbach, L.; Duschinsky, R.; Schnitzer, R. J.; Pleven, E.; Scheiner, J. Nature **1957**, *179*, 663-666.
[3]. Beattie, J. F.; Breault, G. A.; Ellston, R. P. A.; Green, S.; Jewsbury, P. J.; Midgley, C. J.; Naven, R. T.; Minshull, C. A.; Pauptit, R. A.; Tucker, J. A.; Pease, J. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2955-2960.
[4]. Kimura, H.; Katoh, T.; Kajimoto, T.; Node, M.; Hisaki, M.; Sugimoto, Y.; Majima, T.; Uehara, Y.; Yamori, T. *Anticancer Res.* **2006**, *26*, 91-97.
[5]. Lagoja, I. M. Chem. *Biodiver.* **2005**, *2*, 1-50.
[6]. Dudhea, R.; Sharmab, P. K.; Vermae, P.; Chaudhary, A. J. *Adv. Sci. Res.* **2011**, *2*, 10-17.
[7]. Patel, D. H.; Mistry, B. D.; Desai, K. R *Indian J. Hetero. Chem.* **2003**, *13*, 179-80
[8]. Bantawal, S. H.; Manjathuru, M.; Mari, K. S.; Padiyath, K. M. *Bioorg. Med. Chem.* **2006**, *14*, 2040-2047.
[9]. Sharma, P.; Rane, N.; Gurram, V. K. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 4185-4190.
[10]. Adnan, A. B.; Hesham, T. Z.; Sherif, A. F.; Azza, M. B. *Eur. J. Med. Chem*. **2003**, *38*, 27-36.
[11]. Russell, R. K.; Press, J. B.; Rampulla, R. A.; McNally, J. J.; Falotico, R.; Keiser, J. A.; *J. Med. Chem.* **1988**, *31*, 1786-1793.
[12]. Fillios, L. C.; Naito, C.; Andrews, S.; Roach, A. M. *Circ. Res.* **1960**, *8*, 71-77.
[13]. Kappe, C. O. *Tetrahedron* **1993**, *49*, 6937-6963.
[14]. Kreutzberger, A.; Burgwitz, K. *Arch. Pharm*. **1981**, *314*, 394-398.
[15]. Monge, A.; Martinez-Merino, V.; Sanmartin, C.; Fernandez, F. J.; Ochoa, M. C.; Bellver, C. Artigas, P. *Arznei-Forschung*. **1990**, *40*, 1230-1233.
[16]. Yamazi, Y.; Takahashi, M.; Todome, Y. *Proc. Soc. Exp. Biol. Med.* **1970**, *133*, 674-677.
[17]. Prichard, M. N.; Quenelle, D. C.; Hartline, C. B.; Harden, E. A.; Jefferson, G.; Frederick, S. L.; Daily, S. L.; Whitley, R. J.; Tiwari, K. N.; Maddry, J. A.; Secrist, J. A.; Kern, E. R. *Antimicrob. Agents Chemother.* **2009**, *53*, 5251-5285.
[18]. Miyasaka, T.; Tanaka, H.; Baba, M.; Hayakawa, H.; Walker, R. T.; Balzarini, J.; De Clercq, E. *J. Med. Chem*. **1989**, *32*, 2507-2509.
[19]. Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Nitta, I.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka**, T.** *J. Med. Chem.* **1992**, *35*, 337-345.
[20]. Balzarini, J.; Baba, M.; De Clercq, E. *Antimicrob. Agents Chemother.* **1995**, *39*, 998-1002.
[21]. Janssen, P. A. J.; Lewi, P. J.; Arnold, E.; Daeyaert, F.; de Jonge, M.; Heeres, J.; Koymans, L.; Vinkers, M.; Guillemont, J.; Pasquier, E.; Kukla, M.; Ludovici, D.; Andries, K.; de Béthune, M. -P.; Pauwels, R.; Das, K.; Clark Jr. , A. D.; Frenkel, Y. V.; Hughes, S. H.; Medaer, B.; De Knaep, F.; Bohets, H.; De Clerck, F.; Lampo, A.; Williams, P.; Stoffels, P. J. *Med. Chem*. **2005**, *48*, 1901-1909.
[22]. Ludovici, D. W.; de Corte, B. L.; Kukla, M. J.; Ye, H.; Ho, C. Y.; Lichtenstein, M. A.; Kavash, R. W.; Andries, K.; de Bethune, M. P.; Azijn, H.; Pauwels, R.; Lewi, P. J.; Heeres, J.; Koymans, L. M.; de Jonge, M. R.; Aken, K. J. V.; Daeyaert, F. F.; Das, K.; Arnold, E.; Janssen, P. A. *Bioorg. Med. Chem.* **2001**, *11*, 2235-2239.
[23]. Das, K.; Clark, A. D.; Lewi, P. J.; Heeres, J.; de Jonge, M. R.; Koymans, L. M. H.; Vinkers, H. M.; Daeyaert, F.; Ludovici, D. W.; Kukla, M. J.; De Corte, B.; Kavash, R. W.; Ho, C. Y.; Ye, H.; Lichtenstein, M. A.; Andries,

K.; Pauwels, R.; de Béthune, M. -P.; Boyer, P. L.; Clark, P.; Hughes, S. H.; Janssen, P. A. J.; Arnold, E. *J. Med. Chem*. **2004**, *47*, 2550-2560.
[24]. Yan, Z. H.; Wu, H. Q.; Chen, W. X.; Piao, H. R.; He, Q. Q.; Chen, F. E.; De Clercq, E.; Pannecouque, C. *Bioorg. Med. Chem*. **2014**, *22*, 3220-3226.
[25]. Pindola, V. K.; Zarowitz, B. J. *Pharmacother*. **2002**, *22*, 1249-1265.
[26]. Gong, B.; Hong, F.; Kohm, K.; Jenkins, S.; Tulinsky, J.; Bhatt, R.; de Vries, P.; Singer, J. W.; Klein, P. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2303-2308.
[27]. Mayer, T. U.; Kapoor, T. M.; Haggarty, S. J.; King, R. W.; Schreiber, S. L.; Mitchison, T. J. Science **1999**, *286*, 971-974.
[28]. Sharp, D. J.; Rogers, G. C.; Scholey, J. M. *Nature* **2000**, *407*, 41-47.
[29]. Yoon, Y. A.; Park, C. S.; Cha, M. H.; Choi, H.; Sim, J. Y.; Kim, J. G. *Biooorg. Med. Chem. Lett.* **2010**, *20*, 5735-5738.
[30]. Jain, K. S.; Chitre, T. S.; Miniyar, P. B.; Kathiravan, M. K.; Bendre, V. S.; Veer, V. S.; Shahane, S. R.; Shishoo, C. J. *Curr. Sci.* **2006**, *90*, 793-803.
[31]. Al-Masoudi, N. A.; Jafar, N. N. A.; Baqir, S. J.; Pannecouque, C.; Leyssen, P.; Neyts, J. *Antivir. Chem. Chemother.* **2012**, *23*, 103-112.
[32]. Al-Masoudi, N. A.; Kassim, A. G.; Abdul-Reda, N. A. *Nucleos. Nucleot. Nucl.* **2014**, *33*, 141-161.
[33]. Al-Masoudi, N. A.; Marich, Y. A.; Al-Salihi, N. J.; Saeed, B. *Z. Naturforsch.* **2014**, *69b*, 913-923.
[34]. Davis, A. L.; Keeler, J.; Laue, E. D.; Moskau, D. *J. Magn. Reson*. **1992**, *98*, 207-216.
[35]. Lawson, A.; Tinkler, R. B. *Chem. Rev.* **1970**, *70*, 593-618.
[36]. Xu, Y.; Yang, H.; Hu, J.; Wang, X. W; Liu, J. Y. *J. Chinese Pharm. Sci.* **2007**, *16*, 125-127.
[37]. Iijima, I.; Rice, K. C. *J. Heterocycl. Chem.* **1978**, *15*, 1527-1528.
[38]. Davis, A. L.; Keeler, J.; Laue, E. D.; Moskau, D. J. Magn. Reson. **1992**, *98*, 207-216.
[39]. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309-321.
[40]. Hargrave, K. D.; Proudfoot, J. R.; Grozinger, K. G.; Cullen, E.; Kapadia, S. R.; Patel, U. R.; Fuchs, V. U.; Mauldin, S. C.; Vitous, J.; Behnke, M. L.; Klunder, J. M.; Pal, K.; Skiles, J. W.; McNeil, D. W.; Rose, J. M.; Chow, G. C.; Skoog, M. T.; Wu, J. C.; Schmidt, G.; Engel, W. W.; Eberlein, W. G.; Saboe, T. D.; Campbell, S. J.; Rosenthal, A. S.; Adams, J. *J. Med. Chem.* **1991**, *34*, 2231-2241.
[41]. Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; Clair, M. H. St.; Lehrmann, S. N.; Gallo, R.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7096-7100.
[42]. Seeliger, S.; de Groot, B. L. *J. Comput. Aid. Mol. Des.* **2010**, *24*, 417-422.
[43]. Zhan, P.; Liu, X.; Li, Z.; Fang, Z.; Pannecouque, C.; De Clercq, E. *Chem. Biodivers.* **2010**, *7*, 1717-1727.