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ORIGINAL RESEARCH

Synthesis, anti-17β-HSD and antiproliferative activity of new substituted 5-nitrosopyrimidine analogs

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Abstract A new series of 2-amino-4-alkylamino-6-methylamino-5-nitrosopyrimidine derivatives **10–14** have been synthesized from 5-nitrosopyrimidine analog **9** by nucleophilic aromatic substitution reaction with various amines using dimethylformamide as a solvent at 70–90 °C. Similarly, various 4-alkylamino-5-nitrosopyrimidine analogs **16–24** were obtained from **9** and primary and secondary amines using dichloromethane at room temperature. Analogously, treatment of **9** with 2-thioglycolic acid afforded 4-thioalkyl derivative **15**. Treatment of **9** with chloroacetyl chloride (**26**) gave the corresponding chloroacetamido analog **27**, which afforded the desired 2-(benzothiazol-2-ylthio)-*N*-(4-isopropoxy-6-(methylamino)-5-nitrosopyrimidine-2-yl)acetamide (**29**) on treatment with 2-mercaptobenzothiazole (**28**) in the presence of triethylamine and dichloromethane. Condensation of **9** with butyraldehyde in acidic ethanol gave the corresponding 2-butylideneamino analog **30**. Selected examples of the synthesized compounds were evaluated for their 17β-hydroxysteroid dehydrogenase type 1 and 2 (17β-HSD1 and 2) inhibitory activity. Futhermore, same compounds were evaluated for their antiproliferative activity against two solid tumour-derived cell lines consisting Hep-G2 (human hepatocarcinoma) and MCF-7 (breast cancer).

Keywords Aminopyrimidines · Anti-17β-HSD activity · Antiproliferative activity · Nitrosopyrimidines · Nucleophilic substitution reaction

Introduction

Pyrimidines are compounds with in vitro biological activity against a wide spectrum of unrelated viruses, such as poliovirus (Yamazi et al. 1970), herpes (Prichard et al. 2009), and HIV (Miyasaka et al. 1989; Tanaka et al. 1992; Balzarini et al. 1995). 2,4-Diamino-N^4-6-diarylpyrimidines were identified to block the proliferation of tumour cell lines in vivo, especially duodenum cancer (Gong et al. 2004). The pyrimidine antibiotic, bacimethrin (4-amino-5-(hydroxymethyl)-2-methoxypyrimidine) is a known drug against several staphylococcal bacteria (Reddick et al. 2001), meanwhile trimethoprim (2,4-diamio-5-(3,4,5-trimethoxybenzyl)pyrimidine) (Stenbuck and Hood HM inventors 1962; Brumfitt and Hamilton-Miller 1993) is used, in combination with sulfamethoxazole for treatment of urinary tract infections and *Pneumocystis jirovecii pneumonia* (Hughes et al. 1977). In addition, several pyrimidine derivatives exhibited significant antitumor activity e.g., imatinib mesylate (Gleevec) (Pindola and Zarowitz 2002), contains a 2-amino-4-pyridyl substituted pyrimidine moiety, is a novel agent for treatment of chronic Leukemia via the tyrosine

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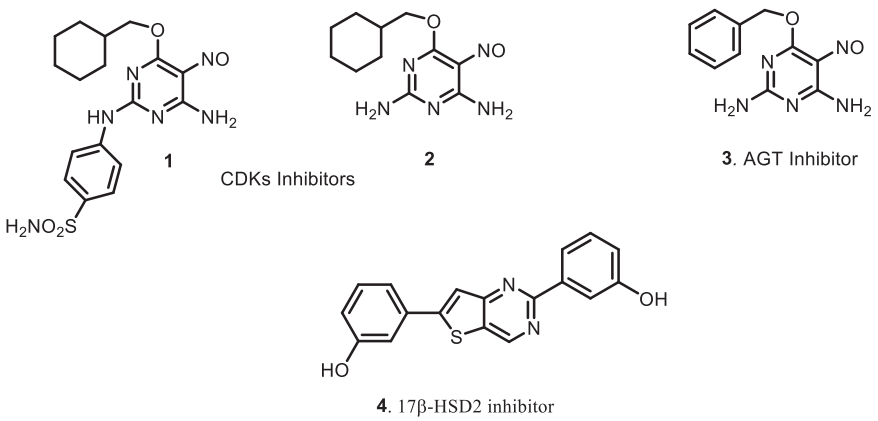


Fig. 1 Some potentially active pyrimidine analogues

kinase inhibition. Recently, Yoon et al. (2010) have reported some novel pyrimidine derivatives as potent acid pump antagonists (APAs). Jain et al. (2006) have reviewed the biological and medicinal significance of pyrimidine analogs extensively. Furthermore, nitrosopyrimidines constitute a class of biologically relevant molecules and some of these analogs exhibited inhibition activity against cyclin-dependent kinases (CDKs) (e.g.: **1** and **2**) (Marchetti et al. 2007; Mesguiche et al. 2003; Sayle et al. 2003; Arris et al. 2000) and of the DNA-repair protein *O*(6)-alkylguanine-DNA alkyl transferase (AGT) **3** (Terashima and Kohda 1998; Chae et al. 1995; Arris et al. 2000) leading to a renewed interest in the synthesis of these pyrimidine derivatives. Marchal et al. (2002, 2010) and Melguizo et al. (2002) have synthesized several alkoxy-5-nitrosopyrimidine analogs, meanwhile some nitrosopyrimidines have been reported as potential antifungal (Olivella et al. 2012) and antibacterial agents (Olivella et al. 2015). Recently, we have tested a series of 5-nitrosopyrimidines against HIV activity, meanwhile one of these analogs exhibited in vitro remarkable activity against HIV-1 (Al-Masoudi et al. 2016a). In addition, Perspicace et al. (2013) have reported new thieno[3,2-*d*]pyrimidines and analogs (e.g: **4**) as inhibitors for 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2), the enzyme catalyses the intracellular conversion of inert cortisone to physiologically active cortisol. Few examples of 17β-HSD2 inhibitors have been described in the literature (Poirier et al. 2001; Bydal et al. 2004).

In respect with the biological significance of pyrimidines and in continuation of our ongoing work (Al-Masoudi et al. 2008, 2011, 2012, 2014a, b, 2015, 2016a, b; Fröhlich et al. 1999; Marich et al. 2014; Jaffer et al. 2013) on the synthesis, antiviral and nitric oxide synthase inhibition activities of various pyrimidine derivatives, we report here the synthesis and the biological activity of new 4-alkylamino-and 4-thioalkyl-5-nitrosopyrimidine derivatives as inhibitors of 17β-HSD1 or 17β-HSD2 enzymes (Fig. 1).

Results and discussion

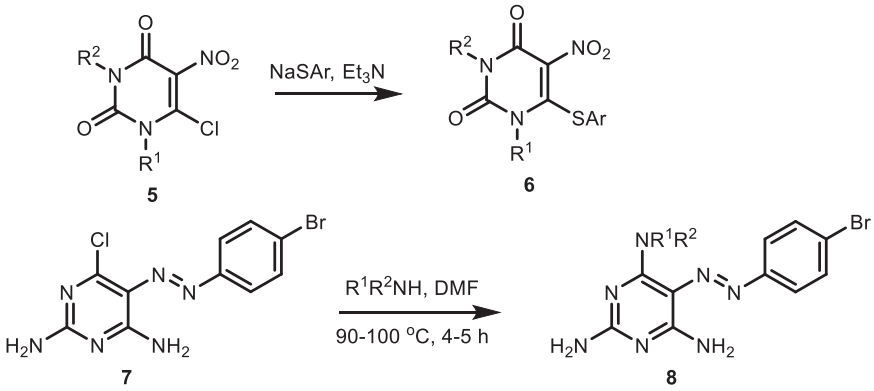
Chemistry

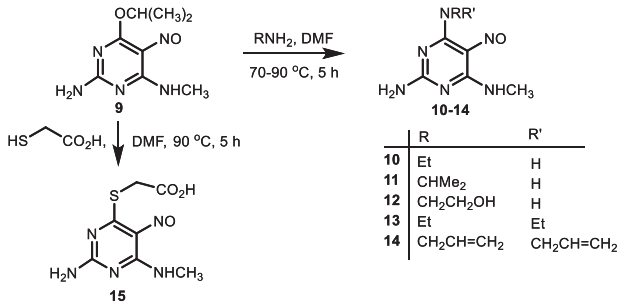
A facile nucleophilic aromatic substitution reaction (S_NAr) can been proceeded smoothly in the presence of a good leaving group and highly electron-deficient residues such as nitro, nitroso and azoaryl residues (Riabova et al. 2008; Hammarström et al. 2003; Lucrezia et al. 2000). In accordance with such interesting S_NAr reaction, we have synthesized a series of pyrimidine derivatives **6** (Al-Masoudi et al. 2008) and **8** (Al-Masoudi et al. 2014b) in good yields by treatment of the nitro-**5** and azoaryl-**7** analogs with arylthiolate anion and alkyl amino groups, respectively (Scheme 1).

These results encouraged us to explore similar aminolysis reaction at C-5 of pyrimidine ring in the presence of adjacent highly electron-deficient nitroso group. In our present work, 2-amino-4-isopropoxy-6-methylamino-5-nitropyrimidine (**9**) has been selected as a key intermediate for the synthesis of new 4-alkylamino and thio analogs, aiming to evaluate their inhibitory activity against HSD-1. Thus, treatment of **9** with various alkyl amines: e.g.: ethylamine, isopropylamine, ethanolamine, diethylamine and *N*-vinylpropenen-2-ene-1-amine in DMF at 70–90 °C afforded mainly, after purification, the 4-alkylamino-5-nitropyrimidine analogs **10–14** (75–53%). Alternatively, treatment of **9** with 2-thioglycolic acid gave the corresponding 4-thioalkyl analog **15** (66%) (Scheme 2).

Next, we examined the reactivity of compound **9** towards the displacement reaction in aprotic solvent at ambient temperature. Thus, subsequent treatment of **9** with the desired primary amines such as: vinyl, propyl, butyl, pentyl, hexyl and cyclohexyl amines or secondary amines e.g.: dimethyl and dipropyl amines as well as piperazine in dichloromethane as a solvent at room temperature for 20 h till 3 days, proceeded smoothly to give the substituted 4-aminoalkyl-5-nitrosopyrimidines **16–24** in 70–61% yield (Scheme 3).

Scheme 1 Nucleophilic displacement of chloro residue by arylthiolate anion and alkylamino groups





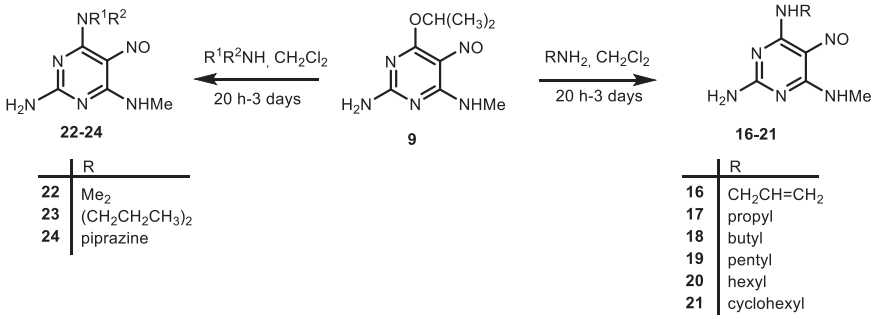
Scheme 2 Synthesis of 2-amino-4-alkylamino- or 4-alkylthio-6-methylamino-5-nitrosopyrimidine analogues

The structures of **10–24** were assigned on the basis of their IR, ^{1}H, ^{13}C and 2D NMR spectra. The ^{1}H NMR spectra showed similar patterns of the aliphatic protons, NH and NH_2 which identified by D_2O exchange. The ^{1}H and ^{13}C NMR spectra of non-symmetrically substituted compounds **10–12** and **16–21** showed two sets of signals, especially for the NH*Me* at C-6 of pyrimidine ring, indicating that, in solution, these compounds existed in equilibrium as α and β rotamers (Fig. 2). This equilibrium is due to two possible N=O···H–N hydrogen bonds existed at both rotamers.

The two singlets at the regions δ 2.93–2.86 and 2.86–2.85 ppm of compounds **10–20** were assigned to two rotamers (α and β) of methyl protons of NHMe group, except **21**, which appeared as a multiplet with cyclohexane protons at δ 2.86 ppm. NCH_2 protons of the alkyl groups at C-4 of the analogs **10–13**, **18–20** and **23** appeared as triplet, quartet or multiplet at the regions δ 3.75–3.23 ppm, except **11** where CH proton of isopropyl group appeared as multiplets of two rotamers at δ 4.47 and 4.25 ppm. Compounds **14** and **16** showed two multiplets at δ 3.79 and 4.0 9 ppm attributed to NCH_2 protons allyl group, meanwhile the multiplets at δ 5.55 and 5.97 ppm were assigned to CH=*CH_2* protons, respectively. Further, the olefinic methylene protons CH=*CH_2* appeared at δ 5.15 and 5.13 ppm, respectively. SCH_2 protons of **15** resonated as a singlet at δ 3.94 ppm, whereas the piperazine protons appeared as two multiplets at δ 4.23 and 2.24 ppm. Other protons of *N*-alkyl groups at C-4 of the pyrimidine ring were fully analysed (*c.f.* Experimental section). In the ^{13}C NMR spectra of **10–24**, C-2 of all pyrimidines resonated at the regions δ 170.1–162.5 ppm, while C-4 and C-6 were resonated at the regions δ 170.6–151.2 ppm and δ 170.3–151.7 ppm, respectively. Interestingly, C-5 of the analogs **11**, **19** and **24** appeared at low field (δ 136.4, 136.7 and 138.8 ppm, respectively), indicating for non-hydrogen bonding between N=O and NH groups, meanwhile C-5 of the other analogs were resonated at the regions δ 70.4–69.6 ppm. Resonances at the regions δ 27.8–26.3 ppm were assigned to methyl carbon atom of NHMe group. Carbon atom of the allylic group (*CH_2*-CH=CH_2) of **14** and **16** appeared at δ 49.2 and 42.5 ppm, respectively, whereas (*CH*=CH_2) carbon atom resonated at δ 134.7 and 116.2 ppm, respectively. In addition, the resonance at δ 116.3 and 116.2 ppm were assigned to the olefinic carbon atoms (CH=*CH_2*), respectively. The carbon atoms of *N*-alkyl groups and piperazine at C-4 of pyrimidine molecule were fully analyzed (*c.f.* Experimental section).

Our efforts have been focused also on the synthesis of 5-nitrosopyrimidines carrying 2-thiobenzothiazole moiety as potential pharmacological compounds. Recently, Shi et al. (2012) have synthesized novel 2-mercaptobenzothiazole derivatives with potential anticancer activity, since one of these analogs; **25** exhibited activity against liver carcinoma of HepG2 cell line with $IC_{50} = 48$ nM. Such result prompted us to modify our 5-nitrosopyrimidine **9** by conjugation with benzothiazole moiety via 2-thiocetamido linkage, aiming to evaluate the inhibitory activity against 17βHSD-1 or 17βHSD-2. Treatment of **9** with chloroacetyl chloride (**26**) in chloroform at room temperature afforded the chloroacetamide **27** (71%), which furnished **29** (57%) on refluxing with 2-mercaptobenzothiazole (**28**) in dichloromethane and triethylamine. Analogously, treatment of **9** with butyraldehyde in boiling ethanol in the presence of acid furnished the shiff base **29** in 55% yield (Scheme 4).

Scheme 3 Synthesis of 2-amino-4-alkylamino-6-methylamino-5-nitrosopyrimidine derivatives



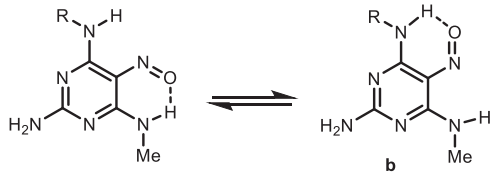


Fig. 2 Rotamers of non-symmetrically substituted 5-nitrosopyrimidines

The structures of **27**, **29** and **30** were assigned on the basis of their IR, ^{1}H, ^{13}C and 2D NMR spectra. In the ^{1}H NMR spectra of **27** and **30**, the multiplets at δ 5.54 and 5.55 ppm were assigned to *CH*Me$_2$ proton, whereas the two singlets at δ 1.40/1.39 ppm and 1.49/1.40 ppm were attributed to two rotamers of ethyl residues of CH*Me$_2$* group, respectively. CH$_2$Cl and NHMe protons of **27** were appeared as singlets at δ 4.81 and 2.86 ppm, respectively, while NH*Me* protons of **30** resonated as two singlets of *α*/*β* rotamers at δ 2.88 and 2.86 ppm. Furthermore, **30** showed a doublet at δ 7.91 ppm ($J = 8.9$ Hz) assigned to CH=N proton at C-2 of pyrimidine backbone, whereas the two multiplets at δ 2.25 and 1.55 ppm were attributed to methylene protons of propyl group (=N*CH$_2$CH$_2$*CH$_3$), respectively. Methyl protons of propyl residue appeared as a triplet at δ 0.88 ppm ($J = 7.3$ Hz). All the new compounds have been identified by their HSQC NMR spectroscopy (Davis et al. 1992).

Compounds **16** and **29** were selected for 2D NMR study. The gradient HMBC (Willker et al. 1993) NMR spectrum of **16** revealed two $^{2}J_{C;H}$ couplings between H-2′ of allyl group at δ_H 5.97 ppm and C-3′ at δ_C 134.7 ppm as well as C-1′ at δ_C 42.5 ppm of the same group. Further, a $^{3}J_{C;H}$ coupling between CH$_2$-1′ of allyl residue at δ_H 4.09 ppm and C-4 of the pyrimidine ring at δ_C 170.6 ppm was observed (Fig. 3). The gradient HMBC NMR spectrum of **29** allowed $^{3}J_{C,H}$ coupling the assignment of C-4 at δ_C 163.3 ppm by correlation with *CH*Me$_2$ proton at δ_H 5.54 ppm. A $^{2}J_{C;H}$ coupling between SCH$_2$ protons at δ_H 3.05 ppm and carbon atom of carbonyl group at δ_C 170.0 ppm, in addition, to a $^{3}J_{C;H}$ coupling of the same protons with C-2′ of the benzothiazole backbone at δ_C 164.5 ppm were witnessed. The aromatic protons C-4′ and C-7′ at the regions δ_H 7.93–7.35 ppm showed two $^{2}J_{C;H}$ correlations with C-3a′ and C-7a′ at δ_C 134.9 and 138.7 ppm, respectively (Fig. 3).

Bioactivity

In vitro inhibition activity of 17β-HSD1 and 17β-HSD2

17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) catalyzes the conversion of the weakly active estrone (E1) to the highly active estradiol (E2), meanwhile 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) catalyzes the conversion of E2 and testosterone (T) into the estrone (E1) and *δ*4-androstene-3,17-dione (*δ*4-AD), respectively (Wu et al. 1993). Recently, 17β-HSD1 came into the focus of interest as a novel therapeutic target for the treatment of estrogen dependent diseases like breast cancer (BC) and endometriosis (Masuzaki and Flier 2003). 17β-HSD2 is expressed in osteoblastic cells (Dong et al. 1998), therefore its inhibition can lead to the desired increase of E2 and T levels in the bone tissue and may thus be a novel approach for the treatment of osteoporosis. Since 17β-HSD2 catalyzes the inactivation of E2 into E1, inhibitory activity toward this enzyme must be avoided. Gargano et al. (2015) and Wetzel et al. (2011) have reported numerous analogs as inhibitors of 17β-HSD1 and 2, leading for treatment of breast cancer and osteoporosis, respectively. However, 17β-HSD1 inhibitors should not inhibit 17-HSD2 and, of course, should not be estrogenic. Our new synthesized compounds were tested for their ability to inhibit 17β-HSD1 and 17β-HSD2. For the determination of 17β-HSD1 and 17β-HSD2 inhibition, tritiated substrates E1 or E2 were incubated with placental enzymes 17β-HSD1 or 17β-HSD2, respectively, cofactor and inhibitor as described by Kruchten et al. (2008) (compound concentration: 1 μM). The inhibition values of the test compounds are shown in Table 1. All compounds showed less than 10% inhibition at 1.0 μM and were considered to be inactive.

Scheme 4 . *Reagents and conditions*: (i) $CHCl_3$, K_2CO_3, 4 h, r.t.; (ii) E_3N, CH_2Cl_2, reflux, 4 h; (iii) EtOH/AcOH, reflux, 3 h

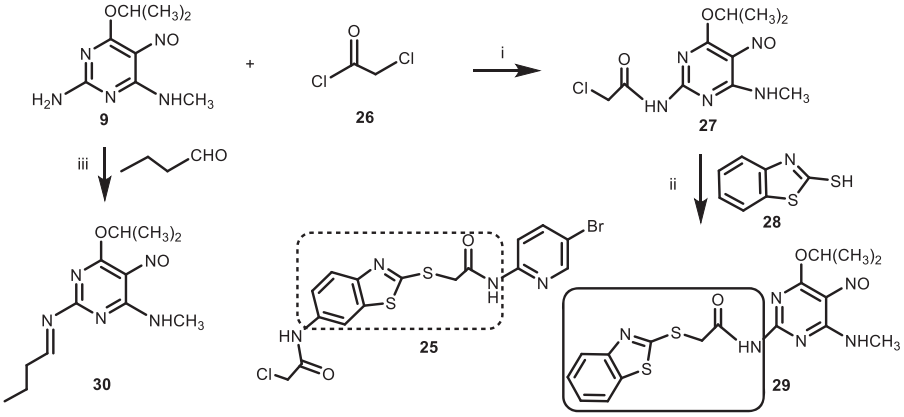


Fig. 3 $J_{C,H}$ correlations in the HMBC NMR spectra of **16** and **29**

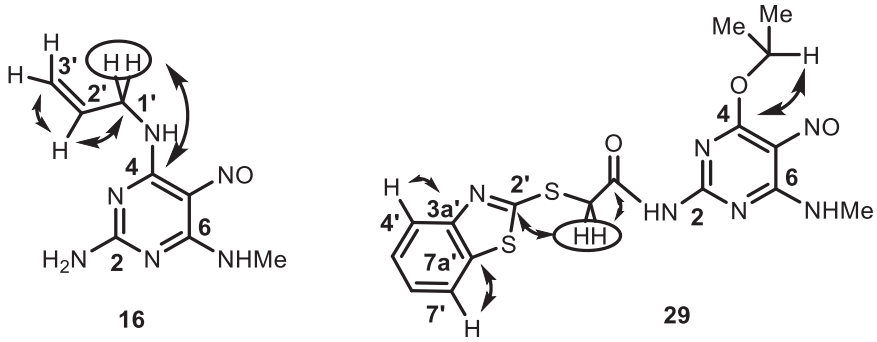


Table 1 17β-HSD1 and 17β-HSD2 inhibitory activity for some nitrosopyrimidines

Comp.	17β-HSD1[a] % inhib. (1 μM)	17β-HSD2[b] % inhib. (1 μM)
9	ni	5.6 ± 0.10
10	ni	4.6 ± 0.06
11	0.7 ± 0.02	1.6 ± 0.04
16	5.8 ± 0.09	6.1 ± 0.12
17	ni	ni
18	ni	6.1 ± 0.21
19	ni	1.9 ± 0.03
20	ni	6.0 ± 0.10
29	ni	6.4 ± 0.11

ni no inhibition

[a] Human placenta, cytosolic fraction, substrate [^{3}H]E1+E1 [500 nM], cofactor NADH [500 μM]

[b] Human placenta, microsomal fraction, substrate [^{3}H]E2+E2 [500 nM], cofactor NAD+ [1500 μM]

[c] Mean values of two determinations, standard deviation less than 10%

In vitro antiproliferative activity

Compounds **9–11**, **16–20** and **29** have been selected for their antiproliferative activity against two solid tumour-derived cell lines consisting Hep-G2 (human hepatocarcinoma) and MCF-7 (breast cancer) using the microculture tetrazolium assay (MTT) method (Alley et al. 1988). Doxorubicin (Uyeki et al. 1981) has been used for comparative purposes of the cytotoxic activities. All compounds were inactive against MCF-7 cell line ($IC_{50} > 50$ μM). However, compounds **16** and **29** exhibited IC_{50} values of 8.2 ± 0.08 μM and 1.6 ± 0.1 μM against Hep-G2 cell lines, respectively.

Both compounds did not show superior activity than doxorubicin against Hep-G2 cell lines with IC_{50} value of 0.03 μM.

Experimental

Chemistry

Melting points are uncorrected and were measured on a Buchi melting point apparatus B-545 (Buchi Labortechnik AG, Switzerland). Microanalytical data were obtained with a Vario, Elementar apparatus (Shimadzu, Japan). The IR spectra were recorded on Schimadzu Fourier Transform Infra-red spectrophotometer (Model 270), using KBr discs. NMR spectra were recorded on 400 and 600 MHz (^{1}H) and at 100 MHz and 150.91 MHz (^{13}C) spectrometers (Bruker, Germany) with TMS as the internal standard and on δ scale

in ppm. The reagents used were of analytical grade while the solvents were purified before use.

*General procedure for the synthesis of 2-amino-4-alkylamino-6-methylamino-5-nitroso- pyrimidine in DMF (**10–14**)*

To a solution of **9** (211 mg, 1.00 mmol) in DMF (10 mL) was added the required amine (1.00 mmol) and the mixture was heated at 70–90 °C for 5 h. Water (10 mL) was added and the mixture was cooled and the precipitate was collected, washed with water, dried and recrystallized from ethanol to give the required product.

*2-Amino-4-ethylamino-6-methylamino-5-nitrosopyrimidine (**10**)*

From ethylamine (45 mg). Yield: 147 mg (75%) as a violet solid, m.p. 121–124 °C; IR (KBr): 3116 (NH_2), 2977 (NH), 2778 (CH), 1646 (C=N), 1570 (C=C); 1524 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.76$, 11.53 ((2xs, 1H, NH (2 isomers)), 8.68 (d, 1H, $J = 5.1$ Hz, *NH*Me) 7.40 (d, 2H, $J = 6.5$ Hz, NH_2), 3.50, 3.47 ((2×q, 2H, $J = 7.0$ Hz, CH_2CH_3 (2 rotamers)), 2.92, 2.86 ((2xs, 1H, NH*Me* (2 rotamers)), 1.17, 1.13 ((2xt, 6H, CH_2CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 164.8$ (C-2), 163.9 (C-4), 152.0, 151.1 (C-6, (2 isomers)), 70.1 (C-5), 35.2, 34.0 (CH_2CH_3 (2 rotamers)), 27.8, 26.3 ((NHMe (2 rotamers)), 15.2, 14.9 (CH_2CH_3 (2 isomers)). Anal. Calcd. for $C_7H_{12}N_6O$ (196.11): C, 42.51; H, 6.16; N, 42.83. Found; C, 42.71; H, 6.00; N, 42.60.

*2-Amino-4-isopropylamino-6-methylamino-5-nitrosopyrimidine (**11**)*

From isopropylamine (59 mg). Yield: 126 mg (60%) as a violet solid, m.p. 146–148 °C, IR (KBr, cm^{-1}): 3332 (NH_2), 3000 (NH), 2870 (CH), 1653 (C=N), 1605 (C=C), 1573 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.77$, 11.75 (2xs, 1H, NH (2 rotamers)), 8.67 (d, 1H, $J = 4.7$ Hz, $NHCH_3$) 7.47 (d, 2H, $J = 6.5$ Hz, NH_2), 4.47, 4.25 (2×m, 1H, $CHMe_2$ (2 rotamers)), 2.93, 2.86 ((2×s, 1H, NH*Me* (2 rotamers)), 1.21, 1.17 (2m, 6H, $CHMe_2$ (2 rotamers)). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 164.9$ (C-4), 163.9 (C-6), 162.5 (C-2), 136.4 (C-5), 41.9 ($CHMe_2$), 27.8 ($NHCH_3$), 22.8 ($CHMe_2$). Anal. Calcd. for $C_9H_{14}N_6O$ (210.24): C, 45.70; H, 6.71; N, 39.97. Found; C, 45.51; H, 6.60; N, 39.65.

*2-((2-Amino-6-(methylamio)-5-nitrosopyrimidin-4-yl) amino)ethan-1-ol (**12**)*

From ethanolamine (61 mg). Yield: 153 mg (72%), as a brown solid, m.p. 138–141 °C; IR (KBr, cm^{-1}): 3325 (OH), 2980 (NH), 2760 (CH), 1660 (C=N), 1600 (C=O), 1560 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.54$, 11.26 (2xs, 1H, NH (2 rotamers)), 8.27 (br s., 1H, *NH*Me), 7.81 (br s., 2H, NH_2), 4.32 (br s., 1H, OH), 3.23 (t, 2H, $J = 6.0$ Hz, CH_2), 2.97 (q, 2H, $J = 6.0$ Hz, CH_2), 2.89, 2.86 ((2xs, 1H, NH*Me* (2 rotamers)), 1.23 (2×t, 6H, 2xCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 167.8$ (C-2), 165.7 (C-4), 162.0 (C-6), 70.4 (C-5), 61.4 (CH_2OH), 44.2 (NCH_2), 27.8, 26.3 (NHMe (2 rotamers)). Anal. Calcd. for $C_7H_{12}N_6O_2$ (212.10): C 39.62, H 5.70, N 39.60. Found; C, 45.51; H, 6.60; N, 39.65.

*2-Amino-4-diethylamino-6-methylamino-5-nitrosopyrimidine (**13**)*

From diethylamine (72 mg). Yield: 148 mg (66%) as a red crystals, m.p. 180–182 °C; IR (KBr, cm^{-1}): 3332 (NH_2), 3116 (NH), 2977 (CH), 1653 (C=N), 1573 (C=C), 1488 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.20$ (br s., 1H, NH), 7.87 (d, 2H, $J = 6.1$ Hz, NH_2), 3.75 (2xq, 4H, $J = 7.1$ Hz, 2×CH_2), 1.23 (2×t, 6H, 2×CH_2CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 170.6$ (C-6), 163.9 (C-4), 162.9 (C-2), 70.2 (C-5), 46.0 (NCH_2), 26.6 (NHMe), 13.8 (NCH_2CH_3). Anal. Calcd. for $C_9H_{16}N_6O$ (224.27): C, 48.20; H, 7.19; N, 37.47. Found; C, 47.91; H, 7.01; N, 37.21.

*2-Amino-4-diallylamino-6-methylamino-5-nitrosopyrimidine (**14**)*

From diallylamine (83 mg). Yield: 131 mg (53%) as a brown solid, m.p. 158–161 °C; IR (KBr, cm^{-1}): 3335 (NH_2), 3120 (NH), 3000 (CH), 1660 (C=N), 1590 (C=C), 1480 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.21$ (br s., 1H, NH), 7.94 (d, 2H, $J = 6.2$ Hz, NH_2), 5.55 (m, 2H, 2×CH_2CH=CH_2), 5.15 (m, 4H, 2×CH_2CH=CH_2), 3.79 (m, 4H, 2×CH_2CH=CH_2), 2.89 (br s, 3H, NH*Me*). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 170.6$ (C-6), 163.9 (C-4), 162.9 (C-2), 134.8 (CH_2CH=CH_2), 118.3 (CH_2CH=CH_2), 70.2 (C-5), 49.2 (CH_2CH=CH_2), 26.7 (NHMe). Anal. Calcd. for $C_{11}H_9N_{16}O$ (248.29): C, 53.21; H, 6.50; N, 6.50. Found; C, 52.89; H, 6.39; N, 6.27.

*2-((2-Amino-6-(methylamio)-5-nitrosopyrimidin-4-yl)thio) acetic acid (**15**)*

To a stirred solution of **9** (200 mg, 0.95 mmol) in dichloromethane (10 mL) containing triethylamine (0.5 mL) was added 2-thioglycolic acid (88 mg, 0.95 mmol) and the mixture was heated at 50 °C for 8 h. After cooling, the mixture was evaporated to dryness and the residue was recrystallized from ethanol to give **15** (160 mg, 66%) as a light brown powder, m.p. 123–126 °C; IR (KBr, cm^{-1}):

3520 (OH), 3250 (NH_2), 2977 (NH), 2780 (CH), 1709 (C=O), 1620 (C=N), 1588 (C=C), 1470 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.30$ (br s., 1H, CO_2H), 11.0 (br s., 1H, NH), 8.11 (br s., 1H, *NH*Me), 7.92 (d, 2H, $J =$ 6.8 Hz, NH_2), 3.94 (s, 2H, SCH_2), 2.90 (br s., 3H, NH*Me*). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 171.5$ (CO_2H), 168.2 (C-6), 163.0 (C-4), 160.8 (C-2), 90.2 (C-5), 31.3 (SCH_2), 28.9 (NHMe). Anal. Calcd. for $C_7H_9N_5O_3S$ (243.24): C, 34.57; H, 3.73; N, 28.79. Found; C, 34.28; H, 3.83; N, 28.92.

*General procedure for the synthesis of 2-amino-4-alkylamino-6-methylamino-5-nitroso- pyrimidine in dichloromethane (**16–24**)*

To a solution of **9** (211 mg, 1.00 mmol) in CH_2Cl_2 (10 mL) was added the corresponding amine (1.00 mmol) and the mixture was stirred at room temperature and the reaction monitored by thin layer chromatography (CH_2Cl_2/MeOH 9:1) until no starting material was observed. The mixture was evaporated to dryness and the residue was suspended in water, and the precipitate was filtered washed with water, dried and recrystallized from ethanol.

*2-Amino-4-allylamino-6-methylamino-5-nitrosopyrimidine (**16**)*

From allylamine (57 mg). Yield: 127 mg (61%) as a violet crystals, m.p. 145–147 °C; IR (KBr, cm^{-1}): 3332 (NH_2), 3178 (NH); 1655 (C=N), 1573 (C=C), 1527 (N=O). ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 11.50$ (br s., 1H, NH), 8.75 (br s, 1H, *NH*Me), 7.87 (d, 2H, $J = 7.8$ Hz, NH_2), 5.97 (m, 1H, CH_2CH=CH_2), 5.13 (m, 2H, CH_2CH=CH_2), 4.09 (m, 2H, CH_2CH=CH_2), 2.86 (br s, 3H, NH*Me*). ^{13}C NMR (150.91, DMSO-d_6): $\delta = 170.6$ (C-4), 164.8 (C-6), 163.4 (C-2), 134.7 (CH_2CH=CH_2), 116.2 (CH_2CH=CH_2), 70.2 (C-5), 42.5 (CH_2CH=CH_2), 27.9 (NHMe). Anal. Calcd. for $C_8H_{12}N_6O$ (208.11): C, 46.15; H, 5.81; N, 40.36. Found; C, 45.98; H, 5.73; N, 40.08.

*2-Amino-6-methylamino-4-propylamino-5-nitrosopyrimidine (**17**)*

From propylamine (59 mg). Yield: 143 mg (68%); as a violet crystals, m.p. 160–162 °C; IR (KBr, cm^{-1}): 3110 (NH_2), 2973 (NH), 2772 (CH), 1650 (C=N), 1562 (C=C); 1520 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.79$, 11.53 (2xs, 1H, NH (2 rotamers)), 8.66 (d, 1H, $J = 4.6$ Hz, *NH*Me), 7.42 (d, 2H, $J = 6.9$ Hz, NH_2), 3.40 (m, 2H, NCH_2-1′), 2.93, 2.85 (2x d, 3H, NH*Me* (2 rotamers)), 1.57 (m, 2H, CH_2-2′), 0.89 (t, 3H, $J = 7.2$ Hz, Me-3′). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 170.6$ (C-6), 164.9 (C-4), 163.9 (C-2), 70.2 (C-5), 42.1 (CH_2-1′), 26.7 (NHMe), 22.3 (CH_2-2′), 11.9 (Me-3′). Anal. Calcd. for $C_8H_{14}N_6O$ (210.24): C, 45.70; H, 6.71; N, 39.97. Found; C, 45.54; H, 6.64; N, 39.74.

*2-Amino-4-butylmino-6-methylamino-5-nitrosopyrimidine (**18**)*

From butylamine (73 mg). Yield: 133 mg (63%) as a violet solid, m.p. 148–150 °C; IR (KBr, cm^{-1}): 3317 (NH_2), 3170 (NH), 1630 (C=N), 1573 (C=C); 1530 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.19$ (br s., 1H, NH), 7.87 (d, 2H, $J = 7.9$ Hz, NH_2), 3.47 (m, 2H, CH_2-1′), 2.92, 2.86 (s, 3H, NH*Me* (2 rotamers)), 1.50-1.38 (m, 4H, CH_2-2′+CH_2-3′), 0.92 (t, 3H, $J = 7.1$ Hz, Me-4′). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 170.6$ (C-6), 164.9 (C-2), 163.9 (C-4), 70.2 (C-5), 52.9 (C-1′), 31.1 (C-2′), 26.7 (NHMe), 20.1 (C-3′), 14.1 (C-4′). Anal. Calcd. for $C_9H_{16}N_6O$ (224.27): C, 48.20; H, 7.19; N, 37.47. Found; C, 47.96; H, 7.01; N, 37.2.

*2-Amino-6-methylamino-4-pentylamino-5-nitrosopyrimidine (**19**)*

From pentylamine (87 mg). Yield: 155 mg (70%) as a pink crystals, m.p. 128–130 °C; IR (KBr, cm^{-1}): 3317 (NH_2), 3186 (NH), 1610 (C=N), 1573 (C=C); 1473 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.79$, 11.53 (2xs, 1H, NH (2 rotamers)), 8.69 (d, 1H, $J = 4.5$ Hz, *NH*Me), 7.41 (d, 2H, $J = 5.4$ Hz, NH_2), 3.45 (m, 2H, NCH_2-1′), 2.93, 2.86 (2xd, 3H, NH*Me* (2 rotamers)), 1.61, 1.52 (m, 4H, CH_2-2′ +CH_2-3′), 1.30 (m, 2H, CH_2-4′), 0.88 (t, 3H, $J = 7.0$ Hz, Me-5′). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 164.8$ (C-4), 163.9 (C-6), 163.4 (C-2), 136.7 (C-5), 40.6 (C-1′), 29.2 (C-2′), 28.7 (C-3′+NHMe), 22.4 (C-4′), 14.4 (C-5′). Anal. Calcd. for $C_{10}H_{18}N_6O$ (238.30): C, 50.40; H, 7.61; N, 35.27. Found; C, 50.21; H, 7.00; N, 33.12.

*2-Amino-4-hexylmino-6-methylamino-5-nitrosopyrimidine (**20**)*

From hexylamine (101 mg). Yield: 162 mg (68%) as a pink crystals, m.p. 118–121 °C; IR (KBr, cm^{-1}): 3332 (NH_2), 3178 (NH), 1593 (C=N), 1567 (C=C), 1473 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.79$, 11.53 (2s, 1H, NH (2 rotamers)), 8.67 (d, 1H, $J = 4.9$ Hz, *NH*Me), 7.41 (d, 2H, $J = 6.9$ Hz, NH_2), 3.37 (m, 2H, CH_2-1′) 2.93, 2.86 (2xd. 3H, NH*Me* (2 rotamers)), 1.59-1.40 (m, 8H, CH_2-2′ - CH_2-5′), 0.86 (t, 3H, $J = 7.2$ Hz, Me-6′). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 170.2$ (C-6), 164.3 (C-4), 162.8 (C-2), 69.6 (C-5), 41.0 (C-1′) 31.0 (C-2′+C-4′), 30.8 (C-3′), 26.0 (NHMe), 21.9 (C-5′), 13.8 (C-6′). Anal. Calcd. for $C_{11}H_{20}N_6O$ (252.32): C, 52.36; H, 7.99; N, 33.31. Found; C, 52.19; H, 7.82; N, 33.09.

*2-Amino-4-cyclohexylamino-6-methylamino-5-nitrosopyrimidine (**21**)*

From cyclohexyamine (99 mg). Yield: 145 mg (61%) as a red powder, m.p. 222–225 °C; IR (KBr, cm^{-1}): 3317 (NH_2), 3163 (NH), 1600 (C=N), 1558 (C=C), 1450 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.51$ (br s., 1H, NH), 8.26 (d, 1H, $J = 4.9$ Hz, *NH*Me), 7.39 (br s., 2H, NH_2), 2.86 (m, 4H, NHCH_3+$H_{cyclohexane}$), 1.86–1.27 (m, 10H, $H_{cyclohexane}$). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta =$ 170.6 (C-6), 164.9 (C-4), 162.5 (C-2), 70.2 (C-5), 49.0 (C-1′), 32.4 (C-2′+C-6′), 27.8 (NHMe), 24.5, 22.2 (C-3′+C-4′+C-5′). Anal. Calcd. for $C_{11}H_{18}N_6O$ (250.31): C, 52.78; H, 7.25; N, 33.58. Found; C 52.56; H, 7.13; N, 33.36.

*2-Amino-4-dimethylamino-6-methylamino-5-nitrosopyrimidine (**22**)*

From dimethylamine (45 mg). Yield: 165 mg (69%) as a violet solid, m.p. 117–120 °C; IR (KBr, cm^{-1}): 3332 (NH_2), 3170 (NH), 1589 (C=N), 1527 (C=C), 1498 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.19$ (br s., 1H, NH), 8.65 (br s., 1H, *NH*Me), 7.91 (d, 2H, $J = 7.9$ Hz, NH_2), 3.18, 3.15 (2×s, 6H, NMe_2), 2.87, 2.85 (2×s., 3H, NH*Me* (2 rotamers)). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 170.0$ (C-6), 163.4 (C-2), 151.2 (C-4), 69.7 (C-5), 39.8, (NMe_2), 26.2 (NHMe). Anal. Calcd. for $C_7H_{12}N_6O$ (196.21): C, 42.85; H, 6.16; N, 42.83. Found; C, 42.66; H, 6.02; N, 42.59.

*2-Amino-6-methylamino-4-dipropylamino-5-nitrosopyrimidine (**23**)*

From dipropylamine (101 mg). Yield: 165 mg (69%) as a violet crystals, m.p. 120–123 °C; IR (KBr, cm^{-1}): IR (KBr, cm^{-1}): ν 3120 (NH_2), 2980 (NH), 1642 (C=N), 1570 (C=C); 1510 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta =$ 11.19 (br s., 1H, NH), 7.89 (d, 1H, $J = 7.9$ Hz, NH_2), 3.44 (m, 4H, 2×$CH_2$$CH_2CH_3$), 2.86 (br s., 3H, NH*Me*), 1.64 (m, 4H, 2×$CH_2CH_2$$CH_3$), 0.90 (2×t, 3H, $J = 7.5$ Hz, 2x$CH_2CH_2CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta =$ 170.0 (C-6), 163.4 (C-2), 151.2 (C-4), 69.7 (C-5), 48.3 ($CH_2CH_2CH_3$), 26.2 (NHMe), 21.7 ($CH_2CH_2CH_3$), 10.9 ($CH_2CH_2CH_3$). Anal. Calcd. for $C_{11}H_{20}N_6O$ (252.32): C, 52.36; H, 7.99; N, 33.31. Found; C, 52.16; H, 7.84; N, 33.04.

*2-Amino-6-methylamino-4-piprazino-5-nitrosopyrimidine (**24**)*

From piperazine (86 mg). Yield: 145 mg (65%) as a violet solid; m.p. 185–188 °C; IR (KBr, cm^{-1}): 3348 (NH_2), 3170 (NH), 1589 (C=N) 1595 (C=C), 1535 (N=O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.19$ (br s., 1H, NH), 8.64 (s, 1H, $NHCH_2$), 7.89 (d, 2H, $J = 8.0$ Hz, NH_2), 4.23 (m, 4H, 2×CH_2), 2.86 (br s., 3H, NH*Me*), 2.24 (m, 4H, 2×CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 170.1$ (C-2), 163.4 (C-4), 151.2 (C-6), 138.8 (C-5), 69.7 (CH_2), 44.1 (CH_2), 26.2 (NHMe). Anal. Calcd. for $C_9H_{15}N_7O$ (237.27): C, 45.56; H, 6.37; N, 41.32. Found; C, 45.35; H, 6.31; N, 41.11.

*2-Chloro-N-(4-isopropoxy-6-(methylamino)-5-nitrosopyrimidin-2-yl)acetamide (**27**)*

2-Chloroacetyl chloride (170 mg, 1.50 mmol) was added dropwise to a mixture of **9** (211 mg, 1.00 mmol) and potassium carbonate (276 mg, 2.00 mmol) in chloroform (20 mL) at room temperature with stirring for 4 h. After removal of the dichloromethane and vacuum filtration, the solid was washed with water and dried under vacuum. Recrystallization from ethyl acetate/ petroleum ether gave **27** (204 mg, 71%) as oil; IR (KBr, cm^{-1}): 3322 (NH_2), 3180 (NH); 1670 (C=O), 1650 (C=N), 1580 (C=C), 1520 (N=O); 1330 (C–O). ^{1}H NMR (400 MHz, DMSO-d_6): $\delta =$ 11.20. (br s., 1H, NH), 8.61 (br s., 1H, NHCO), 5.54 (m, 1H, *CH*Me_2), 4.81 (s, 2H, CH_2Cl), 2.86 (br s., 3H, NH*Me*) 1.40, 1.39 (2×s, 6H, CHMe_2). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 167.2$ (C=O), 162.1 (C-6), 159.3 (C-4), 151.7 (C-2), 130.1 (C-5), 72.1 (*CH*Me_2), 41.6 (CH_2Cl), 26.7 (NHMe), 22.3 (CHMe_2). Anal. Calcd. for $C_{10}H_{14}ClN_5O_3$ (287.70): C, 41.75; H, 4.91; N, 24.34. Found; C, 41.52; H, 4.82; N, 24.21.

*2-(Benzothiazol-2-ylthio)-N-(4-isopropoxy-6-(methylamino)-5-nitrosopyrimidine-2-yl)acetamide (**29**)*

To a mixture of **27** (288 mg, 1.00 mmol) and triethylamine (184 mg, 1.82 mmol) in dichloromethane (30 mL) was added 2-mercaptobenzothiazole (**28**) (152 mg, 0.91 mmol) and the mixture was heated under reflux for 4 h. After cooling, the mixture was evaporated to dryness and the residue was purified on a SiO_2 column chromatography (5 g) using methanol, in gradient (0–10%) and chloroform as eluent to give **29** (238 mg, 57%) as a brown solid, m.p. 152–155 °C; IR (KBr, cm^{-1}): 3317 (NH_{amide}), 3163 (NH), 1774 (C=O), 1615 (C=N), 1589 (C=C), 1526 (N=O). ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 11.19$ (s, 1H, NH), 8.06 (br s., 1H, *NH*Me), 7.93–7.35 (m, 5H, $H_{arom.}$), 5.54 (m, 1H, *CH*Me_2), 3.05 (m, 2H, SCH_2), 2.87 (br s., 3H, NH*Me*), 1.40, 1.38 (2xs, 6H, CHMe_2). ^{13}C NMR (150.91, DMSO-d_6): $\delta = 170.0$ (C=O), 164.5 (C-2′), 163.3 (C-4+C-6), 152.2 (C-2), 151.2 (C-3′), 138.7 (C-7a′), 134.9 (C-3a′+C-5), 124.7, 121.9, 121.3 ($C_{arom.}$), 69.6 (*CH*Me_2), 36.3 (SCH_2), 26.1 (NHMe), 21.7 (CHMe_2). Anal. Calcd. for

$C_{17}H_{18}N_6O_3S_2$ (418.49): C, 48.79; H, 4.34; N, 20.08. Found; C, 48.56; H, 4.22; N, 19.84.

*2-(Butylideneamino)-6-isopropoxy-4-methylamino-5-nitrosopyrimidine (**30**)*

To a solution of **9** (200 mg, 1.00 mmol) in ethanol (15 mL) was added butyraldehyde (0.1 mL, 1.00) followed by three drops of glacial acetic acid and the mixture was heated under reflux for 3 h. (148 mg, 55%) as a brown solid, m.p. 160–164 °C; IR (KBr, cm^{-1}): 2990 (NH), 1620 (C=N), 1590 (C=C); 1530 (N=O). ^{1}H NMR (DMSO-d_6): $\delta =$ 11.23 (br s., 1H, NH), 7.91 (d, 1H, $J = 8.9$ Hz, CH=N); 5.55 (m, 1H, *CH*Me_2), 2.88, 2.87 (2xs, 3H, NH*Me* (2 rotamers)), 2.25 (m, 2H, *CH*$_2$$CH_2$Me), 1.55 (m, 2H, CH_2*CH*$_2$Me), 1.49, 1.40 (2xs, 6H, CH*Me*$_2$), 0.88 (t, 3H, $J = 7.3$ Hz, CH_2CH_2*Me*). ^{13}C NMR (DMSO-d_6): $\delta = 173.2$ (C-4), 170.6 (C-6), 163.9 (CH=N), 151.7 (C-2), 82.3 (C-5), 73.6 (*CH*Me_2), 33.7 (*CH*$_2$$CH_2$Me), 26.7 (NHMe), 22.2 (CH*Me*$_2$), 18.5 (CH_2*CH*$_2$Me), 13.8 (CH_2CH_2*Me*). Anal. Calcd. for $C_{12}H_{19}N_5O_2$ (265.32): C, 54.32; H, 7.22; N, 26.40. Found; C, 54.09; H, 7.11; N, 26.17.

Biological methods

[2, 4, 6, 7-3H]-E2 and [2, 4, 6, 7-3H]-E1 were bought from Perkin Elmer, Boston. Quickszint Flow 302 scintillator fluid was bought from Zinsser Analytic, Frankfurt.

17β-HSD1 and 17β-HSD2 were obtained from human placenta according to previously described (Zhu et al. 1993; Qiu et al. 2002; Kruchten et al. 2008). Fresh human placenta was homogenized and centrifuged. The pellet fraction contains the microsomal 17β-HSD2, while 17β-HSD1 was obtained after precipitation with ammonium sulphate from the cytosolic fraction.

Inhibition of 17β-HSD1

Inhibitory activities were evaluated by an established method (Lin et al. 1992; Sam et al. 1995, 1998) with minor modifications. Briefly, the enzyme preparation was incubated with nicotinamide adenine dinucleotide-hydrogen (NADH) [500 µM] in the presence of inhibitors at 37 °C in a phosphate buffer (50 mm) supplemented with 20% of glycerol and ethylenediaminetetraacetic acid (EDTA) (1 mm). Inhibitor stock solutions were prepared in DMSO. The final concentration of dimethylsulfoxide (DMSO) was adjusted to 1% in all samples. The enzymatic reaction was started by addition of a mixture of unlabelled- and [2, 4, 6, 7-3H]-E1 (final concentration: 500 nM, 0.15 µCi). After 10 min, the incubation was stopped with $HgCl_2$ and the mixture was extracted with diethylether. After evaporation, the steroids were dissolved in acetonitrile. E1 and E2 were separated using acetonitrile/water (45:55) as mobile phase in a C18 reverse phase chromatography column (Nucleodur C18 Gravity, 3 µm, Macherey-Nagel, Düren) connected to a HPLC-system (Agilent 1100 Series, Agilent Technologies, Waldbronn). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad). The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation: *% conversion*=(*% E2*)/(*% E2*+*% E1*)×100. Each value was calculated from at least three independent experiments.

Inhibition of 17β-HSD2

The 17β-HSD2 inhibition assay was performed similarly to the 17β-HSD1 procedure. The microsomal fraction was incubated with NAD+ [1500 µM], test compound and a mixture of unlabelled- and [2, 4, 6, 7-3H]-E2 (final concentration: 500 nM, 0.11 µCi) for 20 min at 37 °C. Further treatment of the samples and HPLC separation were carried out as mentioned above.

The conversion rate was calculated after analysis of the resulting chromatograms according to the following equation: *% conversion*=(*% E1*)/(*% E1*+*% E2*)×100.

Cytotoxicity assays

Cell cultures were seeded at 1 × 105 cells per ml in 96 multiwell plates in specific media [RPMI-1640 medium with 20mM HEPES buffer (Life Technologies)], supplemented with 10% FCS and antibiotic (gentamicin), and incubated at 37 °C in a humidified CO_2 (5%) atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method). Compounds were dissolved in dimethyl sulfoxide at 100 mm and then diluted into culture medium.

Conclusion

In conclusion, a new series of 4-alkylamino and 4-alkylthio-5-nitrosopyrimidine derivatives were prepared from 2-amino-4-isopropoxy-6-methylamino-5-nitropyrimidine, as a key intermediate, via aromatic nucleophilic substitution reaction (S_NAr), since the nitroso group at C-5 of pyrimidine backbone would highly activates it towards such nucleophilic substitution of isopropoxy group. The key intermediate has been further modified by conjugation with 2-mercaptobenzothiazole via a thioacetamide linkage at C-2 of pyrimidine backbone. Compounds **9–11**, **16–20** and **29** were screened for their inhibitory activity against 17β-

hydroxydehyrogenase type 1 (17β-HSD1) and type 2 (17β-HSD2). All these compounds showed less than 10% inhibition for both enzymes at concentration of 1.0 μM. Furthermore, all compounds were tested against two solid tumour-derived cell lines consisting Hep-G2 (human hepatocarcinoma) and MCF-7 (breast cancer). Compound **29** showed a moderate antitumor activity against Hep-G2 ($IC_{50} = 1.6 \pm 0.1$ μM) and led to be promising agent for further structural modification.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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