# Synthesis and Antiviral Activity of Benzyl-Substituted Imidazo[1,5-*a*]-1,3,5-triazine (5,8-Diaza-7,9-dideazapurine) Derivatives

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A variety of imidazo[1,5-*a*]-1,3,5-triazine derivatives carrying *C*-, *O*-, and *S*-benzyl and/or 4-methylbenzyl groups were synthesized and examined for their inhibitory effects on the replication of ortho- and paramyxoviruses. The key compounds 8-R-2-thioxo-2,3-dihydroimidazo[1,5-*a*]-1,3,5-triazin-4(1H)-ones **3a,b,d** were synthesized by chlorotrimethylsilane/HMDS-effected cyclization–rearrangement of the corresponding 6-amino-5-(formylamino)-5-R-2-mercaptopyrimidin-4(5H)-ones **2a,b,d** (R = benzyl, 4-methylbenzyl, and 5-(benzyloxy)pentyl). Compounds **3a,b** were further transformed into 4-thiones **5a,b** and 4-dimethylamino derivatives **7a,b**. Preparation of *S*-methyl, *S*-benzyl, and *S*-(4-methylbenzyl) derivatives **12–19** was carried out by the treatment of thioxo compounds **3b,d**, **5b**, and **8b** in an alcohol/potassium carbonate system with methyl iodide or the appropriate aralkyl bromide. Simultaneous presence of the benzyl and thio structural units was found to be indispensable for any selective biological activity. Some 2-thio substituted compounds were specifically inhibitory to some viruses, e.g., 8-(4-methylbenzyl)-2-[(4-methylbenzyl)thio]imidazo[1,5-*a*]-1,3,5-triazin-4-one (**13**) and 8-[5-(benzyloxy)pentyl]-2-[(4-methylbenzyl)thio]imidazo[1,5-*a*]-1,3,5-triazin-4-one (**15**) inhibited influenza A virus at a concentration of 4.1 and 5.3 $\mu$M, and 2-(benzylthio)-6,8-dimethylimidazo[1,5-*a*]-1,3,5-triazin-4-one (**16**) and 6,8-dimethyl-2-[(4-methylbenzyl)thio]imidazo[1,5-*a*]-1,3,5-triazin-4-one (**17**) inhibited respiratory syncytial virus at a concentration of 21.9 and 15.7 $\mu$M, respectively, that is, at concentrations that were 20–50-fold lower than the cytotoxic concentrations. Compound **13** was inhibitory to respiratory syncytial virus at a concentration of 1.4 $\mu$M, that is, at a concentration that was 180-fold lower than the cytotoxic concentration to MDCK or Vero cells but only 7-fold lower than the cytotoxic concentration to HeLa cells. The 4-thiones **5a,b** were nonselectively inhibitory to ortho- and paramyxoviruses at concentrations that coincided with their cytotoxic concentrations.

In our previous work[1–3] we developed the synthetic routes toward some derivatives of imidazo[1,5-*a*]-1,3,5-triazine (5,8-diaza-7,9-dideazapurine), ring isomers of 9-substituted guanines, 2-thioxanthines, and hypoxanthines. Recently we described the transformation of hypoxanthine analogues into adenine analogues of this type.[4]

In view of the reports on the important interactions of benzyl groups with some proteins, e.g., PNP,[5] and on the potent in vitro antiviral (i.e., antirhinovirus) activity of a series of 9-benzyl-6-(dimethylamino)purines,[6,7] it seemed of interest to synthesize benzyl-substituted imidazo[1,5-a]-1,3,5-triazines and evaluate their antiviral activity.

Our initial synthetic goals were 8-benzyl-4-(dimethylamino)imidazo[1,5-*a*]-1,3,5-triazine (**7a**) and its 8-(4-methylbenzyl) congener **7b**. However, after the antimyxo tests of the initial series of compounds had revealed that only the sulfur-containing compounds showed some promising inhibitory effects, we concentrated on the preparation of further imidazo[1,5-*a*]-1,3,5-triazine derivatives containing the benzyl and thio structural units.

## Chemistry

9-Benzyl-6-(dimethylamino)purine-like compounds **7a,b** were prepared, as shown in Scheme 1, following our reported procedures for the synthesis of imidazo[1,5-*a*]-1,3,5-triazine ring system by cyclization–rearrangement of 5-(acylamino)-5-alkyl-6-amino-2-mercaptopyrimidin-4(5*H*)-ones to form 8-substituted and 6,8-disubstituted 2-thioxo-2,3-dihydroimidazo[1,5-*a*]-1,3,5-triazin-4(1*H*)-ones[1,2] and further transformation of the latter into 4-aminoimidazo[1,5-*a*]-1,3,5-triazines.[4]

Thus ethyl 2-cyano-2-(formylamino)acetate dissolved in anhydrous ethanol containing 1.4 equiv of sodium ethoxide reacted with benzyl bromide (a) and 4-methylbenzyl bromide (b) to give starting C-aralkylated esters **1a,b**, respectively. Reaction of **1a,b** with 1 equiv of thiourea in anhydrous ethanol in the presence of 1.1 equiv of sodium ethoxide afforded 5-(formylamino)-5-aralkylpyrimidinones **2a,b** in 40% and 35% yields, respectively. The reaction was carried out at room temperature for 10 h. After that time some unreacted thiourea was still present, but the extension of the reaction time, raising of the temperature, or using higher excess of reagents resulted in even poorer yields. As side products appeared deformylated 5-amino-5-aralkylpyrimidinones (**2a′,b′**, ca. 30% yield) which were not observed in any other case of the preparation of similar pyrimidinones described so far.[1–3] They were identified by the absence of the formyl group signals in

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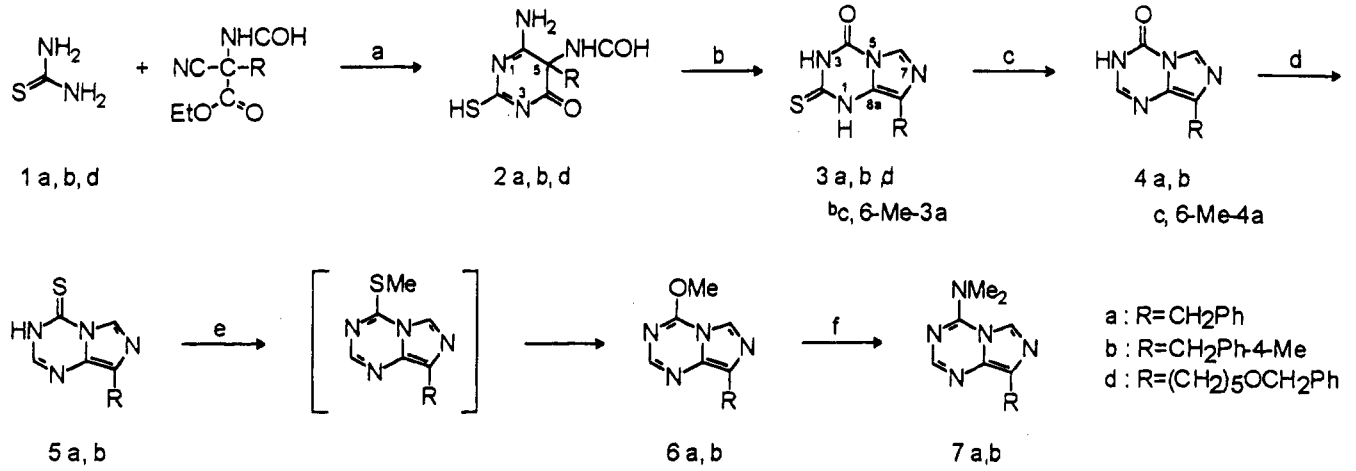
**Scheme 1**[a]

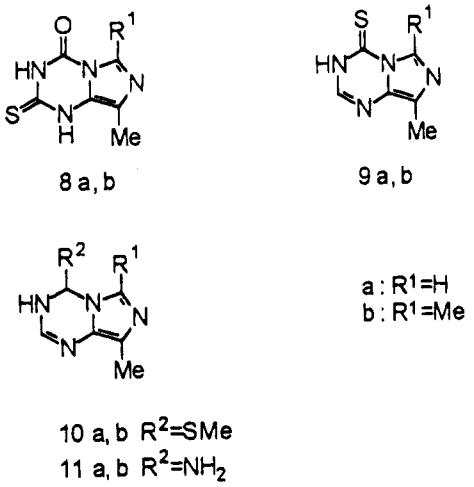



[a] Reagents: (a) Na/EtOH; (b) chlorotrimethylsilane, Py, HMDS; (c) $NH_3$ (aq), Raney Ni deactivation; (d) 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide, xylene; (e) $K_2CO_3$, MeOH, MeI; (f) $Me_2NH$/MeOH. [b] Described earlier in ref 3.

$^1H$ and $^{13}C$ NMR spectra (at ca. 8.0 and 161.1 ppm, respectively) and elemental analysis of 5-benzyl compound **2a'**.

Treatment of **2a,b**, in anhydrous pyridine with chlorotrimethylsilane at room temperature followed by hexamethyldisilazane at reflux resulted in 2-thioxanthine analogues **3a** (90%) and **3b** (80%). The above cyclization–rearrangement was substantiated by changes in $^{13}C$ NMR spectra, the most conspicuous being the disappearance of the signal of tetrasubstituted carbon C-5 at ca. 57.9 ppm. Desulfurization of **3a,b**, by heating their solutions in diluted aqueous ammonia with Raney nickel catalyst, provided hypoxanthine analogues **4a** (65%) and **4b** (60%). The main reaction was accompanied by degradative processes involving the cleavage of the six-membered ring of **4a,b**.[8] These processes turned out to be more important in the case of **4c**, the 6-methyl-substituted derivative of **4a**. On attempt of desulfurization of the previously described[3] **3c**, the corresponding **4c** could be obtained in only 40% yield due to lower rate of its formation and higher rate of degradation. Compound **4c** was also unstable under the conditions of the next stage of the envisaged synthetic route, thiation, and therefore its transformation into the 9-benzyl-6-(dimethylamino)purine analogue was not pursued any further. The direct replacement of a 4-oxo functionality of **4a,b** by a 4-thioxo one was accomplished using 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson reagent)[9] in boiling xylene. Following chromatography on silica gel, the 6-mercaptopurine analogues **5a,b** were isolated in 35% and 85% yields, respectively. The yield appeared to be strongly dependent upon solubility. In diluted solutions the thiation processes are slow enough not to be completed before the decomposition of the product becomes significant. Imidazotriazine-4-thiones **5a,b** when subjected to methyl iodide in methanol in the presence of potassium carbonate at room temperature were first converted into 4-methylthio derivatives, which after 30 min in the reaction medium were completely transformed into 4-methoxy derivatives **6a,b** (60% and 65% yield of isolated products, respectively). Compounds **6a,b** in accord with what we reported earlier on other 4-methoxyimidazo[1,5-*a*]-1,3,5-triazines,[4] were extremely susceptible to nucleophilic displacement; when treated with anhydrous methanolic dimethylamine at room temperature, they formed during 5 min the desired 8-benzyl-4-(dimethylamino)imidazol[1,5-*a*]-1,3,5-triazine (**7a**) (50%) and its 8-(4-methylbenzyl) analogues **7b** (60%).

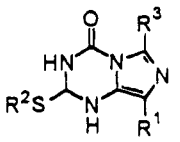
**Chart 1**



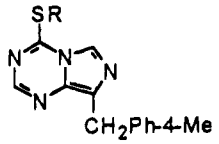

All benzylated imidazo[1,5-*a*]-1,3,5-triazine derivatives newly prepared by the above described synthetic route (with the exception of the unstable 4-methoxy compounds) were examined for their reactivity against various viruses, including ortho- and paramyxoviruses. Some closely related 8-methyl-substituted imidazotriazine derivatives, **8a,b**–**11a,b**, reported earlier[2,4] were also included. A selective antiviral effect was noted only for the sulfur-containing compounds. Taking the most promising 2-thioxo compounds **3a,b** as a lead, we turned towards the preparation of further imidazo[1,5-*a*]-1,3,5-triazine derivatives containing the benzyl and thio structural units.

A reaction involving cyclization–rearrangement was applied to introduce a benzyl group in the form of a 5-(benzyloxy)pentyl substituent into the C-8 position. Thus ethyl 7-(benzyloxy)-2-cyano-2-(formylamino)heptanoate (**1d**) reacted with thiourea in anhydrous ethanol in the presence of sodium ethoxide to afford 5-[(benzyloxy)pentyl]-5-(formylamino)-substituted pyrimidinone **2d** (25% yield after silica gel short column chromatographic separation). Cyclization–rearrangement of **2d** into imidazo[1,5-*a*]-1,3,5-triazine derivative **3d** (80%

**Chart 2**



12 $R^1$=$CH_2$Ph-4-Me; $R^2$=Me; $R^3$=H
13 $R^1$=$CH_2$Ph-4-Me; $R^2$=$CH_2$Ph-4-Me; $R^3$=H
14 $R^1$=$(CH_2)_5OCH_2Ph$; $R^2$=Me; $R^3$=H
15 $R^1$=$(CH_2)_5OCH_2Ph$; $R^2$=$CH_2$Ph-4-Me; $R^3$=H
16 $R^1$=Me; $R^2$=$CH_2$Ph; $R^3$=Me
17 $R^1$=Me; $R^2$=$CH_2$Ph-4-Me; $R^3$=Me



18 R=Me
19 R=$CH_2$Ph-4-Me

yield) proceeded under conditions analogous to those specified above for compounds **3a,b**. Introduction of an ether group into the C-8 position of the imidazo[1,5-*a*]-1,3,5-triazine system via cyclization−rearrangement enlarged the scope of this reaction, which so far has been limited to alkyl, alkenyl, and aralkyl substituents.

Other modifications encompassed methylation and benzylation reactions of the thioxo function of three 2-thioxo-2,3-dihydroimidazo[1,5-*a*]-1,3,5-triazin-4(1*H*)-ones (**3b,d** and **8b**) and an imidazo[1,5-*a*]1,3,5-triazine-4-thione (**5b**). Generally the reactions were performed at room temperature using methyl iodide, benzyl bromide, and 4-methylbenzyl bromide in alcohol in the presence of potassium carbonate. Selective S-substitution of 2-thioxo derivatives was accomplished using 1.0 equiv of an alkylating agent in methanol at room temperature. When an excess of the reagent was used, the reaction had to be carefully followed by TLC in order to be interrupted before the N-3 alkylation set in. Thus treatment of **3b** with methyl iodide and 4-methylbenzyl bromide gave **12** and **13** in 80% and 70% yields, respectively; similarly, **3d** afforded **14** (80%) and 15 (90%). Reaction of **8b** with benzyl bromide and 4-methylbenzyl bromide provided the corresponding **16** (90%) and **17** (94%). The above-mentioned spontaneous transformation of 4-(alkylthio)imidazo[1,5-*a*]-1,3,5-triazines into 4-methoxy derivatives, due to nucleophilic attack of methanol used as an alkylation medium, was avoided by replacing methanol with 2-propanol. The 4-thione **5b** thus subjected to methyl iodide and 4-methylbenzyl bromide was converted into **18** and **19** in 80% and 70% yields, respectively.

Tables 1−3 summarize the physicochemical and UV, $^1$H, and $^{13}$C NMR spectral data for the benzylimidazo-[1,5-*a*]-1,3,5-triazines prepared. The $^1$H and $^{13}$C NMR spectra indicated that the tautomeric preferences in solution for 8-benzyl, 8-(4-methylbenzyl), and 8-[5-(benzyloxy)pentyl] derivatives of thio-substituted imidazo[1,5-*a*]-1,3,5-triazines are 4(3*H*)-oxo-2(1*H*)-thioxo (**3a,b,d**) and 4(3*H*)-thioxo (**5a,b**), similar to the corresponding purines.[10] Analogously to other imidazotriazines, studied in this respect previously,[4] two separate NH signals appeared in the $^1$H NMR spectra of compounds **3a,b,d** (at $\delta_H$ 13.40, 13.36, 13.21 and 12.51, 12.48 and 12.39, respectively). In addition, a difference of 11 ppm between chemical shift value for C-2 in compound **3b** ($\delta_C$ 171.53) and model 2-methylthio derivative **12** (160.72) excluded any significant contribution of the 2-thiol form. Similarly, 13 ppm difference between chemical shift value for C-4 in **5b** and 4-methylthio derivative **18** excluded the 4-thiol form for **5b**.

The 5,5-disubstituted pyrimidinones **2a,b,d**, like their congeners described previously,[11] were found to exist in DMSO-$d_6$ solution in the form of 6-imino-4-oxo-2-thioxo (four separate signals of single NH protons). Nevertheless, throughout this paper, **2a,b,d** are presented as 6-amino-2-mercapto-4-oxo tautomers, as envisaged under the conditions of cyclization−rearrangement.[2]

## Antiviral Activity

Antiviral evaluation of the newly synthesized benzyl derivatives **3**−**5**, **7**, and **12**−**19** together with several of their 8-methyl analogues **8**−**11** prepared earlier[2,4] showed activity against ortho- and paramyxoviruses only for some sulfur-containing compounds (Table 4).

The 4-thio substituted compounds **5a,b**, **10a**, and **18** were quite potent ($IC_{50}$: 0.6−222 $\mu$M) but not selective (differences in activity against influenza A virus vs respiratory syncytial virus, up to 5-fold; inhibitory concentrations coinciding with cytotoxic concentrations). They did not require the presence of a benzyl group to be active: the antiviral activity and the cytotoxicity of the 8-methyl derivative **10a** were similar to those of its 8-(4-methylbenzyl) counterpart **18**. The presence of two benzyl groups in 4-thio compound **19** resulted in a loss of activity.

On the other hand, 2-thio substituted compounds **3a,b,d** and **12**−**17** had $IC_{50}$ values against ortho- and paramyxoviruses in the range of 1.4−775 $\mu$M and were more selective than the 4-substituted derivatives. Differences in activity against influenza A virus versus respiratory syncytial virus were up to 15-fold; virus inhibitory concentrations were generally 15−30-fold lower than cytotoxic concentrations (**3a,b** and **15**−**17**). Five of the tested compounds (**3d** and **12**−**15**) showed distinct differences in cytotoxicity to MDCK (Madin−Darby canine kidney), Vero, and HeLa cells. The most conspicuous in that respect was compound **13** which proved not to be cytotoxic to MDCK and Vero cells at a concentration up to 260 $\mu$M but was cytotoxic to HeLa cells (MTC: 10.3 $\mu$M). When tested in MDCK and Vero cells, compound **13** was found to be more potent against respiratory syncytial virus (RSV) ($IC_{50}$: 1.4 $\mu$M, the concentration 180-fold lower than the MTC) than ribavirin ($IC_{50}$: 11.4 $\mu$M, the concentration 70-fold lower than the MTC). Ribavirin is presently the only drug licensed for clinical use against RSV with limitation to aerosolized form due to the toxicity of the other forms.

For the 2-thio derivatives, the presence of a benzyl substituent was indispensable for acquiring antiviral activity, e.g., the antivirally inactive compound **8b** was upon S-benzylation transformed into compound **16** with marked activity ($IC_{50}$: 20.9 $\mu$M) against respiratory syncytial virus, that is, at a concentration 25−30-fold lower than the cytotoxic concentration. Unlike the loss of activity observed with the 4-thio substituted compounds after introduction of the second 4-methylbenzyl

**Table 1.** Physical Properties, UV Spectral Data, and Elemental Analyses of Benzyl-Substituted Imidazo[1,5-*a*]-1,3,5-triazine Derivatives

| compd | yield, %[a] | recrystn solv | mp, °C | UV (EtOH) $\lambda_{max}$, nm ($\epsilon \times 10^{-3}$) | formula | anal. |
|---|---|---|---|---|---|---|
| **3a** | 90 | EtOH (anhydrous) | 225 dec | 254 (6.4), 292 (10.1) | $C_{12}H_{10}N_4OS$ | C,H,N,S |
| **3b** | 80 | EtOH (anhydrous) | 230 dec | 249 (5.2), 296 (9.1) | $C_{12}H_{12}N_4OS$ | C,H,N,S |
| **3d** | 80 | MeOH or EtOH | 195 | 257 (9.8), 289 (14.6) | $C_{17}H_{20}N_4O_2S$ | C,H,N,S |
| **4a** | 65 | $CHCl_3$–MeOH (4:1) | >260 dec | 266 (8.6), 300 (4.3) | $C_{12}H_{10}N_4O$ | C,H,N |
| **4b** | 60 | $CHCl_3$–MeOH (4:1) | >245 dec | 265 (8.8) 299 (4.3) | $C_{13}H_{12}N_4O$ | C,H,N |
| **4c** | 40 | MeOH | >200 dec | 261 (8.6), 268 (8.9), 304 (4.3) | $C_{13}H_{12}N_4O$ | C,H,N |
| **5a** | 35 | *i*-PrOH | 200 dec | 278 (15.7), 349 (4.4) | $C_{12}H_{10}N_4S$ | C,H,N,S |
| **5b** | 85 | *i*-PrOH | 218 dec | 279 (16.8), 349 (4.6) | $C_{13}H_{12}N_4S$ | C,H,N,S |
| **6a** | 60 | | amorph, unstable | *b* | | *b* |
| **6b** | 65 | | amorph, unstable | *b* | | *b* |
| **7a** | 50 | *i*-PrOH (anhydrous) | 156 | 270 (5.2), 281 (6.1), 293 (4.9), 335 (5.6) | $C_{14}H_{15}N_5$ | C,H,N |
| **7b** | 60 | *i*-PrOH (anhydrous) | 138 | 273 (5.7), 281 (5.9) | $C_{15}H_{17}N_5$ | C,H,N |
| **12** | 85 | *i*-PrOH | 220 dec | 279 (13.2) | $C_{14}H_{14}N_4OS{\cdot}0.5H_2O$ | C,S,H,[c] N[d] |
| **13** | 85 | *i*-PrOH | 205 dec | 280 (13.5) | $C_{21}H_{20}N_4OS{\cdot}0.5H_2O$ | C,H,N,S |
| **14** | 80 | EtOH | 136 | 279 (12.8) | $C_{18}H_{22}N_4O_2S$ | C,H,S,N[e] |
| **15** | 90 | MeOH or EtOH | 142 | 248 (12.1) | $C_{25}H_{28}N_4O_2S$ | C,H,S,N[f] |
| **16** | 90 | MeOH–$H_2O$ (95:5) or *i*-PrOH | >250 dec | 280 (13.5) | $C_{14}H_{14}N_4OS$ | C,H,N,S |
| **17** | 95 | MeOH–$H_2O$ (95:5) or *i*-PrOH | >230 dec | 279 (13.4) | $C_{15}H_{16}N_4OS{\cdot}0.25H_2O$ | C,H,N,S |
| **18** | 80 | *i*-PrOH | 123 | 252 (12.5), 265 (9.8), 275 (8.6), 286 (4.8), 365 (3.1) | $C_{14}H_{14}N_4S$ | C,H,N,S |
| **19** | 70 | *i*-PrOH | 146 | 256 (11.7), 267 (11.5), 276 (10.8), 364 (3.1) | $C_{21}H_{20}N_4S$ | C,H,N,S |

[a] After chromatography. [b] Not determined. [c] H: calcd, 5.08; found, 4.67. [d] N: calcd, 18.98; found, 18.19. [e] N: calcd, 15.64; found, 14.99. [f] N: calcd, 12.50; found, 11.48.

**Table 2.** $^1H$ NMR Data of Benzyl-Substituted Imidazo[1,5-*a*]-1,3,5-triazine Derivatives (Chemical shifts ($\delta_H$, ppm) in DMSO-$d_6$–TMS[a])

| compd | N-1H[b] | N-3H[b] | H-6 | H-2 | C-$CH_2$Ar (O-$CH_2$Ar) | S-Me (S-$CH_2$Ar) | Ar-4-Me | Ar | other signals |
|---|---|---|---|---|---|---|---|---|---|
| **3a** | 13.40 | 12.51 | 8.11 | | 4.01 | | | 7.15–7.27 (m) | |
| **3b** | 13.36 | 12.48 | 8.09 | | 3.93 | | 2.24 | 7.05d,7.13 (d) | |
| **3d** | 13.21 | 12.39 | 8.07 | | (4.34) | | | 7.27–7.37 (m) | 1.29–1.60 [2 m, $CH_2(CH_2)_3CH_2O$], 2.60 [t, C-8-$CH_2(CH_2)_3$], 3.41 [t, $(CH_2)_3CH_2O$] |
| **4a** | | 12.15 | 8.28 | 7.71 | 4.02 | | | 7.10–7.35 (m) | |
| **4b** | | 12.10 | 8.26 | 7.71 | 3.97 | | 2.23 | 7.05d,7.13 (d) | |
| **4c** | | 11.79 | | 7.54 | 3.93 | | | 7.15–7.25 (m) | 2.66 (6-Me) |
| **5a** | | *c* | 8.51 | 7.77 | 4.07 | | | 7.10–7.27 (m) | |
| **5b** | | *c* | 8.33 | 7.68 | 3.98 | | 2.23 | 7.03d,7.12 (d) | |
| **6a** | | | 8.28 | 8.06 | 4.21 | | | 7.10–7.30 (m) | 4.15 (O-Me) |
| **6b** | | | 8.30 | 8.06 | 4.09 | | 2.23 | 7.04d,7.14 (d) | 4.21 (O-Me) |
| **7a** | | | 8.45 | 7.77 | 4.05 | | | 7.10–7.30 | 3.37 [$N(Me)_2$] |
| **7b** | | | 8.45 | 7.77 | 4.00 | | 2.23 | 7.03–7.13 | 3.37 [$N(Me)_2$] |
| **12** | | 12.46 | 8.12 | | 3.93 | 2.41 | 2.23 | 7.05 (d), 7.14 (d) | |
| **13** | | 12.43 | 8.13 | | 3.98 | (4.35) | 2.25, 2.26 | 7.06 (d, 4H), 7.14 (d, 2H), 7.32 (d, 2H) | |
| **14** | | 12.41 | 8.12 | | (4.42) | 2.50[d] | | 7.28–7.35 (m) | 1.27–1.70 [2 m, $CH_2(CH_2)_3CH_2O$], 2.64 [t, C-8-$CH_2(CH_2)_3$], 3.40 [t, $(CH_2)_3CH_2O$] |
| 15 | | *c* | 7.88 | | (4.42) | (4.28) | 2.24 | 7.28–7.35 (m, 7H), 7.08 (d, 2H) | 1.30–1.72 [2 m, $CH_2(CH_2)_3CH_2O$], 2.67 [t, C-8-$CH_2(CH_2)_3$], 3.41 [t, $(CH_2)_3CH_2O$] |
| 16 | | 12.09 | | | | (4.37) | | 7.22–7.50 (m) | 2.21 (8-Me), 2.60 (6-Me) |
| **17** | | 12.00 | | | | (4.41) | 2.26 | 7.11 (d), 7.32 (d) | 2.20 (8-Me), 2.60 (6-Me) |
| 18 | | | 8.38 | 8.18 | 4.11 | 2.76 | 2.23 | 7.04 (d), 7.14 (d) | |
| 20 | | | 8.34 | 8.20 | 4.11 | (4.68) | 2.22, 2.27 | 7.04 (d, 2H), 7.14 (t, 4H) 7.39 (d, 2H) | |

[a] Singlet unless shown otherwise; d, doublet; t, triplet; m, multiplet. [b] Broad. [c] Not observed. [d] Overlapping with DMSO-$d_5$ signal.

group (**5b** → **19**), such modification in the 2-thio-substituted series (**3b** → **13**) resulted in a significant, over 180-fold, increase of the antiviral potency against respiratory syncytial virus.

The benzyl-substituted imidazo[1,5-*a*]-1,3,5-triazine derivatives had no selective activity against human immunodeficiency virus (HIV-1 or HIV-2) (data not shown). None of the compounds proved inhibitory to HIV-1 or HIV-2 at a concentration that was nontoxic to the host cells, except for compound **5a** that inhibited HIV-1 and HIV-2 replication at an $IC_{50}$ of 20.6 and 17.7 $\mu$M, respectively ($CC_{50}$: 35.1 $\mu$M). The two compounds (**5a,b**) that proved to be the most toxic for MDCK, HeLa, and Vero cells were also the most toxic to the host CEM cells (Table 4) that were used in the anti-HIV assays. Interestingly, compounds **12**–**14** proved markedly cytotoxic to human carcinoma (HeLa) cells (MTC: 10.3–20.3 $\mu$M) and human lymphocyte (CEM) cells but not to simian kidney (Vero) and canine kidney (MDCK) cells. It may be worth exploring the reason for this differential toxicity.

In addition, all the compounds were examined and found inactive at the highest subtoxic concentrations tested (≥100–600 $\mu$M) against herpes simplex virus

**Table 3.** $^{13}C$ NMR Data of Benzyl-Substituted Imidazo[1,5-*a*]-1,3,5-triazine Derivatives (Chemical shifts ($\delta_c$, ppm) in DMSO-$d_6$-TMS)

| compd | C-2 | C-4 | C-6 | C-8 | C-8a | C-$CH_2$Ar (O-$CH_2$Ar) | S-Me (S-$CH_2$Ar) | Ar-4-Me | Ar | other signals |
|---|---|---|---|---|---|---|---|---|---|---|
| **3a**[a] | 171.63 | 141.22 | 127.33 | 119.82 | 124.97 | 30.69 | | | 139.94, 128.40, 128.04, 125.75 | |
| **3b** | 171.53 | 141.21 | 127.27 | 120.02 | 124.84 | 30.23 | | 20.49 | 136.84, 134.62, 128.57, 128.26 | |
| **3d** | 171.43 | 141.19 | 126.83 | 121.52 | 124.29 | (71.78) | | | 138.70, 128.08, 127.21, 127.08 | 69.62, 28.98, 28.60, 25.14, 24.70 [$(CH_2)_5$] |
| **4a** | 139.95 | 143.50 | 126.57 | 130.67 | 132.85 | 31.69 | | | 141.22, 128.40, 128.10, 125.79 | |
| **4b**[a] | 139.95 | 143.50 | 126.47 | 130.87 | 132.76 | 31.27 | | 20.48 | 136.89, 134.68, 128.64, 128.27 | |
| **4c** | 139.49 | 145.10 | 137.78 | 128.32 | 132.97 | 31.52 | | | 140.13, 128.48, 128.11, 125.76 | 15.92 (6-Me) |
| **5a** | 137.29 | 168.34 | 128.65 | 128.70 | 131.46 | 31.68 | | | 139.70, 128.47, 128.14, 125.88 | |
| **5b** | 142.44 | 170.13 | 126.51 | 129.04 | 130.69 | 31.45 | | 20.48 | 137.42, 134.48, 128.58, 128.23 | |
| **6a** | 148.38 | 151.20 | 122.13 | 128.84 | 134.71 | 31.81 | | | 140.13, 128.40, 128.11, 125.79 | 56.17 (O-Me) |
| **6b** | 148.22 | 151.79 | 122.15 | 129.05 | 134.55 | 31.59 | | 20.48 | 137.00, 134.69, 128.64, 128.24 | 56.14 (O-Me) |
| **7a** | 148.92 | 149.47 | 124.52 | 126.23 | 135.63 | 31.65 | | | 140.59, 128.34, 128.03, 125.63 | 39.87 ($NMe_2$) |
| **7b** | 148.85 | 149.44 | 124.46 | 126.46 | 135.54 | 31.23 | | 20.48 | 137.52, 134.49, 128.56, 128.22 | 39.85 ($NMe_2$) |
| **12** | 160.72 | 147.74 | 122.54 | 124.11 | 135.96 | 31.63 | 12.98 | 20.50 | 138.22, 134.19, 128.46, 128.27 | |
| **13**[a] | 160.80 | 148.15 | 122.28 | 123.87 | 136.11 | 31.70 | (33.39) | 20.50, 20.58 | 135.57, 134.15, 128.46, 128.26, 138.37, 135.98, 128.77, 128.63 | |
| **14** | 162.01 | 148.86 | 121.59 | 124.22 | 136.22 | (71.70) | 13.00 | | 138.69, 128.10, 127.28, 127.16 | 69.65, 29.01, 28.87, 25.87, 25.33 [$(CH_2)_5$] |
| **15** | 157.57 | 147.11 | 122.96 | 125.97 | 134.87 | (71.69) | (33.49) | 20.59 | 135.84, 128.08, 127.24, 127.15, 138.66, 135.47, 128.86, 128.68 | 69.62, 29.01, 28.83, 25.81, 25.32 [$(CH_2)_5$] |
| **16** | 148.72 | 144.78 | 136.74 | 122.32 | 132.42 | | (33.58) | | 137.10, 129.10, 128.28, 127.19 | 15.62 (6-Me), 11.08 (8-Me) |
| **17** | 150.02 | 145.34 | 136.23 | 121.66 | 132.82 | | (33.38) | 20.58 | 136.32, 134.10, 129.99 128.81 | 15.68 (6-Me), 11.13 (8-Me) |
| **18** | 145.69 | 157.90 | 123.07 | 129.67 | 131.54 | 31.25 | 12.48 | 20.47 | 136.87, 134.74, 128.67, 128.26 | |
| **19**[a] | 145.65 | 156.85 | 123.16 | 129.75 | 131.63 | 31.25 | (33.30) | 20.47, 20.61 | 136.84, 134.74, 128.67, 128.25, 137.02, 132.61, 129.10, 129.06 | |

[a] Assignments on the basis of proton-coupled spectra.

**Table 4.** Activity against Ortho- and Paramyxoviruses and Cytotoxicity of Benzyl-Substituted Imidazo[1,5-*a*]-1,3,5-triazine Derivatives

| | $IC_{50}$,[a] $\mu$M | | | | MTC,[b] $\mu$M, 5 day | | | $CC_{50}$,[c] $\mu$M, 4 day |
|---|---|---|---|---|---|---|---|---|
| compd | influenza virus A, MDCK | influenza virus B, MDCK | respiratory syncytial virus, HeLa | parainfluenza-3 virus, Vero | MDCK | HeLa | Vero | CEM |
| **3a** | 54.2 | ≥581.3 | >775.1 | >775.1 | >775.1 | >775.1 | 581.3 | 430.2 ± 96.1 |
| **3b** | 54.4 | ≥551.4 | 257.3 | >735.2 | >735.2 | ≥735.2 | ≥735.2 | >735.2 |
| **3d** | 174.4 | >290.6 | 23.2 | ND[d] | 290.6 | 87.2 | ND[d] | 258.7 ± 93.0 |
| **4a** | >884.9 | >884.9 | 663.7 | >884.9 | >884.9 | >884.9 | 884.9 | 632.7 ± 358.4 |
| **4b** | >833.3 | >833.3 | ≥833.3 | >833.3 | >833.3 | ≥833.3 | 625.0 | >833.3 |
| **4c** | 625.0 | >833.3 | >416.6 | >416.6 | ≥833.3 | 416.6 | 416.6 | 262.5 ± 87.5 |
| **5a** | 4.9 | 3.3 | 3.3 | 33.0 | 16.5 | 16.5 | 24.7 | 35.1 ± 15.7 |
| **5b** | 2.7 | 0.6 | 0.6 | 6.2 | 3.1 | 6.2 | 4.6 | 32.8 ± 12.8 |
| **7a** | >790.5 | >790.5 | 395.3 | >790.5 | >790.5 | >790.5 | >790.5 | 494.0 ± 59.2 |
| **7b** | ≥749.0 | >749.0 | >749.0 | >374.5 | >749.0 | >749.0 | 374.5 | 411.9 ± 130.7 |
| **8a** | >1098.9 | >1098.9 | >549.5 | >1098.9 | >1098.9 | 549.2 | 1098.9 | 785.7 ± 221.9 |
| **8b** | 510.2 | >1020.4 | >510.2 | >1020.4 | >1020.4 | 510.2 | 1020.4 | 525.5 ± 239.7 |
| **9a** | 180.7 | 240.9 | >602.4 | >602.4 | 602.4 | 602.4 | 602.4 | 789.1 ± 401.8 |
| **9b** | 222.2 | 833.3 | >555.5 | >555.5 | >1111.1 | 555.5 | 555.5 | ≥694.4 |
| **10a** | 22.2 | 22.2 | 111.1 | ≥222.2 | 111.1 | 555.5 | 111.1 | 176.1 ± 90.5 |
| **10b** | 103.0 | 103.0 | >515.4 | >515.4 | 515.4 | 515.4 | 515.4 | 439.6 ± 110.8 |
| **11a** | >1342.2 | >1342.2 | >1342.2 | >671.1 | >1342.2 | >1342.2 | 671.1 | 745.8 ± 95.5 |
| **11b** | >1104.9 | >1104.9 | >1104.9 | >1104.9 | >1104.9 | >1104.9 | >1104.9 | 115.2 ± 44.0 |
| **12** | 27.1 | 67.7 | 4.0 | 135.5 | 67.7 | 20.3 | ≥338.9 | 75.3 ± 3.3 |
| **13** | 4.1 | 51.9 | 1.4 | >103.8 | 259.7 | 10.3 | >259.7 | 44.6 ± 25.1 |
| **14** | 11.1 | 55.8 | 2.2 | >27.9 | 55.8 | 11.1 | >111.7 | 120.5 ± 73.6 |
| **15** | 5.3 | 223.2 | 2.6 | >22.3 | 223.2 | 44.6 | ≥89.2 | 342.6 ± 87.4 |
| **16** | 69.9 | 699.3 | 20.9 | >139.8 | ≥699.3 | ≥524.4 | ≥349.7 | 266.0 ± 45.9 |
| **17** | 65.6 | >328.0 | 15.7 | >131.3 | 328.0 | 328.0 | 328.0 | 88.8 ± 51.8 |
| **18** | 14.8 | 74.0 | 22.2 | >148.1 | 74.0 | 74.0 | ≥370.3 | 388.8 ± 77.7 |
| **19** | >277.7 | >277.7 | ≥277.7 | >27.7 | 277.7 | 277.7 | ≥111.1 | |
| amantadine | 660.9 | >1321.8 | >660.9 | ND[d] | >660.9 | 660.9 | ND[d] | |
| ribavirin | 4.9 | 16.3 | 11.4 | 286.8 | >819.6 | >819.6 | >1639.2 | |

[a] Inhibitory concentration required to reduce virus-induced cytopathicity by 50%; virus-induced cytopathicity was recorded the day CPE reached its maximum. [b] Minimum toxic concentration required to cause a microscopically detectable alteration of normal cell morphology. The results listed are the mean values of two or three independent determinations. [c] 50% cytotoxic concentration or concentration required to inhibit CEM cell proliferation by 50%. [d] Not determined.

[HSV-1 (strain KOS), HSV-2 (strain G), thymidine kinase-deficient ($TK^-$) HSV-1 (strain B2006)], vesicular stomatitis virus, and vaccinia virus in human embryonic skin-muscle (ESM) fibroblasts, polio type 1 and Coxsackie type B24 virus in HeLa cells, and Sindbis virus, Semliki forest virus, and reovirus type 1 in Vero cells (data not shown).

## Conclusion

The 2-(benzylthio)- and 2-(4-methylbenzyl)thio-substituted imidazo[1,5-*a*]-1,3,5-triazine derivatives **13** and **15**–**17** could be considered as potential "lead" compounds against influenza A and/or respiratory syncytial virus.

## Experimental Section

**Chemical Procedures.** Melting points were determined on a Laboratory Devices Mel-Temp II micromelting point apparatus in open capillaries and are uncorrected. UV spectra were measured on a Shimadzu UV 160 spectrophotometer. $^1H$ and $^{13}C$ NMR spectra were recorded with a Varian Unity 300 FT NMR spectrometer operating at 299.949 and 75.429 mHz, respectively. Chemical shifts are expressed in $\delta$ values (ppm) relative to tetramethylsilane as an internal standard. Electron impact mass spectra were obtained at 70 eV on a JEOL JMS-D-100 spectrometer. Analytical TLC and preparative layer chromatography (PLC) were conducted on Merck precoated silica gel $F_{254}$ type 60 plates of layer thickness 0.25 and 2.0 mm, respectively. For preparative short-column chromatography, Merck TLC silica gel $HF_{254}$ type 60 was used. Chloroform–methanol mixtures (measured by volume) were used as chromatographic solvent systems: A (4:1), B (9:1), C (12:1), D (95:5). Elemental analyses were performed by Microanalytical Laboratories of the Institute of Organic Chemistry of the Polish Academy of Sciences in Warsaw, Poland; the results are within 0.4% of the theoretical values unless stated otherwise.

The compounds **3c**,[3] **8a,b**,[2] and **9a,b–11a,b**[4] have been fully described previously.

Starting 2-cyano-2-(formylamino)-2-R-substituted ethyl acetates **1a,b,d** were prepared by reaction of the appropriate bromides with ethyl 2-cyano-2-(formylamino)acetate according to the procedure used previously to obtain ethyl 2-cyano-2-(formylamino)propionate.[2]

**Ethyl 2-cyano-2-(formylamino)-3-phenylpropionate (1a)**: yield 65%; mp 86–87 °C (benzene); $^1H$ NMR (DMSO-$d_6$) $\delta_H$ 1.04 (t, 3H, $CH_3CH_2OCO$), 3.34 (s, 2H, $CH_2Ar$), 4.06 (q, 2H, $CH_3CH_2OCO$), 7.27–7.37 (2m, 5H, Ar), 8.17 (s, 1H, HCO), 9.57 (s, 1H, NH).

**Ethyl 2-cyano-2-(formylamino)-3-(4-methylphenyl)-propionate (1b)**: yield 70%; mp 103–104 °C (benzene); $^1H$ NMR (DMSO-$d_6$) $\delta_H$ 1.07 (t, 3H, $CH_3CH_2O$), 2.29 (s, 3H, Ar-4-Me), 3.34 (s, 2H, $CH_2Ar$), 4.08 (q, 2H, $CH_3CH_2OCO$), 7.16 (s, 4H, Ar), 8.16 (s, 1H, HCO), 9.52 (s, 1H, NH).

**Ethyl 2-cyano-2-(formylamino)-7-(benzyloxy)heptanoate (1d)**: yield 70%, oil; $^1H$ NMR ($CDCl_3$) $\delta_H$ 1.25 (t, 3H, $CH_3CH_2OCO$), 1.34–1.73 [m, 6H, $CH_2(CH_2)_3CH_2O$], 2.06 [t, 2H, $CH_2(CH_2)_3CH_2O$], 3.44 [t, 2H, $CH_2(CH_2)_3CH_2O$], 4.23 (q, 2H, $CH_3CH_2OCO$), 4.44 (s, 2H, $OCH_2Ar$), 7.29 (s, 5H, Ar), 8.08 (s, 1H, HCO).

**6-Amino-5-benzyl-5-(formylamino)-2-mercaptopyrimidin-4(5*H*)-one (2a).** To a stirred solution of sodium ethoxide prepared from sodium (0.5 g, 22 mmol) and EtOH (anhydrous, 50 mL) was added ester **1a** (4.92 g, 20 mmol) followed by thiourea (1.5 g, 20 mmol). The mixture was stirred at room temperature with exclusion of moisture for 10 h. TLC in solvent A showed that after that period substrates were still present in the reaction mixture, but prolongation of the reaction time resulted only in the degradation of the products. The mixture was adjusted to pH 6 with glacial acetic acid causing partial precipitation of all components. The whole mixture was evaporated, and the residue was chromatographed on a silica gel short column in solvent A. The desired product **2a**, slightly more mobile than unreacted thiourea, crystallized on concentration of the chromatographic solvent (2.2 g, 40%). An analytical sample was obtained after recrystallization from ethanol: mp 220 °C dec; $^1H$ NMR (DMSO-$d_6$) $\delta_H$ 3.17 (s, 2H, $CH_2Ar$), 7.04–7.26 (2m, 5H, Ar), 8.00 (s, 1H, HCO), 8.52 (br, 1H, C-6=NH), 8.99 (br, 1H, N-1H), 9.36 (s, 1H, C-5-NH), 11.42 (br, 1H, N-3H); $^{13}C$ NMR (DMSO-$d_6$) $\delta_c$ 43.37 ($CH_2Ar$), 57.94 (C-5), 127.71, 128.07, 129.64, 131.49 (Ar), 161.09 (HCO), 166.87 (C-4), 168.28 (C-6), 186.47 (C-2). Anal. ($C_{12}H_{12}N_4O_2S$) C, H, N.

When the precipitate obtained on neutralization was washed with cold water followed by cold ethanol, a crystalline side product (**2a'**) (1.7 g, 30%) remained: mp 204 °C dec (EtOH); $^1H$ NMR (DMSO-$d_6$) $\delta_H$ 2.97 (s, 2H, $CH_2Ar$), ca. 3.5 (vbr) 7.06–7.28 (2m, 5H, Ar); $^{13}C$ NMR (DMSO-$d_6$) $\delta_C$ 47.52 ($CH_2Ar$), 59.49 (C-5), 127.22, 127.87, 129.76, 133.50 (Ar), 170.93 (C-4), 171.75 (C-6), 186.60 (C-2); MS *m/z* (rel abundance, 70 eV) 248 ($M^+$, 37), 157 (72), 91 (100). Anal. ($C_{11}H_{12}N_4OS \cdot 0.25H_2O$) C, H, S; N: calcd, 22.17; found, 21.07.

**6-Amino-5-(formylamino)-5-(4-methylbenzyl)-2-mercaptopyrimidin-4(5*H*)-one (2b)**: prepared from compound **1b** (5.20 g, 20 mmol) and thiourea, by the procedure used above for conversion of **1a** into **2a**; yield 2.0 g, 35%, after chromatographic separation (solvent A); mp 235 °C dec (EtOH); $^1H$ NMR (DMSO-$d_6$) $\delta_H$ 2.24 (s, 3H, Ar-4-Me), 3.13 (s, 2H, $CH_2Ar$), 6.93, 7.05 (2d, 4H, Ar), 7.99 (s, 1H, HCO), 8.52 (br, 1H, C-6=NH), 8.96 (br, 1H, N-1H), 9.34 (s, 1H, C-5-NH), 11.42 (br, 1H, N-3H); $^{13}C$ NMR (DMSO-$d_6$) $\delta_C$ 20.63 (Ar-4-Me), 43.01 ($CH_2Ar$), 57.89 (C-5), 128.47, 128.65, 129.52, 136.67 (Ar), 161.09 (HCO), 166.94 (C-4), 168.42 (C-6), 186.52 (C-2). Anal. ($C_{13}H_{14}N_4O_2S \cdot 0.25H_2O$) C, H, N, S.

Side product **2b'** (1.8 g, 31%): mp 160 °C dec (EtOH); $^1H$ NMR (DMSO-$d_6$) $\delta_H$ 2.25 (s, 3H, Ar-4-Me), 2.94 (s, 2H, $CH_2$-Ar), 6.94, 7.05 (2d, 4H, Ar); $^{13}C$ NMR (DMSO-$d_6$) $\delta_C$ 20.60 (Ar-4-Me), 46.95 ($CH_2Ar$), 59.44 (C-5), 128.46, 129.68, 130.50, 136.14 (Ar), 171.21 (C-4), 171.90 (C-6), 186.53 (C-2).

**6-Amino-5-[5'-(benzyloxy)pentyl]-5-(formylamino)-2-mercaptopyrimidin-4(5*H*)-one** (2d): prepared from compound **1d** (0.66 g, 2 mmol) and thiourea by the procedure used above for conversion of **1a** into **2a**; $t_R$ 24 h; yield 0.170 g, 24%, after chromatographic separation (solvent A) and recrystallization from methanol; mp 213 °C dec; $^1H$ NMR (DMSO-$d_6$) $\delta_H$ 1.27–1.70 [2m, 6H, $CH_2(CH_2)_3CH_2O$], 1.81 [t, 2H, C-5-$CH_2$-$(CH_2)_3$], 3.88 [t, 2H, $(CH_2)_3CH_2O$], 4.43 (s, 2H, $OCH_2Ar$), 7.28–7.35 (m, 5H, Ar), 7.96 (s, 1H, HCO), 8.51 (br, 1H, C-6=NH), 9.01 (br 1H, N-1H), 9.18 (s, 1H, C-5-NH), 11.66 (br, 1H, N-3H); $^{13}C$ NMR (DMSO-$d_6$) $\delta_C$ 22.10, 25.24, 28.12 [$CH_2(CH_2)_3CH_2O$], 37.70 [$CH_2(CH_2)_3CH_2O$], 57.22 (C-5), 69.24 [$CH_2(CH_2)_3CH_2O$], 71.73 ($OCH_2Ar$), 127.22, 128.08, 138.59 (Ar), 161.07 (HCO), 167.03 (C-4), 169.58 (C-6), 186.92 (C-2). Anal. ($C_{17}H_{22}N_4O_3S$) C, H, N, S.

**8-Benzyl-2-thioxo-2,3-dihydroimidazo[1,5-*a*]-1,3,5-triazin-4(1*H*)-one (3a).** Compound **2a** (2.76 g, 10 mmol) dissolved in dry pyridine (50 mL) and TMSCl (2.20 g, 20 mmol) were stirred together at room temperature for 30 min. HMDS (4.2 mL, 20 mmol) was then added, and the mixture was refluxed under dry nitrogen for 10 min. The volatiles were removed in vacuo, and the residue was coevaporated once with anhydrous EtOH (30 mL). The remaining material was dissolved in anhydrous EtOH (20 mL) and kept in the refrigerator for 1 h. The precipitate which crystallized was filtered and recrystallized from EtOH (anhydrous) to give **3a** (2.30 g, 90%): mp 225 °C dec. Anal. ($C_{12}H_{10}N_4OS$) C, H, N, S.

**8-(4-Methylbenzyl)-2-thioxo-2,3-dihydroimidazo[1,5-*a*]-1,3,5-triazin-4(1*H*)-one (3b)**: prepared from compound **2b** (2.90 g, 10 mmol) by the procedure used above for conversion of **2a** into **3a** (2.10 g, 80%); mp 230 °C dec (EtOH anhydrous). Anal. ($C_{13}H_{12}N_4OS$) C, H, N, S.

**8-[5-(Benzyloxy)pentyl]-2-thioxo-2,3-dihydroimidazo-[1,5-*a*]-1,3,5-triazin-4(1*H*)-one (3d)**: prepared from compound **2d** (0.362 g, 1 mmol) by the procedure used above for conversion of **2a** into **3a** (0.270 g, 80%); mp 195 °C (MeOH or EtOH). Anal. ($C_{17}H_{20}N_4O_2S$) C, H, N, S.

**8-Benzylimidazo[1,5-*a*]-1,3,5-triazin-4(3*H*)-one (4a).** A solution of compound **3a** (0.258 g, 1 mmol) in dilute aqueous ammonia (0.5 mL or 25% aqueous $NH_3$ in 5 mL of $H_2O$) was treated with moiset Raney nickel catalyst (0.5 g) at 100 °C for 10 min. Raney Ni was filtered when the mixture was still warm, and the filtrate was concentrated on a Rotavap to ca. 1 mL which made the product **4a** partly crystallize. The crystals (0.06 g) were collected by filtration, the remaining solution was evaporated to dryness, and the residue was chromatographed on a silica gel short column (solvent A). The faster moving fractions afforded an additional 0.085 g of **4a** making a total of 0.145 g (65%): mp >260 °C dec ($CHCl_3$–MeOH, 4:1). Anal. ($C_{12}H_{10}N_4O$) C, H, N. The slower moving fractions contained traces of a degradation product, a derivative of imidazole.

**8-(4-Methylbenzyl)imidazo[1,5-*a*]-1,3,5-triazin-4(3*H*)-one (4b)**: prepared from compound **3b** (0.272 g, 1 mmol) by the procedure used above for conversion of **3a** into **4a** (0.145 g, 60%); mp >245 °C dec ($CHCl_3$–MeOH; 4:1, or MeOH). Anal. ($C_{13}H_{12}N_4O$) C, H, N.

**8-Benzyl-6-methylimidazo[1,5-*a*]-1,3,5-triazin-4(3*H*)-one (4c)**: prepared from compound **3c** (0.272 g, 1 mmol) by the procedure used above for conversion of **3a** into **4a** (0.095 g, 40%); mp >200 °C dec (MeOH). Anal. ($C_{13}H_{12}N_4O$) C, H, N. The amount of a degradation product was larger than for **4a,b** (15–20%).

**8-Benzylimidazo[1,5-*a*]-1,3,5-triazine-4(3*H*)-thione (5a).** Compound **4a** (0.226 g, 1 mmol) in boiling anhydrous xylene (20 mL, partly dissolved, partly suspended) was treated for 48 h at reflux with 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson reagent) (0.404 g, 1 mmol, in two portions, the second one after 24 h of heating). The mixture was then evaporated under reduced pressure; the residue was dissolved in a small amount of EtOH and separated by PLC (solvent C). The desired product was extracted with MeOH from the faster moving band (0.085 g, 35%): yellow crystals after recrystallization from *i*-PrOH; mp 200 °C dec. Anal. ($C_{12}H_{10}N_4S$) C, H, N, S. The slower moving band afforded starting material **4a** (0.100 g, 40% recovery). Attempts to improve yield by longer reaction and/or excess of reagent were unsuccessful.

**8-(4-Methylbenzyl)imidazo[1,5-*a*]-1,3,5-triazine-4(3*H*)-thione (5b).** Thiation of **4b** (0.240 g, 1 mmol) was carried out as described above for the preparation of **5a**. Starting material was well-soluble in xylene, and after 48 h the reaction was completed. The title compound purified by short-column chromatography (solvent D) precipitated as yellow crystals upon concentration of appropriate fractions (0.215 g, 85%): mp 218 °C dec (*i*-PrOH). Anal. ($C_{13}H_{12}N_4S$) C, H, N, S.

**8-Benzyl-4-methoxyimidazo[1,5-*a*]-1,3,5-triazine (6a).** Methyl iodide (1 mL, 16 mmol) was added to a stirred solution of compound **5a** (0.242 g, 1 mmol) in methanol (20 mL) in the presence of potassium carbonate (0.200 g, 1.4 mmol). After stirring at room temperature for 5 min, TLC (solvent C) indicated complete disappearance of **5a** with the formation of two products migrating close to each other. After 30 min, the product migrating faster (4-S-Me derivative) was completely transformed into the slower migrating one (4-O-Me derivative **6a**). It was isolated from the reaction mixture by PLC. Solvents used for developing ($CHCl_3$–MeOH, 12:1) and extraction of the product from silica gel (MeOH) had to be strictly anhydrous. Evaporation of MeOH gave **6a** as a white hygroscopic gum (0.165 g, 65%; unstable).

**4-Methoxy-8-(4-methylbenzyl)imidazo[1,5-*a*]-1,3,5-triazine (6b)**: prepared from compound **5b** (0.256 g, 1 mmol) by the procedure used above for conversion of **5a** into **6a**; white hygroscopic gum (0.140 g, 60%); unstable.

**8-Benzyl-4-(dimethylamino)imidazo[1,5-*a*]-1,3,5-triazine (7a).** Compound **6a** (0.120 g, 0.5 mmol) was treated with an anhydrous saturated methanolic solution of dimethylamine (10 mL) and stirred at room temperature for 5 min. The solution was concentrated in vacuo at room temperature, applied on a PLC plate, and developed with solvent C. The desired product was extracted with methanol from the fastest moving band. The extract after evaporation gave **7a** as a white solid (0.063 g, 50%): mp 156 °C (anhydrous *i*-PrOH). Anal. ($C_{14}H_{15}N_5$) C, H, N.

**4-(Dimethylamino)-8-(4-methylbenzyl)imidazo[1,5-*a*]-1,3,5-triazine (7b)**: prepared from compound **6b** (0.127 g, 0.5 mmol) by the procedure used above for conversion of **6a** into **7a** (0.080 g, 60%); mp 138 °C (anhydrous *i*-PrOH). Anal. ($C_{15}H_{17}N_5$) C, H, N.

**General Procedure for the 2-S Alkylation and Aralkylation of 8-Substituted and 6,8-Disubstituted 2-Thioxo-2,3-dihydroimidazo[1,5-*a*]-1,3,5-triazin-4(1*H*)ones.** To a stirred solution of 2-thioxo-2,3-dihydroimidazo[1,5-*a*]-1,3,5-triazin-4(1*H*)-one (1 mmol) in methanol (10 mL) was added potassium carbonate (200 mg, 1.4 mmol). The mixture was stirred for 5 min at room temperature, and the corresponding halide (1–3 mmol) was added. The reaction mixture was stirred for 5–30 min, up to the moment when TLC in appropriate solvent indicated complete monosubstitution reaction. Workup procedure differed for individual compounds.

**8-(4-Methylbenzyl)-2-(methylthio)imidazo[1,5-*a*]-1,3,5-triazin-4(3*H*)-one (12).** Compound **3b** (0.272 g, 1 mmol) and methyl iodide (0.18 mL, 3 mmol) were reacted according to the general procedure for 15 min. The residue obtained upon concentration of the reaction mixture in vacuo was purified on a silica gel column with solvent D as eluent to give **12** (0.240 g, 85%): mp 220 °C dec (*i*-PrOH). Anal. ($C_{14}H_{14}N_4OS{\cdot}0.5H_2O$) C, S; H: calcd, 5.08; found, 4.67. N: calcd, 18.98; found, 18.19.

**8-(4-Methylbenzyl)-2-[(4-methylbenzyl)thio]imidazo-[1,5-*a*]-1,3,5-triazin-4(3*H*)-one (13).** Compound **3b** (0.272 g, 1 mmol) and 4-methylbenzyl bromide (0.185 g, 1 mmol) were reacted according to the general procedure for 5 min, $H_2O$ (1 mL) was added to the reaction mixture upon stirring. The resulting precipitate was collected on a filter and dried in vacuo to give **13** (0.150 g, 40%). The fitrate was evaporated to dryness. The residue was purified on a silica gel column (solvent B) to give an additional portion of **13** (0.170 g) making a total of 0.320 g (85%): mp 205 °C dec (*i*-PrOH). Anal. ($C_{21}H_{20}N_4OS{\cdot}0.5H_2O$) C, H, N. S.

**8-[5-(Benzyloxy)pentyl]-2-(methylthio)imidazo[1,5-*a*]-1,3,5-triazin-4(3*H*)-one (14).** Compound **3d** (0.172 g, 0.5 mmol) and methyl iodide (0.09 mL, 1.5 mmol) were reacted according to the general procedure for 10 min. The residue obtained upon concentration of the reaction mixture in vacuo was purified by PLC on silica gel with solvent C. The title product was extracted from the gel with MeOH. The foam obtained after evaporation of MeOH was dissolved in EtOH; the solution was concentrated on a Rotavap at room temperature until the first crystals appeared and then kept in a refrigerator for 2 h to give the desired compound (0.140 g, 80%); mp 136 °C (EtOH). Anal. ($C_{18}H_{22}N_4O_2S$) C, H, S; N: calcd, 15.64; found, 14.99.

**8-[5-(Benzyloxy)pentyl]-2-(4-methylbenzyl)thioimidazo-[1,5-*a*]-1,3,5-triazin-4(3*H*)-one (15).** Compound **3d** (0.172 g, 0.5 mmol) and 4-methylbenzyl bromide (0.093 g, 0.5 mmol) were reacted according to the general procedure for 10 min. The workup of the reaction mixture was analogous to that given above for **14** and gave the title compound (0.400 g, 90%): mp 142 °C (MeOH or EtOH). Anal. ($C_{25}H_{28}N_4O_2S$) C, H, S; N: calcd, 12.50; found, 11.48.

**2-(Benzylthio)-6,8-dimethylimidazo[1,5-*a*]-1,3,5-triazin-4(3*H*)-one (16).** Compound **8b** (0.196 g, 1 mmol) and benzyl bromide (0.12 mL, 1 mmol) were reacted according to the general procedure for 30 min. When the reaction mixture was kept in an open flask for 5–10 min, the desired product **16** precipitated (0.140 g). Concentration of the mother liquors gave an additional 0.120 g of **16**, making a total of 0.260 g (90%): mp >250 °C dec (MeOH–$H_2O$, 95:5, or *i*-PrOH). Anal. ($C_{14}H_{14}N_4OS$) C, H, N, S.

**6,8-Dimethyl-2-[(4-methylbenzyl)thio]imidazo[1,5-*a*]-1,3,5-triazin-4(3*H*)-one (17).** Compound **8b** (0.196 g, 1 mmol) and 4-methylbenzyl bromide were reacted according to the general procedure for 10 min. The desired product **17** precipitated during the reaction (0.160 g). Concentration of the mother liquors gave an additional 120 mg of **17**, making a total of 280 mg (94%): mp >230 °C dec (MeOH–$H_2O$, 95:5, or *i*-PrOH). Anal. ($C_{15}H_{16}N_4OS{\cdot}0.25H_2O$) C, H, N. S.

**8-(4-Methylbenzyl)-4-(methylthio)imidazo[1,5-*a*]-1,3,5-triazine (18).** To a stirred solution of compound **5b** (0.256 g, 1 mmol) in 2-propanol (20 mL) was added potassium carbonate (0.200 g, 1.4 mmol) followed by methyl iodide (0.24 mL, 4 mmol). The reaction mixture was stirred at room temperature for 16 h, after which time it contained homogeneous product (TLC). It was purified by PLC with solvent C as the developing solvent and ethanol for the extraction from the gel to afford the title compound (0.210 g, 80%): mp 123 °C (*i*-PrOH). Anal. ($C_{14}H_{14}N_4S$) C, H, N, S.

**8-(4-Methylbenzyl)-4-[(4-methylbenzyl)thio]imidazo-[1,5-*a*]-1,3,5-triazine (19).** To a stirred solution of compound **5b** (0.256 g, 1 mmol) in 2-propanol (20 mL) was added potassium carbonate (0.200 g, 1.4 mmol) followed by 4-methylbenzyl bromide (0.220 g, 1.2 mmol). The reaction was completed after 10 h of stirring at room temperature. Purification analogous to that for **18** afforded the title compound (0.250 g, 70%): mp 146 °C (*i*-PrOH). Anal. ($C_{21}H_{20}N_4S$) C, H, N, S.

**Antiviral Activity and Cytotoxicity Assays.** These were carried out according to well-established procedures.[12–17] For all antiviral activity assays, except for the anti-HIV activity

assays, antiviral activity measurements were based on the inhibition of virus-induced cytopathicity, which was scored microscopically when in the control (untreated) virus-infected cell cultures the cytopathic effect (CPE) had reached 100%. For the anti-HIV activity assays, CEM cell cultures were suspended at 400 000 cells/mL of culture medium and infected with HIV-1($III_B$) or HIV-2(ROD) strains at 100 $CCID_{50}$/mL. Then, 100 $\mu$L of the infected cell suspension was transferred to 200-$\mu$L plate wells containing 100 $\mu$L of serially diluted test compound solutions. After 4 days of incubation at 37 °C, cell cultures were assessed for syncytium formation as described.[15]

Cytotoxicity tests were based on microscopic evaluation of cell morphology [confluent MDCK (Madin–Darby canine kidney), HeLa (human epithelial carcinoma), and Vero (African green monkey kidney) cells] and viability of proliferating CEM (human T-lymphocytes) cells. For cytotoxicity measurements in MDCK, HeLa, and Vero cells, cytotoxicity of the test compounds was scored in parallel with viral CPE and monitored by the microscopically detectable alteration of the morphology of mock-infected cell cultures following treatment with the compounds. The inhibitory effects of the test compounds on cell proliferation (viability) were determined as follows: 100 $\mu$L of a CEM cell culture containing 40 000 cells was added to 200-$\mu$L plate wells containing 100 $\mu$L of serially diluted test compound solutions. After 4 days of incubation at 37 °C, the number of viable cells was counted with an automated Coulter counter (Coulter Electronics, Howspenden Hertz, U.K.). To this end, the 200-$\mu$L cell suspensions were further suspended in 20 mL of Diluid (J.T. Baker, Deventer, The Netherlands) before being counted.

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