Med Chem Res (2012) 21:703–710
DOI 10.1007/s00044-011-9574-8

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Synthesis and antiviral activity of benzimidazolyl- and triazolyl-1,3,5-triazines

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Received: 4 September 2010 / Accepted: 17 January 2011 / Published online: 4 February 2011


**Abstract** A novel series of 1,3,5-triazine analogs was successfully synthesized through conjugation with benzimidazole or 1,2,4-triazole derivatives via a methylenethio linker. The new analogs were in vitro evaluated against HSV-1 in Vero cells; among these analogs, two compounds exhibited good effect in inhibiting HSV-1 replication (for compound **5p**: $EC_{50} = 3.5$ μg/ml, SI = 358; for compound **5r**: $EC_{50} = 5.0$ μg/ml, SI = 300) in comparison to acyclovir.

**Keywords** 1,3,5-Triazines · Benzimidazoles · 1,2,4-Triazoles · Antiviral · Anti-HSV-1

## Introduction

Substituted 1,3,5-triazine derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activity. Many of these compounds have proved to be active as anticancer (Kumar *et al*., 2010), antitumor (Brzozowski *et al*., 2000; Brzozowski and Saczewski 2002), antimicrobial (Zhou *et al*., 2008; Srinivas *et al*., 2006), antimalarial (Melato *et al*., 2008), antiepileptic (Ma *et al*., 2009), and antiviral (Xiong *et al*., 2008) agents. In addition, these compounds are used in the textile, plastic, and rubber industries. Also, they are used as pesticides, dyestuffs, and surface active agents (Blotny, 2006).

On the other hand, benzimidazole and 1,2,4-triazole moieties are a common structural subunit in a large number of both natural products and synthetic compounds. Compounds containing benzimidazole nucleus exhibit antifungal (Arjmand *et al*., 2005), anticancer (Ram *et al*., 1992; Mann *et al*., 2001), anti-inflammatory (Mader *et al*., 2008), and antiviral (Cheng *et al*., 2005; Garuti *et al*., 2000) activities. 1,2,4-Triazole nucleus is also present in various molecules with diverse pharmacological properties, such as antimicrobial (Padmavathi *et al*., 2008), analgesic, anti-inflammatory (Sakya *et al*., 2008), anticancer (Ding *et al*., 1997), antioxidant (Kuş *et al*., 2008), and antiviral (Küçükgüzel *et al*., 2008; Saito *et al*., 2003) activities.

During the last decade, the study of the biological activities of triazine derivatives has been the aim of many researchers, for instance, diaryltriazine analogs are a class of interesting compounds exhibit antiviral activity (Thakur *et al*., 2006; Xiong *et al*., 2008). Also, the structure activity relationships of heteroaryl-triazines have revealed that the presence of substituted heteroaryl derivatives is an essential feature of their pharmacological action. Moreover, sulfur atom plays an important role in the life processes due to its soft nucleophilicity and reversible redox properties (Ding *et al*., 1997).

These observations motivated the authors to prepare new hybridized molecules containing 1,3,5-triazine coupled with benzimidazole or triazole via a methylenethio linker in an attempt to attain potential active lead compounds possessing antiviral activity.

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## Results and discussion

### Chemistry

A convergent synthesis was designed for the preparation of compounds (**5a**–**r**). The first part of this synthesis involved the preparation of 1*H*-benzo[*d*]imidazole-2(3*H*)-thiones (**1a**, **b**) (Mavrova *et al*., 2005) and 1*H*-1,2,4-triazole-5(4*H*)-thiones (**2a**, **b**) (Gülerman *et al*., 2001; Bayrak *et al*., 2009a, b) as previously reported (Scheme 1). **1a** and **1b** were prepared by refluxing ethanol–water solution of sodium hydroxide, carbon disulfide, and 4-(un)substituted-1, 2-diaminobenzenes. 1,2,4-Triazoline-3-thione derivatives (**2a**, **b**) were obtained by the cyclization of the thiosemicarbazides, prepared from reaction of aryl hydrazides with phenylisothiocyanate, in alkaline medium. For the synthesis of Schiff bases of thio-4*H*-1,2,4-triazoles (**3a**, **b**) (Rao *et al*., 2005; Cansiz *et al*., 2004; Bayrak *et al*., 2009a, b), these 1-aminotriazoles were reacted with aromatic aldehyde in ethanol, thus two compounds were obtained (Scheme 1).

The second part of this synthesis involved the preparation of chloromethyl-1,3,5-triazines (**4a**–**e**) according to the reported procedure (Scheme 2) (Overberger *et al*., 1957; Brzozowski *et al*., 2000; Ma *et al*., 2009). The arylbiguanide hydrochlorides were prepared by heating equimolar amounts of arylamines and cyanoguanidine in aqueous hydrochloric acid. Condensation reaction between substituted biguanides and ethylchloroacetate in anhydrous methanol at room temperature afforded chloromethyl-1,3,5-triazines (**4a**–**e**).

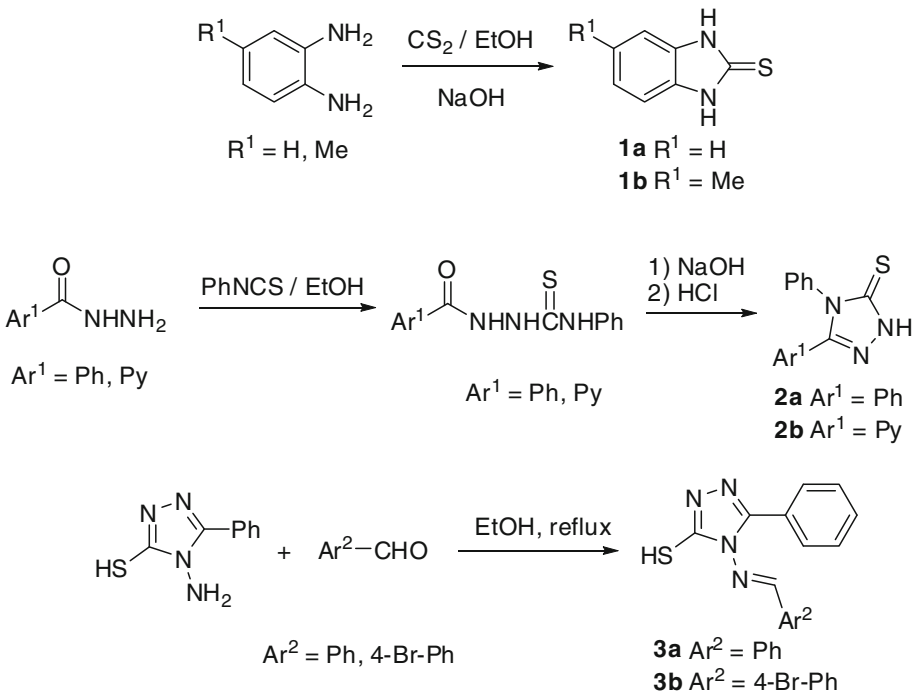
Nucleophilic substitution of a chlorine atom in the second part (**4a**–**e**) with a thiol group in the first part (**1a**, **b**) was carried out in presence of anhydrous $K_2CO_3$ in dry DMF at room temperature to obtain the desired benzimidazole-$SCH_2$-1,3,5-triazine derivatives (**5a**–**j**). 1,2,4-triazole-$SCH_2$-1,3,5-triazine derivatives (**5k**–**r**) were prepared by a similar procedure (Scheme 3). The novel series, 18 synthesized compounds, were purified by recrystallization from appropriate solvents and their physical constants were determined.

The chemical structures of the conjugated products (**5k**–**r**) were identified by spectroscopic methods. For example, the $^1$H NMR spectrum of conjugate **5f** showed two singlets at 2.68 and 4.35 ppm for the $CH_3$ and $SCH_2$ protons, respectively, and a broad singlet at 12.68 ppm for the NH proton in the benzimidazole moiety. Its $^{13}$C NMR spectrum exhibited resonance at 21.7 and 38.3 ppm for the $CH_3$ and $SCH_2$ carbon, respectively, and 149.8 ppm for the -N=C(–N)(–S) carbon and 164.6, 167.2, and 174.1 ppm for 1,3,5-triazine carbons. The mass spectroscopic molecular ion (EI-MS) *m/z*: 363 ($M^+$) observed in the spectra also as well as the elemental analysis data confirm its structure (see "Experimental" section).

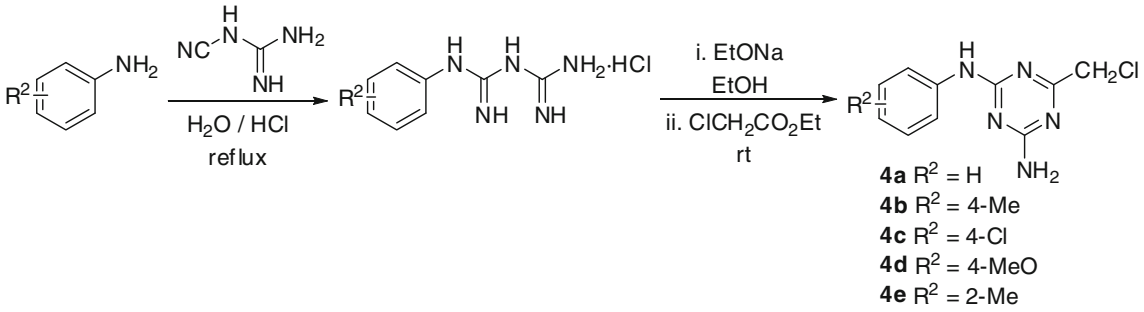
### Biological evaluation

#### *Antiviral activity*

The effective concentrations ($EC_{50}$) of compounds **(5a–r)** required to achieve 50% protection of Vero cells against

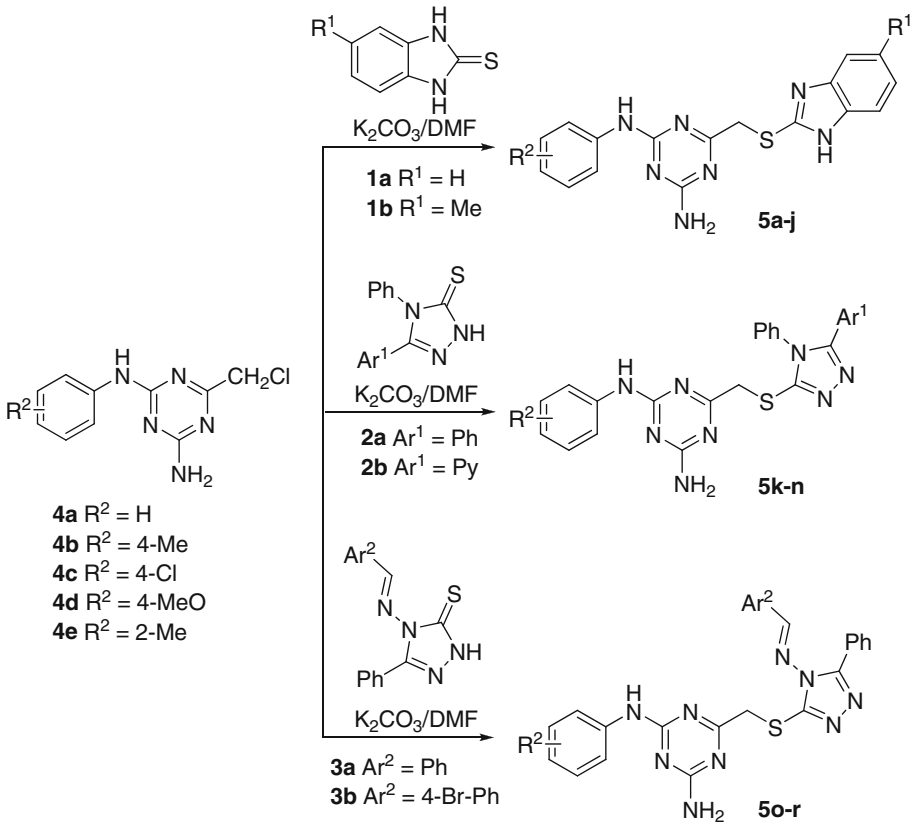



**Scheme 1** Synthesis of benzimidazoles **1a**, **b** and triazoles **2a**, **b** and **3a**, **b**




**Scheme 2** Synthesis of chloromethyl-1,3,5-triazines **4a–e**

**Scheme 3** Synthesis of benzimidazolyl- and triazolyl-1,3,5-triazines **5a–r**




the cytopathic effect of HSV-1 were measured by plaque reduction assay (Boyd *et al.*, 1987; Habbib *et al.*, 2000, 2001).

Standard antiviral agent, acyclovir, was also screened under identical conditions for comparison. Antiviral activities of benzimidazole-1,3,5-triazine derivatives were tested at first (as shown in Table 1), and those of 1,2,4-triazole-1,3,5-triazine derivatives were characterized later (as shown in Table 2). It was observed that the tested compounds (**5a–j**), benzimidazole-triazines, with or without substituent at different positions of the aryl group did not exhibit good antiviral activity ($EC_{50} > 8.5$ μg/ml) against HSV-1 comparable to the reference agent acyclovir ($EC_{50} > 1.5$ μg/ml). However, replacement of benzimidazole moiety with 1,2,4-triazole moiety (**5l–n**) lead to moderate activity ($EC_{50}$ 6.0–7.5 μg/ml). The results show that compound **5n** with *ortho* substitution of methyl group at the aryl ring exhibits better activity than compound **5m** with *para* substitution of methoxy group at the aryl ring.

Improved activity against HSV-1 is observed by the introduction of Schiff base pharmacophore in 1,2,4-triazole at nitrogen atom ($EC_{50}$ values equal to 3.5 and 5.0 μg/ml of **5p** and **5r**, respectively).

**Table 1** Activity of benzimidazole-1,3,5-triazine derivatives against HSV-1 in Vero cells

| Compd. | $R^1$ | $R^2$ | $EC_{50}$ (μg/ml)[a] | $CC_{50}$ (μg/ml)[b] | SI[c] |
|---|---|---|---|---|---|
| **5a** | H | H | >10 | –[d] | –[d] |
| **5b** | H | 4-Me | >10 | – | – |
| **5c** | H | 4-Cl | 8.5 | 1500 | 175 |
| **5d** | H | 4-MeO | >10 | – | – |
| **5e** | H | 2-Me | 9 | >2000 | – |
| **5f** | Me | H | >10 | – | – |
| **5g** | Me | 4-Me | >10 | – | – |
| **5h** | Me | 4-Cl | –[d] | – | – |
| **5i** | Me | 4-MeO | –[d] | – | – |
| **5j** | Me | 2-Me | >10 | – | – |
| Acyclovir | – | – | 1.5 | 1275 | 850 |

[a] Effective concentration required to achieve 50% protection of Vero cells against cytopathic effect of HSV-1

[b] Cytotoxic concentration required to reduce the viability of uninfected Vero cells by 50%

[c] Selectivity index values equal to $CC_{50}/EC_{50}$

[d] Not tested

**Table 2** Activity of 1,2,4-triazole-1,3,5-triazine derivatives against HSV-1 in Vero cells

| Compd | $R^2$ | $Ar^1$ | $Ar^2$ | $EC_{50}$ (μg/ml)[a] | $CC_{50}$ (μg/ml)[b] | SI[c] |
|---|---|---|---|---|---|---|
| **5k** | 4-MeO | Ph | – | >10 | –[d] | –[d] |
| **5l** | 2-Me | Ph | – | 7.5 | 1750 | 234 |
| **5m** | 4-MeO | Py | – | 7 | 1500 | 215 |
| **5n** | 2-Me | Py | – | 6 | 1650 | 275 |
| **5o** | 4-MeO | – | Ph | 8 | 1500 | 188 |
| **5p** | 2-Me | – | Ph | 3.5 | 1250 | 358 |
| **5q** | 4-MeO | – | 4-Br-Ph | –[d] | – | – |
| **5r** | 2-Me | – | 4-Br-Ph | 5 | 1500 | 300 |
| Acyclovir | – | – | – | 1.5 | 1275 | 850 |

[a] Effective concentration required to achieve 50% protection of Vero cells against cytopathic effect of HSV-1

[b] Cytotoxic concentration required to reduce the viability of uninfected Vero cells by 50%

[c] Selectivity index values equal to $CC_{50}/EC_{50}$

[d] Not tested

### *Cytotoxicity*

The cytotoxic activity against Vero cells was examined for only the compounds which showed higher potency against HSV-1 among the tested series (Tables 1, 2). The results revealed that compounds **5p** and **5r** exhibited $CC_{50}$ values of 1250 and 1650 μg/ml and their selectivity index ($SI = CC_{50}/EC_{50}$) were 358 and 300, respectively in comparison with reference agent acyclovir ($CC_{50}$ 1275 μg/ml and SI 850).

## Experimental

### Chemistry

Melting points (°C uncorrected) were determined on *Fisher-Johns* melting point apparatus. IR spectra (KBr) were recorded on Mattson 5000 FTIR spectrophotometer ($\nu$, $cm^{-1}$). $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker 400 MHz spectrometer using DMSO-$d_6$ as a solvent using TMS as an internal standard (chemical shifts in $\delta$, ppm). Mass spectra were recorded on JEOL JMS-600H spectrometer. Elemental analysis was performed at the Microanalytical Center, Cairo University, Egypt.

### *Synthesis of 2-amino-4-arylamino-6-chloromethyl-1,3,5-triazines (**4a–e**)*

To a solution of sodium methoxide in methanol (2.3 g Na and 90 ml methanol), corresponding biguanide hydrochloride (0.1 mol) was added and the reaction mixture was stirred at r.t. for 3 h. Then, ethyl chloroacetate was added dropwise and the reaction mixture was stirred for 48 h. The product that precipitated was collected by filtration, washed with methanol (2 × 5 ml) and water and purified by crystallization from suitable solvent.

### *Synthesis of 2-amino-4-arylamino-6-(benzimidazol-2-ylthio)methyl-1,3,5-triazines (**5a–j**)*

To a mixture of benzimidazole derivatives (0.01 mol) and anhydrous $K_2CO_3$ (1.38 g, 0.01 mol) in anhydrous DMF (10 ml), 6-chloromethyl-1,3,5-triazines (**4a–e**) was added. The reaction mixture was stirred at r.t. for 2 h; then poured on crushed ice. The precipitated product was collected by filtration, washed with water (2 × 5 ml) and purified by recrystallization from suitable solvent.

### *6-[(1H-benzo[d]imidazol-2-ylthio)methyl]-N2-phenyl-1,3,5-triazine-2,4-diamine (**5a**)*

Recrystallization from methanol/DMF gave white solid (212 mg, 61%) of m.p. 130–133°C, IR (KBr): 3570, 3378, 3338, 1629; $^1H$ NMR: 4.38 (s, 2H), 6.95 (d, 1H, $J = 6.8$ Hz), 7.14 (d, 2H, $J = 7.2$ Hz), 7.20 (brs, 4H), 7.40 (d, 1H, $J = 4.4$ Hz), 7.53 (d, 1H, $J = 4.4$ Hz), 7.72 (brs, 2H), 9.57 (s, 1H), 12.66 (s, 1H); $^{13}C$ NMR: 38.3 ($CH_2$), 120.4, 122.6, 123.1, 128.6, 130.8, 140.1, 149.8 (S–C=N), 164.6 (C, triazine), 167.2 (C, triazine), 174.1 (C, triazine); MS (EI) *m/z*: 349 ($M^+$). Anal. Calcd. for $C_{17}H_{15}N_7S$: C, 58.44; H, 4.33; N, 28.06; found: C, 58.56; H, 4.13; N, 28.26.

*6-[(1H-benzo[d]imidazol-2-ylthio)methyl]-N2-p-tolyl-1,3,5-triazine-2,4-diamine (**5b**)*

Recrystallization from ethanol gave white solid (214 mg, 59%) of m.p. 95–98°C; IR (neat): 3568, 3380, 3345, 1625; $^1$H NMR: 2.32 (s, 3H), 4.43 (s, 2H), 7.04 (brs, 2H), 7.13–7.20(m, 4H), 7.47 (brs, 2H), 7.60 (brs, 2H), 9.50 (s, 1H); MS (EI) *m/z*: 363 ($M^+$). Anal. Calcd. for $C_{18}H_{17}N_7S$: C, 59.49; H, 4.71; N, 26.98; found: C, 59.62; H, 4.59; N, 28.84.

*6-[(1H-benzo[d]imidazol-2-ylthio)methyl]-N2-(4-chlorophenyl)-1,3,5-triazine-2,4-diamine (**5c**)*

Recrystallization from DMF gave white solid (218 mg, 57%) of m.p. 118–120°C, $^1$H NMR: 4.39 (s, 2H), 7.13–7.14 (m, 3H), 7.26–7.29 (m, 3H), 7.46 (brs, 2H), 7.74 (brs, 2H), 9.71 (s, 1H), 12.76 (s, 1H); MS (EI) *m/z*: 383 ($M^+$). Anal. Calcd. for $C_{17}H_{14}ClN_7S$: C, 53.19; H, 3.68; N, 25.54; found: C, 53.22; H, 3.59; N, 25.64.

*6-[(1H-benzo[d]imidazol-2-ylthio)methyl]-N2-(4-methoxyphenyl)-1,3,5-triazine-2,4-diamine (**5d**)*

Recrystallization from methanol gave white solid (203 mg, 54%) of m.p. 141–143°C, $^1$H NMR: 3.70 (s, 3H), 4.35 (s, 2H), 6.80 (brs, 2H), 7.12–7.14 (m, 4H), 7.47 (s, 3H), 7.59 (s, 1H), 9.43 (s, 1H), 12.68 (s, 1H); MS (EI) *m/z*: 379 ($M^+$). Anal. Calcd. for $C_{18}H_{17}N_7OS$: C, 56.98; H, 4.52; N, 25.84; found: C, 56.69; H, 4.43; N, 25.56.

*6-[(1H-benzo[d]imidazol-2-ylthio)methyl]-N2-o-tolyl-1,3,5-triazine-2,4-diamine (**5e**)*

Recrystallization from methanol/DMF gave white solid (221 mg, 61%) of m.p. 95–97°C, $^1$H NMR: 2.19 (s, 3H), 4.32 (s, 2H), 7.01 (brs, 3H), 7.07 (d, 1H, $J = 6.8$ Hz), 7.13–7.12 (m, 2H), 7.20 (d, 1H, $J = 6.8$ Hz), 7.32 (d, 1H, $J = 6.8$ Hz), 7.40 (brs, 1H), 7.51 (brs, 1H), 8.91 (s, 1H), 12.68 (s, 1H); MS (EI) *m/z*: 363 ($M^+$). Anal. Calcd. for $C_{18}H_{17}N_7S$: C, 59.49; H, 4.71; N, 26.98; found: C, 59.67; H, 4.61; N, 26.77.

*6-[(5-Methyl-1H-benzo[d]imidazol-2-yl thio)methyl]-N2-phenyl-1,3,5-triazine-2,4-diamine (**5f**)*

Recrystallization from methanol/DMF gave white solid (250 mg, 69%) of m.p. 156–158°C, IR (KBr): 3614, 3390, 3323; $^1$H NMR: 2.68 (s, 3H), 4.35 (s, 2H), 6.95 (d, 1H, $J = 6.8$ Hz), 7.01–7.03 (m, 2H), 7.21–7.24 (m, 4H), 7.34 (d, 1H, $J = 6.8$ Hz), 7.73 (brs, 2H), 9.58 (s, 1H), 12.68 (s, 1H); $^{13}$C NMR: 21.7 ($CH_3$), 38.3 ($CH_2$), 120.4, 122.6, 123.1, 128.6, 130.8, 140.1, 149.8 (S–C=N), 164.6 (C, triazine), 167.2 (C, triazine), 174.1 (C, triazine); MS (EI) *m/z*: 363 ($M^+$). Anal. Calcd. for $C_{18}H_{17}N_7S$: C, 59.49; H, 4.71; N, 26.98; found: C, 59.33; H, 4.84; N, 26.66.

*6-[(5-Methyl-1H-benzo[d]imidazol-2-yl thio)methyl]-N2-p-tolyl-1,3,5-triazine-2,4-diamine (**5g**)*

Recrystallization from methanol gave white solid (219 mg, 58%) of m.p. 120–121°C, IR (KBr): 3614, 3390, 3396; $^1$H NMR: 2.23 (s, 3H), 2.39 (s, 3H), 4.32 (s, 2H), 6.96 (d, 1H, $J = 8.4$ Hz), 6.66–6.77 (m, 2H), 7.22 (brs, 3H), 7.39 (s, 1H), 7.65 (s, 2H), 9.46 (s, 1H), 12.47 (s, 1H); MS (EI) *m/z*: 377 ($M^+$). Anal. Calcd. for $C_{19}H_{19}N_7S$: C, 60.46; H, 5.07; N, 25.98; found: C, 60.73; H, 4.98; N, 26.11.

*N2-(4-chlorophenyl)-6-((5-methyl-1H-benzo[d]imidazol-2-ylthio)methyl)-1,3,5-triazine-2,4-diamine (**5h**)*

Recrystallization from ethanol gave pale brown solid (194 mg, 49%) of m.p. 107–110°C; $^1$H NMR: 2.34 (s, 3H), 4.35 (s, 2H), 6.96 (s, 1H), 7.18 (brs, 3H), 7.31 (d, 2H, $J = 8$ Hz), 7.40 (d, 1H, $J = 8$), 7.72 (brs, 2H), 9.70 (s, 1H), 12.44 (s, 1H); MS (EI) *m/z*: 397 ($M^+$). Anal. Calcd. for $C_{18}H_{16}ClN_7S$: C, 54.34; H, 4.05; N, 24.64; found: C, 54.72; H, 3.87; N, 24.93.

*N2-(4-methoxyphenyl)-6-((5-methyl-1H-benzo[d]imidazol-2-ylthio)methyl)-1,3,5-triazine-2,4-diamine (**5i**)*

Recrystallization from methanol gave white solid (283 mg, 72%) of m.p. 131–133°C, IR (KBr): 3614, 3390, 3396, 1640; $^1$H NMR: 2.40 (s, 3H), 3.71 (s, 3H), 4.33 (s, 2H), 6.76 (brs, 3H), 6.79 (d, 1H, $J = 8$ Hz), 6.96 (d, 1H, $J = 8$ Hz), 7.19–7.37 (m, 2H), 7.52 (d, 2H, $J = 8$ Hz), 9.39 (s, 1H), 12.47 (s, 1H); MS (EI) *m/z*: 393 ($M^+$). Anal. Calcd. for $C_{19}H_{19}N_7OS$: C, 58.00; H, 4.87; N, 24.92; found: C, 57.81; H, 4.67; N, 25.13.

*6-[(5-Methyl-1H-benzo[d]imidazol-2-ylthio)methyl]-N2-o-tolyl-1,3,5-triazine-2,4-diamine (**5j**)*

Recrystallization from methanol/DMF gave white solid (230 mg, 61%) of m.p. 111–113°C, IR (KBr): 3620, 3390, 3370, 1625; $^1$H NMR: 2.19 (s, 3H), 2.39 (s, 3H), 4.29 (s, 2H), 6.98 (d, 2H, $J = 8$ Hz), 7.06–7.08 (m, 2H), 7.12 (d, 1H, $J = 8$ Hz), 7.19–7.26 (m, 2H), 7.33 (d, 1H, $J = 8$ Hz), 7.39 (d, 1H, $J = 8$ Hz), 8.91 (s, 1H), 12.49 (s, 1H); MS (EI) *m/z*: 377 ($M^+$). Anal. Calcd. for $C_{19}H_{19}N_7S$: C, 60.46; H, 5.07; N, 25.98; found: C, 60.24; H, 5.27; N, 25.76.

*Synthesis of 2-amino-4-arylamino-6-(1,2,4-triazol-3-ylthio)methyl-1,3,5-triazines (**5k–r**)*

To a mixture of 1,2,4-triazole derivative (0.01 mol) and anhydrous $K_2CO_3$ (1.38 g, 0.01 mol) in anhydrous DMF (10 ml), 6-chloromethyl-1,3,5-triazines (**4a–d**) was added. The reaction mixture was stirred at r.t. for 2 h; then poured on crushed ice. The precipitated product was collected by filtration, washed with water (2 × 5 ml) and purified by recrystallization from suitable solvent.

*6-[(4,5-Diphenyl-4H-1,2,4-triazol-3-yl thio)methyl]-N2-(4-methoxyphenyl)-1,3,5-triazine-2,4-diamine (**5k**)*

Recrystallization from DMF gave white solid (231 mg, 48%) of m.p. 167–169°C, $^1$H NMR: 3.72 (s, 3H), 4.25 (s, 2H), 6.81 (s, 2H), 7.24 (d, 2H, $J = 8$ Hz), 7.35 (d, 2H, $J = 8$ Hz), 7.51–7.53 (m, 7H), 7.81 (s, 1H), 8.03 (brs, 2H), 9.39 (s, 1H). MS (EI) *m/z*: 482 ($M^+$). Anal. Calcd. for $C_{25}H_{22}N_8OS$: C, 62.22; H, 4.60; N, 23.22; found: C, 62.35; H, 4.67; N, 23.25.

*6-[(4,5-Diphenyl-4H-1,2,4-triazol-3-yl thio)methyl]-N2-o-tolyl-1,3,5-triazine-2,4-diamine (**5l**)*

Recrystallization from DMF gave yellow solid (289 mg, 62%) of m.p. 188–191°C, $^1$H NMR: 2.16 (s, 3H), 4.19 (s, 2H), 6.96 (brs, 2H), 7.07–7.08 (m, 2H), 7.19 (d, 1H, $J = 7.2$ Hz), 7.29 (d, 1H, $J = 7.2$ Hz), 7.36 (brs, 3H), 7.38–7.39 (m, 3H), 7.54–7.56 (m, 3H), 8.87 (s, 1H). MS (EI) *m/z*: 466 ($M^+$). Anal. Calcd. for $C_{25}H_{22}N_8S$: C, 64.36; H, 4.75; N, 24.02; found: C, 64.31; H, 4.85; N, 23.83.

*N2-(4-methoxyphenyl)-6-{[4-phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio]methyl}-1,3,5-triazine-2,4-diamine (**5m**)*

Recrystallization from DMF gave white solid (217 mg, 45%) of m.p. 191–192°C, $^1$H NMR: 3.71 (s, 3H), 4.23 (s, 2H), 6.82 (brs, 2H), 7.11 (brs, 2H), 7.35–7.37 (m, 4H), 7.41–7.42 (m, 2H), 7.55–7.56 (m, 5H), 9.35 (s, 1H). MS (EI) *m/z*: 483 ($M^+$). Anal. Calcd. for $C_{24}H_{21}N_9OS$: C, 59.61; H, 4.38; N, 26.07; found: C, 59.67; H, 4.26; N, 26.27.

*6-{[4-Phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio]methyl}-N2-o-tolyl-1,3,5-triazine-2,4-diamine (**5n**)*

Recrystallization from DMF gave white solid (243 mg, 52%) of m.p. 205–207°C, IR (KBr): 3650, 3335, 1635, 1605. $^1$H NMR: 2.16 (s, 3H), 4.26 (s, 2H), 7.07 (brs, 2H), 7.08–7.12 (m, 2H), 7.19 (d, 1H, $J = 7.2$ Hz), 7.29 (d, 1H, $J = 7.2$ Hz), 7.36–7.38 (m, 4H), 7.39 (brs, 2H), 7.56 (brs, 2H), 9.38 (s, 1H). MS (EI) *m/z*: 467 ($M^+$). Anal. Calcd. for $C_{24}H_{21}N_9S$: C, 61.65; H, 4.53; N, 26.96; found: C, 61.85; H, 4.58; N, 26.86.

*6-{[4-(Benzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio]methyl}-N2-(4-methoxyphenyl)-1,3,5-triazine-2,4-diamine (**5o**)*

Recrystallization from DMF gave pale brown solid (320 mg, 63%) of m.p. 255–257°C, $^1$H NMR: 3.7 (s, 3H), 4.77 (m, 1H), 5.07 (m, 1H), 6.81 (s, 2H), 7.21 (brs, 2H), 7.29 (d, 1H, $J = 8$ Hz), 7.34–7.46 (s, 5H), 7.47–7.71 (m, 5H), 7.97–8.22 (m, 2H), 9.46 (s, 1H). MS (EI) *m/z*: 509 ($M^+$). Anal. Calcd. for $C_{26}H_{23}N_9OS$: C, 61.28; H, 4.55; N, 24.74; found: C, 61.39; H, 4.51; N, 24.65.

*6-{[4-(Benzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio]methyl}-N2-o-tolyl-1,3,5-triazine-2,4-diamine (**5p**)*

Recrystallization from DMF gave white solid (350 mg, 71%) of m.p. 221–222°C, $^1$H NMR: 2.11 (s, 3H), 4.67 (m, 1H), 5.00 (m, 1H), 6.86 (s, 2H), 7.30–7.31 (m, 3H), 7.34–7.36 (m, 3H), 7.40 (brs, 2H), 7.50–7.51 (m, 4H), 7.97–8.12 (m, 2H), 8.74 (s, 1H). MS (EI) *m/z*: 493 ($M^+$). Anal. Calcd. for $C_{26}H_{23}N_9S$: C, 63.27; H, 4.70; N, 25.54; found: C, 63.29; H, 4.61; N, 25.65.

*6-{[4-(4-Bromobenzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio]methyl}-N2-(4-methoxyphenyl)-1,3,5-triazine-2,4-diamine (**5q**)*

Recrystallization from DMF gave white solid (464 mg, 79%) of m.p. 249–251°C, IR (KBr): 3530, 3395, 1635, 1615; $^1$H NMR: 3.70 (s, 3H), 4.77 (s, 1H), 5.12 (m, 1H), 6.83 (s, 2H), 7.13–7.36 (m, 4H), 7.42–7.71 (m, 7H), 7.83 (brs, 1H), 7.92–8.24 (m, 2H), 9.46 (s, 1H). MS (EI) *m/z*: 589 ($M^+$+2), 587 ($M^+$). Anal. Calcd. for $C_{26}H_{22}BrN_9OS$: C, 53.07; H, 3.77; N, 21.42; found: C, 53.17; H, 3.72; N, 21.35.

*6-{[4-(4-Bromobenzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio]methyl}-N2-o-tolyl-1,3,5-triazine-2,4-diamine (**5r**)*

Recrystallization from DMF gave yellow solid (377 mg, 66%) of m.p. 233–235°C, IR (KBr): 3567 ($NH_2$), 3332 (NH), $^1$H NMR: 2.14 (brs, 3H), 4.70 (m, 1H), 4.79 (brs, 1H), 6.89–7.21 (m, 5H), 7.24–7.36 (m, 5H), 7.41–7.64 (m, 4H), 8.02 (s, 1H), 8.07 (brs, 1H), 8.99 (s, 1H). MS (EI) *m/z*: 573 ($M^+$+2), 571 ($M^+$). Anal. Calcd. for $C_{26}H_{22}BrN_9S$: C, 54.55; H, 3.87; N, 22.02; found: C, 54.47; H, 3.79; N, 22.23.

### Antiviral screening

The antiviral activity of the selected compounds against HSV-1 was measured by the plaque reduction assay. Confluent monolayers of Vero cells ($1 \times 10^6$/well) in six-well plates were infected with 100 PFU of HSV-1. After 1 h adsorption period, the cultures were overlaid with Dulbecco's modified eagle medium (DMEM) containing 2% heat-inactivated fetal calf serum (FCS) including various concentrations of the selected compounds. The plates were incubated at 37°C in 5% $CO_2$ incubator for 3 days, then fixed with formaline and stained with crystal violet in methanol. Infectious virus production was quantified by counting the plaques caused by virus-induced cytopathic effect. The activity was calculated as $EC_{50}$; 50% effective concentration.

### Cytotoxicity assay

The cytotoxic activity was examined for only the compounds which showed higher potency among the tested series. Confluent monolayers Vero cells were seeded in 96-well plates at $5 \times 10^4$ cells per well and incubated at 37°C in 5% $CO_2$ incubator. After 1 day, the cells were overlaid with DMEM containing 5% FCS and serial dilutions of the compounds. After 3 days incubation, 10 µl of Alamar blue was added to each well. The plates were incubated for additional 3 h. Optical densities were read at 590 and 620 nm with a microplate reader. The cytotoxicity was calculated as $CC_{50}$; 50% cytotoxic concentration.

## Conclusion

In conclusion, this preliminary study demonstrated that two groups of 1,3,5-triazine derivatives were new versatile scaffolds that exhibited anti-HSV-1 activity. Among these derivatives, compound **5p** showed the best activity (3.5 µg/ml) against HSV-1 in comparison to acyclovir.

**Acknowledgments** This study was supported by Mansoura University research grant. The authors would like to thank Dr El-Sayed E. Habib, Department of Microbiology, Faculty of Pharmacy, Mansoura University for his assistance in performing the biological testing at the Department of Pediatrics, Medical College of Virginia, Virginia Commenwealth University, USA.

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