ACCSCIENCE PUBLISHING

Microbes & Immunity

ORIGINAL RESEARCH ARTICLE

Synthesis and *in vitro* assessment of triazole-based compounds as potential inhibitors of herpes simplex virus type 1

Supplementary Files

1. Step-wise synthesis of pyrazine-fused triazole Schiff base derivatives (5a–5t)

(a) Step 1: Synthesis of triazole intermediate

(i) Reactants: Pyridine-5-carboxamide and hydrazine hydrate.

(ii) Molar ratio: 1:2.

(iii) Solvent: Ethanol.

(iv) Conditions: Reflux for 7 h under stirring.

(v) The hydrazide group provides a precursor for cyclization with carbon disulfide.

(b) Step 2: Cyclization with carbon disulfide

(i) Reactants: Hydrazide intermediate, carbon disulfide, and potassium hydroxide (KOH).

(ii) Conditions: Stirring under heat 85 °C.

(iii) Solvent/base medium: Alcoholic KOH.

(iv) Reaction type: Cyclization and sulfur incorporation.

(v) Introduces the biologically active triazole core with a thiol functional group.

(c) Step 3: Schiff base formation (compounds 4a–4t)

(i) Reactants: Triazole-thiol intermediate (from step 2) and substituted benzaldehydes.

(ii) Conditions: Aqueous basic medium, reflux 55 °C.

(iii) Catalyst/base: KOH.

(iv) Reaction type: Nucleophilic addition followed by condensation (C=N bond formation).

(d) Step 4: Final pyrazine fused cyclization (compounds 5a–5t)

(i) Reactants: Schiff base derivatives (4a–4t) and aryl halides.

(ii) Solvent: Sulphonated dimethylformamide.

(iii) Additives: Ammonium fluoride (used as an activator) and triethylamine (used as a catalyst and base to promote cyclization).

(iv) Reaction type: Cyclization and arylation under nucleophilic substitution conditions.

- Introduction of fused pyrazine ring via electrophilic substitution for enhanced rigidity and biological relevance.

(v) Conditions:

- Conventional heating: Heating under controlled temperature (145 °C).
- Microwave-assisted synthesis: Using an Anton Paar microwave reactor (closed-vessel mode, 120 °C, 15–30 min, automatic internal pressure monitoring, power input 100–200 W depending on scale and reactivity).

Table S1. Schematic representation of pyrazine-fused triazole derivatives (5a–5t)

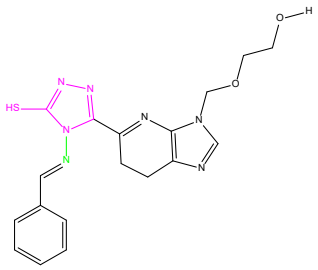
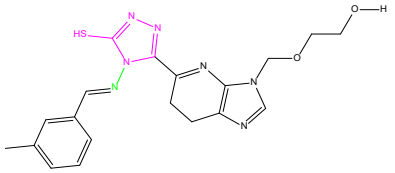
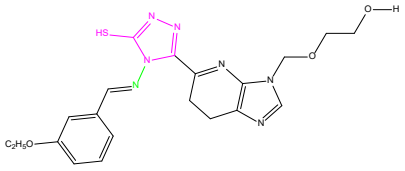
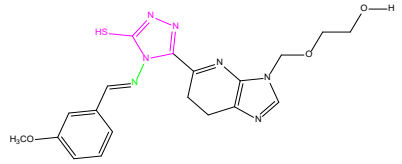
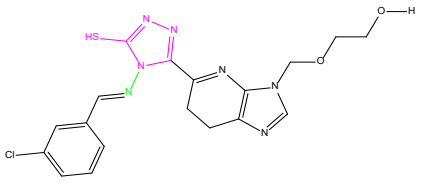
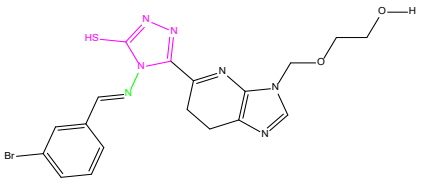
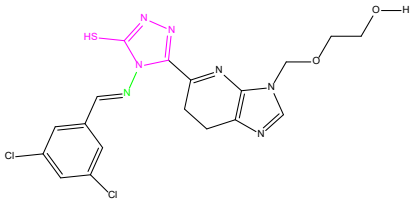
Compound	Schematic representation
5a	
5b	
5c	
5d	
5e	
5f	
5g	

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Table S1. (*Continued*)

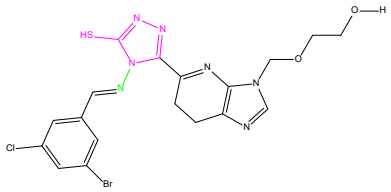
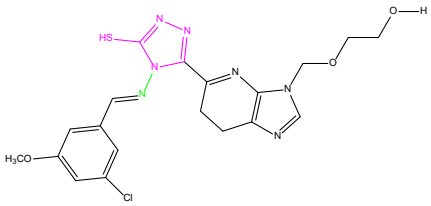
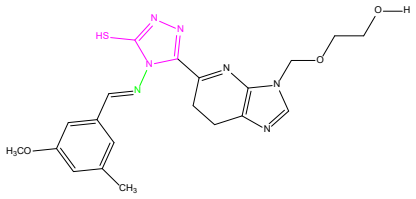
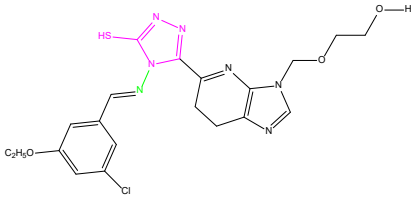
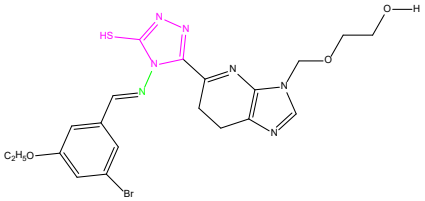
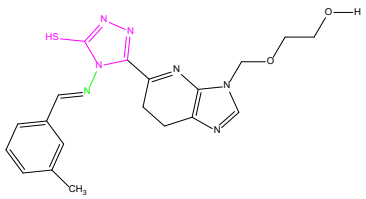
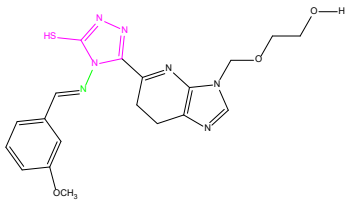
Compound	Schematic representation
5h	
5i	
5j	
5k	
5l	
5m	
5n	

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Table S1. (*Continued*)

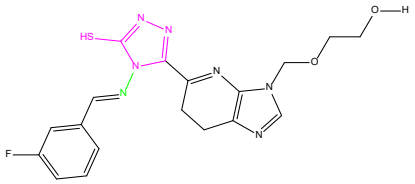
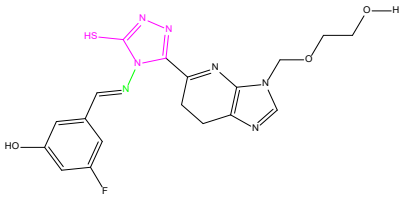
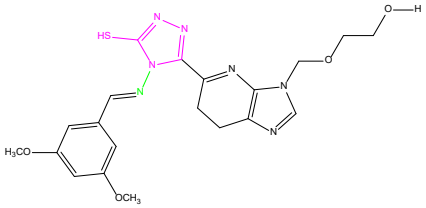
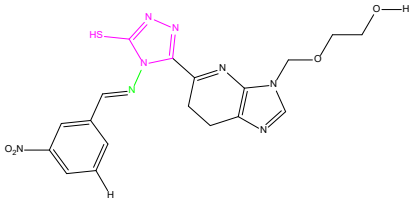
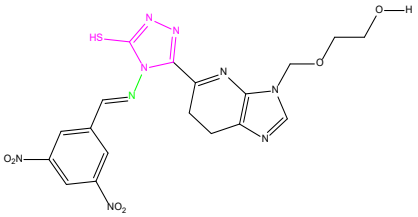
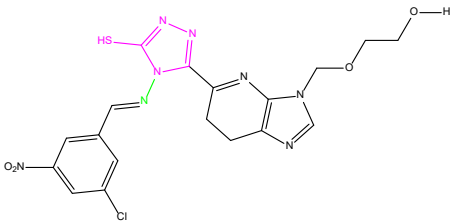
Compound	Schematic representation
5o	
5p	
5q	
5r	
5s	
5t	

Table S2. Summary of physicochemical and analytical data for Schiff base derivatives (5a–5t), confirming structural identity, purity, and synthetic efficiency

Entry	Compound																			
	5a	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**	**5k**	**5l**	**5m**	**5n**	**5o**	**5p**	**5q**	**5r**	**5s**	**5t**
R1	–H	$-CH_3$	$-OC_2H_5$	$-OCH_3$	–Cl	–Br	–Cl	–Cl	$-OCH_3$	$-OCH_3$	$-OC_2H_5$	$-OC_2H_5$	–H	–H	–F	–H	$-OCH_3$	$-NO_2$	$-NO_2$	$-NO_2$
R2	–H	–H	–H	–H	–H	–H	–Cl	–Br	–Cl	$-CH_3$	–Cl	–Br	$-CH_3$	$-OCH_3$	–H	–F	$-OCH_3$	–H	$-NO_2$	–Cl
Molecular formula	$C_{18}H_{19}N_7O_2S$	$C_{19}H_{21}N_7O_2S$	$C_{20}H_{23}N_7O_3S$	$C_{19}H_{21}N_7O_3S$	$C_{18}H_{18}ClN_7O_2S$	$C_{18}H_{18}BrN_7O_2S$	$C_{18}H_{17}C_{12}N_7O_2S$	$C_{18}H_{17}BrClN_7O_2S$	$C_{19}H_{20}ClN_7O_3S$	$C_{20}H_{23}N_7O_3S$	$C_{20}H_{22}ClN_7O_3S$	$C_{20}H_{22}BrN_7O_3S$	$C_{19}H_{21}N_7O_2S$	$C_{19}H_{21}N_7O_3S$	$C_{18}H_{18}FN_7O_2S$	$C_{18}H_{18}FN_7O_2S$	$C_{20}H_{23}N_7O_4S$	$C_{18}H_{18}N_8O_4S$	$C_{18}H_{17}N_9O_6S$	$C_{18}H_{17}ClN_8O_4S$
Molecular weight (g/mol)	397.13	413.48	441.51	427.48	431.09	476.35	466.34	510.80	461.93	441.51	457.95	520.41	411.18	427.48	415.45	415.12	457.51	442.45	487.10	476.90
Melting point (°C)	161	182	168	222	198	200	144	191	226	228	219	245	188	202	192	164	178	189	256	210
R_f value	1.2	1.06	1.54	1.36	1.77	1.23	1.55	0.23	1.47	2.34	1.4	1.3	0.20	0.15	1.36	1.25	1.23	1.87	0.29	0.87
Elemental analysis	C = 54.40, H = 4.82, N = 24.67, O = 8.05, S = 8.05	C = 55.46, H = 5.14, N = 23.83, O = 7.78, S = 7.79	C = 54.41, H = 5.25, N = 22.21, O = 10.87, S = 7.26	C = 53.38, H = 4.95, N = 22.94, O = 11.23, S = 7.50	C = 50.06, H = 4.20, Cl = 8.21, N = 22.70, O = 7.41, S = 7.42	C = 45.39, H = 3.81, Br = 16.77, N = 20.58, O = 6.72, S = 6.73	C = 46.36, H = 3.67, Cl = 15.20, N = 21.03, O = 6.86, S = 6.87	C = 42.33, H = 3.35, Br = 15.64, Cl = 6.94, N = 19.20, O = 6.26, S = 6.28	C = 49.40, H = 4.36, Cl = 7.67, N = 21.23, O = 10.39, S = 6.94	C = 54.41, H = 5.25, N= 2 2.21, O = 10.87, S = 7.26	C = 50.47, H = 4.66, Cl = 7.45, N = 20.60, O = 10.08, S = 6.74	C = 46.16, H = 4.26, Br = 15.35, N = 18.84, O = 9.22, S = 6.16	C = 55.46, H = 5.14, N = 23.83, O = 7.78, S = 7.79	C = 53.38, H = 4.95, N = 22.94, O = 11.23, S = 7.50	C = 52.04, H = 4.37, F = 4.57, N = 23.60, O = 11.23, S = 7.50	C = 52.04, H = 4.37, F = 4.57, N = 23.60, O = 7.70, S = 7.72	C = 52.51, H = 5.07, N = 21.43, O = 13.99, S = 7.01	C = 48.86, H = 4.10, N = 25.33, O = 14.46, S = 7.25	C = 44.35, H = 3.52, N = 25.86, O = 19.69, S = 6.58	C = 45.33, H = 3.59, Cl = 7.43, N = 23.50, O = 13.42, S = 6.92
Conventional time (h)	18	36	15	24	16	12	10	22	20	18.5	28	33	31	8	19	17	6	8	28	16
Microwave time (min)	16	12	14	18	22	10	15	18	16	12	14	23	21	26	10	10	12	19	16	20

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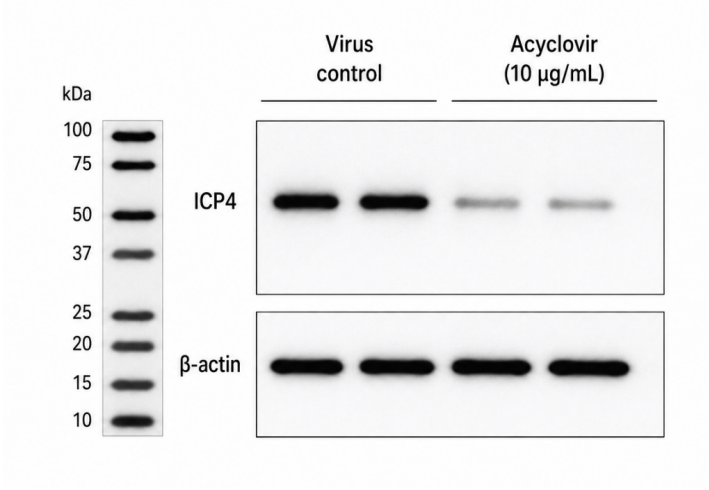

Figure S1. The raw Western blot images are consistent with the observed antiviral trends, showing a progressive reduction in ICP4 band intensity from the virus control to acyclovir and further to the most active compounds (5f and 5t). This decrease indicates suppressed herpes simplex virus type 1 replication. In contrast, β-actin bands remain uniform across all lanes, confirming equal protein loading. Overall, this pattern aligns with the plaque reduction assay, polymerase chain reaction data, and cell viability results, where compounds with stronger antiviral activity correspond to lower viral load, reduced plaque formation, and diminished ICP4 expression. Collectively, these findings demonstrate a clear inverse relationship between antiviral efficacy and ICP4 protein levels.
Abbreviation: ICP4: Infected-cell protein 4.