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ORIGINAL ARTICLE

Synthesis and pharmacological evaluation of novel 2*H*/6*H*-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-*a*]pyridine-9-carbonitrile derivatives

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Abstract In the present study a new series of 3-hydroxy-7-isocyano-6-oxo-8-phenyl-2-(substitutedphenyl(piperidin-1-yl)methyl)-6*H*-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-*a*]-pyridine-9-carbonitrile (**5a–j**) and (*Z*)-2-(4-substitutedbenzylidene)-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2*H*-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-*a*]pyridine-9-carbo-nitrile derivatives (**4a–j**) were synthesized. The newly synthesized compounds were characterized by IR, ^{1}H NMR, LC–MS mass and C, H, N analyses. All newly synthesized compounds were screened for their antibacterial (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Klebsiella pneumoniae*) and antifungal (*Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Penicillium marneffei* and *Trichophyton mentagrophytes*) activity. The results revealed that all synthesized compounds have a significant biological activity against the tested microorganisms. Compounds **4a, 4f, 4i, 4j, 5a, 5i** and **5j** exhibited good antimicrobial activity.

1. Introduction

Triazoles are important classes of heterocyclic compounds. In particular, fused 1,2,4-triazoles express antifungal (El-Hawash et al., 1999), bactericidal (El-Hawash et al., 1999; Brown and Iwai, 1979), anxiolytic (Tarzia et al., 1988; Trust and Albrigh, 1980), anticonvulsant (Tarzia et al., 1989) or herbicidal (Peignier et al., 1991; Cantegriil et al., 1997) activities and can act as antidepressants (Sarges et al., 1990). Therefore, versatile and widely applicable methods for their synthesis are of considerable interest. Most methods for the preparation of fused 1,2,4-triazoles are mainly based on hydrazones as precursors. However, these methods have some restrictions regarding their applicability and the use of toxic reagents like lead tetraacetate (Bower and Doyle, 1957; Pollak and Tisler, 1966) and bromine (Pollak and Tisler, 1966; Gibson, 1963), also the other products were formed in low yield and isolated as salts (Hadi et al., 1992; Al-Najjar et al., 1996). Many 1,2,4-triazine derivatives are well known to possess biological activities, thus they have been found to be useful as herbicides (Neunhoeffer, 1978; Neunhoeffer, 1984). In the last decade they have been screened

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in vitro supporting for their anti-HIV and anti-cancer activities (Abdel-Rahman et al., 1999; Abdel-Rahman et al., 1994; Abdel-Rahman et al., 1999; Abdel-Rahman, 2001). However the aza-Wittig reaction is a powerful tool for the synthesis of five- to seven-membered nitrogen heterocycles (Takeuchi et al., 1989; Eguchi and Goto, 1994; Eguchi et al., 1992; Takeuchi et al., 1989; Molina and Fresneda, 1988; Molina et al., 1990; Molina and Vilaplana, 1990; Wamhoff and Schmidt, 1993; Sato et al., 1993; Molina et al., 1990a; Molina et al., 1990b). Annulation of ring systems with N-heterocycles by means of an aza-Wittig reaction has recently been widely utilized because of the availability of functionalized iminophosphoranes (Palacios et al., 2007; Eguchi, 2006; Braese et al., 2005; Eguchi, 2005; Fresneda and Molina, 2004). Many important monocyclic nitrogen heterocycles such as indole, pyridine, pyrimidine and isoquinoline derivatives have been synthesized *via* the intramolecular aza-Wittig reaction (Takeuchi et al., 1989; Eguchi and Goto, 1994; Eguchi et al., 1992; Takeuchi et al., 1989), as well as by the intermolecular aza-Wittig reaction followed by electrocyclization, intramolecular cycloaddition or heterocyclization (Molina and Fresneda, 1988; Molina et al., 1990; Wamhoff and Schmidt, 1993; Sato et al., 1993; Molina et al., 1990a; Molina et al., 1990b). We have previously published the synthesis of fused pyrimidines based on the tandem aza-Wittig annulation strategy (Barsy and El-Rady, 2006), and as a part of our ongoing studies we now describe a novel one-pot synthesis of 1,2,4-triazolo[1,5-*a*]pyridine and pyrido[1,2-*b*][1,2,4]triazines derivatives in good yield.

2. Experimental

2.1. Materials and reagents

All reagents and solvents were purchased and used without further purification. Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Crude products were purified by column chromatography on silica gel of 60–120 mesh. IR spectra were obtained on a Perkin–Elmer BX serried FT-IR 5000 spectrometer using KBr pellet. NMR spectra were recorded on a Varian 300 MHz spectrometer for ^{1}H NMR. The chemical shifts were reported as ppm down field using TMS as an internal standard. LC–MS Mass spectra were recorded on a MASPEC low resolution mass spectrometer operating at 70 eV.

2.2. General procedure for the preparation of 1-amino-6-(triphenylphosphoranylidene-amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (**2**)

1-Amino-6-(triphenylphosphoranylideneamino)-2-oxo-4-phenyl -1,2-dihydro-pyridine-3,5-dicarbonitrileiminophosphorane (**2**) were synthesized according to the reported method by the reaction with triphenylphosphine/hexachloroethane and triethylamine reagent system (the Appel method, i.e., the modified Kirsanov reaction) (Appel et al., 1970). To a stirred mixture of 1,6-diamino-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile **1**, (0.5 g, 2.0 mmol), hexachloroethane (0.473 g, 2.0 mmol, 1.0 equiv.) and triphenylphosphine (0.52 g, 2.0 mmol, 1.0 equiv.) in anhydrous benzene (50.0 mL) triethylamine (0.4 mL, 4.0 mmol, 2.0 equiv.) were added dropwise. The resultant solution was heated at reflux for 2 h. The mixture was filtered while still hot in order to remove the precipitates and the filtrate was evaporated under reduced pressure to give a solid product which was crystallized from ethanol as colorless crystals; yield: 950 mg (95%); mp 170 °C, IR (KBr, cm^{-1}): 1656 (C═O), 2210 (C═N), 3229, 3259 (NH_2), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.1–7.8 (*m*, 20H, Ar-H), 8.9 (s, 2H, NH_2); LC–MS (*m*/*z*, %): 511 $(M)^+$. Anal. Calcd for $C_{31}H_{22}N_5OP$: C, 72.79; H, 4.34; N,13.69. Found: C, 72.65; H, 4.25; N, 13.57.

2.3. Synthesis of 5-oxo-7-phenyl-2-thiol-3,5-dihydro[1,2,4]-triazolo[1,5-a]pyridine-6,8-dicarbonitrile (**3**)

To a solution of 1-amino-6-(triphenylphosphoranylideneamino)-2-oxo-4-phenyl-1,2-dihydro-pyridine-3,5-dicarbonitrileiminophosphorane **2** (0.5 g, 1.0 mmol) in 15 mL of dry toluene an excess of carbon disulfide (7 mL) was added. The reaction mixture was heated in a closed two-neck round bottom flask at 100 °C for 3 h. The crystals that formed were collected and crystallized from a mixture of DMF and H_2O (1:1) as yellow crystals, yield: 240 mg (83%); mp 215 °C, IR (KBr, cm^{-1}): 1200 (SH), 1655 (C═O), 2210 (C═N), 3120 (NH), ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.5 (s, 1H, SH), 6.9–7.3 (*m*, 5H, Ar-H), 10.3 (s, H, NH); LC–MS (*m*/*z*, %): 293 $(M)^+$. Anal. Calcd for $C_{14}H_7N_5OS$: C, 57.33; H, 2.41; N, 23.88. Found: C, 57.11; H, 2.55; N, 23.57.

2.4. Synthesis of (Z)-2-(4-substitutedbenzylidene)-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**4**)

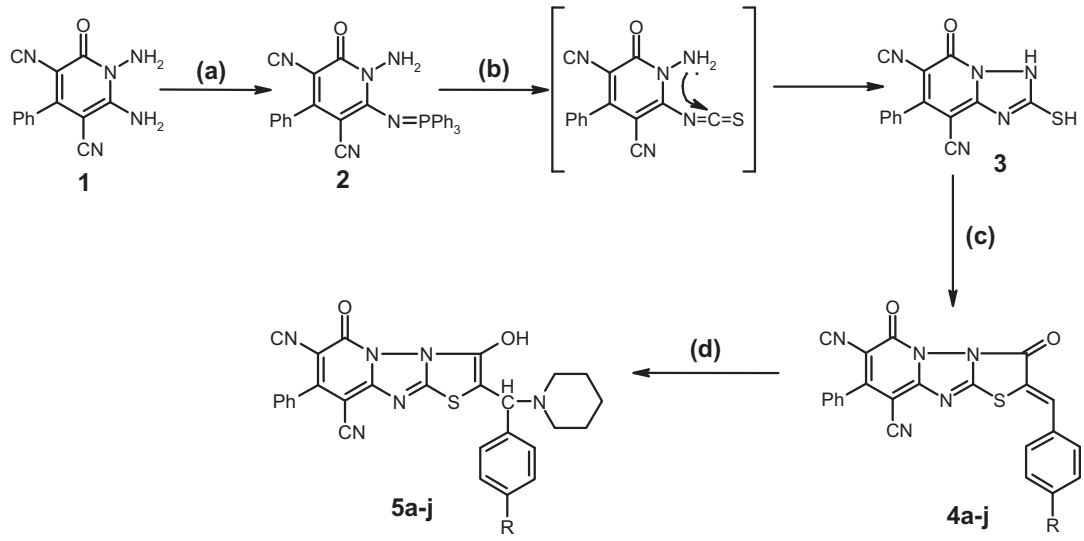
A mixture of 5-oxo-7-phenyl-2-thiol-3,5-dihydro[1,2,4]-triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (**3**) (5 mmol), aromatic aldehydes (5 mmol), chloroacetic acid (5 mmol) and fused sodium acetate (10 mmol) were refluxed in acetic acid/acetic anhydride (25:5 mL) mixture for 3 h. Then the reaction mixture was cooled, filtered and crystallized from acetic acid to give the (*Z*)-2-(4-substitutedbenzylidene)-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2*H*-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-*a*]pyridine-9-carbonitrile **4a–j** in 66–89% yields. The R_f values were measured using benzene/ethyl acetate mixture as an eluent in ratio (9:1). The reaction sequences were outlined in Scheme 1.

2.4.1. (Z)-2-Benzylidene-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**4a**)

White solid in a yield of 87%, mp 146–148 °C; IR (KBr, cm^{-1}): 3032 (Ar-H), 1730 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.20 (s, 1H, CH), 7.60–7.55 (m, 2H, Ar-H), 7.45–7.33 (m, 6H, Ar-H), 7.19–7.17 (m, 2H, Ar-H), LC–MS (*m*/*z*, %): 422 $(M+1)^+$. Anal. Calcd for $C_{23}H_{11}N_5O_2S$: C, 64.25; H, 2.55; N, 16.57. Found: C, 65.55; H, 2.63; N, 16.62.

2.4.2. (Z)-2-(4-Chlorobenzylidene)-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**4b**)

White solid in a yield of 66%, mp 171–172 °C; IR (KBr, cm^{-1}): 3030 (Ar-H), 1730 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz,



S.No	Compound	S.No	Compound
4a & 5a	R=H	**4f & 5f**	R=Br
4b & 5b	R=Cl	**4g & 5g**	R=$OCOCH_3$
4c & 5c	R=NO_2	**4h & 5h**	R=CH_3
4d & 5d	R=OCH_3	**4i & 5i**	R=$CH(CH_3)_2$
4e & 5e	R=$N(CH_3)_2$	**4j & 5j**	R=$C(CH_3)_3$

Scheme 1 (a) PPh_3, C_2Cl_6/TEA, toluene/reflux 2 h; (b) dry toluene, CS_2; (c) RCHO, $ClCH_2COOH$, fused CH_3COONa, reflux; (d) piperidine, THF, rt.

DMSO-d_6): δ 7.80 (s, 1H, CH), 7.68–7.66 (m, 2H, Ar-H), 7.44–7.33 (m, 5H, Ar-H), 7.17–7.15 (m, 2H, Ar-H), LC–MS (m/z, %): 456 $(M+1)^+$. Anal. Calcd for $C_{23}H_{10}ClN_5O_2S$: C, 60.76; H, 2.36; N, 15.52. Found: C, 60.60; H, 2.21; N, 15.36.

*2.4.3. (Z)-2-(4-Nitrobenzylidene)-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**4c**)*

Yellow solid in a yield of 79%, mp 201–202 °C; IR (KBr, cm^{-1}): 3050 (Ar-H), 1730 (C═O), 16,000 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.21–8.20 (m, 2H, Ar-H), 8.03–8.00 (m, 2H, Ar-H), 7.94 (s, 1H, CH), 7.46–7.33 (m, 3H, Ar-H), 7.17–7.15 (m, 2H, Ar-H), LC–MS (m/z, %): 465 $(M+1)^+$. Anal. Calcd for $C_{23}H_{10}N_6O_4S$: C, 59.37; H, 2.29; N, 18.24. Found: C, 59.23; H, 2.16; N, 18.02.

*2.4.4. (Z)-7-Isocyano-2-(4-methoxybenzylidene)-3,6-dioxo-8-phenyl-3,6-dihydro-2H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**4d**)*

White solid in a yield of 81%, mp 183–184 °C; IR (KBr, cm^{-1}): 3030 (Ar-H), 1710 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80 (s, 1H, CH), 7.46–7.33 (m, 5H, Ar-H), 6.71–6.70 (m, 2H, Ar-H), 3.06 (s, 3H, OCH_3). LC–MS (m/z, %): 452 $(M+1)^+$. Anal. Calcd for $C_{24}H_{13}N_5O_3S$: C, 64.12; H, 2.98; N, 15.06. Found: C, 63.85; H, 2.90; N, 15.51.

*2.4.5. (Z)-2-(4-(Dimethylamino)benzylidene)-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**4e**)*

Yellow solid in a yield of 69%, mp 203–204 °C; IR (KBr, cm^{-1}): 3030 (Ar-H), 1710 (C═O), 1580 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80 (s, 1H, CH), 7.46–7.33 (m, 5H, Ar-H), 6.71–6.70 (m, 2H, Ar-H), 3.02 (s, 3H, $N(CH_3)_2$). LC–MS (m/z, %): 465 $(M+1)^+$. Anal. Calcd for $C_{25}H_{16}N_6O_2S$: C, 64.83; H, 3.56; N, 17.89. Found: C, 64.64; H, 3.47; N, 18.07.

*2.4.6. (Z)-2-(4-Bromobenzylidene)-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**4f**)*

White solid in a yield of 89%, mp 194–195 °C; IR (KBr, cm^{-1}): 3030 (Ar-H), 1710 (C═O), 1580 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80 (s, 1H, CH), 7.68–7.66 (m, 2H, Ar-H), 7.44–7.33 (m, 5H, Ar-H), 7.17–7.15 (m, 2H, Ar-H). LC–MS (m/z, %): 502 $(M+2)^+$. Anal. Calcd for $C_{23}H_{10}BrN_5O_2S$: C, 55.43; H, 2.22; N, 13.88. Found: C, 55.21; H, 2.01; N, 14.00.

*2.4.7. (Z)-Methyl 4-((9-cyano-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridin-2-ylidene)methyl)benzoate (**4g**)*

White solid in a yield of 68%, mp 165–166 °C; IR (KBr, cm^{-1}): 3030 (Ar-H), 1760 (OCO), 1730 (C═O), 1580 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80 (s, 1H, CH), 7.67–7.66 (m, 2H, CH), 7.49–7.33 (m, 5H, Ar-H), 7.17–7.15 (m, 2H, Ar-H), 3.89 (s, 3H, $COCH_3$). LC–MS (m/z, %): 480 $(M+1)^+$. Anal. Calcd for $C_{25}H_{13}N_5O_4S$: C, 62.89; H, 2.91; N, 14.73. Found: C, 62.63; H, 2.73; N, 14.61.

*2.4.8. (Z)-7-Isocyano-2-(4-methylbenzylidene)-3,6-dioxo-8-phenyl-3,6-dihydro-2H-thiazolo-[3′,2′:2,3][1,2,4]triazolo [1,5-a]pyridine-9-carbonitrile (**4h**)*

White solid in a yield of 74%, mp 192–193 °C; IR (KBr, cm^{-1}): 3030 (Ar-H), 1730 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz,

DMSO-d_6): δ 7.80 (s, 1H, CH), 7.59–7.57 (m, 2H, Ar-H), 7.40–7.33 (m, 3H, Ar-H), 7.18–7.17 (m, 4H, Ar-H), 2.34 (s, 3H, CH_3). LC–MS (*m*/*z*, %): 436 $(M+1)^+$. Anal. Calcd for $C_{24}H_{13}N_5O_2S$: C, 66.58; H, 3.18; N, 16.27. Found: C, 66.20; H, 3.01; N, 16.08.

*2.4.9. (Z)-7-Isocyano-2-(4-isopropylbenzylidene)-3,6-dioxo-8-phenyl-3,6-dihydro-2H-thiazolo-[3′,2′:2,3][1,2,4]triazolo [1,5-a]pyridine-9-carbonitrile (**4i**)*

White solid in a yield of 72%, mp 210–211 °C; IR (KBr, cm^{-1}): 3030 (Ar-H), 1730 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80 (s, 1H, CH), 7.63–7.62 (m, 2H, Ar-H), 7.40–7.25 (m, 5H, Ar-H), 7.17–7.15 (m, 2H, Ar-H), 2.87–2.70 (m, 1H, CH), 1.20 (d, 6H, CH_3). LC–MS (*m*/*z*, %): 464 $(M+1)^+$. Anal. Calcd for $C_{26}H_{17}N_5O_2S$: C, 67.71; H, 3.88; N, 15.32. Found: C, 67.38; H, 3.70; N, 15.11.

*2.4.10. (Z)-2-(4-(tert-Butyl)benzylidene)-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**4j**)*

White solid in a yield of 75%, mp 201–202 °C; IR (KBr, cm^{-1}): 3030 (Ar-H), 1730 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80 (s, 1H, CH), 7.63–7.62 (m, 2H, Ar-H), 7.40–7.25 (m, 5H, Ar-H), 7.17–7.15 (m, 2H, Ar-H), 1.35 (s, 9H, CH_3). LC–MS (*m*/*z*, %): 478 $(M+1)^+$. Anal. Calcd for $C_{27}H_{19}N_5O_2S$: C, 68.23; H, 4.17; N, 14.72. Found: C, 67.91; H, 4.01; N, 14.67.

*2.5. 3-Hydroxy-7-isocyano-6-oxo-8-phenyl-2-(substitutedphenyl(piperidin-1-yl)methyl)-6H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]-pyridine-9-carbonitrile (**5a–j**)*

To a solution of (*Z*)-2-(4-substitutedbenzylidene)-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2*H*-thiazolo-[3′,2′:2,3][1,2,4]-triazolo[1,5-*a*]-pyridine-9-carbonitrile (**4a–j**) (2 mmol) in tetrahydrofuran (20 mL) a solution of piperidine (3 mmol) was added and the mixture was stirred for 6 h at room temperature under the conditions of Michael addition reaction. The crude product thus formed was crystallized from benzene/cyclohexane mixture to give products **5a–j** in 65–80% yield.

*2.5.1. 3-Hydroxy-7-isocyano-6-oxo-8-phenyl-2-(phenyl(piperidin-1-yl)methyl)-6H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**5a**)*

Colorless crystals in a yield of 67%, mp 130–132 °C; IR (KBr, cm^{-1}): 3100 (O–H), 3030 (Ar-H), 2960 (C–H aliphatic), 1730 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): 7.70–7.30 (m, 10H, Ar-H), 6.90 (s, 1H, CH), 3.7–3.22 (m, 4H, $N(CH_2)_2$), 1.7–1.4 (m, 6H, $(CH_2)_3$). LC–MS (*m*/*z*, %): 507 $(M+1)^+$. Anal. Calcd for $C_{28}H_{22}N_6O_2S$: C, 66.93; H, 5.02; N, 16.95. Found: C, 66.39; H, 4.38; N, 16.59.

*2.5.2. 2-((4-Chlorophenyl)(piperidin-1-yl)methyl)-3-hydroxy-7-isocyano-6-oxo-8-phenyl-6H-thiazolo[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**5b**)*

Colorless crystals in a yield of 70%, mp 159–160 °C; IR (KBr, cm^{-1}): 3180 (O–H), 3050 (Ar-H), 2940 (C–H aliphatic), 1730 (C═O), 1600 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80–7.20 (m, 9H, Ar-H), 6.80 (s, 1H, CH), 3.5–3.2 (m, 4H, $N(CH_2)_2$), 1.6–1.4 (m, 6H, $(CH_2)_3$), LC–MS (*m*/*z*, %): 542 $(M+1)^+$. Anal. Calcd for $C_{28}H_{21}ClN_6O_2S$: C, 62.88; H, 4.36; N, 16.21. Found: C, 62.16; H, 3.91; N, 15.53.

*2.5.3. 3-Hydroxy-7-isocyano-2-((4-nitrophenyl)(piperidin-1-yl)methyl)-6-oxo-8-phenyl-6H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**5c**)*

Yellow crystals in a yield of 69%, mp 175–176 °C; IR (KBr, cm^{-1}): 3120 (O–H), 3030 (Ar-H), 2900 (C–H aliphatic), 1730 (C═O), 1600 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80–7.20 (m, 9H, Ar-H), 6.80 (s, 1H, CH), 3.45, 3.13 (m, 4H, $N(CH_2)_2$), 1.50 (m, 4H, $(CH_2)_2$), 1.33 (m, 2H, CH_2), LC–MS (*m*/*z*, %): 552 $(M+1)^+$. Anal. Calcd for $C_{28}H_{21}N_7O_4S$: C, 61.43; H, 4.29; N, 18.24. Found: C, 60.97; H, 3.84; N, 17.78.

*2.5.4. 3-Hydroxy-7-isocyano-2-((4-methoxyphenyl)(piperidin-1-yl)methyl)-6-oxo-8-phenyl-6H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**5d**)*

Pale yellow crystals in a yield of 71%, mp 130–132 °C; IR (KBr, cm^{-1}): 3150 (O–H), 3030 (Ar-H), 2920 (C–H aliphatic), 1730 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.60–7.30 (m, 9H, Ar-H), 6.90 (s, 1H, CH), 3.65 (s, 3H, OCH_3), 3.62–3.20 (m, 4H, $N(CH_2)_2$), 1.6–1.4 (m, 6H, $(CH_2)_3$), LC–MS (*m*/*z*, %): 537 $(M+1)^+$. Anal. Calcd for $C_{29}H_{24}N_6O_3S$: C, 65.22; H, 4.98; N, 15.89. Found: C, 64.91; H, 4.51; N, 15.66.

*2.5.5. 2-((4-(Dimethylamino)phenyl)(piperidin-1-yl)methyl)-3-hydroxy-7-isocyano-6-oxo-8-phenyl-6H-thiazolo[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**5e**)*

Orange crystals in a yield of 75%, mp 182–183 °C; IR (KBr, cm^{-1}): 3150 (O–H), 3030 (Ar-H), 2920 (C–H aliphatic), 1730 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.70–6.60 (m, 9H, Ar-H), 6.90 (s, 1H, CH), 3.5–3.3 (m, 4H, $N(CH_2)_2$), 2.90 (s, 6H, $N(CH_3)_2$), 1.6–1.30 (m, 4H, $(CH_2)_3$), LC–MS (*m*/*z*, %): 550 $(M+1)^+$. Anal. Calcd for $C_{30}H_{27}N_7O_2S$: C, 65.83; H, 5.26; N, 18.13. Found: C, 65.56; H, 4.95; N, 17.84.

*2.5.6. 2-((4-Bromophenyl)(piperidin-1-yl)methyl)-3-hydroxy-7-isocyano-6-oxo-8-phenyl-6H-thiazolo[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**5f**)*

Pale yellow crtstals in a yield of 72%, mp 174–175 °C; IR (KBr, cm^{-1}): 3150 (O–H), 3030 (Ar-H), 2920 (C–H aliphatic), 1720 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.95–7.25 (m, 9H, Ar-H), 6.85 (s, 1H, CH), 3.65–3.25 (m, 4H, $N(CH_2)_2$), 1.65–1.3 (m, 6H, $(CH_2)_3$), LC–MS (*m*/*z*, %): 587 $(M+2)^+$. Anal. Calcd for $C_{28}H_{21}BrN_6O_2S$: C, 57.91; H, 4.05; N, 14.88. Found: C, 57.44; H, 3.62; N, 14.35.

*2.5.7. Methyl4-((9-cyano-3-hydroxy-7-isocyano-6-oxo-8-phenyl-6H-thiazolo[3′,2′:2,3]-[1,2,4]triazolo[1,5-a]pyridin-2-yl)(piperidin-1-yl)methylbenzoate (**5g**)*

Yellow crystals in a yield of 65%, mp 141–142 °C; IR (KBr, cm^{-1}): 3160 (O–H), 3050 (Ar-H), 1765 (OCO), 1720 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80–7.21 (m, 9H, Ar-H), 6.90 (s, 1H, CH), 3.90 (s, 3H, $COCH_3$), 3.6–3.2 (m, 4H, $N(CH_2)_2$), 1.60–1.2 (m, 6H, $(CH_2)_3$), LC–

MS (*m*/*z*, %): 565 $(M+1)^+$. Anal. Calcd for $C_{30}H_{24}N_6O_4S$: C, 64.25; H, 4.44; N, 15.32. Found: C, 63.82; H, 4.28; N, 14.86.

*2.5.8. 3-Hydroxy-7-isocyano-6-oxo-8-phenyl-2-(piperidin-1-yl (p-tolyl)methyl)-6H-thiazolo[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**5h**)*

Yellow crystals in a yield of 70%, mp 179–180 °C; IR (KBr, cm^{-1}): 3120 (O–H), 3030 (Ar-H), 2920 (C–H, aliphatic), 1730 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.75–7.20 (m, 9H, Ar-H), 6.75 (s, 1H, CH), 3.65–3.35 (m, 4H, $N(CH_2)_2$), 2.30 (s, 3H, CH_3), 1.65–1.2 (m, 6H, $(CH_2)_3$), LC–MS (*m*/*z*, %): 521 $(M+1)^+$. Anal. Calcd for $C_{29}H_{24}N_6O_2S$: C, 67.58; H, 4.97; N, 16.57. Found: C, 66.91; H, 4.65; N, 16.14.

*2.5.9. 3-Hydroxy-7-isocyano-2-((4-isopropylphenyl) (piperidin-1-yl)methyl)-6-oxo-8-phenyl-6H-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**5i**)*

Orange crystals in a yield of 72%, mp 139–140 °C; IR (KBr, cm^{-1}): 3150 (O–H), 3050 (Ar-H), 2920 (C–H, aliphatic), 1730 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.70–7.20 (m, 9H, Ar-H), 6.70 (s, 1H, CH), 3.67–3.25 (m, 4H, $N(CH_2)_2$), 2.80–2.70 (m, 1H, CH), 1.62–1.10 (m, 12H, $(CH_3)_2$ and $(CH_2)_3$). LC–MS (*m*/*z*, %): 549 $(M+1)^+$. Anal. Calcd for $C_{31}H_{28}N_6O_2S$: C, 68.23; H, 4.84; N, 15.92. Found: C, 67.86; H, 5.14; N, 15.32.

*2.5.10. 2-((4-(tert-Butyl)phenyl)(piperidin-1-yl)methyl)-3-hydroxy-7-isocyano-6-oxo-8-phenyl-6H-thiazolo[3′,2′:2,3][1,2,4]triazolo[1,5-a]pyridine-9-carbonitrile (**5j**)*

Pale yellow crystals in a yield of 80%, mp 165–166 °C; IR (KBr, cm^{-1}): 3200 (O–H), 3050 (Ar-H), 2960 (C–H aliphatic), 1730 (C═O), 1590 (C═N), ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.70–7.15 (m, 9H, Ar-H), 6.80 (s, 1H, CH), 3.65–3.20 (m, 4H, $N(CH_2)_2$), 1.9–1.11 (m, 15H, $(CH_2)_3$ and $(CH_3)_3$). LC–MS (*m*/*z*, %): 563 $(M+1)^+$. Anal. Calcd for $C_{32}H_{30}N_6O_2S$: C, 68.76; H, 5.69; N, 15.35. Found: C, 68.31; H, 5.37; N, 14.94.

3. Results and discussion

The IR spectrum of (*Z*)-2-benzylidene-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2*H*-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-*a*]pyridine-9-carbonitrile (**4a**) showed an absorption band at 3032 cm^{-1} indicates the Ar-H stretching. The absorption band at 1730 cm^{-1} due to the presence of C═O stretching of the thiazole ring system. Other prominent absorption band is observed at 1590 cm^{-1} (C═N). The 300 MHz ^{1}H NMR spectrum of compound **4a** showed a singlet at δ 8.20 integrating for one proton, which is attributed to the benzylidene proton. The aromatic protons resonated as three multiplets at δ 7.60–7.55, 7.45–7.33 and 7.19–7.17. Further evidence for the formation of compound **4a** was obtained by recording its mass spectra. The mass spectrum of compound **4a** showed a molecular ion peak at *m*/*z* 422 $(M+1)^+$, which is in consistent with its molecular formula $C_{23}H_{11}N_5O_2S$. The characterization data of (*Z*)-2-(4-substitutedbenzylidene)-7-isocyano-3,6-dioxo-8-phenyl-3,6-dihydro-2*H*-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-*a*]-pyridine-9-carbonitrile (**4a–j**) are given in Section 2.

The IR spectrum of 3-hydroxy-7-isocyano-6-oxo-8-phenyl-2-(phenyl(piperidin-1-yl)methyl)-6*H*-thiazolo-[3′,2′:2,3][1,2,4] triazolo[1,5-*a*]pyridine-9-carbonitrile (**5a**) was showed an absorption band at 3100 cm^{-1}, 3030 cm^{-1} indicated the O–H, Ar-H stretching. The absorption band appeared at 1730 cm^{-1} due to the presence of C═O stretching of the thiazolo ring system. Other prominent absorption band was observed at 1590 cm^{-1} (C═N). The 300 MHz ^{1}H NMR spectrum of compound **5a** showed a singlet at δ 6.90 integrating for one proton, which was attributed to the benzylidene proton. The aromatic protons resonated as multiplet at δ 7.70–7.30 and 3.7–1.4 (m, 10H, $5CH_2$ of piperidine). Further evidence for the formation of compound **5a** was obtained by recording its mass spectra. The mass spectrum of compound **5a** showed a molecular ion peak at *m*/*z* 507 $(M+1)^+$, which was in consistent with its molecular formula $C_{28}H_{22}N_6O_2S$. The characterization data of 3-hydroxy-7-isocyano-6-oxo-8-phenyl-2-(substitutedphenyl-(piperidin-1-yl)-methyl)-6*H*-thiazolo-[3′,2′:2,3][1,2,4]triazolo[1,5-*a*]-pyridine-9-carbonitrile (**5a–j**) were given in Section 2.

4. Pharmacological studies

4.1. Antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Klebsiella pneumoniae* (recultured) bacterial strains by disc diffusion method (Cruickshank et al., 1975; Collins, 1976). The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. The compounds **4a, 4b, 4e, 4f, 4i, 4j, 5a, 5b, 5d, 5g, 5i** and **5j** showed very good activity against all the bacterial strains.

4.2. Antifungal studies

Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method (Khan, 1997; Varma, 1998). The antifungal screening data showed moderate to good activity. Compounds **4a, 4c, 4f, 4i, 4j, 5a, 5c, 5f, 5i** and **5j** emerged as very active against all the fungal strains.

5. Pharmacological assay

5.1. Antibacterial assay

A standard inoculum ($1–2\times10^7$ c.f.u/cm^3 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatman No.1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile disc previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37 °C. The inhibition zones were measured and compared with

Table 1 Antibacterial activity of thiazolotriazinoes (**4a–j**) and (**5a–j**).

Compound no.	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Streptococcus pyogenes*
4a	20 (6.25)	25 (6.25)	29 (6.25)	18 (6.25)	23 (6.25)
4b	23 (6.25)	28 (6.25)	30 (6.25)	18 (6.25)	21 (6.25)
4c	10 (12.5)	–	–	17 (6.25)	11 (6.25)
4d	8 (25)	23 (6.25)	9 (25)	–	8 (12.5)
4e	22 (6.25)	27 (6.25)	32 (6.25)	20 (6.25)	24 (6.25)
4f	21 (6.25)	29 (6.25)	32 (6.25)	20 (6.25)	23 (6.25)
4g	10 (12.5)	15 (25)	–	–	17 (12.5)
4h	12 (12.5)	–	21 (6.25)	–	8 (25)
4i	21 (6.25)	24 (6.25)	29 (6.25)	19 (6.25)	23 (6.25)
4j	21 (6.25)	26 (6.25)	32 (6.25)	21 (6.25)	24 (6.25)
5a	22 (6.25)	26 (6.25)	28 (6.25)	21 (6.25)	24 (6.25)
5b	21 (6.25)	28 (6.25)	30 (6.25)	20 (6.25)	22 (6.25)
5c	–-	10 (6.25)	–	–	10 (6.25)
5d	23 (6.25)	26 (6.25)	29 (6.25)	21 (6.25)	22 (6.25)
5e	8 (25)	–	–	10 (6.25)	12 (12.5)
5f	–	8 (12.5)	10 (12.5)	14 (12.5)	–
5g	19 (6.25)	28 (6.25)	27 (6.25)	21 (6.25)	20 (6.25)
5h	10 (12.5)	–	12 (6.25)	–	8 (25)
5i	20 (6.25)	27 (6.25)	28 (6.25)	20 (6.25)	22 (6.25)
5j	22 (6.25)	28 (6.25)	31 (6.25)	21 (6.25)	24 (6.25)
Standard[a]	24 (6.25)	30 (6.25)	33 (6.25)	23 (6.25)	25 (6.25)

– indicates bacteria is resistant to the compounds at >100 μg/mL, MIC values are given in brackets. MIC (μg/mL) = minimum inhibitory concentration, i.e., lowest concentration to completely inhibit bacterial growth. Zone of inhibition in mm.

[a] Ciprofloxacin was used as standard.

Table 2 Antifungal activity of thiazolotriazinoes (**4a–j**) and (**5a–j**).

Compound no.	*Aspergillus fumigatus*	*Aspergillus flavus*	*Trichophyton mentagrophytes*	*Penicillium marneffei*	*Candida albicans*
4a	22 (6.25)	22 (6.25)	25 (6.25)	22 (6.25)	20 (6.25)
4b	8 (25)	–	12 (12.5)	–	17 (6.25)
4c	22 (6.25)	20 (6.25)	22 (6.25)	25 (6.25)	17 (6.25)
4d	15 (6.25)	–	7 (25)	21 (6.25)	18 (6.25)
4e	5 (25)	18 (6.25)	–	12 (12.5)	17 (6.25)
4f	24 (6.25)	21 (6.25)	21 (6.25)	23 (6.25)	18 (6.25)
4g	11 (12.5)	12 (25)	–	–	14 (12.5)
4h	9 (25)	–	12 (12.5)	9 (25)	10 (12.5)
4i	22 (6.25)	19 (6.25)	20 (6.25)	23 (6.25)	19 (6.25)
4j	21 (6.25)	26 (6.25)	32 (6.25)	21 (6.25)	24 (6.25)
5a	22 (6.25)	22 (6.25)	25 (6.25)	22 (6.25)	20 (6.25)
5b	8 (25)	–	12 (12.5)	–	17 (6.25)
5c	22 (6.25)	20 (6.25)	22 (6.25)	25 (6.25)	17 (6.25)
5d	15 (6.25)	–	7 (25)	21 (6.25)	18 (6.25)
5e	5 (25)	18 (6.25)	–	12 (12.5)	17 (6.25)
5f	24 (6.25)	21 (6.25)	21 (6.25)	23 (6.25)	18 (6.25)
5g	11 (12.5)	12 (25)	–	–	14 (12.5)
5h	9 (25)	–	12 (12.5)	9 (25)	10 (12.5)
5i	22 (6.25)	19 (6.25)	20 (6.25)	23 (6.25)	19 (6.25)
5j	21 (6.25)	26 (6.25)	32 (6.25)	21 (6.25)	24 (6.25)
Standard[b]	25 (6.25)	21 (6.25)	23 (6.25)	25 (6.25)	19 (6.25)

– indicates fungus is resistant to the compounds at >100 mg/mL, MIC values are given in brackets. MIC (mg/mL) = minimum inhibitory concentration, i.e., lowest concentration to completely inhibit fungal growth. Zone of inhibition in mm.

[b] Amphotericin was used as standard.

the controls. Minimum inhibitory concentration (MIC) was determined by broth dilution technique. The nutrient broth, which contained logarithmic serially twofold diluted amount of test compound and controls were inoculated with approximately 5×10^5 c.f.u of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentrations (*MIC*). Ciprofloxacin was used as a standard drug. The diameter of the zone of inhibition and minimum inhibitory concentration values are given in Table 1.

5.2. Antifungal assay

Sabouraud's agar media was prepared by dissolving 1 g peptone, 4 g D-glucose, and 2 g agar in 100 cm^3 distilled water, and adjusting pH to 5.7 using buffer. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loop full of particular fungal strain was transferred to 3 cm^3 saline to get a suspension of corresponding species. 20 cm^3 of agar media was poured in to each Petri dish. Excess of suspension was decanted and the plates were dried by placing in a incubator at 37 °C for 1 h. Using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 d. The inhibition zones in diameter were measured and compared with the controls. The Nutrient Broth, which contained logarithmic serially twofold diluted amount of test compound and controls was inoculated with approximately $1.6–6 \times 10^4$ c.f.u cm^{-3}. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentrations (*MIC*). Amphotericin B was used as the standard drug. The diameter of zone of inhibition and minimum inhibitory concentration values are given in Table 2.

6. Conclusion

The investigation of antibacterial screening data reveals that among the 20 compounds screened, seven compounds showed good bacterial and fungal inhibition almost equivalent to that of standard.

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References

Abdel-Rahman, R.M., 2001. Pharmazie 56, 18.
Abdel-Rahman, R.M., Seada, M., Fawzy, M., 1994. Pharmazie 49, 811.
Abdel-Rahman, R.M., Morsy, J.M., El-Edfawy, S., 1999. Pharmazie 54, 667.
Abdel-Rahman, R.M., Morsy, J.M., Hanafy, F., 1999. Pharmazie 54, 347.
Al-Najjar, A.A., Amer, S.A.R., Riad, M., Elghamry, I., Elnagdi, M.H., 1996. J. Chem. Res., 296–297.
Appel, R., Kleistuk, R., Ziehn, K.D., Knoll, F., 1970. Chem. Ber. 103, 3631.
Barsy, M.A., El-Rady, A.E., 2006. J. Heterocycl. Chem. 43, 523.
Bower, J.D., Doyle, F.P., 1957. J. Chem. Soc., 727–732.
Braese, S., Gil, C., Knepper, K., Zimmermann, V.A., 2005. Angew. Chem., Int. Ed. 44, 5188.
Brown, D.J., Iwai, J., 1979. Aust. J. Chem. 32, 2727–2733.
Cantegriil, R., Chem, A., Mortier, J., Peignier, R., 1997. Eur. Patent 483027; Chem. Abstr., 1997, 117, 131214.
Collins, A.H., 1976. Microbiological Methods, second ed. Butterworth, London.
Cruickshank, R., Duguid, J.P., Marion, B.P., 1975, 12th ed.. In: Medicinal Microbiology, vol. 2 Churchill Livingstone, London.
Eguchi, S., 2005. ARKIVOC 2, 98.
Eguchi, S., 2006. Top. Heterocycl. Chem. 6, 113–156.
Eguchi, S., Goto, S., 1994. Heterocycl. Commun. 1, 51.
Eguchi, S., Yamashita, K., Matsushita, Y., 1992. Synlett, 295.
El-Hawash, S.A., Habib, N.S., Fanaki, N.H., 1999. Pharmazie 45, 808–813.
Fresneda, P.M., Molina, P., 2004. Synlett, 1–17.
Gibson, M.S., 1963. Tetrahedron 19, 1587–1589.
Neunhoeffer, H., 1984. Comprehensive Heterocyclic Chemistry. In: Katritzky, A.R., Rees, C.W. (Eds.), Bolton, A.J. Mckillop, A. (Vol. Eds.), vol. 3, Part 2B, Pergamon Press, Oxford, p. 285.
Hadi, A., Martin, N., Seoane, C., Soto, J.L., 1992. J. Heterocycl. Chem. 29, 1229.
Khan, Z.K., 1997. In vitro and vivo screening techniques for bioactivity screening and evaluation. In: Proceedings of the International Workshop UNIDO-CDRI, p. 210.
Molina, P., Fresneda, P.M., 1988. J. Chem. Soc., Chem. Commun., 1819.
Molina, P., Vilaplana, M.J., 1990. Synthesis, 474.
Molina, P., Alajarin, M., Vidal, A., 1990. Tetrahedron 46, 1063.
Molina, P., Alajarin, M., Vidal, A., 1990a. J. Chem. Soc., Chem. Commun., 7.
Molina, P., Alajarin, M., Vidal, A., 1990b. J. Org. Chem. 55, 6140.
Neunhoeffer, H., 1978. The Chemistry of Heterocyclic Compounds. In: Weissberger, A., Taylor, E.C. (Eds.). Wiley, New York, p. 189, 33.
Palacios, F., Alonso, C., Aparicio, D., Rubiales, G., Delos Santos, J.M., 2007. Tetrahedron 63, 523–575.
Peignier, R., Cheme, A., Cantregril, R., Mortier, A., 1991. Eur. Patent 441718; Chem. Abstr., 1991, 115, 208000.
Pollak, A., Tisler, M., 1966. Tetrahedron 22, 2073–2079.
Sarges, S., Howard, H.R., Browne, R.G., Lbel, L.A., Seymour, P.A., Koe, B.K., 1990. J. Med. Chem. 33, 2240–2254.
Sato, T., Ohmori, H., Ohkuho, T., Motoki, S., 1993. J. Chem. Soc., Chem. Commun., 1802.
Takeuchi, H., Yanagida, S., Ozaki, T., Hagiwara, S., Eguchi, S., 1989. J. Org. Chem. 54, 431.
Takeuchi, H., Hagiwara, S., Eguchi, S., 1989. Tetrahedron 45, 6375.
Tarzia, G., Ocelli, E., Toja, E., Barone, D., Corsico, N., Gallico, L., Luzzani, F., 1988. J. Med. Chem. 32, 1115–1123.
Tarzia, G., Ocelli, E., Barone, D., 1989. Il Farmaco 44, 3–16.
Trust, R.I., Albrigh, J.D. 1980. U.S. Patent 4242515.
Varma, R.S., 1998. Antifungal Agents: Past, Present & Future Prospects. National Academy of Chemistry & Biology, Lucknow, India.
Wamhoff, A., Schmidt, A., 1993. J. Org. Chem. 58, 6976.