One-pot, multi-component synthesis of novel 2-amino-[1,2,4]triazolo[1,5-a] pyrimidine-6-carboxamide derivatives as antiproliferative agents

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Abstract

A novel series of 2-amino-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide derivatives has been achieved successfully *via* an efficient one-pot three-component Biginelli-like heterocyclization reaction between different benzaldehydes, 1H-1,2,4-triazole-3,5-diamine, and *N*-substituted acetoacetamides in the presence of p-toluenesulfonic acid as a catalyst. Moreover, the effects of different conditions on the reaction were well investigated. In addition, cancer cell growth inhibition activity for these target compounds was also explored, and analogue **5l** demonstrated the most potent cytotoxic activity against different cancer cells. These finds indicat that 2-amino-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide core could be well worth further optimization as a potential scaffold for development of anticancer agents.

Introduction

[1, 2, 4]Triazolo[1,5-a]pyrimidines are a well-documented nitrogenous heterocyclic compounds which [1–2], have attracted remarkable research interest in very diverse fields ranging from agriculture to medicine owing to their broad biological activities, including herbicidal [3–6], fungicidal [7], antiparasitic [8], antimicrobial [9], anti-inflammatory [10], antiviral [11], and antitumor activities [12–15]. These diverse pharmacological activities have made [1, 2, 4]triazolo[1,5-a]pyrimidine core a promising bicyclic scaffold to develop novel drugs for treatment of different diseases, and some representative important structures are shown in Fig. 1.

On the other hand, arylamide compounds are well known to exhibit various biological and medicinal activities, particularly as anticancers [16–18]. Our group previously has reported a series of heterocycle-based arylamide derivatives, which exhibited potent antiproliferative activities toward different kinds of human cancer cell lines [19–22]. Inspired by these facts and in continuation of our work on the discovery of new bioactive 1,2,4-triazole-fused heterocycles [23–26], herein we reported an efficient one-pot multi-component synthesis of a series of novel [1, 2, 4]triazolo[1,5-a]pyrimidine/arylamide hybrids **5a**–**v** (Scheme 1). Although an extensive research on the synthesis of [1, 2, 4]triazolo[1,5-a]pyrimidines has been performed, to the best of our knowledge, multi-component reaction for 2-amino-[1, 2, 4]triazolo[1,5-a]pyrimidine-6-carboxamide derivatives has never been reported up to now. Results from preliminary antiproliferative evaluation of these newly synthesized compounds are also described in this work.

Results And Discussion

Chemistry

A series of [1, 2, 4]triazolo[1,5-a]pyrimidine/arylamide hybrids **5a**–**v** were synthesized through a three-component Biginelli-like heterocyclization reaction from different benzaldehydes, 1H-1,2,4-triazole-3,5-diamine, and *N*-substituted acetoacetamides, as shown in Scheme 1. To optimize the reaction conditions for preparation of 2-amino-5-methyl-N-(p-tolyl)-7-(3,4,5-trimethoxyphenyl)-[1, 2, 4]triazolo[1,5-a]pyrimidine-6-carboxamide **5a**, various catalysts, addition of the catalyst, solvent, temperatures and time were

investigated. The results were shown in Table 1 (entries 1–14). It was observed that catalyst and amount of catalyst played a significant role in the model reaction (entries 1–3, 9–10), and the good results were achieved in the presence of p-toluenesulfonic acid (5% entry 3). We further studied the influence of solvents, such as DMF, THF, EtOH, dioxane, DCM and toluene at reflux temperature or 90°C (entries 3–8), and the highest yield of the products was obtained in the presence of DMF (entry 3). In addition, we subsequently explored the reaction at different temperatures and reaction times (entry 9–14), and it was revealed that a considerable increase or a sharp decrease of temperatures decreased the yield slightly, while longer reaction times had no obvious effect on the yields. Finally, the procedure in entry 3 was chosen as the best conditions for an efficient one-pot method. To explore the generally applicability of this protocol, we next synthesized a variety of [1, 2, 4]triazolo[1,5-a]pyrimidines using different types of acetoacetamides containing both electron withdrawing or donating groups in the aromatic ring, as well as hetercycles (Table 2).

Table 1

Optimization of reaction conditions [a]

Entry	Catalyst	Mol (%)	Solvent	Temperature (°C)	Time (h)	Yield [b] (%)
1	CH_3COOH	5%	DMF	90	12	45
2	HCl	5%	DMF	90	12	30
3	TsOH	5%	DMF	90	12	51
4	TsOH	5%	THF	Reflux	12	35
5	TsOH	5%	Dioxane	90	12	40
6	TsOH	5%	EtOH	Reflux	12	25
7	TsOH	5%	DCM	Reflux	12	Trace
8	TsOH	5%	Toluene	90	12	42
9	TsOH	10%	DMF	90	12	47
10	TsOH	1%	DMF	90	12	38
11	TsOH	5%	DMF	80	12	35
12	TsOH	5%	DMF	100	12	45
13	TsOH	5%	DMF	90	16	58
14	TsOH	5%	DMF	90	20	55

[a] Reagents and conditions: 3,4,5-trimethoxybenzaldehyde (0.3 mmol),1H-1,2,4-triazole-3,5-diamine (0.3 mmol), 3-oxo-*N*-p-tolylbutanamide (0.3 mmol), and solvent (5 mL). [b] Isolated yield.

The chemical structures assigned for the reaction products were well characterized with various spectroscopic techniques including ^{1}H NMR, ^{13}C NMR, and HRMS, which were in full agreement with the proposed structures, and given in the Experimental Section.

In vitro **antiproliferative activity**

Antiproliferative activities of all newly synthesized [1, 2, 4]triazolo[1,5-a]pyrimidines **5a**–**v** *in vitro* was investigated at a single concentration of 100 μM through MTT assay against A549 (human alveolar epithelial cells), T47D (human breast cancer cells), and Panc-1 (human pancreatic carcinoma cell line). The results expressed as the percent growth of treated cells compared to untreated control cells, and were summarized in Table 2. Disappointingly, most triazolopyrimidine derivatives demonstrated weak or moderate growth inhibition activity against three tested cancer cell lines. Notably, analogues **5l**, **5n**, **5t**, and **5v** displayed significant cytotoxic activities on A549 and Panc-1 with growth inhibition (GI) percent higher than 50%. It was interesting to obtain that derivatives having electron-withdrawing groups always exerted much lower antiproliferative activity, such as **5h**, **5i**, **5j**, and **5k**. In contrast, compound **5l** with 3-hydroxyl,4-methoxy group as R^2 substituent demonstrated the most potent cell growth inhibition activity toward T47D, Panc-1, and A549 cell lines with IC_{50} values at 58.37, 97.60, and 107.15 μM, respectively,

Due to technical limitations, Table 2 is only available as a download in the supplementary files section.

Conclusion

The present work reported an efficient one-pot three-component Biginelli-like heterocyclization procedure for the preparation of new 2-amino-[1, 2, 4]triazolo[1,5-a]pyrimidine-6-carboxamide derivatives from different benzaldehydes, 1H-1,2,4-triazole-3,5-diamine, and *N*-substituted acetoacetamides in the presence of p-toluenesulfonic acid as a catalyst. Reaction conditions were also optimized, and all the newly prepared derivatives were fully characterized by routine spectral methods. Moreover, these new compounds were evaluated for their *in vitro* cancer cell growth inhibition activity against A549, T47D, and Panc-1 cells. Several compounds demonstrated potent cytotoxic activity toward different cancer cells. Particularly, analogue **5l** having 3-hydroxyl,4-methoxy moiety exhibited the most potent cell growth inhibition activity on T47D and Panc-1 cells with IC_{50} values at 58.37 and 97.60 μM, respectively. In brief, 2-amino-[1, 2, 4]triazolo[1,5-a]pyrimidine-6-carboxamide core could represent a potential scaffold for optimization and development of anticancer agents.

Experimental Protocols

General information

All commercially available starting materials were used without further purification. Melting points (mp) were determined by a Buchi B-545 apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Mercury-Plus 400 spectrometer in $CDCl_3$ or DMSO-d_6 solution and chemical shifts (δ) were expressed as parts per million (ppm) using tetramethylsilane as an internal reference. High-

resolution mass spectra (HRMS) were carried out with an Agilent QTOF 6540 mass spectrometer. *N*-substituted acetoacetamides were synthesized according to the procedures reported by Armaghan [27].

Procedure for the preparation of 2-amino-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide derivatives 5a–v

To a mixture of appropriate aldehyde (0.3 mmol), 1H-1,2,4-triazole-3,5-diamine (0.3 mmol), *N*-substituted acetoacetamides (0.3 mmol) in DMF (5 mL) was added to p-toluenesulfonic acid (0.015 mmol), and the resulting solution was stirred for 16 h at 90°C. After cooling, the reaction mixtures was treated with saturated sodium bicarbonate solution (30 mL), extracted with EtOAc (3 × 20 mL), dried over anhydrous $MgSO_4$, and evaporated in vacuo to give a precipitate, which was purified by silica gel column chromatography to provide the target compounds **5a**–**v** in yields of 43–66%.

*2-amino-5-methyl-N-(p-tolyl)-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5a**).* Yield, 58%; mp: 293.5-295.1°C; ^{1}H NMR (400 MHz, DMSO-d_6)d: 2.24 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 3.68 (s, 3H, CH_3O), 3.69 (s, 6H, 2×CH_3O), 6.44 (s, 2H, NH_2), 7.05 (s, 2H, ArH), 7.10 (d, J = 8.0 Hz, 2H, ArH), 7.36 (d, J = 8.4 Hz, 2H, ArH), 10.39 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.84, 168.25, 163.54, 159.55, 157.64, 147.22, 144.11, 141.06, 138.37, 134.37, 129.54, 124.49, 124.01, 112.30, 65.23, 61.18, 27.79, 25.61. HRMS (ESI) m/z: calcd for $C_{23}H_{24}N_6O_4$ ($M+H^+$) 449.1932 found 449.1931.

*2-amino-N-(3-methoxyphenyl)-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5b**),*Yield: 49%; mp: 226.1-227.5°C; ^{1}H NMR (400 MHz, DMSO-d_6) d: 2.69 (s,3H, CH_3), 3.67 (s, 3H, CH_3O), 3.71 (s, 6H, 2×CH_3O), 3.72 (s, 3H, CH_3O), 6.60 (s, 2H, NH_2), 6.70 (dd, J_1 = 1.6 Hz, J_2 = 8.0 Hz, 1H, ArH), 7.10 (s, 3H, ArH), 7.24 (s, 2H, ArH), 10.60 (s, 1H, NH).^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.13, 163.78, 159.92, 158.75, 154.82, 152.91, 142.51, 139.93, 139.37, 130.11, 124.75, 119.18, 112.02, 109.92, 107.54, 105.59, 60.47, 56.41, 55.41, 23.05. HRMS (ESI) m/z: calcd for $C_{23}H_{24}N_6O_5$ ($M+H^+$) 465.1881 found 465.1879.

*2-amino-N-(4-methoxyphenyl)-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5c**).* Yield, 50%; mp: 290.5-291.3°C; ^{1}H NMR (400 MHz, DMSO-d_6)d: 2.69 (s, 3H, CH_3), 3.69 (s, 3H,CH_3O), 3.71 (s, 6H, 2×CH_3O), 3.73 (s, 3H, CH_3O), 6.59 (s, 2H, NH_2), 6.91 (d, J = 8.8 Hz, 2H, ArH), 7.12 (s, 2H, ArH), 7.47 (d, J = 8.8 Hz, 2H, ArH),10.47 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13C NMR (101 MHz, DMSO) δ 168.45, 163.82, 156.44, 156.27, 154.13, 152.97, 143.62, 139.12, 133.62, 132.06, 121.27, 118.41, 114.41, 106.27, 60.50, 56.18, 55.62, 15.50. HRMS (ESI) m/z: calcd for $C_{23}H_{24}N_6O_5$ ($M+H^+$) 465.1881 found 465.1877.

*2-amino-N-(3,4-dimethylphenyl)-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5d**).* Yield, 55%; mp: 308.1-309.2°C; ^{1}H NMR (400 MHz, DMSO-d_6) d: 2.16 (s, 6H, 2×CH_3), 2.55 (s, 3H, CH_3), 3.68 (s, 3H, CH_3O),3.70 (s, 6H, 2×CH_3O), 6.43 (s, 2H, NH_2), 7.06 (s, 3H, ArH), 7.17 (d, J = 6.4 Hz, 1H, ArH), 7.27 (s, 1H, ArH), 10.40 (s,1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.04, 163.48, 158.80, 154.78, 152.88, 142.43, 139.31, 136.89, 136.57, 132.42, 130.03, 124.84, 120.97, 119.37, 117.35,

107.56, 60.47, 56.42, 23.02, 19.96, 19.21. HRMS (ESI) m/z: calcd for $C_{24}H_{26}N_6O_4$ ($M+H^+$) 463.2089 found 463.2086.

2-amino-N-(3,4-dimethoxyphenyl)-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide ***(5e)***. Yield, 61%; mp: 281.0-282.3°C; ^{1}H NMR (400 MHz, DMSO-d_6) d: 2.57 (s, 3H, CH_3), 3.68 (s, 6H, 2×CH_3O), 3.71 (s, 9H, 3×CH_3O), 6.45 (s, 2H, NH_2), 6.88 (d, J = 8.4 Hz, 1H, ArH), 6.97 (dd, J_1 = 2.0 Hz, J_2 = 8.4 Hz, 1H, ArH), 7.06 (s, 2H, ArH), 7.10 (d, J = 2.0 Hz, 1H, ArH), 10.31 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.09, 163.32, 158.84, 154.80, 152.90, 148.98, 145.97, 142.49, 139.35, 132.28, 124.82, 119.27, 112.48, 111.97, 107.57, 104.84, 60.49, 56.43, 56.14, 55.73, 23.07. HRMS (ESI) m/z: calcd for $C_{24}H_{26}N_6O_6$ ($M+H^+$) 495.1987 found 495.1984.

*2-amino-N-mesityl-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (****5f****).* Yield, 52%; mp: 303.1-304.5°C; ^{1}H NMR (400 MHz, DMSO-d_6)d: 1.81 (s, 6H, 2×CH_3), 2.19 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 3.73 (s, 3H, CH_3O), 3.75 (s, 6H, 2×CH_3O), 6.57 (s, 2H, NH_2), 6.82 (s, 2H, ArH), 7.04 (s, 2H, ArH), 9.59 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.36, 164.09, 157.43, 154.01, 153.08, 143.56, 139.29, 136.18, 134.92, 134.21, 131.66, 128.93, 118.82, 107.17, 60.48, 56.39, 20.77, 18.11, 15.61. HRMS (ESI) m/z: calcd for $C_{25}H_{28}N_6O_4$ ($M+H^+$) 477.2245 found 477.2240.

*2-amino-N-(4-chlorophenyl)-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (****5g****).* Yield, 57%; mp: 289.3-291.5°C; ^{1}H NMR (400 MHz, DMSO-d_6) d: 2.57 (s, 3H, CH_3), 3.67 (s, 3H, CH_3O),3.69 (s, 6H, 2×CH_3O), 6.47 (s, 2H, NH_2), 7.04 (s, 2H, ArH), 7.37 (d, J =8.8 Hz, 2H, ArH), 7.51 (d, J = 8.8 Hz, 2H, ArH), 10.61 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.17, 163.88, 158.71, 154.84, 152.92, 142.59, 139.40, 137.73, 129.24, 128.17, 124.69, 121.18, 118.90, 107.50, 60.48, 56.44, 23.06. HRMS (ESI) m/z: calcd for $C_{22}H_{21}ClN_6O_4$ ($M+H^+$) 469.1386 found 469.1377.

*2-amino-5-methyl-N-(3-nitrophenyl)-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (****5h****).* Yield: 45%; mp: 329.5–330.9°C; ^{1}H NMR (400 MHz, DMSO-d_6) d: 2.72 (s, 3H, CH_3), 3.65 (s, 3H, CH_3O), 3.70 (s, 6H, 2×CH_3O), 6.64 (s, 2H, NH_2), 7.07 (s, 2H, ArH), 7.64 (t, J = 7.6 Hz, 1H, ArH), 7.83 (d, J = 7.2 Hz, 1H, ArH), 7.98 (d, J = 7.2 Hz, 1H, ArH), 8.59 (s, 1H, ArH), 11.07 (s, 1H,NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.56, 165.11, 156.61, 154.23, 153.03, 148.36, 143.92, 139.90, 139.18, 133.49, 130.91, 125.72, 119.23, 117.66, 113.79, 106.24, 60.48, 56.20, 15.53. HRMS (ESI) m/z: calcd for $C_{22}H_{21}N_7O_6$ ($M+H^+$) 480.1626 found 480.1623.

*2-amino-N-(4-fluorophenyl)-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (****5i****).*Yield, 57%; mp: 311.2-312.9°C; ^{1}H NMR (400 MHz,DMSO-d_6) δ: 2.57 (s, 3H, CH_3), 3.68 (s, 3H, CH_3O), 3.69 (s, 6H, 2×CH_3O), 6.46 (s, 2H, NH_2), 7.05 (s, 2H, ArH), 7.15 (t, J = 8.8 Hz, 2H, ArH), 7.48 (d, J = 4.8 Hz, 1H, ArH), 7.50 (d, J = 5.2Hz, 1H, ArH), 10.52 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.14,

163.67, 158.76, 154.83, 152.91, 142.55, 139.39, 135.19, 124.74, 121.56, 118.99, 116.05, 115.82, 107.53, 60.49, 56.44, 23.06. HRMS (ESI) m/z: calcd for $C_{22}H_{21}FN_6O_4$ ($M+H^+$) 453.1681 found 453.1677.

*2-amino-5-methyl-N-(4-nitrophenyl)-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5j**).* Yield, 43%;mp: 319.9-320.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.71 (s, 3H, CH_3), 3.65 (s, 3H, CH_3O), 3.70 (s, 6H, 2×CH_3O), 6.64 (s, 2H, NH_2), 7.05 (s, 2H, ArH), 7.81 (d, *J*= 9.2 Hz,2H, ArH), 8.25 (d, *J*= 9.2 Hz,2H, ArH), 11.18 (s, 1H, NH).^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.57, 165.26, 156.62, 154.23, 153.03, 144.82, 143.92, 143.35, 139.16, 133.44, 125.53, 119.52, 117.63, 106.19, 60.49, 56.23, 15.54. HRMS (ESI) m/z: calcd for $C_{22}H_{21}N_7O_6$ ($M+H^+$) 480.1626 found 480.1618.

*2-amino-N-(4-bromophenyl)-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5k**).*Yield, 54%; mp: 280.1-284.3°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.69 (s, 3H, CH_3), 3.67 (s, 3H, CH_3O), 3.70 (s, 6H, 2×CH_3O), 6.62 (s, 2H, NH_2), 7.08 (s, 2H, ArH), 7.53 (s, 4H, ArH), 10.74 (s, 1H, NH).^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.51, 164.50, 156.47, 154.17, 153.00, 143.73, 139.15, 138.26, 133.51, 132.18, 121.60, 118.05, 116.31, 106.22, 60.50, 56.20, 15.51. HRMS (ESI) m/z: calcd for $C_{22}H_{21}BrN_6O_4$ ($M+H^+$) 513.0881 found 513.0870.

*2-amino-N-(3-hydroxy-4-methoxyphenyl)-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5l**).*Yield, 56%; mp: 249.7-250.3°C;^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.68 (s, 3H, CH_3), 3.69 (s, 3H, CH_3O), 3.72 (s, 9H, 3×CH_3O), 6.58 (s, 2H, NH_2), 6.85 (d, *J* = 8.4 Hz, 1H, ArH), 6.92 (d, *J* = 8.0 Hz, 1H, ArH), 7.12 (s, 2H, ArH), 7.15 (s, 1H,ArH), 9.12 (s, 1H, OH), 10.36 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.43, 163.69, 156.38, 154.11, 152.97, 146.90, 144.88, 143.55, 139.13, 133.60, 132.50, 118.54, 112.89, 110.52, 108.15, 106.26, 60.50, 56.28, 56.19, 15.48. HRMS (ESI) m/z: calcd for $C_{23}H_{24}N_6O_6$ ($M+H^+$) 481.1830 found 481.1823.

*2-amino-N-(4-methoxy-3-nitrophenyl)-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5m**).* Yield, 47%; mp: 286.1-288.2°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.51 (s, 3H, CH_3), 3.67 (s, 3H, CH_3O), 3.72 (s,6H,2×CH_3O),3.90 (s, 3H, CH_3O), 6.63 (s, 2H, NH_2), 7.07 (s, 2H, ArH), 7.37 (d, *J* = 8.8 Hz, 1H, ArH), 7.67 (d, *J* = 7.6 Hz, 1H, ArH), 8.22 (s, 1H, ArH), 10.82 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.52, 164.56, 156.55, 154.20, 153.02, 149.12, 143.86, 139.17, 138.79, 133.52, 131.64, 125.89, 117.75, 116.01, 115.52, 106.23, 60.50, 57.23, 56.21, 15.52. HRMS (ESI) m/z: calcd for $C_{23}H_{23}N_7O_7$ ($M+H^+$) 510.1732 found 510.1724.

*2-amino-N-(3-amino-4-methoxyphenyl)-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5n**).*Yield, 53%; mp: 240.1-241.1°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.67 (s, 3H, CH_3), 3.69 (s, 3H, CH_3O),3.72 (s, 9H, 3×CH_3O), 4.81 (s,2H, NH_2), 6.56 (s, 2H, NH_2), 6.71 (s, 2H, ArH), 6.97 (s, 1H, ArH), 7.13 (s, 2H, ArH), 10.25 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.40, 163.50, 156.31,

154.09, 152.96, 143.60, 143.45, 139.11, 138.18, 133.63, 132.56, 118.74, 110.90, 107.54, 106.26, 106.02, 60.50, 56.21, 55.89, 15.46. HRMS (ESI) m/z: calcd for $C_{23}H_{25}N_7O_5$ ($M+H^+$) 480.1990 found 480.1986.

*2-amino-N-(3-aminophenyl)-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5o**).* Yield, 63%; mp: 272.1-273.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.56 (s, 3H, CH_3), 3.70 (s, 3H, CH_3O),3.71 (s, 6H, 2×CH_3O), 5.11 (s, 2H, NH_2), 6.28 (d, *J* = 7.6 Hz, 1H, ArH), 6.42 (s, 2H, NH_2), 6.53 (d, *J* = 7.6 Hz, 1H, ArH), 6.88 (s, 1H, ArH), 6.91 (d, *J* = 8.0 Hz, 1H, ArH),7.06 (s, 2H, ArH),10.19 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.02, 163.35, 158.75, 154.77, 152.89, 149.55, 142.32, 139.44, 139.35, 129.41, 124.80, 119.54, 110.46, 107.56, 107.43, 105.28, 60.49, 56.43, 23.00. HRMS (ESI) m/z: calcd for $C_{22}H_{23}N_7O_4$ ($M+H^+$) 450.1885 found 450.1886.

*2-amino-5-methyl-N-phenyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5p**).* Yield, 51%; mp: 305.1-306.5°C; ^{1}H NMR (400 MHz, DMSO-d6) δ: 2.58 (s, 3H, CH_3), 3.67 (s, 3H, CH_3O), 3.69 (s, 6H, 2×CH_3O), 6.45 (s, 2H, NH_2), 7.06 (s, 2H, ArH), 7.10 (d, *J* = 7.2 Hz, 1H, ArH), 7.30 (t, *J* = 8.0 Hz, 2H, ArH), 7.48 (d, *J* = 7.6 Hz, 2H, ArH), 10.47 (s, 1H, NH). 13C NMR (100 MHz, DMSO-d6) δ: 168.12, 163.74, 158.77, 154.82, 152.90, 142.51, 139.37, 138.79, 129.27, 124.77, 124.56, 119.71, 119.18, 107.55, 60.47, 56.42, 23.05. HRMS (ESI) m/z: calcd for $C_{22}H_{22}N6O_4$ (M+H+) 435.1776 found 435.1784.

*2-amino-N-benzyl-5-methyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5q**).*Yield, 66%; mp: 230.1-231.5°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.60 (s, 3H, CH_3), 3.74 (s, 6H, 2×CH_3O),3.75 (s, 3H, CH_3O), 4.36 (d, *J* = 5.6 Hz, 2H, CH_2), 6.53 (s, 2H, NH_2), 7.04 (t,*J* = 3.6 Hz, 2H, ArH), 7.07 (s, 2H, ArH), 7.22 (d, *J* = 2.4 Hz, 2H, ArH), 7.23 (s, 1H, NH), 8.96 (t, *J* = 5.6 Hz, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.31, 165.92, 156.64, 154.07, 153.01, 143.45, 139.22, 138.70, 133.76, 128.62, 127.59, 127.34, 118.26, 106.49, 60.57, 56.25, 43.18, 15.32. HRMS (ESI) m/z: calcd for $C_{23}H_{24}N_6O_4$ ($M+H^+$) 449.1932 found 449.1930.

*2-amino-5-methyl-N-phenethyl-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5r**).* Yield, 52%; mp: 252.1-253.5°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.47 (s, 3H, CH_3), 2.61 (t, *J* = 7.2 Hz, 2H, CH_2), 3.39 (d, *J* = 6.0 Hz, 2H, CH_2), 3.71 (s, 3H, CH_3O), 3.83 (s, 6H, 2×CH_3O), 6.51 (s, 2H, NH_2), 7.05 (d, *J* = 7.2 Hz, 2H, ArH), 7.11 (s, 2H, ArH), 7.20 (s, 1H, NH), 7.24 (t, *J* = 7.2 Hz, 2H, ArH), 8.52 (t, *J* = 5.6 Hz, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.28, 165.86, 156.44, 154.05, 152.99, 143.40, 139.43, 139.22, 133.77, 128.95, 128.68, 126.56, 118.43, 106.50, 60.51, 56.34, 40.96, 34.99, 15.19. HRMS (ESI) m/z: calcd for $C_{24}H_{26}N_6O_4$ ($M+H^+$) 463.2089 found 463.2084.

*2-amino-5-methyl-N-(thiazol-2-yl)-7-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5s**).* Yield, 47%; mp: 289.1-291.2°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.51 (s, 3H, CH_3), 3.67 (s, 3H, CH_3O), 3.69 (s, 6H, 2×CH_3O), 6.51 (s,2H, NH_2), 7.01 (s, 2H, ArH), 7.29 (d, *J* = 3.6 Hz, 1H, ArH), 7.48 (d, *J*= 3.6 Hz, 1H, ArH), 12.62 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.22, 164.01, 158.61, 157.67, 155.02,

152.85, 142.83, 139.52, 138.34, 124.42, 117.11, 114.70, 107.56, 60.53, 56.37, 23.10. HRMS (ESI) m/z: calcd for $C_{19}H_{19}N_7O_4S$ ($M+H^+$) 442.1292 found 442.1283.

*2-amino-5-methyl-7-phenyl-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5t**).* Yield, 48%; mp: 293.2-295.1°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.70 (s, 3H, CH_3), 3.67 (s, 3H, CH_3O), 3.72 (s, 6H, 2×CH_3O), 6.61 (s, 2H, NH_2), 6.83 (s, 2H, ArH), 7.46 (s, 2H, ArH), 7.48 (s, 1H, ArH),7.78 (d, *J* = 5.6 Hz, 2H, ArH), 10.42 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.50, 163.93, 157.09, 154.28, 153.19, 143.75, 138.41, 134.85, 134.62, 130.03, 128.75, 118.41, 97.91, 60.49, 56.16, 15.58. HRMS (ESI) m/z: calcd for $C_{22}H_{22}N_6O_4$ ($M+H^+$) 435.1776 found 435.1751.

*2-amino-N-(4-fluorophenyl)-5-methyl-7-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5u**).* Yield, 46%; mp: 285.9-287.1°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.71 (s, 3H, CH_3), 6.62 (s, 2H, NH_2), 7.15 (t, *J* = 8.8 Hz, 2H, ArH), 7.44-7.50 (m, 5H, ArH), 7.76 (d, *J* = 4.0 Hz, 2H, ArH), 10.53 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.51, 164.03, 160.17, 157.77, 157.15, 154.30, 143.80, 138.38, 135.08, 130.01, 128.71, 121.99, 118.21, 115.99, 15.52. HRMS (ESI) m/z: calcd for $C_{19}H_{15}FN_6O$ ($M+H^+$) 363.1364 found 363.1363.

*2-amino-5-methyl-7-phenyl-N-(p-tolyl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (**5v**).*Yield, 60%; mp: 266.1-267.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.24 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 6.60 (s, 2H, NH_2), 7.11 (s, 2H, ArH), 7.35 (s, 2H, ArH), 7.43 (s, 3H, ArH), 7.76 (s, 2H, ArH), 10.38 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.46, 163.87, 157.13, 154.27, 143.72, 138.41, 136.23, 133.71, 129.97, 129.58, 128.67, 120.11, 118.47, 20.86, 15.49. HRMS (ESI) m/z: calcd for $C_{20}H_{18}N_6O$ ($M+H^+$) 359.1615 found 359.1615.

Antiproliferative activity

The cancer cell growth inhibition activity of compounds **5a**–**v** were evaluated *in vitro* toward a panel of three different human cancer cell lines, including T47D, A549, and Panc-1 using the standard MTT assay. T47D and A549 cells were cultured in RPMI-1640, and Panc-1 was cultured with DMEM medium supplemented with 10% FBS. Tested samples were dissolved in dimethyl sulfoxide (DMSO) at 100 mM and compound **5l** was diluted into a series of concentrations with the medium. Exponentially growing cells were then seeded into 96-well plates (2×10^3 cells/well) and incubated at 37 °C for 48 h. The medium was changed, and cells grew with the tested samples, including 0.1% DMSO as a negative control. Subsequently, 10 μL of MTT solution (5 mg/mL) was added into each well, and the plates were incubated for 4 h at 37°C. Finally, absorbance at 570 nm (Abs) of the suspension was obtained with a microplate reader, and inhibition percentage was measured using the following formula: % inhibition = ($Abs_{control}$ − $Abs_{compound}$)/$Abs_{control}$×100%. IC_{50} values of compound **5l** was calculated through the prism statistical package (GraphPad Software, San Diego, CA, U.S.A.).

Declarations

Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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Figures

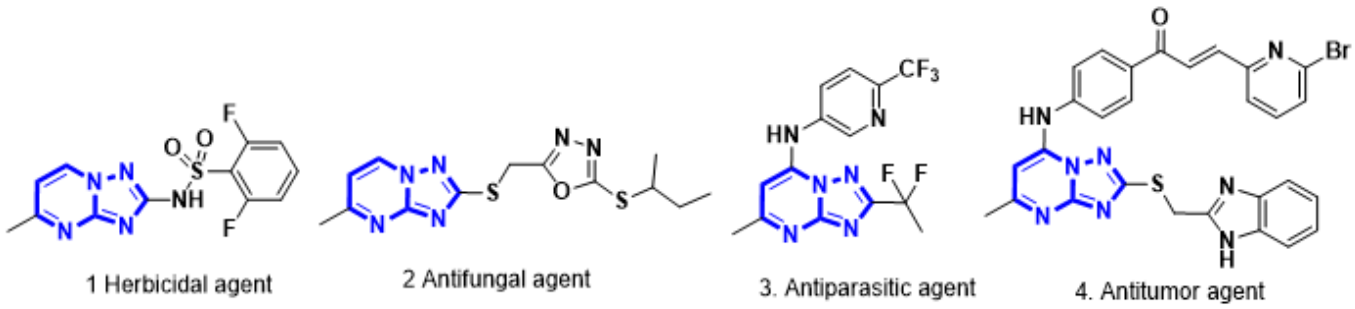

Figure 1

Representative examples of biologically active triazolopyrimidine derivatives

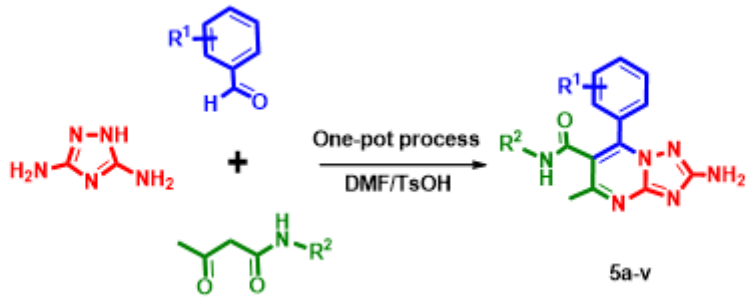

Figure 2

Scheme 1. Synthesis of the target compounds 5a–v.

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