Research Article

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Synthesis and antioxidant activity of 2-methylthio-pyrido[3,2-*e*][1,2,4] triazolo[1,5-*a*]pyrimidines

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Abstract: A series of 2-methylthio-pyrido-triazolopyrimidines (**1-17**) were prepared by the reaction of dimethyl-*N*-cyanoimidodithiocarbonate with hydrazinopyridine carboxylic acid as starting reactants. Their chemical structures were affirmed with HREI-MS, IR and NMR analyses. The target compounds (**1-17**) were evaluated for their antioxidant activity using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging, ferric reduction antioxidant power (FRAP) and reducing power capability (RPC). The results revealed that some pyrido-triazolopyrimidines showed good activity as antioxidant agents, in particular, compounds **12** and **15** were found to possess good antioxidant activity. Butylated hydroxyl toluene (BHT) was used as reference drug.

Keywords: pyrido-triazolopyrimidines; alkylation; thionation; antioxidants; DPPH.

1 Introduction

Extensive research in the organic-medicinal chemistry field has led to the discovery of different classes of bioactive substances, most of them being nitrogen-containing heterocycles. Within the huge number of nitrogenous heterocyclic products, in particular those containing

quinazoline, triazole, triazine, benzoquinazoline and triazoloquinazoline platforms, have been broadly scouted, showing to be highly versatile motif during the identification of new worthy and selective bioactive compounds for medicinal purposes.

One of the most challenging proceedings in the medicinal chemistry range is to explore and elaborate more potent drugs with only slight toxic effects and completely reversible. Thus, it is essential to consider the physiochemical features in the design and development of new bioactive constituents as well as their mechanism of action. Hundreds of thousands of new heterocyclic organic compounds are synthesized per year around the globe; many of them are submitted to biological screening for determination of their activities. These random investigation processes have been misfit, but they have led to discovery of new lead compounds whose structures have been optimized and developed to produce clinical agents.

Triazolo-annelated quinazolines were found to be potent adenosine antagonists, herbicidal and fungicidal agents [1-3]. In addition, further chemical transformation of their inherent lactam groups and substituent types at positions 2 and 5 were offered access to a variety of derivatives that have shown antihistamine, antimicrobial, antiviral, antihypertensive, anti-inflammatory, antitumor and antioxidant activities [4-11]. In light of the above-mentioned facts, we are interested in the conversion of triazolquinazoline moiety into a pyridotriazolopyrimidine scaffold. This scaffold has already been reported as a potent fungicidal and herbicidal agent [2,3] and incorporated in various substances that displayed interesting antimicrobial activity [12]. The emergence of various diseases resulting from oxidative stress such as diabetes, cancer, stroke, and cardiovascular dysfunctions, requires a permanent revaluation search and adjustment of adequate strategy to design and develop new bioactive substances to protect the human body against such diseases. In continuation of our on-going program research dealing with antioxidants, this research deals with the preparation and characterization of 2-methylthio-

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pyrido-triazolopyrimidines (**1-17**); thereafter, evaluating their antioxidant activity using different assays.

2 Experimental

2.1 Chemistry

Employing a Bruker AMX (500, 700 and 150 MHz), a JEOL the MStation JMS-700 and a Perkin Elmer FT-IR Spectrum BX and a STUART Melting point SMP 10 systems to measure NMR, HREI-MS, IR spectra and melting points, respectively. The measured melting points on open glass capillaries were uncorrected. The NMR samples were prepared in DMSO- d_6 and δ -values are given in ppm downfielded from TMS. DC Mikrokarten polygram SIL G/UV254 (Macherey-Nagel Firm, Duren Thickness: 0.25 mm) was used for following-up of the reactions and examining the purity of substances by TLC.

2.1.1 Preparation of 2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*a*]pyrimidin-5(4*H*)-one (**1**)

2-Hydrazinonicotinic acid (5.5 mmol) was mixed with a solution of dimethyl-*N*-cyanoimdidithiocarbonate (5 mmol) in EtOH (20 mL) at RT. Thereafter, Et₃N (20 mmol) was added gradually through for 10 min. After completion, the reaction mixture was stirred overnight at RT. Then, the reaction mixture was acidified by conc. HCl (0.5 ml) and boiled at 80°C for 1-3 h. Pouring the mixture into ice/water, the obtained solid was separated and dried. Yield (74%); mp: > 300°C; IR (KBr) ν/cm^{-1} 1684 (C=O); ¹H NMR (700 MHz, DMSO- d_6): δ 13.22 (1H, br s, -NH), 8.85 (1H, br d, *J* = 4.3 Hz, H-8), 8.55 (1H, br d, *J* = 7.7 Hz, H-6), 7.60 (1H, dd, *J* = 7.8, 4.8 Hz, H-7), 2.63 (3H, s, -SCH₃); ¹³C NMR (175 MHz, DMSO- d_6): δ ppm 163.1 (C-2), 160.20 (C-5), 154.7 (C-3a), 150.8 (C-9a), 146.3 (C-8), 138.4 (C-6), 122.5 (C-5a), 113.2 (C-7), 14.0 (-SCH₃); HRMS (EI), *m/z* Calcd. for C₉H₇N₅OS (M)⁺ 233.0371 found 233.0404.

2.1.2 Preparation of pyrido-triazolopyrimidines 2-11

At RT, K₂CO₃ (0.6 mmol) was added to a solution of **1** (0.5 mmol) in DMF (8 mL) and left to stir for 5 min. Thereafter, alkylhalide (1 mmol) was added and the reaction mixture was left to stir at RT for 18 h. Pouring the mixture into ice/water, the solid was collected, washed with H₂O and dried.

4-Ethyl-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*a*]pyrimidin-5(4*H*)-one (2**)** Yield (72%); mp: 200-202°C; IR (KBr) ν/cm^{-1} 1672 (C=O); ¹H NMR (700 MHz, DMSO- d_6): δ 8.87 (1H, br d, *J* = 4.6 Hz, H-8), 8.60 (1H, br d, *J* = 7.8 Hz, H-6), 7.63 (1H, dd, *J* = 7.6, 4.9 Hz, H-7), 4.17 (2H, q, *J* = 7 Hz, H-1'), 2.65 (3H, s, -SCH₃), 1.29 (3H, t, *J* = 7 Hz, H-2'); ¹³C NMR (175 MHz, DMSO- d_6): δ ppm 163.1 (C-2), 159.0 (C-5), 154.8 (C-3a), 151.5 (C-9a), 145.6 (C-8), 138.7 (C-6), 122.6 (C-5a), 112.6 (C-7), 39.60 (C-1'), 14.0 (-SCH₃), 12.8 (C-2'); HRMS (EI), *m/z* Calcd. for C₁₁H₁₁N₅OS (M)⁺ 261.0684, found 261.0722.

4-Allyl-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*a*]pyrimidin-5(4*H*)-one (3**)** Yield (70%); mp: 165-167°C; IR (KBr) ν/cm^{-1} 1680 (C=O); ¹H NMR (700 MHz, DMSO- d_6): δ 8.88 (1H, br d, *J* = 4.5 Hz, H-8), 8.61 (1H, br d, *J* = 7.8 Hz, H-6), 7.64 (1H, dd, *J* = 7.5 Hz, H-7), 5.96 (1H, m, H-2'), 5.27 (1H, br d, *J* = 17.3 Hz, H-3a'), 5.18 (1H, br d, *J* = 10.4 Hz, H-3b'), 4.74 (2H, d, *J* = 3.7 Hz, H-1'), 2.65 (3H, s, -SCH₃); ¹³C NMR (175 MHz, DMSO- d_6): δ ppm 163.0 (C-2), 159.0 (C-5), 154.9 (C-3a), 151.5 (C-9a), 145.7 (C-8), 138.8 (C-6), 131.6 (C-2'), 122.7 (C-5a), 117.8 (C-3'), 112.50 (C-7), 45.9 (C-1'), 14.0 (-SCH₃); HRMS (EI), *m/z* Calcd. for C₁₂H₁₁N₅OS (M)⁺ 273.0684, found, 273.0731.

4-Benzyl-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*a*]pyrimidin-5(4*H*)-one (4**)** Yield (69%); mp: 190-192°C; IR (KBr) ν/cm^{-1} 1677 (C=O); ¹H NMR (500 MHz, DMSO- d_6): δ 8.87 (1H, br d, *J* = 4.5 Hz, H-8), 8.62 (1H, br d, *J* = 7.8 Hz, H-6), 7.64 (1H, dd, *J* = 7.8, 4.8 Hz, H-7), 7.43 (2H, br d, *J* = 7.6 Hz, H-2'/6'), 7.33 (2H, br t, *J* = 7.4 Hz, H-3'/5'), 7.29 (1H, br t, *J* = 7.1 Hz, H-4'), 5.33 (2H, s, H-7'), 2.65 (3H, s, -SCH₃); ¹³C NMR (175 MHz, DMSO- d_6): δ ppm 163.5 (C-2), 159.4 (C-5), 155.0 (C-3a), 151.8 (C-9a), 145.7 (C-8), 138.8 (C-6), 136.1 (C-1'), 128.9 (C-3'/5'), 128.20 (C-2'/6'), 128.0 (C-4'), 122.7 (C-5a), 112.6 (C-7), 47.2 (C-7'), 14.00 (-SCH₃); HRMS (EI), *m/z* Calcd. for C₁₆H₁₃N₅OS (M)⁺ 323.0841, found 323.0891.

4-(2-Methylbenzyl)-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*a*]pyrimidin-5(4*H*)-one (5**)** Yield (75%); mp: 206-208°C; IR (KBr) ν/cm^{-1} 1680 (C=O); ¹H NMR (700 MHz, DMSO- d_6): δ 8.91 (1H, dd, *J* = 4.6 Hz, H-8), 8.63 (1H, br d, *J* = 7.8 Hz, H-6), 7.66 (1H, dd, *J* = 7.8, 4.8 Hz, H-7), 7.23 (1H, br d, *J* = 7.5 Hz, H-6'), 7.17 (1H, br t, *J* = 7.4 Hz, H-5'), 7.10 (1H, br d, *J* = 7.7 Hz, H-3'), 7.06 (1H, br t, *J* = 7.4 Hz, H-4'), 5.29 (2H, s, H-7'), 2.63 (3H, s, -SCH₃), 2.44 (3H, s, Ar-Me); ¹³C NMR (175 MHz, DMSO- d_6): δ ppm 163.00 (C-2), 159.5 (C-5), 154.9 (C-3a), 151.9 (C-9a), 145.80 (C-8), 138.9 (C-6), 135.7 (C-1'), 133.8 (C-2'), 130.50 (C-3'), 127.6 (C-4'), 126.4 (C-5'), 126.1 (C-6'), 122.8 (C-5a), 112.6 (C-7), 45.2 (C-7'), 19.3 (Ar-Me), 14.00 (-SCH₃); HRMS (EI), *m/z* Calcd. for C₁₇H₁₅N₅OS (M)⁺ 337.0997, found 337.1062.

4-(3-Methylbenzyl)-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*α*]pyrimidin-5(4*H*)-one (6) Yield (79%); mp: 192-194°C; IR (KBr) ν/cm^{-1} 1680 (C=O); ^1H NMR (700 MHz, DMSO- d_6): δ 8.89 (1H, dd, $J = 4.2, 1.6$ Hz, H-8), 8.62 (1H, br d, $J = 7.8$ Hz, H-6), 7.64 (1H, dd, $J = 7.8, 4.8$ Hz, H-7), 7.24 (1H, br s, H-2'), 7.21 (2H, m, H-5',6'), 7.08 (1H, br d, $J = 7.4$ Hz, H-4'), 5.29 (2H, s, H-7'), 2.65 (3H, s, -SCH₃), 2.26 (3H, s, Ar-Me); ^{13}C NMR (175 MHz, DMSO- d_6): δ ppm 163.0 (C-2), 159.40 (C-5), 154.90 (C-3a), 151.90 (C-9a), 145.70 (C-8), 138.80 (C-6), 138.0 (C-1'), 135.9 (C-3'), 128.8 (C-2'), 128.6 (C-5'), 128.5 (C-4'), 125.2 (C-6'), 122.7 (C-5a), 112.60 (C-7), 47.1 (C-7'), 21.40 (Ar-Me), 14.0 (-SCH₃); HRMS (EI), m/z Calcd. for C₁₇H₁₅N₅OS (M)⁺⁺ 337.0997, found 337.1062.

4-(3-Methoxybenzyl)-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*α*]pyrimidin-5(4*H*)-one (7) Yield (81%); mp: 175-177°C; IR (KBr) ν/cm^{-1} 1688 (C=O); ^1H NMR (700 MHz, DMSO- d_6): δ 8.89 (1H, dd, $J = 4.3, 2.1$ Hz, H-8), 8.62 (1H, br d, $J = 7.8$ Hz, H-6), 7.62 (1H, br d, $J = 5.7$ Hz, H-7), 7.24 (1H, br t, $J = 7.8$ Hz, H-5'), 7.02 (1H, br s, H-2'), 6.98 (1H, br d, $J = 7.6$ Hz, H-6'), 6.85 (1H, br d, $J = 8.1$ Hz, H-4'), 5.29 (2H, s, H-7'), 3.72 (3H, s, O-Me), 2.65 (3H, s, -SCH₃); ^{13}C NMR (175 MHz, DMSO- d_6): δ ppm 162.9 (C-2), 159.7 (C-5), 159.4 (C-3'), 154.9 (C-3a), 151.7 (C-9a), 145.8 (C-8), 138.8 (C-6), 137.6 (C-1'), 130.0 (C-5'), 122.7 (C-5a), 120.1 (C-6'), 114.0 (C-4'), 113.2 (C-2'), 112.6 (C-7), 55.5 (O-Me), 47.1 (C-7'), 14.0 (-SCH₃); HRMS (EI), m/z Calcd. for C₁₇H₁₅N₅O₂S (M)⁺⁺ 353.0946, found 353.0998.

3-((2-(Methylthio)-5-oxopyrido[3,2-*e*][1,2,4]triazolo[1,5-*α*]pyrimidin-4(5*H*)-yl)methyl)-benzonitrile (8) Yield (80%); mp: 248-250°C; IR (KBr) ν/cm^{-1} 1678 (C=O); ^1H NMR (700 MHz, DMSO- d_6): δ 8.91 (1H, br s, H-8), 8.61 (1H, br d, $J = 7.0$, H-6), 7.90 (1H, br s, H-2'), 7.80 (1H, br d, $J = 7$ Hz, H-4'), 7.76 (1H, br d, $J = 7$, H-6'), 7.65 (1H, dd, $J = 7.8, 4.9$ Hz, H-7), 7.57 (1H, br t, $J = 7.6$ Hz, H-5'), 5.38 (2H, s, H-7'), 2.64 (3H, s, -SCH₃); ^{13}C NMR (175 MHz, DMSO- d_6): δ ppm 162.9 (C-2), 159.6 (C-5), 154.9 (C-3a), 151.9 (C-9a), 145.9 (C-8), 138.7 (C-6), 137.7 (C-1'), 132.9 (C-6'), 131.7 (C-2'), 131.1 (C-4'), 130.0 (C-5'), 131.1 (C-4'), 122.7 (C-5a), 119.2 (-C $\equiv$ N), 112.8 (C-7), 46.6 (C-7'), 14.0 (-SCH₃); HRMS (EI), m/z Calcd. for C₁₇H₁₂N₆OS (M)⁺⁺ 348.0793, found 348.0821.

4-((2-(Methylthio)-5-oxopyrido[3,2-*e*][1,2,4]triazolo[1,5-*α*]pyrimidin-4(5*H*)-yl)methyl)benzonitrile (9) Yield (70%); mp: 279-281°C; IR (KBr) ν/cm^{-1} 1675 (C=O); ^1H NMR (700 MHz, DMSO- d_6): δ 8.91 (1H, br s, H-8), 8.61 (1H, br d, $J = 5.1$ Hz, H-6), 7.81 (2H, br s, H-3'/5'), 7.65 (3H, m, H-7,2'/6'), 5.41 (2H, s, H-7'), 2.64 (3H, s, -SCH₃); ^{13}C NMR (175 MHz, DMSO- d_6): δ ppm 162.9 (C-2), 159.5 (C-5), 154.9 (C-3a), 151.8 (C-9a), 145.9 (C-8), 141.7 (C-1'), 138.8 (C-6), 132.8 (C-3'/5'), 128.8 (C-2'/6'), 122.7 (C-5a), 119.2 (-C $\equiv$ N), 112.7 (C-7), 110.7 (C-4'), 46.9 (C-7'), 14.0 (-SCH₃); HRMS (EI), m/z Calcd. for C₁₇H₁₂N₆OS (M)⁺⁺ 348.0793, found 348.0821.

4-(4-Chlorobenzyl)-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*α*]pyrimidin-5(4*H*)-one (10) Yield (77%); mp: 250-252°C; IR (KBr) ν/cm^{-1} 1677 (C=O); ^1H NMR (700 MHz, DMSO- d_6): δ 8.90 (1H, br d, $J = 4.1$ Hz, H-8), 8.62 (1H, br d, $J = 7.8$ Hz, H-6), 7.65 (1H, dd, $J = 7.8, 4.8$ Hz, H-7), 7.47 (2H, d, $J = 7.5$ Hz, H-3'/5'), 7.39 (2H, d, $J = 7.5$ Hz, H-2'/6'), 5.31 (2H, s, H-7'), 2.65 (3H, s, -SCH₃); ^{13}C NMR (175 MHz, DMSO- d_6): δ ppm 162.9 (C-2), 159.4 (C-5), 155.0 (C-3a), 151.8 (C-9a), 145.8 (C-8), 138.8 (C-6), 135.1 (C-1'), 132.6 (C-4'), 130.2 (C-2'/6'), 128.8 (C-3'/5'), 122.7 (C-5a), 112.7 (C-7), 46.6 (C-7'), 14.0 (-SCH₃); HRMS (EI), m/z Calcd. for C₁₆H₁₂ClN₅OS (M)⁺⁺ 357.0451, found 357.0503.

2-(3-(2-(Methylthio)-5-oxopyrido[3,2-*e*][1,2,4]triazolo[1,5-*α*]pyrimidin-4(5*H*)-yl)prop-yl)-isoindoline-1,3-dione (11) Yield (71%); mp: 198-200°C; IR (KBr) ν/cm^{-1} 1681 (C=O); ^1H NMR (700 MHz, DMSO- d_6): δ 8.85 (1H, br d, $J = 4.3$ Hz, H-8), 8.55 (1H, br d, $J = 7.8$ Hz, H-6), 7.84 (4H, m, H-5'/6', 4'/7'), 7.60 (1H, dd, $J = 7.8, 4.2$ Hz, H-7), 4.16 (2H, t, $J = 7.0$ Hz, CH₂-8'), 3.70 (2H, t, $J = 7$ Hz, CH₂-10'), 2.14 (2H, quint, $J = 7$ Hz, CH₂-9'), 2.55 (3H, s, -SCH₃); ^{13}C NMR (175 MHz, DMSO- d_6): δ ppm 168.4 (C-1'/3'), 162.9 (C-2), 159.2 (C-5), 154.8 (C-3a), 151.5 (C-9a), 145.5 (C-8), 138.6 (C-6), 134.9 (C-3a'/7a'), 132.0 (C-5'/6'), 123.5 (C-4'/7'), 122.7 (C-5a), 112.6 (C-7), 41.8 (CH₂-8'), 35.7 (CH₂-10'), 26.1 (CH₂-9'), 13.9 (-SCH₃); HRMS (EI), m/z Calcd. for C₂₀H₁₆N₆O₃S (M)⁺⁺ 420.1005, found 420.1063.

2.1.3 Preparation of 2-(Methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*α*]pyrimidine-5(4*H*)-thione (12)

Boiling an equimolar amount of **1** and P₂S₅ (1 mmol) in absolute pyridine (10 mL) for 3-4 h. After that, the reaction mixture was cooled and poured into ice/water, the obtained yellow solid was washed thoroughly with water and dried. Yield (90%); mp: 292-294°C; IR (KBr) ν/cm^{-1} 1248 (C=S); ^1H NMR (700 MHz, DMSO- d_6): δ 8.94 (1H, br d, $J = 4.3$ Hz, H-8), 8.62 (1H, br d, $J = 7$ Hz, H-6), 7.67 (1H, dd, $J = 7.8, 4.6$ Hz, H-7), 2.66 (3H, s, -SCH₃); ^{13}C NMR (175 MHz, DMSO- d_6): δ ppm 186.1 (C-5), 164.0 (C-2), 155.5 (C-3a), 149.8 (C-9a), 143.0 (C-8), 141.6 (C-6), 124.6 (C-5a), 123.2 (C-7), 14.1 (-SCH₃); HRMS (EI), m/z Calcd. for C₉H₇N₅S₂ (M)⁺⁺ 249.0143, found 249.0187.

2.1.4 Preparation of pyrido-triazolopyrimidines 13-16

Over a period of 10 min at RT, K₂CO₃ (1 mmol) was added to a stirred solution of **12** (0.5 mmol) in DMF (8 mL). Then, alkylhalides (1.5 mmol) was added and kept stirring in the

reaction mixture at 80 °C for 20 h. The mixture was poured into ice/water, the solid collected and dried.

5-(Ethylthio)-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*a*]pyrimidine (13) Yield (58%); mp: 159-161°C; ¹H NMR (700 MHz, DMSO-*d*₆): δ 9.06 (1H, br d, *J* = 4.2 Hz, H-8), 8.66 (1H, br d, *J* = 8.1 Hz, H-6), 7.74 (1H, dd, *J* = 8.1, 4.6 Hz, H-7), 3.41 (2H, q, *J* = 7.3 Hz, H-1'), 2.69 (3H, s, -SCH₃), 1.42 (3H, t, *J* = 7.3 Hz, H-2'); ¹³C NMR (175 MHz, DMSO-*d*₆): δ ppm 168.2 (C-5), 165.3 (C-2), 156.1 (C-3a), 155.0 (C-9a), 143.5 (C-8), 135.7 (C-6), 122.8 (C-5a), 113.7 (C-7), 24.7 (C-1'), 14.4 (C-2'), 13.9 (-SCH₃); HRMS (EI), *m/z* Calcd. for C₁₁H₁₁N₅S₂ (M)⁺ 277.0456, found 277.0498.

5-(Allylthio)-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*a*]pyrimidine (14) Yield (50%); mp: 165-167°C; ¹H NMR (700 MHz, DMSO-*d*₆): δ 9.08 (1H, br d, *J* = 4.2 Hz, H-8), 8.70 (1H, br d, *J* = 8.1 Hz, H-6), 7.75 (1H, dd, *J* = 8.1, 4.6 Hz, H-7), 5.99 (1H, m, H-2'), 5.40 (1H, br d, *J* = 17.3 Hz, H-3a'), 5.20 (1H, br d, *J* = 10.4 Hz, H-3b'), 4.20 (2H, d, *J* = 3.7 Hz, H-1'), 2.70 (3H, s, -SCH₃); ¹³C NMR (175 MHz, DMSO-*d*₆): δ ppm 167.6 (C-5), 165.2 (C-2), 156.1 (C-3a), 154.5 (C-9a), 143.3 (C-8), 135.8 (C-6), 132.8 (C-2'), 122.7 (C-5a), 119.7 (C-3'), 113.4 (C-7), 32.8 (-CH₂-1'), 13.9 (-SCH₃); HRMS (EI), *m/z* Calcd. for C₁₂H₁₁N₅S₂ (M)⁺ 289.0456, found 289.0506.

5-(Benzylthio)-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*a*]pyrimidine (15) Yield (54%); mp: 182-184°C; ¹H NMR (700 MHz, DMSO-*d*₆): δ 9.07 (1H, br d, *J* = 4.4 Hz, H-8), 8.69 (1H, br d, *J* = 8.1 Hz, H-6), 7.74 (1H, dd, *J* = 8.1, 4.7 Hz, H-7), 7.53 (2H, br d, *J* = 7.6 Hz, H-2'/6'), 7.37 (2H, br t, *J* = 7.5 Hz, H-3'/5'), 7.30 (1H, br t, *J* = 7.6 Hz, H-4'), 4.71 (2H, s, -CH₂-7'), 2.71 (3H, s, -SCH₃); ¹³C NMR (175 MHz, DMSO-*d*₆): δ ppm 167.7 (C-5), 165.4 (C-2), 156.2 (C-3a), 154.9 (C-9a), 143.6 (C-8), 136.8 (C-1'), 135.8 (C-6), 129.9 (C-3'/5'), 129.1 (C-2'/6'), 128.9 (C-4'), 122.9 (C-5a), 113.2 (C-7), 34.1 (-CH₂-7'), 13.9 (-SCH₃); HRMS (EI), *m/z* Calcd. for C₁₆H₁₃N₅S₂ (M)⁺ 339.0612, found 339.0676.

5-((2-Methylbenzyl)thio)-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*a*]pyrimidine (16) Yield (61%); mp: 179-181°C; ¹H NMR (700 MHz, DMSO-*d*₆): δ 9.07 (1H, br d, *J* = 4.3 Hz, H-8), 8.69 (1H, br d, *J* = 8.1 Hz, H-6), 7.73 (1H, dd, *J* = 8.1, 4.6 Hz, H-7), 7.50 (1H, br d, *J* = 7.4 Hz, H-6'), 7.24 (2H, m, H-3'/5'), 7.19 (1H, br t, *J* = 7.6 Hz, H-4'), 4.69 (2H, s, -CH₂-7'), 2.71 (3H, s, -SCH₃), 2.44 (3H, Ar-CH₃); ¹³C NMR (175 MHz, DMSO-*d*₆): δ ppm 167.9 (C-5), 165.4 (C-2), 156.2 (C-3a), 154.9 (C-9a), 143.6 (C-8), 137.4 (C-1'), 135.8 (C-6), 134.0 (C-2'), 130.9 (C-3'), 130.7 (C-4'), 128.5 (C-5'), 126.7 (C-6'), 122.9 (C-5a), 113.2 (C-7), 32.7 (-CH₂-7'), 19.4 (Ar-CH₃), 13.9 (-SCH₃); HRMS (EI), *m/z* Calcd. for C₁₇H₁₅N₅S₂ (M)⁺ 353.0769, found 353.0832

2.1.5 Preparation of 5-Chloro-2-(methylthio)pyrido[3,2-*e*][1,2,4]triazolo[1,5-*a*]pyrimidine (17)

An amount of 2 mmol from pyrido-triazolopyrimidine (1) was boiled with POCl₃ (2 mL) in C₆H₆ (14 mL) for 5 h; then the solvent was removed by evaporation and the residue neutralized with a saturated aqueous solution of K₂CO₃ (6 ml). The obtained solid was collected and dried. Yield (75%); mp: 250-252°C; ¹H NMR (700 MHz, DMSO-*d*₆): δ 8.82 (1H, br d, *J* = 4.4 Hz, H-8), 8.55 (1H, br d, *J* = 8.1 Hz, H-6), 7.62 (1H, dd, *J* = 8.1, 4.6 Hz, H-7), 2.63 (3H, s, -SCH₃); ¹³C NMR (175 MHz, DMSO-*d*₆): δ ppm 163.1 (C-2), 160.2 (C-3a), 154.7 (C-9a), 150.8 (C-5), 146.3 (C-8), 137.5 (C-6), 122.5 (C-5a), 113.2 (C-7), 14.0 (-SCH₃); HRMS (EI), *m/z* Calcd. for C₉H₆ClN₅S (M)⁺ 251.0032, found 251.0092.

2.2 Antioxidant assays

In our previous paper [8,13], we have reported in detail the methodology routes for determination of DPPH radical scavenging activity %, FRAP values and reducing power of the target compounds.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

3.1 Chemistry

The 3-amino-5-(methylthio)-1,2,4-triazole or 5-amino-*N*-(2,6-dichlorophenyl)-1,2,4-triazole-3-sulfonamide was reacted with 2-chloronicotinic acid to afford the target (1) has been described [2,3]. Herein, the synthesized 2-hydrazinonicotonic acid (A) [14] was reacted with dimethyl-*N*-cyanoimidodithio carbonte to give the intermediate (B) (Scheme 1). Then, the corresponding 2-methylthio-pyrido-triazolopyrimidin-5-one (1) was obtained in a good yield of 74% by treatment of the intermediate B with Conc. HCl. The target 1 was characterized by NMR, IR and MS spectral analyses. The IR spectrum of 1 was characterized by a strong stretching (C=O) peak at 1684 cm⁻¹. Reaction of 1 with alkyl halides furnished the *N*-alkylated pyrido-triazolopyrimidines (2-11) in 69-81% yields (Scheme 1, Table 1). The IR spectra of 2-11 showed the characteristic (C=O) group absorption bands at 1670-1688 cm⁻¹. Boiling equimolar amounts of 1 and P₂S₅ in pyridine for 3-4 h has furnished the respective 2-methylthio-pyrido-triazolopyrimidin-5-thione (12) as

i : Et₃N, EtOH, overnight stirring; ii: conc. HCl, 80 °C; iii: DMF, K₂CO₃, alkyl(heteroalkyl)halides; iv: P₂S₅, pyridine; v: DMF, K₂CO₃, alkylhalides 80 °C; vi: POCl₃, benzene

Scheme 1: Synthesis of the new products **1-17**.

yellow solid in yield of 90%. The structure of product **12** was proved with IR and ¹³C NMR analyses, where C=S group exhibited an intrinsic a weak absorption peak at 1248 cm⁻¹ and a resonance peak at 186.1 ppm, respectively. The expected thioether products (**13-16**) were obtained from the reaction of **12** with the appropriate alkyl halides in basic medium. The carbonyl group of **1** was successfully transformed into imidoyl chloride group in **17** by employing POCl₃ as a chlorinating agent. Complete disappearance of the carbonyl peak at 1684 cm⁻¹ in the IR spectrum of **1**, confirmed the production of **17**. Taking into account the NMR data of structural related compounds [9,15,16], the parent structure 2-methylthio-pyrido-triazolopyrimidine and its derivatives were simply confirmed by ¹H NMR, that showed one AMX spin coupling system and a singlet of a 2-methylthio group at about 2.65 ppm that was recorded at about 14 ppm in all ¹³C NMR spectra. This spin coupling system was interpretable for the three protons of pyridoring, i.e. H-8 and H-6 (often broad doublet, each) and H-7 (dd) at about δ 8.9 – 7.5 ppm. In all ¹³C NMR spectra, the parent structure of all products exhibited nine signals, including the most downfield thionated (C-2) and lactam-

carbonyl carbon (C-5) at about 163 and 160 together with the characteristic three aromatic –CH-carbons at about 146, 139 and 113 ppm for C-8, 6 and 7 (**1** and **2-11**). The C-5 showed a characteristic δ -value at 186.1 ppm for thiocarbonyl function in compound **12**, while in the thioalkyl/aralkyl derivatives **13-16** it was assigned as the most downfield carbon at about 168 ppm.

3.2 Antioxidant activity

The DPPH, FRAP and RPC assays were applied to assess the *in vitro* antioxidant activity of the pyrido-triazolopyrimidines (**1-17**). The obtained results were presented in figures 1-3 and compared with standard synthetic antioxidant. The target pyrido-triazolopyrimidines (**1-17**) showed various antioxidant activities relative to BHT. Figure: 1 presented the pyrido-triazolopyrimidines (**1-17**) inhibitory effects at a concentration of 0.5 mg/ml, the same as DPPH radical. The pyrido-triazolopyrimidines **12** and **15** were found to exhibit high DPPH radical scavenging activity among the tested products, however, lower than BHT.

Table 1: The chemical structures of the products 1-17.

Cpd.	R	Cpd.	R
2,13		7	
3,14		8	
4,15		9	
5,16		10	
6		11	

Pyrido-triazolopyrimidine (17) demonstrated moderate scavenging activity in relation to the compounds 12 and 15. Whereas compounds 1-11 appeared less active (Figure: 1). The reducing power capacities of pyrido-triazolopyrimidines 1-17 were compared with BHT (Figure: 2). Compounds 12, 15 and 17 showed the highest antioxidant activity at a concentration of 0.5 mg/mL in regards to the other pyrido-triazolopyrimidines, whereas 1, 5, 8, 10 and 16 exhibited moderate effects. However, at the same concentration (0.5 mg/mL), the targets 1-17 appeared less active than BHT. Figure 3 showed that the target pyrido-triazolopyrimidines 12 and 15 exhibited the highest activity and their values of FRAP are 973 and 1143 ($\mu\text{mol Trolox}/100\text{ g}$). Compounds 14, 16 and 17 were found to possess relative comparable antioxidant activity to 12 and 15. Meanwhile, compounds 2, 3, 5, 8, 10 and 13 demonstrated moderate activity (a higher FRAP value is the greatest antioxidant activity). Additionally, compounds 1, 4, 6 and 9 showed the lowest activity. From the summarized results, it was noticed that the targets antioxidant is correlated with substituents on the pyrido-triazolopyrimidine ring. As presented in Table 2, the highest DPPH scavenging activity of 12 and 15 when compared with other pyrido-triazolopyrimidines, may be due to the existence of a thiocarbonyl group in 12 and its transformed thioether in 15, however, compound 15 appeared to be the most active. In accordance with our reported antioxidant results [13], compound 12 could have the same behavior as the reported benzotriazoloquinazolines and expected to

Each value is expressed as mean $\pm$ SD (n = 3).

Figure: 1: DPPH free radical scavenging activity of compounds 1-17.

Each value is expressed as mean $\pm$ SD (n = 3).

Figure: 2: Reducing power capability (absorbance at 700 nm) of compounds 1-17.

Each value is expressed as mean $\pm$ SD (n = 3). Antioxidant activity ($\mu\text{mol Trolox}/100\text{ g}$).

Figure: 3: Ferric reducing antioxidant power (FRAP) of compounds 1-17.

undergo sequential proton loss electron transfer (SPLET) mechanism as illustrated in scheme 2. Thus, compound 12 may be showing a lower DE than the others.

Scheme 2: Reasonable mechanism of the redox reaction or antioxidant activity of product **12**.

Generally, in redox reactions of organic compounds, if a C-atom loses a bond to H and gains a bond to a heteroatom (or to another C-atom), it is considered to be an oxidation reaction because the H-element is the least electronegative one. Thus, in the dehydrogenation process the C-atom undergoes an overall loss of electron density and loss of electrons is oxidation. Contrary, in the hydrogenation or reduction of an organic compound a C-atom forms a new bond to H and loses a bond to a heteroatom (or to another C-atom). Accordingly, chemical modification on the parent **1** furnished **2–17** with high diversity in antioxidant activity; in particular, the conversion of C=O in **1** into C=S in **12**, has resulted in the increase of antioxidant effects. Similarly, the formation of **13–16** derivatives from **12** positively improved in the activity profiles, which is probably explained due to the presence of an electron-rich sulfur-containing moiety (-S-R).

4 Conclusions

A total of seventeen new 2-methylthio-pyrido-triazolopyrimidines were successfully prepared by the reaction of dimethyl-*N*-cyanoimidodithiocarbonate and hydrazine-pyridine carboxylic acid as the reactants. In all three assays used, the 5-thione (**12**) and 5-thiobenzyl (**15**) products have shown the strongest antioxidant effects.

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Authors' contributions: First, Third and Fourth authors participated in the designing and writing the proposal of this research point, and synthesis-analysis of the products. The second author conducted the biological studies and presented the results. All authors wrote, read and approved the final manuscript.

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