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DESIGN, EFFICIENT SYNTHESIS, MECHANISM OF REACTION AND ANTIPROLIFERATIVE ACTIVITY AGAINST CANCER AND NORMAL CELL LINES OF A NOVEL CLASS OF FUSED PYRIMIDINE DERIVATIVES

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Abstract: This work concentrated on the utility of hydrazonoyl halides in synthesis of bioactive heterocycles like triazoles, pyrazoles, pyrimidines and their fused derivatives which have a wide spectrum of pharmaceutical value. Herein we discussed the synthesis of new heterocyclic compounds containing fused pyrimidine rings derived from hydrazonoyl halides and their significant pharmaceutical importance as anticancer agents. New fused pyrimidine derivatives bearing 1,2,3-triazole moiety were prepared *via* reaction of enaminone **2** with and 6-amino-2-thioxo-pyrimidin-4-one and then with hydrazonoyl chlorides **6a-h**. In addition, 3-amino-6-(2-oxo-2H-chromen-3-yl)pyridine-2-carbonitrile (**12**) was submitted to react with carbon dioxide to afford 3-(1,2,3,4-tetrahydro-2,4-dithioxopyrido[2,3-*d*]pyrimidin-7-yl)-2H-chromen-2-one (**15**), which act as key molecule for synthesis of new series of fused pyrimidinethione derivatives containing coumarine moiety *via* its reaction with different selected derivatives of hydrazonoyl halides **6a-h**. Structures of the newly synthesized compounds were confirmed using spectral data (IR, ^{1}H NMR and Mass spectrometry) and microanalytical methods. Also, they screened for their anticancer activity.

Keywords: fused pyrimidines, 1,2,3-triazole, coumarin, anticancer activity, antiproliferative activity

Fused heterocyclic compounds formed the widest diversity of heterocycles of chemical and pharmaceutical significance (1). They largely exist in numerous natural products. They are also among the most frequently encountered scaffolds in numerous pharmaceutically relevant compounds and drugs.

One of the most important classes of fused heterocycles is fused pyrimidines. Owing to their very wide spectrum of biological and pharmacological properties, number of heterocyclic condensed pyrimidine derivatives have become of a great attraction in the field of pharmaceutical chemistry research (2-4). Fusion of pyrimidine moiety with different heterocycle gives rise to a new class of heterocyclic compounds with improved biological activity. Coumarins compounds have great practical biological activities (5), so their fusion with pyrimidine moiety results in compounds with significant biological activities (6). Also, heterocycles containing nitrogen and sulfur atoms in the core structure shows various biologically and pharmacologically active compounds. Many of fused pyrimidines like purines, pyridopyrimidines, triazolopyrimidines, furopyrimidines, pyrazolopyrimidines, and quinazolines were found to possess variable pharmacological activities (7, 8). The nitrogen containing heterocycles triazolopyrimidines represent a pharmaceutically important class of compounds because of their diverse range of biological activities such as antitumor (9, 10). Fused pyrimidine derivatives have been reported as anti-inflammatory, analgesic (11), antitubercular (12), anti-microbial (13), anti- HIV (14), cardiovascular (15), calcium-sensing receptor antag-

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onists (16), and as hypnotic drugs for the nervous system (17). The present investigation provides some of the biological and medicinal properties of compounds having fused pyrimidine nucleus as anti-proliferative agents against cancer and normal cell lines.

EXPERIMENTAL

All melting points were determined on an electrothermal apparatus and are uncorrected. IR spectra were recorded (KBr discs) on Shimadzu FT-IR 8201 PC spectrophotometer. ^{1}H NMR spectra were recorded in $(CD_3)_2SO$ solutions on JNM-LA 400 FT-NMR system spectrometer and chemical shifts are expressed in δ ppm units using TMS as an internal reference. Mass spectra were recorded on a GC-MS QP1000 EX Shimadzu. Elemental analyses were carried out at the microanalytical center of Cairo University. Hydrazonoyl halides (18). were prepared as previously mentioned.

Chemistry

3-(Dimethylamino)-1-(5-methyl-1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)prop-2-en-1-one (2)

A mixture of 1-(5-methyl-1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)ethanone(**1**) (10 mM)and dimethylformamide dimethylacetal (11.9 g, 14 mL, 10 mM) were heated under reflux in dry xylene (15 mL) for 4 h the hot solution evaporated to its half volume and then cooled. The resulting solid was collected and recrystallized from benzene to give **2** as orange crystals. yield: 92%, m.p.: 294-296^{o}C; FT-IR (KBr, cm^{-1}): 3032,2986 *v* (CH), 1645 *v* (C=O, conjugated), 1635 *v* (C=N), 1590 *v* (C=C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.36 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.17 (s, 3H, CH_3), 5.08 (d, 1H, CH=), 6.69 (d, 1H, CH=), 7.36-7.8 (m, 4H, Ar-H's); MS: m/z [%] = 279 (M^+, 21%), 264 (14%), 249 (33%), 227 (41%), 211 (55%), 160 (23%), 150 (100%), 70 (25%), 50 (70%). Analysis: calcd. for $C_{11}H_{10}N_3OBr$ (279): C, 47.16; H, 3.60; N, 15.00%; found: C, 46.95; H, 3.45; N, 14.83%.

5-(1-(4-Bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (5)

A mixture of enaminone **8** (3.78 g, 10 mM) and 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (**9**) (1.43 g, 10 mM) in acetic acid (40 mL) was refluxed for 6 h. The reaction mixture was cooled and diluted with methanol and the solid product was collected by filtration and recrystallized from dioxane to give **10** as yellow solid. yield: 90%; m.p.: over 300^{o}C; FT-IR (KBr, cm^{-1}): 3441, 3356 *v* (2NH), 3077, 2904 *v* (CH), 1703 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.88 (s, 3H, CH_3), 7.60-8.71 (m, 4H, Ar-H), 9.12 (s, 1H, pyridine-H), 9.36 (s, 1H, pyridine-H), 12.64 (s, 2H, 2NH); MS: m/z [%] = 415 (M^+, 1%), 384 (64%), 330 (33%), 285 (33%), 271 (48%), 227 (45%), 209 (55%), 158 (59%), 140 (100%), 93 (29%), 52 (34%). Analysis: calcd. for $C_{16}H_{11}N_6OSBr$ (415): C, 46.28; H, 2.67; N, 20.24%; found: C, 46.55; H, 2.35; N, 19.90%.

To a stirred mixture of thione (**10**) (5 mM) in dimethylformamide (20 mL) was added anhydrous potassium carbonate (7.5 mM), and methyl iodide (5 mM). The reaction mixture was stirred overnight at room temperature then poured into ice-water. The solid formed was filtered, washed with water, dried and recrystallized from ethanol/dioxane mixture to give compound **14.**

3-Acetyl-6-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-phenylpyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one (9a)

Yellow solid; yield: 70%; m.p.: 188-190^{o}C; FT-IR (KBr, cm^{-1}): 3090, 2968, 2923 *v*(CH),1716 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.48 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 7.40-8.63 (m, 9H, Ar-H), 8.65 (s, 1H, pyridine-H), 9.04 (s, 1H, pyridine-H); MS: m/z [%]= 541 (M^+, 18%), 469 (74%), 391 (100%), 369 (41%), 297 (15%), 249 (7%), 180 (11%), 120 (11%), 111 (11%), 77 (63%); Analysis: calcd. for $C_{25}H_{17}N_8O_2Br$ (541): C, 55.47; H, 3.17; N, 20.70%; found: C, 55.75; H, 2.92; N, 20.40%.

3-Acetyl-6-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-(p-tolyl)pyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one (9b)

Yellow solid. yield: 70%; m.p.: 120-122^{o}C; FT-IR (KBr, cm^{-1}): 1708 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ =2.24 (s, 1H, CH_3), 2.64 (s, 3H, CH_3), 7.18-8.56 (m, 8H, Ar-H), 8.58 (s, 1H, pyridine-H), 8.91 (s, 1H, pyridine-H); MS: m/z [%] = 555 (M^+, 30%), 535 (36%), 508 (21%), 330 (11%), 104 (100%), 91 (49%), 77 (79%); Analysis: calcd. for $C_{25}H_{19}BrN_8O_2$ (555): C, 56.23; H, 3.45; N, 20.18%; found: C, 56.00; H, 3.10; N, 19.91%.

3-Acetyl-6-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-(4-chlorophenyl) pyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one (9c)

Yellow solid; yield: 80%; m.p.: 90-92^{o}C; FT-IR (KBr, cm^{-1}): 3090, 2969, 2922 *v* (CH),1715 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ =2.26 (s, 1H, CH_3), 7.16-8.46 (m, 8H, Ar-H), 8.38 (s, 1H, pyridine-H), 8.71 (s, 1H, pyridine-H); MS: m/z [%]

= 575 (M^+, 9%), 560 (24%), 104 (100%), 90 (24%), 76 (64%). Analysis: calcd. for $C_{25}H_{16}N_8O_2BrCl$ (575): C, 52.15; H, 2.80; N, 19.46%; found: C, 52.45; H, 2.50; N, 19.15%.

3-Acetyl-6-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-(4-nitrophenyl)pyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one (9d)

Yellow solid; yield: 70%; m.p.: 120-122°C; FT-IR (KBr, cm^{-1}): 3087, 2918 *v* (CH), 1705 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ =2.64 (s, 3H, CH_3), 7.18-8.56 (m, 8H, Ar-H), 8.58 (s, 1H, pyridine-H), 8.91 (s, 1H, pyridine-H); MS: m/z [%] = 586 (M^+, 9%), 148 (12%), 128 (12%), 116 (16%), 104 (49%), 91 (13%), 77 (100%). Analysis: calcd. for $C_{25}H_{16}N_9O_4Br$ (586): C, 51.21; H, 2.75; N, 21.50%; found: C, 51.21; H, 2.45; N, 21.20%.

Ethyl-6-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-5-oxo-1-phenyl-1,5-dihydropyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidine-3-carboxylate (9e)

Yellow solid; yield: 80%; m.p.: 168-170°C; FT-IR (KBr, cm^{-1}): 3069, 2955 *v* (CH), 1716 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.17-1.39 (t, 3H, CH_3), 4.51-4.52 (q, 2H, CH_2), 7.42-9.00 (m, 9H, Ar-H), 9.49 (s, 1H, pyridine-H), 9.56 (s, 1H, pyridine-H); MS: m/z [%]= 571 (M^+, 6%), 560 (16%), 498 (9%), 455 (28%), 434 (28%), 399 (45%), 391 (49%), 363 (34%), 332 (100%), 264 (46%), 220 (30%), 197 (62%), 178 (43%), 157 (83%), 102 (68%). Analysis: calcd. for $C_{26}H_{19}N_8O_3Br$ (571): C, 54.65; H, 3.35; N, 19.61%; found: C, 54.32; H, 3.04; N, 19.32%.

Ethyl 6-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-5-oxo-1-(p-tolyl)-1,5-dihydropyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidine-3-carboxylate (9f)

Yellow solid, yield:80%; m.p.: 198-200°C; FT-IR (KBr, cm^{-1}): 3066, 2939 *v* (CH), 1707 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ =1.18-1.43 (t, 3H, CH_3), 2.38 (s, 3H, CH_3), 4.49-4.54 (q, 2H, CH_2), 7.36-8.60 (m, 8H, Ar-H), 9.49 (s, 1H, pyridine-H), 9.56 (s, 1H, pyridine-H); MS: m/z [%]= 585 (M^++1, 4%), 584 (M^+, 10%), 104 (91%), 91 (47%), 76 (100%). Analysis: calcd. for $C_{27}H_{21}N_8O_3Br$ (585): C, 55.40; H, 3.62; N, 19.14%; found: C, 55.11; H, 3.31; N, 19.05%.

Ethyl 6-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-(4-chlorophenyl)-5-oxo-1,5-dihydropyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidine-3-carboxylate (9g)

Yellow solid, yield:70%; m.p.: 120-122°C; FT-IR (KBr, cm^{-1}): 3090, 2959, 2909 *v* (CH), 1711 *v* (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ =1.15-1.49 (t, 3H, CH_3), 4.59-4.44 (q, 2H, CH_2), 7.34-8.62 (m, 8H, Ar-H), 8.79 (s, 1H, pyridine-H), 9.16 (s, 1H, pyridine-H); MS: m/z [%] = 607 (M^++2, 9%), 605 (M^+, 30%), 585 (36%), 508 (21%), 330 (11%), 104 (100%), 91 (49%), 77 (79%). Analysis: calcd. for $C_{25}H_{18}ClBrN_8O_3$ (605): C, 51.56; H, 2.99; N, 18.50%; found: C, 51.26; H, 3.00; N, 18.13%.

6-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-5-oxo-N,1-diphenyl-1,5-dihydropyrido[2,3-*d*][1,2,4]triazolo[4,3-a]pyrimidine-3-carboxamide (9h)

Yellow solid, yield: 80%; m.p.: 90-92°C; FT-IR (KBr, cm^{-1}): 3030, 2961 *v* (CH), 1699 *v* (C=O) ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.48 (s, 3H, CH_3), 7.11-8.68 (m, 14H, Ar-H), 8.20 (s, 1H, pyridine-H), 11.27 (s, 1H, pyridine-H), 11.73 (s, 1H, NH); MS: m/z [%] = 618 (M^+, 9%), 570 (24%), 104 (100%), 90 (24%), 76 (64%). Analysis: calcd. for $C_{30}H_{20}N_9O_2Br$ (618): C, 58.26; H, 3.26; N, 20.38%; found: C, 58.50; H, 3.00; N, 20.04%.

3-(1,2,3,4-tetrahydro-2,4-dithioxopyrido[2,3-d]pyrimidin-7-yl)-2*H*-chromen-2-one (15).

To a solution of **1** (1.3g, 5 mM) in dry pyridine (20 mL) carbon disulfide (5 mM) was added and the reaction mixture was refluxed on water bath for 6 h. then the reaction mixture was left to cool to room temperature, poured onto ice-cold water and neutralized with diluted hydrochloric acid to complete precipitation. The solid obtained was filtered off, washed, dried well and recrystallized from ethanol to give **15** as orange crystals. yield: 65%; m.p.: 280-282°C; FT-IR (KBr, cm^{-1}): 3330 *v* (NH), 1677 *v* (C=O), 1620 *v* (C=C), 1606 *v* (C=N), 1320 *v* (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.32-7.61 (m, 6H, Ar-H), 8.52 (s, 1H, C_4-H of coumarin), 11.21 (s, 2H, 2NH); MS: m/z [%] = 339 (M^+, 3.7%), 299 (1.12%), 272 (12%), 211 (29.3%), 174 (10.05%), 172 (14%), 145 (43.91%), 77 (75%), 50 (45%); Analysis: calcd. for $C_{16}H_9N_3O_2S_2$(339): C, 56.62; H, 2.67; N, 12.38%; found: C, 56.63; H, 2.65; N, 12.37%.

General procedure for synthesis of 16a-h

A mixture of compound **15** (3.4 gm, 10 mM) and the appropriate of hydrazonoyl halides (10 mM) was boiled under reflux in dioxane (20 mL) containing catalytic amount of triethylamine (1-1.5 mL) for 2-3 h the reaction mixture was left overnight for cooling, the solid collected and recrystallized from

acetic acid to give the corresponding derivatives 1**6a-h** "respectively" as yellow crystals.

3-(3-Acetyl-1-phenyl-5-thioxo-1,5-dihydropyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-8- yl)-2H-chromen-2-one (**16a**).

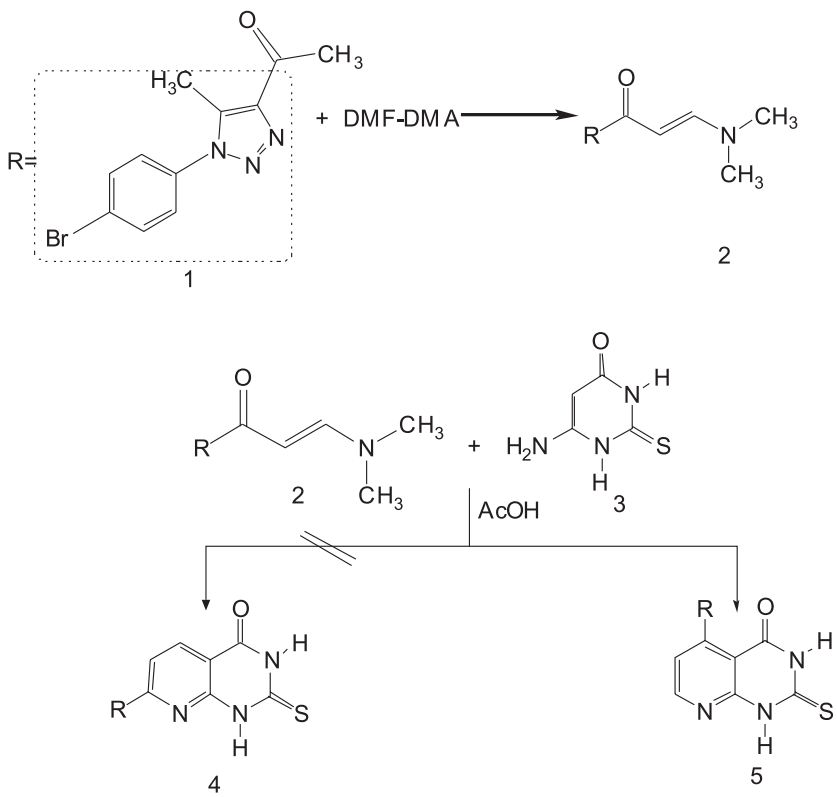
Yield: 64%; m.p.:169-171°C; FT-IR (KBr, cm^{-1}): 1690, 1668 *v* (C=O), 1630 *v* (C=C), 1617 *v* (C=N), 1345 *v* (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.65(s, 3H, CH_3), 7.12-7.6(m, 11H, Ar-H), 8.52(s, 1H, C_4-H of coumarin); MS : m/z [%] = 467 (M^++2, 2.05%), 465 (M^+, 0.5%), 450 (3.07%), 410 (16%), 371 (28%), 320 (4.85%), 238 (1.1%), 227 (1.5%), 176 (5.76%), 150 (16%), 132 (70%), 128 (28%), 77 (100%), 100 (65%), 50 (18%); Analysis: calcd. for $C_{25}H_{15}N_5O_3S$ (465): C, 64.51; H, 3.25; N, 15.05%; found: C, 64.42; H, 3.16; N, 15.03%.

3-(3-Acetyl-5-thioxo-1-(p-tolyl)-1,5-dihydropyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-8-yl)-2H-chromen-2-one (**16b**).

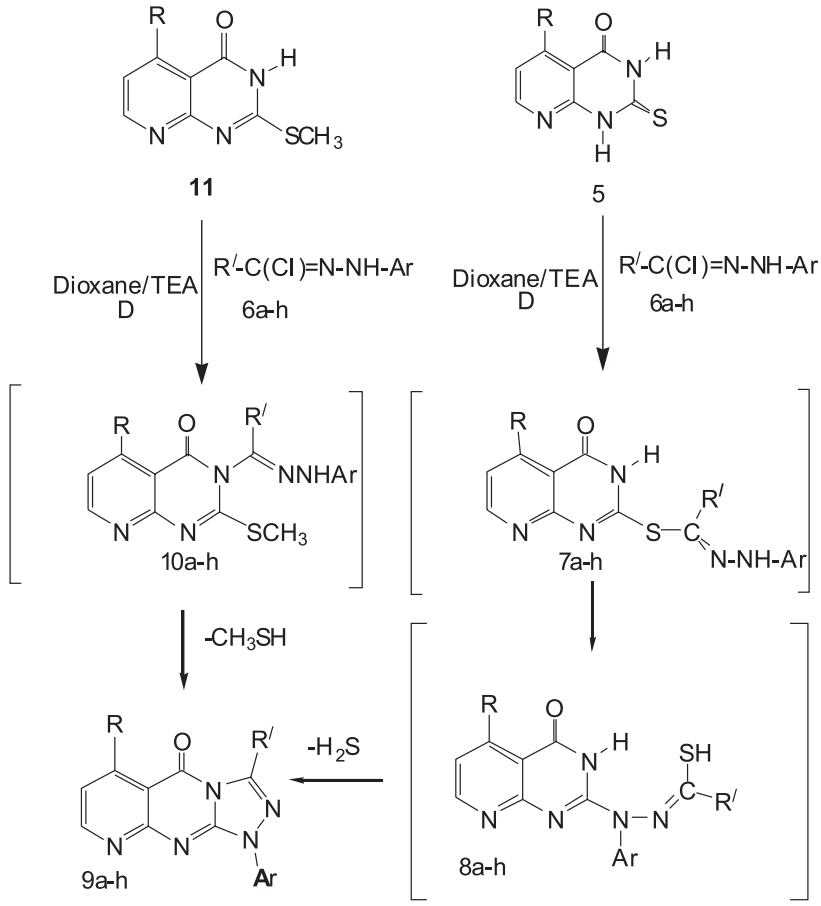
Yield: 81%; m.p.:153-155°C; FT-IR(KBr, cm^{-1}): 1697,1672*v*(C=O),1625 *v*(C=C), 1600 *v*(C=N), 1340 *v*(C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.56(s, 3H, CH_3), 2.68(s, 3H, CH_3), 7.14-7.85(m, 10H, Ar-H), 8.50(s, 1H, C_4-H of coumarin); MS: m/z [%] = 479 (M^+, 5%), 464 (30%), 434 (17.5%), 419 (22%), 345 (27%), 329 (8.5%), 306 (17%), 238 (2%), 222 (11%), 170 (5%), 150 (17%), 128 (23%), 77 (100%), 50 (9%); Analysis: calcd. for $C_{26}H_{17}N_5O_3S$ (479): C, 65.12; H, 3.57; N, 14.61%; found: C, 65.23; H, 3.41; N, 14.45%.

3-(3-Acetyl-1-(4-chlorophenyl)-5-thioxo-1,5-dihydropyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-8-yl)-2H-chromen-2-one (**16c**).

Yield: 76%; m.p.:162-164°C; FT-IR(KBr, cm^{-1}): 1692, 1666 *v* (C=O), 1628 *v* (C=C), 1617 *v* (C=N), 1345 *v* (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.65 (s, 3H, CH_3), 7.50-7.8 (m, 10H, Ar-H), 8.52 (s, 1H, C_4-H of coumarin); MS: m/z [%] = 501 (M^++2, 1%), 484 (5%), 482 (12%), 461 (19%), 450 (2%), 360 (22.06%), 341 (8%), 304 (1.2%), 240 (37%), 225 (1%), 149 (17%), 77 (100%), 65 (8%); Analysis: calcd. for $C_{25}H_{14}ClN_5O_3S$ (499): C, 60.06; H, 2.82; N, 14.01%; found: C, 60.16; H, 2.75; N, 13.82%.



Scheme 1



Ar = XC_6H_4

R/ / X : a, CH_3CO / H; b, CH_3CO / 4-CH_3; c, CH_3CO / 4-Cl; d, CH_3CO / 4-NO_2; c, COOEt / H; f, COOEt / 4-CH_3; g, COOEt / Cl; h, CONHPh / Ph.

Scheme 2

3-(3-Acetyl-1-(4-nitrophenyl)-5-thioxo-1,5-dihydropyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-8-yl)-2H-chromen-2-one (16d).

Yield: 62%; m.p.: 143-145°C; FT-IR (KBr, cm^{-1}): 1689, 1670 *v* (C=O), 1630 *v* (C=C), 1618 *v* (C=N), 1343 *v* (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.63 (s, 3H, CH_3), 7.5-8.02 (m, 10H, Ar-H), 8.52 (s, 1H, C_4-H of coumarin); MS: m/z [%] = 512 (M^++2, 3%), 510 (M^+, 11.05%), 495 (18%), 467 (13.6%), 457 (10%), 346 (7%), 306 (1.08%), 240 (32%), 227 (18%), 170 (5.76%), 150 (14%), 133 (50%), 77 (100%), 65 (81%); Analysis: calcd. for $C_{25}H_{14}N_6O_5S$ (510): C, 58.82; H, 2.76; N, 16.46%; found: C, 58.85; H, 2.84; N, 16.35%.

Ethyl 8-(2-oxo-2H-chromen-3-yl)-1-phenyl-5-thioxo-1,5-dihydropyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidine-3-carboxylate (16e).

Yield: 72%; m.p.: 101-103°C; FT-IR (KBr, cm^{-1}): 1715 *v* (C=O-ester), 1680 *v* (C=O-coumarin), 1630 *v* (C=C), 1612 *v* (C=N), 1350 *v* (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.23-1.31(t, 3H, CH_3CH_2), 4.14-4.22 (q, 2H, CH_2CH_3), 6.82-7.51 (m, 11H, Ar-H), 8.51 (s, 1H, C_4-H of coumarin); MS: m/z [%] = 495 (M^+, 5.3%), 480 (M^+-CH_3,1.4%), 466 (M^+-CH_2CH_3, 3.4%), 450 (12%), 374 (11.2%), 327 (18.2%), 269 (12.1%), 244 (12.12%), 226 (11%), 177 (45%), 165 (36.5%), 150 (42%), 104 (25.5%), 96 (32%), 57 (100%); Analysis: calcd. for

$C_{26}H_{17}N_5O_4S$ (495): C, 63.02; H, 3.46; N, 14.13%; found: C, 62.89; H, 3.39; N, 14.02%.

Ethyl 8-(2-oxo-2H-chromen-3-yl)-5-thioxo-1-(p-tolyl)-1,5-dihydropyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidine-3-carboxylate (16f).

Yield: 81%; m.p.: 96-98^{d}C; FT-IR (KBr, cm^{-1}): 1724 *v* (C=O-ester), 1680 *v* (C=O-coumarin), 1622 *v* (C=C), 1606 *v* (C=N), 1345 *v* (C=S); ^{1}HNMR (400 MHz, DMSO-d_6): δ = 1.20-1.27 (t, 3H, CH_3CH_2), 2.58 (s, 3H, CH_3), 4.11-4.27 (q, 2H, CH_2CH_3), 7.12-7.58 (m, 10H, Ar-H), 8.48 (s, 1H, C_4-H of coumarin); MS: m/z [%] = 511 (M^++2, 1.3%), 494 (M^+-15, 6%), 466 (12%), 445 (2.5%), 435 17.3%), 386 (11.3%), 356 (5.4%), 329 (7.99%), 300 (3.7%), 227 (18.8%), 173 (4.69%), 127 (47%), 91 (71.2%), 77 (37%), 63 (27%) 55 (72%); Analysis: calcd. for $C_{27}H_{19}N_5O_4S$ (509): C, 63.64; H, 3.76; N, 13.74%. found: C, 63.82; H, 3.65; N, 13.63%.

Ethyl 1-(4-chlorophenyl)-8-(2-oxo-2H-chromen-3-yl)-5-thioxo-1,5-dihydropyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidine-3-carboxylate (16g).

Yield: 79%; m.p.:121-123^{o}C; FT-IR (KBr, cm^{-1}): 1715 *v* (C=O-ester), 1680 *v* (C=O-coumarin), 1620 *v* (C=C), 1612 *v* (C=N), 1350 *v* (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.21-1.3 (t, 3H, CH_3CH_2), 4.13-4.23(q, 2H, CH_2CH_3), 7.3-7.7 (m, 10H, Ar-H), 8.5 (s, 1H, C_4-H of coumarin); MS: m/z [%]= 530 (M^++1, 10.5%), 529 (M^+, 4.09%), 497 (9.12%), 456 (1.2%), 402 (2%), 369 (0.5%), 336 (11.6%), 325 (20.5%), 247 (12.06%), 217 (11.2%), 188 (9.03%), 174 (19.2%), 144(35%), 119(27%), 97 (45%); Analysis: calcd. for $C_{27}H_{16}ClN_5O_4S$ (529): C, 58.93; H, 3.04; N, 13.22%; found: C, 58.89; H, 3.12; N, 13.14%.

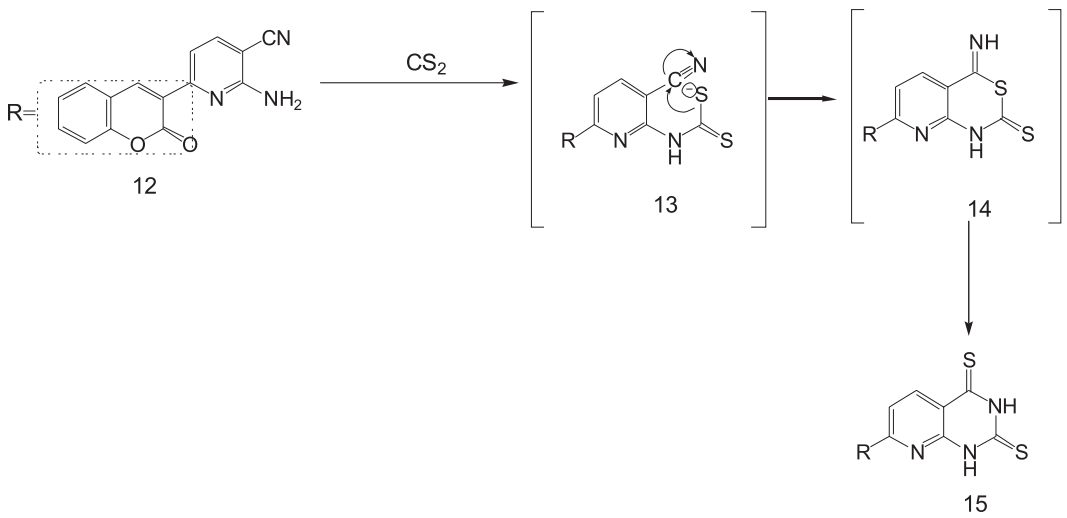
8-(2-Oxo-2H-chromen-3-yl)-N,1-diphenyl-5-thioxo-1,5-dihydropyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidine-3-carboxamide (16h).

Yield: 83%; m.p.:134-136^{o}C; FT-IR (KBr, cm^{-1}): 3330 *v* (NH), 1680 *v* (broad, C=O), 1620 *v* (C=C), 1612 *v* (C=N), 1345 *v* (C=S); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.85-7.42 (m, 16H, Ar-H),11.06 (s, 1H, NH), 8.51 (s, 1H, C_4-H of coumarin); MS: m/z [%] = 544(M^++2, 2.05%), 540 (M^+, 0.5%), 482 (7.03%), 465 (12.06%), 454 (2.3%), 341 (26.7%), 328 (4.85%), 304 (11%), 238 (3.96%), 225 (1%), 170 (5.76%), 149 (13%), 131 (50%), 128 (42%), 77 (100%), 50 (8%); Analysis: calcd. for $C_{30}H_{18}N_6O_3S$ (542): C, 66.41; H, 3.34; N, 15.49%; found: C, 66.56; H, 3.25; N, 15.39%.

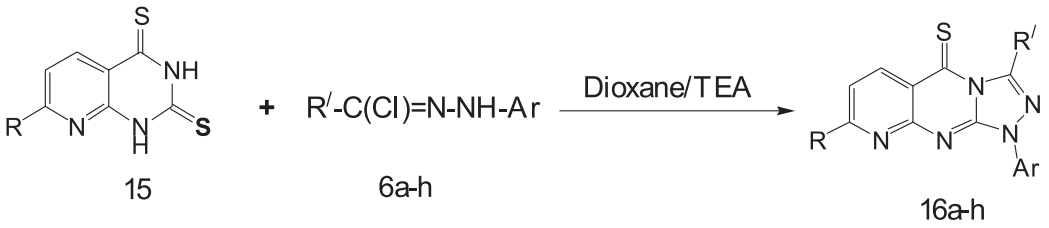
Antiproliferative activity

Cells

Cell lines: MCF-7 (human breast cancer) and BALB/3T3 (murine fibroblast) are being maintained in the Institute of Immunology and Experimental Therapy, Wroclaw, Poland. All cancer cell lines were obtained from American Type Culture Collection (Rockville, Maryland, USA) and are being maintained in the Institute of Immunology and Experimental Therapy (Wrocław, Poland). MCF-7 cells were cultured in Eagle medium (IIET, Wroclaw, Poland) supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 8 ug/mL of insulin



Scheme 3



$Ar = XC_6H_4$

$R^{/}/X$: a, CH_3CO/ H ; b, CH_3CO/4-CH_3 ; c, CH_3CO/ 4-Cl; d, CH_3CO / 4-NO_2; e, COOEt/H ; f, COOEt/ 4-CH_3 ; g, COOEt /Cl ; h, CONHPh /Ph.

Scheme 4

and 1% mem non-essential amino acid solution 100x (all from Sigma–Aldrich Chemie GmbH, Steinheim, Germany). BALB/3T3 cell line was cultured in DMEM (Gibco, UK) supplemented with 2 mM L-glutamine, 10% fetal bovine serum (GE Healthcare, Logan, UT, USA). All culture media were also supplemented with antibiotics: 100 µg/mL streptomycin (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and 100units/mL penicillin (Polfa Tarchomin SA, Warsaw, Poland). All cell lines were grown at 37°C with 5% CO_2 humidified atmosphere.

Compounds

Prior to usage, the compounds were dissolved in DMSO (stock solution 10 mg/mL) and culture medium (1 : 9) to the concentration of 1 mg/mL and subsequently diluted in culture medium to reach the required concentrations (ranging from 100 to 0.1 µg/mL, only one compound 1a-K was tested in different range of concentrations from 10 to 0.01 µg/mL, because of small amount of tested compound).

An anti-proliferative assay *in vitro*

24 hours before addition of the tested compounds, the cells were plated in 96-well plates (Sarstedt, Germany) at density of 1×10^4 cells per well. The assay was performed after 72 hours exposure to varying concentrations of the tested compounds. The *in vitro* cytotoxic effect of all compounds was examined using the SRB assay.

Cytotoxic test SRB

The details of this technique were described by Skehan et al. (19). The cells were attached to the bottom of plastic wells by fixing them with cold 50% TCA (trichloroacetic acid, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) on the top of the culture medium in each well. The plates were incubated at 4°C for 1 hour and then washed five times with tap water. The cellular material fixed with TCA was stained with 0.4% sulphorhodamine B (SRB, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) dissolved in 1% acetic acid (POCH, Gliwice, Poland) for 30 min. Unbound dye was removed by rinsing (five times) in 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (POCH, Gliwice, Poland) for determination of the optical density (ë = 540 nm) in Synergy H4 multi-mode microplate reader (BioTek Instruments USA).

RESULTS AND DISCUSSION

Due to the electrophilicity of the enaminone **2** (synthesized *via* the reaction of **1** (20) with DMF-DMA), we studied its reactivity towards some nitrogen nucleophiles like 6-amino-2-thioxopyrimidin-4-one **3**. Thus, reaction of enaminone **2** with compound **3** under reflux in acetic acid led to formation of product which could be formulated as **4** or **5**. The possible isomeric structure **5** was discarded based on the data obtained from H^1NMR spectra. Thus, the H^1NMR spectrum for the product **4** revealed in addition to the peaks of the signals of aromatic protons and the two NH protons, two doublet signals at δ 7.60-8.71 and 9.12, 9.36 ppm attributed to the two protons of the pyridine ring. The formation of product and not the possible isomeric product **5** (21) indicated that the reaction starts with initial nucleophilic attack of the amino group of compound **3** to

the olefinic carbon attached to the $-NMe_2$ group in enaminone **2** followed by elimination of dimethylamine and subsequent ring closure *via* elimination of water to give the pyridine ring (Scheme 1).

Next, we examined the reactivity of product **5** towards hydrazonoyl chlorides **6a-h** since the structure of compound **5** contains a thiourea residue. Thus, reaction of 5-(1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one **5** with hydrazonoyl chlorides **6a-h** in refluxing dioxane in the presence of triethylamine led to the formation of the respective products **9** through the non isolated intermediates **7** and **8** (Scheme 2). 1,3,6-Trisubstituted-pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidine-5-ones **9** were also obtained by reaction of 2-methylthio-5-substituded-pyrido[2,3-*d*]pyrimidin-4-one **11** with hydrazonoyl chlorides **6a-h** under the same reaction conditions (Scheme 2). The mechanism of formation of products **9** was previously discussed (22, 23). The structure assigned for the products **9** was based on both microanalytical and spectral data. For example, the IR spectra in each case showed the absence of the absorption bands assigned for the 2 NH groups. Also, the ^{1}H NMR revealed the absence of the signals due to the 2 NH protons and instead revealed the signals attributed to the protons of the R substituent in the assigned structure **9** (23) (see Experimental part).

Treatment of aminocyanopyridin **12** (prepared as previously mentioned (25)) with carbon disulfide in pyridine afforded the required compound **15** through two intermediates **13, 14**, whose subsequent rearrangement occurred (Dimroth rearrangement) (26, 27). Dimroth rearrangement is an isomerization process in which exo and endocyclic heteroatoms are translocated on a heterocyclic ring system. Also it is considered to be amidine rearrangement. Unexpected product formed and the possible occurrence of rearrangement should be kept in mind

Table 1. Value of IC_{50} [µg/mL].

Compounds	IC_{50} ± SD [µg/mL]			
	MCF-7		BALB/3T3	
Cisplatin	2.97	± 0.727	2.18	± 0.600
5	27.79	± 5.998	49.91	± 3.095
9a	Nd		Nd	
9b	Nd		Nd	
9c	Nd		Nd	
9d	Nd		Nd	
9e	Nd		Nd	
9f	Nd		Nd	
9g	73.13	± 12.203	Nd	
9h	Nd		Nd	
15	17.68	± 0.742	46.42	± 2.83
16a	14.20	± 0.638	Nd	
16b	26.90	± 4.129	43.23	± 4.349
16c	54.47	± 4.9	Nd	
16d	Nd		Nd	
16e	41.81	± 3.182	Nd	
16f	2.78	± 0.542	6.64	± 0.877
16g	Nd		Nd	
16h	65.36	± 4.785	Nd	
	Iinhibition of proliferation for DMSO ± SD [%]			
Dissolvent	MCF-7		BALB/3T3	
DMSO	31.63	± 3.967	6.04	± 2.068
Concentration of DMSO: 1%				

wherever nucleophilic substitution reaction occurred on those heterocycles. In this type of rearrangement the driving force is to convert the moiety (two sulfur atoms attached to the same carbon, which is low stable) to two C=S groups (separated with NH group, which is more stable)) leads to the formation of the target fused pyrimidinedithone **15**. (Scheme 3).

Then, it was found that compound **15** have a thiourea residue. So, its reactivity toward hydrazonyl chlorides was interested to be studied. When compound **15** was submitted to react with selected derivatives of the hydrazonoyl chlorides **6a-h** in dioxane containing triethylamine as a catalyst it give the respective substituted triazolopyridopyrimidinethione derivatives **16a-h** (Scheme 4)

The structures of the new derivatives were deduced from correct microanalytical and spectral data (IR, ^{1}H NMR, Mass spectrometry)

A respective examples; IR spectrum of compound **16a** devoid any signals for NH groups. In addition, its ^{1}H NMR showed singlet signal at 2.65 ppm for three protons of CH_3 group.

On the other hand, IR spectrum of compound **16e** showed strong stretching absorption band at1715 *vmax* for carbonyl ester and its H^{1}NMR spectrum reveled the absence of any signals for the 2NH groups and showed triplet signal at 1.23-1.31ppm attributed to the three protons of CH_2CH_3 group and quartet signal at 4.14-4.22 ppm for two protons of CH_2CH_3.

Also, The structure were confirmed by their mass spectrum (*m*/*z*) [M$^+$], which agrees with their molecular formula (see Experimental part).

Antiproliferative activity

The results were calculated as the IC_{50} (inhibitory concentration 50%), the concentration of tested compound which inhibits 50% of the cells population. IC_{50} values were calculated for each experiment separately and mean values ± SD are presented in the table. Each compound at each concentration was tested in triplicate in a single experiment, which was repeated 3–5 times. The results of the studies on anti-proliferative activity of tested compounds are summarized in Table 1.

In case of first part of compounds from **5** to **9h** the **5** reveal the highest antiproliferative activity (IC_{50} = 27.79 µg/mL) from all tested compounds against breast cancer cell line MCF-7 but it is also active (IC_{50} = 49.91 µg/mL) against normal cell line BALB/3T3. **9g** show lower antiproliferative activity (IC_{50} = 73.13 µg/mL) than **5** but it is not active against BALB/3T3 in used range of concentration.

In case of next part of compounds from **15** to **16h** the highest antiproliferative activity against breast cancer cell line MCF-7 shows **16f** compound its IC_{50} value is 2.78 µg/mL but it is also active against normal cell line BALB/3T3 and in this case IC_{50} values is 6.64 µg/ml. High antiproliferative activity shows also **16a** its IC_{50} value is 14.20 µg/mL and this compound is not active against normal cell line BALB/3T3 in used range of concentration. **15**, **16b**, **16c**, **16e** and **16h** reveal antiproliferative activity weaker than **16a** and **16f** compounds. **16d** and **16g** do not show activity against MCF-7 and BALB/3T3 in used concentrations.

CONCLUSION

The newly synthesized compounds seem to be interesting for pharmaceutical studies. They revealed high potency as anti-cancer agents. Also, the results obtained from the testing compounds showed reasonable therapeutic indices especially those of potent activities and this beside their lower possible side effects due to no action on Notch intracellular domain responsive genes.

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