International Journal of Advanced Research and Publications
ISSN: 2456-9992

A Novel Synthesis Of Fused Pyrazolopyrimidine: Pyrazolo-Triazolo-Pyrimidine For Anticancer Evalution.

A.Y. Hassan, Mona. S. Kadh, N.M. Saleh, E.S. Abou-Amra.

Prof. of Organic Chemistry,
Faculty of Science (Girls), Al-Azhar University.
helali_aisha@yahoo.com

Prof. of Organic Chemistry,
Faculty of Science (Girls), Al-Azhar University.
mkadh@yahoo.com

Assist. Prof. of Organic Chemistry,
Faculty of Science (Girls) Al-Azhar University.
drnashwamostafa@azhar.edu.eg

Assist. lecturer of Organic Chemistry Department,
Faculty of Science (Girls) Al-Azhar University.
eman.sadek.1612@gmail.com

Abstract: Some novel fused pyrazolo-triazolo-pyrimidine (**2- 5)**were designed and prepared via reaction of 5-amino-6-hydrazineyl-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (**1**) with some electrophilic and nucleophilic reagents. The structures of target compounds were confirmed by elemental analyses and spectral data. The newly synthesized compounds were selected by the NCI – Maryland-U.S.A. and were tested for their anticancer activity in an initial single high dose in the full NCI 60 cell line panel.

Keywords: pyrazolo [3,4-d] pyrimidine,pyrazolotriazolopyrimidine,anticanceractivity.

1. Introduction

Pyrazolo[3,4-d]pyrimidines and their correlating fused heterocycles are of significant chemical and pharmaceutical interest as purine parallel [1] and abundant of their derivatives were reported to have antiviral, [2,3] anticancer, [4] anti-inflammatory, [5,6] antimycobacterial, [7] antimicrobial, [8,9] activities and xanthine oxidase inhibitor [7] like allopurinol which was first synthesized by Robins in 1956,[10] and is still the drug for the treatment of hyperuricemia and gouty arthritic disease.[11] Moreover, pyrazolotriazolopyrimidines expected to possess notable chemical and pharmacological activities [8,12–15] and represented an attractive key intermediate for obtaining and selective human A3 adenosine receptors. [14] Therefore, based on the previous reports we interested in this paper on the synthesis of fused pyrazolo-triazolopyrimidine derivatives and exploring novel anticancer agents of some new compounds conserving the pyrazolopyrimidine nucleus.

2. Results and discussion

Synthesis of the objective compounds was carried out according to the proceedings light in schemes **1**. As explained in Figure **1** treating of 5-aminopyrazole-4-carboxylate with thiocarbohydrazide (TCH) in ethanolic sodium ethoxide solution under reflux conferred compound 1 as the start of all preparations. ^{1}HNMR spectrum of **1** showed five deuterium exchangeable signals at δ (5.29, 5.69, 6.29, 6.32, 8.19) ppm due to $=NNH_2$(tautomer), NH-pyrimidine(tautomer), NH_2, NH_2(tautomer) and $NHNH_2$ protons, respectively. Cyclocondensation of **1** with ethyl pyridine-2-carboxylate or 2,2,2-trichloro-acetamide under refluxing in ethanolic sodium ethoxide solution produced compound **2** with releasing molecules of H_2O and EtOH, while resulted compound **3** with elimination of H_2O and NH_3 molecules, respectively. IR and ^{1}HNMR of **2** and **3** showed no signal for NH of 6-hydrazineyl pyrimidine and this an evidence on cyclization. ^{13}C NMR spectrum of **3** showed signals at δ (87.0, 156.0) ppm assignable to CCl_3 and C=N triazole carbons, respectively besides the expected signals for aromatic carbons. The mass spectra of **2** and **3** also support their chemical formula and showed a molecular ion peak at m/z = 344 ($M^{+\cdot}$) and m/z = 383($M^{+\cdot}$ - 1), respectively. Fusion of **1** with salicylaldehyde produced compound **4** after removal H_2O molecule. IR spectrum of **4** showed peaks at (3424, 3320) cm^{-1} for OH and NH_2 groups, its ^{1}HNMR spectrum revealed two deuterium exchangeable signals due to NH-triazole and OH moieties at δ (8.70, 9.90) ppm, respectively , also displayed a singlet signals at δ 4.17 ppm for CH-triazole proton, its ^{13}CNMR spectrum showed signals at δ 85.3 ppm assignable to C_{12} triazole carbon besides the expected signals for aromatic and others carbons. The mass spectrum of **4** also support its chemical formula and showed a molecular ion peak at m/z = 361 ($M^{+\cdot}$). The reaction of **1** with cyanamide by fusion can be occurred according to nucleophilic addition of cyanamide NH_2 on carbonyl oxygen of **1** and elimination of H_2O molecule followed by nucleophilic attack of NH_2 pyrimidine on cyanamide C≡N which lead to cyclization and formation of compound **5**. The IR spectrum of **5** showed the absence of the amidic carbonyl group. Also, its ^{13}CNMR spectrum confirmed the structure as there are no signal for C=O and showed a signal assignable to C=N triazole carbon at δ 161.1 ppm besides the expected signals for aromatic carbons. The mass spectrum of **5** also support its chemical formula and showed a molecular ion peak at m/z = 281 ($M^{+\cdot}$). (Scheme **1**)

3. Biological evaluation

3.1. NCI results.

The synthesized Compounds were selected by the National Cancer Institute (NCI, Bethesda, USA) at one dose (10^{-5}M) in the full 60 human cancer cell lines orderly into subpanels derived from nine various human cancer forms: leukemia, melanoma, lung, colon, renal, ovarian, breast, prostate and CNS. The anticancer activity of the synthesized compounds is presented in Table (**1**).

4. Conclusion

Compounds **2**, **3**, **4** and **5** were synthesized by the reaction of 5-amino-6-hydrazineyl-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one **1** with some electrophilic and nucleophilic reagents by refluxing or fusion and theyexerted mild anticancer activity against certain cancer cell lines.

5. Experimental

5.1. Chemistry

Melting points were determined on Electro thermal LA 9000 SERIS. Infrared (IR) spectra were recorded on Schimadzu FT-IR Affinity-1 spectrometer .^{1}H ,^{13}C NMR spectra were recorded on Jeol spectrometer (500 MHz) and Bruker high performance digital FT-NMR spectrometer avance III (400 MHz) using deuterated dimethylsulfoxide (DMSO‑d6) as a solvent. The Mass spectra were carried out using a Schimadzu GC/ MS-QP-5050A mass spectrometer at 70 ev in the regional center for mycology and biotechnology, at Al-Azhar University.

5.1.1.5-amino-6-hydrazineyl-1-phenyl-1,5-dihydro-4H-pyrazolo [3,4-d] pyrimidin-4-one**&**(Z)-5-amino-6-hydrazine-ylidene-1-phenyl-1,5,6,7-tetrahydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (**1**, $C_{11}H_{11}N_7O$) An equimolar mixture of 5-amino pyrazole-4-carboxylate (2.31g, 10 mmol) and thio carbohydrazide (TCH) (1.06g,10 mmol) in ethanolic sodium ethoxide solution [(prepared by dissolving sodium metal (0.24 g, 10 mmol) in absolute ethanol (30 ml)] was heated under reflux for 8 h. The reaction mixture was cooled, poured on to crushed ice, the precipitate was collected by filtration, washed with water, dried and crystallized from ethanol to afford compound **1** as white solid (1.93g ,75%). M.p.: 185-187^{o}C. ^{1}H NMR (500 MHz, DMSO-d_6):5.29 (s,2H, =NNH$_2$(tautomer),D$_2$O exchangeable); 5.69 (s,1H, NH-pyrimidine(tautomer),D$_2$O exchangeable); 6.29, 6.32 (2s,2H, NH$_2$(tautomer),D$_2$O exchangeable);7.37-7.69 (m,5H,Ar-H); 8.19 (s,3H, NHNH$_2$,D$_2$O exchangeable); 8.44 (s,1H, =CH-pyrazole).Anal. Calcd for $C_{11}H_{11}N_7O$ (257.26); C, 51.36; H, 4.31; N, 38.11. Found: C, 51.47; H, 4.37; N, 38.33.

5.1.2. General method for preparation of compounds **2** and **3**. An equimolar mixture of compound **1** (2.57g, 10 mmol) and ethyl pyridine-2-carboxylate (1.51g, 1.34 ml) or 2,2,2-trichloro-acetamide (1.62g, 1.08ml) (10 mmol) in ethanolic sodium ethoxide solution [(prepared by dissolving sodium metal (0.24 g, 10 mmol) in absolute ethanol (30 ml)] was heated under reflux for 40 h. The reaction mixture was cooled, poured on to crushed ice, the precipitate was collected by filtration to afford compounds **2** and **3**, respectively.

5.1.2.1. 8-amino-1-phenyl-7-(pyridin-2-yl)-1,8-dihydro-4H-pyrazolo[3,4-d][1,2,4]triazolo[1,5-a] pyrimidin-4-one(**2**, $C_{17}H_{12}N_8O$), white solid, crystallized from acetone (2.03g ,59%). M.p.: 234-236^{o}C. IR (KBr, cm^{-1}): 3432,3322(NH$_2$); 3049(CH-aromatic); 1660(C=O); 1619(C=N); 1548(C=C).^{1}H NMR (500 MHz, DMSO-d_6): 6.28 (s,2H, NH$_2$, D$_2$O exchangeable);7.38-7.66(m, 9H, Ar-H); 8.18 (s,1H, =CH-pyrazole). MS (m/z, %): 344.04 (M$^{+\cdot}$, 17); 56.48 (100). Anal. Calcd for $C_{17}H_{12}N_8O$ (344.34); C, 59.30; H, 3.51; N, 32.54. Found: C, 59.41; H, 3.58; N, 32.63.

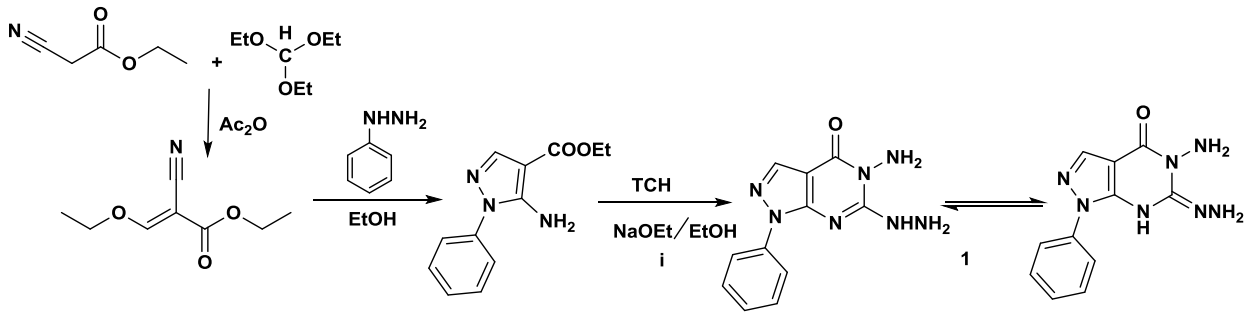
5.1.2.2. 8-amino-1-phenyl-7-(trichloromethyl)-1,8-dihydro-4H-pyrazolo[3,4-d][1,2,4]triazolo [1,5-a] pyrimidin-4-one (**3**, $C_{13}H_8Cl_3N_7O$), off white crystals, crystallized from ethanol (1.99g , 52%). M.p.: 144-146^{o}C. IR (KBr, cm^{-1}): 3431,3322(NH$_2$); 3048(CH-aromatic); 1660(C=O-amidic); 1618(2C=N); 1547(C=C). ^{1}H NMR (500 MHz, DMSO-d_6): 6.20 (s, 2H, NH$_2$, D$_2$O exchangeable) 7.38-7.55 (m, 5H, Ar-H); 8.13(s,1H,=CH-pyrazole).^{13}C NMR (500MHz, DMSO-d_6): 87.0(C_{13}-Cl$_3$);110.2(C_{11}-fused pyrazolopyrimidine) ;124.1, 126.0, 129.6, 139.2 (phenyl $C_{16\text{-}21}$); 140.0 (C_7-fused pyrazolopyrimidine); 152.2(C_5=N-fused triazolopyrimidine); 156.0(C_3=N triazole); 162.0(C=O pyrimidine). MS (m/z, %): 383.52 (M$^{+\cdot}$ - 1, 17); 185.08 (100). Anal. Calcd for $C_{13}H_8Cl_3N_7O$ (384.61); C, 40.60; H, 2.10; Cl, 27.65; N, 25.49. Found: C, 40.63; H, 2.17; Cl, 27.74; N, 25.52.

5.1.3. General method for preparation of compounds **4** and **5**. An equimolar mixture of compound **1** (2.57g, 10 mmol) and salicylaldehyde (1.22g, 1.06ml) or cyanamide (0.42g, 0.32 ml) (10 mmol) was heated until the contents melted, the reaction was maintained at temperature 200^{o}C for 6 h. The fused mass thus obtained was treated with ethanol, collected by filtration and washed by ethanol, methanol, ethyl acetate, acetone and hexane to afford compound **4** and **5**, respectively.

5.1.3.1.8-amino-7-(2-hydroxyphenyl)-1-phenyl-1,6,7,8-tetrahydro-4H-pyrazolo [3,4-d] [1,2,4] triazolo [1,5-a]pyrimidin-4-one (**4**, $C_{18}H_{15}N_7O_2$), brown powder (2.02g, 56%). M.p.: >360 ^{o}C. IR (KBr, cm^{-1}):3424,3320(OH, NH$_2$); 3220(NH) 3068(CH-aromatic); 2962 (CH-aliphatic); 1650 (C=O amidic); 1624(2C=N); 1597(C=C).^{1}H NMR (400 MHz, DMSO-d_6): 4.17 (s,1H, CH-triazole); 4.42 (s,2H,NH$_2$,D$_2$O exchangeable) 7.02-7.63 (m, 9H,Ar-H); 8.35 (s,1H,=CH-pyrazole); 8.70 (s,1H,NH,D$_2$O exchangeable); 9.90 (s,1H, OH, D$_2$O exchangeable).^{13}C NMR (500MHz, DMSO-d_6): 85.3(C_{12}-triazole);110.0(C_4-pyrazole);115.5, 122.7, 129.0, 129.3, 141.0, 154.2 (phenyl $C_{14\text{-}19}$, $C_{21\text{-}26}$); 141.0 ($C_{5,8}$pyrazole); 152.3(C_{10}=N pyrimidine); 160.8(C=O).MS (m/z, %): 361.23 (M$^{+\cdot}$, 9); 158.77 (100). Anal. Calcd for $C_{18}H_{15}N_7O_2$ (361.37); C, 59.83; H, 4.18; N, 27.13. Found: C, 59.89; H, 4.22; N, 27.18.

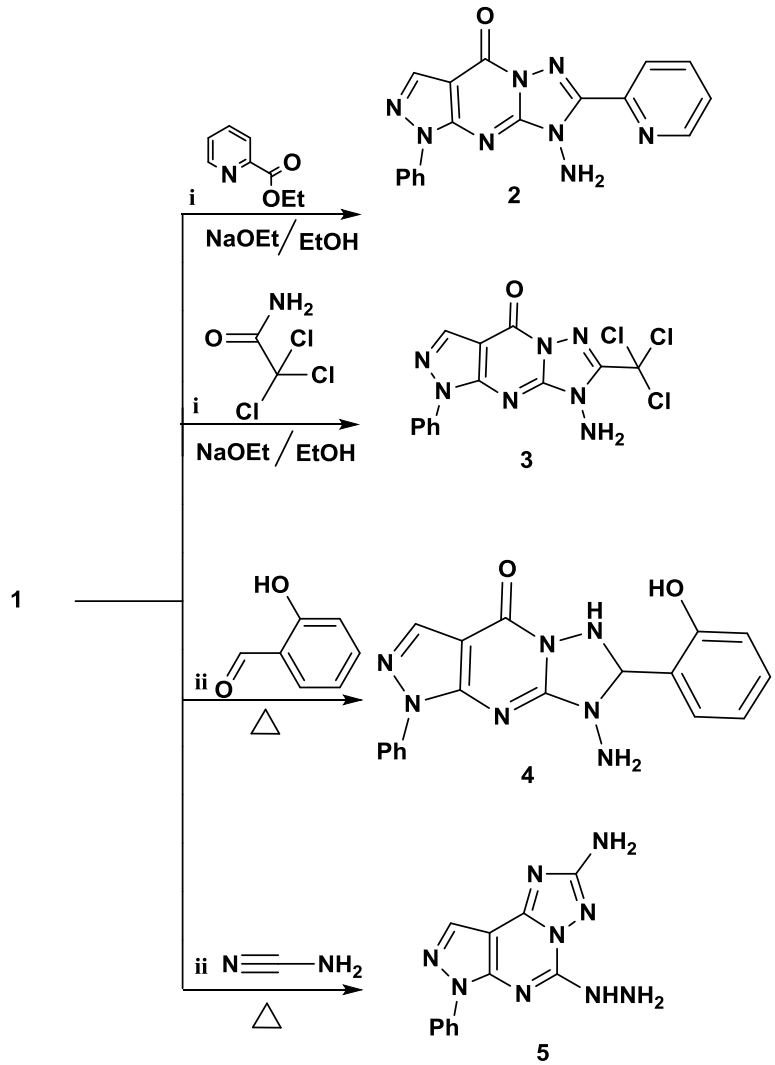
5.1.3.2.5-hydrazineyl-7-phenyl-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-2-amine (**5**,$C_{12}H_{11}N_9$), off white solid (1.51g, 54%). M.p.: >360^{o}C. IR (KBr, cm^{-1}): 3334,3149,3120 (NH$_2$, NH); 3060 (CH-aromatic);1631 (4C=N); 1540 (C=C).^{1}H NMR (400 MHz, DMSO-d_6): 6.15 (s,2H, NH$_2$, D$_2$O exchangeable); 6.97 (s, 3H, NHNH$_2$, D$_2$O exchangeable); 7.12-7.91 (m, 5H,Ar-H); 8.04 (s,1H,=CH-

pyrazole); 10.40 (s,1H,NH(tautomer),D_2O exchangeable).^{13}C NMR (500MHz, DMSO-d_6): 104.2 (C_6-fused pyrazolopyrimidine); 118.5, 126.1, 128.2, 139.4 (phenyl C_{14-19}); 136.0 (C_7=N pyrazole); 148.1(C_{10}-fused pyrazolopyrimidine); 161.1(C_3=N triazole); 169.3(C_{12}=N pyrimidine). MS (m/z, %): 281.04 ($M^{+\cdot}$, 7); 43.14 (100). Anal. Calcd for $C_{12}H_{11}N_9$ (281.28); C, 51.24; H, 3.94; N, 44.82. Found: C, 51.31; H, 3.97; N, 44.85.



Conditions: (i) reflux 8h.

Figure 1.



Conditions:(i) reflux, 40h; (ii) fusion 6h.

Scheme (1)

5.2. NCI methodology

All compounds submitted to the NCI 60 Cell screen are tested at a single high dose (10^{-5} M) as previously reported[16].

Acknowledgments

The authors wish to express all thanks to National Institute of Health (NIH), Bethesda, MD, USA, for the screening studies of anticancer activity reported in the paper.

Table (1): *Evaluation of anticancer activity (Percentage of Growth inhibition) of the selected compounds from scheme **1** for most sensitive cell lines.*

Comp. No.	NCI-No.	Mean Growth%	Range of Growth %	Panel Subpanel cell line (Growth inhibition percent)
2	812489	100.14	40.38 79.56 to 119.94	CNS Cancer: SNB-75 (20.44). Renal Cancer: CAKI-1 (16.88), UO-31 (17.30).
3	812490	100.05	55.26 81.01 to 136.27	CNS Cancer: SNB-75 (15.00). Ovarian Cancer: SK-OV-3 (15.39).Renal Cancer: CAKI-1 (16.09), UO-31 (18.99).
4	812486	94.84	43.19 70.44 to 113.63	Non-Small Cell Lung Cancer: HOP-92 (25.80), NCI-H23 (15.42). CNS Cancer: SNB-75 (28.02). Melanoma: MALME-3M (14.70), UACC-62 (17.22). Ovarian Cancer: IGROV1 (29.56). Renal Cancer: CAKI-1 (22.39), UO-31 (22.62). Breast Cancer: MDA-MB-231/ATCC (16.63), HS 578T (25.16).
5	812438	100.37	54.55 68.58 to 123.13	Ovarian Cancer: IGROV1 (31.42). Renal Cancer: CAKI-1(16.08),UO-31(30.79).

Range: Variation in value between the most sensitive cell lines and the most inefficient one.

References

[1] M. Chauhan, & R. Kumar, "Medicinal attributes of pyrazolo[3,4-d]pyrimidines: A review," Bioorganic & Medicinal Chemistry, 21(18), 5657–5668, 2013.

[2] E. R. El-Bendary, & F. A. Badria, "Synthesis, DNA-binding, and Antiviral Activity of Certain Pyrazolo[3,4-d]pyrimidine Derivatives," Archiv Der Pharmazie, 333(4), 99–103, 2000.

[3] J.-H. Chern, K.-S. Shia, T.-A. Hsu, C.-L. Tai, C.-C. Lee, Y.-C. Lee, S.-R. Shih,"Design, synthesis, and structure–activity relationships of pyrazolo[3,4-d]pyrimidines: a novel class of potent enterovirus inhibitors," Bioorganic & Medicinal Chemistry Letters, 14(10), 2519–2525, 2004.

[4] L. Ballell, R.A. Field, G.A.C. Chung, &R.J.Young, Bioorganic & Medicinal Chemistry Letters,17, 1736, 2007.

[5] S. Alcaro, A. Artese, M. Botta, A.T. Zizzari, F. Orallo, F. Ortuso, M. Yáñez, "Hit Identification and Biological Evaluation of Anticancer Pyrazolopyrimidines Endowed with Anti-inflammatory Activity," ChemMedChem, 5(8), 1242–1246, 2010.

[6] K. R. A. Abdellatif, & R.B. Bakr, "New advances in synthesis and clinical aspects of pyrazolo[3,4-d]pyrimidine scaffolds," Bioorganic Chemistry, 78, 341–357, 2018.

[7] S. Gupta, L.M. Rodrigues, A.P. Esteves, A.M.F. Oliveira-Campos, M.S.J. Nascimento, N. Nazareth, H. Cidade, M.P. Neves, E. Fernandes, &M. Pinto, et al,"Synthesis of N-aryl-5-amino-4-cyanopyrazole derivatives as potent xanthine oxidase inhibitors." European Journal of Medicinal Chemistry, 43(4), 771–780, 2008.

[8] A. F. Eweas, S.A. Swelam, O.A. Fathalla, N.M. Fawzy, & S.I. Abdel-Moez,"Synthesis, anti-microbial evaluation, and molecular modeling of new pyrazolo[3,4-d]pyrimidine derivatives," Medicinal Chemistry Research, 21(11), 3848–3857, 2011.

[9] S.B. Yewale, S.B. Ganorkar, K.G. Baheti, & R.U. Shelke, "Novel 3-substituted-1-aryl-5-phenyl-6-anilinopyrazolo[3,4-d]pyrimidin-4-ones:Docking, synthesis and pharmacological evaluation as a potential anti-inflammatory agents," Bioorganic & Medicinal Chemistry Letters, 22(21), 6616–6620, 2012.

[10] S. Kobayashi, "The Synthesis and Xanthine Oxidase Inhibitory Activity of Pyrazolo[3, 4-d]pyrimidines," CHEMICAL & PHARMACEUTICAL BULLETIN, 21(5), 941–951, 1973.

[11] N. Boechat, L.C.S. Pinheiro, T.S. Silva, A.C.C. Aguiar, A.S. Carvalho, M.M. Bastos, C.C.P. Costa, S. Pinheiro, A.C. Pinto, & J.S. Mendonça, et al, "New Trifluoromethyl Triazolopyrimidines as Anti-Plasmodium falciparum Agents," Molecules, 17(7), 8285–8302, 2012.

[12] A. Rashad, M. Hegab, R. Abdel-Megeid, M. Ali, & F. Abdel-Megeid, "Synthesis and Antitumor Evaluation of Some Newly Synthesized Pyrazolopyrimidine and Pyrazolotriazolopyrimidine Derivatives," Phosphorus, Sulfur, and Silicon and the Related Elements, 185(1), 74–83, 2010.

[13] M.M. Kandeel, A. M. Kamal, E.K.A. Abdelall, & H.A.H. Elshemy, "Synthesis of novel chromene derivatives of expected antitumor activity" European Journal of Medicinal Chemistry, 59, 183–193, 2013.

[14] B. Cacciari, C. Bolcato, G. Spalluto, K.-N. Klotz, M. Bacilieri, F. Deflorian, & S. Moro, "Pyrazolo-triazolo-pyrimidines as adenosine receptor antagonists: A complete structure–activity profile," Purinergic Signalling, 3(3), 183–193, 2006.

[15] E. Al-Afaleq, & S. Abubshait,"Heterocyclic o-Aminonitriles: Preparation of Pyrazolo[3,4-d]-pyrimidines with Modification of the Substituents at the 1- Position," Molecules, 6(7), 621–638, 2001.

[16] A. Monks, D, Scudiero, P. Skehan, R, Shoemaker, K. Paull, D. Vistica, H. Curtis, L. John, C. Paul, V-W. Anne, G-G. Marcia, C. Hugh, M. Joseph & M. Boyd,"Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines'' JNCI Journal of the National Cancer Institute, 83(11), 757–766, 1991.