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Cytotoxic activity of some benzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives against the human cancer cell lines HepG2 and MCF-7

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Abstract

Different benzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives were synthesized in high yields and purity with short reaction times by the reaction of 2-aminobenzimidazole, aldehydes and active carbonyl compounds in the presence of Silica sulfuric acid/ethylene glycol (SSA/EG) as a catalyst. Cytotoxicity of the synthesized benzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives **1-6** was carried out against the two human cancer cell lines, hepatocellular carcinoma (HepG2) and breast carcinoma (MCF-7) as well as Vero cells (normal cells).

Keywords *:*Benzo[4,5]imidazo[1,2-*a*]pyrimidine; Cytotoxicity; human cancer cell lines; Hepatocellular carcinoma; breast carcinoma.

1. Introduction

According to the WHO report published in 2018, the major cause of the death is cancer, where the number of cancer cases will be increased to 22 million by 2030 [1]. Chemotherapeutic drugs such as doxorubicin, thiotepa, cisplatin, paclitaxel and chlorambucil were failed to arrest the growth of cancer cells completely. The traditional chemotherapy is facing the lacking of selectivity towards tumor cells [2] and the resistance of cancer cells to drugs that are structurally and mechanistically distinct [3]. Accordingly, there is an emergent need to develop some new versatile chemotherapeutic agents. Continuous exploration and discover of anti-cancer drugs with low toxicity and high efficiency are still important approaches in anti-cancer drug research and development [4-10].

Dihydropyrimidine derivatives have attracted much attention as important structural motifs in medical chemistry. They have significant therapeutic and biological activities, such as T cell activation [11], antineoplastic activity [12], as well as DNA-topoisomerase I [13], TIE-2 and VEGFR2 inhibitory activities [14].Moreover, benzo[4,5]imidazo[1,2-a]pyrimidine derivatives have been reported to possess pharmacological and therapeutic properties [15,16]. So in the current study, we aim to study the cytotoxic activity of the synthesized benzo[4,5]imidazo[1,2-a]- pyrimidine derivatives towards different cancer cell lines.

2. Results and discussion

2.1. Chemistry

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2-Aminobenzoimidazole was used a precursor for synthesizing different types from benzo[4,5]imidazo-[1,2-*a*]pyrimidine derivatives *via* one-pot three component reaction in the presence of silica sulfuric acid/ ethylene glycol (SSA/EG) as a catalyst.

Thus, when 2-amino- benzimidazole was left to react with acetyl acetone and aromatic aldehydes in the presence of SSA/EG afforded the corresponding benzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives **1a-g** in good yields.

Also, reaction of benzoylacetone with different aldehydes and 2-amino- benzimidazole in the presence of SSA/EG, afforded the corresponding benzo[4,5]imidazo- [1,2-*a*]pyrimidine derivatives **2a-g** in yields. In continuation with our work in this area, we decided to prepare and evaluate the biological activity of some new benzo[4,5]-imidazo[1,2-*a*]-pyrimidine derivatives. Thus, 2-aminobenzimidazole and 1,3-diphenylpropane-1,3-dione were reacted by different aldehydes in the presence of SSA/EG. The reactions were finished at specified time and afforded the corresponding benzo[4,5]imidazo[1,2-*a*]-pyrimidine derivatives **3a-g** in good yields [17].

In addition, for obtaining another type of benzo[4,5]imidazo[1,2-*a*]pyrimidine, condensation reaction of 2-aminobenzimidazole and malononitrile with aromatic aldehydes was carried out to afford the corresponding 2-amino-4-aryl-1,4-dihydrobenzo[4,5] imidazo[1,2-*a*]pyrimidine-3-carbonitrile derivatives **4a-f** in good yields. Another types of benzo[4,5]-imidazo[1,2-*a*]pyrimidine derivative were synthesized for their expected pharmacological activities.

Thus, the three-component condensation of ethyl cyanoacetate with 2-aminobenzimidazole and different aldehydes was applied. The obtained products were confirmed as 1,2-dihydrobenzo[4,5]-imidazo[1,2-*a*]pyrimidine-3-carbonitrile derivatives **5a-g** [18].

Moreover, the benzo[4,5]imidazo[1,2-*a*]-pyrimidine derivatives **6a-g** were synthesized *via* reacting the 2-aminobenzimidazole and ethyl acetoacetate with different aldehydes in the presence of SSA/EG at the specified times in good yields [19] (Scheme 1).

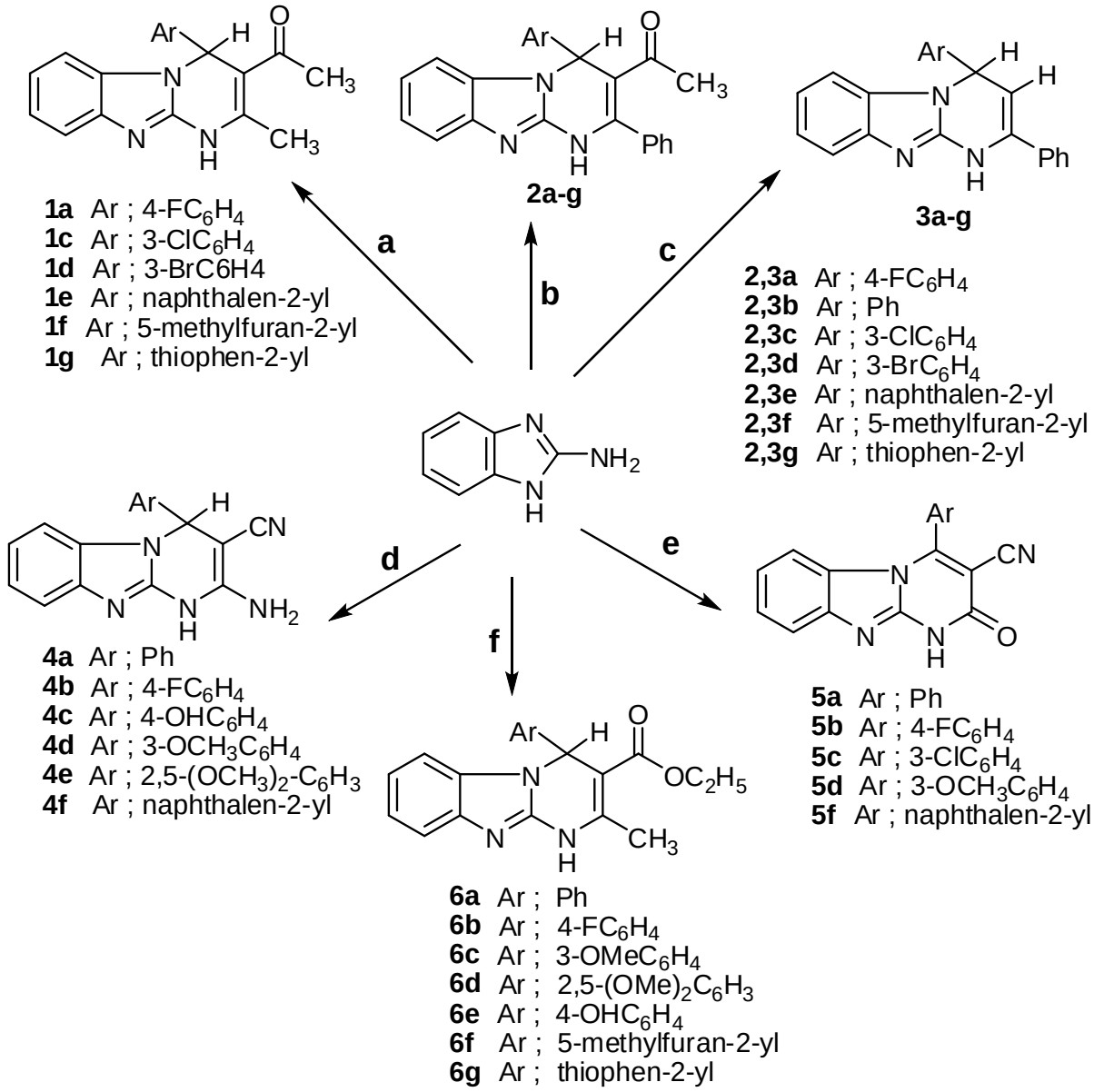
2.2. Evaluation of the biological activity.

Target of the present work is to evaluate the antitumor activity of various synthesized benzo[4,5]imidazo[1,2-*a*]pyrimidinederivatives**1-6** with studying effect of substituent on the activity. The cytotoxic activity was evaluated by using CV assay [20] against Vero cells and the two human cancer cell lines, hepatocellular carcinoma (HepG2) and breast carcinoma (MCF-7). Doxorubicin was used as a reference drug, which consider as one of the most effective anti-cancer agents.

Cytotoxic activity for the tested compounds is reported in Table 1. Upon using the general structure provided in Scheme 1, certain aspects of the structure activity relationships for the synthesized benzo[4,5]imidazo- [1,2-*a*]pyrimidine derivatives were more clearly highlighted.

From the cytotoxicity assay results presented in Table 1, it was noticed that compound **2e** showed remarkable cytotoxicity (≥75%) effect and was able to inhibit the proliferation of the cancer cells HepG2 and a moderate activity towards MCF-7tumor cell lines, whereas the cytotoxicity of the other compounds ranged from moderate (70 – 40 %) to weak (< 40%) effects.

- Compounds **1a,d,e,3d,5d,6d** showed moderate activity against the two tested cell lines (HepG2 and MCF-7).
- Compounds **1c,4c,4d,5b,6b,6c,6g** showed moderate activity against the tested cell line HepG2.
- Compounds **2d, 3e** showed moderate activity against the tested cell line MCF-7.
- Compounds **3b,3c,3g,4a,4b,4e,4f,5a,5f,6e,6f** showed low activity against the two tested cell lines (HepG2 and MCF-7).
- Compounds **2d,3e** showed low activity against the tested cell line HepG2.
- Compounds **1c,4c,4d,5b,6b,6c,6g** showed low activity against the tested cell line MCF-7.



Scheme 1: Synthesis of benzo[4,5]imidazo[1,2-a]pyrimidine derivatives: Reagents and reaction conditions: (a) aldehyde (0.01 mol) and acetyl acetone (0.01 mol) in ethylene glycol (5 mL) containing SSA (42.6 mg, 11 mol %) at 120 °C, 1-40 min. in 60-93 yields; (b) aldehyde (0.01 mol) and benzoyl acetone (1 mmol) in ethylene glycol (5 mL) containing SSA (42.6 mg, 11 mol %) at 120 °C, 1-40 min. in 58-97 yields;(c) aldehyde (0.01 mol) and 1,3-diphenylpropane-1,3-dione (0.01 mol) in ethylene glycol (5 mL) containing SSA (42.6 mg, 11 mol %) at 120 °C, 15-60 min., in 76-97% yields; (d) aldehyde (0.01 mol) and malononitrile(0.01 mol) in ethylene glycol (5 mL), containing SSA (42.6 mg, 11 mol %) at 120 °C, 5-15 min.in 83-97% yields.; (e) aldehyde (0.01 mol) and ethyl cyanoacetate(0.01 mol) in ethylene glycol (5 mL) containing SSA (42.6 mg, 11 mol %) at 120 °C, 35-80 min. in moderate yields; (f) aldehyde (0.01 mol) and ethyl acetoacetate (0.01 mol) in ethylene glycol (5 mL) containing SSA (42.6 mg, 11 mol %) at 120 °C, 10-35 min. in 70-94% yields.

Table 1: The cytotoxic activities *in vitro*aganist Vero cells and 2 human cancer cell lines, hepatocellular carcinoma (HepG2) and breast carcinoma (MCF-7).

Compound No.	Cytotoxicity %		
	Vero cells	HepG2	MCF-7
1a	23.00	56.70	50.00
1c	23.00	59.50	23.50
1d	24.60	53.80	41.40
1e	7.00	53.00	62.10
1f	0.00	0.00	0.00
1g	0.00	0.00	0.00
2a	0.00	0.00	0.00
2b	0.00	0.00	0.00
2c	0.00	0.00	0.00
2d	20.00	29.30	43.30
2e	28.50	76.00	51.40
2f	0.00	0.00	0.00
2g	0.00	0.00	0.00
3a	0.00	0.00	0.00
3b	10.00	14.60	26.70
3c	7.00	17.80	15.00
3d	12.00	54.00	46.00
3e	22.00	25.50	49.10
3f	0.00	0.00	0.00
3g	5.00	22.00	10.00
4a	0.00	17.00	10.00
4b	10.00	19.50	16.50
4c	12.50	50.00	37.60
4d	18.00	58.00	31.10
4e	13.60	15.00	19.30
4f	8.00	26.30	13.40
5a	9.00	21.00	10.00
5b	19.00	47.00	30.00
5c	0.00	0.00	0.00
5d	20.00	67.00	65.00
5f	15.00	25.00	20.00
6a	0.00	0.00	0.00
6b	12.80	45.00	22.10
6c	22.00	47.30	36.50
6d	20.00	48.00	53.00
6e	10.00	21.00	25.00
6f	20.00	30.00	22.50
6g	17.80	50.80	23.40

Remarkable cytotoxic activity (>75% of cell population), Moderate Cytotoxicity (75- 40 % of cell population), Low Cytotoxicity (40-0.1% of cell population), No Cytotoxicity (0% of cell population)[16].

IC_{50}value of the remarkable compound **2e** was found to be more toxic to cancer cells than normal cells. In this regards, "therapeutic index" is an important parameter to select samples for developing drugs. Compound **2e** was not as active as standard drug (doxorubicin) Table 2 and Figs. 1-3.

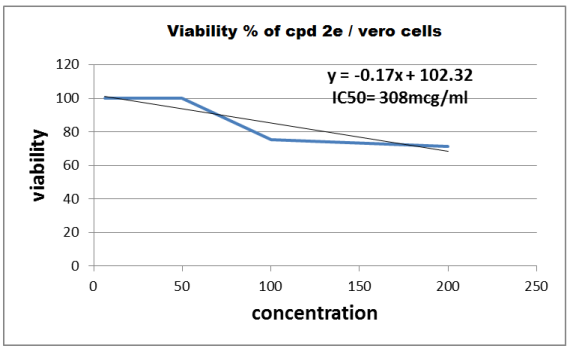


Fig 1: IC_{50} value of compound**2e** in Vero cell line.

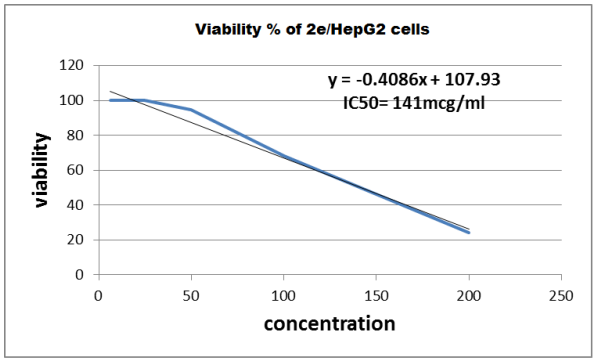


Fig 2: IC_{50} value of compound**2e** in HepG2 cell line.

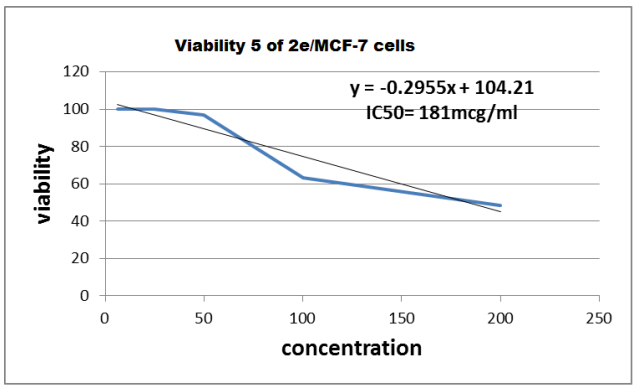


Fig 3: IC_{50} value of compound**2e** in MCF7 cell line.

Table 2:IC_{50}& SI of compound **2e**

Compd. No.	IC_{50} (µg/mL)			Selective index	
	Vero	HepG2	MCF-7	HepG2	MCF-7
2e	308	141	181	2.18	1.7
Doxorubicin	--	12	6		

- If the compound IC_{50} is > 90 µg/mL, the compoundis classified as not cytotoxic
- If the IC_{50} is between 2 and 89 µg/mL, thecompound is classified as moderately cytotoxic.
- If the IC_{50} is < 2µg/mL, the compound is classified as cytotoxic.

Experimental Section:

***In vitro* cytotoxicity crystal violet assay in Vero, HepG2 and MCF-7 cell lines**

Crystal violet (CV) cell cytotoxicity assay is one of the common methods used to detect cell viability or drug cytotoxicity. CV is a triarylmethane dye that can bind to ribose type molecules such as DNA in nuclei. Normally, dead adherent cells will detach from cell culture plates and will be removed from viable cell population during washing steps. CV staining can be used to quantify the total DNA of the remaining population and thus determine cell viability. The CV staining is directly proportional to the cell biomass and can be measured at 490 nm. CV staining is a quick and versatile assay for screening cell viability under diverse stimulation or inhibition conditions [21].

The present work aimed to screen 38 compounds for testing their potential to inhibit the viability of cells in two human cancer cell lines namely breast and hepatic carcinomas cell lines. The *in vitro* cytotoxic effect ofthe compoundswas tested against African Green Monkey kidney (Vero cells) and two human tumor cell lines: human hepatocellular carcinoma (HepG2) and human breast carcinoma (MCF-7).

Preparation of cell lines: Vero cells were seeded in 75 cm^2 tissue culture flasks containing 20 ml liquid growth medium of Dulbecco's modification of Eagle medium supplemented with the following L-glutamine, 10% FBS, 1% Antibiotic (penicillin/streptomycin) and 1% HEPES buffer and maintained at pH 7.2 under aseptic conditions inside a biology safety cabinet. Vero cells were grown at 37 ºC in humidified atmosphere. Vero cells were subcultured when they were in a semi-confluent state. Similarly, HepG2 and MCF-7 cells were seeded in 75 cm^2 tissue culture flasks containing 20 mL liquid growth medium of RPMI.

Preparation of stock compounds: To prepare final concentration of each compounds(20 mg/mL), 0.002 g of each compound was dissolved in 100 µL DMSO (100%), and then kept in -20 ºC till being used.

Cell viability assay: The anticancer activity of samples on MCF7, HepG2 & Vero cells was determined by CV assay [15]. Cells (7×10^3/well) were plated in 0.1 mL of growth medium/well in 96-well plates. After 24hrs incubation, tested compounds were added and the working concentration was prepared in 2% MM: 200µg/ml. Doxorubicin was used as the standard drug to treat cancer. After 24 hours of incubation, cell viability was determined using crystal violet. Twenty µL CV (5 mg/mL) was added to all wells of the plate. Viable cells stained purple color. The plate was transferred to an ELISA reader and the OD (optical density) values were read at 490nm.

Determination of IC_{50} values: IC_{50} values were calculated for active extracts possessing ≥ 75 % cytotoxicity. Measurements were performed and the concentration required for a 50 % inhibition of viability (IC_{50}) was determined graphically. Standard Graph was plotted by taking concentration of the compound in X axis and relative cell viability in Y axis.

Cell viability (%) = Mean OD/Control OD x 100%

Cell cytotoxicity (%) = 100 – cell viability (%)

Selectivity index (SI): The SI indicates the cytotoxic selectivity (i.e safety). This value is the ratio of the concentration of the compound at which 50% of the normal cell line survived in normal cell line to that of the same compound at which 50% of cancer cell death occurred in cancer cell lines.

SI = IC_{50} of compound in a normal cell line/ IC_{50} of the same compound in cancer cell line.

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Conflicts of interest

There are no conflicts to declare.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal. "Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries". Cancer J. Clin., 68 (2018), 394-424.

[2] C. Holohan, S. Van Schaeybroeck, D.B. Longley, P.G. Johnston. "Cancer drug resistance: an evolving paradigm". Nat. Rev. Canc., 13 (2013), 714-726.

[3] Y. Wang, Y.A. Suh, M.Y. Fuller, J.G. Jackson, S. Xiong, T. Terzian, A. Quintas-Cardama, J.A. Bankson, A.K. El-Naggar, G. Lozano. "Restoring expression of wild-type p53 suppresses tumor growth but does not cause tumor regression in mice with a p53 missense mutation". J. Clin. Investig., 121 (2011), 893-904.

[4] S. Y. Abbas, K. A. M. El-Bayouki, W.M. Basyouni, E.A. Mostafa. "New series of 4(3*H*)-quinazolinone derivatives: syntheses and evaluation of antitumor and antiviral activities".Med. Chem. Res., 27 (2018), 571-582.

[5] Y. A. Ammar, A. M. Sh. El-Sharief, A. Belal, S. Y. Abbas, Y. A. Mohamed, A. B. M. Mehany, A. Ragab. "Design, synthesis, antiproliferative activity, molecular docking and cell cycle analysis of some novel (morpholinosulfonyl) isatins with potential EGFR inhibitory activity". Eur. J. Med. Chem., 156 (2018), 918-932.

[6] M. Eldeeb, E. F. Sanad, A. Ragab, Y. A. Ammar, K. Mahmoud, M. M. Ali, N. M. Hamdy, Anticancer Effects with Molecular Docking Confirmation of Newly Synthesized Isatin Sulfonamide Molecular Hybrid Derivatives against Hepatic Cancer Cell Lines, Biomedicines 10 (2022) 722

[7] S. A. Ibrahim, A.Ragab, H. A. El-Ghamry, Coordination compounds of pyrazolone-based ligand: Design, characterization, biological evaluation, antitumor efficiency, and DNA binding evaluation supported by in silico studies, Appl Organomet Chem., 36 (2022) e6508

[8] A.S. Al Wasidi, A.S. Hassan, A.M. Naglah. In vitro cytotoxicity and druglikeness of pyrazolines and pyridines bearing benzofuran moiety. J. Appl. Pharm. Sci., 10 (2020) 142-148.

[9] N. M. Morsy, A. S. Hassan, T. S. Hafez, M. R. H. Mahran, I. A. Sadawe, A. M. Gbaj, Synthesis, antitumor activity, enzyme assay, DNA binding and molecular docking of Bis-Schiff bases of pyrazoles, Journal of the Iranian Chemical Society, 18 (2021) 47–59

[10] H. R. Elgiushy, S. H. Mohamed, H. Taha, H. Sawaf, Z. Hassan, N. A. Abou-Taleb, E. M. El-labbad, A. S. Hassan, K.A.M. Abouzid, S. F. Hammad, Identification of a promising hit from a new series of pyrazolo[1,5-a] pyrimidine based compounds as a potential anticancer agent with potent CDK1 inhibitory and pro-apoptotic properties through a multistep in vitro assessment, Bioorganic Chemistry 120 (2022) 105646

[11] J.J. Nunes, X.T. Zhu, P. Amouzegh, C. Ghiron, D.N. Johnston, E. C. Power. "2-Amino-4-Hydroxy-5-Pyrimidinecarboxamide Derivatives and Related Compounds As Inhibitors Of T Cell Activation For The Treatment Of Inflammatory Diseases". WO. Pat., 2005009443 (2005).

[12] J.P. Whitten, M. Schwaebe. "Tetracyclic imidazole analogs". WO. Pat., 2008060693 (2008).

[13] N. Zanatta, S.S. Amaral, A. Esteves-Souza, A. Echevarria, P.B. Brondani, D.C. Flores, H.G. Bonacorso, A.F.C. Flores, M.A.P. Martins. "Synthesis and Characterization of Some Novel 2-(Trifluoromethyl) pyrimido[1,2-a]-benzimidazoles and Pyrimido[1,2-a]-benzimidazol-2H)-ones of Biological Interest". Synthesis, 14 (2006), 2305-12.

[14] M. Cheung, P.A. Harris, M. Hasegawa, S. Ida, K. Kano, N. Nishigaki, H. Sato, J.M. Veal, Y. Washio R.I. West. "Benzimidazole derivatives useful as tie-2 and/or vegfr-2 inhibitors". WO. Pat., 2002044156 (2002).

[15] J.J. Nunes, X.T. Zhu, M. Ermann, C. Ghiron, D.N. Johnston, C.G.P. Saluste. "Substituted heterocyclic compounds and methods of use". WO Pat., 2005021551, March 10, 2005. Chem. Abstr. 142 (2005), 298123.

[16] M.W. Martin, J. Newcomb, J.J. Nunes, C. Boucher, L. Chai, L.F. Epstein, T. Faust, S. Flores, P. Gallant, A. Gore, Y. Gu, F. Hsieh, X. Huang, J.L.S. Kim, K. Morgenstern, A. Oliveirados-Santos, V.F. Patel, D. Powers, P. Rose, Y. Tudor, S.M. Turci, A.A. Welcher, D. Zack, H.L. Zhao, L. Zhu, X.T. Zhu, C. Ghiron, M. Ermann, D. Johnston C.G.P. Saluste."Structure-Based Design of Novel 2-Amino-6-phenyl-pyrimido[5′,4′:5,6]pyrimido-[1,2-*a*]benzimidazol-5(6*H*)-ones as Potent and Orally Active Inhibitors of Lymphocyte Specific Kinase (Lck): Synthesis, SAR, and In vivo Anti-Inflammatory Activity". J. Med. Chem., 51 (2008), 1637-48.

[17] W.M. Basyouni, N.M. Abdelazeem, S.Y. Abbas, K.A.M. El-Bayouki, M.Y. El-kady. "Silica Sulfuric Acid / Ethylene Glycol: An Efficient Eco-Friendly Catalyst for One-Pot Synthesis of New Benzo[4,5]imidazo[1,2-a]pyrimidines". Organic Preparations and Procedures International, 52 (2020), 249–260.

[18] W.M. Basyouni, S.Y. Abbas, N. M. Abdelazeem, Kh.A.M. El-Bayouki, and M.Y. El Kady. "Silica sulfuric acid/ethylene glycol as an efficient catalyst for the synthesis of benzo[4,5]imidazo[1,2-a]pyrimidine-3carbonitrile derivatives". *Synth. Comm.*, 49 (2019), 3112–3120.

[19] N.M. Abdelazeem, W.M. Basyouni, S.Y. Abbas, Kh. A.M. El-Bayouki, M.Y. El-kady. "Silica sulfuric acid / ethylene glycol: An Efficient Eco-friendly Catalyst for One-pot Synthesis of Tricyclic and Tetarcyclic Dihydropyrimidine Derivatives". Egypt. J. Chem., 62 (2019), 2265 – 2276.

[20] M. Feoktistova,; P. Geserick, and M. Leverkus. "Crystal Violet Assay for Determining Viability of Cultured Cells". Cold Spring Harb. Protoc., 4 (2016), 343-346.

[21] S.M.A. Moustafa, B.M. Menshawi, G.M. Wassel, Kh. Mahmoud, M.M. Mounier. "Screening of some plants in Egypt for their cytotoxicity aganist four Human Cancer cell lines". Int. J. Pharm. Tech. Res., 6 (2014), 1074-1084.