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Synthesis and biological evaluation of some new imidazo[1,2-c]pyrimido [5,4-e]pyrimidin-5-amine derivatives

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ABSTRACT

*The pyrimidine ring is a skeleton of particular interest that it is present in many compounds exhibiting biological and pharmaceutical activity. In the present work, some new pyrimidine derivatives were prepared via the formation of 5-oxo-4H-benzopyran (3), and its transformation to pyrimidine derivative (5) by reaction with benzamidine HCl. Compound **5** was subjected to react with thiophosgene to give isothiocyanate derivative **6**. Phenacyl amine **7** was prepared by reaction of phenacyl bromide with hexamethylenetetramine under Delepine reaction condition. The reaction of compounds **6** and **7** in ethanol and drops of TEA afforded the imidazopyrimidinopyrimidine **8**. Compound **8** was subjected to react with excess of $POCl_3$ to give its corresponding 5-chloro-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidine **(9).** Then compound **9** was reacted with different amines to give N-(aryl)-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido [5,4-e]pyrimidin-5-amine **(10 a-k).** The new imidazopyrimidinopyrimidine derivatives, **10a-k**, were first assessed for their anti-tumor properties towards two cancer cell lines, namely human hepatic malignancy (HepG2) and breast (MCF-7) by utilizing an MTT assay at a sole strength of 10 µM. Compounds **10d**, **10e**, **10f**, **10h,** and **10i** displayed very strong activity, which contains in general, electron-withdrawing groups.*

1. Introduction

Over the last hundred years, there have been considerable advances in the utility of nitrogen heterocyclic derivatives in the medicinal chemistry sector [1-11]. Pyrimidine derivatives perform as essential pharmacophores in therapeutic agent research and demonstrate a broad spectrum of biological properties, encompassing anti-bacterial [12], anti-convulsant [13], anti-fungal [14], anti-human immunodeficiency virus (HIV) [15], anti-hypertensive [16], anti-inflammatory [4], anti-malarial [17] and anti-tumor functions [3, 18, 19]. The imidazole derivatives also exhibit notable anti-bacterial [20], anti-fungal [21], anti-HIV [22], anti-hypertensive [23], anti-inflammatory [24], and anti-tumor [25-27] characteristics.

Anticancer drugs are a very important subject of frontier research over the world. In the last 20 years, many anticancer drugs including nitrogen heterocyclic scaffolds, for example, Tozasertib VX-680 or MK-0457 Antitumor and Barasertib Anticancer (bearing pyrazole ring), Uramustine, Tegafur, Fluorouracil and Methotrexate (bearing a pyrimidine ring), Crizotinib (Xalkori) Anticancer, Imatinib mesylate (Gleevec anticancer) and Oxisuran (bearing Pyridine ring), zoledronic acid (bearing an imidazole Skeleton). Many years ago, the discovery of nitrogen heterocyclic compounds, that can be used as anticancer agents, has been investigating by our research group. The previous studies reported several heterocyclic compounds displaying important anticancer activities [28-32].

In view of the previous studies, novel N-(aryl)-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido [5,4-e]pyrimidin-5-amine (10 a-k) derivatives were designed by combining pyrimidine, pyrimidine, and imidazole skeleton. The target compounds were synthesized and their biological activities were evaluated.

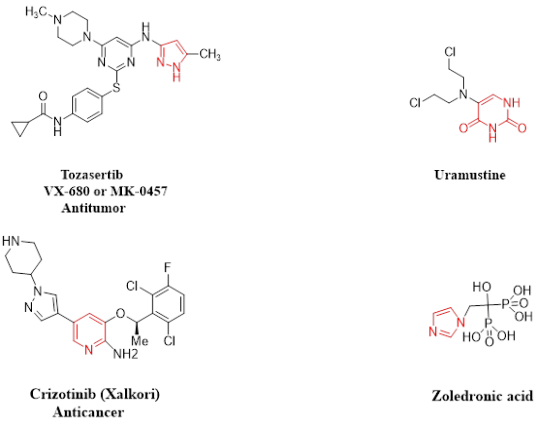

Figure 1. Heterocyclic Nitrogen compounds having anticancer activity.

2. Results and discussion

2,8-Diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidine-5(6H)-thione derivative **(8)** was designed by combining imidazole and pyrimidine skeletons. Also, the objective compounds were prepared, and their biological activities were estimated.

The pyrimidine ring is a skeleton of particular interest if it is present in many compounds exhibiting biological and pharmaceutical activity [33]. In the present work, some new pyrimidine derivatives were prepared via the formation of 5-oxo-4*H*-benzopyran, and its transformation to pyrimidine system by reaction with benzamidine HCl [34]. 2-Amino-4-p-tolyl-3-cyano-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydrobenzo-4*H*-pyran **(3)** was synthesized from the corresponding *p*- tolylidenemalononitrile **(2)** and dimedone [35, 36]. Compound **3** showed in its IR spectrum the cyano and carbonyl groups absorption bands at 2220 and 1680 cm^{-1}, respectively. The ^{1}H-NMR spectrum of compound **3** showed the proton on C_4-H as a singlet at δ 4.29 ppm. The two protons on C-6 appear as an AB system with a coupling constant J=16.00 Hz, demonstrating that these two geminal protons are not equivalents. However, the protons on C-8 seem as a

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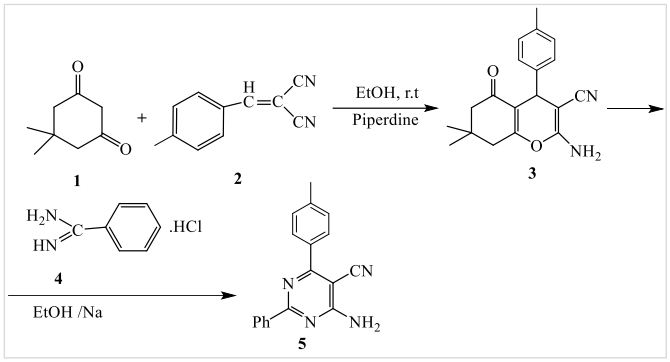
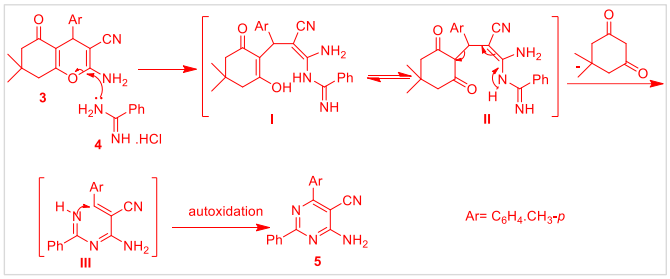
broad singlet at δ =2.39 ppm. The ^{13}CNMR spectrum show two olefinic double bonds between C-2, δ= 159.2 ppm, and C3, δ 58.1 ppm, and C-8a, δ=155.1 ppm and C-4a, δ=113.9 ppm in compound **3**, clearly showing the attendance of push–pull effect that responsible for the δ values showed this olefinic carbon atom. Further reaction of **3** with an excess of benzamidine hydrochloride leads to the respective 4-amino-6-*p*-tolyl-2-phenylpyrimidine-5-carbonitrile **(5)** (Scheme **1**). The IR spectrum of **5** shows the NH_2 group as two bands around 3400 and 3320 cm^{-1}, the conjugated cyano group at 2219 cm^{-1} and several bands in the aromatic region. The ^{1}HNMR spectrum shows the NH_2 protons as a singlet at δ 6.89 ppm and the expected signals corresponding to the protons of monosubstituted and δ 7.17-8.20 ppm of a benzene ring. The ^{13}CNMR spectrum of **5** exhibits signals due to the heterocyclic ring. The cyano group appears at δ 115.5 ppm. The proposed reaction mechanism for the formation of compound 5 can be shown as follows:

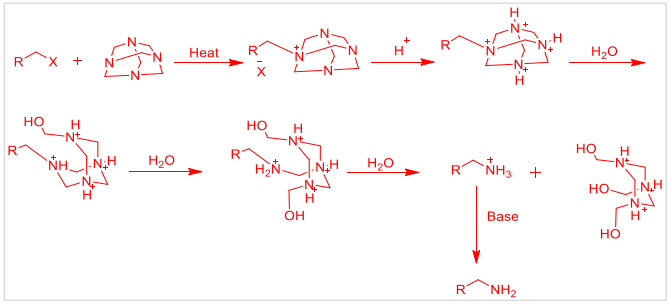


Scheme 1. Synthesis of compounds **3-5**.



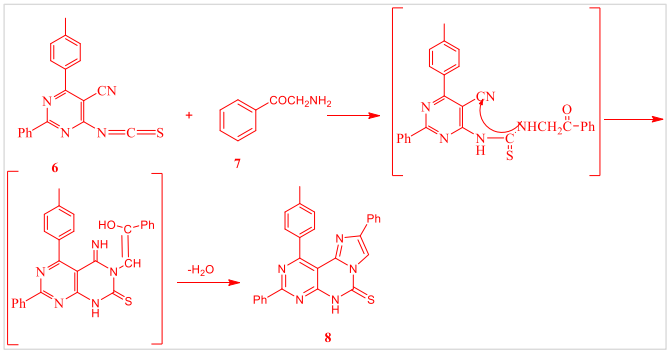
Scheme 2. Reaction mechanism of formation compound 5

Compound **5** was subjected to react with thiophosgene in chloroform either in presence of 75% acetic acid and 4N HCl or in presence of trimethylamine to give isothiocyanate derivative **6,** its IR showed bands in the region of 685-720 cm^{-1} may be according to –C-S- vibration. Isothiocyanate **6** exhibited a broad and very strong band centered at 2100 cm^{-1} according to the isothiocyanate group. This agrees with the observation of Williams [37, 38] on phenylisothiocyanate. Isothiocyanate 6 also shows a weak or medium weak band between 1050-1090 cm^{-1}, this is maybe due to isothiocyanate symmetric stretching vibration. Phenacyl amine **7** was prepared by reaction of phenacyl bromide with hexamethylenetetramine under Delepine reaction condition. It is the synthesis of primary aliphatic amines by acidic hydrolysis of quaternary amines prepared from urotropine (hexamethylene) and alkyl halides [39] General Delepine reaction mechanism can be shown as follow:

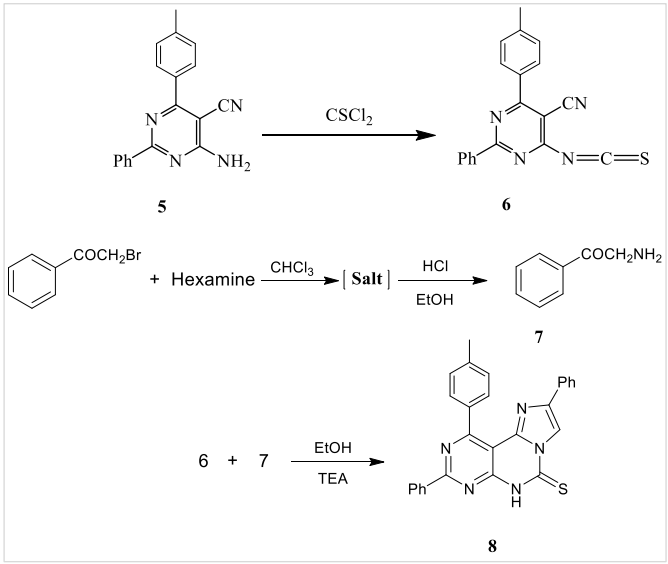


Scheme 3. Conversion of alkyl halide to alkyl amine

In addition of hexamine to the phenacyl bromide, the signal at δ= 4.56 ppm (CH_2-Br) moved to 3.91 ppm. The peak at δ= 4.7 ppm (hexamine protons) disappeared. This product was not crystallized due to thermal instability. Hydrolysis of this product with 2N HCl-EtOH afforded 60% yield of phenacylammonium chloride salt. The reaction of compound **6** and **7** in ethanol and drops of TEA afforded the imidazopyrmidinopyrimidine **8** according to the following reaction mechanism:



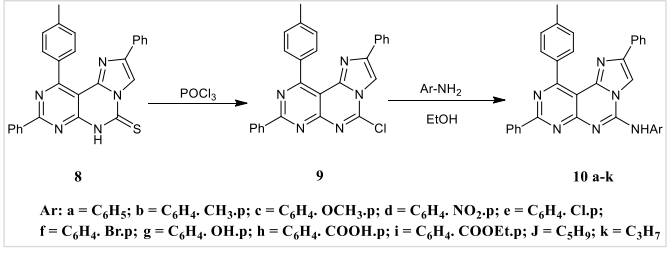
Scheme 4. Reaction mechanism of formation compound 8



Scheme 5. Synthesis of compounds **6-8**.

Structure **8** was established its correct structure spectroscopically, IR spectrum of **8** demonstrated absorption bands at 3150 and 1630 cm^{-1}attributed to NH, C=N, and a strong absorption band at 1350 due to C=S. ^{1}HNMR was characterized by a peak at δ 7.24 ppm due to imidazole methine and deshielded NH proton at δ 12.75 ppm, while its ^{13}CNMR displayed band at δ 183 ppm due to C=S. Moreover, mass spectrum was also demonstrated molecular ion peak at *m/z* = 445 due to its correct molecular formula.

Compound **8** was subjected to react with excess of $POCl_3$ to give its corresponding 5-chloro-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidine **(9).** Then compound **9** was reacted with different amines to give N-(aryl)-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido [5,4-e]pyrimidin-5-amine **(10 a-k).**



Scheme 6. Synthesis of compounds **9, 10 a-k**.

The IR spectrum of compound **9** indicated no absorption band around 3000 cm^{-1} corresponding to the NH function group, and instead displayed a band at 670 cm^{-1} due to the C-Cl function group. The ^{1}HNMR spectrum of compound **9** showed a singlet signal at δ 8.80 ppm attributed to the CH proton of the imidazole ring. The ^{13}CNMR spectrum indicated a characteristic signal at δ 160.3 ppm due to the C-

Cl carbon atom. However, the IR spectrum of compound **10a** once again showed absorption at 3200 cm^{-1} owing to the NH function group. The ^{1}HNMR spectrum of compound **10a** displayed two characteristic singlet signals at δ 9.45 and 8.82 ppm according to NH and imidazole CH protons, respectively. The ^{13}CNMR spectrum showed a characteristic signal at δ 107.6 and 169.3 ppm referred to imidazole CH and C-NH carbon atoms, respectively.

Pharmacological activity:

Cytotoxicity assay:

The new imidazopyrimidinopyrimidine derivatives, **10a-k**, were first assessed for their anti-tumor properties towards two cancer cell lines, namely human hepatic malignancy (HepG2) and breast (MCF-7) by utilizing an MTT assay at a sole strength of 10 µM.

Cytotoxic consequences have been documented in the form of the concentration necessary to inhibit malignant cell growth by 50% following administration of cells to the substances being tested. 5-fluorouracil (5-FU) has been deployed to form a positive control. The newly manufactured imidazopyrimidinopyrimidine substances, **10a-k**, have been assessed with a view to establish their cytotoxicity towards various malignant cell strains, in particular MCF-7 and HepG2. Furthermore, their influence on caspase-3 and Bcl-2 molecular biomarker expression was also assessed. The Bcl-2 class of proteins, which includes protein Bcl-2, an anti-apoptotic compound, is a family of indispensable proteins that are concerned with the ascendency of predetermined cell death [40, 41]. The caspase group, e.g., caspase-3, is a further sizeable category of regulatory genes that are implicated in apoptosis [42, 43]. The fundamental procedure for the commencement of cellular death was nominated by the assessment of protein expression in compounds associated with the apoptotic path exploiting Western blot techniques. These involve Bcl-2 and caspase-3 proteins. In vitro experiments have shown that the imidazopyrimidinopyrimidine derivatives, **10a-k**, have anti-tumor properties through the inhibition of free radical generation and the transfer of estrogen metabolism in the direction of the metabolite 2-hydroxyestrone, which has fewer estrogenic properties. Additionally, there is a halt at the G1/S stage of the cell cycle. Once this has occurred, cells are preserved in the stopped phase until the inhibitory agent is withdrawn. When the manufactured pharmaceuticals, **10a-k**, are used to halt the reproductive cycle, cells require washing before they can resume mitosis and proliferation.

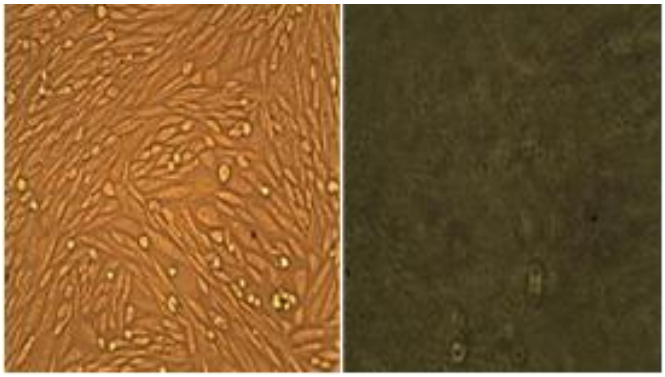
Furthermore, it was experiential that the synthesized compounds **10a-k**, act as apoptosis-inducing compounds. Once released from the cell cycle, cells can be seen entering programmed cell death in a coordinated fashion. If the period of arrest is prolonged, cellular necrosis, as opposed to apoptosis, occurs in combination with apoptosis, thus diminishing the migration of cancer cells, their invasion, and neovascularization. Following the 24-hour development period with the varying imidazopyrimidinopyrimidine derivatives, **10a-k**, an MTT assay was utilized to assess viability. The in vitro cytotoxicity assay for both MCF-7 and HepG2 cell lines revealed that overall, most of the substances demonstrated greater cytotoxicity against the liver cancer cells rather than the malignant breast cells. The most powerful cytotoxic impact on the HepG2 cells was seen with **10d, 10e, 10f, 10h**, and 1**0i**. The HepG2 cell line was therefore chosen to explore the underlying molecular mechanism further. Bcl-2 and caspase-3 expression was appraised, these represented markers for anti-apoptosis and apoptosis, respectively. Cells received the gauged IC_{50} of each pharmaceutical and were then harvested after 48 hours of incubation. Protein extraction was then conducted for measurement. **10d, 10e, 10f, 10h**, and **10i** were associated with Bcl-2 expression down-regulation compared with control cells. Caspase-3 expression was inactivated following administration of these substances, a finding which could underlie their possible anti-tumor effects. The imidazopyrimidinopyrimidine derivatives, **10a-k**, have been estimated to diminish the growth of differing tumor cell lines, e.g., MCF-7 and HepG2, when dispensed at concentrations of 10 µM. The newly synthesized compounds **10d, 10e, 10f, 10h**, and **10i** were observed to be potent against both cell lines in a concentration spectrum for IC_{50} of 3-9 µM. Such data propose that the synthesized compounds with electron-withdrawing substituent derivatives trigger a chain of biochemical alterations, including the simultaneous activation of intracellular caspase and attendant apoptosis. In contrast, **10a-c, 10j**, and **10k** demonstrated the least potent cytotoxic effects with the IC_{50} ranging between 36 and 54 µg/mL (Table 1), a likely sign of a large number of electron-donating moieties within their construction.

Structure activity relationship:

Nucleotides are well known as the chemical building blocks of DNA. There are four categories of nitrogen bases located in nucleotides which are: adenine (A), thymine (T), guanine (G), and cytosine (C). The heterocyclic adenine base always pairs with thymine, while guanine always pairs with cytosine through a hydrogen bond. The cytotoxic activity of the tested compounds for dissimilar cell lines based on two factors [44, 45], namely, the formation of an intermolecular hydrogen bond with DNA bases, and the positive charge on the tested compounds enticed to the negative charge on the cell wall (Table 1). By comparison, the investigational cytotoxic activity of the newly synthesized compounds in this study to their chemical structures, the following structure-activity relationship was nominated. Thus, compound **10d**, **10e**, **10f**, **10h**, and **10i** displayed very strong activity, which contains in general, nitro group, chlorine and bromine atoms, carboxylic and ester groups as electron-withdrawing groups which may be added to any unsaturated moiety in DNA or forming a hydrogen bond with either one of the nucleobases of the DNA and instigates its damage. Compound **10g** exhibited strong activity due to the presence of hydroxyl groups which may be added to any unsaturated moiety in DNA or forming a hydrogen bond with either one of the nucleobases of the DNA and causes its damage. Compounds **10e** and **10f** exposed very strong activity due to the existence of strong electron attracting atoms (halogen atoms) which rendered the molecule positivity charged forming electrostatic attraction with the DNA nucleobases.

Table 1. IC_{50} values of the compounds **10 a-k**

Compound	IC_{50} (µM)	
	HepG2	MCF-7
5-Fu	60.7±1.3	41.5±0.3
10a	86.3±1.7	97.8±2.9
10b	62.7±1.2	76.2±2.3
10c	39.7±1.2	53.9±2.5
10d	14.2±0.7	15.1±0.5
10e	22.6±1.2	17.6±0.9
10f	19.2±1.2	26.0±0.7
10g	23.6±1.1	25.7±0.8
10h	14.0±0.6	11.5±0.8
10i	16.1±2.1	17.7±0.8
10j	149.1±2.9	99.3±2.0
10k	105.7±3.5	88.0±2.9



HEPG-2 MCF-7

HEPGII: Hepatoma cells; **MCF-7**: Cells from breast cancer.

3. Materials and Methods

Melting points majored with a Gallenkamp apparat and uncorrected. All spectroscopical measurements were carried out according to the previously reported work [46].

Synthesis of 2-amino-4-p-tolyl-3-cyano-5,6,7,8-tetrahydro-7,7dimethyl-5oxo-4H-benzopyran (3):

A mixture of dimedon (1.4 g, 0.01 mol), p-tolyledinemalononitrile (1.68 g, 0.01 mol) and a few amounts of triethylamine dissolved in ethanol (50 mL). The mixture was then stirred at room temperature for one hour, a precipitate was produced the solid which received by

filtration. After that, more purification was done by recrystallization from ethanol.

Yield 80%, 223°C; IR (kBr) γ_{max}, cm^{-1}: 3400 and 3300 (NH_2), 2220 (CN), 1680 (CO), 1630 (C=C). ^{1}HNMR (DMSO-d_6) δ (ppm): 0.99 (s, 6H, $2CH_3$), 2.08 (d, 1H, C_6-Ha, J=16.00 Hz), 2.15 (d, 1H, C_6-Hb, J=16.00 Hz), 2.39 (s, 2H, C_8-H), 4.29 (s, 1H, C_4-H), 6.80 (s, 2H, NH_2), 7.05-7.31 (m, 5H, Ar-H); ^{13}CNMR (DMSO–d_6) δ (ppm): 21.3, 24.4, 32.4, 38.8. 39.2, 51.5, 58.1, 113.9, 119.2, 128.9, 135.4, 141.1, 155.1, 159.2, 198.9. MS (EI): m/z (%): 308 (M^+, 100), 293 (M^+-CH_3, 30), 217 ([M=C_6H_4-CH_3] +, 80); 161 (20). Anal. Calcd. for $C_{19}H_{20}N_2O_2$ (308.38): C, 74.00; H, 6.54; N, 9.08%. Received; C, 74.36; H, 6.92; N, 9.43%.

Synthesis of 4 -amino- 6- p-tolyl- 2-phenylpyrimidine-5-carbonitirile (5):

A mixture of sodium metal (0.23 g, 0.01 mol) in dry ethanol (20 mL) was cooled in ice-cold water and benzamidine hydrochloride was added (1.56 g, 0.01 mol) with stirring for 15 min. The separated sodium chloride was filtered, and the resulting solution compound 3 (3.08 g, 0.01 mol) was added. The mixture was heated in a pressure tube in a silicon oil bath for 6 hours. The reaction left to cool, filter the solid material, and recrystallized to receive compound **5**.

Yield 70%, m.p. 240°C; IR (KBr) γ_{max}, cm^{-1}: 3400 and 3320 (NH), 2219 (CN), 1620 and 1580 (C=C); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.37 (s, 3H, CH_3), 6.89 (s, 2H, NH_2), 7.17-8.20 (m, 9H, Ar-H). ^{13}CNMR (DMSO-d_6) (ppm): 21.3, 39.2, 87.4, 115.5, 125.8, 127.5, 129.4, 131.1, 132.6, 134.8, 165.1, 167.7, 168.2. MS (EI): m/z (%): 268 (100), 285 (60), 224 (20), 183 (50), 104 (30), 91(10). Anal. Calcd. for $C_{18}H_{14}N_4$ (286.33): C, 75.5; H, 4.93; N, 19.57%. Received: C, 75.62; H, 4.81; N, 19.72%.

Synthesis of 4-isothiocyanato-2-phenyl-6-(p-tolyl)pyrimidine-5-carbonitrile (6):

Method A: A mixture of thiophosgene (0.2 mL, 0.002 mol) in chloroform (50 mL) is added dropwise to a stirred mixture of 4 -amino- 6- p-tolyl- 2-phenylpyrimidine-5-carbonitirile **(5)** (0.572 g, 0.002 mol) in 75 % acetic acid (20 mL) and 4N HCl (10 mL), stirring is continued until the reaction is complete 5 h as judged by the disappearance of the orange color of the chloroform layer mixture. After that, the organic layer is separated then washed with water, dried ($MgSO_4$), and the solvent removed under vacuo. The residue is crystallized from diluted acetone. Yield 63%; mp. 235 °C.

Method B: Thiophosgene (1.5 mL, 0.017 mol**)** in chloroform (50 mL) is added dropwise to a stirred mixture of **5** (2.2 g, 0.008 mol) and triethylamine (5 mL) in chloroform (130 mL). Stirring was continued for 1 h, followed by heating for 25 min, the reaction solution is washed with water, the organic layer then separated, dried with $MgSO_4$ and the solvent removed. The obtained solid material is crystallized from diluted acetone. Yield 63%; mp. 240 °C.

Yield 82%, m.p. 240°C; IR (KBr) γ_{max}, cm^{-1}: 2219 (CN), 2100 (NCS), 1050-1090 (NCS stretching vibration), 1620 (C=N), 1580 (C=C) and 685-720 (C-S-Vibration); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.37 (s, 3H, CH_3), 7.17 (d, 2H, Ar-H), 7.50 (m, 3H, Ar-H), 7.59 (d, 2H, Ar-H), 8.36 (d, 2H, Ar-H). ^{13}CNMR (DMSO-d_6) (ppm): 21.3, 100.7, 115.5, 125.7, 127.5, 129.2, 129.5, 131.1, 131.7, 132.6, 134.7, 136.9, 164.4, 167.0, 169.9. MS (EI): m/z (%): 328 (M^+, 28). Anal. Calcd. for $C_{19}H_{12}N_4S$ (328.39): C, 69.49; H, 3.68; N, 17.06; S, 9.76%. Found: C, 69.36; H, 3.45; N, 16.99; S, 9.66%.

Synthesis of phenacylamine.HCl (7):

Hexamethylenetetramine (0.28 g, 0.002 mol) was dissolved in 5 mL chloroform. After that, phenacyl bromide (0.39 g, 0.002 mol) was added all at once; the suspension was stirred for 24 hours and the quaternary salt was formed, washed with 7 mL chloroform, and left to dry at 60 °C under reduced pressure (20 mmHg) for 4 h. The product was dissolved with 9 mL methanol and 5 mL 50% HCl acid at 20°C for two days. Then, 6 mL methanol was added to the present solid, and the suspension was heated at 50°C for 24 h and then reflux for 1.5 h, cooled to 20°C, and evaporated at a bath temperature of 50°C to produce a mushy solid, that recrystallized from water to form 0.7 g phenacylamine hydrochloride, in a yield of 60%, m.p. 197°C. [Lit. 194°C] [47].

Yield 60%, m.p. 197°C; ^{1}HNMR (DMSO-d_6) δ (ppm): 3.91 (s, 2H, CH_2), 7.55-8.12 (m, 5H, Ar-H), 8.72 (s, 2H, NH_2). ^{13}CNMR (DMSO-d_6) (ppm): 51.1, 128.6, 128.8, 133.1, 135.1, 195.3. MS (EI): m/z (%): 328 (M^+, 28). Anal. Calcd. for $C_{19}H_{12}N_4S$ (328.39): C, 69.49; H, 3.68; N, 17.06; S, 9.76%. Found: C, 69.36; H, 3.45; N, 16.99; S, 9.66%.

Synthesis of 2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido [5,4-e]pyrimidine-5(6H)-thione (8):

A mixture of compound **6** (0.32 g, 0.001 mol) and compound **7** (0.13 g, 0.001 mol) was dissolved in ethanol 50 mL and triethylamine (1 mL) was refluxed for 3 hours. The crude product was then filtered, dried, and recrystallized from ethanol to produce compound **8**.

Yield 60%, m.p. 282°C; IR (KBr) γ_{max}, cm^{-1}: 3150 (NH), 1630 (C=N), 1590 (C=C), 1350 (C=S); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.31 (s, 3H, CH_3), 7.13 (d, 2H, Ar-H), 7.24 (s, 1H, imidazole C-H), 7.47-7.70 (m, 8H, Ar-H), 7.60 (d, 2H, Ar-H), 8.34 (d, 2H, Ar-H), 12.75 (s, 1H, NH). ^{13}CNMR (DMSO-d_6) (ppm): 21.5, 112.4, 120.0, 125.7, 127.5, 128.7, 128.8, 129.2, 129.5, 131.1, 131.7, 133.0, 134.7, 140.1, 143.7, 154.2, 161.8, 164.2, 183.0. MS (EI): m/z (%): 445 (M^+, 48). Anal. Calcd. for $C_{27}H_{19}N_5S$ (445.54): C, 72.79; H, 4.30; N, 15.72; S, 7.20%. Found: C, 72.66; H, 4.24; N, 15.36; S, 7.17%.

Synthesis of 5-chloro-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidine (9):

A mixture of compound **8** (0.44 g, 0.001 mol) and excess $POCl_3$ (30 mL) was warmed in a pressure tube at 150 °C in a silicon oil bath. The reaction solution was poured into crushed ice. The solid filtered off, washed with ice water and left to dried. Compound **9** was obtained and recrystallized from ethanol.

Yield 60%, m.p. 121°C; IR (KBr) γ_{max}, cm^{-1}:1635 (C=N), 1600 (C=C), 670 (C-Cl); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.34 (s, 3H, CH_3), 7.15 (d, 2H, Ar-H), 7.49 (m, 6H, Ar-H), 7.60 (d, 2H, Ar-H), 8.14 (d, 2H, Ar-H), 8.19 (d, 2H, Ar-H), 8.80 (s, 1H, imidazole C-H). ^{13}CNMR (DMSO-d_6) (ppm): 21.7, 107.5, 120.2, 125.7, 127.5, 128.7, 128.8, 129.2, 129.5, 130.0, 131.1, 131.7, 133.0, 134.7, 143.4, 158.3, 160.3, 163.4, 164.4. MS (EI): m/z (%): 447 (M^+, 35), 448 (M^++1, 18). Anal. Calcd. for $C_{27}H_{18}ClN_5$ (447.93): C, 72.40; H, 4.05; N, 15.64%. Found: C, 72.27; H, 3.99; N, 15.55%.

Synthesis of N-(aryl)-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido

[5,4-e]pyrimidin-5-amine (10 a-k): General procedure:

An equimolar amount of compound **9** (0.44 g, 0.001 mol) and appropriate amine (0.001 mol) was heated in absolute ethanol (40 mL) for 4-6 h. The reaction product was filtered, dried, and recrystallized from ethanol to give compounds **10 a-k**.

N,2,8-Triphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidin-5-amine (10a):

Yield 82%, m.p. 192°C; IR (KBr) γ_{max}, cm^{-1}: 3200 (NH), 1632 (C=N), 1595 (C=C); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.34 (s, 3H, CH_3), 7.15 (d, 2H, Ar-H), 7.2-7.74 (m, 11H, Ar-H), 7.59 (d, 2H, Ar-H), 8.12 (d, 2H, Ar-H), 8.18 (d, 2H, Ar-H), 8.82 (s, 1H, imidazole C-H), 9.45 (s, 1H, NH). ^{13}CNMR (DMSO-d_6) (ppm): 21.9, 107.6, 110.5, 117.8, 122.6, 125.7, 127.5, 128.8, 129.2, 129.5, 130.0, 131.1, 131.7, 133.0, 134.7, 138.9, 143.4, 157.3, 163.4, 164.4, 169.3. MS (EI): m/z (%): 504 (M^+, 40). Anal. Calcd. for $C_{33}H_{24}N_6$ (504.60): C, 78.55; H, 4.79; N, 16.66%. Found: C, 78.38; H, 4.66; N, 16.48%.

2,8-Diphenyl-N,10-di-p-tolylimidazo[1,2-c]pyrimido[5,4-e]pyrimidin-5-amine (10b):

Yield 77%, m.p. 163°C; IR (KBr) γ_{max}, cm^{-1}: 3190 (NH), 1625 (C=N), 1600 (C=C); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.20 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 7.06 (d, 2H, Ar-H), 7.17 (d, 2H, Ar-H), 7.20 (d, 2H, Ar-H), 7.50 (m, 6H, Ar-H), 7.60 (d, 2H, Ar-H), 8.12 (d, 2H, Ar-H), 8.20 (d, 2H, Ar-H), 8.82 (s, 1H, imidazole C-H), 9.45 (s, 1H, NH). ^{13}CNMR (DMSO-d_6) (ppm): 21.9, 107.6, 110.5, 120.3, 125.7, 127.5, 128.8, 129.2, 129.5, 129.8, 130.0, 131.1, 131.2, 131.7, 133.0, 134.7, 135.9, 143.4, 157.3, 163.4, 164.4, 169.3. MS (EI): m/z (%): 518 (M^+, 28). Anal. Calcd. for $C_{34}H_{26}N_6$ (518.62): C, 78.74; H, 5.05; N, 16.20%. Found: C, 78.68; H, 4.99; N, 16.14%.

N-(4-Methoxyphenyl)-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidin-5-amine (10c):

Yield 69%, m.p. 210°C; IR (KBr) γ_{max}, cm^{-1}: 3200 (NH), 1630 (C=N), 1590 (C=C); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.30 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 6.93 (d, 2H, Ar-H), 7.13 (d, 2H, Ar-H), 7.50 (m, 6H, Ar-H), 7.59 (d, 2H, Ar-H), 7.64 (d, 2H, Ar-H), 8.14 (d, 2H, Ar-H), 8.19 (d, 2H, Ar-H), 8.82 (s, 1H, imidazole C-H), 9.42 (s, 1H, NH). ^{13}CNMR (DMSO-d_6) (ppm): 21.5, 55.3, 107.5, 110.5, 115.1, 121.7, 125.7, 127.5, 128.7, 129.2, 129.5, 130.0, 131.1, 131.2, 131.7, 133.0, 134.7, 143.4, 153.3, 157.3, 163.4, 164.4, 169.3. MS (EI): m/z (%): 534 (M^+, 40). Anal. Calcd. for $C_{34}H_{26}N_6O$ (534.62): C, 76.39; H, 4.90; N, 15.72%. Found: C, 76.21; H, 4.77; N, 15.68%.

N-(4-Nitrophenyl)-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidin-5-amine (10d):

Yield 77%, m.p. 190°C; IR (KBr) γ_{max}, cm^{-1}: 3200 (NH), 1630 (C=N), 1590 (C=C), 1530, 1350 (Symm. & asymm. NO_2); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.30 (s, 3H, CH_3), 7.13 (d, 2H, Ar-H), 7.2-7.74 (m, 11H, Ar-H), 7.60 (d, 2H, Ar-H), 8.14 (d, 2H, Ar-H), 8.20 (d, 2H, Ar-H), 8.82 (s, 1H, imidazole C-H), 9.42 (s, 1H, NH). ^{13}CNMR (DMSO-d_6) (ppm): 21.8, 107.4, 110.8, 119.2, 124.7, 125.7, 127.5, 128.8, 129.2, 129.5, 130.2, 131.1, 131.7, 133.2, 134.7, 137.9, 143.4, 145.3, 157.3, 163.4, 164.4, 169.3. MS (EI): m/z (%): 549 (M^+, 42). Anal. Calcd. for $C_{33}H_{23}N_7O_2$ (549.59): C, 72.12; H, 4.22; N, 17.84%. Found: C, 72.00; H, 4.07; N, 17.76%.

N-(4-Chlorophenyl)-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidin-5-amine (10e):

Yield 64%, m.p. 176°C; IR (KBr) γ_{max}, cm^{-1}: 3200 (NH), 1630 (C=N), 1590 (C=C), 670 (C-Cl); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.29 (s, 3H, CH_3), 7.18 (d, 2H, Ar-H), 7.5-7.78 (m, 11H, Ar-H), 7.55 (d, 2H, Ar-H), 8.16 (d, 2H, Ar-H), 8.18 (d, 2H, Ar-H), 8.82 (s, 1H, imidazole C-H), 9.44 (s, 1H, NH). ^{13}CNMR (DMSO-d_6) (ppm): 21.9, 107.6, 110.5, 122.1, 125.7, 127.5, 127.7, 128.6, 129.2, 129.5, 129.6, 130.4, 131.2, 131.6, 133.2, 134.7, 137.0, 143.5, 157.5, 163.2, 164.3, 169.5. MS (EI): m/z (%): 538 (M^+, 52), 539 (M^++1, 27). Anal. Calcd. for $C_{33}H_{23}ClN_6$ (539.04): C, 73.53; H, 4.30; N, 15.59%. Found: C, 73.44; H, 4.11; N, 15.47%.

N-(4-Bromophenyl)-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidin-5-amine (10f):

Yield 78%, m.p. 187°C; IR (KBr) γ_{max}, cm^{-1}: 3200 (NH), 1630 (C=N), 1590 (C=C), 720 (C-Br); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.29 (s, 3H, CH_3), 7.18 (d, 2H, Ar-H), 7.50-7.78 (m, 11H, Ar-H), 7.55 (d, 2H, Ar-H), 8.16 (d, 2H, Ar-H), 8.18 (d, 2H, Ar-H), 8.82 (s, 1H, imidazole C-H), 9.44 (s, 1H, NH). ^{13}CNMR (DMSO-d_6) (ppm): 21.5, 107.3, 110.2, 116.7, 118.5, 125.7, 127.5, 128.5, 129.2, 129.5, 130.3, 131.0, 131.5, 132.4, 133.3, 134.5, 137.9, 143.2, 157.3, 163.6, 164.2, 169.5. MS (EI): m/z (%): 582 (M^+, 44), 583 (M^++1, 40). Anal. Calcd. for $C_{33}H_{23}BrN_6$ (583.49): C, 67.93; H, 3.97; N, 14.40%. Found: C, 67.78; H, 3.88; N, 14.27%.

4-((2,8-Diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidin-5-yl)amino)phenol (10g):

Yield 61%, m.p. 166°C; IR (KBr) γ_{max}, cm^{-1}: 3400 (OH), 3150 (NH), 1620 (C=N), 1600 (C=C); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.29 (s, 3H, CH_3), 6.90 (d, 2H, Ar-H), 7.18 (d, 2H, Ar-H), 7.38 (d, 2H, Ar-H), 7.50 (m, 6H, Ar-H), 7.82 (d, 2H, Ar-H), 8.16 (d, 2H, Ar-H), 8.21 (d, 2H, Ar-H), 8.85 (s, 1H, imidazole C-H), 9.44 (s, 1H, NH), 9.99 (s, 1H, OH). ^{13}CNMR (DMSO-d_6) (ppm): 21.3, 107.7, 110.3, 116.5, 122.1, 125.5, 127.5, 128.8, 129.3, 129.6, 130.1, 131.1, 131.5, 131.7, 133.1, 134.7, 143.4, 148.5, 157.2, 163.4, 164.3, 169.3. MS (EI): m/z (%): 520 (M^+, 38). Anal. Calcd. for $C_{33}H_{24}N_6O$ (520.60): C, 76.14; H, 4.65; N, 16.14%. Found: C, 76.22; H, 4.44; N, 16.16%.

4-((2,8-Diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidin-5-yl)amino)benzoic acid (10h):

Yield 72%, m.p. 193°C; IR (KBr) γ_{max}, cm^{-1}: 3390 (OH), 3180 (NH), 1720 (CO), 1620 (C=N), 1610 (C=C); ^{1}HNMR (DMSO-d_6) δ (ppm): 2.29 (s, 3H, CH_3), 7.19 (d, 2H, Ar-H), 7.30-7.72 (m, 10H, Ar-H), 8.03 (d, 2H, Ar-H), 8.13 (d, 2H, Ar-H), 8.20 (d, 2H, Ar-H), 8.85 (s, 1H, imidazole C-H), 9.44 (s, 1H, NH), 11.20 (s, 1H, OH). ^{13}CNMR (DMSO-d_6) (ppm): 21.6, 107.4, 110.8, 111.2, 120.6, 125.8, 127.7, 128.5, 129.2, 129.5, 130.4, 131.1, 131.7, 133.2, 134.8, 137.0, 143.5, 144.1, 157.4, 163.2, 164.4, 169.2. MS (EI): m/z (%): 548 (M^+, 61). Anal. Calcd. for $C_{34}H_{24}N_6O_2$ (548.61): C, 74.44; H, 4.41; N, 15.32%. Found: C, 74.37; H, 4.38; N, 15.22%.

Ethyl 4-((2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidin-5-yl)amino)benzoate (10i):

Yield 74%, m.p. 210°C; IR (KBr) γ_{max}, cm^{-1}: 3410 (OH), 3200 (NH), 1725 (CO), 1615 (C=N), 1595 (C=C); ^{1}HNMR (DMSO-d_6) δ (ppm): 1.32 (t, 3H, CH_3), 2.40 (s, 3H, CH_3), 4.32 (q, 2H, CH_2), 7.15 (d, 2H, Ar-H), 7.50-7.81 (m, 12H, Ar-H), 8.13 (d, 2H, Ar-H), 8.18 (d, 2H, Ar-H), 8.80 (s, 1H, imidazole C-H), 9.40 (s, 1H, NH). ^{13}CNMR (DMSO-d_6) (ppm): 14.1, 21.3, 60.9, 107.5, 110.5, 111.4, 120.1, 125.7, 127.5, 128.7, 129.2, 129.5, 130.0, 130.7, 131.1, 131.6, 133.0, 134.6, 143.2, 143.4, 157.3, 163.4, 164.5, 165.9, 169.9. MS (EI): m/z (%): 576.23 (M^+, 47). Anal. Calcd. for $C_{36}H_{28}N_6O_2$ (576.66): C, 74.98; H, 4.89; N, 14.57%. Found: C, 74.77; H, 4.82; N, 14.47%.

N-Cyclopentyl-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidin-5-amine (10j):

Yield 77%, m.p. 166°C; IR (KBr) γ_{max}, cm^{-1}: 3210 (NH), 1620 (C=N), 1590 (C=C); ^{1}HNMR (DMSO-d_6) δ (ppm): 1.50-1.80 (m, 8H, 4 CH_2), 2.40 (s, 3H, CH_3), 2.68 (t, 1H, CH), 5.60 (s, 1H, NH), 7.18 (d, 2H, Ar-H), 7.40-7.58 (m, 6H, Ar-H), 7.65 (d, 2H, Ar-H), 8.13 (d, 2H, Ar-H), 8.20 (d, 2H, Ar-H), 8.80 (s, 1H, imidazole C-H). ^{13}CNMR (DMSO-d_6) (ppm): 21.7, 23.8, 32.8, 56.4, 107.3, 110.7, 125.4, 127.5, 128.9, 129.2, 129.7, 130.1, 131.3, 131.5, 133.2, 134.6, 143.5, 157.2, 163.6, 164.3, 169.4. MS (EI): m/z (%): 496 (M^+, 60). Anal. Calcd. for $C_{32}H_{28}N_6$ (496.62): C, 77.39; H, 5.68; N, 16.92%. Found: C, 77.27; H, 5.56; N, 16.88%.

N-Cyclopropyl-2,8-diphenyl-10-(p-tolyl)imidazo[1,2-c]pyrimido[5,4-e]pyrimidin-5-amine (10k):

Yield 59%, m.p. 153°C; IR (KBr) γ_{max}, cm^{-1}: 3210 (NH), 1620 (C=N), 1590 (C=C); ^{1}HNMR (DMSO-d_6) δ (ppm): 0.57 (m, 2H, CH_2), 0.82 (m, 2H, CH_2), 2.25 (pent, 1H, CH), 2.40 (s, 3H, CH_3), 5.60 (s, 1H, NH), 7.18 (d, 2H, Ar-H), 7.40-7.58 (m, 6H, Ar-H), 7.65 (d, 2H, Ar-H), 8.13 (d, 2H, Ar-H), 8.20 (d, 2H, Ar-H), 8.80 (s, 1H, imidazole C-H). ^{13}CNMR (DMSO-d_6) (ppm): 7.8, 21.5, 26.5, 107.2, 110.9, 125.5, 127.4, 128.5, 129.5, 129.8, 130.2, 131.2, 131.6, 133.3, 134.4, 143.4, 157.4, 163.4, 164.4, 169.3. MS (EI): m/z (%): 468 (M^+, 38). Anal. Calcd. for $C_{30}H_{24}N_6$ (468.56): C, 76.90; H, 5.16; N, 17.94%. Found: C, 76.78; H, 5.10; N, 17.77%.

Pharmacological activity:

Cytotoxicity assay:

Eleven substances were investigated for their cytotoxic effects on two human cancer cell lines, i.e. HepG-2, from hepatocellular carcinoma, and MCF-7, from breast malignancy. Cell lines were acquired from the ATCC through the Holding Company for Biological Products and Vaccines (Cairo, Egypt).

5-Fluorouracil (5-FU) was utilized as a traditional anti-tumor agent to act as a control. Reagents deployed included RPMI-1640 medium, MTT, DMSO and 5-FU (Sigma Co., St. Louis, MO, USA), together with fetal bovine serum (GIBCO, Paisley, United Kingdom). The varied cell lines described above [48, 49] were employed to evaluate the inhibitor impact of the substances under investigation on cellular proliferation with the use of an MTT assay. This colorimetric analysis is founded on the transformation of the yellow form of tetrazolium bromide to a purple formazan type; this reaction is catalyzed by mitochondrial succinate dehydrogenase, which is present in live cells.

RPMI-1640 medium and 10% fetal bovine serum were employed for cellular culture. Penicillin (100 units/mL) and streptomycin (100 μg/mL) were added and the cells were cultured at 37° C in a 5% CO_2 incubator. A 96-well plate was used for seeding the cell lines [50] at a density of 1.0×10^4 cells/well. These were cultured for 48 hours in a 5% CO_2 incubator at a temperature of 37° C. Following incubation, varying concentrations of the substances were administered, and further incubation was initiated for 24 hours. 20 μL of MTT solution at 5 mg/mL was then added; the cells were incubated for a further 4 hours. 100 μL volume of DMSO was then placed in each well to act as a solvent to the generated purple formazan.

A colorimetric assay was performed using an absorbance wavelength of 570 nm and a plate reader (EXL 800, BioTech,

Winooski, VT, USA). The relative cell viability (%) was computed according to the following equation:

A_{570} of treated samples/A_{570} of untreated sample) × 100.

4. Conclusions

The objective of the present study was to synthesize and investigate the anticancer activity of some new imidazopyrimidopyrimidine derivatives with the hope of discovering new structures that leads to serving as anticancer agents. The results of the anticancer screening showed that compounds **10d**, **10e**, **10f**, **10h**, and **10i** exhibited the highest *in vitro* cytotoxic activity when compared with the other tested compounds and 5-Fu as a reference drug. In particular, compounds **10d** and **10h** proved to be the most active member in this study with special effects against human HepG2 and MCF-7.

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