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Original Article

Synthesis and cytotoxic evaluation of some novel 3-[2-(2-phenyl-thiazol-4-yl)-ethyl]-3H-pyrido[2,3-d]pyrimidin-4-one derivatives

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Abstract

Background and purpose: Pyridopyrimidine and its derivatives have a variety of chemical and biological significances. Thiazole-containing compounds have also been reported to have a wide range of biological activities. Due to the valuable cytotoxic effects of both thiazole and pyridopyrimidinone derivatives, a series of pyridopyrimidinone-thiazole hybrids were synthesized in the present study.
Experimental approach: Briefly, different acyl chlorides were reacted with 2-amino nicotinic acid followed by anhydride acetic to give the corresponding pyridobenzoxazinones. The aminothiazole derivative **G** was also prepared *via* a multistep procedure and incorporated into the benzoxazinones to furnish the target pyridopyrimidinone, **K1-K5**. Furthermore, the cytotoxic activity of the final compounds was determined against MCF-7 and HeLa cell lines using MTT assay.
Findings/Results: The results indicated that aromatic substitution on C2 of pyridopyrimidine nucleus was in favor of cytotoxic activity on both cell lines, of which, compound **K5** bearing a chlorophenyl group showed the highest cytotoxicity.
Conclusion and implications: The results of the present study are valuable in terms of synthesis of hybrid molecules and also cytotoxic evaluations which can be useful for future investigations about the design of novel pyridopyrimidinone-thiazole hybrids possessing better cytotoxic activities.

Keywords: Cytotoxicity; Pyridopyrimidine; Thiazole.

INTRODUCTION

Nowadays, cancer is one of the principal causes of death throughout the world, especially in developed countries. Cancer is generally characterized by the loss of control of cell proliferation leading almost often to death if patients are untreated. Surgery, radiotherapy, and chemotherapy, alone or in combination, are important ways to combat these life-threatening diseases. However, two major drawbacks for chemotherapy are drug resistance and toxicities which encourage medicinal chemists to continuously pursue the design and development of new chemotherapeutics based on well-known scaffolds.

Pyridopyrimidine and its derivatives have a variety of biological significances including antimicrobial (1,2), analgesic (2,3), antiallergic (2,3), antitumor (2,4-7), antihypertensive (2,8), antileishmanial (9), antifolate (2), anti-inflammatory (2,3), diuretic (10), potassium-sparing (2), antifungal (2), and anti-HIV (2) activities.

Thiazole-containing compounds have also been reported to have a wide range of biological activities including antitumor (2,11-13), anti-inflammatory, analgesic, antibacterial (14-16), and antifungal (14,16) effects.

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Thiazole is widely used in anticancer drug design and development. Several thiazole-containing anticancer drugs like bleomycin, tiazofurin, dabrafenib (17), and dasatinib (18) have been reported. Ritonavir (anti-HIV), meloxicam (anti-inflammatory), nizatidine (anti-peptic ulcer), and penicillins (antibiotic) are some other examples of thiazole-bearing products with biological significances (12,14).

Hybridization of two or more pharmacophores into a single molecule is an approach towards the discovery of new compounds with the expectation of additional potentiality to render synergistic effects. Therefore, the introduction of more than one pharmacophore or scaffold in a single molecule, each with a different mechanism of action, can be an effective approach for multi-target drug design, for example in cancer treatment. Hybrid pharmacophores may be attached to different locations in the active sites leading to the elimination of drug resistance. Also, this method can reduce anticancer side effects (12,19,20).

Palbociclib, a new pyridopyrimidine anticancer drug bearing a pyridopyrazine side chain, has been approved in recent years for the treatment of hormone receptor-positive, human epidermal growth factor receptor 2 negative advanced or metastatic breast cancer. Palbociclib is an orally available, highly selective inhibitor of CDK4 and CDK6, serine-threonine kinases that regulate the cell cycle progression (21,22,23).

We have previously reported the synthesis of some 3-(2-(2-phenylthiazol4-yl)ethyl)-quinazoline-4(3H) ones (24) and some 6-nitro derivatives of thiazole-containing 4-(3H)-quinazolinones (12) with potential anticancer activities against a panel of cell lines. Due to the structural similarity of quinazolinone and its isostere pyridopyrimidinone and also the valuable cytotoxic effects of both thiazole and pyridopyrimidinone bearing compounds, in this study, a series of pyridopyrimidinone-thiazole hybrids were synthesized and their cytotoxic activities were determined against MCF-7 and HeLa cell lines using MTT assay.

MATERIALS AND METHODS

Chemicals and Instruments

All starting materials, reagents, and solvents were purchased from commercial suppliers like Merck (Germany) and Aldrich (USA) companies. The purity of the synthesized compounds was proved by thin-layer chromatography (TLC) using various solvents. Merck silica gel 60 F254 plates were applied for analytical TLC. 1HNMR spectra were recorded using a Bruker 400 MHz spectrometer (Germany) and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. The infrared (IR) spectra were obtained on a Shimadzu 470 spectrophotometer (Japan) (potassium bromide disks). Melting points were determined using an electrothermal melting point analyzer apparatus and are uncorrected. MCF-7 (breast cancer) and HeLa (cervical cancer) cell lines were purchased from the Pasteur Institute of Iran (Tehran, I.R. Iran).

Preparation of compounds

3-(2-(2-Phenylthiazol-4-yl)ethyl)pyrido [2,3-d]pyrimidin-4(3H)-one derivatives **K1-K5** were prepared from two separate reactions steps to produce the primary amine 2-(2-phenyl-thiazol-4-yl)-ethylamine **G** and benzoxazinone derivatives **J1-J5,** respectively, as depicted in Fig. 1. The primary amine **G** was synthesized through a five-step route. In the first step, 4-phthalimido-2-butanone **B** was prepared by the addition of methyl vinyl ketone to phthalimide **A** which was brominated in the second step to give 1-bromo-4-N-phthalimido-2-butanone **C**. Nucleophilic substitution of thiobenzamide **E** to the brominated intermediate **C** followed by ring closure furnished the thiazole derivative **F.** The thiazole derivative **F** was reacted with hydrazine hydrate to produce the free amine, 2-phenyl-4-(2-aminoethyl) thiazole **G** (12). The pyridooxazinones **J1-J5** with different substituents at position 2 were synthesized from the reaction of 2-amino nicotinic acid **H** and different acyl chlorides. The reaction of the primary amine **G** with these pyridooxazinones **J1-J5** yielded the final compounds **K1-K5** as explained below.

Details of preparation procedures and chemistry of synthesized compounds

Procedure for the preparation of N-acyl nicotinic acids (I1-I5). Each acyl chloride (0.37 mol) was added in a drop-wise manner to a mixture of compound **H** (0.25 mol) in dimethylformamide (125 mL) at such a rate that the temperature of the mixture did not rise above 40 °C.

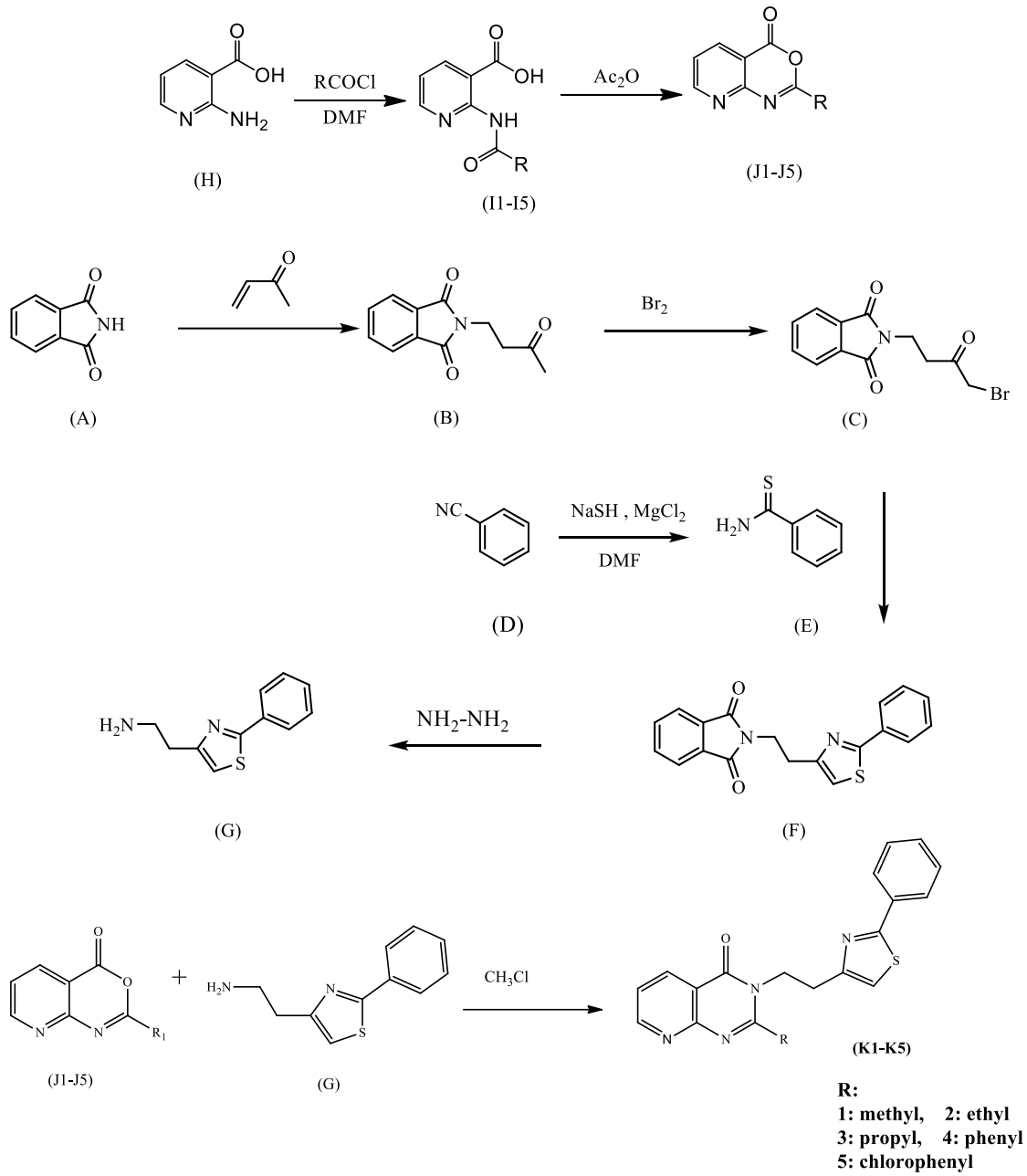


Fig. 1. General reaction scheme for the preparation of target compounds **K1-K5.**

The mixture was stirred at room temperature for at least an additional 3 h. Completion of the reaction was determined by TLC and the mixture was poured into water (1 L) and stirred for 1 h. The precipitated product was collected by filtration, washed with cold water, and dried under reduced pressure yielding **I1-I5** as white or pale yellow powders (60-75%).

Procedure for the preparation of 2-substituted-pyridooxazinone (J1-J5) (25)

Each N-acyl nicotinic acid **I** (0.125 mol) was dissolved in acetic anhydride (90 mL) and, while stirring, slowly heated to 170-180 °C in a round-bottom flask equipped with a claisen-distillation head. Completion of the reaction was confirmed by TLC, and the produced acetic acid was distilled under reduced pressure. The residue was then cooled and the product was washed with n-hexane to give compounds **J1-J5** as pale yellow solids (60-75%) which were used directly for the next step.

Procedure for the preparation of 3-(2-(2-phenylthiazol-4-yl)ethyl)pyrido[2,3-d]pyrimidin-4(3H)-one derivatives (K1-K5)

To prepare 3-(2-(2-phenylthiazol-4-yl)ethyl)pyrido[2,3-d]pyrimidin-4(3H)-one derivatives ***K1-K5***, 0.5 mmol of the

corresponding pyridooxazinones ***J1-J5*** was refluxed with 1 mmol of the free amine **G** in glacial acetic acid (15 mL) for 6-7 h. After completion of the reaction, acetic acid was evaporated under reduced pressure and the residue was purified with preparative TLC to obtain the final products ***K1-K5*** as white or yellowish crystals (25-40%).

Cytotoxicity assay

MCF-7 and HeLa cells were grown in RPMI 1640 medium completed with 5% v/v fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin and maintained at 37 °C in a humidified atmosphere (90%) containing 5% CO_2. The medium was changed every two to three days and sub-cultured when the cell population density reached 70-80% confluence. After 2-3 subcultures, 180 µL of the cell suspensions (5×10^4 cells/mL) were seeded in 96 well plates and incubated for 24 h (26). The stock solutions of the final compounds **K1-K5** (10 mM, 1 mL) were prepared using a minimum volume of dimethyl sulfoxide (DMSO) and serial dilutions appropriately performed by the medium to reach the desired concentrations for MTT assay. After 24 h incubation, 20 µL of different concentrations of final compounds were added as such to have final concentrations (in the wells) of 1, 10, 50, 100, 200 µM for HeLa and 100, 200, 250, 300, 350 µM for MCF7 cells, respectively. Paclitaxel at 1 µM was used as a standard anticancer drug for comparison. The wells containing only the cell suspension and the wells containing the medium alone were regarded as the negative control and the blank, respectively. The microplates were further incubated for 48 h (19,26).

To evaluate cell survival, treated cells were incubated with 20 µL of MTT solution (5 mg/mL in phosphate buffer solution) for 4 h, afterwards, the culture medium was aspirated and 150 µL of DMSO was added and pipetted up and down to dissolve formazan crystals. The absorbance of each well was measured at 540 nm using the enzyme-linked immunosorbent assay (ELISA) plate reader Awareness USA (19,26). Each experiment was performed triplicate and repeated in three different days.

The percentage of cell viability was calculated using the following equation:

$$Cell\ Suurvival\ \% = \frac{\text{MA of the drug treated wells} - \text{MA of the blank}}{\text{MA of the negative control well} - \text{MA of the blank}} \times 100$$

where, MA is mean absorbance. IC_{50} values were calculated by plotting the log10 percent of cell viability against compound concentrations (13).

Statistical analysis

The results were reported as mean ± SD. The IC50 of each compound was determined using the achieved dose-percent of inhibition curve. Analysis of variance (ANOVA) followed by Tukey's posthoc test was used to determine the differences between various groups, *P* values ≤ 0.05 were considered significant.

RESULTS

Chemistry

*2-(2-Phenyl-thiazol-4-yl)-ethylamine (**G**)*

Brownish powder MP: 70-72 °C, yield 68%. ^{1}HNMR (400 MHz-DMSO-d6) δ (ppm): 2.58 (2H, s, NH2), 3.01 (2H, t, *J* = 6.4 Hz, CH1), 3.18 (2H, t, *J* = 6.4 Hz, CH2), 6.99(1H, s, CH4), 7.42-7.48 (3H, m, CH9, CH10, CH11), 7.92-7.98 (2H, m, CH8, CH12).

*2-Methyl-3-(2-(2-phenylthiazol-4-yl)ethyl) pyrido[2,3-d]pyrimidin-4(3H)-one (**K1**)*

White powder MP: 115-117 °C, yield 65%. IR (KBr, cm^{-1}) v_{max} = 3067 (C-H, Ar), 2932 (C-H), 1696 (C=O), 1527 (C=C, Ar). ^{1}HNMR (400 MHz-DMSO-d_6) δ (ppm): 1.18(3H, s, CH20), 3.15 (2H, t, *J* = 7.2 Hz, CH10), 4.04 (2H, t, *J* = 7.2 Hz, CH9), 6.92 (1H, s, CH11), 7.25-7.30 (3H,m, CH16,CH17, CH18), 7.6-7.65 (2H, m, CH15, CH19), 7.68-7.77 (3H, m, CH5, CH6, CH7), IR (KBr, cm^{-1}) v_{max} = 3006 (C-H, Ar), 2922 (C-H), 1684 (C=O), 1527 (C=C, Ar).

*2-Ethyl-3-(2-(2-phenylthiazol-4-yl)ethyl) pyrido[2,3-d]pyrimidin-4(3H)-one (**K2**)*

White powder MP: 118-120 °C, yield 72%. IR (KBr, cm^{-1}) v_{max} = 3047 (C-H, Ar), 2918 (C-H), 1683 (C=O), 1567 (C=C, Ar). ^{1}HNMR (400 MHZ-DMSO-d_6) δ (ppm): 0.89(3H, t,

J = 4.8 Hz, CH21), 1.31 (2H, q, *J* = 5.6 Hz, CH20), 3.25(2H, *t*, *J* = 7.2 Hz, CH10), 4.13(2H, t, *J* = 7.2 Hz, CH9), 7.01 (1H, s, CH11), 7.34-7.40 (3H, m, CH16, CH17, CH18), 7.70-7.75 (2H, m, CH15, CH19), 7.78-7.88 (3H, m, CH5, CH6, CH7).

*2-Propyl-3-(2-(2-phenylthiazol-4-yl)ethyl) pyrido[2,3-d]pyrimidin-4(3H)-one (**K3**)*

Milky color powder MP: 131-133 °C, yield 58%. IR (KBr, cm^{-1}) v_{max} = 3080 (C-H, Ar), 2926 (C-H), 1712 (C=O), 1577 (C=C). ^{1}HNMR (400 MHZ-DMSO-d_6) δ (ppm): 0.09(3H, t, *J* = 6.8 Hz, CH22), 0.88 (2H, hex, *J* = 6.0 Hz, CH21), 1.30 (2H, t, *J* = 6.8 Hz, CH20), 3.25(2H, t, *J* = 7.2 Hz, CH10), 4.13 (2H, t, J = 7.2 Hz, CH9), 7.01 (1H, s, CH11), 7.33-7.40 (3H, m, CH16, CH17, CH18), 7.70-7.75 (2H, m, CH15, CH19), 7.78-7.87 (3H, m, CH5, CH6, CH7).

*2-Phenyl-3-(2-(2-phenylthiazol-4-yl)ethyl) pyrido[2,3-d]pyrimidin-4(3H)-one (**K4**)*

Yellow crystals MP: 162-165 °C, yield 75%. IR (KBr, cm^{-1}) v_{max} = 3100 (C-H, Ar), 2970 (C-H), 1698 (C=O), 1597 (C=C). ^{1}HNMR (400 MHZ-DMSO-d_6) δ (ppm): 3.16(2H, t, *J* = 7.2 Hz, CH10), 4.04(2H, t, *J* = 7.2Hz, CH9), 6.92(1H, s, CH11), 7.25-7.30 (5H, m, CH16, CH17, CH18, CH20, CH24), 7.62-7.64 (3H, m, CH21, CH22, CH23), 7.70-7.78 (5H, m, CH5, CH6, CH7, CH15, CH19).

*2-(4-Chlorophenyl)-3-(2-(2-phenylthiazol-4-yl)ethyl)pyrido[2,3-d]pyrimidin-4(3H)-one (**K5**)*

Pale gray powder MP: 173-175 °C, yield 64%. IR (KBr, cm^{-1}) v_{max} = 3060(C-H, Ar), 2863 (C-H), 1684 (C=O), 1588 (C=C). ^{1}HNMR (400 MHZ-DMSO-d_6) δ (ppm): 3.31(2H, t, *J* = 8.8 Hz, CH10), 3.93 (2H, s, CH9), 6.94 (1H, s, CH11), 7.28-7.34 (5H, m, CH16, CH17, CH18, CH20,CH24), 7.63-7.70 (5H, m, CH5, CH6, CH7 ,CH15, CH19), 7.73-7.76 (2H, m, CH21, CH23).

Cytotoxic effect of synthesized compounds

The results of the MTT assay for evaluation of cytotoxic effects of compounds **K1-K5** are presented in Figs. 2 and 3 on MCF-7 and HeLa cell lines, respectively, which showed significant toxic effects ($P < 0.5$) compared with the negative control group on both cell lines. IC_{50} of the target compounds against MCF-7 and HeLa cell lines are also listed in Table 1.

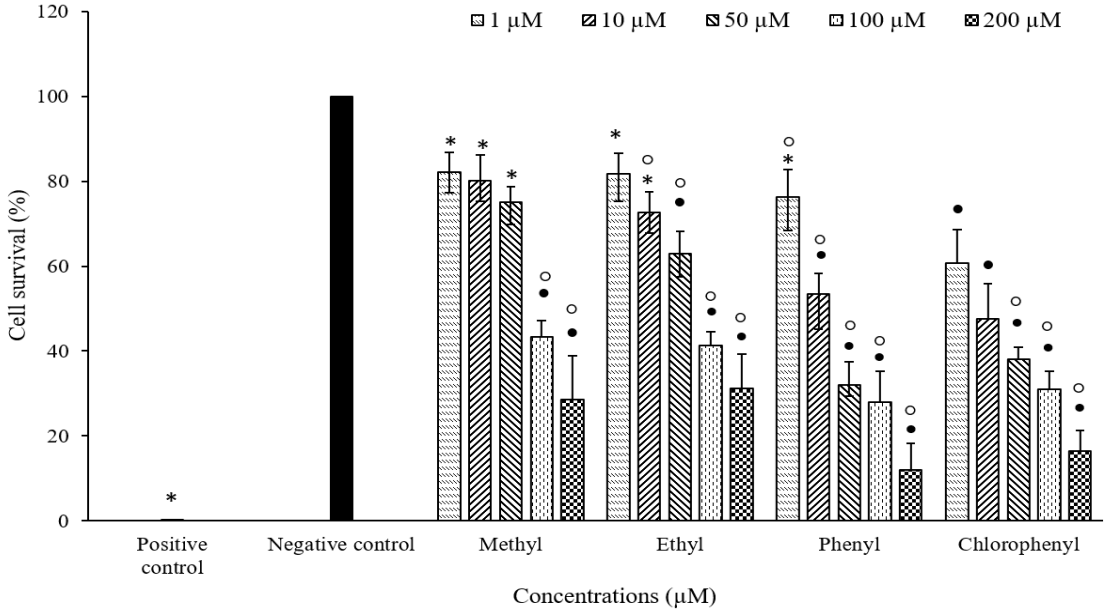


Fig. 2. Cytotoxic effects of compounds **K1-K5** on HeLa cells following exposure to the different concentrations (μM) of compounds. Cell survival was determined using the MTT method. Data are presented as mean ± SD **P* < 0.05 and •*P* < 0.001 indicate significant differences compared to the negative control group; and °*P* < 0.05 versus the lowest concentration of each compound. n = 3 × 3. Paclitaxel at 1 μM used as the positive control.

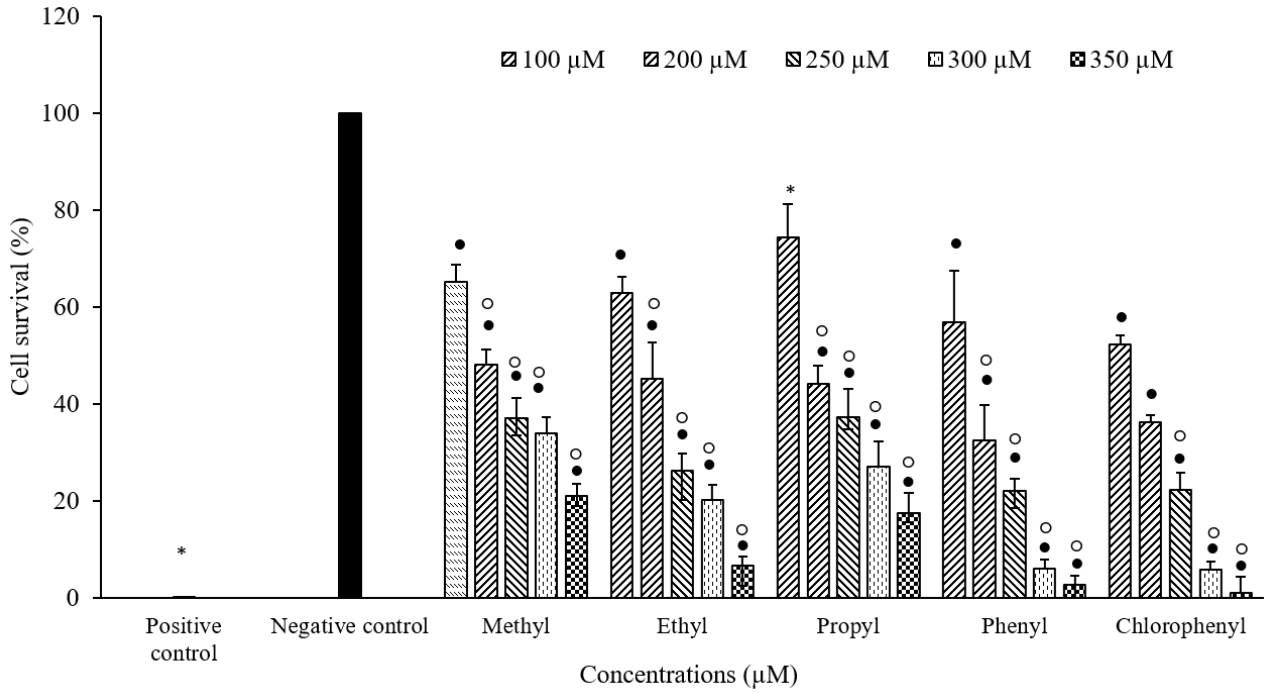
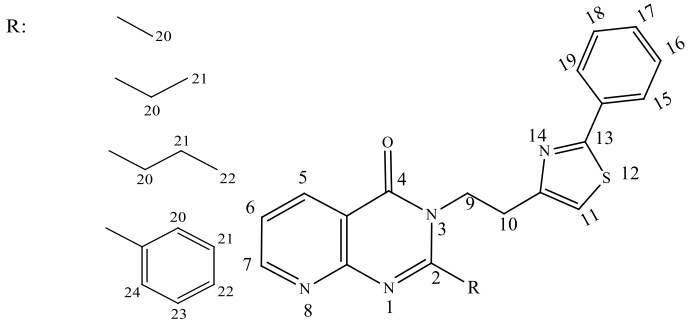


Fig. 3. Cytotoxic effects of compounds **K1-K5** on MCF7 cells following exposure with the different concentrations (µM) of the compounds. Cell survival was determined using the MTT method. Data are presented as mean ± SD **P* < 0.05 and •*P* < 0.001 indicate significant differences compared to the negative control group; and °*P* < 0.05 versus the lowest concentration of each compound. n = 3 × 3. Paclitaxel at 1 µM used as the positive control.

Table 1. The IC_{50} values (µM) of compounds **K1-K5** against MCF-7 and HeLa cell lines using MTT assay.



Target compounds	R	IC_{50} (µM)	
		MCF-7	HeLa
K1	Methyl	188	113
K2	Ethyl	162	105
K3	Propyl	195	157
K4	Phenyl	125	36
K5	4-Chloro phenyl	119	15

DISCUSSION

In this study, some pyridopyrimidines as biologically active scaffolds were conjugated with another well-known moiety (thiazole ring) in a multi-step reaction procedure to produce some interesting novel compounds. Next, all synthesized compounds were tested for their cytotoxic effects on two human carcinoma cell lines, including MCF-7 and HeLa. Different pyridooxazinones were used to prepare pyridopyrimidines. In these reactions, almost any primary amine may be added to pyridooxazinones to achieve overall replacement of the ring-O by the ring-N with the formation of pyridopyrimidines.

Preparation of pyridooxazinones has been reported in several literatures using different

methods. It can be produced *via* one or two-step(s) procedures using nicotinic acid or its derivatives as starting materials in high yields (27,28). As pyridooxazinones are highly reactive they should be used immediately after preparation. In this study, pyridooxazinones were prepared using a two-steps procedure. The final pyridopyrimidines contained 4-ethyl-2-phenylthiazole group on position 3 of their structures.

For the preparation of these pyridopyrimidines, a primary amine-containing thiazole (compound **G**, Fig. 1) was synthesized. The most practical method to prepare thiazoles is the Hantzsch reaction which involves the condensation of α-haloketones and thiourea or thioamides in refluxing alcohol (29). Phthalimide as an NH2-synthon was used here for the preparation of the amine **G**. Application of phthalimide in Gabriel synthesis for the preparation of primary amines is well-documented (33). After alkylation, the resulting alkyl phthalimide is reacted with hydrazine hydrate to give the desired primary amine **G** and phetalazine by-product (30). Finally, the reaction of compound **G** with different pyridooxazinones resulted in the preparation of the new pyridopyrimidines **K1**-**K5**.

By comparison of the 1HNMR spectra of compound **G** and the target compounds **K1**-**K5**, a single peak at 2.58 ppm belonging to the NH_2 group of **G** is observed while after the reaction of **G** with the pyridooxazinones this peak was disappeared in the 1HNMR spectra of the target compounds to confirm that the reaction of the amine and pyridooxazinones was performed. Also, the triplet peaks belonging to the methylene groups of **G** especially the NH_2-CH_2- peak at 3.01 ppm was down fielded to about 4 ppm confirming the insertion of the amine to give electron-withdrawing amide bond in compounds **K1**-**K5**.

The results of cytotoxic evaluation revealed that phenyl- and 4-chlorophenyl- substituted derivatives showed the highest cytotoxic activity against both cell lines, especially the HeLa cells, while aliphatic substituted compounds showed lower activity, particularly on MCF-7 cells. Finally, compound **K5** with 4-chlorophenyl substituent exhibited the highest potency against MCF-7 and HeLa cells with IC_{50}s of 119 μM and 15 μM, respectively.

CONCLUSION

In summary, some novel pyridopyrimidinone-thiazole hybrids were synthesized and their *in vitro*-cytotoxic activities were evaluated against MCF-7 and HeLa cell lines. The results of cytotoxic evaluation represented that compounds with phenyl and 4-chlorophenyl substitutes showed the highest cytotoxic activity against both cell lines, especially the HeLa cells, and finally compound **K5** with 4-chlorophenyl substituent exhibited the highest potency against both cell lines. The results of the present study are valuable in terms of synthesis of hybrid molecules and also cytotoxic evaluations and can be useful for future investigations about the design of novel pyridopyrimidinone-thiazole hybrids possessing better cytotoxic activities.

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Conflict of interest statement

The authors declared no conflicts of interest in this study.

Authors' contribution

M. Rahmani Khajouei and Gh. Khodarahmi contributed to the concept and idea development. The experimental studies were performed by A. Ghaderi and supervised by M. Rahmani Khajouei and Gh. Khodarahmi. Data analysis, manuscript preparation, editing and review were conducted by M. Rahmani Khajouei and Gh. Khodarahmi.

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