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SYNTHESIS AND ANTI-CANCER ACTIVITY OF 3-CHLORO-N-PHENYLBENZAMIDE

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Abstract: Targeted chemotherapy is one of the popular cancer treatments. Many studies had shown that enzyme IKKβ is one of the key elements which essential for activation of cell proliferation in the NF-κB signaling pathways. Therefore, this enzyme was utilized as target for activity inhibition. The 3-Chloro-*N*-phenylbenzamide, an aryl amide compound, was designed and synthesized as a small molecule aimed to act as IKKβ inhibitor and possess anti-proliferative activity. The compound was simulated and docking with IKKβ enzyme by computer-aided process to evaluate for its potential therapeutic properties. The structure of this compound was characterized by spectroscopic methods. The results from infrared spectrum showed a singlet peak of secondary amine at wavenumber 3,352 cm^{-1} and a carbonyl amide peak at wavenumber 1,659 cm^{-1}. The ^1H Nuclear Magnetic Resonance spectrum showed two distinct regions of 10 protons; the –NH peak at 10.37 ppm and another nine aromatic protons in the range of 7.10 – 8.03 ppm. The fragmentation pattern from mass spectrum showed the remarkable base peak at m/z 135.2 which is the typical of benzoyl fragment peak with the molecular ion peak also corresponding with the molecular weight of compound which equals to 231. An MTT assay indicated the ability of the synthesized compound that can inhibit the growth of cervical cancer cell line (SiHa) with an IC_{50} of 22.4 µM. The result emphasized the possibility of targeted cancer therapy of this compound.

Keywords: Aryl amide, Anticancer, Targeted chemotherapy, Benzamide, IKKβ inhibitor

INTRODUCTION

Chemotherapy is one of the important approaches used to treat cancer, however, anticancer drugs in current clinical use generally possess excessive organ toxicity, lack of cell specificity, short circulation half-life, and some may induce resistance in the target cells. Therefore, there is still need to develop a safe and effective drug for cancer patients (World Cancer Report, 2014; Plummer *et al*., 2016; Camidge, 2014; National Cancer Institute, 2016).

In an attempt to develop the targeted anticancer drug, we have chosen IKKβ as our targeted molecule since it evidenced that this enzyme significantly involved in cell apoptosis through the NF-Kβ pathway (Laurent *et al*., 2007; Tanaka *et al*., 2006; Miller *et al*., 2010; Gamble *et al*., 2012; Jost *et al*., 2007; Kanduri *et al*., 2011; Florian *et al*., 2007; Yanting *et al*., 2016; Sawada *et al*., 2016). This pathway can be rapidly and transiently activated by a wide variety of substances, such as mitogens, cytokines, and microbial components. NF-κB activation is dependent on a specific IκB kinase (IKK) complex composed of two catalytic subunits, IKKα (IKK1) and IKKβ (IKK2), and the regulatory subunit NF-κB essential modulator (NEMO/IKKγ). Upon activation, IKK phosphorylates specific serine residues in IκB proteins, triggering their ubiquitination and degradation by the proteasome, thus allowing the NF-κB dimers to translocate to the nucleus to regulate gene expression.

IKK is a crucial protein kinase that activates NF-κB translocating from cytoplasm to nucleus for DNA binding. Therefore, inhibition of NF-κB signaling has been considered a promising way to overcome tumor cell-intrinsic resistance to killing by cytotoxic agents. In fact, pharmacologic inhibitors of IKK were found to increase the apoptotic sensitivity of melanoma and other chemoresistant tumor cells to doxorubicin treatment (Miller *et al*., 2010; Jost *et al*., 2007; Kanduri *et al*., 2011; Florian *et al*., 2007; Yanting *et al*., 2016).

In searching for aryl amide as our lead compound, we came across with IMD 0354, a small molecule reported as IKKβ inhibitor, which even though possessed good activity but still toxic (Yanting *et al*., 2016; Sawada *et al*., 2016; Tanaka *et al*., 2006; Abdelaziz *et al*., 2015; El-Hashash *et al*., 2018). Therefore, we choose this compound as our lead to design the desired compound using molecular modeling process in choosing the appropriate structure. In this study, we choose 3-Chloro-*N*-phenylbenzamide as our targeted compound in order to investigate the influences of hydroxyl group together with the other ring substituents.

MATERIALS AND METHODS

Cells and drugs

The human SiHa cervical cell line was purchased from ATCC (Manassas, VA). Doxorubicin was purchased from LC Laboratories (Woburn, MA).

Melting points were measured using Differential Scanning Calorimeter, DSC 204 F1 Phoenix®, (Netzsch, USA). Infrared measurements (KBr disc) was carried out using Thermo Spectra-Tech, P/N 700-0085, Ver3.9 10/01 spectrophotometer (Shelton, USA). ^{1}H-NMR and ^{13}C-NMR experiments were carried out using Bruker AVF-400 (Bruker, Karlsruhe, Germany). Chemical shifts (δH) are reported relative to TMS as the internal standard. High-resolution mass spectra was recorded using a GC-MS/MS TQ, 7890GC/ 7000 GC/MS triple Quad (Agilent technologies, USA). Analytical thin layer chromatography (TLC) (Merck KGaA, Darmstadt, Germany) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products. Aniline, 3-chlorobenzoyl chloride and oxalyl chloride were purchased from Sigma-Aldrich, USA. All other reagents and solvents were AR grade and were purified and dried by standard techniques.

Synthesis of Aryl Amide

A suspension of acid (5.14 mmol) in toluene (15 ml) at 0^{o}C was treated with oxalyl chloride (0.64 ml, 7.20 mmol) followed by 3 drops of dimethylformamide. The reaction mixture was stirred at room temperature under N_2 atmosphere for 20 minutes after that it was raised to reflux for 1.5 hours still under N_2 atmosphere. The solvent was removed and the mixture was concentrated in vacuo. The resulting solids were again dissolved in dichloromethane (5 mL) and treated with the mixture of amine (0.96 ml, 7.71 mmol) and triethylamine (0.72 ml, 5.14 mmol) in dichloromethane (10 mL) and stirred overnight under N_2 atmosphere. The mixture was diluted with dichloromethane (100 mL), washed with 1 M NaOH (30 mL), then water (100 mL x 3). The organic layer was dried with anhydrous $MgSO_4$ and solvent was removed with rotary evaporator to obtained crude product. The crude product was purified by column chromatography with silica and used 7:3 hexane: dicholoromethane as eluent. The product was obtained as a white solid (92 % yield).

3-Chloro-N-phenylbenzamide. White crystals (yield 92%), m.p. (DSC) 141.7 °C; IR (KBr, ν cm^{-1}) 3,352 (NH), 1,659 (C=O); ^{1}H-NMR (DMDO-d6) δ ppm: 7.13 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.37 (t, 2H, *J* = 7.6 Hz, Ar-H), 7.81(d, 2H, *J* = 7.6 Hz, Ar-H), 7.94 (s, 1H, *J* = 7.6 Hz, Ar-H), 7.56 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.66 (d, 1H, *J* = 8.0 Hz, Ar-H), 8.03 (s, 1H, Ar-H), 10.37(s, 1H, NH)); ^{13}C-NMR (DMSO-d6) δ ppm: 120.94, 124.38, 126.94, 127.91, 129.10,

130.82, 131.82, 133.71, 137.41, 139.37, 164.52 (C=O); HRMS (ESI) m/z calcd for [M + H]+ ($C_{13}H_1ClNO$): 231.68, 233.04, found: 231.1, 233.1, 139.1, 111.0; Anal. Calcd. for $C_{13}H_1ClNO$ (231.1): C, 67.40; H, 4.35; N, 6.05; found C, 67.44; H, 4.29; N, 6.06.

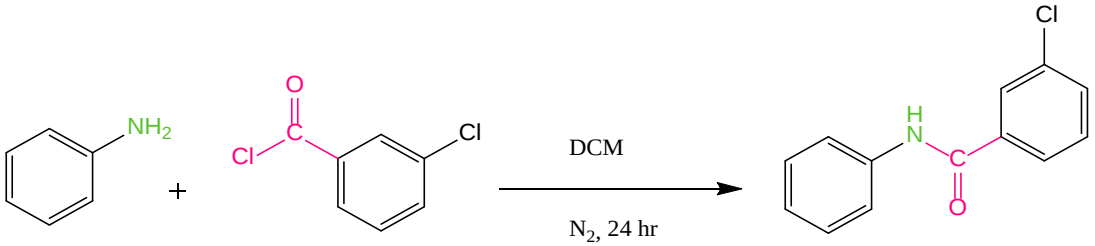
Viability assay (MTT)

The in vitro anti-proliferative activity of the newly prepared aryl amide was examined against human cervical cancer cell line (SiHa). The assay was carried out, as triplicates, utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. The doxorubicin was also included in this study as a reference drug. The results were expressed as median growth inhibitory concentration (IC_{50}) values that represent the compound concentrations required to afford a 50% inhibition of cell growth after 48 and 72 h of incubation, compared to the untreated controls (Table 1).

RESULTS AND DISCUSSION

Chemistry

The target aryl amide (choosen from modeling parameter, Fig. 1) was synthesized by amidation reaction. The one-step synthesis was carried out using aniline and 3-chlorobenzoyl chloride as starting materials. The mixture solution was run in dry dichloromethane and under nitrogen for 24 hours to give the desired compound as white powder with 92 % yield.



Scheme I. The synthetic reaction of 3-Chloro-*N*-phenylbenzamide

The postulated structure of the newly synthesized aryl amide was determined via spectroscopic methods and is in full agreement with its spectral and elemental analyses data (Figures 2-8).

Biological Evaluation

MTT assay was utilized to examine the in vitro anti-proliferative activity of the newly prepared aryl amide compound with the human cervical cancer cell line (SiHa). The 50% inhibitory concentration (IC_{50}) was estimated, after 48 and 72 h for SiHa cell, from graphic plots of the dose response curve for each conc. using SoftMax Pro 6.3 software (Molecular Devices, LLC, San Jose, California, United States). The data presented are the mean of at least three separate experiments (Figure 9).

Table 1. IC_{50} values (μM) for 3-Chloro-*N*-phenylbenzamide and doxorubicin

Compound	IC_{50} (μM)
3-Chloro-*N*-phenylbenzamide	22.4
Doxorubicin	0.9

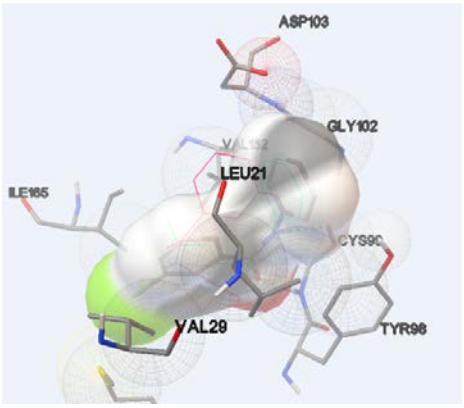


Figure 1. Docking of 3-Chloro-*N*-phenylbenzamide with 4KIK

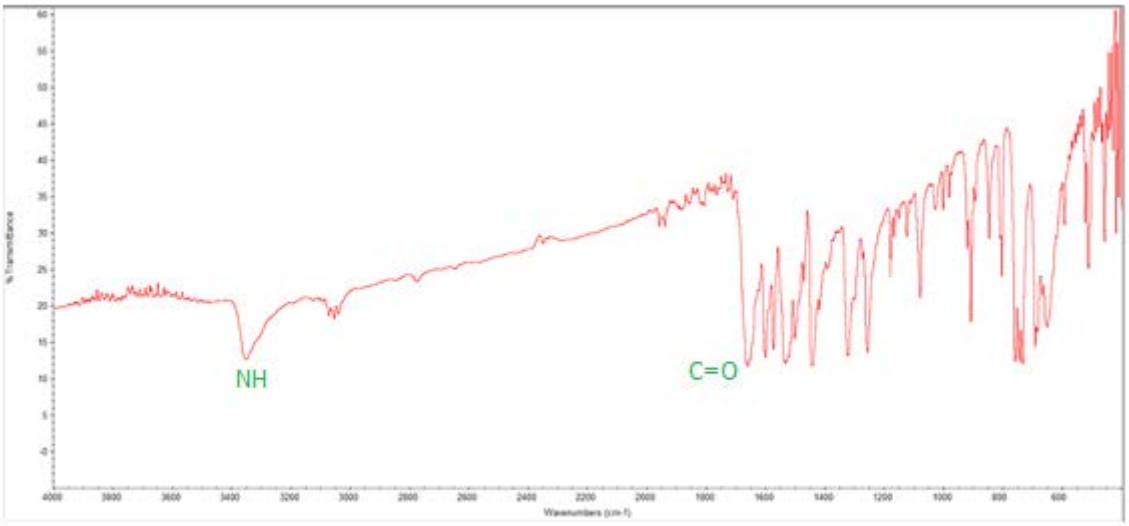


Figure 2. Infrared spectrum of 3-Chloro-*N*-phenylbenzamide (KBr)

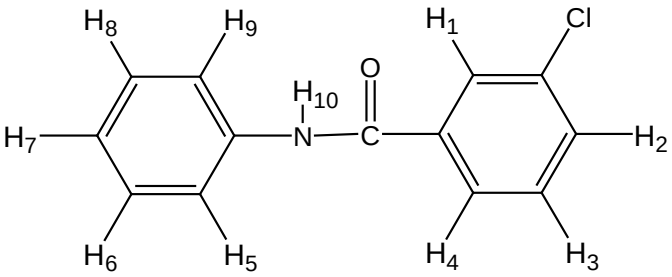


Figure 3. Assigned protons for ^{1}H NMR spectrum of 3-Chloro-*N*-Phenylbenzamide

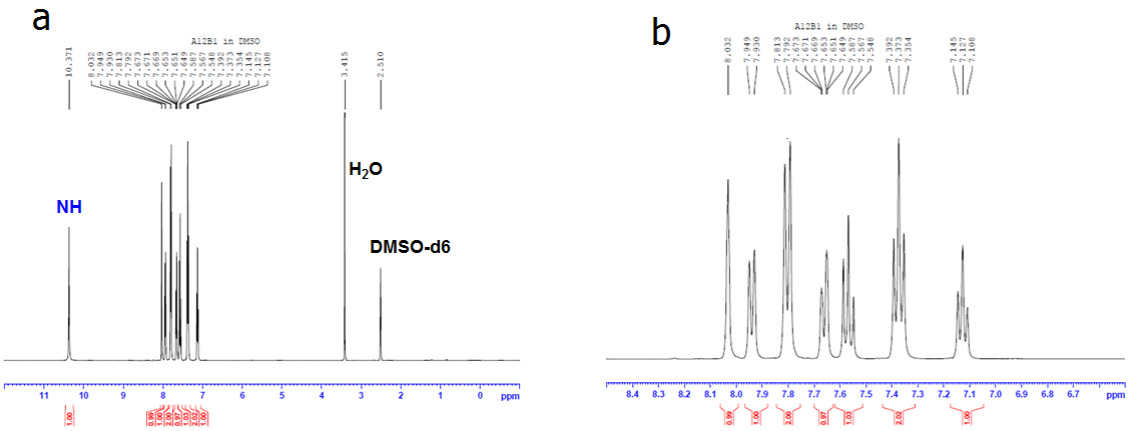


Figure 4. **(a)** ^{1}H NMR spectrum of 3-Chloro-*N*-phenylbenzamide
(b) Expanded ^{1}H NMR spectrum of Aromatic region of 3-Chloro-*N*-phenylbenzamide

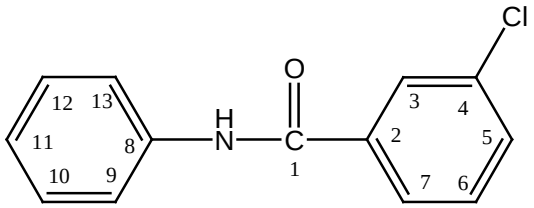


Figure 5. Assigned protons for ^{13}C NMR spectrum of 3-Chloro-*N*-Phenylbenzamide

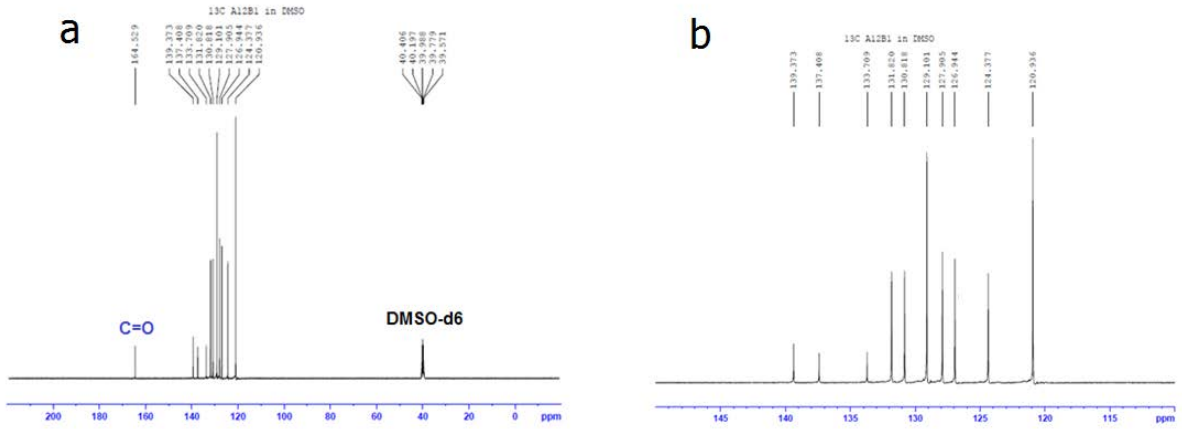


Figure 6. **(a)** ^{13}C NMR spectrum of 3-Chloro-*N*-phenylbenzamide.
(b) Expanded ^{13}C NMR spectrum of Aromatic region of 3-Chloro-*N*-phenylbenzamide

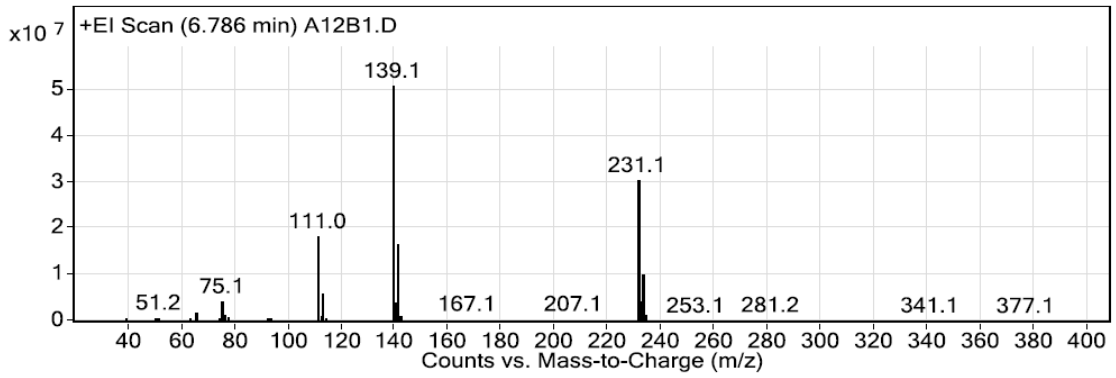


Figure 7. Mass spectrum of 3-Chloro-*N*-phenylbenzamide

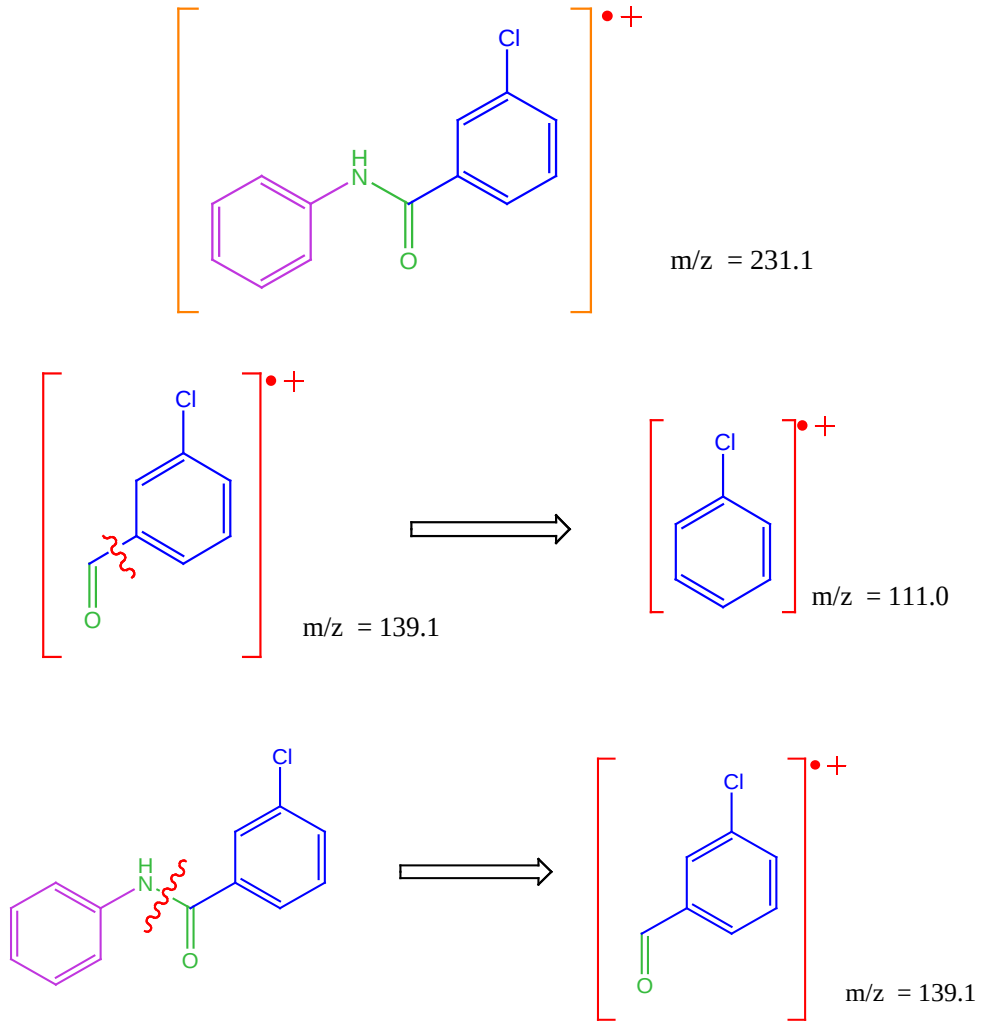


Figure 8. Fragmentation pattern of 3-Chloro-*N*-phenylbenzamide

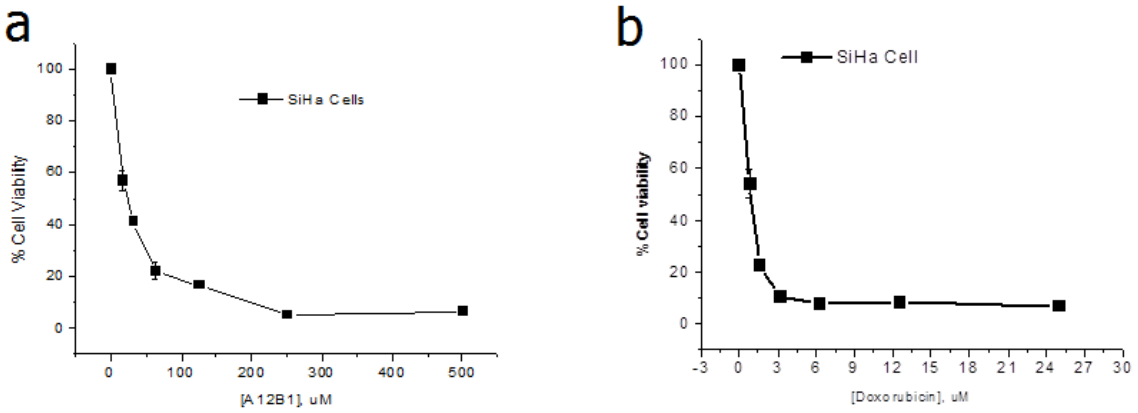


Figure 9. The efficacy of 3-Chloro-*N*-phenylbenzamide **(a)** and Doxorubicin **(b)** on inhibition of SiHa cell growth

CONCLUSION

The synthesis of a novel aryl amide compound; 3-Chloro-*N*-phenylbenzamide was achieved in one-pot amidation reaction by converting the carboxylic acid compound to acyl chloride followed with the addition of amine compound without any separation. Column chromatography was applied to purified the desired compound using 7:3 hexane: dichloromethane as eluent to give the purified compound as white solid with melting point of 141.7 ^{o}C determined by Differential Scanning Calorimetry (DSC). The structure of newly synthesized compound was characterized by Infrared (IR), Nuclear Magnetic Resonance (NMR), Mass Spectroscopic Methods and Elemental analysis and all of the results were in agreement with its proposed structure. The compound was then evaluated for its anti-proliferative activity towards the cervical cancer SiHa cell line and possessed an IC_{50} of 22.4 µM. using doxorubicin as reference. The results of our research indicate that the synthesized compound represents a particularly challenging target which can be regarded as the promising drug candidates for development of anticancer targeted therapy.

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