Journal of Engineering and Applied Sciences 11 (4): 800-804, 2016
ISSN: 1816-949X

Nano Studies on a Synthesized Dihydroimidazo[2,1-a]isoquinoline Derivative and Evaluation of its Cytotoxicity Properties on Human Breast Cancer T-47D Cell Lines

Marzieh Dorvar and Samira Arab-Salmanabadi
Department of Chemistry, Shahr-e-Qods Branch, Islamic Azad University,Tehran, Iran

Abstract: In the present research, nano studies on synthesised Dihydroimidazo[2,1-a]isoquinoline derivative named(Z)-methyl2-(1-(benzo[d]thiazol-2-yl)-2oxo-1,2-dihydroimidazo[2,1-a]isoquinolin-3(10bH)-ylidene)acetate are described. Determination of particles size was suitably characterized by means of X-ray Diffraction patterns (XRD) and Scanning Electron Microscopy (SEM). In addition, anticancer activity on human breast cancer T-47D cell lines was investigated.

Key words: Nano research, cytotoxicity, MTT assay, Dihydroimidazo [2,1-a]isoquinoline, characterization

INTRODUCTION

Nanotechnology and nanoparticle synthesis has been commonly focused by researchers worldwide (Shafaei-Ghomi and Ghasemzadeh, 2015; Palaniappan *et al*., 2015; Liu *et al*., 2016). MCR.s (Multi-Component Reactions) are appropriate methods to achieve novel nano-structured molecules (Zolfigola *et al*., 2015). MCRs were created as effective methods for synthesis of complex molecules (Pollegatti *et al*., 2011). The reaction is known to produce complex, diverse molecules in a one-pot condition. MCRs are considered as special methods in 'Diversity Oriented Synthesis' (DOS) and 'Biology-Oriented Synthesis' (BIOS) design strategies for achieving higher degree of scaffold diversification (Shinde *et al*., 2014). However, processing multi-component fine powders are extremely challenging and usually results in a non-homogeneous multiphase compound (Vasylkiv *et al*., 2007). One of the most important reactions at this type is synthesis with N-heterocycles compounds. N-heterocycles are special group of heterocyclic compounds because of their biological properties (Adib *et al*., 2011; Verma *et al*., 2013).

Dihydroimidazoisoquinolines are fused compounds that have been well-recognized for their pharmacological and biological activities such as antiulcer, hypnotic, anticonvulsant, sedative, antihypertensive, vasodilator, anti-inflammatory, anti-biosis and fibrinogen receptor antagonists and many other biological activities (Chang *et al*., 2012; Li *et al*., 2009; Norris *et al*., 2001; Zhang *et al*., 2007). For example, benz-imidazo[2,1-a]isoquinolines and imidazoquinoxalines have potential anticancer activities (Maleki and Rezayan, 2014).

Cancer has become the second cause of mortality in the world. Thus of potent and specific anticancer agents is urgently needed, not only against cancer but also against problems like severe toxicity as well as resistance to the existing drugs. Millions of organic chemical compounds are synthesized, hundreds of thousands of which have been tested to find new prospective leads for different pharmacy therapeutic areas (Arab-Salmanabadi *et al*., 2014). Diagnosis and treatment of cancer has been arguably the fastest developing area of modern day biomedical research. Selectivity of anticancer drugs towards tumour cells over normal cells is a key factor for achieving therapeutic efficacy and highly potent tubulises may turn out to be extremely effective tools in this regard (Shankar *et al*., 2013). Breast cancer is a leading cause of morbidity and mortality worldwide with over a million cases yearly (El-Ansary *et al*., 2014). In our previous research (Arab-Salmanabadi *et al*., 2015), we synthesised a new Dihydroimidazo[2,1-a]isoquinoline Derivative named (Z)-methy l2-(1-(benzo [d] thiazol-2-yl)-2oxo-1,2-dihydroimidazo[2,1-a]isoquinolin-3(10bH)-ylidene)acetate) 4 via a multicomponent reaction (Fig. 1). In the present research, Nano studies on this novel compound 4 are described. Determination the particles size was suitably characterized by using of XRD and SEM analyses. In addition anticancer activity on human breast cancer T-47D cell lines was investigated.

Corresponding Author: Samira Arab-Salmanabadi, Department of Chemistry, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran

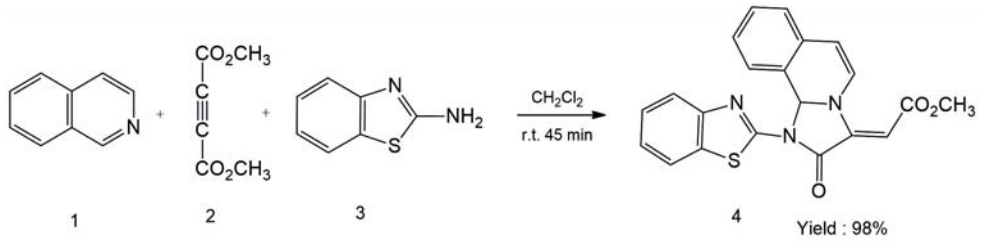

Fig. 1: Syenthesis of compound 4

MATERIALS AND METHODS

Compound 4 was prepared by known method (Arab-Salmanabadi *et al*., 2015) and other Chemicals were purchased from Merck and Fluka.

The powders were performed with a STOE theta-theta (XRD). Then they were characterized by scanning electron microscopy (KYKY-em3200). Samples were coated with gold at 10 mA for 2 min prior to SEM analysis. Spectral data (IR, ^{1}H-NMR, ^{13}C-NMR, Mass Spectroscopy, elemental analysis and X-ray analysis) of the synthesized compound 4 was given in our previous research (Arab-Salmanabadi *et al*., 2015). Cytotoxicity was calculated based on MTT assay.

To a mixture of isoquinoline 1 and dialky lacetylenedicarboxylates 2 in CH_2Cl_2, 2-aminobenzothiazole 3 was added at room temperature. The reaction was complete over 45 min to produce compound 4 in excellent yields (Arab-Salmanabadi *et al*., 2015).

RESULTS AND DISCUSSION

Chemistry: Our new synthetic method leading to the formation of the title compound is given in Fig. 1. The reaction between isoquinoline 1, dialkylacetylenedicarboxylates 2 and 2-aminobenzothiazole 3 leads to compound 4 (Arab-Salmanabadi *et al*., 2015).

The structure of compound 4 was characterized from its elemental analysis, IR and high-field ^{1}H and ^{13}CNMR spectra and clearly indicated the formation of synthesized this compound (Arab-Salmanabadi *et al*., 2015).

Characterization of z)-methyl 2-(1-(benzo [d] thiazol-2-yl)-2-oxo-1,2-dihydroimidazo[2,1-a]isoquinolin-3(10bh)ylidene)acetate nanoparticles: In Fig. 2, the indexed X-ray diffraction (XRD) pattern of compound 4 is shown. The length breadth of the Bragg peak depends on both instrument and sample dependent effects. In order to reduce these contributions, a diffraction pattern from the line broadening of standard material could be collected. Equation 1 is applied to estimate the instrument corrected broadening β corresponding to the diffraction peak of compound 4 nanoparticles (Eq. 1):

$$\beta = \beta_1 - \beta_2 \quad (1)$$

Using Debye-Scherrer equation (Eq. 2), crystallite sizes (D_c) of compound 4 nanoparticles were estimated (Eq. 2):

$$D_c = K\lambda / \beta\cos\theta \quad (2)$$

θ is Bragg angle of diffraction peak (in radians), K is the so-called shape factor that its value is normally 0.9, also λ represents X-ray wavelength and D is the size of the crystal particles, β (peak width at half maximum intensity, in radians or FWHM) is diffraction peak broadening correction of Cu1-X$Co_xFe_2O_4$.

The X-ray powder diffraction data of compound 4 nanoparticles for most intense reflection, that are shown. The sharp diffraction peak located at 2θ = 25.79° is chosen to calculate the crystallite size. The results agreed favourably with the calculated values and the estimated average crystallite sizes of compound 4 by Debye Scherrer equation were obtained to be 67.9 nm.

Surface morphology of the compound 4 nanoparticles was investigated by Scanning Electron Microscopy (SEM) images in Fig. 3. The grain micro-structure of the nanoparticles was seen by SEM micrographs. These micrographs provide a better view of the grain development and grain sizes. The particle size and external morphology of the fine calcined powders were obtained by SEM micrographs of compound 4 nanoparticles that are shown in Fig. 3. The grain average sizes measured of compound 4 nanoparticles are 28.53 nm.

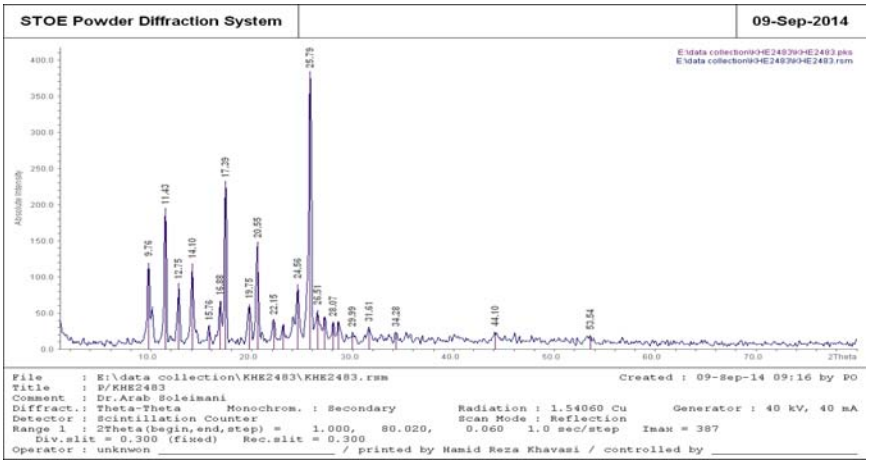

Fig. 2: X-ray powder diffraction data of Z)-Methyl 2-(1-(benzo[d]thiazol-2-yl)-2-oxo-1,2-dihydroimidazo[2,1-a]isoquinolin-3(10bH)-ylidene)acetate nanoparticles for most intense reflection

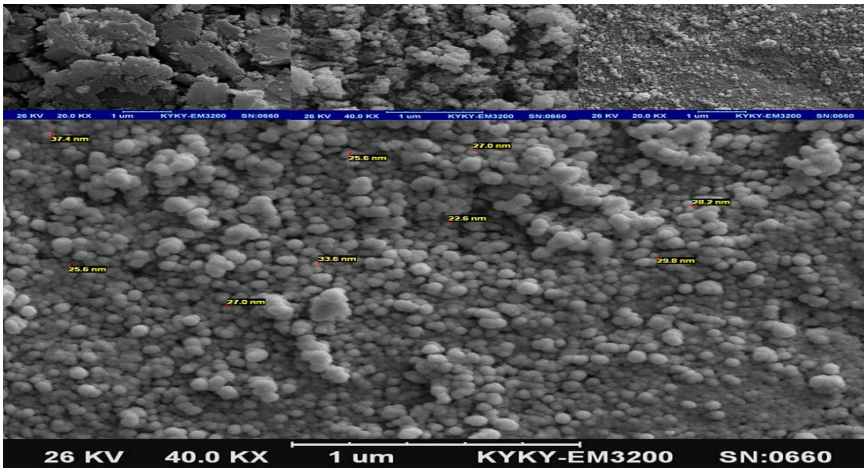

Fig. 3: SEM images of Z)-Methyl 2(1(benzo[d]thiazol-2-yl)-2-oxo-1, 2-dihydroimidazo [2,1-a]isoquinolin 3(10bH)-ylidene) acetate nanoparticles

There probably are some large particles represent to synthesis the aggregation of nanoparticles compounds due to the high level of energy and the surface tension of the nanoparticles.

Biological activity

Assessment of cytotoxic activity by cell viability assay (MTT): The cytotoxic on T-47D cells was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on 96 well plates. Cells at dilution of 1×10^4 at each well were cultured in DMEM medium containing 10% calf fetal serum and 1% penicillin/ streptomycin under 10% CO_2 at 37°C. After 24 h of the cells growth, the supernatant is taken away, afterwards the cells were treated at different concentrations (1.5, 0.75, 0.375, 0.187 μg μ^{-1}) of compound 4. After 48 h of incubation, half of them was removed, in each well 100 μL of MTT reagent was added. They were further incubated for 3 h. The MTT reagent was removed from each well. The formed crystalline formazan was solved with 200 μL of 100% isopropanol. The absorption was read in Elisa reader (BioTek) at 570 nm. Results were the mean values from at least three different experiments in triplicate. Inhibitory concentration of compound 4 was determined by Pharm Software.

The MTT assay is a colorimetric assay for evaluating cell viability. Tetrazolium dye reduction is dependent on NAD(P)H-dependent oxidoreductase enzymes under defined conditions, it reflects the number of viable cells present. These enzymes are

Table 1: Cytotoxic activity of synthesized compound against T-47D cell line at various concentrations

Imidazoisoquinolin concentration	1.5 μg μL^{-1}	0.75 μg μL^{-1}	0.375 μg μL^{-1}	0.187 μg μL^{-1}
Mean OD (570 nm) (Triplicate) for imidazoisoquinolin	0.193	0.478	0.516	0.568
OD 570 nm mean (Triplicate) control	0.602	0.602	0.602	0.602

Cytotoxicity % = (1-mean OD test/mean OD control)×100

capable of reducing the yellow tetrazolium dye to its insoluble purple formazan in living cells. The XTT, MTS and the WSTs are closely related to tetrazolium. They're connected with the intermediate electron acceptor, 1-methoxy PMS. With WST which is cell-impermeable, plasma membrance electron transport causes reduction of outside the cell. Tetrazolium dye examinations can also be used to measure cytotoxicity (destruction of viable cells) or cytostatic activity (shift from proliferative to resting status) of potential medicinal factors and toxic materials.

The half maximal Inhibitory Concentration (IC_{50}) is an index of the effectiveness of a substance in inhibiting a specific biological or biochemical function. IC_{50} shows the number of a particular drug or other substance (inhibitor) which needed to inhibit a given biological process (or component of a process, i.e., an enzyme, cell, cell receptor or microorganism) by half. It is commonly used as a measure of antagonist drug potency in pharmacological research. According to the FDA, IC_{50} illustrates the concentration of a drug that is necessary for 50% inhibition in vitro. It resembles EC_{50} for agonist drugs. EC50 also shows the plasma concentration required for obtaining 50% of a maximum effect *in vivo* (Table 1).

CONCLUSION

In this research, we have developed a rapid and efficient method for synthesizing a nano medicinal compound that indicates good cytotoxicity. This synthesis method involves several advantages including the simplicity of performance, good yields of product and relatively short reaction time. The synthesized compound can be used as a template for future development through medication to design more potent and selective cytotoxic agent. Also the synthesized compound was also screened for its size in Nano-meter range.

ACKNOWLEDGEMENTS

The researchers gratefully acknowledge the Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran for financial support. Also, Nano biotechnology Department, Pasteur Institute of Iran is gratefully acknowledged for providing necessary research of biological studies.

REFERENCES

Adib, M., B. Mohammadi, S. Ansari, H.R. Bijanzadeh and L.G. Zhu, 2011. Solvent-free reaction between acenaphthoquinone, various benzils and ammonium acetate: Synthesis of 9, 10-diaryl-7H-benzo[d, e]imidazo[2, 1-a] isoquinolin-7-ones. Tetrahedron Lett., 52: 2299-2301.

Arab-Salmanabadi, S., M. Dorvar and B. Notash, 2015. Synthesis of novel functionalized dihydroimidazo[2, 1-a]isoquinolines and dihydroimidazo[2, 1-a] quinolines: Single crystal X-ray studies of (Z)-methyl 2-(1-(benzo[d]thiazol-2-yl)-2-oxo-1,2-dihydroimidazo [2, 1-a] isoquinolin-3(10bH)-ylidene) acetate. Tetrahedron Lett., 71: 1292-1296.

Arab-Salmanabadi, S., M.H. Farjam and M. Chiani, 2014. Characterization, cytotoxicity and antimicrobial studies of (Z)-2-(3-(butylamino)-4-oxopent-2-en-2-yl)-2-hydroxy-1H-indene 1, 3 (2H)-dione. Adv. Environ. Biol., 8: 115-120.

Chang, M.Y., M.H. Wu and Y.L. Chen, 2012. Synthesis of dihydrobenzoimidazo[2, 1-a]isoquinolines. Tetrahedron Lett., 53: 4156-4160.

El-Ansary, A.K., A.M. Kamal and M.A. Al-Ghorafi, 2014. Synthesis and evaluation of 4-anilinoquinazoline bioisosteres as potential anti-breast cancer agents. Eur. J. Med. Chem., 86: 202-210.

Li, G., R. Kakarla, S.W. Gerritz, A. Pendri and B. Ma, 2009. A facile one-step synthesis of 5-chloro-imidazo[1, 5-a]quinazoline by microwave irradiation. Tetrahedron Lett., 50: 6048-6052.

Liu, D., D. Dong, Y. He, J. Liu and B. Liu, 2016. One-step synthesis of C 60 nano-assemblies at different temperatures. Mater. Design, 93: 343-346.

Maleki, A. and A.H. Rezayan, 2014. Synthesis of pyrido [20,10:2,3]imidazo[4,5-c]iso-quinolines via a one-pot, three-component reaction. Tetrahedron Lett., 55: 1848-1850.

Norris, D., P. Chen, J.C. Barrish, J. Das, R. Moquin, B.C. Chen and P. Guo, 2001. Synthesis of imidazo[1, 5-a] quinoxalin-4 (5H)-one template via a novel intramolecular cyclization process. Tetrahedron Lett., 42: 4297-4299.

Palaniappan, P., G. Sathishkumar and R. Sankar, 2015. Fabrication of nano-silver particles using Cymodocea serrulata and its cytotoxicity effect against human lung cancer A549 cells line. Spectrochimica Acta Part A. Mol. Biomolecular Spectroscopy, 138: 885-890.

Pellegatti, L., E. Vedrenne, M.A. Hiebel, F. Buron and S. Massip *et al*., 2011. Synthesis of [1,2,4,5]tetrazino [60,10:2,3]imidazo[4,5-c]isoquinolin-5-ones by microwave-assisted three-component reaction. Tetrahedron Lett., 52: 5224-5228.

Safaei-Ghomi, J. and M.A. Ghasemzadeh, 2015. An efficient multi-component synthesis of 14-aryl-14H-dibenzo [a, j] xanthene derivatives by AgI nanoparticles. J. Saudi Chem. Soc., 19: 642-649.

Shankar, P.S., S. Bigotti, P. Lazzari, I. Manca, M. Spiga, M. Sani and M. Zanda, 2013. Synthesis and cytotoxicity evaluation of diastereoisomers and N-terminal analogues of tubulysin-U. Tetrahedron Lett., 54: 6137-6141.

Shinde, A.H., M. Srilaxmi, B. Satpathi and D.S. Sharada, 2014. A highly efficient synthesis of imidazo-fused polyheterocycles via Groebke-Blackburn-Bienayme reaction catalyzed by $LaCl_3.7H_2O$. Tetrahedron Lett., 55: 5915-5920.

Vasylkiv, O., Y. Sakka and V.V. Skorokhod, 2007. Nano-explosion synthesis of multi-component ceramic nano-composites. J. Eur. Ceramic Soc., 27: 585-592.

Verma, A.K., R.R. Jha, V.K. Sankar and R.P. Singh, 2013. Selective synthesis of 4, 5-dihydroimidazo-and imidazo [1, 5-a] quinoxalines via modified Pictet-Spengler reaction. Tetrahedron Lett., 54: 5984-5990.

Zhang, Y., Q. Zhou, R.A. Houghten and Y. Yongping, 2007. Solid-phase synthesis of 3-alkyl-8-arylamino-1H-imidazo[4, 5-g]quinazolin-2(3H)-thiones and 3-alkyl-8-arylamino-1H-imidazo[4, 5-g]quinazolin-2(3H)-ones. Tetrahedron Lett., 48: 7042-7045.

Zolfigola, M.A., S. Baghery, A.R. Moosavi-Zare and S.M. Vandat, 2015. Synthesis and characterization of new 1-(α-aminoalkyl)-2-naphthols using pyrazine-1, 4-diium trinitromethanide {[1, 4-DHPyrazine][$C(NO_2)_3$]$_2$} as a novel nano-structured molten salt and catalyst in compared with Ag–TiO_2 nano composite. J. Mol. Catalysis A-Chem., 409: 216-226.