**Synthesis of Novel Phthalimido Oxime Esters and Evaluation of Their Cytotoxicity**

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*Abstract*: A series of novel optically pure oxime esters derivatives were synthesized by the reaction of substitute keto oximes with various *N*-substituted α-amino acids chlorides in the presence of triethylamine and dichloromethane at 0°C, and their structures were characterized by IR, and 1D-NMR methods. The synthesized compounds were tested for their ability to inhibit the proliferation of human colon cancer cells and human epithelial cells. Some of them have been revealed a significant cytotoxic effect.

*Keywords*: oxime esters, α-amino acids, stereoselective, cytotoxic, biological activity, enantiomer.

RUNNING TITLE: SYNTHESIS OF OXIME ESTERS.

INTRODUCTION

Cancer remains a serious human health problem, despite considerable progress in the understanding of its biology and pharmacology. The main problem is that cancer is not one disease, but a group of diseases affecting different organs and systems of the body.

Cancer develops due to abnormal and uncontrolled cell division, frequently at a rate greater than that of most normal body cells.1

For some types of disseminated cancers, chemotherapy is the only effective therapy because it distributes anticancer drugs through the circulatory system. Oxime esters are a small, but important, class of biologically useful compounds for synthesis of fragrances,2 crop protection, and therapeutic studies.3 They are useful building blocks in peptide synthesis.4 Oxime esters are selective covalent inhibitors of serine hydrolase retinoblastoma-binding protein 9 (RBBP9) and cleave DNA under photolytic conditions.5,6 They also possess fungicidal,7 herbicidal,8 insecticidal, and antitumor activity.9 Oxime esters of dihydrocoumaric acid have been synthesized and they are reported to have antibacterial activity.10 Aromatic benzophenone oxime esters and dibenzosuberone oxime esters are pharmacologically important.11 Vanillin derived piperidin-4-one oxime esters have been tested for antioxidant and antimicrobial potential.12 The oxime esters derived from nafimidone have been tested as potential anticonvulsant compounds.13

Several methods have been developed for the preparation of oxime esters derivatives.3 The most common method is the condensation of acid chlorides with oximes under basic conditions or the use of acid anhydrides in presence of strong acids.2, 6a, b, 10, 12, 13

Oxime esters can be prepared using α,β-unsaturated aldehydes and oximes using a *N*-heterocyclic carbene as a redox esterification catalyst,14 or by treatment of alkyl- or aryl-substituted oximes with aliphaticor aromatic acids in the presence of *N*-[3-(methylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride(EDCI) reagent.15

A large number of researches on their synthesis and biological activities have been reported during the last thirty years.16 However, no attention has been paid to the stereoselective synthesis of chiral oxime esters derivatives. So in continuation of our research, which is aimed the preparation of natural and non-natural compounds of biomedical importance,17 and in connection with ongoing investigations on the reactivity of natural amino acids,18 we report herein an efficient and easy methodology for the synthesis of series of new optically pure oxime esters **5a-k** starting from the commercially available acetophenone derivatives **1a-f** and natural amino acids **3a-d** which are of considerable interest as chiral pool agents since they are easily accessible and inexpensive enantiomerically pure compounds.

EXPERIMENTAL

Reagent grade chemicals and solvents were purchased from commercial supplier and used without purification. TLC was performed on silica gel F254 plates (Merck). Silica gel (100-200 mesh) was used for column chromatographic purification. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX spectrometer. 1H NMR and 13C NMR spectral data were recorded on Advance Bruker 300 spectrometer (300 MHz) with CDCl3 as solvent and TMS as internal standard. *J* values are in Hz.

*General procedure for the preparation of oximes (****2a-f****)*

Acidic hydroxylamine (NH2OH.HCl, 0.1 mol) was added dropwise to a stirred solution of substituted acetophenone (0.02 mol) in 95% EtOH (150 mL) and pyridine (8 mL, 0.1 mol) at room temperature. The resulting mixture was refluxed for 0.5 -2h (until the starting material was completely consumed as indicated by TLC), and cooled in cold water for1 h. The precipitate was collected by suction, washed with warm water (3\*50 mL) and dried in a vacuum oven. The crude compound was recrystallized in ethanol to give a white solid.

*Synthesis of α N-Phthilimido amino acids (****4a-d****)*

A solution of amino acid (1 equiv.) in toluene was added to a solution of phthalic anhydride (1 equiv.) and triethylamine (1.2 equiv.). Then the reaction mixture was refluxed on water bath for near about 4-5 hrs. After the reaction was completed, the resulting solution was separated. The organic phase was washed with water until neutral, dried over MgSO4, and filtered. The filtrate was evaporated and purified by column chromatography on silica gel to give compound (**4a-d)**.

*Synthesis of oxime esters (****5a-k****)*

A mixture of *N*-Phthaloyl-*L*-amino acid **4a-d** (1 equiv.) and thionyl chloride (2 mL, slow addition) were mixed together and the contents were heated at 55°C for 4h. The reaction mixture was cooled to room temperature and kept in an ice bath. Then, to a solution of oxime **2** (1 equiv.), triethylamine (1.2 equiv.) in dichloromethane (CH2Cl2, 30 mL) were added subsequently to the reaction mixture and the contents were stirred at room temperature for 2h. When the reaction was complete, checked by thin-layer chromatography (TLC) analysis, the solvent was removed under reduced pressure and extracted with dichloromethane (3\*30 mL). The organic layer was dried over anhydrous MgSO4 and concentrated under reduced pressure. The residue thus obtained was purified by silica gel chromatography (hexane/ ethylacetate) to afford the desired oxime esters derivatives (**5a-k).**

*Cell lines and culture medium*

The human colon carcinoma cells (*Caco*-*2*; *ECACC*. *86010202*) and the epidermoid carcinoma epithelial cells (*Hep-2 ; ATCC CCL-23*)  were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% of fetal bovine serum, 1% non-essential amino acids and 1% penicillin/streptomycin (Invitrogen). At 85-90% confluence, cells were harvested using 0.25% trypsin/ EDTA solution and sub-cultured onto 96-well plates according to the experimental requirements.

*Cytotoxicity Screening Assay*

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay described earlier19 was used to screen the cytotoxic activity of isolated compound. Briefly, the *Caco-2* and *Hep-2* cell lines (1 x 105cells/well) were grown overnight on 96-well flat bottom cell culture plates, incubated 24 h. When a partial monolayer had formed, the supernatant was flicked off, the monolayer washed once with medium and 100 μl of different concentrations (10, 5, 2.5 and 1.25 mg/mL) of pure compounds were added to the cells.in the microtitre plates. After 24 h, the cells were washed and treated with 0.01 mL MTT reagent (Invtrogen) prepared in 5.0 mg/mL phosphate buffered saline (PBS) per well. Plates were incubated at 37 °C in a 5% CO2 atmosphere for 4 h, and 0.1 mL dimethylsulfoxide (DMSO) was added. After an overnight incubation at 37 °C, the absorbance was measured at 550 nm using an ELISA reader (Thermo scientific Multiskan FC) and was compared with the control cultures without compound. Results were generated from 3 independent experiments and each experiment was performed in triplicate. The percentage growth inhibition was calculated using following formula,

% cell inhibition= 100-{(At-Ab)/ (Ac-Ab)}x100

Where,

At= Absorbance value of test compound

Ab= Absorbance value of blank

Ac=Absorbance value of control

Stock solutions (5 mg/mL) of pure compounds were prepared in dimethylsulfoxide (DMSO) and the final concentration of this solvent was kept constant at 0.25 %. Serial dilutions with culture media were prepared just prior to addition to test.

*Statistical analysis*

The results are expressed as mean ± SEM. Data were statistically analyzed by one-way analysis of variance (ANOVA) to determine differences among groups and Tukey test as a post-hoc. All the statistical analysis was conducted using Statistical Package for Social Science (SPSS for Windows; v19.0, USA) and differences were considered statistically significant when p<0.05.

Analytical and spectral data of the synthesized compounds are given in supplementary material to this paper.

RESULTS AND DISCUSSION

The substituted oximes **2a-f** was synthesized according to the literature procedure20 shown in **Scheme 1**. The condensation of acetophenone derivatives **1a-f** with hydroxylamine hydrochloride and pyridine gave white shiny coloured oxime **2a-f** with 90% yield.

The α *N*-Phtalimido amino acids (Compounds **4a-d**) were synthesized according to our method described in the literature18a by allowing phthalic anhydride to react with a number of commercially available amino acids in refluxing apolar solvents such as toluene in the presence of triethylamine and separating the formed water (**scheme 1**).

The treatment of **4a-d** with thionyl chloride followed by treatment with keto oxime in anhydrous dichloromethane in the presence of Et3N at 0°C to room temperature, provide the corresponding *N*-substituted phthaloyl derivatives **5a-k** with average yield of 76% in two steps and after purification. Their structures were established with IR, 1HNMR, 13C NMR and mass spectrometry.



Scheme 1.Synthesis of oxime esters 5a-k.

The antiproliferative potential of the synthesized compounds **5a**, **5b**, **5c**, **5d**, **5f**, **5h**, **5i** and **5j** was determined *in vitro* against two cancer cell lines such as *Hep-2* and *Caco-2*. The cytotoxicity values were obtained as % inhibition of different concentrations and are summarized in **table** **1** and **2**.

**Table 1:**Cytotoxic activity of some derivatives against Caco-2 cellsa

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Concentrations mg/mL | | | |
| Compounds | **10** | **5** | **2.5** | **1.25** |
| 5a | < 20% | < 20% | < 20% | < 20% |
| 5b | 59.67±1.91 | 40.98±1.22 | 22.56±2.03 | 25.17±1.15 |
| 5c | 52.94±1.22 | 43.01±2.16 | 41.37±0.9 | 35.01±0.85 |
| 5d | 56.19±0.78 | 44.41±1.08 | 37.96±1.2 | 29.06±0.63 |
| 5f | < 20% | < 20% | < 20% | < 20% |
| 5h | < 20% | < 20% | < 20% | < 20% |
| 5i | 41.03±3.54 | 40.15±1.56 | 28.92±1.94 | 14.02±0.76 |
| 5j | < 20% | < 20% | < 20% | < 20% |

aThe 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay described earlier19 was used to screen the cytotoxic activity of isolated compound

**Table 2:** Cytotoxic activity of some derivatives against Hep-2 cells

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Concentrations mg/mL | | | |
| Compounds | **10** | **5** | **2.5** | **1.25** |
| 5a | < 20% | < 20% | < 20% | < 20% |
| 5b | 48.22 ±1.12 | 20.15±0.86 | 11.02±1.61 | 7.89±0.74 |
| 5c | 57.85.±2.13 | 43.21±1.92 | 21.11±0.83 | 27.02±2.35 |
| 5d | 61.02 ±1.69 | 56.12±2.01 | 31.12±0.77 | 10.25±0.92 |
| 5f | < 20% | < 20% | < 20% | < 20% |
| 5h | < 20% | < 20% | < 20% | < 20% |
| 5i | 53.91 ±1.6 | 50.77±2.51 | 17.01±1.38 | 9.49±1.02 |
| 5j | < 20% | < 20% | < 20% | < 20% |

The results exhibit that only four compounds **5b**, **5c**, **5d** and **5i** have moderate potency of around 40% inhibition at 10 mg/mL against *Hep-2* and *Caco-2*, while the other compounds **5a**, **5f**, **5h** and **5j** were inactive against the two cancer cell lines (inhibition effect < 20%). It was observed that when the methoxy group was attached at the *para* position of the phenyl ring (compound **5b**) the activity reduced to 48.22% against *Hep-2* cell line. Replacing the substituent at *para* position by an electron withdrawing group has caused decrease in the anticancer activity as compared to compound **5b** against *Caco-2* Cell line. This can be justified by the fact that compounds bearing electron withdrawing groups like fluoro (F) (**5c**) and chloro (Cl) (**5d**) substituents at the *para* position of the phenyl ring have exhibited activity at inhibitory ratios values 52.94 and 56.19% respectively against *Caco-2* cell line and 57.85 and 61.02% respectively against *Hep-2* cell line. Compound **5i** also showed activity against *Hep-2* at 53.91% and 41.03% against *Caco-2* cell lines. It can be concluded that the cytotoxicities of resulting oxime esters derivatives are significantly correlated with the nature of the substituent group.

CONCLUSIONS

In summary, we have prepared a new series of optically pure phthalimido oxime esters derivatives and their cytotoxic activities against two human cancer cells lines *Caco-2* cells and *Hep-2* were evaluated. Some derivatives exhibited strong cytotoxic activity, therefore, the further structural modification and anti-tumor activity study *in* *vivo* would be in progress. Our findings could provide new evidence showing the relationship between the chemical structure and biological activity and may be useful for the design of novel chemotherapeutic drugs.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compounds are available electronically at the pages of journal website: <http://www.shd-pub.org.rs/index.php/JSCS>[,](http://www.shd.org.rs/JSCS/) or from the corresponding author on request.

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