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Synthesis of polyfunctionalized piperidone oxime ethers and their cytotoxicity on HeLa cells

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ABSTRACT

A series of twenty 2,6-diarylpiperidin-4-one *O*-methyloximes were synthesized with fluoro/chloro/bromo/methyl/methoxy/ethoxy/isopropyl substituents on various positions of the phenyl at C-2 and C-6 in association with/without methyl substituent on the secondary amino group and methyl/ethyl/isopropyl substituents on the active methylene centers. Regardless of their substitution all compounds predominantly exist in the chair conformation except **3m**, which adopts a twist-boat conformation. All the synthesized compounds were evaluated for their in vitro antiproliferative activity against human cervical carcinoma (HeLa) cell line. The cytotoxicity of the test compounds was determined by measuring the number of live cells after 24 h of treatment by MTT assay method. This preliminary SAR suggests some lead molecules **3c–f**, **3j–k**, **4d–g**, and **4i** with a scope of further structural optimization of the piperidone pharmacophore toward the development of anticancer drug synthesis.

Nitrogen containing heterocycles always signified a subject of great interest due to their ubiquity in nature and massive presence as part of the skeletal backbone of many therapeutic agents.[1] Of these heterocycles, piperidone pharmacophore is very momentous by its broad-spectrum of biological actions.[2] Introduction of various substituents on the piperidone heterocycle and reduction of the carbonyl as oxime functionality improved the biological efficacy.[3] As a part of our ongoing research[4] toward the development of new piperidone based compounds with structure diversity, for this study, 2,6-diphenylperidin-4-one *O*-methyloxime **3a**, 3-alkyl analogs **3b–j**, 3,5-dimethyl analogs **3k–o** and some *N*-methyl analogs **4d–i** were synthesized as depicted in Scheme 1.

All compounds were characterized by their analytical and spectral data, and thus their stereochemistries are established.[5] Compound **3a** has no substitution on the heterocycle other than the phenyl rings at C-2 and C-6, which paved the secondary amino group. The vicinal couplings clearly suggest that **3a** adopts a chair conformation with an equatorial orientation of the phenyl rings on both sides of the secondary amino group. Introduction of a methyl group at C-3 of **3a** along with/without halo/alkyl/alkoxy substituents on the phenyl at C-2 and C-6 (**3b–h**) did not alter the stereochemistry. Likewise, the introduction of methyl group at ring nitrogen of **3d–h** also did not alter the stereochemistry of **4d–h**. Both the methyl groups at N-1 and C-3 preferred the equatorial disposition. Similarly to methyl, the introduction of ethyl **3i** and isopropyl **3j** groups at C-3 of **3a** also did not affect the stereochemistry significantly. They also adopt the analogous stereochemistry as **3b**. However, the decrease in the $^{3}J_{2a,3a}$ of **3j** indicates that there may be a least possible population of boat conformation for **3j** and its *N*-methyl analog **4i**.

On the other hand, the incorporation of methyl group on both sides of the active methylene centers C-3 and C-5 partially/completely modify the stereochemistry of the synthesized compounds **3k–o**. Although the 3,5-dimethyl substituted piperidones **1k–o** exist in the chair conformation with an equatorial orientation of all substituents, the oxime derivatives **3k**, **3l**, **3n** and **3o** underwent epimerization to retain the chair conformation. One of the methyl groups, which is *syn* to the oxime moiety was epimerized to adopt an axial orientation at C-5 to stabilize the chair conformation. In fact, in order to relieve from the severe allylic 1,3-interaction between the methyl at C-5 and N–O, the methyl at C-5 was epimerized. But a prominent change was noticed on compound **3m**, which underwent a conformational transformation instead of the epimerization observed in analogous compounds and thus adopts a twist-boat conformation.

All the synthesized compounds **3a–4i** were evaluated for their in vitro antiproliferative activity against human cervical carcinoma (HeLa) cell line. The cytotoxicity of the test compounds was determined by measuring the number of live cells after 24 h of treatment by MTT assay.[6] The IC_{50} values of all compounds are summarized along with their stereochemical structures in Table 1 for better

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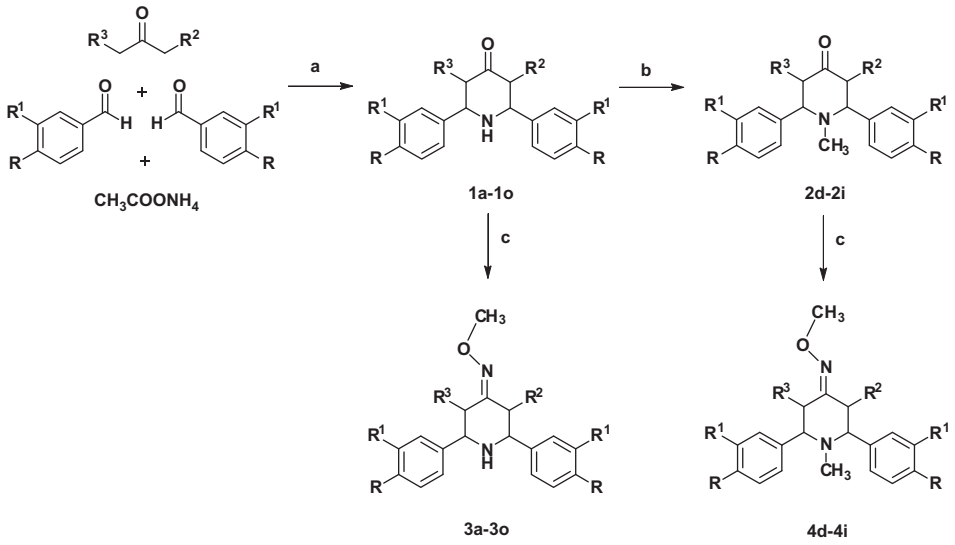

Entry	R	R^1	R^2	R^3	Entry	R	R^1	R^2	R^3
a	H	H	H	H	**i**	H	H	CH_2CH_3	H
b	H	H	CH_3	H	**j**	H	H	$CH(CH_3)_2$	H
c	Br	H	CH_3	H	**k**	H	F	CH_3	CH_3
d	Cl	H	CH_3	H	**l**	CH_3	H	CH_3	CH_3
e	F	H	CH_3	H	**m**	$CH(CH_3)_2$	H	CH_3	CH_3
f	H	F	CH_3	H	**n**	OCH_3	H	CH_3	CH_3
g	CH_3	H	CH_3	H	**o**	OCH_2CH_3	H	CH_3	CH_3
h	OCH_3	H	CH_3	H					

Scheme 1. Reagents and conditions: (a) Ethanol/warm; (b) methyl iodide/anhydrous K_2CO_3/dry acetone/reflux; (c) methoxylamine hydrochloride/sodium acetate trihydrate/ethanol/reflux.

structure–activity comprehension. Besides, the standard drugs Camptothecin and Etoposide were also analyzed under identical conditions and their IC_{50} values are also reproduced in the Table 1.

The HeLa cell line was obtained from American Type Culture Collection (Manassas, VA, USA). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from BioWhittaker®, whereas fetal bovine serum (FBS) and other cell culture materials were purchased from Gibco BRL Life Technologies, USA. Paraformaldehyde and Bisbenzimide Hoechst 33342 stain were procured from Sigma–Aldrich Corp., St. Louis, MO, USA, and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasolium bromide] was purchased from Biosesang Inc., Korea.

Cells were cultured in T-75 tissue culture flasks (Nunc, Denmark) at 37 °C in a 5% CO_2 humidified incubator using appropriate media supplemented with DMEM containing 10% heat-inactivated FBS, 100 units/mL Penicillin and 100 μg/mL Streptomycin. Cells were seeded in a 96 well microtiter plate containing 100 μL medium at a final density of 2×10^4 cells/well at identical conditions. After overnight incubation, the cells were treated with different concentrations of test compounds (6.25–100 μg/mL) or DMSO (carrier solvent) in a final volume of 200 μL. After 24 h, 10 μL of MTT (5 mg/mL) was added to each well and the plate was incubated at 37 °C in the dark for 4 h. Then the media along with MTT was removed and the formazan crystals were solubilized by adding DMSO (100 μL/well). Finally, the reduction of MTT was quantified by reading the absorbance at 570 nm by GENios® microplate reader (Tecan Austria GmbH). Effects of the test compounds on cell viability were calculated using cells treated with DMSO as control. The data were subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The IC_{50} (inhibition of cell viability to 50%) concentrations were calculated using the respective regression equation as shown in Table 1.

A careful analysis of Table 1 provides the structure–activity correlations as indicated by their IC_{50} values. Compound **3a** is a simple piperidone molecule and it has no substitution on the phenyl as well as active methylene centers and ring nitrogen, which shows activity at an IC_{50} of 121 μM. The introduction of a methyl group on one of the active methylene centers of the piperidone moiety **3a** affords **3b**, which fairly improves the efficacy as noticed IC_{50} of 113 μM. However, the replacement of methyl by ethyl **3i** shows an incredible improvement in its efficacy (IC_{50} 57 μM). Similarly, the replacement of methyl by isopropyl **3j** exhibits activity at an IC_{50} of 49 μM. Further the incorporation of a methyl group at the ring nitrogen of **3i** improved the IC_{50} of **4i** from 57 to 41 μM.

A remarkable improvement is observed by the introduction of a bromo substituent on *para*-position of the phenyl at C-2 and C-6 (**3c**), which discloses an excellent inhibition of the growth of the HeLa cells at an IC_{50} of 25.02 μM in 24 h. This IC_{50} is nearly three-fold higher than the standard drug Camptothecin (8.93 μM); however, it is very closer and even comparable to the Etoposide standard (23.33 μM). The replacement of *para*-bromo