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RESEARCH ARTICLE

Derivatives of β-Phenylisoserine Side Chain of Paclitaxel (Taxol) with Varying Hydrophobicity

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Abstract

Paclitaxel (Taxol) displays anticancer activity by binding to β-tubulin and interrupting the cell cycle at the G_2-M phase. It is known from previous studies that the Paclitaxel-binding pocket of β-tubulin is very hydrophobic in nature. Structure-activity studies have shown that β-phenylisoserine side chain at C-13 of Paclitaxel is essential for anticancer activity. We set out to develop structural analogues of β-phenylisoserine with varying hydrophobicity using co-catalyzed oxidation and microwave mediated ring-opening strategies. It is expected that an increase in the hydrophobicity of β-phenylisoserine side chain can increase the binding of Paclitaxel derivatives to β-tubulin and thus increase the potency of these compounds as anti-cancer drugs.

Introduction

Paclitaxel (Taxol)[1)] belongs to a family of compounds called Taxanes. It inhibits cell replication by binding to the N-terminal region of β-tubulin (Figure 1) and promoting the formation of highly stable microtubules that resist depolymerization. Paclitaxel is used to treat carcinomas produced in ovaries, breasts, head, neck and lung, as well as in skin and mucous membrane, more commonly observed in patients suffering from AIDS.[2,3,4)] Paclitaxel is isolated from the bark of thewestern pacific yew tree. It contains 10-deacetylbaccatin-III, a naturalsubstance isolated from the leaves of the *Taxusbaccata*species and β-phenylisoserine sidechain.[5,6)]Paclitaxel and a closely related analogue Taxotere (Figure2; generic name docetaxel) are presently the only taxoids in clinical use.The low abundance of the majority of other reported taxanes limits their availability for pharmacological evaluation. Infact, the limited supply of *T. brevifolia* along with the low yield of the drug was the initial obstacle in the clinical development of Taxol.

The β-phenylisoserine side chain at C-13 of Paclitaxel is essential for the anticancer activity of the taxoid family. Structure-activity studies have shown that the presence of the β-phenylisoserine side chain (as N-benzoyl-(2R,3S)-3-phenylisoserine) with correct absolute stereochemistry is significant for strong cytotoxicity.[7)]The development of methodologies for synthesis of β-phenylisoserine derivatives is a hot field of investigation to obtain important lead structures for use as potential therapeutic agents.

We set out to develop structural analogues of β-phenylisoserine with varying hydrophobicity (Schematic 1) using our own co-catalyzed oxidation and microwave mediated ring-opening strategies.Several methodologies have been discovered for the synthesis of these derivatives. However most of them are associated with harsher reaction conditions, long reaction time and difficult work up procedure as well as lower yield.[8)]In this study, we used a methodology where microwave irradiation under solvent free condition promoted the synthesis of a library of compounds containing β-phenylisoserine structural moiety as the core structure.

Materials

NMR Spectra were measured using Joel LA 400 FT NMR and Varian Gemini 2000 FT NMR machines in $CDCl_3$. Chemical Shifts were recorded as δ downfield from the internal standard (TMS). Thin layer chromatography (TLC) was carried out on aluminium sheets pre-coated with silica gel 60 F_{254} (Merck). The plates were visualized by UV light followed by development on iodine vapours. Column Chromatography was performed on ACME silica gel eluant (60-120 mesh). Mass spectra (MS) were recorded on Hewlett Packard GC MS model no. 5989 mass spectrometer with ionization electron beam energy of 70 eV. Optical rotation was measured on Autopol II/ Autopol III polarimeters. IR spectra were recorded on Perkin-Elmer 1600 FT IR spectrophotometer. HPLC analysis were done with water 745 integrator, water 510 pump and detected with Shimadzu SPD-10 AVP, UV VIS detector. The microwave oven used for the experiment was MLS 1200 mega high performance microwave digestion unit.

Methods

General Procedure for Synthesis of Ethyl-∝-Hydroxy-β-amino-γ- phenylpropionate(*2a-d*):To a solution of epoxide (1 mmol) and amine (4 mmol) in dichloromethane (5 ml), neutral alumina (Al_2O_3) was added (which acts as a solid support). This slurry was transferred to a Teflon vessel. The vessel was kept under microwave oven for 10-15 minutes at 50 watt (60 watt in case of 2d) and 50 °C. After the completion of the reaction (monitored by TLC: product formation at R_f = 0.7; ethyl acetate: hexane-3: 2, in case of 2d R_f = 0.7; ethyl acetate: hexane-1: 4) ethyl acetate (20 mL) was added and stirred for 30 minutes. The alumina was filtered off and the solvent was removed *in vacuo*. This mixture was then subjected to preparative TLC (silica gel; ethyl acetate:hexane- 3:2, in case of 2-D silica gel; ethyl acetate:hexane- 1:4 to get brown colored Bestatin analogues.

General Procedure for Synthesis of trans-Amino 3-phenylglycidate: To a solution of epoxide (1 mmol) and amine (4 mmol) in dichloromethane (5 mL) neutral alumina (Al_2O_3) was added (which acts as a solid support). This slurry was transferred to a Teflon vessel. The vessel was kept under microwave oven for 10-20 minutes at 60 watts and 50 °C. After the completion of the reaction ethyl acetate (20 mL) was added and stirred for 30 minutes. The alumina was filtered off and the solvent was removed in vacuo. The product was generally isolated by preparative thin layer chromatography (glass plate coated by silica gel) using gradient elution from ethyl acetate to hexane (1:9 to 1:4, depending on the polarities of amines).

General Procedure for Synthesis of β-phenylisoserine derivatives (*5*):To a solution of epoxide (1 mmol) and amine (4 mmol) in dichloromethane (5 ml) neutral alumina (Al_2O_3) was added (which acts as a solid support). This slurry was transferred to a Teflon vessel. The vessel was kept under microwave oven for 15-20 minutes at 50 watt and 50 °C (at 100 watt and 80 °C in case of aliphatic amines). After the completion of the reaction ethyl acetate was added and stirred for 30 minutes. The alumina was filtered off and the solvent was removed *in vacuo*. This residue was taken in CCl_4 solution. To this CCl_4 solution hexane was added to precipitate the product and leaving the amine and the epoxide in the mother liquor. This brown powder was then subjected to column chromatography (silica gel; ethyl acetate: hexane-1: 5-1: 3) to get brown colored Bestatin analogs (two diastereomers).

Results

Initially we studied the reactivity pattern of various aromatic and aliphatic amines with ethyl-3-phenylglycidate **1**. Typically, neutral alumina was added to a solution of epoxide (1mmol) and amine (4 mmol) in dichloromethane (5mL). The solvent was evaporated and the slurry was transferred to a Teflon vessel. The vessel was kept in the microwave oven for 10 minutesusing a power of 50 watts and a temperature of 50 °C (Schematic 2).

After the completion of reaction ethyl acetate (4mL/mmol) was added to the reaction mixture and stirred for 30 minutes. The alumina was filtered off and the solvent was removed from the filtrate under vacuum. The product was further purified via preparative thin layer chromatography to give the corresponding β-phenylisoserine derivative in good to excellent yields **2**. The results of various representative examples are compiled in Chart 3. As seen from the result in Schematic 3, ethyl-3-phenylglycidate **1** and different aryl amines react to give corresponding β-phenylisoserine derivatives in good yields (entries 1-3). Interestingly, these microwave assisted opening didn't produce the other regioisomer.

After seeing the effect of aromatic amines on this epoxide **1,** we next explored the reaction of **1** with aliphatic amines (Schematic4). First ethyl-3-phenylglycidate was treated with benzylamine under similar conditions as described above but at little higher power i.e. 60 watt. After isolation of product **3**aby preparative TLC surprisingly the product was found to be *trans*-amidation product instead of open product in 82 % yield (Schematic

3, entry 4). However no epoxide-opened product could be obtained even after prolonged reaction time. The reason for getting *trans*-amidation product was not clear at this point and more investigations were needed to know the exact course of reaction.

The epoxide **1** was similarly treated with allylamine, isopropylamine, cyclohexylamine and *n*-butylamine at 50 °C using a power of 60 watts for 10-15 minutes under similar conditions as described before. In each case single *trans*-amidation product **3** was obtained in excellent yield (Schematic 3, entries 5-8). Interestingly the reaction of epoxide **1** with α-methylbenzylamine again gave the corresponding open product (Schematic 3, entry 9) in high yield (60%).

After getting this set of results, various aromatic amines were reacted with the methyl-N- (3-phenylglycidyl) to get derivatives of various amino acids**4**, which were obtained using an earlier reported protocol.[9,10] The reactions were carried out at 50-80 °C using a power of 50-100 watt for 10-20 minutes (Schematic 5). Interestingly, as seen from the results, epoxy derivatives of N-cinnamoyl dipeptides **4**produced only open chain products **5** with both aromatic and aliphatic amines, which were characterized as two diastereomers of corresponding β-phenylisoserine derivatives (Table 2, entries 1-13) in 59 to 73 % yields.

Discussion

Paclitaxel is produced by endophytic fungi[11] in many members of the Taxaceae family including the pacific yew,*Taxusbrevifolia*. Other natural productshave also been discovered that stabilizemicrotubules includingepothilones A and B from a myxobacterium,*Sorangiumcellulosum*,eleutherobin from a marine soft coral and discodermolidefrom a deep-sea sponge. These molecules mimic an endogenous cellular factor that helps stabilize microtubules in normalcytoplasm.The natural reagent might be a small molecule orpart of a microtubule-associated protein (MAP).The mechanism of action of Taxol involves stabilization of microtubules which causes inhibition of depolymerization back to tubulin.[12] Investigations by Horwitz and co-workers have revealed that Taxol promotes the stabilization of microtubules by inhibiting depolymerization of microtubules to soluble tubulin.[13,14] Taxol inhibits cell proliferation at the G_2-M phase of the cell cycle in contrast to other anticancer drugs which act at the G_1-S phase. Studies on structure–activity relationship of Taxol derivatives have proved that a β-phenylisoserine moiety at C-13 and the oxetane ring are important forbiological activity.[5] Paclitaxel is especially effective as an anticancer agent because tubulin is not its only target but it also binds to a protein called Bcl-2,[15,16] which blocks the process of apoptosis, or cell death. Therefore, Paclitaxel plays a dual role in destroying dividing cells; first by stabilizing assembled microtubules and disrupting mitosis, and second by inhibiting Bcl-2 and allowing apoptosis to proceed. A molecular docking study has shown that Paclitaxel binds to a hydrophobic pocket (Figure 3) in the second globular domain of β tubulin, facing the central hole in a microtubule. Figure 4 shows the β-phenylisoserine moiety in close proximity to the β strands B9 and B10 and the α helix H7. Also visible in the figure is Val 23 close to the phenyl ring.[17]

β-Phenylisoserine derivatives are considered an important synthetic target.[18,19,20] Three approaches have been used to synthesize these compounds: a) Sharpless asymmetric amino-hydroxylation of cinnamic acid esters;[18,19,20,21] b) asymmetric carbon-carbon bond formation;[22] c) using other chiral sources, such as 2,3-dihydroxyesters from Sharpless asymmetric dihydroxylation[23,24] or amino-acids.[25,26] The use of inorganic solid support as catalyst has also been developed for dry media reaction, resulting in the higher selectivity, milder conditions and ease of handling.[27] Our choice of using solid-support likes neutral alumina, which is known to catalyse opening of epoxide[28] eliminated the use of cobalt catalyst.[29] Over the last few years, there has been growing interest in the use of microwave heating in organic synthesis.[30] The use of these reaction conditions has several advantages over conventional reaction conditions such as short reaction time, ease of work up, reduction in the usual thermal degradation and better selectivity. In microwave-assisted reaction,the reactants are irradiated in a sealed teflon vessel.The absence of solvent reduces the risk of explosion and exposure when reaction takes place in a microwave oven.[31]

Conclusion

A series of β-phenylisoserinederivatives of varying hydrophobicityhave been successfully synthesized using a new kind of microwave-assisted epoxide ring opening reaction. The highlights of this reaction are speed and high yields. A future direction can be to use these β-phenylisoserine side chains tosynthesize differentkinds of Taxanes and to conduct structure-activity relationship studies for these novel compounds. It is expected that these new compounds can be even more potent than Paclitaxelwhich can lead to the development of new anti-cancer drugs against breast and ovarian cancers.

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