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# Adamantyl-tethered-biphenylic compounds induce apoptosis in cancer cells by targeting Bcl homologs

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ABSTRACT

Bcl homologs prominently contribute to apoptotic resistance in cancer cells and serve as molecular targets in treatment of various cancers. Herein, we report the synthesis of biphenyl-adamantane derivatives by a ligand free palladium on carbon based Suzuki reaction using diisopropylamine as a base for the coupling of adamantane based aryl chloride with a variety of aryl boronic acids. Among the biphenyl derivatives synthesized, compound 3′-(adamantan-1-yl)-4′-methoxy-[1,1′-biphenyl]-3-ol (AMB) displayed cytotoxic activity against hepatocellular carcinoma cell lines without significantly affecting the normal cell lines. Further, AMB caused increased accumulation of the HCC cells in subG1 phase, decreased the expression of Bcl-2, Bcl-xL, cyclin D1, caspase-3, survivin and increased the cleavage of PARP in a time-dependent manner. In silico molecular interaction studies between Bcl homologs and AMB showed that the biphenyl scaffold is predicted to form π–π interactions with Phe-101 and Tyr-105 and the adamantyl fragment is predicted to occupy another hydrophobic region in the kink region of the binding groove. In summary, we report on the synthesis and biological characterization of adamantyl-tethered biphenylic compounds that induce apoptosis in tumor cells most likely by targeting Bcl homologs.


Aged cells, damaged cells, auto-reactive cells and cells with irreparable DNA damage are eliminated from the body of all the multicellular organisms by a complex process of programmed cell death or apoptosis.[1] In multicellular organisms, a balance is maintained between the rate of cell division and cell death.[2] In case of oncogenesis, cells exhibit density independent proliferative potential and resistance to apoptotic signals.[3] Bcl-2 family proteins play a key role in modulation of apoptosis and more than 20 members of the Bcl-2 family have been discovered and broadly categorized into pro and antiapoptotic proteins.[4,5] BAD, BAK, BAX, PUMA, NOXA are proapoptotic and Bcl-2, Bcl-xL, Bcl-w, Mcl-1, Bfl-1 are predominant antiapoptotic proteins of the Bcl-2 protein family.[6–8] The equilibrium is maintained between pro and antiapoptotic proteins to ensure homeostasis.[9,10] Overexpression of antiapoptotic Bcl-2 family proteins has been demonstrated to contribute to prolonged cell survival, resistance to apoptosis, cancer development and progression, and decreased sensitivity to chemotherapeutics in cancer cells.[11,12] The pro-apoptotic BAD protein interact with the hydrophobic binding groove of Bcl homologs (Bcl-2 and Bcl-xL)[13] and disrupts the integrity of mitochondria to initiate the release of cytochrome C into the cytoplasm to induce apoptosis.[14,15] Therefore, targeting Bcl-2/Bcl-xL proteins using small molecules that can interact with their hydrophobic socket may serve as agents with therapeutic potential to induce apoptosis in cancer cells.

Several heterocycles targeting Bcl homologs have been extensively investigated and few of them have been promoted to clinics.[16,17] Navitoclax (ABT-263) is a well-characterized small molecule inhibitor of Bcl-2 that has been synthesized following a structure based drug design approach.[18] Further structure based

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design yielded the potent selective inhibitors of Bcl-2 called ABT-199 and ABT-737.[19,20] In addition, biphenyl based compounds have been studied extensively and were shown to exhibit very good antitumor activity against various human malignancies.[21,22] Honokiol and magnolol are the biphenyl containing natural compounds isolated from *Magnolia officinalis* with an excellent antineoplastic activity against several cancer types.[23,24] The synthetic derivatives of eugenol have been demonstrated to induce antiproliferative activity on neuroectodermal tumor cells.[25] However, the mechanism of action of these compounds is not clearly understood. In order to investigate the underlying mechanism of biphenyl derivatives in imparting anticancer activity and in continuation of our effort to synthesize various organic compounds[26–31], we synthesized a series of adamantyl tethered biphenyl derivatives. Adapalene is a topical drug in which adamantane substitution at *meta* position to the aryl system is seen. Therefore, we considered *meta* position as choice of substitution and synthesized biphenyl-adamantanes and evaluated for their antiproliferative activity against hepatocellular carcinoma cell lines. We also followed an in silico approach to explain the role of biphenyls in apoptosis induction of cancer cells.

The synthetic route for biphenyl-based small molecule libraries is outlined in Figure 1A. Here we attempted the synthesis of targeted compounds by a ligand free palladium on carbon based Suzuki reaction using diisopropylamine as a base for the coupling of adamantane based aryl chloride with a variety of aryl boronic acids.[32–34] Most of the reactions were completed in 4 h with excellent yields. On the other hand, ortho-substituted boronic acids showed prolonged reaction time with an exception of ortho methyl phenyl boronic acid which completed in 4 h. Interestingly, fused aromatic- and chloro substituted-boronic acids proved to be excellent substrates under the given experimental conditions. The characterization information and the structures of various biphenyl-adamantanes were provided as Supplementary Table 1.

*AMB suppresses the viability of hepatocellular carcinoma (HCC) cells in a dose- and time-dependent manner:* We investigated the potential effect of novel biphenyl derivatives on HepG2 cells using a MTT assay as described previously.[35–37] We found compounds **3f** (AMB), **3i** and **3m** to be potent cytotoxic agents against HepG2 cells and further evaluation revealed that the compounds **3i** and **3m** significantly induce cytotoxicity against normal hepatocytes (LO2) whereas AMB did not affect the viability of non-transformed cells. Therefore, 3′-(adamantan-1-yl)-4′-methoxy-[1,1′-biphenyl]-3-ol (**3f**, AMB) was found to be most effective cytotoxic agent among the newly synthesized compounds with the $IC_{50}$ of 26.1 μM. Further, the effect of lead compound on the viability of a panel of three HCC cell lines (HepG2, Huh7 and Hep3B) at different dose (0, 10, 25, 37.5 and 50 μM) and time points (0, 24, 48 and 72 h) was also investigated. We found that AMB mitigated the proliferation of HepG2, Huh7 and Hep3B cell lines in a dose- and time-dependent manner (Fig. 1B). Navitoclax was used as positive control and observed the reduction in the percentage of cell viability in dose-dependent manner (Fig. 1B). All the synthesized compounds were screened for their cytotoxic activity on LO2 cells. However, except

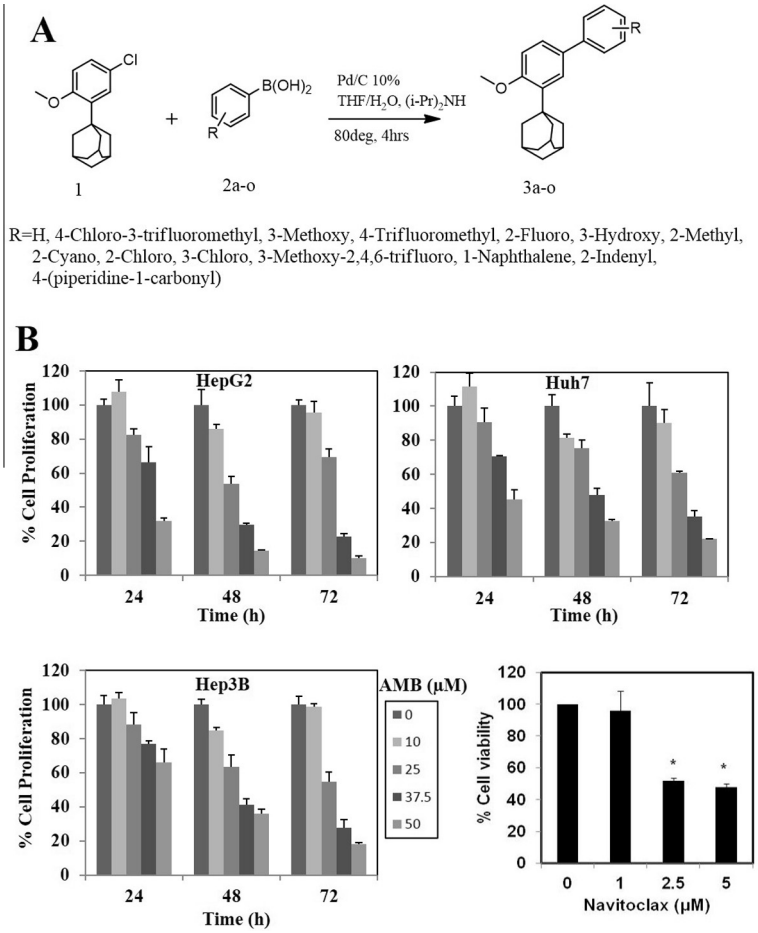


**Figure 1.** (A) Schematic representation for the synthesis of biphenyl based small molecules. (B) HCC cells ($2.5 \times 10^4$/mL, HepG2, Huh7 and Hep3B) were plated in triplicate, treated with indicated concentrations of AMB, and then subjected to MTT assay after 24, 48 and 72 h to analyze proliferation of cells. AMB suppresses the viability of various HCC cell lines in a dose- and time-dependent manner. Navitoclax, a small molecule inhibitor of Bcl-2/Bcl-xL/Bcl-W also suppressed the viability of HepG2 cells in a dose-dependent manner $^*p < 0.05$.