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Synthesis of *2H*-1,3-benzoxazin-4(3*H*)-one derivatives containing indole moiety: Their in vitro evaluation against PDE4B

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ABSTRACT

A number of *2H*-1,3-benzoxazin-4(3*H*)-one derivatives containing indole or benzofuran moieties were synthesized by using Pd/C–Cu mediated coupling-cyclization strategy as a key step. The *o*-iodoanilides or *o*-iodophenol were coupled with 3-{2-(prop-2-ynyloxy)ethyl}-2*H*-benzo[*e*][1,3]oxazin-4(3*H*)-one using 10%Pd/C–CuI–PPh_3 as a catalyst system and Et_3N as a base to give the target compounds. All the synthesized compounds were tested for their PDE4B inhibitory potential in vitro using a cell based cAMP reporter assay. Some of them showed fold increase of the cAMP level when tested at 30 μM. A representative compound showed encouraging PDE4B inhibitory properties that were supported by its docking results.

While asthma affects ~300 million people worldwide at present, chronic obstructive pulmonary disease (COPD) is projected to be the 3rd leading cause of death globally by 2020.[1] Inhibitors of phosphodiesterase 4 (PDE4) are considered as beneficial to treat COPD or asthma.[1,2] However, the first-generation (e.g., rolipram) as well as second generation PDE4 inhibitors (e.g., cilomilast and roflumilast) suffered from side effects like nausea and emesis.[1,2] Encouragingly, roflumilast (Daxas®, Nycomed) has been launched in Europe for the treatment of chronic bronchitis in 2009 and in US (Daliresp, Forest Lab) for exacerbations during COPD in 2012. Nevertheless, it is desirable to devote continued efforts towards identification of newer class of PDE4 inhibitors having fewer side effects.

In the inflammatory cells cAMP (cyclic adenosine monophosphate) plays the role of a negative regulator of the primary activating pathways such as cytokine release by T-cells. Levels of cAMP on the other hand are regulated by cAMP-specific phosphodiesterases (PDE) isozyme for example, PDE4 predominantly expressed in inflammatory and immune cells in addition to brain.[1,2] PDEs the super family of enzymes (that hydrolyze the phosphodiester bond of cAMP and cGMP) can be subdivided into 11 different groups or isozymes, for example, PDE1 to PDE11. Inhibition of the PDE4 effectively elevates the intracellular cAMP levels, thereby activating specific protein phosphorylation cascades that in turn inhibits the release of inflammatory mediators such as cytokines [tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), interleukin-12 (IL-12), leukotriene B4 (LTB4), interferon-γ (IFN-γ)], as well as activation of inflammatory cells.[3] The cAMP specific PDE4 isozymes[4] (which require a divalent metal ion, for example, Zn for catalysis) are encoded by four genes (A-D) that give rise to four isoforms, for example, PDE4A to PDE4D.[5] Since knockout mice studies have revealed that PDE4B ablation suppresses TNF-α production, hence inhibition of PDE4B has been proposed to be beneficial for the development of more effective anti-inflammatory drugs to combat with COPD and asthma.

Due to their wide range of pharmacological properties 2-substituted indoles have been explored as a number of potential therapeutic agents[6] including PDE4 inhibitors.[7] The 2*H*-benzo[*e*][1,3]oxazin-4(3*H*)-one nucleus on the other hand has been found to be integral part of several bioactive agents.[8,9] Thus, combination of both in a single entity via linking them through an appropriate linker should provide a new framework from which the molecules derived might show interesting pharmacological activities. Prompted by this idea we designed the template **C** (when X = NR or O) from **A** and **B**. Initially, a number of virtual molecules derived from **C** were tested in silico against several drug targets including

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PDE4B without performing their actual chemical synthesis. Accordingly, the compound **D** emerged as a virtual hit against PDE4B in this study. Docking studies (Fig. 2, see also Fig. S-1 in ESI) [performed using Chemical Computing Group's Molecular Operating Environment (MOE) software 2008.10 Version, 'DOCK' application module, see the ESI] indicated that compound **D** utilized the conserved active binding site with its benzooxazinone carbonyl and *N*-methanesulfonyl oxygen participated in H-bonding interactions with the PDE4B protein. The carbonyl oxygen formed H-bond with the His234 of metal binding pocket and sulfonyl oxygen formed H-bond with the Gln443 of Q pocket in the active site. These interactions were similar to that of the known inhibitor rolipram (see Fig. S-2 in ESI). Indeed, the dock score of compound **D** (−19.79 K cal/mol) was comparable with that of rolipram (−24.62 K cal/mol). The compound **D** has also shown an arene-arene interaction with the Phe446 residue of PDE4B (Fig. 2, see also Fig. S-1 in ESI). Encouraged by these observations we then decided to synthesize a library of small molecules based on **C** and screen them against PDE4B in vitro. As part of our ongoing effort on the identification of novel PDE4 inhibitors[10] we now report the results of our recent study. To the best of our knowledge the in vitro pharmacological properties of these *2H*-1,3-benzoxazin-4(3*H*)-one derivatives (**C**) containing indole or benzofuran moieties have not been explored earlier.

Several elegant and attractive methods[11–13] have been reported for the construction of indole ring including the transition metal mediated methods of which the palladium catalyzed reactions gained particular attention. As a less expensive catalyst system the use of Pd/C–CuI–PPh_3 has also gained considerable interest for the efficient synthesis of various heterocyclic structures[14] including indoles.[15,16] Compared to other Pd catalysts Pd/C is stable, easy to handle and separable from the product and is recyclable.[14] The use of Pd/C catalyzed reaction therefore is advantageous. All these features prompted us to explore a Pd/C mediated construction of indole ring as a key step for the synthesis of our target compounds **C** and **D** (or **3**) (Scheme 1).

The starting alkyne that is, 3-{2-(prop-2-ynyloxy)ethyl}-*2H*-benzo[*e*][1,3]oxazin-4(3*H*)-one (**2**) required for the synthesis of our target compound **3** was prepared according to a reported procedure as shown in Scheme 2.[8] Thus, the methyl salicylate **5** obtained from **4** was converted to 2-hydroxy-*N*-(2-hydroxyethyl) benzamide (**6**) which was then treated with paraformaldehyde under acidic conditions followed by the hydrolysis of the intermediate formed to give 3-(2-hydroxyethyl)-*2H*-benzo[*e*][1,3]oxazin-4(3*H*)-one (**7**). The subsequent propargylation of **7** afforded the desired terminal alkyne **2** that was used for the coupling-cyclization with a range of *o*-iodoanilides and *o*-iodophenol (**1**) in the presence of 10% Pd/C, PPh_3, CuI and Et_3N in MeOH (Table 1).

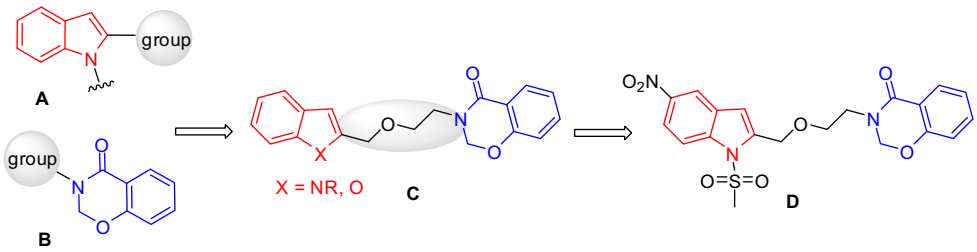

Figure 1. Design of novel and potential bioactive molecules C (and D) from A and B.

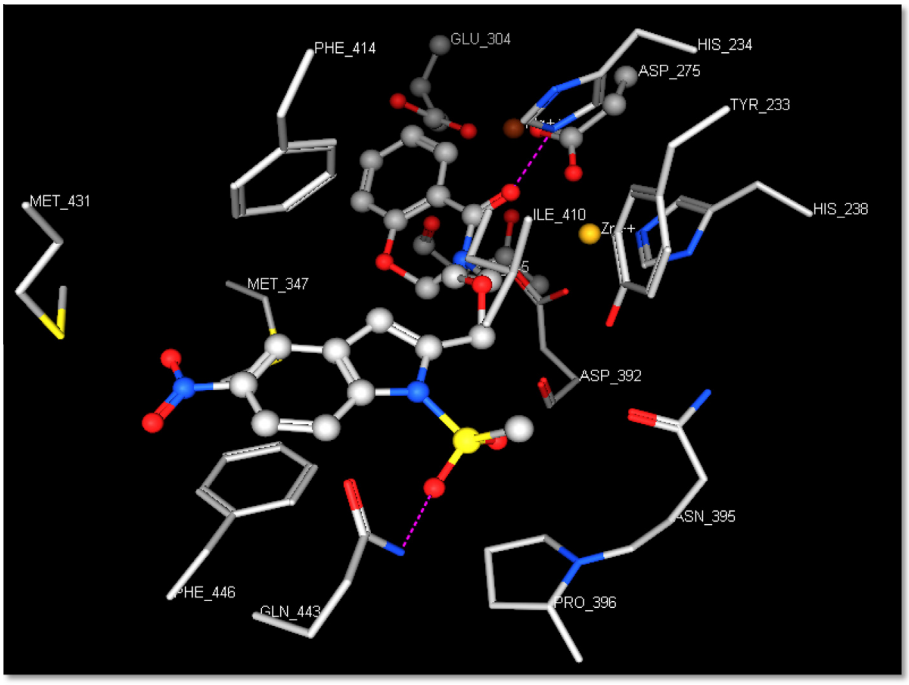

Figure 2. Binding mode of D in the PDE4B (PDB code-1XMY).