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Original article

# Rational design, synthesis and anti-proliferative evaluation of novel 1,4-benzoxazine-[1,2,3]triazole hybrids

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ABSTRACT

A series of novel 1,2,3-triazole-1,4-benzoxazine hybrids **5a**–**n** were efficiently synthesized employing click chemistry approach and evaluated for anti-proliferative activity against four cancer cell lines such as HeLa (cervical), MIAPACA (pancreatic), MDA-MB-231 (breast) and IMR32 (neuroblastoma). Compounds **5n** and **5g** exhibited promising anti-proliferative activity with $GI_{50}$ values ranging from **1.2** to **2.5** μM and **0.1**–**1.1** μM respectively against all cell lines, like HeLa, MDA-MB-231, MIAPACA and IMR32, while compound **5l** showed significant activity against MDA-MB-231 and IMR32 with $GI_{50}$ values ranging from **1.1** and **1.4** μM. This is the first report on the synthesis and *in vitro* anti-proliferative evaluation of 1,2,3-triazole-1,4-benzoxazine hybrids.



## 1. Introduction

The 2*H*-1,4-benzoxazin-3-(4*H*)-one scaffold has been extensively studied as an important heterocyclic system for building natural and designed synthetic compounds [1]. The 2*H*-1,4-benzoxazin-3-(4*H*)-ones and 3,4-dihydro-2*H*-1,4-benzoxazines are frequently utilized as suitable scaffolds for the design of biologically active compounds, having various properties such as antibacterial [2], bacterial histidine protein kinases [3], for treating infections caused by *Mycobacterium* [4], for treating cardiovascular disease, myocardial necrosis or arrhythmia [5], peroxisome proliferator activated receptor (PPAR) agonist, diabetes, hyperlipidaemia[6], neuroprotectants [7], inhibitors of nitric oxide synthase, inflammatory, autoimmune, cardiovascular disorders [8,9], inhibitors of the coagulation serine proteases [10], anxiety and depression [11].

In general, 1,2,3-triazoles have received attention not only in organic chemistry but also in medicinal chemistry due to their easy synthesis by click chemistry bearing attractive features as well as numerous biological activities [12–16]. In particular, by combining 1,2,3-triazoles with other pharmacophores *via* click chemistry, a number of compounds with potent antitumour activity were synthesized. For example, a series of 1,2,3-triazole bearing podophyllotoxins proved more potent than etoposide in selected human cancer cell lines [17], a library of 1,2,3-triazole analogues of combretastatin A-4 displayed potent cytotoxic activity against several cancer cell lines with $IC_{50}$ values in nano-molar range [18], a family of bifunctional hybrids of 1,2,3-triazole-tethered β-lactam-chalcones exhibited moderate to good cytotoxic activity [19] and *N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)arylamide was identified as a novel and proprietary antitumour molecule with $IC_{50}$ of 46 μM against MCF-7 cancer cell line [20].

As shown in Fig. 1, compound (I) and (II) is a potential antibacterial agent and also acts as an inhibitor of bacterial histidine protein kinase [2,4]. The compound (III) possesses D2 receptor antagonistic activity and is a potential antipsychotic agent [21]. 1,4-Benzoxazinone (IV) inhibits the coagulation serine proteases factor Xa, thrombin and factor VIIa [10], and 1,4-benzoxazinone (VI) is a potential agent for treating anxiety and depression [11]. Myocardial necrosis or arrhythmia and 1,4-benzoxazine derivative (V) possesses peroxisome proliferator activated receptor (PPAR) agonist activity and could be used in treating diabetes, hyperlipidaemia and other diabetic complications [6].

As shown in Fig. 2, compound (a) is an aromatase inhibitor which could reduce the growth stimulatory effect of oestrogen-dependent breast cancer [22], compound (b) effectively inhibited

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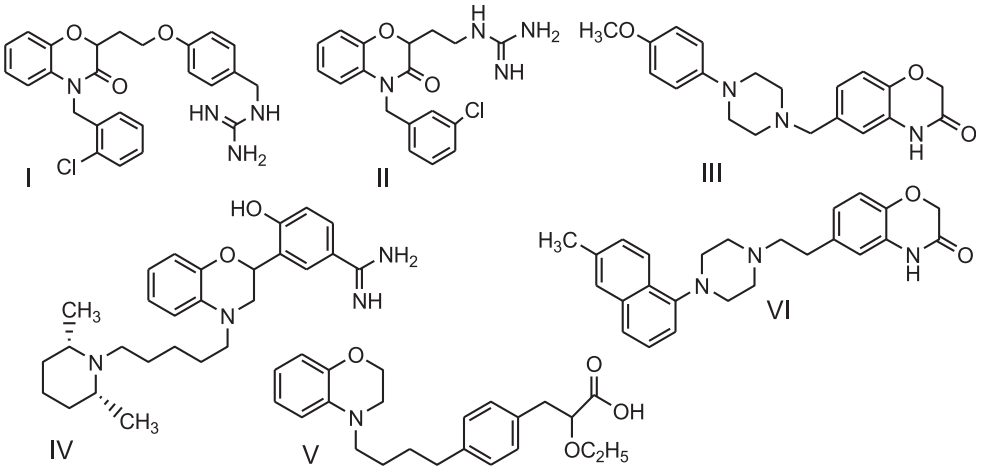


**Fig. 1.** Representative examples of biologically active 1,4-benzoxazine derivatives.

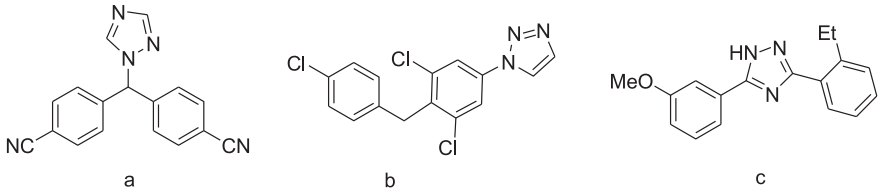


**Fig. 2.** Triazole based under clinical trial cancer therapy agents.

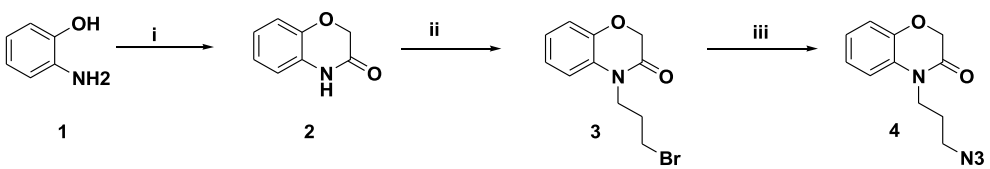
epithelia proliferation [23], while contragestazol (c) exhibited outstanding effect on the suppression of Oophoroma cells [24]. All these compounds are under clinical trials for cancer therapy.

Considering the above facts, it is of our interest to integrate both 1,4-benzoxazine and triazole pharmacophore units in one molecular platform to generate a newer scaffold for biological evaluation [25–30]. These 1,2,3-triazoles were efficiently prepared through Cu(I) catalysed click chemistry. In continuation to our ongoing research activities [31–38], to discover and develop tumour growth inhibitors and apoptotic inducers as potential new anti cancer-agents, we herein report an efficient method for the synthesis of novel 1,4-benzoxazine-1,2,3-triazole hybrids **5a**–**n** in excellent yields. The synthesized hybrids **5a**–**n** were evaluated for their *in vitro* anti-proliferative activity against four human cancer cell lines such as HeLa (cervical), MIAPACA (pancreatic), MDA-MB-231 (breast) and IMR32 (neuroblastoma) using an SRB cell proliferation assay to estimate the viability or growth. Significantly, the compounds **5n** and **5g** showed promising anti-proliferative activity with $GI_{50}$ values ranging from **1.2** to **2.5** μM and **0.1**–**1.1** μM respectively against all cell lines, like HeLa, MDA-MB-231, MIAPACA and IMR32 human cancer cell lines.

## 2. Results and discussion

### 2.1. Chemistry

The synthesis of the desired 4-(3-azidopropyl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-ones was performed in three steps starting from amino phenol using 1,3-dibromo propane and sodium azide as intermediates. The first synthetic step involved the subsequent acylation of aminophenol with TEBA, $NaHCO_3$ and chloroacetyl chloride in chloroform, about 12 h and "*in situ*" cyclization delivered benzoxazinone **2** [39]. In the presence of $K_2CO_3$ and catalytic amount of TBAI, a secondary amine **2** was alkylated with 1,3-dibromo propane in DMF solution about 1 h to give the corresponding tertiary amine **3** under room temperature conditions, in good yields. Organic azide **4** were generated *in situ* by reacting alkyl bromide **3** with $NaN_3$ at 120 °C in DMF solution for 12 h, and subsequently, copper salt, sodium ascorbate, and alkynes (procured from commercial sources) were added without isolating the intermediate organic azides (Scheme 1). Finally, using a one-pot protocol, 1,4-benzoxazin-1,2,3-triazole hybrids **5a**–**n** were



***Reagent & conditions***: i) TEBA, $NaHCO_3$, Chloroacetyl chloride, $CHCl_3$,12 h, reflux, 55°C ii) 1,3-dibromo propane, TBAI, $K_2CO_3$, DMF, RT, 1 h, iii) $NaN_3$, TBAB, DMF, 12 h, reflux, 120°C

**Scheme 1.** Synthesis of 1,4-benzoxazine azide **4**.