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Potential anti-proliferative agents from 1,4-benzoxazinone-quinazolin-4(3*H*)-one templates

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ABSTRACT

A novel synthetic protocol has been developed for the synthesis of 1,4-benzoxazinone-acetylphenylallyl quinazolin-4(3*H*)-one hybrids **7a**–**n** by employing Pd-catalyzed C—H arylation in presence of 5–10% phosphine ligand in good to excellent yields and evaluated for their anti-proliferative activity against three cancer cell lines such as A549 (lung), HeLa (cervical), MDA-MB-231 (breast). Compounds **7d**, **7f**, **7l** and **7n** exhibited promising anti-proliferative activity with GI_{50} values ranging from 0.37 to 2.73 μM respectively against A549, HeLa, and MDA-MB-231, while compound **7f** showed significant activity against MDA-MB-231 with GI_{50} value 0.58 μM, **7j** showed significant activity against A549 with GI_{50} value 0.32 μM and **7l** showed significant activity against HeLa with GI_{50} value 0.37 μM. This is the first report on the synthesis and *in vitro* anti-proliferative evaluation of 1,4-benzoxazinone-acetylphenylallyl quinazolin-4(3*H*)-one hybrids.

Research and development of potent and effective anticancer agents represents one of the most important advances in therapeutics, not only in the control of serious infections, but also in the prevention and treatment of some infectious complications of therapeutic modalities such as cancer chemotherapy, surgery and hospital acquired infections. The majority of pharmaceuticals and biologically active agrochemicals are heterocyclic while countless additives and modifiers used in industrial applications ranging from cosmetics, reprography, information storage and plastics are heterocyclic in nature.[1] Nitrogen containing heterocyclic compounds have maintained the interest of researchers through decades of historical development of organic synthesis. Among the nitrogen heterocycles, 1,4-benzoxazinone is the important scaffold present in various agrochemicals and primarily used by plants as natural defense chemicals.[2,3]

In particular, the utility of the 1,4-benzoxazin-3-(4*H*)-one scaffold as a privileged structure for the generation of drug-like libraries in drug-discovery programs has been amply demonstrated. Benzoxazinone based compounds form an important class of benzofused heterocycles with a wide spectrum of biological activities.[4] Many compounds are in the development phase as potential new drugs acting against different targets. In particular, as shown in Fig. 1 compound (**I**) exhibited anti-proliferative activity against a wide range of human tumor cell lines with GI_{50} mean values at low micromolar level. Compound (**II**) Cappamensin A, was isolated from the roots of *Capparis sikkimensis* displayed significant *in vitro* antitumor activity in various human cell lines[5] and both the derivatives of compound (**III**) are potential non-nucleoside SGLT2 inhibitors for the treatment of type 2 diabetes.[6] Compound SLV-314 (**IV**) was evaluated on binding to dopamine D2 receptors and serotonin reuptake sites. These classes of compounds proved to be potent *in vitro* dopamine D2 receptor antagonist and in addition were highly active as serotonin reuptake inhibitors[7] and a series of benzoxazine-rhodanine (**V**) have been developed as potent inhibitors of $PI3K_{\gamma}$ in enzymatic and cell based assays.[8] The compound (**VI**) displayed significant anticonvulsant activity.[9] Moreover, benzoxazinone derivatives are also known to exhibit other activities such as anti-inflammatory,[10] antiulcer,[11] antipyretic,[12] antifungal,[13] potassium channel modulators,[14] anti-rheumatic agents,[15] and plant resistance factors against microbial diseases and insects.[16]

Heck cross coupling reaction has become powerful method for the clean construction of innumerable class of chemical architectures. It has emerged as a powerful tool for the construction of carbon-carbon double bonds. It is extensively used in the area of small molecule, natural product and polymer synthesis.[17–23] In this regard, a direct C—H functionalization of the allylquinazolinones

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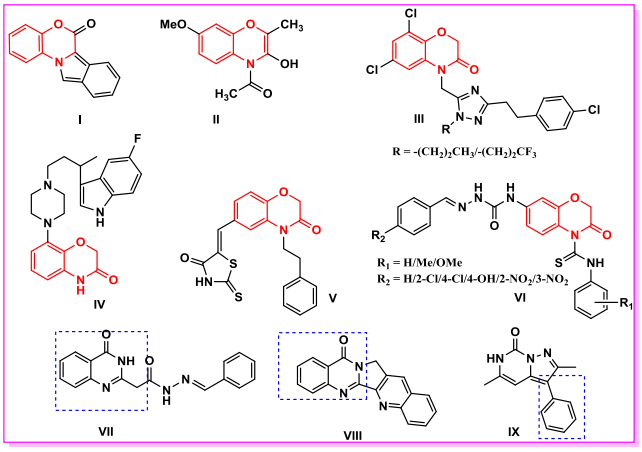


Fig. 1. Structures of 1,4-benzoxazinone, quinazolinone and Pd-coupling based bioactive compounds.

would be a more desirable alternative. Direct C—H arylation protocol has been developed as an attractive, atom-economical alternative to avoid unwanted side products.

Here, we envisioned the use of coupling reactions through C—H activation by utilizing a Pd catalyst and successfully synthesized the benzoxazinone bearing quinazolinone class antiproliferative agents. In this paper, we reveal the palladium catalyzed intermolecular C—H arylation as a key step from 4-(2-(4-bromophenyl)-2-oxoethyl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one to synthesize 1,4-benzoxazine-acetylphenylallyl quinazolin-4(3*H*)-one hybrids as a new class of anti proliferative agents.

As shown in Fig. 1 Compound (**VII**), which is a quinazolinone derived Schiff's base, was designed and synthesized as novel antioxidant and anti-inflammatory agent.[24] The cytotoxic alkaloid Luotonin A (**VIII**) and its derivatives infused with quinazolinone moiety are clinically proven anti-cancer agents[25] and compound (**IX**) a novel precursor for MJL1-109-2, a known nonpeptide CRHR-1 antagonist, was successfully synthesized employing Pd-catalyzed C—H arylation (Fig. 1).[26]

Molecular hybridization, which covalently combines two or more drug pharmacophores into a single molecule, is an effective tool to design highly active novel entities. The clubbed pharmacophores may act on multiple therapeutic targets and offer the advantage of overcoming inevitable drug resistance.[27] A literature survey revealed that modification on quinazolinone pharmacophore may result in increase of its biological potencies. Considering the above facts, it is of our interest to integrate both 1,4-benzoxazinone and allyl quinazolinone pharmacophore units in one molecular platform to generate a newer scaffold for anti-proliferative evaluation. Inspired by the applications of quinazolinone derivatives in the field of medicinal chemistry, especially in the treatment of cancer, we aimed to develop novel benzoxazinone bearing quinazolinone derivatives as antitumor agents by Heck-cross coupling. In continuation to our ongoing research activities,[28a–g] to discover and develop tumor growth inhibitors and apoptotic inducers as potential new anti cancer-agents, we herein reported an efficient method for the synthesis of novel 1,4-benzoxazine-acetylphenylallyl quinazolin-4(3*H*)-one hybrids **7a-n** in good to excellent yields (Fig. 2) and evaluated for their *in vitro* anti-proliferative activity against three human cancer cell lines such as A549 (lung), HeLa (cervical) and MDA-MB-231 (breast) using a SRB cell proliferation assay to estimate the viability or growth.

One of the key intermediate 4-(2-(4-bromophenyl)-2-oxoethyl)-2*H* benzo[*b*][1,4] oxazin-3(4*H*)-one (**2**) was accomplished by reaction of 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one with

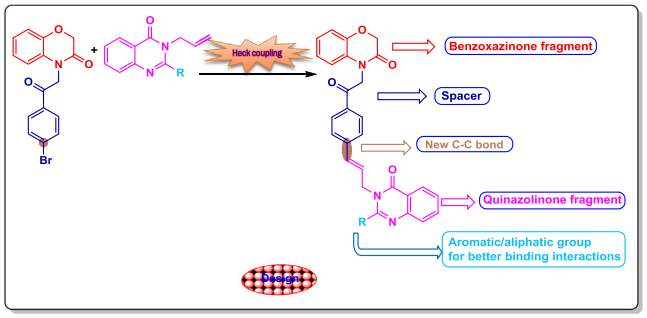
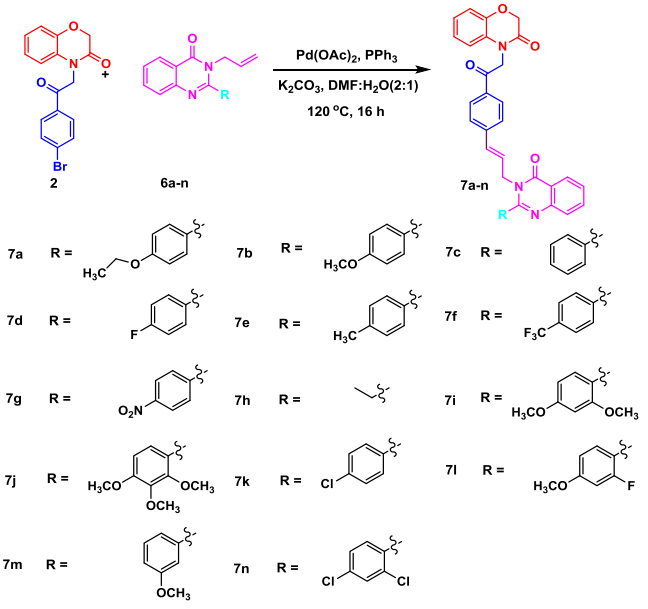


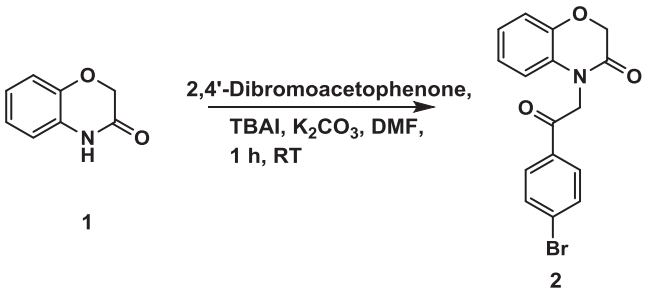
Fig. 2. Design strategy and anti-proliferative evaluation of new analogues **7a–n** against A549, HeLa and MD-MB-231.

2,4′-dibromo acetophenone. The synthetic step involved in the presence of K_2CO_3 and catalytic amount of TBAI, a secondary amine **1** was reacted with 2,4′-dibromo acetophenone in DMF solution about 1 h to give the corresponding tertiary amine **2** under room temperature, in good yield (Scheme 1). 3-Allyl-2-phenylquinazolin-4(3*H*)-one, another key fragment for the synthesis of these novel quinazolinone analogues, was synthesized by the condensation of anthranilamide (**3**) with various benzaldehydes (**a–n**) in presence of phase transfer catalyst TBAHS in methanol at 80 °C for 2 h to afford 2-phenyl-2,3-dihydro quinazolinones **4a–n** and subsequently in the second step compounds **4a–n** were refluxed with $KMnO_4$ in acetone at 56 °C for 1 h, to give compounds **5a–n**. Then it was followed by the reaction with allylbromide in presence of K_2CO_3 at room temperature for 8 h to yield 3-allyl quinazolinone derivatives **6a–n** (Scheme 2).

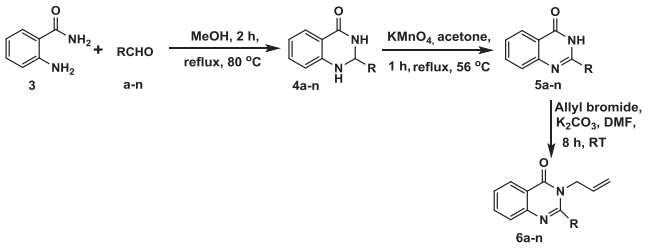
Synthesis of targeted 1,4-benzoxazinone-acetylphenylallyl quinazolin-4(3*H*)-one hybrids **7a–n** were achieved by Pd catalyzed Heck-cross coupling reaction [$Pd(OAc)_2$, PPh_3, K_2CO_3, DMF:H_2O (2:1), at 120 °C for 16 h] of 4-(2-(4-bromophenyl)-2-oxoethyl)-2*H* benzo[*b*][1,4]oxazin-3(4*H*)-one (**2**) with substituted 3-allylquinazolinones **6a–n** (Scheme 3). With the compound 4-(2-(4-bromophenyl)-2-oxoethyl)-2*H* benzo[*b*][1,4]oxazin-3(4*H*)-one (**3**) in hand, we carried out an intermolecular coupling reactions with substituted allylquinazolinones through C—H arylation.



Scheme 1. Synthesis of 4-(2-(4-bromophenyl)-2-oxoethyl)-2*H*-benzo[*b*] [1,4]oxazin-3(4*H*)-one (**2**).



Scheme 2. Synthesis of 3-allyl quinazolin-4(3*H*)-ones **6a–n**.



Scheme 3. Synthesis of 1,4-benzoxazinone-acetylphenylallyl quinazolin-4(3*H*)-one hybrids **7a–n**.

The synthesized 1,4-benzoxazinone-acetylphenylallyl quinazolin-4(3*H*)-one hybrids **7a–n** were confirmed on the basis of their spectral data. In ^{1}H NMR spectra, the characteristic doublet signal appeared for *trans* proton **7a-n** in the range of δ 4.93–6.47 ppm with coupling constant 15.8–17.8 Hz with disappearance of *cis* proton doublet. The structures for all these compounds were further confirmed by HRMS analysis. For instance, **7a** displayed a molecular ion peak at *m*/*z* 572.21800 $[M+H]^+$ suggesting the molecular formula of $C_{35}H_{29}O_5N_3$. Additionally, the IR spectra for the target compounds **7a–n** exhibited characteristic absorption bands at 3000–3100 cm^{-1}, 1662–1680 cm^{-1}, 1500 cm^{-1} and 1050 cm^{-1} which corresponded to =C—H, C=O, C=C, and C—N respectively.

The *in vitro* anti-proliferative activity of the designed compounds **7a–n** were evaluated against a panel of three different human cancer cell lines, A549 (lung), HeLa (cervical) and MDA-MB-231 (breast) summarized in Table 1. The compounds were picked for an advanced assay against these three human cancer cell lines at five different concentrations (0.01, 0.1, 1, 10, 100 μM). GI_{50} (growth inhibitory activity) was calculated and these values corresponded to the concentration of the compound causing 50% decrease in the net cell growth as compared to the standard drugs, Nocodazole and Combretastatin. Results were calculated for each of these parameters if the level of activity was reached; however, if the effect was not achieved, the value was expressed as greater or less than the maximum or minimum concentration tested.

Table 1
[a]GI_{50} values of the tested compounds against three human cancer cell lines.

Compound	A549	HeLa	MDA-MB-231
7a	1.0 ± 0.01	1.05 ± 0.02	1.13 ± 0.02
7b	1.5 ± 0.02	1.03 ± 0.02	3.6 ± 0.09
7c	1.0 ± 0.01	1.37 ± 0.05	13.4 ± 1.9
7d	0.81 ± 0.01	1.02 ± 0.01	1.52 ± 0.06
7e	0.9 ± 0.03	1.4 ± 0.05	3.4 ± 0.03
7f	0.93 ± 0.02	1.32 ± 0.02	**0.58 ± 0.02**
7g	1.0 ± 0.04	0.66 ± 0.04	8.8 ± 0.6
7h	0.91 ± 0.01	0.88 ± 0.04	6.14 ± 0.3
7i	1.36 ± 0.1	0.59 ± 0.05	6.03 ± 0.42
7j	**0.32 ± 0.01**	1.51 ± 0.01	6.76 ± 0.15
7k	0.76 ± 0.03	1.1 ± 0.09	2.0 ± 0.07
7l	0.67 ± 0.03	**0.37 ± 0.04**	2.4 ± 0.05
7m	0.61 ± 0.01	0.55 ± 0.04	13.0 ± 1.7
7n	0.91 ± 0.01	0.62 ± 0.01	2.73 ± 0.02
Nocodazole[b]	**<0.01**	**<0.01**	**<0.01**
Combretastatin[b]	**0.056 ± 0.001**	**0.084 ± 0.002**	**0.046 ± 0.001**

[a] GI_{50}: 50% Growth inhibition, concentration of drug (in μM) resulting in a 50% reduction in net protein increase compared with control cells.
[b] Positive controls.

Based on Table 1, the synthesized compounds **7a–n** showed significant to moderate cancer cell growth inhibition with GI_{50} values ranging from 0.32 to 13.4 μM. In particular, Compounds **7d**, **7f**, **7l** and **7n** exhibited promising anti-proliferative activity with GI_{50} values ranging from 0.37 to 2.73 μM respectively against all cell lines, like A549, HeLa, and MDA-MB-231. It was observed that, modification on quinazolinone pharmacophore [NO_2, CF_3, 2,4-dimethoxy, trimethoxy, 2-F, 4-OCH_3, 3-OCH_3, 2,4-DiCl] was associated with a significant increase in the growth inhibitory effect against A549, HeLa, and MDA-MB-231 human cancer cell lines.

In conclusion, we have developed a novel and efficient protocol for the synthesis of 1,4-benzoxazinone-acetylphenylallyl quinazolin-4(3*H*)-one hybrids **7a–n** under palladium catalyst. This sequence tolerates a wide range of substituted allyl quinazolinones, which proceed very cleanly, and provide the title compounds in good to excellent yields and the entire series of synthesized derivatives were evaluated for anti-proliferative activity against three different human cancer cell lines, namely A549, HeLa and MDA-MB-231 (lung, cervical and breast cancer respectively). In general, the majority of target compounds displayed moderate to promising activity against the tested cancer cell lines. Based on the activity results, compound **7f** showed significant activity against MDA-MB-231 with GI_{50} value 0.58 μM and **7j** showed significant activity against A549 with GI_{50} value 0.32 μM and **7l** showed significant activity against HeLa with GI_{50} value 0.37 μM. We succeeded in the modification on quinazolinone moiety along with 1,4-benzoxazinone and double bond linker which played a crucial role in exhibiting promising anti-proliferative activities. In light of the above, these studies might provide insights to develop new drug leads in the pursuit of more effective anti-proliferative agents.

Acknowledgements

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A. Supplementary data

Experimental section and copies of the ^{1}H, ^{13}C NMR and ESI-MS spectra for some of the important compounds. Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2017.10.044.

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General procedure for synthesis of 1,4-benzoxazinone-acetylphenylallyl quinazolin-4(3H)-one hybrids 7a–n by Heck reaction:

To a suspension of compound allylquinazolinones (**6a**–**n**, 1.1 mmol), 4-bromobenzoxazinone (**2**) (1 mmol), K_2CO_3 (1.2 mmol), PPh_3 (0.1 mmol) and $Pd(OAc)_2$ (0.1 mmol) in DMF:H_2O (2:1, 200 mL) was refluxed for 16 h at 120 °C. Then the solvent was evaporated under *vacuum*. The residue obtained was extracted with diethyl ether and water. The combined organic phases was washed with water, dried over sodium sulphate and evaporated under *vacuum*. The residue obtained was purified over column chromatography over silica gel and recrystallized from ethanol to afford the following compounds.

2-(4-Ethoxyphenyl)-3-((E)-3-(4-(2-(2,3-dihydro-3-oxobenzo [b] [1,4]oxazin-4-yl) acetyl) phenyl)allyl) quinazolin-4(3H)-one (***7a***):

Light brown solid (75% yield): m.p: 120 °C; IR (KBr, *ν*): 3451, 2927, 1680, 1601, 1500, 1469, 1398, 1282, 1233, 1109, 1050, 979, 753, 697, 478 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.25 (t, *J* = 13.8 Hz, 3H, CH_3), 4.00–4.09 (m, 2H, CH_2), 4.45–4.49 (m, 1H, N-CH_2), 4.72 (s, 2H, N-CH_2), 5.01–5.06 (m, 1H, N-CH_2), 5.3 (s, 2H, CH_2-O), 6.08 (d, *J* = 16.0 Hz, 1H, *trans*-H), 6.28–6.33 (m, 1H, *allylic*-H), 6.59 (d, *J* = 7.6 Hz, 1H, Ar-H), 6.91–7.09 (m, 5H, Ar-H), 7.34–7.37 (m, 3H, Ar-H), 7.48–7.56 (m, 2H, Ar-H), 7.76–7.81 (m, 2H, Ar-H), 7.93 (d, *J* = 8.3 Hz, 2H, Ar-H), 8.38 (d, *J* = 7.9 Hz, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.3, 47.7, 48.1, 67.3, 114.5, 117.0, 117.2, 122.7, 124.0, 126.7, 126.8, 127.3, 127.7, 129.1, 129.4, 131.8, 132.2, 133.1, 134.2, 140.0, 145.0, 156.3, 161.9, 165.0, 191.0; MS (ESI): *m/z* 572 $[M+H]^+$; HRMS: Calcd for $C_{35}H_{29}O_5N_3$: 572.21800, Found: 572.21695.