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Quinazolinones–Phenylquinoxaline hybrids with unsaturation/saturation linkers as novel anti-proliferative agents

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ABSTRACT

A new series of novel quinazolinones with allylphenyl quinoxaline hybrids **9a**–**n** were efficiently synthesized in good yields by the reaction of 3-allyl-2-methylquinazolin-4(3*H*)-one (**5a**–**n**) with bromophenyl) quinoxaline (**8**) utilizing Pd catalyzed Heck-cross coupling 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 **9a**, **9e**, **9g** and **9h** exhibited promising anti-proliferative activity with GI_{50} values ranging from **0.06** to **0.2** μM against four cell lines, while compounds **9e** and **9k** showed significant activity against HeLa and MIAPACA cell lines and compounds **9b**, **9d**, **9h** and **9j** showed selective potency against IMR32 and MDA-MB-231 cell lines. This is the first report on the synthesis and in vitro anti-proliferative evaluation of *E*-2-(4-substituted)-3-(3-(4-(quinoxalin-2-yl)phenyl)allyl) quinazolin-4(3*H*)-ones (**9a**–**n**). Docking results indicate a sign of good correlation between experimental activity and calculated binding affinity (dock score), suggesting that these compounds could act as promising DNA intercalates.

The quinazolinone moiety containing natural products (Luotonin, Rutaecarpine, Tryptanthrin, Chloroqualone, Alloqualone, etc.) represent the medicinally and pharmaceutically important class of compounds,[1,2] because of their diverse range of biological activities such as anti-cancer, diuretic, anti-inflammatory, anti-convulsant and anti-hypertensive[3–6] activity. In recent years, quinazolinone embedded numerous natural products have been identified.[7–15] The cytotoxic alkaloid Luotonin A and its derivatives infused with quinazolinone moiety are clinically proven as anti-cancer agents (Fig. 1).[16–24] Previous studies have clearly demonstrated that Luo functions as DNA topoisomerase-I poison.[25] Considering the potent bioactivities of the compounds possessing Luo pharmacophore, we were interested to synthesize novel Luo analogues and evaluated their anti-cancer activities such as cytotoxicity, cell cycle regulation and mechanistic aspects.

A hybrid anti-cancer agent, which combines different heterocyclic compounds in one, which increases the cytotoxicity and enhances specificity was recently demonstrated.[26] The initial study proved that pyrroloquinoline nucleus leads to the selective inhibition of tumor growth.[27–32] Recently, as part of our on-going programme to discover and develop tumor growth inhibitors and apoptosis inducers as a potential new anti-cancer agents, we have identified several classes of molecules that includes 1*H*-pyrazole-5-carboxylic acid, 1*H*-pyrrolo[3,4-*b*]quinolin-3(2*H*)-one, analogues of (+)-varitriol, novel derivatives of benzosuberones with thiazolidine-2,4-diones and fused coumarin moieties.[33] In the present investigation, we have discovered a novel quinazolinones bearing allylphenyl quinoxaline hybrids, by employing Heck-cross methodology to elicit combined anti-tumour efficacy/cytotoxicity against different cancer cell lines in vitro (HeLa, MIAPACA, MDA-MB-231and IMR32). Significantly, the compounds **9k**, **9h** have shown promising cytotoxicity against the HeLa cancer cell lines with GI_{50} values of 0.06 and 0.2 μM, respectively and the compounds **9a**, **9g**, **9k** and **9h** displayed the most potent selectivity against HeLa and MDA-MB-231 cell lines with GI_{50} values of 0.08 and 0.4 μM, respectively. Quinazolinone derivatives are reported as anti-cancer agents and many of them showed good DNA intercalation.[34,35] To assess the possible intercalating potency of the synthesized compounds a molecular docking studies were performed using DNA structure obtain from PDB. The protein structure bound to respinomycin D was downloaded, as it belongs to anthracycline family of antitumor antibiotics. Since doxorubicin also belongs to the same family it has been taken as standard.

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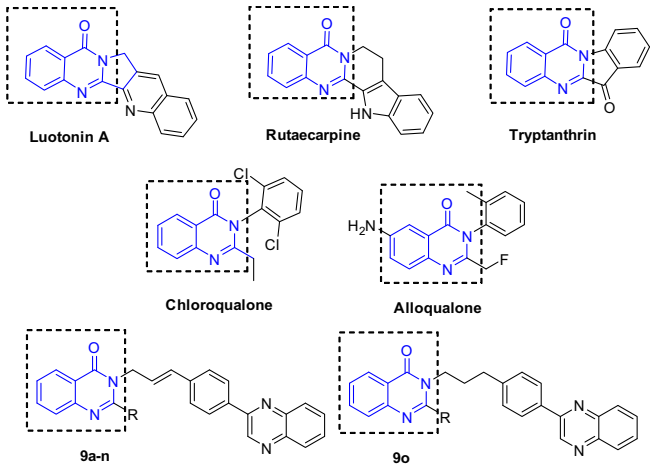


Figure 1. Quinazolinone scaffold containing natural products and its designed conjugates (**9a**–**n**, **9o**).

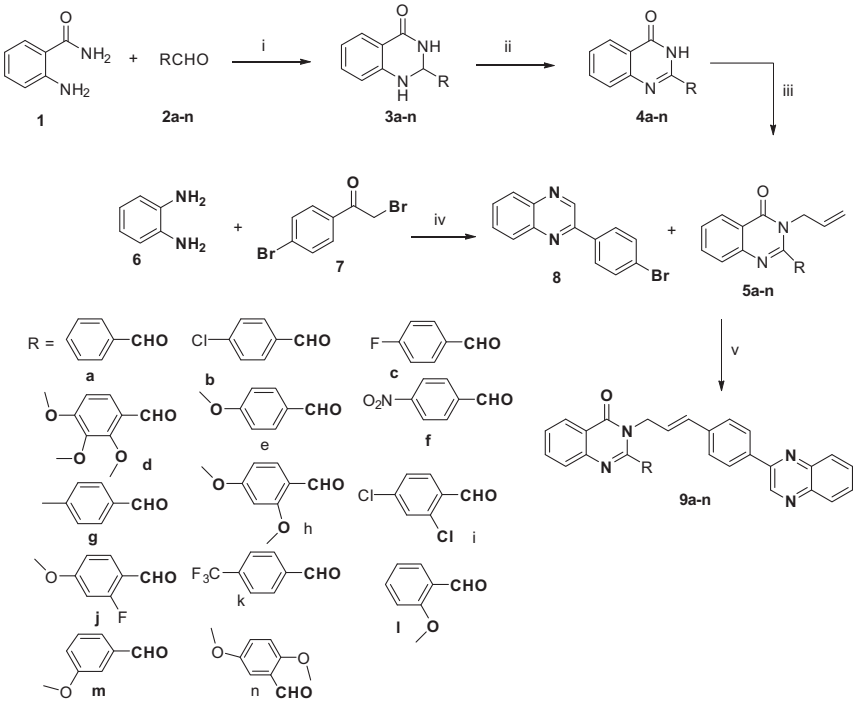
The clinical agent doxorubicin is well-studied of this class but has a relatively simple molecular architecture in which the pendant daunosamine sugar residue is in the DNA minor groove. The compounds **9a**–**9o** were evaluated in silico (docking) to identify their hypothetical binding mode using the NMR structure of the respinomycin D intercalation complex with a double stranded DNA molecule (AGACGTCT)2 complex in solution[36] derived from NOE restraints and molecular dynamics simulations.

Synthesis of targeted (*E*)-2-(4-substituted)-3-(3-(4-(quinoxalin-2-yl)phenyl)allyl)quinazolin-4(3*H*)-ones (**9a**–**n**) were achieved by Pd catalyzed Heck-cross coupling reaction [$Pd(OAc)_2$, PPh_3, K_2CO_3, DMF/H_2O (8:2), at 120 °C for 4 h] of halo-qunaxolines with substituted 3-allylquinazolinones. The 2-(4-bromophenyl)quinoxaline (**8**) was readily prepared by the reaction of *ortho*-phenylene diamine (**6**) with various phenacyl bromides (**7**) in presence of catalytic amounts of Zr/WO_3 at 80 °C. It was further coupled with 3-allylquinazolinone derivatives **5a**–**n**; which were obtained by the reaction of various benzaldehydes (**2a**–**n**) with anthranilamide (**1**) in presence of phase transfer catalyst TBAHS in methanol, followed by oxidation of 2-phenyl-2,3-dihydro qunazolinones (**3a**–**n**) and allylation with allylbromide in presence of K_2CO_3 at room temperature for 2-phenyl quinazolin-4(3*H*)-ones (**4a**–**n**) (Scheme 1).

Under similar Heck-coupling conditions [$Pd(OAc)_2$, PPh_3, K_2CO_3, DMF/H_2O (8:2), 120 °C for 4 h] when 2-(4-bromophenyl)quinoxaline (**8**) was reacted with 3-allyl-2-methylquinazolin-4(*H*)-one (**5**) saturated 2-methyl-3-(3-(4-(quinoxalin-2-yl)phenyl)propyl) quinazolin-4(3*H*)-one (**9o**) was obtained with an yield of 67% (Scheme 2).

All the newly synthesized compounds were characterized by using ^{1}H NMR, ^{13}C NMR, IR and mass spectrometry. Spectral data of all synthesized compounds were in good agreement with the proposed structures. IR spectrum revealed the presence of C=O bond (1680–1700 cm^{-1}) and CH (2928–2934 cm^{-1}) functional groups of compounds **9a**–**n**, which were further confirmed by ^{1}H NMR. The characteristic proton of quinoxaline displayed at δ 9.0–9.3 ppm and double bond protons appeared as a multiplet at δ 6.50–6.30 ppm with coupling constant between 17 and 16 Hz. Compound **9o** indicating the reduction of double bond showed two additional triplets and one multiplet, one triplet at δ 4.16–4.01 ppm, another triplet at δ 2.8–2.6 ppm and one proton appeared as a multiplet at δ 2.2–2.1 ppm. The molecular formula of all the synthesized compounds was confirmed by HRMS data analysis. For instance, **9a** displayed a molecular ion peak at *m*/*z* 467.18664 $[M+H]^+$ suggesting the molecular formula of $C_{31}H_{23}ON_4$.

Effects of the compounds on the viability of human cancer cells: The in vitro anti-proliferative activity of the designed compounds **9a**–**n** was evaluated against four different human cancer cell lines, HeLa (cervical), MIAPACA (pancreatic), MDA-MB-231 (breast) and IMR32 (neuroblastoma) summarized in Table 1. The compounds were picked up for an advanced assay against these four human



Scheme 1. Synthesis of (*E*)-2-(4-substituted)-3-(3-(4-(quinoxalin-2-yl)phenyl)allyl)quinazolin-4(3*H*)-ones (**9a**–**n**). *Reagents and conditions:* (i) MeOH, reflux, 2 h; (ii) $KMnO_4$, acetone, reflux, 1 h; (iii) allyl bromide, K_2CO_3, DMF, rt, 8 h; (iv) Zr/WO_3, 80 °C, 30 min; (v) $Pd(OAc)_2$, PPh_3, K_2CO_3, DMF/H_2O (8:2), 120 °C, 4 h.