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Bioorganic & Medicinal Chemistry Letters 17 (2007) 208–213
Bioorganic & Medicinal Chemistry Letters

2-Arylidenedihydroindole-3-ones: Design, synthesis, and biological activity on bladder carcinoma cell lines

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Received 29 August 2006; revised 19 September 2006; accepted 20 September 2006
Available online 16 October 2006

Abstract—2-Arylidenedihydroindole-3-ones were assayed for their antiproliferative and apoptotic abilities as potential drug candidates to treat bladder tumor. These compounds were tested on cell lines obtained from bladder tumors of various stages [superficial (pTa and pT1) *vs.* invasive (⩾pT2)]. The most active compound (**3c**) inhibited the proliferation, induced apoptosis, and decreased the expression of *p*-Stat5 and *p*-Pyk2 in DAG-1 and RT112 lines in which the FGFR3 is either mutated or overexpressed. Knowing that FGFR3 is involved in cell proliferation, differentiation, and migration through cell signaling pathways including *p*-Stat5 way via *p*-Pyk2, let us assume that compound **3c** may probably act through FGFR3 pathway.

Bladder cancer is the fourth most common malignancy in men and the ninth most common in women in the Western world. More than 90% of bladder tumors are urothelial cell carcinomas (UCC).[1] The carcinoma can be classified in two development stages [superficial (pTa and pT1) vs invasive (⩾pT2)] and three grades (G1 to G3).[2] The clinical course of superficial (pTa and pT1) papillary urothelial cell carcinoma is characterized by a high risk of recurrence (>70%) and a propensity to progress in invasive tumors (10–15%). Muscle-invasive tumors (⩾pT2) have a poorer prognosis because 50% of these patients will relapse with metastatic disease within 2 years of treatment.[3–5]

Fibroblast growth factor receptors (FGFRs) are tyrosine kinase receptors that integrate many different intracellular signals affecting cell growth, differentiation, migration, and angiogenesis. Activation of FGFR3 induces phosphorylation residues in the intracellular domain and causes cellular proliferation and tumor development. Depending on the cellular context, these effects are activated through various signal transduction pathways.[6] FGFR3 can interact with various proteins such as SH2-B, *p*-Stat5 through *p*-Pyk2 (a member of the focal adhesin kinase family), and PI3kinase.[7–9] FGFR3 mutations and surexpression of wild type FGFR3 are consequently frequent events in low stage UCC and superficial bladder tumors.[10]

Currently, drugs available for treating UCC-related cancer need to be taken in high doses to be efficient. In this context, molecules targeting cells where FGFR3 is mutated or overexpressed can be of high interest as potential drug candidates for treating bladder carcinoma. In this regard, several molecules acting as FGFR3 inhibitors have been recently reported. Such inhibitors include: PKC412, benzimidazole quinolinones, derivatives of arylaminooxazole, CHIR-258, and imidazolidinediones.[11–15]

The naturally occurring flavonoids are known to either induce apoptosis or to modulate cell proliferation of UCC.[16] Flavonoid-rich nutrition is highly recommended for preventing bladder cancer. In earlier studies, it was demonstrated that aurones, a flavonoid-subgroup, were able to interact with ATP-binding site of P-glycoprotein

Keywords: 2-Arylidenedihydroindole-3-ones; Bladder carcinoma; Cell signaling; Antiproliferative.
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doi:10.1016/j.bmcl.2006.09.057

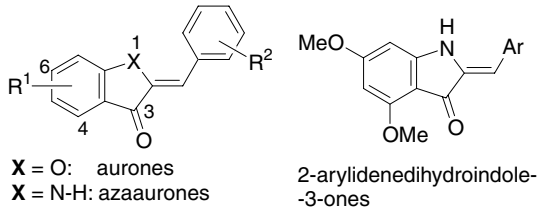
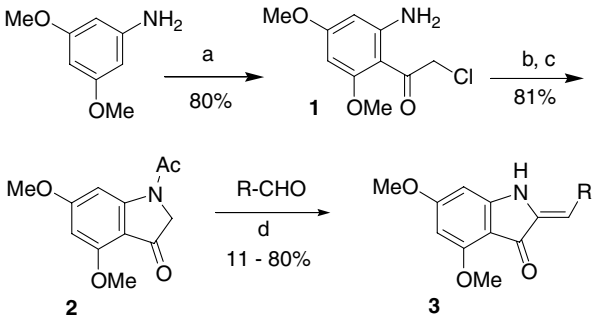


Figure 1. Structures of investigated molecules.



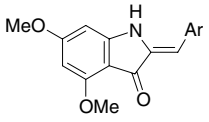
Scheme 1. Reactions and conditions: (a) Cl–CH_2–CN, $ZnCl_2$/BCl_3, CH_2Cl_2 (reflux) then HCl (2 N), reflux; (b) CH_3CO–Cl, 85 °C; (c) K_2CO_3, acetone, reflux; (d) KOH, H_2O/MeOH, 50 °C.

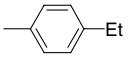
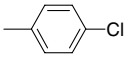
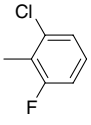
and to inhibit kinases.[17,18] Therefore, we tested aurone family members for their antiproliferative effect on UCC, because no prior studies had investigated them as agents for UCC treatment. Especially, we studied 2-arylidene-2,3-dihydro-1*H*-indole-3-ones (azaaurones) as structural analogs of the naturally occurring aurones (Fig. 1). In this series, we maintained the 4,6-dimethoxy-2,3-dihydro-1*H*-indole-3-one moiety and varied the arylidene group. The arylidene moieties were chosen on the basis of literature reports, where halogenated aryls and imidazoles were used as structural features in a number of kinase inhibitors.[19–22]

Azaaurones **3** were prepared from indolin-3-one **2** by base-catalyzed condensation with an arylaldehyde (Scheme 1). The condensation affords exclusively *Z*-azaaurones as confirmed by ^{1}H NMR.[23] Oxindole **2** was prepared starting from 3,5-dimethoxyaniline and chloroacetonitrile as previously reported.[24]

In this study, we have used three bladder tumour cell lines that mimic the bladder tumour progression process. These lines were obtained from bladder tumors of various grades and various stages (RT112 was from a pT1G2, DAG-1[25] from a pT1G3 and J82 from a pT2G3 tumors). DAG-1 cells featured the S249C mutation on exon 7 and J82 cells the K650E mutation on exon 15, whereas RT112 line overexpressed the wild type FGFR3.[26] Flow cytometry analysis showed a more important FGFR3 expression in RT112 (39 ± 2 a.u.) cells than in DAG-1 (28 ± 3 a.u.) and J82 (19 ± 4 a.u.) cells (data not shown). A preliminary screening using an in vitro test to assess the ability of a set of aurones

Table 1. The effect of azaaurones (at 10 μM) on cell viability in DAG-1, RT112, and J82 cell lines after 72 h incubation



Compound (10 μM)	Ar	Cell lines		
		DAG-1	RT112	J82
3a		98 ± 3	97 ± 3	99 ± 4
3b		99 ± 4	93 ± 3	94 ± 4
3c		58 ± 2	48 ± 3	97 ± 4
3d		91 ± 3	93 ± 5	95 ± 4
3e		90 ± 5	92 ± 3	89 ± 4
3f		89 ± 4	92 ± 4	90 ± 3
Control	Without compound	100 ± 3	100 ± 2	100 ± 2

Results are expressed in percentage of viable cells.