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Highly selective anthraquinone-chalcone hybrids as potential antileukemia agents

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ABSTRACT

A series of 23 novel anthraquinone-chalcone hybrids containing amide function was synthesized and structurally characterized. Sixteen compounds exerted strong cytotoxic activities against K562, Jurkat and HL-60 leukemia cell lines and significantly lower cytotoxic effects against normal MRC-5 cells, indicating very high selectivity in their anticancer action. The compounds **6g**, **6u** and **6v** activate apoptosis in K562 cells through the extrinsic and intrinsic apoptotic pathway. The compound **6e** triggered apoptosis in K562 cells only through the extrinsic apoptotic pathway. Treatment of K562 cells with each of these four compounds caused decrease in the expression levels of MMP2, MMP9, and VEGF, suggesting their anti-invasive, antimetastatic and antiangiogenic properties. The compounds **6g** and **6v** downregulated expression levels of miR-155 in K562 cells, while compounds **6e** and **6u** upregulated miR-155 levels in treated cells, in comparison with control cells. The structure-based 3-D QSAR models for **6f**, **6e**, **6i** and **6l** describe pro-apoptotic activity against caspase-3.

Anticancer chalcones inhibit various molecular targets like ABCG2, 5α-reductase, histone deacetylases, p53 degradation, angiogenesis, tubulin and kinases. Human ABCG2, a member of ATP-binding cassette (ABC) transporters having a protecting role in cancer stem cells was inhibited by chalcones bearing quinoxaline unit.[1] Shimizu et al. isolated geranylated chalcones from *Artocarpus incises* for effective blocking intracellular 5α-reductase to prevent proliferation of prostate cancer by arresting male androgen biosynthesis.[2] Coumarin-chalcone hybrids showed histone-deacetylase inhibitory activity when tested against leukemia K562 and U-937 cell lines.[3] Boronic-chalcones act as putative MDM2 antagonists interfering with p53-MDM2 interaction.[4] 4′-Hydroxy chalcone suppressed several steps of angiogenesis, including endothelial cell proliferation, migration and tube formation.[5] Matrix metalloproteinases-2/9, a family of enzymes that have ability to degrade many molecules of the extracellular matrix can be inhibited by prenylated chalcones containing hydroxyl groups on A or B ring.[6] 3,4,5-Trimethoxychalcones bind with microtubular protein tubulin and prevent its polymerization, which is essential phase for mitosis.[7] Chromenylchalcones successfully inhibit aurora kinase, a family of serine/threonine kinases, responsible for cell cycle control.[8]

Anthraquinone is well known scaffold in anthracycline drugs such as daunorubicin, doxorubicin, mitoxantrone and ametantrone that are widely used for the treatment of cancer.[9] They act as intercalators,[10] inhibitors of telomerase,[11] DNA topoisomerase II[12] and ROS inducers in a redox cycle system by the presence of quinone intermediates.[13] The natural anthraquinone emodin induces apoptosis in human acute promyelocytic leukemia HL-60 cells through activation of the caspase-3 cascade, but independent of ROS production.[14]

Although anthracyclines are effective against a wide variety of cancer cells, their clinical use is reduced due to low selectivity and high cardiotoxicity.[9] Incorporating anthraquinone ring in a chalcone system combined with an aromatic amide function, we tried to obtain hybrid molecules with better selectivity and synergistic or additive pharmacological properties. Thus, a series of new anthraquinone-chalcone hybrids having amide function were synthesized and screened for their anticancer and antidiabetic activity.

The synthesis of novel anthraquinone-chalcone hybrids containing amide function **6a–w** is presented in Scheme 1, while experimental details are provided in Supplementary material. The commercially available anthracene **1** was acylated using acetyl chloride to 1-acetylanthracene **2**[15] which was then oxidized by chromium (VI) oxide in glacial acetic acid yielding 1-acetylanthraquinone **3**.[16] The ketone **3**

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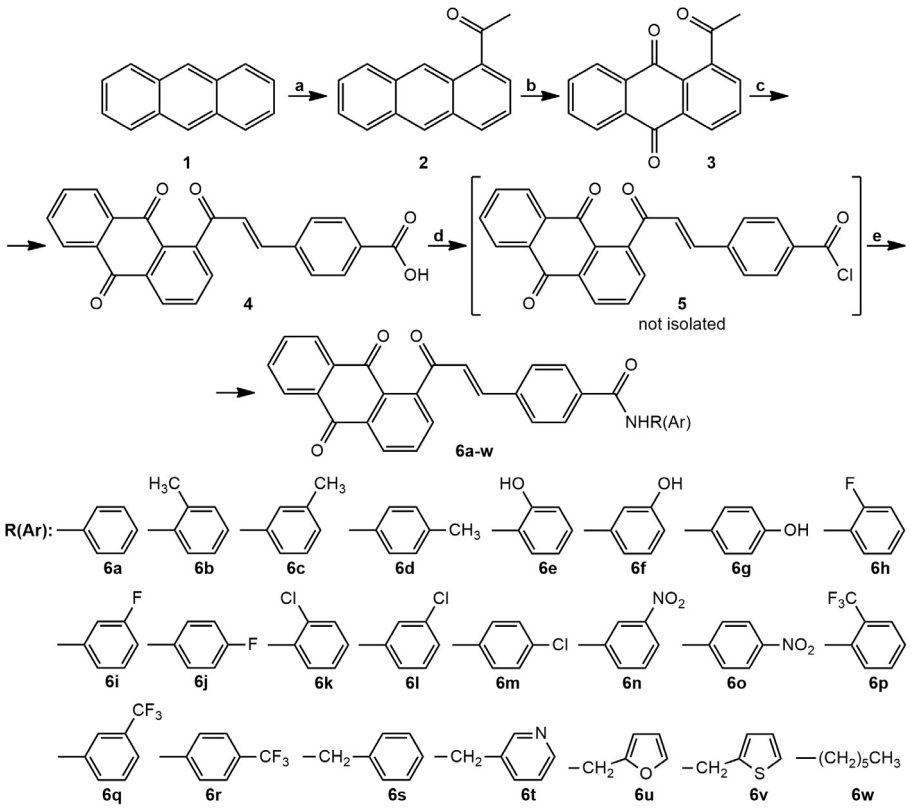

Scheme 1. Reagents and conditions: a) CH_3COCl, $AlCl_3$, CH_2Cl_2, 2 h, 0 °C, 5 M HCl; b) CrO_3, CH_3COOH, 5 min reflux, H_2O; c) 4-formylbenzoic acid, NaOH, MeOH, 2 h, reflux, HCl; d) $SOCl_2$, DMF, CH_2Cl_2, 2 h, rt; e) primary amine, THF, 6 h, reflux, H_2O.

was recrystallized from ethanol and condensed with 4-formylbenzoic acid in the presence of NaOH as a base catalyst, affording anthraquinone-chalcone carboxylic acid **4**. The obtained intermediate **4** was then converted into acyl chloride using $SOCl_2$, and, without isolation, reacted with selected primary amines in dry tetrahydrofuran giving the final hybrids **6a-w** in moderate to good yields (51–88%).

The structure of all compounds was confirmed by means of ^1H and ^{13}C NMR spectroscopy, IR and elemental analysis (see Supplementary material). The olefinic protons of the chalcone double bond in hybrid compounds **6a–w**, as well as their precursor **4**, appeared as an AB system. On the basis of coupling constant values (J = 16.0–16.8 Hz), all of the compounds were isolated and characterized in *E*-isomeric form. The signal for amide proton appears as a sharp singlet for compounds **6a–r** at the highest ppm values (9.70–10.82 ppm), while for compounds **6s–w** it exists as a triplet in slightly lower ppm range (8.53–9.20 ppm) due to the coupling with protons from adjacent methylene group.

The cytotoxicity of anthraquinone-chalcone hybrids containing amide function **6a-w** was evaluated against three human leukemia cancer cell lines (K562, Jurkat and HL-60), and normal human lung fibroblasts MRC-5 using MTT cell survival test. The obtained IC_{50} values are shown in Table 1. Sixteen of the tested compounds showed good cytotoxic potential against all three cancer cell lines. As it can be seen from Table 1, there is no significant difference between the influence of electron-donating and electron-withdrawing groups of the aniline scaffold on the cytotoxic action against cancer cells. The derivatives **6e** and **6f** containing *ortho*- and *meta*-hydroxyphenyl group showed the best cytotoxic activity against K562 and HL-60 cell lines, with IC_{50} values of 3.87 and 3.69 μM for K562 cells and 1.89 and 1.68 μM for HL-60 cells, respectively. The compounds containing aromatic or heterocyclic ring linked to methylene group (**6s–v**), as well as derivative **6w** containing alkyl group, also exerted potent antiproliferative activities against tested cell lines. The derivatives **6i** and **6 l** containing electron-withdrawing groups (F and Cl, respectively) at *meta*-position of the aniline moiety, showed significantly lower activity against K562 cell line, compared to other tested compounds.

Table 1
The cytotoxic activity of the investigated compounds **6a–w**.[a]

Compd.	IC_{50} ± SD (μM)			
	K562	Jurkat	HL-60	MRC-5
6a	10.93 ± 1.70	4.83 ± 0.77	6.81 ± 1.16	187.52 ± 6.90
6b	29.01 ± 1.64	15.83 ± 1.69	17.53 ± 2.04	101.62 ± 4.46
6c	25.03 ± 1.87	14.62 ± 1.99	17.56 ± 0.36	99.84 ± 3.80
6d	5.39 ± 0.59	3.81 ± 0.05	7.06 ± 1.03	> 200
6e	3.87 ± 0.17	3.23 ± 0.04	1.89 ± 0.22	60.36 ± 4.17
6f	3.69 ± 0.21	3.22 ± 0.14	1.68 ± 0.08	17.07 ± 3.01
6g	5.99 ± 0.24	3.66 ± 0.41	3.50 ± 0.67	190.74 ± 7.73
6h	5.20 ± 0.39	4.22 ± 0.51	3.73 ± 1.43	126.84 ± 20.52
6i	78.18 ± 1.56	27.64 ± 3.27	44.51 ± 5.18	191.12 ± 2.23
6j	31.84 ± 3.41	28.14 ± 2.39	22.82 ± 2.42	> 200
6k	4.67 ± 0.35	3.37 ± 0.15	4.22 ± 0.70	133.50 ± 2.64
6l	95.11 ± 6.92	5.73 ± 0.06	36.66 ± 2.81	183.24 ± 23.70
6m	8.53 ± 0.41	4.24 ± 0.63	5.71 ± 0.15	86.71 ± 10.20
6n	23.14 ± 2.19	3.81 ± 0.16	4.06 ± 1.59	197.12 ± 4.08
6o	25.52 ± 0.90	5.15 ± 0.51	3.57 ± 1.25	175.62 ± 10.72
6p	5.24 ± 0.92	3.29 ± 0.02	3.55 ± 0.69	124.93 ± 3.51
6q	7.14 ± 0.66	3.15 ± 0.51	2.81 ± 1.13	166.36 ± 23.77
6r	12.84 ± 1.03	5.21 ± 1.30	4.24 ± 0.69	105.75 ± 11.71
6s	3.96 ± 0.95	2.84 ± 0.56	3.85 ± 0.75	48.99 ± 4.16
6t	4.40 ± 0.41	3.14 ± 0.11	2.40 ± 0.69	8.68 ± 0.24
6u	4.45 ± 0.73	4.25 ± 0.67	3.16 ± 1.12	161.01 ± 6.57
6v	4.35 ± 0.47	4.25 ± 0.78	4.43 ± 0.78	188.28 ± 11.20
6w	5.08 ± 0.66	3.85 ± 0.60	5.39 ± 0.85	> 200
DOX[b]	0.14 ± 0.03	0.11 ± 0.03	0.05 ± 0.01	0.29 ± 0.05

[a] Results are mean values ± SD of three independent experiments.
[b] Doxorubicin.

All tested compounds, except **6f** and **6t**, showed significantly lower toxicity towards normal MRC-5 cells, compared to our previously