CYTOTOXIC EVALUATION OF SUBSTITUTED BENZALDEHYDES

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A series of fifty-four commercial aldehydes have been synthesized and evaluated for their activity against peripheral blood mononuclear cells (PBMC) and four human cancer cell lines, exhibiting potent citotoxicity (IC_{50} ranging from 0.36 to 4.75 μg mL^{-1}). The structure-activity relationship (SAR) analysis indicated that the number, the positions and the type of substituents attached into the aromatic and heteroaromatic ring are critical for the biological activity. The aldehydes **24**, **26**, **48** and **49** displayed a potent citotoxicity activity compared to the reference drug doxorubicin being, therefore, this discovery important for the rational design of new compounds against cancer.

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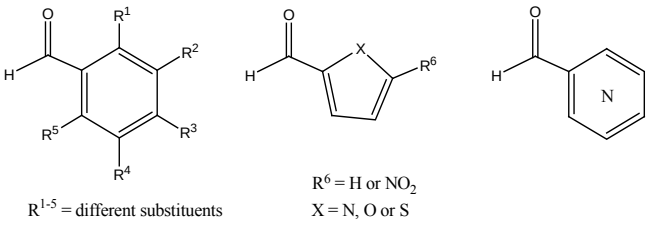
INTRODUCTION

The aldehyde function has a great versatility in organic synthesis since it is able to perform a large number of chemical transformations such as the preparation of alcohols, ethers, carboxylic acids, esters and alkanes. Aldehydes can also be used to form carbon-carbon bond, and may be used to obtain various systems and heteroaromatic compounds. In medicinal chemistry aldehydes are used in the formation of Schiff bases,[1] amines,[2] hydrazones and *N*-acylhydrazones,[3-8] which are present in many compounds with application in different diseases. One of them is cancer, which the National Institutes of Health (NIH) estimates that the over-all costs of cancer in 2007 were $226.8 billion[9] being a leading cause of death worldwide and accounted for 7.6 million deaths (13 % of all deaths) in 2008.[10] Due to the interest of our group in this field and the importance of the aldehydes[11] for the preparation of its respective hydrazones and *N*-acylhydrazones, classes of compounds which have potent activities against cancer, the aim of this article was to evaluate fifty-four commercial aldehydes against peripheral blood mononuclear cells (PBMC) and four human cancer cell lines. Although these commercial benzaldehydes have been used by research groups in the search of new anticancer drugs, they had not been tested previously against cancer cell lines. Considering that, the evaluation of commercial aldehydes common used in medicinal chemistry could be valuable information for the rational design of new compounds against cancer.

MATERIAL AND METHODS

Cytotoxicity Against Cancer Cell Lines

Initially, as a pre-screening procedure, all commercial aldehydes **1**-**54** (Scheme **1**) were tested *in vitro* only against three human cancer cells: SF-295 (glioblastoma), OVCAR-8 (ovary) and HCT-116 (colon) (National Cancer Institute, Bethesda, MD) at 5μg mL^{-1} by using MTT assay (Table **1**). Afterward, the compounds were classified by their growth inhibition (GI) percentage, at least in one cell line, as active (100 % *GI*), moderately active (75 % < *GI* < 100 %), or inactive (*GI* < 50 %).



Scheme 1. Commercial aldehydes used in medicinal chemistry tested against cancer cell lines.

The aldehydes **24**, **26**, **48** and **49** which displayed more than 80 % of GI, were selected for *in vitro* anticancer activities evaluation against PBMCs and four human cancer cell lines: OVCAR-8, SF-295, HCT-116, HL-60, using the MTT cell proliferation assay. The concentrations that induce 50% inhibition of cell growth (IC_{50}) in μg mL^{-1} are reported in Table **2**.

Table 1. Growth Inhibition Percentage (*GI* %) for three human tumors cells line by the MTT Assay of commercial aldehydes **1-54**.

Com-pound	R on benzaldehyde	Growth Inhibition[a] (*GI*, %)					
		OVCAR-8	**SD**	**HCT-116**	**SD**	**SF-295**	**SD**
1	H	0.00	0.00	13.12	2.78	18.12	2.14
2	2-NO_2	6.91	1.89	43.00	4.19	55.60	4.72
3	3-NO_2	37.23	3.75	50.97	1.10	35.32	17.31
4	4-NO_2	17.92	5.97	33.18	19.74	64.53	2.68
5	2,6-diNO_2	69.07	1.51	87.71	2.25	68.16	2.42
6	2-F	14.54	0.21	19.86	1.95	51.17	2.92
7	3-F	0.00	0.00	9.90	0.38	24.09	7.22
8	3-Cl	0.00	0.00	12.81	2.78	17.12	5.89
9	4-Cl	0.15	0.34	9.49	9.65	20.20	6.16
10	2,3-diCl	36.62	5.26	55.34	0.45	51.65	5.30
11	2,4-diCl	39.25	3.10	62.77	1.65	69.99	1.52
12	2,6-diCl	1.63	0.39	6.93	2.96	28.59	2.26
13	3,4-diCl	23.12	2.97	31.79	2.05	60.50	10.50
14	2-Br	0.00	0.00	9.05	0.92	38.21	9.49
15	3-Br	4.74	3.00	10.63	3.01	46.78	4.18
16	4-Br	0.00	0.00	5.53	1.49	33.69	11.00
17	4-CH_3	3.63	4.36	32.23	20.53	63.13	1.00
18	2-CN	2.52	2.73	5.36	4.14	42.51	0.43
19	3-CN	19.25	0.53	67.51	0.62	50.39	1.31
20	4-CN	62.66	5.48	65.89	3.66	59.11	19.73
21	2-OH	4.24	0.68	0.53	0.06	19.24	2.98
22	3-OH	4.22	0.63	9.50	1.81	34.73	0.17
23	4-OH	6.70	0.63	9.94	1.27	48.77	1.73
24	2.3-diOH	84.41	3.39	64.38	6.07	80.42	0.13
25	2,4-diOH	22.73	3.39	18.09	1.28	31.02	13.92
26	2,5-diOH	84.07	2.37	63.32	5.75	84.57	0.31
27	3,4-diOH	47.10	3.79	46.10	14.13	68.18	2.13
28	3,4,5-triOH	41.27	2.53	48.81	5.16	67.79	0.58
29	2-OCH_3	19.08	6.50	54.21	0.65	69.60	4.66
30	3-OCH_3	16.32	0.21	44.31	25.59	52.58	4.07
31	4-OCH_3	4.48	4.29	4.64	10.92	22.67	20.48
32	2,6-diOCH_3	70.69	2.00	76.19	0.02	68.00	3.93
33	2,4-diOCH_3	8.62	5.95	27.22	4.53	51.46	4.57
34	2,3-diOCH_3	0.00	0.00	19.39	10.49	42.56	8.73
35	2,5-diOCH_3	45.49	3.51	46.04	5.51	55.18	4.71
36	3,4-diOCH_3	11.79	2.51	16.54	0.93	25.11	22.17
37	3-OEt	41.62	4.83	42.95	7.83	65.86	10.39
38	4-OEt	0.38	3.44	0.00	0.00	21.92	7.12
39	2-OH; 3-OCH_3	44.82	1.21	42.55	7.24	62.61	1.12
40	2-OH; 4-OCH_3	4.90	1.95	13.64	6.17	56.52	3.74
41	2-OH; 4-CH_3	7.44	0.46	32.41	1.77	43.71	5.08
42	2-OH; 5-CH_3	0.00	0.00	17.16	2.71	40.80	8.67
43	2-OH; 5-NO_2	57.43	4.23	39.17	32.34	78.17	0.16
44	3-NO_2; 4-Cl	71.26	0.12	63.26	2.90	62.79	4.31
45	3-Cl; 4-OH	11.89	3.65	19.57	1.33	60.08	1.48
46	4-N$(CH_3)_2$	5.18	1.66	0.00	0.00	22.49	8.85
47	4-N$(Et)_2$	9.56	0.18	4.98	0.27	44.78	2.26
Compound No. and name		**OVCAR-8**	**SD**	**HCT-116**	**SD**	**SF-295**	**SD**
48	5-NO_2-2-furaldehyde	85.60	2.08	82.88	3.28	75.83	1.89
49	5-NO_2-2-thiophene-C(O)H	86.38	1.77	80.38	0.80	89.46	0.13
50	2-thiophene-C(O)H	0.00	0.00	12.45	3.97	18.47	2.09
51	2-pyrrole-carboxaldehyde	0.00	0.00	11.19	1.52	18.66	0.95
52	2-pyridine-carboxaldehyde	4.41	0.90	13.72	1.53	32.53	1.59
53	3-pyridine-carboxaldehyde	8.61	0.28	13.60	4.55	40.64	13.09
54	4-pyridine-carboxaldehyde	6.09	3.17	23.02	3.66	26.02	2.41

Table 2. Cytotoxic activity of the aldehydes **24**, **26**, **48** and **49** [IC_{50} (μg mL^{-1})] on human tumor cell lines.

Compound	HCT-116 IC_{50} (CI 95 %)*	OVCAR-8 IC_{50} (CI 95 %)*	HL-60 IC_{50} (CI 95 %)*	SF-295 IC_{50} (CI 95 %)*	PBMCIC_{50} (CI 95 %)*
2,3-dihydroxybenz-aldehyde	2.299 (1.929 to 2.741)	1.807 (1.558 to 2.096)	1.741 (0.5464 to 5.546)	4.750 (4.422 to 5.102)	>5
2,5-dihydroxybenz-aldehyde	1.931 (1.075 to 3.467)	1.284 (1.096 to 1.503)	0.8470 (0.2356 to 3.045)	3.826 (3.348 to 4.371)	2.839 (1.931 to 4.174)
5-nitro-2-furaldehyde	1.199 (0.9509 to 1.511)	1.563 (1.353 to 1.805)	0.3605 (0.1578 to 0.8235)	3.823 (3.538 to 4.131)	2.137 (1.296 to 3.525)
5-nitro-2-thiophene-carboxaldehyde	1.199 (0.5633 to 0.6610)	0.7508 (0.6526 to 0.8637)	0.3761 (0.1723 to 0.8211)	2.866 (2.618 to 3.139)	1.935 (1.151 to 3.253)
Doxorubicin	0.125 (0.09 to 0.17)	0.265 (0.17 to 0.305)	0.02 (0.01 to 0.02)	0.23 (0.19 to 0.25)	0,8 (0,72 to 0,88)

* Data are presented as IC_{50} values and 95 % confidence intervals (*IC* 95 %) obtained by nonlinear regression for all cell lines colon (HCT-116), ovary (OVCAR-8), leukemia (HL-60), glioblastoma (SF-295) and normal lymphocytes (PBMCs) from three independent experiments. Doxorubicin was used as positive control. Experiments were performed in triplicate. IC_{50} = concentrations that induce 50 % inhibition of cell growth in μg mL^{-1}.

RESULTS AND DISCUSSION

The evaluation of commercial benzaldehydes **1**-**54** tested against four human cancer cell lines suggested that the number, the positions and the type of substituent attached into the aromatic and heteroaromatic ring are critical for the biological activity (Figure 1).

For example, the structure-activity relationship (SAR) analysis indicated that the number and the positions of hydroxyl group into aromatic ring are critical for the biological activity. Dihydroxyl groups in 2,3 (**24**) or 2,5 (**26**) positions displayed good cytotoxic activities, however when these groups are in 2,4 (**25**); 3,4 (**27**) or 3,4,5 (**28**) positions no activity were detected. It is important to mention that when hydroxyl groups in 2,3 (**24**) or 2,5 (**26**) positions are replaced by dimethoxy groups **34** and **35**, respectively the aldehydes were inactive. The presence of a methoxy group and one hydroxyl group such as the aldehyde **34** leads to loss of activity. In this context, when one of hydroxyl group was replaced by methyl (**42**) or nitro group (**43**) the aldehydes were inactive. Another information is that aldehydes with only one hydroxyl group into the ring in the positions 2 (**20**), 3 (**21**) or 4 (**22**) were inactive.

Other substituent present in the aromatic aldehydes (mono or disubstituted) such as F, Cl, Br, CN, NO_2, OEt, $N(Me)_2$ or $N(Et)_2$ leads to loss of activity.

The cytotoxic evaluation of the heteroaromatic aldehydes suggested that the size, the substituents and its positions into the ring are important for the biological evaluation. For example, five-membered ring were more active than six being the aldehydes **48** and **49** were the most actives. Among them, **49** (5-nitro-thiophene carboxaldehyde) displayed the best result, indicating that the nitro group in the five position and the sulfur are important for the anticancer activity. The SAR for this class of compounds is summarizes in Figure 1.

EXPERIMENTAL

Cytotoxicity Against Cancer Cell Lines

The commercial aldehydes **1**-**54** (1.715–5.0 μg mL^{-1}) were tested for their cytotoxic activity against PBMCs (normal lymphocytes) and four human cancer cell lines: OVCAR-8 (ovary), SF-295 (glioblastoma), HCT-116 (colon), HL-60 (leukaemia) (National Cancer Institute, Bethesda, MD). All cell lines were maintained in RPMI 1640 medium supplemented with 10 % fetal bovine serum, 2 mM glutamine, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37 °C with 5 % CO_2. Each compound was dissolved with DMSO, until reaching a concentration of 1 mg mL^{-1}. The final concentration of DMSO in the culture medium was kept constant, below 0.1 % (v/v). The commercial aldehydes **1**-**54** were incubated with the cells for 72 h. The negative control received the same amount of DMSO (0.001 % in the highest concentration). The cell viability was determined by reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a blue formazan product as described by Mosmann.[12]

Cytotoxicity Against Normal Peripheral Blood Mononuclear Cells

Fresh peripheral human heparinized blood was collected from healthy donors with no recent or chronic smoking, drinking or drug intake. Peripheral blood mononuclear cells (PBMCs) were obtained by a standard method of density-gradient centrifugation over Histopaque®-1077. The PBMCs were cultivated with RPMI 1640 supplemented with 20% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, 100 g/ml streptomycin and phytohemagglutinin (final concentration: 2 %) at 37 ºC with 5% CO_2.[13-15] The study was approved by the Ethical Committee of Federal University of Ceará State, Brazil. For all experiments, cell viability was performed by using a hemocytometer and Trypan Blue assay.

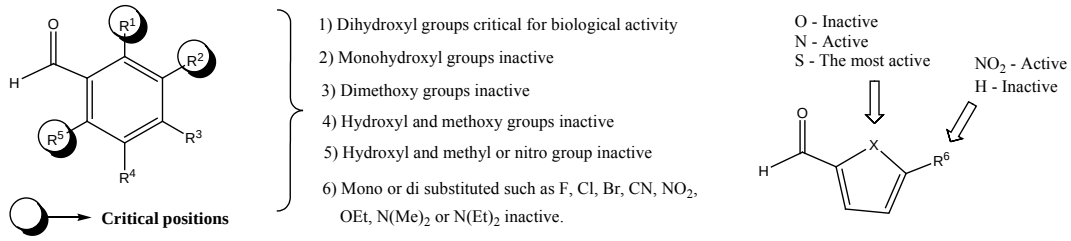


Figure 1. SAR of the benzaldehydes series tested against cancer cell lines.

CONCLUSION

In this work we report the cytotoxicity activity of a series of fifty-four commercial aldehydes, which have been evaluated for their activity against peripheral blood mononuclear cells (PBMC) and four human cancer cell lines. The SAR of this class indicated that the number, the positions and the type of substituents attached into the aromatic and heteroaromatic ring are critical for the biological activity. The position 2, 3 and 5 in aromatic ring and the dihydroxyl groups are critical for biological activity. In the heteroaromatic aldehydes the size, substituents and the heteroatom are important for cytotoxic activity. In this context, the compound **49** displayed a potent cytotoxicity activity compared to the reference drug doxorubicin. Considering the data of this current study, our findings are valuable information for the rational design of new compounds against cancer.

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