BIOLOGICAL EVALUATION OF PYRAZINAMIDE DERIVATIVES AS AN ANTICANCER CLASS

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A series of fifty one pyrazinyl derivatives have been synthesized and evaluated for their activity against four cancer cell lines, exhibiting good citotoxicity (IC_{50} ranging from 1.1 to 5.6 μg mL^{-1}). The structure-activity relationship (SAR) analysis indicated that the hydroxyl group located in *ortho* position is critical for the biological activity of these compounds. The presence of hydroxyl groups on benzene ring plays an important role in the anticancer activity of this series, feature especially observed in disubstituted derivatives.

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Introduction

The class of substances known as heteroaromatics is extremely important in drug discovery being present in many drugs used against different types of diseases. In this context could be mention the nucleus pyrazine, which possessed a wide range of biological activities being found in nature and in many drugs.[1]

For example, 2-isopropyl-3-methoxy pyrazine is an aroma compound found in coffee; pyrazinamide is a first line anti-tuberculosis drug;[2,3] oltipraz is a schistosomicide and also used for tumor prevention;[4,5] telaprevir is used to treat hepatite C;[6] bortezomib is used against multiple myeloma[7,8] and glipizide, an oral anti-diabete drug[9] (Figure **1**).

Due to the importance of pyrazine nucleus in drug discovery and our continuous search of new potent and safe anticancer agents the aim of this work is to present a series of fifty-one pyrazine hydrazone derivatives design by molecular hybridization (Scheme **1**), which were tested against four cancer cell lines with good results.

The reason to synthesized pyrazine hydrazone derivatives is because hydrazone functional group is also described with a wide range of pharmacological activities, such as anticancer agents.[10] It is important to mention that cancer disease is a leading cause of death worldwide and accounted for 7.6 million deaths (13 % of all deaths) in 2008[11] being urgently need new drugs and strategies to fight against this disease.

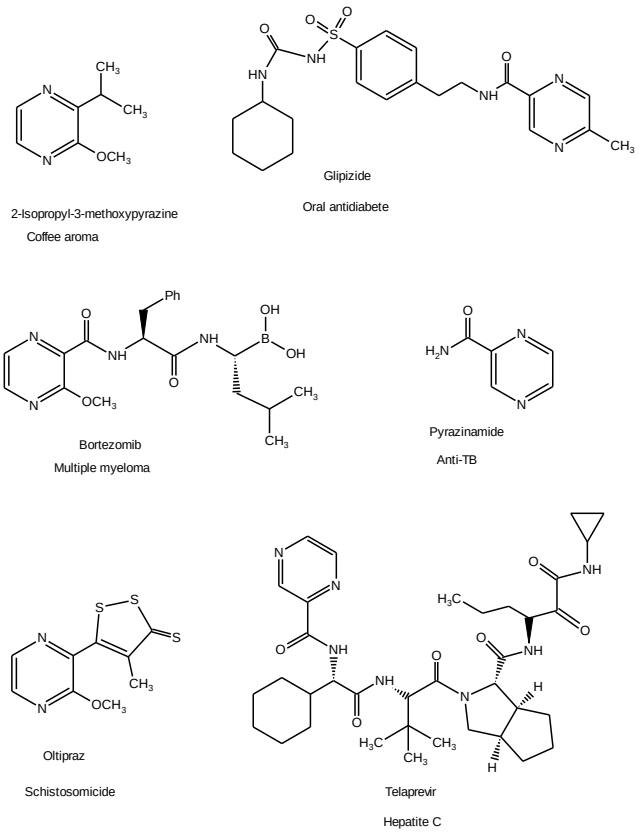
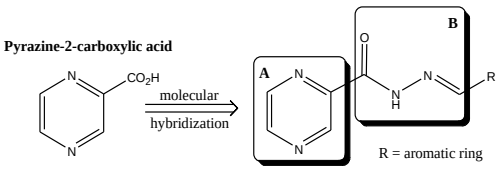


Figure 1. Biological activities of pyrazinyl derivatives



Scheme 1. Pyrazine hydrazone derivatives

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Table 1. Growth Inhibition Percentage (*GI*, %) for three tumors cells line by the MTT Assay of compounds **1-51**.

Compound	R[b]	Growth Inhibition[a] (%)					
		SF-295	SD	HCT-116	SD	OVCAR-8	SD
1	H	49.40%	0.91%	50.06%	4.76%	66.30%	2.86%
2	2-NO_2	26.12%	3.83%	25.57%	2.81%	0.00%	0.00%
3	3-NO_2	16.,27%	1.32%	25.44%	0.91%	0.00%	0.00%
4	4-NO_2	24.66%	3.39%	28.37%	0.43%	5.83%	4.58%
5	2-F	35.49%	0.88%	11.21%	1.16%	0.00%	0.00%
6	3-F	41.03%	0.94%	16.26%	2.93%	11.43%	3.51%
7	4-F	17.43%	1.07%	17.12%	1.71%	0.00%	0.00%
8	2-Cl	32.20%	1.53%	13.37%	2.87%	0.00%	0.00%
9	3-Cl	36.02%	4.14%	13.80%	0.55%	11.97%	1.22%
10	4-Cl	47.60%	1.69%	9.23%	3.72%	8.41%	4.73%
11	3-Br	29.54%	1.55%	23.63%	3.11%	11.92%	4.35%
12	4-Br	16.81%	7.97%	22.85%	2.62%	10.79%	1.37%
13	2-CN	37.44%	2.26%	30.53%	1.04%	9.55%	6.03%
14	3-CN	26.34%	1.16%	22.25%	3.72%	0.00%	0.00%
15	4-CN	19.82%	1.69%	6.12%	3.23%	0.00%	0.00%
16	2-OH	42.32%	2.36%	53.17%	3.42%	52.37%	1.94%
17	3-OH	32.73%	1.63%	19.32%	2.87%	0.00%	0.00%
18	4-OH	41.43%	0.13%	12.25%	2.50%	2.80%	1.36%
19	3-OCH_3	9.62%	0.69%	23.33%	0.12%	8.31%	5.49%
20	4-OCH_3	31.54%	2.28%	13.45%	3.60%	0.00%	0.00%
21	3-OCH_2CH_3	32.65%	4.89%	30.27%	0.79%	15,16%	5.11%
22	4-OCH_2CH_3	0.70%	3.14%	23.11%	1.99%	2.7%	1.07%
23	2,3-diOH	90.41%	0.40%	53.21%	43.18%	51.78%	2.17%
24	2,4-diOH	74.24%	0.20%	74.37%	1.04%	94.48%	3.15%
25	2,5-diOH	64.07%	3.24%	49.70%	4.25%	74.24%	0.27%
26	3,4-diOH	47.61%	1.21%	27.44%	1.76%	34.61%	0.27%
27	2,3-diOCH_3	30.15%	1.46%	15.58%	7.77%	13.14%	0.41%
28	2,4-diOCH_3	49.69%	3.14%	22.39%	3.31%	6.17%	8.35%
29	2,6-diOCH_3	28.72%	4.25%	19.46%	1.24%	0.00%	0.00%
30	3,4-diOCH_3	38.59%	3.44%	23.12%	5.38%	7.53%	3.42%
31	3,5-diOCH_3	41.10%	3.74%	13.16%	1.24%	6.17%	0.96%
32	3,4,5-triOCH_3	31.45%	2.82%	17.38%	1.83%	6.96%	1.91%
33	2-OH; 3-OCH_3	90.84%	1.01%	89.75%	1.86%	91.58%	2.33%
34	2-OH; 4-OCH_3	87.33%	0.71%	92.46%	3.62%	93.03%	1.92%
35	3-OH; 4-OCH_3	39.24%	6.98%	10.53%	1.86%	4.24%	0.68%
36	3-OCH_3; 4-OH	53.55%	4.76%	25.17%	0.62%	13.44%	3.29%
37	2,3-Cl	48.83%	2.13%	25.83%	19.57%	12.08%	0.55%
38	2,4-Cl	29.50%	0.71%	10.23%	5.38%	0.00%	0.00%
39	2,6-Cl	30.36%	6.98%	2.76%	1.24%	4.04%	8.08%
40	3,4-Cl	46.68%	3.95%	18.14%	1.45%	0.00%	0.00%
41	2-OH; 5-NO_2	84.11%	1.01%	72.47%	1.18%	78.89%	1.86%
42	2,4-CH_3	57.99%	0.10%	15.80%	1.45%	4.04%	3.70%
43	2,4-F	39.67%	5.36%	10.89%	1.12%	0.00%	0.00%
44	4-N(OCH_2CH_3)$_2$	55.50%	3.58%	67.83%	2.32%	87.06%	2.29%
45	4-N(OCH_3)$_2$	41.83%	0.56%	19.02%	1.34%	21.04%	0.76%
46	2-furaldehyde	22.66%	1.60%	21.17%	2.68%	0.00%	0.00%
47	5-nitro-2-furaldehyde	41.48%	8.60%	85.68%	1.25%	88.57%	1.09%
48	5-amino-2-furaldehyde	23.28%	5.96%	24.19%	6.71%	1.35%	7.86%
49	5-nitro-2-thiophene-carboxaldehyde	30.12%	4.58%	68.26%	1.44%	26.81%	1.52%
50	2-pyridine carboxaldehyde	26.12%	1.60%	21.00%	2.56%	9.22%	1.64%
51	2-pyrrole carboxaldehyde	7.04%	0.94%	23.50%	0.37%	0.00%	0.00%

[a]Experiments were performed in triplicate. [b]In case of compounds **1-45**, R=substituents on a phenyl ring. SD – Standard Deviation.

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Table 2. Cytotoxic activity of compounds **33**, **34** and **41** [IC_{50} (μg mL^{-1})] on tumor cell lines.

Compound	HCT-116	OVCAR-8	HL-60	SF-295
	IC_{50}, SD	IC_{50}, SD	IC_{50}, SD	IC_{50}, SD
33	4.099 3.213 to 5.228	1.564 0.8915 to 2.745	2.977 2.489 to 3.562	2.063 1.192 to 3.570
34	3.223 2.247 to 4.623	1.190 1.004 to 1.410	2.269 1.890 to 2.723	1.117 0.8968 to 1.390
41	4.595 4.121 to 5.122	5.606 4.436 to 7.086	3.828 3.184 to 4.603	1.550 1.024 to 2.345
Doxorubicin	0.125 (0.09 – 0.17)	0.265 (0.17 – 0.305)	0.02 0.01-0.02	0.23 0.19-0.25

* Data are presented as IC_{50} values and 95 % confidence intervals obtained by nonlinear regression for all cell lines colon (HCT-116), ovarium (OVCAR-8), (leukemia (HL-60), glioblastoma (SF-295), from three independent experiments. Doxorubicin (Dox) 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

Chemistry

These compounds have been previously synthesized, characterized and evaluated for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis* H37Rv, displaying promising results also in this field.[12-14] Briefly, the synthesis of desired compounds involved the reaction of appropriate benzaldehydes and and 2-pyrazinecarbo-hydrazide, ethanol/water 1:1 ratio at room temperature for 4-24 hours. The compounds were obtained in 50-90 % yields.

Cytotoxicity Against Cancer Cell Lines

All compounds **1-51** were tested *in vitro* against three cancer cells: SF-295 (glioblastoma), HCT-116 (colon) and OVCAR-8 (human ovary) (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 %).

Compounds **33**, **34** and **41** which displayed more than 84% of GI, were selected for *in vitro* anticancer activities evaluation against four human cancer cell lines: HCT-116 (colon), OVCAR-8 (human ovary), HL-60 (leukemia) and SF-295 (glioblastoma), using the MTT assay. A common feature of these active compounds is an *ortho*-hydroxy group in the phenyl ring, indicating the importance of this substituent in the biological activities. It is important to mention that the mono-substituted 2-hydroxy derivative, compound **16**, is only moderately active against these cell lines. This result suggests the importance of a second group into the ring, such as 3-methoxy (**33**), 4-methoxy (**34**) and 5-nitro (**41**) group, and that steric and/or electronic effects could play a critical role in anticancer activities.

The concentrations that induce 50 % inhibition of cell growth (*IC*50) in μg mL^{-1} are reported in Table **2**.

Conclusion

In this work we report a cytotoxicity activity of a series of fifth one pyrazinyl hydrazone derivatives, which have been evaluated for their activity against four human cancer cell lines. This study reveals the importance of hydroxyl substituent of aromatic ring located in ortho position, specially in dissubstituted compounds, indicating that the number, the positions and the type of substituents attached to aromatic ring can be critical for the biological activity and a good starting point to the discovery of new prototypes against cancer.

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