Short Communication

Biological Evaluation of Isoniazid Derivatives as an Anticancer Class

Felipe A. R. RODRIGUES 1, Augusto C. A. OLIVEIRA 1, Bruno C. CAVALCANTI 1, Claudia PESSOA 1, Alessandra C. PINHEIRO 2, Marcus V. N. DE SOUZA * 2

1 Laboratório de Oncologia Experimental, Universidade Federal do Ceará, Fortaleza, CE, Brazil.
2 FioCruz-Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos-Far-Manguinhos, Rua Sizenando Nabuco, 100, Manguinhos, 21041-250 Rio de Janeiro, RJ, Brazil.

* Corresponding author. E-mail: marcos_souza@far.fiocruz.br (M. V. N. de Souza)

Sci Pharm. 2014; 82: 21–28    doi:10.3797/scipharm.1307-25
Published:  September 22nd 2013   Received:  July 31st 2013
Accepted:  September 22nd 2013

This article is available from: http://dx.doi.org/10.3797/scipharm.1307-25

Abstract
A series of thirty-two isoniazid derivatives have been evaluated for their activity against four human cancer cell lines with potent cytotoxicity (IC50 ranging from 0.61 to 3.36 μg/mL). The structure-activity relationship (SAR) analysis indicated the number, the positions, and the types of substituents attached to the aromatic ring as being critical factors for the biological activity. Briefly, we observed that the presence of a hydroxyl group on the benzene ring plays an important role in the anticancer activity of this series, especially when it is located in ortho-position. Among the thirty-two compounds, three displayed good cytotoxic activity when compared to the reference drug doxorubicin and are thus being considered leading compounds of this new class.

Keywords
Antitumor activity • Isoniazid • Hydrazone • Drugs • Cytotoxicity

Introduction
Nicotinic acid (pyridine-3-carboxylic acid), its derivatives and isomers form an important class of heterocyclic compounds with a wide range of applications, among which the use thereof as starting materials for the synthesis of biological active compounds such as Nevirapine, namely an anti-HIV drug [1]. Nicotinic acid, also known as vitamin B3 and niacin, as well as its amide niacinamide are found in several aliments and animals,
and play a critical role in different biological processes [2]. This class of heterocyclic compounds also showed a broad spectrum of biological activities, such as anti-carcinogenic [3], antioxidant [4], anti-inflammatory [5], and anti-bacterial ones [6]. For instance, we should mention isoniazid (isonicotinylhydrazine), an important first-line anti-tuberculosis drug, which keeps an analogy with isonicotinic acid, an isomer of nicotinic acid (Figure 1) [7, 8]. However, in spite of the relevance of isoniazid in tuberculosis treatment in the last twenty years, this drug has rarely been studied in the field of cancer [9], even after promising perspectives of analogues of isoniazid in this field, which can be illustrated by the work of Malhotra and co-workers [10]. This disease, which according to an estimate from the National Institute of Health (NIH), implied in overall costs of $226.8 billion in 2007 [11], accounted for 7.6 million deaths (13% of all deaths) in 2008 [12], being to date a leading cause of death worldwide. Therefore, in view of such an urgent need of new drugs against this disease, an important strategy for drug discovery has been recently developed, namely drug repositioning, which is defined as the study aimed at the application of available drugs for other diseases [13, 14]. This study of isoniazid and its derivatives in the cancer field, which shall be grounded in our experience with TB drugs [15–20], is particularly motivated by the lack of studies in drug discovery focused on isoniazid derivatives against cancer. In this context, the aim of this work was the antitumoral evaluation against human cancer cell lines of thirty-two isoniazid hydrazone derivatives designed by molecular hybridization (Scheme 1), which display potent and promising results (Tables 1 and 2). Hydrazones are also described to possess a wide range of pharmacological activities, such as being anticancer agents [21, 22].

![Fig. 1. Structures of Nicotinic acid and Isoniazid](image)

**Results and Discussion**

**Chemistry**

All the isonicotinohydrazides derivatives 1–32 were synthesized by our research group and tested against *M. tuberculosis* [15–18]. Briefly, the synthesis of desired compounds involved the reaction of appropriate benzaldehydes and isoniazid, in THF under reflux or room temperature for 4–12 hours. The compounds were obtained in 75–99% yields.

![Sch. 1. Isoniazid hydrazone derivatives designed by molecular hybridization](image)
Cytotoxicity Against Cancer Cell Lines

All compounds 1–32 were tested in vitro against three human cancer cells: OVCAR-8 (ovary), SF-295 (glioblastoma), and HCT-116 (colon) (National Cancer Institute, Bethesda, MD) at 5 μg/mL by using the 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%).

Tab. 1. Growth Inhibition Percentage (GI %) for three Tumors Cell Lines by the MTT Assay of compounds 1–32.

| Cpd. | R       | Growth Inhibitiona (%) |
|------|---------|------------------------|
|      |         | OVCAR-8  | SD | SF-295  | SD | HCT-116 | SD |
| 1    | H       | 0.00%    | 0.00% | 19.31%  | 1.54% | 0.00%    | 0.00% |
| 2    | 2-NO2   | 0.74%    | 1.40% | 26.54%  | 3.79% | 9.31%    | 6.01% |
| 3    | 3-NO2   | 2.00%    | 0.89% | 29.81%  | 1.81% | 0.00%    | 0.00% |
| 4    | 4-NO2   | 0.00%    | 0.00% | 28.76%  | 0.82% | 0.00%    | 0.00% |
| 5    | 2-F     | 0.00%    | 0.00% | 23.04%  | 0.00% | 0.00%    | 0.00% |
| 6    | 3-F     | 0.00%    | 0.00% | 34.53%  | 0.25% | 8.04%    | 1.17% |
| 7    | 4-F     | 0.00%    | 0.00% | 24.38%  | 4.70% | 1.70%    | 6.19% |
| 8    | 2-Cl    | 0.00%    | 0.00% | 32.20%  | 8.00% | 0.00%    | 0.00% |
| 9    | 3-Cl    | 0.00%    | 0.00% | 34.41%  | 4.70% | 2.78%    | 3.23% |
| 10   | 4-Cl    | 0.00%    | 0.00% | 28.47%  | 7.34% | 5.00%    | 0.63% |
| 11   | 2-Br    | 62.18%   | 5.97% | 65.02%  | 1.20% | 66.87%   | 4.13% |
| 12   | 3-Br    | 0.00%    | 0.00% | 34.41%  | 3.71% | 6.27%    | 1.53% |
| 13   | 3-CN    | 0.00%    | 0.00% | 40.13%  | 2.56% | 17.94%   | 1.50% |
| 14   | 4-CN    | 0.00%    | 0.00% | 31.38%  | 0.41% | 4.74%    | 0.63% |
| 15   | 2-OH    | 100.00%  | 1.78% | 80.47%  | 3.38% | 80.58%   | 0.54% |
| 16   | 3-OH    | 0.00%    | 0.00% | 22.34%  | 3.46% | 0.00%    | 0.00% |
| 17   | 4-OH    | 0.00%    | 0.00% | 7.36%   | 6.68% | 0.00%    | 0.00% |
| 18   | 2,3-diOH| 100.00%  | 0.13% | 86.53%  | 1.90% | 79.44%   | 0.36% |
| 19   | 3,4-diOH| 36.31%   | 1.65% | 37.39%  | 0.33% | 26.07%   | 4.04% |
| 20   | 2-OCH3  | 2.27%    | 5.84% | 43.80%  | 0.33% | 0.00%    | 0.00% |
| 21   | 3-OCH3  | 1.19%    | 1.27% | 22.34%  | 1.81% | 3.66%    | 0.90% |
| 22   | 4-OCH3  | 5.95%    | 0.02% | 23.45%  | 4.04% | 0.00%    | 0.00% |
| 23   | 2,3-diOCH3 | 0.65% | 1.78% | 26.95%  | 0.25% | 0.00%    | 0.00% |
| 24   | 2,4-diOCH3 | 19.87% | 2.29% | 43.74%  | 6.18% | 0.00%    | 0.00% |
| 25   | 2,5-OCH3 | 49.52% | 1.91% | 52.78%  | 6.43% | 66.05%   | 1.17% |
| 26   | 2,6-diOCH3 | 23.65% | 1.67% | 35.17%  | 5.94% | 72.46%   | 2.51% |
| 27   | 3,5-diOCH3 | 7.48% | 0.25% | 39.13%  | 5.11% | 15.85%   | 1.23% |
| 28   | 2,3,4-triOCH3 | 0.00% | 0.00% | 32.90%  | 1.57% | 0.00%    | 0.00% |
| 29   | 2-OCH2CH3 | 2.63% | 0.51% | 37.91%  | 0.25% | 0.00%    | 0.00% |
| 30   | 3-OCH2CH3 | 0.00% | 0.00% | 9.69%   | 4.70% | 0.00%    | 0.00% |
| 31   | 2-OH; 3-OCH3 | 100.00% | 1.27% | 86.77%  | 0.08% | 88.64%   | 0.27% |
| 32   | 3-OH; 4-OCH3 | 18.89% | 6.48% | 43.86%  | 1.79% | 5.76%    | 1.48% |

*Experiments were performed in triplicate. SD ... Standard Deviation.
Compounds 15, 18, and 31, which displayed more than 96% 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. The concentrations that induce 50% inhibition of cell growth (IC50) in μg/mL are reported in Table 2.

### Table 2. Cytotoxic activity of compounds 18, 31, and 15 [IC50 (μg/mL)] on tumor cell lines*.

| Cpd. | HCT-116 | OVCAR-8 | HL-60 | SF-295 |
|------|---------|---------|-------|--------|
|      | IC50    | IC50    | IC50  | IC50   |
|      | SD      | SD      | SD    | SD     |
| 15   | 2.025   | 2.021   | 2.452 | 3.366  |
|      | 1.427 to 2.873 | 1.857 to 2.199 | 2.174 to 2.766 | 1.814 to 6.245 |
| 18   | 1.367   | 0.6182  | 0.6173| 0.9670 |
|      | 1.106 to 1.690 | 0.5522 to 0.6922 | 0.5421 to 0.7028 | 0.8281 to 1.129 |
| 31   | 1.718   | 1.242   | 1.932 | 1.912  |
|      | 1.133 to 2.606 | 1.059 to 1.455 | 1.646 to 2.268 | 1.621 to 2.256 |
| Doxorubicin | (0.09–0.17) | (0.17–0.305) | 0.01–0.02 | 0.19–0.25 |

* Data are presented as IC50 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. IC50 = concentrations that induce 50% inhibition of cell growth in μg/mL.

The structure-activity relationship (SAR) analysis indicated that the number, the positions, and the types of substituents attached to the aromatic ring are critical for the biological activity. The disubstituted derivatives displayed the best results appearing as the most active groups attached to the ring, namely the hydroxy, methoxy, chloro, and nitro groups. In general, we observed that the presence of hydroxyl groups on the benzene ring plays an important role in the anticancer activity of this series, especially when it is located in ortho-position. It is worth mentioning that, given that hydroxyl groups located in ortho-position in hydrazone systems are good ligands for metals, the action mechanism of this class could possibly be based on the formation of complexes that are likely to inactivate enzymes involved in abnormal cell division. As another peculiarity about the SAR from this class, we should mention that when comparing one of the leads from this class to the bioisostere (E)-N’-(2-hydroxybenzylidene)pyrazine-2-carbohydrazide [20, 23], this lead displayed a stronger antitumor activity, thus indicating that the inclusion of another nitrogen into the ring and/or the position of the group acylhydrazone decreases the potency of the pyridine hydrazone class. This bioisostere is an analogue of the first-line drug pyrazinamide, which is also an important first-line anti-tuberculosis drug (Figure 2) [8].
Experimental

**General Procedure for the Synthesis of Isoniazid Hydrazone Derivatives 1–32**

To a stirred solution of isoniazid (1.0 mmol) in ethanol (10 mL) was added the appropriate amount of benzaldehyde (1.05 mmol), and the reaction mixture was stirred for 4–12 hours at room temperature or under reflux. The reaction mixture was concentrated under reduced pressure, and the residue was purified by washing with cold ethanol (3 x 10 mL), thus affording the isoniazid hydrazone derivatives 1–32 in 75–99% yield.

**Cytotoxicity Against Cancer Cell Lines**

Compounds 1–32 (1.715–5.0 µg/mL) were tested for their cytotoxic activity against 3–4 human cancer cell lines: OVCAR-8 (ovary), SF-295 (glioblastoma), HCT-116 (colon), and HL-60 (leukemia) (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 penicillin, and 100 µg/mL streptomycin at 37 °C with 5% CO₂. Each compound was dissolved with DMSO until reaching a concentration of 1 mg/mL. The final concentration of DMSO in the culture medium was kept constant, below 0.1% (v/v). Compounds 1–32 were incubated with the cells for 72 hours. 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-2H-tetrazolium bromide (MTT) to a blue formazan product as described by Mosmann [24].

**Conclusion**

In this work, we report the potent cytotoxic activity of a series of thirty-two isoniazid hydrazone derivatives, which have been evaluated for their activity against four human cancer cell lines. The SAR of this class indicated that the number, the positions, and the types of substituents attached to the aromatic ring are critical for the biological activity. Another peculiarity about the SAR from this class concerns the fact that bioisostere (E)-N’-(2-hydroxybenzylidene)pyrazine-2-carbohydrazide displayed a lower antitumor activity, which indicates that the inclusion of another nitrogen into the ring and/or the position of the group acylhydrazone decreases the potency of the pyridine hydrazone class.
In comparison to the reference drug doxorubicin, compound 18 displayed a good cytotoxic activity, thus suggesting that compounds based on the isoniazid drug could be a good starting point for the discovery of new leading compounds against cancer.

Acknowledgement

The authors are grateful to the Brazilian agencies Capes (Coordenadoria de Apoio à Pesquisa e Ensino Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), and FUNCAP/CE (Fundação de Fundação Cearense de Pesquisa) for fellowships and financial support.

Authors’ Statement

The authors declare no conflict of interest.

References

[1] Grozinger KG, Fuchs V, Hargrave KD, Mauldin S, Vitous J, Campbell S, Adams J. Synthesis of nevirapine and its major metabolite. J Heterocycl Chem. 1995; 32: 259–263. http://dx.doi.org/10.1002/jhet.5570320144

[2] Hankes LV. In: Handbook of vitamins, nutritional, biochemical and clinical aspects. Marcel Dekker: New York, 1984; pp.329–377.

[3] Arigon J, Bernhart C, Bouaboula M, Casellas P, Combet R, Jegham S, Hllairet S, Fraisse P. Nicotinamide derivatives, preparation thereof and therapeutic use thereof. WO 2009074749A2, 2009.

[4] Aanandhi MV, Mansoori MH, Shanmugapriya S, George S, Shanmugasundaram P. Synthesis and in vitro antioxidant activity of substituted pyridinyl 1,3,4 oxadiazole derivatives. Res J Pharm Biol Chem Sci. 2010; 1: 1083–1090.

[5] Kheradmand A, Navipour L, Shafaroodi H, Saeedi-Motahar G, Shafiee A. Design and synthesis of niflumic acid-based -acylhydrazone derivatives as novel anti-inflammatory and analgesic agents. Med Chem Res. 2013; 22: 2411–2420. http://dx.doi.org/10.1007/s00044-012-0235-3

[6] De Souza MVN, Fernandes EL, Pais KC, Vasconcelos TA, Wardell SMSV, Wardell JL. Cyclisation of 2-chloro-N-(methyl-2-pyridinyl)nicotinamides to methyl 5-oxo-5,6-dihydro-dipyrido[1,2-a:3′,2′-e]pyrimidin-11-ium chlorides. J Chem Res. 2006; 2: 93–97. http://dx.doi.org/10.3184/030823406776331007

[7] De Souza MVN. Current Status and future prospects for new therapies for pulmonary tuberculosis. Curr Opin Pulm Med. 2006; 12: 167–171. http://dx.doi.org/10.1097/01.mcp.0000219264.42686.c9

[8] De Souza MVN, Ferreira ML, Pinheiro AC, Saraiva MF, Almeida MV, Valle MS. Synthesis and Biological Aspects of Mycolic Acids: An Important Target Against Mycobacterium tuberculosis. ScientificWorldJournal. 2008; 8: 720–751. http://dx.doi.org/10.1100/tsw.2008.99
[9] Black M, Hussain H. Hydrazine, Cancer, the Internet, Isoniazid, and the Liver. Ann Intern Med. 2000; 133: 911–913. http://dx.doi.org/10.7326/0003-4819-133-11-200012050-00016

[10] Kumar H, Malhotra D, Sharma R, Sausville E, Malhotra M. Synthesis, characterization and evaluation of Isoniazid analogues as potent anticancer agents. Pharmacologyonline. 2011; 3: 337–343.

[11] American Cancer Society. http://www.cancer.org/ (Accessed July, 2013)

[12] World Health Organization. http://www.who.int/gho/ncd/mortality_morbidity/cancer/en/index.html (Accessed July, 2013)

[13] Ma D, Chan DS, Leung C. Drug repositioning by structure-based virtual screening. Chem Soc Rev. 2013; 42: 2130–2141. http://dx.doi.org/10.1039/c2cs35357a

[14] Liu Z, Fang H, Reagan K, Xu X, Mendrick DL, Slikker Jr W, Tong W. In silico drug repositioning – what we need to know. Drug Discov Today. 2013; 18: 110–115. http://dx.doi.org/10.1016/j.drudis.2012.08.005

[15] De Souza MVN, Vasconcelos TA, Mello SCP, Wardell SMSV, Peralta MA, Ferreira B, Henriques MGMO, Neves Jr I, Lourenço MCS. Synthesis and Anti-Mycobacterial Activity of N’-[E)-(Disubstituted-Phenyl)Methylidene] Isonicotino-Hydrazide Derivatives. Lett Drug Des Discov. 2005; 2: 563–566. http://dx.doi.org/10.2174/157018005774479131

[16] De Souza MVN, Neves Jr I, Miranda GBP, Lourenço MCS, Vasconcelos TA, Pais KC, Wardell JL, Wardell SMSV, Alcântara Jr JP. Synthesis and in vitro anti-tubercular activity of a series of N’(disubstitutedbenzoyl) Isoniazid derivatives. Lett Drug Des Discov. 2006; 3: 424–428. http://dx.doi.org/10.2174/157018006777805549

[17] Ferreira ML, Cardoso LNF, Gonçalves RSB, Silva ET, Lourenço MCS, De Souza MVN. Synthesis and antitubercular evaluation of N’-[E)-(hydroxy, methoxy and ethoxy-substituted-phenyl) methylidene]isonicotinohydrazide derivatives. Lett Drug Des Discov. 2008; 5: 137–140. http://dx.doi.org/10.2174/157018008783928472

[18] Lourenço MCS, De Souza MVN, Pinheiro AC, Ferreira ML, Goncalves RSB, Nogueira TCM, Peralta MA. Evaluation of anti-tubercular activity of nicotinic and Isoniazid analogues. Arkivoc. 2007; 15: 181–191. http://dx.doi.org/10.3998/ark.5550190.0008.f18

[19] Carvalho SA, Da Silva EF, De Souza MVN, Lourenço MCS, Vicente FRC. Synthesis and antimycobacterial evaluation of new trans-cinnamic acid hydrazide derivatives. Bioorg Med Chem Lett. 2008; 18: 538–541. http://dx.doi.org/10.1016/j.bmcl.2007.11.091

[20] Lima CHS, Henriques MGMO, Candéa ALP, Lourenço MCS, Bezerra FAFM, Kaiser CR, De Souza MVN. Antimycobacterial evaluation of N’-(E)-heteroaromatic pyrazine-2-carboxyhydrazide derivatives. Med Chem (Hilversum). 2011; 7: 245–249. http://dx.doi.org/10.2174/157340611795564303
[21] Narang R, Narasimhan B, Sharma S.
A review on biological activities and chemical synthesis of hydrazide derivatives.
Curr Med Chem. 2012; 19: 569–612.
http://dx.doi.org/10.2174/092986712798918789

[22] Rollas S, Küçükgüzel SG.
Biological activities of hydrazones derivatives.
Molecules. 2007; 12: 1910–1939.
http://dx.doi.org/10.3390/12081910

[23] Vergara FMF, Da Silva CHL, Henriques MGMO, Candéa ALP, Lourenço MCS, Ferreira ML, Kaiser CR, De Souza MVN.
Synthesis and antimycobacterial activity of N’-[(E)-(monosubstituted-benzylidene)]-2-pyrazinecarbohydrazide derivatives.
Eur J Med Chem. 2009; 44: 4954–4959.
http://dx.doi.org/10.1016/j.ejmech.2009.08.009

[24] Mosmann TJ.
Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays.
J Immunol Meth. 1983; 65: 55–63.
http://dx.doi.org/10.1016/0022-1759(83)90303-4