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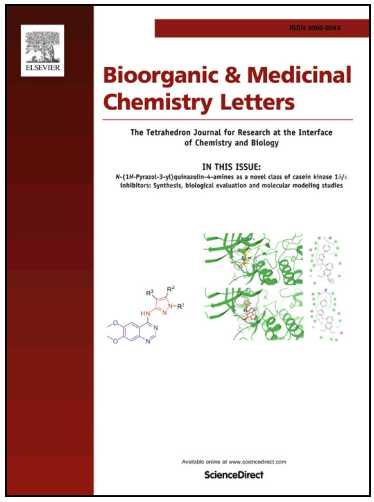

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Synthesis and *in vitro* antiproliferative activities of (5-aryl-1,2,4-oxadiazole-3-yl) methyl D-ribofuranosides

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ABSTRACT

The emergence of multidrug resistance cell lines is one of the major obstacles in the success of cancer chemotherapeutic treatment. Therefore, it remains a big challenge the development of new and effective drugs to defeat cancer. The presence of nitrogen heterocycles in the architectural design of drugs has led to the discovery of new leading compounds. Herein, we report the synthesis, characterization and *in vitro* antiproliferative activity against six cancer cell lines of D-ribofuranosides derivatives bearing a 1,2,4-oxadiazolic ring, with the aim of developing new active compounds. Most of these derivatives exhibit significant antiproliferative activities in the micromolar range. Noteworthy, the most potent compound of the series showed better selectivity towards the more resistant colon cancer cell line WiDr.

The synthesis of small molecules with antitumor activity remains the focus of attraction of many research groups. Despite ongoing developments in cancer treatments this disease remains persistent and prevalent, and therefore there is a high demand to develop new therapeutic agents that are more effective, specific and with less side effects than current chemotherapeutic drugs. As the metabolic profiles of cancer cells are distinct from those of normal cells (Warburg effect[1] and lipogenesis), the metabolic pathways involved in these processes could be the target for new cytotoxic therapies that are selective for a broad line of cancer cells.[2]

Nucleoside analogs have been in clinical use for nearly 50 years and have become key pieces in the treatment of cancer or viral infections. The approval of several additional drugs over the past decade demonstrates that this family still possesses strong potential.[3]

Several recent studies confirm the validity of the use of carbohydrates to improve antitumor activity. For example, carbohydrate-based sulfamates were shown to be excellent carbonic anhydrase (CA) IX inhibitors with Ki values of 1.9–2.4 nM, with 2–3 orders of magnitude selectivity for the inhibition of CA IX over CA I and CA II.[4]

Our previous efforts to obtain effective antitumor agents showed that 5-deoxy-5-*S*-(1,2,4-triazol-3-yl)-2,3-*O*-cyclopentylidene-β-D-ribofuranoside derivatives **1-4** (Figure 1) had a moderate inhibitory activity against BW 5147 lymphoma cell line (**1-2**)[5] and prostate cancer cell line (PC3) (**1-4**).[6] In the latter, compounds **1**, **3** and **4** were found to arrest cells at the G_0/G_1 phase of the cell cycle, while compound **2** induced apoptosis. In these cases, five-membered heterocyclic rings of known medicinal relevance (triazole, isoxazole and isoxazoline)[7] were selected to synthesize the novel diheterocyclic D-ribofuranoside derivatives **1-4**.

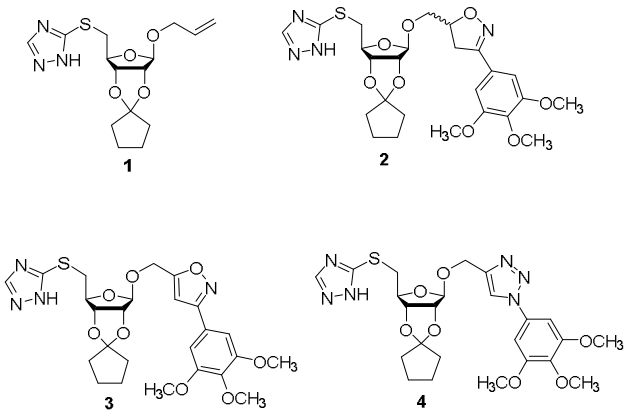

Figure 1. Structure of diheterocyclic D-ribofuranoside derivatives **1-4**.

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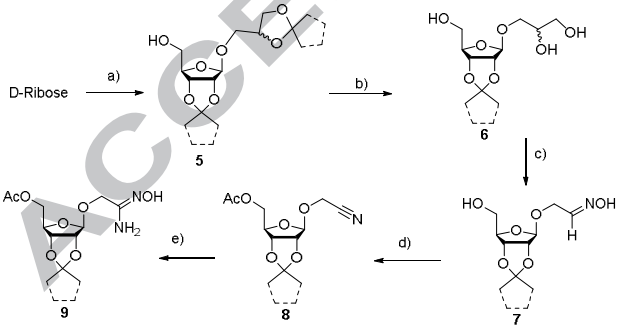
Oxadiazole is a molecular scaffold with versatile biological behavior. Particularly, the 1,2,4-oxadiazole ring is an interesting pattern for the synthesis of novel bioactive compounds and they are often used with the intention of being bioisosteric replacements for ester and amide functionalities.[8] Some drugs containing this heterocyclic ring are commercially available, such as prenoxdiazine (anti-tussive), oxolamina (anti-inflammatory) or irrigor (vasodilator). Besides this, the development of novel biologically active compounds containing a 1,2,4-oxadiazole ring were recently reported. 3,5-Disubstituted-1,2,4-oxadiazole is the principal scaffold of a new generation of agonists of the sphingosine 1-phosphate receptor 1 (S1P1), exhibiting high potency and selectivity.[9] Pyrazole-pyrimidine derivatives bearing 1,2,4-oxadiazole were tested in vitro for anti-tumor activity in a panel of 12 human tumor cell lines, finding that N^3-(4-(3-(tert-butyl)-1,2,4-oxadiazol-5-yl)phenyl)-6-phenyl-1*H*-pyrazolo[3,4-d]pyrimidine-3,4-diamine has a moderate overall potency, combined with good selectivity towards the renal cancer cell line RXF 393.[10] 4-Chloro-benzamides derivatives containing the 1,2,4-oxadiazole core, afforded a new class of receptor tyrosine kinase inhibitors, showing comparable activity to the multi-targeted tyrosine-kinase inhibitor Ponatinib.[11]

Considering that the 1,2,4-oxadiazolic ring is an important part of many biologically active compounds and the potential antitumor activity of the diheterocyclic 2,3-*O*-cyclopentylidene-β-D-ribofuranoside compounds, we envisioned the preparation of a small and focused library of compounds bearing both structural features. The antiproliferative activity was explored against a panel of six human solid tumor cell lines. The results were compared to those obtained for compounds **2-4**.

Previous studies[5] suggested that a 1,2,4-triazole linked to the C-5 position of ribose by a sulfur improved the antiproliferative activity. Therefore, we designed an alternative synthetic route to incorporate a 1,2,4-oxadiazolic ring to the D-ribofuranoside scaffold preserving the thioheterocyclic group at C-5. In addition, an analogous set of compounds substituting the 1,2,4-triazole group by 5-amino-1,3,4-thiadiazole, a core structural component in drug arrays,[12] was synthesized.

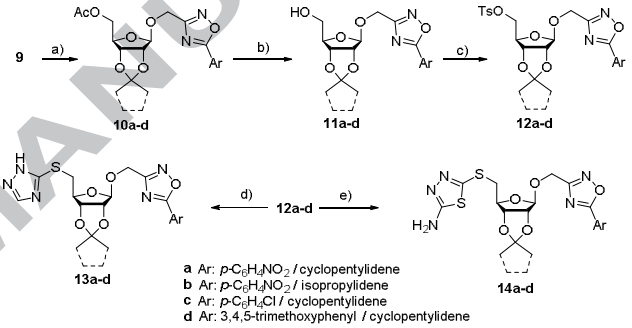
The synthetic route consists of two parts. The first one comprises the transformation of commercially available D-ribose into the appropriate intermediate to introduce the heterocyclic rings (Scheme 1). The second relates to the introduction of both heterocyclic groups (Scheme 2).



Scheme 1. Reagents and conditions, yield %: a) $CuSO_4$, cyclopentanone or acetone, glycerol, H_2SO_4, 40°C, 4 days, 34% (5a), 25% (5b); b) AcOH 70%, 2 h, 89% (6a), 90% (6b); c) i. $NaIO_4$, EtOH/H_2O, 1 h; ii. $NH_2OH{\cdot}HCl$, NaOH, H_2O/EtOH, 87% (7a), 97% (7b); d) Ac_2O, pyridine, 73% (8a), 57% (8b); e) $NH_2OH{\cdot}HCl$, NaOH, H_2O/MeOH, 65°C, 89% (9a), 87% (9b).

The synthesis started with the simultaneous protection of D-ribose and glycerol in a one-step reaction to afford compound **5** as a diastereomeric pair (Scheme 1). The cyclopentylidene group of the glycerol moiety was selectively removed in acetic acid 70% at room temperature for 2 h yielding compound **6**. Compound **7** was obtained by cleavage of diol **6** with sodium periodate followed by reaction with hydroxylamine. Cyanomethyl derivative **8** was achieved by treating compound **7** with acetic anhydride in pyridine at 50 °C for 3 h. Finally, a methanol solution of cyanomethyl derivative **8** was refluxed for 3 h to give the 2-amino-2-(hydroxyimino)ethyl derivative **9**.

The 1,2,4-oxadiazoles **10a-d** (Scheme 2) were obtained by reacting 2-amino-2-(hydroxyimino)ethyl derivative **9** with a variety of substituted benzoyl chlorides in dichloromethane, using DIPEA as base, at room temperature, overnight and in the dark. Given that reports in the literature show that electron withdrawing groups (Cl and NO_2) attached at position 4 of the phenyl group enhanced the cytotoxic activity,[13] we speculated that the introduction of these substituents could be promising to obtain compounds with better antiproliferative activities. Next, compounds **10a-d** were converted to their corresponding tosyl derivatives **12a-d** by successive deprotection with NaOMe/MeOH (1.3 M) and tosylation steps. Finally, compounds **13a-d** and **14a-d** were obtained by the nucleophilic displacement of the tosyl group with sodium 1,2,4-triazole-3-thiolate and sodium 1,3,4-thiadiazole-5-amino-2-thiolate, respectively.



Scheme 2. Reagents and conditions, yields %: a) i. substituted benzoyl chlorides, DIPEA, CH_2Cl_2; ii. toluene, reflux, 18-46%; b) NaOMe/MeOH, 74-96%; c) TsCl, pyridine, 80-95%; d) sodium 1,2,4-triazole-3-thiolate, DMF, 50°C, 60-77%; e) sodium 5-amino-1,3,4-thiadiazole-2-thiolate, DMF, 50°C, 56-86%.

The diheterocyclic compounds **13-14** were evaluated for their in vitro antiproliferative activity against the human cancer cell lines A549 (lung), HeLa (cervical), HBL-100 (breast), SW1573 (lung), T-47D (breast), and WiDr (colon).[14] In addition, compounds **2-4** and selected intermediates (**10-12**) were tested for comparison purposes. The results, expressed as GI_{50} values, are shown in Table 1.

From the results of biological activity some preliminary structure-activity relationships can be inferred. When compared to the prototype compounds **2-4**, the 1,2,4-oxadiazolic analog **13d** showed overall a slight increase in the antiproliferative activity. A comparable positive effect was observed for the corresponding 5-amino-1,3,4-thiadiazole derivative **14d**. As anticipated by other authors,[13] compounds with electron withdrawing groups on the phenyl ring (**13a**, **13c**, **14a** and **14c**) presented an improvement on the biological activity, based on GI_{50} values. From this group, compound **13c** resulted the most active of the series, with GI_{50} values against all cell lines in the range 4.5-24 μM. Noteworthy, **13c** was more active in the most resistant cell line to conventional antitumor drugs WiDr. Next, the replacement of the cyclopentadienyl group (**13a**, **14a**) by an isopropylidene substituent (**13b**, **14b**) produced a remarkable loss in activity. Finally, when considering the synthetic intermediates

10-**12**, we observe that only the tosyl derivative showed inactive (GI_{50} > 100 μM).

Table 1. Antiproliferative activity (GI_{50}) against human solid tumor cell lines.[a]

Compound	*A549*	*HBL-100*	*HeLa*	*SW1573*	*T-47D*	*WiDr*
2	37 ± 4.9	43 ± 3.0	38 ± 3.0	>100	55 ± 8.9	53 ± 8.2
3	59 ± 8.6	87 ± 15	67 ± 2.2	97 ± 4.8	94 ± 1.3	74 ± 9.7
4	33 ± 4.0	84 ± 13	30 ± 9.6	>100	41 ± 8.8	26 ± 2.3
10a	29 ± 6.0	23 ± 6.4	17 ± 2.0	29 ± 0.4	52 ± 3.6	36 ± 2.8
11a	32 ± 4.1	17 ± 2.6	15 ± 2.0	27 ± 2.4	50 ± 9.4	41 ± 5.4
11b	99 ± 1.7	99 ± 2.3	99 ± 0.8	60 ± 11	>100	>100
12a	>100	>100	>100	>100	>100	>100
13a	10 ± 2.4	24 ± 1.1	20 ± 0.5	15 ± 5.0	20 ± 3.5	20 ± 5.0
13b	90 ± 14	>100	89 ± 15	>100	>100	>100
13c	13 ± 0.4	24 ± 3.3	14 ± 7.2	10 ± 4.9	10 ± 1.5	4.5 ± 1.6
13d	28 ± 0.8	34 ± 3.8	37 ± 12	36 ± 2.4	44 ± 17	42 ± 6.0
14a	14 ± 2.5	26 ± 1.6	28 ± 3.1	16 ± 4.0	19 ± 4.0	26 ± 4.2
14b	>100	>100	>100	>100	>100	>100
14c	15 ± 2.1	30 ± 1.4	16 ± 2.6	17 ± 3.1	18 ± 2.5	15 ± 4.1
14d	36 ± 13	33 ± 4.3	31 ± 6.8	38 ± 0.5	42 ± 2.6	41 ± 1.1

[a] Values are expressed in μM and are means of two to three experiments ± standard deviation.

In conclusion, we have synthesized a small and focused library of diheterocyclic-ribose derivatives containing a 5-aryl-1,2,4-oxadiazole unit. These compounds as well as some of their precursors were evaluated for their cell proliferation inhibition properties against six human cancer cell lines. Our results correlate to previous studies, which suggested that compounds containing a sulfur-linked 1,2,4-triazole group on position 5 of the ribose framework improved the antiproliferative activity. Although several of the synthesized derivatives exhibited moderate potency against all cell lines, the 1,2,4-triazole group resulted more effective than the 5-amino-1,3,4-thiadiazole ring. The *p*-chlorophenyl turned out to be the most effective aryl group. Compound **13c** was found to be the most active in the series showing a moderate overall potency with good selectivity towards the colon cell line WiDr (GI_{50}= 4.5 μM).

Acknowledgments

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References and notes

1. Vander Heiden, M. G.; Cantley, L. C.; Thompson, C. B. *Science* **2009**, *324*, 1029.
2. Flaveny, C. A.; Griffett, K.; El-Gendy, B. E.-D. M.; Kazantzis, M.; Sengupta, M.; Amelio, A. L.; Chatterjee, A.; Walker, J.; Solt, L. A.; Kamenecka, T. M.; Burris, T. P. *Cancer Cell* **2015**, *28*, 1.
3. Jordheim, L. P.; Durantel, D.; Zoulim, F.; Dumontet, C. *Nat. Rev. Drug Disc.* **2013**, *12*, 447.
4. Moeker, J.; Mahon, B. P.; Bornaghi, L. F.; Vullo, D.; Supuran, C. T.; McKenna, R.; Poulsen, S.-A. *J. Med. Chem.* 2014, 57, 8635.
5. Avanzo, R.E.; Anesini, C.; Fascio, M.L.; Errea, M.I.; D'Accorso, N.B. *Eur. J. Med. Chem.* **2012**, *47*, 104.
6. Gueron, G.; Avanzo, R. E.; Schuster, F.; Carabelos, M. N.; Vazquez, E.; Fascio, M. L,; D'Accorso, N. B. *Biomed. Pharmacother.* **2014**, *68,* 847.
7. Akhtar, J.; Khan A. A.; Ali, Z.; Haider, R.; Yar, M. S. *Eur. J. Med. Chem.* **2017**, *125*, 143.
8. McBriar, M. D.; Clader, J. W.; Chu, I.; Del Vecchio, R. A.; Favreau, L.; Greenlee, W. J.; Hyde, L. A.; Nomeir, A. A.; Parker, E. M.; Pissarnitski, D. A.; Song, L.; Zhang, L.; Zhao, Z. *Bioorg Med Chem Lett.* **2008**, 18, 215.
9. Gilmore, J. L.; Sheppeck, J. E.; Watterson, S. H.; Haque, L.; Mukhopadhyay, P.; Tebben, A. J.; Galella, M. A.; Shen D. R.; Yarde, M.; Cvijic, M. E.; Borowski, V.; Gillooly, K.; Taylor, T.; McIntyre, K. W.; Warrack, B.; Levesque, P. C.; Li, J. P.; Cornelius, G.; D'Arienzo, C.; Marino, A.; Balimane, P.; Salter-Cid, L.; Barrish, J. C.; Pitts, W. J.; Carter, P. H.; Xie, J.; Dyckman, A. J. *J. Med. Chem.* **2016**, *59*, 6248; b) Rosenberg, A. J.; Liu, H.; Jin, H.; Yue, X.; Riley, S.; Brown, S. J.; Tu, Z. *J. Med. Chem.* **2016**, *59*, 6201.
10. Maftei, C. V.; Fodor, E.; Jones, P. G.; Daniliuc, C. G.; Heiko Franz, M.; Kelter, G.; Fiebig, H.- H.; Tamma, M.; Neda, I. *Tetrahedron* **2016**, *72*, 1185.
11. Han, M.; Li, S.; Ai, J.; Sheng, R.; Hu, Y.; Hu, Y.; Geng, M. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 5679.
12. Jain, A. K., Sharma, S.; Vaidya, A.; Ravichandran, V.; Agrawal, R. K. *Chem. Biol. Drug Des.* **2013**, *81*, 557.
13. Shi, W.; Hu, J.; Bao, N.; Li, D.; Chen, L.; Sun, J. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 147.
14. Nieto, D.; Bruña, S.; González-Vadillo, A. M.; Perles, J.; Carrillo-Hermosilla, F.; Antiñolo, A.; Padrón, J. M.; Plata, G. B.; Cuadrado, I. *Organometallics* **2015**, *34*, 5407−5417.

Supplementary Material

Materials and methods, synthesis, compound characterization, and ^{1}H- and ^{13}C-NMR spectra.

Graphical Abstract

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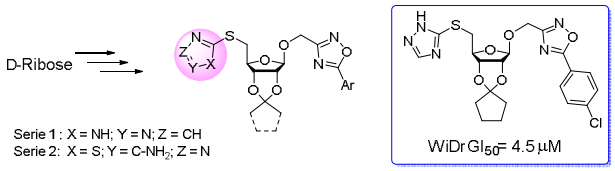

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