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Design, synthesis and evaluation of novel levoglucosenone derivatives as promising anticancer agents

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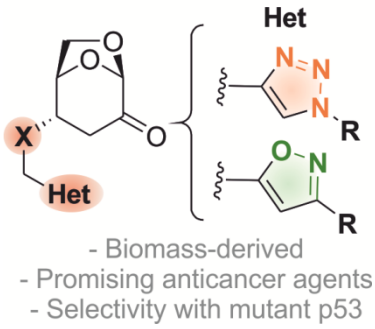
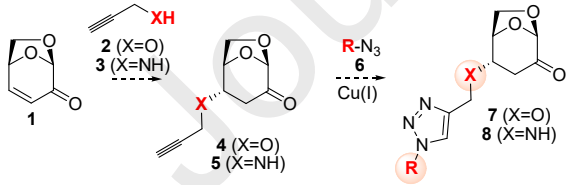


A series of levoglucosenone-derived 1,2,3-triazoles and isoxazoles featuring a flexible spacer between the heteroaromatic and anhydropyranose cores have been designed and synthesized following an hetero Michael // 1,3-dipolar cycloaddition path. The use of a design of experiments approach allowed the optimization of the oxa-Michael reaction with propargyl alcohol as nucleophile, a key step for the synthesis of the target compounds. All of the compounds were tested for their anticancer activity on MDA-MB-231 cells, featuring mutant p53. The results highlighted the importance of the introduction of the flexible spacer as well as the higher activity of oxa-Michael isoxazole-derivatives. The most prominent compounds also showed anti-proliferative activities against lung and colon cancer cell lines. The compounds showed enhanced cytotoxic effects in the presence of mutant p53, determined both by endogenous mutant p53 knock down (R280K) and by reintroducing p53 R280K in cells lacking p53 expression.

Levoglucosenone (**1**), is the main product of the pyrolytic transformation of acid-pretreated cellulosic materials.[1] Its structural rigidity and versatile functionality were exploited in the synthesis of natural products,[1] valuable intermediates,[2] new tools of asymmetric synthesis,[3] and development of greener solvents.[4] It also emerges as a prominent pharmacophore in medicinal chemistry.[1] In particular, some of its derivatives have shown anticancer activities related with the restoration of p53,[5] a transcription factor acting as a tumor suppressor which can induce apoptosis or senescence in cases of persistent oncogenic stress or DNA damage.[6] Mutation of the p53 gene (*TP53*) is the most common genetic alteration in human cancer (more than 50% in some cases).[6c] In most cases, missense mutations are found in the DNA binding domain. As a consequence, human tumors often show abundant expression of point mutant p53 proteins which have lost tumor suppressor function. The possibility to restore wt (wild type) function by a refolding process induced upon the interaction of mutant p53 proteins with small organic molecules was proposed. In this way, a robust cytotoxic response may be unleashed in tumor cells.Several small molecules able to restore wt p53-associated responses were identified .[6d] However, direct interaction with the mutant protein may not to be required in all cases. Even though the underlying mechanisms are still under investigation, this leading strategy in anticancer drug development resulted in the discovery of PRIMA-1 and other compounds which have reached clinical trials.[6e]

Recently we reported the synthesis of levoglucosenone-derivatives featuring triazol units attached to the C-4 position. Some derivatives exhibited satisfactory *in vitro* anti-proliferative activity against MDA-MB-231 breast cancer cell line, and showed enhanced activity in the presence of mutant p53, suggesting a possible path to be further exploited.[5f]

This exciting discovery motivated us to explore structural modifications for SAR studies. We foresaw evaluating the effect of introducing a flexible spacer between the anydrosugar and triazole cores, which would significantly change the conformational freedom and topology of the molecule. This modification could be done by exploiting the known reactivity of **1** as Michael acceptor (Scheme 1), allowing access to additional elements of diversity, such as the nature of the heteroatom at C-4) and the possibility to synthesize other heteroaromatic cores.



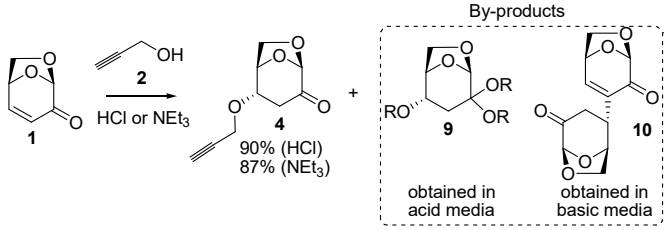
Scheme 1. Synthesis of novel levoglucosenone derivatives.

Despite its great synthetic utility, the oxa-Michael reaction is one of the less studied variants of the Michael-type reactions. The major drawbacks are related with the poor nucleophilicity of alcohols and the reversibility of the addition stage.[7] Levoglucosenone is a reactive enone system suitable for 1,4-conjugate additions with a variety of reagents,[1] but the reaction using alcohols has been almost unexplored. Initially we carried out the base-catalyzed addition of **2** to levoglucosenone using the procedure reported by Shafizadeh *et al* for methanol as nucleophile (0.6 eq. NEt_3, r.t., 3 h, 100%),[8a] but the results obtained were disappointing. The desired product **4** was obtained in low yield (<40%), with a significant dimerization of levoglucosenone (*vide infra*). We also tested the conditions reported by the same group for the oxa-Michael reaction with benzyl alcohol (BnOK/BnOH, r.t., 5 min),[8b] but the isolated yields of **4** were still very low. On the basis of the difficulties encountered to efficiently achieve

this transformation with known procedures, we undertook the optimization of the acid promoted and base promoted oxa-Michael reaction of **1** and **2** with the aid of a design of experiments approach (DOE), which allows the exploration of the multidimensional chemical space on a more rational basis.[9] Since the oxa-Michael reactions can be typically carried out in acid media (activation of the Michael acceptor) or basic media (activation of the Michael donor),[7] we decided to evaluate both conditions.

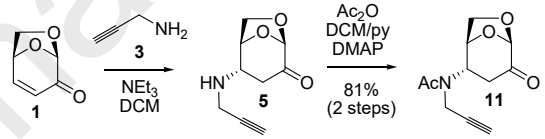
Combining a survey of inexpensive promoters, variable selection by factorial designs, and final optimization through central composite analysis (detailed in the ESI), the desired **4** could be successfully obtained in high yields (Scheme 2). The by-products (ketal **9** or dimer **10**) were kept as traces at the optimized protocols. In the acid-catalyzed process, HCl was the most promising acid promoters. Interestingly, the central composite analysis yielded a second-order polynomial model with a strong quadratic behavior with factor A (HCl normality). Using concentrated HCl solutions decreased the yield of **4** through formation of the by-product **9**, whereas this side reaction could be suppressed by the water content provided by more diluted HCl. However, highly diluted HCl feedstocks somehow displaced the equilibrium towards **1**. The optimal reaction conditions were predicted around 1.5 eq. HCl 5N, 16 h, 25 ºC, affording a 90% of **4** (higher than those obtained in all previous experiments), demonstrating the power of the conducted DOE methodology. The value was reproducible after several replications, and showing minor changes upon scaling. To the best of our knowledge, this is the first report of the acid-catalyzed oxa-Michael reaction using levoglucosenone as acceptor.

In the case of base-catalyzed conditions, the central composite design uncovered a linear relationship with negative slopes between reaction time and Nº eq. of NEt_3 (the most promising base under study) with the yield of **4**. This prompted us to evaluate the reactions at descending quantities of NEt_3 keeping the reaction time fixed at 6 min (shorter reaction times resulted operationally impractical). In perfect agreement with the DOE predictions, the yields of **4** increased gradually to reach a maximum around 0.1-0.15 eq (see SI). Even lower amounts of NEt_3 resulted in a dramatic reduction in the catalytic activity. The optimal reaction conditions were then set at 0.1 eq. NEt_3, 6 min, 25 °C, affording an overall yield of 87%. These results were in clear contrast to the experimental reaction conditions found for Shafizadeh *et al.* using methanol as nucleophile (0.6 eq. NEt_3, 3 h),[8a] suggesting that the nature of the alcohol is closely related with the optimal reaction parameters.



Scheme 2. Design of experiments-optimized synthesis of **4**. Optimal conditions: 1.5 eq. HCl 5N, 16 h, 25 ºC (acid conditions); or 0.1 Eq. NEt_3, 6 min, 25 ºC (basic conditions).

The synthesis of **5** was more straightforward, obtaining the desired product in ca. 100% conversion. However, after purification by column chromatography, compound **5** experienced significant retro-aza-Michael decomposition. Such behavior (particularly important with aliphatic amines) was recently reported by Greatrex and co-workers.[2b] To overcome this limitation, we foresaw that acetylation of the secondary amine in **5** would reduce the tendency for the retro-aza-Michael decomposition. Upon treating the reaction crude of **5** with freshly distilled Ac_2O, the acetamide **11** could be isolated in 81% overall yield after column chromatography (Scheme 3). As in the case of **4**, the 1,6-anhydro bridge allowed excellent levels of diastereoselectivity, obtaining only the isomers resulting from the attack of the nucleophiles through the α face of the enone.



Scheme 3. Synthesis ofacetamide **11**

Once the two propargyl precursors were synthesized, the 1,3-dipolar cycloaddition with organic azides was carried out to obtain the desired 1,2,3-triazoles. The aliphatic azides were prepared by standard S_N2 reactions of NaN_3 and the corresponding primary or secondary alkyl chloride or bromide, whereas the aromatic azides were synthesized via the Sandmeyer reaction. The azides were chosen to provide diversity of alkyl, vinyl and aryl groups with different substitution patterns and electronic characters. Using a modified protocol of Kim (0.5 mmol of **4**/**11**, 1.5 eq. azide, 40 mol% sodium ascorbate, 10 mol% $CuSO_4.5H_2O$, CH_2Cl_2:H_2O 1:1),[10] the desired triazoles were obtained in good yields (Table 6).

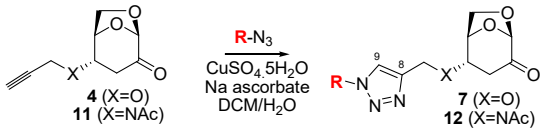
The pharmacokinetic properties of new compounds represent an obstacle that must be overcome to reach the clinical trials. Hence, an early estimation of the ADME properties is mandatory, allowing a preliminary drug-likeness evaluation in a simple and rapid fashion. Using the online open access tool Molinspiration (www.molinspiration.com) we computed the following molecular descriptors: the partition coefficient (log P), the molecular weight (MW), the topological polar surface area (TPSA), the number of hydrogen bond acceptors (nON), the number of hydrogen bond donors (nOHNH), and the number of rotatable bonds (nrotb). All of the compounds in the library show no violation of the Lipinski´s rule of five. All of them have a MW lower than 500 kDa, a logP lower than 5, nON less than 10 and nOHNH less than 5. Moreover, they all showed a polar surface area lower than 140 $Å^2$ and 10 or less rotable bonds, which indicate good cell permeability. Altogether, this *in silico* study supported the library choice.

The *in vitro* antiproliferative activity was studied on the MDA-MB-231 cell line, originally derived from Triple Negative Breast cancer (TNBC). This cell line lacks a wt allele at the *TP53* locus but retained a mutated one, allowing exclusive expression of endogenous p53 R280K mutant protein. Upon treatment with each individual compound for 48 h at different concentrations, living cells were quantified using the MTT viability assay and normalized comparing with untreated cells. The half maximal inhibitory concentration (IC_{50}) obtained for all compounds are also shown in Table 1.

Interestingly, while none of the nitrogenated compounds **12** exhibited interesting levels of cytotoxicity (IC_{50}> 50 µM), most of the oxigenated counterparts were active in the range 18.6-36.0 µM, comparable with the cytotoxicity of PRIMA-1 determined on the same line and similar experimental conditions.[11] The lack of activity of the alkyne precursors (IC_{50} > 100 µM) suggested that the incorporation of the triazole unit was important in terms of cytotoxicity, whereas the larger amount of bioactive compounds (regarding the original series) validated our structural hypothesis.

To analyze the influence of the nature of the heterocycle, we foresaw the synthesis and evaluation of isoxazole analogues, considered a prime scaffold for drug discovery.[12]

Table 1. Synthesis and evaluation of triazoles **7** and **12** on survival of MDA-MB-231 breast cancer cells.[a]

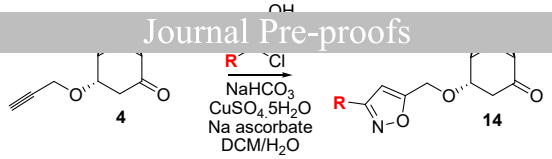


Entry	X	R	Product	Yield	IC_{50} (µM)
1	O	-Bn	**7a**	78%	>50
2	O	-CH_2CO_2Me	**7b**	58%	21.3
3	O	-$CH_2C(O)Ph$	**7c**	79%	25.9
4	O	-Octyl	**7d**	85%	>50
5	NAc	-Bn	**12a**	67%	>50
6	NAc	-CH_2CO_2Me	**12b**	77%	>50
7	NAc	-$CH_2C(O)Ph$	**12c**	76%	>50
8	NAc	-*n*Octyl	**12d**	91%	>50
9	O	-4-NO_2Ph	**7e**	81%	24.8
10	O	-4-ClPh	**7f**	77%	26.1
11	O	-Ph	**7g**	70%	25.2
12	O	-4-FPh	**7h**	78%	23.7
13	O	-Butyl	**7i**	66%	27.9
14	O	-$CH_2C(Me)=CH_2$	**7j**	74%	26.7
15	O	-2-ClPh	**7k**	81%	26.4
16	O	-2-BrPh	**7l**	79%	22.5
17	O	-4-CO_2HPh	**7m**	75%	23.2
18	O	-α-naphtyl	**7n**	96%	21.9
19	O	-2,3-dichloroPh	**7o**	55%	26.0
20	O	-CH_2(4-OCH_3)Ph	**7p**	~100%	34.8
21	O	-CH_2(4-NO_2)Ph	**7q**	~100%	25.4
22	O	-CH_2(β-naphtyl)	**7r**	98%	23.7
23	O	-α-cyclohexanone	**7s**	67%	36.0
24	O	fluorenil	**7t**	87%	18.6
25	O	-cyclohexyl	**7u**	48%	24.3

[a]General procedure: 0.5 mmol of **4** or **11**, 1.5 eq. azide, 40 mol% sodium ascorbate, 10 mol% $CuSO_4.5H_2O$, CH_2Cl_2:H_2O 1:1). [b]Isolated yield obtained after column chromatography. [c]IC_{50}: concentration achieving 50 % reduction in viability determined by MTT assay.

Taking advantage of the alkyne precursor **4**, the synthesis of the targets were carried out following the known 1,3-dipolar cycloaddition with nitrile oxides.[13] Starting from commercially available aldehydes, the imidoyl chlorides **13** were easily prepared in 2 steps (Scheme 4). Exposure of **13a** (R=Ph) with $NaHCO_3$ afforded the corresponding nitrile oxide, which further reacted with alkyne **4** under Sharpless conditions (Cu(I), t-BuOH/H_2O) to give **14a** in low yield (36%).[13] Analysis of the reaction mixture by ^1H NMR evidenced significant retro-oxa-Michael decomposition. To prevent this side reaction, we next evaluated the CH_2Cl_2/H_2O solvent system with the aim of minimizing the contact between **4** and the base in the bifasic system. To our delight, this simple modification allowed the isolation of **14a** as the only detectable isomer in very good yield. We used this modified procedure for the synthesis of a small library of isoxazoles featuring diversity (Table 2). In all cases the corresponding 3,5-disubstituted isoxazoles were obtained as single regioisomers, with high conversion (as determined by TLC analysis of the reaction mixtures) and isolated yields varying from discrete to very good depending on the nature of the substituent at the isoxazole core. The modest yields observed in some cases were mainly attributed to partial decomposition that took place during the chromatographic purification stage. In general, aromatic fragments afforded cleaner reactions than aliphatic or vinylic moieties, which is consistent with the relative stability of the corresponding imidoyl chloride.[13]

Table 2. Synthesis and evaluation of isoxazoles **14** on survival of MDA-MB-231 breast cancer cells.[a]



Entry	R	Product	Yield	IC_{50} (μM)
1	-Ph	**14a**	76%	21.9
2	-4-OMe-Ph	**14b**	70%	21.9
3	-4-NO_2-Ph	**14c**	52%	16.5
4	2-Naphtyl	**14d**	59%	21.2
5	-CH=CH-Ph	**14e**	88%	22.9
6	3,5-$(CF_3)_2$-Ph	**14f**	62%	23.9
7	-4-Cl-Ph	**14g**	82%	17.9
8	-cyclohexyl	**14h**	65%	15.8
9	-CH=CH-4-OMe-Ph	**14i**	35%	26.4
10	-5-Cl-2-furyl	**14j**	66%	13.8

[a]General procedure: 0.25 mmol of **4,** 1 eq. of imidoyl chloride, 10 mol% of sodium ascorbate, 2 mol% of $CuSO_4.5H_2O$ and 4.5 eq of $KHCO_3$ in DCM:H_2O 1:1[b]Isolated yield obtained after column chromatography . [c]IC_{50}: concentration achieving 50 % reduction in viability determined by MTT assay.

The biological assays performed with compounds **14a-j** showed that all isoxazoles displayed cytotoxicity, with IC_{50} values ranging 13.8-26.4 μM. These values were lower than those obtained with the 1,2,3-triazole derivatives **7**, suggesting that the pendant isoxazole ring is a more prominent structural motif. This can be evidenced by comparing the activities observed for compounds with the same R substituent at the aromatic fragment (for example, **14g**, **14h** and **14c** with **7f**, **7u** and **7e**, respectively), being the isoxazoles ~43% more potent (on average) than the corresponding triazole analogues. In fact, the most active compound synthesized in this work was **14j**, featuring a 5-Cl-2-furyl substituent, with an IC_{50} value of 13.8 μM. In order to expand our observations, we selected compounds **14a**, **14c**, **14e**, **14g**, **14h** and **14j**, as well as compounds **7e-g**, **7j**, **7t**, and **7u** for further studies. The selection was made to include the most prominent members of each library, and to cover different elements of structural diversity.

First, we tested the anti-proliferative activity in other cancer cell lines, such as lung (H1299) and colon (HT29). As shown in Table 3, the selected compounds exhibited interesting results, with IC_{50} values slightly lower than those observed with MDA-MB-231 cells. Isoxazoles were still more active than triazoles, being compounds **14h** and **14j** the most prominent members of the library.

Table 3. Anti-proliferative activity of selected compounds against lung (H1299) and colon (HT29) cancer cell lines.

		IC_{50} (μM)		
Entry	**Compound**	**MDA-MB-231**	**H1299**	**HT29**
1	**7e**	24.8	10.3	18.3
2	**7f**	26.1	10.6	14.7
3	**7g**	25.2	10.9	13.4
4	**7j**	26.7	14.3	17.2
5	**7t**	18.6	9.0	15.6
6	**7u**	24.3	12.0	11.9
7	**14a**	22.0	9.8	16.9
8	**14c**	23.0	11.2	19.8
9	**14e**	15.9	7.1	13.7
10	**14g**	18.3	9.0	14.5
11	**14h**	15.2	6.1	9.0
12	**14j**	13.6	7.6	10.1

To further characterize the selected compounds, we tested their effect on the non-tumorigenic Vero cell line in similar conditions. We found that in all cases the IC_{50} increased comparing with the three tumorigenic cell lines, indicating a selective antiproliferative effect towards malignant cells (see ESI). Comparing with H1299 and HT29 cells, IC_{50} values changed more than three-fold for several compounds. Although the differences were less marked when we compared Vero with MDA-MB-231 cells, compounds **7t** and **7u** showed a clear increase of more than two-fold. Interestingly, these same compounds also showed the highest selectivity index values (SI: $IC_{50\ non\ tumor}/IC_{50\ tumor}$) comparing Vero with H1299 and HT29 cells (with up to almost 5- and 6-fold IC_{50} increase in the cases of **7u** and **7t**, respectively. Thus, our results indicate that some of the synthesised compounds have a selective antiproliferative effect against tumor cells, particularly **7t** and **7u**.

Finally, we wondered if the cytotoxic effect depends on the presence of mutant p53. To test this hypothesis, similar survival assays were performed upon p53 R280K knock down. MDA-MB-231 cells were transduced with plasmids coding for a specific shRNA for p53 (shp53) or an unrelated sequence as a control (shControl).[14] The reduction in mutant p53 levels was confirmed by western blot.

We found that survival was significantly increased upon mutant p53 knock down when cells were treated with all compounds tested (Figure 1a), supporting the idea that mutant p53 is necessary to achieve higher activity. To further explore the dependence of the cytotoxic effect on the presence of mutant p53 we analysed the effect of reintroducing p53 R280K in cells lacking expression of any p53 form. To that end we used H1299 cells, which lost expression of any p53 form due to a spontaneous deletion of the gene. Cells were transduced with plasmids coding for p53 R280K of GFP as a control and the expression of mutant p53 was confirmed by western blot. In line with our results, we found that cytotoxicity was significantly increased in cells expressing mutant p53 in all cases (Figure 1b). Therefore, our results showed that the analysed compounds displayed a selective effect on mutant p53 expressing cells. This is a relevant feature, since p53 mutants are absent in normal cells. Consequently, selective action on mutant p53 expressing cells may enhance specificity of the treatment, reducing the risk of adverse effects.

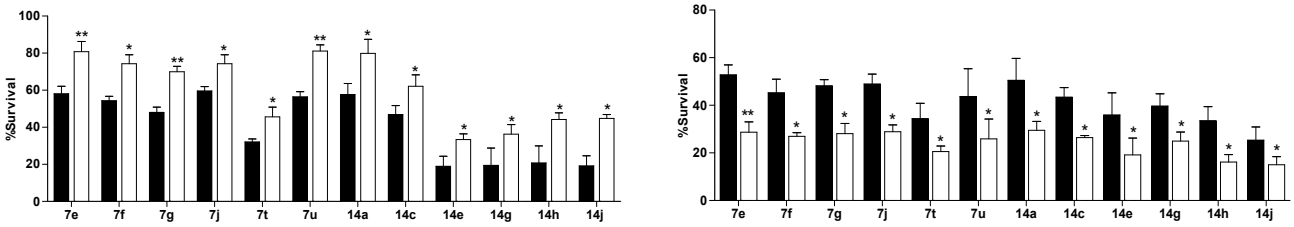


Figure 1. a) Cytotoxicity of selected triazoles and isoxazoles is reduced in MDA-MB-231 cells upon mutant p53 knock down. Survival assays for the indicated compounds (25 μM) on control cells (shControl, black bars) or cells with stable knock down of p53 R280K (shp53,white bars). b) Cytotoxicity of selected triazoles and isoxazoles is enhanced upon introduction of p53 R280K in p53 null H1299cells. Survival assays for the indicated compounds (10 μM) on p53 null H1299 lung carcinoma cells (black bars) or H1299 cells expressing p53 R280K (white bars). In both cases, survival was normalized to DMSO control treatment and expressed as mean value and standard error of the mean (sem). One tail T-test, n=3, * p<0,05, ** p<0,01.

In conclusion, novel levoglucosenone-derived compounds have been designed and synthesized through an hetero Michael addition and 1,3-dipolar cycloaddition sequence. The oxa-Michael reaction with propargyl alcohol, key to obtain the most active compounds, was optimized with the aid of a design of experiments approach. All compounds were further evaluated as anticancer agents against MDA-MB-231 cell line. The incorporation of a flexible spacer proved to be relevant in terms of the biological activity, as well as the incorporation of an oxygen atom at the C-4 position of the anhydro pyranose. On the other hand, the nature of the heteroaromatic ring at the pendant side chain was also evaluated, being isoxazole more active than the 1,2,3-triazole analogues. The most active compounds were tested against two additional cancer cell lines showing very good results. A higher antiproliferative activity against tumor cells was observed for several of the identified compounds. Among them, **7t** and **7u** are particularly interesting since they showed the highest selectivity for tumor cells, suggesting that they could provide a useful therapeutic window. In addition, the identified compounds showed a significant dependence on the presence of mutant p53, a specific marker of tumor cells, further underlining the potential of these compounds as leading molecules for antitumoral strategies.

The mechanism of action of compounds like PRIMA-1 and PRIMA-1^{MET}, originally identified with the aim to reactivate wt p53-like functions in cell expressing mutant p53, is still a matter of debate. A direct interaction between derivatives of these compounds and mutant p53 was demonstrated.[15] However, mechanisms that do not require direct interaction with mutant p53 were also proposed.[16] In particular, PRIMA-1 and PRIMA-1^{MET} are able to enhance oxidative stress or activation of the Unfolded Protein Response, even in cells lacking p53 or bearing the wt form. In some cases, the presence of mutant p53 may promote cytotoxicity by sensitizing cells to the stress conditions imposed by the presence of drug treatment. Collectively, the available evidence suggests that these compounds may activate several mechanisms, inducing a response that is dose and context dependent. In spite of its complexity, the presence of pleiotropic effects may also represent an advantage, since they may allow to expand the application to wt or null p53 tumors, or to engage in synergistic responses with other anti-cancer agents. In the case of the compounds characterized in this work, we cannot exclude the possibility that they act through more than one mechanism, which may even not require direct interaction with mutant p53. Moreover, different mechanisms may be activated, depending on the specific chemical properties of each compound. Future studies aimed to understand the involvement of mutant p53-dependent mechanisms in the observed effects will be important to understand the potential application of these compounds. Nevertheless, our results showed at least partial selectivity towards the presence of mutant p53, which may represent a potential advantage to increase the selective action on tumor cells. In this regard, these compounds provide useful information to further explore structural features able to enhance activity and selectivity.

Acknowledgement

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Supplementary Data. Experimental details, optimization of the oxa-Michael reaction between **1** and **2**, analytical data, copies of NMR spectra of all compounds and HPLC data of selected compounds.

References

1) a) *Levoglucosenone and Levoglucosans: Chemistry and Applications*; Witczak, Z. J., Ed.; ATL Press: Mount Prospect, USA, 1994. b) M. B. Comba, Y. Tsai, A. M. Sarotti, M. I. Mangione, A. G. Suárez and R. A. Spanevello, *Eur. J. Org. Chem.*, 2018, 590–604. c) M. S. Miftakhov, F. A. Valeev and I. N. Gaisina, *Russ. Chem. Rev.*, 1994, **63**, 869-882.

2) a) K. P. Stockton, C. J. Merritt, C. J. Sumby and B. W. Greatrex, *Eur. J. Org. Chem.*2015, 6999–7008. b) S. W. Kim, E. T. Ledingham, S. Kudo, B. W. Greatrex and J. Sperry, *Eur. J. Org. Chem.*2018, 2028–2038. c) M. B. Comba, M. I. Mangione, A. G. Suárez, A. M. Sarotti and R. A. Spanevello, *Eur. J. Org. Chem.*2018, 6848–6856.

3) G. G. Gerosa, R. A. Spanevello, A. G. Suárez and A. M. Sarotti, *J. Org. Chem.*, 2015, **80**, 7626–7634.

4) J. Sherwood, A. Constantinou, L. Moity, C. R. McElroy, T. J. Farmer, T. Duncan, W. Raverty, A. J. Hunt and J. H. Clark, *Chem. Commun.*, 2014, **50**, 9650–9652.

5) a) C. Müller, M. A. Gomez-Zurita Frau, D. Ballinari, S. Colombo, A. Bitto, E. Martegani, C. Airoldi, A. S. van Neuren, M. Stein and J. Weiser, *ChemMedChem*, 2009, **4**, 524–528. b) J.Westman, K. Wiman and N. Mohell, Patent NumberWO2007139497. c) G. F. Giri, M. Danielli, R. A. Marinelli and R. A. Spanevello, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 3955–3957. d) Z. J. Witczak, J. Sarnik, A. Czubatka, E. Forma and T. Poplawski, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 5606–5611. e) J. Sarnik, A. Czubatka-Bienkowska, A. Macieja, R. Bielski, Z. J. Witczak and T. Poplawski, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 1215–1219. f) Y. H. Tsai, C. M. Borini Etichetti, C. Di Benedetto, J. E. Girardini, F. T. Martins, R. A. Spanevello, A. G. Suárez and A. M. Sarotti, *J. Org. Chem.*, 2018, **83**, 3516–3528.

6) a) L. Römer, C. Klein, A. Dehner, H. Kessler and J. Buchner, *Angew. Chem. Int. Ed.*, 2006, **45**, 6440–6460. b) E. B. Kastenhuber and S. W. Lowe, *Cell*, 20[illegible]kowski, *Nature*, 2013, **502**, 333. d) M. N. Saha, L. Qiu and H. Chang, *J. Hematol. Oncol.*, 2013, **6**, 23. e) 28) J. E. Girardini, C. Marotta and G. Del Sal, *Pharmacol. Res.*, 2014, **79**, 75–87.
7) C. F. Nising and S. Bräse, *Chem. Soc. Rev.*, 2012, **41**, 988–999.
8) a) F. Shafizadeh, R. H. Furneaux and T. T. Stevenson, *Carbohydr. Res.*, 1979, **71**, 169–191. b) R. H. Furneaux, G. J. Gainsford, F. Shafizadeh and T. T. Stevenson, *Carbohydr. Res.*, 1986, **146**, 113–128.
9) E. Morgan, *Chemometrics: Experimental Design*, Wiley, New York, 1995.
10) Lee, B. Y.; Park, S. R.; Jeon, H. B.; Kim, K. S. *Tetrahedron Lett.* 2006, **47**, 5105.
11) J. Soares, L. Raimundo, N. A. L. Pereira, Â. Monteiro, S. Gomes, C. Bessa, C. Pereira, G. Queiroz, A. Bisio and J. Fernandes, *Oncotarget*, 2016, **7**, 4326.
12) a) H. Z. Zhang, Z. L. Zhao and C. H. Zhou, *Eur. J. Med. Chem.*, 2018, **144**, 444–492. b) J. Zhu, J. Mo, H. zhi Lin, Y. Chen and H. peng Sun, *Bioorganic Med. Chem.*, 2018, **26**, 3065–3075.
13) F. Himo, T. Lovell, R. Hilgraf, V. V Rostovtsev, L. Noodleman, K. B. Sharpless and V. V Fokin, *J. Am. Chem. Soc.*, 2005, **127**, 210–216.
14) J. E. Girardini, M. Napoli, S. Piazza, A. Rustighi, C. Marotta, E. Radaelli, V. Capaci, L. Jordan, P. Quinlan and A. Thompson, *Cancer Cell*, 2011, **20**, 79–91.
15) J. M. R. Lambert, P. Gorzov, D. B. Veprintsev, M. Söderqvist, D. Segerbäck, J. Bergman, A. R. Fersht, P. Hainaut, K. G. Wiman and V. J. N. Bykov. *Cancer Cell* 2009, **15**, 376–388.
16) For a recent review, see: A. Perdrix, A. Najem, S. Saussez, A. Awada, F. Journe, G. Ghanem and M. Krayem. *Cancers* 2017, ***9***, 172.

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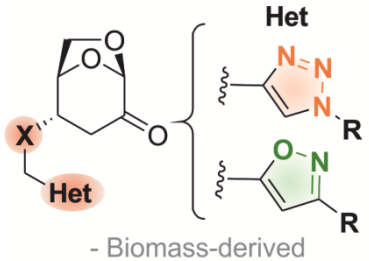
March 12th 2020

Dear Editor,

We have no competing interests to declare.

Yours sincerely,

Dr. Ariel M. Sarotti



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