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Oxa/thiazole-tetrahydropyran triazole-linked hybrids with selective antiproliferative activity against human tumour cells†

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Inspired by diverse marine bioactive compounds, the principle of molecular hybridization was applied to produce a series of new compounds combining diverse heterocyclic systems (oxa/thiazoles and tetrahydropyrans) *via* a triazole ring, attempting to increase the activity of individual building blocks. These new compounds exhibit a highly interesting antiproliferative activity against different human tumour cells and good selectivity when compared to normal cells. The formation of reactive oxygen species and the interaction with P-gp were also evaluated for the lead compounds.

Introduction

In the literature of marine metabolites, there are a large number of products that include heterocycles such as oxazoles and thiazoles that exhibit powerful bioactivities.[1] In particular, oxa/thiazole marine metabolites with potent cytotoxic activity are usually large molecules and have complex structures such as enigmazole A (**1**)[2] and phorboxazole A (**2**)[3] (Fig. 1). However, there are also small molecule metabolites containing these heterocycles with relevant bioactivities such as the potent anticancer compound streptochlorin (**3**)[4] and the neuroactive thiazoline pulicatin D (**4**).[5] Moreover, there are reports of several small compounds containing oxa/thiazoles with interesting antiproliferative activities.[6] Due to our interest in tetrahydropyran rings,[7–9] the metabolites enigmazole A (**1**) and phorboxazole A (**2**) draw our attention.

In the search for new bioactive products, the use of the molecular hybridization concept was envisioned to produce novel entities. Molecular hybridization is a powerful approach for obtaining original lead structures valuable in the field of drug discovery.[10] This strategy has been used extensively in the preparation of new antimalarial,[11] antibacterial[12] and anticancer agents.[13] Compounds obtained in this way show improved activity, selectivity or reduced side effects. Remarkably, it has also been shown that it is possible to obtain active hybrid drugs linking substructures or small fragments of bioactive compounds.[14,15]

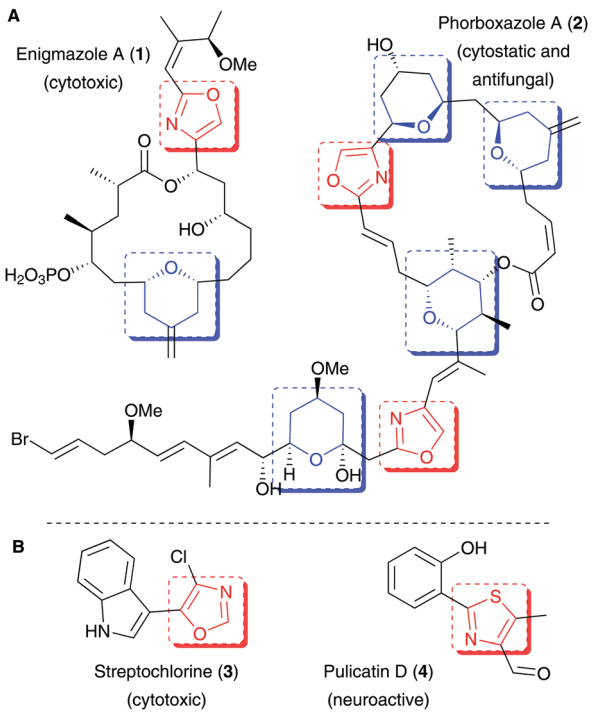

Fig. 1 (A) Structures of bioactive natural products containing oxazole and tetrahydropyran rings. (B) Structures of small bioactive natural products containing oxazole or thiazole rings.

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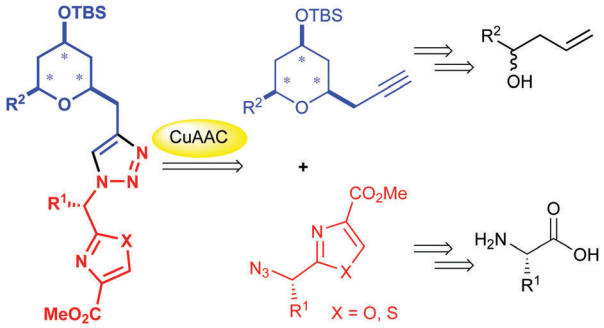
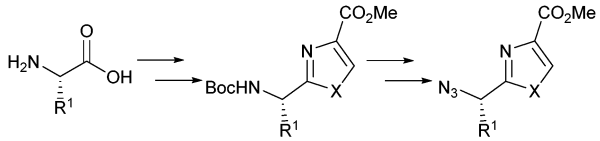


Fig. 2 Retrosynthetic analysis of hybrid compounds. * Relative stereochemistry.



5a: R^1 = H, X = O **6a**: R^1 = H, X = O
5b: R^1 = Bn, X = O **6b**: R^1 = Bn, X = O
5c: R^1 = (*S*)-*sec*-Bu, X = O **6c**: R^1 = (*S*)-*sec*-Bu, X = O
5d: R^1 = H, X = S **6d**: R^1 = H, X = S
5e: R^1 = Bn, X = S **6e**: R^1 = Bn, X = S

Scheme 1 Synthesis of an oxa/thiazole block.[18]

Inspired by this background, we decided to explore hybrids that include oxa/thiazole scaffolds coupled with fragments containing tetrahydropyran rings. Herein, we described the design, preparation and preliminary biological evaluation of a small and focused library of triazole-linked oxa/thiazole–tetrahydropyran hybrids.

Some hybrid compounds containing these kinds of heterocycles have been previously prepared in order to obtain bioactive products. Nevertheless, these compounds used tetrahydropyrans derived from carbohydrates, linked mostly with simple thiazoles, which highly differ from the ones reported here.[16] When considering the design of hybrid compounds, the first decision is related to the selection of the best strategy to link both fragments. Traditionally, we can consider three options, namely, fusion, merging and linking through a spacer. In the particular case of our hybrids, we decided to explore the latter. Thus, the building blocks containing oxa/thiazoles produced from amino acids and tetrahydropyrans prepared by Prins cyclization could be linked *via* the copper(I) catalyzed version of the azide–alkyne [3+2] cycloaddition (CuAAC) (Fig. 2).[17] We have explored this method[18] earlier and considered that it would allow us to combine the building blocks in a simple way. From the two possible options, the azide moiety was located on the oxa/thiazole block and the ethynyl moiety on the tetrahydropyran block, based on the aforementioned information.

Results and discussion

Chemistry

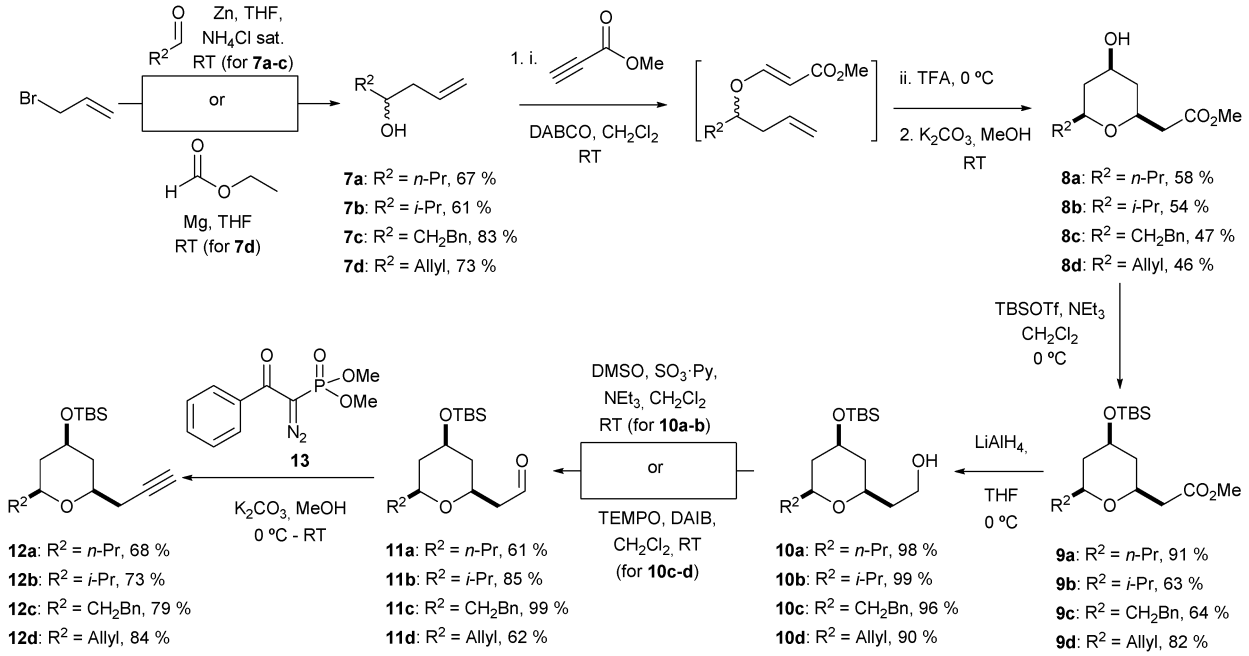
The first step was the preparation of the building blocks. Briefly, the oxa/thiazole blocks were synthesised as described previously[18] starting from commercially available amino acids alanine, phenylalanine and isoleucine. The method allowed us to produce enantiomerically pure oxazoles **6a–c** and thiazoles **6d–e** (Scheme 1).

The tetrahydropyran containing blocks were obtained using Hart's strategy based on the Prins cyclization of enol ethers with trifluoroacetic acid (TFA) as a promoter (Scheme 2).[19] Allylic alcohols **7a–c** were prepared from the corresponding aldehydes by a Barbier reaction. For the symmetrical alcohol **7d**, a double Grignard addition on ethyl formate was utilized as shown in Scheme 2. At this stage, we define the substituent R^2 of the final compounds. Then, allylic alcohols **7a–d** were reacted with methyl propiolate using 1,4-diazabicyclo[2.2.2]octane (DABCO) as catalyst to produce the corresponding enol ethers,[20] followed by the *in situ* addition of TFA to promote the Prins cyclization in a sequential reaction.

The resulting intermediates were treated without purification with K_2CO_3 in dry MeOH to obtain the desired deprotected 4-hydroxytetrahydropyrans **8a–d** (all *cis* compounds) as racemates in three steps and in good yields. The relative configuration was confirmed using the trifluoroacetic ester of **8a** by means of coupling constants and NOE analysis (see the ESI†). Then, the protection of the free secondary alcohol using *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) was performed, followed by the reduction of methyl esters **9a–d** with $LiAlH_4$, producing the primary alcohols **10a–d** in high yields. Reaction of **10a** and **10b** using Parikh–Doering oxidation gave the desired aldehydes **11a–b**. Unfortunately, for **10c–d** no reaction occurred under these conditions, and a different approach for these two compounds was attempted. Oxidations using diverse conditions such as P_2O_5 or IBX were assayed but the reaction did not proceed. Fortunately, it was achieved using (diacetoxyiodo)benzene (DAIB) as catalyst and with a small excess of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO), affording the desired aldehydes **11c–d**. The aldehydes were converted in good yields into the corresponding alkynes **12a–d** using the Taber modification of the Bestmann–Ohira reagent (**13**).[21] With both building blocks in hand, we produced the desired hybrid compounds using CuAAC (**14a–p**). The products were obtained in moderate to excellent yields as inseparable diastereomeric mixtures for compounds derived from **6b**, **6c** and **6e**, and as enantiomeric mixtures for compounds derived from **6a** and **6d** (Table 1).

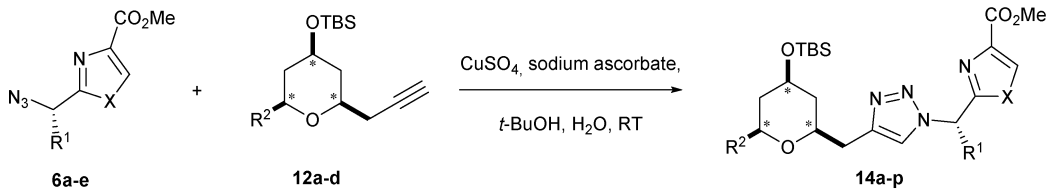
Antiproliferative activity

The antiproliferative activity of compounds **14a–p** and a subset of their precursors was evaluated against the human solid tumour cell lines A549 (non-small cell lung), HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast) and WiDr (colon). The results of growth inhibition after 48 h of exposure are given as GI_{50} (Table 2). The standard anticancer drugs cisplatin (CDDP) and 5-FU (5-fluorouracil) were used as reference drugs. Overall, the hybrid compounds (**14a–p**) showed interesting activities against all cell lines, whilst the oxa/thiazole precursors **5–6** were inactive (GI_{50} > 100 μM).



Scheme 2 Synthesis of racemic tetrahydropyran blocks.

Table 1 Synthesis of hybrid compounds



6a-e + 12a-d → 14a-p

Oxa/thiazole block	Tetrahydropyran block	R^1	R^2	X	Hybrid (yield %)
6a	**12a**	H	*n*-Pr	O	**14a** (52)
6b	**12a**	Bn	*n*-Pr	O	**14b** (78)
6c	**12a**	(*S*)-*sec*-Bu	*n*-Pr	O	**14c** (81)
6d	**12a**	H	*n*-Pr	S	**14d** (55)
6e	**12a**	Bn	*n*-Pr	S	**14e** (82)
6a	**12b**	H	i-Pr	O	**14f** (32)
6b	**12b**	Bn	i-Pr	O	**14g** (66)
6c	**12b**	(*S*)-*sec*-Bu	i-Pr	O	**14h** (97)
6e	**12b**	Bn	i-Pr	S	**14i** (86)
6a	**12c**	H	CH_2Bn	O	**14j** (49)
6b	**12c**	Bn	CH_2Bn	O	**14k** (73)
6c	**12c**	(*S*)-*sec*-Bu	CH_2Bn	O	**14l** (85)
6e	**12c**	Bn	CH_2Bn	S	**14m** (82)
6b	**12d**	Bn	Allyl	O	**14n** (58)
6c	**12d**	(*S*)-*sec*-Bu	Allyl	O	**14o** (43)
6e	**12d**	Bn	Allyl	S	**14p** (93)

*Relative stereochemistry.

In contrast, the tetrahydropyran precursors (**9a–12a**) we tested gave dual results.

On the one hand, intermediates **9a–10a** induced antiproliferative effects in all cell lines and in the range of 18–40 μM. On the other hand, compounds **11a–12a** were inactive.

Noteworthily, compound **15** (see the ESI†), which is the deprotected analog of **14b** and was prepared in previous attempts to produce the desired hybrid compounds, lacks activity against all the human tumour cell lines tested. This reinforces our previous findings that the presence of the TBS group might contribute to the activity of these products, at least when considering its influence on the lipophilicity of the end products.[22]

Hybrid compound **14n** displayed the most potent activity (3.1–6.6 μM) against all cell lines and was selected initially as the lead compound to infer some structure–activity relationships. Taking the data as a whole (Fig. 3), we can observe that

Table 2 Calculated lipophilicity (*C* log *P*) and antiproliferative activity (GI_{50}) against human cells of hybrid compounds **14a–p** and selected intermediates. Values are given in μM and represent mean values of at least three independent experiments ± standard deviation

Compound	*C* log *P*	A549	HBL-100	HeLa	SW1573	T-47D	WiDr	BJ-hTert
5a	0.96	>100	>100	>100	>100	>100	>100	
5b	2.68	>100	>100	>100	>100	>100	>100	
5c	2.68	>100	>100	>100	>100	>100	>100	
5d	1.34	>100	>100	>100	>100	>100	>100	
5e	3.08	>100	>100	>100	>100	>100	>100	
6a	−0.14	>100	>100	>100	>100	>100	>100	
6b	1.60	>100	>100	>100	>100	>100	>100	
6c	1.59	>100	>100	>100	>100	>100	>100	
6d	0.25	>100	>100	>100	>100	>100	>100	
6e	1.98	>100	>100	>100	>100	>100	>100	
9a	3.98	32 ± 6.0	33 ± 8.3	32 ± 6.7	24 ± 6.7	35 ± 4.5	40 ± 8.9	
10a	3.92	23 ± 0.6	21 ± 2.3	19 ± 1.7	18 ± 0.3	28 ± 5.3	20 ± 1.8	
11a	3.93	>100	>100	>100	>100	>100	>100	
12a	4.33	>100	>100	>100	>100	>100	>100	
14a	2.73	17 ± 1.5	19 ± 1.2	18 ± 0.4	16 ± 2.6	20 ± 1.7	18 ± 1.0	
14b	4.91	21 ± 5.9	5.2 ± 0.6	36 ± 7.1	4.2 ± 0.4	12 ± 1.2	9.1 ± 4.5	>100
14c	4.90	12 ± 3.7	6.0 ± 2.1	27 ± 3.4	4.6 ± 1.5	14 ± 1.1	16 ± 1.0	
14d	3.11	16 ± 0.9	18 ± 0.1	17 ± 1.7	15 ± 1.8	16 ± 3.7	16 ± 1.9	
14e	5.30	24 ± 2.4	20 ± 2.3	37 ± 8.3	11 ± 2.3	23 ± 1.9	28 ± 6.0	
14f	2.01	45 ± 3.2	38 ± 2.2	32 ± 2.2	36 ± 4.0	43 ± 5.9	35 ± 2.4	
14g	3.89	10 ± 1.8	13 ± 1.8	7.7 ± 2.6	7.5 ± 2.8	10 ± 0.6	7.2 ± 3.5	
14h	4.64	5.9 ± 0.4	4.4 ± 1.2	5.5 ± 0.63	7.0 ± 1.5	8.5 ± 0.4	7.6 ± 1.2	53 ± 1.9
14i	4.76	17 ± 3.7	16 ± 5.5	5.8 ± 1.6	7.5 ± 2.1	13 ± 4.0	12 ± 2.4	
14j	3.03	12 ± 0.9	14 ± 1.7	11 ± 1.0	10 ± 1.5	17 ± 0.4	14 ± 1.5	
14k	4.91	17 ± 4.2	88 ± 5.1	8.7 ± 1.5	22 ± 5.7	41 ± 16	29 ± 6.7	
14l	4.80	9.2 ± 3.3	13 ± 1.6	5.8 ± 0.6	9.0 ± 1.6	13 ± 2.1	15 ± 4.6	>100
14m	5.77	10 ± 3.1	>100	25 ± 15	6.2 ± 0.8	>100	>100	
14n	3.54	4.6 ± 0.7	6.6 ± 1.9	4.3 ± 1.0	4.5 ± 0.2	5.5 ± 0.5	3.1 ± 0.4	41 ± 7.0
14o	3.43	8.0 ± 3.2	4.5 ± 0.2	4.2 ± 0.5	6.4 ± 0.6	10 ± 1.1	6.9 ± 2.1	53 ± 7.6
14p	4.40	11 ± 2.7	13 ± 3.3	7.3 ± 2.6	7.7 ± 1.3	13 ± 1.0	9.8 ± 2.5	52 ± 5.8
15	0.68	>100	>100	>100	>100	>100	>100	
CDDP	0.04	21 ± 0.6	1.9 ± 0.2	2.0 ± 0.3	3.0 ± 0.4	15 ± 2.3	26 ± 5.3	14 ± 2.4
5-FU	−0.89	n.t.	5.5 ± 2.3	15 ± 4.7	4.3 ± 1.6	47 ± 18	49 ± 6.7	5.5 ± 0.5

oxazole derivatives are in general more active than their corresponding thiazole analogs. When considering the substituent R^1, it appears that glycine derivatives (R^1 = H) produced less active compounds (**14a**, **14d**, **14f**, **14j**). In contrast, for Bn (**14b**, **14e**, **14g**, **14i**, **14k**, **14m**, **14n**, **14p**) or (*S*)-*sec*-butyl (**14c**, **14h**, **14l**, **14o**) analogs there is no clear selectivity. Finally, the allyl group at R^2 gave the best activity values.

In order to look for selectivity, we tested the six most potent compounds of the series (**14b**, **14h**, **14l**, **14n–p**) against the (non-tumour) human fibroblast cell line BJ-hTert. The results (Table 2) show that compounds **14b** and **14l** were inactive and therefore appear as promising leads for further investigation. In fact, our compounds display a better selectivity profile than the standard drugs CDDP and 5-FU. The remaining analogs exhibited GI_{50} values against BJ-hTert cells in the range of 41–53 μM, which still represent selectivity toward tumour cells.

Oxidative stress measurement in HeLa cells

In order to determine whether the antiproliferative activity of the hybrid compounds was related to the production of reactive oxygen species (ROS), we run the ROS-Glo™ H_2O_2 assay. This luminescence test allows the detection of H_2O_2 directly, minimizing the false hit rate. In addition, ROS in cells are converted to H_2O_2 (the longest-lived ROS). Therefore, an increase in H_2O_2 can reflect a general increase in the ROS level. We applied this experiment to HeLa cells, which were exposed to compounds **14b** and **14l** at a low and a high dose (1 and 10 μM), and an incubation time of 48 h. The results of relative ROS production (0.9 at 1 μM, 1.2 at 10 μM) reveal that no relevant increase of ROS production was observed.

Interaction with P-glycoprotein

To determine whether or not P-glycoprotein (P-gp) could affect the activity of **14b** and **14l**, we determined the GI_{50} values after 48 h of exposure to the abovementioned compounds in wild type and P-gp overexpressing SW1573 cells, and in the absence or presence of the P-gp transport inhibitor verapamil (at a fixed concentration of 10 μM). The standard microtubule interacting drugs, known substrates of P-gp, paclitaxel and vinblastine were used for comparison purposes. Table 3 shows the GI_{50} values obtained after 48 h of drug exposure. For better comparison of the data, we defined the resistance factor (R_f) for a given compound as the ratio of GI_{50} values against the P-gp overexpressing and the wild-type cell lines, respectively. In the absence of verapamil, we observed that **14b** and **14l** were not affected by P-gp overexpression (R_f = 2 and 1, respectively). In contrast, vinblastine was the most affected (R_f = 2388). Co-treatment with verapamil produced a decrease in R_f for both antimitotic drugs. However, for **14b** and **14l**, the R_f value remained constant. These results indicate that the activity of **14b** and **14l** is not affected by the overexpression of P-gp.

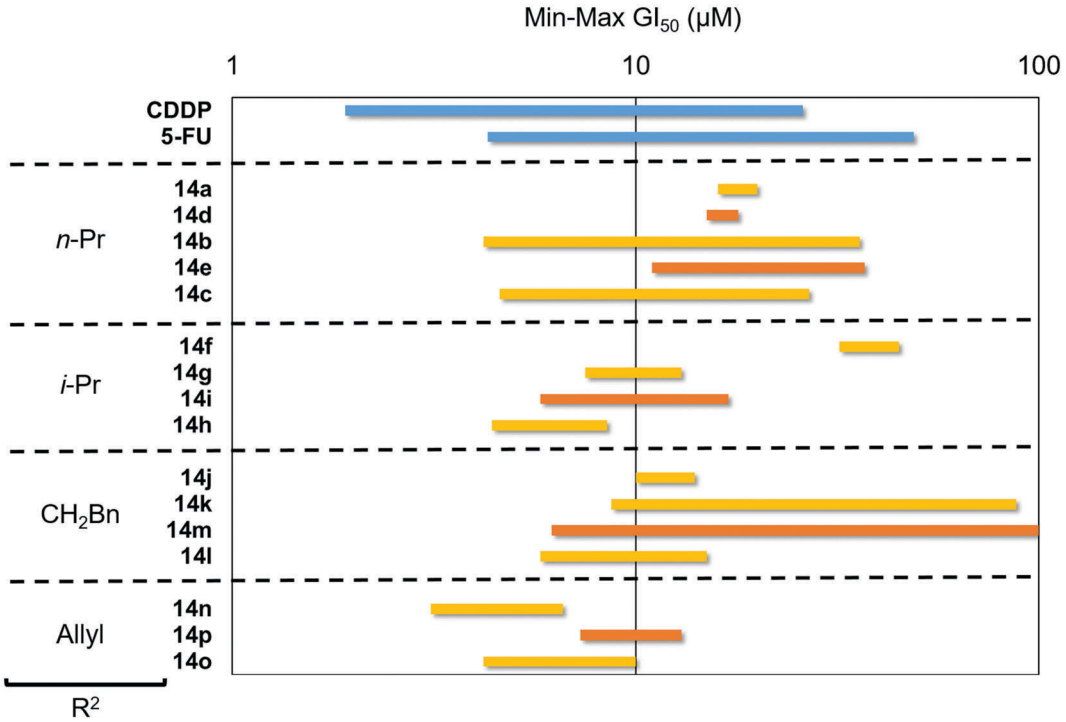


Fig. 3 GI_{50} range plot of tested compounds. X = O, yellow bars. X = S, orange bars.

Table 3 Anti-proliferative activity (GI_{50}) of **14b** and **14l** and tubulin-interacting drugs in SW1573 and SW1573/P-gp cell lines. Values are given in nM and represent the mean values of at least three independent experiments ± standard deviation

	− Verapamil			+ Verapamil		
	SW1573	SW1573/P-gp	R_f[a]	SW1573	SW1573/P-gp	R_f
14b	12 041 ± 938	28 074 ± 1238	2	11 912 ± 3608	29 340 ± 9860	2
14l	27 206 ± 3034	21 805 ± 9678	1	21 464 ± 3665	31 616 ± 14 164	1
Paclitaxel	1.5 ± 0.5	196 ± 53	128	1.6 ± 0.2	4.2 ± 0.9	3
Vinblastine	0.9 ± 0.3	2051 ± 682	2388	0.8 ± 0.2	1.0 ± 0.5	1

[a] R_f represents the ratio between GI_{50} SW1573/P-gp and SW1573.

Conclusions

A small library of oxa/thiazole-tetrahydropyran triazole-linked hybrid compounds has been synthesized. The building blocks were connected by a molecular hybridization process using CuAAC. The results on the antiproliferative activity of the hybrid compounds showed that the molecular hybridization process allows obtaining active compounds from inactive building blocks. Further mechanistic studies demonstrated good selectivity for compounds **14b** and **14l** against non-tumour cells and that the abovementioned derivatives were not substrates for P-gp extrusion. In summary, we have exemplified that CuAAC represents a plausible strategy to bind blocks of heterocyclic structures to expand the chemical space of bioactive compounds. Future work will shed light on the role of the stereochemistry of the tetrahydropyran block in the antiproliferative activity.

Conflicts of interest

The authors confirm that this article has no conflict of interest.

Acknowledgements

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