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N-1,2,3-Triazole-Isatin Derivatives: Anti-proliferation effects and target identification in solid tumour cell lines

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Natalia Busto[a], Joana Leitão-Castro[b], Alfonso T. García-Sosa[c], Francisco Cadete[b] Carolina S. Marques[d], Renata Freitas[b], Anthony J. Burke[d.ef*]

[a] Departamento de Química, Universidad de Burgos, Plaza Misael Bañuelos s/n, 09001 Burgos, Spain.
[b] I3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen, 208 | 4200-135 Porto, Portugal
[c] Institute of Chemistry, University of Tartu, Ravila 14 A, Tartu 50411, Estonia.
[d] LAQV-REQUIMTE, University of Évora, Institute for Research and Advanced Studies, Rua Romão Ramalho, 59, 7000 Évora, Portugal.
[e]Chemistry Department, School of Science and Technology, University of Évora, Rua Romão Ramalho 59, 7000-671 Évora, Portugal.

* Corresponding authors current address, Faculty of Pharmacy, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal ajburke@ff.uc.pt.

Abstract

Molecular hybridization approaches have become an important strategy in medicinal chemistry, and to this end, we have developed a series of novel *N*-1,2,3-triazole-isatin hybrids that are promising as tumour anti-proliferation agents. Our isatin hybrids presented high cytotoxic activity against colon cancer cell line SW480, lung adenocarcinoma cell line A549, as well as breast cancer cell lines MCF7 and MDA-MB-231. All tested compounds demonstrated better anti-proliferation (to 1-order of magnitude) than the *cis*-platin (CDDP) benchmark. In order to explore potential biological targets for these compounds, we used information from previous screenings and identified as putative targets the histone acetyltransferase P-300 (EP300) and the acyl-protein thioesterase 2 (LYPLA2), both known to be involved in epigenetic regulation. Advantageous pharmacological properties were predicted for these compounds such as good total surface area of binding to aromatic and hydrophobic units in the enzyme active site. In addition, we found down-regulation of LYPLA2 and EP300 in both the MCF7 and MDA-MB-231 breast cancer cells treated with our inhibitors, but no significant effect was detected in normal breast cells MCF10A. We also observed upregulation of EP300 mRNA expression in the MCF10A cell line for some of these compounds and the same effect for LYPLA2 mRNA in MCF7 for one of our compounds. These results suggest an effect at the transcriptional regulation level and associated with oncological contexts.

Keywords

Isatin; oxindole; 1,2,3-triazole; breast cancer; epigenetic regulation; EP300.

1. Introduction

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Cancer is the second leading cause of death worldwide, after cardiovascular disease, being the main or secondary source of premature death in most countries globally. In 2018, 9.6 million people died from cancer-related illnesses and many more deaths are expected if we cannot stop its relentless onslaught.[1] Lung, breast, and colorectal cancers are the three most incident cancers and among the five leading causes of cancer-related death. In contrast, 40% of cancers are controllable if detected and properly treated at an early stage, particularly in the case of lymphomas, breast, colorectal and cervical cancers. Therefore, early intervention and detection, as well as the development of new therapeutics, play an important role in the reduction of the burden caused by cancer.

Molecular hybrid constructs can reduce side effects and overcome drug resistance – which incidentally is important in the context of cancer treatments – and for which the individual pharmacophores may have their own mechanism of action. The 1,2,3-triazole unit is a privileged pharmacophore that is present in many bioactive compounds,[2] including some molecular hybrids developed within our group.[3] It is stable towards hydrolysis and can increase the lipophilicity of the compounds, along with isatin and its analogs, presenting a rich therapeutic spectrum[4]. The isatin-oxindole-1,2,3-triazole hybrid combination shows an interesting and broad biological activity spectrum that includes anti-tumour activity.[5] We have recently shown the importance of this class in both butyrylcholinesterase (BuChE) inhibition and β-amyloid anti-aggregation.[6]

Several anti-tumour 1,2,3-triazole-isatin compounds were reported in lung and colorectal cancer (Fig.1). Aouad *et al.* have shown the potential of certain isatin-triazole-benzothiazole compounds for tackling SW182 colorectal cancer lines and H199 lung cancer cell lines.[7] This study demonstrated that the most likely target of these compounds was the minor groove of DNA. Nagarsenkar *et al.* reported on benzylidene-based hybrids with anti-tumour activity that included A549 cell line anti-proliferation.[8] The mode of action appeared to be through interaction with the mitochondrial-mediated intrinsic pathway, thereby inducing apoptosis. Sing *et al.* reported on a di-isatin-1,2,3-triazole hybrid that showed activity in A549 cell lines.[9a] Furthermore, Solomon *et al* demonstrated that isatin–benzothiazole analogs present high cytotoxic activity against breast cancer cell lines[9b], while similar results were obtained using oxindole-benzofuran hybrids[9] and 1,2,3-triazole-linked quinoline-isatin molecular hybrids[9d].

Considering the potential of these compounds as anti-tumour agents, we assessed their activity in preclinical cell-line cultures derived from human colon cancer cells (SW480), lung adenocarcinoma cells (A549), and breast cancer cells (MCF7, MDA-MB-231) and controls (MCFA10). Our results suggest a generic anti-proliferative effect that could be linked with deregulation of the epigenetic factors EP300 and LYPLA2.

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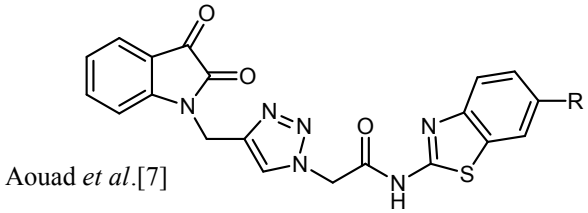
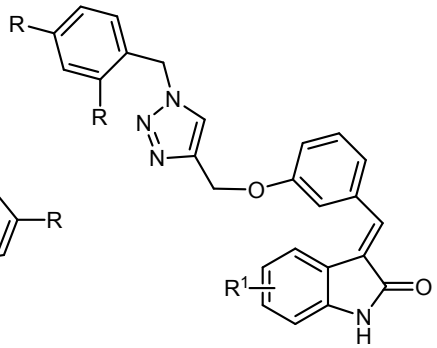
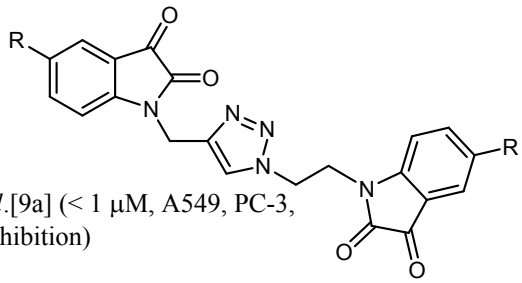


Aouad *et al*.[7]

R = H (H199; SW182, > 50% inhibition)
R = Me (H199; SW182, > 50% inhibition)
R = SO_2CH_3 (H199; SW182, > 50% inhibition)



Nagarsenkar *et al*.[8](A549 moderate inhibition; 26.7μM)



Sing *et al*.[9a] (< 1 μM, A549, PC-3, THP-1 inhibition)

Figure 1: Some examples of lung and colorectal anti-tumour isatin-1,2,3-triazole molecules.

2. Results and Discussion

2.1. Chemistry

Our goal was to assay isatin-triazoles **(1)** and 3-aryl-2-hydroxyoxindole-triazoles/acetal-protected-oxindole triazoles **(2)** (Table 1) against different solid tumours. The key 1,2,3-triazole unit was installed using the Sharpless-Meldal versatile copper-catalyzed alkyne-azide cycloaddition (CuAAC) that worked very robustly in these reactions, and in the case of those compounds containing the aryl and hydroxyl units at the stereogenic center C-3 (**(2a)-(2j)**) via rhodium catalyzed arylboronic acid addition to the isatin carbonyl unit at C-3. The chiral ligand of choice from extensive ligand screening was found to be BINAP. Full accounts of the synthesis of these compounds, and the purity are available in our previous report.[10]

2.2. Tumour anti-proliferation effects

2.2.1 Bioassay screening against solid tumours

As part of an ongoing effort to develop and screen novel compounds with anti-tumour activity, we subjected nine of the compounds shown in Table 1 to anti-tumour activity screening against lymphoma cell lines.[11,12] The effect on cell proliferation was assessed on four established human cell lines derived from DLBCL (DOHH-2, VAL, OCI-LY-10, and SU-DHL-2) using readily established procedures. [11,12] The cell-cycle studies in OCI-LY-10 showed arrested cell proliferation at the sub-G_0 phase in particular hybrids.[13] Furthermore, this seems to indicate that epigenetic transcription factor perturbations, from possible enzyme inhibition or gene down-regulation, could be the reason for this observation and is of interest to the discussion on the possible targets in the solid-tumour proliferation studies discussed below (section 2.3).

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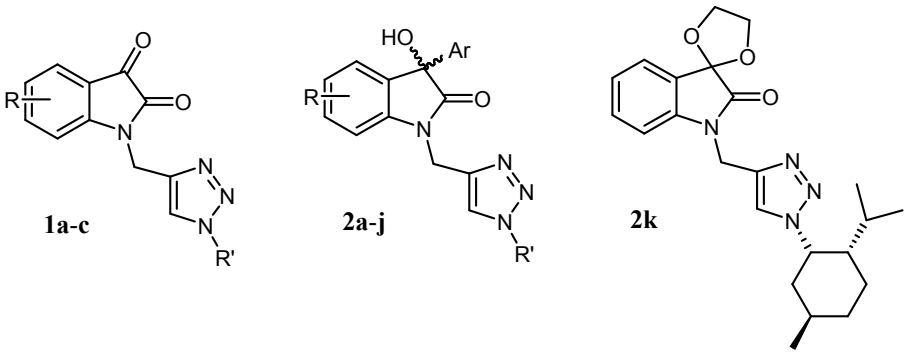
Table 1. Chemically modified oxindole derivatives designed as promising anti-tumour therapeutics.



Compound	R	R'	Ar
(1a)	H	Bn	-
(1b)	H	(1*S*,2*S*,5*R*)-neomenthyl	-
(1c)	5-Me	Bn	-
(2a)	H	Bn	C_6H_5
(2b)	H	Bn	2-naphthyl
(2c)	H	Bn	4-MeC_6H_4
(2d)	H	Bn	4-FC_6H_4
(2e)	H	Bn	4-MeOC_6H_4
(2f)	H	Bn	3-thienyl
(2g)	H	Bn	3-HOC_6H_4
(2h)	H	Butyl	C_6H_5
(2i)	H	(1*S*,2*S*,5*R*)-neomenthyl	C_6H_5
(2j)	5-Br	Bn	C_6H_5

The cytotoxicity evaluation of our compounds against solid tumour cell lines, namely human colon cancer cells (SW480) and lung adenocarcinoma cells (A549), was performed using MTT assays. The human embryonic kidney cell line Hek293 was also included as a reference model to determine the cytotoxicity of the compounds against non-tumour cells. Compounds **(1a-c)**, **(2a-h)**, **(2j),** and **(2k)**, in different concentrations, were tested against these cell lines over a 72h incubation period. The IC_{50} values are indicated in Table.2.

The compounds (*S*)-**(2b)** and (*R*)-**(2b)** as well as **(1b)** revealed very good dose-dependent anti-tumour activity in the case of SW480 (Table 2, 9.9 μM, 8.7 μM, and 8.9 μM, respectively)**.** Interestingly, **(1b)** likewise exhibited satisfactory results in the lymphoma cell lines OCI-LY-10 and SU-DHL-2.[12] Both compounds (*S*)-**(2b)** and (*R*)-**(2b)** also had the best anti-proliferation effect in the A549 cell line (28.3 and 23.5 μM, Table 2, respectively).

Concerning the activity within the enantiomeric series, we observed that the *S*-configured enantiomers were more potent in both tumour cell lines (Table 2), as depicted in the cases:

(2a) (compare entry 4 and 13 in Table 2 – SW480); **(2d)** (compare entry 6 and 16 in Table 2- SW480); **(2e)** (compare entry 7 and 17 in Table 2 – SW480); **(2J)** (compare entry 11 and 20 in Table 2 - SW480 and A549). Regarding **(2b)**, the *R*-enantiomer was more active in the same cell lines, whilst (*S*)-**(2f)** displayed stronger effects in the SW480 cell line (compare entries 8 and 18 in Table 2), while giving equal potency in the A549 cell line. As regards the cytotoxicity against the Hek293 cell line, the *R*-enantiomer was generally more predominant in its cytotoxicity. The possible targets of these tumour cell lines are discussed below (section 2.3).

To further assess the cytotoxic effects of these compounds on solid tumours, compounds **(1a)**, **(1b)**, **(1c)**, (*S*)-**(2b),** and (*R*)-**(2j)** were selected and their effects evaluated on breast cancer cell lines MCF7 and MDA-MB-231, and in a control cell line (MCF10A) representative of non-tumorigenic breast tissue, also using MTT assays. The calculated IC_{50} values are shown in Table 2. What was remarkable here, was that all compounds showed much better anti-proliferative effects, to about one order of magnitude, than the *cis*-platin (CDDP) bench-mark.

The *N*-1,2,3-triazole-isatin derivatives presented slightly higher cytotoxic activity in the MCF7 cell line than in the MDA-MB-231. Compounds **(1a)** and **(1b)** showed the strongest cytotoxic activity for MCF7, whilst **(1b)** and (*R*)-**(2j)** seemed to be the most effective against MDA-MB-231. Cytotoxic activity of our compounds, particularly **(1b)** and (*R*)-**(2j)**, was also found in MCF10A cells, derived from non-malignant fibrocystic breast cells and commonly considered as representatives of the non-tumorigenic condition. This result suggests that the cytotoxicity of our compounds is not specific for malignant cells but also for cells with altered growth. In addition, a considerable proportion of the tested compounds showed lower IC_{50} values, in the tumour cell lines compared to the normal Hek293 cell line, suggesting that these compounds might have minor side effects when cell growth is not altered.

2.2.2 Structure activity relationships

The molecules with the free carbonyl unit, i.e. **(1a)**-**(1c),** showed inhibitions of < 3 μM against MCF7, MCF10A, and the MDA-MB-231 cell lines. However, in the case of the oxindole derivatives, due to lack of available samples we only tested one enantiomeric series; i.e. the enantiomers (*S*)- and (*R*)-**(2j).** The compound (*R*)-(**2j**) was more potent against SW480 and A549 than its enantiopode, but in the case of the other cell lines, notably; Hek293, MCF7, MDA-MB-231 and MCF10A it was the opposite (Table 2, entries 11 and 20). Both (*S*)-**(2b)** and (*R*)-**(2b)** showed <10 μM inhibition against the SW480 cell line, indicating that the 2-

napthyl unit may be an important pharmacophore in the case of the oxindole derivatives. Lack of samples also limited the test (*R*)-**(2b)** against MCF7, MCF10A and MDA-MB-231.

2.3 *In silico* studies

To identify the possible biological targets for our compounds, we performed an *in silico* investigation to pinpoint putative targets. To do this, we carried out a screening assay with compounds **(1a)** and **(1c)** using the "*similarity ensemble approach"* (SEA)[13] and the SwissADME[14] tools. Using the SEA platform, the screening revealed that compounds **(1a)** and **(1c)** showed strong affinities for the histone acetyltransferase P-300 (EP300). The (*S*)-**(2b)** and (*S*)-**(2j)** compounds were predicted, by the same platform, to target acyl-protein thioesterase 2 (LYPLA2 or ATP2).

EP300 is a 2400 amino acid multidomain protein that has a catalytic site containing HAT domains [15] and that functions as a master transcriptional regulator implicated in several cancers, including prostate[16], neuroblastomas[17], and breast cancer[18]. It is responsible for cancer cell survival and sustained proliferation and, therefore, is considered a potential anti-cancer therapeutic agent. It works by acetylating key histones, influencing the transcription of oncogenes and tumour suppressor genes. For lung adenocarcinoma cells, it was suggested that EP300 may promote snail-dependent EMT (epithelial-mesenchymal transition) by acetylating snail at K187 site[19] and it was also reported that the inhibitor C646[20] could radiosensitize A549 cells by enhancing mitotic catastrophe[21]. It is also worth pointing out that small molecules targeting EP300, such as L002[18], CCS1477[23a], NEO2734[23b], and NEO1132[23b] have shown preclinical anti-lymphoma activity. As far as we are aware, neither 1,2,3-triazoles, isatins/oxindoles or their hybrids have been reported to inhibit this enzyme, the predominant inhibitors being benzimidazoles, benzodiazepinones, indoles, pyrazolones, and oxazolidinedione. [24]

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Table 2. IC_{50} of the target compounds against SW480, A549, Hek293, MCF7, MCF10A in MTT assay for a 72h incubation period.

Entry	Com-pound	%ee[a,b]	SW480 (μM)	A549 (μM)	Hek293 (μM)	MCF7 (μM)	MDA-MB-231 (μM)	MCF10A (μM)
1	**(1a)**	-	32.2 ± 2.9	54.7 ± 2.1	63.7 ± 4.5	1.39 ± 0.2	2.95 ± 0.4	1.80± 0.3
2	**(1b)**	>98	8.9 ± 0.8	53.8 ± 1.6	23.1 ± 1.4	1.34 ± 0.2	1.79 ± 0.2	1.08± 0.2
3	**(1c)**	-	26.6 ± 2.3	41.5 ± 2.3	26.9 ± 1.7	1.50 ± 0.2	2.40 ± 0.7	1.54± 0.2
4	**(*S*)-(2a)**	80	62.4 ± 4.2	>100	>100			
5	**(*S*)-(2b)**	86	9.9 ± 1.6	28.3 ± 1.5	12.4 ± 1.1	1.75 ± 0.2	3.10 ± 0.4	1.33 ± 0.2
6	**(*S*)-(2d)**	78	28.2 ± 3.1	72.1 ± 4.5	50.3 ± 2.2			
7	**(*S*)-(2e)**	87	36.6 ± 1.1	95.9 ± 5.1	38.0 ± 2.3			
8	**(*S*)-(2f)**	75	29.5 ± 2.8	>100	64.0 ± 4.7			
9	**(*S*)-(2g)**	86	31.8 ± 2.3	>100	33.7 ± 1.5			
10	**(*S*)-(2h)**	74	>100	>100	> 100			
11	**(*S*)-(2j)**	95	18.9 ± 1.7	29.3 ± 3.2	87.3 ± 6.4	2,039±0.3	1,622±0.2	1,105±0.2
12	**(2k)**	98	>100	>100	>100			
13	**(*R*)-(2a)**	74	75.3 ± 3.5	>100	>100			
14	**(*R*)-(2b)**	86	8.7 ± 0.5	23.5 ± 1.4	14.0 ± 1.1			
15	**(*R*)-(2c)**	67	> 100	>100	> 100			
16	**(*R*)-(2d)**	76	32.1 ± 1.6	77.3 ± 3.9	53.5 ± 2.8			
17	**(*R*)-(2e)**	87	75.8 ± 4.3	>100	>100			
18	**(*R*)-(2f)**	89	62.5 ± 3.7	98.0 ± 4.7	46.4 ± 2.7			
19	**(*R*)-(2h)**	94	>100	>100	>100			
20	**(*R*)-(2j)**	92	28.7 ± 1.8	65.9 ± 2.8	36.1 ± 1.9	1.49 ± 0.3	1.41 ± 0.2	1.02 ± 0.2
21	**CDDP**[c]		12.8 ± 1.2	6.0 ± 0.9	15.9 ± 2.0	14.65 ± 3.89	18.7 ± 4.77	33.76 ± 2.05

[a] Determined using HPLC with a chiral column.[10]
[b] This was the assigned configuration to carbon-3 of the oxindole unit.
c Cisplatin

To determine if there was possible binding between compounds (**1a**), (**1b**), (**1c**), (*S*)-(**2b**), (*R*)-(**2b**), (*S*)-(**2j**) (which showed better inhibition for SW480 and A549 when compared to its enantiopode; Table 2), and the acetyltransferase EP300 protein, we conducted a molecular docking study. In the case of these compounds, docking with Glide XP[25] against the co-crystallized protein-allosteric inhibitor CPI-076 complex (6pgu from the RCSB Protein Data Bank[15]) returned docking scores that most closely resembled those of the co-crystallized ligand (CPI-076, -5). Scores (in kcal/mol) were (**1a**) -4.51, (**1b**) -4.09, (**1c**) -3.70, (*S*)-(**2b**) -5.02, (*R*)-(**2b**) -2.86, (*R*)-(**2j**) -5.13, (*S*)-(**2j**) -4.62, (*S*)-(**2a**) -1.49 and (*R*)-(**2a**) -4.36. In the case of (**2b**) it was the (*S*) enantiomer that gave the better calculated binding energy with the enzyme than its (*R*) enantiopode, contrary to the bioactivity data, which could indicate that EP300 is not the main target for (**2b**). But the opposite was observed for the (**2j**)

enantiomers. It should be noted that the scores for the (**2j**) enantiopodes actually parallel the bioassay results for these enantiomers in the case of Hek293, MCF7, MDA-MB-231 and MCF10A, indicating that EP300 may be the biological target in these cell-lines. The docked predicted binding-pose of (**1a**) in acetyltransferase P-300 is shown in Figure 2, where van der Waals and $\pi-\pi$ interactions are observed, as well as direct hydrogen bonds, including bonds through bridging water molecules known to be important in some protein-ligand complexes.[26]

As was the case for our compound (**1a**)**,** Liu *et al*.[27] observed that 4-(3-cyclopropyl-4-((5-(4,5-dimethyl-2-(trifluoromethyl)phenyl)thiophen-2-yl)methylene)-5-oxo-4,5-dihydro-1H-pyrazol1-yl) benzoic acid showed good binding with the Try1436 residue when docked with EP300 (PDB 7BIY) (see Fig. 2). These results support the initial *in silico* screenings implying that the EP300 protein could be the target of compounds (**1a**) and (**1c**).

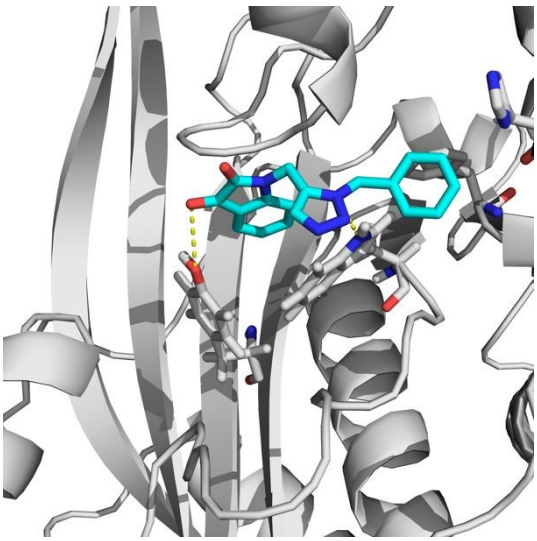

Figure 2. Docked compound **(1a)** (cyan) in the EP300 binding site (in white, 6gpu) showing hydrogen bonds with Tyr1397 and Trp1436, as well as $\pi-\pi$ contacts with Trp1436, Phe1595, and His1591. Oxygen atoms are in red, nitrogen atoms are in blue.

Post-translational S-palmitoylation fixes target proteins to membranes via high-energy thioester bond formation.[16] LYPLA2 displays Ras depalmitoylase and lysophospholipase activity *in vitro*, which seems to be exclusive from APTs. It should be noted that inhibition of Ras palmitoylation leads to attenuated growth signalling and transformation in mutant cells.[16] Furthermore, the Ras proteins are well-known instigators of many cancers and are attractive targets for the development of anti-cancer therapeutics. Although this protein is implicated in cancers such as renal cell carcinoma[28], melanoma[29], and chronic lymphocytic

leukaemia (CLL)[30], we are unaware of its involvement in colorectal cancers. In the case of lung cancer, high protein expression levels of LYPLA1 were detected in A549 cells.[31]

Compounds (**1a**), (**1b**), (**1c**), and (*R*)-(**2b**) show appreciable anti-proliferative activities in these tumour cell lines. However, apart from the fact that (*R*)**-**(**2b**) is the enantiomer of (*S*)**-**(**2b**), none of these compounds were detected as valid hits on the SwissADME suit screen. Due to structural analogy with the hit compounds (*S*)-(**2b**) and (*S*)-(**2j**), we suggest that they may target the LYPLA2 enzyme, or even the initial mRNA, inhibiting tumour proliferation.

Compounds (**1b**), (*S*)-(**2b**), (*R*)-(**2b**), (*S*)-(**2j**), and (*R*)-(**2j**) were docked with LYPLA2 (PDB 5syn) and the predicted docking score for the co-crystallized ligand with the enzyme was -9.86 kcal/mol, -6.58 for (*R*)-(**2a**), -4.30 for (*S*)-(**2a**), -9.49 for (**1a**), -5.62 for (**1b**), -8.67 for (**1c**) -9.76 for (*S*)-(**2b**), -6.14 for (*R*)-(**2b**), -8.13 for (*R*)-(**2j**), -9.1 for (*S*)-(**2j**), respectively. Although it should be noted that it is not expected that there be a meaningful correlation between the docking scores and the bioassay inhibitions (particularly between enantiomers) but rather a general trend of the possibility of binding *versus* non-binding, some of the observed results particularly for (**2b**), (**2b**) and (**2j**) (where the trend in predicted binding energies fails to match with the bioassay results, may indicate that LYPLA2 is not the principle target for these inhibitors. Details of the principal contact points are illustrated in Fig.3 and 4, respectively, and they show binding with water bridges that had previously been observed by Won *et al.* [16] Of note was the close proximity of both (*S*)-**(2b)** and (*R*)**-(2j)** with the Leu33 residue, which was also observed in the case of the ML349 sulfone in the study of Won *et al*.[16] It is interesting to gather proof of evidence that our compounds potentially bind to this esterase protein target, considering that they have also been shown to be good cholinesterase binders.[6]

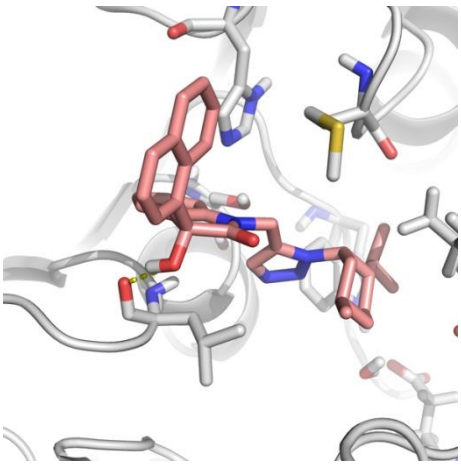

Figure 3. Docked compound (*S*)**-(2b)** (in salmon) in the binding site of LYPLA2 (in white, 5syn) showing hydrogen bonds with Leu33, and bridges with HOH431, as well as π–π contacts with Trp148 and hydrophobic contacts with Leu33 and Leu81. Oxygen atoms in red, nitrogen atoms in blue, sulfur in yellow, bromine in carmine

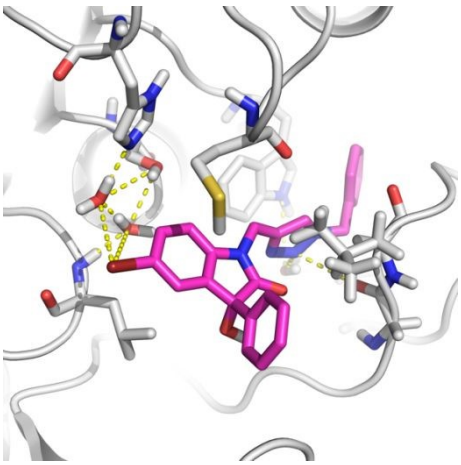

Figure 4. Docked compound (*R*)-**(2j)** (in cyan) in the binding site of LYPLA2 (in white, 5syn) showing hydrogen bonds with Gly80, and bridges with HOH429, HOH431, and HOH456, as well as π–π contacts with Trp148 and hydrophobic contacts with Leu33 and Leu81. Oxygen atoms in red, nitrogen atoms in blue, sulfur in yellow, bromine in carmine

2.4 Calculated Physicochemical Properties

Molecular properties for selected compounds were calculated using SwissADME database (Table 3) and these returned promising results, with properties within Lipinski's rules, as well as passing PAINS (Pan-assay interference compounds)[32] filters to determine possible pan-assay, non-specific interfering compounds (PAINS are compounds that can give false-positive results in high-throughput screens and should be identified and avoided if possible). The latter two compounds on the list, as expected, showed the best lipophilicities for membrane penetration and good total surface area of binding to aromatic and hydrophobic units in the enzyme active site.

Table 3. Molecular properties for selected compounds.

Entry	Compound	MW	MLOGP	#H-bond acceptors	#H-bond donors	TPSA	PAINS #alerts
1	**(1a)**	318.33	1.59	4	0	68.09	0
2	**(1b)**	366.46	2.50	4	0	68.09	0
3	**(1c)**	332.36	1.82	4	0	68.09	0
4	**(*S*)-(2b)**	494.63	4.20	4	1	71.25	0
5	**(*R*)-(2j)**	475.34	3.35	4	1	71.25	0

MW = molecular weight; #H-bond = number of hydrogen bond; TPSA = topological surface area (Å^2)

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2.5 Gene expression studies of the predicted biological targets

To determine whether EP300 and LYPLA2 expression varied upon treatment, we exposed cell lines MCF7, MDA-MB-231, and MCF-10A to the compounds tested in section 2.2.4, in the concentrations that produced 50% inhibition of cell viability. Afterwards, RNA was extracted and transcribed into cDNA, which was posteriorly used to perform qPCR analyses. Cells treated with DMSO were used as vehicle control, and the obtained results are displayed in Fig.5 and Fig.6.

The data shows that *N*-1,2,3-triazole-isatin derivatives **(1a)**-**(1c)** significantly lower *EP300* gene expression in breast cancer cell lines MCF7 and MDA-MB-231, compared to the vehicle control treatment DMSO (Fig.5). Surprisingly, all these compounds significantly upregulated EP300 mRNA expression in the MCF10A cell line (Fig. 5 (C)). The chiral non-racemic *N*-1,2,3-triazole-oxindole derivative (*S*)-**(2b)** caused significant downregulation of LYPLA2 expression on MDA-MB-231 and MCF10A and (*R*)-**(2j)** on both MCF7 and MDA-MB-231 (Fig.5). Again, surprisingly, (*S*)-**(2j)** showed significant upregulation of LYPLA2 mRNA over the negative control in MCF7 (Fig. 6C) but (*R*)-**(2j)** showed no significant difference with the control in the MCF10A cell line.

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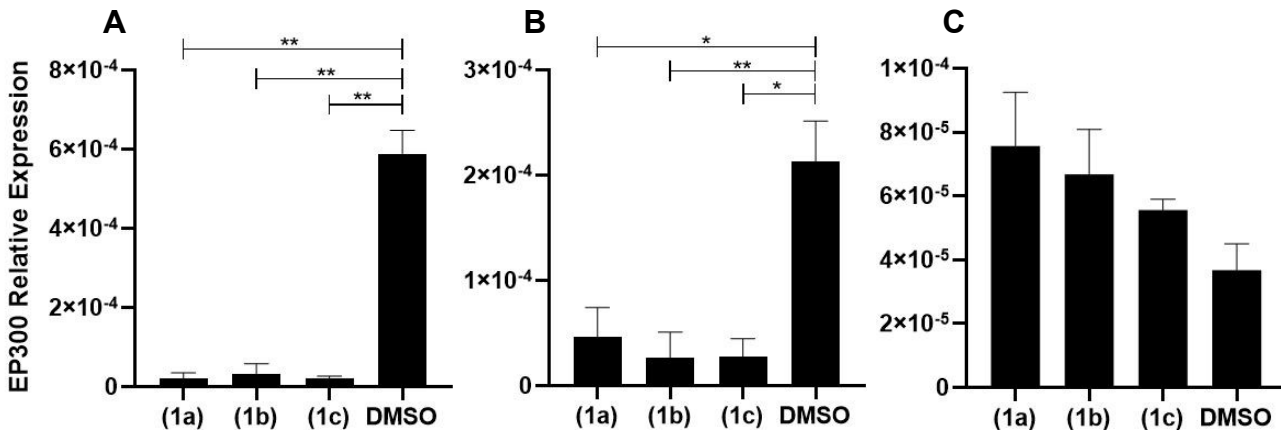

Figure 5. EP300 mRNA expression levels analyzed by qPCR in breast cancer cell lines MCF7 (A), MDA-MB-231 (B) and MCF10A (C) upon treatment with compounds (1a), (1b) and (1c). Y-axis depicts the ratios between EP300 expression compared to GAPDH expression. Statistical analyses were done using Forsythe and Welch ANOVA test with Dunnett T3 correction. *p-value<0.05 and **p-value<0.01

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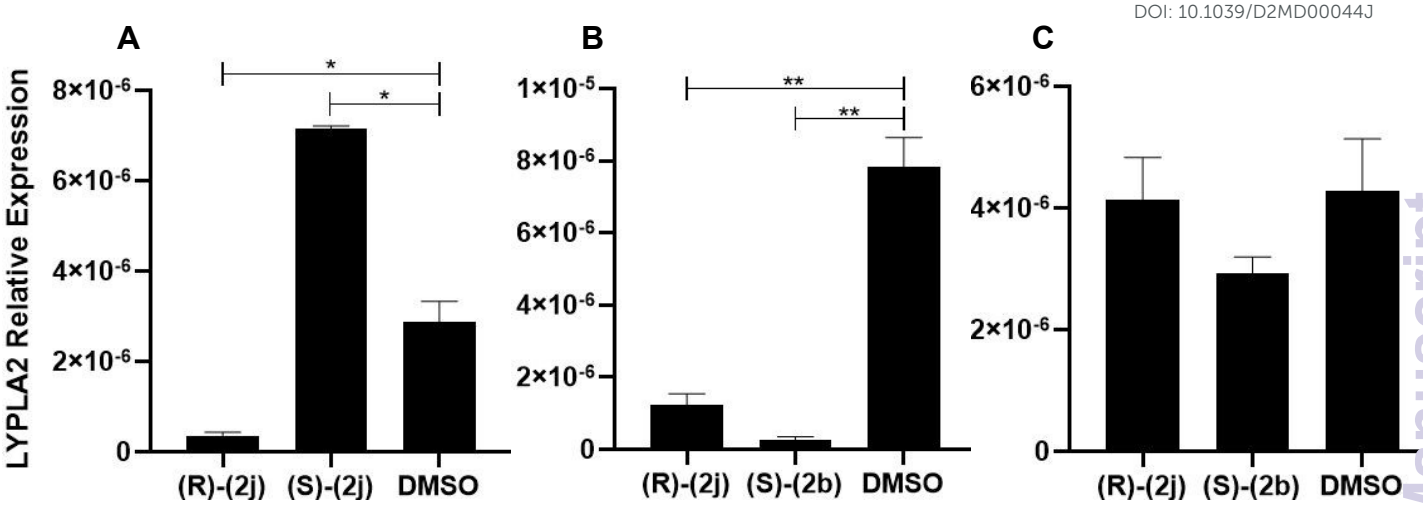

Figure 6. LYPLA2 mRNA expression levels analyzed by qPCR in breast cancer cell lines MCF7 (A), MDA-MB-231 (B) and MCF10A (C) upon treatment with compounds (*R*)-(2j) and (*S*)-(2b). Y-axis depicts the ratios between LYPLA2 expression compared to GAPDH expression. Statistical analyses were done using Forsythe and Welch ANOVA test with Dunnett T3 correction. *p-value<0.05 and **p-value<0.01.

3. Conclusions

Herein, we developed novel *N*-1,2,3-triazole-isatin hybrids and described their activities as tumour anti-proliferation agents using cell-based assays with colon (SW480), lung (A549), malignant (MCF-7 and MDA-MB-231) and non-malignant (MCF10A) breast cells. All these compounds showed good pharmacological profiles and drug-likeness properties. Virtual screening using the SwissADME database suggested that the possible targets for the most potent inhibitors **(1a)**, **(1b)**, **(1c)**, (*S*)-**(2b),** and (*R*)**-(2j)**, found from the cell-based assays, could be EP300 and/or LYPLA2. Prior cell-cycle studies carried out in the case of the DLBCLs, suggest the involvement of our inhibitors at an epigenetic level, indicating possible targeting of these enzymes. Importantly, we detected downregulation of EP300 and LYPLA2 on breast cancer cells MCF-7 and MDA-MB-231, and surprisingly upregulation in MCF10A (for compounds **(1a)**-**(1c)** and in MCF7 (for compound (*S*)-**(2j)**). Docking of these compounds in these enzymes also suggests an effect on these epigenetic regulators in oncogenic contexts. Thus, the developed *N*-1,2,3-triazole-isatin hybrids can give rise to promising therapeutic agents for the treatment of a variety of cancer types and diseases

associated with altered cell growth. Our current objective is to investigate our best inhibitors in suitable pre-clinical models.

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Conflict of Interest

There is no conflict of interest to declare.

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