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ARTICLE

Synthesis of novel Perillyl-Dihydropyrimidinone Hybrids designed for antiproliferative activity

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A series of fifteen novel dihydropyrimidinone hybrid compounds were synthesized via multicomponent reaction combined with Huisgen reaction in good yields. The antiproliferative activity was investigated against nine tumor cell lines and four hybrid compounds (TGI < 10μM) showed promising antiproliferative activity against OVCAR-3 (ovarian), UACC-62 (melaoma) and U251 (glioma) tumor cell lines. Several hybrid compounds assayed have high TGI values (TGI 147.92-507.82) for the human keratinocytes cell line (HaCat),which reveals selectivity to cancer cells.

Introduction

Multifunctional Drugs and Hybrid Compounds

The modern pharmaceutical industry has faced unprecedented challenges regarding the development of new drugs. Although the amount of funds spent in scientific research has doubled since 1991, the approval of new drugs decreased around 50% over the same period. While most diseases involve multiple pathogenic factors, the decline in new drug development can be attributed in part to the "one drug and one target paradigm," which defines the development of a specific drug to each target.[1] Additionally, many of traditional treatments for multifactorial diseases, using a single drug, have proven to be inefficient because of the inability of the drug to act at different and specific sites inside the body.[2] Alternatively, the use of drug cocktails represents a breakthrough. However, several drawbacks associated with undesired side effects and low patient compliance, prevent their use as standard protocol.[3]

To circumvent this problem, a new conception for drugs has been postulated, in which the objective is to create a "single chemical entity" with multiple associated biological activities, as exemplified by a pioneer work in the treatment of neurodegenerative disorders.[4] Searching for these structures, the goal is to find molecules that can act on various targets or molecular receptors, and this process is called "*one-compound-multiple-targets strategy*".[5] Molecules that are able to on more than one molecular targets are called "multifunctional compounds" (MFC) and have brought promising results in terms of improving the therapeutic potential with synergistic effects and minimizing side effects. It is also possible to highlight the lower risk of drug-drug interaction in relation to drug cocktails.[6]

To have access to such compounds, it is necessary to use strategies able to join two structures with different activities in a single chemical entity. The MFCs are classified as hybrid drugs when two or more drugs with different activities are linked through a stable or metabolizable connection (linker). In this case, the chemical structures of the original molecules remain essentially the same. On the other hand, the MCFs are designed as chimeric drugs when the new chemical entity is formed by the fusion of two or more pharmacophores from different molecules, which hold only parts of the original parent structures (Figure 1).[7]

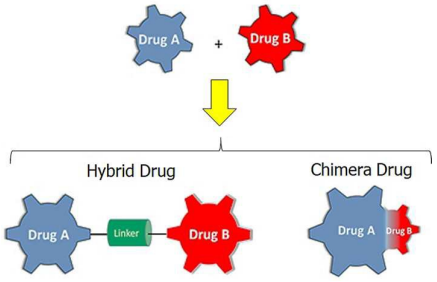

Figure 1. Picture definition of Hybrid Drugs and Chimera Drugs

Although both types of MFCs (hybrids and chimeras) have their advantages and disadvantages, the central idea of the association of two pharmacophores is to increase the potency of both and/or reduce their dosage.[8] Recent reports in the literature have proven

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the success of hybridization of several drugs, improving the characteristics of each original compound. For example: tacrine was the first drug to combat the Alzheimer Disease (AD) by the inhibition of acetylcholinesterase enzyme (AChE). However, poor selectivity and hepatoxicity were observed as injurious collateral effects due to oxidative stress.[9] The hybridization of tacrine with the antioxidant compounds such as ferulic or caffeic acids led to a new hybrid compounds with an increase of antioxidant properties and a high selectivity on the inhibition of AChE. Additionally, the hybrid compounds have been found to inhibit AChE-induced αβ aggregation inhibitory activity.[10]

This strategy was also successfully applied in the field of anticancer agents.[11] A synergistic effect can be achieved when the two pattern molecules have similar effects. The biologically active β-lactams and chalcones, used to form hybrid compounds via formation of 1,2,3-triazole linker, showed antiproliferative activity against A-549 (lung), THP-1 (leukemia) and Caco-2 tumor cell lines, at low concentrations (Figure 2).[12]

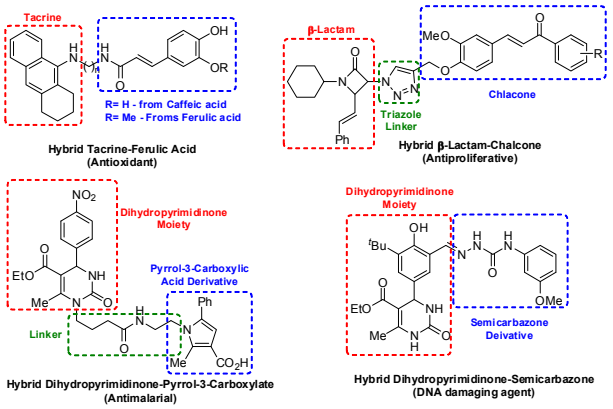

Figure 2. Biologically active hybrid compounds

The dihidropyrimidinones were also investigated as a component of new hybrid compounds. The hybrid dihydropyrimidinone-pyrrol-3-carboxylate is a promising antimalarial compound due to high inhibitory activity on the Hsp70 molecular chaperones of *Plasmodium falciparum* (malaria). The chaperones are a family of proteins that prevent a wrong folding of a protein, helping to maintain its functional structure. Additionally, it induces the protein destruction if the correct conformation cannot be achieved.[13] Another example is the hybrid dihydropyrimidinone-semicarbazone, which was found to be a highly selective human DNA ligase 1 inhibitor (hLig1). This property resulted in an antiproliferative activity against tumour cells being a promising prototype as hybrid anticancer compound (see Figure 2).[14]

Dihidropyrimidinones, Perillyl Alcohol and Antiproliferative Activities

The dihydropyrimidinones (DHPMs) are recognized as effective bioactive agents with a large scope of pharmacological activities, including anticancer properties.[15]

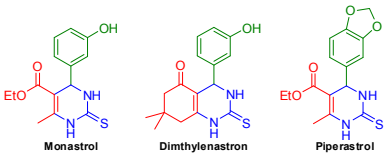

Figure 3. Antiproliferative ~~D~~dihydropyrimidinones

Monastrol,[16] dimethylenastron[17] and piperastrol[18] are three notorious members of these extensive class of bioactive heterocycles and their antiproliferative activity against cancer cell lines have been established (Figure 3). Their antiproliferative activities were attributed to its ability to inhibit the enzyme Eg5 Kinesin, responsible for the mobility of organelles during the mitotic cycle of cell division. Inhibition of this enzyme blocks the formation of the bipolar spindle and causes disruption in the cell cycle.[19] Armed with this background, we choose the dihidropyrimidinone scaffold as constitutive half-part of the new hybrid compounds to be synthesized. On the other hand, the other part of the hybrid molecule was chosen based on the antiproliferative activities exhibited by perillyl alcohol.[20] Perillyl alcohol is a natural occurring monocyclic terpene, found in the essential oils extracted from lavender, lemongrass, and peppermint, among others. Its antitumoral properties have been extensively studied in the last decades as active compound against various cancer cell lines, such as pancreatic,[21] liver,[22] melanoma,[23] colon,[24] leukemia[25] and remarkable activity against gliomas.[26] Furthermore, perillyl alcohol has been used clinically as nasal spray applications to minimize the injurious effects of recurrent malignant glioma.[27] However, there are few reports of synthesis and applications of hybrid compounds based on perillyl moiety (Figure 4).[28]

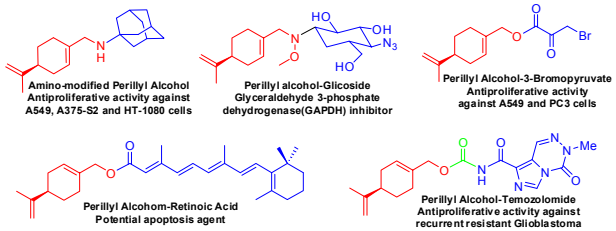

Figure 4. Hybrid compounds from ~~P~~perillyl ~~A~~alcohol

Thus, the present study reports the synthesis and the cytotoxic activities of a series of novel hybrid compounds derived from dihydropyrimidinones and (*S*)-perillyl alcohol against a set of tumour cell lines. The hybridization process was performed using the Huisgen reaction to create a stable 1,2,3-triazolyl linker (Figure 5).[29]

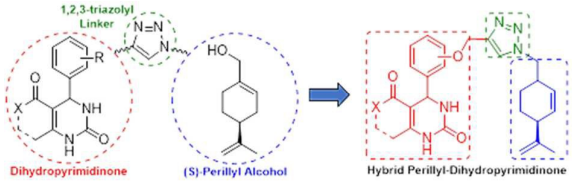

Figure 5. Strategy to hybridize DHPMs and ~~P~~perillyl alcohol via 1,2,3-~~T~~triazolyl linker

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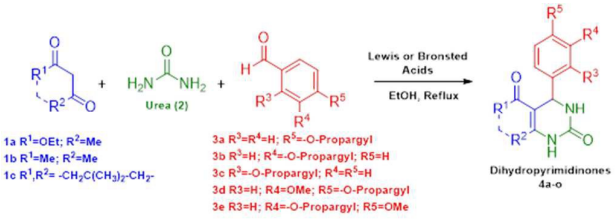
Results and Discussion

Synthesis of Propargyl Dihydropyrimidinones

The synthesis of dihydropyrimidonones **4a-o** was accomplished via the multicomponent Biginelli reaction through a cyclocondensation of ethyl acetoacetate **1a**, acetylacetone **1b** or dimedone **1c**, as the 1,3-dicarbonyl compounds, urea (**2**), and a series of propargyloxy benzaldehydes **3a-e**, under Bronsted or Lewis acids catalysis[30] (Scheme 1, Table 1). The aldehydes were easily prepared, in good yields, by *O*-alkylation reactions from the corresponding hydroxy-substituted benzaldehydes and propargyl bromide.[31]



Scheme 1. Synthesis of Propargyloxy Dihydropyrimidinones

Table 1. Synthesis of propargyloxy-Dihydropyrimidinones

1	3	DHPM						Y-%
			R^1	R^2	R^3	R^4	R^5	
1a	3a	4a	EtO	Me	H	H	OPg[a]	90
1a	3b	4b	EtO	Me	H	OPg[a]	H	75
1a	3c	4c	EtO	Me	OPg[a]	H	H	83
1a	3d	4d	EtO	Me	H	OPg[a]	OMe	86
1a	3e	4e	EtO	Me	H	OMe	OPg[a]	75
1b	3a	4f	Me	Me	H	H	OPg[a]	69
1b	3b	4g	Me	Me	H	OPg[a]	H	67
1b	3c	4h	Me	Me	OPg[a]	H	H	67
1b	3d	4i	Me	Me	H	OPg[a]	OMe	62
1b	3e	4j	Me	Me	H	OMe	OPg[a]	64
1c	3a	4k	$-CH_2C(CH_3)_2CH_2-$		H	H	OPg[a]	67
1c	3b	4l	$-CH_2C(CH_3)_2CH_2-$		H	OPg[a]	H	74
1c	3c	4m	$-CH_2C(CH_3)_2CH_2-$		OPg[a]	H	H	70
1c	3d	4n	$-CH_2C(CH_3)_2CH_2-$		H	OPg[a]	OMe	72
1c	3e	4o	$-CH_2C(CH_3)_2CH_2-$		H	OMe	OPg[a]	81

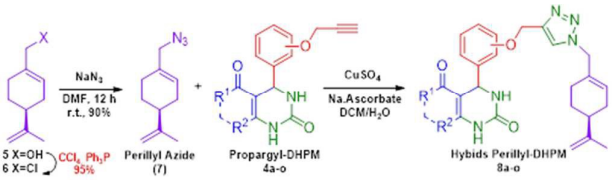
[a] OPg= OCH_2CCH

The propargyloxy dihydropyrimidinones were purified through column chromatography on silica gel (**4a-j**) or simple crystallization (**4k-o**). All compounds were fully characterized by conventional spectroscopic methods including HRMS for the novel compounds. The data were in accordance with the proposed structures. The main ^{1}H NMR spectroscopic evidences of the DHPM formation are the broad singlets at 9.20 ppm and 7.70 ppm, respectively, assigned to N-H groups. The singlet around 5.10 ppm assigned to the benzylic hydrogen also corroborates the formation of the DHPM ring.

Synthesis of Hybrids Perillyl-Dihydropyrimidinones

The chosen strategy to hybridize molecules was the regioselective copper(I) catalyzed [3+2] cycloaddition of alkynes and azide compounds.[32] Thus, to perform the molecular hybridization with propargyloxy dihydropyrimidinones **4a-o**, the perillyl azide **7** was required. It was not possible to prepare the azido compound from the perillyl alcohol mesylate. Therefore, the perillyl alcohol (**5**) was converted into perillyl chloride (**6**), via Appel's reaction[28a,33] and then it was transformed into the corresponding perillyl azide (**7**). The overall yield for these two steps was 85%.

With the propargyloxy-dihydropyrimidinones **4a-o** and the perillyl azide (**7**) in hands, we proceeded to the synthesis of the hybrids perillyl-dihydropyrimidinone **8a-o** through the copper-catalyzed Huisgen reaction. (Scheme 2). The results for the synthesis of hybrids perillyl-DHPM are shown in the Table 2, below



Scheme 2. Synthesis of Perillyl Azide and the Cu(I) catalyzed Huisgen reaction

The hybrids perillyl-dihydropyrimidinones **8a-o** were purified through column chromatography on silica gel. The yields were calculated after the isolation of the pure products. All compounds were fully characterized by conventional spectroscopic methods including HRMS for the novel compounds and the data were compatible with the proposed structures (Table 2).

Table 2. Synthesis of hybrids perillyl-dihydropyrimidinones

Entry	Perillyl-DHPM Hybrids						Y-%
		R^1	R^2	R^3	R^4	R^5	
1	8a	EtO	Me	H	H	Xc*	90
2	8b	EtO	Me	H	Xc*	H	79
3	8c	EtO	Me	Xc*	H	H	85
4	8d	EtO	Me	H	Xc*	OMe	73
5	8e	EtO	Me	H	OMe	Xc*	71
6	8f	Me	Me	H	H	Xc*	72
7	8g	Me	Me	H	Xc*	H	71
8	8h	Me	Me	Xc*	H	H	68
9	8i	Me	Me	H	Xc*	OMe	67
10	8j	Me	Me	H	OMe	Xc*	76
11	8k	$-CH_2C(CH_3)_2CH_2-$		H	H	Xc*	84
12	8l	$-CH_2C(CH_3)_2CH_2-$		H	Xc*	H	68
13	8m	$-CH_2C(CH_3)_2CH_2-$		Xc*	H	H	80
14	8n	$-CH_2C(CH_3)_2CH_2-$		H	Xc*	OMe	71
15	8o	$-CH_2C(CH_3)_2CH_2-$		H	OMe	Xc*	79

The appearance of a low field singlet (7.40-8.20 ppm) assigned to the triazolic hydrogen along with characteristic signals of DHPM and terpene ring in the ^{1}H NMR spectra, corroborate the obtention

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of the desired compounds. All spectroscopic data were compatible with the proposed structures.

Next, the fifteen synthesized new hybrid molecules were screened for their antiproliferative activities against nine different tumor cell lines, using the Total Growth Inhibition (TGI) as a parameter of the antiproliferative activity.

***In vitro* antiproliferative activity of hybrid compounds**

In vitro studies of cytotoxicity are widely used in the initial drug screening for anticancer drug development.[34] Furthermore, these studies are important to identify whether the tested substance interferes with either cell metabolism or cell survival, and it can be indicative of the mechanism of action by its comparison with known chemotherapeutics.

The antiproliferative activities of hybrid compounds **8a-o** were evaluated against the human cancer cell lines: UACC-62 (melanoma), U251 (glioma), MCF7 (breast), NCI/ADR-RES (multidrug resistant breast), 786-0 (kidney), NCI-H460 (lung), PC-3 (prostate), OVCAR-3 (ovarian), HT29 (colon). The TGI values for HaCaT (normal human keratinocytes) were evaluated to determinate the action against the non-tumoral cells. Doxorubicin (DOX) was employed as the positive control (Table 3).

Table 3. In vitro antiproliferative activity (TGI)[a] of hybrid compounds 8a-o.

Hybrid	UACC-62	U251	MCF7	NCI/ADR-RES	786-0	NCI-H460	PC-3	OVCAR-3	HT29	HaCaT
8a	19.18	12.43	43.14	>100	97.08	>100	92.21	6.90	77.13	165.69
8b	22.92	12.19	42.52	>100	39.67	>100	>100	18.45	73.42	507.82
8c	>100	>100	>100	>100	>100	>100	>100	81.19	>100	294.37
8d	37.75	98.09	>100	>100	>100	>100	>100	13.45	>100	79.75
8e	26.73	11.34	82.50	>100	87.34	>100	54.39	53.76	>100	75.76
8f	20.50	19.99	28.97	>100	23.26	78.17	49.27	32.65	62.63	44.47
8g	31.56	30.52	42.96	>100	44.08	>100	49.76	13.33	86.22	115.66
8h	34.23	30.21	68.70	>100	80.31	83.14	62.01	34.31	79.55	29.51
8i	>100	>100	>100	84.90	>100	>100	>100	>100	95.99	486.19
8j	3.83	77.91	53.26	80.17	88.09	>100	85.69	48.03	94.95	147.92
8k	29.94	6.61	>100	>100	53.16	>100	23.98	29.34	>100	436.18
8l	20.92	25.97	30.97	>100	>100	51.61	21.87	>100	48.99	36.44
8m	26.47	26.98	44.95	>100	26.67	38.72	21.68	11.81	28.95	78.72
8n	62.01	8.28	44.81	>100	>100	>100	79.92	>100	>100	451.02
8o	86.16	44.04	91.94	>100	>100	>100	>100	94.99	>100	71.34
Dox	1.66	0.09	0.99	0.78	0,02	0.46	15.69	0.04	0.88	1.47

[a] The concentration that elicits total growth inhibition (TGI in μM) was determined from a non-linear regression analysis using ORIGIN 8.0® (OriginLab Corpo ration). The experiments were conducted in triplicate. Dox: doxorubicin, positive control.

The TGI (concentration that produces total growth inhibition, or cytostatic effect) was determined through a non-linear regression analysis. The values presented in Table 2 were obtained from the tumor cells lines mentioned above. After careful analysis, we were able to identify four compounds with TGI values less than 10 μM. Compound **8a** exhibited TGI value of 6.90 μM for the OVCAR-3 tumor cell line, while the hybrid **8j** showed TGI value of 3.83 μM for the UACC-62 cell line. Hybrids **8k** and **8n** displayed TGI values of 6.62 μM and 8.28 μM, respectively, both for glioblastoma multiform (U256) tumor cell line (Figure 6).

Additionally, a series of seven hybrids compounds revealed TGI range values between 10-20 μM, being considered promising compounds: **8a** (UACC-62 and U251), **8b** (U251 and OVCAR-3), **8d** (OVCAR-3), **8e** (U251), **8f** (U251), **8g** (OVCAR-3) and **8m** (OVCAR-3).

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Interesting to note, these compounds were mainly selective to three tumour cell lines. For UACC-62, hybrid compounds **8a** and **8j** were the most active, while compounds **8a**, **8b**, **8e**, **8f**, **8k**, **8n** were more active to U251. For OVCAR cell line, compounds **8a**, **8b**, **8d**, **8g** and **8m** showed the best activities. Thus, U251 and OVCAR-3 were the tumour cell lines that showed greater sensitivity for most compounds.

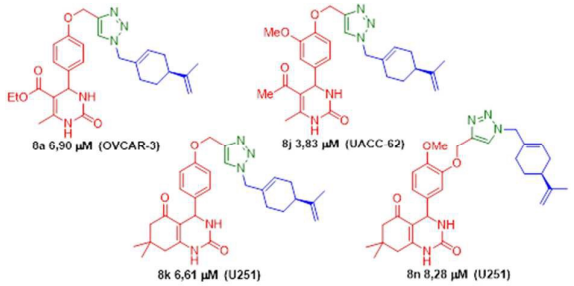

Figure 6. The most potent perillyl-dihydropyrimidinone hybrids.

Compounds **8b**, **8i**, **8k** and **8n** showed high TGI values for HaCaT. Noteworthy are compounds **8b**, **8k** and **8n** with antiproliferative activity against tumor cell line U251 and TGI value for HaCaT of 507.82 μM, 436.18 μM and 451.02 μM, respectively. The ratio of HaCaT/U251 values to compounds **8b**, **8k** and **8n** is 41.66, 65.98 and 54.47, respectively, and makes evident the high selectivity of these compounds relative to U251 tumor cell line.

Previous comparative studies of monastrol and oxo-monastrol regarding the antiproliferative activities,[18] indicated that thio-derivatives were always cytotoxic while the oxo-analogues were cytostatic. One of the main conclusions from this initial study was the influence of sulfur atom in monastrol on the potency of antiproliferative activity. Comparison between monastrol and oxo-monastrol showed significant difference in growth inhibition profiles. For example, monastrol was more potent than oxo-monastrol at concentrations of 25 μM (Figure 7 and 8, respectively).

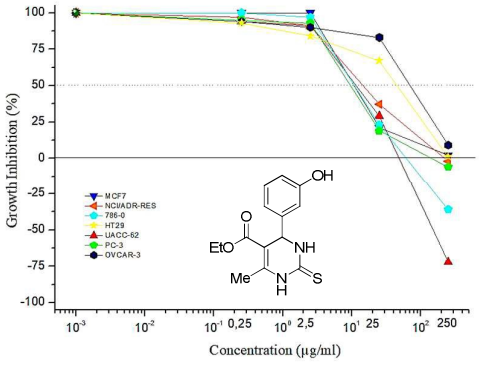

Figure 7. Profile of oxo-monastrol against different tumor cell lines

Although the activity of thio-dihydropyrimidinones hybrids were not evaluated in the present study, the results obtained for perillyl-dihydropyrimidinone **8b** (an oxo-derivative) showed growth inhibition profile similar to those of Monostrol (thio-derivative) rather than to oxo-monastrol, at the concentration of 25 μM (Figure 9). Thus, the presence of the perillyl group in the hybrid structures may have brought about an increase in the antiproliferative potencies of oxo-derivative **8b** as well as other perillyl-dihydropyrimidinone hybrids described herein.

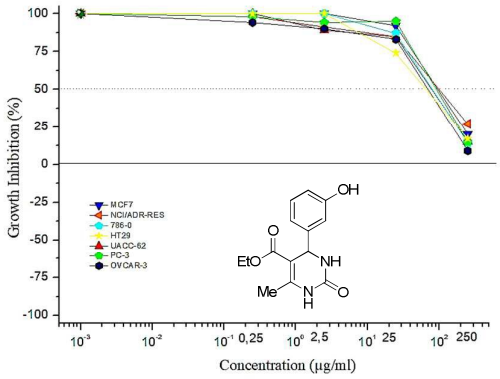

Figure 8. Profile of oxo-monastrol against different tumor cell lines

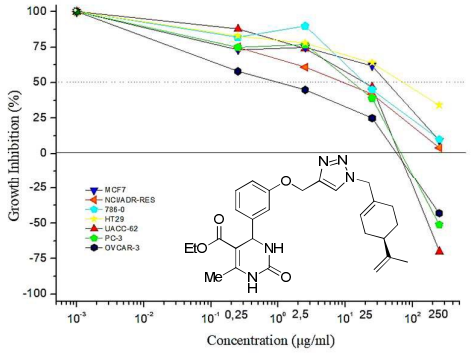

Figure 9. Profile of hybrid **8b** against different tumor cell lines

The antiproliferative activity of these DHPM hybrids against glioblastomas were not entirely surprise. Early studies conducted by our research group, showed the DHPMs were active against human U138-MG and Rat-C6 tumour cell lines.[35] In other study, it was demonstrated the DHPM was able to inhibit the Ecto-5'Nucleotidase/CD73,[36] an ecto-enzyme responsible for hydrolysing the nucleoside monophosphates such as AMP to adenosine.[37] More recently, hybrids of monastrol-fatty acids exhibited antiproliferative activity against glioblastomas,[38] as well as the Hantzsch dihydropyridine-fatty acids hybrids showed similar properties.[39]

Experimental Section

Chemistry

General procedure for synthesis of Propargyloxy Benzaldehydes 3a-e.

A mixture of hydroxy aldehydes (5.0 mmol), propargyl bromide (10 mmol), potassium carbonate (10 mmol) and acetone (50 mL) was stirred under reflux for 1-4 h period, monitored by TLC. After, the crude mixture was filtered, and the solvent was retired under vacuum to yield the propargyloxy benzaldehydes **3a-e** with

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satisfactory level of purity. The characterization of compound **3a-e** were confirmed by comparison with the reported data. The compounds **3a-e** were used in the next step without further purification.

4-(prop-2-yn-1-yloxy)benzaldehyde (3a):[40] Yield 95%; yellow solid; m.p. 71°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.90 (s, 1H); 7.86 (d, 2H, *J*= 8.8 Hz); 7.09 (d, 2H, *J*= 8.6 Hz); 4.78 (d, 2H, *J*= 2.5 Hz); 2.58 (t, 1H, *J*= 2.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.8; 162.3; 131.9; 130.5; 115.1; 77.5; 76.4; 55.9; IR (ν_{max} cm^{-1}): 3413, 3214, 2834, 2744, 2115, 1686, 1606, 1256, 1160, 1003, 827.

3-(prop-2-yn-1-yloxy)benzaldehyde (3b):[40] Yield 85%; colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.98 (s, 1H); 7.53-7.46 (m, 3H); 7.23 (ddd, 1H, *J*= 7.8; 2.8 and 1.5 Hz); 4.76 (d, 2H, *J*= 2.5 Hz); 2.56 (t, 1H, *J*= 2.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 191.7; 157.9; 137.6; 130.0; 123.9; 121.9; 113.4; 77.8; 76.1; 55.8; IR (ν_{max} cm^{-1}): 3284, 2836, 2740, 2123, 1701, 1588, 1266, 1246, 1038, 791, 681.

2-(prop-2-yn-1-yloxy)benzaldehyde (3c):[40] Yield 95%; Yellow solid; m.p. 66°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.50 (s, 1H); 7.87 (dd, 1H, *J*= 7.6 and 2.0 Hz); 7.60-7.56 (m, 1H); 7.14-7.08 (m, 2H); 4.85 (d, 2H, *J*= 2.3 Hz); 2.57 (t, 1H, *J*= 2,3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.4; 160.0; 135.6; 128.4; 125.4; 121.6; 113.1; 77.6; 76.4; 56.3; IR (ν_{max} cm^{-1}): 3266, 2879, 2119, 1686, 1599, 1478, 1458, 1227,758.

4-methoxy-3-(prop-2-yn-1-yloxy)benzaldehyde (3d):[41] Yield 97%; Yellow solid; m.p. 71°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.86 (s, 1H); 7.55-7.51 (m, 2H); 7.01 (d, 1H, *J*= 8.1 Hz); 4,82 (d, 2H, *J*= 2.3 Hz); 3.96 (s, 3H); 2.55 (t, 1H, *J*= 2,5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 190.6; 154.8; 147.2; 129.8; 127.2; 111.9; 110.8; 77.6; 76.4; 56.5; 56.1; IR (ν_{max} cm^{-1}): 3225, 2936, 2836, 2122, 1669, 1588, 1508, 1438, 1257, 1126, 1015, 714, 634.

3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde (3e):[40] Yield 96%; Yellow solid; m.p. 88°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.86 (s, 1H); 7.45 (dd, 1H, *J*= 2.0 and 8.1 Hz); 7.43 (d, 1H, *J*= 1.8 Hz); 7.14 (d, 1H, *J*= 8.3 Hz); 4.85 (d, 2H, *J*= 2.4 Hz); 3.93 (s, 3H); 2.56 (t, 1H, *J*= 2.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.8; 152.0; 150.0; 130.8; 126.1; 112.5; 109.4; 77.4; 76.6; 56.5; 55.9; IR (ν_{max} cm^{-1}): 3245, 3006, 2845, 2121, 1685, 1584, 1504, 1263, 1132, 1001, 800, 740.

General procedure for synthesis of Propargyloxy DHPMs 4a-j

The aldehyde (2.0 mmol), urea (2.4 mmol), 1,3-dicarbonyl compound (2.0 mmol), $CeCl_3.7H_2O$ (20 mol%) and ethanol (2 mL) were stirred under reflux conditions for 3h-6h period, monitored by TLC. After, the solvent was evaporated under vacuum and the crude mixture was purified by chromatography in column of silica gel using a hexane-ethyl acetate as eluent to isolate the desired DHPMs **4a-j** in pure form. The characterization of compound **4a** was confirmed by comparison with the reported data.

Ethyl 6-methyl-2-oxo-4-[4-(prop-2-yn-1-yloxy)phenyl]-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4a):[42] Yield 75%; White solid; m.p. 153ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.18 (br s, 1H); 7.69 (br s, 1H); 7.15 (d, 2H, *J*= 8.8 Hz); 6.92 (d, 2H, *J*= 8.8 Hz); 5.08 (d, 1H, *J*= 3.3 Hz); 4.76 (d, 2H, *J*= 2.3 Hz); 3.98 (q, 2H, *J*= 7.0 Hz); 3.55 (t, 1H, *J*= 2.3 Hz); 2.24 (s, 3H); 1.10 (t, 3H, *J*= 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-*d6*): δ 165.8; 156.8; 152.6; 148.6; 138.3; 127.8; 115.1; 99.9; 79.8; 78.6; 59.7; 55.8; 53.8; 18.2; 14.6; IR (ν_{max} cm^{-1}): 3276, 3247, 3123, 2978, 2116, 1707, 1655, 1224, 1100, 790, 645; HRMS calc. for [$C_{17}H_{18}N_2O_4$+Na]: 337.1159; Found: 337.1152.

Ethyl 6-methyl-2-oxo-4-[3-(prop-2-yn-1-yloxy)phenyl]-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4b): Yield 90%; white solid; m.p. 156ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.21 (br s, 1H); 7.75 (br s, 1H); 7.26 (t, 1H, *J*= 7.8 Hz); 6.90-6.81 (m, 3H); 5.11 (d, 1H, *J*= 3.3 Hz); 4,75 (d, 2H, *J*= 2.3 Hz); 4,00 (q, 2H, *J*= 7.3 Hz); 3.55 (t, 1H, *J*= 2.5Hz); 2,24 (s, 3H); 1.11 (t, 3H, *J*= 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 165.8; 157.7; 152.7; 149.0; 146.8; 130.0; 119.5; 113.8; 113.4; 99.6; 79.6; 78.6; 59.8; 55.8; 54.1; 18.2; 14.6; IR (ν_{max} cm^{-1}): 3279, 3247, 3118, 2977, 2928, 2117, 1717, 1699, 1643, 1225, 1097, 777; HRMS calc. for [$C_{17}H_{18}N_2O_4$+Na]: 337.1159; Found: 337.1158.

Ethyl 6-methyl-2-oxo-4-[2-(prop-2-yn-1-yloxy)phenyl]-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4c): Yield 83%; Pale green solid; m.p. 155ºC; ^{1}H NMR (300 MHz, DMSO-*d6*): δ 9.14 (br s, 1H); 7.21-7.27 (m, 2H); 7.06-7.10 (m, 2H); 6,92 (t, 1H, *J*= 7.0 Hz); 5.49 (d, 1H, *J*= 2.2 Hz); 4.85 (dd, 1H, *J*= 2.3; 16.1 Hz); 4.78 (dd, 1H, *J*= 16,1 and 2.6 Hz); 3.99-3.84 (m, 2H); 3.58 (t, 1H, *J*= 2.3 Hz); 2.28 (s, 3H); 1.03 (t, 3H, *J*= 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-*d6*): δ 165.3; 154.6; 152.1; 149.0; 132.3; 128.5; 127.4; 121.0; 112.6; 97.6; 79.5; 78.2; 59.0; 55.8; 48.8; 17.8; 14.1; IR (ν_{max} cm^{-1}): 3252, 3115, 2981, 2928, 2123, 1707, 1668, 1637, 1488, 1228, 1088, 759; HRMS calc. for [$C_{17}H_{18}N_2O_4$+Na]: 337.1159; Found: 337.1156.

Ethyl 4-[4-methoxy-3-(prop-2-yn-1-yloxy)phenyl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4d): Yield 75%; Yellow solid; m.p. 149ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.40 (br s, 1H); 7.04 (d, 1H, *J*= 1.8 Hz); 6.92 (dd, 1H, *J*= 8.3 and 1.8 Hz); 6.81 (d, 1H *J*= 8.3 Hz); 5.97 (br s, 1H); 5.36 (d, 1H, *J*= 2.5 Hz); 4.73 (d, 2H, *J*= 2.0 Hz); 4.09 (q, 2H, *J*= 7.3 Hz); 3.84 (s, 3H); 2.49 (t, 1H, *J*= 2.4 Hz); 2.34 (s, 3H); 1.18 (t, 3H, *J*= 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 165.7; 153.6; 149.4; 146.8; 146.3; 136.3; 120.2; 113.3; 111.7; 101.4; 78.5; 76.0; 60.0; 57.0; 55.9; 55.1; 18.6; 14.2; IR (ν_{max} cm^{-1}): 3268, 3237, 2976, 2122, 1701, 1642, 1511, 1219, 1089, 1018, 798; HRMS calc. for [$C_{18}H_{20}N_2O_5$+Na]: 367.1264; Found: 367.1259.

Ethyl 4-[3-methoxy-4-(prop-2-yn-1-yloxy)phenyl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4e): Yield 86%; Yellow solid; m.p. 119ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.17 (br s, 1H); 7.70 (br s, 1H); 6.96 (d, 1H, *J*= 8.3Hz); 6.87 (d, 1H, *J*= 1.8Hz); 6.71 (dd, 1H, *J*= 8.3 and 1.8 Hz); 5.10 (d, 1H, *J*= 3.0 Hz); 4.73 (d, 2H, *J*= 2.0 Hz); 4.00 (q, 2H, *J*= 7.0 Hz); 3.73 (s, 3H); 3.53 (t, 1H, *J*= 3.5 Hz); 2.24 (s, 3H); 1.11 (t, 3H, *J*= 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 165.4; 152.3; 149.0; 148.3; 145.7; 138.6; 117.7; 114.2; 110.8; 99.3; 79.4; 78.2; 59.2; 56.1; 55.5; 53.5; 17.8; 14.2; IR (ν_{max} cm^{-1}): 3236, 3102, 2937, 2122, 1695, 1654, 1510, 1220, 1096, 786, 642; HRMS calc. for [$C_{18}H_{20}N_2O_5$+Na]: 367.1264; Found: 367.1269.

5-acetyl-6-methyl-4-(4-(prop-2-yn-1-yloxy)phenyl)-3,4-dihydropyrimidin-2(1H)-one (4f): Yield 67%; Yellow solid; m.p. 187ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.14 (br s, 1H); 7.75 (br s, 1H); 7.17 (d, 2H, *J*= 8.6 Hz); 6.93 (d, 2H, *J*= 8.6 Hz); 5,20 (d, 1H, *J*= 3.3 Hz); 4.76 (d, 2H, *J*= 2.3 Hz); 3.53 (t, 1H, *J*= 2.5 Hz); 2.28 (s, 3H); 2.09 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 194.4; 156.4; 152.1; 147.9; 137.2; 127.6; 114.8; 109.7; 79.3; 78.2; 55.4; 53.3; 30.2; 18.9; IR (ν_{max} cm^{-1}): 3297,

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2940, 2117, 1674, 1613, 1238, 1029; HRMS calc. for $[C_{16}H_{16}N_2O_3+Na]$: 307.1053; Found: 307.1047.

5-acetyl-6-methyl-4-(3-(prop-2-yn-1-yloxy)phenyl)-3,4-dihydropyrimidin-2(1H)-one (4g): Yield 69%; Pale yellow solid; m.p. 190ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9.17 (br s, 1H); 7.80 (br s, 1H); 7.26 (t, 1H, *J*= 7.8 Hz); 6.90-6.84 (m, 3H); 5.24 (d, 1H, *J*= 3.3 Hz); 4.75 (d, 2H, *J*= 2.0 Hz); 3.54 (t, 1H, *J*= 2.3 Hz); 2.29 (s, 3H); 2.12 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d6*): δ 194.3; 157.3; 152.2; 148.3; 145.8; 129.6; 119.3; 113.6; 112.9; 109.4; 79.2; 78.3; 55.4; 53.6; 30.3; 18.9; IR (ν_{max} cm^{-1}): 3278, 3254, 2959, 2129, 1709, 1680, 1606, 1379, 1225, 1029, 760; HRMS calc. for $[C_{16}H_{16}N_2O_3+Na]$: 307.1053; Found: 307.1048.

5-acetyl-6-methyl-4-(2-(prop-2-yn-1-yloxy)phenyl)-3,4-dihydropyrimidin-2(1H)-one (4h): Yield 67%; Yellow solid; m.p. 182ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9.16 (br s, 1H); 7.34 (br s, 1H); 7.27 (td, 1H, *J*= 7.5 and 1.5 Hz); 7.11 (d, 1H, *J*= 7.8 Hz); 7.07 (dd, 1H, *J*= 7.6 and 1.5 Hz) 6.94 (t, 1H, *J*= 7.3 Hz); 5.56 (d, 1H, *J*= 3.3 Hz); 4.89 (dd, 2H, *J*= 16.1 and 2.3 Hz); 4.84 (dd, 2H, *J*= 16.1 and 2.3 Hz); 3.59 (t, 1H, *J*= 2.3 Hz); 2.29 (s, 3H); 2.03 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 194.4; 154.3; 152.1; 148.2; 131.7; 128.8; 127.1; 121.3; 112.8; 107.8; 79.4; 78.3; 55.8; 48.7; 29.7; 18.7; IR (ν_{max} cm^{-1}): 3297, 2948, 2117, 1674, 1613, 1225, 1029; HRMS calc. for $[C_{16}H_{16}N_2O_3+Na]$: 307.1053; Found: 307.1049.

5-acetyl-4-(4-methoxy-3-(prop-2-yn-1-yloxy)phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one (4i): Yield 64%; Yellow solid; m.p. 143ºC; $_1$H NMR (400 MHz, DMSO-*d6*): δ9.13 (br s, 1H); 7.73 (br s, 1H); 6.97 (d, 1H, *J*= 1.8 Hz); 6.93 (d, 1H, *J*= 8.3 Hz); 6.81 (dd, 1H, *J*= 8.3 and 1.8 Hz); 5.19 (d, 1H, *J*= 3.3 Hz); 4.69 (d, 2H, *J*= 2.3 Hz); 3.73 (s, 1H); 3.51 (t, 1H, *J*= 2.3 Hz); 2.28 (s, 3H); 2.08 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 194.4; 152.1; 148.7; 148.0; 146.3; 136.5; 119.7; 113.5; 112.2; 109.3; 79.2; 78.4; 56.3; 55.6; 53.2; 30.1; 18.8; IR (ν_{max} cm^{-1}): 3303, 3272, 3117, 2941, 2125, 1691, 1618, 1505, 1226, 1133, 1019; HRMS calc. for $[C_{17}H_{18}N_2O_4+Na]$: 337.1159; Found: 337.1167.

5-acetyl-4-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one (4j): Yield 62%; Yellow solid; m.p. 183ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9.15 (br s, 1H); 7.76 (br s, 1H); 6.96 (d, 1H, *J*= 8.3 Hz); 6.93 (d, 1H, *J*= 2.0 Hz); 6,70 (dd, 1H, *J*= 8.3 and 2.0 Hz); 5.21 (d, 1H, *J*= 3.5 Hz); 4.74 (d, 2H, *J*= 2.5 Hz); 3.74 (s, 3H); 3.51 (t, 1H, *J*= 2.4 Hz); 2.29 (s, 3H); 2.10 (s, 3H); ^{13}C MNR (75 MHz, DMSO-*d6*): δ194.5; 152.1; 149.2; 148.0; 145.8; 137.8; 117.8; 114.2; 111.1; 109.3; 79.4; 78.2; 56.1; 55.5; 53.5; 30.2; 18.8; IR (ν_{max} cm^{-1}): 3380, 3266, 2947, 2114, 1672, 1590, 1232, 1139, 1015; HRMS calc. for $[C_{17}H_{18}N_2O_4+Na]$: 337.1159; Found: 337.1153.

General Procedure for synthesis of Propargyloxy DHPMs 4k-o

The aldehyde (**3a-e**, 2.0 mmol), urea (2.4 mmol), HCl (3 drops) and ethanol (2 mL) were stirred under reflux for 1 h period. Next, dimedone (2.0 mmol) was added in 5 portions, during a total time of 2.5 h, maintaining the reaction mixture at 60 ^{o}C under stirring. The end of the reaction was monitored by TLC and the solvent was retired under vacuum. The crude product was purified through recrystallization from hot ethanol to give the DHPMs in the pure form.

7,7-dimethyl-4-(4-(prop-2-yn-1-yloxy)phenyl)-4,6,7,8-tetrahydroquinazoline-2,5(1H,3H)-dione (4k): Yield 74%; Pale yellow solid; m.p. 223ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9,42 (br s, 1H); 7.69 (br s, 1H); 7.15 (d, 2H, *J*= 8,8 Hz); 6.91 (d, 2H, *J*= 8.8 Hz); 5.10 (d, 1H, *J*= 2.8 Hz); 4.75 (d, 2H, *J*= 2.5 Hz); 3.52 (t, 1H, *J*= 2.3 Hz); 2.40 (d, 1H, *J*= 17.4 Hz); 2.28 (d, 1H, *J*= 17.1 Hz); 2.18 (d, 1H, *J*= 16.1 Hz); 2.03 (d, 1H, *J*= 16.1 Hz); 1.01 (s, 3H); 0.90 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d6*): δ193.4; 156.7; 152.7; 152.3; 130.1; 127.8; 115.0; 108.0; 79.8; 78.6; 55.8; 51.9; 50.3; 39.6; 32.8; 29.2; 27.4; IR (ν_{max} cm^{-1}): 3303, 3241, 3069, 2965, 2872, 2129, 1693, 1625, 1502, 1373, 1250, 1023, 826; HRMS calc. for $[C_{19}H_{20}N_2O_3+Na]$: 347.1366; Found: 347.1362.

7,7-dimethyl-4-(3-(prop-2-yn-1-yloxy)phenyl)-4,6,7,8-tetrahydroquinazoline-2,5(1H,3H)-dione (4l): Yield 67%; Pale yellow solid; m.p. 203ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9.45 (br s, 1H); 7.74 (br s, 1H); 7.24 (t, 1H, *J*= 7.9 Hz); 6.88-6.81 (m, 3H); 5.12 (d, 1H, *J*= 2.8 Hz); 4.73 (d, 2H, *J*= 2.5 Hz); 3.53 (t, 1H, *J*= 2.3 Hz); 2.40 (d, 1H, *J*= 17.1 Hz); 2.28 (d, 1H, *J*= 17.1 Hz); 2.19 (d, 1H, *J*= 16.1 Hz); 2.05 (d, 1H, *J*= 16.4 Hz); 1,02 (s, 3H); 0.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 193.4; 157.7; 153.1; 152.4; 146.6; 129.9; 119.6; 113.9; 113.2; 107.6; 79.7; 78.7; 55.8; 52.2; 50.3; 39.6; 32.8; 29.2; 27.4; IR (ν_{max} cm^{-1}): 3272, 2952, 2123, 1680, 1607, 1373, 1232, 1035, 760; HRMS calc. for $[C_{19}H_{20}N_2O_3+Na]$: 347.1366; Found: 347.1367.

7,7-dimethyl-4-(2-(prop-2-yn-1-yloxy)phenyl)-4,6,7,8-tetrahydroquinazoline-2,5(1H,3H)-dione (4m): Yield 70%; Pale yellow solid; m.p. 218ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9.38 (br s, 1H); 7.25-7.20 (m, 2H); 7.07 (m, 2H); 6.90 (t, 1H, *J*= 7.3 Hz); 5.39 (d, 1H, *J*= 1.8 Hz); 4.80 (d, 1H, *J*= 15.9 Hz); 4.76 (d, 1H, *J*= 15.9 Hz); 3.56 (s, 1H); 2.40 (d, 1H, *J*= 17.1 Hz); 2.33 (d, 1H, *J*= 17.4 Hz); 2.16 (d, 1H, *J*= 16.1 Hz); 2,01 (d, 1H, *J*= 16.1 Hz); 1.03 (s, 3H); 0.98 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ192.6; 155.0; 153.1; 151.7; 132.0; 128.5; 128.2; 120.8; 112.8; 105.4; 79.5; 78.2; 55.9; 48.9; 48.5; 39.5; 32.2; 28.7; 27.3; IR (ν_{max} cm^{-1}): 3416, 3290, 3210, 3100, 2960, 2125, 1705, 1613, 1385, 1237, 746; HRMS calc. for $[C_{19}H_{20}N_2O3+Na]$: 347.1366; Found: 347.1362.

7,7-dimethyl-4-(4-methoxy-3-(prop-2-yn-1-yloxy)phenyl)-4,6,7,8-tetrahydroquinazoline-2,5(1H,3H)-dione (4n): Yield 81%; White solid; m.p. 236ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9.44 (br s, 1H); 7.71 (br s, 1H); 6.95 (d, 1H, *J*= 1.8 Hz); 6.92 (d, 1H, *J*= 8.3 Hz); 6.80 (dd, 1H, *J*= 8.3 e 1.8 Hz); 5.10 (d, 1H, *J*= 2.8 Hz); 4.68 (d, 2H, *J*= 2.3 Hz); 3.73 (s, 3H); 3.53 (t, 1H, *J*= 2.4 Hz); 2.41 (d, 1H, *J*= 17.1 Hz); 2.28 (d, 1H, J= 17.4 Hz); 2.20 (d, 1H, J= 16.1 Hz); 2.05 (d, 1H, J= 15.9 Hz); 1.02 (s, 3H); 0.94 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ192.9; 152.3; 151.9; 148.6; 146.3; 137.0; 119.6; 113.2; 111.9; 107.4; 79.2; 78.3; 56.4; 55.6; 51.4; 49.9; 39.2; 32.3; 28.8; 27.0; IR (ν_{max} cm^{-1}): 3256, 2974, 2153, 1676, 1603, 1376, 1231, 1138, 1014, 776, 694, 642; HRMS calc. for $[C_{20}H_{22}N_2O_4+Na]$: 377.1472; Found: 377.1471.

7,7-dimethyl-4-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-4,6,7,8-tetrahydroquinazoline-2,5(1H,3H)-dione (4o): Yield 72%; Pale green solid; m.p. 215ºC; ^{1}H NMR (300 MHz, DMSO-*d6*): δ9.45 (br s, 1H); 7.73 (br s, 1H); 6.95 (d, 1H, *J*= 8.3 Hz); 6.86 (d, 1H, *J*= 1.8 Hz); 6.71 (dd, 1H, *J*= 8.3 and 1.8 Hz); 5.11 (d, 1H, *J*= 3.0 Hz); 4.73 (d, 2H, *J*= 2.5 Hz); 3.71 (s, 3H); 3.53 (t, 1H, *J*= 2.3 Hz); 2.41 (d, 1H, *J*= 17.1 Hz); 2.28 (d, 1H, *J*= 17.4 Hz); 2.20 (d, 1H, *J*= 16.1 Hz); 2.05 (d, 1H, *J*= 16.1 Hz); 1.02 (s, 3H); 0.94 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ193.0; 152.5; 151.9; 149.0; 145.6; 138.2; 117.8; 114.0; 110.7; 107.3; 79.4;

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78.2; 56.1; 55.4; 51.5; 49.9; 39.2; 32.3; 28,8; 26,9; IR (ν_{max} cm^{-1}): 3324, 3272, 2962, 2122, 1676, 1614, 1366, 1222, 1125, 1007, 758; HRMS calc. for [$C_{20}H_{22}N_2O_4$+Na]: 377.1472; Found: 377.1469.

Procedure for synthesis of Perillyl Azide (7)

To a mixture of (s)-perillyl chloride (5.0 mmol) and dimethylformamide (1.5 mL) was added sodium azide (15.0 mmol) and the reaction was stirred for 12h at room temperature. The end of the reaction was monitored by TLC and water (5.0 mL) was added in one portion. The aqueous phase (H_2O/DMF) was washed with hexane (3 x 15.0 mL). The organic phase was separated and washed with NaCl saturated solution, dried over anhydrous magnesium sulfate. After simple filtration, the filtrate was removed over vacuum* to yield the crude perillyl azide (**7**) in 90%. The perillyl azide was used in a next step without further purification. *This process should be carefully controlled to avoid the loss of mass.

(S)-1-(azidomethyl)-4-(prop-1-en-2-yl)cyclohex-1-ene (7): Yield 90%; Colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.76 (br s, 1H); 4.76-4.73 (m, 2H); 3.70 (d, 1H, *J*= 13.4 Hz); 3.65 (d, 1H, *J*= 13.1 Hz); 2.19-2.10 (m, 4H); 2.05-1.96 (m, 1H); 1.90-1.86 (m, 1H); 1.75 (s, 3H); 1.55-1.49 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.3; 134.1; 126.4; 108.9; 57.4; 40.7; 30.4; 27.3; 27.0; 20.7; IR (ν_{max} cm^{-1}): 3085, 2923, 2096, 1645, 1439, 1242, 891.

General procedure for synthesis of Hybrids Perillyl-DHPM 8a-o

The DHPM (**4a-o**) (0.5 mmol), perillyl azide (**7**, 0.6 mmol), dichloromethane (5.0 mL) and water (5.0 mL) were mixed in flask and then, copper sulfate pentahydrate (10.0 mol%) and sodium ascorbate (10.0 mol%) were added in this order. The reaction was kept under stirring at room temperature for 24h. The end of the reaction was monitored by TLC. Next, EDTA 0.1M (10.0 mL) was added and mixture was stirred for 15 min. The aqueous phase was washed with dichloromethane (3 x 10 mL). The organic phase separated and was washed with NaCl saturated solution. After separation, the organic layer was dried over anhydrous magnesium sulfate and filtered. The filtrate was removed under vacuum and the crude solid was purified by chromatography in column of silica gel using hexane-ethyl acetate as eluent to give a Perillyl-DHPM hybrid in pure form.

5-etoxycarbonyl-6-methyl-4-(4-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-3,4-dihidropirimidin-2-(1H)-one (8a): Yield 79%; White solid; m.p. 80ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.50 (br s, 1H); 7.58 (s, 1H); 7,23 (d, 2H, *J*= 8.8 Hz); 6.91 (d, 2H, *J*= 8.8 Hz); 6.02 (br s, 1H); 5.76 (br s, 1H); 5.34 (d, 1H, *J*= 2.4 Hz); 5.17 (s, 2H); 4.85 (s, 2H); 4.78 – 4.65 (m, 2H), 4.08 (m, 2H); 2.32 (s, 3H); 2.17-2.10 (m, 2H); 1,94-1,80 (m, 4H); 1.72 (s, 3H); 1.52-1.41 (m, 1H); 1.16 (t, 3H, *J*= 7.0 Hz); ^{13}C NMR (100 MHz, CDCl3): δ 165.7; 157.9; 153.5; 149.0; 146.2; 144.2; 136.7; 131.8; 127.8; 127.4; 122.4; 114.8; 109.0; 101.4; 62.1; 59.9; 56.5; 55.0; 40.4; 30.4; 27.0; 26.3; 20.7; 18.5; 14.1; IR (ν_{max} cm^{-1}): 3230, 3102, 2936, 1693, 1638, 1600, 1427, 1227, 1082, 777; HRMS calc. for [$C_{27}H_{33}N_5O_4$+H]: 492.2605; Found: 492.2607.

5-etoxycarbonyl-6-methyl-4-(3-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-3,4-dihidropirimidin-2-(1H)-one (8b): Yield 90%; White solid; m.p. 74ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (br s, 1H); 7.59 (s, 1H); 7.21 (t, 1H, *J*= 7.8 Hz); 6.95 (s, 1H); 6.91 (d, 1H, *J*= 7.8 Hz); 6.88 (d, 1H, *J*= 7.8 Hz); 6.10 (br s, 1H); 5.76 (br s, 1H); 5.36 (s, 1H); 5.17 (s, 2H); 4.84 (s, 2H); 4.73 (s, 1H); 4.69 (s, 1H); 4.06 (q, 2H, *J*= 7.3 Hz); 2.33 (s, 3H); 2.21-2.11 (m, 2H); 2.04-1.93 (m, 4H); 1.72 (s, 3H); 1.49-1.41 (m, 1H); 1.15 (t, 3H, *J*= 7.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.6; 158.5; 153.4; 149.0; 146.7; 145.3; 144.1; 131.8; 129.7; 127.3; 122.5; 119.4; 113.8; 113.4; 109.0; 100.9; 62.1; 59.9; 56.4; 55.4; 40.4; 30.4; 27.0; 26.3; 20.7; 18.6; 14.1; IR (ν_{max} cm^{-1}): 3232, 3101, 2818, 1695, 1634, 1596, 1446, 1224, 1084, 773; HRMS calc. for [$C_{27}H_{33}N_5O_4$+H]: 492.2605; Found: 492.2609.

5-etoxycarbonyl-6-methyl-4-(2-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-3,4-dihidropirimidin-2-(1H)-one (8c): Yield 85%; White solid; m.p. 182ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (br s, 1H); 7.62 (s, 1H); 7.22 (td, 1H, *J*= 7.8 and 1.8 Hz); 7.06 (dd, 1H, *J*= 7.6 and 1.8 Hz); 7.01 (d, 1H, *J*= 7.8 Hz); 6.90 (t, 1H, *J*= 7.6 Hz); 5.99 (br s, 1H); 5.74 (br s, 2H); 5.28 (s, 2H); 4.87 (d, 1H, *J*= 16.1 Hz); 4.83 (d, 1H, *J*= 16.1Hz); 4.73 (s, 1H); 4.69 (s, 1H); 4.04 (q, 2H, J= 7.0 Hz); 2.39 (s, 3H); 2.21-2.10 (m, 2H); 1.98-1.79 (m, 4H); 1.72 (s, 3H); 1.50-1.40 (m, 1H); 1.09 (t, 3H, *J*= 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8; 155.5; 153.7; 149.0; 148.2; 143.9; 131.8; 130.4; 129.0; 127.3; 127.0; 122.5; 121.2; 112.2; 109.0; 98.2; 62.3; 59.8; 56.5; 50.1; 40.4; 30.4; 27.0; 26.3; 20.7; 18.5; 14.1; IR (ν_{max} cm^{-1}): 3397, 3113, 2980, 1724, 1694, 1639, 1592, 1446, 1221, 1080, 753; HRMS calc. for [$C_{27}H_{33}N_5O_4$+H]: 492.2605; Found: 492.2605.

5-etoxycarbonyl-6-methyl-4-(4-methoxy-3-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-3,4-dihidropirimidin-2-(1H)-one (8d): Yield 71%; yellow solid; m.p. 182ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.17 (br s, 1H); 8.10 (s, 1H); 7.70 (br s, 1H); 7.01 (d, 1H, *J*= 1.8 Hz); 6.91 (d, 1H, *J*= 8.6 Hz); 6.76 (dd, 1H, *J*= 8.3 and 1.8 Hz); 5.66 (br s, 1H); 5.10 (d, 1H, *J*= 3.0 Hz); 5.07 (d, 1H, *J*= 11.8 Hz); 5.04 (d, 1H, *J*= 11.8 Hz); 4.91 (s, 2H); 4.69 (s, 2H); 3.99 (q, 2H, *J*= 7.3 Hz); 3.71 (s, 3H); 2.25 (s, 3H); 2.14-2.04 (m, 2H); 1.94-1.86 (m, 3H); 1.75-1.72 (m, 1H); 1.69 (s, 3H); 1.39-1.33 (m, 1H); 1.10 (t, 3H, *J*= 7.0 Hz); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 165.4; 152.3; 148.9; 148.5; 148.3; 147.1; 142.6; 137.3; 132.5; 125.5; 124.7; 118.8; 112.6; 112.0; 109.1; 99.3; 61.9; 59.2; 55.5; 55.1; 53.4; 40.0; 29.9; 26.8; 26.0; 20.6; 17.8; 14.2; IR (ν_{max} cm^{-1}): 3260, 3115, 2929, 1692, 1511, 1454, 1256, 1225, 1084, 1001, 754; HRMS calc. for [$C_{28}H_{35}N_5O_5$+Na]: 544.2530; Found: 544.2527.

5-etoxycarbonyl-6-methyl-4-(3-methoxy-4-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-3,4-dihidropirimidin-2-(1H)-one (8e): Yield 73%; yellow solid; m.p. 78ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.16 (br s, 1H); 8.13 (s, 1H); 7.68 (br s, 1H); 7.07 (d, 1H, *J*= 8.3 Hz); 6.87 (d, 1H, *J*= 2.0 Hz); 6.73 (dd, 1H, *J*= 8.3 and 2.0 Hz); 5.67 (br s, 1H); 5.12 (d, 1H, *J*= 3.3 Hz); 5.08 (s, 2H); 4.91 (s, 2H); 4.71 (s, 2H); 4.01 (q, 2H, *J*= 7.0 Hz); 3.72 (s, 3H); 2.25 (s, 3H); 2.17-2.05 (m, 2H); 1.93-1.89 (m, 3H); 1.77-1.73 (m, 1H); 1.70 (s, 3H); 1.42-1.32 (m, 1H); 1.12 (t, 3H, *J*= 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-*d6*): δ 165.4; 152.2; 148.9; 148.8; 148.2; 146.6; 142.7; 138.0; 132.5; 125.4; 124.6; 117.8; 113.7; 110.7; 109.0; 99.3; 61.8; 59.2; 55.4; 55.0; 53.5; 40.0; 29.8; 26.7; 26.0; 20.6; 17.7; 14.1; IR (ν_{max} cm^{-1}): 3232, 3105, 2929, 1698, 1640, 1515, 1449, 1220, 1082, 774; HRMS calc. for [$C_{28}H_{35}N_5O_5$+Na]: 544.2530; Found: 544.2533.

5-acetyl-6-methyl-4-(4-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-3,4-dihidropirimidin-2-(1H)-one (8f): Yield 71%;

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pale green solid; m.p. 92ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.14 (br s, 1H); 8.14 (s, 1H); 7.76 (br s, 1H); 7.17 (d, 2H, J= 8.6 Hz); 6.98 (d, 2H, J= 8.8 Hz); 5.65 (br s, 1H); 5.21 (d, 1H, J= 3.3 Hz); 5.11 (s, 2H); 4.90 (s, 2H); 4.69 (s, 2H); 2.28 (s, 3H); 2.14-2.03 (m, 5H); 1.94-1.84 (m, 3H); 1.75-1.72 (m, 1H); 1.69 (s, 3H); 1.41-1.31 (m, 1H); ^{13}C NMR (75 MHz, DMSO-*d6*): δ 194.3; 157.3; 152.0; 148.9; 147.8; 142.7; 136.7; 132.5; 127.6; 125.4; 124.5; 114.6; 109.6; 109.0; 61.1; 55.0; 53.3; 40.0; 30.2; 29.8; 26.7; 25.9; 20.5; 18.8; IR (ν_{max} cm^{-1}): 3242, 3112, 2922, 1699, 1609, 1509, 1429, 1227, 1168, 1006, 781; HRMS calc. for [$C_{26}H_{31}N_5O_3$+Na]: 484.2319; Found: 484.2317.

5-acetyl-6-methyl-4-(3-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-3,4-dihidropirimidin-2-(1H)-one (8g): Yield 72%; white solid; m.p. 145ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9.17 (br s, 1H); 8.14 (s, 1H); 7.81 (br s, 1H); 7.25 (t, 1H, *J*= 7.8 Hz); 6.96 (dd, 1H, *J*= 8.1 and 2.3 Hz); 6.87-6.84 (m, 2H); 5,66 (br s, 1H); 5.24 (d, 1H, *J*= 3.5 Hz); 5.11 (s, 2H); 4.90 (s, 2H); 4.69 (s, 2H); 2.29 (s, 3H); 2.14-2.04 (m, 5H); 1.95-1.88 (m, 3H); 1.76-1.69 (m, 4H); 1.41-1.31 (m, 1H); ^{13}C NMR (75 MHz, DMSO-*d6*): δ 194.2; 158.1; 152.1; 148.9; 148.2; 145.8; 142.6; 132.4; 129.6; 125.4; 124.6; 118.9; 113.4; 113.0; 109.4; 109.0; 61.0; 55.0; 53.6; 40.0; 30.2; 29.8; 26.7; 26.0; 20.5; 18.9; IR (ν_{max} cm^{-1}): 3270, 3110, 2928, 1690, 1599, 1446, 1240, 1013, 806, 776; HRMS calc. for [$C_{26}H_{31}N_5O_3$+Na]: 484.2319; Found: 484.2310.

5-acetyl-6-methyl-4-(2-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-3,4-dihidropirimidin-2-(1H)-one (8h): Yield 68%; white solid; m.p. 139ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9.13 (br s, 1H); 8.17 (s, 1H); 7.27-7.25 (m, 2H); 7.20 (dd, 1H, *J*= 8.3 and 1.1 Hz); 7.07 (dd, 1H, *J*= 7.6 e 1.8 Hz); 6.92 (td, 1H, *J*= 7.3 e 1.0 Hz); 5.66 (br s, 1H); 5.60 (d, 1H, *J*= 3.3 Hz); 5.29 (d, 1H, *J*= 12.6 Hz); 5.24 (d, 1H, *J*= 12.6 Hz); 4.91 (s, 2H); 4.69 (s, 2H); 2.28 (s, 3H); 2.13-2.03 (m, 2H); 1.96-1.87 (m, 6H); 1.74-1.68 (m, 4H); 1.40-1.30 (m, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 194.5; 154.8; 152.1; 148.9; 148.2; 143.1; 132.5; 131.7; 128.8; 127.0; 125.4; 124.3; 121.0; 113.0; 109.0; 107.7; 61.8; 55.1; 48.6; 40.0; 29.8; 29.6; 26.7; 25.9; 20.5; 18.6; IR (ν_{max} cm^{-1}): 3413, 3207, 3080, 2934, 1685, 1636, 1597, 1430, 1232, 764, 583; HRMS calc. for [$C_{26}H_{31}N_5O_3$+Na]: 484.2319; Found: 484,2320.

5-acetyl-6-methyl-4-(4-methoxy-3-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-3,4-dihidropirimidin-2-(1H)-one (8i): Yield 76%; yellow solid; m.p. 154ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9.14 (br s, 1H); 8.09 (s, 1H); 7.75 (br s, 1H); 7.04 (d, 1H, *J*= 1.6 Hz); 6.90 (d, 2H, *J*= 8.3 Hz); 6.76 (dd, 1H, *J*= 8.3 and 1.6 Hz); 5.66 (br s, 1H); 5.20 (d, 1H, *J*= 3.0 Hz); 5.08 (s, 2H); 4.90 (s, 2H); 4.69 (s, 2H); 3.71 (s, 1H); 2.29 (s, 3H); 2.13-2.04 (m, 5H); 1.94-1.88 (m, 3H); 1.75-1.69 (m, 4H); 1.41-1.31 (m, 1H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 194.5; 152.2; 149.0; 148.5; 148.1; 147.2; 142.6; 136.6; 132.5; 125.5; 124.8; 119.0; 112.8; 112.1; 109.3; 109.1; 61.8; 55.6; 55.1; 53.6; 40.0; 30.2; 29.9; 26.8; 26.0; 20.6; 18.9; IR (ν_{max} cm-1): 3232, 3125, 2929, 1695, 1598, 1510, 1422, 1223, 1128, 757; HRMS calc. for [$C_{27}H_{33}N_5O_4$+Na]: 514.2425; Found: 514.2422.

5-acetyl-6-methyl-4-(3-methoxy-4-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-3,4-dihidropirimidin-2-(1H)-one (8j): Yield 67%; pale green solid; m.p. 93ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9.15 (br s, 1H); 8.13 (s, 1H); 7.77 (br s, 1H); 7.07 (d, 1H, *J*= 8.3 Hz); 6.93 (d, 2H, *J*= 2.0 Hz); 6.71 (dd, 1H, *J*= 8.3 and 2.0 Hz); 5.67 (br s, 1H); 5.23 (d, 1H, J= 3.3 Hz); 5.09 (s, 2H); 4.91 (s, 2H); 4.70 (s, 2H); 3.73 (s, 1H); 2.30 (s, 3H); 2.14-2.05 (m, 5H); 1.95-1.89 (m, 3H); 1.76-1.70 (m, 4H); 1.42-1.32 (m, 1H); ^{13}C NMR (75 MHz, DMSO-*d6*): δ 194.4; 152.1; 149.0; 148.9; 148.0; 146.8; 142.8; 137.2; 132.5; 125.4; 124.6; 118.0; 113.7; 111.0; 109.2; 109.0; 61.8; 55.4; 55.0; 53.6; 40.0; 30.1; 29.8; 26.7; 26.0; 20.6; 18.8; IR (ν_{max} cm-1): 3232, 3134, 2929, 1701, 1600, 1514, 1417, 1226, 1131, 754; HRMS calc. for [$C_{27}H_{33}N_5O_4$+Na]: 514.2425; Found: 514.2431.

7,7-dimethyl-4-(4-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-4,6,7,8-tetrahydroquinazoline-2,5(1H,3H)-dione (8k): Yield 68%; white solid; m.p. 223ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.42 (br s, 1H); 8.13 (s, 1H); 7.70 (br s, 1H); 7.15 (d, 2H, *J*= 8.6 Hz); 6.96 (d, 2H, *J*= 8.6 Hz); 5.65 (s, 1H); 5.09 (br s, 3H); 4.89 (s, 2H); 4.69 (s, 2H); 2.40 (d, 1H, *J*= 17.4 Hz); 2.27 (d, 1H, *J*= 17.1 Hz); 2.20-2.00 (m, 4H); 1.94-1.88 (m, 3H); 1.76-1.69 (m, 4H); 1.39-1.33 (m, 1H); 1.01 (s, 3H); 0.90 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d6*): δ 192.8; 157.1; 152.1; 151.9; 148.9; 142.7; 137.3; 132.5; 127.3; 125.4; 124.5; 114.4; 109.1; 107.6; 61.1; 55.0; 51.3; 49.8; 40.0; 32.3 (x2); 29.8; 28.8; 26.9; 26.7; 26.0; 20.6; IR (ν_{max} cm^{-1}): 3310, 3099, 2938, 1681, 1641, 1606, 1453, 1372, 1244, 1044, 818, 778, 557; HRMS calc. for [$C_{29}H_{35}N_5O_3$+H]: 502.2813; Found: 502.2813.

7,7-dimethyl-4-(3-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-4,6,7,8-tetrahydroquinazoline-2,5(1H,3H)-dione (8l): Yield 84%; white solid; m.p. 192ºC; NMR 1H (400 MHz, DMSO-*d6*): δ 9.45 (br s, 1H); 8.14 (s, 1H); 7.75 (br s, 1H); 7.23 (t, 1H, *J*= 8.1 Hz); 6.93 (dd, 2H, *J*= 8.1 and 2.0 Hz); 6.85-6.82 (m, 2H); 5.66 (s, 1H); 5.14 (d, 1H, *J*= 2.8 Hz); 5.09 (s, 2H); 4.90 (s, 2H); 4.70 (s, 2H); 2.40 (d, 1H, *J*= 17.1 Hz); 2.30 (d, 1H, *J*= 17.4 Hz); 2.20-2.04 (m, 4H); 1.95-1.85 (m, 3H); 1.76-1.72 (m, 1H); 1.69 (s, 3H); 1.41-1.31 (m, 1H); 1.01 (s, 3H); 0.90 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 192.9; 158.0; 152.5; 151.9; 148.9; 146.1; 142.6; 132.5; 129.4; 125.4; 124.6; 118.7; 113.2; 112.8; 109.0; 107.2; 61.0; 55.0; 51.7; 49.8; 40.0; 32.3 (x2); 29.8; 28.6; 27.0; 26.7; 26.0; 20.6; IR (ν_{max} cm^{-1}): 3251, 2911, 1692, 1610, 1378, 1234, 1009, 764; HRMS calc. for [$C_{29}H_{35}N_5O_3$+H]: 502.2813; Found: 502.2816.

7,7-dimethyl-4-(2-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-4,6,7,8-tetrahydroquinazoline-2,5(1H,3H)-dione (8m): Yield 80%; white solid; m.p. 198ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.34 (br s, 1H); 8.14 (s, 1H); 7.22-7.14 (m, 3H); 7.04 (dd, 1H, *J*= 7.6 e 1.5 Hz); 6.88 (t, 1H, J= 7.3 Hz); 5.67 (br s, 1H); 5.48 (d, 1H, J= 2.3 Hz); 5.24 (d, 1H, *J*= 12.3 Hz); 5.20 (d, 1H, *J*= 12.6 Hz); 4.91 (s, 2H); 4.69 (s, 2H); 2.38 (d, 1H, *J*= 17.4 Hz); 2.32 (d, 1H, *J*= 17.1 Hz); 2.16-1.84 (m, 7H); 1.74-1.68 (m, 4H); 1.41-1.30 (m, 1H); 1.02 (s, 3H); 0.98 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 192.6; 155.3; 153.0; 151.7; 148.9; 143.3; 132.4; 132.0; 128.5; 127.5; 125.4; 124.2; 120.7; 113.0; 109.0; 105.6; 62.0; 55.1; 49.9; 47.5; 40.0; 39.2; 32.3; 29.8; 28.5; 27.4; 26.7; 25.9; 20.6; IR (ν_{max} cm^{-1}): 3355, 3225, 2913, 1695, 1645, 1459, 1371, 1240, 1054, 796, 747, 558; HRMS calc. for [$C_{29}H_{35}N_5O_3$+Na]: 524.2632; Found: 524.2626.

7,7-dimethyl-4-(4-methoxy-3-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-4,6,7,8-tetrahydroquinazoline-2,5(1H,3H)-dione (8n): Yield 79%; white solid; m.p. 200ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ9.41 (br s, 1H); 8.11 (s, 1H); 7.70 (br s, 1H); 7.00 (d, 1H, *J*= 1.8 Hz); 6.90 (d, 1H, *J*= 8.3 Hz); 6.76 (dd, 1H, *J*= 8.3 and 1.8 Hz); 5.67 (br s, 1H); 5.11 (d, 1H, *J*= 2.8 Hz); 5.06 (d, 1H, *J*= 11.8 Hz); 5.01

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(d, 1H, *J*= 14.9 Hz); 4.90 (s, 2H); 4.70 (s, 2H); 3.70 (s, 3H); 2.40 (d, 1H, *J*= 17.4 Hz); 2.32 (d, 1H, *J*= 17.1 Hz); 2.21-2.04 (m, 4H); 1.94-1.90 (m, 3H); 1.76-1.69 (m, 4H); 1.42-1.31 (m, 1H); 1.02 (s, 3H); 0.94 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d6*): δ 193.0; 152.4; 151.9; 148.9; 148.3; 147.0; 142.5; 137.0; 132.5; 125.5; 124.7; 118.8; 112.5; 111.9; 109.0; 107.3; 61.8; 55.5; 55.0; 51.4; 49.9; 40.0; 39.1; 32.3; 29.8; 28.6; 27.1; 26.7; 26.0; 20.6; IR (ν_{max} cm^{-1}): 3265, 3138, 2933, 1692, 1642, 161, 1442, 1374, 1246, 1129, 1002, 770; HRMS calc. for [$C_{30}H_{37}N_5O_4$+H]: 554.2738; Found: 554.2735.

7,7-dimethyl-4-(3-methoxy-4-((1-perillyl-methyl)-1H-1,2,3-triazol-4-yl)-methoxyphenyl)-4,6,7,8-tetrahydroquinazoline-2,5(1H,3H)-dione (8o): Yield 71%; peach solid; m.p. 188ºC; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.44 (br s, 1H); 8.12 (s, 1H); 7.73 (br s, 1H); 7.05 (d, 1H, *J*= 8.3 Hz); 6.85 (d, 1H, *J*= 1.8 Hz); 6.71 (dd, 1H, *J*= 8.3 e 1.8 Hz); 5.66 (br s, 1H); 5.10-5.06 (m, 3H); 4.90 (s, 2H); 4.69 (s, 2H); 3.68 (s, 3H); 2.42 (d, 1H, *J*= 17.1 Hz); 2.27 (d, 1H, *J*= 17.4 Hz); 2.21 (d, 1H, *J*= 16.1 Hz); 2.14-2.03 (m, 3H); 1.94-1.88 (m, 3H); 1.75-1.69 (m, 4H); 1.41-1.30 (m, 1H); 1.02 (s, 3H); 0.93 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ 193.0; 152.4; 152.0; 148.9; 148.8; 146.6; 142.8; 137.7; 132.5; 125.4; 124.6; 117.9; 113.4; 110.5; 109.1; 107.4; 61.7; 55.4; 55.0; 51.4; 49.9; 40.0; 32.3 (x2); 29.8; 28.9; 26.8; 26.7; 26.0; 20.6; IR (ν_{max} cm^{-1}): 3229, 3102, 2936, 1678, 1630, 1517, 1449, 1373, 1246, 1138, 1020, 603; HRMS calc. for [$C_{30}H_{37}N_5O_4$+H]: 554.2738; Found: 554.2737.

Biological Assays

In vitro antiproliferative assay

The antiproliferative activity of EERP was evaluated in vitro against nine different human cancer cell lines (U251 (glioma), UACC-62 (melanoma), MCF-7 (breast), NCI-ADR/RES (multidrug-resistant ovary carcinoma), OVCAR-3 (ovary), 786-0 (renal), NCI-H460 (non-small cell lung cancer), PC-3 (prostate), and HT-29 (colon), kindly provided by Frederick Cancer Research Development Center, National Cancer Institute, Frederick, MA, USA. The antiproliferative activity of EERP was also evaluated in vitro against spontaneously transformed keratinocytes from histologically normal skin (HaCaT cells) provided by Dr. Ricardo Della Coletta (University of Campinas). Doxorubicin was employed as the positive control.

Cells in 96-well plates (100 µL cells/well) were exposed to EERP at 0.25, 2.5, 25 and 250 µg/mL in DMSO/RPMI at 37°C, 5% of CO2 in air for 48 h. Doxorubicin was used as standard (0.025, 0.25, 2.5 and 25 µg/mL). Final DMSO concentration (0.2%) did not affect cell viability. Before (T0 plate) and after (T1 plates) sample addition, cells were fixed with 50% trichloroacetic acid and cell proliferation was determined by spectrophotometric quantification (540 nm) of cellular protein content using sulforhodamine B assay. The TGI (concentration that produces total growth inhibition or cytostatic effect) were determined through non-linear regression analysis using the concentration-response curve for each cell line in ORIGIN 8.0® (OriginLab Corporation).

Conclusions

The synthesis of novel perillyl-dihidropyrimidinone hybrid molecules was accomplished through the multicomponent Biginelli reaction combined the Huisgen triazole ring formation reaction in reasonable to good yields. A series of four compounds showed promising antitumoral activity against UACC-62, U251 and OVCAR-3, with TGI values lower than 10 µM (**8a**, **8j**, **8k**, **8n**) while a series of seven compounds showed antiproliferative activity against UACC-62, U251 and OVCAR-3, with TGI values between 10-20 µM (**8a**, **8b**, **8d**, **8e**, **8f**, **8g**, **8m**). Additionally, four compounds showed high TGI value for non-tumoral cell line HaCaT with high TGI values between 436.18-507.82 µM. These preliminary results are promising and indicate the need for subsequent studies to determine the mechanism of action, which are under current investigation in our laboratory.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 H. Y. Zhang, *FEBS Lett.*, 2005, **579**, 5260.
2 G. M. J. Maggiora, *Comput. Aided Mol. Des.*, 2011, **25**, 699.
3 Y. Bansal, O.Silakari, *Eur. J. Med. Chem.*, 2014, **76**, 31.
4 M. B. H. Youdim, J. J. Buccafusco, *Trends Pharmacol. Sci.*, 2005, **26**, 27.
5 C. T. Keith, A. A. Borisy, B. R. Stockwell, *Nat. Rev. Drug Discov.* 2005, **4**, 71.; J. L. Medina-Franco, M. A. Giulianotti, G. S. Welmaker, R. A. Houghten, *Drug Discov. Today*, 2013, **18**, 495.
6 R. Morphy, Z. J. Rankovic, *J. Med. Chem.,* 2005, **48**, 6523.
7 T. Karoli, S. K. Mamidyala, J. Zuegg, S. R. Fry, E. H. L. Tee, T. A. Bradford, P. K. Madala, J. X. Huang, S. Ramu, M. S. Butler, M. A. Cooper, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 2428; F. W. Muregi, A. Ishih, *Drug Dev. Res.*, 2010, **71**, 20.
8 C. Liu, X. Gu, Y. Z. Zhu, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6942
9 M. I. Fernandez-Bachiller, C. Perez, G. C. Gonzalez-Munoz, S. Conde, M. G. Lopez, M. Villarroya, A. G. Garcıa, M. I. J. Rodrıguez-Franco, *J. Med. Chem.,* 2010, **53**, 4927.
10 R. Pi, X. Mao, X. Chao, Z. Cheng, M.; Liu, M. Duan, M. Ye, M. Chen, Z. Mei, P. Liu, W. Li, Y. Han, *PLoS ONE*, 2012, **7**, 31921; X. Chao, X. He, Y. Yang, X. Zhou, M. Jin, S. Liu, Z. Cheng, P. Liu, P. Wang, L. Yu, Y. Tan, Y. Huang, J. Qin, S. Rapposelli, R. Pi, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 6498.
11 S. Fortin, G. Beérubé, *Expert Opin. Drug Discov.*, 2013, **8**, 1029.
12 P. Singh, R. Raj, V. Kumar, M. P. Mahajan, P. M. S. Bedi, T. Kaur, A. K. Saxena, *Eur. J. Med. Chem.*, 2012, **47**, 594.

View Article Online
DOI: 10.1039/C8MD00270C

13 A. N. Chiang, J-C. Valderramos, R. Balachandran, R. J. Chovatiya, B. P. Mead, C. Schneider, S. L. Bell, M. G. Klein, D. N. Huryn, X. S. Chen, B. W. Day, D. A. Fidock, P. Wipf, J. L. Brodsky, *Bioorg. Med. Chem.*, 2009, **17**, 1527.

14 K. V. Sashidhara, L. R. Singh, M. Shameem, S. Shakya, A. Kumar, T. S. Laxman, S. Krishna, M. I. Siddiqi, R. S. Bhattac, D. Banerjee, *Med. Chem. Commun.*, 2016, **7**, 2349.

15 For recent Reviews see: B. Mohammadi, F. K. Behbahani, *Mol. Divers.*, 2018, **22**, 405; L. H. S. Matos, F. T. Masson, L. A. Simeoni, M. Homem-de-Mello, *Eur. J. Med. Chem.,* 2018, **143**, 1779; R. Kaur, S. Chaudhary, K. Kumar, M. K. Gupta, R. K. Rawal, *Eur. J. Med. Chem.*, 2017, **132**, 108; A. de Fatima, T. C. Braga, L. S. Neto, B. S. Terra, B. G. F. Oliveira, D. L. Silva, L. V. J. Modolo, *RSC Adv.*, 2015, **6**, 363.

16 T. U. Mayer, T. M. Kapoor, S. J. Haggarty, R. W. King, S. L. Schreiber, T. J. Mitchison, *Science* 1999, **286**, 971.

17 M. Gartner, N. Sunder-Plassmann, J. Seiler, M. Utz, I. Vernos, T. Surrey, A. Giannis, *ChemBioChem*, 2005, **6**, 1173.

18 D. Russowsky, R. F. S. Canto, S. A. A. Sanches, M. G. M. D'Oca, A. de Fatima, R. A. Pilli, L. K. Kohn, M. A. Antonio, J. E. de Carvalho, *Bioorg. Chem.*, 2006, **34**, 173.

19 V. Sarli, A. Giannis, *Clin. Cancer Res.*, 2008, **14**, 7583; B. C. Guido, L. M. Ramos, D. O. Nolasco, C. C. Nobrega, B. Y. G. Andrade, A. Pic-Taylor, B. A. D. Neto, J. R. Corrêa, *BMC Cancer* 2015, **15**, 283.

20 T. C. Chen, C. O. Da Fonseca, A. H. Schönthal, *Am. J. Cancer Res.*, 2015, **5**, 1580.

21 S. Sarkar, B. Azab, B. A. Quinn, X. Shen, P. Dent, A. L. Klibanov, L. Emdad, S. K. Das, D. Sarkar, P. B. Fisher, *Curr. Mol. Med.*, 2014, **14**, 125.

22 S. Sultana, S. Nafees, A. Q. Khan, *Hum. Exp. Toxicol.* 2013, **32**, 1179.

23 I. V. Lebedeva, Z-Z. Su, N. Vozhilla, J. Chatman, D. Sarkar, P. Dent, M. Athar, P. B. Fisher, *Cancer Res.,* 2008, **68**, 7439.

24 S. Bardona, V. Foussard, S. Fournel, A. Loubat, *Cancer Lett.*, 2002, **181**, 187.

25 M. B. Sahin, S. M. Perman, G. Jenkins, S. S. Clark, *Leukemia* 1999, **13**, 1581.

26 H-Y. Cho, W. Wang, N.; Jhaveri, S. Torres, J. Tseng, M. N. Leong, D. J. Lee, A. Goldkorn, T. Xu, N. A. Petasis, S. G. Louie, A. H. Schonthal, F. M. Hofman, T. C. Chen, *Mol. Cancer Ther.*, 2012, **11**, 2462; J. Fernandes, C. O. Da Fonseca, A. Teixeira, C. R. Gattass, *Oncol. Rep.*, 2005, **13**, 943; S. Afshordel, B. Kern, J. Clasohm, H. König, M. Priester, J. Weissenberger, D. Kögel, G. P. Eckerta, *Pharmacol. Res.*, 2015, **91**, 69.

27 C. O. Da Fonseca, R. M. Teixeira, J. C. T. Silva, J. S. G. Fischer, O. C. Meirelles, J. A. Landeiro, T. Quirico-Santos *Anticancer Res.*, 2013, **33**, 5625; C. O. Da Fonseca, R. Linden, D. Futuro, C. R. Gattass, T. Quirico–Santos, *Arch. Immunol. Ther. Exp.*, 2008, **56**, 267; C. O. Da Fonseca, I. P. Soares, D. S. Clemençon, S. Rochlin, L. Cardeman, T. Quirico-Santos, *J. Histol. Histopathol.*, 2015, **2**, 12. (http://dx.doi.org/10.7243/2055-091X-2-12).

28 Z. Hui, M. Zhang, L. Cong, M. Xia, J. Dong, *Molecules* 2014, **19**, 6671; N. S. Nandurkar, J. Zhang, Q. Ye, L. V. Ponomareva, Q-B. She, J. S. Thorson, *J. Med. Chem.*, 2014, **57**, 7478; T. C. Chen, J. Yu, E. N. Nigjeh, W. Wang, P. T. Myint, E. Zandi, F. M. Hofman, A. H. Schönthal, *Cancer Lett.*, 2017, **400**, 161; B. C. Das, S. M. Mahalingam, L. Panda, B. Wang, P. D. Campbell, T. Evans, *Tetrahedron Lett.*, 2010, **51**, 1462; T. C. Chen, , C. O. Da Fonseca, A. H. Schönthal, *Int. J. Mol. Sci.*, 2016, **17**, 1463

29 D. Dheer, V. Singh. R. Shankar, *Bioorg. Chem.*, 2017, **71**, 30.

30 D. Russowsky, F. A. Lopes, V. S. S. Silva, K. F. S. Canto, M. G. M. D'Oca, M. N.Godoi, *J. Bras. Chem Soc.*, 2004, **15**, 165; b) V. P. de Souza, V. Vendrusculo, A. M. Moraás, L. Steffens, F. S. Santos, D. J. Moura, F. S. Rodembusch, D. Russowsky, *New J. Chem.*, 2017, **41**, 15305; G. C. O. Silva, J. R. Correa, M. O. Rodrigues, H. G. O. Alvim, B. C. Guido, C. C. Gatto, K. A. Wanderley, M. Fioramonte, F. C. Gozzo, R. O. M. A. de Souza, B. A. D. Neto, *RSC Adv.*, 2015, **5**, 48506.

31 J. B. Giguère, D. Thibeault, F. Cronier, J. S. Marois, M. Auger, J. F. Morin, *Tetrahedron Lett.*, 2009, **50**, 5497.

32 V. Moro, P. C. Ferreira, P. Migowski, F. S. Rodembusch, J. Dupont, D. S. Lüdtke, *Tetrahedron*, 2013, **69**, 201.

33 R. Appel, *Angew. Chem. Int. Ed. Engl.*, 1975, **14**, 801.

34 D. W. Zaharevitz, S. L. Holbeck, C. Bowerman, P. A. Svetlik, *J. Mol. Graph. Model.*, 2002, **20**, 297.

35 R. F. S. Canto, A. Bernardi, A. M. O. Battastini, D. Russowsky, V. L. Eifler-Lima, *J. Braz. Chem. Soc.*, 2011, **22**, 1379; C. S. Stuepp, F. Figueiró, F. B. Mendes, E. Braganhol, A. Bernardi, R. L. Frozza, C. G. Salbego, R. F. S. Canto, D. Russowsky, V. L. Eifler-Lima, A. M. O. Battastini, *Anticancer Res.*, 2013, **33**, 4463.

36 F. Figueiró, F. B. Mendes, P. F. Corbelini, F. Janarelli, E. H. F. Jandrey, D. Russowsky, V. L. Eifler-Lima, A. M. O. Battastini, *Anticancer Res.*, 2014, **34**, 1837.

37 H. Zimmermann, M. Zebisch, N. Strater, *Purinergic Signal.*, 2012, **8**, 437.

38 T. G. M. Treptow, F. Figueiró, E. H.F. Jandrey, A. M.O. Battastini, C. G. Salbego, J. B. Hoppe, P. S. Taborda, S. B. Rosa, L. A. Piovesan, C. R. M. D'Oca, D. Russowsky, M. G. M. D'Oca, *Eur. J. Med. Chem.*, 2015, **95**, 552.

39 D. C. Cabrera, S. B. Rosa, F. S. Oliveira, M. A. G. Marinho, C. R. M. D'Oca, D. Russowsky, A. P. Horn, M. G. M. D'Oca, *Med. Chem. Commun.*, 2016, **7**, 2167.

40 R. H. Hans, E. M. Guantai, C. Lategan, P. J. Smith, B. Wan, S. G. Franzblau, J. Gut, P. J. Rosenthal, K. Chibale, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 942.

41 S. C. Zammit, A. J. Cox, R. M. Gow, Y. Zhang, R. E. Gilbert, H. Krum, D. J. Kelly, S. J. Williams, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 7003.

42 R. González-Olvera, V. Román-Rodríguez, G. Negrón-Silva, A. Espinoza-Vázquez, F. Rodríguez-Gómez, R. Santillan, *Molecules*, 2016, **21**, 250.

View Article Online
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