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Synthesis of Novel 1,2,3-Triazole-Dihydropyrimidinone Hybrids Using Multicomponent 1,3-Dipolar Cycloaddition (Click)–Biginelli Reactions: Anticancer Activity

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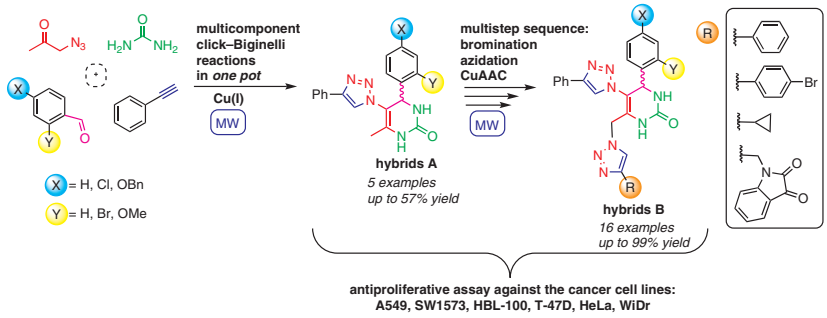

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Abstract In this work, 21 novel (1,4-disubstituted 1,2,3-triazole)-dihydropyrimidinone (1,2,3-trzl-DHPM) type hybrids were synthesized and characterized. These were divided into two types: hybrids A (5 in total) containing the dihydropyrimidinone heterocyclic ring decorated with a 1,4-disubstituted 1,2,3-triazole in the C-5 position [these compounds were accessed by a multicomponent copper(I)-catalyzed azide alkyne cycloaddition (CuAAC) (or click)–Biginelli reactions with satisfactory yields (39–57%)] and hybrids B (16 in total) containing two 1,2,3-triazole units in the C-5 and C-6 methyl position of the DHPM. Hybrids B were synthesized *via* functionalization of the C-6 methyl group of hybrids A, a multistep sequence of reactions was used that included bromination, azidation, and a CuAAC. Hybrids B were obtained in very good to excellent yields (up to 99%). Some hybrids A and B were evaluated for their antiproliferative activity against different cancer cell lines that included A549 and SW1573 (non-small-cell lung), HBL-100 and T-47D (breast), HeLa (cervix) and WiDr (colon). Three of these hybrids were potent cell proliferation inhibitors of non-small-cell lung cancer, cervix cancer, breast cancer, and colon cancer.

Key words hybrids, 1,2,3-triazole, dihydropyrimidinone, click reaction, Biginelli reaction, one-pot, cancer, antiproliferative

Dihydropyrimidinone (DHPM) and 1,2,3-triazole (1,2,3-trzl) moieties are important pharmacophores in medicinal chemistry. There currently exists an enormous number of compounds containing these heterocycles which exhibit diverse and potent biological activities, such as antitumor, antiviral, antifungal, anti-inflammatory, among others.[1,2] The well-known drug monastrol provided the first impulse for the development of a wide diversity of DHPM derivatives. Monastrol is an antimitotic inhibitor, that specifically inhibits Eg5 kinesin.[3] Cefatrizine is a β-lactum antibiotic containing the 1,2,3-triazole unit.[4]

The combination of these two distinct heterocyclic rings in a single molecule can allow the drug to act on multiple targets simultaneously. The development of hybrids containing a DHPM or a 1,2,3-triazole ring gained interest over the last number of years due to their structural characteristics. The 1,2,3-triazole ring is very robust, it is also resistant to hydrolysis, oxidation and reductions, it can act as both a hydrogen-bond donor and an acceptor, be involved in π–π-stacking interactions, and as bioisosteres of some important functional groups such as amides and esters.[5] Hybrids containing a DHPM or 1,2,3-triazole ring have made an important contribution in drug discovery for cancer treatment.[1,2,5]

Recently, there has been a growing interest in synthesizing new 1,2,3-triazole-dihydropyrimidinone hybrids for different applications, mainly in the field of medicinal chemistry.[6] There exists a variety of these hybrids in the literature where the dihydropyrimidinone has been decorated with one or two 1,2,3-triazole units in five out of the six possible positions of the ring, i.e., N1, N3, C4, C5, and C6.[6] In most hybrids the 1,2,3-triazole unit is connected to the DHPM ring by a spacer. The classical multicomponent Biginelli and CuAAC (click) reactions are important tools for obtaining these diversity-interesting hybrids. The Biginelli reaction is a very useful reaction. It is possible to obtain a wide diversity of compounds by using aromatic or aliphatic aldehydes, derivatives of urea or thioureas, and CH acidic carbonyl building blocks.[7] Copper-catalyzed alkyne–azide cycloaddition (CuAAC), which is considered possibly one of

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the best click reactions, was introduced simultaneously and independently by Sharpless and Meldal. This is a very important reaction for obtaining the 1,4-regioisomer of 1,2,3-triazole.[8]

The main synthetic strategy used in the literature for obtaining (1,4-disubstituted 1,2,3-triazole)-DHPM hybrids was to synthesize the dihydropyrimidinone by a multicomponent Biginelli reaction followed by the introduction of the 1,4-disubstituted 1,2,3-triazole unit using the CuAAC reaction.[6a–c,6e–j] A one-pot synthesis of 1,4-disubstituted 1,2,3-triazole-dihydropyrimidinone hybrids has been described in the literature. In this case, *O*-propargylbenzaldehydes were used as precursors upon which the 1,4-disubstituted 1,2,3-triazole unit could be introduced in the C-4 position of the DHPM.[6d] In this work, our objective was to synthesize two types of hybrid, the mono- and di(1,4-disubstituted 1,2,3-triazole)-DHPM hybrids **A** and **B** (Figure 1), respectively. These hybrids were designed based on their structural characteristics, polarity, rigidly, capacity to establish hydrogen bonds, and π–π stacking with a wide range of molecular targets. Hybrids **A1–5** contain the 1,4-disubstituted 1,2,3-triazole unit in the C-5 position of the DHMP ring and the other hybrids **B1–16** contain two 1,2,3-triazole units linked in the C-5 and C-6 methyl group positions of DHPM.

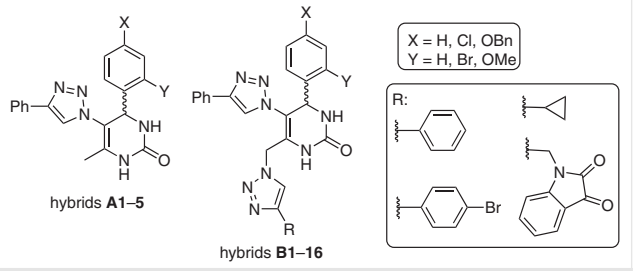


Figure 1 Hybrids **A** and **B**

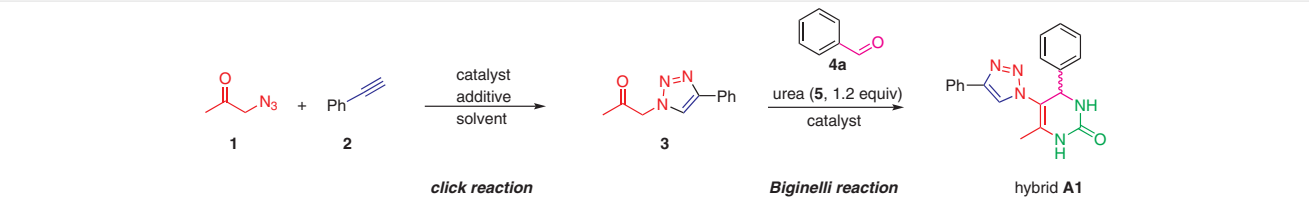
Novel hybrids **A** and **B** are potential anticancer therapeutic agents due their structural features. In this work they were evaluated for their anticancer activity, in a series of antiproliferative assays *in vitro* against six different cancer cell lines: A549 and SW1573 (non-small-cell lung), HBL-100 and T-47D (breast), HeLa (cervix) and WiDr (colon).

Our first objective was the synthesis of hybrids **A**, which was achieved by reacting the acidic carbonyl building block, 1-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propan-2-one (α-ketotriazole, **3**), with 1-azidopropan-2-one (**1**) and phenylacetylene (**2**). This was presumed to take place via a sequential CuAAC followed by a Biginelli reaction (Scheme 1). The precursor **3** was synthesized in excellent yields (99%), two different copper catalysts were used, CuI and $CuSO_4{\cdot}5H_2O$ (Table 1, entries 1–3). The click reactions carried out with CuI and DIPEA/AcOH[9] additives in dichloromethane (DCM) were performed in both conventional heating and microwave conditions.[10] The results obtained were very similar, but the reaction under microwave irradiation was superior in that the reaction was complete in only 10 min. The reaction with $CuSO_4{\cdot}5H_2O$ and L-ascorbic acid (reducing agent) in dimethylformamide (DMF) under microwave conditions also gave an excellent yield (98%) but with a longer reaction time. The multicomponent Biginelli reaction was performed with several catalysts, like Lewis acids and Brønsted acids, which included: TMSOTf, transition metal and rare-earth metal containing salts such as CuI, $CuSO_4{\cdot}5H_2O$, $FeCl_3{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$, $Yb(OTf)_3$, $CeCl_3$, and acetic acid. However, the cyclocondensation only worked with CuI and $CuSO_4{\cdot}5H_2O$. The yields were also low, and the best result obtained was with the CuI catalyst (Table 1, entries 4 and 5).

On the basis of these results we decided to conduct a sequential one-pot click–Biginelli process with all four components that included: 1-azidopropan-2-one (**1**), phenylacetylene (**2**), benzaldehyde (**4a**) and urea (**5**) catalyzed by CuI or $CuSO_4{\cdot}5H_2O$ (Scheme 2 and Table 2).

In the one-pot sequential approach, the click reaction of 1-azidopropan-2-one (**1**) and phenylacetylene (**2**) catalyzed by Cu(I) produced the 1-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propan-2-one (**3**, Scheme 2), this was followed by the Cu(I)-catalyzed Biginelli reaction of intermediate **3** with benzaldehyde (**4a**) and urea (**5**) to afford the desired product hybrid **A1**. Both CuI and $CuSO_4{\cdot}5H_2O$ were tested under different reaction conditions, both in batch and under microwave irradiation.

The best result was obtained using CuI under MW conditions (57% yield, Table 2, entry 1). Increasing the catalyst load of the conventional synthesis from 10 mol% to 15 mol% resulted in a decrease of the yields (Table 2, entries 4 and 6). Looking at the reactions under microwave conditions with 5 mol% and 10 mol% of catalyst (Table 2, entries 1 and 3), the yield decreased by half. In both cases the decrease of the yields with the increase of the catalyst load was probably due to the decomposition of the intermediate (*N*-acyl iminium **6**, Scheme 2), because α-ketotriazole **3** was recovered



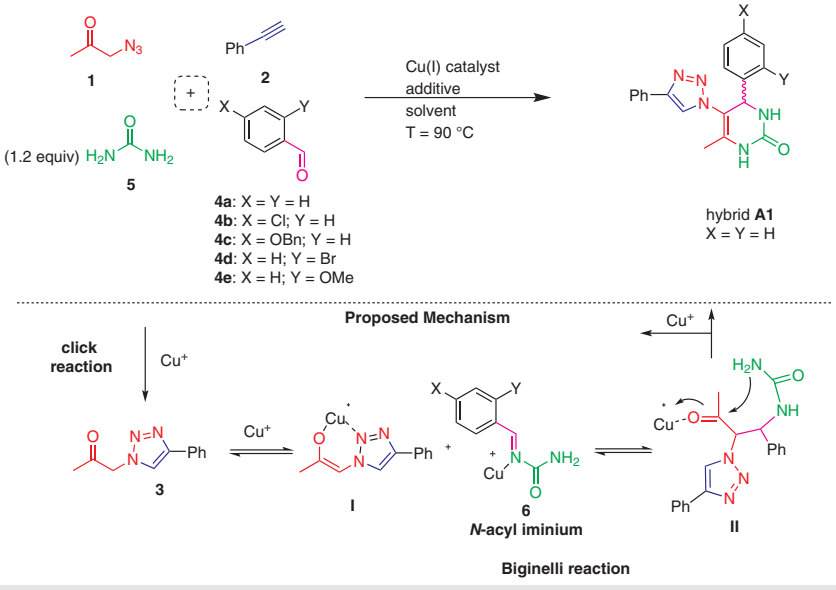
Scheme 1 First synthetic approach for obtaining hybrid **A1**

Table 1 Results for the Non-sequential CuAAC (Click) and Biginelli Reactions

Entry	Catalyst (mol%)	Additive (mol%)	Solvent	Conditions	Temp (°C)	Time	Yield (%)
Click Reaction – Product 1-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)propan-2-one (**3**)							
1	CuI (10)	DIPEA/AcOH	MeCN	conventional	Rt	24 h	98
2	CuI (10)	DIPEA/AcOH	MeCN	MW	90	10 min	99
3	$CuSO_4 \cdot 5H_2O$ (5)	L-ascorbic acid (20)	DMF	MW	90	25 min	98
Biginelli Reaction – Product Hybrid **A1**							
4	CuI (10)	–	MeCN	conventional	85	4 d	49
5	CuI (5)	–	MeCN	MW	90	24 h	50
6	$CuSO_4 \cdot 5H_2O$ (5)	L-ascorbic acid (20)	DMF	MW	90	24 h	15
7	AcOH	–	AcOH	conventional	100	4 d	0
8	TMSOTf (10)	–	MeCN	conventional	90	20 h	0
9	$FeCl_3 \cdot 6H_2O$ (5)	HCl (5)	EtOH	conventional	90	4 d	0
10	$Yb(OTf)_3$ (5)	HCl (5)	EtOH	conventional	90	4 d	0
11	$NiCl_2 \cdot 6H_2O$ (5)	HCl (5)	EtOH	conventional	90	4 d	0
12	$CeCl_3$ (5)	HCl (5)	EtOH	conventional	90	4 d	0

at the end of the reaction. A number of solvents were also screened, but we found that the sequence only works in acetonitrile (MeCN), ethanol, or without a solvent (Table 2, entries 7 and 9). In an attempt to improve the yield of the reaction, a catalytic amount of Fe(0) was added to the reaction with CuI. The objective was to use Fe(0) as a reducing agent and to keep the Cu(I) species active. Unfortunately, the yields obtained were lower, only 35% (under microwave conditions) and 42% (under conventional conditions; Table 2, entries 13 and 14). The reaction mechanism proposed for the Cu(I)-catalyzed click–Biginelli reaction sequence is depicted in Scheme 2. In the case of the click reaction, this should occur *via* the Huisgen 1,3-dipolar cycloaddition.[12] The Biginelli reaction is presumed to proceed through the *N*-acyl iminium **6** intermediate [formed *in situ* from the reaction of benzaldehyde (**4a**) and urea (**5**)] – which is assumed to be stabilized by the Cu(I) ion – which reacts with the α-ketotriazole enolate (**I**), derived from **3** via tautomerism to give **II** that suffers cyclization and dehydration to afford the hybrid **A1**.[13]

In order to test the scope of the reaction we used these optimized conditions for the synthesis of four other hybrids, using four benzaldehyde derivatives that included: *p*-chlorobenzaldehyde (**4b**), *p*-benzyloxybenzaldehyde (**4c**),

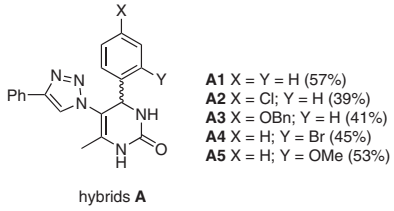


Scheme 2 One-pot synthesis of 1,2,3-triazole-dihydropyrimidinone hybrids **A1**

Table 2 Results for the Sequential CuAAC (Click) and Biginelli Reactions

Entry	Catalyst (mol%)	Additive (mol%)	Solvent	Conditions	Time	Yield (%)
1	CuI (5)	DIPEA (4), AcOH (4)	MeCN	MW	24 h	57[11]
2	$CuSO_4{\cdot}5H_2O$ (5)	L-ascorbic acid (20)	DMF	MW	24 h	15
3	CuI (10)	DIPEA (4), AcOH (4)	MeCN	MW	24 h	29
4	CuI (10)	DIPEA (4), AcOH (4)	MeCN	conventional	67 h	42
5	$CuSO_4{\cdot}5H_2O$ (10)	L-ascorbic acid (20)	DMF	conventional	5 d	0
6	CuI (15)	DIPEA (4), AcOH (4)	MeCN	conventional	4 d	25
7	CuI (10)	DIPEA (4), AcOH (4)	EtOH	conventional	4 d	16
8	CuI (10)	DIPEA (4), AcOH (4)	H_2O	conventional	4 d	0
9	CuI (10)	DIPEA (4), AcOH (4)	–	conventional	4 d	24
10	CuI (10)	DIPEA (4), AcOH (4)	toluene	conventional	4 d	0
11	CuI (10)	DIPEA (4), AcOH (4)	THF	conventional	4 d	0
12	CuI (5)	–	AcOH	conventional	6 d	0
13	CuI (5)	DIPEA (4), AcOH (4), Fe (5)	MeCN	MW	25 h	35
14	CuI (5)	DIPEA (4), AcOH (4), Fe (5)	MeCN	conventional	3 d	42

2-bromobenzaldehyde (**4d**), and 2-methoxybenzaldehyde (**4e**, Figure 2). Other benzaldehyde derivatives, such as: 4-nitrobenzaldehyde, 2,6-dichlorobenzaldehyde, 2-chloro-5-nitrobenzaldehyde, and 4-chloro-3-nitrobenzaldehyde were used, but unfortunately, they did not furnish the desired products. A putative explanation for the low yields obtained for the hybrids **A** is due to the low reactivity of the acidic carbonyl building block, α-ketotriazole **3**.



A1 X = Y = H (57%)
A2 X = Cl; Y = H (39%)
A3 X = OBn; Y = H (41%)
A4 X = H; Y = Br (45%)
A5 X = H; Y = OMe (53%)

Figure 2 Hybrids **A1–5**[11]

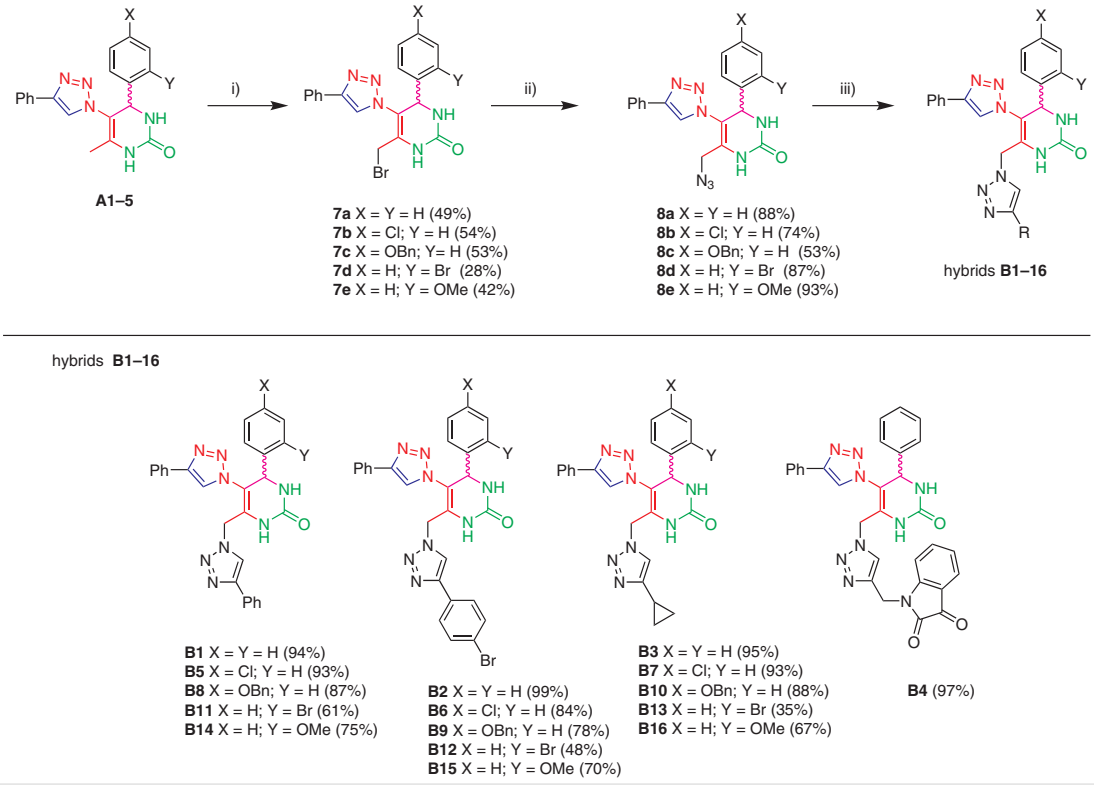
The chemical structures of hybrids **A** were characterized by ^{1}H NMR and ^{13}C APT NMR, all data are available in the Supporting Information. In the ^{1}H NMR spectrum of hybrid **A1**, it was possible to find the characteristic signals for the 1,2,3-triazole and the DHPM rings. The peaks with chemical shifts δ = 5.4, 8.5, and 8.9 ppm were attributed to the CH and NH from DHPM and the peak at δ = 7.7 ppm (singlet) is attributed to the CH from the 1,2,3-triazole ring. In the case of the ^{13}C APT NMR spectrum, it is possible to identify some characteristic carbons. In the case of the CHs of the 1,2,3-triazole and DHPM rings, the carbon signals appeared at δ = 123.9 and 58.7 ppm, respectively. For hybrid **A1** we have recorded a high resolution mass spectrum (HRMS) using the ESI-TOF method to determine the exact mass of $[M + H]^+$ of 410.0619 providing the molecular formula $C_{19}H_{16}BrN_5O$ (calcd 410.0610), which is in agreement with the identified structure.

The synthetic pathway to the hybrids **B1–16**, i.e. 5,6-di(1,4-disubstituted 1,2,3-triazole)dihydropyrimidinones, consisted in the functionalization of the C-6 methyl position of the DHPM ring of hybrids **A** through a sequential pathway consisting of: (i) bromination, (ii) azidation, and (iii) CuAAC (Scheme 3). The most challenging reaction was the bromination at the C-6 methyl position of the DHPM ring. In fact, this is the strategy that is mostly used in the literature for the functionalization of the DHPM.[6a,14]

In our case, the bromination reaction of the C-6 methyl group of DHPM was performed with several bromination reagents, such as Br_2, *N*-bromosuccinimide, and tetrabutylammonium tribromide ($TBABr_3$). Only the Br_2 (yields up to 20%) and $TBABr_3$ (yields up to 54%) reagents furnished the desired products **7a–e**.[15] The bromination reaction with $TBABr_3$ had several advantages compared to molecular bromine; which included better yields, easier handling, and better control of the exact quantity of bromine added.

In the second step, the azidation of 6-bromomethyl with NaN_3, the reaction was carried out under microwave irradiation, the azide intermediates **8a–e** were obtained with very good yields (up to 93% yield).[16] The last step was the formation of the 1,2,3-triazole *via* the click reaction. The click reaction was catalyzed by $CuSO_4{\cdot}5H_2O$ (with L-ascorbic acid as reductant) and involved the cycloaddition of **8a–e** with a variety of alkynes.

The reactions were performed under microwave irradiation using four different alkynes: phenylacetylene (**2**), cyclopropylacetylene (**9**), 4-bromophenylacetylene (**10**), and 1-(prop-2-yn-1-yl)isatine (**11**). The sixteen hybrids **B1–16** were obtained in very good to excellent yields (up to 99%).[17]



Scheme 3 Synthetic strategy for obtaining the hybrids **B1–16**. Reaction conditions: (i) $TBABr_3$ (1.5 equiv), dichloromethane, rt, 2 h; (ii) NaN_3 (1.5 equiv), DMF, 60 °C, microwaves, 30 min; (iii) alkyne (1 equiv), DMF, 90 °C microwaves, 10 min.[15–17]

All intermediates **7a–e**, **8a–e** and the hybrids **B1–16** were fully characterized by ^{1}H NMR, ^{13}C NMR (all spectra are available in the Supporting Information), and mass spectra. In relation to the intermediates **7a–e**, the ^{1}H NMR spectra are very similar to hybrids **A1–5**. The major difference was the disappearance of the signal corresponding to the C-6 methyl group at δ = 1.6 ppm, and the appearance of two doublets with δ = 3.91 and 4.02 ppm (J = 11 Hz), which corresponds to the 6-$BrCH_2$ substituent. In the case of the azide intermediates **8a–e**, the methylene group has the two doublets with δ = 3.82 and 3.91 ppm (J = 14 Hz), which were more shielded due to the effect of the azide group. In the structural characterization of the hybrid **B1** by ^{1}H NMR spectroscopy, the C-6 methylene protons suffer a deshielding effect by the 1,2,3-triazole ring. The two doublets appear with chemical shifts at δ = 5.01 and 5.14 ppm and cou-

Table 3 Antiproliferative Activity (GI_{50}) of Hybrid Compounds **A1–3**, **B1–4**, and **B7**

Compound	GI_{50} ± SD (μM)					
	Non-small-cell lung		Breast		Cervix	Colon
	A549	SW1573	HBL-100	T-47D	HeLa	WiDr
A1	27 ± 2.5[a]	17 ± 1.6[a]	32 ± 6.2[b]	30 ± 4.0[b]	25 ± 6.8[b]	36 ± 8.1[b]
A2	34 ± 3.7[b]	30 ± 11[b]	38 ± 3.6[b]	37 ± 6.7[b]	34 ± 6.8[b]	39 ± 6.3[b]
A3	18 ± 2.2[b]	14 ± 5.2[b]	24 ± 3.8[b]	25 ± 4.9[b]	17 ± 2.1[b]	25 ± 1[b]
B1	58 ± 18[b]	28 ± 7.1[b]	39 ± 6[b]	47 ± 7[b]	29 ± 3.7[b]	57 ± 19[a]
B2	26 ± 3.9[b]	30 ± 13[b]	52 ± 1.5[a]	37 ± 2.4[a]	32 ± 5.4[b]	38 ± 4.2[b]
B3	43 ± 2.6[b]	37 ± 0.76[a]	77 ± 4.7[b]	48 ± 0.59[a]	50 ± 5.6[b]	56 ± 2.8[a]
B4	>100[b]	32 ± 8.2[a]	50 ± 4.0[a]	>100[b]	35 ± 3.6[b]	>100[b]
B7	17 ± 1.1[b]	15 ± 6.3[b]	20 ± 2.3[b]	32 ± 4.4[b]	22 ± 1.8[b]	18 ± 1.5[b]

[a] GI_{50} values are means of two independent experiments.
[b] GI_{50} values are means of three independent experiments.

pling constants of 15 Hz. It seems that the C–H proton of DHMP – that couples with N–H – appears as a doublet with a coupling constant of 2 Hz. The characteristic protons of the 1,2,3-triazole rings appear at δ = 7.90 and 8.43 ppm.

For intermediates **7a**, **8a**, and hybrid **B1** we have recorded high-resolution mass spectra (HRMS) using the ESI-TOF method to determine the exact mass of $[M + H]^+$ of each (see ref. 15–17).

Eight of the novel hybrid **A** and **B** compounds described in this work were evaluated for antiproliferative activity *in vitro* against six different human cancer cell lines: A549 and SW1573 (non-small-cell lung), HBL-100 and T-47D (breast), HeLa (cervix), WiDr (colon), the results of GI_{50} (growth inhibition of 50%) are given in Table 3. Only three hybrids showed significant antiproliferative effect with GI_{50} values below 20 μM.

These included hybrids **A** (**A1–3** with one 1,2,3-triazole unit) where the best growth inhibitors were **A1** and **A3**. **A1** was limited to non-small-cell lung cancer – SW1573 with GI_{50} 17 μM and in the case of hybrid **A3** was shown to be active against non-small-cell lung cancer – A549 (GI_{50} = 18 μM), SW1573 (GI_{50} = 14 μM) and cervix cancer – HeLa (GI_{50} = 17 μM). Hybrid **A3** bearing a *p*-benzyloxyphenyl substituent in the C4-position of DHPM showed antiproliferative effects in various cancer cell lines.

In the case of hybrids **B** (**B1–4** and **B7** with two 1,2,3-triazole units), the best growth inhibitor was the hybrid **B7**, showing the best IG_{50} values for non-small-cell lung cancer – SW1573 (GI_{50} = 15 μM) and A549 (GI_{50} = 17 μM), colon cancer WiDr (GI_{50} = 18 μM), and breast cancer HBL-100 (GI_{50} = 20 μM). The only difference between hybrids **B3** and **B7** is the fact that the latter contains a Cl atom instead of a hydrogen, and it seems that this halogen enhances the antiproliferative effect in all cancer cell lines. In the T-47D cell line all compounds had low antiproliferative effects. Interestingly, both hybrids type **A** and **B** have compounds with anticancer activity.

The first family of (4-phenyl-1,2,3-triazole)dihydropyrimidinone hybrids **A1–5** with the 1,2,3-triazole ring directly linked to the C-5 position of DHPM were synthesized in good overall yields using the combination of the CuAAC and Biginelli multicomponent reactions in a one-pot sequential manner. The second family of di(1,4-disubstituted 1,2,3-triazole)dihydropyrimidinone hybrids **B1–16** were successfully synthesized in excellent yields *via* functionalization of C-6 methyl group position of the DHPM from hybrids **A**. The best bromination method found involved the $TBABr_3$ reagent affording the brominated intermediates **7a–e** in good yields. Azidation with NaN_3 furnished the new azide intermediates **8a–e** also in very good yields. The last step, i.e., the introduction of the 4-substituted 1,2,3-triazole ring in the C-6 position of hybrids **A**, was carried out using the CuAAC reaction. Hybrids **B1–16** were obtained in excellent yields. The overall yields were generally good (up to 43%). Three of the eight tested hybrids **A1**, **A3**, and **B7** were shown to be the best antiproliferative agents in the non-small-cell line cancer SW1573 showing IG_{50} values of 17, 14, and 15 μM, respectively. Hybrid **A3** was shown to be active also in A549 and HeLa cancer cell line. Hybrid **B7** was also active against A549, HBL-100, and WiDr cancer cell lines. In conclusion, both families have much potential for development as anticancer drugs and further studies are in progress.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0039-1690781.

References and Notes

(1) (a) Kaur, R.; Chaudhary, S.; Kumar, K.; Gupta, M. K.; Rawal, R. K. *Eur. J. Med. Chem.* **2017**, *132*, 108; and citations therein. (b) Matos, L. H. S.; Masson, F. T.; Simeoni, L. A.; Homem-de-Mello, M. *Eur. J. Med. Chem.* **2018**, *143*, 1779; and citations therein.

(2) (a) Dheer, D.; Singh, V.; Shankar, R. *Bioorg. Chem.* **2017**, *71*, 30; and citations therein. (b) Xu, Z.; Zhao, S.-J.; Liu, Y. *Eur. J. Med. Chem.* **2019**, *183*, 1117002; and citations therein.

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

(4) Dunn, G. L.; Hoover, J. R. E.; Berges, D. A.; Taggart, J. J.; Davis, L. D.; Dietz, E. M.; Jakas, D. R.; Yim, N.; Actor, P.; Uri, J. V.; Weisbac, J. A. *J. Antibiot.* **1976**, *29*, 65.

(5) (a) Bozorov, K.; Zhao, J.; Aisa, H. A. *Bioorg. Med. Chem.* **2019**, *27*, 3511. (b) Bonandi, E.; Christodoulou, M. S.; Fumagalli, G.; Perdicchia, D.; Rastelli, G.; Passarella, D. *Drug Discovery Today* **2017**, *22*, 1572.

(6) (a) Khanetskyy, B.; Dallinger, D.; Kappe, C. O. *J. Comb. Chem.* **2004**, *6*, 884. (b) Liu, C.; Zhao, X.; Li, Y. *J. Heterocycl. Chem.* **2011**, *48*, 92. (c) Liu, C.; Wang, J.-D. *Molecules* **2010**, *15*, 2087. (d) Salehi, P.; Dabiri, M.; Koohshari, M.; Movahed, S. K.; Bararjanian, M. *Mol. Diversity* **2011**, *15*, 833. (e) Quan, Z.-J.; Xu, Q.; Zhang, Z.; Da, Y.-X.; Wang, X.-C. *Tetrahedron* **2013**, *69*, 881. (f) Kaoukabi, H.; Kabri, Y.; Curti, C.; Taourirte, M.; Rodriguez-Ubis, J. C.; Snoeck, R.; Andrei, G.; Vanelle, P.; Lazrek, H. B. *Eur. J. Med. Chem.* **2018**, *155*, 772. (g) Olvera, R. G.; Rodrígu, V. R.; Negrón-Silva, G. E.; Espinoza-Vázquez, A.; Rodríguez-Gómez, F. J.; Santillan, R. *Molecules* **2016**, *21*, 250. (h) Venugopala, K. N.; Rao, G. B. D.; Bhandary, S.; Pillay, M.; Chopra, D.; Aldhubiab, B. E.; Attimarad, M.; Alwassil, O. I.; Harsha, S.; Mlisana, K. *Drug Des., Dev. Ther.* **2016**, *10*, 2681. (i) Teleb, M.; Rizk, O. H.; Zhang, F.-X.; Fronczek, F. R.; Zamponi, G. W.; Fahmy, H. *Bioorg. Chem.* **2018**, *83*, 354. (j) Mostafa, A. S.; Selim, K. B. *Eur. J. Med. Chem.* **2018**, *156*, 304. (k) Shamsiya, A.; Damodaran, B. *ChemistrySelect* **2019**, *4*, 3076.

(7) (a) Biginelli, P. *Gazz. Chim. Ital.* **1893**, *23*, 360. (b) Kappe, C. O. *Tetrahedron* **1993**, *49*, 6937. (c) Saini, A.; Kumar, S.; Sandhu, J. S. *J. Indian Chem. Soc.* **2007**, *84*, 959. (d) Wan, J.-P.; Liu, Y. *Synthesis* **2010**, 3943. (e) Suresh Sandhu, J. S. *ARKIVOC* **2012**, *(i)*, 66.

(8) (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596. (b) Tornoe, C.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.

(9) Shao, C.; Wang, X.; Zhang, Q.; Luo, S.; Zhao, J.; Hu, Y. *J. Org. Chem.* **2011**, *76*, 6832.

(10) (a) Appukkuttan, P.; Dehaen, W.; Fokin, V. V.; Van der Eycken, E. *Org. Lett.* **2004**, *6*, 4223. (b) González-Olvera, R.; Urquiza-Castro, C. I.; Negrón-Silva, G. E.; Ángeles-Beltrán, D.; Lomas-Romero, L.; Gutiérrez-Carrillo, A.; Lara, V. H.; Santillanc, R.; Morales-Serna, J. A. *RSC Adv.* **2016**, *6*, 63660. (c) Espinoza-Vázquez, A.; Rodríguez-Gómez, F. J.; González-Olvera, R.; Angeles-Beltrán, D.; Mendoza-Espinosa, D.; Negrón-Silva, G. E. *RSC Adv.* **2016**, *6*, 72885.

(11) **General Procedure for One-Pot Syntheses of Hybrids A1–5**
The reaction was carried out in a Biotage microwave reactor in a 20 mL vial equipped with a magnetic stirring bar. The reagents were added in the following order: CuI (45 mg, 0.24 mmol, 5 mol%), acetonitrile (MeCN, 3 mL) , DIPEA (34 µL, 0.2 mmol, 4%), acetic acid (11 µL, 0.2 mmol, 4%), phenylacetylene **2** (0.55 mL, 5 mmol, 1 equiv), 1-azidopropan-2-one (**1**, 500 mg, 5 mmol, 1 equiv) dissolved in 2 mL of MeCN. The benzaldehyde derivative **4a–e** (5 mmol, 1 equiv) and the urea **5** (363 mg, 6 mmol, 1.2 equiv) were added immediately. The sealed-vial was placed in the reactor under the following conditions: 24 h, 90 °C, pre-stirring 60 s, normal adsorption. When the reaction was complete, the reaction mixture was poured onto 100 mL of ice-water, and after 2 h, the precipitated product was filtered off. Purification was achieved through crystallization.
Compound **A1**: beige solid (949 mg, yield 57%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.69 (s, 3 H), 5.36 (s, 1 H, CH), 7.15 (d, *J* = 7 Hz, 2 H, Har), 7.24–7.34 (m, 4 H, Har), 7.42 (t, *J* = 8 Hz, 2 H, Har), 7.69 (s, 1 H, CHtrzl), 7.77 (d, *J* = 7.2 Hz, 2 H, Har), 8.46 (s, 1 H, NH), 8.89 (S, 1 H, NH) ppm. ^{13}C-APT-NMR (100 MHz, DMSO-d_6): δ = 14.6, 58.7, 108.0, 123.9, 125.6, 127.1, 128.4, 128.4, 129.0, 129.4, 130.8, 133.3, 142.6, 146.2, 152.4 ppm. HRMS (ESI-TOF): *m/z* calcd for $C_{19}H_{17}N_5ONa$ [M + Na]$^+$: 354.1325; found: 354.1335.

(12) (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004. (b) Singh, M. S.; Chowdhury, S.; Koley, S. *Tetrahedron* **2016**, *72*, 5257.

(13) Kappe, C. O. *J. Org. Chem.* **1997**, *62*, 7201.

(14) (a) Dallinger, D.; Kappe, C. O. *Pure Appl. Chem.* **2005**, *77*, 155. (b) Kheder, N. A.; Mabkhot, Y. N.; Farag, A. M. *Heterocycles* **2009**, *78*, 937. (c) Mondal, M. A.; Khan, A. A.; Mitra, K. *J. Chem. Sci.* **2018**, *130*, 151.

(15) **General Procedure for the Synthesis of Brominated Intermediates 7a–e**
To a 50 mL round-bottom flask with a magnetic stirring bar, hybrids **A1–5** (1 equiv), CH_2Cl_2 (15 mL), and $TBABr_3$ (1.5 equiv) were added. The mixture was stirred for 2 h. For the workup, a small amount of Na_2SO_3 was added to the reaction mixture, and it was extracted with a solution of sat. aq $NaHCO_3$. The organic layer was dried with $MgSO_4$, filtered, and concentrated under vacuum. The mixture was purified by column chromatography.
Compound **7a**: white solid (121 mg, yield 49%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.91 and 4.02 (2d, *J* = 11 Hz, 2 H, $BrCH_2$), 5.40 (d, *J* = 2 Hz, 1 H, CH-DHPM), 7.17 (d, *J* = 7 Hz, 2 H, Har), 7.25–7.35 (m, 4 H, Har), 7.44 (d, *J* = 7.5 Hz, 2 H, Har), 7.78 (d, *J* = 7 Hz, 2 H, Har), 7.86 (s, 1 H, Htrzl), 8.61 (s, 1 H, NH), 9.16 (d, *J* = 2 Hz, 1 H, NH) ppm. ^{13}C APT NMR (100 MHz, DMSO-d_6): δ = 24.54, 58.02, 110.11, 122.81, 125.20, 126.63, 128.20, 128.23, 128.72, 128.98, 130.04, 132.83, 141.31, 146.14, 151.82 ppm. HRMS (ESI-TOF): *m/z* calcd for $C_{19}H_{16}BrN_5O$ [M + H]$^+$: 410.0610; found: 410.0619.

(16) **General Procedure for the Synthesis of Azide Intermediates 8a–e**
The reaction was carried out in a Biotage microwave reactor in a 5 mL vial equipped with a magnetic stirrer. Added to the vial were the 1,2,3-trzl-DHPM-Br (**7a–e**), DMF (3 mL), and NaN_3 (1.5 equiv). The sealed vial was placed in the reactor under the following conditions: 30 min, 60 °C, pre-stirring 60 s, normal adsorption. When the reaction was complete, H_2O (5 mL) was added to the reaction mixture, and it was extracted with AcOEt. The organic layer was dried with MgSO4, filtered, and concentrated under vacuum. The crude product was purified by column chromatography and characterized.
Compound **8a**: white solid (135 mg, yield 88%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.82 and 3.91 (2 d, *J* = 14 Hz, 2 H, CH_2), 5.47 (d, *J* = 2 Hz, 1 H, CH-DHPM), 7.19 (d, *J* = 7 Hz, 2 H, Har), 7.25–7.37 (m, 4 H, Har), 7.43 (t, *J* = 7.6 Hz, 2 H, Har), 7.79 (d, *J* = 9 Hz, 2 H, Har), 7.88 (br s, 1 H, Htrzl), 8.63 (s, 1 H, NH), 9.24 (d, *J* = 2 Hz, 1 H, NH) ppm. ^{13}C APT NMR (100 MHz, DMSO-d_6): δ = 46.52, 58.02, 110.61, 123.10, 125.18, 126.66, 128.16, 128.22, 128.68, 128.96, 130.12, 130.96, 141.41, 146.27, 151.79 ppm. HRMS (ESI-TOF): *m/z* calcd for $C_{19}H_{16}N_8ONa$ [M + Na]$^+$: 395.1339; found: 395.1347.

(17) **General Procedure for the Synthesis of Hybrids B1–16**
The reaction was carried out in a Biotage microwave reactor in a 3 mL vial equipped with a magnetic stirring bar. To the vial, $CuSO_4{\cdot}5H_2O$ (5 mol%), L-ascorbic acid (20 mol%), DMF (3 mL), 1,2,3-trzl-DHPM-N_3 (**8a–e**, 1 equiv) and alkyne **2**, **9**, **10**, or **11** (1 equiv) were added. The sealed vial was placed in the reactor under the following conditions: 10 min, 90 °C, pre-stirring 60 s, normal adsorption. When the reaction was complete, AcOEt (5 mL) and H_2O (5 mL) were added to the reaction mixture, and this was extracted with AcOEt. The organic phase was collected and dried with $MgSO_4$, filtered, and concentrated under vacuum. The crude product was purified by recrystallization.
Compound **B1**: beige solid (179 mg, yield 94%). Overall yield for the three-step reactions was 41%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 5.01 and 5.14 (2 d, *J* = 15 Hz, 2 H, CH_2), 5.49 (d, *J* = 2 Hz, 1 H, CH-DHPM), 7.21–7.35 (m, 7 H, Har), 7.40–7.45 (m, 4 H, Har), 7.75 (d, *J* = 7.6 Hz, 2 H, Har), 7.80 (d, *J* = 8 Hz, 2 H, Har), 7.90 (s, 1 H, Htrzl), 8.43 (s, 1 H, Htrzl), 8.62 (s, 1 H, NH), 9.22 (d, *J* = 2 Hz, 1 H, NH) ppm. ^{13}C APT NMR (100 MHz, DMSO-d_6): 46.30, 58.19, 111.36, 121.97, 123.18, 125.19, 126.74, 128.01, 128.16, 128.26, 128.70, 128.96, 130.06, 130.09, 130.44, 141.31, 146.06, 146.24, 151.65 ppm. HRMS (ESI-TOF): *m/z* calcd for $C_{27}H_{22}N_8ONa$ [M + Na]$^+$: 497.1809; found: 497.1820.

