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# Synthesis of hybrid perillyl-4*H*-pyrans. Cytotoxicity evaluation against hepatocellular carcinoma HepG2/C3A cell line

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**Abstract**

A series of 15 new hybrid perillyl-4*H*-pyrans compounds was straightforwardly synthesized by a strategy combining the multicomponent reaction and the copper-catalyzed alkyne-azide cycloaddition (CuAAC). The 2-amino-4*H*-pyrans-3-carbonitrile containing the alkyne moiety was prepared via multicomponent reaction between 1,3-dicarbonyl, a propargyloxy aromatic aldehyde and malononitrile or ethyl α-cyanoacetate. The alkyne derivative was sequentially reacted with the perillyl azide component through the copper-catalyzed [3+2] Huisgen cycloaddition reaction. The antiproliferative activity of hybrid compounds were evaluated against the human hepatoma HepG2/C3A cell line.

## 1 | INTRODUCTION

The modern pharmaceutical industry has faced unprecedented challenges regarding the development of new drugs. Although investments have increased steadily over the past 20 years, new drug approvals have declined by about 50% over the same period. Considering that many human diseases involve multiple factors, the decline in the development of new drugs can be attributed, in part, to the "one-drug-one-target paradigm," which is the development of specific drugs for specific molecular targets.[1] Moreover, many traditional treatments of multifactorial diseases using a single drug have been proved inefficient due to the inability of drug to act in different sites of the organism.[2] Alternatively, the use of cocktails of drugs represents a breakthrough. However, several drawbacks associated with severe side effects or low patient compliance prevent its indiscriminate use.[3] In order to overcome this problem, a new concept for drugs has been postulated: "a single chemical entity able to act on various targets simultaneously."[4,5] These multifunctional compounds (MFCs) have brought promising results as synergistic effects and minimization of side effects on therapeutics.[6]

One of the ways to achieve compounds with these special properties is the "molecular hybridization" in

which two active compounds are connected through a linker.[7,8] The MFCs are classified as "hybrid drugs" when two or more molecules, with different activities, are linked through a stable or metabolizable linker, where the original molecules remain essentially unaltered. On the other hand, "chimeric drugs" are designed when only parts of the original molecules are chemically connected forming a new molecule (Figure 1).[9,10]

The multicomponent reactions (MCRs) have emerged as a powerful tool addressed to discovery of new drugs. The MCRs are a special kind of one-pot reactions allowing the formation of several chemical bonds from consecutive and ordered events of a single process, where three or more reagents are mixed concomitantly in the same reaction flask. It occurs with high atom economy, since collateral products are not formed, and the final product contains all or almost all the atoms of the starting reagents.[11] Through this strategy, molecules with a high degree of complexity could be prepared in a direct way without the tedious processes of isolation and the purification of intermediates.[12] As a superior synthetic strategy, it brings advantages such as operational simplicity, functional and structural diversity and atom efficiency, among others.[13,14] Particularly, the MCRs have been successfully applied in the synthesis of heterocyclic compounds allowing an elegant and rapid synthesis of polyfunctionalized structurally complex heterocycles.[15]

Among the heterocycles, the synthesis of 4*H*-pyrans have attracted great interest because they constitute the central unit in many natural products. Furthermore, a variety of pharmacological properties such as: antispasmodic activity,[16] anticoagulants,[17] antirheumatic,[18] enhancers in treatment of neurodegenerative diseases,[19] excitatory amino acid transporters (EAAT)[20] or AIDS-Associated Dementia[21] have been reported in the literature. Additionally, 4*H*-pyrans derivatives were identified as apoptotic inducers or anticancer active compounds.[22–24]

Based on this information, we envisioned the preparation of hybrid pyran compounds starting from 2-amino-3-cyano-4*H*-pyrans and perillyl alcohol. The choice of perillyl alcohol was due their interesting biological activity. Recent studies revealed a promising anticancer activity,[25] preventing skin melanoma[26] or antimetastatic agent in brain gliomas.[27] Additionally, their obtention from a natural source turns itself as a component from a renewable font.

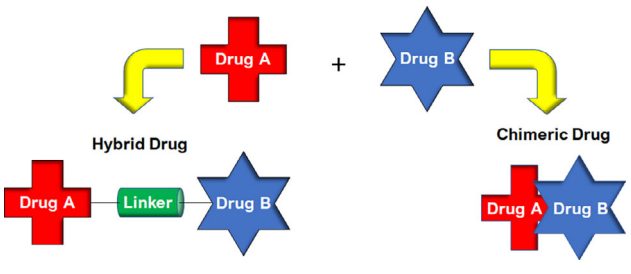



**FIGURE 1** Pictorial representation of the hybrid drugs and chimeric drugs

The chosen method to connect both molecules was planned by the creation of a stable triazolyl linker via "Click" reaction.[28] The copper-catalyzed azide-alkyne cycloaddition (CuAAC, copper catalyzed Huisgen reaction) is a main method to construct the triazolyl linker.[29,30] This methodology was elegantly demonstrated by Pal and coworkers synthesizing a plethora of different complex molecules with interesting biological activities.[31–33]

The sequential combining of multicomponent and other reactions, such as CuAAC reactions, has also proven to be an efficient and versatile strategy to prepare complex hybrid molecules[34,35] as was demonstrated in a recent review article.[36] In a previous report, we have applied this approach successfully in the synthesis of perillyl-dihydropyrimidinones hybrids as a potent antiproliferative compounds against cancer cell lines.[37] Thus, a series of perillyl-4*H*-pyran hybrids were synthesized combining multicomponent synthesis of 4-arylpropargyloxy-4*H*-pyrans followed by a copper-catalyzed Huisgen reaction with an azido derivative (Figure 2).

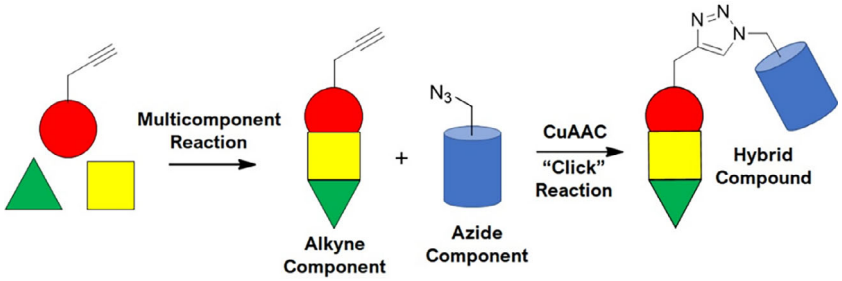
## 2 | RESULTS AND DISCUSSION

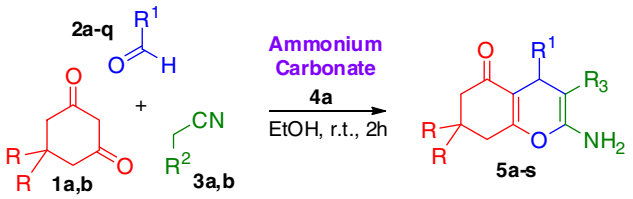
### 2.1 | Synthesis

The multicomponent approach to prepare the 4-aryl-4*H*-pyrans was extensively discussed in the literature a plethora of catalysts and reaction conditions. Homogeneous Bronsted base catalysts,[38] Lewis acid,[39] ionic liquids[40] or ammonia[41] are some of them. Heterogeneous catalysis was successfully applied such as heteropoly acids,[42] MgO,[43] metal/silica nanocomposites[44] and silica/magnetic nanoparticle.[45]

Several reports have discussed the use of ammonium acetate as catalyst in the multicomponent synthesis of 4-aryl-4*H*-pyrans and their derivatives.[46,47] Despite promiscuous use of ammonium acetate in organic synthesis, recently, the ammonium carbonate has been used as reagent or catalyst in the synthesis of heterocyclic compounds such as Paal-Knorr pyrrole synthesis and Bucherer-Bergs hydantoin synthesis. In early work, we observed a superior reactivity of ammonium carbonate in the multicomponent synthesis of γ-nitroamides or Hantzsch dihydropyridines, instead ammonium acetate or ammonium formate.[48] This has encouraged us to

**FIGURE 2** General strategy for synthesis the hybrid compounds




**SCHEME 1** Synthesis of 4-aryl-4*H*-pyran derivatives **5a–r**

investigate the use of ammonium carbonate, as catalyst, to promote the synthesis of 4-aryl-4*H*-pyran derivatives. Thus, we carried out the reaction between the 1,3 diketones **1a,b**, aldehydes **2a-q** and malononitrile **3a** or the ethyl α-cyanoester **3b** under catalysis of ammonium carbonate [$(NH_4)_2CO_3$, **4a**, 20 mol%]. For comparative purpose, the reactions also were performed in presence of ammonium formate ($NH_4HCO_2$, **4b**) and ammonium acetate ($NH_4CH_3CO_2$, **4c**) in ethanol for 2 hours at room temperature (Scheme 1). The reaction afforded the 4-aryl-4*H*-pyrans derivatives **5a-r** in good yields. The results are shown in the Table 1.

All compounds were easily isolated from washing the solid product with water and EtOH or, in some cases, by recrystallization. The compounds were fully characterised through the usual $^1$H NMR, $^{13}$C NMR and IR spectroscopic methods, including HRMS for the novel compounds. The data were in accordance with the proposed structures.

The relative efficiency of different ammonium salts as catalyst (20 mol%), can be seen comparatively at entries 1, 2 and 3 in the Table 1. The use of ammonium carbonate (**4a**) showed a superior activity, based on the yield of compound **5a** (95%, Entry 3). This higher activity can be attributed to the fact 1 M equivalent of **4a** releases 2 equivalents of ammonia, which acts as the base and by decomposition of carbonic acid into $CO_2$. The ammonium salts **4b** or **4c** release only 1 M equivalent of ammonia per equivalent of salt and the yield of reaction decreases (entries 2 and 3, respectively). The reaction with 1,3-ciclohexanedione (**1b**) also was effective for the preparation of **5b** (90%; Entry 4). A conjunct of aromatic **2b-k** was reacted with dimedone (**1a**) and malononitrile (**3a**) under the same conditions of ammonium carbonate catalysis. In all cases, good yields (85%-94%) were observed (Entries 5-13). The heteroaromatic aldehydes such as 2-thiophenecarboxaldehyde (**2l**), 2-furancarboxaldehyde (**2m**) were equally effective to afford the respective substituted 4-aryl-4*H*-pyrans (Entries 14 and 15, respectively). On the other hand, the use of aliphatic aldehydes **2n–q**, showed low yields (Entries 16-19) in the formation of compounds **5n-q**, respectively. These results were understood by the fact the aliphatic aldehydes are more reactive than the aromatic ones, and the occurrence of side reactions could be favored. The catalyst **4a** was also able to promote the reaction of dimedone (**1a**), benzaldehyde (**2a**) and ethyl α-cyanoester **3b** to afford the respective 4-aryl-4*H*-pyran **5r** in 80% yield (Entry 20).

### 2.1.1 | A tentative mechanism

Although complex equilibria and/or competitive reactions may be a part of the mechanisms can occur in a multicomponent process, simple approximations can be suggested within certain parameters, allowing a quick view about the transformations of reagents into products.[51] Several plausible base-catalyzed mechanistic proposals for the multicomponent reaction to access the 2-amino-3-cyano-4*H*-pyrans derivatives have appeared in the literature.[50,52] A tentative mechanism starts as an equilibrium of the ammonium carbonate furnishing the basic ammonia, responsible to promote the Knoevenagel-like condensation of the aldehyde **2a** and malononitrile **3a**, to afford the benzylidene adduct **6**. Additionally, the ammonia induces the formation of enolate **7**. A final event involves the reaction of intermediate **6** and enolate **7** leading to the cyclic intermediate **8**, followed by a hydrogen tropism to afford the 2-amino-3-cyano-4*H*-pyran **5a** (Scheme 2).

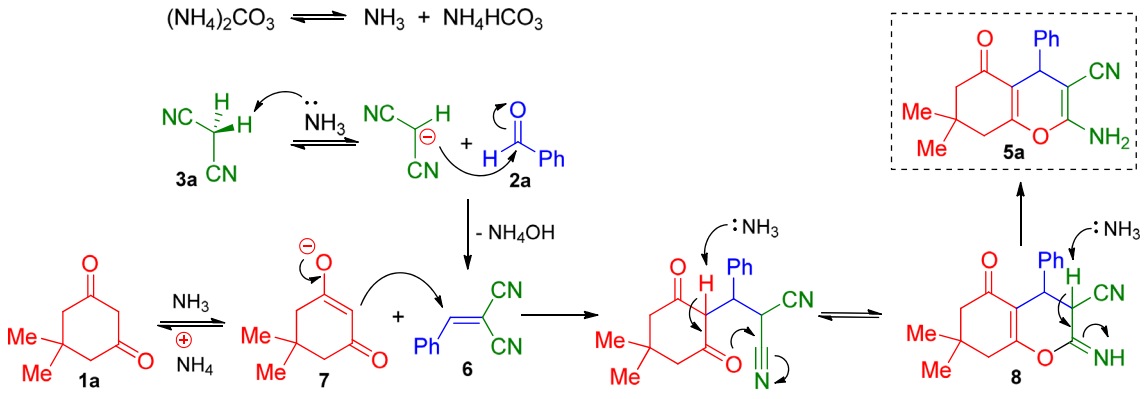
### 2.1.2 | Synthesis of hybrid molecules

To prepare the hybrid compounds, molecules with an alkyne portion and other with an azide portion are

**TABLE 1** Synthesis of 4*H*-pyrans under ammonium salts catalysis

| | Carbonyl 1 | | Aldehyde 2 | | Nitrile 3 | Cat. | | 5[a] Yield | | Refs. |
|---|---|---|---|---|---|---|---|---|---|---|
| Entry | R | | $R^1$ | | $R^2$ | 4 | | (%) | | |
| 1 | **1a** | Me | **2a** | Ph | **3a** | CN | **4a** | **5a** | 95 | [22] |
| 2 | **1a** | Me | **2a** | Ph | **3a** | CN | **4b** | **5a** | 85 | [23] |
| 3 | **1a** | Me | **2a** | Ph | **3a** | CN | **4c** | **5a** | 70 | [23] |
| 4 | **1b** | H | **2a** | Ph | **3a** | CN | **4a** | **5b** | 90 | [49] |
| 5 | **1a** | Me | **2b** | 4-F—$C_6H_4$ | **3a** | CN | **4a** | **5c** | 90 | [50] |
| 6 | **1a** | Me | **2c** | 4-Cl—$C_6H_4$ | **3a** | CN | **4a** | **5d** | 93 | [24] |
| 7 | **1a** | Me | **2d** | 3,4-$(Cl)_2$—$C_6H_3$ | **3a** | CN | **4a** | **5e** | 91 | [25] |
| 8 | **1a** | Me | **2f** | 4-MeO—$C_6H_4$ | **3a** | CN | **4a** | **5f** | 89 | [23] |
| 9 | **1a** | Me | **2g** | 3,4-$(MeO)_2$—$C_6H_3$ | **3a** | CN | **4a** | **5 g** | 91 | [23] |
| 10 | **1a** | Me | **2h** | 3,4,5-(MeO)—$C_6H_2$ | **3a** | CN | **4a** | **5h** | 92 | [23] |
| 11 | **1a** | Me | **2i** | 3-$NO_2$—$C_6H_4$ | **3a** | CN | **4a** | **5i** | 86 | [23] |
| 12 | **1a** | Me | **2j** | 4-CN—$C_6H_4$ | **3a** | CN | **4a** | **5j** | 89 | [23] |
| 13 | **1a** | Me | **2k** | 4-*N*,*N*$(Me)_2C_6H_4$ | **3a** | CN | **4a** | **5k** | 89 | [23] |
| 14 | **1a** | Me | **2l** | 2-Thiophene | **3a** | CN | **4a** | **5l** | 91 | [23] |
| 15 | **1a** | Me | **2m** | 2-Furan | **3a** | CN | **4a** | **5m** | 93 | [49] |
| 16 | **1a** | Me | **2n** | —$CH(CH)_3$ | **3a** | CN | **4a** | **5n** | 52 | [26,27] |
| 17 | **1a** | Me | **2o** | —$CH_2CH(CH_3)_2$ | **3a** | CN | **4a** | **5o** | 63 | [28] |
| 18 | **1a** | Me | **2p** | —$CH_2(CH_2)_3CH_3$ | **3a** | CN | **4a** | **5p** | 83 | [22] |
| 19 | **1a** | Me | **2q** | —$CH_2(CH_2)_5CH_3$ | **3a** | CN | **4a** | **5q** | 78 | [29] |
| 20 | **1a** | Me | **2a** | Ph | **3b** | $CO_2Et$ | **4a** | **5r** | 80 | [22] |

[a]$^1$H NMR and $^{13}$C NMR as presented in the Data S1.




**SCHEME 2** A tentative mechanistic pathway to formation of compound **5a**

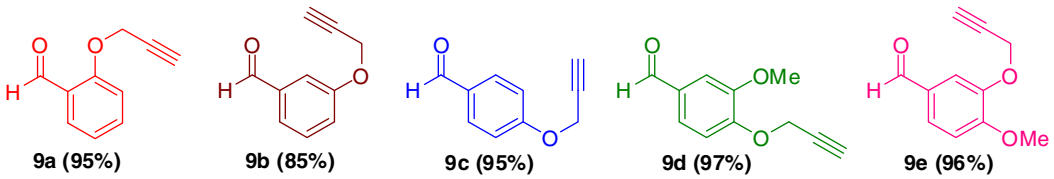


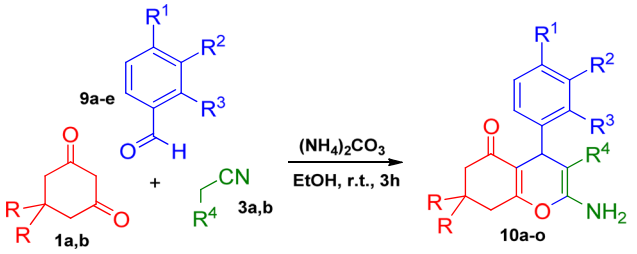

**FIGURE 3** Structures of propargyloxy benzaldehydes **9a–e**

needed. We choose to have the alkyne portion linked to the pyran moiety. For this, salicylic aldehyde, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, vanillin and isovanillin were reacted with propargyl bromide to afford the respective propargyloxy benzaldehydes **9a–e**, respectively, in good yields (Figure 3).

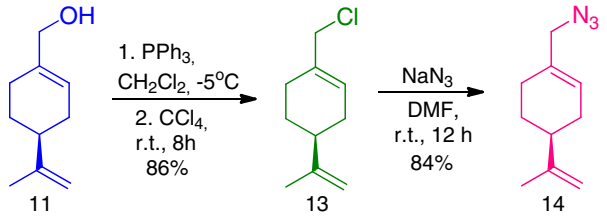
With the propargyl aldehydes **9a–e** in hands, a series of 4-arylpropargyloxy-4*H*-pyran derivatives **10a–e** was synthesized via multicomponent reaction with 1,3-dikenotes **1a,b**, malononitrile **3a** or α-cyanoester **3b** (Scheme 3). The results are shown in Table 2, below.

The 4-arylpropargyloxy-4*H*-pyrans **10a–o** were obtained in good yields. All compounds were fully characterised by usual $^{1}$H NMR, $^{13}$C NMR and I.R. spectroscopic including the HRMS spectrometry for novel compounds. Next, the azide derivative **14** was prepared starting from the perillyl alcohol (**11**). The perillyl alcohol was firstly transformed into perillyl chloride (**13**) in presence of carbon tetrachloride ($CCl_4$, **12**) through the Appel reaction in 86% yield after purification.[53] The compound **13** was converted into perillyl azide (**14**) in presence of $NaN_3$ in 84% yield and was used in the crude form in the next step (Scheme 4).

The next step was the construction of the triazole linkage between both molecules with alkyne and azide moieties through the Copper-catalyzed Alkyne-Azide Cycloaddition (CuAAC, Huisgen reaction). Originally, the reaction was developed using Cu(I) species as the active catalyst.[54] After, some expeditious modification permitted the use of Cu(II) species in presence sodium ascorbate, as reducing agent, to generate in situ the active Cu(I) specie.[55] Heterogeneous catalysis, greener process or ultrasound-promoted reaction also brought technical improvements performing this methodology.[56–58] Thus, the 4-arylpropargyloxy-4*H*-pyrans **10a–o** were used as substrate for the preparation of hybrid compounds by reaction with perillyl azide (**14**) under catalysis of




**SCHEME 3** Synthesis of 4-arylpropargyloxy-4H-pyran derivatives **10a–o**

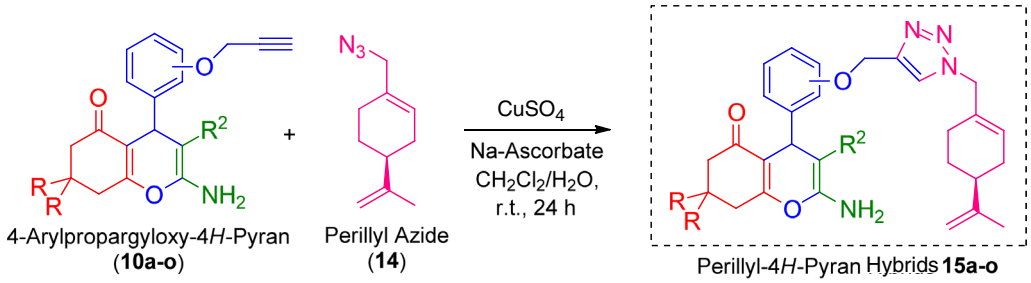



**SCHEME 4** Preparation of perillyl azide (**14**) from perillyl alcohol

**TABLE 2** Synthesis 4-arylpropargyloxy-4*H*-pyran derivatives

| | 1 | | Aldehydes 9 | | | | 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Entry | R | | | $R^1$ | $R^2$ | $R^3$ | $R^4$ | 10 Yield (%) | | |
| 1 | **1a** | Me | **9c** | H | H | OPg | **3a** | CN | **10a** | 90 |
| 2 | **1a** | Me | **9b** | H | OPg | H | **3a** | CN | **10b** | 85 |
| 3 | **1a** | Me | **9a** | OPg | H | H | **3a** | CN | **10c** | 89 |
| 4 | **1a** | Me | **9d** | OPg | OMe | H | **3a** | CN | **10d** | 88 |
| 5 | **1a** | Me | **9e** | OMe | OPg | H | **3a** | CN | **10e** | 86 |
| 6 | **1a** | Me | **9c** | H | H | OPg | **3b** | $CO_2Et$ | **10f** | 79 |
| 7 | **1a** | Me | **9b** | H | OPg | H | **3b** | $CO_2Et$ | **10g** | 82 |
| 8 | **1a** | Me | **9a** | OPg | H | H | **3b** | $CO_2Et$ | **10h** | 86 |
| 9 | **1a** | Me | **9d** | OPg | OMe | H | **3b** | $CO_2Et$ | **10i** | 88 |
| 10 | **1a** | Me | **9e** | OMe | OPg | H | **3b** | $CO_2Et$ | **10j** | 81 |
| 11 | **1b** | H | **9c** | H | H | OPg | **3a** | CN | **10k** | 87 |
| 12 | **1b** | H | **9b** | H | OPg | H | **3a** | CN | **10l** | 85 |
| 13 | **1b** | H | **9a** | OPg | H | H | **3a** | CN | **10m** | 85 |
| 14 | **1b** | H | **9d** | OPg | OMe | H | **3a** | CN | **10n** | 93 |
| 15 | **1b** | H | **9e** | OMe | OPg | H | **3a** | CN | **10o** | 97 |

Abbreviations: Pg, propargyl.




**SCHEME 5** Synthesis of perillyl-4*H*-pyran hybrid compounds **15a–o**

**TABLE 3** Synthesis of perillyl-4*H*-pyran hybrids

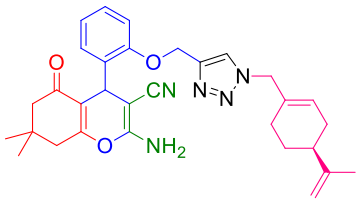
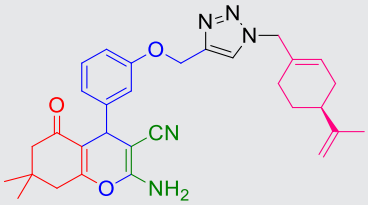
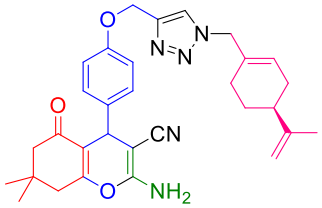
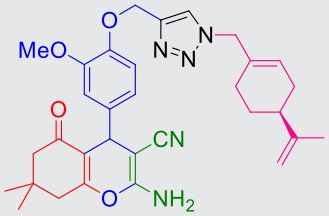
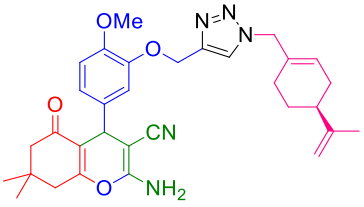
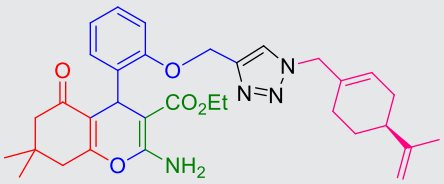
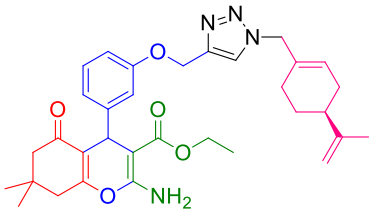
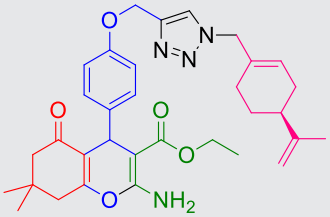
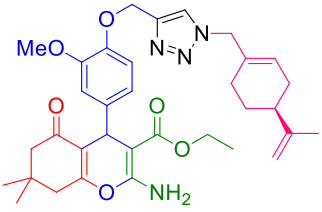
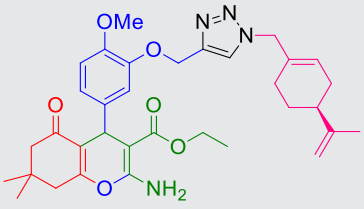
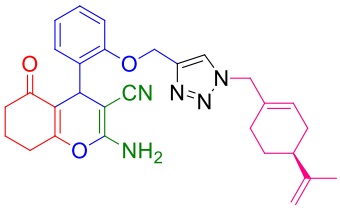
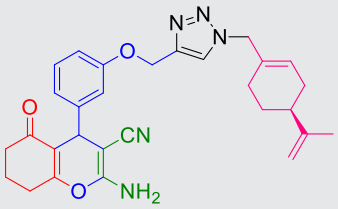
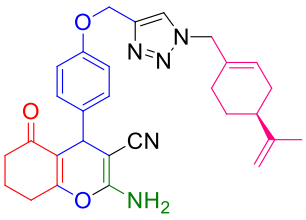
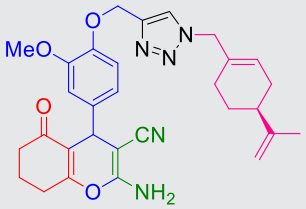
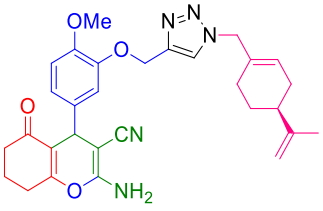
| Entry | Compound | Hybrid structure | Yield (%) |
|---|---|---|---|
| 1 | **15a** |  | 81 |
| 2 | **15b** |  | 79 |
| 3 | **15c** |  | 76 |
| 4 | **15d** |  | 82 |
| 5 | **15e** |  | 78 |

**TABLE 3** (Continued)

| Entry | Compound | Hybrid structure | Yield (%) |
|---|---|---|---|
| 6 | **15f** |  | 89 |
| 7 | **15g** |  | 81 |
| 8 | **15h** |  | 77 |
| 9 | **15i** |  | 84 |
| 10 | **15j** |  | 82 |
| 11 | **15k** |  | 80 |

(Continues)

**TABLE 3** (Continued)

| Entry | Compound | Hybrid structure | Yield (%) |
|---|---|---|---|
| 12 | **15l** |  | 72 |
| 13 | **15m** |  | 69 |
| 14 | **15n** |  | 83 |
| 15 | **15o** |  | 76 |

$CuSO_4$/Na-ascorbate system (Scheme 5). The results are shown in Table 3, below.

The formation of hybrid compounds by the formation of triazole-link via CuAAC reactions was achieved in good yields and the relative positions of substituents on the aromatic ring did not influenced significantly the yields. The perillyl-4*H*-pyran hybrids derived from dimedone component, present different substituents at C3 position. In the reaction with malononitrile, the hybrids **15a–e** present a cyano-group at this position Table 3, entries 1-5, respectively). On the other hand, the reaction with ethyl 2-cyanoacetate afforded an ethoxy-carbonyl group at C3 position (Entries 6-10, respectively). The hybrids **15k–o** are derived from 1,3-cyclohexanedione and malononitrile (Entries 11-15, respectively). All compounds were characterized through the usual $^1$H NMR, $^{13}$C NMR, I.R. spectroscopy including the HRMS analysis of novel compounds. All recorded data was in accordance with the proposed structures.

## 2.2 | Biology

### 2.2.1 | Cytotoxicity against hepatoma cell line HepG2/C3A

In order to investigate the toxicity of new hybrid compounds, preliminarily, we performed the MTT assay of compounds **15a–j** against the human hepatoma cell line HepG2/C3A. The human hepatoma cell line HepG2/C3A is a sub-clone of the hepatoma derived HepG2 cell line

**TABLE 4** Cell viability percent values against the HepG2/C3A cell line

| Entry | Hybrid | Cell viability (%)[a] | | | | |
|---|---|---|---|---|---|---|
| | | 20 μM | 40 μM | 60 μM | 80 μM | 100 μM |
| 1 | **15a** | 76.0 ± 2.7 | 61.7 ± 5.2 | 55.7 ± 3.1 | 60.9 ± 5.0 | 60.8 ± 6.6 |
| 2 | **15b** | 90.1 ± 9.6 | 73.9 ± 5.3 | 69.4 ± 4.7 | 66.6 ± 4.9 | 71.2 ± 6.1 |
| 3 | **15c** | 102.2 ± 2.7 | 93.8 ± 1.0 | 86.9 ± 1.5 | 91.1 ± 4.5 | 86.0 ± 1.0 |
| 4 | **15d** | 95.9 ± 4.7 | 97.2 ± 4.2 | 92.3 ± 2.4 | 90.9 ± 4.7 | 91.3 ± 5.9 |
| 5 | **15e** | 92.8 ± 5.1 | 100.6 ± 8.9 | 98.6 ± 8.4 | 93.1 ± 5.6 | 88.4 ± 5.5 |
| 6 | **15f** | 100.5 ± 4.8[b] | 86.3 ± 3.8 | 79.4 ± 4.4 | 68.7 ± 4.7 | 68.5 ± 3.3 |
| 7 | **15g** | 79.9 ± 5.8 | 77.1.9 ± 7.0 | 82.3 ± 5.6 | 88.5 ± 4.1 | 98.4 ± 4.0[c] |
| 8 | **15h** | 105.6 ± 1.6[b] | 100.9 ± 3.2[b] | 98.0 ± 4.1 | 93.1 ± 5.9 | 90.3 ± 4.8 |
| 9 | **15i** | 103.3 ± 4.2[b] | 104.0 ± 4.6[b] | 93.0 ± 3.0 | 88.9 ± 2.2 | 95.3 ± 4.9[c] |
| 10 | **15j** | 94.4 ± 1.4 | 88.2 ± 1.2 | 80.8 ± 3.7 | 85.0 ± 3.3 | 79.2 ± 1.7 |

[a]CV (%) of camptothecin 29.7 ± 1.2 (2 μM) as the control.
[b]Not active.
[c]Discrepant data due to the formation of crystals.

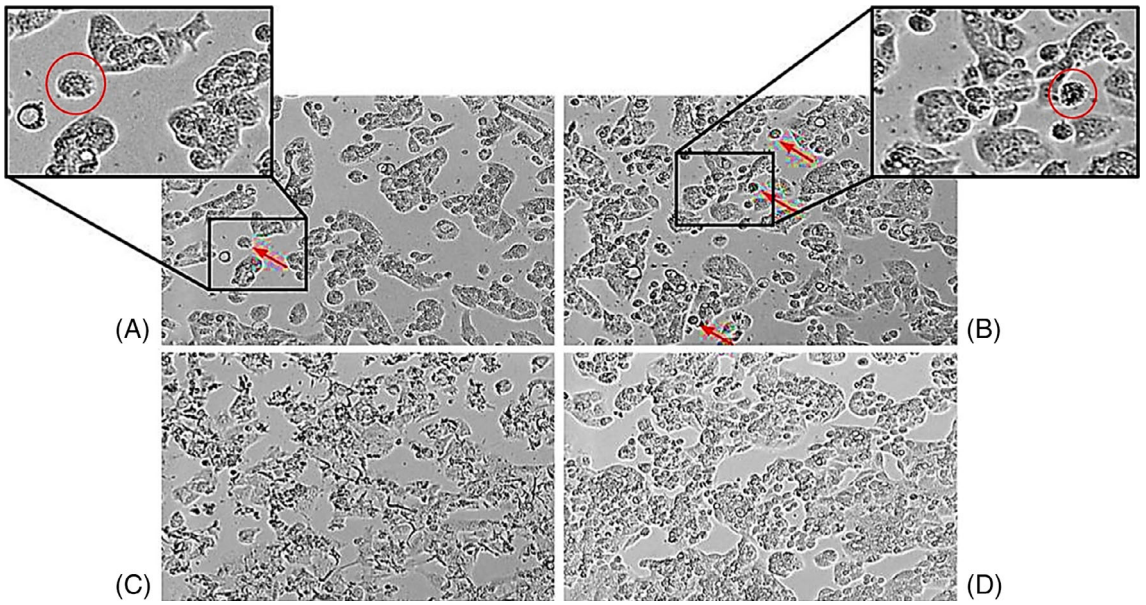



**FIGURE 4** Images from optical microscopy of morphological changes of HepG2/C3A cells after 24 hours treatment: A, incubated with **15a** (40 μM), B, Incubated with **15f** (80 μM), C, incubated with Camptothecin (2 μM). D, Control (HepG2/C3A)

and exhibits phenotypic responses and high sensitivity to toxic compounds. For this reason, they have been widely used as in vitro models for the study of hepatocellular functions, as well as in toxicity studies.

In this work, the cytotoxicity was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay described by Mosmann[59] with little modifications.

The assay was based on the reduction of the yellowish tetrazolium salt into insoluble formazan crystals of purple color by the mitochondrial enzyme succinate dehydrogenase. Greater reduction reaction shows great number of viable cells, thus indicating lower cytotoxicity. The MTT assay was performed in four replicates. The cytotoxicity was expressed as cell viability (C.V.) as a function of different concentrations of 20, 40, 60, 80, and 100 μM. The Table 4 shows the percent cell viability for the hybrid compounds **15a–j**.

High values of cell viability were observed for all studied compounds against the HepG2/C3A cell line. The

more active hybrid compounds were **15a** (55.7% at 60 μM), **15b** (66.6% at 80 μM) and **15f** (68.7% at 80 μM and 68.5% at 100 μM (Table 4, entries 1, 2 and 6, respectively). Some compounds were not active at low concentration (entries 6, 8 and 9, respectively). In two cases, the formation of small crystals of compounds was observed, and the C.V. (%) values can be uncorrected due to low solubility of compounds in the culture medium (entries 7 and 9, respectively).

The images from optical microscopy showed the morphological changes of cellular culture after the incubation of perillyl-4*H*-pyran hybrids (Figure 4). The Figure 4A,B show the changes of cell morphology observed after 24 hours of incubation with compound **15a** (40 μM) and **15f** (80 μM), respectively. Figure 4C show the morphology changes after treatment with Camptothecin (2 mM). The culture of HepG2/C3A cell line, used as a control, is showed in Figure 4D. The perillyl-4*H*-pyran hybrids treatments clearly reduced the cell confluency and cause apoptosis morphology, as pyknotic nuclei and membrane breakdown, mainly in compound **15f** as showed in detail in Figure 4B.

## 3 | CONCLUSION

The synthetic strategy based on the combining multicomponent reaction and CuAAC has been proved a powerful tool to prepare structurally complex molecules in a quick and straightforward way. The use of ammonium carbonate, as an environmental benign catalyst, proved to be efficient for the preparation of 4*H*-pyran derivatives via multicomponent process. Thus, a series of structurally novel perillyl-4*H*-pyran hybrid compounds were synthesized in few steps and good yields. The cytotoxicity of hybrid compounds was evaluated against HepG2/C3a cell line. The morphological changes of tumoral cells showed the presence of pyknotic nuclei as evidence of apoptosis process. Unfortunately, a poor cytotoxicity was observed against HepG2/C3A cell line. New investigations with different tumoral cell lines are under investigations.

## 4 | EXPERIMENTAL

### 4.1 | Chemistry

#### 4.1.1 | General procedure for the synthesis of 4*H*-pyrans 5a–m

To a round-bottom flask equipped with a magnetic stirring bar were added dimedone (1 mmol, 0.14 g), malononitrile (1 mmol, 0.07 g), aldehyde (1 mmol), ammonium carbonate (0.2 mmol, 0.02 g) and ethanol (5 mL). The reaction was stirred for 2-3 hours at room temperature and was monitored by TLC. After completion of the reaction, the solvent was evaporated and the solid obtained was washed with cold ethanol, filtered and dried in a vacuum pump. The crude products show a good level of purity, confirmed by the melting point compared with corresponding literature.

#### 4.1.2 | General procedure for synthesis of the 4*H*-pyrans 5n–q

To a round-bottom flask equipped with a magnetic stirring bar were added malononitrile (1 mmol, 0.07 g), aliphatic aldehyde (1 mmol), ammonium carbonate (0.2 mmol, 0.02 g) and ethanol (5 mL). The reaction was stirred for 0.5 hours at room temperature and was monitored by TLC. After forming the intermediate, dimedone (1 mmol, 0.14 g) was added. The reaction was stirred for 3-4 hours and was monitored by TLC. After completion of the reaction, the solvent was evaporated and the solid obtained was washed with cold ethanol, filtered and dried in a vacuum pump.

#### 4.1.3 | General procedure for synthesis of the 4*H*-pyran 5r

To a round-bottom flask equipped with a magnetic stirring bar were added ethyl 2-cyanoacetate (1 mmol, 0.11 g), benzaldehyde (1 mmol, 0.11 g), ammonium carbonate (0.2 mmol, 0.02) and ethanol (5 mL). The reaction was stirred for 1 hour under refluxing ethanol and was monitored by TLC. After forming the intermediate, dimedone were added (1 mmol, 0.14 g). The reaction was stirred for 3-4 hours and was monitored by TLC. After completion of the reaction, the solvent was evaporated and the solid obtained was recrystallized from hot ethanol. The solid was filtered, washed with cold ethanol, and dried in a vacuum pump.

#### 4.1.4 | General procedure for synthesis 4-arylpropargyloxy 4*H*-pyrans 10a–e,k–o

In a round-bottomed flask were added dimedone (1 mmol, 0.14 g), malononitrile (1, mmol, 0.07 g), aldehyde 1 mmol (**9a-d**), ammonium carbonate (0.2 mmol, 0.02 g) and 5 mL of ethanol as the solvent. The reaction was stirred for 3-4 hours, at room temperature, and was monitored by TLC. After the finish of reaction was, the

solvent was evaporated in the rotary evaporator, and then the solid obtained was washed with ethanol, filtered and dried in a rotary evaporator.

*2-amino-7,7-dimethyl-5-oxo-4-{4-[(prop-2-yn-1-yl)oxy] phenyl}-5,6,7,8-tetrahy dro-4H-1-benzopyran-3-carbonitrile (**10a**)*

Yield: 89%, white solid, m.p. 212°C. $^1$H NMR (DMSO-*d*6, 400 MHz): δ 0.95 (s, 3H), 1.03 (s, 3H), 2.10 (d, *J* = 16.0 Hz, 1H), 2,24 (d, *J* = 16.0 Hz, 1H), 2.50 (2H), 3.54 (t, J = 4.0 Hz, 1H), 4.13 (s, 1H), 4.74 (d, ***J*** = 4.0 Hz, 2H), 6.89 (d, J = 8.0 Hz, 2H), 6.96 (s, 2H), 7,05 (d, J = 8.0 Hz, 2H). $^{13}$C NMR (DMSO-*d*6, 100 MHz): δ 26.9, 28.3, 31.8, 34.8, 39.7, 50.0, 55.3, 58.5, 78.1, 79.4, 112.9, 114.5, 119.8, 128.2, 137.6, 155.9, 158.4, 162.2, 195.7. IR ($\nu_{max}$ cm$^{-1}$): 3358, 3181, 2957, 2192, 2118, 1642, 1204. HRMS calc. for [$C_{21}H_{20}N_2O_3$+Na]: 371.1366, found: 371.1364.

*2-amino-7,7-dimethyl-5-oxo-4-{3-[(prop-2-yn-1-yl)oxy] phenyl}-5,6,7,8-tetrahy dro-4H-1-benzopyran-3-carbonitrile (**10b**)*

Yield: 85%, white solid, m.p. 172°C-173°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 0.95 (s, 3H), 1.02 (s, 3H), 2.11 (d, J = 16.0 Hz, 1H), 2.23 (d, J = 16.0 Hz, 1H), 2.50 (2H), 3.54 (t, J = 4.0 Hz, 1H), 4.13 (s, 1H), 4.72 (d, J = 4.0 Hz, 2H), 6.67-6.82 (m, 3H), 7.00 (s, 2H), 7.21 (t, J = 8.0 Hz, 1H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 26.9, 28.3, 31.8, 35.4, 39.7, 49.9, 55.4, 58.1, 78.2, 79.2, 112.1, 112.5, 114.2, 119.7, 120.1, 129.3, 146.4, 157.3, 158.5, 162.6, 195.8. IR ($\nu_{max}$ cm$^{-1}$): 3451, 3302, 2948, 2183, 1670, 1362, 1036. HRMS calc. for [$C_{21}H_{20}N_2O_3$+Na]: 371.1366, found: 371.1365.

*2-amino-7,7-dimethyl-5-oxo-4-{2-[(prop-2-yn-1-yl)oxy] phenyl}-5,6,7,8-tetrahdro-4H-1-benzopyran-3-carbonitrile (**10c**)*

Yield: 90%, white solid, m.p. 209°C-210°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 0.97 (s, 3H), 1.04 (s, 3H), 2.06 (d, J = 16.0 Hz, 1H), 2.24 (d, J = 16.0 Hz, 1H), 2.50 (2H), 3.56 (t, J = 4.0 Hz, 1H), 4.43 (s, 1H), 4.67 4.77 (m, 2H), 6.84 (s, 2H), 6.89 (t, J = 7.0 Hz, 1H), 7.00-7.04 (m, 2H), 7.14-7.19 (m, 1H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 26.9, 28.6, 31,0, 31.8, 39.8, 50.1, 56.1, 57.1, 78.1, 79.4, 111.8, 112.8, 119.9, 121.1, 127.8, 129.2, 132.5, 155.1, 159.0, 163.1, 195.7. IR ($\nu_{max}$ cm$^{-1}$): 3460, 3311, 3256, 3191, 2966, 2973, 2192, 1689, 1381, 1017. HRMS calculated for [$C_{21}H_{20}N_2O_3$+Na]: 371.1366, found: 371.1364.

*2-amino-7,7-dimethyl-5-oxo-4-{3-methoxy-4-[(prop-2-yn-1-yl)oxy]phenyl}-5,6, 7,8-tetrahydro-4H-1-benzopyran-3-carbonitrile (**10d**)*

Yield: 88%, pale yellow solid, m.p. 180°C-182°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 0.97 (s, 3H), 1.03 (s, 3H), 2.11 (d, J = 16.0 Hz, 1H), 2.25 (d, J = 16.0 Hz, 1H), 2.50 (2H), 3.53 (t, J = 4.0 Hz, 1H), 3.70 (s, 3H), 4.12 (s, 1H), 4.71 (d, J = 2.0 Hz, 2H), 6.62-6.65 (m, 1H), 6.70 (d, J = 2.0 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.97 (s, 2H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 27.2, 28.9, 32.2, 35.5, 39.7, 50.4, 55.9, 56.5, 58.9, 78.7, 80.0, 111.6, 113.1, 114.4, 119.3, 120.3, 138.9, 145.7, 149.3, 158.8, 162.9, 196.2. IR ($\nu_{max}$ cm$^{-1}$): 3349, 3163, 2976, 2192, 1633, 1017. HRMS calc. for [$C_{22}H_{22}N_2O_4$+Na]: 401.1472, found: 401.1473.

*2-amino-7,7-dimethyl-5-oxo-4-{4-methoxy-3-[(prop-2-yn-1-yl)oxy]phenyl}-5,6, 7,8-tetrahydro-4H-1-benzopyran-3-carbonitrile (**10e**)*

Yield: 86%, pale yellow solid, m.p. 187°C-189°C. $^1$H MNR (DMSO-*d6*, 400 MHz): δ 0.98 (s, 3H), 1.04 (s, 3H), 2.11 (d, J = 16.0 Hz, 1H), 2,25 (d, J = 16.0 Hz, 1H), 2.50 (2H), 3.50 (t, J = 4.0 Hz, 1H), 3.73 (s, 3H), 4,12 (s, 1H), 4.68 (d, J = 2.0 Hz, 2H), 6.73-6.79 (m, 2H), 6.90 (d, J = 2.0 Hz, 1H), 6.94 (s, 2H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 27.4, 28.8, 32.2, 35.4, 39.7, 50.5, 56.0, 56.9, 58.8, 78.8, 79.6, 112.4, 113.3, 114.2, 120.3, 121,1, 137.6, 146.8, 148.6, 158.9, 162.7, 196.4. IR ($\nu_{max}$ cm$^{-1}$): 3377, 3293, 3181, 2966, 2192, 1652, 1362, 1017. HRMS calc. for [$C_{22}H_{22}N_2O_4$+Na]: 401.1472, found: 401.1469.

*2-amino-5-oxo-4-[2-(prop-2-yn-1-yloxy)phenyl]-5,6,7,8-tetrahydro-4H-chromene −3-carbonitrile (**10k**)*

Yield: 83%, white solid, m.p. 193°C-195°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 1.83-1.99 (m, 2H), 2.20-2.34 (m, 2H), 2.53-2.66 (m, 2H), 3.54 (t, J = 2.0 Hz, 1H), 4.14 (s, 1H), 4.74 (d, J = 4.0 Hz, 2H), 6.89 (d, J = 8.0 Hz, 2H), 6.96 (s, 2H), 7.08 (d, J = 8.0 Hz, 2H). $^{13}$C MNR (DMSO-*d6*, 100 MHz): δ 19.8, 26.4, 34.6, 36.3, 55.3, 58.3, 78.1, 79.4, 114.0, 114.6, 119.8, 128.2, 137.7, 155.9, 158.4, 164.2, 195.9, IR ($\nu_{max}$ cm$^{-1}$): 3452, 3219, 2950, 2193, 2119, 1691, 1205. HRMS calc. for [$C_{19}H_{16}N_2O_3$+Na]: 343.1053, found: 343.1057.

*2-amino-5-oxo-4-[3-(prop-2-yn-1-yloxy)phenyl]-5,6,7,8-tetrahydro-4H-chromene −3-carbonitrile (**10l**)*

Yield: 85%, White solid, m.p. 207°C-208°C, $^1$H NMR (DMSO-*d6*, 400 MHz): δ 1.84-2.00 (m, 2H), 2.23-2.36 (m, 2H), 2.55-2.68 (m, 2H), 3.55 (t, J = 2.0 Hz, 1H), 4.16 (s, 1H), 4.74 (d, J = 4.0 Hz, 2H), 6.71 (t, J = 2.0 Hz, 1H), 6.77-6.84 (m, 2H), 6.99 (s, 2H), 7.22 (t, J = 8.0 Hz, 1H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 19.8, 26.5, 35.2, 36.3, 55.3, 58.0, 78.2, 79.2, 112.2, 113.6, 114.0, 119.7, 120.1, 129.3, 146.4, 157.3, 158.5, 164.6, 195.8. IR ($\nu_{max}$ cm$^{-1}$): 3340, 3265, 3153, 2192, 1642, 1362, 1036. HRMS calc. for [$C_{19}H_{16}N_2O_3$+Na]: 343.1053, found: 343.1053.

*2-amino-5-oxo-4-[4-(prop-2-yn-1-yloxy)phenyl]-5,6,7,8-tetrahydro-4H-chromene −3-carbonitrile (**10m**)*

Yield: 83%, white solid, m.p. 193°C-194°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 1.83-1.99 (m, 2H), 2.20-2.34

(m, 2H), 2.53-2.66 (m, 2H), 3.54 (t, J = 2.0 Hz, 1H), 4.14 (s, 1H), 4.74 (d, J = 4.0 Hz, 2H), 6.89 (d, J = 8.0 Hz, 2H), 6.96 (s, 2H), 7.08 (d, J = 8.0 Hz, 2H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 19.8, 26.4, 34.6, 36.3, 55.3, 58.3, 78.1, 79.4, 114.0, 114.6, 119.8, 128.2, 137.7, 155.9, 158.4, 164.2, 195.9. IR ($\nu_{max}$ cm$^{-1}$): 3452, 3219, 2950, 2193, 2119, 1691, 1205. HRMS calc. for [$C_{19}H_{16}N_2O_3$+Na]: 343.1053, found: 343.1057.

*2-amino-4-[3-methoxy-4-(prop-2-yn-1-yloxy)phenyl]-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**10n**)*

Yield: 93%, pale yellow solid, m.p. 203°C-205°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 1.85-1.99 (m, 2H), 2.24-2.35 (m, 2H), 2.56-2.67 (m, 2H), 3.54 (t, J = 2.0 Hz, 1H), 3.73 (s, 3H), 4.15 (s, 1H), 4.72 (d, J = 2.0 Hz, 2H), 6.64 (dd, J = 2.0 Hz and J = 6.0 Hz, 1H), 6.75 (d, J = 2.0 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 6.98 (s, 2H). $^{13}$C-RMN (DMSO-*d6*, 100 MHz): δ 19.8, 26.5, 34.9, 36.4, 55.5, 56.1, 58.3, 78.2, 79.5, 111.4, 113.8, 114.2, 118.7, 119.9, 138.5, 145.2, 148.9, 158.4, 164.4, 195.9. IR ($\nu_{max}$ cm$^{-1}$): 3396, 3311, 3265, 3191, 2929, 2192, 2118, 1652, 1362, 1129. HRMS calc. for [$C_{20}H_{18}N_2O_4$+Na]: 373.1159, found: 373.1160.

*2-amino-4-[4-methoxy-3-(prop-2-yn-1-yloxy)phenyl]-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**10o**)*

Yield: 97%, pale yellow solid, m.p. 181°C-183°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 1.85-2.00 (m, 2H), 2.22-2.35 (m, 2H), 2.54-2.65 (m, 2H), 3.52 (t, J = 2.0 Hz, 1H), 3.73 (s, 3H), 4.13 (s, 1H), 4.69-4.70 (m, 2H), 6.74-6.79 (m, 2H), 6.89 (d, J = 8.0 Hz, 1H), 6.93 (s, 2H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 19.9, 26.5, 34.8, 36.4, 55.6, 56.3, 58.3, 78.3, 79.2, 112.0, 113.5, 113.9, 119.8, 120.5, 137.2, 146.3, 148.1, 158.5, 164.3, 195.8. IR ($\nu_{max}$ cm$^{-1}$): 3405, 3321, 3256, 3191, 2938, 2826, 2192, 1652, 1372, 1017. HRMS calc. for [$C_{20}H_{18}N_2O_4$+Na]: 373.1159, found: 373.1155.

### 4.1.5 | General procedure for synthesis 4-arylpropargyloxy 4*H*-iyrans 10f–j

In a round-bottomed flask were added dimedone (1 mmol, 0.14 g), malononitrile (1, mmol, 0.07 g), aldehyde 1 mmol (**9a–d**), ammonium carbonate (0.2 mmol, 0.02 g) and 5 mL of ethanol as the solvent. The reaction was stirred for 1 hour under refluxing ethanol and was monitored by TLC. After forming the intermediate, dimedone were added (1 mmol, 0.14 g). The reaction was stirred for 3-4 hours and was monitored by TLC. After completion of the reaction, the solvent was evaporated and the solid obtained was recrystallized from hot ethanol. The solid was filtered, washed with cold ethanol and dried in a vacuum pump.

*Ethyl 2-amino-7,7-dimethyl-5-oxo-4-{4-[(prop-2-yn-1-yl) oxy]phenyl}-5,6,7,8-te trahydro-4H-1-benzopyran-3-carboxylate (**10f**)*

Yield: 86%, white solid, m.p. 120°C-121°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 0.90 (s, 3H), 1.03 (s, 3H), 1.10 (t, J = 8.0 Hz, 3H), 2.06 (d, J = 16.0 Hz, 1H), 2.25 (d, J = 16.0 Hz, 1H), 2.46 (d, J = 16.0 Hz, 1H), 2.53 (d, J = 16.0 Hz, 1H), 3.52 (brs, 1H), 3.89-4.01 (m, 2H), 4.46 (s, 1H), 4.70 (d, J = 4.0 Hz, 2H), 6.81 (d, J = 8.0 Hz, 2H), 7.05 (d, J = 8.0 Hz, 2H), 7.53 (s, 2H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 14.3, 26.6, 28.6, 31.9, 32.4, 39.6, 50.0, 55.3, 58.8, 78.1, 78.1, 79.5, 114.0, 115.7, 128.6, 139.3, 155.3, 159.1, 162.0, 168.1, 195.9. IR ($\nu_{max}$ cm$^{-1}$): 3489, 3248, 2950, 2110, 1691, 1652, 1196. HRMS calc. for [$C_{23}H_{25}N_1O_5$+Na]: 418.1625, found: 418.1625.

*Ethyl 2-amino-7,7-dimethyl-5-oxo-4-{3-[(prop-2-yn-1-yl) oxy]phenyl}-5,6,7,8-te trahydro-4H-1-benzopyran-3-carboxylate (**10g**)*

Yield: 82%, white solid, m.p. 138°C-140°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 0.91 (s, 3H), 1.03 (s, 3H), 1.11 (t, J = 8.0 Hz, 3H), 2.07 (d, J = 16.0 Hz, 1H), 2.6 (d, J = 16.0 Hz, 1H), 2.48 (d, J = 16.0 Hz, 1H), 2.54 (d, J = 16.0 Hz, 1H), 3.55 (t, J = 2.0 Hz, 1H), 3.089-4.00 (m, 2H), 4.48 (s, 1H), 4.66-4.75 (m, 2H), 6.72-6.76 (m, 3H), 7.14 (t, J = 8.0 Hz, 1H), 7.56 (s, 2H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 14.3, 26.6, 28.6, 31.9, 33.1, 39.5, 50.0, 55.3, 58.8, 77.6, 78.2, 79.3, 111.5, 114.9, 115.4, 120.6, 128.7, 148.0, 156.9, 159.2, 162.3, 168.0, 195.8. IR ($\nu_{max}$ cm$^{-1}$): 3498, 3321, 3265, 2957, 2127, 1698, 1362, 1045. HRMS calc. for [$C_{23}H_{25}N_1O_5$+Na]: 418.1625, found: 418.1627.

*Ethyl 2-amino-7,7-dimethyl-5-oxo-4-{2-[(prop-2-yn-1-yl) oxy]phenyl}-5,6,7,8-tetrahydro-4H-1-benzopyran-3-carboxylate (**10h**)*

Yield: 79%, pale yellow solid, m.p. 160°C-161°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 0.90 (s, 3H), 1.03 (s, 3H), 1.07 (t, J = 8.0 Hz, 3H), 1.99 (d, J = 16.0 Hz, 1H), 2.22 (d, J = 16.0 Hz, 1H), 2.41 (d, J = 18.0 Hz, 1H), 2.52 (d, J = 18.0 Hz, 1H), 3.52 (t, J = 2.0 Hz, 1H), 3.79-3.91 (m, 2H), 4.57 (s, 1H), 4.62-4.71 (m, 2H), 6.80-6.84 (m, 1H), 6.91 (d, J = 8.0 Hz, 1H), 7.06-7.10 (m, 1H), 7.16 (dd, J = 6.0 Hz and 2.0 Hz, 1H), 7.47 (s, 2H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 14.2, 26.6, 28.8, 31.3, 31.8, 39.8, 50.2, 55.6, 58.5, 75.9, 77.8, 79.5, 112.2, 113.3, 119.9, 127.1, 131.8, 132.9, 155.6, 159.7, 162.5, 168.4, 195.7. IR ($\nu_{max}$ cm$^{-1}$): 3377, 3302, 2957, 1689, 1381, 1204, HRMS calc. for [$C_{23}H_{25}N_1O_5$+Na]: 418.1625, found: 418.1623.

*Ethyl 2-amino-7,7-dimethyl-5-oxo-4-{3-methoxy-4-[(prop-2-yn-1-yl)oxy]phenyl}-5,6,7,8-tetrahydro-4H-1-benzopyran-3-carboxylate (**10i**)*

Yield: 81%, pale yellow solid, m.p. 128°C-130°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 0.93 (s, 3H), 1.03 (s, 3H), 1.13 (t, J = 8.0 Hz, 3H), 2.08 (d, J = 16,0 Hz, 1H), 2.26 (d, J = 16.0 Hz, 1H), 2.50 (1H), 2,55 (d, J = 18.0 Hz, 1H), 3.51 (t, J = 2.0 Hz, 1H), 3.70 (s, 3H), 3.87-3.99 (m, 2H), 4.46 (s, 1H), 4.68 (d, J = 2.0 Hz, 2H), 6.60 (dd, J = 6.0 and 2.0 Hz, 1H), 6.74 (d, J = 2.0 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 7.52 (s, 2H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 14.3, 26.5, 28.7, 31.9, 32.7, 39.6, 50.0, 55.4, 56.0, 58.8, 78.0, 78.1, 79.5, 112.1, 113.7, 115.6, 119.1, 140.2, 144.7, 148.3, 159.1, 162.2, 168.1, 195.9. IR ($\nu_{max}$ cm$^{-1}$): 3405, 3256, 2948, 2118, 1717, 1512, 1250, 1008. HRMS calc. for [$C_{24}H_{27}N_1O_6$+Na]: 448.1731, found: 448.1731.

*Ethyl 2-amino-7,7-dimethyl-5-oxo-4-{4-methoxy-3-[(prop-2-yn-1-yl)oxy]phenyl} -5,6,7,8-tetrahydro-4H-1-benzopyran-3-carboxylate (**10j**)*

Yield: 88%, Pale yellow solid, m.p. 153°C-155°C. $^1$H NMR (DMSO-*d6*, 400 MHz): δ 0.92 (s, 3H), 1,04 (s, 3H), 1.13 (t, J = 8.0 Hz, 3H), 2.07 (d, J = 16.0 Hz, 1H), 2.26 (d, J = 16.0 Hz, 1H), 2.50 (2H), 3,51 (t, J = 2.0 Hz, 1H), 3.69 (s, 3H), 3.89-4.02 (m, 2H), 4.45 (s, 1H), 4.58-4.69 (m, 2H), 6.71 (dd, J = 6.0 e 2.0 Hz, 1H), 6.81-6.83 (m, 2H), 7.50 (s, 2H). $^{13}$C NMR (DMSO-*d6*, 100 MHz): δ 14.3, 26.7, 28.6, 31.8 32.5, 39.7, 50.0, 55.5, 56.4, 58.8, 78.0, 78.2, 79.3, 111.6, 114.7, 115.7, 120.9, 138.8, 145.8, 147.6, 159.2, 162.1, 168.0, 195.3. IR ($\nu_{max}$ cm$^{-1}$): 3423, 3302, 2957, 1679, 1362, 1027. HRMS calc. for [$C_{24}H_{27}N_1O_6$+Na]: 448.1731, found: 448.1731.

### 4.1.6 | Procedure for the synthesis of (*S*)-perillyl azide 14

In a round-bottomed flask containing perillyl chloride (**13**, 5.0 mmol) and dimethylformamide (1.5 mL) was added sodium azide (15.0 mmol) in one portion. The reaction was stirred for 12 hours, at room temperature, and the end of the reaction was confirmed by TLC. Then, water (5.0 mL) was added, and the aqueous phase was washed with hexane (3 × 15.0 mL) and separated. The organic phases were combined, washed with saturated NaCl solution, dried over anhydrous magnesium sulfate, filtered, and the filtrate was evaporated, yielding the perillyl azide (**14**) in 84% and was used in the next step in the crude form.

*(4S)-1-(azidomethyl)-4-(prop-1-en-2-yl)cyclohex-1-ene (**14**)*

Yield: 84%, Colorless liquid. $^1$H NMR ($CDCl_3$, 400 MHz): δ 1.47-1.57 (m, 1H), 1.75 (s, 3H), 1.84-1.90 (m, 1H), 1.96-2.06 (m, 1H), 2.10-2.23 (m, 4H), 3.64 (d, J = 12.0 Hz, 1H), 3.69 (d, J = 12.0 Hz, 1H), 4.76 (d, J = 1 Hz, 1H), 4.75 (t, J = 2.0 Hz, 1H), 5.75 (s, 1H). $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 20.7, 27.0, 27.3, 30.4, 40.7, 57.4, 108.8, 126.3, 132.1, 149.3. IR ($\nu_{max}$ cm$^{-1}$): 3085, 2923, 2096, 1645, 1439, 1242, 891.

### 4.1.7 | General procedure for the synthesis of perillyl-4*H*-pyran hybrids 15a–j

In a round-bottomed flask, were added the propargyloxy 4*H*-pyran (**10a-o**, 0.5 mmol), the perillyl azide (**14**, 0.6 mmol, 0.10 g), dichloromethane (5.0 mL), water (5.0 mL), copper sulfate pentahydrate (0.05 mmol, 0.02 g) and sodium ascorbate (0.05 mmol, 0.01 g). The reaction was kept under stirring at room temperature for 24 hours, and the end of the reaction was confirmed by TLC. Then, 10 mL of a 0.1 M solution of EDTA was added and the stir was continued for 10 minutes. The aqueous phase was extracted with dichloromethane (3 × 10 mL). The organic phases were combined, washed with saturated NaCl solution, dried over anhydrous magnesium sulfate and filtered. The filtrate was reduced under vacuum to give a solid that was purified by column chromatography using a gradient of hexane-ethyl acetate as eluent.

*2-amino-7,7-dimethyl-5-oxo-4-{2-[(1-(S)-perillyl-methyl)-1H-1,2,3-triazol-4-yl]me thoxyphenyl}-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**15a**)*

Yield: 81%, pale yellow solid, m.p. 91°C-93°C. $^1$H NMR ($CDCl_3$, 400 MHz): δ 1.05 (s, 3H, —$CH_3$), 1.09 (s, 3H, —$CH_3$), 1.40-1.47 (m, 1H, perillyl-H), 1.72 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.78-1.83 (m, 1H, perillyl-H), 1.93-2.02 (m, 3H, $(CH_3)_2C$—$\underline{CH_2}$— + perillyl-H), 2.09-2.12 (m, 2H, perillyl-H), 2.14-2.19 (m, 2H, perillyl-H), 2.34 (s, 2H, —$CH_2$—C═O), 4.42 (s, 2H, —$NH_2$), 4.69 (s, 1H, ═C—CH—C═), 4.72 (d, J = 12.0 Hz, 2H, —C═$CH_2$), 4.84 (d, J = 16.0 Hz, 1H, —$OCH_2$—), 5.22-5.32 (m, 2H, —N—$CH_2$—C—), 5.75 (s, 1H, —C═CH—), 6.88-6.94 (m, 2H, aromatic-H), 7.11-7.18 (m, 2H, aromatic-H), 7.73 (s, 1H, —C═CH—N—). $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 20.7, 26.3, 27.0, 27.6, 28.9, 30.4, 30.7, 32.1, 40.4, 50.6, 56.3, 61.9, 62.2, 77.2, 109.0, 112.2, 112.8, 119.2, 121.1, 123.3, 127.2, 128.3, 129.4, 131.1, 132.0, 144.1, 149.0, 155.5, 158.0, 162.2, 195.9. IR ($\nu_{max}$ cm$^{-1}$): 3433, 2948, 2192, 1689, 1362, 1213. HRMS calc. for [$C_{31}H_{35}N_5O_3$+Na]: 548.2632, HRMS found: 548.2625.

*2-amino-7,7-dimethyl-5-oxo-4-{3-[(1-(S)-perillyl-methyl)-1H-1,2,3-triazol-4-yl]me thoxyphenyl}-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**15b**)*

Yield: 79%, pale yellow solid, m.p. 176°C-178°C. $^1$H NMR ($CDCl_3$, 400 MHz): δ 1.05 (s, 3H, —$CH_3$), 1.11 (s, 3H,

—$CH_3$), 1.41-1.51 (m, 1H, perillyl-H), 1.73 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.79-1.84 (m, 1H, perillyl-H), 1.93-2.03 (m, 3H, $(CH_3)_2C$—$\underline{CH_2}$— + perillyl-H), 2.11-2.17 (m, 2H perillyl-H), 2.18-2.27 (m, 2H perillyl-H), 2.41 (d, J = 18.0 Hz, 1H, —$CH_2$—C═O), 2.51 (d, J = 18.0 Hz, 1H, —$CH_2$—C═O), 4.38 (s, 1H, ═C—CH—C═), 4.62 (brs, —$NH_2$), 4.72 (d, J = 16.0 Hz, 2H, —C═$CH_2$), 4.85 (s, 2H, —$OCH_2$—), 5.20 (s, 2H, —N—$CH_2$—C—), 5.77 (s, 1H, —C═CH—), 6.81-6.88 (m, 3H, aromatic-H), 7.21 (t, J = 8.0 Hz, 1H, aromatic-H), 7.60 (s, 1H, —C═CH—N—). $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 20.7, 26.3, 27.1, 27.9, 28.6, 30.4, 32.2, 35.4, 40.4, 40.6, 50.6, 56.5, 62.0, 63.1, 77.2, 109.0, 113.4, 113.7, 114.2, 120.6, 122.6, 122.6, 127.4, 129.6, 131.8, 144.9, 149.1, 157.5, 158.3, 161.7, 195.9. IR ($\nu_{max}$ $cm^{-1}$): 3460, 3221, 3191, 2966, 2192, 1670, 1372, 1027. HRMS calc. for [$C_{31}H_{35}N_5O_3$+Na]: 548.2632, found: 548.2629.

*2-amino-7,7-dimethyl-5-oxo-4-{4-[(1-(S)-perillyl-methyl)-1H-1,2,3-triazol-4-yl]me thoxylphenyl}-5,6,7,8-tetrahydro-4H-cromene-3-carbonitrile (**15c**)*

Yield: 76%, pale yellow solid, m.p. 86°C-88°C. $^1$H NMR ($CDCl_3$, 400 MHz): δ 1.03 (s, 3H, —$CH_3$), 1.10 (s, 3H, —$CH_3$), 1.41-1.51 (m, 1H, perillyl-H), 1.73 (s, 3H, $H_2C$═C—$CH_3$), 1.80-1.84 (m, 1H, perillyl-H), 1.94-2.05 (m, 3H, $(CH_3)_2C$—$CH_2$— + perillyl-H), 2.11-2.17 (m, 2H, perillyl-H), 2.21-2.26 (m, 2H, perillyl-H), 2.44 (s, 2H, —$CH_2$—C═O), 4.36 (s, 1H, ═C—CH—C═), 4.59-4.62 (m, 2H, —$NH_2$), 4.72 (d, J = 16.0 Hz, 2H, —C═$CH_2$), 4.83 (s, 2H, —$OCH_2$—), 5.17 (s, 2H, —N—$CH_2$—C—), 5.77 (s, 1H, —C═CH—), 6.90 (d, J = 8.0 Hz, 2H, aromatic-H), 7.16 (d, J = 8.0 Hz, 2H, aromatic-H), 7.56 (s, 1H, —C═CH—N—). $^{13}$C NMR ($CDCl_3$, 100 MHz): δ 20.7, 26.3, 27.0, 27.6, 28.8, 30.4, 32.1, 34.7, 40.4, 50.6, 56.5, 62.1, 63.4, 77.2, 109.0, 114.1, 114.7, 118.8, 122.4, 127.4, 128.7, 131.8, 136.1, 144.4, 149.1, 157.2, 161.3, 196.1. IR (νmax $cm^{-1}$): 3340, 3172, 2948, 2360, 2183, 1679, 1558, 1372, 1222. HRMS calc. for [$C_{31}H_{35}N_5O_3$+Na]: 548.2632, found: 548.2632.

*2-amino-7,7-dimethyl-5-oxo-4-{3-methoxy-4-[(1-(S)-4-perillyl-methyl)-1H-1,2,3-triazol-4-yl]methoxyphenyl}-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**15d**)*

Yield: 82%, pale yellow solid, m.p. 89°C-90°C. $^1$H RMN ($CDCl_3$, 400 MHz): δ 1.03 (s, 3H, -$CH_3$), 1.09 (s, 3H, —$CH_3$), 1.39-1.49 (m, 1H, perillyl-H), 1.71 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.77-1.82 (m, 1H, perillyl-H), 1.91-2.01 (m, 3H, $(CH_3)_2C$—$\underline{CH_2}$— + perillyl-H), 2.09-2.13 (m, 2H, perillyl-H), 2.14-2.22 (m, 2H, perillyl-H), 2.43 (s, 2H, —$CH_2$—C═O), 3.85 (s, 3H, —$OCH_3$), 4.32 (s, 1H, ═C—CH—C═), 4.69-4.72 (m, 2H, —C═$CH_2$), 4.76 (brs, 2H, —$NH_2$), 4.82 (s, 2H, —$OCH_2$—), 5.23 (s, 2H, —N—$CH_2$—C—), 5.74 (s, 1H, —C═CH—), 6.65-6.68 (m, 1H, aromatic-H), 6.82 (d, J = 2.0 Hz, 1H, aromatic-H), 6.92 (d, J = 8.0 Hz, 1H, aromatic-H), 7.58 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 20.4, 26.0, 26.7, 27.2, 28.6, 30.1, 31.8, 34.7, 40.1, 40.3, 50.3, 55.5, 56.1, 62.8, 62.9, 76.9, 108.6, 111.3, 113.6, 118.5, 118.9, 122.3, 127.1, 131.4, 136.7, 144.2, 146.2, 148.7, 148.9, 157.2, 161.2, 195.2. IR ($\nu_{max}$ $cm^{-1}$): 3331, 3172, 2948, 2183, 1689, 1512, 1362, 1036. HRMS calc. for [$C_{32}H_{37}N_5O_4$+Na]: 578.2738, HRMS found: 578.2741.

*2-amino-7,7-dimethyl-5-oxo-4-{4-methoxy-3-[(1-(S)-4-perillyl-methyl)-1H-1,2,3-triazol-4-yl]methoxyphenyl}-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**15e**)*

Yield: 78%, pale yellow solid, m.p. 93°C-94°C. $^1$H RMN ($CDCl_3$, 400 MHz): δ 1.03 (s, 3H, —$CH_3$), 1.07 (s, 3H, —$CH_3$), 1.37-1.47 (m, 1H, perillyl-H), 1.70 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.76-1.80 (m, 1H, perillyl-H), 1.89-1.99 (m, 3H, $(CH_3)_2C$—$\underline{CH_2}$— + perillyl-H), 2.07-2.11 (m, 2H, perillyl-H), 2.14-2.18 (m, 2H, perillyl-H), 2.38 (d, J = 16.0 Hz, 1H, —$CH_2$—C═O), 2.56 (d, J = 16.0 Hz, 1H, —$CH_2$—C═O), 3.80 (s, 3H, —$OCH_3$), 4.30 (s, 1H, ═C—CH—C═), 4.69 (d, J = 12.0 Hz, 2H, —C═$CH_2$), 4.80 (brs, 2H, —$NH_2$), 4.85 (brs, 2H, —$OCH_2$—), 5.26 (s, 2H, —N—$CH_2$—C—), 5.72 (s, 1H, —C═CH—, 6.76-6.78 (m, 1H, aromatic-H), 6.84-6.86 (m, 2H, aromatic-H), 7.59 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 20.9, 26.6, 27.3, 28.2, 28.8, 30.7, 32.4, 35.2, 40.8, 50.9, 56.1, 56.7, 63.1, 63.1, 63.2, 77.5, 109.2, 111.8, 114.0, 119.2, 121.2, 123.2, 127.6, 132.0, 136.4, 144.5, 147.6, 148.8, 149.3, 158.0, 161.9, 196.4. IR ($\nu_{max}$ $cm^{-1}$): 3340, 3181, 2948, 2332, 2201, 1689, 1372, 1138, 1017. HRMS calc. for [$C_{32}H_{37}N_5O_4$+Na]: 578.2738, HRMS found: 578.2735.

*Ethyl-2-amino-7,7-dimethyl-5-oxo-4-{2-[(1-(S)-perillyl-methyl)-1H-1,2,3-triazol-4-yl]methoxyphenyl}-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate (**15f**)*

Yield: 80%, pale yellow solid, m.p. 80°C-81°C. $^1$H RMN ($CDCl_3$, 400 MHz): δ 0.96 (s, 3H, —$CH_3$), 1.05 (s, 3H, —$CH_3$), 1.13 (t, J = 8.0 Hz, 3H, —$OCH_2\underline{CH_3}$), 1.38-1.50 (m, 1H, perillyl-H), 1.72 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.79-1.86 (m, 1H, perillyl-H), 1.95-2.01 (m, 3H, $(CH_3)_2C$—$\underline{CH_2}$— + perillyl-H), 2.07-2.11 (m, 2H, perillyl-H), 2.12-2.14 (m, 2H, perillyl-H), 2.19-2.23 (m, 2H, —$CH_2$—C═O), 3.92-4.04 (m, 2H, —$COOCH_2$—), 4.69 (s, 1H, ═C—CH—C═), 4.73-4.79 (m, 2H, —C═$CH_2$), 4.81-4.92 (m, 2H, —$OCH_2$—), 5.14 (s, 2H, —N—$CH_2$—C—), 5.76 (s, 1H, —C═CH—), 6.03 (brs, 2H, —$NH_2$), 6.86 (t, J = 8.0 Hz, 2H, aromatic-H), 7.09 (t, J = 8.0 Hz, 1H, aromatic-H), 7.33 (d, J = 8.0 Hz, 1H, aromatic-H), 7.68 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 14.1, 20.7, 26.3, 27.0, 27.1, 29.1, 30.4, 30.4, 32.1, 40.3, 40.4, 50.7, 56.3, 59.5, 77.2, 79.3, 109.1, 111.3, 111.4, 114.4, 120.2, 127.0, 127.1, 127.4, 132.2, 132.4, 148.8, 148.8, 156.1, 158.6, 161.9, 169.5, 196.6. IR ($\nu_{max}$ $cm^{-1}$): 3480, 3274, 2957,

1698, 1185, 1027. HRMS calc. for [$C_{33}H_{40}N_4O_5$+Na]: 595.2891, HRMS found: 595.2896.

*Ethyl-2-amino-7,7-dimethyl-5-oxo-4-{3-[(1-(S)-perillyl-methyl)-1H-1,2,3-triazol-4-yl]methoxyphenyl}-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate (**15g**)*

Yield: 72%, pale yellow solid, m.p. 67°C-68°C. $^1$H RMN ($CDCl_3$, 400 MHz): δ 0.97 (s, 3H, —$CH_3$), 1.07 (s, 3H, —$CH_3$), 1.15 (t, J = 8.0 Hz, 3H —$OCH_2\underline{CH_3}$), 1.39-1.50 (m, 1H, perillyl-H), 1.71 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.78-1.82 (m, 1H, perillyl-H), 1.92-2.02 (m, 3H, $(CH_3)_2C$—$\underline{CH_2}$— + perillyl-H), 2.09-2.16 (m, 2H, perillyl-H), 2.19-2.23 (m, 2H, perillyl-H), 2.38 (d, J = 18.0 Hz, 1H, —$CH_2$—C═O), 2.45 (d, J = 18.0 Hz, 1H, —$CH_2$—C═O), 3.99-4.09 (m, 2H, —$COOCH_2$—), 4.66-4.73 (m, 3H, —C═$CH_2$ + ═C—CH—C═), 4.83 (s, 2H, —$OCH_2$—), 5.12-5.19 (m, 2H, —N—$CH_2$—C—), 5.75 (s, 1H, —C═CH—), 6.23 (brs, 2H, —$NH_2$), 6.71-6.73 (m, 1H, aromatic-H), 6.88-6.90 (m, 2H, aromatic-H), 7.11 (t, J = 8.0 Hz, 1H, aromatic-H), 7.59 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 14.2, 20.6, 26.3, 27.0, 27.5, 28.8, 30.4, 32.1, 33.7, 40.4, 50.7, 56.4, 59.6, 61.9, 77.2, 80.4, 108.9, 112.2, 114.8, 116.5, 121.3, 122.5, 127.3, 128.6, 131.8, 144.4, 147.5, 149.0, 157.8, 158.3, 161.4, 169.0, 196.3. IR ($\nu_{max}$ $cm^{-1}$): 3386, 2948, 2351, 1689, 1558, 1213. HRMS calc. for [$C_{33}H_{40}N_4O_5$+Na]: 595.2891, HRMS found: 595.2884.

*Ethyl-2-amino-7,7-dimethyl-5-oxo-4-{4-[(1-(S)-perilil-methyl)-1H-1,2,3-triazol-4-yl]methoxyphenyl}-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate (**15h**)*

Yield: 69%, pale yellow solid, m.p. 74°C-76°C. $^1$H RMN ($CDCl_3$, 400 MHz): δ 0.94 (s, 3H, —$CH_3$), 1.06 (s, 3H, —$CH_3$), 1.14 (t, J = 8.0 Hz, 3H, —$OCH_2\underline{CH_3}$), 1.39-1.49 (m, 1H, perillyl-H), 1.69 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.76-1.81 (m, 1H, perillyl-H), 1.91-2.01 (m, 3H, $(CH_3)_2C$—$\underline{CH_2}$— + perillyl-H), 2.09-2.15 (m, 2H, perillyl-H), 2.18-2.23 (m, 2H, perillyl-H), 2.39 (s, 2H, —$CH_2$—C═O), 3.92-4.00 (m, 2H, —$COOCH_2$—), 4.62 (s, 1H, ═C—CH—C═), 4.69 (d, J = 12.0 Hz, 2H, —C═$CH_2$), 4.81 (brs, 2H, —$OCH_2$—), 5.11 (s, 2H, —N—$CH_2$—C—), 5.74 (s, 1H, —C═CH—), 6.24 (s, 2H, —$NH_2$), 6.80 (d, J = 8.0 Hz, 2H, aromatic-H), 7.15 (d, J = 8.0 Hz, 2H, aromatic-H), 7.55 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 14.1, 20.6, 26.2, 26.9, 28.9, 30.3, 32.1, 32.8, 40.3, 50.5, 56.3, 59.4, 61.9, 77.2, 80.5, 108.9, 113.8, 115.4, 116.6, 122.3, 127.2, 129.1, 131.7, 138.7, 144.4, 148.9, 156.4, 158.2, 161.2, 169.0, 196.2. IR ($\nu_{max}$ $cm^{-1}$): 3405, 3311, 2957, 1689, 1381, 1036. HRMS calc. for [$C_{33}H_{40}N_4O_5$+Na]: 595.2891, HRMS found: 595.2889.

*Ethyl-2-amino-7,7-dimethyl-5-oxo-4-{3-methoxy-4-[(1-(S)-4-perillyl-me- thyl)-1H-1,2,3-triazol-4-yl] methoxyphenyl}-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate (**15i**)*

Yield: 83%, pale yellow solid, m.p. 62°C-64°C. $^1$H RMN ($CDCl_3$, 400 MHz): δ 0.99 (s, 3H, —$CH_3$), 1.10 (s, 3H, —$CH_3$), 1.18 (t, J = 8.0 Hz, 3H, —$OCH_2\underline{CH_3}$), 1.42-1.49 (m, 1H, perillyl-H), 1.73 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.79-1.84 (m, 1H, perillyl-H), 1.93-2.00 (m, 3H, $(CH_3)_2C$—$\underline{CH_2}$— + perillyl-H), 2.14-2.16 (m, 2H, perillyl-H), 2.17-2.22 (m, 2H, perillyl-H), 2.33 (d, J = 8.0 Hz, 1H, —$CH_2$—C═O), 2.37 (d, J = 8.0 Hz, 1H, —$CH_2$—C═O), 3.85 (s, 3H, —$OCH_3$), 4.02-4.08 (m, 2H, —$COOCH_2$—), 4.65 (s, 1H, ═C—CH—C═), 4.70-4.73 (m, 2H, —C═$CH_2$), 4.82 (brs, 2H, —$OCH_2$—), 5.23 (d, J = 4.0 Hz, 2H, —N—$CH_2$—C—), 5.75 (s, 1H, —C═CH—), 6.16 (s, 2H, —$NH_2$), 6.70 (dd, J = 6.0 and 2.0 Hz, 1H, aromatic-H), 6.87-6.90 (m, 2H, aromatic-H), 7.59 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 14.3, 20.7, 26.4, 27.3, 29.1, 30.4, 31.2, 32.2, 33.3, 40.5, 50.7, 55.9, 56.4, 59.6, 63.4, 77.2, 80.8, 109.0, 112.6, 113.7, 115.6, 116.8, 119.9, 122.5, 127.4, 131.8, 139.8, 144.8, 145.9, 149.1, 158.3, 161.3, 169.1, 196.5, IR ($\nu_{max}$ $cm^{-1}$): 3423, 2938, 2341, 1689, 1372, 1027. HRMS calc. for [$C_{34}H_{42}N_4O_6$+Na]: 625.2997, HRMS found: 625.2996.

*Ethyl-2-amino-7,7-dimethyl-5-oxo-4-{4-metoxi-3-[(1-(S)-perillyl-methyl)-1H-1,2,3-triazol-4-yl]methoxyphenyl}-5,6,7,8-tetrahydro-4H-chromene-3-carbo-xylate (**15j**)*

Yield: 76%, pale yellow solid, m.p. 70°C-72°C. $^1$H RMN ($CDCl_3$, 400 MHz): δ 0.99 (s, 3H, —$CH_3$), 1.09 (s, 3H, —$CH_3$), 1.17 (t, J = 8.0 Hz, 3H, —$OCH_2\underline{CH_3}$), 1.41-1.49 (m, 1H, perillyl-H), 1.72 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.77-1.82 (m, 1H, perillyl-H), 1.92-2.02 (m, 3H, $(CH_3)_2C$—$\underline{CH_2}$— + perillyl-H), 2.12-2.14 (m, 2H, perillyl-H), 2.16-2.23 (m, 2H, perillyl-H), 2.40 (d, J = 18.0 Hz, 1H, —$CH_2$—C═O), 2.54 (d, J = 18.0 Hz, 1H, —$CH_2$—C═O), 3.80 (s, 3H, —$OCH_3$), 3.99-4.09 (m, 2H, —$COOCH_2$—), 4.63 (s, 1H, ═C—CH—C═), 4.71 (d, J = 14.0 Hz, 2H, —C═$CH_2$), 4.83 (s, 2H, —$OCH_2$—), 5.24 (t, J = 14.0 Hz, 2H, —N—$CH_2$—C—), 5.74 (s, 1H, —C═CH—), 6.17 (s, 2H, —$NH_2$), 6.73 (d, J = 8.0 Hz, 1H, aromatic-H), 6.87-6.92 (m, 2H, aromatic-H), 7.63 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 14.3, 20.7, 26.3, 27.0, 27.6, 28.8, 30.4, 32.2, 33.2, 40.4, 50.7, 55.8, 56.4, 59.6, 63.1, 77.2, 80.7, 109.0, 111.0, 114.2, 116.6, 121.5, 122.7, 127.3, 131.8, 138.7, 144.5, 146.9, 147.7, 149.1, 158.2, 161.4, 169.1, 196.5. IR ($\nu_{max}$ $cm^{-1}$): 3414, 3293, 2948, 1689, 1195, 1027. HRMS calc. for [$C_{34}H_{42}N_4O_6$+Na]: 625.2997, HRMS found: 625.2999.

*2-amino-5-oxo-4-{2-[(1-(S)-perillyl-methyl)-1H-1,2,3-triazol-4-yl]methoxyphe nyl}-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**15k**)*

Yield: 79%, pale yellow solid, m.p. 132°C-133°C. $^1$H RMN ($CDCl_3$, 400 MHz): δ 1.37-1.47 (m, 1H, perillyl-H), 1.71 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.77-1.80 (m, 1H, perillyl-H), 1.92-1.96 (m, 3H, perillyl-H), 1.97-1.98 (m, 2H, perillyl-H), 2.08-2.19 (m, 2H, —$CH_2$—$\underline{CH_2}$—C—), 2.29-2.31 (m, 2H, —$CH_2$—$\underline{CH_2}$—$CH_2$—), 2.45 (s, 2H, —$CH_2$—C═O), 4.60 (s, 2H, —$NH_2$), 4.68 (s, 1H, ═C—CH—C═), 4.71 (brs, 2H, —C═$CH_2$), 4.82 (brs, 2H, —$OCH_2$—), 5.20-5.28 (m, 2H, —N—$CH_2$—C—), 5.73 (s, 1H, —C═CH—), 6.86-6.93 (m, 2H, aromatic-H), 7.09-7.16 (m, 2H, aromatic-H), 7.76 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 20.1, 20.6, 26.2, 26.8, 26.9, 30.3, 30.6, 30.6, 36.7, 40.3, 56.3, 61.7, 61.7, 61.9, 108.9, 112.1, 113.9, 121.0, 127.1, 128.2, 129.2, 131.2, 131.8, 131.9, 149.0, 155.5, 158.0, 164.0, 196.1 IR ($\nu_{max}$ $cm^{-1}$): 3396, 3311, 3181, 2938, 2192, 1661, 1362, 1204. HRMS calc. for [$C_{29}H_{31}N_5O_3$+Na]: 520.2319, HRMS found: 520.2322.

*2-amino-5-oxo-4-{3-[(1-(S)-perillyl-methyl)-1H-1,2,3-triazol-4-yl]methoxyphe nyl}-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**15l**)*

Yield: 81%, pale yellow solid, m.p. 146°C-148°C. $^1$H-RMN ($CDCl_3$, 400 MHz): δ 1.40-1.50 (m, 1H, perillyl-H), 1.72 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.80-1.83 (m, 1H, perillyl-H), 1.88-1.96 (m, 2H, perillyl-H), 1.99-2.04 (m, 3H, perillyl-H), 2.11-2.21 (m, 2H, —$CH_2$—$\underline{CH_2}$—C—), 2.29-2.40 (m, 2H, —$CH_2$—$\underline{CH_2}$—$CH_2$—), 2.49-2.66 (m, 2H, —$CH_2$—C═O), 4.38 (s, 1H, ═C—CH—C═), 4.69 (s, 2H, —$NH_2$), 4.74 (d, J = 16.0 Hz, 2H, —C═$CH_2$), 4.84 (brs, 2H, —$OCH_2$—), 5.19 (s, 2H, —N—$CH_2$—C—), 5.76 (s, 1H, —C═CH—), 6.81-6.89 (m, 3H, aromatic-H), 7.20 (t, J = 8.0 Hz, 1H, aromatic-H), 7.61 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 20.0, 20.7, 20.7, 26.3, 26.9, 27.1, 30.4, 35.3, 36.7, 40.4, 56.4, 62.0, 62.8, 62.8, 109.0, 113.3, 114.2, 114.9, 120.6, 122.6, 127.3, 129.5, 131.8, 145.0, 149.0, 157.6, 158.3, 163.5, 195.9. IR ($\nu_{max}$ $cm^{-1}$): 3451, 3321, 3200, 2929, 2174, 1679, 1353, 1008. HRMS calc. for [$C_{29}H_{31}N_5O_3$+Na]: 520.2319, HRMS found: 520.2327.

*2-amino-5-oxo-4-{4-[(1-(S)-perillyl-methyl)-1H-1,2,3-triazol-4-yl]methoxyphe nyl}-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**15m**)*

Yield: 77%, pale yellow solid, m.p. 129°C-130°C. $^1$H RMN ($CDCl_3$, 400 MHz): δ 1.41-1.51 (m, 1H, perillyl-H), 1.73 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.80-1.84 (m, 1H, perillyl-H), 1.94-1.99 (m, 3H, perillyl-H), 2.00-2.07 (m, 2H, perillyl-H), 2.11-2.23 (m, 2H, —$CH_2$—$\underline{CH_2}$—C—), 2.29-2.41 (m, 2H, —$CH_2$—$\underline{CH_2}$—$CH_2$—), 2.51-2.65 (m, 2H, —$CH_2$—C═O), 4.39 (s, 1H, ═C—CH—C═), 4.61 (s, 2H, —$NH_2$), 4.72 (d, J = 16.0 Hz, 2H, —C═$CH_2$), 4.85 (s, 2H, —$OCH_2$—), 5.17 (s, 2H, —N—$CH_2$—C—), 5.77 (s, 1H, —C═CH—), 6.91 (d, J = 8.0 Hz, 2H, aromatic-H), 7.17 (d, J = 8.0 Hz, 2H, aromatic-H), 7.61 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 20.1, 20.7, 26.3, 27.0, 27.1, 29.7, 30.4, 34.6, 36.8, 40.4, 56.5, 62.2, 62.3, 63.6, 109.0, 114.8, 115.4, 127.4, 128.7, 131.8, 136.1, 149.1, 156.6, 157.3, 163.0, 164.7, 196.1. IR ($\nu_{max}$ $cm^{-1}$): 3340, 3134, 2938, 2341, 2174, 1670, 1353. HRMS calc. for [$C_{29}H_{31}N_5O_3$+Na]: 520.2319, HRMS found: 520.2314.

*2-amino-5-oxo-4-{3-methoxy-4-[(1-(S)-perillyl-methyl)-1H-1,2,3-triazol-4-yl]me thoxylphenyl}-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**15n**)*

Yield: 84%, pale yellow solid, m.p. 118°C-120°C. $^1$H RMN ($CDCl_3$, 400 MHz): δ 1.38-1.49 (m, 1H, perillyl-H), 1.71 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.77-1.81 (m, 1H, perillyl-H), 1.87-1.95 (m, 3H, perillyl-H), 1.98-2.07 (m, 2H, perillyl-H), 2.09-2.20 (m, 2H, —$CH_2$—$\underline{CH_2}$—C—), 2.28-2.41 (m, 2H, —$CH_2$—$\underline{CH_2}$—$CH_2$—), 2.50-2.61 (m, 2H, —$CH_2$—C═O), 3.86 (s, 3H, —$OCH_3$), 4.35 (s, 1H, ═C—CH—C═), 4.68-4.72 (m, 2H,—C═$CH_2$), 4.77 (s, 2H, —$NH_2$), 4.81 (brs, 2H, —$OCH_2$—), 5.23 (s, 2H, —N—$CH_2$—C—), 5.74 (s, 1H, —C═CH—), 6.65 (dd, J = 6.0 and 2.0 Hz, 1H, aromatic-H), 6.85 (d, J = 2.0 Hz, 1H, aromatic-H), 6.93 (d, J = 8.0 Hz, 1H, aromatic-H), 7.58 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 20.1, 20.7, 26.3, 26.8, 26.9, 27.0, 30.3, 34.8, 36.7, 40.4, 55.9, 56.4, 62.8, 63.2, 108.9, 111.7, 111.8, 114.0, 114.1, 115.1, 119.1, 127.4, 131.7, 137.1, 146.4, 146.5, 149.0, 149.1, 157.5, 163.2, 196.2, IR ($\nu_{max}$ $cm^{-1}$): 3368, 2957, 2201, 1679, 1362, 1008, HRMS calc. for [$C_{30}H_{33}N_5O_4$+Na]: 550.2425, HRMS found: 550.2419.

*2-amino-5-oxo-4-{4-methoxy-3-[(1-(S)-perillyl-methyl)-1H-1,2,3-triazol-4-yl]me thoxylphenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**15o**)*

Yield: 82%, pale yellow solid, m.p. 83°C-84°C. $^1$H RMN ($CDCl_3$, 400 MHz): δ 1.37-1.47 (m, 1H, perillyl-H), 1.70 (s, 3H, $H_2C$═C—$\underline{CH_3}$), 1.77-1.80 (m, 1H, perillyl-H), 1.89-1.97 (m, 3H, perillyl-H), 1.97-2.03 (m, 2H, perillyl-H), 2.08-2.18 (m, 2H, perillyl-H), 2.29-2.35 (m 2H, —$CH_2$—$\underline{CH_2}$—C—), 2.48-2.56 (m, 2H, —$CH_2$—$\underline{CH_2}$—$CH_2$—), 2.66-2.71 (m, 2H, —$CH_2$—C═O), 3.81 (s, 3H, —$OCH_3$), 4.31 (s, 1H, ═C—CH—C═), 4.69 (d, J = 14.0 Hz, 2H, —C═$CH_2$), 4.80 (s, 2H, —$NH_2$), 4.83 (s, 2H, —$OCH_2$—), 5.28 (d, J = 2.0 Hz, 2H, —N—$CH_2$—C—), 5.72 (s, 1H, —C═CH—), 6.78 (d, J = 8.0 Hz, 1H, aromatic-H), 6.87 (d, J = 8.0 Hz, 2H, aromatic-H), 7.59 (s, 1H, —C═CH—N—). $^{13}$C RMN ($CDCl_3$, 100 MHz): δ 20.0, 20.6, 26.3, 27.0, 27.0, 30.3, 34.8, 36.7, 40.4, 55.8, 56.4, 62.8, 62.9, 108.9, 111.4, 113.5, 114.8, 118.9, 120.9, 122.9, 127.3, 131.7, 136.0, 144.2, 147.2, 148.4, 149.0, 157.6, 163.4, 196.2, IR

($\nu_{max}$ $cm^{-1}$): 3423, 2929, 2341, 2183, 1652, 1567, 1148. HRMS calc. for [$C_{30}H_{33}N_5O_4$+Na]: 550.2425, HRMS found: 550.2422.

## 4.2 | Biologic assays

### 4.2.1 | Culture cell

For this work, was utilized the human hepatocarcinoma cell line HepG2/C3a, acquired from the Bank of Cells of Rio de Janeiro (BCRJ), free of mycoplasma was used. Cells were cultured in DMEM medium (Gibco), supplemented with 10% fetal bovine serum (Gibco) and antibiotic/antimycotic (Gibco), and maintained at 37°C, 5% $CO_2$, and 95% relative humidity.

### 4.2.2 | Cytotoxicity assay

Cytotoxicity was assessed by the MTT assay with $2.5 \times 10^4$ cells per well, were seeded in a 96 well culture dish and left for 24 hours for cell stabilization. After this time the cells were treated with compounds **15a–j** for 24 h.

After the incubation time, the compounds were removed, and the MTT solution (0.5 mg/mL, Invitrogen) was added and the plates were incubated for 4 hours in an oven at 37°C. Subsequently, the MTT solution was removed, the formazan crystals were dissolved in DMSO and the plates read in spectrophotometer (TP Reader, Thermo Plate) at 540 nm. The treatments were done at concentrations of 20, 40, 60, 80, and 100 μM, respectively. The percentage of cell viability was calculated by the formula (Treated Absorbance/Control Absorbance) × 100. The compound camptothecin (2 μM) was used as reference.

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