



# Novel molecules containing structural features of NSAIDs and 1,2,3-triazole ring: Design, synthesis and evaluation as potential cytotoxic agents

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## ABSTRACT

For the first time the template containing structural features of more than one NSAIDs and the 1,2,3-triazole ring was explored for the identification of potential cytotoxic agents. These new and complex molecules were predicted to be effective inhibitors of PDE4B by molecular modelling studies *in silico*. The multi-step synthesis of these compounds were carried out starting from the well-known drug nimesulide and involved the use of copper-catalyzed azide-alkyne cycloaddition (CuAAC) approach as the key step. Mainly two types of compounds e.g. 1-aryl-1H-1,2,3-triazoles and N-aryl-2-(1H-1,2,3-triazol-1-yl)acetamide derivatives were synthesized by using this method in good yields. The *in vitro* screening of these compounds against two cancer cell lines e.g. HCT-15 (human colon cancer cell line) and NCI-H226 (human lung cancer cell line) using a colorimetric MTT assay allowed identification of two preliminary hit molecules i.e. **8a** and **8f**. The SAR (Structure Activity Relationship) analysis indicated that the presence of an amide linker between the aryl ring and the 1,2,3-triazole moiety was favorable for the activities. The compound **8a** and **8f** showed significant inhibition of PDE4B *in vitro* and good interactions with this protein *in silico* suggesting PDE4B as their potential target. The usefulness and concerns of these molecules in the light of computational ADME prediction were analyzed. Overall, novel molecules were identified as potential cytotoxic agents for further study.

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## 1. Introduction

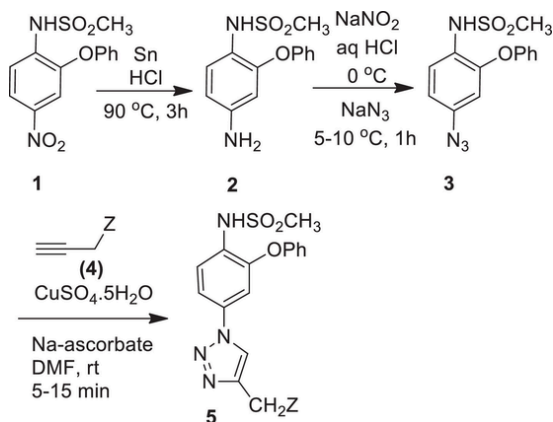
Assembly of two or more frameworks in a single molecular entity (molecular hybridization) has become an interesting strategy in the identification of promising new chemical entities (NCEs) or bioactive agents in various therapeutic areas including cancer. Triazole being bio-isosters of amide, ester and carboxylic acid in addition to its ability to mimic various amino acids has been explored widely for this purpose [1]. Till date numerous polyfunctionalized or densely substituted molecules containing the 1,2,3-triazole framework has been reported for the identification of various bioactive compounds including the antiproliferative agents. For example, molecules based on C-14 dehydroabiatic acid (DHAA)-1,2,3-triazole hybrid (A, Fig. 1) were synthesized and evaluated against SK-OV-3 (ovary), PC-3 (prostate), MDA-MB-231 (breast) and MCF-7 (breast) cancer cell lines [2]. Similarly, molecules

based on diphenyl-1H-pyrazole-1,2,3-triazole hybrid linked through acrylate moiety (B, Fig. 1) were explored as apoptosis inducing cytotoxic and anti-inflammatory agents [3]. Molecules based on benzo-suberone-1,2,3-triazole hybrid (C, Fig. 1) were evaluated for their anti-proliferative activities [4]. Notably, besides the presence of 1,2,3-triazole ring all these molecules contain either an amide or ester as a linker that allowed successful hybridization of different frameworks. Nevertheless, the molecular hybridization of more than two frameworks is rather uncommon in the literature. On the other hand, due to our interest in the identification of potential anti-proliferative agents we have explored the nimesulide-1,2,3-triazole hybrid (e.g. D, Fig. 2) previously [5]. In further continuation of this research we also reported the synthesis and biological evaluation of complex molecules [6-8] containing 1,2,3-triazole ring along with the framework of certain NSAIDs (Nonsteroidal anti-inflammatory drugs). Indeed, the PDE4B (Phosphodiesterase 4B) inhibition along with cytotoxic properties of a series of 2,2,4-trimethyl-1,2-dihydroquinolonyl substituted 1,2,3-triazole derivatives were evaluated previously [9]. Prompted by the earlier report on

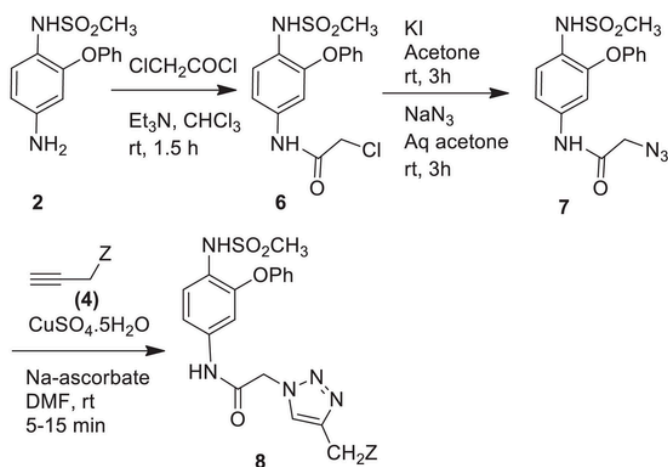
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E-mail address: [sarbani277@yahoo.com](mailto:sarbani277@yahoo.com) (S. Pal).



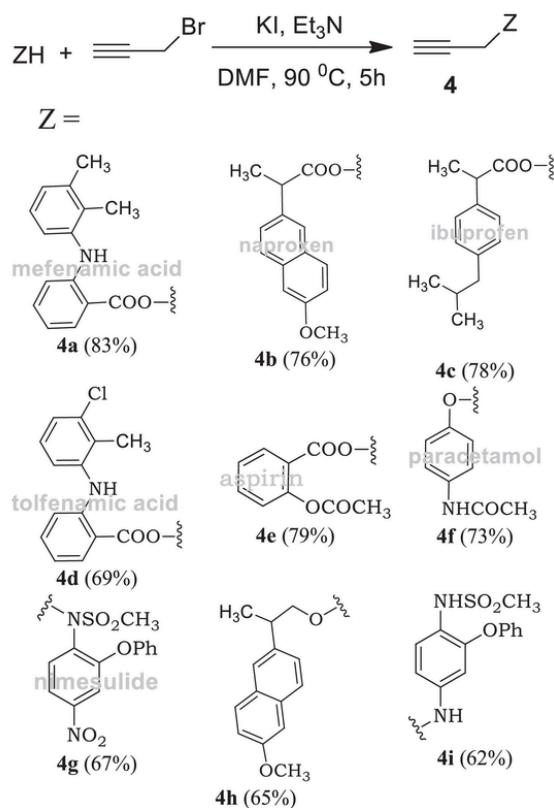


**Scheme 1.** Synthesis of 1-aryl-1H-1,2,3-triazole derivatives **5** based on **E** (Fig 2).



**Scheme 2.** Synthesis of *N*-aryl-2-(1H-1,2,3-triazol-1-yl)acetamide derivatives **8** based on **F** (Fig 2).

the construction of a 1,2,3-triazole ring is commonly performed by using the click chemistry approach that involves copper (I)-catalyzed 1,3-cycloaddition reaction between a terminal alkyne and an azide [17]. Being known as copper-catalyzed azide-alkyne cycloaddition (CuAAC) this approach has found enormous applications in organic synthesis and medicinal chemistry [1-9]. This CuAAC based approach has been used as a key step in the current synthesis of target compounds. The required azide **3** and **7** were synthesized from nimesulide (**1**). Thus the aniline derivative (**2**) obtained from **1** via reduction of its nitro group [18] was transformed into the azide **3** following the known and traditional method (Scheme 1). In another effort the aniline **2** was *N*-acylated using chloroacetyl chloride [19] to give the compound **6** that on treatment with KI followed by NaN<sub>3</sub> afforded the azide **7** (Scheme 2). These azides were separately coupled with an appropriate terminal alkyne (**4**) under CuAAC conditions. A mixture of CuSO<sub>4</sub>·5H<sub>2</sub>O and sodium ascorbate was used as a pre-catalyst system in this coupling reaction to generate the Cu(I) species *in situ* that actually catalyzed the reaction. The required terminal alkynes (**4a-i**) were prepared *via* the reaction of propargyl bromide with various drugs e.g. mefenamic acid, naproxen, ibuprofen, tolfenamic acid, acetylsalicylic acid (aspirin), nimesulide, and acetaminophen (paracetamol) or compound **2** or a reduced product of naproxen in DMF at 90 °C (Scheme 3). All the terminal alkynes obtained were employed to react with the azide **3** or **7** under the CuAAC conditions separately and individually. The duration of the reaction was 5-15 min depending on the nature and type of terminal alkynes used. The reaction proceeded well in all these cases affording the de-



**Scheme 3.** Preparation of alkyne **4**.

sired product in good yield (Table 1). Thus a variety of compound **5** and **8** were prepared using the method described in Schemes 1 and 2.

All the synthesized compounds were characterized by spectral (NMR, IR and MS) data. For example, <sup>1</sup>H NMR spectra of a representative compound **5c** (Fig. 4A) showed (i) a doublet at δ 0.87 (for 2CH<sub>3</sub>) and a quintet at δ 1.81 (for CH) due to the *i*-propyl group, (ii) a doublet at δ 2.41 due to the CH<sub>2</sub> protons between the *i*-propyl and phenyl group, (iii) a doublet at δ 1.48 and a quartet at δ 3.71 due to the CH<sub>3</sub> group attached to the benzylic carbon and the benzylic proton, (iv) one singlet at δ 3.08 due to the -SO<sub>2</sub>CH<sub>3</sub> protons and (v) one singlet at δ 5.24 and δ 7.01 due to the CH<sub>2</sub> group attached to the triazole ring and the triazole ring proton. In the <sup>13</sup>C NMR spectra, the Me of *i*-propyl group and the methanesulfonamide moiety and that attached to the benzylic carbon appeared at 22.4, 39.9 and 18.3 ppm. Further, the CH<sub>2</sub>, OCH<sub>2</sub>, and -O-C=O group appeared at 44.9, 57.8 and 174.6 ppm, respectively. A similar <sup>1</sup>H and <sup>13</sup>C NMR spectral analysis can be presented for another compound **8c** (Fig. 4B).

Next, the synthesized compounds were tested *in vitro* against two cancer cell lines e.g. HCT-15 (human colon cancer cell line) and NCI-H226 (human lung cancer cell line) using a colorimetric MTT assay. The well-known anticancer agent doxorubicin was used as a reference compound in this assay. The results of active compounds that showed moderate to good effects are presented in Table 2. Among them the compound **8a** was found to be most active against HCT-15 whereas the compound **8f** was identified as the best active agent against NCI-H226 cell line. Notably, **8a** was not so effective against NCI-H226 whereas **8f** was least effective against HCT-15 indicating their selectivity towards the particular cancer cell line. Among the rest of the compounds, **5d** and **5e** showed some effects against HCT-15 and NCI-H226, respectively. From the viewpoint of SAR (Structure Activity Relationship) the *N*-aryl-2-(1H-1,2,3-triazol-1-yl)acetamide derivatives (**8**) appeared as a better active class of compounds over 1-aryl-1H-1,2,3-triazole derivatives (**5**) suggesting that the presence of amide linker between the aryl ring and the 1,2,3-triazole moiety was favorable for the observed activi-



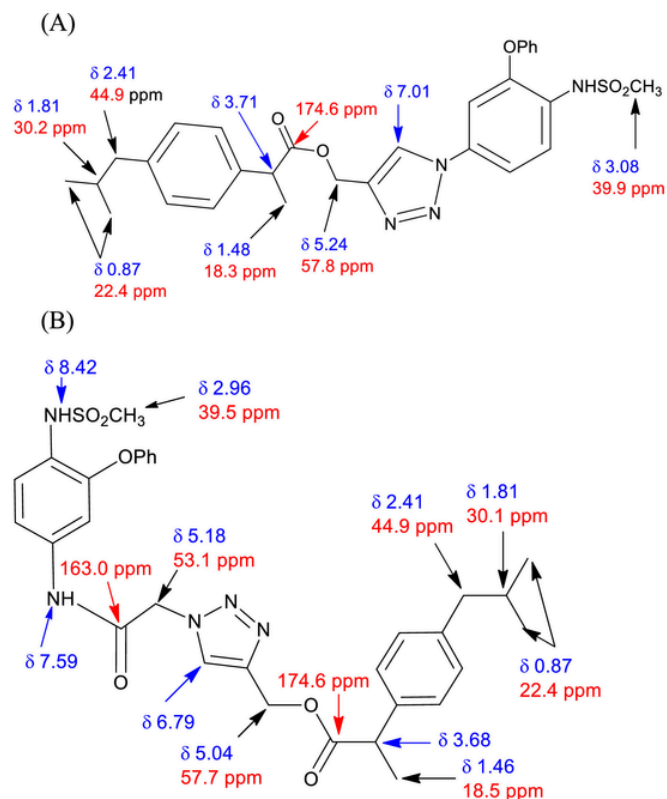


Fig. 4. Partial representation of <sup>1</sup>H and <sup>13</sup>C NMR spectral data of compound (A) 5c and (B) 8c.

Table 2

The % control growth of cancer cells at 10<sup>-4</sup> M concentration of test compounds.

Compound	HCT-15	NCI-H226
5c	53.6	57.9
5d	50.7	59.0
5e	57.7	51.6
8a	44.1	52.3
8b	55.2	69.1
8f	65.2	28.3
Doxorubicin	23.1	27.6

Values are average of three independent determinations

The molecule also participated in several van der Waals / hydrophobic contacts within the cut-off distance of 4 Å among which the contact with PHE678 (a key residue in the CR3 region) is mention worthy. The other noticeable hydrophobic contacts of **8f** were observed (i) with the hydrophobic residues like ILE582, PHE586, PHE618, MET603, LEU674, MET519 etc and (ii) with the hydrophobic regions of polar residues e.g. SER454, ASN455, SER520, ASN567 etc. Notably, the reference compound rolipram participated in two H-bonds with GLN615 and HIS406 at a distance of 2.9 and 3.09 Å (the second one being somewhat weaker H-bond), and two aromatic pi interactions with PHE618 and PHE586 (Fig. 6, see also Fig S-3 in suppl data). Additionally, it participated in hydrophobic contacts (at a distance cut-off 4 Å) mainly with residues PHE678, TYR405, MET519, THR579, ASN567, etc.

Having identified the compound **8a** and **8f** as potential hits it was necessary to conducted some initial assessment about ADME (absorption, distribution, metabolism, and excretion) or pharmacokinetic properties of these compounds and compared with that of rolipram. Thus the computational ADME prediction of these compounds was carried out using Swiss ADME web-tool [21] and results are summarized in Table 3 (among the various descriptors only notable one are listed in the table). Because of relatively high molecular weight it was expected

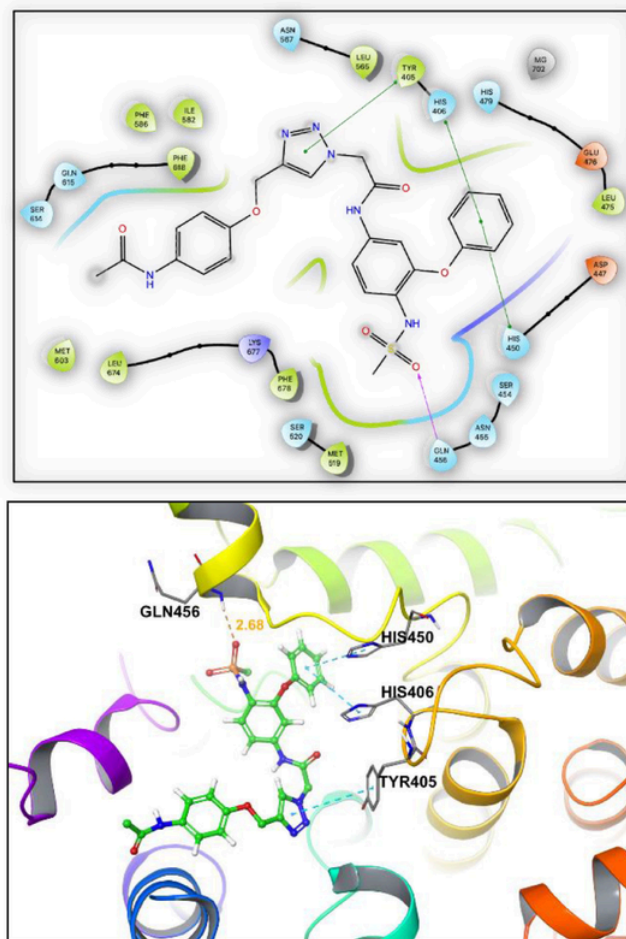


Fig. 5. The 2D (H-bond presented in magenta and pi-pi in green color) and 3D interaction diagrams (where H-bond and pi-pi stacking are shown in orange and cyan dashed lines, respectively) of molecule **8f** with PDE4B (PDB ID: 4MYQ) prepared using Maestro visualizer (Schrödinger, LLC).

that unlike rolipram these molecules would not show favorable drug likeness and high GI absorption. However, this type of concerns could be found for a number of drugs like macrolide antibiotics or anticancer drug Taxol that still entered into the market and are currently in patient's use. Moreover, both **8a** and **8f** were predicted to be a non-penetrant of BBB. Finally, a non-Pgp substrate was predicted for **8a** though not for **8f**.

### 3. Conclusions

In conclusion, novel molecules possessing structural features of more than one NSAIDs and the 1,2,3-triazole ring was explored for the identification of potential cytotoxic agents. These new and complex molecules were predicted to be effective inhibitors of PDE4B by molecular modelling studies *in silico*. The multi-step synthesis of these compounds were carried out starting from the well-known anti-inflammatory drug nimesulide and involved the use of copper-catalyzed azide-alkyne cycloaddition (CuAAC) approach as the key step. Mainly two types of compounds e.g. 1-aryl-1H-1,2,3-triazoles and N-aryl-2-(1H-1,2,3-triazol-1-yl)acetamide derivatives were synthesized by using this method in good yields. The *in vitro* screening of these compounds against two cancer cell lines e.g. HCT-15 (human colon cancer cell line) and NCI-H226 (human lung cancer cell line) using a colorimetric MTT assay allowed identification of two preliminary hit molecules i.e. **8a** and **8f**. The SAR (Structure Activity Relationship) analysis indicated that the presence of an amide linker between the aryl ring and

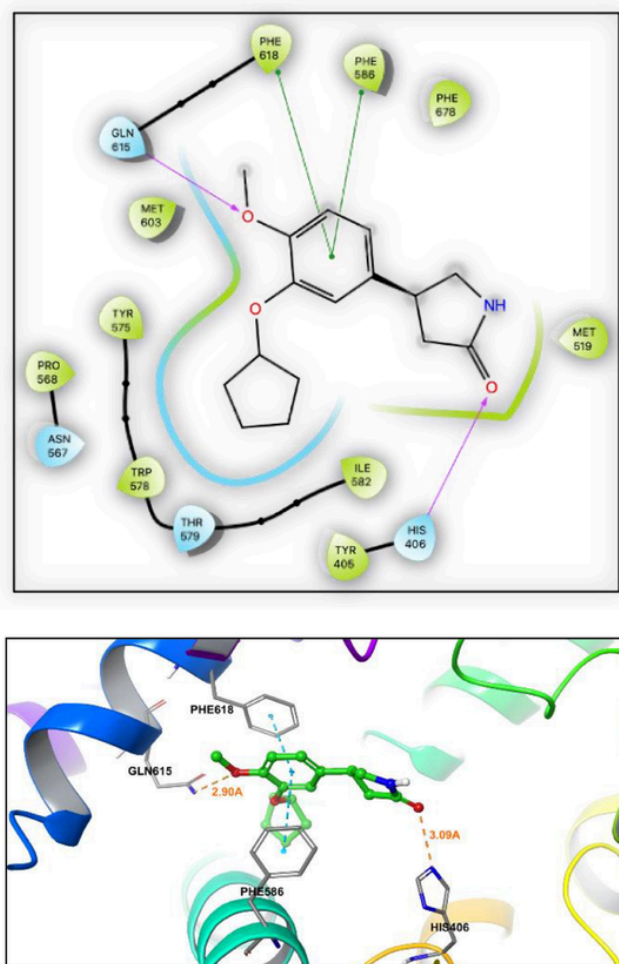


Fig. 6. 2D followed by 3D interaction diagram of reference compound rolipram with PDE4B (PDB ID: 4MYQ).

the 1,2,3-triazole moiety was favorable for the activities. The compound **8a** and **8f** showed significant inhibition of PDE4B *in vitro* and good interactions with this protein *in silico* suggesting PDE4B as their potential target. The usefulness and concerns of these molecules in the light of computational ADME prediction were analysed and discussed. Overall, the current research involving the synthesis and identification of novel molecules as potential cytotoxic agents for further study would attract interest.

#### Author statement

Jyoti Mareddy was involved in the preparation, isolation, purification and characterization of all the target compounds presented in the current manuscript.

Kazi Amirul Hossain, N. Sudhakar Yadav and Venkanna Banothu were involved in performing all the *in silico* studies as well as *in vitro* assays.

Jaya Shree Anireddy and Sarbani Pal were responsible for conceptualization, coordination and overall supervision of the entire work presented in the submitted manuscript.

#### Supplementary data

Supplementary data associated with this article can be found, in the on line version, at xxxxxxxxx

Table 3

Computational ADME prediction of **8a**, **8f** and the reference compound rolipram.

Properties	Molecules		
(i) Physicochemical	<b>8a</b>	<b>8f</b>	rolipram
Molecular Weight (g/mol)	640.71	550.59	275.34
Consensus Log P <sup>a</sup>	4.28	2.08	2.44
Log S (ESOL) <sup>b</sup>	-6.75 (poorly soluble)	-4.00 (moderately soluble)	-2.90 (soluble)
(ii) Pharmacokinetics			
GI <sup>c</sup> absorption	Low	Low	High
BBB <sup>d</sup> penetration	No	No	Yes
P-gp <sup>e</sup> substrate	No	Yes	Yes
(iii) Drug likeness			
Lipinski rule	No; 2 violations: MW <sup>f</sup> > 500, N or O <sup>g</sup> > 10	No; 2 violations: Rotors <sup>h</sup> > 10, TPSA <sup>i</sup> > 140	No violation
Veber rule	No; 2 violations: MW > 500, N or O > 10	No; 2 violations: Rotors > 10, TPSA > 140	No violation

<sup>a</sup> Log P: Lipophilicity.

<sup>b</sup> Log S (ESOL): water solubility, calculated by ESOL method which is a Quantitative Structure-Property Relationship (QSPR) based model.

<sup>c</sup> GI: Gastrointestinal.

<sup>d</sup> BBB: Blood Brain Barrier

<sup>e</sup> P-gp: permeability glycoprotein.

<sup>f</sup> MW: molecular weight,

<sup>g</sup> N or O: Nitrogen/Oxygen indicates number of H-bond acceptor,

<sup>h</sup> Rotors: total number of rotatable bonds,

<sup>i</sup> TPSA: Total polar surface area.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.131222.

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