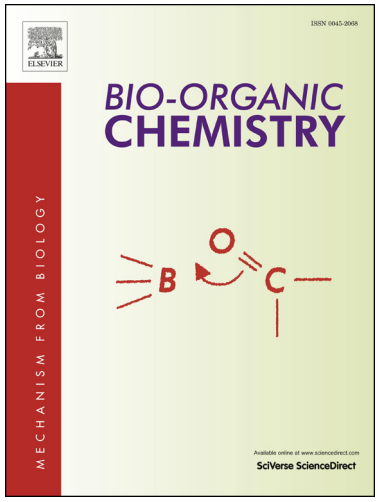

Preliminary Communications

Novel alkynyl substituted 3,4-dihydropyrimidin-2(1*H*)-one derivatives as potential inhibitors of chorismate mutase

V. Mallikarjuna Rao, P. Mahesh Kumar, D. Rambabu, Ravikumar Kapavarapu, S. Shobha Rani, Parimal Misra, Manojit Pal

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Novel alkynyl substituted 3,4-dihydropyrimidin-2(1*H*)-one derivatives as potential inhibitors of chorismate mutase

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Abstract: A series of novel alkynyl substituted 3,4-dihydropyrimidin-2(1*H*)-one (DHPM) derivatives were designed, synthesized and evaluated *in vitro* as potential inhibitors of chorismate mutase (CM). All these compounds were prepared *via* a multi-component reaction (MCR) involving sequential I_2-mediated Biginelli reaction followed by Cu-free Sonogashira coupling. Some of them showed promising inhibitory activities when tested at 30 µM. One compound showed dose dependent inhibition of CM with IC_{50} value of 14.76 ± 0.54 µM indicating *o*-alkynylphenyl substituted DHPM as a new scaffold for the discovery of promising inhibitors of CM.

Keywords: pyrimidinone, alkyne, iodine, palladium, chorismate mutase

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1. **Introduction**

Dihydropyrimidinone (DHPM) framework has been found to be integral part of several biologically active marine alkaloids [1-4]. Among them, the most potent are the crambine [1] and batzelladine [2]. In addition to their potent HIV gp-120 CD4 inhibitory activities [1,5], these compounds also showed antiviral, antitumor, antibacterial and anti-inflammatory activities [6,7]. Among various DHPM derivatives known the 4-substituted 3,4-dihydropyrimidin-2(1*H*)-ones have attracted particular attention due to their wide range of biological activities [7]. They have a similar pharmacological profile to that of classical dihydropyridine based calcium channel modulators [8-10]. Nevertheless, DHPMs have attracted our attention because of the fact that several DHPMs have been examined for their antimicrobial properties in the recent years [11-13]. Structurally, all these derivatives differed with respect to the substituents on the two key positions i.e. C-4 and C-5 positions of the dihydropyridine ring.

Despite the availability of a range of effective antibiotics and drugs, the threat of infectious diseases or death due to microbial infections, still poses considerable health problems. Tuberculosis (TB) still remains a leading cause of death worldwide due to a number of factors such as (a) long duration of treatment (6–9 months), (b) increased incidence of (multi or extensive) drug resistance, (c) co-morbidity with HIV-AIDS and (d) declined effort in anti-infective drug discovery research. Indeed, TB kills more than two million people a year worldwide. Thus the discovery [14], development and introduction of new treatments for tuberculosis have become an essential goal of current pharmaceutical research. *Mycobacterium tuberculosis* chorismate mutase (*MtbCM or CM) catalyzes the rearrangement of chorismate to prephenate in the biosynthetic pathway to form phenylalanine and tyrosine after chorismate being formed by the action of chorismate synthase, an enzyme of the shikimate pathway main trunk (Fig. 1). In bacteria, MtbCM plays a key role in the synthesis of aromatic amino acids necessary for the survival of organism and therefore inhibition of MtbCM may hinder the supply of nutrients to the organism. Due to the absence of this pathway in animals but not in bacteria CM is considered as a promising target for the identification of new drugs [15]. However, only a few small molecules have been reported to possess inhibitory activity against CM [16-18]. In continuation of our efforts on the identification of novel inhibitors of CM [19-24] we became interested in evaluating the library of small molecules based on 4-substituted 3,4-dihydropyrimidin-2(1*H*)-ones **B** derived from known DHPM framework **A** (Fig. 2). We anticipated that introduction of an alkynyl moiety possessing an appropriate polar / functional

Insert **Fig. 1** here

Insert **Fig 2** here**.**

Insert **Fig. 3** here**.**

group at the *o*-position of the C-4 aryl ring may favor the interaction of the resulting molecule with the CM protein. This was supported by the docking studies of a representative molecule **C** (glide score = -17.23) which showed H-bonding interactions involving the OH group of the alkynyl side chain in addition to the two C=O moieties (of the central ring and ester) with Arg49, Lys60, Glu109 and Gln76 residues of the chorismate mutase protein (Fig. 3). Notably, shifting of the alkynyl moiety from *o*- to *m*- or *p*- position or its complete removal decreased the interactions with the protein *in silico*. These observations strengthened our initial thought on focusing on **B** containing alkynyl moiety at the *o*-position of the C-4 aryl ring. Herein we report our preliminary findings on the CM inhibiting properties of a series of alkynyl substituted DHPM derivatives *in vitro*. To the best of our knowledge this is the first report that discloses DHPM derivatives as potential inhibitors of CM.

2. Results and discussion

2.1. Chemistry

Generally, DHPM derivatives are conveniently prepared *via* a widely used multi component reaction (MCR), called Biginelli reaction [25] that involves the cyclocondensation of an aldehyde, a *β*-ketoester, and urea (or thiourea) to give dihydropyrimidin-2(1*H*)-ones (or thio analogues) [6,7]. A large number of reports are now available on Biginelli reaction [9] to improve the reaction conditions and product yields along with variations in all three reactants. Recently, we have explored further application of Biginelli reaction beyond three component limit. Thus, an overall four component reaction was developed involving a sequential phosphorous acid-mediated Biginelli followed by Cu-free Sonogashira or Heck or Suzuki reaction [26]. The use of elemental iodine on the other hand has been explored for successful Biginelli reaction earlier [27]. In view of the easy availability and inexpensive as well as safer

Insert **Scheme 1** here**.**

nature of I_2 we modified our earlier strategy by replacing the phosphorous acid with I_2 and found that the new strategy worked well (vide infra). We therefore used this modified method to synthesize our target alkynyl derivatives **B** (or **5**, Scheme 1) that were designed for the potential inhibition of CM. Thus, a mixture of aldehyde **1** (1.0 mmol), ethyl or *t*-butyl or methyl acetoacetate **2** (5.0 mL), urea **3** (1.25 mmol) and I_2 (10 mol %) was stirred at room temperature for 5 min. Subsequently, the reaction mixture was heated to 100-120 °C for 1-2 h and then cooled to 50 °C. To this was added pyrrolidine (3 mL), $PdCl_2(PPh_3)_2$ (2 mol%) and the alkyne **4** (1.5 mmol) with stirring and the mixture was stirred at 80-85 °C for 1-2 h. After usual work up and purification the desired product **5** was isolated in good yield. All the compounds synthesized were well characterized by IR absorbsions near 2227 (-C≡C-), 1700 (C=O) and 1650 (C=O) cm^{-1} and appearance of a peak near δ 5.6 corresponding to C-4 hydrogen [24].

2.2. Pharmacology

All the synthesized DHPM derivatives were tested for their inhibitory potential against *Mycobacterium tuberculosis* H37Rv chorismate mutase (CM). The assay [28,29] involved determination of activity of enzyme CM which catalyzes the conversion of chorismate to prephenate. Thus determination of activity of CM is based on the direct observation of conversion of chorismic acid to prephenate spectrophotometrically at OD_{274}. This reaction was performed in the presence of test compounds to determine their CM inhibiting activities. A known inhibitor of CM i.e. 4-(3,5-dimethoxyphenethylamino)-3-nitro-5-sulfamoylbenzoic acid [16] was prepared and used as a reference compound the IC_{50} value of which was found to be less than 10 µM. Compounds **5c, 5d, 5e** and **5f** showed 60, 41, 63 and 57% inhibition of CM compared to other molecules when tested (see ESI for the procedure) at 30 µM (Table 1) whereas rest of the compounds were either less active or inactive. Notably, compounds containing alkynyl side chain possessing a hydroxy group showed superior activities than

others. A hydrophobic chain or a bulky group at the alkynyl moiety was found to be unfavorable for activities. In general, a bulky ester group attached to the central DHPM ring (e.g. **5k-m**) was also found to be unfavorable. Based on the preliminary data generated compound **5e** was chosen for further evaluation *in vitro*. In a dose response study, compound **5e** showed inhibition of CM in a dose dependent manner with an IC_{50} value of 14.76 ± 0.54 µM (Fig. 4). Thus, alkynyl substituted DHPM framework appeared as a new scaffold for the development of novel inhibitors of chorismate mutase. Since tuberculosis is a leading cause of death worldwide, the present class of compounds are of further interest as potential antitubercular agents.

Insert **Table 1** here.

Insert **Fig. 4** here.

3. Conclusions

In conclusion, we have described the design, synthesis and *in vitro* evaluation of novel *o*-alkynylphenyl substituted 3,4-dihydropyrimidin-2(1*H*)-one derivatives as potential inhibitors of chorismate mutase. A series of small molecules synthesized *via* a MCR involving sequential iodine-mediated solvent-free Biginelli reaction followed by copper-free Sonogashira coupling in a single pot were screened against CM. Some of them showed promising inhibitory activities when tested at 30 µM and one compound showed dose dependent inhibition of CM with IC_{50} value of 14.76 ± 0.54 µM. Overall, this research has identified alkynyl substituted DHPM as a new scaffold for the development of new inhibitors of chorismate mutase.

Acknowledgements

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Figure captions

Fig. 1. Conversion of chorismate to prephanate by CM in the shikimate pathway

Fig 2. Design of DHPM based novel inhibitors of chorismate mutase

Fig. 3. Docking of molecule **C** into CM (PDB code-2FP2)

Fig. 4. Dose dependent inhibition of CM by the compound **5e**

Figures

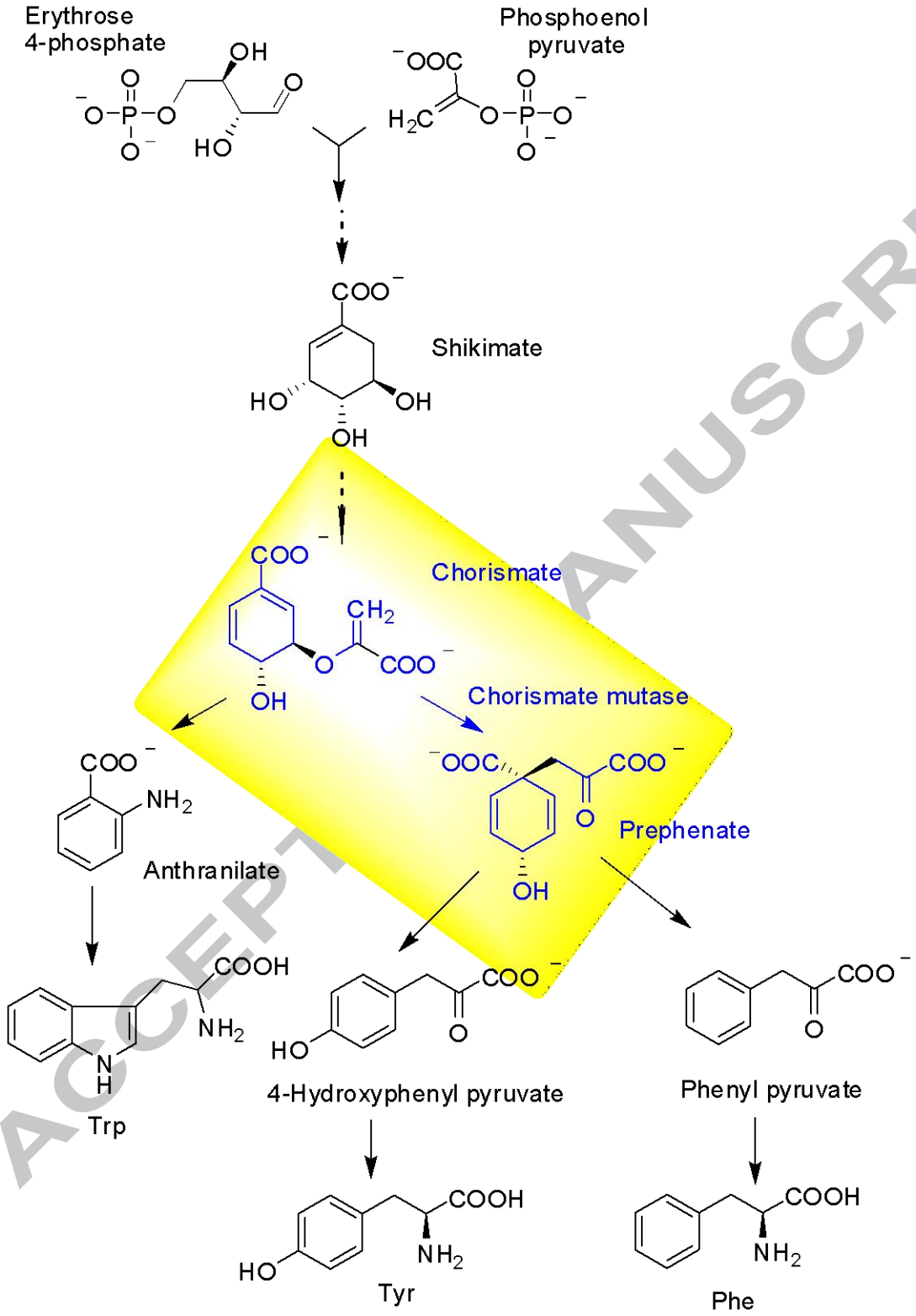
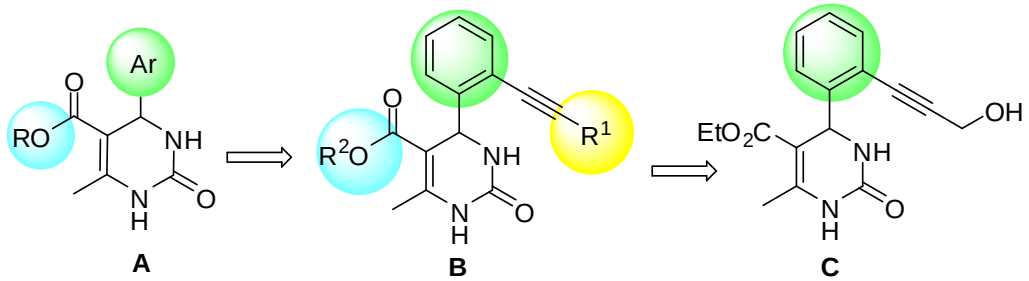


Fig. 1.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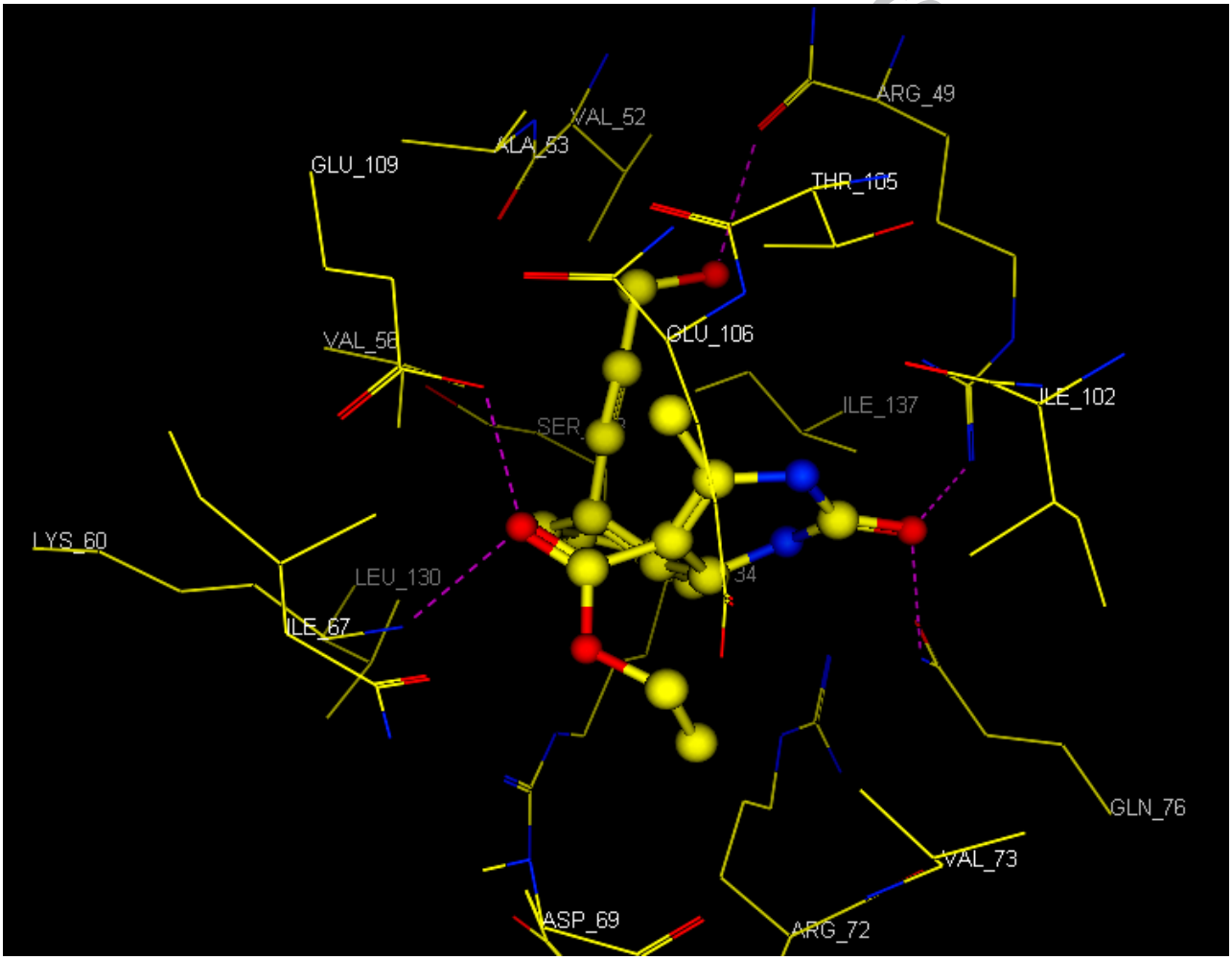
NH
N
H
O
A
R2O
R1
B
EtO2C
OH
C

Fig 2.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LEU_130
ILE_67
GLN_76
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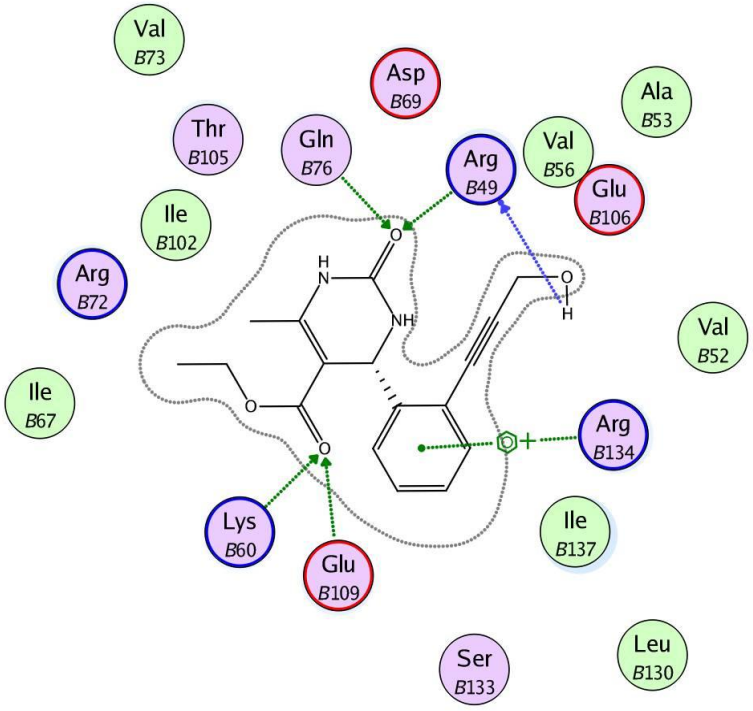
ASP_69
ARG_72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Glu B106
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Ile B67
Arg B134
Lys B60
Ile B137
Glu B109
Ser B133
Leu B130

Fig. 3.

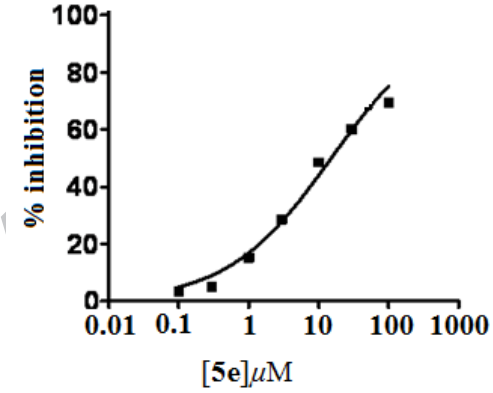
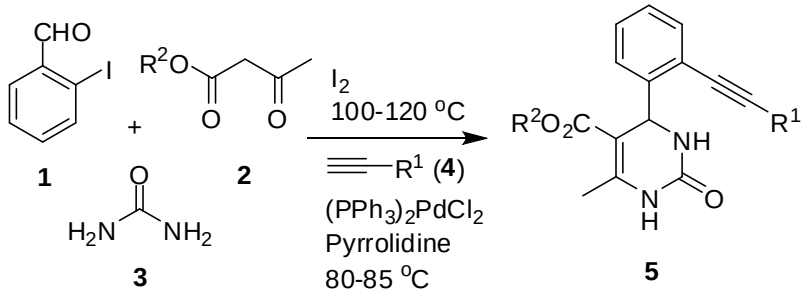

% inhibition
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
[5e]μM

Fig. 4.

Schemes

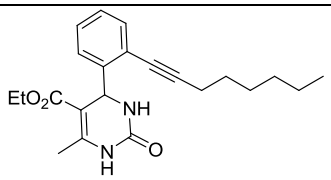
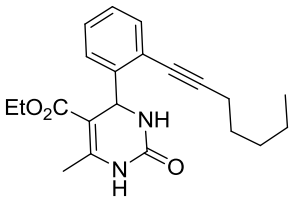
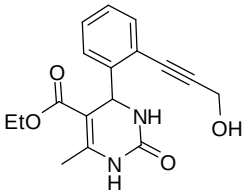
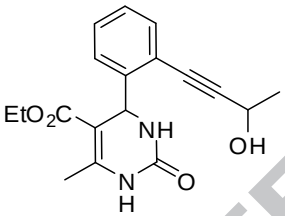
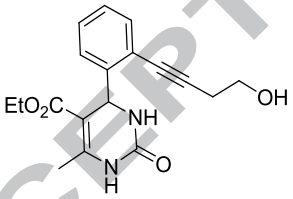
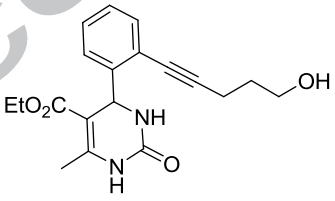
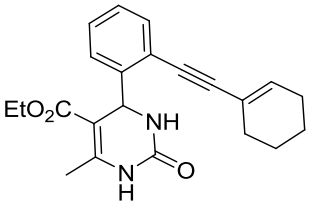


R^1	R^2	**5**	% yield
n-Hexyl	Et	**5a**	85
n-Pentyl	Et	**5b**	87
-CH_2OH	Et	**5c**	84
-CH(OH)CH_3	Et	**5d**	80
-$(CH_2)_2$OH	Et	**5e**	83
-$(CH_2)_3$OH	Et	**5f**	85
1-cyclohexenyl	Et	**5g**	90
1-hydroxycyclohexanyl	Et	**5h**	86
phenyl	Et	**5i**	81
4-(n-pentyl)phenyl	Et	**5j**	85
n-Pentyl	t-Bu	**5k**	87
1-cyclohexenyl	t-Bu	**5l**	82
1-hydroxycyclohexanyl	t-Bu	**5m**	84
1-hydroxycyclohexanyl	Me	**5n**	82
1-cyclohexenyl	Me	**5o**	81
n-Hexayl	Me	**5p**	84
4-Methyl phenyl	Me	**5q**	83

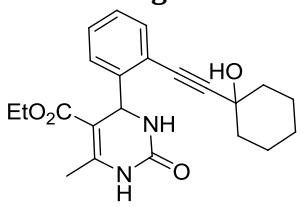
Scheme 1. Synthesis of *o*-alkynylphenyl substituted dihydropyrimidin-2(1*H*)-one derivatives (**5**) *via* a MCR involving sequential iodine-mediated Biginelli reaction followed by Cu-free Sonogashira coupling.

Tables

Table 1. Inhibition of chorismate mutase by compound **5** *in vitro*.

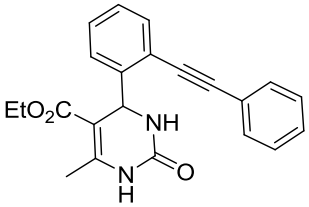
Entry	Compound **5**	% inhibition @ 30 μM
1.	 **5a**	31
2.	 **5b**	29
3.	 **5c**	60
4.	 **5d**	41
5.	 **5e**	63
6.	 **5f**	57
7.		27

8. **5g**



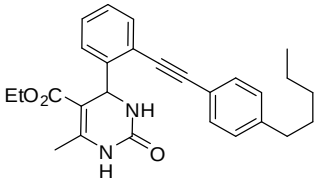
5h 20

9. **5h**



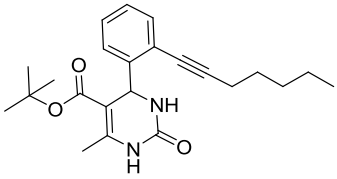
5i 17

10. **5i**



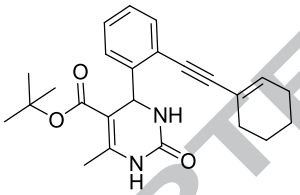
5j 19

11. **5j**



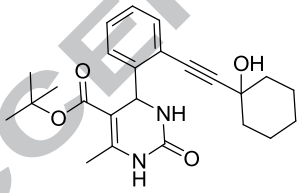
5k 30

12. **5k**



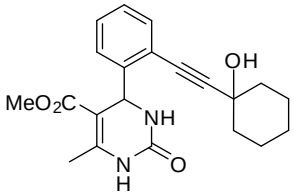
5l 22

13. **5l**

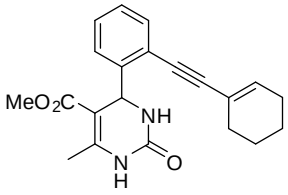
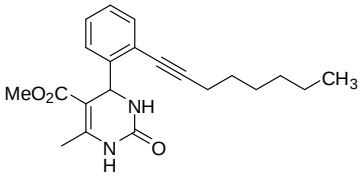
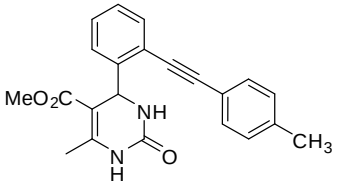


5m 18

14. **5m**



5n 25

15.	 **5o**	21
16.	 5p	28
17.	 **5q**	16

[a]Data presented are average of three experiments.

Graphical Abstract

Novel alkynyl substituted 3,4-dihydropyrimidin-2(1*H*)-one derivatives as potential inhibitors of chorismate mutase

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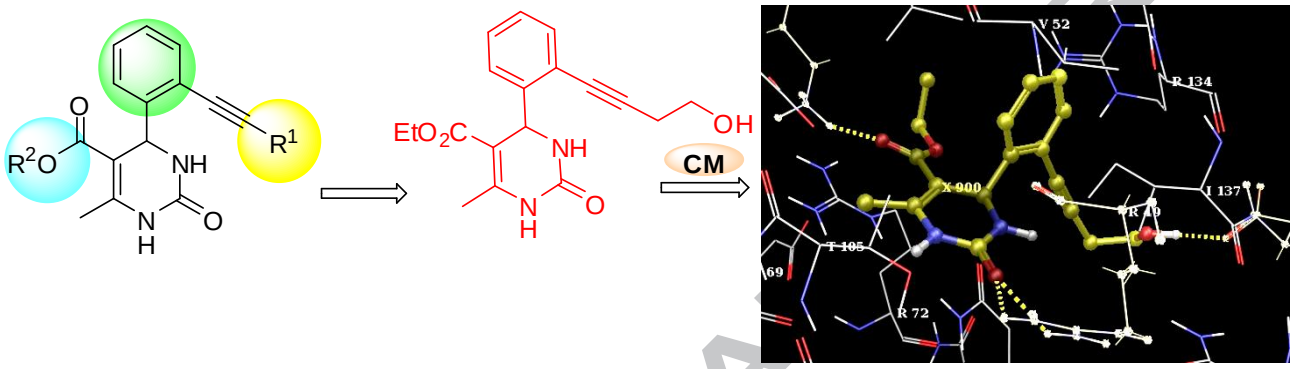

Highlights

- Alkynyl substituted DHPMs are synthesized via an alternative MCR.
- The MCR involved I_2-mediated Biginelli followed by Sonogashira reaction.
- These compounds showed chorismate mutase (CM) inhibitory properties in vitro.
- One compound showed dose dependent inhibition of CM (IC_{50} ~ 14.76 ± 0.54 μM)