Bioorganic & Medicinal Chemistry Letters 30 (2020) 127112

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Ultrasound assisted rapid synthesis of mefenamic acid based indole derivatives under ligand free Cu-catalysis: Their pharmacological evaluation☆

Rapolu Venkateshwarlu[a,b], Shambhu Nath Singh[a], Vidavalur Siddaiah[b], Hindupur Ramamohan[a], Rambabu Dandela[c,*], Kazi Amirul Hossain[d], P. Vijaya Babu[d], Manojit Pal[d,*]

[a] Custom Pharmaceutical Services and Dr. Reddy's Laboratories Ltd., Bollaram Road, Miyapur, Hyderabad 500049, India
[b] Department of Organic Chemistry & FDW, Andhra University, Visakhapatnam 530003, Andhra Pradesh, India
[c] Department of Industrial and Engineering Chemistry, Institute of Chemical Technology, Indianoil Odisha Campus, Samantpuri, Bhubaneswar 751013, India
[d] Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Hyderabad 500046, India

ARTICLE INFO

Keywords:
Mefenamic acid
Indole
Ultrasound
Cu
PDE4

ABSTRACT

An improved and rapid synthesis of mefenamic acid based indole derivatives has been achieved *via* the ligand free Cu-catalyzed coupling-cyclization method under ultrasound irradiation. This simple, straightforward and inexpensive one-pot method involved the reaction of a terminal alkyne derived from mefenamic acid with 2-iodosulfanilides in the presence of CuI and K_2CO_3 in PEG-400. The reaction proceeded *via* an initial C−C bond formation (the coupling step) followed by C−N bond formation (the intramolecular cyclization) to afford the mefenamic acid based indole derivatives in good to acceptable yields. Several of these compounds showed inhibition of PDE4 *in vitro* and the SAR (Structure Activity Relationship) within the series is discussed. The compound **3d** has been identified as a promising and selective inhibitor of PDE4B (IC_{50} = 1.34 ± 0.46 μM) that showed TNF-α inhibition *in vitro* (IC_{50} = 5.81 ± 0.24 μM) and acceptable stability in the rat liver microsomes.

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are well-known group of agents used to treat and prevent inflammation. Several studies have indicated that NSAIDs possess ability to inhibit the viability of colon,[1–4] breast,[5] prostate,[6] and stomach[7] cancer cells *in vitro*. The mefenamic acid (A, Fig 1), one of the prominent members of NSAIDs showed anti-proliferative effects/apoptosis against human liver cancer cell lines[8] and cytotoxic effects against colon cancer cell lines (HCT 116 and CaCo-2).[9] Prompted by these earlier observations we have reported the synthesis and anti-proliferative effects of a series of mefenamic acid based novel indole derivatives against oral (CAL 27) and breast (MCF-7) cancer cell lines. One of these compounds **A** (Fig 1) showed promising growth inhibition (55.56 ± 6.05%) of CAL 27 cancer cells at 10 μM but no effect on normal (HEK293T) cells indicating its potential selectivity towards oral cancer.[10] However, no further studies especially on exploring the pharmacological target or mechanism of action of this compound were performed at that time. Herein we report not only the further pharmacological evaluation of this class of compounds but also an improved synthesis for their more convenient access compared to that reported earlier.[10]

In our another study earlier we have observed that compounds possessing PDE4 inhibitory properties have potential to show significant growth inhibition of oral cancer cells (CAL 27).[11] This prior observation encouraged us to assess the PDE4 inhibitory potential of compound **A** at the initial stage. In order to verify the prediction that compound **A** might inhibit PDE4 the molecule **A** along with mefenamic acid **B** and the reference compound rolipram **C** was docked into the PDE4B (ID: 4MYQ) *in silico* (Fig 2 and Table 1). With a strong binding affinity (−11.7 Kcal/mol that was better than rolipram's −9.3 Kcal/mol) the molecule **A** showed two H-bonds with HIS406 and HIS450, and one aromatic pi interaction with PHE618. Additionally, it formed several other non-bonded contacts (like hydrophobic/VdW) with hydrophobic residues ILE582, PHE586, LEU674, MET519, LEU565, PHE678, TYR405 and hydrophobic regions of polar or charged residues like; ASN567, SER454, LYS677, THR517, GLN615 etc. The mefenamic acid on the other hand missed those two H-bonds and showed fewer aromatic pi interactions with residues PHE618 and PHE586 (binding affinity −8.5 Kcal/mol) (Fig 3). Though it formed hydrophobic contacts with hydrophobic residues ILE582, TRP578, TYR575 and

☆ IPDOIPM clearance number IPDOIPM-00640

* Corresponding authors.
E-mail addresses: r.dandela@iocb.ictmumbai.edu.in (R. Dandela), manojitpal@rediffmail.com (M. Pal).

https://doi.org/10.1016/j.bmcl.2020.127112
Received 25 January 2020; Received in revised form 13 March 2020; Accepted 13 March 2020
Available online 16 March 2020
0960-894X/

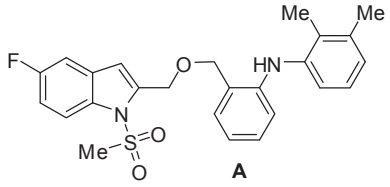
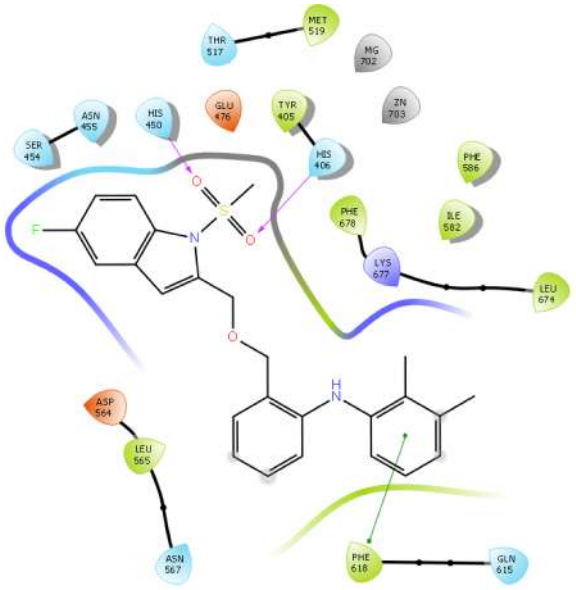


Fig. 1. The mefenamic acid based indole derivative (**A**) that showed promising effects on oral cancer cell line.



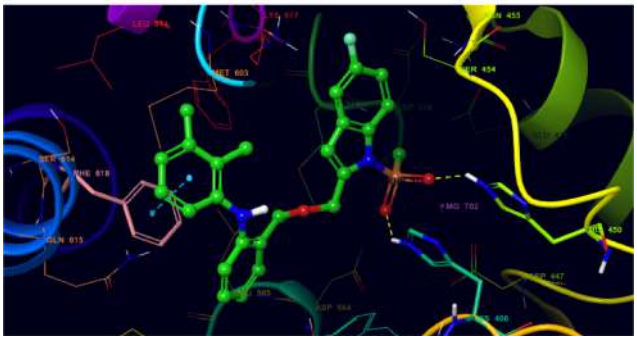
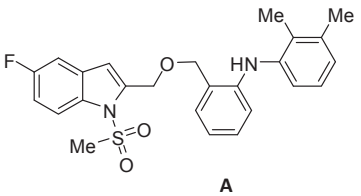
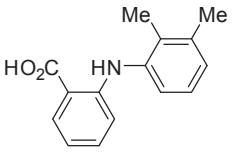
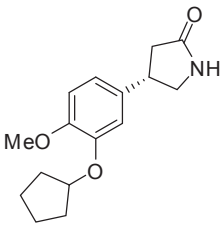


Fig. 2. 2D (H-bond in magenta color, pi-pi in green) and 3D interaction diagram between PDE4B (ID: 4MYQ) and the molecule **A**, prepared by using in Maestro visualizer (Schrödinger, LLC) (H-bond and pi-pi stacking are shown in yellow and cyan dashed lines, respectively).

hydrophobic regions of polar or charged residues like ASN567, THR579, GLN615 etc the numbers however were relatively low. Overall, the docking study suggested potential PDE4 inhibitory properties of molecule **A** that might be better than mefenamic acid, the molecule of its origin. It was therefore become essential to test the molecule **A** and its analogues against PDE4.

Table 1
Docking of molecules into PDE4B.

Molecules	Binding affinity (kcal/mol) for PDE4B (ID: 4MYQ)
 **A**	−11.7
 **B**	−8.5
 **C**	−9.3

*B = Mefenamic acid and C = Rolipram.

The use of transition metal catalyzed approaches for the synthesis of 2-subsituted indole derivatives *via* the one-pot coupling/cyclization strategy has become a popular method.[12–25] Generally, a 2-haloanilide is coupled with an appropriate terminal alkyne in the presence of one or more transition metal catalysts under suitable reaction conditions. The use of an appropriate Pd-salt as a catalyst and Cu-salt as a co-catalyst is common for this purpose. In our earlier method we employed 10% Pd/C–PPh_3–CuI as a catalyst system in combination with Et_3N in MeOH for the synthesis of **A** (Fig 1) and related analogues. However, the use of bimetallic salts as catalysts and expensive PPh_3 as a ligand appeared to be drawbacks of this methodology. Moreover, the use of Et_3N and MeOH was not an environmentally friendly option at all. We therefore became interested in exploring a faster as well as more environmentally friendly method for the synthesis of **A** and it's derivatives. Thus the mefenamic acid based indole derivatives were synthesized via a Cu-catalyzed one-pot method involving the coupling of terminal alkyne **1** (prepared from mefenamic acid following the known method)[10] with 2-iodoanilides (**2**) under ultrasound irradiation (Scheme 1). The reaction was performed in PEG-400 and was free from the use of any ligand. The details of this

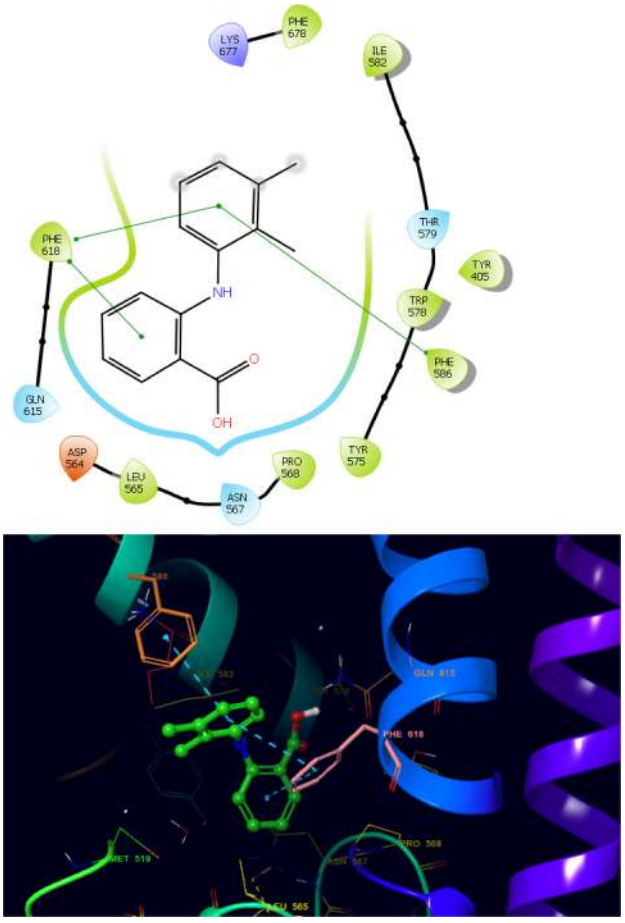
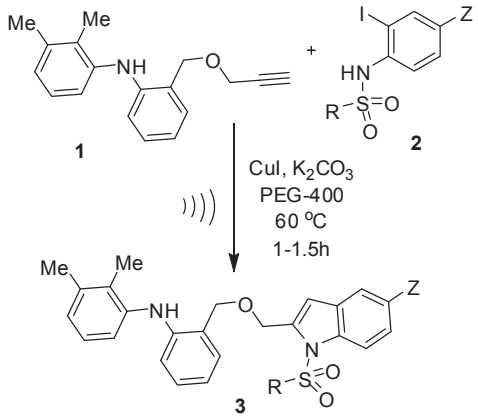
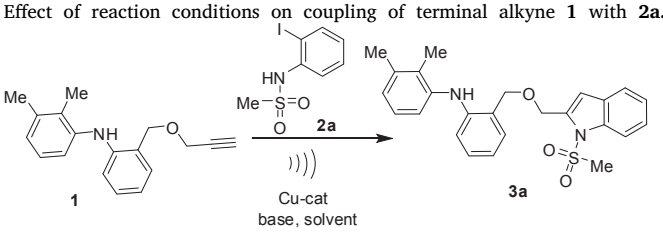


Fig. 3. 2D and 3D interaction diagram between PDE4B and mefenamic acid **B** (pi-pi stacking are shown in cyan dashed lines).



Scheme 1. Ultrasound assisted synthesis of mefenamic acid based indole derivatives (**3**) under ligand free Cu-catalysis.

Table 2
Effect of reaction conditions on coupling of terminal alkyne **1** with **2a.**[a]

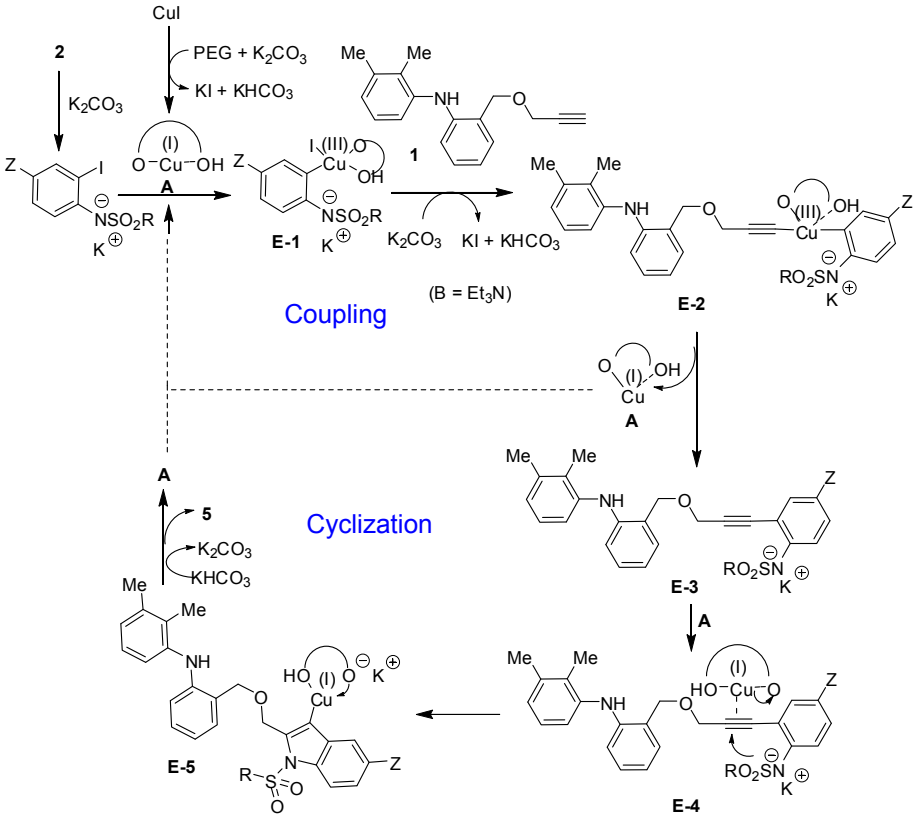


Entry	Cu-cat (mol%)	Base	Solvent	Time	Yield[b]
1.	CuI (10)	K_2CO_3	PEG-400	5	47
2.	CuI (20)	K_2CO_3	PEG-400	1	78
3.	CuI (30)	K_2CO_3	PEG-400	1	75
4.	CuI (20)	K_2CO_3	EtOH	3	62
5.	CuI (20)	K_2CO_3	n-BuOH	3	59
6.	CuI (20)	Et_3N	PEG-400	5	60
7.	CuBr	K_2CO_3	PEG-400	7	54
8.	CuCl	K_2CO_3	PEG-400	7	45
9.	No catalyst	K_2CO_3	PEG-400	7	No reaction
10.	CuI (20)	K_2CO_3	PEG-400	12	43[c]

[a] All reactions were carried out using alkyne **1** (1 equiv.), **2a** (1 equiv.), a Cu-catalyst and base (2 equiv.) in a solvent (5.0 mL) at 60 °C under ultrasound irradiation.
[b] Isolated yields.
[c] The reaction was performed in the absence of ultrasound.

study are presented.

Over the years the ultrasound assisted reactions have attracted considerable interest in organic synthesis and enormous applications of these reactions have been reported.[26] This is because the ultrasound assisted reactions (i) are considered as green approaches in organic synthesis,[27] (ii) play important role in waste minimization and reduction of energy requirements[28] and (iii) are efficient (e.g. shorter reaction time, milder conditions, higher yields etc) for the synthesis of various organic molecules.[29,30] The PEG-400 on the other hand is considered as an environmentally friendly solvent[31] due to its high boiling, non-hazardous and polar nature that allows its easy recovery (from the reaction mixture) and recyclability. Due to our long standing interest in the use of ultrasound assisted reactions as well as use of PEG-400 as a solvent we decided to explore these reaction conditions in our current effort. Accordingly, to establish the optimized reaction conditions the coupling of alkyne **1** with 2-iodosulfanilide **2a** was examined under various reaction conditions and the results are summarized in Table 2. The reaction proceeded well when carried out using 10 mol% CuI as a catalyst and K_2CO_3 as a base in PEG-400 under ultrasound using a laboratory ultrasonic bath SONOREX SUPER RK 510H model producing irradiation of 35 kHz (entry 1, Table 2). However, the desired product **3a** was obtained in 47% yield after 5 h. The use of higher quantity of CuI e.g. 20 mol% improved the product yield significantly and the reaction was completed within 1 h (entry 2, Table 2). We were



Scheme 2. Proposed reaction mechanism for the Cu-catalyzed formation of **3** *via* the coupling-cyclization under ultrasound irradiation.

delighted with this observation and continued our effort for possibility of further increase in yield of **3a**. Accordingly, the quantity of CuI used was increased from 20 mol% to 30 mol% but no further improvement in product yield was observed (entry 3, Table 2). Change of solvent from PEG-400 to EtOH or *n*-BuOH (entry 4 and 5, Table 2) or base from K_2CO_3 to Et_3N (entry 6, Table 2) did not improve the product yield. The use of other catalysts e.g. CuBr or CuCl was also examined but found to be less effective (entry 7 and 8, Table 2). Notably, the reaction did not proceed in the absence of catalyst (entry 9, Table 2) indicating key role played by the Cu-sat in the current coupling-cyclization method. The reaction was also performed under silent condition when the desired indole **3a** was obtained in 43% yield after 12 h (entry 10, Table 2). All these reactions were performed at 60 °C. The decrease of reaction temperature to 50 °C or less increased the reaction time and reduced the product yield. On the other hand increase of temperature to 80 °C or above did not improve the product yield. Nevertheless, the condition of entry 2 of Table 2 (i.e. the combination of CuI and K_2CO_3 in PEG-400 at 60 °C under ultrasound) appeared to be optimum and was used for the preparation of analogues of **3a**.

To prepare a range of analogues of **3a** various *o*-iodosulphanilides (**2a–m**) were employed to react with the alkyne **3** under the optimized conditions (Table 3). All these ultrasound assisted reactions proceeded smoothly to afford the desired indole derivatives (**3**). The presence of substituent like Me, F, Cl, Br and Et on the anilide ring was well tolerated in this Cu-catalyzed coupling-cyclization reaction. The common spectral (^{1}H and ^{13}C NMR and Mass) data were used to characterize the indole derivatives (**3**) synthesized (see the Supplementary data file).

A plausible reaction mechanism for the Cu-catalyzed formation of **3** *via* the coupling-cyclization under ultrasound irradiation is depicted in Scheme 2. The PEG appeared to play a dual role i.e. as a solvent as well as ligand in the present reaction.[32,33] Initially, a Cu(I) complex (**A**) formed *via* the interaction of CuI with PEG under ultrasound, underwent oxidative addition with the 2-iodoanilide (**2**) to afford the arene-Cu(III) species **E-1**. Subsequently, the alkyne **1** reacted with **E-1** in the presence of K_2CO_3 leading to the arene-Cu(III)-alkyne species **E-2**, which on reductive elimination furnished the alkynyl derivative **E-3** with the regeneration of active Cu(I) catalyst **A**. The **E-3** then underwent intramolecular ring closure in the presence of **A** in a regioselective manner to give the desired indole **3**. It is evident from Table 2 that the current Cu-catalyzed reaction was accelerated considerably by the

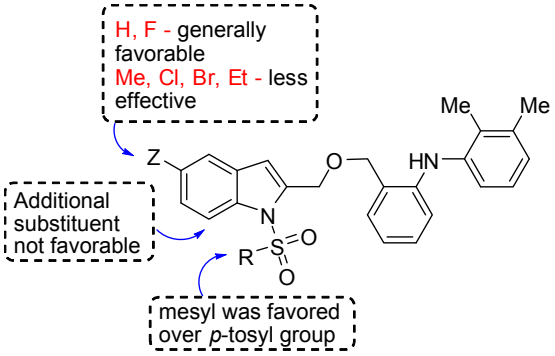


Fig. 4. Summary of SAR for PDE4B inhibitory activities of compound **3.**

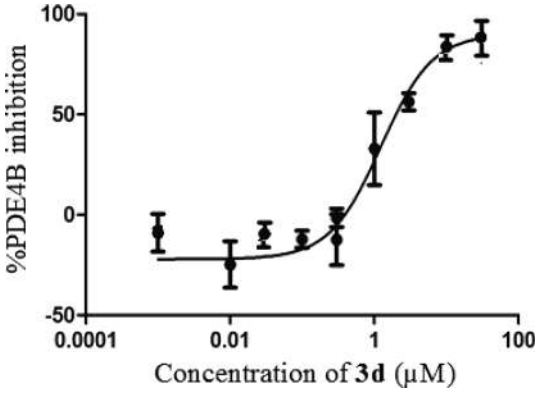
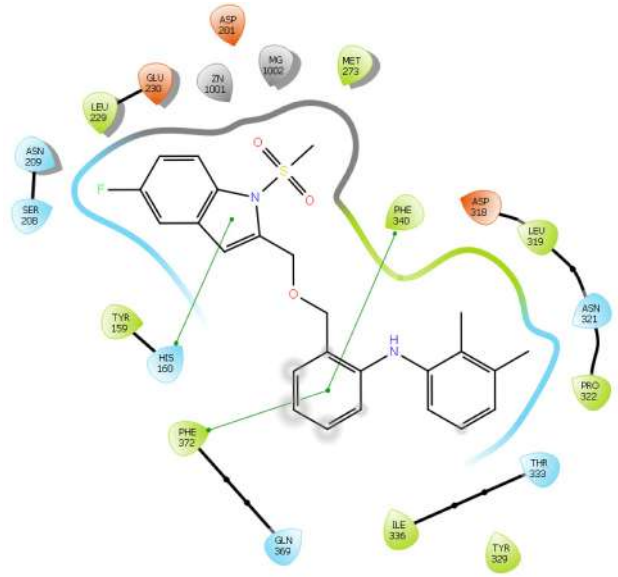


Fig. 5. Concentration dependent study of compound **3d** against PDE4B.

ultrasound irradiation. Indeed, ultrasound causes compression of the liquid and then rarefaction (expansion), in which a sudden pressure drop forms small, oscillating bubbles of gaseous substances. These bubbles expand with each cycle of the applied ultrasonic energy until they reach an unstable size and then they can collide and/or collapse violently. This effect causes the increase of local temperature within the reaction medium (via the violent collapse of the cavitation bubbles) that eventually facilitate crossing of activation energy barrier.[34] Thus the conversion of reactants to intermediates and subsequently to product(s) take place within short period of time. The participation of ultrasound in various steps of Scheme 2 explains the rapid formation of indole **3** from the alkyne (**1**) and 2-iodoanilide (**2**).

All the synthesized compounds (**3a-m**) were evaluated for their PDE4B inhibitory properties *in vitro* at 10 μM using an enzyme based assay.[35] Rolipram, a well-known inhibitor of PDE4 was used as a reference compound. Notably, compounds containing mesyl group at indole nitrogen atom (e.g. **3a-f**) showed superior activities over those (**3 g-m**) containing *p*-tosyl group at the same position (Table 4). Among the *N*-mesyl indole derivatives the compound **3a**, **3b**, **3d** and **3e** showed PDE4B inhibition > 50% and the compound **3d** was identified as most active one among them. A brief overview of SAR (Structure-Activity-



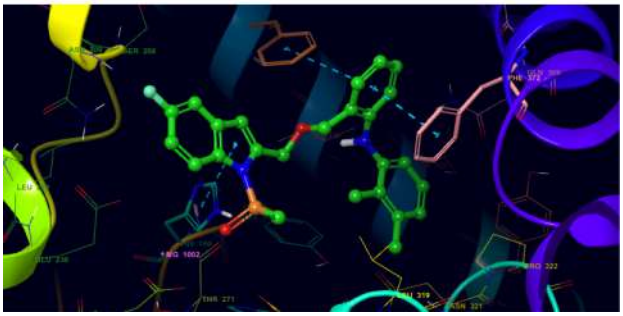


Fig. 6. 2D and 3D interaction between PDE4D (ID: 5K32) and the molecule **3d** (or **A**) (pi-pi stacking are shown in cyan dashed lines).

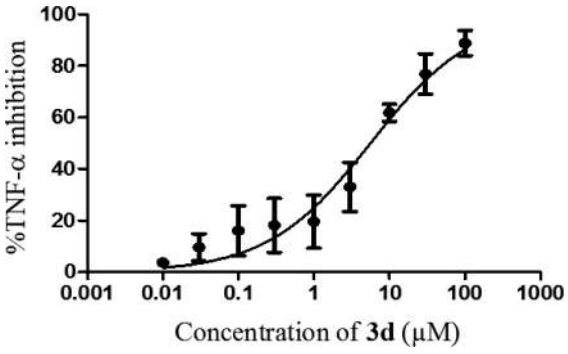


Fig. 7. *In vitro* TNF-α inhibition of compound **3d**.

Table 5
Computational ADME prediction of **3d**, mefenamic acid and rolipram

Properties	Molecules		
(i) Physicochemical	**3d**	Mefenamic acid	Rolipram
Molecular Weight (g/mol)	452.54	241.29	275.34
Consensus Log P[a]	5.04	3.29	2.44
Log S (ESOL)[b]	−5.96 (moderately soluble)	−4.86 (moderately soluble)	−2.90 (soluble)
(ii) Pharmacokinetics			
GI[c] absorption	Low	High	High
BBB[d] permeation	No	Yes	Yes
P-gp[e] substrate	No	No	Yes
(iii) Druglikenss			
Lipinski rule	1 Violation (log P > 5)	No violation	No violation
Veber rule	No violation	No violation	No violation
Bioavailability score	0.55	0.56	0.55

[a] Log P: Lipophilicity.
[b] Log S (ESOL): water solubility, calculated by ESOL method which Quantitative Structure-Property Relationship (QSPR) based model.
[c] GI: Gastrointestinal.
[d] BBB: Blood Brain Barrier.
[e] P-gp: permeability glycoprotein.

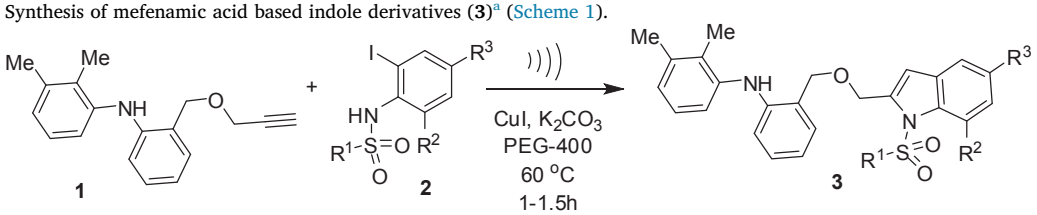
Relationship) is presented in Fig 4. In general, the bulkiness of the group present at the indole nitrogen appeared to be crucial for activity. A smaller group such mesyl moiety was favored over the bulkier moiety i.e. p-tosyl group. The size and nature of substituent present at the C-5 position of the indole ring also seemed to be influential for PDE4 inhibition. The presence of "H" or "F" at this position was more favorable than other substituents e.g. Me, Cl, Br or Et that are relatively bigger in size. Moreover, the presence of additional substituent at C-7 position of the indole ring was also found to be less effective. Notably, the mediocre activity of mefenamic acid indicated the key role played by the appropriately substituted indole ring in compound **3** that was corroborated by the outcome of docking studies (Fig 2 and 3). Nevertheless, a concentration response study was performed using the compound **3d** (Fig. 5) and the IC_{50} value was found to be 1.34 ± 0.46 μM that was in the same order of rolipram (IC_{50} = 1.03 ± 0.23 μM). The IC_{50} value of **3a**, **3b** and **3e** was found to be 1.76 ± 0.98, 4.13 ± 0.25 and 4.48 ± 0.36, respectively. It was then desirable to examine the potential of **3d** to inhibit PDE4D (one of the four sub types of PDE4 i.e. PDE4A, B, C and D) that was thought to be responsible for emetic side effects shown by the existing PDE4 inhibitors.[36–38] Due to their PDE4B inhibition the compound **3a**, **3b** and **3e** were also included along with **3d** and mefenamic acid in this assay (Table 4). All these compounds showed some inhibition of PDE4D. The compound **3d** showed 42% inhibition at 30 μM but 15% inhibition at 10 μM indicating its selectivity (~4.5 fold) towards PDE4B over PDE4D. In order to understand its interaction with PDE4D the compound **3d** was docked into PDE4D (ID: 5 K32) *in silico* (Fig 6). The compound **3d** showed fewer interactions with PDE4D (mostly aromatic pi-interactions involving the residues such as PHE340, PHE 372 and HIS 160 without any H-bonding) compared to PDE4B providing the plausible reasons in support of its selectivity towards PDE4B over D.

We have observed that the mefenamic acid based indole derivative **3d** (or **A**, Fig 1) that showed promising effects on oral cancer cell line in our earlier study possess PDE4 inhibitory properties. To assess its potential for anti-inflammatory effects the compound **3d** was evaluated in a separate study when it showed concentration dependent inhibition of TNF-α *in vitro* (Fig. 7) with an IC_{50} value 5.81 ± 0.24 μM.

Next, as part of common Med Chem strategy it was desirable to gain some preliminary idea about ADME (absorption, distribution, metabolism, and excretion) or pharmacokinetic properties of **3d**. Thus the computational ADME prediction of compounds **3d** and mefenamic acid along with the known inhibitor rolipram was performed using SwissADME web-tool[39] and results are presented in Table 5 (among the various descriptors only notable one are listed in the table). Indeed, the desirable ADME was predicted for compound **3d** and except the low GI absorption, no BBB (Blood Brain Barrier) penetration and no P-gp substrate potential have been predicted for **3d** with comparable bioavailability score to the reference compound rolipram. Nevertheless, the compound **3d** was taken for *in vitro* microsomal stability study that was performed using the rat liver microsomes. At a concentration of 5 μM the compound **3d** showed acceptable stability after 60 min [i.e. the mean % of **3d** remaining after 60 min compared to 0 min ~64.9; half-life ($t_{1/2}$, min) ~110 and intrinsic clearance (CL_{int}, μL/min/mg) ~25.2]. Overall, the compound **3d** could be a promising inhibitor of PDE4 and appeared to have medicinal value especially from the viewpoint of developing dual anticancer-anti-inflammatory agent.

In conclusion, a rapid synthesis of mefenamic acid based indole derivatives has been achieved *via* the ligand free Cu-catalyzed coupling-cyclization method under ultrasound irradiation. This simple, straightforward and inexpensive one-pot method involved reaction of a terminal alkyne derived from mefenamic acid with 2-iodosulfanilides in the presence of CuI and K_2CO_3 in PEG-400. The reaction proceeded *via* initial C—C bond formation (the coupling step) followed by C—N bond formation (the intramolecular cyclization) to afford the mefenamic acid based indole derivatives in good to acceptable yield**.** Several of these compounds showed inhibition of PDE4 when tested at 10 μM *in vitro*. The SAR (Structure Activity Relationship) within the series is discussed. The compound **3d** has been identified as a promising and selective

Table 3
Synthesis of mefenamic acid based indole derivatives (**3**)[a] (Scheme 1).



Entry	*o*-Iodoanilide (**2**) R^1, R^2, R^3	Products (**3**)	Time (h)	Yield[b] (%)
1	Me, H, H **2a**	**3a**	1	78
2	Me, H, Me **2b**	**3b**	1	67
3	Me, Me, Me **2c**	**3c**	1.5	67
4	Me, H, F **2d**	**3d**	1.5	71
5	Me, H, Cl **2e**	**3e**	1	69
6	Me, H, Br **2f**	**3f**	1	73
7	*p*-MeC_6H_4, H, H **2g**	**3g**	1.5	72
8	*p*-MeC_6H_4, H, Me **2h**	**3h**	1	73
9	*p*-MeC_6H_4, Me, Me **2i**	**3i**	1.5	65
10	*p*-MeC_6H_4, H, F **2j**	**3j**	1	73
11	*p*-MeC_6H_4, H, Cl **2k**	**3k**	1.5	76
12	*p*-MeC_6H_4, H, Br **2l**	**3l**	1.5	69
13	*p*-MeC_6H_4, H, Et **2m**	**3m**	1	71

[a] All the reactions were carried out by using alkyne **1** (1.0 mmol), an appropriate 2-iodosulfanilide (**2**, 1.0 mmol), CuI (20 mol%) and K_2CO_3 (2.0 mmol) in PEG-400 (5.0 mL) at 60 °C under ultrasound.
[b] Isolated yield.

inhibitor of PDE4B (IC_{50} = 1.34 ± 0.46 μM) that was supported by *in silico* docking studies. This compound showed anti-inflammatory activity potential *via* the inhibition of TNF-α *in vitro* (IC_{50} = 5.81 ± 0.24 μM). A favourable ADME (absorption, distribution, metabolism, and excretion) or pharmacokinetic properties were predicted for **3d** *in silico* that was supported by its acceptable stability in the rat liver microsomes. Thus, the compound **3d** appeared to be interesting hit molecule especially from the viewpoint of developing a dual anticancer-anti-inflammatory agent. Overall, the current study not only disclosed a ultrasound assisted rapid synthesis of mefenamic acid based indole derivatives under ligand free Cu-catalysis but also revealed a new template for the design and identification of future inhibitors of PDE4.

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.

Acknowledgement

The authors thank Dr. K. V. L. Parsa and his group for *in vitro* assay and the management of Dr. Reddy's Lab Ltd, Hyderabad, India, for continuous support and encouragement.

Table 4
In vitro evaluation of compounds against PDE4B and PDE4D2

Entry	Compound	% inhibition	
		PDE4B at 10 μM	PDE4D2 at 30 μM
1	**3a**	61.45	40.86
2	**3b**	55.13	38.91
3	**3c**	37.26	ND
4	**3d**	67.99	42.05
5	**3e**	54.93	33.64
6	**3f**	40.42	ND
7	**3g**	39.91	ND
8	**3h**	36.21	ND
9	**3i**	23.06	ND
10	**3j**	34.56	ND
11	**3k**	26.82	ND
12	**3l**	24.57	ND
13	**3m**	32.09	ND
14	Mefenamic acid	38.91	35.54
15	Roflumilast (10 nM)	91.64	88.32

[a]Data represent the mean values of three independent determinations.
ND = not determined.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127112.

References

1. Hara A, Yoshimi N, Niwa M, Ino N, Mori H. Apoptosis induced by NS-398, a selective cyclooxygenase-2 inhibitor, in human colorectal cancer cell lines. *Jpn J Cancer Res.* 1997;1997(88):600–604.
2. Richter M, Weiss M, Weinberger I, Furstenberger G, Marian B. Growth inhibition and induction of apoptosis in colorectal tumor cells by cyclooxygenase inhibitors. *Carcinogenesis.* 2001;2001(22):17–25.
3. Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath Jr CW. Aspirin use and risk of fatal cancer. *Cancer Res.* 1993;1993(53):1322–1327.
4. Thun MJ, Namboodiri MM, Heath Jr CW. Aspirin use and reduced risk of fatal colon cancer. *N Engl J Med.* 1991;1991(325):1593–1596.
5. Harris RE, Namboodiri KK, Farrar WB. Nonsteroidal antiinflammatory drugs and breast cancer. *Epidemiology.* 1996;1996(7):203–205.
6. Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, Chen CS. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. *J Biol Chem.* 2000;2000(275):11397–11403.
7. Sawaoka H, Kawano S, Tsuji S, et al. Cyclooxygenase-2 inhibitors suppress the growth of gastric cancer xenografts via induction of apoptosis in nude mice. *Am J Physiol.* 1998;1998(274):G1061–G1067.
8. Woo DH, Han IS, Jung G. Mefenamic acid-induced apoptosis in human liver cancer cell-lines through caspase-3 pathway. *Life Sci.* 2004;75:2439–2449.
9. Somchit MN, Faizah S, Zuraini A, Khairi HM, Hasiah AH, Zakaria ZA. Selective *In vitro* cytotoxic effects of piroxicam and mefenamic acid on several cancer cells lines. *Res J Pharmacol.* 2009;3:15–18.
10. Babu PV, Ashfaq MA, Kumar KS, Mukkanti K, Pal M. Mefenamic acid based novel indole analogues: their synthesis and anti-proliferative effects. *Arab J Chem.* 2015. https://doi.org/10.1016/j.arabjc.2015.05.018.
11. Babu PV, Mukherjee S, Deora GS, et al. Ligand/PTC-free intramolecular Heck reaction: synthesis of pyrroloquinoxalines and their evaluation against PDE4/luciferase/oral cancer cell growth in vitro and zebrafish in vivo. *Org Biomol Chem.* 2013;11:6680–6685.
12. Knölker HJ, Reddy KR. Isolation and synthesis of biologically active carbazole alkaloids. *Chem Rev.* 2002;102:4303–4427.
13. Horton DA, Bourne GT, Smythe ML. The combinatorial synthesis of bicyclic privileged structures or privileged substructures. *Chem Rev.* 2003;103:893–930.
14. Cacchi S, Fabrizi G. Synthesis and functionalization of indoles through palladium-catalyzed reactions. *Chem Rev.* 2005;105:2873–2920.
15. Zeni G, Larock RC. Synthesis of heterocycles via palladium π-alkyne chemistry. *Chem Rev.* 2004;104:2285–2309.
16. Cacchi S, Fabrizi G, Parisi LM. 2-Aryl and 2-heteroaryl indoles from 1-alkynes and o-iodotrifluoroacetanilide through a domino copper-catalyzed coupling-cyclization process. *Org Lett.* 2003;5:3843–3846.
17. Pal M, Subramanian V, Batchu VR, Dager I. Synthesis of 2-substituted indoles via Pd/C-catalyzed reactions in water. *Synlett.* 2004:1965–1969.
18. Cacchi S, Fabrizi G, Parisi LM, Bernini R. 2-Aryl and 2-Heteroaryl Pyrrolo[2,3-b] quinoxalines via Copper-Catalyzed Reaction of 1-Alkynes with 2-Bromo-3 trifluoroacetamidoquinoxaline. *Synlett.* 2004:287–290.
19. Slough GA, Krchňák V, Helquist P, Canham SM. Synthesis of readily cleavable immobilized 1,10-phenanthroline resins. *Org Lett.* 2004;6:2909–2912.
20. Liu F, Ma D. Assembly of conjugated enynes and substituted indoles via CuI/amino acid-catalyzed coupling of 1-alkynes with vinyl iodides and 2-bromotrifluoroacetanilides. *J Org Chem.* 2007;72:4844–4850.
21. Oskooie HA, Heravi MM, Behbahani FK. A facile, mild and efficient one-pot synthesis of 2-substituted indole derivatives catalyzed By $Pd(PPh_3)_2Cl_2$. *Molecules.* 2007;12:1438–1446.
22. Layek M, Lakshmi U, Kalita D, et al. Pd/C-mediated synthesis of indoles in water. *Beilstein J Org Chem.* 2009;5:46. https://doi.org/10.3762/bjoc.5.46.
23. Nakhi A, Prasad B, Reddy U, et al. A new route to indolesvia in situdesilylation–Sonogashira strategy: identification of novel small molecules as potential anti-tuberculosis agents. *Med Chem Comm.* 2011;2:1006–1010.
24. Rao RM, Reddy Ch, Alinakhi U, et al. Sequential coupling/desilylation–coupling/cyclization in a single pot under Pd/C–Cu catalysis: Synthesis of 2-(hetero)aryl indoles. *Org Biomol Chem.* 2011;9:3808–3816.
25. Prasad B, Adepu R, Sandra S, et al. $AlCl_3$ mediated unexpected migration of sulfonyl groups: regioselective synthesis of 7-sulfonyl indoles of potential pharmacological interest. *Chem Commun.* 2012;48:10434–10436.
26. Mason TJ. Ultrasound in synthetic organic chemistry. *Chem Soc Rev.* 1997;26:443–451.
27. Cella R, Stefani HA. Ultrasonic reactions. In: Zhang W, Cue BW, eds. *Green techniques for organic synthesis and medicinal chemistry.* Chichester, UK: John Wiley & Sons, Ltd; 2012https://doi.org/10.1002/9780470711828.ch13.
28. Pizzuti L, Franco MSF, Flores AFC, Quina FH, Pereira CMP. Recent advances in the ultrasound-assisted synthesis of azoles. *Green Chem - Environ Benign Approaches.* 2012. https://doi.org/10.5772/35171.
29. Puri S, Kaur B, Parmar A, Kumar H. Applications of ultrasound in organic synthesis - a green approach. *Curr Org Chem.* 2013;17:1790–1828.
30. Kumar Reddy DN, Chandrasekhar KB, Siva Ganesh YS, et al. FeF3-catalyzed MCR in PEG-400: ultrasound assisted synthesis of N-substituted 2-aminopyridines. *RSC Adv.* 2016;6:67212–67217. https://doi.org/10.1039/c6ra14228a.
31. Chen J, Spear SK, Huddleston JG, Rogers RD. Polyethylene glycol and solutions of polyethylene glycol as green reaction media. *Green Chem.* 2005;7:64.
32. Declerck V, Martinez J, Lamaty F. Microwave-assisted copper-catalyzed heck reaction in PEG solvent. *Synlett.* 2006;18:3029.
33. Mao J, Guo J, Fang F, Ji S-J. Highly efficient copper(0)-catalyzed Suzuki-Miyaura cross-coupling reactions in reusable PEG-400. *Tetrahedron.* 2008;64:3905.
34. Suslick KS, Hammerton DA, Cline RE. Sonochemical hot spot. *J Am Chem Soc.* 1986;108:5641–5642.
35. Wang P, Myers JG, Wu P, Cheewatrakoolpong B, Egan RW, Billah MM. Expression, purification, and characterization of human cAMP-specific phosphodiesterase (PDE4) sub-types A, B, C, and D. *Biochem Biophys Res Commun.* 1997;234:320–324.
36. Kodimuthali A, Jabaris SSL, Pal M. Recent advances of phosphodiesterase 4 inhibitors for the treatment of asthma and chronic obstructive pulmonary disease. *J Med Chem.* 2008;18:5471–5489.
37. Lin CH, Chang SH, Fang JY. Recent advances using phosphodiesterase 4 (PDE4) inhibitors to treat inflammatory disorders: animal and clinical studies. *Curr. Drug Ther.* 2016;11:21–40.
38. Li H, Zuo J, Tang W. Phosphodiesterase-4 inhibitors for the treatment of inflammatory diseases. *Front Pharmacol.* 2018;9:1048. https://doi.org/10.3389/fphar.2018.01048.
39. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep.* 2017;7:1–13.