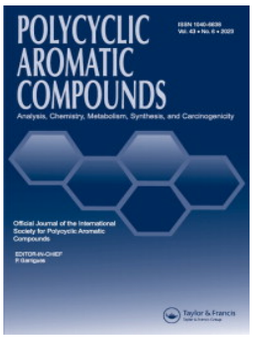

Polycyclic Aromatic Compounds

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Design, Synthesis and Bioevaluation of Highly Functionalized 1,2,3-Triazole-Guanidine Conjugates as Anti-Inflammatory and Antioxidant Agents

Madiha A. Siddiqui[a], Amol A. Nagargoje[a], Mubarak H. Shaikh[b], Rashiqua A. Siddiqui[c], Amit A. Pund[d], Vijay M. Khedkar[e], Ashish Asrondkar[f], Prathmesh P. Deshpande[a], and Bapurao B. Shingate[a]

[a]Department of Chemistry, Dr. Babasaheb Ambedkar, Marathwada University, Aurangabad, India; [b]Department of Chemistry, Radhabai Kale Mahila Mahavidyalaya, Ahmednagar, India; [c]Department of Microbiology, Government Institute of Science Aurangabad, Aurangabad, India; [d]Department of Chemistry, Shivaji Arts, Commerce and Science College Kannad, Aurangabad, India; [e]Department of Pharmaceutical Chemistry, School of Pharmacy, Vishwakarma University, Pune, India; [f]Haffkine Institute for Training Research and Testing Parel, Mumbai, India

ABSTRACT

In search of new biologically potent molecules a small focused library of new guanidine-1,2,3-triazole hybrid derivatives were synthesized *via* Organocatalytic enolate- mediated azide-carbonyl [3 + 2] cycloaddition yielding a highly functionalized triazole core structure. The synthesis of all the derivatives were confirmed by spectral analysis ^{1}H NMR, ^{13}C NMR and MS. The new guanidine-1,2,3-triazole conjugates were found to exhibit promising anti-inflammatory and antioxidant activity. The anti-inflammatory activity screened by membrane stabilization method summarizes the four potential conjugates **5c**, **5f**, **5h** and **5g** to be potent in comparison with standard drug Diclofenac sodium (**DFS**). The conjugates were also assessed for antioxidant potential by DPPH method. Among all the synthesized compounds, the compounds **5d**, **5f**, **5g** and **5h** exhibited potent antioxidant activity. Molecular docking study was performed to gain insight into the putative binding mode and binding strength of these compounds with the target enzyme Cyclo-oxygenase (COX-2) enzyme. The *in vitro* and *in silico* studies together with simpler designing and synthesis strategy *via* Organocatalytic enolate- mediated azide-carbonyl [3 + 2] cycloaddition followed by a two-component reaction with guanidine in basic conditions rationalize guanidine-1,2,3-triazole hybrid derivatives as easily assessable novel therapeutic agents.

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1,2,3-Triazole; molecular hybridization; anti-inflammatory; COX-2 Inhibitor; Antioxidant

CONTACT Bapurao B. Shingate bapushingate@gmail.com Department of Chemistry, Dr. Babasaheb Ambedkar, Marathwada University, Aurangabad, Maharashtra 431004, India

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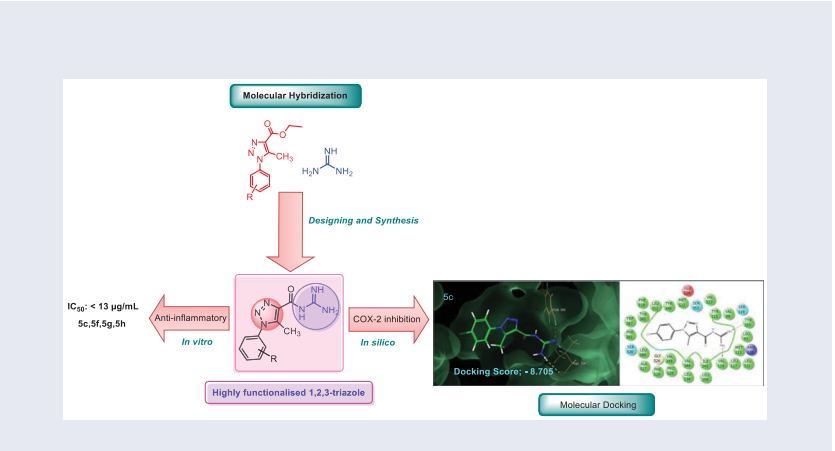


Introduction

Inflammation is a generic response of the body to invasiveness such as pathogens, irritants, and certain conditions of cell damage. Acute or chronic in either form, the response evoked by inflammation plays a dominant pathological role in various diseases like arthritis, cancer, tumor proliferation, stroke, neurodegenerative and cardiovascular diseases.[1] The onset of inflammation is observed with the increased rates of arachidonic acid oxidation. Cyclooxygenase, a membrane-bound heme protein (i.e. it contain Heme Prosthetic Group in its structure) controls the complex conversion of arachidonic acid to prostaglandins and thromboxane that are autocrine and paracrine lipid mediators of many physiological and pathological responses.[2] Cyclooxygenases exist in two isoforms, constitutive COX-1 which provides cytoprotective prostaglandins in GI tract and thromboxane in blood platelets and inducible COX-2, expressed in inflammatory lesions and tumors but barely in the normal cells.[3] These two isoforms mainly differ in substrate and inhibitor selectivity and follow the identical catalytic mechanism that produces same product *via* same reaction.[4,5] The COX-1 is responsible for homeostatic conditions governing the gastrointestinal and renal integrity whereas COX-2 induces inflammatory conditions. Based on pharmacodynamic features at a therapeutic dose, NSAIDs are classified as either nonselective COX-1/COX-2 inhibitors or selective COX-2 inhibitors. Conventional nonselective inhibitors, for example, SC-560 and indomethacin disturb the homeostatic conditions by COX- 1 inhibition and result in gastric ulceration, kidney failure and GI toxicity.[6,7] Also, the inhibition of COX-1 counteracts the biosynthesis of thromboxane causing platelet dysfunctions ultimately increasing the bleeding time.[8] Later developed COX-2 selective inhibitors, for example, celecoxib and rofecoxib are associated with adverse cardiovascular complications.[9] Hence improving the COX-2 inhibitors represents a formidable challenge. Figure 1 represents some marketed COX-2 inhibitors.

Central five-membered heterocyclic or carbocyclic motifs are common structural features of many COX-2 inhibitors (Figure 2). COX-2 inhibitors are generally flexible with regard to carbocyclic/heterocyclic core motif for efficient binding. Among the multitude of known heterocyclics, only a scant of literature reports the 1,2,3-triazole core in panel of anti-inflammatory drug candidates.[10] Moreover, free radicals are widely believed to be involved in the etiology of inflammatory diseases as active component in the cyclooxygenase and lipoxygenase mediated conversion of arachidonic acid into proinflammatory intermediates.[11] The reactive oxygen species are released from the activated neutrophils and macrophages and lead to tissue injury by damaging macromolecules and lipid peroxidation of membrane. Hence the antioxidant agents that can scavenge free radicals effectively can be beneficial in the treatment of inflammatory disorders.

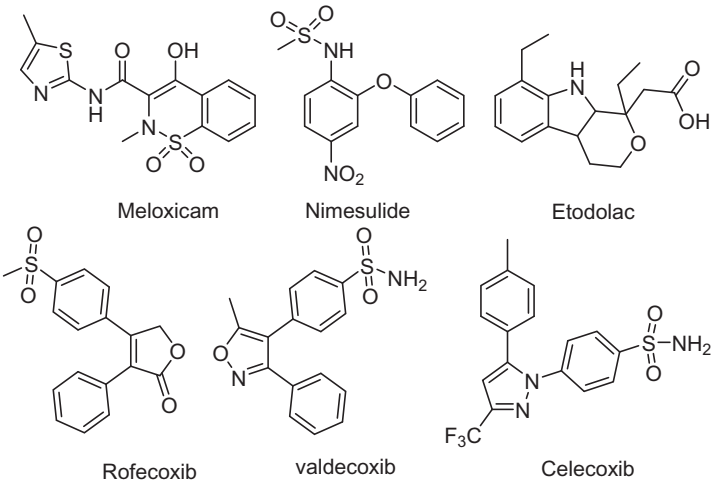


Figure 1. Marketed COX-2 inhibitors.

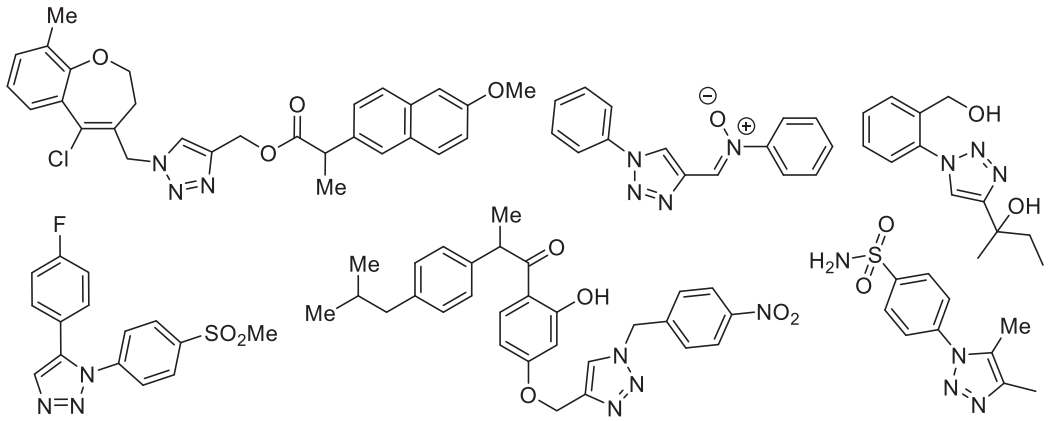


Figure 2. 1,2,3-Triazole as anti-inflammatory agent.

1,2,3-Triazole possess favorable therapeutic index in the context of medicinal chemistry, the large dipole, *H*-boding capabilities, and remarkable metabolic stability toward enzymatic degradation. Hybrid triazole compounds have been deemed the current medicine regime and are being applied for the discovery of new drugs aimed at treating multifactorial diseases like cancer, HIV, neurodegenerative disorders and infectious disease like tuberculosis, fungal and bacterial infections.[12] Recently a hybrid of 1,2,3-triazole and benzoxepine or quinolone heterocyclics,[13] 1,2,3-triazole substituted *N*-aryl/alkyl nitrones,[14] 24 derivatives of 1,2,3-triazole,[15] 1,2,3-triazole methane sulfonyl phenyl derivatives,[16] conjugate of 1,2,3-triazole and ibuprofen,[17] and poly functionalized 1,2,3-triazole benzene sulfonamides[18] as potent anti-inflammatory agents had been reported. Their anti-inflammatory activity was found to be due to the selective inhibition of COX-2 enzyme. Some significant derivatives of discussed work are shown in Figure 2.

Pharmaceutical outcome of 1,2,3-triazoles is consistent with their 1,4-,1,5- or 1,4,5-substitution patterns, among them fully decorated 1,2,3-triazoles have significant role in pharmaceutical chemistry.[19–21] Recently compounds containing guanidine functionality have attracted considerable synthetic interest as hydrophilic functionality to introduce in bioactive scaffolds.[22] T. Yu and coworkers have reported the small molecular antimicrobial peptide (AMP) mimics with guanidine moiety as cationic functional group, and it has been observed to potentiate the antimicrobial

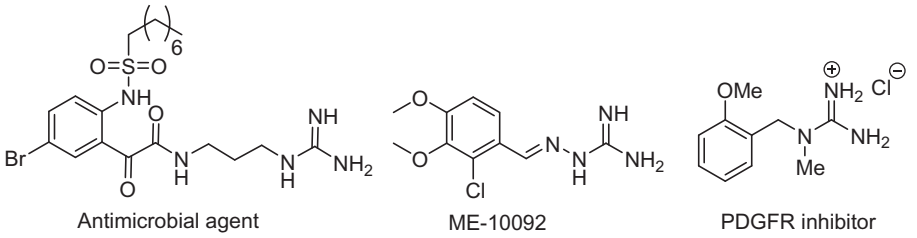


Figure 3. Biologically potent guanidine derivatives.

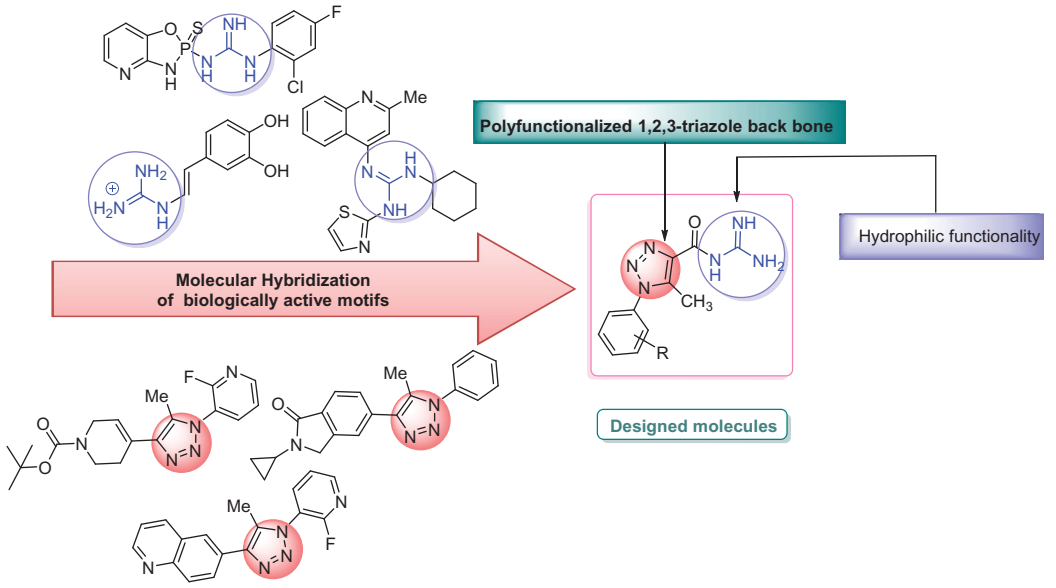


Figure 4. Designing of fully decorated 1,2,3-triazole-gauanidine conjugates.

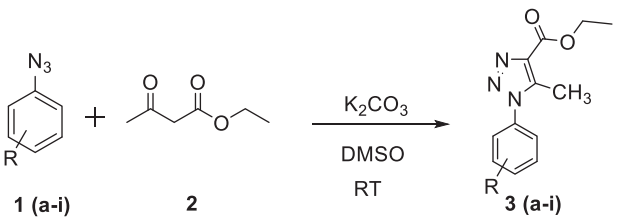
activity of parent compound[23] (Figure 3). Dambrova et al. studied the anti-inflammatory and antinociceptive effect of NF-KB inhibitory activity of guanidine-based compound ME10092.[24] Recently J. Medved and coworkers have developed inhibitor of platelet-derived growth factor receptor PDGFRα with guanidine moiety appended to an aromatic core.[25]

In view of the reported biological importance of guanidine,[26–28] 1,2,3-triazole, specially, fully decorated 1,2,3-triazoles,[29–31] we speculated that molecular hybridization of both the scaffolds would result in better biological activity (Figure 4). In continuation to earlier work on the triazole heterocyclic moiety[32–40] in this study, we report the synthesis of novel guanidine derivatives of 1,2,3-triazole as anti-inflammatory agents with potential antioxidant activity. The mechanism of inhibition of inflammation is studied *via in silico* study of docking interactions with COX-2 enzyme.

Result and discussion

Synthesis

Initially, a panel of 1,2,3-triazole were synthesized *via* organocatalytic enolate- mediated azide-carbonyl [3 + 2] cycloaddition popularly known as Ramachary-Bressy-Wang, [3 + 2] cyclo-addition reaction.[41] A modified version of the synthetic route to fully decorated 1,2,3-triazole reported by R. Nelson and mates[42] in 2016 is attempted in the synthesis of 1,4,5-trisubstituted 1,2,3-triazole



Scheme 1. Synthesis of 5-methyl-1-phenyl-1*H*-1,2,3-triazolecarboxylates **3(a–i)**.

carboxylates (Scheme 1; Table 1). The synthetic strategy provides a simple access to fully substituted 1,2,3-triazoles from commercial substrates in moderate to excellent yields. After the successful synthesis of 1,4,5-trisubstituted 1,2,3-triazole carboxylates **3(a–i)**, these derivatives were treated with guanidine in strongly basic environment that supports nucleophilic attack *via* guanidine to afford the novel *N*-carbamimidoyl-5-methyl-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide derivatives **5(a–i)** in 76–84% yields (Scheme 2; Table 2).

Biological activity

Anti-inflammatory activity

According to these results, the newly synthesized triazole-guanidine conjugates exhibit promising anti-inflammatory activity compared with standard drug **DFS** (Diclofenac sodium). The triazole-guanidine conjugates **5c**, **5f**, **5g** with C-4 chloro, C-3 methyl and C-2 bromo substituents on phenyl ring respectively, are found to be most potent anti-inflammatory agents among the series with IC_{50} values ranging 10–13 μg/mL closer to standard drug DFS (IC_{50} 13.24 ± 0.98). The compounds **5b** (C-3 chloro) and **5d** (C-2 nitro) prevent the HRBC membrane lysis with potential measured in terms of IC_{50} 15.51 ± 1.33 and 16.47 ± 1.40. Compounds **5a** and **5i** were found to be the least efficient anti-inflammatory agents with IC_{50} in the range 18–19 μg/mL.

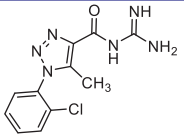
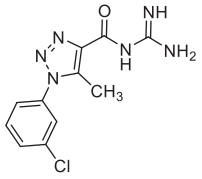
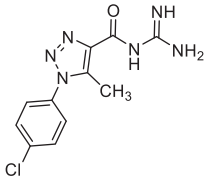
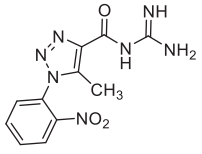
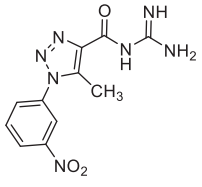
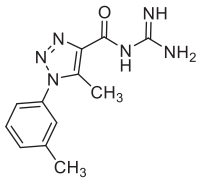
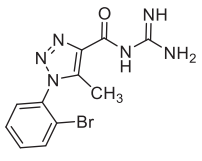
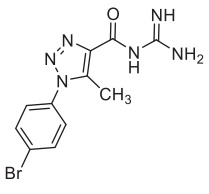
The structure-activity relationship (SAR) revealed that the introduction of chloro, methyl and bromo group at C-4, C-3 and C-2 positions activate the conjugates with IC_{50} 10.11 ± 1.24, 11.86 ± 2.13 and 13.69 ± 1.59 respectively. But the substitution of C-3 and C-2 with chloro and nitro group resulted in next lower inhibition compared to standard drug **DFS** (IC_{50} 13.24 ± 0.98). C-4 methoxy and unsubstituted derivatives of the triazole-guanidine conjugates were the least potent inhibitors (Table 2).

1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity

The antioxidant potential of synthesized derivatives is evaluated in terms of IC_{50} concentrations, i.e. a concentration required to scavenge 50% of the radicals. A lower IC_{50} value indicates the greater antioxidant activity. *N*-carbamimidoyl-5-methyl-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide possess moderate to good antioxidant activity. The conjugate **5f** and **5h**, were found to be more potent as compared to standard drug Ascorbic acid (Table 2). Compounds **5f** and **5h** displayed excellent activity with IC_{50} values 12.42 ± 0.78 and 13.9 ± 0.49 μ;g/mL, respectively, and were found to exhibit better activity than standard drug Ascorbic acid. The conjugate **5d** and **5g**, were found to be equally potent with IC_{50} values 14.67 ± 0.01 and 14.63 ± 0.81 μ;g/mL in comparison with Ascorbic acid (Table 2).

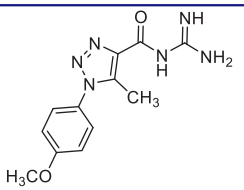
The structure-activity relationship (SAR) revealed that the introduction of Methyl substituents at the C-3 position of the phenyl ring in triazole-guanidine conjugates (**5f**) enhances the radical scavenging ability (IC_{50}12.42 ± 0.78) hence exhibiting good antioxidant potential. The next highest antioxidant potential in terms of RSA was observed for **5h** (IC_{50} 13.9 ± 0.49) with C-4 Bromo substitution at phenyl ring. The substitution on phenyl ring at positions C-2 (**5d** and **5g**) by nitro

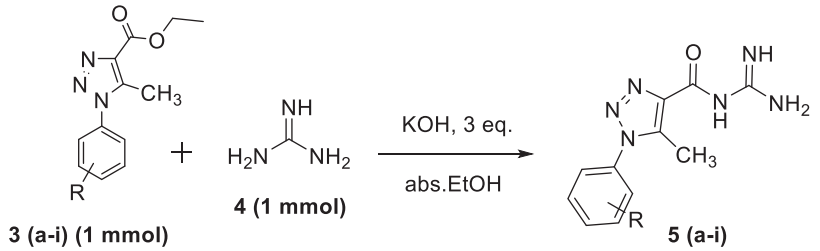
Table 1. Synthesis of *N*-carbamimidoyl-5-methyl-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide.

Entry	Compound	Structure	Yield (%)
1	**5a**		83
2	**5b**		81
3	**5c**		84
4	**5d**		76
5	**5e**		82
6	**5f**		80
7	**5g**		82
8	**5h**		83

(*continued*)

Table 1. Continued.

Entry	Compound	Structure	Yield (%)
9	**5i**		80



Scheme 2. *N*-carbamimidoyl-5-methyl-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide **5(a–i)**.

Table 2. Screening of anti-inflammatory activity and antioxidant potential of synthesized conjugates (**5a–i**).

Compound	DPPH IC_{50} (μg/mL)	Anti-inflammatory activity μg/mL (IC_{50})	Glide score	Glide energy (kcal/mol)	Hydrogen bond (Å)
5a	22.7 ± 0.49	19.67 ± 2.15	−8.166	−35.826	Tyr355 (2.205), Val116 (2.006)
5b	15.8 ± 0.94	15.51 ± 1.33	−8.442	−40.866	Tyr355 (2.195), Val116 (2.008)
5c	18.3 ± 0.08	10.11 ± 1.24	−8.705	−44.144	Tyr355 (2.169), Val116 (2.019)
5d	14.67 ± 0.01	16.47 ± 1.40	−8.335	−39.475	Val116 (2.155)
5e	24.02 ± 0.32	17.63 ± 0.76	−8.302	−38.192	Tyr355 (2.170), Val116 (1.761)
5f	12.42 ± 0.78	11.86 ± 2.13	−8.681	−43.55	Tyr355 (2.183), Val116 (2.011)
5g	14.63 ± 0.81	13.69 ± 1.59	−8.511	−41.035	Tyr355 (2.105)
5h	13.9 ± 0.49	12.16 ± 0.63	−8.575	−42.112	Val116 (2.116)
5i	28.9 ± 0.94	18.5 ± 1.64	−8.181	−36.037	–
AA	14.80 ± 0.17	–	–	–	–
DFS	–	13.24 ± 0.98	–	–	–

Values are expressed as mean ± standard deviation ($n = 3$). **AA**: Ascorbic acid; **DFS**: Diclofenac sodium.

(**5d**) and bromo(**5g**) group exhibits better radical scavenging ability with IC_{50}14.67 ± 0.01 and 14.63 ± 0.81.

Furthermore, the trends in radical scavenging ability and hence antioxidant data (Table 2) revealed that substitution with methoxy group and C-2 Chloro substituted phenyl ring was inactivating the conjugates with lowest radical scavenging ability and hence lowest antioxidant potential (IC_{50}28.9 ± 0.94 and 22.7 ± 0.49) for **5i** and **5a** respectively. The substitution with a chloro group at the C-3and C-4 positions (**5b** and **5c**) are found to be somewhat less potent than standard drug AA with antioxidant potential RSA (IC_{50}15.8 ± 0.94 and 18.3 ± 0.08) (Table 2).

Molecular docking

All the 1,2,3-triazole-guanidine conjugates (**5a–i**) were found to be docked well into the active site of COX-2 enzyme engaging in multiple bonded and non-bonded interactions with residues

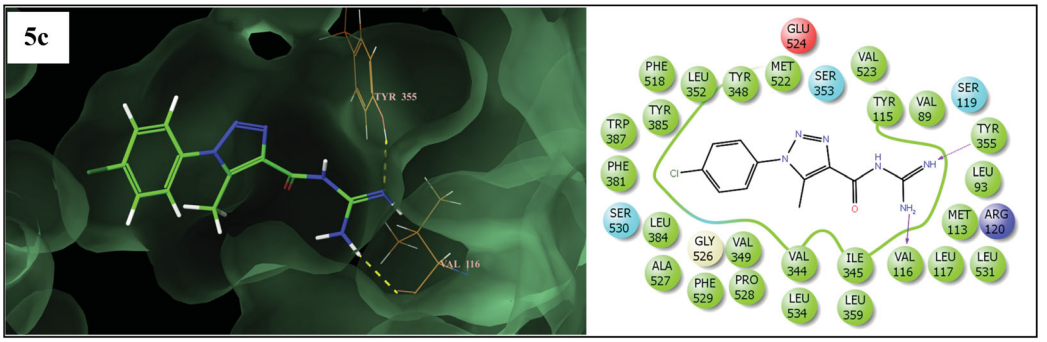


Figure 5. Binding mode of **5c** into the active site of COX-2 (on right side: the pink lines signify hydrogen bonding interactions).

lining the active site (Table 2). A linear correlation was observed between their binding affinities (Glide dock scores) and observed anti-inflammatory activities. Therefore, to understand the various thermodynamic interactions which could contribute to their enhanced binding affinity, a detailed per-residue interaction analysis was carried out which for one of the most active analogs **5c** (Figure 5) revealed that the molecule was deeply embedded into the active site of enzyme (Glide dock score: −8.705 and Glide binding energy: −44.144 kcal/mol).

The enhanced binding affinity is attributed to significant van der Waals interactions observed with *N*-carbamimidoyl-carboxamide side chain through Leu531 (−2.004 kcal/mol), Leu359 (−1.189 kcal/mol), Tyr355 (−1.668 kcal/mol), Arg120 (−1.714 kcal/mol) and Val116 (−1.154 kcal/mol) residues while the central 1–5-methyl-1*H*-1,2,3-triazole core was seen to be engaged in similar interactions with Val523 (−2.489 kcal/mol), Met522 (−1.577 kcal/mol), Ser353 (−1.289 kcal/mol) and Val349 (−3.665 kcal/mol) residues. Even the 4-chlorophenyl side chain exhibited significant van der Waals interactions with Ser530 (−1.424 kcal/mol), Ala527 (−4.585 kcal/mol), Gly526 (−1.962 kcal/mol), Phe518 (−1.038 kcal/mol), Trp387 (−1.583 kcal/mol), Tyr385 (−1.453 kcal/mol), Leu384 (−1.064 kcal/mol), Phe381 (−1.165 kcal/mol) and Leu352 (−2.062 kcal/mol) residues. While these van der Waals interactions were found to be the major driving force in the mechanical interlocking of the molecule, even some significant electrostatic interactions observed with Ser530 (−2.276 kcal/mol), Glu524 (−1.267 kcal/mol), Val523 (−1.043 kcal/mol), Glu520 (−1.797 kcal/mol), Arg513 (−1.976 kcal/mol), Glu510 (−2.356 kcal/mol), Tyr385 (−1.595 kcal/mol), Tyr355 (−2.005 kcal/mol), Ser353 (−1.153 kcal/mol), Tyr348 (−1.404 kcal/mol) and Val116 (−1.872 kcal/mol) residues could assist the molecule to snuggly fit into the active site. Furthermore, the compound was seen to be engaged in close hydrogen bonding interactions with Tyr355 (2.169 Å) and Val116 (2.019 Å) through the carbamimidoyl side chain which serve as 'anchor' to guide the orientation of a ligand into the 3 D space of active site. Other molecules in the series were also observed to be engaged similar network of interactions with COX-2 enzyme (SI: Figure 6–13) which suggest that the 1,2,3-triazole-guanidine scaffold could serve as pertinent starting point for structure-based lead optimization.

Experimental

All the chemicals and reagents were of analytical grade and purchased from Sigma Aldrich, Alfa Aesar, Spectrochem and used without further purification. Thin-layer chromatography (TLC) was performed on pre-coated 0.25 mm silica gel plates (60 F-254) using UV light as visualizing agent. Melting points were determined by an open capillary method and are uncorrected. ^{1}H NMR spectra were recorded in DMSO-d_6 on Bruker 500 MHz instruments. ^{13}C NMR spectra were recorded on a Bruker 126 MHz instrument using DMSO-d_6. Chemical shifts (δ) were reported as

parts per million (ppm) in δ scale downfield from TMS (internal standard). Mass spectra were recorded under electrospray ionization MS.

General procedure for the preparation of 5-methyl-1-phenyl-1H-1,2,3-triazolecarboxylates 3(a–i)

Excess of K_2CO_3 (3 equivalents) is added to a solution of phenyl azide (1 mmol) with ethyl acetoacetate (EAA) (1 mmol) in DMSO and stirred at room temperature. The resulting reaction was monitored by TLC. After the completion of the reaction, cold water (30 mL) was added to give a precipitate that was filtered off and washed successively with cold water and then vacuum dried. The products were purified by crystallization with EtOH.

General procedure for the preparation of N-carbamimidoyl-5-methyl-1-phenyl-1H-1,2,3-triazole-4-carboxamide derivatives 5(a–i)

In a round-bottom flask equipped with a magnetic stirring bar, a mixture of 1,4,5-trisubstituted 1,2,3-triazole carboxylates (**3a–i**) (1 mmol) and guanidine **4** (1 mmol) in excess of ethanolic KOH solution was stirred at RT. Progress of the reaction is monitored by TLC. After the completion of the reaction, the reaction mixture was poured on 50 mL ice water, acidified with dil. HCl and the resulted solidified product were filtered, dried, and recrystallized by EtOH to get pure *N*-carbamimidoyl-5-methyl-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide derivatives **5(a–i)**.

N-carbamimidoyl-1-(2-chlorophenyl)-5-methyl-1H-1,2,3-triazole-4-carboxamide (5a)

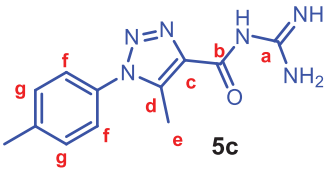
Brown solid; M.P.: 78–80 °C; ^{1}H NMR (500 MHz, DMSO, δ ppm): δ 7.75 (s, 1H), 7.70 (s, 1H), 7.65–7.63 (m, 1H), 7.59–7.57 (m, 1H), 2.52 (s, 3H); ^{13}C NMR (125 MHz, DMSO, δ ppm): 166.1, 158.9, 143.2, 135.3, 134.9, 133.9, 129.6, 129.6, 127.1, 126.9, 9.70; MS, calculated mass for $C_{11}H_{11}ClN_6O$: 278.0683 and found $[M+H]^+$: 279.1.

N-carbamimidoyl-1-(3-chlorophenyl)-5-methyl-1H-1,2,3-triazole-4-carboxamide (5b)

Off cream solid; M.P.: 130–132 °C; ^{1}H NMR (500 MHz, DMSO, δ ppm): 7.75 (s, 1H), 7.65 (s, 2H), 7.58 (s, 1H), 2.52 (s, 3H).^{13}C NMR (125 MHz, DMSO, δ ppm): 165.9, 158.8, 143.3, 137.3, 135.1, 133.7, 131.2, 129.3, 125.0, 123.9, 9.690; MS, calculated mass for $C_{11}H_{11}ClN_6O$: 278.0683 and found $[M+H]^+$: 279.0.

N-carbamimidoyl-1-(4-chlorophenyl)-5-methyl-1H-1,2,3-triazole-4-carboxamide (5c)

White solid; M.P.: 171–172 °C; MS, calculated mass for $C_{11}H_{11}ClN_6O$: 278.0683 and found $[M+H]^+$: 279.1.



5c

^{1}H NMR (500 MHz, DMSO, δ ppm): 7.75 (s, 2H), 7.65 (dd,2H), 2.59 (s,3H)

^{13}C NMR (125 MHz, DMSO, δ ppm): 166.0, 158.8, 143.5, 136.1, 135.2, 134.7, 133.9, 129.6, 126.9, 9.7.

HMBC data result for compound 5c

Correlation of ^{1}H NMR and ^{13}C NMR

^{1}H NMR		^{13}C NMR		
H at position	ppm value	C at position	ppm value	HMBC result
e	2.59 (s,3H)	e	9.7	H_e is correlated with C_e
		c	143.5	H_e is correlated with C_c
		d	136.1	H_e is correlated with C_d
f	7.65 (dd,2H)	f	134.7, 133.9	H_f is correlated with C_f
		i	135.2	H_f is correlated with C_i
g	7.75 (s, 2H)	g	129.6, 126.9	H_g is correlated with C_g

Conclusion: As per correlation of HMBC confirmed the structure of compound **5c**.

N-carbamimidoyl-1-(2-nitrophenyl)5-methyl–1H-1,2,3-triazole-4-carboxamide (5d)

Bright white solid; M.P.: 126–128 °C; ^{1}H NMR (500 MHz, DMSO, δ ppm): 8.31 (d, 1H), 8.00 (m, 1H), 7.94 (m, 2H), 2.51 (s, 3H); ^{13}C NMR (125 MHz, DMSO, δ ppm): 171.0, 162.9, 145.4, 143.1, 137.6, 134.7, 131.9, 129.5, 128.1, 125.7, 9.7; MS, calculated mass for $C_{11}H_{11}N_7O_3$: 289.0923 and found $[M+H]^+$: 290.1.

N-carbamimidoyl-1-(3-nitrophenyl)5-methyl–1H-1,2,3-triazole-4-carboxamide (5e)

White crystalline solid; M.P.: 130–132; ^{1}H NMR (500 MHz, DMSO, δ ppm): δ 8.30 (s, 1H), 8.28 (s, 1H), 8.01 (s, 1H), 7.99 (s, 1H), 7.91 (s, 1H), 2.53 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ 166.1, 158.98, 143.4, 137.5, 135.3, 133.9, 131.3, 129.6, 125.2, 124.0, 9.860; MS, calculated mass for $C_{11}H_{11}N_7O_3$: 289.0923 and found $[M+H]^+$: 290.1.

N-carbamimidoyl-1-(3-tolyl)-5-methyl-1H-1,2,3-triazole-4-carboxamide (5f)

Light orange solid; M.P.: 124–125 °C; ^{1}H NMR (500 MHz, DMSO, δ ppm): 8.42 (d, 1H), 7.94 (s, 2H), 7.72 (s, 1H), 2.68 (s, 3H), 2.60 (s, 3H); ^{13}C NMR (125 MHz, DMSO, δ ppm): 171.5, 166.2, 163.0, 158.9, 143.3, 139.2, 135.0, 133.7, 129.96, 125.2, 20.69, 9.7; MS, calculated mass for $C_{12}H_{14}N_6O$: 258.1229 and found $[M+H]^+$: 259.1.

N-carbamimidoyl-1-(2-bromophenyl)-5-methyl-1H-1,2,3-triazole-4-carboxamide (5g)

Faint pink solid; M.P.: 184–186 °C; ^{1}H NMR (500 MHz, DMSO, δ ppm): 7.85 (s, 1H), 7.67 (s, 2H), 7.55 (t,1H), 2.50 (s, 3H); ^{13}C NMR (125 MHz, DMSO, δ ppm): 171.0, 162.9, 145.4, 143.1, 137.6, 134.7, 131.9, 129.5, 128.1, 125.7, 9.75; HRMS, calculated mass for $C_{11}H_{11}BrN_6O$: 322.0178 and found $[M+H]^+$: 323.6.

N-carbamimidoyl-1-(4-bromophenyl)-5-methyl-1H-1,2,3-triazole-4-carboxamide (5h)

White crystalline solid; M.P.: 127–129 °C;^{1}H NMR (500 MHz, DMSO, δ ppm): 7.82 (s, 2H), 7.57 (s, 2H), 2.59 (s, 3H); ^{13}C NMR (125 MHz, DMSO, δ ppm): 166.0, 158.8, 143.3, 135.4, 135.1, 132.5, 132.5, 127.4, 127.1, 122.4, 9.70; MS, calculated mass for $C_{11}H_{11}BrN_6O$: 322.0178 and found $[M+H]^+$: 323.2.

N-carbamimidoyl-1-(4-methoxyphenyl)-5-methyl-1H-1,2,3-triazole-4-carboxamide(5i)

White crystalline solid; M.P.: 127–129 °C;^{1}H NMR (500 MHz, DMSO, δ ppm): 7.48 (s, 2H), 7.13 (s, 2H), 3.84 (s, 3H), 2.43 (s, 3H). ^{13}C NMR (125 MHz, DMSO, δ ppm): 166.2, 158.8, 143.1, 138.9, 134.9, 133.7, 129.9, 125.2, 124.9, 20.71, 10.28, 9.730; HRMS, calculated mass for $C_{12}H_{14}N_6O_2$: 274.1178 and found $[M+H]^+$: 275.1.

Experimental procedure for biological activity

Anti-inflammatory activity

Anti-inflammatory agents inhibit the cyclooxygenase enzymes which are responsible for the conversion of arachidonic acid to prostaglandins. Because Human Red Blood Cell (HRBC) membranes are similar to these lysosomal membrane components,[43] the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure in estimating anti-inflammatory activity. Thus, HRBC membrane stabilization method[44] was used to estimate *in vitro* anti-inflammatory activity of all synthesized compounds (Table 1, **5(a–i)**). **DFS** stabilizes the membrane, thereby reducing the hemolysis. Thus, with the increase in the component prevented from leaking the concentration of DFS increases, the Optical Density (O.D.) decreases thereby decreasing the effect of the tonicity caused by hypo saline.

The anti-inflammatory activities of various compounds are assessed by in vitro HRBC membrane stabilization method. Blood is collected from healthy volunteers. The collected blood was mixed with equal volume of Alsever's solution and centrifuge with the Iso saline. To 1 mL of HRBC suspension equal volume of test drug in different concentration are added. All the assay mixture is incubated at 37 °C for 30 min and centrifuged. The hemoglobin content in the supernatant solution is estimated by using spectrometer at 560 nm. The percentage of hemolysis was calculated.

$$\%\ \text{Hemolysis} = \left(\text{Optical density of Test sample}/\text{Optical density of Control}\right) \text{x } 100$$

Antioxidant activity

Antioxidant activities of the synthesized compounds **5a–i** were measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.[45] DPPH radical scavenging activity is the most commonly used method for screening the antioxidant potential of diverse synthetic heterocyclic scaffolds as well as natural antioxidants. Ascorbic acid (AA) has been used as a standard drug for the comparison of antioxidant activity and the observed results are summarized in (Table 2).

The antioxidant activity of synthesized compounds have been evaluated *in vitro* as per Braca et al. using 1, 1-Diphenyl-2picrylhydrazyl (DPPH) radical scavenging assay,[46] the results were equated with standard antioxidant Ascorbic acid (AA). In this method, the hydrogen atom or electron donation ability of the derivatives can be measured from the bleaching of the purple-coloured methanol solution of 1, 1-Diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. Aliquots of 30 μL of various concentrations (10,20, 30, 40, and 50 μg/mL) of compounds were added in 3 mL DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (0.004%) and absorbance was taken at λmax = 520 nm after 30 min using Ascorbic acid as reference and methanol as control. The following equation calculated the percentage scavenging of free radical production from DPPH.

$$\%\,\text{Of Scavenging} = \left[(\text{A control} - \text{A sample})/\text{A blank}\right] \text{X } 100,$$

where 'A control' is the absorbance of the control reaction (containing all reagents except the test

compound), and 'A sample' is the absorbance of the test compound. Tests were carried out in triplicate, and the results were given as means ± standard deviations.

Molecular docking

In order to gain mechanistic insight into the anti-inflammatory activity exhibited by the 1,2,3-triazole-guanidine conjugates (**5a–i**), molecular docking study was performed against Cyclo-oxygenase 2 (COX-2) (pdb id: 1PXX), the enzyme catalyzing the bio conversion of arachidonic acid to inflammatory prostaglandins (PGs) that mediates inflammation and pain. The *in silico* binding affinity study was performed using the standard protocol implemented in GLIDE (Grid-Based Ligand Docking with Energetics) module of the Small Drug Discovery Suite (Schrödinger, LLC, New York, NY).[47–49] The X-ray crystal structure of cyclooxygenase-2 (COX-2) enzyme was retrieved from the Protein Data Bank (www.rcsb.org/1PXX) and refined using the *protein preparation* wizard which involves eliminating the crystallographically observed water molecules (since no water molecule was found to be conserved in ligand-protein interaction), adding hydrogen atoms, assignment of charge and protonation state. The refined structure was then subjected to energy minimization, to relieve the steric clashes among the residues due to addition of hydrogen atoms, using OPLS-2005 force field until the average RMSD of the non-hydrogen atoms reached 0.3 Å. The shape and properties of the active site of COX-2 were defined and setup for docking using the *receptor grid generation* panel which creates a 10X10X10Å box that was centered on the native ligand in the crystal complex. The 3 D structures of 1,2,3-triazole-guanidine conjugates **5(a–i)** were sketched in the *build* panel of Maestro and optimized using *ligand preparation* tool. With this initial setup, the molecular docking study was performed using Schrödinger's proprietary extra precision (i.e. with GlideXP) scoring function to gauze the binding affinity of the molecules. The output files were in terms of the docking poses were visualized and analyzed for the key elements of interaction with the enzyme using Maestro's Pose Viewer utility.

Conclusion

A new series of highly functionalized triazoles, *N*-carbamimidoyl-5-methyl-1-phenyl-1*H*-1,2,3-triazole-4-carboxamide derivatives **5(a–i)** were designed and synthesized. The designing strategy affords densely functionalized 1,2,3-triazoles, which are amenable to further modifications due to the presence of guanidine group. The synthesized derivatives were studied for anti-inflammatory activity using membrane stabilization method. Compound **5c** showed the most potential anti-inflammatory activity. The conjugates **5c**, **5f**, **5h** and **5g** were found to be potent with closer IC_{50} values in comparison with standard drug DFS. Even the molecular docking study showed that these compounds possess good to an excellent binding affinity for COX-2 engaging in multiple bonded and non-bonded interactions. The results have shown that the compounds **5d**, **5f**, **5g** and **5h** are good synthetic antioxidants compared with all synthesized compounds. Hence the synthesized derivatives of triazole-guanidine can be considered as potential lead molecule to prevent the oxidative and inflammatory process.

Disclosure statement

The authors declare no conflict of interest, financial or otherwise.

ORCID

Mubarak H. Shaikh http://orcid.org/0000-0002-1190-2371
Vijay M. Khedkar http://orcid.org/0000-0001-9982-7785

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