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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and SAR studies of potent H^+/K^+-ATPase and anti-inflammatory activities of symmetrical and unsymmetrical urea analogues

Kadalipura P. Rakesh[1] · Nanjudappa Darshini[1] · Sunnadadoddi L. Vidhya[2] · Rajesha[2] · Ningegowda Mallesha[1]

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Abstract A sequence of symmetrical and unsymmetrical urea derivatives **1–24** were synthesized and characterized by standard spectroscopic techniques. The synthesized analogues were tested for their in vitro H^+/K^+-ATPase and anti-inflammatory activities. The majority of the compounds showed outstanding activity, compared to that of omeprazole and indomethacin, usual standard drugs of antiulcer and anti-inflammatory, respectively. In particular, hydroxy, methyl, and methoxy derivatives **13–24** were the most active compounds possessing a significant amplify for diverse substituents on the benzene ring thus, contributing positively to gastric ulcer inhibition. Compounds **1–3** and **22–24** showed excellent anti-inflammatory activity due to the presence of electron-withdrawing groups (Cl and F) on the molecule.

Keywords Ureas's · SAR · H^+/K^+-ATPase · anti-inflammatory

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✉ Rajesha
rajeshamg7@gmail.com

✉ Ningegowda Mallesha
research@sriramchem.com

[1] SRI RAM CHEM, R & D Centre, Plot No. 31, JCK industrial Park, Belagola Industrial Area, Mysuru 570016 Karnataka, India

[2] Bharathi College PG and Research Center, Bharathinagara, Maddur, Mandya district, Karnataka 571422, India

Introduction

Urea is a useful molecule that is generally present in natural products and frequently displays a broad scope of biological activities (Fournier et al. 1991). The substituted urea is a most importance in medicinal chemistry and it is used for the synthesis of various organic or heterocyclic compounds with diversified biological activities including antimicrobial (Umadevi et al. 2012; Gulkok et al. 2012), antiviral (Rajtar et al. 2013), anticonvulsant (Ramesh et al. 2013), anti-proliferative (Cho et al. 2012), antitumor (Lu et al. 2013), antiulcernic (Vijayakumar et al. 2013), antimalaria (Anderson et al. 2013). Some of the urea derivatives are thiourea, phenyl urea, sulfur ureas are found to exhibit a potent inhibiting effect on HIV-1 protease enzyme and as anticancer and sedative-hypnotic activities. They are also served as an extensive application as fertilizers, agro-chemicals, and synthetic intermediates for many drugs (Viana et al. 2013).

Gastric ulcers are commonly occurring diseases and believed to result from an imbalance between the aggressive and defensive forces in the stomach (Gustavsson et al. 1983). Newly, agents have been recognized that totally suppress acid secretion by inhibition of the gastric proton pump H^+/K^+-ATPase. Such inhibition leads to intense and persistent achlorhydria, which outcome in ulcer healing rates significantly more rapid than those achievable by H_2 antagonists. H^+/K^+-ATPase catalyzes the terminal step in gastric acid, whereas histamine plays crucial role in acid secretion. As a result, inhibition of H^+/K^+-ATPase can supply an essentially greater reduction in gastric acid secretion (Sachs et al. 1976; Forte et al. 1980; Lind et al. 1983).

Non-steroidal anti-inflammatory drugs (NSAIDs) are most commonly used in therapeutics, mainly for the action

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of pain and inflammation in arthritis for decades. NSAIDs reduce the pain and swelling related with arthritis by blocking the metabolism of arachidonic acid by cyclooxygenase enzyme (COX) thereby the production of prostaglandin (Vane 1971). COX activity originates from two diverse and separately regulated enzymes, termed COX-1 and COX-2 (Yokoyama and Tanabe 1989; Hla and Neilson 1992). COX-1 is nominated as housekeeping enzyme because of its physiological and homeostatic functions, whereas COX-2 is expressed in pathological state and triggers inflammatory signals, thus selected as an adaptive enzyme. Both of these enzymes share more than sixty percent of homology with respect to their structure (Kurumbail et al. 1996). Overall, these explanations recommend that COX-1 and COX-2 serve up different physiological and pathophysiological functions. Therefore, although there are a number of anti-inflammatory drugs are available in the market, development of novel compounds having anti-inflammatory agents with improved profile is still a necessity.

Hence, with this observations and earlier investigations from our research group (Rakesh et al. 2015a, 2016a, 2016b, 2015b), the present work was undertaken with a view to assess the H^+/K^+-ATPase and anti-inflammatory agents of the title compounds.

Experimental

Materials

All chemicals and reagents obtained from Sigma Aldrich (India), Merck (India), and Avra Synthesis (India) were used without further purification. Melting points were determined on a Superfit melting point apparatus (India) and are uncorrected. 1H nuclear magnetic resonance (NMR) (400 MHz) and 13C NMR (100 MHz) spectra were recorded on a Agilent Technologies (USA) using dimethyl sulfoxide (d6) or $CDCl_3$ as solvent. High-resolution mass spectroscopic analysis was performed on a Bruker Micro-TOF QII mass spectrometer in positive mode. Progress of the reaction was monitored by TLC using silica gel 60 F254, with the solvent system comprising hexane and ethylacetate in the ratio 04:01 and the compounds on the TLC plates were detected by under ultraviolet light and iodine vapor.

General synthesis procedure of *N*'-substituted ureas (1–24)

The ureas (**1–24**, 1 mmol) was separately dissolved in 5 mL of THF and added anhydrous sodium hydride (1 mmol) to the solution, after 5 min, isocyanates (1 mmol) were added to the solutions, after completion of the reaction (monitored by TLC), reaction mass was poured in to ice cold water and then extracted with EtOAc, the organic layer was washed with water and dried over anhydrous sodium sulfate. The organic solvent was removed under vacuum to get crude products (3a-r). The solid product was further purified by column chromatography by using hexane and ethyl acetate (95:5) as eluent to give pure products.

Biological activity

Gastric H^+/K^+-ATPase activity

Isolation of parietal cells from sheep stomach The fundic stomach slice of sheep quickly after sacrificing was collected and was rinsed with Krebs ringer buffer (250 mM sucrose, 2 mM $MgCl_2$, 1 mM EGTA, and 2 mM Hepes-Tris of pH 7.4). The top layer was pinned with the help of needles on the dissection table. Mucosal scrapings were hanging in 10 volumes of Krebs ringer buffer (pH = 7.4) containing sucrose (250 Mm) and homogenized with 20 strokes of a mortar driven Teflon pestle homogenizer. The tissues were discarded and the filterate was subjected to sub cellular fractionation. The pellets so obtained were dissolved in 2 mL of sucrose-EGTA buffer and was used as enzyme sample.

Protein estimation

Protein was calculated by using Lowry method (Lowry et al. 1951) and bovine serum albumin as standard (0–75 µg). Eight clean and dry test tubes were taken and aliquots of various concentrations of the synthesized derivatives were made. To the 7th and 8th test tube, the unknown sample (5 and 10 µL of the cells isolated from the sheep stomach) for which the protein content was added. In every test tube, the solution was made up to 1 mL by the addition of distilled water followed by the addition of 5 mL of Lowry's reagent. All the test tubes are incubated at room temperature (rt) for 8–10 min and 0.5 mL of Folin-Ciocalteu reagent was added and again incubation at rt for 30 min. Absorbance of each solution was read at 670 nm against the blank solution. A graph was plotted by using concentration of protein on *x*-axis and OD on *y*-axis. From the standard graph obtained, the unknown concentration of protein sample was calculated and found to contain 21 mg of protein per 8 g of tissue homogenate.

Inorganic phosphorus estimation

Inorganic phosphorus was estimated according to literature reported method (Fiske and Subbarow 1925). Aliquots of working standard solution (40 µg/mL) were added into eight

fresh and dry test tubes in the volume of 0 to 1 mL; 5 and 10 µL of the enzyme sample were taken in test tube 7th and 8th, respectively. The volumes of all test tubes were made up to 8.6 mL using 10% trichloroacetic acid (TCA). Ammonium molybdate (1 mL) and 8-Anilino-1-naphthalenesulfonic acid ammonium salt, reagent (0.4 mL) were added to all test tubes. All test tubes were allowed to stand for 10 min at rt. Then the color was developed and read at its λ_{max} of 660 nm.

ATPase activity

ATPase activity was determined as described by using reported method (Im et al. 1985). Basal Mg^{2+} dependent ATPase activity was calculated in 1 mL of the reaction includes of 2 mmol/L ATP and 50 mmol/L Tris-HCl buffer (pH = 7.5). K^+ stimulated and HCO_3^- stimulated ATPase activity in the basal medium. The ATPase reaction was started by the addition of the substrate (ATP), carried out at 37 °C for 10–15 min and closed with 1 mL cold 20% TCA. Liberated inorganic phosphate from ATP was estimated by using literature method (Fiske and Subbarow 1925).

Activity of the enzyme sample in the presence of ATP is 0.066 µmoles.

Activity of the enzyme sample in the absence of ATP is 0.042 µmoles.

100% activity of the enzyme is 0.066–0.042 µmoles = 0.024 µmoles.

Anti-inflammatory activity

Human erythrocyte suspension

The human blood was collected from a healthy volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and collected in heparinzed vacutainer. The collected healthy human blood was washed 0.9% saline and centrifuged for 10 min at 3000 rpm. The packed cells were washed with 0.9% saline and 40% v/v suspension made by isotonic phosphate buffer of 154 mM NaCl in 10 mM sodium phosphate buffer at pH 7.4 used as Stock erythrocyte or RBC suspension.

Hypotonic solution-induced haemolysis

The activity of the synthesized compounds was performed according to the reported method (Shinde et al. 1999). The test sample consisted of stock erythrocyte (RBC) suspension 0.5 mL mixed with 5 mL of hypotonic solution (50 mM NaCl in 10 mM sodium phosphate buffered saline at pH 7.4) containing different concentrations of sample (20, 40, 60, 80, and 100 µg/mL). The control consists of 0.5 mL RBC suspension mixed with 5 mL of hypotonic buffered solution alone. The mixtures were incubated for 10 min at room temperature, centrifuged for 10 min at 3000 rpm and supernatant was measured by spectrophotometrically at 540 nm. The % inhibition of haemolysis was calculated from the following formula.

$$\%\ \text{Inhibition of haemolysis} = \left[\frac{A_1 - A_2}{A_1}\right] \times 100$$

Where:

A_1 = Absorbance of hypotonic buffered solution alone

A_2 = Absorbance of test /standard sample in hypotonic solution

Results and discussion

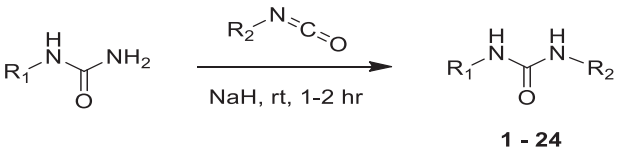
Chemistry

All the urea are prepared as per our recently reported protocol (Rakesh et al. 2016a, 2016b), amides are reacted with isocyante in presence of sodium hydride at room temperature for 1–2 h. Corresponding urea obtained in reasonable to excellent yield (Scheme 1) and obtained products are well characterized with NMR and mass spectroscopic techniques. Obtained derivatives are tabulated Table 1.

Biological activity

H^+/K^+-ATPase activity

We have synthesized disubstituted urea's (**1–24**) from this work and tested for their capacity to reduce H^+/K^+-ATPase using standard drug omeprazole. The obtained results (IC_{50} values) are tabulated in Table 1 and the data represents average values from triplicate runs. A close test of the outcome results revealed some interesting facts with respect to structure–activity relationship. It is evident from the results that electron-donating and electron-withdrawing groups is important for inhibition of H^+/K^+-ATPase activity. Investigations have shown that H^+/K^+-ATPase inhibition is associated with the modification of electron-donating and electron-withdrawing groups in the benzene ring, this could be the reason for higher activity of electron-donating (OH, OCH_3, and CH_3) analogue compared to electron-withdrawing (Cl and F) analogues. Based on our



Scheme 1 Synthesis of symmetrical and unsymmetrical urea from sodium hydride and isocyanate

Table 1 Antiulcer activity and anti-inflammatory activities of the synthesized compounds

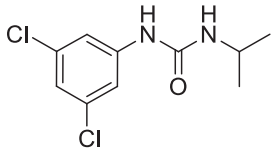
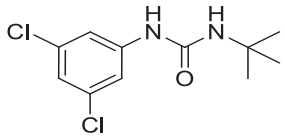
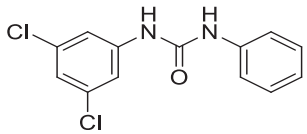
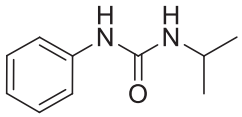
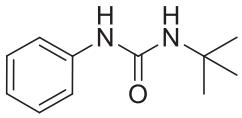
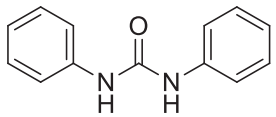
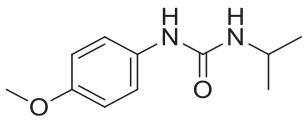
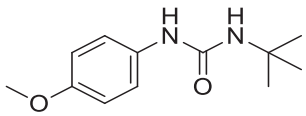
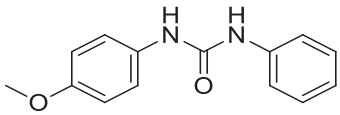
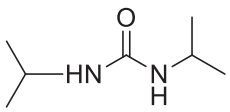
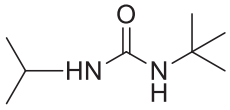
Entry	Structure	Antiulcer activity[a] (IC_{50} μg/mL)	Anti-inflammatory activity[a] (IC_{50} μg/mL)
1		62.4 ± 1.23	34.4 ± 0.12
2		58.5 ± 0.45	28.3 ± 1.24
3		52.3 ± 1.24	20.7 ± 1.74
4		78.8 ± 0.75	60.6 ± 1.07
5		70.4 ± 1.44	56.3 ± 0.12
6		66.1 ± 0.58	52.0 ± 0.73
7		36.1 ± 1.47	48.3 ± 0.43
8		30.0 ± 0.33	50.1 ± 0.92
9		22.9 ± 0.14	48.2 ± 0.47
10		Nil	Nil
11		Nil	Nil

Table 1 continued

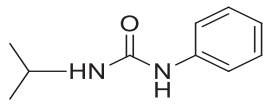
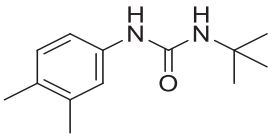
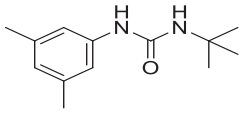
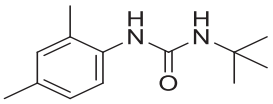
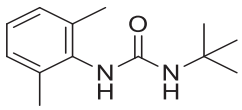
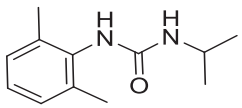
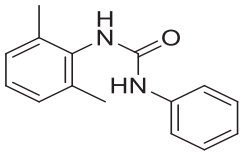
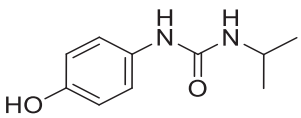
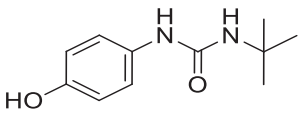
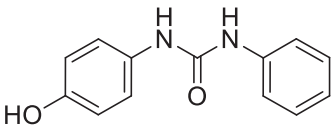
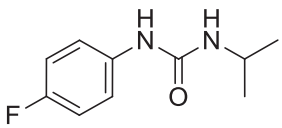
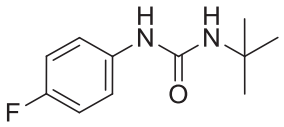
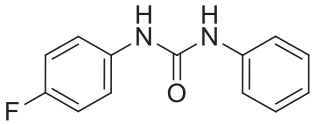
Entry	Structure	Antiulcer activity[a] (IC_{50} μg/mL)	Anti-inflammatory activity[a] (IC_{50} μg/mL)
12		90.5 ± 0.49	86.1 ± 1.44
13		34.3 ± 0.14	50.1 ± 0.14
14		32.2 ± 1.40	42.2 ± 0.12
15		36.7 ± 0.58	42.1 ± 0.44
16		30.1 ± 0.11	44.4 ± 0.24
17		34.9 ± 0.11	38.2 ± 0.47
18		10.1 ± 0.45	36.1 ± 0.14
19		38.4 ± 0.22	58.1 ± 0.41
20		34.3 ± 0.43	50.6 ± 0.41
21		18.4 ± 0.14	46.7 ± 0.17
22		60.4 ± 0.88	40.0 ± 0.42

Table 1 continued

Entry	Structure	Antiulcer activity[a] (IC_{50} μg/mL)	Anti-inflammatory activity[a] (IC_{50} μg/mL)
23		56.1 ± 0.47	36.4 ± 0.41
24		48.5 ± 1.54	20.3 ± 0.61
Omerazole		38.2 ± 0.85	–
Indomethacin		–	44.8 ± 0.25

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases

earlier results (Rakesh et al. 2016), compounds with electron-donating groups on the phenyl ring showed excellent activity. Compounds with electron-withdrawing groups on phenyl ring showed moderate or less activity. The same development has been noticed here also.

Among the derivatives, diphenyl urea **6** displayed activity with $IC_{50} = 66$ μg/mL which is less potent than omeprazole ($IC_{50} = 38$ μg). It can be inferred that compounds without substitution on the aryl ring showed lesser activity. Therefore, the effect of substituents on the phenyl ring and different aromatic and aliphatic isocyanates roles was further investigated. When a methoxy, hydroxyl, and methyl groups was placed on the phenyl ring, derivatives exhibited good activity. Compound **7–9** and **13–21** showed good activity with IC_{50} values are lower than that of the reference standard drug omperazole (38 μg/mL). Further, the substitution of halogens (Cl and F), compounds (**1–3** and **22–24**) showed moderate or least H^+/K^+-ATPase activity. We have also interestingly to design both sides of aliphatic groups (**10** and **11**) and tested the activity, the obtained results are nil or poor H^+/K^+-ATPase activity.

In vitro anti-inflammatory activity

All the synthesized compounds were further tested for their in vitro anti-inflammatory activity and results are presented in Table 1. A few numbers of compounds have been noticed for good to moderate activity compared to standard drug indomethacin. The compounds **1–3** and **22–24** showed excellent activity with IC_{50} values are lower than the standard indomethacin (42 μg/mL). It is evident from the results that the compounds containing electron-withdrawing groups Cl and F (**1–3** and **22–24**) are good anti-inflammatory agents and electron-donating groups OH and OCH_3 (**7–8** and **19–21**) are less activity and methyl (CH_3) groups (**13–18**) in the molecule showed moderate activity. Two sides of aliphatic groups (**10** and **11**) are also showed very less or poor activity.

Conclusion

In the present study, we have tested for their in vitro H^+/K^+ ATPase and anti-inflammatory activity of disubstituted urea's analogues. Compounds **7–9** and **13–21** with OH, OCH_3, and CH_3 groups in benzene ring showed excellent H^+/K^+ ATPase activity than omperazole. Compounds **1–3** and **22–24** with Cl and F in benzene ring showed good anti-inflammatory activity than indomethacin. Finally, we have synthesized simple and small active analogues, which shown potent H^+/K^+ ATPase and anti-inflammatory activity.

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Conflict of interest The authors declare that they have no competing interests.

References

Anderson JW, Sarantakis D, Terpinski J, Kumar TR, Tsai HC, Kuo M, Ager AL et al. (2013) Novel diaryl ureas with efficacy in a mouse model of malaria. Bioorg Med Chem Lett 23:1022–1025

Cho HJ, EI-Gamal MI, Oh CH, Lee SH, Kim G, Jun HH, Hong SC, Kyung HY (2012) Synthesis and antiproliferative activity of new aminoisoquinolinylurea derivatives against melanoma cell line. Bull Kore Chem Soc 33:3635–3639

Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorous. J Biol Chem 66:375–400

Forte JG, Machen TF, Obrink KJ (1980) Mechanism of gastric H^+ and -Cl transport. Annu Rev Physiol 42:111–126

Fournier J, Bruneau C, Dixneuf H, Lécolier S (1991) Ruthenium catalyzed synthesis of symmetric *N*, *N*′-disubstituted ureas

directly from carbon dioxide and amines. J Org Chem 56:4456–4458

Gulkok Y, Bicer T, Onurdag K, Ozgen S, Sahin MF, Dogruer DS (2012) Synthesis of some new urea and thiourea derivatives and evaluation of their antimicrobial activities. Turk J Chem 36:279–291

Gustavsson S, Adam HO, Loof L, Nyberg A, Nyren O (1983) Rapid healing of duodenal ulcers with omeprazole: double-blind dose-comparative trial. Lancet 322:124–125

Hla T, Neilson K (1992) Human cyclooxygenase2 Cdna. Proc Natl Acad Sci 89:7384–7388

Im WB, Sih JC, Blakeman DP, McGrath JP (1985) Omeprazole, a specific inhibitor of gastric H^+/K^+-ATPase, is a H^+-activated oxidizing agent of sulfhydryl groups. J Biol Chem 260:4591–4597

Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, Gildehaus D et al. (1996) Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nat New Biol 384:644

Lind T, Cederberg C, Eberved G, Haglund U, Olbe L (1983) Effect of omeprazole –a gastric proton pump inhibitor –on pentagastrin stimulated acid secretion in man. Gut 24:270–276

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Lu CS, Tang K, Li Y, Jin B, Yin DL, Chen XG, Ma C, Huang HH (2013) Synthesis and in vitro antitumor activities of novel benzyl urea analogues of sorafenib. Yao Xue Xue Bao 48:709–717

Rajtar B, Szacon E, Swiatek L, Rzadkowska M, Matosiuk D, Malgorzata PD (2013) Antiviral activity of 1-(1-arylimidazolidine-2-ylidene)-3-(4-chlorobenzyl)urea derivatives. J Pre Clin and Clin Res 7:104–106

Rakesh KP, Manukumar HM, Gowda DC (2015a) Schiff's bases of quinazolinone derivatives: synthesis and SAR studies of a novel series of potential anti-inflammatory and antioxidants. Bioorg Med Chem Lett 5:1072–1077

Rakesh KP, Ramesha AB, Shantharam CS, Mantelingu K, Mallesha N (2016a) An unexpected reaction to methodology: an unprecedented approach to transamidation. RSC Adv 6:108315–108318

Rakesh KP, Shantharam CS, Manukumar HM (2016b) Synthesis and SAR studies of potent H^+/K^+-ATPase inhibitors of quinazolinone-schiff's base analogues. Bioorg Chem 68:1–8

Rakesh KP, Suhas R, Chandan S, Manukumar HM, Gowda DC (2015b) Quinazolinones linked amino acids derivatives as a new class of promising antimicrobial, antioxidant and anti-inflammatory agents. Eur J Chem 6:254–260

Ramesh D, Gangadhar S, Katti SA (2013) Synthesis, characterization and anticonvulsant activity of 1-(aryl)-3-(diphenylmethyl) urea derivatives. Int J Res Pharm Chem 3:748–752

Sachs G, Chang HH, Rabon E, Shackman R, Lewin M, Saccomani G (1976) A noneletrogenetic H^+ pump in plasma membranes of hog stomach. J Biol Chem 251:7690–7698

Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN (1999) Membrane stabilizing activity a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia 70:251–257

Umadevi P, Deepti K, Srinath I, Vijayalakshmi G, Tarakaramji M (2012) Synthesis and in-vitro antibacterial activity of some new urea, thiourea and thiosemicarbazide derivatives. Int J Pharm Pharma Sci 4:379–383

Vane JR (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. New Biol 231:232–235

Viana GM, Aguiara LC, Ferrão JA, Simas ABC, Vasconcelos MG (2013) The use of aqueous potassium dichloroiodate for the synthesis of ureas. Tetra Lett 54:936–940

Vijayakumar S, Bongu S, Gomathi P (2013) Anti-ulcerogenic activity of unsymmetrically substituted urea derivative (1-benzyl-3-(4-methylphenyl) urea. Afri J Pharm Pharmacol 7:2522–2527

Yokoyama C, Tanabe T (1989) Cloning of human gene encoding prostaglandin endoperoxide synthase and primary structure of the enzyme. Biochem Biophys Res Commun 165:888–894