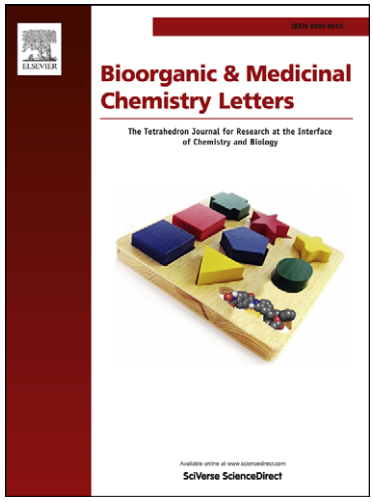


Design, synthesis, characterization and anti-inflammatory evaluation of novel pyrazole amalgamated flavones

Hemant V. Chavan, Babasaheb P. Bandgar, Laxman K. Adsul, Valmik D. Dhakane, Pravin S. Bhale, Vishnu N. Thakare, Vijay Masand

PII: S0960-894X(13)00004-8
DOI: http://dx.doi.org/10.1016/j.bmcl.2012.12.094
Reference: BMCL 19979

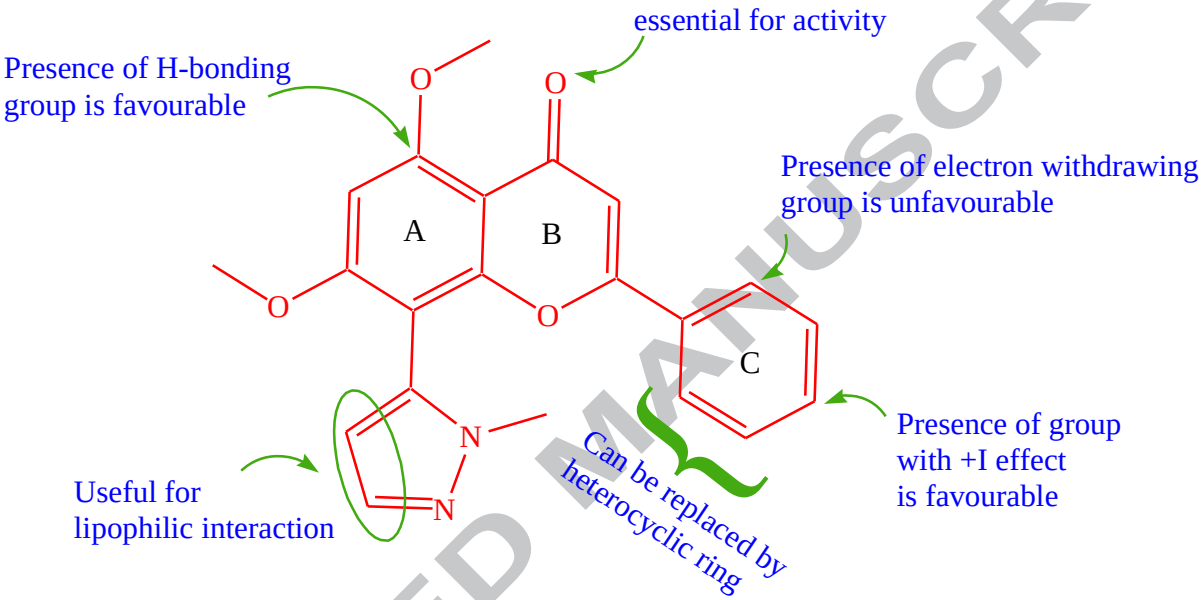
To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 7 October 2012
Revised Date: 19 December 2012
Accepted Date: 28 December 2012

Please cite this article as: Chavan, H.V., Bandgar, B.P., Adsul, L.K., Dhakane, V.D., Bhale, P.S., Thakare, V.N., Masand, V., Design, synthesis, characterization and anti-inflammatory evaluation of novel pyrazole amalgamated flavones, *Bioorganic & Medicinal Chemistry Letters* (2013), doi: http://dx.doi.org/10.1016/j.bmcl.2012.12.094

Graphical Abstract

Design, synthesis, characterization and anti-inflammatory evaluation of novel pyrazole amalgamated flavones



A series of novel pyrazole amalgamated flavones have been designed synthesized and evaluated for their anti-inflammatory potential.

Design, synthesis, characterization and anti-inflammatory evaluation of novel pyrazole amalgamated flavones

Hemant V. Chavan,[a] Babasaheb P. Bandgar,[a,*] Laxman K. Adsul,[a] Valmik D. Dhakane,[a] Pravin S. Bhale,[a] Vishnu N. Thakare,[b] Vijay Masand[c]

[a] Medicinal Chemistry Research Laboratory, School of Chemical Sciences, Solapur University, Solapur-413 255, Maharashtra, India.

[b] Department of Pharmacology, Sinhgad Institute of Pharmaceutical Sciences, Kusgaon (Bk), Lonavala-410 401, Maharashtra, India.

[c] Department of Chemistry, Vidyabharati Mahavidyalaya, Camp, Amravati-444 602, Maharashtra, India.

*Corresponding author: Tel. /Fax: 0091-217-2351300; E-Mail: bandgar_bp@yahoo.com

A B S T R A C T

A series of novel pyrazole amalgamated flavones has been designed and synthesized from 1-methyl-5-(2,4,6-trimethoxy-phenyl)-1*H*-pyrazole **6**. The structures of regioisomers **6** and **7** were resolved by 2D ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC experiments. The newly synthesized compounds were tested for their *in vitro* COX inhibition and *in vivo* carrageenan induced hind paw edema in rats and acetic acid induced vascular permeability in mice. Although the compounds have inhibitory profile against both COX-1 and COX-2, some of the compounds are found to be selective against COX-2, supported by inhibition of paw edema and vascular permeability. Docking studies were also carried out to determine the structural features which sway the anti-inflammatory activity of the tested compounds. The keto and phenolic –OH are major factors that are prominently involved in interaction with COX-2 active site.

Keywords: Pyrazole, Flavones, HMBC, HSQC, COSY, Anti-inflammatory activity, Molecular docking.

Flavonoids are natural phytoconstituents having various biological and pharmacological activities.[1] The flavonoid family includes chalcone, flavan-3-ol, flavanone, flavone, flavonol and various biflavonoids and found to have elicited anti-inflammatory activity *in vitro* and *in vivo*.[2,3] The anti-inflammatory activity of flavonoids is due to its multiple cellular mechanisms of action. In addition to their well-known anti-oxidative action, some flavonoids exert inhibitory activity against eicosanoid and nitric oxide (NO) metabolism. For example, some compounds inhibit phospholipase A2s (PLA2), cyclooxygenase (COX), lipoxygenase (LOX) and nitric oxide synthase (NOS).[4-6] Furthermore, flavonoids also found to inhibit and down-regulate expression of other pro-inflammatory mediators *viz.* matrix metalloproteinase (MMP).[7] The flavones are characterized by the planar structure because of the double bond in the central aromatic ring. Specifically, these flavonoids inhibit the expression of the inducible forms of COX and NOS, interleukins, tumor necrosis factor-α (TNF-α) and adhesion molecules in inflammatory cells and tissues. Many flavonoids and biflavonoids affect multiple points in the aforementioned pathways, which suggests that they are anti-inflammatory agents with multiple action mechanisms.[8] One of the best described flavonoids, quercetin, is a member of this group.

Pyrazoles are an important class of compounds for new drug development that attracted much attention due to their broad spectrum of biological activities, such as anti-inflammatory,[9-12] antifungal,[13] anticancer,[14-16] and antiviral activities.[17,18]. Pyrazole derivatives also act as antiangiogenic agents,[19] kinase inhibitor for treatment of type-2 diabetes, hyperlipidemia, obesity,[20] and thrombopiotinmimetics.[21] Recently urea derivatives of pyrazoles have been reported as potent inhibitors of p38 kinase.[22] Among the highly marketed COX-2 inhibitors that comprise the pyrazole nucleus, celecoxib is the one which is treated as a safe anti-inflammatory and analgesic agent. It is considered as a typical model of the diaryl heterocyclic template that is known to selectively inhibit the COX-2 enzyme. Some other examples of pyrazole derivatives such as deracoxib, SC-558, ramifenazone, famprofazone, rimonbant, fipronil, pyraclonil (**Figure 1**) have been reported as potent NSAIDs.[23-27]

In continuation of our ongoing research programme on the development of novel anti-inflammatory agents,[28-30] and based on the diverse biological activities of flavonoids and pyrazoles, in the present study we have synthesized some new synthetic flavones amalgamated with pyrazole scaffold, as potential anti-inflammatory agents.

Synthesis of title compounds **10a-n** was achieved by the oxidation of chalcones **9a-n** with iodine in DMSO. Claisen-Schmidt condensation of 1-(2-hydroxy-4,6-dimethoxy-3-(1-

methyl-1*H*-pyrazol-5-yl)phenyl)ethanone **8** with various aromatic / heteroaromatic aldehydes in presence of NaOH in ethanol at room temperature furnished chalcones **9a-n** in good to excellent yields. 1-(2-Hydroxy-4,6-dimethoxy-3-(1-methyl-1*H*-pyrazol-5-yl)phenyl)ethanone **8** was readily prepared by the Friedel-Craft acetylation of key intermediate 1-methyl-5-(2,4,6-trimethoxyphenyl)-1*H*-pyrazole **6** using acetic anhydride in presence of boron trifluoride etherate under nitrogen atmosphere (**Scheme 1**).

The regioselective synthesis of key intermediate 1-methyl-5-(2, 4, 6-trimethoxyphenyl)-1*H*-pyrazole **6** was carried out using the synthetic strategies illustrated in **Scheme 2**. Xanthoxyline **2** was synthesized by the Friedel-Craft acetylation of 1,3,5-trimethoxybenzene **1** by adopting the literature precedent, and successive O-methylation of the resulting xanthoxyline **2** using dimethyl sulphate and fused potassium carbonate in acetone under reflux condition gave 1-(2,4,6-trimethoxyphenyl)ethanone **3** in excellent yield.[31] 2-Formylation of acetophenone **3** with ethyl formate and sodium hydride gave 3-oxo-3-(2,4,6-trimethoxyphenyl)propanal **4**, and without further purification subsequent treatment of **4** with hydrazine hydrate afforded the corresponding 5-(2,4,6-trimethoxyphenyl)-1*H*-pyrazole **5** in 96% yield. Treatment of compound **5** with methyl iodide and sodium hydride in dry DMF under nitrogen atmosphere afforded a mixture of 1-methyl-5-(2,4,6-trimethoxyphenyl)-1*H*-pyrazole **6** and 1-methyl-3-(2,4,6-trimethoxyphenyl)-1*H*-pyrazole **7**, which were easily separated by silica gel coloumn chromatography.

The structures of the regioisomers **6** and **7** were determined using 1D ^{1}H, ^{13}C NMR spectra, 135-DEPT and 2D NMR experiments (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC and NOESY), which were scanned on Bruker AV-400 and 500 MHz instrument. These two regioisomers differ only in the position of methyl group on pyrazole nucleus, therefore, the ^{1}H and ^{13}C NMR chemical shifts were found to be almost similar. Thus, the structures of these two regioisomers were only assigned with the help of HMBC, HSQC and COSY correlations. The most important HMBC and COSY correlations are summarized in **Figure 2** and the complete chemical shift assignment is shown in **Table 4** and **Table 5** (Supporting information). According to a Heteronuclear Multiple Bond Correlation (HMBC) experiment performed with regioisomer **6**, the HMBC correlation observed between H-13 (δ_H 3.65) and C-3 (δ_C 135.5) and with regioisomer **7,** the HMBC correlation observed between H-13 (δ_H 3.97) and C-1 (δ_C 130.12) allowing to unambiguous identification of the regioisomer **6** and **7**. Additionally, Heteronuclear single Quantum Coherence (HSQC) experiment was used to assign proton signals to the corresponding carbon signals. There is a strong ^{1}H-^{1}H COSY correlation between H-1 and H-2.

All the synthesized compounds (**10a-n**) were screened for their inhibitory potential against the COX-1 and COX-2 enzymes at 100 μM by using Colorimetric COX (ovine) Inhibitor Screening assay kit (Catalogue No. 760111, Cayman Chemicals Co, USA). Indomethacin was used as a reference compound. The results are listed in **Table 1**. The results showed that most of the synthesized compounds have an inhibitory profile against both COX-1 and COX-2, some are found to be more selective towards COX-2 (**10h**, **10e**, and **10i**) by a small percentage of inhibition. The entire series of flavones exhibited significant inhibitory profile against COX-2 (38-51%). Compounds carrying methoxy groups on phenyl ring (**10h** and **10e)** appeared as the most active compounds against COX-2 enzyme. Moreover, compounds containing furan and thiophene heterocycles instead of phenyl ring (**10i** and **10j** respectively) showed significant inhibitory activity on COX-2 enzyme with inhibiting COX-1 enzyme less than indomethacin (**Table 1**). In addition, substitution of fluorine to the second / fourth position of phenyl ring (**10n**, **10b,** and **10f**) resulted in a remarkable decrease in COX-2 inhibitory activity.

The synthesized compounds were also tested for their *in vivo* anti-inflammatory potential by using carrageenan hind paw and acetic acid vascular permeability methods. From the experimental findings, significant ($p < 0.01$) increase in paw volume was observed in rats administered with carrageenan alone when compared with normal rats (**Table 2**). Increase in paw edema indicates the inflammation due to release of inflammatory mediators (prostaglandins, serotonin, histamine, etc.) at various time intervals. The synthesized compounds *viz.* **10h, 10e** and **10i** are found to significantly inhibit ($p<0.01$) the edema formation (**Table 2**). Inhibition of paw edema formation at 6 h is greater that might be due to the inhibition of COX-II**,** which is known to be released at 4-6 h after the carrageenan injection. In acetic acid induced vascular permeability in mice, significant inhibition of dye leakage into peritoneal cavity indicated the anti-inflammatory effects of **10h, 10e** and **10i** (**Table 3**). Since vascular permeability is one of the sign of inflammation, increased vascular permeability in control group indicated the inflammatory response. Prevention and/or inhibition of vascular permeability with **10h, 10e** and **10i** certainly elicited the potent anti-inflammatory activity. Therefore, the compounds showing significant COX-2 inhibitory potential (**10h, 10e** and **10i**) also display potent anti-inflammatory activity *in vivo*.

The docking study was performed with MOE software program to understand the orientations of the compounds in the active site of COX-2. We docked all the synthesised compounds **10a-n** and indomethcin in the active site of COX-2. In COX-2, the binding site is comparatively long, slender, hydrophobic channel extending from the membrane-binding

region of the protein. A second cavity branched off from the main channel that lead to the cyclooxygenase active site is observed in COX-2. A similar pocket also exists in COX-1 but it is relatively small and less accessible due to presence of bulkier isoleucine at position number 523. The well known anti-inflammatory agent Indomethacin binds to Val102, Arg106, Val335, Leu338, Tyr341, Leu345, Trp373, Met508, Val509, Gly512, and Ala513 residues of COX-2. To get clear idea, we docked all the flavone derivatives in the pocket of COX-2.

Hydrophobic as well as H-bonding interactions are involved in binding of most active compound **10h** with COX-2 (**Figure 3**). It appears that the water present inside the active site of COX-2 plays crucial role.[45, 46] The two oxygen atoms, one from ketone group of ring **2** and -OMe from ring **1** adjacent to it, act as H-bond acceptor by interacting with TyrA 341 (a neutral amino acid with polar but non-ionized side chain) with a distance of 3.34A^o (48%) and 2.69 A^o (99%), respectively. Neutral amino acids with nonpolar side chain ValA335, MetA99, LeuA517, AlaA513 surronds the ring 3 that indicates the presence of lipophilic groups on this ring is important for binding with receptor. This fact is confirmed when we compare efficiency of compound **10h** and CLogP values (**Table 1**) with other derivatives in which lipophilic groups are absent on ring 3. From **Figure 3**, it is clear that the binding pocket is large and compound **10h** is small, this means new larger groups can be introduced on ring 3 to increase the interaction between drug and receptor. This suggests that compound **10h** is attractive target as " lead compound". The docking and structure activity relationship (SAR) analysis reveals that pyrazole play crucial role due to its lipophilic character and the nitrogens are unable to act as H-bond acceptor. The summary of SAR study is shown in **Figure 4**.

In conclusion, a series of new pyrazole amalgamated flavones were synthesized in order to evaluate their inhibitory activities on COX-1 and COX-2 enzymes. Among the synthesized compounds, **10h**, **10e**, and **10i** showed significant inhibitory profiles against COX-2, indicating that they are selective inhibitors for COX-2. Moreover, inhibitory effects of **10h**, **10e**, and **10i** are also supported by their effects in inhibition of carrageenan induced paw edema and prevention of dye leakage into peritoneal cavity due to the reduction of vascular permeability which might be due to release of prostaglandins and other inflammatory mediators. The binding mode of the tested compounds inside the active site of COX-2 enzyme was predicted using docking technique. Neutral amino acids with nonpolar side chain ValA335, MetA99, LeuA517, AlaA513 surronds ring 3 of compound **10h**, indicating the presence of lipophilic groups on this ring is important for binding with receptor. It is also

clear that the binding pocket of COX-2 is large and compounds are small, this means introduction of new larger groups on ring 3 may be useful to propose new molecules with enhanced COX-2 selectivity.

Acknowledgements

Author H.V.C. thankful to Council of Scientific and Industrial Research (CSIR), New Delhi, Govt. of India for financial support in the form of SRF. Authors are also grateful to Dr. S. R. Naik and Dr. S. B. Bhise, Sinhgad Institute of Pharmaceutical Sciences, Lonavala, and Pune for providing necessary facility for conducting pharmacological experiments.

References and Notes

1. Havsteen, B. *Biochem. Pharmacol*. **1983**, *32*, 1141-1148.
2. Middleton, F. A.; Strick, P. L. *Brain Res. Brain Res. Rev.* **2000**, *31*, 236–250.
3. Rajnarayana, K.; Reddy, M. S.; Chaluvadi, M. R.; Krishna, D. R. *Ind. J. Pharmacol*. **2001,** *33*, 2-16.
4. Le Marchand, L. *Biomed. Pharmacother.* **2002**, 56, 296-301.
5. Middleton, E. J. Adv. *Exp. Med. Biol.* **1998**, *439*, 175–82.
6. Bitis, L.; Kultar, S.; Melikoglu, G.; Ozsoy, N.; Can, A. *Natural Product Radiance* **2003,** *24*, 580-589.
7. Nijveldt, R. J.; Nood, E.; Hoorn, D.; Boelens, P. G.; Norren, K.; Leeuwen P. *Am. J. Clin. Nutr.* **2001**, *74*, 418–425.
8. Kim, H. P.; Son, K. H.; Chang, H. W.; Kang, S. S. *J. Pharmacol. Sci.* **2004**, *96*, 229-245.
9. Nakamura, T.; Sato, M.; Kakinuma, H.; Miyata, N.; Taniguchi, K.; Bando, K.; Koda, A.; Kameo, K. *J. Med. Chem.* **2003**, *46*, 5416-5427.
10. Cheng, H.; DeMello, K. M. L.; Li, J.; Sakya, S. M.; Ando, K. et.al. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2076-2080.
11. Bekhit, A. A.; Ashour, H. M. A.; Ghany, Y. S. A.; Bekhit, A. E. A.; Baraka, A. *Eur. J. Med. Chem.* **2008**; *43*, 456–463
12. Ahlstrom, M. M.; Ridderstrom, M.; Zamora, I.; Luthman, K. *J. Med. Chem.* **2007**, *50*, 4444-4452.
13. Prakash, O.; Kumar, R.; Parkash, V. *Eur. J. Med. Chem.* **2008**, *43*, 435–440.

14. Brough, P. A.; Aherne, W.; Barril, X.; Borgognoni, J.; Boxall, K.; Cansfield, J. E.; Cheung, K. M.; Collins, I.; Davies, N. G.; Drysdale, M. J.; Dymock, B.; Eccles, S. A.; Finch, H.; Fink, A.; Hayes, A.; Howes, R.; Hubbard, R. E.; James, K.; Jordan, A. M.; Lockie, A.; Martins, V.; Massey, A.; Matthews, T. P.; McDonald, E.; Northfield, C. J.; Pearl, L. H.; Prodromou, C.; Ray, S.; Raynaud, F. I.; Roughley, S. D.; Sharp, S. Y.; Surgenor, A.; Walmsley, D. L.; Webb, P.; Wood, M.; Workman, P.; Wright, L. *J. Med. Chem.* **2008**, *51*, 196-218.
15. Vera-DiVaio, M. A. F.; Freitas, A. C. C.; Castro, H. C. A.; de Albuquerque, S.; Cabral, L. M.; Rodrigues, C. R.; Albuquerque, M. G.; Martins, R. C. A.; Henriques, M. G.; Dias, L. R. S. *Bioorg. Med. Chem.* **2009**, *17*, 295–302;
16. Hsu, T. C.; Robins, R. K.; Cheng, C. C. *Science* **1956**, *13*, 848–868.
17. Storer, R.; Ashton, C. J.; Baxter, A. D.; Hann, M. M.; Marr, C. L. P.; Mason, A. M.; Mo, C. L.; Myers, P. L.; Noble, S. A.; Penn, C. R.; Weir, N. G.; Woods, J. M.; Coe, P. L. *Nucleosides Nucleotides Nucleic Acids* **1999**, *18*, 203–216.
18. Genin, M. J.; Biles, C.; Keiser, B. J.; Poppe, S. M.; Swaney, S. M.; Tarpley, W. G.; Yagi, Y.; Romero, D. L. *J. Med. Chem.* **2000**, *43*, 1034–1040.
19. Qiao, J. X.; Pinto, D. J.; Orwat, M. J.; Han, W.; Friedrich, S. R. PCT Int. Appl. WO 03 99, 276 (*Chem. Abstr.* **2004**, *140*, 16722g).
20. Brown, M. L.; Cheung, M.; Dickerson, S. H.; Drewy, D. H.; Lackey, K. E.; Peat, A. J.; Thomson, S. A.; Veal, J. M.; Wilson, J. L. R. PCT Int. Appl. WO 2004, 9596 (*Chem. Abstr.* **2004**, 140, 128436y).
21. Heerding, D.A. PCT Int. Appl. WO 03 103686 (*Chem. Abstr.* **2004**,*140*, 42170v).
22. Duma, J.; Hatoum-Mokdad, H.; Sibley, R.; Riedl, B.; Scott, W. J.; Monahan, M. K.; Lowinger, T. B.; Brennan, C.; Natero, R.; Turner, T.; Johnson, J. S.; Schoenlebar, R.; Bhargava, A.; Wilhelm, S. M.; Housley, T. J.; Ranges, G. E.; Shrikhande, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2051–2054.
23. Reynold, J. E. F. (Ed.), The Extra Pharmacopia, 30th ed. Martindale, Pharmaceutical Press, London, 1993, p. 1;
24. Amir, M.; Kumar, S. *Ind. J. Chem. Sect. B.* **2005**, *44B*, 2532-2537.
25. Gursoy, A.; Demirayak, S.; Capan, G.; Erol, K.; Vural, K. *Eur. J. Med. Chem.* 2000, *35*, 359-364;
26. Kumar, A. Sharma, S. Bajaj, K. Bansal, D. Sharma, S. Archana, K. Saxena, K. Lata, S. Gupta, B. Srivastava, V. K. *Ind. J. Chem. Sect. B.* **2003**, *42B*, 1979-1984;
27. Dadiboyena, S.; Nefzi, A. *Eur. J. Med. Chem.* **2011**, *46*, 5258-5275.

28. Bandgar, B. P.; Gawande, S. S.; Bodade, R. G. Gawande, N. M. Khobragade, C. N. *Bioorg. Med. Chem*. **2009**, *17*, 8168-8173.

29. Bandgar, B. P.; Gawande, S. S.; Bodade, R. G.; Totre, J. V.; Khobragade, C. N. *Bioorg. Med. Chem.* **2010**, *18*, 1364-1370.

30. Bandgar, B. P.; Patil, S. A.; Gacche, R. N.; Korbad, B. L.; Kinkar, S. N.; Jalde, S. S.; Hote, B. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 730-733.

31. Ahluwalia, V. K. Intermediates for Organic Synthesis; I. K. International Publisher: New Delhi, **2005**. p 140

32. **Preparation of 5-(2,4,6-Trimethoxy-phenyl)-1*H*-pyrazole (5):** Ethyl formate (2.96 gm, 3.21 ml, 30 mmol) was added to a suspension of sodiun hydride (60 % in oil, 0.8 gm, 20 mmol) in tetrahydrofuran (3 ml) at room temperature. After stirring for 10 min, a solution of 1-(2,4,6-trimethoxy-phenyl)-ethanone **3** (2.1 gm, 10 mmol) in THF (15 ml) was added, and the mixture was stirred for 1 h. To the reaction mixture was then added 1M HCl (20 ml), and extracted with diethyl ether (3 x 25 ml). Organic layer was concentrated under reduced pressure. To this hydrazine hydrate (5 gm, 4.84 ml, 100 mmol) was added, and the mixture was stirred for 30 min. The reaction mixture was made alkaline by adding of 6 N NaOH (20 ml) and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous magnesium sulphate, evaporated in vacuo, and recrystallized from ethanol to obtain colorless crystals of title compound **5**. Yield: 85%; MP: 138-140^{o}C; IR (KBr, cm^{-1}): 3293, 3018, 2969, 2939, 2344, 1752, 1607, 1586, 1451, 1204; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.85 (s, 3H, O*CH*$_3$), 3.92 (s, 6H, O*CH*$_3$), 6.22 (s, 2H, Ar*H*), 6.82 (d, 1H, J = 1.6 Hz, C=*CH*), 7.6 (d, 1H, J = 1.6 Hz, N=*CH*), 11.38 (bs, 1H, *NH*); MS (ESI): m/e 235 (M+1).

33. **Preparation of 1-methyl-5-(2,4,6-trimethoxy-phenyl)-1*H*-pyrazole (6):** 5-(2,4,6-Trimethoxy-phenyl)-1*H*-pyrazole **5** (2.34 gm, 10 mmol) was dissolved in dry DMF (15 ml) under N_2 atmosphere. Cooled the flask in an ice bath and methyl iodide (2.84 gm, 1.25 ml, 20 mmol) was added to it. To this solution, sodium hydride (60 % in oil, 0.48 gm, 12 mmol) was added in portions and the resulted solution was then allowed to stir at 0^{o}C for 15 min. The reaction mixture was poured over crushed ice and the resulted solid was filtered off, recrystallized from ethanol to afford the title compound **6** in pure form. The filtrate was then extracted three times with ethyl acetate. The combined extracts were washed with water. After drying over anhydrous $MgSO_4$, the solvent was distilled off under reduced pressure. The resulting residue was purified by silica gel

column chromatography using petroleum ether: ethyl acetate as eluent (9:1) to obtain compound **7** in pure form.

1-methyl-5-(2,4,6-trimethoxy-phenyl)-1H-pyrazole **(6):** Yield: 82%; MP: 147-149^{o}C; IR (KBr, cm^{-1}): 3064, 2962, 2943, 2841, 1612, 1584, 1549, 1473, 1458, 1234, 1161; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.65 (s, 3H, N*CH*$_3$), 3.75 (s, 6H, 2xO*CH*$_3$), 3.87 (s, 3H, O*CH*$_3$), 6.20 (m, 3H, 2xAr*H*, Pyr-*H*), 7.55 (s, 1H, Pyr-*H*); ^{13}C NMR (125 MHz, $CDCl_3$): 36.66 (m, CH_3), 55.40 (m, CH_3), 55.75 (s, CH_3), 90.54 (s, CH), 100.69 (w, C), 107.44 (m, CH), 135.52 (w, C), 137.91 (m, CH), 159.32 (m, C), 162.25 (w, C); 135-DEPT: 36.65 (+), 55.40 (+), 55.75 (+), 90.53 (+), 107.44 (+), 137.89 (+); MS (ESI): m/e 249 (M+1).

1-methyl-3-(2,4,6-trimethoxy-phenyl)-1H-pyrazole **(7):** Yield: 18%; MP: 104-106^{o}C; IR (KBr, cm^{-1}): 3130, 2992, 2956, 2834, 1613, 1586, 1505, 1474, 1224, 1161; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.76 (s, 6H, 2xO*CH*$_3$), 3.85 (s, 3H, O*CH*$_3$), 3.97 (s, 3H, N*CH*$_3$), 6.20 (s, 2H, 2xAr*H*), 6.31 (d, J = 2.8 Hz, 1H, Pyr-*H*), 7.41 (d, J = 2.8 Hz, 1H, Pyr-*H*); ^{13}C NMR (100 MHz, $CDCl_3$): 38.92 (m, CH_3), 55.27 (m, CH_3), 55.91 (s, CH_3), 90.55 (s, CH), 104.51 (w, C), 107.81 (m, CH), 130.13 (m, CH), 144.40 (w, C), 159.37 (m, C), 161.07 (w, C); 135-DEPT: 38.94 (+), 55.28 (+), 55.93 (+), 90.56 (+), 107.81 (+), 130.11 (+); MS (ESI): m/e 249 (M+1).

34. **Preparation of 1-[2-Hydroxy-4,6-dimethoxy-3-(2-methyl-2H-pyrazol-3-yl)-phenyl]-ethanone (8):** Compound **6** (2.48 gm, 10 mmol) was dissolved in 25 ml of dry CH_2Cl_2 under N_2 atmosphere and flask was cooled in an ice bath. To this solution, $BF_3.OEt_2$ (11.36 gm, 10.05 ml, 80 mmol) was added followed by a drop wise addition of acetic anhydride (5.1 gm, 4.72 ml, 50 mmol). The resulted solution was then allowed to stir for 24 h at room temperature. The reaction mixture was diluted with water, rendered alkaline with Na_2CO_3, and extracted with CH_2Cl_2. Removal of solvent in vacuo gave white solid that was recrystallized from methanol to obtain compound **8** in pure form.

Yield: 95%; MF / FWt: $C_{14}H_{16}N_2O_4$ / 276; MP: 221-223 ^{o}C; IR (KBr, cm^{-1}): 3461, 2940, 2370, 1625, 1597, 1416, 1277, 1133; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.69 (s, 3H, CO*CH*$_3$), 3.71 (s, 3H, N*CH*$_3$), 3.88 (s, 3H, O*CH*$_3$), 4.02 (s, 3H, O*CH*$_3$), 6.08 (s, 1H, Ar*H*), 6.26 (d, 1H, *J* = 1.6 Hz, C=*CH*), 7.59 (d, 1H, *J* = 1.6 Hz, N=*CH*); MS (ESI): m/e 277 (M+1)

35. **General procedure for the preparation of pyrazole-based chalcones (9a-n):** 1-(2-Hydroxy-4,6-dimethoxy-3-(1-methyl-1*H*-pyrazol-5-yl)phenyl)ethanone **8** (0.276 gm, 1

mmol) was dissolved in ethanol (10 ml) under stirring. To this was added NaOH (0.12 gm; 3 mmol, with a minimum of water) and stirred for 5 minutes. To this reaction mixture, the aromatic aldehyde **9a-n** (1.2 mmol) was then added and stirring continued at room temperature for 24 h. Reaction was monitored by TLC. After completion of reaction, the mixture was poured over crushed ice and acidified with acetic acid. The separated solid was filtered and washed well with water. Crude product was dried and recrystallized from ethanol to obtain the desired product in pure form.

(*E*)-1-(2-hydroxy-4,6-dimethoxy-3-(1-methyl-1*H*-pyrazol-5-yl)phenyl)-3-phenylprop-2-en-1-one (9a): Yield: 98%; MF / FWt: $C_{21}H_{20}N_2O_4$ / 364; MP: 178-180 ^oC; IR (KBr, cm^{-1}): 3439, 2986, 2944, 2344, 1628, 1578, 1556, 1335, 799, 758; ^1H NMR (400 MHz, DMSO-d^6): δ 3.55 (s, 3H, N*CH*$_3$), 3.89 (s, 3H, O*CH*$_3$), 4.08 (s, 3H, O*CH*$_3$), 6.15 (s, 1H, Ar*H*), 6.43 (d, 1H, *J* = 1.6 Hz, Pyr-*H*), 7.41 (d, 1H, *J* = 1.6 Hz, Pyr-*H*), 7.48 (m, 3H, Ar*H*), 7.77 (m, 3H, 2xAr*H*, CH=*CH*CO), 7.91 (d, 1H, *J* = 15.6 Hz, *CH*=CHCO), 14.O3 (bs, 1H, *OH*); MS (ESI): m/e 365 (M+1)

36. **General procedure for the synthesis of pyrazole amalgamated flavones (10a-n):** To the solution of (*E*)-1-(2-hydroxy-4,6-dimethoxy-3-(1-methyl-1*H*-pyrazol-5-yl)phenyl)-3-phenylprop-2-en-1-one **9a-n** (1 mmol) in DMSO (10 ml), I_2 (0.025 g, 0.1 mmol) was added. The mixture was heated at 150^{o}C for 3h. The completion of reaction was monitored by TLC. After completion of reaction, the reaction mixture was poured into cold water. Product precipitated was filtered off, washed with sodium thiosulphate, dried and recrystallized from methanol to obtain the title compounds **10a-n** in pure form.

5,7-dimethoxy-8-(1-methyl-1*H*-pyrazol-5-yl)-2-phenyl-4*H*-chromen-4-one (10a): Yield: 96 %; MF / FWt: $C_{21}H_{18}N_2O_4$ / 362; MP: 194-196 ^oC; IR (KBr, cm^{-1}): 3019, 2945, 2848, 2400, 1644, 1586, 1536, 1488, 1216, 769, 689; ^1H NMR (400 MHz, $CDCl_3$): δ 3.69 (s, 3H, N*CH*$_3$), 3.95 (s, 3H, O*CH*$_3$), 4.10 (s, 3H, O*CH*$_3$), 6.38 (d, *J* = 2Hz, 1H, Pyr-*H*), 6.49 (s, 1H, Ar*H*), 6.69 (s, 1H, C=*CH*CO), 7.40 (m, 3H, Ar*H*), 7.52 (d, *J* = 8 Hz, 2H, Ar*H*), 7.65 (d, *J* = 2 Hz, 1H, Pyr-*H*); MS (ESI): m/e 363 (M+1).

37. ***In vitro* COX inhibition assay:** The assay was performed by using colorimetric COX (ovine) inhibitor screening assay kit.[38] Briefly, the reaction mixture contains, 150 µl of assay buffer, 10 µl of heme, 10 µl of enzyme (either COX-1 or COX-2), and 50µl of sample (0.1mM). The assay utilizes the peroxidase component of the COX catalytic domain. The peroxidase activity can be assayed colorimetrically by monitoring the appearance of oxidized N, N, N, N'-tetramethyl-*p*-phenylenediamine (TMPD) at

590nm. Indomethacin (0.1 mM) was used as a standard drug. The percent COX inhibition was calculated using following equation,

$$\text{COX inhibition activity (\%)} = 1 - \frac{T}{C} \times 100$$

Where T= Absorbance of the inhibitor well at 590 nm

C= Absorbance of the 100% initial activity without inhibitor well at 590 nm

38. Maurias, M. *Bioorg. Med. Chem.* **2004**, 12, 5571-5578.

39. **Carrageenan induced hind paw edema in rats:** Rats were divided into various groups (n=6) and allowed to free access to water *ad libitum*. Different groups of rats administered with indomethacin (100 mg/kg, b.w.) and various synthesized compounds **10a-10n** (50 mg/kg,) orally. One group of rats served as a control and administered with gum acacia (1 %, w/v; 10 ml/kg, b.w., p.o.). One hour after the drug administration, to all groups of rats, hind paw edema was induced by the method of winter et.al.[40] by injecting 0.1 ml of 1 % (w/v) solution of carrageenan subcutaneously into the subplanter region of hind paw. The hind paw edema volume was measured by volume displacement method using plethysmometer (UGO, Besile 7140, Italy) by immersing the paw till the level *lateral malleolus* at various time intervals (1, 3 and 6 h) after carrageenan injection.

40. Winter, C.A.; Risley, E.A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med*. **1962**, 111, 544-547.

41. **Acetic acid induced vascular permeability in mice:** Mice were divided in to various groups (n=6) and allowed to free access to water *ad libitum*. Different groups of mice administered with indomethacin (10 mg/kg) and selected test compounds from carrageenan group (compounds eliciting significant paw edema inhibition) at dose of 100 mg/kg orally. Control group received gum acacia (2 %, 10 mL/kg, p.o.). One hr of drug treatment mice were injected with 0.25 mL (0.6 %, v/v) solution of acetic acid by intraperitoneally (i.p.). Immediately, 10 mL/kg (10 %, w/v) solution of Evan's blue was injected intravenously through tail vain. After 30 minutes of Evan's blue injection, mice were sacrificed with excess of ether anaesthesia. The abdomen was cut open and exposed the viscera. The mice were held by a flap of abdominal wall and the viscera irrigated with 5 mL normal saline over a Petri dish. The perfusate was collected and then filtered through Whatmann filter paper, and the final volume of filtrate was made up to 10 mL. The dye leaking out into peritoneal cavity in diluted fluid was measured spectrophotomeically at 620 nm.[42]

42. Whittle, B. A.; *Br J Pharmacol.* **1964**, 22, 246-253.

43. **Experimental animals:** Wistar rats of either sex were obtained from National Toxicology Center, Pune, India. The rats were housed and maintained in clean polypropylene cages under standard conditions: humidity (50 + 5 %), temperature (25 ± 2^0 C) and (12 h light/12 h dark cycle), fed with standard diet (Amrut laboratory animal feed, Sangli, Maharashtra, India) and water *ad libitum*. Experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (SIPS/IAEC/2011-12/17) and conform to the Indian National Science Academy Guidelines for the use and care of experimental animals in research. The animal house registration number with Government of India is 962/c/06/CPCSEA.

44. **Molecular modeling:** In present work, we have used MOE 2008, which has improved flexible docking as well as integration with a graphical interface along with other modules, such as analysis, molecular mechanics, and molecular dynamics. To get fruitful results, we docked the ligands into active site of receptor COX-2. A good number of X-ray crystallographically resolved 3D structures are available for COX-2, PDB ID-3NTG was chosen on the basis of following two reasons: (1) X-ray diffraction resolution, 2.14 in present case. (2) The pdb 3NTG contains crystal structure of COX-2 with selective compound 23d-(R). The experimental conformation of the "template" ligand 23d-(R) was analysed to get idea about active site and then deleted, leaving space for docking of candidate ligand to be docked into the protein. Before the actual docking, protein structure was first repaired followed by appropriate protonation in absence of ligand using the Protonate 3D module. The health of protein was checked by plotting Ramchandran plot (**Figure 5**). Protein prepared in this manner were applied directly for docking. During docking, the default parameters and settings using triangle match placement method with London dG scoring were used for the docking except that the number of retain were 20 instead of 30.

45. Niu Huang, N.; Shoichet, B. K. *J. Med. Chem.* 2008, 51, 4862–4865.

46. Kroemer, R. T. *Curr. Protein Pept. Sci.* 2007, 8, 312-328.

Table 1. *In vitro* COX-1 and COX-2 enzyme inhibitory activities of flavones bearing pyrazole scaffold (**10a-n**)

Compound	% inhibition of COX (100 μM)[a]		CLogP
	COX-1	COX-2	
10a	54.1	41.75	3.284
10b	54.1	43.33	3.427
10c	51.58	38.06	3.570
10d	46.08	42.88	4.460
10e	48.42	50.12	2.944
10f	52.76	40.60	3.500
10g	51.58	42.80	3.251
10h	45.67	51.20	2.165
10i	20.38	46.48	2.670
10j	59.99	46.28	3.142
10k	54.1	42.27	4.147
10l	47.38	43.33	3.027
10m	48.86	41.75	4.210
10n	52.18	37.95	3.570
Indomethacin	70.07	43.33	--

[a]The determination was performed in duplicate for two independent experiments;

Table 2. Effects of various synthesized compounds on carrageenan-induced paw edema in rats with different time intervals

Treatment and dose (100 mg/kg.p.o.)	Paw volume (ml) Mean± SEM at various time intervals (n=6)		
	1 h	3 h	6 h
Normal group	0.40±0.04	0.35±0.04	0.38±0.06
Carrageenan control	0.94±0.05$^{\#}$	1.17±0.1$^{\#}$	1.69±0.08$^{\#\#}$
Indomethacin	0.86±0.05	0.95±0.07*	0.78±0.05**
10a	0.77±0.1	0.91±0.1	1.22±0.1
10b	0.76±0.1	1.05±0.2*	1.17±0.1*
10c	0.78±0.3	1.01±0.4	1.44±0.9
10d	0.89±0.4	1.11±0.6	1.32±0.5
10e	0.79±0.5	0.96±0.7*	0.99±0.5**
10f	0.79±0.5	0.99±0.7	1.20±0.5
10g	0.86±0.1	0.92±0.1	1.07±0.3*
10h	0.83±0.4	0.95±0.6*	0.97±0.6**
10i	0.87±0.6	0.94±0.4*	0.88±0.7**
10j	0.91±0.2	1.18±0.6	1.55±0.6
10k	0.81±0.2	0.99±0.03*	1.11±0.1*
10l	1.04±0.06	1.00±0.04	1.02±0.04*
10m	0.79±0.4	1.46±0.08	1.01±0.6*
10n	0.88±0.3	1.24±0.2	1.44±0.3

$^{\#\#}p < 0.01$, $^{\#}p < 0.05$ when compared with normal control group; $^{**}p < 0.01$, $^{*}p < 0.05$ when compared with carrageenan group

Table 3. Effects of various prodrugs on acetic acid induced vascular permeability in mice

Treatment and dose (100 mg / kg.p.o.)	Amount of dye leakage (µg / mL) Mean±SEM	Inhibition of dye leakage (%)
Normal control	0.42±0.02	--
Vehicle treated control	22.03 ±1.42$^{\#\#}$	--
indomethacin	6.52±0.47**	70.40
10b	10.59±1.7**	51.92
10e	7.01±0.8**	68.17
10g	16.44±2.6*	25.32
10h	6.11 ±1.0**	72.26
10i	8.7±0.9**	60.50
10k	14.3±2.5*	35.08
10l	14.7±1.4*	33.27
10m	9.3±2.1**	57.78

$^{\#\#}p < 0.01$ when compared with normal control group; $^{**}p < 0.01$, $^{*}p < 0.05$ when compared with vehicle treated control group

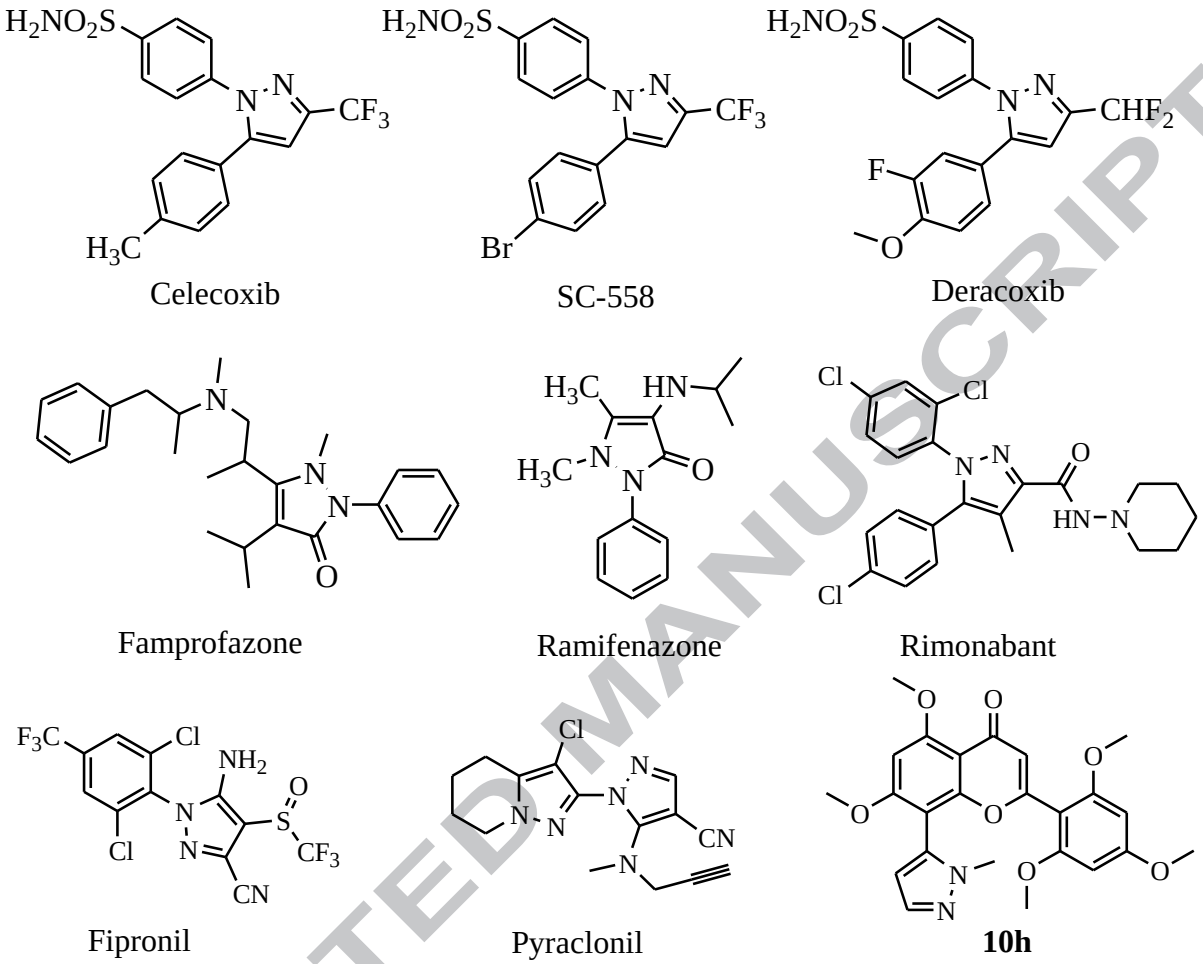
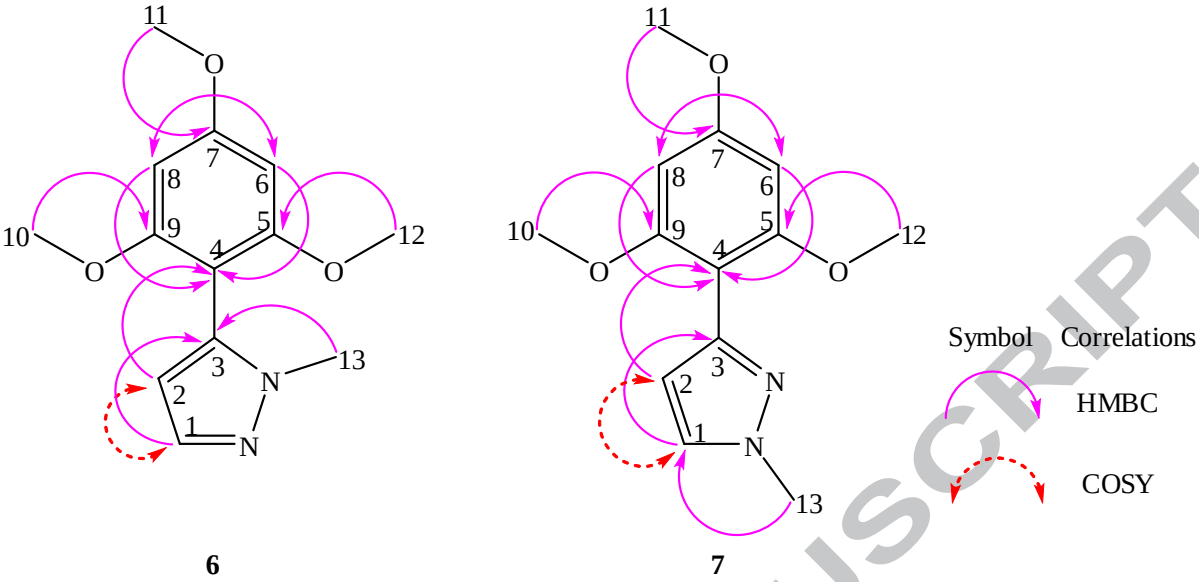
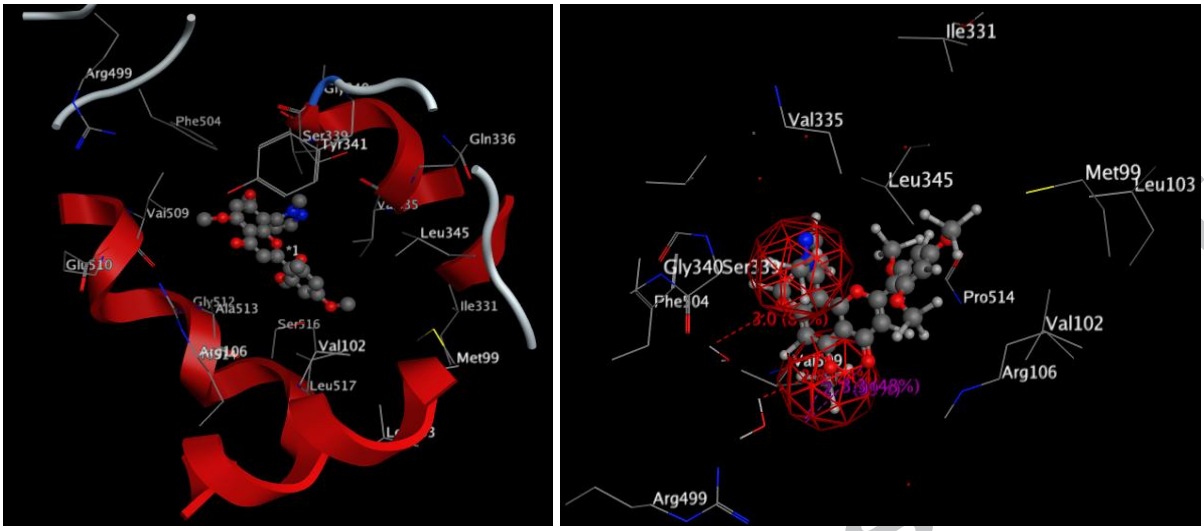


Figure 1. Structures of some known pyrazole NSAIDs and compound **10h**

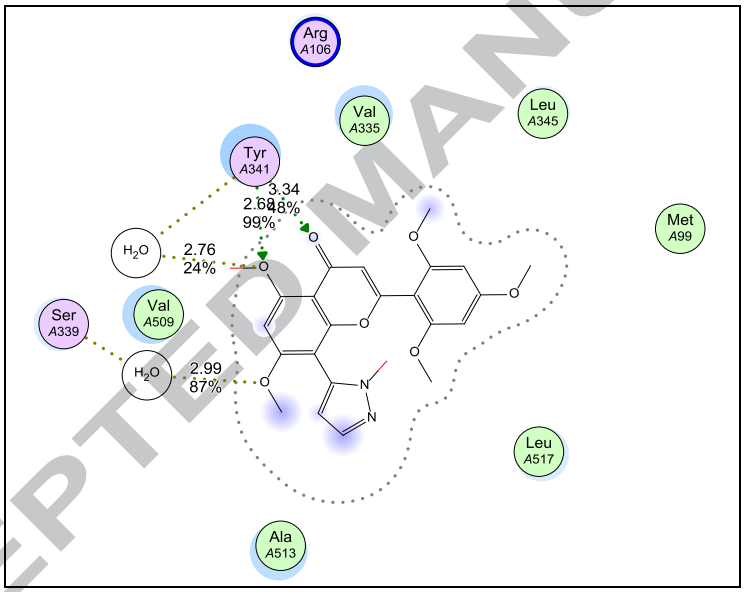


6 **7**

Figure 2. HMBC (H to C) and ^{1}H-^{1}H COSY correlations



(a)



(b)

Figure 3. **(a) 3D (b) 2D representation for docking pose of 10h in active site of COX-2.**

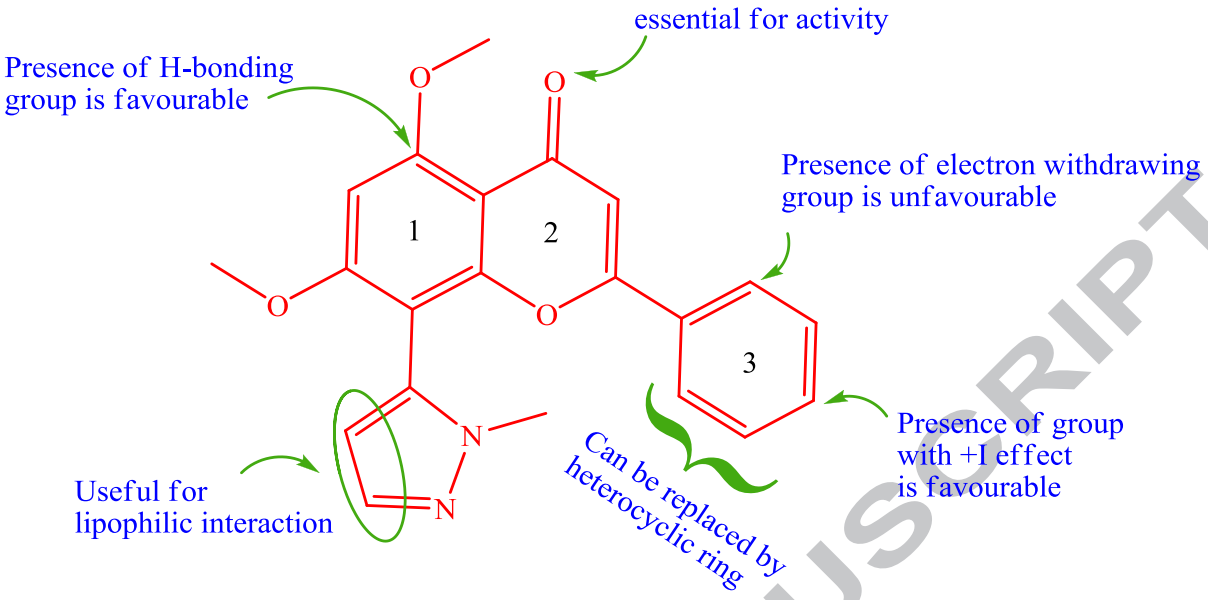


Figure 4. Summary of SAR study of flavone derivatives for COX-2 selectivity

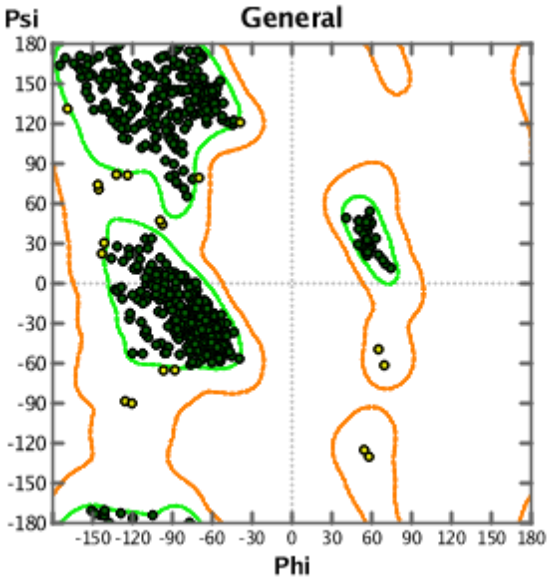
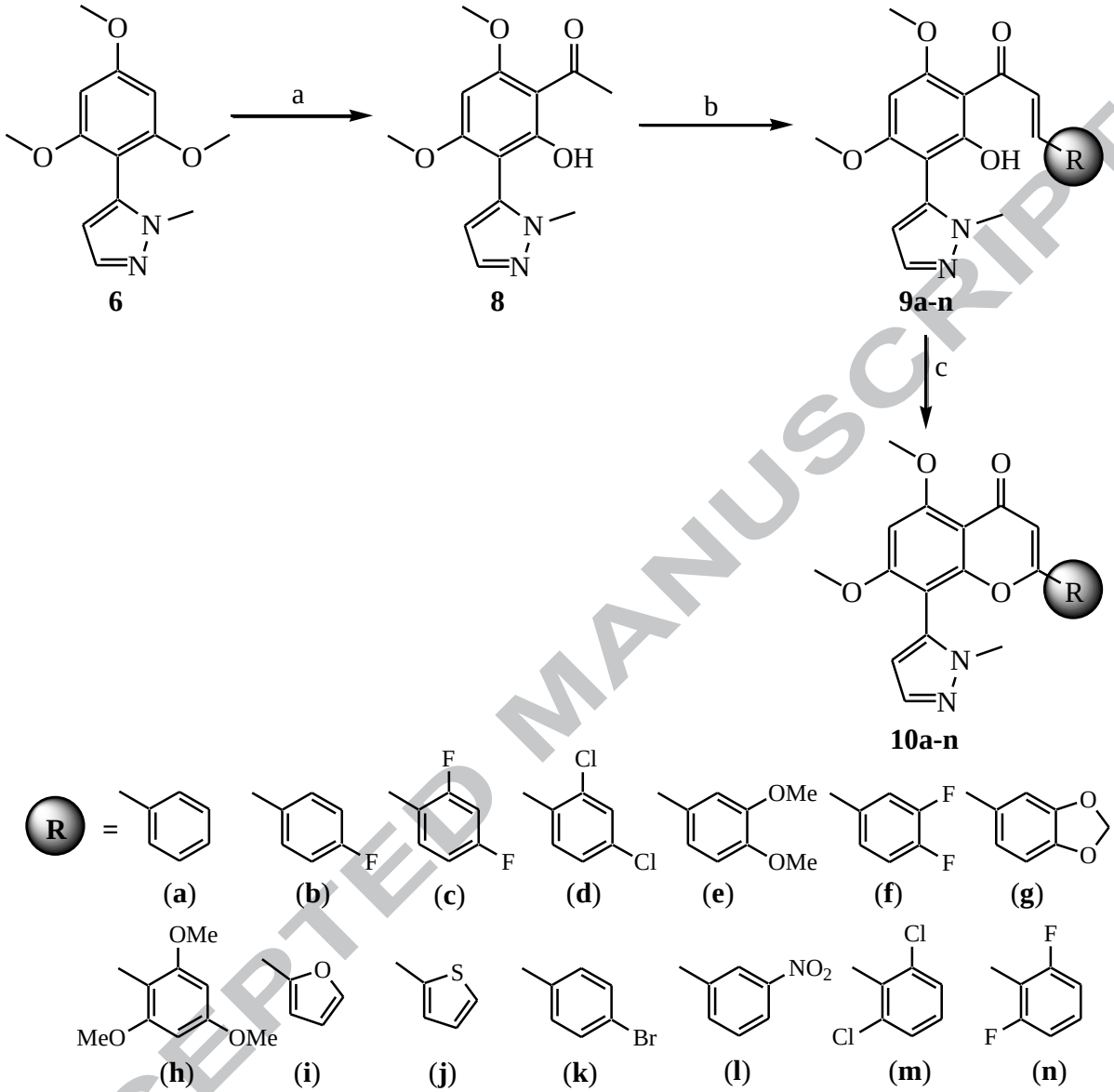
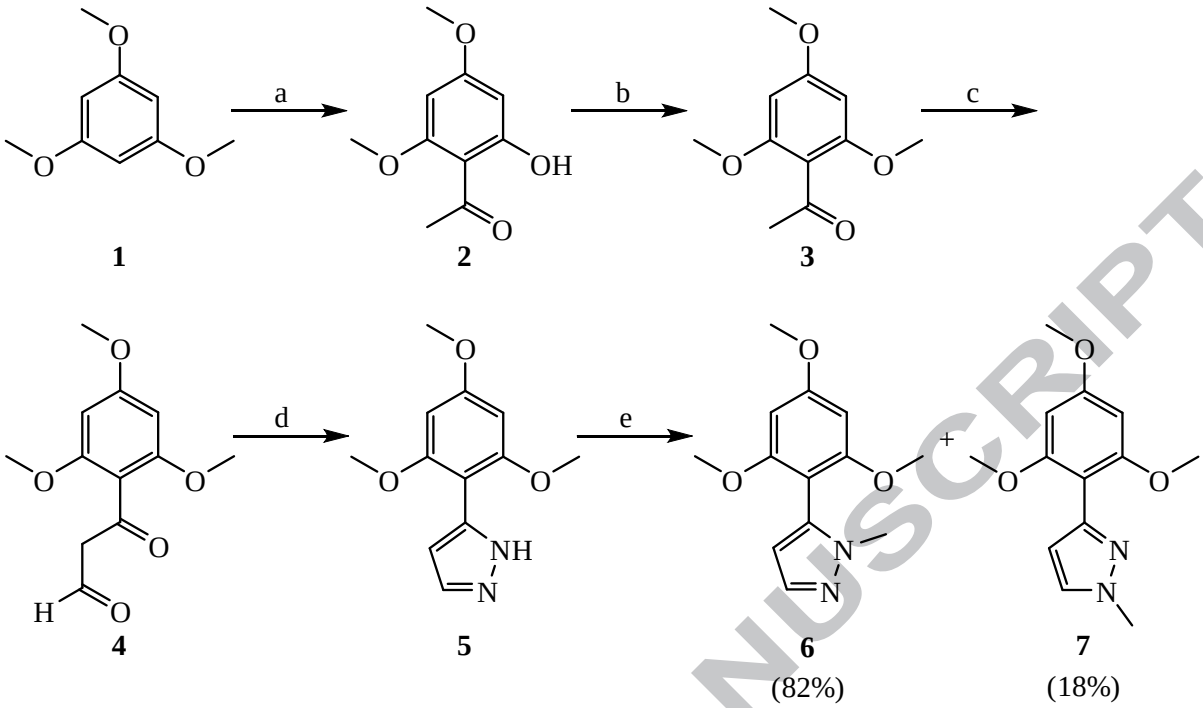


Figure 5. Ramchandran plot for 3NTG after optimization



Scheme 1. Reagents and conditions: (a) Ac_2O, $BF_3.OEt_2$, 0^oC-rt, N_2, 3 h ; (b) RCHO, NaOH, EtOH, rt, 24 h; (c) I_2, DMSO, reflux, 12 h.



Scheme 2. Reagents and conditions: (a) CH_3COCl, $AlCl_3$ (anh.), Et_2O, N_2, 0 ^oC - rt; (b) Me_2SO_4, K_2CO_3, acetone, reflux, 12 h; (c) HCOOEt, NaH, THF, N_2, rt; (d) $NH_2NH_2.H_2O$, rt; (e) MeI, NaH, DMF, N_2, 0 ^oC