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Full Length Research Paper

Synthesis and evaluation of chalcone analogues and pyrimidines as cyclooxygenase (COX) inhibitors

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A series of chalcone analogues was synthesized and used as precursor for the synthesis of novel series of pyrimidines. Both groups have been evaluated for their effects on the cyclooxygenases (COXs) that are imperative enzymes in the genesis of prostaglandin H2, which is an antecedent for the biosynthesis of prostaglandins, thromboxanes and prostacyclins. The results depicted that chalcones and pyrimidines are very active inhibitors according to the pattern of substitution. Compounds C4, C5, P4 and P5 with methoxylation and nitro substitutions showed best results to inhibit COX-2.

Key words: COX inhibitors, chalcones, pyrimidines, anti-inflammatory agents.

INTRODUCTION

Cyclooxygenases (COXs) are imperative enzymes in the genesis of prostaglandin H2 which is an antecedent for the biosynthesis of prostaglandins, thromboxanes, and prostacyclins (Hamberg et al., 1974). There are two isoforms of COX enzymes: Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) correspondingly (Fu et al., 1990). The foremost tasks assigned to COX-1 enzyme include the defence of gastric mucosa, platelet aggregation, and renal blood flow while the COX-2 enzyme is inferable and articulated during inflammation, ache and oncogenesis (Smith et al., 1996). As COX-2 plays its role in inflammation and pain, molecules that restrain its enzymatic action would be of remedial worth. It is established that the enzymatic activity of cyclooxygenases is repressed by numerous non-steroidal anti-inflammatory drugs (NSAIDs) (Meade et al., 1993). These drugs comprise aspirin and indomethacin which are non-discriminating and hold back COX-1 and COX-2 together. Aspirin inhibits COX-1 more strappingly than COX-2 and inhibition of COX-1 by aspirin decreases the creation of PGE2 and PGI2 which has an unfavourable ulcerogenic outcome (Allison et al., 1992). Therefore, in the past few years, selective COX-2 inhibitors, that attain the similar anti-inflammatory efficacy like conventional NSAIDs but lessen the risk of superfluous side effects, have been developed. On the other hand, clinical studies have recommended that selective COX-2 inhibitors could source archetypal COX-mediated side effects like gastrointestinal damage, amplified systemic blood pressure, and hypersensitivity (De Gaetano et al., 2003).Chalcones play pivotal role in the biosynthesis of flavonoids and isoflavonoids (Avila et al., 2008). Flavonoids are the constituents of daily diet. Chemically, chalcones consist of a three carbon α, β-unsaturated carbonyl system. Consequently, these are the condensation moieties of aromatic aldehyde with acetophenones in the attendance of alkali (Nowakowska, 2007). They undergo an assortment of chemical reactions and are useful in synthesis of pyrazoline, isoxazole and a variety of heterocyclic compounds as hexanones. Chalcones play an important role in synthesizing a range of therapeutic compounds. They have shown promising healing efficacy for the treatment of a number of diseases. Chalcone derivatives have

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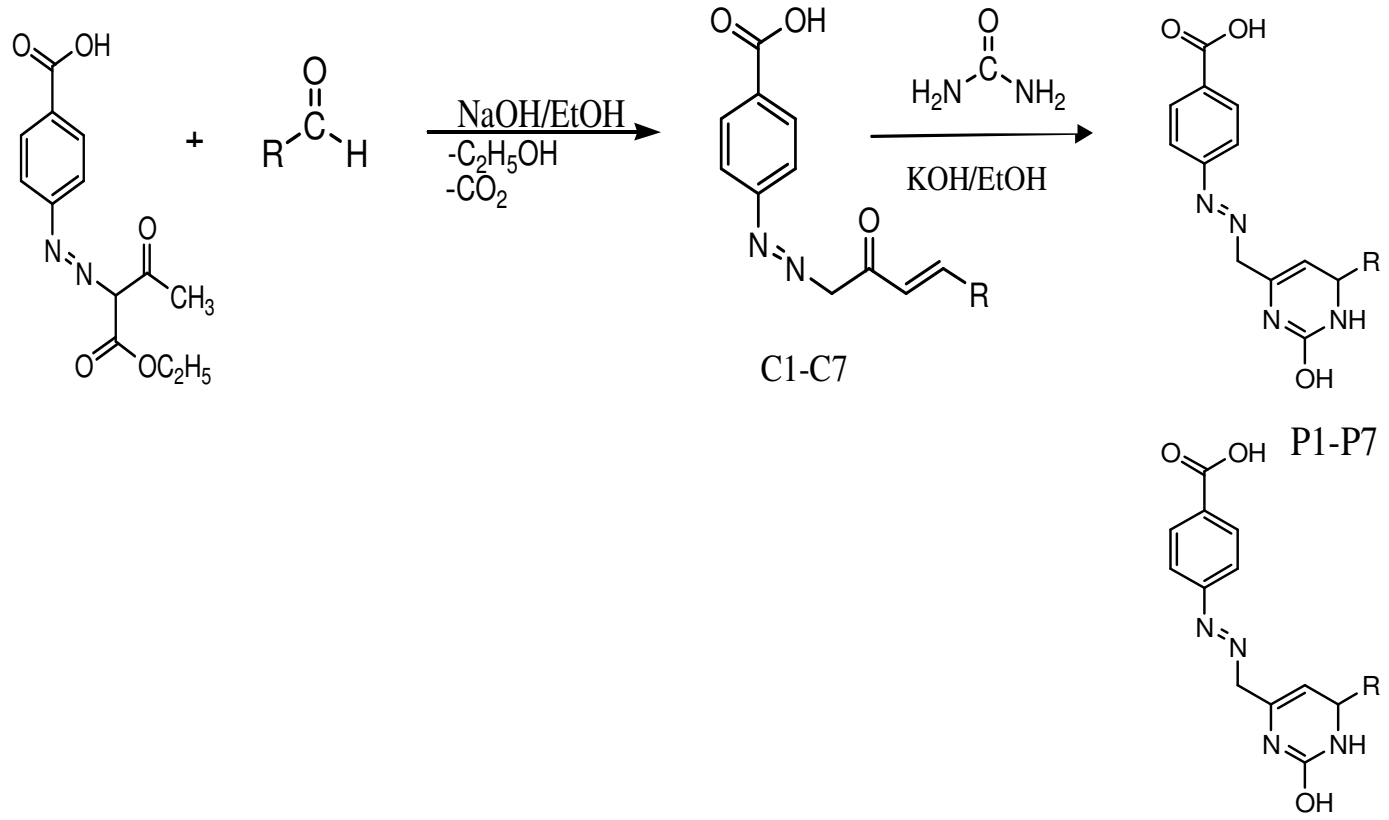

Scheme 1. Synthesis of chalcone analogues and related pyrimidines.

achieved spotlight as they have simple structures, and miscellaneous pharmacological actions. Amazing activities of these compounds include anti-inflammatory (Rajendra et al., 2009), antifungal (Lahtchev et al., 2008) antibacterial (Dominguez et al., 2005), antimalaria (Valla et al., 2006), antitumor (Seo et al., 2010), antimicrobial (Tomar et al., 2007; Nowakowska et al., 2008), antiviral (Trivedi et al., 2007), antituberculosis (Lin et al., 2002), antioxidant (Vogel et al., 2008; Gacche et al., 2008), antimitotic (Ducki et al., 1998), antileishmanial (Boeck et al., 2006), antiplatelet (Zhao et al., 2005), nematicidal (Drissa et al., 2011) and anticancer activities (Won et al., 2005; Ducki, 2007).

Therefore, chalcones are a class of significant curative potential. As far as anti-inflammatory activity is concerned, a number of studies indicated that chalcone derivatives strongly inhibit NO synthesis by various cellular mechanisms, iNOS down-regulation and/ or iNOS inhibition (Arockia et al. 2002; Chiaradia et al., 2008; Suzuki et al., 2005). Though, chalcone derivatives also inhibited 5-lipoxygenase as well as cyclooxygenase-2 (COX-2) catalyzed prostaglandin production (Dao et al., 2004; Kim et al., 2007). In this research, a series of chalcone analogues was synthesized and then using these chalcone analogues as base, a new series pyrimidines was synthesized and evaluated for their effects on the COX-1 and COX-2.

MATERIALS AND METHODS

Chemistry

Chemicals were obtained from E. Merck (Germany) and S. D. Fine Chemicals (India). Purity of compounds was determined by thin layer chromatography by using toluene–ethylformate–formic acid (5:4:1) and benzene–methanol (8:2) solvent system. Compounds were analysed by the IR spectra (KBr pellets), Perkin-Elmer 1720 FT-IR spectrometer and ^{1}HNMR spectra by TMS as the inner standard in DMSO-d_6/ $CDCl_3$. Whereas, Bruker AC 200, DPX 300 and ARX 500 recorded the ^{13}C-NMR (75 and 125 MHz) spectra at 25°C in $CDCl_3$.

Synthesis of chalcone analogues and pyrimidines

All chalcones analogues and pyrimidines were synthesized as already reported earlier by authors (Amit et al., 2008) Briefly, a combination of ethyl 2-(4-carboxyphenylazo) acetoacetate (0.01 mol) and an aldehyde (0.01 mol) in oxygen-free ethanol, a solution of sodium hydroxide in oxygen-free distilled water was added, with constant stirring of the flask. For the consequent 24 h, the reaction mixture was stirred on a magnetic stirrer and then poured on to crushed ice. The removed solid mass was filtered, washed with water and crystallized from ethanol to acquiesce the desired product (C1-C7) as yellow crystals (Scheme 1), and for pyrimidines a mixture of 0.01 mol urea, 1 g KOH and 0.01 mol of the

required chalcone (C1-C7) in 20 ml ethanol was heated in a reflux condenser for 6 h, cooled and poured onto trodden ice. So, the harvested solid product was filtered and recrystalized from ethanol.

3-(2'-Nitrophenyl)-1-[(4)-carboxyphenylazomethyl]-2-propene-1-one (C4)

Yield 72%, mp. 181 to 183°C, IR (KBr): 1365 (NO_2), 1489 (N=N), 1692 (C=O), 1713 (C=O of acid), 3039 (CH); 1H–NMR (DMSO-*d*6): δ 3.10 (s, 2H, CH_2), 6.78 (d, 1H, Hα), 7.14 (d, 1H, Hβ), 7.40 to 8.10 (m, 8H, aromatic), 11.20 (s, 1H, OH); ^{13}C-NMR (CDCl3): δ 58.2, 121.4, 122.7, 126.6, 127.8, 129.1, 130.4, 131.1, 135.1, 143.2, 146.8, 156.9, 170.8, 197.8. Mass (*m/z*): 339. Anal. (%) for $C_{17}H_{13}N_3O_5$, Calcd. C, 60.18; H, 3.86; N, 12.38; O, 23.58; Found: C, 60.07; H, 3.73; N, 12.26; O, 23.46.

3-(4'-Methoxyphenyl)-1-[(4)-carboxyphenylazomethyl]-2-propene-1-one (C5)

Yield 70%, mp. 203 to 205°C, IR (KBr): 1159 (C-O-C), 1488 (N=N), 1682 (C=O), 1707 (C=O of acid), 3039 (CH); 1H–NMR (DMSO-*d*6): δ 3.10 (s, 2H, CH_2), 3.73 (s, 1H, OCH_3), 6.37 (d, 1H, Hα), 6.89 (d, 1H, Hβ), 6.92 to 8.10 (m, 8H, aromatic), 11.13 (s, 1H, OH). ^{13}C-NMR ($CDCl_3$): δ 55.5, 58.4, 114.8, 122.9, 126.8, 127.4, 127.6, 127.8, 131.1, 143.1, 156.8, 160.3, 169.8, 197.2. Mass (*m/z*): 324. Anal. (%) for $C_{18}H_{16}N_2O_4$, Calcd. C, 66.66; H, 4.97; N, 8.64; O, 19.73; Found: C, 66.55; H, 4.86; N, 8.52; O, 19.61.

6-(2'-Nitrophenyl)-4[(4)-carboxyphenylazomethyl]-2-hydroxyl-1,6-dihydropyrimidine (P4)

Yield 76%, mp. 227 to 229°C, IR (KBr): 1563 (C=C ring skeleton Ar. moiety), 1380 (NO_2), 1412 (C=C ring skeleton pyrimidine moiety), 1483 (N=N), 1698 (C=O of acid), 3209 (N-H), 3402 (OH); 1H–NMR (DMSO-*d*6): δ 2.42 (s, 2H, CH_2), 4.47 (d, 1H, NH-CH), 4.80 (s, 1H, OH), 5.26 (s, 1H, NH), 5.91 (d, 1H, CH), 7.11 to 8.92 (m, 8H, Ar-H), 12.19 (s, 1H, OH); ^{13}C-NMR ($CDCl_3$): δ 38.4, 63.6, 118.4, 121.4, 122.8, 127.4, 127.9, 131.2, 134.6, 138.3, 139.4, 148.1, 156.9, 163.9, 172.1. Mass (*m/z*): 381. Anal. (%) for $C_{18}H_{15}N_5O_5$, Calcd. C, 56.69; H, 3.96; N, 18.37; O, 20.98; Found: C, 56.69; H, 3.96; N, 18.37; O, 20.98.

6-(4'-Methoxyphenyl)-4[(4)-carboxyphenylazomethyl]-2-hydroxyl-1,6 dihydropyrimidine(P5)

Yield 73%, mp. 219 to 221°C, IR (KBr): 1560 (C=C ring skeleton Ar. moiety), 1411 (C=C ring skeleton pyrimidine moiety), 1487 (N=N), 1693 (C=O of acid), 3210 (N-H), 3400 (OH); 1H–NMR (DMSO-*d*6): δ 2.34 (s, 2H, CH_2), 3.72 (s, 1H, OCH_3), 4.46 (d, 1H, NH-CH), 4.85 (s, 1H, OH), 5.19 (s, 1H, NH), 5.92 (d, 1H, CH), 6.42 to 8.06 (m, 8H, Ar-H), 12.32 (s, 1H, OH); ^{13}CNMR ($CDCl_3$): δ 46.9, 56.4, 62.3, 114.9, 117.9, 122.8, 127.6, 128.7, 131.4, 136.3, 140.3, 156.4, 159.2, 163.9, 169.8. Mass (*m/z*): 366. Anal. (%) for $C_{19}H_{18}N_4O_4$, Calcd. C, 62.29; H, 4.95; N, 15.29; O, 17.47; Found: C, 62.29; H, 4.95; N, 15.29; O, 17.47.

Biological assay

The COXs inhibition assay was performed as mentioned in the assay procedure instructions of 'Colorimetric COX (ovine) inhibitor Screening Assay Kit', Cayman Chemical Company, MI, USA. Briefly, the reaction mixture of 100% initial activity wells contained 150 µl of assay buffer, 10 µl of heme and 10 µl of relative (COX-1 or COX-2) enzyme solution. While the reaction mixture of inhibitor wells was comprised of 150 µl of assay buffer, 10 µl of heme, and 10 µl of enzyme (COX-1 or COX-2), 10 µl of the test samples (1 mM). The plates were carefully shaken for 5 s and were incubated for 5 min at 25°C. After 5 min incubation, 20 µl of the colorimetric substrate solution was added to all the wells, followed by the addition of 20 µl of arachidonic acid to all the wells. The plates were shaken gently for few seconds and again incubated for 5 min at 25°C. The absorbance of all the wells was read at 590 nm using Thermo make Automatic Ex-Microplate Reader. The COXs inhibition activity (%) was calculated using following formula:

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

where T = absorbance of the inhibitor well at 590 nm, and C = absorbance of the 100% initial activity without inhibitor well at 590 nm.

RESULTS AND DISCUSSION

In the typical anti-inflammatory pharmaceutical study, COX-2 enzyme inhibition as a target for design of anti-inflammatory agents has remained a momentous matter. Besides, most of the conventional NSAIDs have been formulated as selective inhibitors of COX-2. The COX inhibition assay was performed as mentioned in the assay procedure instructions of 'Colorimetric COX (ovine) inhibitor Screening Assay Kit', Cayman Chemical Company, MI, USA. All the samples were investigated to be good to brilliant inhibitors of COX-2. Intriguingly, the mainstream samples exhibited COX-2 inhibition better than aspirin (a known COX-2) inhibitor. C4, C5, P4 and P5 were most active compounds even than control. By and large, the range of effective COX-2 inhibition was such that compounds with methoxy substitution were more active than those bearing nitro substitution whilst all other samples displayed modest to excellent to COX-2 inhibition. All compounds were also checked for inhibition of COX-1, an enzyme recognized for its gastro-protective properties. The results in Table 1, obviously illustrate that the compounds showing effectual COX-2 inhibition are less reactive to COX-1. The existence of methoxy and nitro substitution as compounds C5, P5, C4 and P4 of chalcones and pyrimidines were established to be complimentary for COX-2 inhibition. Especially, nitro substitution at position 2 is more active than at position 3. Nevertheless, it is certainly hard to underlay a common structure activity relationship with other samples which showed reasonable to good inhibition of COX-2.

Conclusion

In summary, it can be concluded that chalcones and pyrimidines have potential as selective COX-2 inhibitors and the derivatives with excellent inhibitory activity of COX-2 are also less active against COX-1. So by using different substitution patterns good COX-2 inhibitors can

Table 1. COX-1 and COX-2 inhibitory activity of chalcone analogues and related pyrimidines derivatives.

Compound	R	Molecular formula	Percentage Inhibition	
			COX-1	COX-2
C1	Phenyl	$C_{17}H_{14}N_2O_3$	10.12	23.51
C2	2-Hydroxyphenyl	$C_{17}H_{14}N_2O_4$	09.22	19.11
C3	4- Hydroxyphenyl	$C_{17}H_{14}N_2O_4$	13.89	21.31
C4	2-Nitrophenyl	$C_{17}H_{13}N_3O_5$	23.11	69.77
C5	4-Methoxyphenyl	$C_{18}H_{16}N_2O_4$	19.01	73.33
C6	3- Nitrophenyl	$C_{17}H_{13}N_3O_5$	21.91	31.22
C7	2-Chlorophenyl	$C_{17}H_{13}N_2O_3Cl$	17.65	33.11
P1	Phenyl	$C_{18}H_{16}N_4O_3$	04.92	16.28
P2	2-Hydroxyphenyl	$C_{18}H_{16}N_4O_4$	13.55	18.55
P3	4- Hydroxyphenyl	$C_{18}H_{16}N_4O_4$	05.22	19.43
P4	2-Nitrophenyl	$C_{18}H_{15}N_5O_5$	19.32	58.74
P5	4-Methoxyphenyl	$C_{19}H_{18}N_4O_4$	12.88	74.99
P6	3- Nitrophenyl	$C_{18}H_{15}N_5O_5$	12.65	39.67
P7	2-Chlorophenyl	$C_{18}H_{15}N_4O_3Cl$	07.21	22.18

Values are means of three experiments, acetyl salicylic acid (34.71% inhibition) and selective COX-1 inhibitor (32.4%) were used as positive controls for COX-2 and COX-1, respectively.

be designed and some of these can be selected for further investigations because of their good results in this study.

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