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Highthroughputput Screening (HTS) Assays for Cyclooxygenase-2 and 5-Lipoxygenase, the Targets for Inflammatory Disorders

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Inflammation is the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants. As eicosanoids formed via the cyclooxygenase (COX) and lipoxygenases (LOX) pathway are the major players in inflammation. COX-1, COX-2 and 5-LOX have been employed as the targets in developing anti-inflammatory drugs. The present study is an attempt to develop high-throughput screening (HTS) compatible assay methods for COX-1, COX-2 and 5-LOX, to screen compound libraries and identify potential anti-inflammatory drug candidates. HTS involves testing of compound libraries against a biological target using a quantitative bioassay. Its purpose is to identify "hits" that modulate the activity of a biological target, which forms the starting point for a collaborative discovery effort between medicinal chemists and biologists. TMPD (Tetramethyl-p-Phenylene diamine) assay compatible for HTS was developed to screen the compounds against COX-1 and COX-2. The spectrophotometric assay involving co-oxidation of TMPD during the reduction of PGG_2 to PGH_2 was standardized for screening compound libraries. Nearly 10,000 compounds were screened against COX-1 and 72 potent molecules were identified. 2000 compounds were screened against COX-2 and 3 hits were found. Similarly FOX (Ferrous oxidation-xylenol orange) assay compatible for HTS was developed for screening of compounds against 5-LOX, based on the complex formation of Fe^{3+} /xylenol orange with absorption at visible light. Nearly 10,000 compounds were screened against 5-LOX on HTS platform and 5 hits were found. The hits, thus identified through the *in vitro* assays, were further evaluated for their efficacy on cell lines and animal models.