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Indomethacin from Anti-Inflammatory to Anticancer Agent

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Abstract

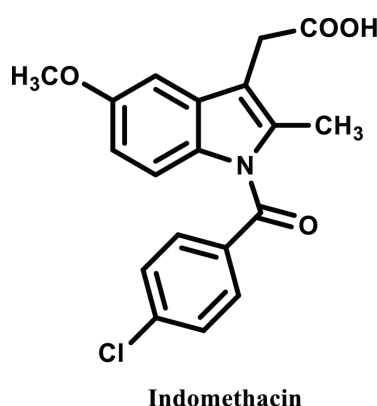
The chapter “Indomethacin from Anti-inflammatory to Anticancer Agent” covers the recent reports regarding the implication of COX-2/PGE2 in multiple cancer cell proliferation to emphasize the anticancer potential of COX-inhibitors including indomethacin and to reveal that the reduction of PGE2 production interferes with the cancer cell proliferation belongs to multiple cancer cell types. Impressively, indomethacin is involved in antiproliferative and apoptotic actions against cancer cell types via COX-2-independent mechanisms to highlight indomethacin as promising anticancer agent with dual actions to control the cancer cell proliferation. The cardiovascular complications result from diaryl heterocycle sulfonamide/methylsulfone selective COX-2 inhibitors upon reduction in PGE2 and PGI2 production that affects the vascular tone limits the use of Celecoxib as chemopreventive agent against recurrence of colorectal carcinoma cells. Kinetic profile of indomethacin against COX-2 showed obvious difference from that of selective COX-2 inhibitors in which it recovered completely from the enzyme after long time of incubation while COX-2 inhibitors did not recover to impress that this might be implicated in the cardiovascular toxicity of the selective inhibitors. This raised the concern to develop the indomethacin from nonselective COX- to selective COX-2-inhibitors and to assert whether the cardiac complications are from pharmacological class effect or chemical class effect.

Keywords: indomethacin, COX-2-independent mechanism, apoptosis, antiproliferative, kinetic profile

1. Introduction

Indomethacin is indole-3-acetic acid derivative, classified as nonsteroidal anti-inflammatory drug (NSAID). The drug is primarily used for the treatment of painful inflammatory conditions

that involves gout and osteoarthritis [1]. The mechanistic role of indomethacin in inhibition of pain has been verified by being nonselective inhibitor to cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isozymes [2]. The enzymatic activity of COX involves bis-oxygenation of arachidonic acid to (prostaglandin G₂) PGG₂, which then reduced to PGH₂ in a peroxidase reaction by the same protein [3]. COX-1 is constitutively expressed in most tissues, to which the production of prostaglandins is attributed to; and COX-2, which is induced by cytokines, mitogens and endotoxins in inflammatory cells, is implicated to the elevated levels of prostaglandins during the inflammation. Prostaglandins are hormone-like mediators involved in the induction of pain, fever and inflammation [2]. The inhibition of indomethacin to the two COX isozymes with minimal selectivity to COX-2 made the drug have serious complications such as gastric ulcers and renal toxicity upon long-term oral administration [4, 5].



Indomethacin and the other NSAIDs were found to have significant anticancer activity against wide variety of cancer cell types, *in vitro* and *in vivo* [6–10]. Moreover, epidemiological studies reported that the use of such type of drugs is linked to the reduction of cancer risk [11–13]. Indomethacin performs its anticancer activity in different fashions, inhibits proliferation via induction of apoptotic death of tumor cells [6, 9, 10], reduces tumorigenesis by enhancing the immune response [14, 15] and inhibiting the angiogenesis [16, 17] as well.

Interestingly, to mention that the mechanism to which the anticancer activity of NSAIDs including indomethacin attributed is the reduction of PGE₂; a type of prostaglandins generated from the bis-oxygenation of arachidonic acid by COX-2. PGE₂ contributes to the cell proliferation, cell cycle proliferation and cell cycle progression through various cell signaling mechanisms leads to induction of oncogenic genes and eventually overexpression of proliferative proteins [18–22]. Recently, extensive studies on various cancer cell types including colorectal carcinoma (CRC) justified the efficacy of indomethacin to reduce the levels of antiapoptotic proteins and progressive cell proliferation represented by tumor size by COX-independent mechanisms [23–26].

After emerge and marketing of celecoxib; selective COX-2 inhibitor in December 1998, rofecoxib was released in 1999 worldwide then lumiracoxib and etoricoxib (**Figure 1**) that are marketed in Europe. Those inhibitors are still marketed for the treatment of inflammatory

disorders except for rofecoxib and lumiracoxib (the only carboxylic coxib) that were withdrawn due to observation of cardiovascular complications from the recommended daily dose with rofecoxib [27] and observation of liver failure with lumiracoxib [28]. Selective COX-2 inhibitors were launched to treat the individuals who cannot tolerate severe gastrointestinal responses of NSAIDs. A few years later, extensive preclinical and clinical data generated to report the role of COX-2 in tumor growth and/or metastasis [29]. Studies on experimental animals showed that selective COX-2 inhibitors including celecoxib block the formation, growth and metastases of multiple tumor types [30]. Consistently, celecoxib demonstrated dramatic chemopreventive efficacy against colon polyps and reduced the incidence of recurrent adenomas of any type by 45% and of high risk lesions by 66% over a 400 mg dose twice daily for 3 years [31, 32]. One of the complications that should be tackled in the near future for selective COX-2 inhibitors celecoxib in specific is the cardiovascular complications that comes after administration of 400 mg twice daily to be the same as the dose recommended for chemopreventive effect to control the recurrence of CRC [32, 33]. The magnitude of cardiovascular complications of celecoxib limits its use for colon cancer prevention since the development of colon cancer is a slow process, so, the patients with polyps would need to take celecoxib for a long period of time to achieve the target protective effect. Accordingly, a question should be admitted, and should have an evidenced answer: Does the cardiovascular problems of selective COX-2 inhibitors class of anti-inflammatory agent come out of pharmacological class effect or chemical class effect? To my knowledge, we cannot confirm that it is pharmacological class effect and not chemical class effect because the chemical structure of COX-2 inhibitors that share the CVS side effects are Y-shaped diaryl-heterocycle sulfonamide/methylsulfonyl. Thus, it is required to develop new chemical class of selective COX-2 inhibitors help us be provided with verified answer to such important question. The answer of the question would raise the concern to the main reason(s) of CVS complications to tackle and eventually modify the strategy toward generation of selective COX-2 inhibitors with chemopreventive benefits against CRC and other cancer cell types.

Based on the above findings indomethacin, as nonselective COX-inhibitor could be considered strategic lead compound that worth it studying and developing to line it among the chemotherapeutic agents used against cancer to be either prophylactic or therapeutic treatment and/or even adjuvant therapy upon combination with other anticancer agents to

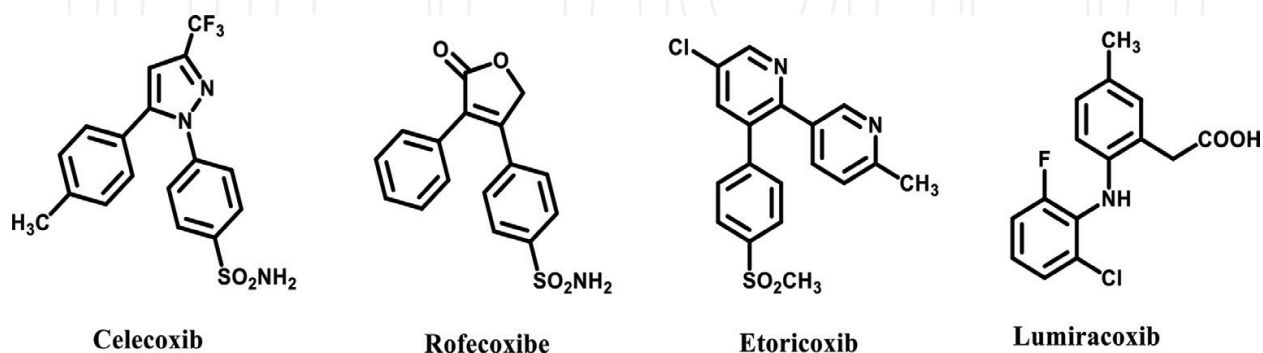


Figure 1. Diarylsulfonamide/methylsulfone selective COX-2 inhibitors.

synergize the chemotherapeutic effect [25]. The subjected insight in this book chapter regarding indomethacin could be easily justified on scientific bases: (1) indomethacin is the most NSAID that is intensively studied as chemopreventive and chemotherapeutic agent against multiple cancer cell types among the other drugs of the same class to show observable results. (2) Indomethacin as a different chemical class when compared to selective COX-2 inhibitors, developing indomethacin-based selective COX-2 inhibitor to excel celecoxib would benefit in asserting whether the cardiovascular system (CVS) problems are originated from chemical class effect. In case, the reason of the cardiovascular system (CVS) complications is attributed to the chemical class effect and indomethacin-based developed structures are devoid of the complications, the patients would be largely benefited from such class of compounds, and it would be chemopreventive agents used for long time without developing cardiovascular system (CVS) complications. (3) Kinetic profile of indomethacin inhibition to COX-2 shows recovery after long time of tight binding to the enzyme [34], and on the other hand, selective COX-2 inhibitors' kinetic profile shows no recovery even after long time of tight binding [35]. This obvious difference between indomethacin and selective COX-2 inhibitors in performing the functionally irreversible inhibition effect to COX-2 has to lead us highlighting indomethacin as promising base to build upon it the developed structures in a way to generate selective COX-2 inhibitors with minimized serious side effects observed with the diaryl heterocycle sulfonamide/methylsulfone class of compounds.

The book chapter covers progressively and in detail some critical topics served in concluding future trends in regard to developing indomethacin to be effective chemopreventive and treatment of various cancer cell types without induction of severe cardiovascular system (CVS) complications: *the implication of COX-2/PGE2 in the anticancer activity of COX-inhibitors*, *COX-2-independent mechanisms of anticancer activity of indomethacin*, and *significance of indomethacin's anticancer activity over the other NSAIDs and selective COX-2 inhibitors*.

2. Implication of COX-2/PGE2 in anticancer activity of COX-inhibitors

PGE2 implicated in promoting cell proliferation of human esophageal squamous cell carcinoma. The study started with observation of expression and upregulation of c-Myc, an oncogenic transcription factor, and then a link was expected to exist between PGE2 and c-Myc but requires a reliable elucidation. Deeper studies revealed that PGE2 substantially increased the proliferation of cultured esophageal squamous cell carcinoma cells and increased mRNA and protein expression of c-Myc. Moreover, knockdown of c-Myc by RNA interference significantly attenuated PGE2-induced cell proliferation. Furthermore, a mechanistic study described that stability and nuclear accumulation of c-Myc oncogenic protein is attributed PGE2 via phosphorylation on serine 62 that induced by extracellular signal regulated kinase (ERK)-dependent manner and this was confirmed when PGE2 activation of ERK was fully abolished by protein kinase C (PKC) inhibitors. Consistently, PGE2 receptor (EP2) agonist resulted in the same effect on expression of c-Myc as PGE2 and knockdown of EP2 receptor by EP2 small interfering RNA (siRNA) delayed PGE2-induced c-Myc expression to verify the association of PGE2 to c-Myc protein expression in esophageal squamous cancer cell proliferation [18].

It was reported for celecoxib to be effective after *Helicobacter Pylori* eradication therapy in improving gastric precancerous lesions and stops progression into cancer. The therapeutic effect of celecoxib is explained in the study by measuring the expression and activity of COX-2 for patients with gastric precancerous lesions received celecoxib up to 3 months to be compared with those received placebo for the same period of time. The measurements were determined by immunostaining and PGE2 assay, cell proliferation by Ki-67 immunostaining, apoptosis by TUNEL staining and angiogenesis by microvascular disease (MVD) assay using CD31 staining. The results showed that there was a significant elevation in COX-2 protein expression in gastric precancerous lesions when compared with that resulted from chronic gastritis with consequent increase in cell proliferation and angiogenesis. Patients who were treated with celecoxib showed significant improvement in gastric precancerous lesions (sites of dysplasia) with 84.6% regressed dysplasia, while those treated with placebo showed 60% suggesting that celecoxib was effective on the regression of dysplasia. On the other hand, celecoxib effectively suppressed cell proliferation, induced cell apoptosis and inhibited angiogenesis exhibited by decreased MVD. Interestingly, COX-2 inhibition was accompanied by up-regulation of PPAR γ expression that is protective protein with reported antineoplastic effects [36].

Overexpression of COX-2 frequently occurred in head and neck squamous cell carcinoma (HNSCC). COX-2 promotes the release of pro-inflammatory mediator PGE2 which binds to cell surface G-protein coupled receptors EP1–4 to exert its pharmacological effects. Upon studying the biochemical functions of PGE2 and its cell receptors in HNSCC cellular proliferation, it was found that COX-2 and cell receptors EP1, EP2 and EP3 were constitutively expressed in tumoral lesions of HNSCC. An important finding was declared in the study states that small concentration of selective COX-2 inhibitors succeed to suppress PGE2 without inhibition of cell proliferation. However, exogenous addition of EP3-specific agonists with PGE2 induces DNA synthesis in all HNSCC cell lines. Thus, it could be suggested that EP3 receptor subtype of PGE2 should be regarded for future strategies targeting HNSCC prevention [19].

Another study defined a critical mechanism to justify the role of PGE2 in promoting CRC cell division in which prometastatic adaptor protein human enhancer filamentation 1 (HEF1) links between PGE2 and cell cycle machinery in CRC cells. PGE2 induces expression of HEF1 mRNA and protein in CRC. Knockdown of HEF1 suppresses PGE2-induced cell proliferation and cell cycle progression. CRC cells were examined and found that there is 50% elevated levels of HEF1 when compared to normal tissues. Further, HEF1 promotes cell cycle progression of colorectal carcinogenesis via interaction with and activation of cell cycle kinase Aurora A to report that PGE2 is inducer to crucial downstream mediator, HEF1 in colorectal carcinogenesis [20].

Small noncoding RNA, microRNAs (miRNAs) have a key role in stopping the translation and accelerate the degradation of mRNA that regulates the cellular growth and survival through gene suppression. miRNA has a significant contribution in controlling disease progression in pancreatic cancer cells (PaCa). Elevated levels of COX-2 were observed with PGE2 and decrease in miRNA increased the cancer growth and metastases of PaCa. Restoration of miRNA-143 (miR-143) in human PaCa cells reduced COX-2 and inhibited cell proliferation. Mitogen activated kinase (MAPK) was correlated to not detecting miR-143 in some pancreatic cancer cell subtypes to justify the implication of MAPK activation in regulating miR-143 beside COX-2 and PGE2 [21].

$\alpha 7$ Nicotinic acetylcholine receptor (nAChR) protein is significantly biosynthesized via cholinergic signaling in nonsmall cell lung cancer (NSCLC) beside COX-2-driven PGE2. The mechanism by which PGE2 promoted NSCLC cell proliferation over $\alpha 7$ nAChR induction showed the positive effect of PGE2 on $\alpha 7$ nAChR expression, promotor activity and cell signaling pathways. The association of the two stimulatory factors to cell growth of NSCLC cells was confirmed upon attenuation of PGE2-induced cell proliferation via $\alpha 7$ nAChR siRNA or acetylcholine transferase. Moreover, PGE2 induced $\alpha 7$ nAChR production was blocked by EP4 receptor antagonist and EP4 siRNA. Furthermore, it was recorded that blocking c-Jun, critical transcription factor, activated by c-Jun N-terminal kinase (JNK), phosphoinositol 3-kinase (PI3K) and protein kinase A (PKA), led to abolishing the PGE2-induced $\alpha 7$ nAChR production and consequent cell growth. It is worthy to mention that activation of JNK, PI3K and PKA resulted from acting of PGE2 on EP4 receptor subtype [22].

Chemopreventive effects of indomethacin was observed for 4-hydroxybutyl(butyl) nitrosamine(OH-BBN)-induced urinary bladder cancers in mice. The study came over conducting three experiments in which the indomethacin was continually administered prior to week 1 or following week 13 OH-BBN dosing for 32 weeks, 1 week after intake of OH-BBN at week 13 for 12 weeks and 30 weeks, and 1 week after intake of OH-BBN at week 13 for 61 weeks, respectively. The chemopreventive effect of indomethacin was observably impressive to show development of palpable bladder masses 3% of animals in case of experiment 1, 77% decrease in palpable masses and 82% decrease in palpable and microscopic masses in case of experiment 2, 26% developed palpable mass under treatment of indomethacin and 66% in control group in case of experiment 3 [26].

3. COX-2-independent mechanisms of anticancer activity of indomethacin

Apoptosis is a programmed cell death induced intrinsically by mitochondrial-mediated or extrinsically by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated signals [37]. Caspase activation is generally accompanied by apoptosis that is dependent on mitochondrial mediated or classical extrinsic TRAIL- or death receptor (DR)-mediated signaling [25]. Tse et al. reported the capability of indomethacin to make tumor cells responsive to TRAIL-mediated apoptosis signals through upregulation of TRAIL receptor (DR 5) and down-modulation of survivin, antiapoptotic protein [38]. The report provided convincing mechanism to the indomethacin-induced process to overcome TRAIL-resistant melanomas. It is well known that indomethacin enhances mitochondrial oxidative stress and the production of reactive oxygen species (ROS) that modulate mitochondrial-mediated signaling [39]. ROS induces the transcription factor, C/enhancer-binding homologous protein that leads to upregulation of DR 5 on tumor cells. Moreover, ROS has a role in down-modulation of surviving via inhibition of transcription of the known regulator, NF-kB [37]. The report suggests that indomethacin could successfully sensitize TRAIL-resistant melanoma cells.

The ability of indomethacin to work against HCT116 human CRC cells does not express COX was reported using proteomic approach to identify the mechanism by which indomethacin

inhibit the CRC growth. The total proteins from indomethacin-treated and untreated cancer cells were separated by immobilized pH gradient-based two-dimensional gel electrophoresis. The different proteins produced throughout the test were identified by peptide mass fingerprint (PMF) based on matrix-assisted laser desorption/ionization time of flight mass spectrometry. The results revealed that indomethacin induced HCT116 apoptosis and inhibited cell growth by downregulation Wnt1-inducible signaling pathway protein 1, Bcl-2-related protein A1 and mitogen-activated protein kinase [24].

c-AMP activates PKA in and c-AMP-response element binding (CREB) protein in melanogenesis. CREB plays an important role in binding to the promotor of the microphthalmia-associated transcription factor (Mitf) gene and consequently activates Mitf gene transcription [40, 41]. Thus, Mitf has a crucial role in transcription of melanogenic genes and activates melanogenic gene transcription of tyrosinase as well. Indomethacin was studied to investigate the effect on melanogenesis in B16F1 melanoma cells. The study resulted in indomethacin inhibited α -melanocyte stimulating hormone that enhances melanin synthesis in melanoma cells., suppressed tyrosinase and Mitf protein levels, reduced tyrosinase promoter activity, lowered mRNA of melanogenic genes, including Mitf gene [23].

AMP-protein kinase (AMPK) is a key factor of master regulation of cellular energy homeostasis [42]. When AMPK is activated, it induces block of cell cycle and apoptotic cell death in different types of cancer cells including gliomas, the primary tumors of central nervous system [43–47]. The apoptotic death and inhibition of growth of cancer cell actions of AMPK are mediated by one of the signaling pathway in which mammalian target of rapamycin (mTOR) is inhibited. It is worthy to note that mTOR is a catalytic core for formation of two definite complexes, mTOR complex 1 (mTORC1) and 2 (mTORC2) and both are sensitive targets to rapamycin, allosteric inhibitor of the complexes [48]. mTORC1 has a supporting role in protein synthesis and cell proliferation. mTOR performs its biological functions by phosphorylating ribosomal p70S6 kinase and translational repressor 4E-BP1 [48]. AMPK phosphorylates raptor and/or tuberous sclerosis complex-mediated inhibition of mTOR stimulator Ras homolog enriched in brain (RHEB) [49]. Beside the role of mTORC1 in cell proliferation, it causes major and observable negative regulation to intracellular degradation of unnecessary and dysfunctional cellular components through lysosomal machinery which is a kind of cytotoxic mechanism [50]. Indomethacin was reported as growth inhibitor to CRC cells by mTOR inhibition [51]. For the glioma cells U251, indomethacin showed superior *in vitro* antiglioma action and restriction to cell proliferation to the other COX-inhibitors when tested against the same cancer cells. The antiproliferative and proapoptotic actions of indomethacin against U251 was evidenced by G2M cell cycle arrest P21 associated with caspase-3/9 activation, DNA fragmentation that were displayed by indomethacin-treated glioma cells [52]. Also, indomethacin stimulates AMPK phosphorylation in glioma cells and the implication of this pathway in antiglioma effect of indomethacin was confirmed by knockdown of AMPK via RNA interference to alter the AMPK activity and antiglioma actions of indomethacin. Generally, AMPK seemed not sufficient antiproliferative pathway to restrict glioma cell proliferation. Therefore, it is expected that treating glioma cells with indomethacin had a synergistic effect came from its AMPK-dependent and -independent pathways in inhibiting the cancer cell growth and proliferation of gliomas.

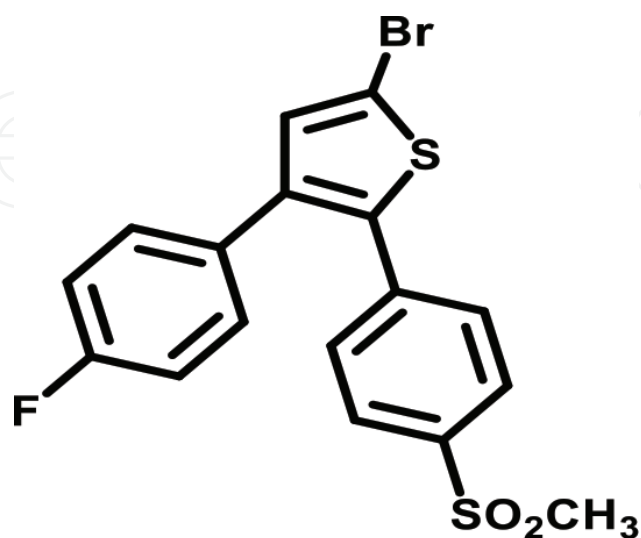
4. Significance of indomethacin and its developed structures' anticancer activity over the other NSAIDs and selective COX-2 inhibitors

According to the literature scan had been done on indomethacin as nonselective COX-inhibitor and antiproliferative agent, it could be observed that indomethacin is the most NSAID that attracted the interest of researchers to study, investigate, identify more about the definite mechanisms and cellular signaling pathways involved in the antiproliferative and apoptotic effects of indomethacin. This might be attributed to two apparent points: one is the observable inhibition of cell growth, reduction of tumor size and implication in the programmed cell death of multiple tumor cell types including glioma and glioblastomas that require lipophilicity for cell penetration. The second is that indomethacin exhibited its anticancer activity against wide variety of cancer cell types by COX-2/PGE2-dependent and -independent mechanisms. This adds great advantage to indomethacin over the other nonselective COX-inhibitors because in that way, indomethacin has dual actions by which it could exert its cytotoxic activity effectively.

Regarding the selective COX-2 inhibitors, the prophylactic actions of celecoxib against recurrence of colon polyps is defined by researchers as dramatic to indicate the capability of celecoxib and other COX-2 inhibitors to block the cancer cell growth and metastases as well. One serious complication that is developed upon long-term therapy of celecoxib that limits its use as chemopreventive therapy for CRC is the cardiovascular toxicity that results from critical reduction in PGE2 and prostacyclin (PGI₂) production. Those types of prostaglandins are COX2-dependent product responsible for regulating vascular tone and atherosclerosis [53]. The problem is absolutely the same as all the selective COX-2 inhibitors since they share the same pharmacological action in which production of prostaglandins is significantly diminished.

Some differential points related to the kinetic profile of both indomethacin [34] and selective COX-2 inhibitors [35] were worth it stopping at to help us draw a future plan to develop indomethacin's chemical structure in a way to enhance COX-2 inhibition activity like selective COX-2 inhibitors and be devoid of cardiovascular complications as well. Nonselective inhibitors including indomethacin perform its inhibition action against the enzyme through 2-step inhibition mechanism involving slow and time-dependent step due to tight reversible binding to the enzyme to be considered as functionally irreversible. But selective COX-2 inhibitors inhibit the enzyme through 3-step inhibition mechanism involving time-dependent step that represents the tightly bound complex of inhibited enzyme. The observation that should be highlighted for both types of COX-inhibitors while monitoring the kinetic model of inhibition mechanism is that indomethacin carboxylic acid is not essential for the tight binding and time-dependent step of enzyme inhibition because the esterified counterpart did not abolish this step or even reduce the tightness of binding to human COX-2 and the formation of the complex maintained functionally irreversible [54, 55]. Further, indomethacin recovered intact after prolonged time of incubation with the enzyme, this suggests that enzyme inhibition came over conformational change not covalent bond formation [34]. On the other hand, DuP 697, selective COX-2 inhibitor showed the same time-dependent step that was responsible for

drug's selective inhibition of human COX-2 but impressively the inhibitor did not show successful recovery even upon dialysis but the inhibitor is freed to inhibit another enzyme under the effect of denaturation to confirm that the tight binding of inhibitor to the enzyme was not based on formation of covalent bond [35].



DuP 697

The detrimental differences in regard to the kinetic profile of indomethacin, nonselective COX-inhibitor and DuP 697, selective COX-2 inhibitor for inhibition of COX-2 raised my concern with that emerge of developed selective COX-2 inhibitors based on indomethacin would definitely help us answer two important questions:

- i. *Is the cardiovascular toxicity of selective COX-2 inhibitors pharmacological class effect of chemical class effect?* The change of chemical class of selective COX-2 inhibitors from the traditional diaryl heterocycle sulfonamide/methylsulfone to indomethacin-based structures and identifying the kinetic profile of the new class of selective inhibitors would provide a strong evidence on the real reasons of cardiovascular problems after administration of selective COX-2 inhibitors to discover whether it lies behind the kinetic mode of enzyme inhibition which depends on the chemical structure or it lies behind the selective action of the drug against COX-2. In case of similar kinetic profile for the generated new inhibitors to the traditional class and no recovery to the inhibitor is shown, so, it will imply that it is a general feature to the selective inhibitors whatever is the chemical structure. It could be drawn that the inability of selective inhibitors to get recovered from COX-2 enzyme might be a significant reason to the CVS complications of the inhibitors. In case, the new structures saved the kinetic profile of the original lead compound, indomethacin, so, it would be worth it monitoring the development of CVS problems after long-term administration of the newly developed indomethacin-based compounds. There are two reports based on epidemiological studies stated clearly that prolonged use of NSAIDs is associated by small increase in CVS risk [56, 57]. This attracted our attention to comment on that this happens though NSAIDs inhibit COX-2 with the same efficacy as selective

COX-2 inhibitors. Thus, it is suggested that chemical structure and/or binding mode most likely play a significant role in determining the kinetic mode of enzyme inhibition. But, we are still in serious need to an evidence results from experimental studies to assertively answer the above question.

- ii. *Would the anticancer activity of indomethacin be enhanced with the new indomethacin-based compounds?* Improvement of the selective inhibition of COX-2 in comparison to indomethacin is supposed to potentiate the antiproliferative and apoptotic activities upon enhanced diminishing to COX-2/PGE2, combining this important signaling pathway with the COX-2-independent mechanisms of anticancer activities that previously described in this book chapter for indomethacin in Section 2 “COX-2-independent mechanisms of anticancer activity of indomethacin”.

Several attempts for generation of indomethacin-based analogs of selective COX-2 inhibition activity [58–60], but the publication that I had to put it in focus in this regard is that described the design and synthesis of indomethacin-based analogs of potentially selective COX-2 inhibition activity and observed diminishing to PGE2 [61]. The successful generation of developed indomethacin structures with selective COX-2 inhibition activity was iteratively reported in the literature but picking this publication to comment on among the others was based on the obvious selectivity index of the generated analogs that excelled celecoxib, dramatic lowering to plasma levels of PGE2 when compared to indomethacin, the innovative perspective upon

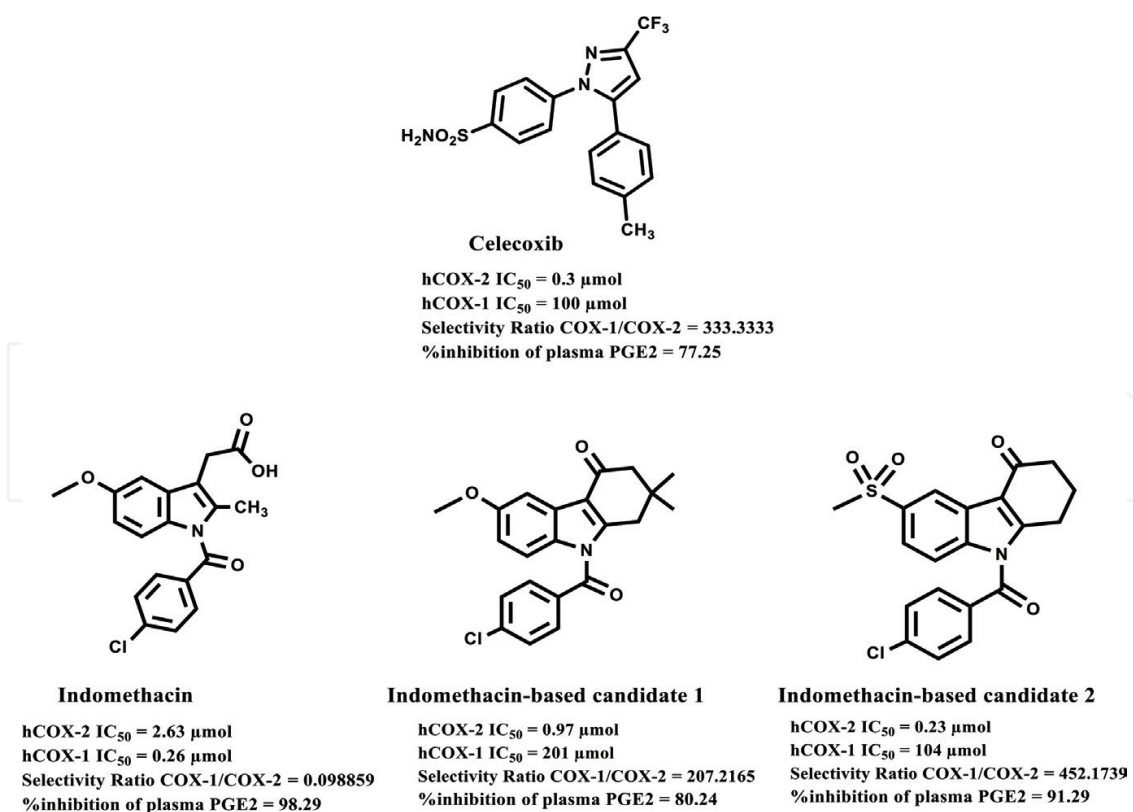


Figure 2. Biological data of the new indomethacin-based selective COX-2 inhibitors 1 and 2 in comparison with indomethacin and celecoxib.

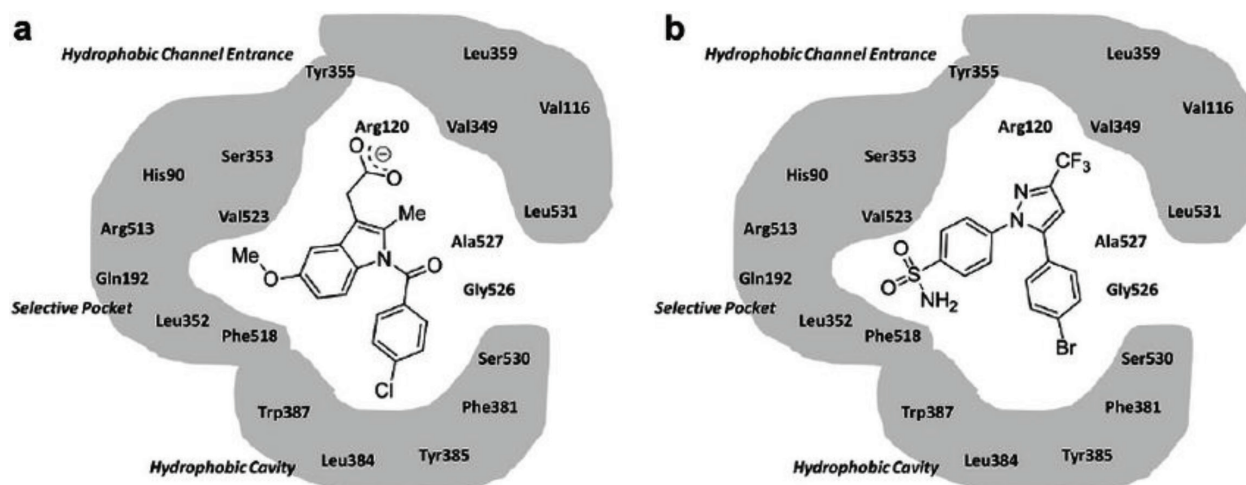


Figure 3. Schematic representation of SC-558 (COX-2 selective inhibitor) binding to COX-2 (a) and indomethacin (nonselective inhibitor) (b).

which the design and modification of the analogs are designed, and above all of this the biological profiling through multiple *in vitro* and *in vivo* tests that done for the analogs to impress the discovery of interesting tetrahydrocarbazole candidates. Accordingly, the newly selective COX-2 inhibitors worth it promoting to study how far it is implicated in CVS toxicity upon long-term therapy against either cancer or inflammatory diseases.

The new tetrahydrocarbazole selective COX-2 inhibitors **(1)** and **(2)** (**Figure 2**) generated were based on enlarging the size of indomethacin indole ring to occupy the wider catalytic pocket of COX-2 than COX-1 by 25% [62] and reducing the opportunity of the ring-extended candidates to interact with COX-1 to raise the selectivity. Introduction of methylsulfonyl group to replace methoxy group of indomethacin at position 6 to enhance the interaction of the designed inhibitor **(2)** (**Figure 3**) with the polar side pocket of COX-2 (selective pocket) that is critical for COX-2 inhibition activity [63]. Deletion of carboxylic acid from the new candidates **(1)** and **(2)** reduce the possibility of the inhibitor to interact with COX-1 via formation of salt bridge with Arg120 amino acid (**Figure 3**) [64] that is critical for conformational change and inhibition of the isoenzyme. Impressively, the biological results throughout *in vitro* testing represented by enzymatic assays against human COX-1 (hCOX-1) and hCOX-2 and *in vivo* testing represented by % inhibition of plasma PGE2 and others revealed the successful verification of the proposed hypothesis suggested to enhance the COX-2 inhibition selectivity. Methoxy derivative **(1)** (**Figure 2**) gave selectivity index against COX-2 (207.2165) to excel both that of indomethacin (0.98859) and celecoxib (333.3333) standard drugs. For the methylsulfonyl derivative **(2)** (**Figure 2**), it excelled the standard materials at much higher value (452.1739). Moreover, the diminishing of plasma levels of PGE2 was very observable in comparison to indomethacin (98.29%) and celecoxib (77.25%) (**Figure 2**). Thus, kinetic profile of the enzyme inhibition of the new candidate **(2)** in the near future would answer the questions described in this section on the book and eventually be able to judge on the chemopreventive of the new selective COX-2 inhibitor and antiproliferative activity as well.

5. Conclusions

Generation of indomethacin-based analogs to indomethacin aiming at enhancing the selective COX-2 inhibition would definitely help us answer an important question concerning the real reason of cardiovascular toxicity of selective COX-2 inhibitors, whether it is pharmacological class effect or chemical class effect. Moreover, enhancing the selectivity of indomethacin against COX-2 among the other NSAIDs providing a candidate privileged with potential anti-inflammatory activity devoid of gastrointestinal side effects and what is more important is obtaining newly developed structure carries effective antiproliferative and apoptotic activity standing for the dual actions reported for indomethacin as a lead compound based on that it performs its anticancer activity by both COX-2-dependent and COX-2-independent mechanisms. Further, the CVS toxicity is expected to be minimized upon enhancing the selective COX-2 inhibition of indomethacin due to observation that there might be a difference in the kinetic mode of enzyme inhibition between diaryl heterocycle sulfonamide/methylsulfone chemical class of selective COX-2 inhibitors and the new indomethacin-based chemical class of compounds that may permit successful recovery of the new inhibitors from the enzyme after long incubation period.

Nomenclature

NSAID	nonsteroidal anti-inflammatory drug
COX	cyclooxygenase
PG	prostaglandin
CRC	colorectal carcinoma
CVS	cardiovascular
ERK	extracellular signal regulated kinase
PKC	protein kinase C
siRNA	small interfering RNA
MVD	microvascular d
HNSCC	head and neck small cell carcinoma
HEF1	human enhancer filamentation 1
miRNA	microRNA
MAPK	mitogen activated kinase
NSCLC	nonsmall cell lung cancer
JNK	c-Jun N-terminal kinase

PI3K	phosphoinositol 3-kinase
PKA	protein kinase A
OH-BBN	4-hydroxybutyl(butyl)nitrosamine
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
DR	death receptor
ROS	reactive oxygen species
PMF	protein mass fingerprint
CREB	c-AMP-response element binding
AMPK	adenosine monophosphate kinase
mTOR	mammalian target of rapamycin
RHEB	Ras homolog enriched in brain

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