Mechanism of Action of Key Enzymes Associated with Cancer Propagation and their Inhibition by Various Chemotherapeutic Agents

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Abstract: The propagation of cancer, which is basically the consequence of uncontrolled multiplication of cells, is a complicated process involving the participation of a number of enzymes. The molecular level understanding of the chemistry of these enzymes is the starting step towards the development of anti-cancer drugs and a collective view of these enzymes (responsible for cell multiplication) could help in the development of multiple target ligands. In this review, the mechanistic chemistry of the key enzymes viz. ribonucleotide reductase, thymidylate synthase, thymidylate phosphorylase, topoisomerase II, closely involved at various stages of cell multiplication and hence responsible for the propagation of cancer, and some of their suitable inhibitors have been discussed. Further, this review will elucidate the chemistry of lactate dehydrogenase and cyclooxygenase, the enzymes responsible for providing the extra energy to the cancer cells and initiating the growth of tumors through the formation of mutagens, respectively.

Key Words: Cell multiplication, cancer, enzymes, active site residues, mechanism of action, inhibitors, multiple target ligands.

INTRODUCTION

The participation of a number of enzymes at different stages of development of cancer, survival of cancer cells even under anaerobic conditions (capability of cancer cells to produce ATP by conversion of glucose to lactate in contrast to the normal cells), difficulty in its detection at early stage and ultimately the hurdle of multi drug resistance [1, 2] has made cancer as the most dreaded disease. Amongst the various approaches for the treatment of cancer, chemotherapy has been widely practiced and continues to generate new avenues of research for the development of highly potent, safe and economical anti-cancer drugs. However, the combined and continues efforts of biologists, theoretical chemists, synthetic chemists, pharmacologists etc. for the last 60 years, since the first time nitrogen mustard was used as anticancer agent [3, 4], are still in process for developing a hardcore chemotherapeutic agent. Because it is the uncontrolled multiplication of the cells, initiated by certain stimuli, which is responsible for the progression of the disease, the anticancer drugs target the process of cell multiplication in one way or the other. Therefore, the development of novel drugs for cancer therapy necessitates the molecular level understanding of the various stages of cell multiplication including the mechanistic function of each enzyme.

In the present contribution, a brief description of the cell multiplication cycle is succeeded by a discussion on the mechanism of action of key enzymes associated with cancer propagation and the available inhibitors of each enzyme. It was envisaged that a collective understanding of the mode of action of these enzymes and their corresponding inhibitors will help in the development of multiple target directed drug/s which are proving to be more safe and economical [5, 6].

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Cell Multiplication Cycle

The division of a parent cell into two daughter cells is a complicated process requiring the participation of several enzymes (Fig. **1**). It is worth to mention here that the overexpression of any one of these enzymes in the cancerous cells results in their faster multiplication in comparison to the normal cells. Based upon certain metabolic differences and for ease of understanding, the process of cell division could be divided into three phases, viz., G1 phase, S phase and G2 phase (Fig. (**1**)) [7, 8]. G1 phase prepares the raw material for cell multiplication; in the S phase the duplication of cell organelle takes place and in G2 phase the cell organelles and other material become organized for separation into the daughter cells (mitosis). Various enzymes taking part in these cellular processes are shown in (Fig. (**1**)).

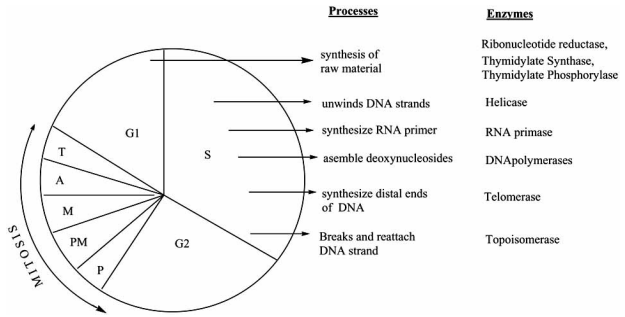

Fig. (1). A schematic view of cell multiplication showing various steps and the catalyzing enzymes.

The rate controlling step of cell division is the duplication of master molecule DNA which takes place in the S phase from the material prepared in G1 phase. In order to inhibit the multiplication of cancer cells, the enzymes responsible for the preparation of raw material (deoxyribonucleotides) for DNA (G1 phase) and the one participating in the rate limiting step of DNA duplication (Topoisomerase II

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in S phase) are the key targets. Keeping in mind the volume of the review, other enzymes participating in the S phase are not included in the discussion.

Enzymes Catalyzing the Process for Synthesis of Raw Material for DNA and its Replication

In an antecedent order, some of the key enzymes taking part in the synthesis of raw material for DNA and its replication are:

1. Ribonucleotide reductase
2. Thymidylate synthase
3. Thymidylate phosphorylase
4. Topoisomerase II

Besides the discussions on the mechanistic roles of these four enzymes, two other enzymes viz. lactate dehydrogenase and cyclooxygenase-2, whose inhibition represents another significant approach towards the control of cancer propagation, have also been included in this review.

1. RIBONUCLEOTIDE REDUCTASE (RNR)

The synthesis of deoxyribonucleotides, the building blocks of DNA, by the reduction of ribonucleotides catalyzed by RNR [9] constitutes the limiting step for the multiplication of DNA and hence the cell division. RNR is an iron containing enzyme consisting of two subunits R1 and R2 [10]. The aminoacid residues present in the active site of the enzyme, surrounding the substrate Guanosine Diphosphate (GDP) have been shown in (Fig. (**2**)). The binding of the substrate in the active site has been affected by the H-bond interactions of the phosphate residue with E623, T624, S625 and ribose residue with E441, N437, C225. Moreover, for the binding of substrate to take place, two aminoacid residues viz. C225 and C462 which during the later part of the process get linked through S-S bond, must be present in the reduced form.

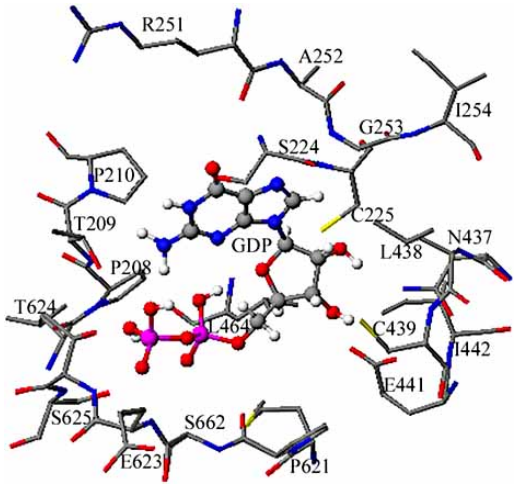


Fig. (2). Active site aminoacid residues of RNR surrounding the natural substrate GDP (pdb ID 4R1R [9]). Hydrogens have been omitted for clarity.

A free radical stabilized by iron exists on Y122 in the resting stage of the enzyme. This radical through a specific pathway [11-14] constituted by N237, W48, Y356, Y731, Y730 is transferred to C439 which is present at a distance of 2.53 Å from C-3' H of GDP and is in an excellent position for removing this hydrogen as a radical. During the active phase of the enzyme, C439 radical takes up a hydrogen radical from C-3' of ribose unit (**1**, Scheme **1**) and the thiol group of C225 (present in R1 subunit) protonates the C2'-OH of ribose followed by loss of water and transfer of a hydrogen from the thiol group of C462 (present in R1 subunit) (**2**-**3**, Scheme **1**). It results in the formation of a disulphide bond between C225 and C462 residues of RNR and the CHOH group of ribose at C-2' changes to CH_2 group forming deoxyribose (**3**, **4** Scheme **1**) [9].

The most prominent feature of RNR mediated reduction of ribonucleotides is the stabilization of the Y122 radical by Fe^{II} and Fe^{III} participation. The removal of iron from the active site of the enzyme could deprive it from its catalytic properties.

Ribonucleotide Reductase as Target for Cancer Chemotherapy

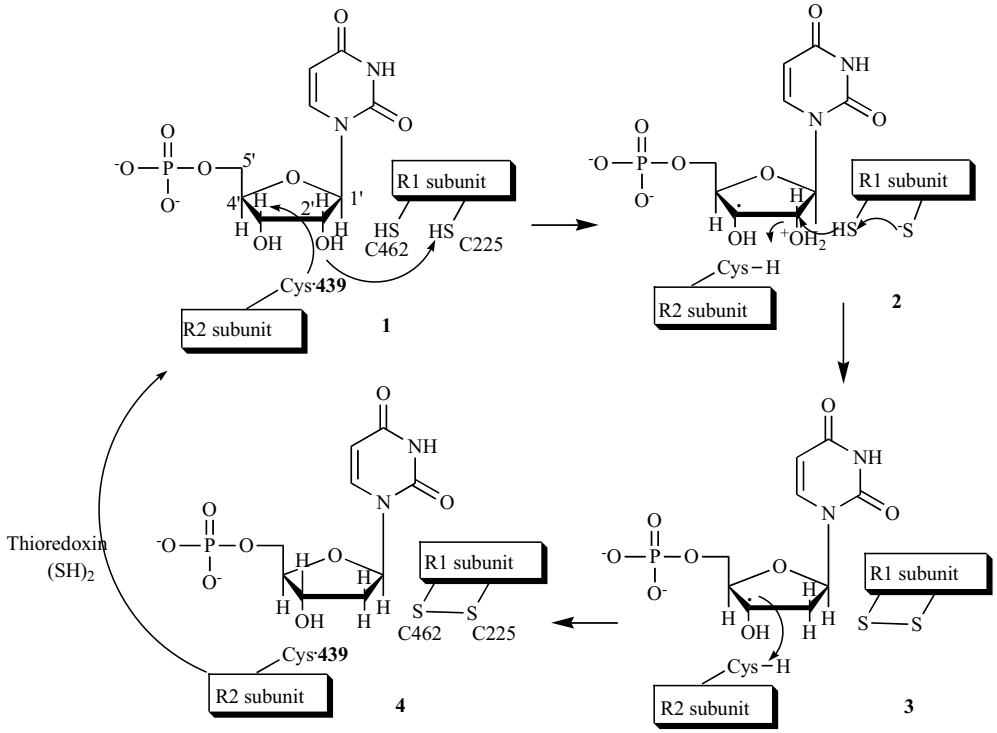
Ribonucleotide reductase (RNR), catalyzing a key step in DNA replication, has been made the target for the chemotherapy of cancer. The activity of the enzyme starts with the generation of a radical at Y122, stabilized by the co-factor iron; a straightforward strategy to inhibit the activity of this enzyme is to trap the co-factor. The compounds investigated as RNR inhibitors (Chart **1**), [15-21] possess the functional group capable of chelating iron. Amongst a series of pyridoxal benzoyl hydrazones, salicylaldehyde benzoyl hydrazones and 2-hydroxy-1-naphthyldehyde benzoyl hydrazones, the compounds **5**-**10** show maximum potency in terms of chelation with iron and antiproliferative activities. Di-pyridin-2-yl-methylene hydrazide derivatives (**11**, **12**; Chart **1**) have also been identified as the most effective chelators. Still better chelation of compounds **13** and **14** with iron has improved their antiproliferative activities and led to the clinical use of desferrioxamine (DFO) (**13**) [15-17] while triapine (**14**) [18-19] has entered phase I and II clinical trials.

2. THYMIDYLATE SYNTHASE (TS)

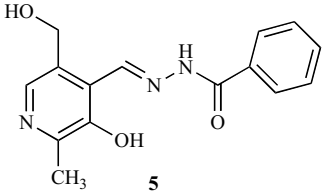
Thymidylate, the raw material for the replication of DNA, is made available by the conversion of deoxyuridine 5'-monophosphate (dUMP) to deoxythymidine 5'-monophosphate (dTMP) through the mediacy of enzyme TS [22-24]. The crystal structure of the enzyme along with the substrate (Fig. (**3**)) demonstrates the role of different aminoacid residues towards the activity of the enzyme. The substrate dUMP is stabilized in the active site of the enzyme through H-bonds.

The phosphate moiety of dUMP forms H-bonds with R23 and S219 aminoacid residues, OH group present at C-3' of deoxyribose unit is H-bonded to H259 and the uracil moiety is held by three H-bonds from C-2 and C-4 carbonyl oxygens and N-3 hydrogen with D221 and N229 aminoacid residues. The sulphur atom of C198, responsible for initiating the reaction, is present at a distance of 3.60 Å from C-6 of uracil (Fig. (**3**)).

The process of conversion of dUMP to dTMP starts with the reaction of sulfhydryl group of cysteine198 of TS at C-6



Scheme 1. Mechanism of action of Ribonucleotide reductase.



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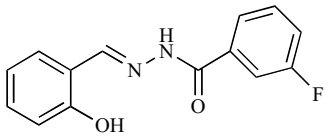
Effect on internalized iron uptake from Tf by SK-N-MC NB cells 24 %



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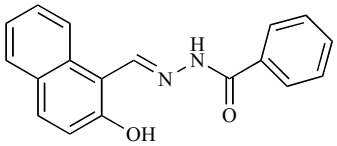

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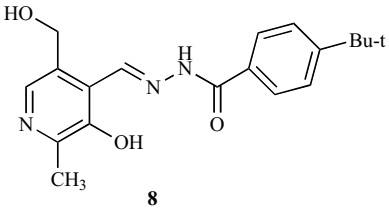

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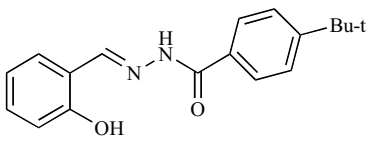
ID50 (μM) 7



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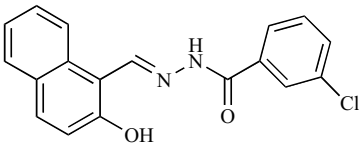

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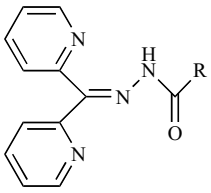
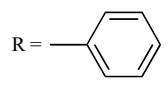
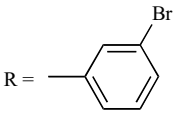


Di-pyridin-2-yl-methylele hydrazide (**11**)



11a



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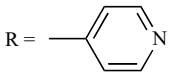

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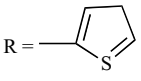

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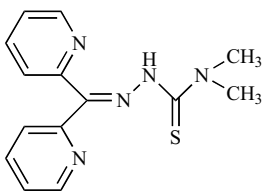
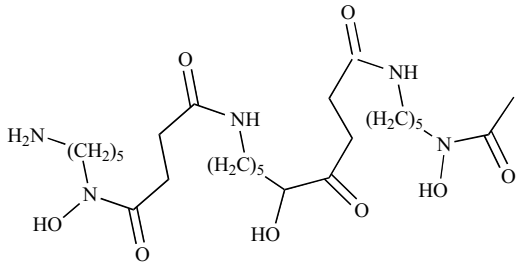


Dp44mT (**12**)

IC_{50} (μM):	**11a**	**11b**	**11c**	**11d**
SK-N-MC	3.13	0.30	0.65	0.65
SK-Mel-28	3.60	0.89	1.56	1.41
MCF-7	2.40	0.39	1.56	0.78

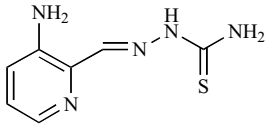
Average IC_{50} 0.03 μM over 28 cell types

(Chart 1. Contd….)



DFO (**13**)

IC_{50}: SK-N-MC neuropithelioma=5±2 μM
SK-Mel-28 melanoma =15±7 μM
MCF-7 Breast cancer = 14±9 μM



Triapine (**14**)

IC_{50}: SK-N-MC neuropithelioma=0.26±0.01 μM
SK-Mel-28 melanoma = 2.6±0.6 μM
MCF-7 Breast cancer = 3.0±1.5 μM

Chart 1. Inhibitors of RNR.

of uracil **17** which converts the C-5 carbon to a nucleophilic enolate **18**. Enolate **18** reacts with iminium ion **16** to take up a methylene unit followed by the transfer of a proton to the methylene unit at C-5 of pyrimidine, thereby forming deoxythymidine monophosphate **19** (Scheme **2**) [22].

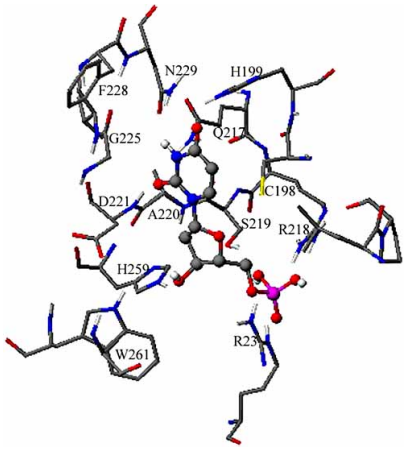


Fig. (3). The active site residues of TS holding the substrate dUMP (pdb ID 2G8D). H_2O molecules and Hydrogens have been suppressed for clarity.

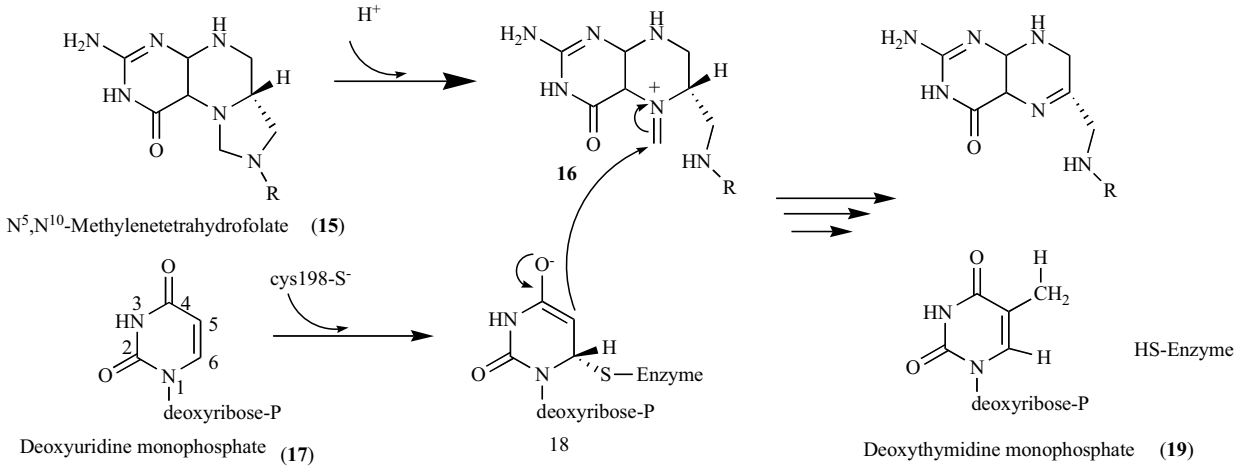
Thymidylate Synthase as Target for Cancer Chemotherapy

Thymidylate synthase has long been considered as a target of choice for the practice of chemotherapy of cancer. The first step in the TS catalyzed conversion of dUMP to dTMP i.e., the activation of C-5 position of uracil by the reaction of $^{-}$S-cys198 at C-6 of uracil could be blocked by using C-5 and /or C-6 substituted uridine analogues. Several such types of compounds (**20**-**24**, Chart **2**) have been investigated for their anticancer activities especially targeting TS, out of which 5-fluorouracil [25-29] is clinically used.

Since the structure of TS has been explored in detail and based upon the structure activity relationship studies of the known TS inhibitors, more potent and safe anti-cancer agents could be developed for successful practice of chemotherapy of cancer [30].

3. THYMIDYLATE PHOSPHORYLASE (TP)

Thymidine phosphorylase plays a catalytic role in the reversible conversion of pyrimidine nucleosides to pyrimidines and 2-deoxyribose-1-phosphate thereby maintaining a balance of nucleotide pool [31]. For example the transfer of deoxyribose unit of uridine to thymine results in the forma-



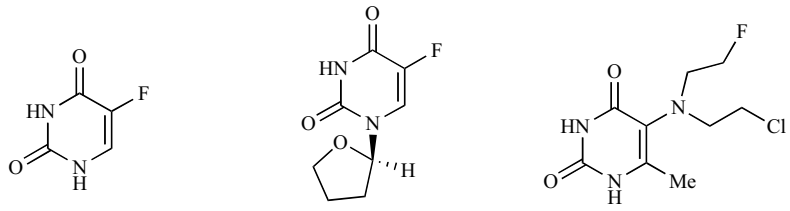
N^5,N^{10}-Methylenetetrahydrofolate (**15**)

Deoxyuridine monophosphate (**17**)

18

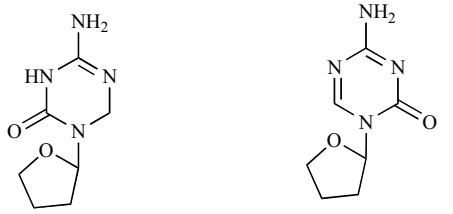
Deoxythymidine monophosphate (**19**)

Scheme 2. Mechanism of action of TS.



5-Fluorouracil (**20**) Ftorafur (**21**) Fluorodopan (**22**)

Average GI_{50} over 59 human tumor cell lines	0.899 µM	616 µM	177 µM



Dihydroazacytidine (**23**) 5-Azacytidine (**24**)

Average GI_{50}	21 µM	36.8 µM

Chart 2. Inhibitors of TS.

tion of thymidine which after phosphorylation serves as the raw material for DNA replication. Moreover, 2-deoxy-D-ribose produced by the catalytic action of TP (identical to platelet derived endothelial cell growth factor, PD-ECGF) [32, 33] acts as an endothelial-cell chemo-attractant [34], responsible for the formation of new blood vessels (angiogenesis) during the tumor growth.

The crystal structures of two TPs bound to different substrates are shown in (Fig. (**4a**)) and (Fig. (**4b**)). The presence of different aminoacid residues in the active sites of two TPs, when uracil and phosphate (Fig. (**4a**)) and uracil and deoxyribose phosphate (Fig. (**4b**)) are the substrates, shows the flexibility of the two pockets of the enzyme. The uracil moiety in both crystal structures, (Fig. (**4a**) and (**4b**)), is present in upper pocket where it is surrounded by R1168, Y1165, I1180, L1114, I1184, G1111 and R168, G96, F162, Q166, E196 respectively. The phosphate moiety is present in the lower pocket, formed by H1082 and S1083 (Fig. (**4a**)) and M197, T94 and lower part of E196 (Fig. (**4b**)). The distance between N-1 of uracil and C-2' of sugar unit is 3.7 Å (Fig. (**4b**)).

During the active phase of the enzyme, the two binding pockets approach each other and the phosphate unit is transferred to the ribose part of nucleoside cleaving the pyrimidine-ribose linkage (**25**-**28**, Scheme **3**) [36].

Thymidylate Phosphorylase as Target for Cancer Chemotherapy

The catalytic activity of TP, maintaining the nucleotide pool of the cell and providing the endothelial cell chemoattractant in the form of 2-deoxy-D-ribose, is a potential target of anti-cancer drugs. The inhibitors of TP are mainly the

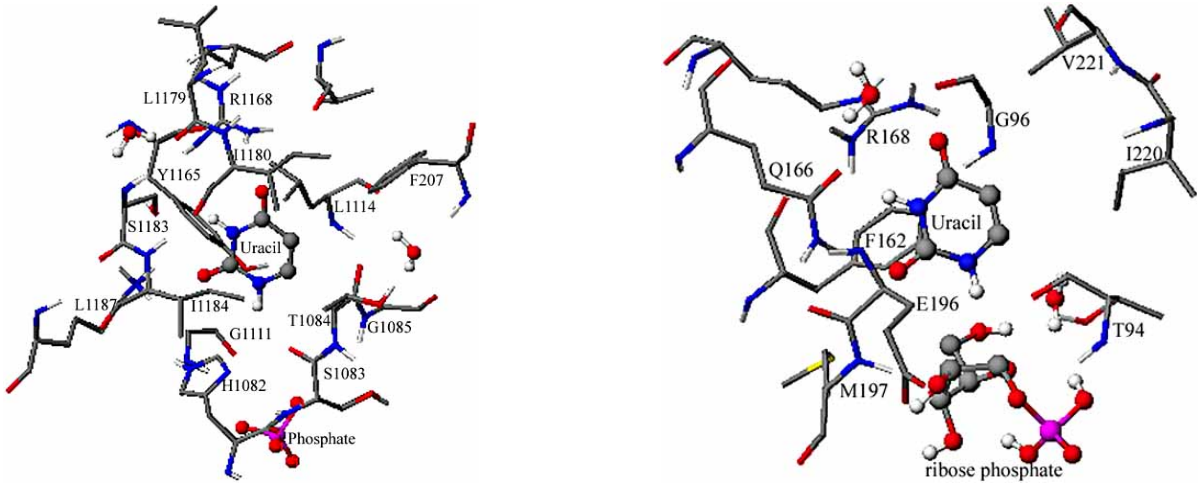
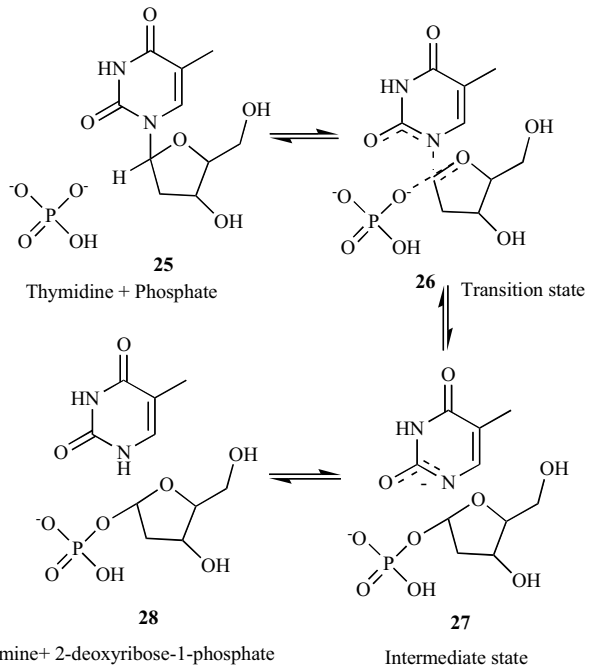


Fig. (4). a) Uracil and phosphate bound to the active site residues of TP (pdb ID 1BRW [35]. b) uracil and deoxyribosephosphate bound to the active site of TP (pdb ID 1TGY). Hydrogens have been suppressed for clarity.



Scheme 3. Mechanism of action of TP.

analogues of its natural substrate and primarily belong to the uracil type molecules with various substituents at C-5 and / or C-6 positions. Compounds like **29** [37] and **30** [38] (Chart **3**) are the potent inhibitors of TP causing a substantial suppression of the tumor growth by inhibiting angiogenesis. Based upon the docking studies, better TP inhibitors **31**, **32** [39] (Chart **3**) have been developed. A number of C-5 and C-6 substituted uracils (**33**, **34** Chart **3**) [40-42], *N*-1 and *N*-1, *C*-5 substituted uracils [43, 44] and purine based compounds (**35**, Chart **3**) [45] have been investigated for TP inhibition.

Unfortunately, none of the compounds investigated as TP inhibitors could reach at the clinical stage so far.

4. TOPOISOMERASE II (TOPO II)

The human genome is currently known to contain five topoisomerases: topoisomerase I, topoisomerase II α and II β and topoisomerase III α and III β. Each enzyme serves an irreplaceable function. The enzymatic activity of topoisomerase was first discovered in 1971 and the name topoisomerase comes from their ability to change the three dimensional structure of DNA molecules without changing the chemical structure. Topo II seems to function predominantly during the separation of daughter DNA strands [46-48], the last step in DNA replication. Due to the *theta* shaped structure of TopoII, its operation parallels with opening and closing of molecular gates and functions as homo- or heterodimers and requires ATP for catalysis [49]. The crystal structure of Topo II with dexrazoxane (a Topo II inhibitor) (Fig. (**5**)) shows the presence of Y28, Y144, H20, T27, L148, Q365 and N142 aminoacid residues in the active site of the enzyme.

The catalysis of Topo II involves binding of one DNA duplex by the dimer followed by the formation of an ATP dependent clamp around another DNA duplex [51]. A transient DNA break is made during which the Topo II enzyme is covalently bound to the DNA *via* an active site tyrosine residue. Another DNA strand is passed through the transient break and then resealing of the broken strand takes place. The whole catalytic process of Topo II is completed in six distinct steps:

1. binding of enzyme to the DNA
2. production of double strand DNA break prior to strand passage
3. strand passage event which occurs in the presence of ATP

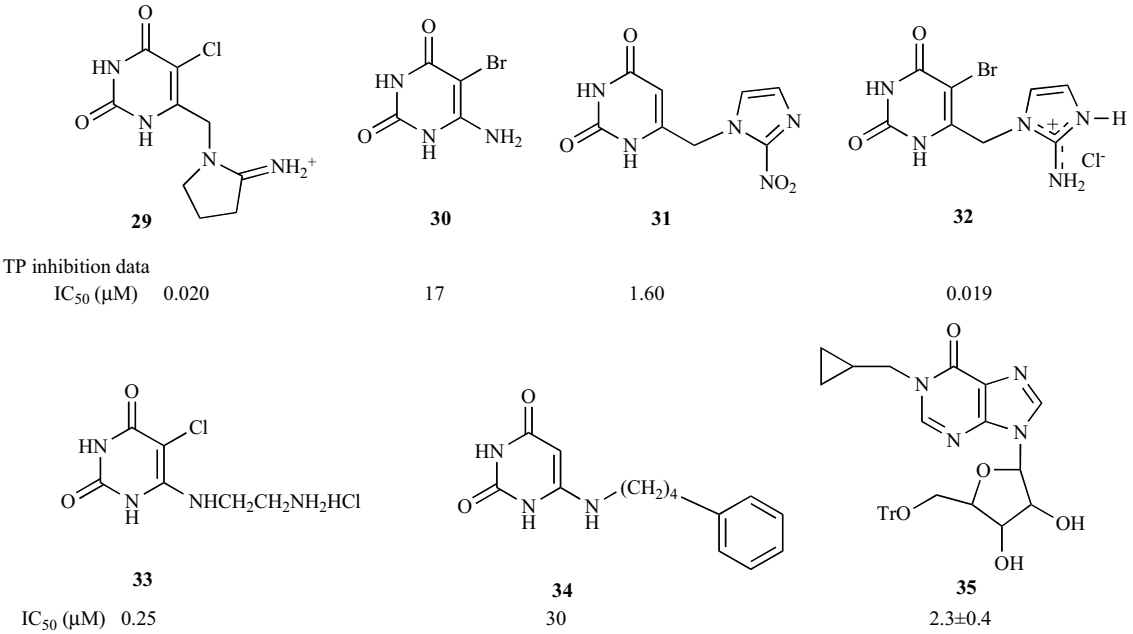


Chart 3. Inhibitors of TP.

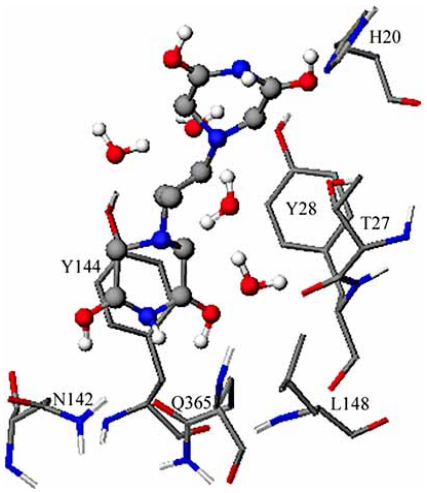


Fig. (5). Active site residues of Topo II bond to dexrazoxane (pdb ID 1QZR [50]).

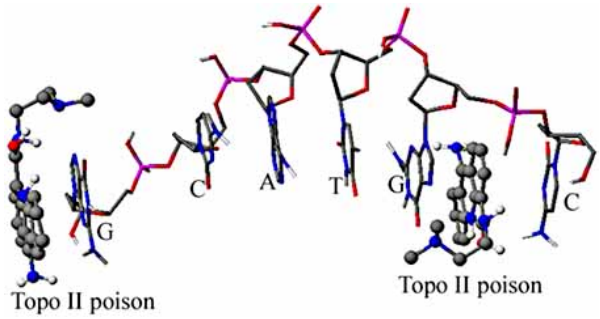


Fig. (6). Topo II poison (9-amino-[N-(2-dimethylamino)ethyl] acridine-4-carboxamide) stacking between the nucleobases of DNA strand (pdb ID 465D [54]).

4. relegation of the DNA break occurring after the strand passage
5. hydrolysis of ATP
6. dissociation and release of DNA from the topoisomerase

This is a concerted cleaving and resealing reaction. Passage of the second duplex through the gap in the first duplex alters DNA topology. The clamp mechanism minimizes the risk of improper resealing of the transient double strand break made by the enzyme. Blocking of this whole process i.e., the Topo II activity will stop the replication of DNA and being advantageously used for the chemotherapy of cancer.

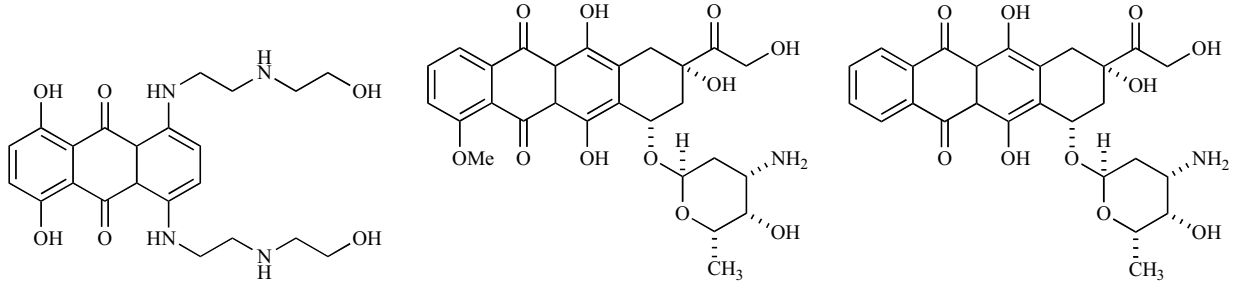
Topoisomerase II as Target for Cancer Chemotherapy

The blockage of tyrosine residues by Topo II poison (Fig. (**5**)) stops the interaction of Topo II with DNA and hence the activity of the enzyme. Various anticancer drugs targeting Topo II viz. 5-fluorouracil, doxorubicin, daunorubicin, mitoxantrone and others [52] increase the life time of the intermediate stage being formed by the interaction of DNA-Topo II enzyme. This is done either by increasing the cleavage of DNA double strand or by inhibiting the resealing of DNA strands. In both cases, the drug's interference with the catalytic activity of the enzyme deprives the cell of the enzyme's ability to decatenate chromosomal DNA strands prior to mitosis and hence the cell growth is altered. The compounds which intercalate with DNA or DNA-Topo II complex (Fig. (**6**)) could block the separation of DNA strands. The use of mitoxantrone [53] (**36**, Chart **4**), capable of intercalating with DNA or DNA-Topo II complex, as anticancer drug has been followed by the synthesis and investigations of a number of anthraquinone like compounds [55-58]. The FDA approval of doxorubicin (**37,** Chart **4**), idarubicin (**38,** Chart **4**), etoposide etc. as anticancer agents led to the development of number of other analogues in order to improve the clinical efficacy and to overcome the problem of drug resistance [59, 60].

The major drawback of anthracendiones mentioned above including mitoxantrone is their cardiac toxicity which might be due to the reduction of *C*-10 carbonyl group and that in turn helps in the generation of oxygen free radicals. As an alternative, certain naturally occurring as well as synthesized anthrones find common use as antitumor drugs. Aloe, a widely used folk medicine for centuries, contains two diastereomeric anthrones: Aloin A and Aloin B (Fig. (**7**)) as the principle components [61] while a number of their analogues have been investigated for anti-psoriatic and DNA-Topo II inhibition properties.

5. LACTATE DEHYDROGENASE (LDH)

One of the metabolic differences between the cancer cells and the normal cells is the altered metabolism process of the



Mitoxanttrone (**36**)

Average GI_{50} over 59 human tumor cell lines 0.0020 µM

Doxorubicin (**37**)

0.0663 µM

Idarubicin (**38**)

0.038 µM

Chart 4. Inhibitors of Topo II.

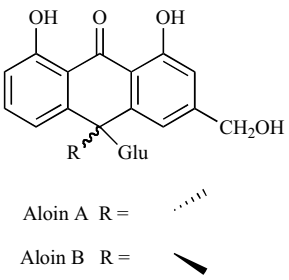


Fig. (7). Anthrones obtained from the leaf exudates of Aloe sp.

former whereby they produce most of the ATP by the conversion of glucose to lactate in contrast to the normal cells which produce ATP from glucose by oxidation-phosphorylation process [62]. The increased glucose uptake could protect the cancer cells from apoptosis as well as oxidative stress [63, 64]. Enzyme LDH, catalyzing the conversion of pyruvate to lactate in the glycolytic pathway of metabolism (Scheme **4**), gets up regulated [65-67] in the cancer cells. During the metabolic phase, the pyruvate (**39**), through its carboxyl group, forms a salt bridge with R171 residue (**40**) of LDH and the electrophilicity of its carbonyl carbon gets increased due to H- bonding with H195 (**41**) and R109 (Fig. (**8**)). The NADH (**42**) present at a distance of 3.6 Å from the carbonyl carbon of pyruvic acid (Fig. (**8**)) helps in the reduction of pyruvate to lactate (**43**) (Scheme **4**).

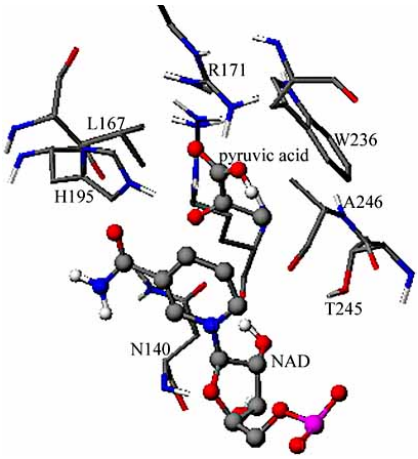
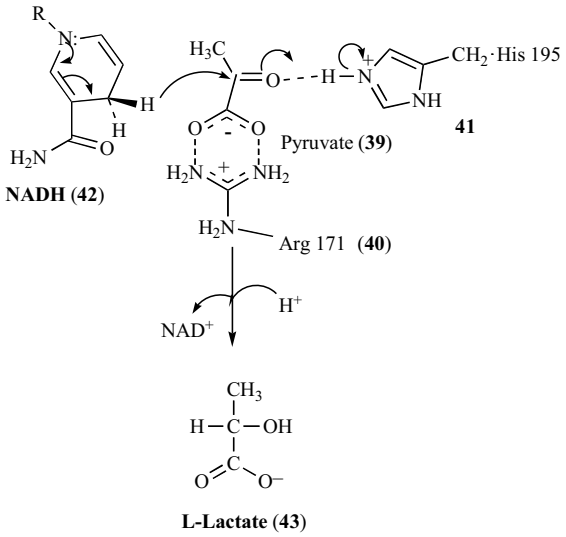


Fig. (8). Active site aminoacid residues of LDH with pyruvate and NAD^+(pdb ID 2FM3). Hydrogens have been omitted for clarity.

Lactate Dehydrogenase as Target for Cancer Chemotherapy

In order to avoid apoptosis and oxidative stress, the cancer cells are dependent on anaerobic type of glycolytic pathway resulting in the production of ATP by the conversion of glucose to lactate. In order to minimize the generation of ATP in the cancer cells, it is desirable to block the glucose to lactate pathway of glycolysis where the enzyme LDH could be made the target. Based upon the anti-malarial activity of gossypol (**44**, Chart **5**), a number of naphthol derivatives have been investigated [68] out of which compounds **45**-**50** (Chart **5**) show appreciable inhibition of LDH. An oxa-spiro compound has also been reported to be a highly potent inhibitor of LDH [69].



Scheme 4. Mechanism of conversion of pyruvate to lactate catalyzed by LDH. A hydride is the nucleophile that reacts with the carbonyl group of pyruvate.

6. ARACHIDONIC ACID METABOLISM AND CANCER: ROLE OF COX-2 IN CANCER PROPAGATION

The prostaglandins produced from arachidonic acid by the mediacy of enzymes like phospholipases (PLA2), lipoxygenases (LOX), cyclooxygenases (COX-1, COX-2) regulate various pathophysiological processes such as inflammatory reaction, gastro-intestinal cytoprotection and ulceration, hemostasis and thrombosis as well as renal haemodynamics [70]. However, the over expression of any one of these enzymes leads to the rapid formation of prostaglandins causing certain unwanted effects like inflammation, psoriasis etc. A high level of COX-2 has been observed in cancerous cells [71, 72]. The peroxidase part of COX, due to the formation of radicals, might be responsible for initiating tumor growth by converting procarcinogens to carcinogens but the substantial decrease in tumor growth after administering celecoxib (**51**, Chart **6**) (COX-2 inhibitor) indicates the role of COX-2 in tumor promotion rather than in tumor initiation [73]. Another role of COX-2 in the cancer cells is to activate epidermal growth factor receptor (EGFR) [74]. A detailed description of the role of COX-2 in promoting cancer has been given in recent reviews [75, 76].

During the metabolic phase of arachidonic acid, COX-2 holds arachidonic acid through a salt bridge formation between the carboxyl group of arachidonic acid and the guanidine moiety of R120 of COX-2 (Fig. (**9**)) [77, 78].

In order to block the catalytic activity of COX-2, the inhibitor must be capable of interacting with aminoacid residues in the active site of the enzyme along with the interactions with guanidine moiety of R120, consequently avoiding the contact of arachidonic acid and COX-2 during the turn over phase of the enzyme. The crystal structure of COX-2 active site with SC-558 (**53**, Chart **6**) (an inhibitor of COX-2) has been shown in (Fig. (**10**)) in which the guanidine moiety of R120 is blocked by the substituent present at C-3 of pyrazole.

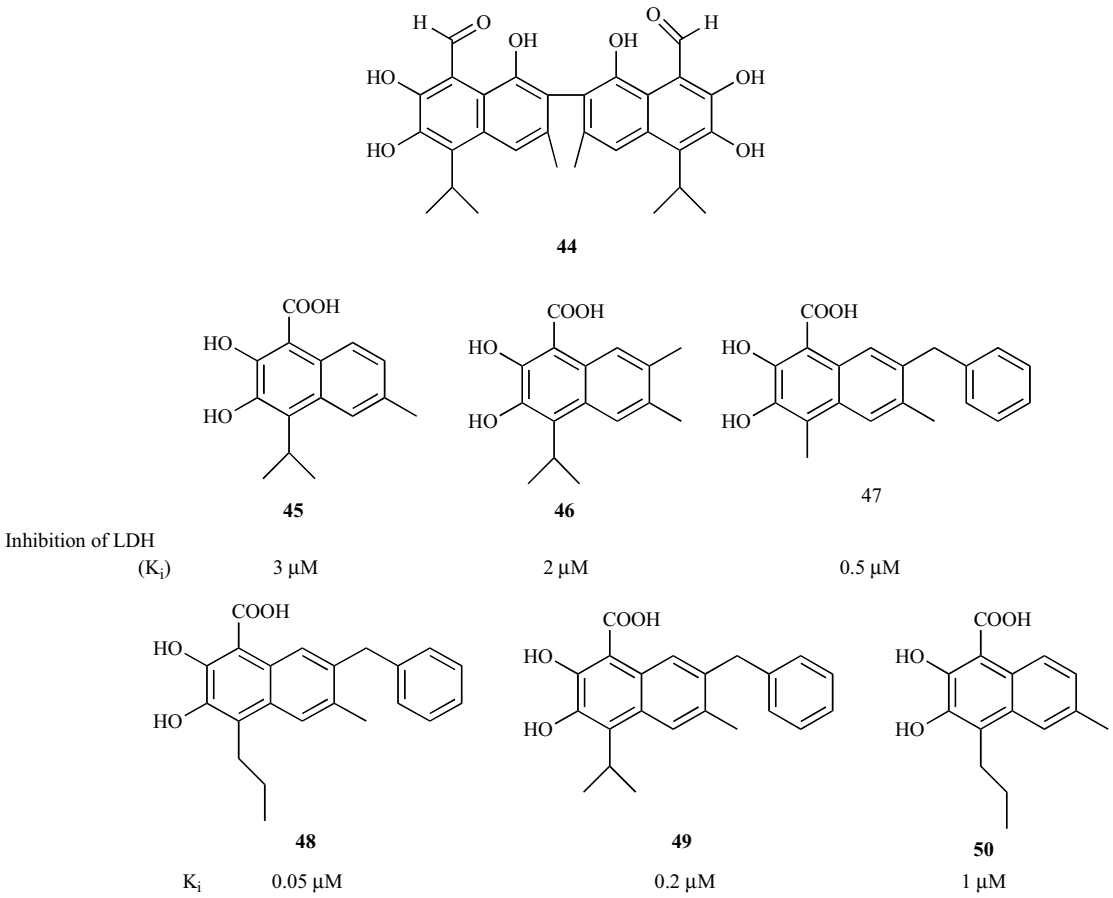


Chart 5. Inhibitors of LDH.

COX-2 Inhibitors in Combination with Anticancer Drugs

Once the role of COX-2 has been established in promoting the tumor growth, the selective COX-2 inhibitors have been tried in combination with anticancer agents for minimizing the propagation of cancer [80]. Some highly potent

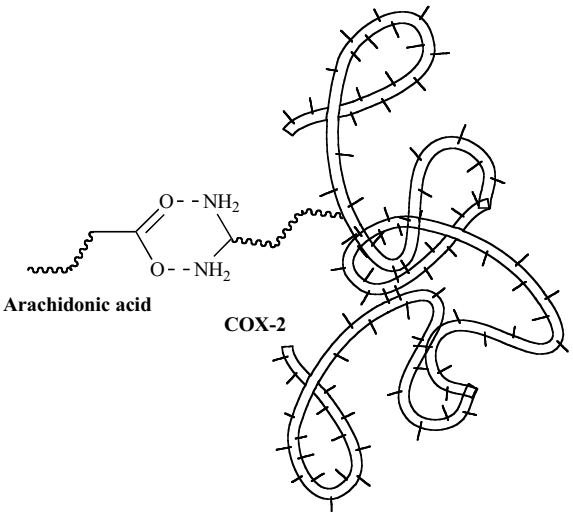


Fig. (9). A schematic representation of binding of R120 residue of COX-2 with carboxyl group of arachidonic acid during the turn over phase.

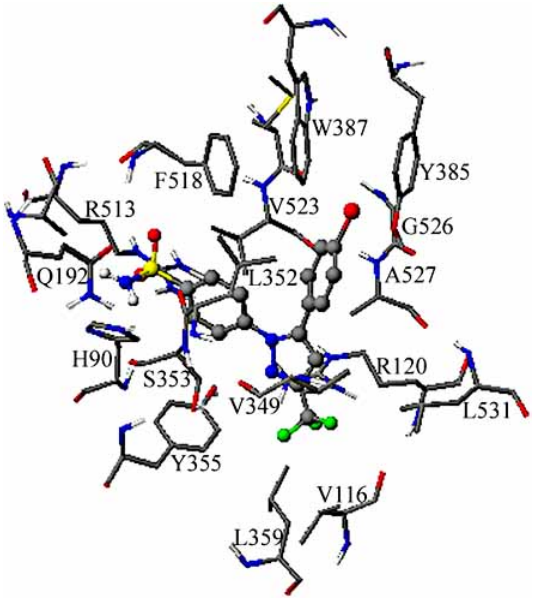
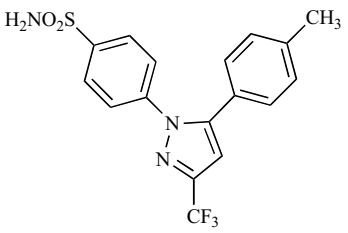
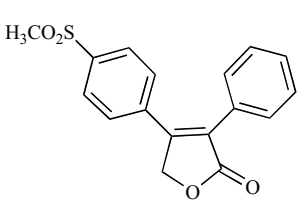


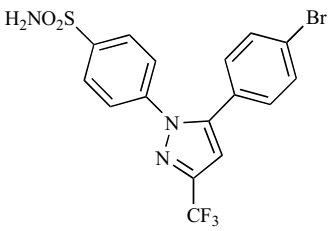
Fig. (10). Active site residues of COX-2 with SC-558 (a selective inhibitor of COX-2) (pdb ID 6COX [79]). Hydrogens are suppressed for clarity.



Celecoxib (**51**)



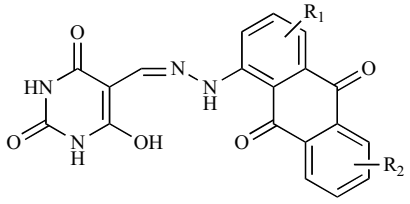
Rofecoxib (**52**)



SC-558 (**53**)

Anti-proliferation activity		
LNCaP cell line	50.4 μM	0 % inhibition
PC 3 cell line	47.0 μM	15 % inhibition

Chart 6. Inhibitors of COX-2 showing anti-proliferative activities.



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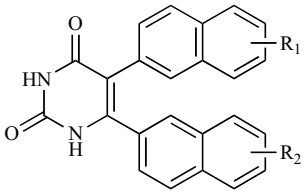
May target TS, TP, RR, Topo II



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May target TS, TP, LDH, COX-2

Chart 7. Proposed design of multiple targeting molecules.

COX-2 inhibitors have been shown in Chart **6**. The detailed descriptions of COX-2 inhibitors which are available in the literature [81-83] will be beyond the limits of this review. However, the basic problem with the existing COX-2 inhibitors is their cardiac toxicity; withdrawal of rofecoxib (52, Chart **6**) has created doubts for others also and necessitated the search for more potent and safe COX-2 inhibitors.

CONCLUDING REMARKS

This overview of the mechanism of action of the key enzymes involved in the cell multiplication (propagation of cancer) and those closely associated with the propagation ofcancer assesses the complexity of the disease. The effective chemotherapy of cancer demands the inhibition of more than one enzyme at a time which requires either the use of combination of drugs or a "magic bullet" capable of targeting more than one enzyme. We hope that the collective discussion of the key enzymes presented in this review could be helpful in designing the new molecules capable of delivering highly efficacious anti-cancer activities. Combining the structural features of the inhibitors (Charts **1-6**) of the enzymes discussed in this review, some multiple target directed molecules (**54**, **55**; Chart **7**) could be developed. Such type of drugs will be more safe, effective and economical.

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