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(Review)

Digging into biochemical and signal transduction of old drugs: Xanthines and their association to cancer

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ABSTRACT

The arena of anticancer drug research has identified xanthine derivatives as intriguing molecules with diverse antimutagenic capabilities against a variety of cancer disorders. Xanthines are purine bases, which are known to act as non-selective and multitargeting agents of several cellular proteins. Natural xanthine analogues were reported to inhibit adenosine and phosphodiesterase subfamilies. Additionally, the effect toward several biochemical and signal transduction changes were studied to observe the efficiency of xanthine derivatives as anticancer agents and/or adjuvants. Several xanthine-based compounds were synthesized and introduced to obtain more selective and potent agents, which are demonstrated as research tools and possible therapeutic agents. The present minireview article illustrates the potential searching for efficacious rule of xanthine derivatives regarding several malignancies covering leukemia, melanoma, colon, CNS, ovarian, renal, prostate, non-small cell lung carcinoma and breast cancers. Further, an attempt to explain the biochemical effect of xanthines toward the complex signaling network of tumor cells was highlighted.

Keywords

Xanthines, purine, anticancer, biomedical modulators, chemotherapy adjuvants

1. Introduction

The increasing complexity of cancer diseases prompted an increase in the development of diverse anticancer agents with varied modes of action. Regarding the biochemical changes of tumor circumferences, the extensive available data of existed drugs represent a pivotal base for the design of novel promising candidates. Xanthines are purine bases, which are known to act as non-selective and multitargeting agents of several cellular proteins [1]. Some xanthines play a role as bronchodilators [2], diuretics [3], natriuretic [4], analgesic adjuvants [5] lipolytic agents [6], cognition enhancers [7], and for treatment of cerebral ischemia [8], Parkinson's disease [9], renal failure [10]. Furthermore, xanthines were studied for their activity as cancer chemotherapy adjuvants [11, 12]. Numbers of xanthine analogs have been synthesized and introduced to obtain more selective and potent agents for use as research tools and as possible therapeutic agents [12]. The adopted substitutions (methyl or alkyl) at several positions and chemical spaces around the xanthine motif played a major involvement in the tumor microenvironment (**Figure 1**).

2. Antitumor activity of methylxanthine derivatives

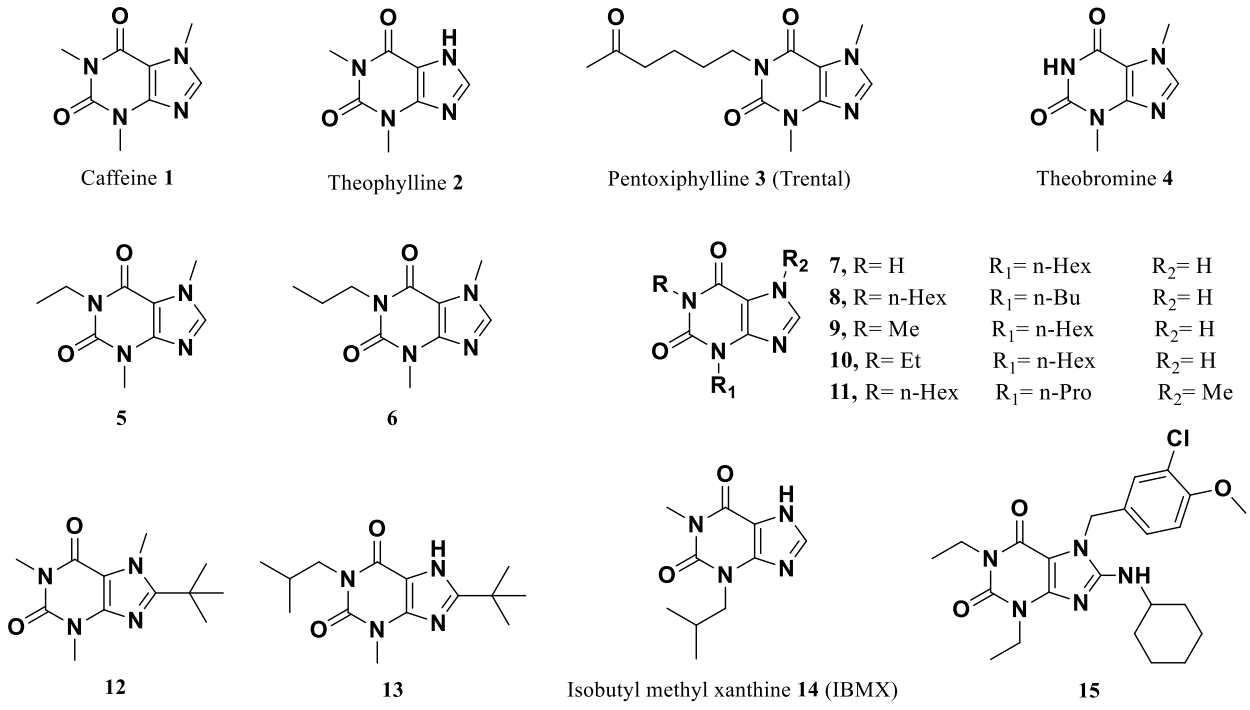

Figure 1. Representation of xanthine derivatives chemical structures.

Caffeine 1 is a methylxanthine with several biological actions. Caffeine regulates both innate and adaptive immunological responses by inhibiting cAMP-phosphodiesterase, according to a number of in vitro and in vivo investigations. Another set of effects was mediated by caffeine via its inhibitory action on adenosine receptors, which could have a direct impact on human tumour neovascularization. Numerous studies have proved that caffeine affects the antitumor activity of DNA-intercalating drugs [13]. During the last decades, there have been extensive researches into the different mechanisms that are responsible for the previous actions of caffeine [14, 15].

Theophylline **2** is an old drug with narrow therapeutic index that has been used in bronchial asthma and COPD [16]. However, as a research tool, theophylline showed inhibition of the Bcl-2 oncogene expression, and an increased expression of c-myc (regulator gene) in chronic lymphocytic leukemia and lymphoma cells. Further, no effect on the oncogene expression and rate of apoptosis were demonstrable in the control normal B lymphocytes [17, 18].

Lentini *et. al.* [19] demonstrated that caffeine **1** and theophylline **2** possess the capacity to inhibit not only cell proliferation, but also the metastatic behavior of melanoma cancer cells. Pentoxifylline **3** (Trental) is a hemorheological drug that is used in the treatment of peripheral vascular disease and other conditions with insufficient regional microcirculation [20-22]. Pentoxifylline 3 works primarily by enhancing erythrocyte flexibility, lowering blood viscosity and decreasing the risk of platelet aggregation and thrombus formation [23]. Further, it prevents shunt thrombosis formation in dialysis patients [24]. Moreover, pentoxiphylline **3** is an oxohexyl-substituted analogue of theobromine **4**, and it has gained wide clinical use for the treatment of intermittent claudication [25, 26]. Caffeine **1** and pentoxifylline **3** inhibited the antigen-elicited proliferation of lymphocytes at concentration 100–300 mM [27]. Similarly, a variety of xanthine derivatives were found to block the antigen-induced activation of tumor mast cell line, apparently by inhibiting the antigen-binding to immunoglobulin E [28]. The topical application of caffeine **1** was reported to inhibit the formation of UVB-induced skin tumors in mice, where, the induction of apoptosis was suggested as one of the responsible mechanisms [29]. Studies showed that various concentrations of caffeine **1** induce apoptosis in several cell lines, including 10 mM in human neuroblastoma cells [30], 4 mM in human pancreatic adenocarcinoma cells [31], 5 mM in human A549 lung adenocarcinoma cells [32], and 450 μM

in mouse epidermal JB6 Cl 41 cells. The treatment with caffeine **1** for 24 h was demonstrated to induce apoptosis in JB6 cells after 72 h of serum starvation [33]. Caffeine **1** (5 mM) has been reported to induce TP53-independent G1 phase arrest in a human lung adenocarcinoma cell line [32].

Hashimoto *et. al.* [34] showed that caffeine **1** (5 mM) seems to cause G0/G1 phase arrest through inhibition of CDK2 activation, which is a key kinase in the G1 to S transition. Caffeine **1** at 1 mM inhibited cell proliferation (75%) in response to fetal bovine serum. The pretreatment with caffeine **1** was reported to significantly inhibit the activation of cyclin D1-CDK4 in a dose dependent manner. Thus, it was suggested that not only the induction of apoptosis, but also the inhibition of proliferation in the G_0 (quiescent) cells could explain the anticarcinogenic effect of caffeine **1**.

3. Methylxanthine derivatives as cancer chemotherapy adjuvants

Several studies were reported to provide better understanding toward the mechanism whereby methylxanthines enhance the lethality of alkylating agents, DNA-intercalating agent and radiation in human tumor cells. According to Fingert *et. al.* [35], an enhanced cytotoxicity of alkylating agents was observed when T24 human bladder tumor cells in culture were exposed to nontoxic concentrations of methylxanthines such as caffeine **1** or pentoxifylline **3**. The tumor cell lethality of alkylating agents such as nitrogen mustard and thiotepa was increased up to 10-folds by using caffeine **1** or pentoxifylline **3** (1 mM) during the first cell cycle (16-24 h). Interestingly, cycloheximide, a protein synthesis inhibitor, abolished the enhanced lethality produced by methylxanthine. This finding suggested the role of methylxanthine in mediating particular cellular proteins that are responsible for enhancing the chemotherapeutic activity of anticancer agents. These methylxanthines produced no significant cell lethality in control plates without thiotepa [35].

Moreover, the extension of G2 phase of the cell cycle has been generally observed in many normal or malignant cells exposed to radiation, alkylating agents, or other antineoplastic drugs [36-38]. According to this model, methylxanthines do not necessarily hinder biological DNA repair processes per se; rather, they act by shortening the time available for repair, presumably through a protein that is affected directly or indirectly by DNA damage and controls the transition from G2 to mitosis [36, 39]. In BHK cells, methylxanthines allowed G2-delayed

cells to reach mitosis without finishing the repair process. Consequently, they resulted in shattered chromosomes, nuclear fragmentation, and cell death [40]. *In vivo* treatment of combination of thiotepa and pentoxiphylline **3** showed greater antitumor effect against human cancer cells compared to thiotepa alone [41]. Additionally, pentoxiphylline **3** enhanced the systemic therapy of human cancer xenografts, and acted selectively to decrease the recovery capacity of cancer versus normal cells. These results demonstrated potentiality for applications in clinical therapy of metastatic disease.

In a study of biochemical modulators, caffeine **1** showed a specific strong enhancement of the cytotoxic effect of cisplatin toward tumor cells [42]. It was found that caffeine **1** inhibited adriamycin (doxorubicin) efflux from tumor cells. Consequently, caffeine **1** enhanced the antitumor effect of adriamycin by inducing specific increase of drug concentration in tumor cells [43]. This effect has been clearly observed both *in vivo* and *in vitro*, and could be aspect of mechanisms whereby caffeine **1** enhances the antitumor activity of adriamycin. The effect of the methylxanthine derivatives on adriamycin induced changes in tumor weight, where the combination of both of them enhanced by 2.1-folds the efficacy of adriamycin. Additionally, combination of both pentoxiphylline **3** and theobromine **4** with adriamycin have an increased potency by 3.1-folds and 3.2-folds, respectively [44].

Similarly, theophylline **2**, pentoxifylline **3** and theobromine **4** inhibited adriamycin efflux in Ehrlich ascites carcinoma cells and P388 leukemic cells. Noteworthy, the previous findings were selective only to tumor tissue, without any effect on normal healthy cells. However, the study also clarified that inhibition of chemotherapy efflux from tumor cells alone is not sufficient to explain the enhancement in antitumor activity [44-47].

Iliakis *et. al.* [48] reported that caffeine **1** seems to inhibit the effect of DNA repair, and Tsuchiya *et. al.* [42, 49] suggested that this agent is a potential enhancer of antitumor agents. The antagonism of mitotic arrest in the G2 checkpoint by caffeine **1** or a less toxic xanthine analogue suggests that they could be useful as an adjuvant for cancer chemotherapy [50, 51]. Cancer cells that lack P53 cannot utilize the G1 checkpoint and, thus, only the G2 checkpoint is available for DNA repair. Pentoxifylline **3** has the same effect, as caffeine [52, 53], and the basis for the ability of methylxanthines to prevent G2 arrest is unknown. It has been proposed that they activate the regulatory P34 cdc protein kinase, which is involved in induction of cancer cells apoptosis [54].

Different xanthine derivatives have been assessed for their effect toward P53-defective tumor cells relative to prevention of G2 arrest and thus DNA repair. Caffeine **1** and nine other xanthine derivatives showed activity with IC_{50} values less than 2 µM. Among them, compounds **3**, **5** and **6** were the most active [12, 55]. Particularly, compound **3** was found to inhibit DNA biosynthesis in combination with vincristine, *in vitro* [56]. Moreover, in clinical trials, caffeine **1** potentiated the effect of chemotherapy regarding induction of a high-rate complete response in patients with osteosarcoma, as well as a high rate of good local response in patients with high-grade soft tissue sarcomas [57-59]. Furthermore, it produced a good response in metastatic carcinoma and lymphoma [60].

4. Methylxanthine derivatives as radiosensitizer agents

Several methylxanthine-derived drugs, including caffeine **1**, theophylline **2** and pentoxifylline **3**, were found to sensitize cancer cells prior radiation at low millimolar concentrations [50, 54, 61, 62]. Although the exact mechanism remains unclear, the radio-sensitizing effect of these drugs could be related to inhibition of one or more components of the DNA damage-response (DDR) and cell cycle checkpoint machinery [63]. Other studies were carried out to determine the effect of methylxanthines as enhancers of the cellular radiation response. Thus, Kim *et. al.* [64] suggested that methylxanthines could be considered as a potential adjuvant agents for clinical radiotherapy. Kelland and Steel [65] found that caffeine **1** modified the initial slope of the acute survival curve in a human cervical carcinoma cell line. The result was a reduced survival. Wolloch *et. al.* [66] got similar results in an ovarian cell line.

The precise mechanism is still under debate, but reports have shown that the radio-sensitizing effect of caffeine **1** is associated with the disruption of multiple DDR cell cycle checkpoints and DNA repair [67-69]. Undoubtedly, the effect of caffeine is to a great extent cell-type dependent. Sarkaria *et. al.* [63] also stated that the radio-sensitizing effects of caffeine **1** are related to the inhibition of ATM (Ataxia–telangiectasia mutated) and ATR (Ataxia–telangiectasia and Rad3 related) protein kinases, which has been proposed as a key mechanism for prevention of arrest in G2 phase. Both proteins ATM and ART are relevant targets for the development of novel anticancer agents.

ATM and ATR act as apical regulators of DNA double strand breaks and replication stress responses, respectively, with overlapping but non-redundant activities [70]. Highly selective small molecule inhibitors of ATM and ATR are currently in preclinical and clinical development. Wherein, preclinical data have provided a strong rationale for clinical testing of

these compounds both in combination with radio- or chemotherapy, and in synthetic lethal approaches to treat tumors with deficiencies in certain DDR components [71].

In general overview of cell cycle checkpoint signaling that are induced by ATM- and ATR-dependent DNA damage, cell cycle progression is halted primarily through their phosphorylation of P53, CHK1 (checkpoint kinase1) and CHK2 (checkpoint kinase 2). Subsequently, G1/S cell cycle arrest is primarily mediated through a P53-dependent increase in P21, a cyclin-dependent kinase inhibitor. Key targets of CHK1/2 include the cdc25 (cell division cycle 25) phosphatases that control the activity of specific cyclin–CDK complexes, which in turn regulate progression through S-phase and entry into mitosis. Phosphorylation of cdc25 phosphatases by CHK1 or CHK2 inhibits their activity ensuring that CDK–cyclin complexes are not activated. This leads to cell cycle arrest either in S-phase or at the G2/M boundary [71]. In this regard, it was revealed that caffeine **1** suppresses ATM/P53 signaling pathway in gamma-irradiated human T-lymphocyte leukemic MOLT-4 cells. P21 up-regulation was repressed as a result of inhibited P53 phosphorylation on Ser15 and, interestingly, ATM-independent Ser392 residue phosphorylation, implying that caffeine **1** may have another physiological target (protein kinase) [72]. Notably, they observed a significant P53-independent decrease in anti-apoptotic Mcl-1 (myeloid cell leukemia-1) expression. Thus, caffeine **1** is suggested to increase the G2/M block override, diminish repair of gamma-irradiation induced DNA damage. Subsequently, it contributes to induction of apoptosis. Ultimately, caffeine **1** and similar methylxanthines promote the cytotoxic effect of ionizing radiation and provide a platform for the development of anticancer therapeutics known as radio-sensitizers [72].

Interestingly, the therapeutic efficacies of certain DNA-damaging anticancer drugs are causally related to the loss of normal DNA damage checkpoint controls during the process of carcinogenesis [73, 74]. Thus, agents that interfere with checkpoint related proteins may show selectivity for tumor cells bearing intrinsic defects in specific checkpoint pathways. Indeed, this prediction is supported by observation of the P53-deficient cells, which are preferentially sensitized to radiation-induced killing by methylxanthines [54, 61, 62]. Therefore, P53 mutation status may provide a potential strategy for treatment with ATM or ATR inhibitors, but P53 status remains a complex biomarker to interpret [75]. Despite these positive results in various tumor model systems, the clinical use of methylxanthines such as caffeine, theophylline *etc.*, is

severely limited by their neurological and cardiac toxicities [76, 77]. The performed studies in cell culture have predicted that the 0.4-1 mM concentrations are needed to enhance tumor cell lethality, which exceeds the maximally tolerated serum levels by 20-fold, and it override the border of lethal serum level in humans [35, 41, 76-78].

5. Methylxanthine derivatives as antitumor agents

Rogozin *et. al.* [79] investigated the potential chemo-preventive activities of 50 different 1,3,7-trialkylxanthines, which resemble caffeine in their structures but differ in the length of alkyl side chains. Results of this study indicated that various trialkyl-substituted xanthine derivatives inhibited epidermal growth factor (EGF) induced neoplastic transformation of JB6 P+ cells. Wherein, The JB6 P+ cell system is a well-developed model for studying the tumor promotion process and the potential effect of antitumor promoting agents [80-82]. Xanthine derivatives **7-11** (**Figure 1**) were substantially more effective than caffeine **1** or theophylline **2** in suppressing AP-1 (activator protein 1) transactivation in JB6 P+ cells, which may account for their antitumor-promoting activity. Interestingly, the inhibitory activity toward cell transformation increases proportional to the increased number of carbons in the alkyl chain at R and R_1. The most effective xanthines for inhibiting cell transformation are compounds **8** and **11**, which involved a hexyl group at R, in addition to compound **10** that has a hexyl group at R_1 [79]. Although the study used xanthine derivatives with a small variation in the number of carbons at R_2 (zero to four), whereas the number of carbons at R or R_1 varied from zero to six, it gives a glimpse about enhancing the antitumor activities of xanthine analogues. In another study aimed to evaluate the effect of 8-alkyl xanthine derivatives as potential cytotoxic agents, compound **12** showed anticancer activity against prostate cancer cell line DU145 with growth inhibition 64 % at 1.0 mM concentration. While compound **13** showed growth inhibition 42 % on the same cell line at the same dose level [83].

6. Phosphodiesterase inhibition and xanthine derivatives

Methylxanthines, such as the previously mentioned along with isobutyl methylxanthine (IBMX, **14**), can inhibit cell proliferation presumably through inhibition of Phosphodiesterase (PDE), resulting in elevation of cAMP [12, 84, 85]. The basic role of PDE family can be simplified by demonstration of its roots (**Figure 2**).

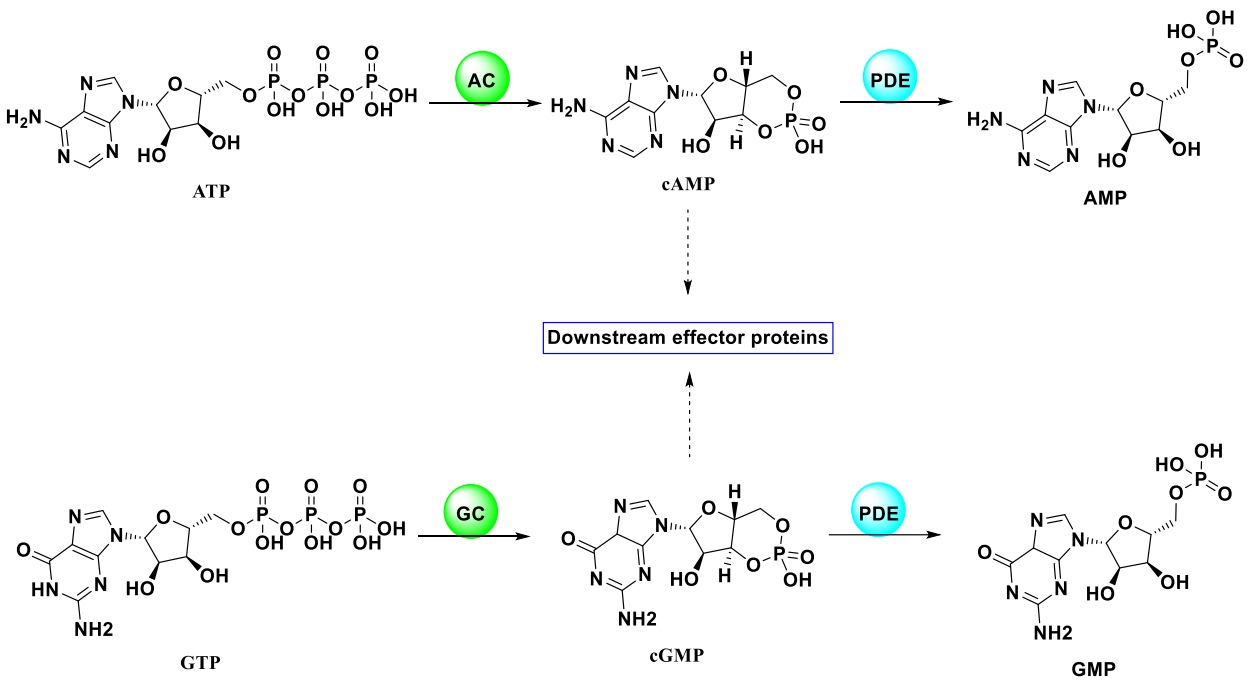

Figure 2. Overview of PDE involvement in the regulation of second messengers cAMP and cGMP.

Adenosine triphosphate (ATP) is a potent and long-lasting inhibitor of human tumor cell development, with the impact appearing to require permeabilization of the tumor cell membrane *via* Ca^{+2} and Na^{+} channel activation. [86]. ATP has a wide range of effects, including cytostatic, cytotoxic, anti-cachexic, tumor blood flow modulation, and enhancement of superoxide production. The duration of cytotoxic effect of ATP appears to involve in the ability of nucleotide to facilitate its own localized release [86, 87], and it could be mechanistically related to P_2X_7 receptor-mediated apoptosis [88]. *In vitro*, ATP can transform leukemia cells into white blood cells and has been proposed as an adjunct therapy to retinoic acid in the treatment of breast and prostate cancer. A phase II clinical trial of ATP for the treatment of small cell lung cancer showed increasing weight gain, improving performance status and quality of patient life [89]. Cyclic adenosine monophosphate (cAMP) is important in many biological processes. It is derived from ATP and used for intracellular signal transduction in many different organisms. The main target of cAMP action in cell is cAMP-dependent protein kinase, which exists as two different isozymes, designated as type 1 (PKA-1) and type 2 (PKA-2). PKA-1 acts as a positive growth regulator, while PKA-2 inhibits cell division [90].

cAMP is a positive intracellular signal for cell proliferation in many differentiated cells. However, in many tumor cells, it is a negative messenger for proliferation, showing a much lower basal level than in normal cells [91]. In malignant tissues, concentration of cAMP is lower in transformed cells by oncogenic viruses than in untransformed cells, and is lower in certain tumors grown than in the corresponding normal tissue [92]. The growth of four tumorigenic cell lines (Flamnion, HEp-2, HeLa line 229 and strain L; NCTC clone 929) were inhibited (70-89 %) by cAMP (0.3 mM), whereas a non-malignant cell line (WI-38) was affected only slightly (13 %) [93]. Thus, the action of cAMP shows sort of selectivity toward tumor cells. Exogenous cAMP or agents that increase the intracellular concentration of this cyclic nucleotide (i.e., PDE inhibitors) decrease the rate of tumor growth and induce morphological and biochemical differentiation [92]. High intracellular levels of cAMP can effectively kill the cancer cells *in vitro* but substances elevating cAMP (cAMP analogues) are not recommended to be used as anticancer drugs as they are highly cytotoxic [94].

Thus, PDEs are connected to a wide range of physiological functions and continue to be a major target for drug development. Phosphodiesterase hydrolyzes the second messenger cAMP, as mentioned previously, and cGMP (cyclic guanosine monophosphate, **Figure 2**) that are formed by adenylate cyclase (AC) and guanylate cyclase (GC), respectively. Both are involved in a variety of cellular responses and extracellular agents such as hormones and neurotransmitters control [95, 96]. There are at least, 11 families of PDEs, some of which (PDE4, 7, and 8) are specific for cAMP and others (PDE5, 6, and 9) for cGMP. Additional family members (PDE1-3, 10, and 11) have a dual specificity. Additionally, PDE subtypes that occur within the same isoform, such as the five subtypes PDE4A, PDE4B, PDE4D2, PDE4D3, and PDE4D5 for PDE4, induce further complexity to the nature of this pathway [95, 96]. The induction of apoptosis by PDE inhibitors is selective toward malignant cells without effecting normal ones. Results of an *in vitro* study have revealed that the proportional apoptosis of pretreated malignant B-Cell with methylxanthine derivative (72 h) increases by 87 % compared to 16 % in normal B-cells [97, 98]. The selective toxicity of PDE inhibitors towards malignant cells is further confirmed by Sarfati *et. al.* [99], who showed that the normal B cells isolated from control donors were totally resistant to PDE-induced apoptosis, while it was selective for the leukemic B cells. Furthermore, Inhibitors of PDE7 have been suggested to reveal a broad

application in the treatment of lymphoid malignancies, in addition to T cell-dependent diseases such as asthma, rheumatoid arthritis [100, 101]

Caffeine **1** and theophylline **2** are non-selective and relatively weak PDE inhibitors with IC_{50} values greater than 500 µM and 100 µM, respectively. Although IBMX **14** showed more potent activity against PDE (IC_{50} = 10 µM), it is relatively non-selective [102]. IBMX **14** at non-cytotoxic concentrations (10^{-4} M) inhibits the tumor colony formation of cloned line LL1 (Lewis lung carcinoma) cells. In an animal study using C57BL/6J mice, the administration of IBMX **14** resulted in a dose-dependent decrease (2- to 10-folds) in formation of lung nodules and 10-fold reduction in the number and size of lung metastases [103]. Moreover, PDE5 inhibitors are suggested to be multitargeting agents that have promising results in the treatment of several tumors. However, the distinct role of PDE5 inhibitors against cancer is not fully understood. The increased apoptosis in different tumor cell types following treatment with PDE5 inhibitors was reported [104]. Mediating caspase dependent apoptosis, and therefore cell growth arrest are the possible mechanisms for the anticancer effect *via* PDE5 inhibition, which could be linked to the concomitant increase in modulation of downstream pathways through the increased cGMP-PKG levels [104]. Interestingly, PDE5 inhibitors alter the tumor microenvironment by augmenting endogenous antitumor immunity *via* reduction of myeloid derived suppressor cell function [105]. It was suggested that PDE5 inhibitors could interfere with the efflux functions of ABC transporters.

Consequently, PDE inhibitors sensitize cancer cells toward cytotoxic agents that are substrates to ABC transporters [106-108]. These novel functions of PDE5 inhibitors could explain the effect sildenafil and vardenafil, the FDA approved PDE5 inhibitors, on brain tumor models. Wherein, doxorubicin and Herceptin (trastuzumab, monoclonal antibody) were transported efficiently across the blood brain tumor barrier after the addition of sildenafil and vardenafil, respectively [109]. Furthermore, the combination chemotherapy with PDE5 inhibitors was found to produce reactive oxygen species that led to apoptosis, which proves the beneficial treatment of broad range of cancers [110-112]. Interestingly, compound **15** represents a potent and selective ethylxanthine based PDE5 inhibitor (PDE5 IC_{50} = 0.6 nM, PDE6/PDE5 = 101) [113] compared to sildenafil IC_{50} value of 3.6 nM (platelets) and 3.0 nM (corpus cavernosum) [114].

7. Recent methylxanthine anticancer candidates

Despite the fact that no methylxanthine-based medications have received clinical approval for the treatment of cancer disorders to date, the exceptional biochemical manifestations of these chemicals stimulate ongoing research to develop more effective and selective anticancer prospects. [115]. **Figure 3** illustrates several methylxanthine based candidates that show promising anticancer activity.

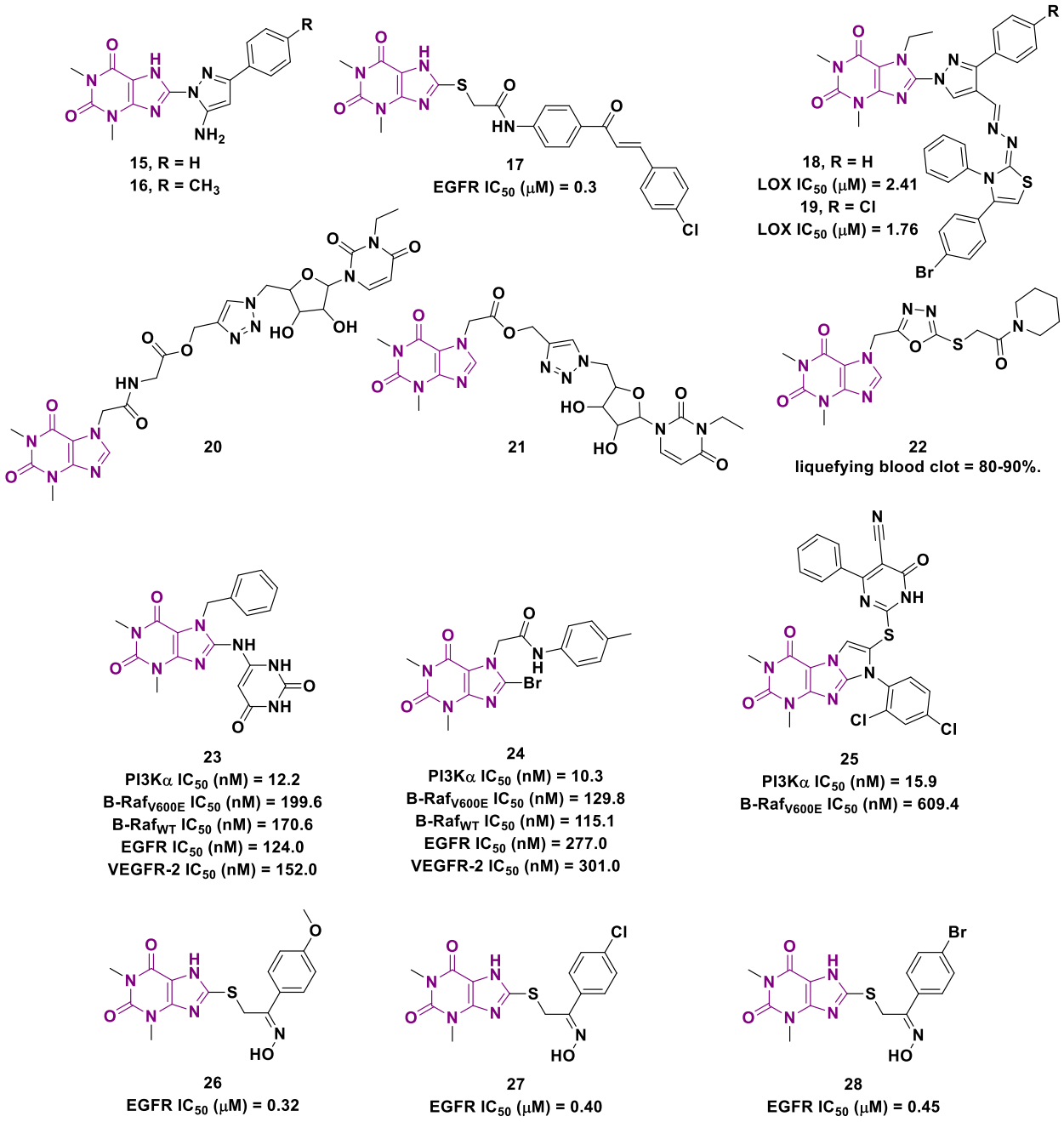

Figure 3. Representative chemical structure of recent methylxanthine derivatives, with illustration of their available protein activity.

Among a small library of methylxanthines featuring thiazolidine moiety at C-8, compounds **15** and **16** (**Figure 3**) showed potent anticancer activity in NCI-60 cell line screening against colon HCT-15 cancer cell line with GI_{50} of 0.47 and 0.8 μM, respectively. Compound **15** retained further GI_{50} of 0.78 μM against renal CAKI-1 cancer cell line, and compound **16** achieved highly potent anticancer activity against leukemia K-562 cell line with GI_{50} value of less than 0.01 μM [116]. Compound **17** was discovered among novel dimethylxanthine derivatives that were hybridized with chalcones, which shows a potent anticancer activity against pancreas Panc-1, breast MCF-7, colon HT-29 and epithelial A-549 cancer cell lines (IC_{50} = 1.7-1.7 μM) [117]. Afifi *et. al.* synthesized a series of xanthine derivatives that were hybridized with variety of heterocyclic rings. Although all the synthesized derivatives retained moderate anticancer activity against lung A549, colon Caco-2, prostate PC3, breast MCF-7, and liver HepG-2 cell lines, they revealed interesting *in vitro* antioxidant activity. Compounds **18** and **19** are representative examples of these derivatives. The former displayed twice the activity of ascorbic acid (reference standard) as NO scavenger. Compound **19** showed an enhanced activity over the reference standard against 15-lipoxygenase enzyme (LOX) with IC_{50} of 1.76 *vs.* 3.98 μM of Zileuton (**Figure 3**) [118]. Methylxanthine as a scaffold was explored by introducing a large substituted 1,2,3-triazole featuring nucleoside moieties at N-7, which unfolds an efficient antiproliferative activity of compounds **20** and **21** (**Figure 3**) against several cell lines; lung A549, colon HT-29, breast MCF-7 and melanoma A375, with IC_{50} of 1.89-5.81 μM [119]. Among methylxanthine-oxadiazole hybridized derivatives, compound **22** revealed thrombolytic activity, in addition to anticancer activity (**Figure 3**) [120].

The further activities that are exerted by methylxanthine derivatives suggest the scaffold suitability to achieve multiprotein targeting with efficient poly pharmacology profile [115, 121]. Furthermore, different design approaches were utilized to gain much more potencies against wide spectrum of malignancies. Compounds **23**, **24** and **25** were discovered among a series of methylxanthines that were designed using an advanced structure- and ligand-based approaches to achieve multi-kinase targeting (**Figure 3**). Their anticancer results revealed mean GI of 40.92, 79.32 and 53.45 %, respectively, in NCI-60 screening program that involves 60 different tumor

cell lines belonging to nine subpanels. Compounds **23** and **24** exerted a promising *in vitro* activity against several oncoproteins such as PI3K, B-Raf, EGFR, and VEGFR-2, which accounts for their potent anticancer activities. compound **23** showed NSCLC HOP-92, CNS SNB-75, renal A498 GI_{50} of 7.51, 12.5 and 7.97 µM, respectively. Whilst, compound **24** surpassed the FDA approved sorafenib activity regarding several cell lines such as CNS SNB-75 (GI_{50} = 1.43 *vs.* 2.96 µM of sorafenib), Ovarian OVCAR-3, OVCAR-4 and SK-OV-3 (GI_{50} = 2.31, 2.1 and 2.15 *vs.* 2.94, 3.51 and 2.57 µM of sorafenib, respectively) and Renal 786-0, A498, TK-10, UO-31 (GI_{50} = 2.32, 2.01, 2.29 and 2.13 *vs.* 3.36, 2.26, 2.37 and 2.57 µM of sorafenib) [122].

Hisham *et. al.* achieved the synthesis of new 1-methyl and 1,3-dimethyl-8-alkylthioxanthine with promising anticancer activity. Among the them, compounds **26-28** showed superior activity than their non-oxime congeners and their 1-methyl derivatives against the human tumor Panc-1, MCF-7, HT-29 and A-549 cell lines (IC_{50} = 0.80-1.80 µM). This activity was found correlating with EGFR inhibition (**Figure 3**).

8. Conclusion

The immense potential of purine in drug discovery of anticancer agent puts xanthine-based derivatives among the scope of investigation in this regard. Although the primary natural xanthines; caffeine, theobromine and theophylline were unable to achieve anti-carcinogenic effect at reasonable phycological concentration, several promising and potent activities were revealed by higher substitutions at positions 7 and/or 8 of xanthine ring. The emerging of focused understanding toward methylxanthine derivatives' basic roles has opened up an exciting opportunity to develop potent anticancer compounds with promising targeting profile. There is urgent need for more clarification regarding the molecular mechanism that is responsible for the molecular action of xanthines toward tumor cells. The available research results are incapable to answer deeper questions regarding the diverse antimutagenic effect against various tumor phenotypes. Ultimately, xanthine scaffold represents a general framework that can be utilized to modulate specific protein targets, and subsequently, could result in more satisfactory treatment of several diseases including cancer.

- **Conflict of Interest**

  There is no conflict of interest.

References

[1] J.W. Phillis. The pharmacology of purines in the CNS: Interactions with psychoactive agents, in: Purines, Springer, 1985 45-56.
[2] A.L. Harris, Connell, M. J., Ferguson, E. W., Wallace, A. M., Gordon, R. J., Pagani, E. D., and Silver, P. J. Role of low Km cyclic AMP phosphodiesterase inhibition in tracheal relaxation and bronchodilation in the guinea pig, Journal of pharmacology and experimental therapeutics, 251 (1989) 199-206.
[3] H. Osswald, J. Schnermann. Methylxanthines and the kidney, in: Methylxanthines, Springer, 2011 391-412.
[4] V. Zanardo, Dani, C., Trevisanuto, D., Meneghetti, S., Guglielmi, A., Zacchello, G. and Cantarutti, F. Methylxanthines increase renal calcium excretion in preterm infants, Neonatology, 68 (1995) 169-174.
[5] J. Sawynok. Pharmacological rationale for the clinical use of caffeine, Drugs, 49 (1995) 37-50.
[6] U. Schwabe, and Ebert, R. Different effects of lipolytic hormones and phosphodiesterase inhibitors on cyclic 3′, 5′-AMP levels in isolated fat cells, Naunyn-Schmiedeberg's archives of pharmacology, 274 (1972) 287-298.
[7] S.H. Snyder. Adenosine receptors and the actions of methylxanthines, Trends in neurosciences, 4 (1981) 242-244.
[8] V.M. Sciotti, Roche, F.M., Grabb, M.C. and Van Wylen, D.G. Adenosine receptor blockade augments interstitial fluid levels of excitatory amino acids during cerebral ischemia, Journal of cerebral blood flow & metabolism, 12 (1992) 646-655.
[9] S. Ferré, Popoli, P., Gimenez-Llort, L., Rimondini, R., Müller, C.E., Strömberg, I., Ögren, S.O. and Fuxe, K. Adenosine/dopamine interaction: implications for the treatment of Parkinson's disease, Parkinsonism & related disorders, 7 (2001) 235-241.
[10] K.L. Berens, J.D. Langston, K.M. Wasan, D.R. Luke. Influence of pentoxifylline and related analogues in endotoxemic renal failure, Circulatory shock, 34 (1991) 344-348.
[11] A.a.P. Tenzer, M. Potentiation of DNA-damage-induced cytotoxicity by G2checkpoint abrogators, Current medicinal chemistry-anti-cancer agents, 3 (2003) 35-46.
[12] J.W. Daly. Alkylxanthines as research tools, Journal of the autonomic nervous system, 81 (2000) 44-52.
[13] M.a.S. Sabisz, A. Modulation of cellular response to anticancer treatment by caffeine: inhibition of cell cycle checkpoints, DNA repair and more, Current pharmaceutical biotechnology, 9 (2008) 325-336.
[14] J.W. Daly, and Fredholm, B. B. Mechanisms of action of caffeine on the nervous system, Coffee, tea, chocolate, and the brain, 2 (2004) 1-11.
[15] J.W. Daly. Mechanism of action of caffeine, in, Raven Press, New York, 1993.
[16] P.J. Barnes. Theophylline: new perspectives for an old drug, Am. J. Respir. Crit. Care Med., 167 (2003) 813-818.
[17] D. Makower, Malik, U., Novik, Y. and Wiernik, P.H. Therapeutic efficacy of theophylline in chronic lymphocytic leukemia, Medical oncology, 16 (1999) 69-71.
[18] R.a.L. Vassallo, J.J., Theophylline: recent advances in the understanding of its mode of action and uses in clinical practice, in: Mayo clinic proceedings, Elsevier, 1998, 346-354.
[19] A. Lentini, Kleinman, H.K., Mattioli, P., Autuori-Pezzoli, V., Nicoli, L., Pietrini, A., Abbruzzese, A., Cardinali, M. and Beninati, S. Inhibition of melanoma pulmonary metastasis by

methylxanthines due to decreased invasion and proliferation, Melanoma research, 8 (1998) 131-138.
[20] D. Moher, Pham, B., Ausejo, M., Saenz, A., Hood, S. and Barber, G.G. Pharmacological management of intermittent claudication, Drugs, 59 (2000) 1057-1070.
[21] C.P.a.W. amlaska, E.A. Pentoxifylline, Journal of the american academy of dermatology, 30 (1994) 603-621.
[22] A. Ward, and Clissold, S.P. Pentoxifylline, Drugs, 34 (1987) 50-97.
[23] B. Angelkort. Thrombozytenfunktion, plasmatische blutgerinnung und fibrinolyse bei chronisch arterieller verschlusskrankheit, Med welt, 30 (1979) 1239.
[24] A. Radmilović, Borić, Z., Naumović, T., Stamenković, M. and Muśikić, P. Shunt thrombosis prevention in hemodialysis patients—A double-blind, randomized study: Pentoxifylline vs placebo, Angiology, 38 (1987) 499-506.
[25] D.M. Aviado, and Dettelbach, H.R. Pharmacology of pentoxifylline a hemorheologic agent for the treatment of intermittent claudication, Angiology, 35 (1984) 407-417.
[26] H.J. Fingert, Pu, A.T., Chen, Z., Googe, P.B., Alley, M.C. and Pardee, A.B. In vivo and in vitro enhanced antitumor effects by pentoxifylline in human cancer cells treated with thiotepa, Cancer research, 48 (1988) 4375-4381.
[27] L.A. Rosenthal, Dennis D. Taub, Marlena A. Moors, and Kenneth J. Blank. Methylxanthine-induced inhibition of the antigen-and superantigen-specific activation of T and B lymphocytes, Immunopharmacology, 24 (1992) 203-217.
[28] H. Ali, Müller,C. E., John, W. D., and Michal, A. B. Methylxanthines block antigen-induced responses in RBL-2H3 cells independently of adenosine receptors or cyclic AMP: evidence for inhibition of antigen binding to IgE, Journal of pharmacology and experimental therapeutics, 258 (1991) 954-962.
[29] Y.P. Lu, Lou, Y.R., Xie, J.G., Peng, Q.Y., Liao, J., Yang, C.S., Huang, M.T. and Conney, A.H. Topical applications of caffeine or (−)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice, Proceedings of the national academy of sciences, 99 (2002) 12455-12460.
[30] M.H. Jang, Shin, M.C., Kang, I.S., Baik, H.H., Cho, Y.H., Chu, J.P., Kim, E.H. and Kim, C.J. Caffeine induces apoptosis in human neuroblastoma cell line, J Korean Med Sci, 17 (2002) 674-678.
[31] A.A. Beenagururajanna, Yi-Weili, O.A., Vaitkevicius, V.K., and Sarkar, F.H. Molecular effects of taxol and caffeine on pancreatic cancer cells, International journal of molecular medicine, 4 (1999) 501-508.
[32] W. Qi, Qiao, D. and Martinez, J.D. Caffeine induces TP53-independent G1-phase arrest and apoptosis in human lung tumor cells in a dose-dependent manner, Radiation research, 157 (2002) 166-174.
[33] Z. He, Ma, W.Y., Hashimoto, T., Bode, A.M., Yang, C.S. and Dong, Z. Induction of apoptosis by caffeine is mediated by the p53, Bax, and caspase 3 pathways, Cancer research, 63 (2003) 4396-4401.
[34] T. Hashimoto, He, Z., Ma, W.Y., Schmid, P.C., Bode, A.M., Yang, C.S. and Dong, Z. Caffeine inhibits cell proliferation by G0/G1 phase arrest in JB6 cells, Cancer research, 64 (2004) 3344-3349.
[35] H.J. Fingert, Chang, J.D. and Pardee, A.B. Cytotoxic, cell cycle, and chromosomal effects of methylxanthines in human tumor cells treated with alkylating agents, Cancer research, 46 (1986) 2463-2467.

[36] R.A. Tobey. Different drugs arrest cells at a number of distinct stages in G2, Nature, 254 (1975) 245-247.
[37] J. Wennerberg, Alm, P., Biörklund, A., Killander, D., Långström, E. and Tropé, C. Cell cycle perturbations in heterotransplanted squamous-cell carcinoma of the head and neck after Mitomycin C and Cisplatin treatment, International journal of cancer, 33 (1984) 213-222.
[38] L.L. Vindeløv, Hansen, H.H., Gersel, A., Hirsch, F.R. and Nissen, N.I. Treatment of small-cell carcinoma of the lung monitored by sequential flow cytometric DNA analysis, Cancer research, 42 (1982) 2499-2505.
[39] S.K. Das, Lau, C.C. and Pardee, A.B. Abolition by cycloheximide of caffeine-enhanced lethality of alkylating agents in hamster cells, Cancer research, 42 (1982) 4499-4504.
[40] C.C. Lau, A.B. Pardee. Mechanism by which caffeine potentiates lethality of nitrogen mustard, Proceedings of the national academy of sciences, 79 (1982) 2942-2946.
[41] H.J. Fingert, A.T. Pu, Z. Chen, P.B. Googe, M.C. Alley, A.B. Pardee. In vivo and in vitro enhanced antitumor effects by pentoxifylline in human cancer cells treated with thiotepa, Cancer Research, 48 (1988) 4375-4381.
[42] K. Tomita, and Tsuchiya, H. Enhancement of cytocidal and antitumor effect of cisplatin by caffeine in human osteosarcoma, Clinical therapeutics, 11 (1988) 43-52.
[43] Y. Sadzuka, Mochizuki, E. and Takino, Y. Mechanism of caffeine modulation of the antitumor activity of adriamycin, Toxicology letters, 75 (1995) 39-49.
[44] Y. Sadzuka, Iwazaki, A., Miyagishima, A., Nozawa, Y. and Hirota, S. Eflects of Methylxanthine derivatives on adriamycin concentration and antitumor activity, Japanese journal of cancer research, 86 (1995) 594-599.
[45] Y. Sadzuka, Iwazaki, A., Miyagishima, A., Nozawa, Y. and Hirota, S. In vitro enhancement of cis-platinum antitumor activity by caffeine and pentoxifylline in a human ovarian cell line, Gynecologic oncology, 43 (1991) 37-45.
[46] E. Petru, Boike, G. and Sevin, B.U. Potentiation of cisplatin cytotoxicity by methylxanthines in vitro, Journal of cancer research and clinical oncology, 116 (1990) 431-433.
[47] A. Viladkar, Juvekar, A., Chitnis, M. and Advani, S. Amelioration of doxorubicin resistance by pentoxifylline in human chronic myeloid leukemia cells in vitro, Selective cancer therapeutics, 7 (1991) 119-126.
[48] G. Iliakis, Nusse, M., Ganapathi, R., Egner, J. and Yen, A. Differential reduction by caffeine of adriamycin induced cell killing and cell cycle delay in Chinese V79 cell, International Journal of radiation oncology* biology* physics, 12 (1987) 1987-1995.
[49] H. Tsuchiya, Tomita, K., Yasutake, H., Ueda, Y., Tanaka, M. and Sasaki, T. Growth inhibition and differentiation of murine melanoma B16-BL6 cells caused by the combination of cisplatin and caffeine, Japanese journal of cancer research, 80 (1989) 1246-1251.
[50] S.R.R. Musk, and Steel, G.G. Override of the radiation-induced mitotic block in human tumour cells by methylxanthines and its relationship to the potentiation of cytotoxicity, International journal of radiation biology, 57 (1990) 1105-1112.
[51] R. Rowley, Zorch, M. and Leeper, D.B. Effect of caffeine on radiation-induced mitotic delay: delayed expression of G2 arrest, Radiation research, 97 (1984) 178-185.
[52] S. Fan, Smith, M.L., Rivert, D.J., Duba, D., Zhan, Q., Kohn, K.W., Fornace, A.J. and O'Connor, P.M. Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline, Cancer research, 55 (1995) 1649-1654.
[53] K.J. Russell, Wiens, L.W., Demers, G.W., Galloway, D.A., Le, T., Rice, G.C., Bianco, J.A., Singer, J.W. and Groudine, M. Preferential radiosensitization of G1 checkpoint—Deficient cells

by methylxanthines, International journal of radiation oncology* biology* physics, 36 (1996) 1099-1106.
[54] S.L. Yao, Akhtar, A.J., Mckenna, K.A., Bedi, G.C., Sidransky, D., Mabry, M., Ravi, R., Collector, M.I., Jones, R.J., Sharkis, S.J. and Fuchs, E.J. Selective radiosensitization of p53–deficient cells by caffeine–mediated activation of p34cdc2 kinase, Nature medicine, 2 (1996) 1140-1143.
[55] X. Jiang, Lim, L.Y., Daly, J.W., Li, A.H., Jacobson, K.A. and Roberge, M.I.C.H.E.L. Structure-activity relationships for G2 checkpoint inhibition by caffeine analogs, International journal of oncology, 16 (2000) 971-979.
[56] M.P. Chitnis, Viladkar, A.B. and Juvekar, A.S. Inhibition of DNA biosynthesis by vincristine and pentoxifylline in murine P388 leukemia cells resistant to doxorubicin, Neoplasma, 37 (1989) 619-626.
[57] H. Tsuchiya, Yasutake, H., Yokogawa, A., Baba, H., Ueda, Y. and Tomita, K. Effect of chemotherapy combined with caffeine for osteosarcoma, Journal of cancer research and clinical oncology, 118 (1992) 567-569.
[58] H. Tsuchiya, Tomita, K., Moril, Y., Asada, N., Morinaga, T. Kitano, S. and Yamamoto, N. Caffeine-Assisted Chemotherapy and Minimizer! Tumor, Anticancer research, 18 (1998) 657-666.
[59] H. Tsuchiya, Yamamoto, N., Asada, N., Terasaki, T., Kanazawa, Y., Takanaka, T., Nishijima, H. and Tomita, K. Caffeine-potentiated radiochemotherapy and function-saving surgery for high-grade soft tissue sarcoma, Anticancer research, 20 (1999) 2137-2143.
[60] M. Hayashi, Tsuchiya, H., Yamamoto, N., Karita, M., Shiral, T., Nishida, H., Takeuchi, A. and Tomita, K. Caffeine-potentiated chemotherapy for metastatic carcinoma and lymphoma of bone and soft tissue, Anticancer research, 25 (2005) 2399-2405.
[61] K.J. Russell, Wiens, L.W., Demers, G.W., Galloway, D.A., Plon, S.E. and Groudine, M. Abrogation of the G2 checkpoint results in differential radiosensitization of G1 checkpoint-deficient and G1 checkpoint-competent cells, Cancer research, 55 (1995) 1639-1642.
[62] S.N. Powell, DeFrank, J.S., Connell, P., Eogan, M., Preffer, F., Dombkowski, D., Tang, W. and Friend, S. Differential sensitivity of p53 (-) and p53 (+) cells to caffeine-induced radiosensitization and override of G2 delay, Cancer research, 55 (1995) 1643-1648.
[63] J.N. Sarkaria, Busby, E.C., Tibbetts, R.S., Roos, P., Taya, Y., Karnitz, L.M. and Abraham, R.T. Inhibition of ATM and ATR kinase activities by the radiosensitizing agent, caffeine, Cancer research, 59 (1999) 4375-4382.
[64] S.H. Kim, Khil, M.S., Ryu, S. and Kim, J.H. Enhancement of radiation response on human carcinoma cells in culture by pentoxifylline, International journal of radiation oncology* biology* physics, 25 (1993) 61-65.
[65] L.R. Kelland, and Steel, G.G. Modification of radiation dose-rate sparing effects in a human carcinoma of the cervix cell line by inhibitors of DNA repair, International journal of radiation biology, 54 (1988) 229-244.
[66] E.H. Wolloch, Sevin, B.U., Perras, J.P., Gottlieb, C.F., Pollack, A., Ramos, R., Schiano, M.A. and Averette, H.E. Cell kinetic perturbations after irradiation and caffeine in the BG-1 ovarian carcinoma cell line, Gynecologic oncology, 43 (1991) 29-36.
[67] N.A. Asaad, Zeng, Z.C., Guan, J., Thacker, J. and Iliakis, G. Homologous recombination as a potential target for caffeine radiosensitization in mammalian cells: reduced caffeine radiosensitization in XRCC2 and XRCC3 mutants, Oncogene, 19 (2000) 5788-5800.

[68] G. Deplanque, Ceraline, J., Mah-Becherel, M.C.M., Cazenave, J.P., Bergerat, J.P. and Klein-Soyer, C. Caffeine and the G2/M block override: a concept resulting from a misleading cell kinetic delay, independent of functional p53, International journal of cancer, 94 (2001) 363-369.
[69] W.D. Block, Merkle, D., Meek, K. and Lees-Miller, S.P. Selective inhibition of the DNA-dependent protein kinase (DNA-PK) by the radiosensitizing agent caffeine, Nucleic acids research, 32 (2004) 1967-1972.
[70] C.E. Helt, Cliby, W.A., Keng, P.C., Bambara, R.A. and O'Reilly, M.A. Ataxia telangiectasia mutated (ATM) and ATM and Rad3-related protein exhibit selective target specificities in response to different forms of DNA damage, Journal of biological chemistry, 280 (2005) 1186-1192.
[71] A.M. Weber, and Ryan, A.J. ATM and ATR as therapeutic targets in cancer, Pharmacology & therapeutics, 149 (2015) 124-138.
[72] A. Tichý, Muthná, D., Vávrová, J., Pejchal, J., Šinkorová, Z., Zárybnická, L. and Řezáčová, M. Caffeine-suppressed ATM pathway leads to decreased p53 phosphorylation and increased programmed cell death in gamma-irradiated leukaemic molt-4 cells, Journal of applied biomedicine, 9 (2011) 49-56.
[73] T. Waldman, Lengauer, C., Kinzler, K.W. and Vogelstein, B. Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21, Nature, (1996) 713-716.
[74] T. Waldman, Zhang, Y., Dillehay, L., Yu, J., Kinzler, K., Vogelstein, B. and Williams, J. Cell-cycle arrest versus cell death in cancer therapy, Nature medicine, 3 (1997) 1034-1036.
[75] M. Olivier, Hollstein, M. and Hainaut, P. TP53 mutations in human cancers: origins, consequences, and clinical use, Cold spring harbor perspectives in biology, 2 (2010) a001008.
[76] D.J. Dobmeyer, Stine, R.A., Leier, C.V., Greenberg, R. and Schaal, S.F. The arrhythmogenic effects of caffeine in human beings, New england journal of medicine, 308 (1983) 814-816.
[77] P.W.a.R. Curatolo, D. The health consequences of caffeine, annals of internal medicine, 98 (1983) 641-653.
[78] H.J. Fingert, Kindy, R.L. and Pardee, A.B. Enhanced lethality by methylxanthines in human bladder cancer cells treated with thiotepa, The journal of urology, 132 (1984) 609-613.
[79] E.A. Rogozin, Lee, K.W., Kang, N.J., Yu, H., Nomura, M., Miyamoto, K.I., Conney, A.H., Bode, A.M. and Dong, Z. Inhibitory effects of caffeine analogues on neoplastic transformation: structure–activity relationship, Carcinogenesis, 29 (2008) 1228-1234.
[80] L.R. Bernstein, and Colburn, N.H. AP1/jun function is differentially induced in promotion-sensitive and resistant JB6 cells, Science, 244 (1989) 566-569.
[81] Z. Dong, Birrer, M.J., Watts, R.G., Matrisian, L.M. and Colburn, N.H. Blocking of tumor promoter-induced AP-1 activity inhibits induced transformation in JB6 mouse epidermal cells, Proceedings of the national academy of sciences, 91 (1994) 609-613.
[82] Z. Dong, and Cmarik, J.L. Harvesting cells under anchorage-independent cell transformation conditions for biochemical analyses, Science signaling, 2002 (2002) pl7-pl7.
[83] R. Suravajhala, Suri, N., Bhagat, M. and Saxena, A.K. Biological evaluation of 8-alkyl xanthines as potential cytotoxic agents, Advances in biological chemistry, 3 (2013) 314-319.
[84] F. Levi-Schaffer, and Touitou, E. Xanthines inhibit 3T3 fibroblast proliferation, Skin pharmacology and physiology, 4 (1991) 286-290.

[85] C. Windmeier, and Gressner, A.M. Effect of pentoxifylline on the fibrogenic functions of cultured rat liver fat-storing cells and myofibroblasts, Biochemical pharmacology, 51 (1996) 577-584.
[86] E. Rapaport. Anticancer activities of adenine nucleotides in tumor bearing hosts, Drug development research, 28 (1993) 428-431.
[87] P. Bodin, and Geoffrey, B. ATP-stimulated release of ATP by human endothelial cells, Journal of cardiovascular pharmacology, 27 (1996) 872-875.
[88] F. Di Virgilio. The P2Z purinoceptor: an intriguing role in immunity, inflammation and cell death, Immunology today, 16 (1995) 524-528.
[89] S.S. Bhagwat, and Williams, M. P2 purine and pyrimidine receptors: emerging superfamilies of G-protein-coupled and ligand-gated ion channel receptors, European journal of medicinal chemistry, 32 (1997) 183-193.
[90] M.V.a.C.-C. Nesterova, Y.S., Significance of protein kinase A in cancer, in: Apoptosis, cell signaling, and human diseases, Springer, 2006 3-30.
[91] M. Drees, Zimmermann, R. and Eisenbrand, G. 3′, 5′-Cyclic nucleotide phosphodiesterase in tumor cells as potential target for tumor growth inhibition, Cancer research, 53 (1993) 3058-3061.
[92] W.N. Hait, and Weiss, B. Increased cyclic nucleotide phosphodiesterase activity in leukaemic lymphocytes, (1976) 321-323.
[93] M.L. Heidrick, and Ryan, W.L. Cyclic nucleotides on cell growth in vitro, Cancer research, 30 (1970) 376-378.
[94] L. Hirsh, Dantes, A., Suh, B.S., Yoshida, Y., Hosokawa, K., Tajima, K., Kotsuji, F., Merimsky, O. and Amsterdam, A. Phosphodiesterase inhibitors as anti-cancer drugs, Biochemical pharmacology, 68 (2004) 981-988.
[95] P. Raboisson, Lugnier, C., Muller, C., Reimund, J.M., Schultz, D., Pinna, G., Le Bec, A., Basaran, H., Desaubry, L., Gaudiot, F. and Seloum, M. Design, synthesis and structure–activity relationships of a series of 9-substituted adenine derivatives as selective phosphodiesterase type-4 inhibitors, European journal of medicinal chemistry, 38 (2003) 199-214.
[96] M. Legraverend, and Grierson, D.S. The purines: potent and versatile small molecule inhibitors and modulators of key biological targets, Bioorganic & medicinal chemistry, 14 (2006) 3987-4006.
[97] F. Mentz, Mossalayi, M.D., Ouaaz, F., Baudet, S., Issaly, F., Ktorza, S., Semichon, M., Binet, J.L. and Merle-Beral, H. Theophylline synergizes with chlorambucil in inducing apoptosis of B-chronic lymphocytic leukemia cells, Blood, 88 (1996) 2172-2182.
[98] S.H. Shafer, Phelps, S.H. and Williams, C.L. Reduced DNA synthesis and cell viability in small cell lung carcinoma by treatment with cyclic AMP phosphodiesterase inhibitors, Biochemical pharmacology, 56 (1998) 1229-1236.
[99] M. Sarfati, Mateo, V., Baudet, S., Rubio, M., Fernandez, C., Davi, F., Binet, J.L., Delic, J. and Merle-Béral, H. Sildenafil and vardenafil, types 5 and 6 phosphodiesterase inhibitors, induce caspase-dependent apoptosis of B-chronic lymphocytic leukemia cells, Blood, 101 (2003) 265-269.
[100] R. Lee, Wolda, S., Moon, E., Esselstyn, J., Hertel, C. and Lerner, A. PDE7A is expressed in human B-lymphocytes and is up-regulated by elevation of intracellular cAMP, Cellular signalling, 14 (2002) 277-284.

[101] P. Wang, Wu, P., Egan, R.W. and Billah, M.M. Cloning, characterization, and tissue distribution of mouse phosphodiesterase 7A1, Biochemical and biophysical research communications, 276 (2000) 1271-1277.
[102] D. Ukena, Schudt, C. and Sybrecht, G.W. Adenosine receptor-blocking xanthines as inhibitors of phosphodiesterase isozymes, Biochemical pharmacology, 45 (1993) 847-851.
[103] P. Janik, Assaf, A. and Bertram, J.S. Inhibition of growth of primary and metastatic Lewis lung carcinoma cells by the phosphodiesterase inhibitor isobutylmethylxanthine, Cancer research, 40 (1980) 1950-1954.
[104] A.K. Tiwari, and Chen, Z.S. Repurposing phosphodiesterase-5 inhibitors as chemoadjuvants, Frontiers in pharmacology, 4 (2013) 1-3.
[105] P. Serafini, Meckel, K., Kelso, M., Noonan, K., Califano, J., Koch, W., Dolcetti, L., Bronte, V. and Borrello, I. Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function, The Journal of experimental medicine, 203 (2006) 2691-2702.
[106] P.R. Ding, Tiwari, A.K., Ohnuma, S., Lee, J.W., An, X., Dai, C.L., Lu, Q.S., Singh, S., Yang, D.H., Talele, T.T. and Ambudkar, S.V. The phosphodiesterase-5 inhibitor vardenafil is a potent inhibitor of ABCB1/P-glycoprotein transporter, Plos one, 6 (2011) e19329.
[107] Z. Shi, Tiwari, A.K., Shukla, S., Robey, R.W., Singh, S., Kim, I.W., Bates, S.E., Peng, X., Abraham, I., Ambudkar, S.V. and Talele, T.T. Sildenafil reverses ABCB1-and ABCG2-mediated chemotherapeutic drug resistance, Cancer research, 71 (2011) 3029-3041.
[108] J.J. Chen, Sun, Y.L., Tiwari, A.K., Xiao, Z.J., Sodani, K., Yang, D.H., Vispute, S.G., Jiang, W.Q., Chen, S.D. and Chen, Z.S. PDE5 inhibitors, sildenafil and vardenafil, reverse multidrug resistance by inhibiting the efflux function of multidrug resistance protein 7 (ATP-binding Cassette C10) transporter, Cancer science, 103 (2012) 1531-1537.
[109] K.L. Black, Yin, D., Ong, J.M., Hu, J., Konda, B.M., Wang, X., Ko, M.K., Bayan, J.A., Sacapano, M.R., Espinoza, A. and Irvin, D.K. PDE5 inhibitors enhance tumor permeability and efficacy of chemotherapy in a rat brain tumor model, Brain research, 1230 (2008) 290-302.
[110] C.M. Whitehead, Earle, K.A., Fetter, J., Xu, S., Hartman, T., Chan, D.C., Zhao, T.L., Piazza, G., Klein-Szanto, A.J., Pamukcu, R. and Alila, H. Exisulind-induced apoptosis in a non-small cell lung cancer orthotopic lung tumor model augments docetaxel treatment and contributes to increased survival, Molecular cancer therapeutics, 2 (2003) 479-488.
[111] L. Pusztai, Zhen, J.H., Arun, B., Rivera, E., Whitehead, C., Thompson, W.J., Nealy, K.M., Gibbs, A., Symmans, W.F., Esteva, F.J. and Booser, D. Phase I and II study of exisulind in combination with capecitabine in patients with metastatic breast cancer, Journal of clinical oncology, 21 (2003) 3454-3461.
[112] A. Das, Durrant, D., Mitchell, C., Mayton, E., Hoke, N.N., Salloum, F.N., Park, M.A., Qureshi, I., Lee, R., Dent, P. and Kukreja, R.C. Sildenafil increases chemotherapeutic efficacy of doxorubicin in prostate cancer and ameliorates cardiac dysfunction, Proceedings of the national academy of sciences, 107 (2010) 18202-18207.
[113] Y. Wang, Chackalamannil, S., Hu, Z., Boyle, C.D., Lankin, C.M., Xia, Y., Xu, R., Asberom, T., Pissarnitski, D., Stamford, A.W. and Greenlee, W.J. Design and synthesis of xanthine analogues as potent and selective PDE5 inhibitors, Bioorganic & medicinal chemistry letters, 12 (2002) 3149-3152.
[114] N.K. Terrett, Bell, A.S., Brown, D. and Ellis, P. Sildenafil (Viagra TM), a potent and selective inhibitor of type 5 cGMP phosphodiesterase with utility for the treatment of male erectile dysfunction, Bioorganic & medicinal chemistry letters, 6 (1996) 1819-1824.

[115] A.R. Mohamed, A.M. El Kerdawy. A Perspective Study on the RTK, PI3K, B-Raf, CDK and the Multi-Protein Targeting in Medicinal Chemistry, Chemistry Biodiversity, 19 (2022) e202200328.
[116] S.M. Rida, F.A. Ashour, S.A. El-Hawash, M.M. El-Semary, M.H. Badr. Synthesis of Some Novel Substituted Purine Derivatives As Potential Anticancer, Anti-HIV-1 and Antimicrobial Agents, Archiv der Pharmazie: An International Journal Pharmaceutical Medicinal Chemistry, 340 (2007) 185-194.
[117] H.A. Abou-Zied, B.G. Youssif, M.F. Mohamed, A.M. Hayallah, M. Abdel-Aziz. EGFR inhibitors and apoptotic inducers: Design, synthesis, anticancer activity and docking studies of novel xanthine derivatives carrying chalcone moiety as hybrid molecules, Bioorg. Chem., 89 (2019) 102997.
[118] O.S. Afifi, O.G. Shaaban, H.A. Abd El Razik, S.E.-D.A.S. El, F.A. Ashour, A.A. El-Tombary, M.M. Abu-Serie. Synthesis and biological evaluation of purine-pyrazole hybrids incorporating thiazole, thiazolidinone or rhodanine moiety as 15-LOX inhibitors endowed with anticancer and antioxidant potential, Bioorganic chemistry, 87 (2019) 821-837.
[119] R.R. Ruddarraju, A.C. Murugulla, R. Kotla, M.C.B. Tirumalasetty, R. Wudayagiri, S. Donthabakthuni, R. Maroju, K. Baburao, L.S. Parasa. Design, synthesis, anticancer, antimicrobial activities and molecular docking studies of theophylline containing acetylenes and theophylline containing 1, 2, 3-triazoles with variant nucleoside derivatives, European journal of medicinal chemistry, 123 (2016) 379-396.
[120] I. Shahzadi, A.F. Zahoor, A. Rasul, N. Rasool, Z. Raza, S. Faisal, B. Parveen, S. Kamal, M. Zia-ur-Rehman, F.M. Zahid. Synthesis, anticancer, and computational studies of 1, 3, 4-oxadiazole-purine derivatives, Journal of Heterocyclic Chemistry, 57 (2020) 2782-2794.
[121] A.R. Mohamed, A. Mostafa, M.A. El Hassab, G.M. Hedeab, S.H. Mahmoud, R.F. George, H.H. Georgey, N.M.A. Gawad, M.K. El-Ashrey. Insights into targeting SARS-CoV-2: design, synthesis, in silico studies and antiviral evaluation of new dimethylxanthine derivatives, RSC Medicinal Chemistry, (2023).
[122] A.R. Mohamed, A.M. El Kerdawy, R.F. George, H.H. Georgey, N.M.A. Gawad. Design, synthesis and in silico insights of new 7, 8-disubstituted-1, 3-dimethyl-1H-purine-2, 6 (3H, 7H)-dione derivatives with potent anticancer and multi-kinase inhibitory activities, Bioorganic Chemistry, 107 (2021) 104569.