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Genotoxic potentials and eukaryotic DNA topoisomerase I inhibitory effects of some benzoxazine derivatives

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Abstract Benzoxazines are heterocyclic compounds which have been used as intermediates in the synthesis of many heterocyclic structures of biological importance as it has been reported that some of the benzoxazines were effective in promoting apoptosis and inhibiting cell proliferation. Present study contains experimental data that showed genotoxic potentials and inhibitory effects on eukaryotic DNA topoisomerase I of 16 newly synthesized benzoxazine derivatives. By rec assay, the bacterial genotoxicity assay, only four tested compounds were found genotoxic at different concentrations and four compounds showed reverse effect. RC_{50} values evaluated by rec assay revealed that BS5 was the most genotoxic and BS4 was the most cytotoxic compound at micromolar concentration. Compounds were also tested for their inhibitory effects on eukaryotic DNA topoisomerase I enzyme and it was found that 14 of the compounds had inhibitory effects on eukaryotic DNA topoisomerase I enzyme. The most active compounds, BS18 and BS4, showed higher inhibitory activities than the positive control drug camptothecin which is a well-known commercial topoisomerase I inhibitor.

Keywords Rec assay · Topoisomerase I · Benzoxazine · Anticancer drugs

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Introduction

Cancer is a disease that leads mortality in the world wide. The discovery and development of new treatments are urgently needed due to the problems of current treatments, such as toxicities and drug-resistance (Krishna and Mayer, 2000). It has been reported that the antitumor efficacy of chemotherapeutic agents correlated with their growth-inhibiting, differentiation-inducing or apoptosis-inducing abilities (Viala *et al.*, 2004). There are several cytotoxic or genotoxic anticancer agents with respect to following strategy: "Cancer cells have problem with the DNA repair system or cell cycle control, so it is more sensitive to DNA damage of the other body cells" (Zhang *et al.*, 2000). Genotoxic drugs affect both normal and cancer cells, but the selectivity associated with sensitivity of rapidly dividing cells such as cancer cells (Zhang *et al.*, 2000; McGovern and Jacobson-Kram, 2006). The importance of cancer cell-specific mechanism intended agents such as inhibitors of DNA topoisomerases which are the major class of anticancer drugs is increasing (Larsen and Gobert, 1999; Li and Liu, 2001). A number of anticancer drugs were in clinical use in the 1970s before their cytotoxic actions were linked to topoisomerases such as fluoroquinolone antibacterial agents and camptothecin (CPT) (Lesher *et al.*, 1962; Wall *et al.*, 1966).

DNA topoisomerases regulate the conformational or topological changes of DNA by catalyzing the concerted breakage and rejoining of DNA strands during normal cell growth (Topcu, 2001; Maxwell and Bates, 2009). DNA generated key cellular processes like replication, transcription, recombination, repair, and chromatin assembly are related with the conformational changes of the topology of DNA and topoisomerases (Nitiss, 1998; Pommier, 2013). There are two types of DNA topoisomerases which

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have been isolated from prokaryotes and eukaryotes (Stewart *et al.*, 1998). Type I DNA topoisomerases (topo I) act by making a transient break in one strand of DNA, whereas type II DNA topoisomerases (topo II) introduce transient double-strand breaks (Berger *et al.*, 1996).

Topo I can relax positive and negative supercoils; it is responsible for relieving the torsional stress associated with DNA replication, transcription, and chromatin condensation (Champoux, 2001; Topcu, 2001; Baker *et al.*, 2009).

Anti-topoisomerase inhibitors are split into two main classes. Topoisomerase poisons target the topoisomerase-DNA complex (cleavable complex), which is normally temporary and breaks DNA ends bounded immediately after altering of topological state and they can trap this complex to cause DNA single breaks. If the amount of these DNA breaks is intolerable, stability of DNA could be disrupted and apoptosis might be induced in the cells.

Catalytic inhibitors can bind either enzyme or DNA to influence the catalytic activity of the enzyme. Both kinds of inhibitors are commercially available for cancer treatment (Bassi and Palitti, 2000; Leppard and Champoux, 2005).

Camptothecin is the best known topo I inhibitor. In animal studies, CPT exhibited potent antitumor activity against a broad spectrum of tumors (Gottlieb and Luce, 1972; Muggia *et al.*, 1972). Studies suggested that CPT was not only stabilized the cleavable complex but also inhibited the relegation step of catalytic cycle of topo I (Hsiang *et al.*, 1985; Svejstrup *et al.*, 1991).

Some new fused heterocyclic compounds such as benzazoles and benzoxazines which are the analogs of flouroquinolones were investigated for induction and inhibition of apoptosis on tumor cells (L5718, mouse lymphoma cell line containing the human mdr-1 gene) by our research team (Varga *et al.*, 2005) depending on the idea that substituted benzoxazoles and related fused-heterocyclic compounds such as benzimidazoles, benzothiazoles, and benzoxazines have shown antibacterial and antifungal (Temiz-Arpaci *et al.*, 2002a, b; Yalcin *et al.*, 2003; Yildiz-Oren *et al.*, 2004a, b), antiviral (Plemper *et al.*, 2004), topoisomerase inhibiting (Alper *et al.*, 2003; Pinar *et al.*, 2004), and antitumor activities (Shi *et al.*, 1996; DeLuca and Kerwin, 1997; Reynolds *et al.*, 1999; Nofal *et al.*, 2000; Sato *et al.*, 2001, Xiang *et al.*, 2012). In addition, it has been reported that some of the benzoxazines were effective in promoting apoptosis and inhibiting cell proliferation (Varga *et al.*, 2005; Liu *et al.*, 2009). Consequently, these derivatives were subjected to application of anticancer drug development (Topcu, 2001).

In this research, a series of previously synthesized benzoxazine derivatives (as seen Table 1) showing antimicrobial activity (Yalcın *et al.*, 2003; Alper-Hayta *et al.*, 2006) was evaluated in connection with anticancer perspective. For this purpose, we used *Bacillus subtilis* (*B. subtilis)* spore microplate rec assay method, a bacterial genotoxicity test system to examine the tested compounds whether they have genotoxic potential and DNA topo I relaxation assay was used to understand if these compounds were the inhibitors of eukaryotic topo I.

Results and discussion

Rec assay

Genotoxic potentials of benzoxazine derivatives were tested by rec assay. Evaluated RC_{50} values for both *Bacillus* strains are shown in Table 1. According to RC_{50} values, BS5 was found to be the strongest genotoxic active compound, whereas compounds BS4, BS7, and BS9 revealed some genotoxic effects. On the other hand, the reverse effect was obtained with BS12, BS13, BS16, and BS17. The remaining eight compounds did not present any genotoxic potential.

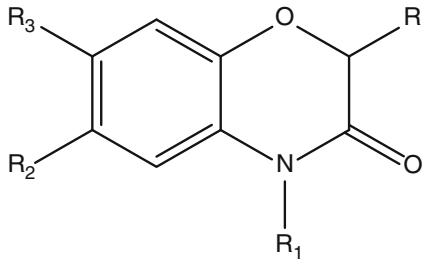
Rec assay gives information for both genotoxic and cytotoxic potentials of compounds. Obtained data suggested that BS4 was the most cytotoxic compound (RC_{50} of rec^+: 430 μM) although it was not the most genotoxic one. In contrast, BS5 was the most genotoxic compound (R_{50}: 2.81), however it showed no cytotoxic effect at low concentrations. According to the expectation that cytotoxic agents have to be the most effective in low concentrations, it was found that BS4 is the most effective cytotoxic derivative among the other tested compounds.

For genotoxicity assessment, ratio of rec^+ and rec^- strain was compared with survival when exposed to these compounds. Survival differences between strains arised from efficiency of post-replicational repair mechanisms. Therefore, it was suggested that genotoxic effective compounds caused damages on DNA such as base changes, stranded breaks, crosslinks.

By rec assay, it was expected that rec^+ strain had to be more resistant than rec^- strain when encountered a genotoxic agent. In contrast, some compounds which induced a reverse effect, cause higher survival ratio for rec^- strain than rec^+. In this case, it could be supposed that RecE gene defect could be reversed in rec^- strain. According to this probability, it was necessary that both strains could have the same survival ratio. Therefore, it was suggested that the compound-induced reverse effect could also induce some mutations on DNA. But it has not been a clear report for reverse effect. Therefore, further studies are needed to shed light on what was responsible for the reverse effect.

Structure–activity relationship (SAR) analysis on rec assay results revealed that holding by a NO_2 group at the benzene ring on position R_3 might induce the genotoxic potential of the compounds. Both genotoxically active

Table 1 Rec assay and DNA-topoisomerase I relaxation assay results of tested benzoxazine derivatives



Compounds	R	R_1	R_2	R_3	RC_{50} Rec^+ (μM)	RC_{50} Rec^- (μM)	R_{50}	Result	IC_{50} (μM)[a]
BS1	$CHd_2COOC_2H_5$	H	H	H	10,400	9,160	1.14	(−)	5,469
BS2	$CH_2COOC_2H_5$	H	CH_3	H	5,810	5,450	1.06	(−)	2,790
BS4	$CH_2COOC_2H_5$	H	$COOC_2H_5$	H	730	420	1.71	(+)	212
BS5	$CH_2COOC_2H_5$	H	H	NO_2	28,360	10,060	2.81	(++)	12,090
BS6	$CH_2COOC_2H_5$	H	H	NH_2	6,816	4,892	1.39	(−)	1,390
BS7	$CH_2COOC_2H_5$	H	Cl	NO_2	1,210	660	1.82	(+)	NE
BS8	$CH_2COOC_2H_5$	CH_3	H	H	2,730	2,140	1.28	(−)	1,826
BS9	$CH_2COOC_2H_5$	CH_3	CH_3	H	2,040	1,310	1.55	(+)	NE
BS10	$CH_2COOC_2H_5$	CH_3	Cl	H	1,800	1,440	1.25	(−)	25,508
BS12	$CH_2COOC_2H_5$	CH_3	H	NO_2	1,421	4,442	0.33	(*r*)	3,578
BS13	$CH_2COOC_2H_5$	CH_3	Cl	NO_2	1,220	2,430	0.5	(*r*)	1,317
BS14	$CH_2COOC_2H_5$	C_2H_5	H	H	2,620	2,420	1.08	(−)	5,239
BS15	$CH_2COOC_2H_5$	C_2H_5	Cl	H	5,600	5,600	1.0	(−)	4,084
BS16	$CH_2COOC_2H_5$	C_2H_5	H	NO_2	920	1,980	0.46	(*r*)	4,535
BS17	$CH_2COOC_2H_5$	C_2H_5	Cl	NO_2	320	1,300	0.25	(*r*)	7,890
BS18	OH	H	H	H	6,730	4,790	1.4	(−)	253
4NQO					6.89	1.63	4.22	(++)	
CPT									497

NE not effected, CPT camptothecin

[a] DNA-topoisomerase I inhibition

compounds such as BS5 and BS7 have NO_2 groups at R_3 position as well as a hydrogen atom on position R_1. When the compounds have alkyl substituents as methyl or ethyl groups at R_1 position instead of hydrogen attached to the nitrogen atom in the oxazin moiety of the compounds, such as BS12, BS13, BS16, and BS17 which induce the reverse effect. This observation showed that having hydrogen atom on the nitrogen of the oxazin ring moiety is essential for the activity to achieve a hydrogen-bond interaction by the active side of the target.

When the structure of the most cytotoxic compound BS4 was analyzed, it was seen that an electron withdrawing group $COOC_2H_5$ at R2 position had to be located unlike the other tested compounds. This group could also play a significant role in reducing the electron density of the benzene ring like NO_2 group to achieve an electron recipient effect to the molecule to attack the nucleophilic side of DNA.

DNA topoisomerase I inhibitor activity

We performed DNA topoisomerase I relaxation assay on the test compounds. The main logic of this assay relies on that in the presence and absence of a compound, enzyme converts supercoiled DNA to its relaxed form in a particular time. If drug interrupts this process, DNA remains in its supercoiled form. In this point, supercoiled DNA band intensities could be compared with its control (untreated supercoiled DNA band). According to IC_{50} values, 14 of the tested 16 compounds revealed inhibitory effect at various concentrations (Table 1). Among these 14 compounds, BS4 (IC_{50}: 212 μM) and BS18 (IC_{50}: 253 μM) were found to be the most effective topo I inhibitors, even they were more active than reference drug CPT (IC_{50}: 497 μM). Electrophoregram of BS4 could be seen in Fig. 1. The obtained gel electrophoresis results showed that all of the inhibitory effective compounds inhibited

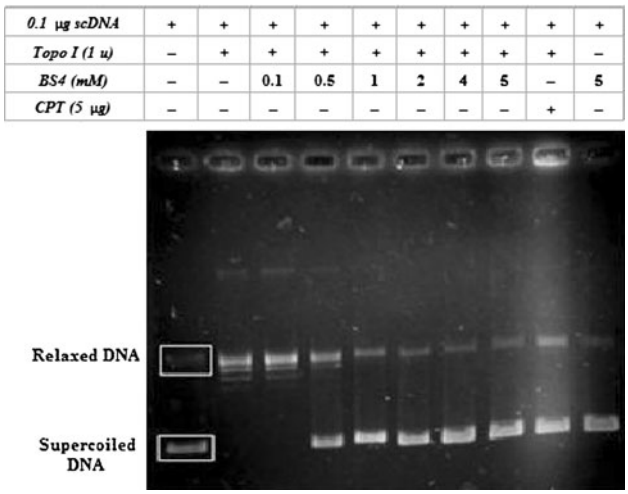


Fig. 1 An electrophoregram result of BS4 for topoisomerase I inhibition. All wells contain 0.1 µg supercoiled pBR322 plasmid DNA and 1 unite of topoisomerase I except wells 1 and 10. BS4 was added at 0.1, 0.5, 1,2,4,5 mM concentration into the *lines* 2–8, respectively. 5 mM of BS4 was added into the DNA without enzyme in *line* 10. 5 µg CPT was used as reference compound in the 9th well

conversion to relax DNA from supercoiled DNA and band intensities of the supercoiled DNA of wells with compounds were higher than the control well. As a result of relaxation assay, the investigated benzoxazine derivatives could be topo I catalytic inhibitors.

Conclusion

In this study previously synthesized 16 benzoxazine derivatives which were originally designed to be anticancer agents were investigated about their biological activities by two different assays. Rec assay was used to examine a number of environmental mutagens in a variety of substances such as food additives, pesticides, and metal compounds (Sharma and Sobti, 2000; Suksamrarn *et al.*, 2003; Ozaki *et al.*, 2004) as well as anticancer agents due to genotoxic effects (Gümüş *et al.*, 1996). Topo I inhibitors are potential anticancer agents because of their crucial role on DNA metabolism. Among the tested compounds, BS4 (ethyl 2-(2-ethoxy-2-oxoethyl)-3-oxo-3,4-dihydro-2*H*-1,4 benzoxazine-6-carboxylate), BS5 (ethyl(7-nitro-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-2-yl) acetate), and BS18 (2-hydroxy-2*H*-1,4-benzoxazine-3(4*H*)-one) were found as the remarkable compounds. By rec assay results, BS5 was found to be the strongest genotoxic effect while BS4 was evaluated genotoxic and the most cytotoxic compound. BS4 and BS18 exhibited lower IC_{50} values than the reference drug CPT, for eukaryotic DNA topo I inhibitor activity. In fact, BS4 showed the most preferred outcome in both assays because it displayed strong genotoxic, cytotoxic, and topo I inhibitory activities. In conclusion, BS4 might be a new anticancer agents and it might cause DNA damages like base substitutions, cross-links or breaks.

Materials and methods

Chemicals

Tested benzoxazine derivatives were previously synthesized by our group at Ankara University, Faculty of Pharmacy (Yalcin *et al.*, 2003; Alper-Hayta *et al.*, 2006). The chemical structure of the compounds could be seen in Table 1. Both bacterial strains *B. subtilis* H17 (arg^-, trp^-, $recE^+$) and *B. subtilis* M45 (arg^-,trp^-, $recE^-$) were obtained from the National Institute of Genetics, Mishima, Shizuoka-Ken, Japan. pBR322 plasmid DNA and 6× loading buffer (bromphenol blue + xylene cyanole) were purchased from MBL Fermentas. Calf thymus DNA topo I enzyme and DNA topo I reaction buffer were purchased from Amersham Biosciences UK. Agarose, CPT, ethidium bromide, 4-nitroquinoline 1-oxide (4NQO) were purchased from Sigma. All the other chemicals were of analytical grade.

Genotoxicity study

Rec assay was performed according to the spore method by Kada with some modifications (Kada *et al.*, 1972, 1980). The rationale of the *B. subtilis* rec assay is based on the relative difference of survival of a DNA repair-recombination proficient strain M45 (rec^+) and its deficient strain H17 (rec^-). Strictly, rec^- strain is $recE^-$ and $uvrABC^+$, while rec^+ strain is $recE^+$ and $uvrABC^+$. Both strains can induce the uvrABC excision repair system after DNA damages. However, in the presence of genotoxins excision repair system is overwhelmed and this leads rec^- strain to death easily. The difference between their survivorships is therefore, simply interpreted as the results of the loss of recE-mediated repair in rec^- (Kada *et al.*, 1972, 1980).

Bacillus subtilis as Gram-positive bacteria is more sensitive to chemicals than Gram-negative bacteria. In addition, the usage of spore forms of *B. subtilis* in the experimental procedures is much more advantageous comparing to its vegetative forms. The fact was that spore forms were more resistance to drugs than vegetative forms. In this respect, rec assay might be used to detect genotoxicity at smaller concentrations (Kada *et al.*, 1972, 1980; Takigami *et al.*, 2002).

Preparation of spores

The spores were prepared by spreading overnight broth cultures of both strains in sterile plates on modified

Schaeffer's agar medium. The plates were incubated at 37 °C for 3 and 5 days for rec^- (M45) and rec^+ (H17) strains, respectively. After incubation, spores were scrapped up, washed, and resuspended in fresh minimal salt solution. Thereafter, they were treated with 2 mg/ml lysozyme for 30 min and subsequently with 1 % SDS for 30 min. The detergent was removed with subsequent washings with sterile distilled water. Spores were resuspended in sterile distilled water and stored at 4 °C until to use (Sharma and Sobti, 2000).

Microplate technique of B. subtilis rec assay

Bacillus subtilis rec assay was miniaturized with satisfactory performance using 96-well microplate. Test compounds were prepared with DMSO which were diluted with the ratio of ½ and triplicates of each concentration and negative control DMSO was pipetted into their corresponding well. Subsequently, *B. subtilis* spore suspensions in broth (10^8 spore/ml) were added in each well. Then, the microplate was incubated at 37 °C for overnight. Optic density values of bacterial growth were measured at 620 nm in Elisa reader. Each value was compared to the negative control values which was assumed to be 100 % of survival to calculate growth inhibition percentage. 4NQO was used as the reference substance (Takigami *et al.*, 2002).

Statistical analysis of rec assay

S-probit analysis was used to determine genotoxic potentials of the compounds. A survival curve was drawn with sample concentration on the abscissa and survivorship on the ordinate. The area enclosed between survival curves of rec^+ and rec^- corresponds to genotoxicity. With the Probit scale transformation, the two curves were converted to two linear functions. Then the enclosed area between the two lines can be calculated by a simple integration. This integrated area designated as S-probit, is a quantitative index for evaluating genotoxic potential.

RC_{50} values for rec^- and rec^+ strains represent the concentration of a compound, where the strain survives 50 %. It is respected that RC_{50} value of rec^+ is higher than rec^- because rec^- strain have *recE* gene mutation. Consequently, higher RC_{50} values mean higher genotoxicity.

In contrast, several compounds could induce higher survival effect on rec^- than rec^+ strain. In such cases the effect of compound was called reverse effect.

Ratio of RC_{50} of rec+/RC_{50} of rec^- was used to calculate R_{50} value of a compound. R_{50} values show a range to determine genotoxic potential of a compound. The criteria consists of four ranges of genotoxicity shown on Table 2 and also contains R_{50} values (Takigami *et al.*, 2002).

Table 2 Criterion of genotoxicity in rec assay

R_{50}	Result
>2	Strong genotoxic response (++)
1.99–1.5	Genotoxic response (+)
1.49–0.85	Non-genotoxic (−)
<0.85	Reverse effect (*r*)

DNA topoisomerase I assay

We implemented relaxation assay to test the DNA topo I inhibition effect of the compounds. Relaxation activity of DNA topo I was determined by measuring the conversion of supercoiled pBR322 plasmid DNA to its relaxed form. The reaction mixture contained 1 µl of 10× DNA topo I reaction buffer [35 mM Tris–HCI pH 8.0, 72 mM KCI, 5 mM $MgCl_2$, 5 mM DTT, 50 mM spermidine, % 0.1 BSA], 0.1 µg pBR322 plasmid DNA, 1 unite topo I enzyme, and different concentrations of test compounds in a total volume of 10 µl. Initially tested compound was incubated with enzyme for 5 min at 37 °C. When DNA was added another 1 h incubation at 37 °C was provided. The mixture was incubated for 1 h at 37 °C and 1 µl 1 % SDS and 3 µl 6× loading buffer were added to terminate the reaction. The samples were immediately run 2 h at 45 V onto 1 % agarose gel, which was prepared of TAE (pH 8.0) buffer. Gels were stained by 1 µg/ml of ethidium bromide and photographed under UV light (Halligan *et al.*, 1985; Stewart and Champoux, 2001). After the electrophoresis, gels were stained with ethidium bromide (1 µg/ml) and photographed under UV light.

Statistical analysis of DNA topoisomerase I assay

Optical intensity of the newly formed bands was used as a measure of the enzyme activity. Moreover, topo I inhibition percentage of a compound was calculated by comparing supercoiled DNA band intensities of control and the compound. Optical intensity of each concentration of a compound was compared to the control to calculate inhibition percentages. This percentages were used to estimate 50 % inhibitor concentration (IC_{50}) of a compound by S-probit analysis. CPT was used as the reference substance for each experiments. If inhibition was not obtained at any concentration of a tested compound it was assumed to have no inhibitory activity (NE) on eukaryotic DNA topo I.

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References

Alper S, Temiz-Arpaci O, Sener-Aki E, Yalcin I (2003) Some new bi- and ter-benzimidazole derivatives as topoisomerase I inhibitors II. Farmaco 58:497–507

Alper-Hayta S, Akı-Sener E, Tekiner-Gulbas B, Yıldız I, Temiz-Arpacı O, Yalcın I, Altanlar N (2006) Synthesis, antimicrobial activity and QSARs of new benzoxazine-3-ones. Eur J Med Chem 41:1398–1404

Baker NM, Rajan R, Mondragon A (2009) Structural studies of type I topoisomerases. Nucleic Acids Res 37(3):693–701

Bassi Li, Palitti F (2000) Anti-topoisomerase drugs as potent inducers of chromosomal aberrations. Genet Mol Biol 23(4):1065–1069

Berger JM, Gamblin SJ, Harrison SC, Wang JC (1996) Structure and mechanism of DNA topoisomerase II. Nature 379:225–232

Champoux JJ (2001) DNA topoisomerases: structure, function and mechanism. Annu Rev Biochem 70:369–413

DeLuca M, Kerwin (1997) The total synthesis of UK-1. Tetrahedron Lett 38:199–202

Gottlieb JA, Luce JK (1972) Treatment of malignant melanoma with CPT. Cancer Chemother Rep 56:103–105

Gümüş F, Izgü F, Algül Ö (1996) Synthesis and structural characterızatıon of some 5(6)-substituted-2-hydroxymethylbenzimidazole derivatives and their platinum (II) complexes and determination of their in vitro antitumor activities by 'Rec-Assay' test. J Pharm Sci 21(1):7–15

Halligan BD, Edwards KA, Liu LF (1985) Purification and characterization of type II DNA topoisomerase from bovine calf thymus. J Biol Chem 260(4):2475–2482

Hsiang YH, Hertzberg R, Hecht S, Liu LF (1985) CPT induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 260:14873–14878

Kada T, Tutikawa K, Sadaie Y (1972) In vitro and host-mediated "rec-assay" procedures for screening chemical mutagens, and phloxine, a mutagenic red dye detected. Mutat Res 16:165–174

Kada T, Hirano K, Sirasu Y (1980) Screening of environmental chemical mutagens by the rec-assay system with *Bacillus subtilis*. Chem Mutagen 6:149–173

Krishna R, Mayer LD (2000) Multidrug resistance (MDR) in cancer mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. Eur J Pharm Sci 11:265–283

Larsen AK, Gobert C (1999) DNA topoisomerase I in oncology: Dr Jekyll or Mr Hyde? Pathol Oncol Res 5(3):171–178

Leppard JB, Champoux JJ (2005) Human DNA topoisomerase I: relaxation, roles, and damage control. Chromosoma 114(2):75–85

Lesher GY, Froelich EJ, Gruett MD, Bailey JH, Brundage RP (1962) Naphthyridine derivatives. A new class of chemotherapeutic agents. J Med Chem 5:1063–1065

Li T, Liu LF (2001) Tumor cell death induced by topoisomerase-targeting drugs. Annu Rev Pharmacol Toxicol 41:53–77

Liu X, Zhao J, Xu J, Zhao B, Zhang Y, Zhang S, Miao J (2009) Protective effects of a benzoxazine derivative against oxidized LDL-induced apoptosis and the increases of integrin beta4, ROS, NF-kappaB and P53 in human umbilical vein endothelial cells. Bioorg Med Chem Lett 19(10):2896–2900

Maxwell T, Bates A (2009) Topo 2008: DNA topoisomerases in biology and medicine. Nucleic Acids Res 37:3

McGovern T, Jacobson- Kram D (2006) Regulation of genotoxic and carcinogenic impurities in drug substances and products. Trends Anal Chem 25(8):790–795

Muggia FM, Creaven PJ, Hansen HH, Cohen MH, Selawry OS (1972) Phase I clinical trial of weekly and daily treatment with CPT (NSC-100880): correlation with preclinical studies. Cancer Chemother Rep 56:515–521

Nitiss JL (1998) Investigating the biological functions of DNA topoisomerases in eukaryotic cells. Biochim Biophys Acta 1400:63–81

Nofal ZM, El-Zahar M, Abd El-Karim SS (2000) Novel coumarin derivatives with expected biological activity. Molecules 5:99–113

Ozaki A, Yamaguchi Y, Fujita T, Kuroda K, Endo G (2004) Chemical analysis and genotoxicological safety assessment of paper and paperboard used for food packaging. Food Chem Toxicol 42:1323–1337

Pinar A, Yurdakul P, Yildiz I, Temiz-Arpaci O, Acan NL, Aki- Sener E, Yalcin I (2004) Some fused heterocyclic compounds as eukaryotic topoisomerase II inhibitors. Biochem Biophys Res Commun 317:670–674

Plemper RK, Erlandson KJ, Lakdawala AS, Prussia A, Boonsombat J, Aki-Sener E, Yalcin I, Yildiz I, Temiz-Arpaci O, Tekiner B, Liotta DC, Snyder JP, Compans RW (2004) A target site for template-based design of measles virus entry inhibitors. Proc Natl Acad Sci USA 101(15):5628–5633

Pommier Y (2013) Drugging topoisomerases: lessons and challenges. ACS Chem Biol 8:82–95

Reynolds MB, DeLuca M, Kerwin (1999) The novel bis(benzoxazole)cytotoxic natural product UK-1 is a magnesium ion-dependent DNA binding agent and inhibitor of human topoisomerase II. Bioorg Chem 27:326–337

Sato S, Kajiura T, Noguchi M, Takehana K, Kobayashi T, Tsuji T (2001) AJI9561, a new cytotoxic benzoxazole derivative produced by *Streptomyces* sp. J Antibiot 54:102–104

Sharma MK, Sobti RC (2000) Rec effect of certain textile dyes in *Bacillus subtilis*. Mutat Res 465:27–38

Shi DF, Bradshaw TD, Wrigley S, McCall CJ, Leieveld P, Fichtner I, Stevens MFG (1996) Synthesis of 2-(4-aminophenyl)benzothiazoles and evaluation of their activities against breast cancer cell lines in vitro and in vivo. J Med Chem 39:3375

Stewart L, Champoux JJ (2001) Assaying DNA topoisomerase I relaxation activity. Methods Mol Biol 95:1–11

Stewart L, Redinbo MR, Qiu X, Hol WGJ, Champoux JJ (1998) A model for the mechanism of human topoisomerase I. Science 279:1535–1540

Suksamrarn A, Poomsing P, Aroonrerk N, Punjanon T, Suksamrarn S, Kongkun S (2003) Antimycobacterial and antioxidant flavones from *Limnophila geoffrayi*. Arch Pharm Res 26(10):816–820

Svejstrup JQ, Christiansen K, Gromova II, Andersen AH, Westergaard O (1991) New technique for uncoupling the cleavage and religation reactions of eukaryotic topoisomerase I: the mode of action of CPT at a specific recognition site. J Mol Biol 222:669–678

Takigami H, Matsui S, Matsuda T, Shimizu Y (2002) The *Bacillus subtilis* rec-assay: a powerful tool for the detection of genotoxic substances in the water environment. Prospect for assessing potential impact of pollutants from stabilized wastes. Waste Manage (Oxford) 22:209–213

Temiz-Arpaci O, Aki-Sener E, Yalcin I, Altanlar N (2002a) Synthesis and microbiological activity of some novel N-[2-(psubstituted-phenyl)-5-benzoxazolyl]-cyclohexyl carboxamide, -cyclohexyl acetamide and -cylohexyl propionamide derivatives. Farmaco 57:771–775

Temiz-Arpaci O, Aki-Sener E, Yalcin I, Altanlar N (2002b) Synthesis and antimicrobial activity of some 2-[p-substitutedphenyl] benzoxazol-5-yl-arylcarboxyamides. Arch Pharm Med Chem 335(6):283–288

Topcu Z (2001) DNA topoisomerases as targets for anticancer drugs. J Clin Pharm Ther 26:405–416

Varga A, Aki-Sener E, Yalcin I, Temiz-Arpaci O, Tekiner-Gulbas B, Cherepnev G, Molnar J (2005) Induction of apoptosis and necrosis by resistance modifiers benzazoles and benzoxazines on

tumour cell line mouse lymphoma L5718 Mdr+cells. In Vivo 19:1087–1092

Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, Athman R, Memet S, Huerre MR, Coyle AJ, DiStefano PS, Sansonetti PJ, Labigne A, Bertin J, Philpott DJ, Ferrero RL (2004) Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. Nat Immunol 5:1166–1174

Wall M, Wani MC, Cooke CE, Palmer KH, McPhail AT, Sim GA (1966) Plant anti-tumor agents I: the isolation and structure of camptothecin, a novel alkaloidal leukemia and antitumor inhibitor from *Camptotheca acuminata*. J Am Chem Soc 88: 3888–3890

Xiang P, Zhou T, Wang L, Sun C, Hu J, Zhao Y, Yang L (2012) Novel benzothiazole, benzimidazole and benzoxazole derivatives as potential antitumor agents: synthesis and Preliminary in vitro biological evaluation. Molecules 17:873–883

Yalcın I, Tekiner-Gulbas B, Yıldız I, Temiz-Arpacı O, Akı-Sener E, Altanlar N (2003) Synthesis and antimicrobial activity of some novel 2,6,7-trisubstituted-2H-3,4-dihydro-1,4-benzoxazin-3-one derivatives. Indian J Chem 42B:905–909

Yildiz-Oren I, Yalcin I, Aki-Sener E, Ucarturk N (2004a) Synthesis and structure–activity relationships of new antimicrobial active multisubstituted benzazole derivatives. Eur J Med Chem 39: 291–298

Yildiz-Oren I, Tekiner-Gulbas B, Yalcin I, Temiz-Arpaci O, Aki-Sener E, Alatanlar N (2004b) Synthesis and antimicrobial activity of new 2-[p-substituted-benzyl]-5-[substituted-carbonylamino] benzoxazoles. Arch Pharm Med Chem 337:402–410

Zhang W, Stoica G, Tasca SI, Kelly KA, Meininger CJ (2000) Modulation of tumor angiogenesis by stem cell factor. Cancer Res 60:6757–6762