PHOTOCHEMICAL ELECTRON TRANSFER REACTIONS OF TALOTREXIN

Anamika Gupta*,Waseem Ahmad, Jawaid Iqbal and Mohd. Rehan Zaheer

Department of Chemistry, Aligarh Muslim University, Aligarh. 202002 (U.P.) India.
remiya59@rediffmail.com,

Abstract

Talotrexin is an antifolate drug with promising antitumor activity. The present study deals with the photodegradation of talotrexin. An aqueous solution of talotrexin was irradiated under aerobic as well as in anaerobic condition with UVA light in a photochemical reactor. Progress of the reaction was monitored by thin layer chromatography. At the end of three major photoproducts were isolated as 2, 4-diaminopteridine-6-carboxalic acid (2), 2, 4-diamino-6-(hydroxymethyl) pteridine (4) and 2-((4-(4-aminobenzamido) - 4-carboxy butyl) carbamoyl) benzoic acid (3) by eluting with chloroform-petrol on silica column.

Key words: Talotrexin, electron transfer, photodegradation, phototoxicity.

Introduction

The dated interest of photochemists in the properties of the electronically excited states of compounds of pharmaceutical use has been rapidly increasing during the last decade [1,2]. The sensitivity of several drugs to ambient light, particularly in the spectral regions of UVA (320–400 nm) and UVB (290–320 nm) [3,4] may pose risks in their use because electronic photoexcitation can lead to their simple degradation and then to a simple decrease in their pharmaceutical efficiency, and also to the formation of side products that can have toxic effects [5,6]. The interaction of drugs with UVA and UVB radiations is usually responsible for a series of photosensitizing effects, i.e. phototoxicity, photoallergy, photomutagenesis and photocarcinogenesis [7,8]. Photoinduced electron transfer (PET) is the branch of photochemistry that exploits the ability of certain photoexcitied molecules to act as strong oxidizing or reducing species, and induce a permanent chemical change in a ground state molecule through an electron transfer mechanism [9]. Photoinduced electron transfer (PET) processes have attracted growing interest in the last decade and many reactions such as cycloadditions, cycloreversions, oxygenations and photodegradation of drugs have been documented [10]. Photoinduced electron-transfer reaction is one of the most elementary chemical processes and plays important roles in many photosensitization phenomena [11].The primary event in any photosensitization process is the absorption of a photon, and the following free radical (via electron transfer) and singlet oxygen generation (through energy transfer) by photo-excited drug molecules may appear to be the principal intermediate species in the phototoxic response [12]. Thus to assess potential drug phototoxicity, it is critical to determine the mechanism of drug photo degradation. Folic acid antagonists, often called *antifolates*, are cytotoxic drugs [13] used as antineoplastic [14], antimicrobial [15], anti-inflammatory [16] and immune-suppressive agents [17]. Antifolates are compounds commonly used to treat various forms of cancer such as breast cancer, head and neck cancer, bladder cancer, acute lymphocytic leukemia, non-Hodgkin's lymphoma, choricarcinoma, and osteogenic sarcoma [18]. They are also being used in the treatment of non-cancerous diseases such as malaria [19], bacterial infections [20], psoriasis and rheumatoid arthritis [21]. They act as antitumor agents by suppressing the effects of folic acid and its derivatives on cellular processes [22]. Although it is a very useful but it can produce photosensitizing disorders such as photomutagenic, photocarcinogenic and photoallergy [23]. Nearly 50 years after their first use as anticancer agents, the antifolates remain a diverse and growing class of drugs with great promise and potential for improving our ability to treat a broad range of human diseases. Talotrexin (PT-523, N (alpha)-(4-amino-4-deoxypteroyl – N (delta) -hemiphthaloyl- L-ornithine)) is a newer antifolate and potent antagonist of DHFR. It combines characteristics of both the classical and nonclassical antifolates [24]. It has demonstrated enhanced antitumor activity in a broad spectrum of cancer models by targeting the enzyme DHFR to prevent DNA synthesis in tumor cells and inhibit tumor growth [25]. Preclinical studies suggest that talotrexin, as compared to methotrexate, the most widely used antifolate, enters into cells up to 10 times more efficiently and demonstrates 10- to 100-fold more potency in overcoming polyglutamation, a well-established mechanism of antifolate resistance [26]. Several antifolate drugs are known to demonstrate phototoxicity [27]. Interest in the photoreactivity of talotrexin arises from the clinical and pharmacological reports of toxic effects associated with the use of this drug [28].

The aim of this study is to contribute to the knowledge of the photochemical process involved in the photodegradation of talotrexin and the possible implications in the phototoxic activity. Herein we have elucidated the photobehaviour of the novel antifolate drug talotrexin under both aerobic and anaerobic conditions in UVA Light. Photolysis of talotrexin (1) resulted in the formation of three major photodegradation products, identified as 2, 3 and 4 from their spectral (IR, ^{1}H- NMR, ^{13}C-NMR, mass spectra) properties (Scheme -1).Photoproducts are presumably produced by electron transfer followed by aerial oxidation and through the cleavage of C-N bond of talotrexin (1).

Experimental

Apparatus and Chemicals

All chemicals used were of analytical grade. Pure Talotrexin was obtained from Varda Biotech (P) Ltd India. Riboflavin was purchased from Sigma Aldrich (India). Photochemical reactions were carried out in quartz fitted immersion well photochemical reactor equipped with 400W medium pressure mercury vapour lamp with continuous supply of water. IR spectra were recorded as KBr discs on a Perkin Elmer model spectrum RXI. [1]H-N M R and ^{13}C-NM R Spectra were recorded on a Bruker Avance-DRX-300 Spectrometer using TMS as internal standard and DMSO as solvent. E/MS were obtained on a VG-ZAB-HS mass spectrometer. High resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer at 70 e V ionization voltage. Column Chromatography was performed on silica gel 60 (70-230 mesh); Thin layer chromatography (TLC) was carried on Merck silica gel 60 F $_{254}$ (0.2 mm thick plates).

Photo irradiation procedure

An aqueous solution of Talotrexin (1) was irradiated with UVA light in a Rayonet photochemical reactor (The Southern New England Ultraviolet Co; Model RPR-208 equipped with four RUL-360 nm fluorescence lamps) for the complete conversion of reactants. Progress of the reaction was monitored by thin layer chromatography (chloroform-methanol, 98:2). After the completion of reaction (when desired conversions have reached) the solvent was removed in a rotary evaporator and products were purified by silica gel column chromatography.

Irradiation of Talotrexin under aerobic condition.

An aqueous solution of talotrexin (1) (170 mg, 0.30 mM) with riboflavin as sensitizer under aerobic condition was irradiated for 3 h at 354 nm. After following the steps described in general photoirradiation procedure, 2, 4-diaminopteridine-6-carboxalic acid (2, 53 mg) was obtained as main product with a trace amount of 2-((4-(4-aminobenzamido) - 4-carboxy butyl)Carbamoyl) benzoic acid (3, 35 mg) as detected on TLC.

2, 4-diaminopteridine-6-carboxalic acid (2):

Yield: 53 mg (31%) HRMS calcd.For(M$^+$) $C_7H_6N_6O_2$ 206.1615 found 206.1601 IR(KBr) 1595, 2600, 3450 cm$^-$^{1}, ^{1}H-NMR (DMSO, δ, ppm); 10.9 (s,1H,COOH), 8.59 (s,1H, H-7), 5.30 (s, 4H, 2NH$_2$), ^{13}C-NMR (DMSO, δ, ppm); 165.6 (COOH), 161.4 (C-2), 155.7(C-8a), 155.4(C-4), 146.4 (C-7), 143.8(C-6), 122.7 (C-4a), Ms:m/z: 206 (M$^+$), 161(M$^+$- 45).

2-((4-(4-aminobenzamido) - 4-carboxy butyl) Carbamoyl) benzoic acid (3)

Yield: 35mg (20%) HRMScalcd. For (M$^+$) $C_{20}H_{21}N_3O_6$ 399.3972 found 399.3968 IR (KBr) 3410, 3050, 1725, 1680 cm^{-1}, ^{1}H-NMR (DMSO, δ, ppm): 8.12 (m, 2H, H-2 & H-6 of benzoic acid), 7.98 (m, 1H, H-1 of amino benzamido), 760 (m, 1H, H-4 of benzoic acid), 7.47(m, 2H, H-3& H-5 of benzoic acid), 6.80 (m, 2H, H-2 & H-6 of amino benzamido), 6.34 (m, 2H, H-3 & H-5 of aminobenzamido), 4.46 (t, 1H, H-4 of carboxy butyl), 4.0 (2H, NH$_2$), 3.20 (t, 2H, H-1 of carboxy butyl), 1.78 (m, 2H, H-3 of carboxy butyl), 1.55 (m, 2H, H-2 of carboxy butyl); ^{13}C-NMR (DMSO, δ, ppm):174.5(COOH of carboxy butyl), 170.4 (COOH of benzoic acid), 168.3(COOH of carbamoyl group), 146.8 (C-4 of aminobenzamido), 133.7(C-4 of benzoic acid), 130.4(C-2 & C-6 of benzoic acid), 129.2(C-1 of benzoic acid),129.6(C-2 &C-6 of aminobenzamido), 128.6 (C-5 of benzoic acid), 128.2 (C-6 of amino benzamido) 127.8(C-1 of amino bezamido), 115.8 (C-3 & C-5 of amino benzamido), 53.4 (C-4 of carboxybutyl), 48.8(C-1 of carboxy butyl), 28.4 (C-3 of carboxy butyl), 21.9(C-2 of carboxy butyl); MS: m/z: 355(M$^+$), 310 (M$^+$- 45).

Irradiation of Talotrexin under anaerobic condition.

An aqueous solution of talotrexin (1) (170 mg, 0.30 mM) under anaerobic condition was irradiated for 4 h at 354 nm. After following the steps described in the photoirradiation procedure, 2, 4-diamino-6-(hydroxymethyl) pteridine (4, 47 mg) was obtained as main product with a trace amount of 3, as detected on TLC

2, 4-diamino-6-(hydroxymethyl) pteridine (4):

Yield: 47 mg (28%) HRMS calcd. For (M$^+$) $C_7H_8N_6O$ 192.178 found 192.174 IR (KBr) 1595, 3450, 3585cm$^-$^{1},^{1}H-NMR (DMSO, δ, ppm) 8.54 (s, 1H, H-7), 5.30 (s, 4H, 2NH$_2$), 4.79 (s, 2H, 6CH$_2$OH),^{13}C-NMR (DMSO, δ, ppm);162.3 (C-2), 155.4 (C-4), 153.7(C-6), 149.4 (C-8a), 144.8 (C-7), 124.1(C-4a), 65.0 (CH$_2$OH), Ms: m/z: 192 (M$^+$), 175(M$^+$- 17), 161 (M$^+$- 31).

Results and discussion

Irradiation of an aqueous solution of talotrexin with medium pressure mercury vapour lamp in an immersion well type photo reactor gave three photoproducts. 2, 4-diaminopteridine-6-carboxalic acid (2) and 2-4-diamino-6-(hydroxymethyl) pteridine (4) was obtained under aerobic and anaerobic condition respectively. 2-((4-(4-aminobenzamido) - 4-carboxy butyl) Carbamoyl) benzoic acid (3) was obtained under both aerobic and anaerobic conditions. (Scheme 1)

The spectral features correlated to the assigned structure of the main photoproducts and were done in comparison with the spectra of the starting drug. The ^{1}H-NMR spectrum of photoproduct (2) showed signals similar to those of parent drug talotrexin, except for the proton signals of 2-((4-(4-aminobenzamido)-4-carboxy butyl) carbamoyl) benzoic acid moiety. A new signal that appeared at δ 10.9 ppm was assigned to the proton of newly generated -COOH group at C-6 that resulted by the cleavage of C-N bond of the 4(2, 4-diaminonpteridin-6yl) methyl) amino moiety in the starting drug. The ^{13}C-NMR spectrum of
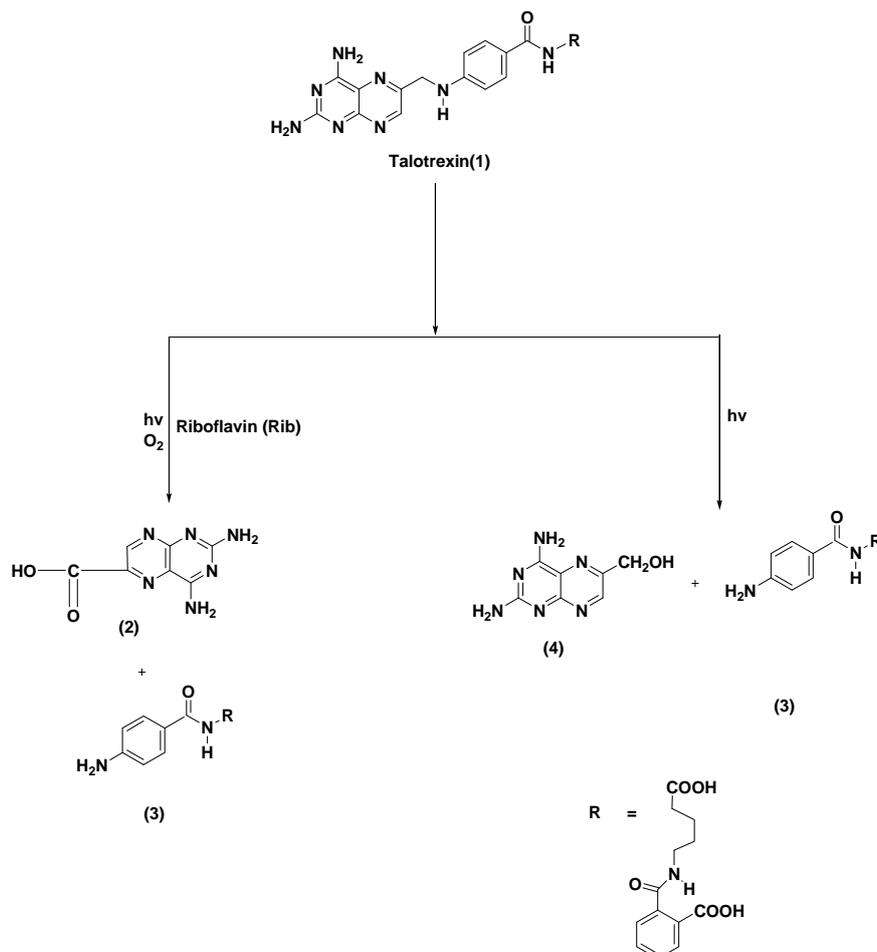
Scheme 1. Photodegradation products of Talotrexin

photoproduct (2) also showed signals similar to those of talotrexin except for the carbon signals of 2-((4-(4-aminobenzamido)-4-carboxy butyl) carbamoyl) benzoic acid moiety. A new signal that appeared at δ 165.6 ppm was assigned to the carbon of –COOH group present at C-6. The ^{1}H-NMR spectrum of photoproduct (4) showed signals similar to those of parent drug talotrexin except for the proton signals of 2-((4-(4-aminobenzamido)-4-carboxy butyl) carbamoyl) benzoic acid moiety. A new signal that appeared at δ 2.0 ppm was assigned to the proton of newly generated –CH$_2$OH group at C-6 that resulted by the cleavage of C-N bond of the 4(2, 4-diaminonpteridin-6yl) methyl) amino moiety in the starting drug. In ^{13}C NMR spectrum of photoproduct (4) the signals of 2-((4-(4-aminobenzamido)-4-carboxy butyl) carbamoyl) benzoic acid moiety did not appear as they were in the starting drug.

The Mechanism of the formation of different talotrexin photoproducts are depicted in scheme-2 and 3. Under aerobic condition photoproducts can be prepared as when an aqueous solution of talotrexin (**1**) with riboflavin as a sensitizer was irradiated triplet excited state of sensitizer (Riboflavin) formed. Triplet excited state of sensitizer then undergo deactivation by an electron transfer to talotrexin and form corresponding radical ions, sens$^{\cdot-}$ and talotrexin$^{\cdot+}$. Talotrexin radical cataion then undergo deprotonation to yield an enamine which on hydrolysis gave 2-((4-(4-aminobenzamido) - 4-carboxy butyl) Carbamoyl) benzoic acid (**3**) and an aldehyde. The aldehyde is finally oxidized due to aerial oxidation and gave photoproduct 2, 4-diaminopteridine-6-carboxalic acid (**2**) (Scheme-2).
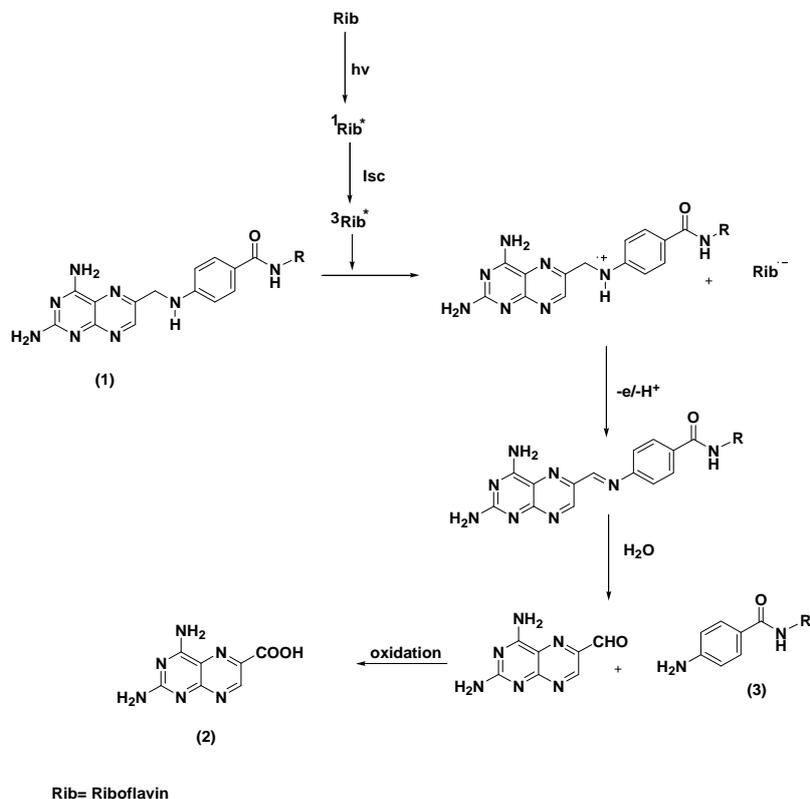
Scheme 2. Mechanistic pathway of photodegradation of Talotrexin under aerobi condition

Alternatively the electron transfer from sens$^{\cdot-}$ to O_2 regenerates Sensitizer and forms $O^{\cdot-}$ (Superoxide radical). The superoxide radical may disproportionate to form H_2O_2. In anaerobic condition photo excited talotrexin undergo intramolecular electron transfer to form radical ion pair which after hydrolysis gave photoproduct 2,4-diamino- 6-hydroxymethylpteridine and 2-((4-(4-aminobenzamido)-4-carboxy butyl) carbamoyl) benzoic acid (**3**) (scheme-3).

Scheme 3. Mechanistic pathway of photodegradation of Talotrexin under an aerobic Condition

Photoproduct 2, 4-diamino-6- hydroxyl methyl pteridine (DHP) exhibits phototoxic effect caused by oxidation of biomacromolecules through photoinduced electron transfer [28].

Conclusion

In conclusion, this work describes that electron transfer play a significancant role in both the phototherapeutic and phototoxic effects of talotrexin. Our results are also of interest in the context of the photodecomposition of talotrexin in aqueous solution and the recent observations of the biological activity of talotrexin decomposition products.

References

[1]   C. Scaiano, S.Giuffrida. Transient photochemistry of naphazoline in a protein environment. New J. Chem, 1999, 23: 1159-1162.
[2]   S. Sortino, S. Petralia, R. Darcy, R.Donohueb, A. Mazzaglia. Photochemical outcome modification of diflunisal by a novel cationic amphiphilic Cyclodextrin., New J. Chem, 2003, 27: 602-608.
[3]   S. Onoue, M.Ochi, G.Gandy, Y.Seto, N. Igarashi, Y. Yamauchi S. Yamada. Photochemical outcome modification of diflunisal by a novel cationic amphiphilic Cyclodextrin. Pharm Res. 2010, 27: 1610–1619.
[4]   J.Eriksson, J.Svanfelt, L.A.Kronberg. Photochemical Study of Diclofenac and Its Major Transformation Products. Photochem.Photobio. 2010, 86: 528-533.
[5]   D.Guidi, S.Ragusa, M.T.Cambria, A.Belvedere, A.Catalfo, A.Cambria, Photosensitizing Effect of Some Nonsteroidal Antiinflammatory Drugs on Natural and Artificial Membranes: Dependence on Phospholipid Composition. Chem. Res. Toxicol, 2005, 18: 204-212.
[6]   M. A. Miranda. Photosensitization by drugs. Pure Appl. Chem, 2001, 73:481-486.
[7]   G. Condorelli, L.Costanzo, G.D.Guidi, S.Giuffrida, S. Sortino. Molecular Mechanism of drug photosensitization Part 7. Photocleavage of DNA sensitized by suprofen. Photochem.Photobiol, 1995, 62: 155-161.
[8]   S. Sortino, G .Cosa, J .C Scaiano. pH effect on the efficiency of the photodeactivation pathways of naphazoline: a combined steady state and time resolved study. New J. Chem, 2000, 24: 59-163.
[9]   M .Zlotorzynska, G .M. Sammis. Photoinduced Electron-Transfer-Promoted Redox Fragmentation of N Alkoxy phthalimides. Org. Lett. 2011, 13: 6264-6267.
[10]   H .R. Memarian, I. Mohammadpoor-Baltork, K. Bahrami. Photoinduced Electron Transfer Reactions of Aryl Benzyl SulfidesPromoted by 2, 4, 6-Triphenylpyrilium Tetrafluoroborate (TP⁺ BF₄⁻). Bull. Korean Chem. Soc, 2006, 27:106-110.
[11]   J. Kou, H. Zhang, Y .Yuan, Z. Li, Y. Wang, T .Yu, Z. Zou. Efficient Photodegradation of Phenanthrene under Visible Light Irradiation via Photosensitized Electron Transfer. J. Phys. Chem. C. 2008, 112: 4291-4296.
[12]   S .Onoue, Y. Yamauchi, T .Kojima, N .Igarashi, Y. Tsuda. Analytical Studies on Photochemical Behavior of Phototoxic Substances; Effect of Detergent Additives on Singlet Oxygen Generation. Pharmaceutical Research. 2008, 25: 861-868.
[13]   E. Liani. L .Rothem, M. A. Bunni, C. A. Smith, G. Jansen, Y. G. Assaraf. Loss of folylpoly-ϒ-glutamate synthetase activity is a dominant mechanism of resistance to polyglutamylation dependent novel antifolates in multiple human leukemia sublines. Int. J. Cancer. 2003, 103, 587-599.
[14]   P. Mayer-Kuckuk, D. Banerjee, S. Malhotra, M. Cells exposed to antifolates show increased cellular levels of proteins fused to dihydrofolate reductase: A method to modulate gene expression. PNAS, 2002, 99, 3400-3405.
[15]   S .Ogwang., H .T Nguyen, M. Sherman, S. Bajaksouzian M. R. Jacobs, W. H Boom, G. Zhang, L. Nguyen. Bacterial Conversion of Folinic Acid Is Required for Antifolate Resistance. J. Biol. Chem, 2011, 286, 15377-15390,.

[16] K.Urakawa, M. Mihara, T. Suzuki, A.Kawamura, K. Akamatsu,Y.Takeda,N .Kamatani. Polyglutamation of antifolates is not required for induction of extracellular release of adenosine or expression of their anti inflammatory effects. Immunopharmacology. 2000, 48: 137-144.

[17] M. Mihara, T. Suzuki, E. Kaneko, N .Takagi, Y.Takeda. Immunosuppressive properties of MX-68, a novel unpolyglutamatable antifolate. Biol Pharm Bull, 1997, 20: 1071-1075.

[18] G.S.A Longo-Sorbello, J .R. Bertino. Current understanding of methotrexate pharmacology and efficacy in, acute leukemias. Use of newer antifolates in clinical trials. Haematologica, 2001, 86: 121–127.

[19] S .K Prajapati, H. Joshi, V. Dev, V. K Dua. Molecular epidemiology of Plasmodium vivax anti-folate resistance in India.Malaria Journal, 2011, 10: 102-109.

[20] C .Clark, L .M Ednie, G. Lin, K. Smith, K. Kosowska- Shick, P. McGhee, B. Dewasse, L. Beachel, P. Caspers B .Gaucher, G. Mert, S. Shapiro, P .C. Appelbaum. "Antistaphylococcal Activity of Dihydrophthalazine Antifolates, a Family of Novel Antibacterial Drugs. antimicrob agents ch, 2009, 53:1353-1361,

[21] J .W. V. Heijden, R. Oerlemans, B. A. C. Dijkmans, H. Qi, C .J. V. Laken, W .F Lems, A. L Jackman, M. Kraan P. P. Tak, M .Ratnam, G. Jansen. Folate Receptor β as a Potential Delivery Route for Novel Folate Antagonists to Macrophages in the Synovial Tissue of Rheumatoid Arthritis Patients. Arthritis & rheumatism, 2009,60: 12-21.

[22] J.J. McGuire. Anticancer antifolates: Current status and future directions. Curr. Pharm. Design, 2003, 9: 2593–2613.

[23] M. L. Dantola, M .P Denofrio, B. Zurbano, C .S Gimenez P. R. Ogilby, C. Lorente, A. H Thomas. Mechanism of photooxidation of folic acid sensitized by unconjugated pterins. Photochem. Photobiol. Sci, 2010, 9: 1604-1612.

[24] R .E Norris, P .C Adamson. Clinical potency of methotrexate, aminopterin, talotrexin and pemetrexed childhood leukemias. Cancer Chemother Pharmacol, 2010, 65, 1125–1130.

[25] N. Hagner, M. Joerger. Cancer chemotherapy: targeting folic acid synthesis. Cancer Management and Research. 2010, 2: 293–301.

[26] C .S Rocha Lima, S .V Orlov, J .Garst, G. M Manikhas A. Dowlati, J.A Quesada, C. Andrews, M. L Ramirez, G Choy, G..Berk. A phase I study of talotrexin (PT-523) in patients with relapsed or refractory non-small cell lung cancer (NSCLC). Journal of Clinical Oncology. 2006, 24: 7142-7146.

[27] H .Okamoto, A. Fukuda, K. Mizuno, N. Matsuyoshi S.Fujii K Imamura. Reactivation of phototoxicity test for psoralens plus ultraviolet A by low-dose methotrexate. Photoimmunol Photomed, 1994, 10: 134-136.

[28] K. Hirakawa, M .Aoshima, Y. Hiraku, S. Kawanishi. Photohydrolysis of Methotrexate Produces Pteridine Which Induces Poly-G– specific DNA Damage through Photoinduced Electron Transfer. Photochem Photobiol, 2002, 76: 467–472.