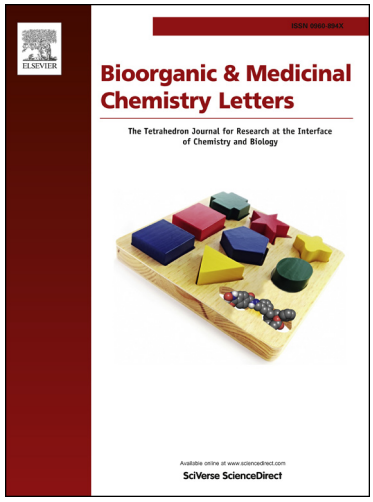

Synthesis, *in vitro* antitumor activity, dihydrofolate reductase inhibition, DNA intercalation and structure-activity relationship studies of 1,3,5-triazine analogues

Prinka Singla, Vijay Luxami, Kamaldeep Paul

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Synthesis, *in vitro* antitumor activity, dihydrofolate reductase inhibition, DNA intercalation and structure-activity relationship studies of 1,3,5-triazine analogues

Prinka Singla, Vijay Luxami and Kamaldeep Paul*

School of Chemistry and Biochemistry, Thapar University, Patiala-147001, India

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ABSTRACT

A series of triazine-benzimidazoles with 4-fluoroaniline substitution has been designed and synthesized. These compounds were further substituted with different primary and secondary amines. The structures of newly synthesized compounds were confirmed by ^{1}H, ^{13}C NMR, mass spectrometry and, in case of compound **18**, by single crystal X-ray diffraction analysis. The newly synthesized compounds were evaluated against 60 human tumour cell lines at one dose and five dose concentration levels. Compounds **7**, **8** and **22** have been found to be the most active antitumor agents with GI_{50} values of 1.77, 1.94 and 2.87 μM respectively. The synthesized compounds were then evaluated for their inhibitory activity to mammalian dihydrofolate reductase. Compound **22** was depicted as the most active compound for the inhibition of dihydrofolate reductase with IC_{50} value of 2.0 nM. DNA binding studies were also revealed strong interacting properties of triazine derivatives towards calf thymus-DNA.

Folate metabolism has been recognized as an attractive target for cancer chemotherapy because of its indispensable role in the biosynthesis of nucleic acid precursors.[1] Dihydrofolate reductase (DHFR) which catalyzes the reduction of folate or 7, 8-dihydrofolate to tetrahydrofolate and intimately couples with thymidylate synthase has been of particular interest. Inhibition of DHFR results in depletion of intracellular reduced folates, which are necessary for one carbon transfer reactions. One carbon transfer reactions are important for the biosynthesis of thymidylate, purine nucleotides, methionine, serine, glycine and many other compounds necessary for RNA, DNA and protein synthesis.[2] Therefore, DHFR represents an attractive target for developing antitumor agents. Several DHFR inhibitors, have found clinical utility as antitumor agents.[3] Because of the frequent occurrence of tumor resistance and ineffectiveness against many solid tumors, various heterocycles have been reported to improve the antitumor spectrum of activity and to circumvent tumor resistance.[4] However, none of these modified analogues showed better DHFR inhibitory or antitumor activity than MTX. *s*-Triazines (1,3,5-triazine) bearing amino groups at C2- and C4-positions have been attracting considerable attention due to their chemotherapeutic potential.[5–10] Substituted 1,3,5-triazine moiety has immense synthetic importance and bioactivity viz., anticancer, antimalarial, antimicrobial, antiviral, antiprotozoal, and antileshmanial. Moreover, triazine analogues also showed dihydrofolate reductase inhibitory properties for an anticancer activity.[11] Derivatives of 1,3,5-triazine, for instance, cycloguanil (**A**), chlorocycloguanil (**B**), clociguanil (**C**), are

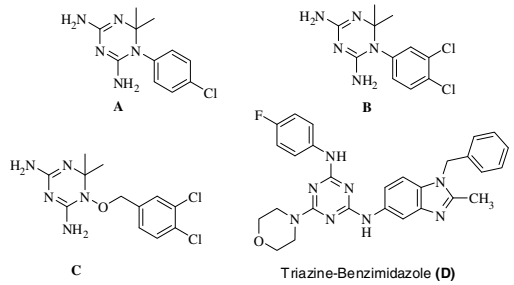

Figure 1. DHFR inhibitors: cycloguanil (**A**), chlorocycloguanil (**B**), clociguanil (**C**) and triazine-benzimidazole (D)

approved drugs for the inhibition of dihydrofolate reductase (Figure 1) and selectively inhibited biochemical processes.[12,13]

On the other hand, benzimidazole, the simplest fused imidazole ring, is a versatile nucleus due to its presence in a wide range of bioactive compounds such as antiparasitic, anticonvulsant, analgesic, antihistaminic, antiulcer, antihypertensive, antiviral, anticancer, antifungal, anti-inflammatory, antioxidant, immunomodulator and anticoagulant.[14] Benzimidazole is also responsible for targeting histone deacetylase 2, the inhibition of phosphodiesterase IV, inhibition of proton pumps and DNA intercalating agent.[15] In order to explore further structure–activity relationships for these classes of compounds, we have combined the two active structural analogues, *s*-triazine and benzimidazole that is enriched with electronic environment to get single molecular framework. The previous results indicated that the 4-fluoroaniline with substitution of secondary amine, morpholine (Figure 1, **D**) showed broad spectrum of antitumor activity

*Corresponding author. Tel.: +91-9465670595; fax: +91-175-236-4498; e-mail: kpaul@thapar.edu