Review Article

Systematic Review of General Cancer Cell Survival Using Target Enzyme MTH1 and its Activity

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ABSTRACT

Cancers have dysfunctional redox regulation and resulting in a reactive oxygen species production and damaging both DNA and free dNTPs. The MTH1 protein sanitizes and oxidized the dNTP pools to prevent the incorporation of damaged bases during DNA replication and its substrates include 8-oxo-dGTP and 2-OH-dATP. Although MTH1 is non-essential in normal cells, Cancer cells require MTH1 activity to avoid the incorporation of oxidized dNTPs mainly resulting in DNA damage and cell death. Recent reports also validated its inhibition as a potential broad spectrum target in oncology and it is described as small molecules that are able to engage this target with anti-proliferative effect. MTH1 is also a member of Nudix phosphohydrolase super family of enzymes (NUDT1). Here we have tabulated 32 compounds out of which 5 compounds are shown to possess excellent MTH1 activity.

Keywords: Purine Derivatives, Anticancer, MTH1 (NUDT1).

INTRODUCTION

The Nudix pyrophosphatase MTH1 is DNA- damage and it is preventing the enzyme. It recognizes and disables the oxidized nucleotides the removal of a pyrophosphate from the damaged nucleotide[1, 2] which subsequently preventing the incorporation in DN or RNA[3]. Free nucleotides are approximately 13,000 times more susceptible to oxidation when compared to nucleic bases and can ultimately lead to point mutations. The enzyme is reported and recognizes a range of substrates including 2-hydroxy-dATP, 2-hydroxy-rATP, 8-oxo-dGTP and 8-oxo-dATP[4, 5, 6].

Cancer cells often exhibit a high level of oxidative stress than is seen in normal tissue and its result is changed in the metabolic pathways, leading an elevated level of oxidized nucleotides. MTH1 is hypothesized to be essential for the survival of tumor cells[7] inhibition of MTH1 has been proposed to cause elevated incorporation of oxidized bases in RNA or DNA, and thereby elevated the levels of mutagenic stress in cancer cells and leading to cell death[8,9]. MTH1 is usually involved in general cancer cell survival.

In a purine macro cycles, compound 1 is exceedingly potent against MTH1 (IC_{50} = o.ooo5µm) and possesses high cellular permeability and good pharmacokinetic properties in rats. Therefore these compounds have high amenability of the target protein towards different chemo types. The biochemical and cellular engagement of MTH1 with selected compounds were independently confirmed by surface Plasmon resonance (compound 1,12-13). The impact on cell viability was observed with table 2 compounds 1 and 12[10]. In compound 17 – (TH287) was (rapidly metabolized in a human and mouse liver microsomes) both *in-vitro* and in vivo via N-dealkylation of the amino methyl substituent[11].

Chemistry

In table 1 Compounds, 26-32 are available from commercial vendors

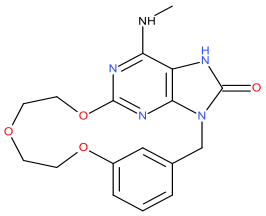
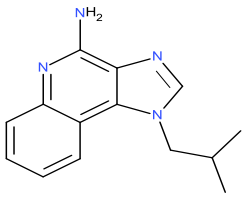
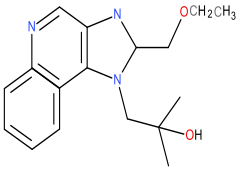
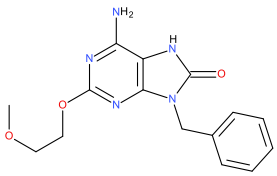
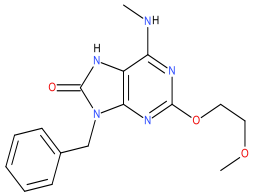
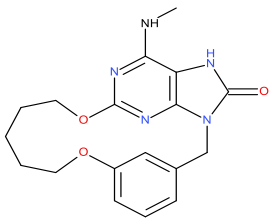
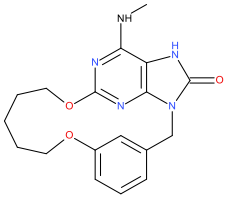
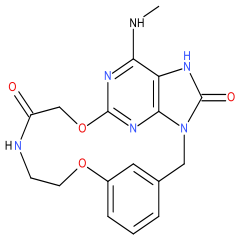
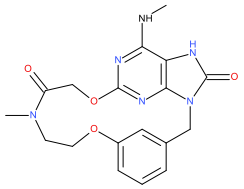
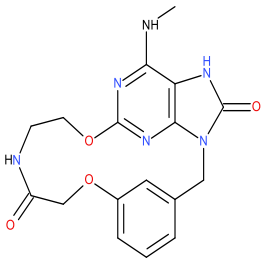
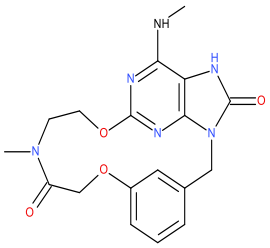
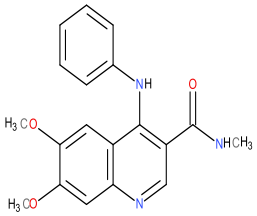
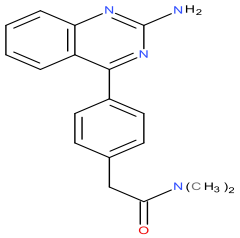
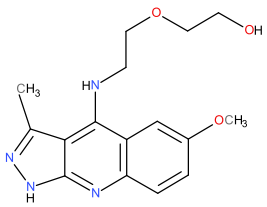
Substrate Km determination

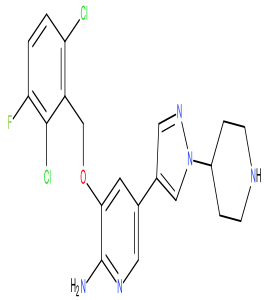
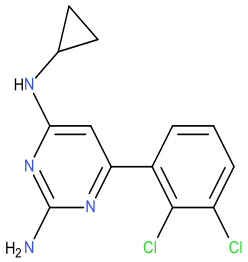
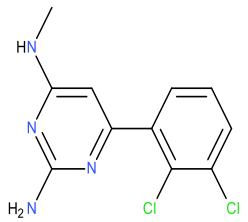
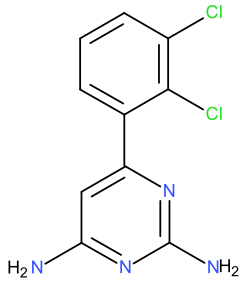
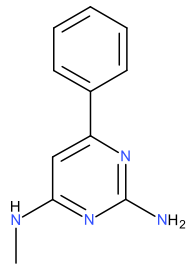
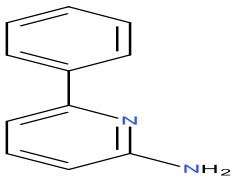
MTH1 substrate Km determination assay conditions were 0.2 nm MTH1 and a concentration the range of substrate from 0–300 µm, diluted in MTH1 reaction buffer. Substrates 8-oxo-dGTP, 8-oxo-rGTP, 8-oxo-dATP, 8-oxo-rATP and 2-OH-rATP, all oxidized ATP-derived its substrates were tested and using an assay format based on an enzymatic hydrolysis of the nucleotide substrate by purified human recombinant MTH1 to form the corresponding monophosphate nucleotide and pyrophosphate[12]. An excess of inorganic pyrophosphatase was added to the assay, which allows the quantification of released inorganic phosphate as a measure of product levels in a coupled enzymatic assay[13].

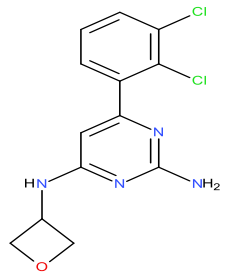
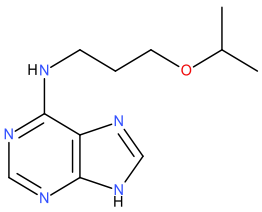
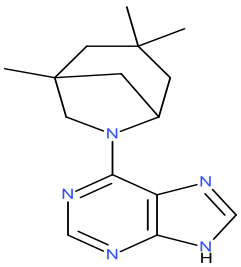
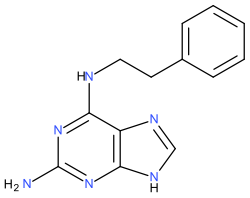
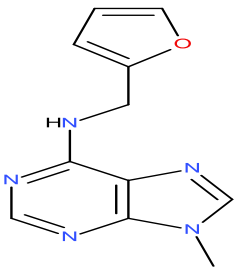
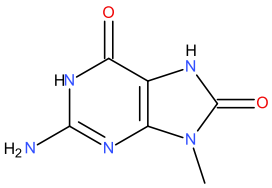
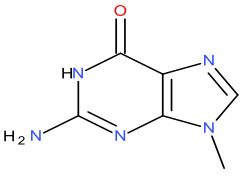
Table 1 shows the MTH1 inhibitory potencies of its range of TLR7 modulators known in the literature. Imiquimod in table 1 compound 2 itself has reasonable submicromolar potency, but Resiquimod, table 1 compound 3 which is known to be a much more potent TLR7 agonist and it shows slightly reduced affinity for MTH1. In table 1 compound 4 is from a distinct series of orally bioavailable purine TLR7 agonists, that elicits a TLR7 –mediated cellular response with similar potency[14].The closely related series of TLR7 agonist purposes and indicate that the exocyclic NH_2 motif is critical for TLR7 activity[15].

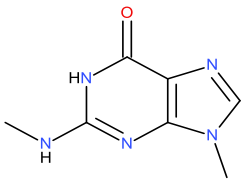
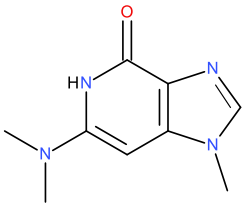
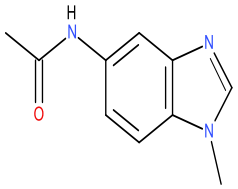
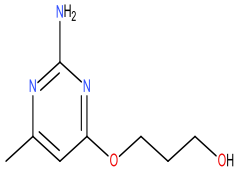
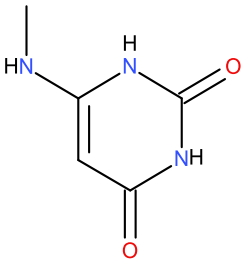
RESULTS AND DISCUSSIONS

Table 1: MTH1 inhibitor with their corresponding IC50 values the enzymatic assay using 8-oxo-dGTP as the substrate.

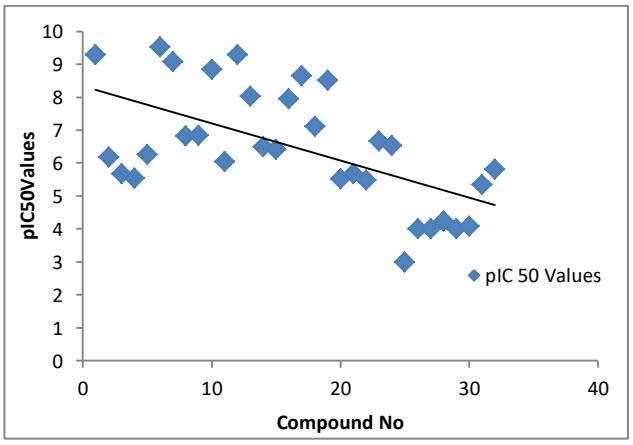
Compound no	Structure	IC_{50} VALUES (µm)	pIC_{50} VALUES (µm)
1.		0.0005 (15)	9.30
2.		0.646 (4)	6.19
3.		2.056 (25)	5.67
4.		2.809 (26)	5.55
5.		0.536 (27)	6.27
6.		0.0003 (28)	9.52
7.		0.0008 (29)	9.09
8.		0.153 (30)	6.82
9.		0.140 (31)	6.85
10.		0.0014 (32)	8.85
11.		0.888 (33)	6.05
12.		0.0005 (6)	9.30
13.		0.009 (7)	8.04
14.		0.32 (3)	6.49

15.		0.39 (4)	6.41
16.		0.011 (2)	7.96
17.		0.0022 (1)	8.66
18.		0.077 (5)	7.11
19.		0.003 (1)	8.52
20.		3 (1)	5.52

21.		2.1 (4)	5.68
22.		3.3 (NPD15095)	5.48
23.		0.21 (NPD7155)	6.67
24.		0.29 (NPD9948)	6.54
25.		1000 (NPD8880)	3.0
26.		100 (1)	4
27.		100 (2)	4

28.		59 (3)	4.23
29.		100 (4)	4
30.		80 (5)	4.09
31.		4.5 (6)	5.35
32.		1.5 (7)	5.82

The above following compounds and pIC_{50} values are plotted as a graph



In the above given table of 32 compounds, the following five compounds were found to have high MTH1 inhibitor activity that is Compound 1(IC_{50}= 0.0005µm), Compound 12 (0.0005µm),Compound 13(0.009µm), Compound 17 (IC_{50}= 0.0022µm), Compound 19 (IC_{50} =0.003)

CONCLUSIONS

MTH1 inhibition has been proposed to "eradicate cancer" through a novel non-oncogene addiction by targeting its function of hydrolyzing oxidized nucleotides, preventing their incorporation of DNA during replication and suppressing the accumulation of otherwise lethal levels of DNA damage. However, it is not known at what level so oxidized nucleotides are expected to be lethal to cells . Indeed, deleting the bacterial MTH1 does not lead its cellular lethality despite the accumulation of thousands of mutations[16]. When incorporated in DNA, the oxidation product of dGTP, 8- oxo-dGTP, can be translated to an adenine rather than a cytosine moiety during replication, thereby leading to a transversion[17]. Being the enantiomer Compound 15 is in an acceptable property range, but its weaker activity, combined with its potential for additional off-target kinase activities, may contribute to the observed anti proliferative activity[18]. MTH1-deprived clones proved to be as viable as their wild-type counterparts, and the growth of both types of cell line diminished similarly upon treatment with inhibitors 16 and 15[19].

As the Nudix family of enzymes to which MTH1 belongs comprises more than 20 genes and other DDR pathways (eg: base excision repair) exist which repair oxidized bases in DNA. In selective small molecule inhibitors, the MTH1 does not support its claimed role of MTH1 being essential for cancer - cell survival.

Abbreviations: MTH1- Multi forms of human polypeptides, TLR7- Toll like Receptor

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