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Pharmocophoric Studies of Anti-Telomerase Drugs

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Abstract:
Human Telomeres are the repetitive DNA sequences of TTAGGG/ AATCCC that protects chromosomes from the loss of essential genes. Telomeres are replicated by telomerase, a special polymerase that has own RNA template. Telomerase is universal marker of human cancer. The cancer cells have neoplastic immortalization rendered by upregulation of telomerase. Rationally, telomerase is an attractive target of cancer therapeutics. Anti-telomerase drugs are the novel enzyme inhibitors. We made a non-computational effort for the basic pharmocophoric design. It is based on the binding functions, , mode of actions, structural resemblances and correlation of reported IC_{50} values. Pyrimidine nucleosides, non-nucleosides, HIV-RTIs, Cartenoids, Flavonoids, synthetic chrome and phenyl derivatives were pharmocophorically studied. A chemical hybrid structure for anti-telomerase drug was proposed.

Keywords: *Human cancer, Binding functions, Telomerase, Pharmocophoric, IC_{50} values, Planarity, Chemical Hybrid.*

1. Objective of Study

Telomerase[1-5] is a specific target for anti-cancer therapy. Anti-telomerases are the most modern and novel therapeutical agents. We, therefore studied, variety of structures to design a rational anti-cancer hybrid drug of pharmocophoric character

2. Introduction

Human telomeres are repetitive DNA sequences of TTAGGG/AATCCC[6-7] that protect chromosomes from loss of essential genes. Telomeres are replicated by telomerase, a special polymerase that has own RNA template. Telomere replication follows the conventional semi-conservative polymerase mechanism.

Telomerase is universal marker of human cancer[8]. Telomeres are molecular clocks that count the number of cell-division and determine the occurrence of cellular senescence and crisis. The cancer cells have neoplastic immortalization rendered by upregulation of telomerase. The tumorogenesis involves replicative senescence (irreversible growth arrest due to telomere shortening) and mitotic catastrophe (abnormal DNA damage leading to cellular mortality). The net consequence is that check point genes in cell-cycle fail in normal cell due to critical telomere shortening.

Human cancer cells are characterized by:-

- Molecular abbreviations due to telomeric uncapping. Cell Proliferation is beyond regulatory mechanism.
- Rate of mitosis follows first order of cytokinetics, over expression of telomerase activity
- Telomere shortening becomes critical
- Telomeres do not deplete cancer cells
- Cell unables to distinguish between double stranded breaks and natural chromosomes.

Rationally telomerase is an attractive target for cancer therapeutics[8]. Anti-telomerase drugs are novel enzyme inhibitors[9-11].

Telomerase is made of reverse transcriptase (TERT) and RNA template[12-15] that dictates the synthesis of G-rich strands of telomere terminal repeats whereas retroviral reverse transcriptase has large hydrophobic amino acids and tyrosine residues.

3. Theoretical Methodology

The pharmacophore models for nucleosides, non-nucleosides, and G-quadruplex iHibitors derived by using Discovery Studio 3 software[16-17]. We made a non-computational effort for the basic pharmacophoric designing. The pharmacophoric studies are principally based on the binding functions and mechanism of actions. The structural diversity of anti-telomerases suggests that binding and interaction with active site of reverse transcriptase may have slight variations in amino acids at site of action. The hypothetical deduction of pharmacophoric structure[18] within catalyst limits allows hydrophobic aliphatic, aromatic-pi-stacking, H-bond donor and H-bond acceptor binding features. The pharmacophoric contributions made by nucleosides, non-nucleosides, cartenoids, flavonoids and substituted aryl compounds as telomerase iHibitors in cancer cells, were interpreted in the terms of binding features, structural resemblances and their correlations with reported IC_{50} or EC_{50} values and possibly with mode of actions. HIV- RT shares telomerase's enzymological composition. Therefore, HIV-1 RTIs considered plausible drugs for anti-telomerase action.

4. Discussion and Result

4.1. Pyrimidine Nucleosides[19-21]

They have structural resemblance with HIV-RTIs. Epigenetic modifiers, Azacitidine and Decitabine share common nucleoside chemistry except variations in ribose moiety of Laminvudine (2/-deoxy-3/-thiarabiose). They act as demethylating agents (Methylation of telomeric DNA is iHibited). Their IC_{50} (nM) values for telomerase inhibition are:-

- AZT (HIV-RTIs), Zidovudine 140 nM
- Lamivudine (HIV-RTIs) 163 nM
- 5-Azacytidine (epigenetic modifier) 370 nM
- Decitabine (epigenetic modifier) 438 nM

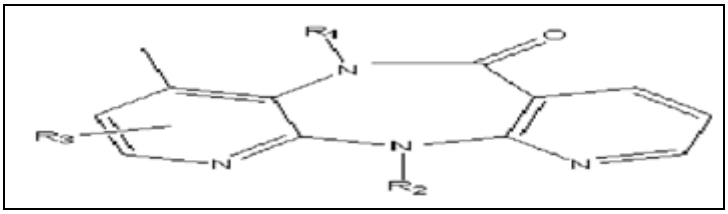
The pharmacophoric contribution is insignificant due to their anti-metabolite action. RT is common site of action for telomerase and HIV-RTIs. The wide difference in **IC_{50}** values may be due to different mode of actions. RTIs produce bioactivated triphosphate metabolite which causes termination of growing DNA chain. HIV-1 RTIs are good therapeutical candidates for combinational therapy with epigenetic modifiers.

4.2. Non-Nucleoside HIV-RTIS[19,22]

They have exquisite selectivity. They do not require bioactivation by kinases to triphosphate and do not attach into growing DNA chain. They bind to allosteric site of RT that distinct from the substrate binding site (nucleoside triphosphate). They bind RT near catalytic site and instantly denature it by the non-competitive mechanism.

Nevirapine and their analogs were considered, they share telomerase iHibition activity by blocking transcriptational activity of TERT gene expression.

The IC_{50} values (Table-1) indicated that isopropyl, 4-methyl and pyridyl (Biosteric to aryl) impart the best activity. The pharmacophoric contributions of five analogs have common numbers of aromatic-pi-stacking, H-bond donor and H-bond acceptor functions. They are heteroaryls (-NH, C=O) and heteroatoms (N, O). The aliphatic hydrophobic variation ranges from methyl to ethyl. Cyclopropyl appears to be quite favorable. Alkyl groups at R_1 and R_3 may be in close proximity, so their binding experiences steric hindrance. The absence of alkyl groups at these positions eliminates steric bulk.

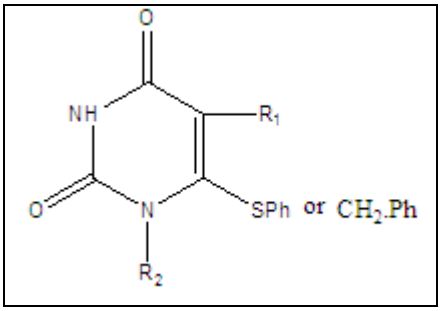


NEVIRAPINE ANALOGS

ANALOGS	R_1	R_2	R_3	IC_{50} values(nM)
1	H	c-Pr	4-Me	0.084
2	CH_3	C_2H_5	H	0.125
3	CH_3	C_2H_5	2-Me	0.17
4	CH_3	C_2H_5	3-Me	0.76
5	H	C_2H_5	4-Cl	0.095

Table 1: IC_{50} values of Nevirapine analogs HIV-1 RT inhibition[22]

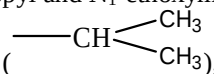
The analogs of 6- phenylthiothymine[22] have good HIV-1-RT inhibitory activity.



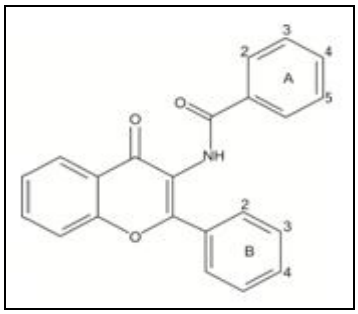
ANALOGS	R1	R2	IC_{50} values(nM)
1	CH_3	$CH_2.O.CH_2.Ph$	0.088
2	C_2H_5	$CH_2.O.CH_2.CH_3$	0.019
3	i-Pr	$CH_2.O.CH_2.CH_3$	0.012
4	i-Pr	$CH_2.O.CH_2.Ph$	0.88
5	i-Pr	$CH_2.O.CH_2CH_3$	0.0041

Table 2: IC_{50} values of 6- phenylthiothymine analogs

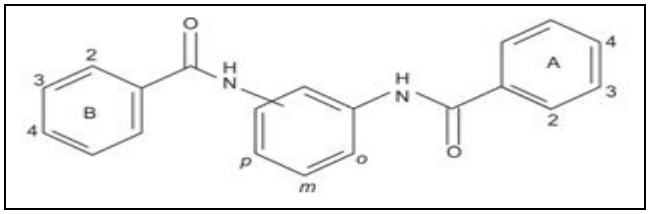
The replacement of 6-thiophenyl by $-CH_2.Ph$, with C_5-isopropyl and N_1-ethoxymethyl gave an analog having IC_{50} value 0.004.

The pharmacophoric contributions of aliphatic hydrophobic (), H-bond acceptor (C=O) and aromatic stacking aryl group are favorable for binding at hydrophobic molecular surface of the enzyme.

Recently, synthetic chrome and phenyl derivatives showed promising telomerase inhibition[23].



-OH position of A moiety	-OH position of B Moiety	IC_{50} (µM)
3,4	3,4	0.38



substituted position	-OH position of A moiety	-OH position of B moiety	IC_{50} (µM)
A. o	3,4	3,4	0.72
B. m	2,3	2,3	0.67

The synthetic chromonal compound has aromatic-pi-stacking, three lone pairs on oxygen atom for H-bond acceptor and one H-bond donor. The phenyl analogs A and B have same aromatic-pi-stacking, two hetero atoms of oxygen as H-bond acceptors and two –H as H-bond donors. The ortho phenolic groups are less favorable for the activity than meta phenolic groups. The aliphatic hydrophobic groups are missing in both categories of synthetic compounds. It may be obvious reason for their large $\mathbf{IC_{50}}$ values than non-nucleosides.

Cartenoids[24-26]: - All the cartenoids telomerase inhibitor have all trans-pi-bonds in the aliphatic chain. The chemopreventive Vitamins A, E, K, curcumin have methyl hydrophobic (-OH), H-bond donor, aryl-pi-stacking pharmacophoric functionalities. The absence of amino group reduces the H-bond donor ability.

Flavonoids[27-29]: - Genestein has chrome nucleus and phenolic groups. The pharmacophoric contribution is exclusively aryl-pi-stacking and H-bond acceptor heteroatom oxygen. They are not very effective telomerase inhibitors but their anti-oxidative action may involve chelation with Mg^{++} and Mn^{++} cations which are important for telomerase reverse transcriptase mechanism.
The pharmocophoric conclusions derived by theoretical studies of four chemical categories are: -

- Thymine DNA base without pentose sugar has promising telomerase inhibition.
- Tricyclic diazepine with 6-membered heterocyclic (preferably pyridyl) with strained cyclopropyl and absence of steric bulk around -4-hydrophobic groups favor anti-telomerase action.
- Ortho phenolic, amido, and aryl groups offer optimal aromatic-pi-stacking, H-bond acceptor and H-bond donor abilities
- The dominance of planarity in aliphatic chain due to trans-pi-bonds or unsaturated functions contribute to DNA interaction.

An ideal chemical hybrid structure for telomerase inhibition in cancer cells should have functionalities, imparting three types of bioactions in monotherapy or combinational therapy. They are: -

- Telomerase inhibition
- Antioxidative
- Immunopotentiation

The telomeric shortening, free radical damage to mitochondrial integrity and over expression of hTERT in cancer cells, should be therapeutically curbed.
The pharmocophoric composition of anti-telomerase drug should have binding mode of four functions: -

- Aliphatic Hydrophobic: - Methyl, Isopropyl
- Aromatic-pi-stacking: - Aryl or isosteric aryl
- H-bond acceptor: - Heteroatoms, N, O
- H-bond donor: - NH, OH

5. Conclusion

Telomerase is universal marker of human cancer. The cancer cells have neoplastic immortalization due to upregulation of this enzyme. Telomerase is, therefore, an attractive target for anti-cancer design. The pharmocophoric studies of various telomerase inhibitors were made by non-computational approach to ascertain basic binding functionalities for enzyme inhibition. The pharmocophoric composition of an anti-telomerase drug should be chemobiologic hybrid of three bioactions and four binding functionalities.

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