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Design, Synthesis and biological evaluation of diazeno-thiazole derivatives as ribonucleotide reductase inhibitors

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Abstract: Ribonucleotide reductase(RNR) is a metalloenzyme that catalyses the rate limiting step in DNA synthesis and repair. It causes the reduction of ribonucleotide to 2'-deoxyribonuclotides which are used as precursors for DNA synthesis, thus offering a good target for inhibition of cell synthesis. Experimental results have been proven that RNR inhibitors can be used as antiviral, anticancer or antibacterial agents. Here we report the synthesis of a novel class of diazeno-thiazole derivatives as potent RNR inhibitors. A series of forty molecules were synthesized and evaluated for their RNR inhibitory properties. All compounds were found to be good inhibitors of the RNR. Compound 3iwas found to be most active showing an IC_{50} value of 0.8 µm. The established SAR study indicated the presence of a polar bridge with an adjacent flexible aromatic ringprerequisite for RNR inhibitory activity. Moreover, compounds having an additional 4-chloro phenyl ring were found to be most potent.

Key words: Ribonucleotide reductase(RNR); diazeno-thiazole derivatives; RNR inhibitor

1. Introduction

Ribonucleotide reductase (RNR) is an iron dependent enzyme, catalysing the reductive step of ribonucleotides to deoxyribonucleotides (dNTPs) conversion. These dNTPs are involved in the synthesis of precursors required for DNA synthesis and repairing of all living cells.[1-3] RNR regulates the total DNA synthesis so that constant DNA to cell mass ratio is maintained during cell division and cell repair.[4] There are three different types of RNR.[5, 6] Class I RNR is functioning in eukaryotic cells, viruses and some bacteria. It is made up of two non-identical proteins R1 and R2. The latter is a small protein in which each polypeptide chain contains a differic cluster and neighbouring tyrosyl residue. The tyrosyl free radical is generated by conversion of ferrous to ferric and causes reduction of NDP under aerobic conditions. The reduction of ribonucleotides is the rate limiting step in DNA synthesis. Therefore, inhibition of RNR stops the DNA synthesis and lead to termination of cell proliferation. Moreover, it is noted that RNR shows low activity in resting cells and more activity in rapidly growing (malignant) cells. Shao[7] Thus,this can provide the practically assured therapeutic target for cancer therapy. In the last few decades,the use of RNR inhibitors, such as hydroxyurea,[8] trimodox,[9] triapine,[10, 11] methisazone, PIH and cytarabine[9] has been established in the treatment of various types of cancer such as non-small cell lung cancer, adenocarcinoma of pancreas, bladder cancer, leukemia and some solid tumors.[12]

Moorthey *et al*. reviewed the structural features of semicarbazones and thiosemicarbazones prerequisite for RNR inhibitory activity by 3D QSAR studies.[9] It was postulated that presence of polar bridges having H-bond donor or acceptor with flexible geometry is important to coordinate with iron. The adjacent aromatic ring with steric bulkiness is favourable for the hydrophobic interactions with tyrosine residue. Moreover, one additional H-bond acceptor coordinates with the metal ion and H-bond donor with flexible geometry is important for activity. By taking these ideas in mind, we hypothesized that hybridizing these two structures might give a new scaffold which could have RNR inhibitory activity (Figure 1). We designed the new class of diazeno-thiazole that could be used for RNR inhibitory activity.

2. Result and Discussion

Inspired by our new lead molecule a series of forty molecules (Table1) were synthesized through the reactions outlined in Scheme1 as reported by our group earlier.[13] This was achieved by reaction of thiosemicarbazide with acetophenone or benzaldehyde in methanol. The catalytic amount of acetic acid was used to drive the reaction smoothly. The hydrazine carbothio amide thus obtained was further treated with phenacyl bromide to get the desired product (**3a-3an**). All the compounds were characterized by Mass and NMR spectroscopy.

All the synthesized compounds were screened for anticancer activity against HL-60 cell line at three different time intervals 24, 48 and 72 hrs as shown in

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Table 1. Seven compounds (**3o, 3p, 3x, 3f, 3i, 3z, 3r**) were found to be most active with IC_{50} values at low micromolar range. The compound **3i** is the most potent with an IC_{50} value of 0.8 µM. It was found to have all the structural features as reviewed by Moorthy *et al*. Substitution of R with methyl and additional functional groups at *para*- position of ring A (R_1) was found to decrease the potency of the compounds

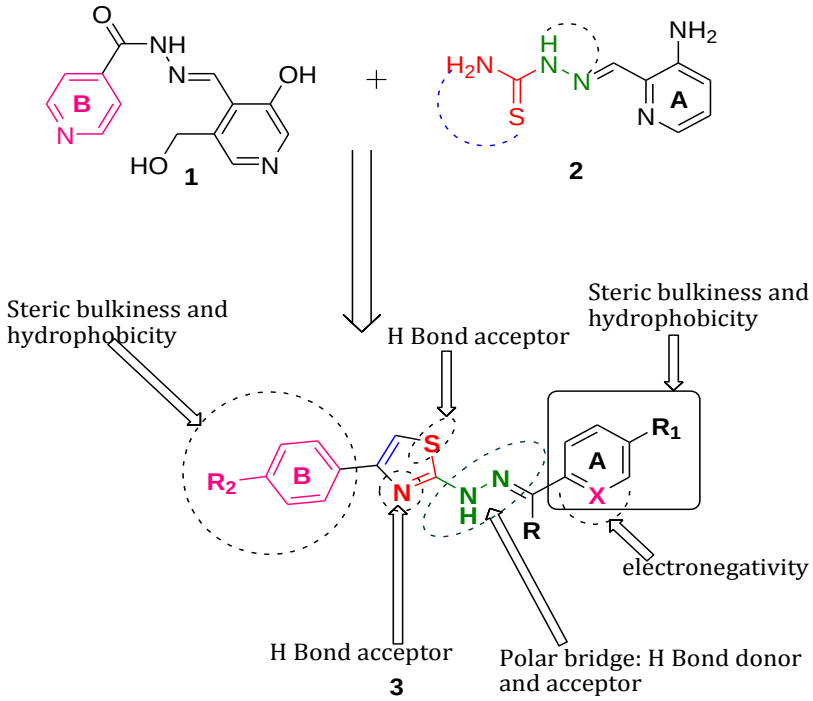


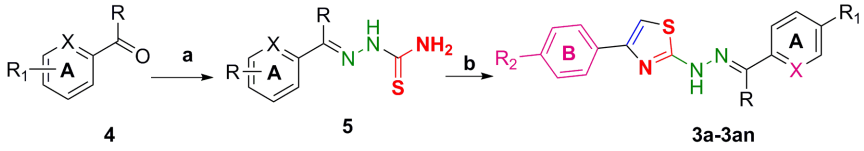
Figure 1. Chemical structures of potent RNR inhibitors **1** and **2** design of 'diazeno-thiazole' 3 scaffold as a prospective RNR inhibitor

(**3a, 3b, 3c, 3d**), Bio-isosteric replacement of C2' with N of ring A retained the potency of compound (**3f**). Moreover, electron donating or withdrawing group at R_1 did not display any improvement in activity (**3g**, **3h**, **3j, 3l, 3k, 3m**). Replacement of R_2 chloro with phenyl imparts hydrophobicity to the compounds but it did not enhance the potency of molecules (**3u, 3v, 3w, 3n**) except for compounds **3o** and **3p** where it has OH and OCH_3 at R_1 position with IC_{50} values of 2.8 and 5.3 µM, respectively. Substitutions other than at *para*-position in ring A (R_1) cause decrease in biological activity (**3aa, 3ab**, and **3ac-3an**).

Molecular modelling studies performed by using the crystal structure of ribonucleotide reductase (PDB id: 1W68), revealed that compound **3i** displayed two polar H-bonding interaction with Tyr324 and one with Ser264. It is also having hydrophobic interactions with Phe245, Phe348. The compound positioned in the active site pocket in such a way that thiazole moiety had л-л interaction with Phe241 and ring B with Tyr324. As Tyr324 is adjacent to ferrous ion, it played a vital role in generation of tyrosyl free radical and localization of free radical required for the reduction of NDP. The two polar H-bonding interactions and one л-л interaction with Tyr324 hypothesized to inhibit effectively the generation of free tyrosyl radical or localization of radical. Additionally, one H-bonding interaction with Ser264 and two hydrophobic interactions would stabilise the binding of compound **3i** with active site residues. Overall, these interactions of diazeno-thiazole derivates with RNR would explain the potent inhibition of HL-60 cell lines by this series of compounds.

3. Conclusion

At last, hybridizing and altering the structure of triapine and PIH we got novel diazeno-thiazole derivatives as RNR inhibitors. A series of forty molecules were synthesized and evaluated against the HL-60 cell line. The compound **3i** was found to be most active IC_{50} value of 0.8 µM. Bio-isosteric replacement of C2 with N led to the decrease in activity (**3l**). It was observed that on replacing of 4-Cl group with phenyl ring of ring B, the molecules lost their activity. Any substitutions at other than *para*-position in ring A decreased the potency of molecule. Structural features and modelling studies revealed that this series of compounds may act by inhibiting RNR and are required to be confirmed by molecular level studies. The potent compounds are under rigorous biological screening to unravel their mechanism of action and will be reported shortly.



Scheme 1. Reagents and conditions:(a) MeOH, NH_2-CS-NH-NH_2, (AcOH glacial), rt, 2 hrs; (b) MeOH, R_2-C_6H_4-CO-CH_2Br, rt, 30-60 min stirring.

Table 1. Growth inhibitory activity of compounds 3a-3an against HL-60 cell line

Sr No.	Code	X	R	R^1	R^2	HL60 Cell Line IC_{50} µM		
						72 hrs	48 hrs	24 hrs
1.	3a	CH	$-CH_3$	-H	-Cl	60	70	70
2.	3b	CH	$-CH_3$	4-Cl	-Cl	75	95	/
3.	3c	CH	$-CH_3$	4-OH	-Cl	50	50	70
4.	3d	CH	$-CH_3$	$4-OCH_3$	-Cl	70	77	/
5.	3e	CH	$-CH_3$	$4-NO_2$	-Cl	90	/	/
6.	3f	N	$-CH_3$	-H	-Cl	5.4	5.4	7.4
7.	3g	CH	-H	-H	-Cl	65	80	/
8.	3h	CH	-H	4-Cl	-Cl	60	75	/
9.	3i	CH	-H	4-OH	-Cl	800nM	1	90
10.	3j	CH	-H	$4-OCH_3$	-Cl	-	-	-
11.	3k	CH	-H	$4-NO_2$	-Cl	60	60	/
12.	3l	N	-H	-H	-Cl	1.4	2	12
13.	3m	CH	$-CH_3$	-H	$-C_6H_5$	5.7	5.7	9.9
14.	3n	CH	$-CH_3$	4-Cl	$-C_6H_5$	-	-	-
15.	3o	CH	$-CH_3$	4-OH	$-C_6H_5$	2.8	3.6	75.8
16.	3p	CH	$-CH_3$	$4-OCH_3$	$-C_6H_5$	5.3	7.4	37.8
17.	3q	CH	$-CH_3$	$4-NO_2$	$-C_6H_5$	9	9.9	93.3
18.	3r	N	$-CH_3$	-H	$-C_6H_5$	2	2	5
19.	3s	CH	-H	-H	$-C_6H_5$	-	-	-
20.	3t	CH	-H	4-Cl	$-C_6H_5$	-	-	-
21.	3u	CH	-H	4-OH	$-C_6H_5$	10	42.4	/
22.	3v	CH	-H	$4-OCH_3$	$-C_6H_5$	-	-	-
23.	3w	CH	-H	$4-NO_2$	$-C_6H_5$	7.1	7.6	67.8
24.	3x	N	-H	-H	$-C_6H_5$	3.2	4	6.5
25.	3y	CH	-H	3- OCH_3-4-OH	$-C_6H_5$	-	-	-
26.	3z	CH	-H	3- OCH_3-4-OH	-Cl	3.6	4	34.9
27.	3aa	CH	-H	4- OCH_3-3-OH	$-C_6H_5$	-	-	-
28.	3ab	CH	-H	4- OCH_3-3-OH	-Cl	25	55	/
29.	3ac	CH	$-CH_3$	$2-OCH_3$	-Cl	30	60	/
30.	3ad	CH	$-CH_3$	$2-CH_3$	-Cl	40	60	/
31.	3ae	CH	$-CH_3$	3-OH	-Cl	30	55	95
32.	3af	CH	$-CH_3$	3- OCH_3-4-OH	-Cl	6.2	7.9	/
33.	3ag	CH	$-CH_3$	$3-NO_2$	-Cl	65	80	/
34.	3ah	CH	-H	$3-SO_3H$	-Cl	-	-	-
35.	3ai	CH	$-CH_3$	2-OH	-Cl	55	85	/
36.	3aj	CH	$-CH_3$	$3-OCH_3$	-Cl	50	65	100
37.	3ak	CH	$-CH_3$	$3-CH_3$	-Cl	60	70	/
38.	3al	CH	-H	3-OH	-Cl	17	40	65
39.	3am	CH	-H	2-OH	-Cl	/	/	/
40.	3an	CH	-H	2-Cl	-Cl	50	65	/

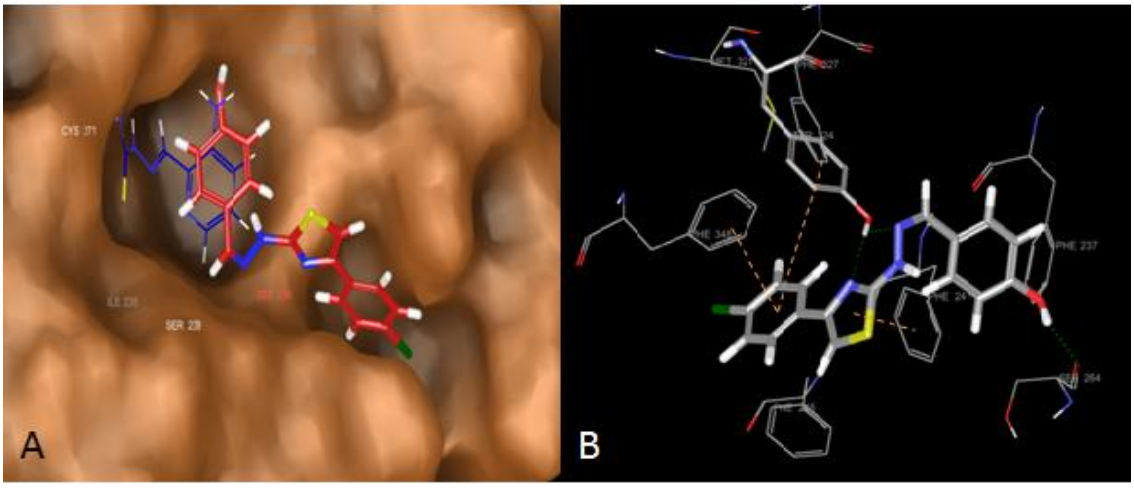


Figure 2. A. Molecular surface diagram of 3i (red colour) and triapine (Blue colour); B. 3D interactive diagram of 3i (Green line; polar hydrogen bond, orange line hydrophobic interaction) (PDB id: 1W68)

4. Experimental

Materials and methods: All the reagents and chemicals were of LR grade and purchased from Sigma-Aldrich (Munich, Germany). Solvents were used without purification unless otherwise specified. Reactions were monitored using precoated TLC plates (Merck) either in Iodine chamber or in UV chamber. The intermediates were characterized by their melting point using Optimelt (Stanford Research System, Sunnyvle, CA 94089) and IR Spectra (IR Prestige 21, Shimadzu Corporation, Japan). Final compounds were characterized by their ^{1}HNMR (300 MHz, Varion) and ESI-MS (Waters-Q-T of Premier-HAB213). Computational studies were carried out in Dell Precision Workstation with Intel Core 2 Quad processor and 8 GB RAM.

4.1. Molecular docking studies

The crystal structure of ribonucleotide reductase enzyme was retrieved from the Protein Data Bank (www.rcsb.org, PDB id: 1W68). It was prepared for docking simulation using Protein Preparation Wizard implemented in Maestro v8.5 (Schrodinger, Inc., New York, NY, 2005) with default parameters. A grid was generated using Glide→ Grid Generation tool implemented in Maestro v8.5 by specifying active site residues. Triapine has been used as standard molecule for comparison. All the ligands sketched using built panel implemented in Maestro v8.5. They were then prepared for docking simulation using Ligprep utility implemented in Maestro v8.5. Glide→Docking module was then used to run the docking simulation with XP protocol. In all the cases default parameters were used.

4.2. General procedure for the synthesis of (2*E*)-2-benzylidene/-(1-phenylethylidene)-hydrazinecarbothio amide

To a solution of acetophenone/ benzaldehyde (0.01 M) and thiosemicarbazide (0.012 M) in methanol (25 mL), a catalytic amount of acetic acid glacial (0.2 mL) was added. The mixture was then stirred for a period of 2-4 h. The solution was then diluted with ice water (50 mL) and the solid obtained was filtered, dried and recrystallized from hot methanol.

4.3. General procedure for the synthesis of 2-[(2*E*)-2-benzylidene/-(1-phenylethylidene)-hydrazinyl]-4-phenyl -1,3-thiazole (3a-3an)

To a solution of hydrazinecarbothioamide (0.01 M) in methanol (25 mL), Appropriate phenacyl bromide (0.01 M) was added after which the mixture was stirred for a period of 30-60 min. The solution was diluted with ice water (50 mL) and the solid obtained was filtered, dried and recrystallized from methanol.

4.4. Cell culture

The human HL-60 promyelocytic leukemia cell line was purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). Cells were grown in RPMI 1640 medium with L-Glutamine and 25mM HEPES supplemented with 10% heat inactivated foetal calf serum (FCS), 1% L-Glutamine and 1% Penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO_2 using a Heraeuscytoperm 2 incubator (Heraeus, Vienna, Austria). Cell counts were determined using a microcell counter CC-110 (SYSMEX, Kobe, Japan). Cells growing in the logarithmic phase of growth were used for all experiments described below.

4.5. Growth inhibition assay

HL-60 cells ($0.1x10^6$ per mL) were seeded in $25cm^2$Nunc tissue culture flasks and incubated with increasing concentrations of drugs at 37 °C under cell culture conditions. Stock solutions were diluted in DMSO. Cell counts and IC_{50} values (IC_{50} = 50% growth inhibition of tumor cells) were determined after 24, 48, and 72 hours using the microcell counter CC-110. All experiments were performed in triplicate and repeated twice.

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References

1. Graslund, A.; Sahlin, M. Electron paramagnetic resonance and nuclear magnetic resonance studies of class I ribonucleotide reductase. Annu Rev Biophys Biomol Struct 1996, 25 (1), 259-286.
2. Reichard, P.; Ehrenberg, A. Ribonucleotide reductase--a radical enzyme. Science 1983, 221 (4610), 514-519.
3. Gräslund, A.; Ehrenberg, A. Tyrosyl radical, diiron center and enzyme mechanism in ribonucleotide reductase. Appl Magn Reson 2007, 31 (3), 447-455.
4. Herrick, J.; Sclavi, B. Ribonucleotide reductase and the regulation of DNA replication: an old story and an ancient heritage. Mol Microbiol 2007, 63 (1), 22-34.
5. Nordlund, P.; Reichard, P. Ribonucleotide reductases. Annu Rev Biochem 2006, 75, 681-706.
6. Jordan, A.; Reichard, P. Ribonucleotide reductases. Annu Rev Biochem 1998, 67 (1), 71-98.
7. Shao, J.; Zhou, B.; Chu, B.; Yen, Y. Ribonucleotide reductase inhibitors and future drug design. Curr Cancer Drug Targets 2006, 6 (5), 409-431.
8. Seiwert, T. Y.; Haraf, D. J.; Cohen, E. E.; Stenson, K.; Witt, M. E.; Dekker, A.; Kocherginsky, M.; Weichselbaum, R. R.; Chen, H. X.; Vokes, E. E. Phase I study of bevacizumab added to fluorouracil-and hydroxyurea-based concomitant chemoradiotherapy for poor-prognosis head and neck cancer. J Clin Oncol 2008, 26 (10), 1732-1741.
9. SHN Moorthy, N.; MFSA Cerqueira, N.; J Ramos, M.; A Fernandes, P. Development of ribonucleotide reductase inhibitors: a review on structure activity relationships. Mini Rev Med Chem 2013, 13 (13), 1862-1872.
10. Nutting, C.; Van Herpen, C.; Miah, A.; Bhide, S.; Machiels, J.-P.; Buter, J.; Kelly, C.; De Raucourt, D.; Harrington, K. Phase II study of 3-AP Triapine in patients with recurrent or

metastatic head and neck squamous cell carcinoma. Ann Oncol 2009, mdn775.

11. Ma, B.; Goh, B. C.; Tan, E. H.; Lam, K. C.; Soo, R.; Leong, S. S.; Wang, L. Z.; Mo, F.; Chan, A. T.; Zee, B. A multicenter phase II trial of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine®) and gemcitabine in advanced non-small-cell lung cancer with pharmacokinetic evaluation using peripheral blood mononuclear cells. Invest New Drugs 2008, 26 (2), 169-173.

12. Cerqueira, N. M.; Pereira, S.; Fernandes, P. A.; Ramos, M. J. Overview of ribonucleotide reductase inhibitors: an appealing target in anti-tumour therapy. Curr Med Chem 2005, 12 (11), 1283-1294.

13. Jadav, S. S.; Kaptein, S.; Timiri, A.; De Burghgraeve, T.; Badavath, V. N.; Ganesan, R.; Sinha, B. N.; Neyts, J.; Leyssen, P.; Jayaprakash, V. Design, synthesis, optimization and antiviral activity of a class of hybrid dengue virus E protein inhibitors. Biorg Med Chem Lett 2015, 25 (8), 1747-1752.