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Design, Synthesis and Anti-proliferative Activity of Noval 1,2,4-Triazine and Pyrrolidin-2-one Derivatives

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ABSTRACT

1,2,4-Triazine and pyrrolidin-2-one derivatives with 1,4 substituted Salicyaldehydes were designed and synthesized in order to obtain new compounds with potential anti-proliferative activity. The structures of all synthesized compounds were confirmed by means of various spectroscopic analytical techniques. The two dimensional chemical structures of compounds were submitted to National Cancer Institute (NCI), under the Developmental Therapeutic Program (DTP). The compounds were selected for screening based on novelty, size and their physicochemical properties like log P, number of rotatable bonds, molecular weight etc. The selected samples were synthesized and send to NCI. The experimental drugs were analyzed at a single dose of 10^{-5} M against 60 different human tumor cancers of the lung, colon, brain, ovary, breast, prostate and kidney. Compounds TP24, PTM6 and PTM8 showed maximum activity out of all synthesized compounds. Molecular docking studies of most active compounds were also performed and revealed that they bind to podophyllotoxin binding pocket of the gamma tubulin protein.

Keywords: Anti-proliferative agents, Cancer cell lines, 1,2,4-Triazine, Pyrrolidin-2-one

INTRODUCTION

Cancer is the second most common cause of human death in the world with 11 million new incidents each year. It is responsible for one in eight deaths worldwide. There are over 100 different types of cancers. Various genes monitor and control the cell proliferation and growth. The genes which mediate and promote cell proliferation and growth are oncogenes whereas, which inhibit cell proliferation and growth are tumor suppressor genes. Lack of equilibrium between oncogenes and tumor suppressor genes leads to cancer. The main characteristic of auto-cancer cells are insensitivity to growth signal inhibition, escape of programmed cell death, unlimited replicative potential, sustained angiogenesis, Tissue invasion and metastasis and instability of genome of cancer that allows cancer progression [1].

1-3 Substituted Triazine derivatives reported to have diverse chemical reactivity and broad spectrum of biological activity such as antitumor, antimicrobial, antitubercular, antimalarial, anticonvulsant, anthelmintic, analgesic and anti-inflammatory activity. Pyrrolidin-2-one has been reported to have anti-inflammatory, anticonvulsant and protein kinase inhibition potential [2-7].

γ-tubulin is a potential target protein for new anticancer drugs and It was found that γ-tubulin activates during cell division and overexpressed in cancer cells. Glaziovianin A is reported to inhibit γ-tubulin protein in cancer cells. Field mapping of glaziovianin A was used to design its field based analogs with similarity more than 0.50 using Forge V 10, Cresset software. All the compounds follows rule of 5. Field alignment studies of most active compounds are showing in Figure 1. The data, of all synthesized compounds, was submitted to National Cancer Institute (NCI) for anti-proliferative activity. The compounds were selected for screening based on novelty, size and their physicochemical properties like log P, Number of rotatable bonds, molecular weight etc. The selected samples were synthesized and send to NCI. The experimental drugs were analyzed at a single dose of 10^{-5} M against 60 different human tumor cancers of the lung, colon, brain, ovary, breast, prostate and kidney. As per the protocol of NCI, Eleven representative compounds, of the series, were selected and granted NSC D-codes. Compound TP24 (NSC D-783148), PTM6 (NSC D-783152), PTM8 (NSC D-782530) exhibited the best result at single dose [8-14].

MATERIALS AND METHODS

Designing of compounds

Molecular field mapping and alignment approach was used to design all the compounds using Forge V 10, Cresset software. 2D structures of the

analogs were made and converted to 3D structures. Conformers of the analogs were generated, energy minimized and aligned with field points of reference compound. The field alignment score of each designed compound was calculated in comparison to field pattern of reference compound. Physical properties, total no of conformers, Molecular weight, total number of atoms, 2D structure similarities, SlogP, Topological Surface Area (TPSA), flexibility of bonds and rule of five (rof 5) are also calculated and compared to reference compound [15,16].

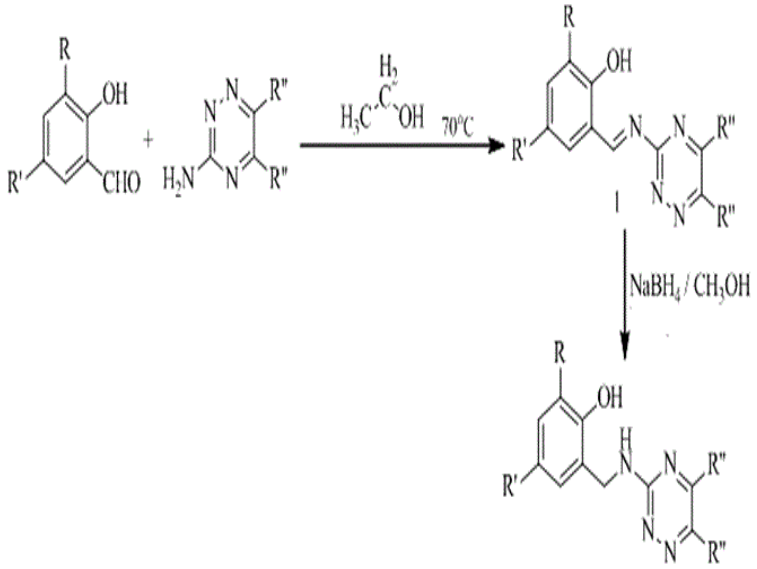
Chemistry

The laboratory grade & analytical grade chemicals and solvents were bought from authorized distributors of Fisher Scientific, Sigma Aldrich, S.D. Fine Chemicals and CDH companies. The open tube capillary method was used to determine the melting points of synthesized compounds and uncorrected. The reaction completion and purity of compounds was done by Thin Layer Chromatography (TLC). TLC solvent systems; chloroform: methanol in the ratio 6:4, ethyl acetate: Hexanes in the ratio 5:5 were used to develop the TLC for compounds of Schemes 1 and 2 respectively. The TLC spots were visualized by using iodine vapors/UV light (254 nm). Bruker AMX 400 was used to obtain Proton Nuclear Magnetic Resonance (H-NMR) and Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) spectrum. Bruker ATR instrument was used to obtain IR spectrums. Proton spectrum and 13C spectrums were recorded at 400 MHz and 100 MHz respectively. An API 3000 MS Q3 (Shimadzu) spectrometer was used to record Mass spectrum.

General method for the synthesis of 1,2,4-triazine derivatives

Step 1: 1,2,4-triazin-3-amine (1.0 mol) and respective aldehyde compound (2 mol) were dissolved in 20 ml ethanol in two separate beakers. Respective aldehyde solution (20 ml) was added drop by drop to the solution of 1,2,4-triazin-3-amine (20 ml). The resulting mixture was transfer to round bottom flask and refluxed for 4 h. The completion of reaction was monitored by TLC. The mixture was cooled; Schiff's base was separated, filtered and dried. The dried Schiff's base was dissolved in a minimum quantity of chloroform and petroleum ether (1:1) and allowed to set at 10°C for 12 h. Microcrystalline powder was separated out.

Step 2: Schiff base (25 mmol) was dissolved in methanol (20 ml) by gentle warming and stirring in a beaker. In another beaker, Sodiumborohydride (79 mmol) was dissolved in 5 molar solution of sodium hydroxide in water. Sodiumborohydride solution was slowly added to the solution of Schiff base and resulting mixture was stirred for 2 h at 20-25°C. The reaction was monitored by TLC. After completion of the reaction, the solution was diluted with water and extracted with ethyl acetate or dichloromethane (3 × 60 ml) using a separating funnel. The ethyl acetate or dichloromethane extracts were passed through anhydrous sodium sulphate bad, and concentrated under reduced pressure. Further purification was done by using mixture of solvents ethyl acetate and petroleum ether (50:50) (Scheme 1) [10,17].



Code	Subst.	R	R'	R''
TP11	2-OH	3-OCH3	H	CH_3
TP13	2-OH	Cl	Cl	CH_3
TP14	H	H	5-OCH_3	CH_3
TP17	3-OH	4-OCH_3	-	CH_3
TP 22	2-OH	Cl	Cl	H
TP24	H	H	4-OCH_3	H

Scheme 1: Synthesis route of 1,2,4-triazine derivatives

2-(((5,6-dimethyl-1,2,4-triazin-3-yl)amino)methyl)-6-methoxyphenol (TP11): Brown colour solid, M.P. 134-136°C, m/z: m/z: 233.09 (100.0%), 234.09 (12.7%); ($C_{10}H_{11}N_5O_2$) Molecular weight: 233.23. ATR FTIR (cm^{-1}):3330 (-NH- str.), 3588–3200 (-OH- str., Broad), 1650-1550 (C-N- str.), 2145-2120 (N=C=N- str.). ^{1}H-NMR (300 MHz, DMSO-d_6), δ=6.83-6.60(m, 2H), 6.72 (dd, J=6.4, 2.7 Hz, 1H), 6.32-6.22 (m, 2H), 4.80-4.83 (dd, J=5.5, 0.9 Hz, 2H), 3.87 (s, 3H), 2.86 (s, 3H), 2.75 (s, 3H); ^{13}C-NMR (300 MHz, DMSO-d_6), δ=160.38, 158.85, 147.45, 146.24, 144.88, 126.39, 122.41, 122.26, 110.83, 56.01, 40.35, 21.48, 18.07. Elemental Analysis: C, 51.50; H, 4.75; N, 30.03; O, 13.72; Found: C, 51.51; H, 4.72; N, 30.10; O, 13.71.

2,4-dichloro-6-(((5,6-dimethyl-1,2,4-triazin-3-yl)amino)methyl)phenol (TP13): Brown colour solid, M.P. 141-142°C, m/z: 298.04 (100.0%), 300.04 (64.3%), 299.04 (14.5%), 302.03 (10.2%), 301.04 (8.4%), 303.04 (1.4%); ($C_{12}H_{12}Cl_2N_4O$), Molecular weight: 299.16. ATR FTIR (cm^{-1}):3330 (-NH- str.), 3588-3200 (-OH- str., Broad), 1650-1550 (C-N- str.), 2145-2120 (N=C=N- str.).^{1}H-NMR (300 MHz, DMSO-d_6), δ=8.06 (s, 1H), 7.20 (d, J=2.2 Hz, 1H), 7.07-7.09 (dt, J=2.2, 1.0 Hz, 1H), 6.32-6.36 (t, J=5.5 Hz, 1H), 4.86-4.88 (dd, J=5.5, 1.0 Hz, 2H), 2.83 (s, 3H), 2.71 (s, 3H).; ^{13}C-NMR (300 MHz, DMSO-d6) δ 160.38, 158.85, 149.37, 146.24, 128.22, 127.66, 127.47, 127.30,120.98, 39.33, 21.48, 18.07. Elemental Analysis: C, 48.18; H, 4.04; Cl, 23.70; N, 18.73; O, 5.35; Found: C, 48.17; H, 4.06; Cl, 23.68; N, 18.73; O, 5.25.

N-(4-methoxybenzyl)-5,6-dimethyl-1,2,4-triazin-3-amine (TP 14): Brown colour solid, M.P. 154-155°C, m/z: 244.13 (100.0%), 245.14 (14.3%), 245.13 (1.5%), 246.14 (1.2%); ($C_{13}H_{16}N_4O$) Molecular weight: 244.29. ATR-FTIR (KBr) (cm^{-1}):3330 (-NH- str.), 3588-3200 (-OH- str., Broad), 1650-1550 (C-N- str.), 2145-2120 (N=C=N- str.). ^{1}H-NMR (300 MHz, DMSO-d_6), δ=7.17 (dt, J=8.5, 1.0 Hz, 2H), 6.94-6.84 (m, 2H), 5.82 (t, J=5.5 Hz, 1H), 4.77 (dt, J=5.5, 1.0 Hz, 2H), 3.79 (s, 3H), 2.81 (s, 3H), 2.70 (s, 3H). ^{13}C-NMR (300 MHz, DMSO-D_6), δ=160.54, 158.92, 158.85, 146.24, 133.08, 129.04, 113.68, 55.32, 44.61, 21.48, 18.07. Elemental Analysis: C, 63.91; H, 6.60; N, 22.93; O, 6.55; Found: C,

63.92; H, 6.61; N, 22.91; O, 6.45.

5-(((5,6-dimethyl-1,2,4-triazin-3-yl)amino)methyl)-2-methoxyphenol (TP17): Brown colour solid, M.P. 133-134°C, m/z: 260.13 (100.0%), 261.13 (14.3%), 262.13 (1.5%), 261.12 (1.5%); ($C_{13}H_{16}N_4O_2$), Molecular weight: 260.29. ATR FTIR (cm^{-1}): 3330 (-NH- str.), 3588–3200 (-OH- str., Broad), 1650–1550 (C-N- str.), 2145-2120 (N=C=N- str.). ^{1H}-NMR (300 MHz, DMSO-d_6), δ=7.80 (s, 1H), 6.73 (dt, J=9.7, 1.1 Hz, 3H), 5.93 (t, J=5.5 Hz, 1H), 4.85-4.87 (dt, J=5.5, 0.7 Hz, 2H), 3.81 (s, 3H), 2.82 (s, 3H), 2.70 (s, 3H); ^{13}C-NMR (300 MHz, DMSO-d_6), δ=160.54, 158.85, 146.43, 146.24, 146.12, 133.65, 119.92, 114.57, 112.72, 56.17, 44.68, 21.48, 18.07. Elemental Analysis: C, 59.99; H, 6.20; N, 21.52; O, 12.29; Found: C, 59.96; H, 6.18; N, 21.50; O, 12.27.

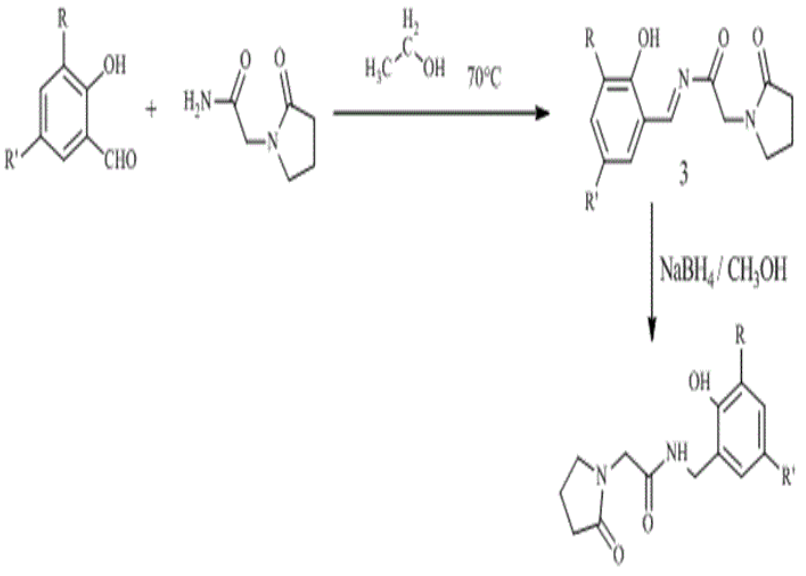
2-(((1,2,4-triazin-3-yl)amino)methyl)-4,6-dichlorophenol (TP 22): Brown colour solid, M.P. 109-110°C, m/z: 270.01 (100.0%), 272.00 (63.9%), 271.01 (10.9%), 274.00 (10.3%), 273.01 (7.0%), 271.00 (1.5%), 275.00 (1.3%); ($C_{10}H_8Cl_2N_4O$) Molecular Weight: 270.10. ATR FTIR (cm^{-1}): 3330 (-NH- str.), 3588-3200 (-OH- str., Broad), 1650-1550 (C-N- str.), 2145-2120 (N=C=N- str). ^{1}H-NMR (300 MHz, DMSO-d_6), δ=9.00 (d, J=1.3 Hz, 1H), 8.31 (d, J=1.3 Hz, 1H), 7.97 (s, 1H), 7.20 (d, J=2.2 Hz, 1H), 7.09 (dt, J=2.1, 1.0 Hz, 1H), 6.92 (t, J=5.5 Hz, 1H), 4.86 (dd, J=5.5, 1.0 Hz, 2H). ^{13}C-NMR (300 MHz, DMSO-D_6), δ=162.07, 149.60, 149.37, 138.06, 128.22, 127.66, 127.47, 127.30, 120.98, 39.36. Elemental Analysis: C, 44.30; H, 2.97; Cl, 26.15; N, 20.67; O, 5.90 found: C, 44.29; H, 2.87; Cl, 26.05; N, 20.57; O, 5.89.

N-(4-methoxybenzyl)-1,2,4-triazin-3-amine (TP24): Brown colour solid, M.P. 121-122°C, m/z: 216.10 (100.0%), 217.10 (13.4%); ($C_{11}H_{12}N_4O$) Molecular Weight: 216.24. ATR FTIR (cm^{-1}):3330 (-NH- str.), 3588-3200 (-OH- str., Broad), 1650-1550 (C-N- str.), 2145-2120 (N=C=N- str.). 1H NMR (300 MHz, DMSO-d_6), δ=9.08 (d, J=1.2 Hz, 1H), 8.37 (d, J=1.3 Hz, 1H), 7.25-7.29 (dt, J=8.4, 1.0 Hz, 2H), 6.91-6.87 (m, 2H), 6.38-6.42 (t, J=5.5 Hz, 1H), 4.74-4.77 (dt, J=5.5, 1.0 Hz, 2H), 3.78 (s, 3H). ^{13}C-NMR (300 MHz, DMSO-D_6), δ=162.18, 158.92, 149.60, 138.06, 133.08, 129.04, 113.68, 55.32, 44.64. Elemental Analysis: C, 61.10; H, 5.59; N, 25.91; O, 7.40; Found: C, 61.00; H, 5.59; N, 25.89; O, 7.39.

General method for the synthesis of pyrrolidin-2-on derivatives

Step 1: 2-(2-oxopyrrolidin-1-yl) acetamide (1.0 mol) and respective aldehyde compound (2 mol) were dissolved in 20 ml ethanol in two separate beakers. Respective aldehyde solution (20 ml) was added drop by drop to the solution of 2-(2-oxopyrrolidin-1-yl) acetamide (20 ml). The resulting mixture was transfer to round bottom flask and refluxed for 4 h. The completion of reaction was monitored by TLC. The mixture was cooled; Schiff's base was separated, filtered and dried. The dried Schiff's base was dissolved in a minimum quantity of chloroform and petroleum ether (1:1) and allowed to set at 10°C for 12 h. Micro-crystalline powder was separated out.

Step 2: Schiff base (25 mmol) was dissolved in methanol (20 ml) by gentle warming and stirring in a beaker. In another beaker, Sodiumborohydride (79 mmol) was dissolved in 5 molar solution of sodium hydroxide in water. Sodiumborohydride solution was slowly added to the solution of Schiff base and resulting mixture was stirred for 2 h at 20-25°C. The reaction was monitored by analysis of TLC. After completion of the reaction, the solution was diluted with water and extracted with ethyl acetate or dichloromethane (3× 60 ml) using a separating funnel. The ethyl acetate or dichloromethane extracts were passed through anhydrous sodium sulphate bad, and concentrated under reduced pressure. Further purification was done by using a mixture of solvents ethyl acetate and petroleum ether (50:50) (Scheme 1) [10,17].



Code	Subst.	R	R'	R''
PTM1	2-OH	3-Cl	5-Cl	-
PTM2		3-OCH_3	4-OH	-
PTM5		H	4-OCH_3	-
PTM6	2-OH	3-OCH_3	H	-
PTM8	2-OH		5-OCH_3	

Scheme 2: Synthesis route of pyrrodine-2-one derivatives

N-(3,5-dichloro-2-hydroxybenzyl)-2-(2-oxopyrrolidin-1-yl)acetamide (PTM1): Pale white colour solid, M.P. 153-144°C, m/z: 405.94 (100.0%), 403.94 (51.0%), 407.93 (48.2%), 406.94 (14.3%), 404.94 (7.3%), 408.94 (7.0%), 407.94 (1.7%). ($C_{13}H_{14}C_{l2}N_2O_3$, Molecular weight: 317.17). ATR FTIR (cm^{-1}): 3330 (-NH- str.), 3588-3200 (-OH- str., Broad), 16300-1690 (C=O- str.), 2275-2250 (O=C-N- str.); ^{1}H-NMR (300 MHz, DMSO-d_6), δ=8.18-8.22 (t, J=6.2 Hz, 1H), 7.96 (s, 1H), 7.16-7.10 (m, 2H), 4.38-4.41 (dd, J=6.1, 1.0 Hz, 2H), 4.04 (s, 2H), 3.36-3.40 (t, J=4.9 Hz, 2H), 2.29-2.36 (d, J=5.5, 1.3 Hz, 2H), 1.89-1.82 (m, 2H); ^{13}C-NMR (300 MHz, DMSO-D_6), δ=176.59, 169.82, 149.06, 128.22, 127.66, 126.89, 120.98, 47.92, 46.04, 38.75, 30.72, 18.11. Elemental Analysis: C, 38.45; H, 3.48; Br, 39.35; N, 6.90; O, 11.82, found: C, 38.35; H, 3.38; Br, 39.15; N, 6.90; O, 11.62.

N-(4-hydroxy-3-methoxybenzyl)-2-(2-oxopyrrolidin-1-yl)acetamide (PTM2): Yellowish white colour solid, M.P. 146-147°C, m/z: 278.13 (100.0%), 279.13 (15.5%), 280.13 (2.0%). ($C_{14}H_{18}N_2O_4$, Molecular weight: 278.30). ATR FTIR (cm^{-1}): 3330 (-NH- str.), 3588-3200 (-OH- str.,

Broad), 1630-1690 (C=O- str.), 2275-2250 (O=C-N- str.); ^{1}H-NMR (300 MHz, DMSO-d_6), δ=7.43 (t, J=6.2 Hz, 1H), 7.26 (s, 1H), 6.719-6.7 (m, 3H), 4.24-4.26 (dt, J=6.2, 0.9 Hz, 2H), 4.04 (s, 2H), 3.83 (s, 3H), 3.40 (t, J=4.9 Hz, 2H), 2.30-2.34 (t, J=5.5 Hz, 2H), 1.82-189 (ddd, J=10.4, 5.5, 4.9 Hz, 2H); ^{13}C-NMR (300 MHz, DMSO-D_6), δ=176.59, 169.91, 147.45, 145.52, 132.88, 121.35, 115.61, 111.70, 55.98, 47.92, 46.04, 43.29, 30.72, 18.11. Elemental Analysis C, 60.42; H, 6.52; N, 10.07; O, 23.00; Found: C, 60.24; H, 6.20; N, 10.1; O, 22.90.

N-(4-methoxybenzyl)-2-(2-oxopyrrolidin-1-yl)acetamide (PTM5): Yellowish white colour solid, M.P. 137-138°C, m/z 262.13 (100.0%), 263.14 (15.5%), 264.14 (1.7%). ($C_{14}H_{18}N_2O_3$, Molecular weight: 262.13). ATR FTIR (cm^{-1}): 3330 (-NH- str.), 3588-3200 (-OH- str., Broad), 1630–1690 (C=O- str.), 2275-2250 (O=C-N- str.); ^{1}H-NMR (300 MHz, DMSO-d_6), δ=7.21-7.25 (t, J=6.2 Hz, 1H), 7.11-7.15 (dt, J=8.4, 1.0 Hz, 2H), 6.92-6.87 (m, 2H), 4.10-4.16 (dt, J=6.1, 1.0 Hz, 2H), 4.03 (s, 2H), 3.78 (s, 3H), 3.36-3.40 (t, J=4.9 Hz, 2H), 2.30-2.34 (t, J=5.5 Hz, 2H), 1.82-1.89 (ddd, J=10.4, 5.5, 4.9 Hz, 2H); ^{13}C-NMR (300 MHz, DMSO-D_6), δ=176.59, 169.91, 158.85, 133.38, 128.72, 113.83, 55.32, 47.92, 46.04, 43.87, 30.72, 18.11. Elemental Analysis C, 64.10; H, 6.92; N, 10.68; O, 18.30; Found: C, 64.09; H, 6.91; N, 10.65; O, 18.26.

N-(2-hydroxy-3-methoxybenzyl)-2-(2-oxopyrrolidin-1-yl)acetamide (PTM6): Yellowish white colour solid, M.P. 131-132°C, m/z: 278.13 (100.0%), 279.13 (15.5%), 280.13 (2.0%). ($C_{14}H_{18}N_2O_4$, Molecular weight: 278.30). ATR FTIR (cm^{-1}): 3330 (-NH- str.), 3588-3200 (-OH- str., Broad), 1630-1690 (C=O- str.), 2275-2250 (O=C-N- str.). ^{1}H-NMR (300 MHz, DMSO-d6), δ=8.01 (t, J=6.2 Hz, 1H), 6.87-6.72 (m, 2H), 6.70 (dd, J=7.3, 1.9 Hz, 1H), 6.32 (s, 1H), 4.32-4.34 (dd, J=6.1, 0.9 Hz, 2H), 4.03 (s, 2H), 3.87 (s, 3H), 3.37- 3.40 (t, J=4.9 Hz, 2H), 2.26-2.36 (td, J=5.5, 1.3 Hz, 2H), 1.82-1.89 (qd, J=5.5, 4.8 Hz, 2H); ^{13}C-NMR (300 MHz, DMSO-D_6), δ=176.59, 169.82, 147.45, 144.56, 126.57, 122.41, 121.91, 110.83, 56.01, 47.92, 46.04, 39.78, 30.72, 18.11. Elemental Analysis C, 60.42; H, 6.52; N, 10.07; O, 23.00; Found: C, 60.24; H, 6.20; N, 10.1; O, 22.90.

N-(2-hydroxy-5-methoxybenzyl)-2-(2-oxopyrrolidin-1-yl)acetamide (PTM8): Yellowish white colour solid, M.P. 135-137°C, m/z: 278.13 (100.0%), 279.13 (15.5%), 280.13 (2.0%). ($C_{14}H_{18}N_2O_4$, Molecular Weight: 278.30). ATR FTIR (cm^{-1}): 3330 (-NH- str.), 3588–3200 (-OH- str., Broad), 1630–1690 (C=O- str.), 2275-2250 (O=C-N- str.); ^{1}H-NMR (300 MHz, DMSO-d_6), δ=8.26 (s, 1H), 7.98-8.02 (t, J=6.2 Hz, 1H), 7.34-7.37 (dd, J=8.4, 2.3 Hz, 1H), 6.88-6.79 (m, 2H), 4.29- 4.31 (dd, J=6.2, 1.0 Hz, 2H), 3.97 (s, 2H), 3.77 (s, 3H), 3.36-3.40 (t, J=4.9 Hz, 2H), 2.30-2.34 (t, J=5.5 Hz, 2H), 1.82-1.89 (ddd, J=10.4, 5.5, 4.9 Hz, 2H); ^{13}C-NMR (300 MHz, DMSO-D_6), δ=176.59, 169.82, 151.53, 148.40, 126.99, 116.22, 113.95, 113.52, 55.29, 47.92, 46.04, 39.93, 30.72, 18.11. Elemental Analysis C, 60.42; H, 6.52; N, 10.07; O, 23.00; Found: C, 60.24; H, 6.20; N, 10.1; O, 22.90.

Criterion for submission and selection of compounds for testing in the NCI screens

The 2 D structures of compounds were submitted to National cancer institute, under the Developmental Therapeutic Program (DTP). The compounds were selected for screening based on novelty, size and their physicochemical properties like log P, Number of rotatable bonds, molecular weight etc.

The selected samples were synthesized and send to NCI. The experimental drugs were analyzed at a single dose of 10^{-5} M against 60 different human tumor cancers of the lung, colon, brain, ovary, breast, prostate and kidney. The tumor cell lines of the cancer screening panel were grown in Roswell Park Memorial Institute medium containing 5% Fetal Bovine Serum (FBV) and 2 millimolar L-glutamine. The cell lines were inoculated into 96-wells high throughput screening plate at strength ranging from 5,000 to 40,000 cells per well. The above 96-wells plate was incubated at favourable growing conditions, 37°C temperature, 95% air, 5% CO_2 and 100% humidity. Two walls of each cell line were stabilized with cold Tricarboxylic Acid (TCA), which represents cell strength for each cell line at the moment of experimental sample addition (Tz). The experimental sample and 50 µg/ml gentamicin were also added to each wells of 96 wells plate and incubated for another 48 hours under the same conditions. After 48 h, cold TCA was added to each well and the plate was cooled at 4°C for 60 min.

The supernatant liquid of each well was disposed of and the plate was rinsed with water and dried. The staining reagent, 100 µl, 0.4% sulforhodamine B solution in 1% acetic acid, was added to each well and the plates were incubated at 37°C for 10 min. Unreacted staining reagent was washed out with 1% acetic acid. The plates were dried and stained cells were dispersed in 10 mM trizma base. The trizma base acquired the colour of live cells. The colour intensity of trizma base was measured by reading absorbance on a plate reader at a wavelength of 515 nm [18], Formula:

$$[(-Ti-Tz/(C-Tz)] \times 100 \text{ for concentration for which } Ti \geq Tz$$

$$[(-Ti-Tz/(C-Tz)] \times 100 \text{ for concentration for which } Ti < Tz$$

The absorbance, Measurements [Time zero, (Tz), Control growth, (C) and Test growth in the presence of drug at the 10^{-5} M concentration level (Ti)] was read and used to calculate the percentage growth

Molecular modelling

The receptor model was built by using AutoDock Tools 1.4.6 and MGL Tools 1.5.4 packages. 3D ligand structures were prepared by optimizing and defining geometry and partial charge by using MMFF94 and Gasteiger respectively. Molecular Docking were carried out on 1SA1 protein model. Proteins were obtained from Protein data bank RCSB portal. All type of charges, H_2 atoms, and factors of solvation were defined and applied by using AutoDock tools. Auto grid program was utilized to create grid maps of grid points (xx Angstrom) and Angstrom spacing (0.375). The van der Waals & electrostatic charges were assessed and defined as per dielectric constant. The Lamarckian genetic algorithm and the Solis & Wets local exploration methods were used for defining binding pockets on protein. The population size of 150, translational step of 0.2 A°, & quaternion and torsion steps of 5 were added and maximum of 250,000 energy assessments were prepared for molecular docking studies [19-22].

RESULTS AND DISCUSSION

3Designing of compounds

Field analysis of the designed compounds was performed and compared with known antiangiogenic and anticancer compounds Honokiol and SU4312. The field point pattern is a sophisticated ‘pharmacophore’ which can be used to define a template for protein binding. There are four molecular fields which represent the binding properties of a ligand: positive electrostatic (colored red), negative electrostatic (colored blue), Van der Waals attractive i.e. ‘steric’ (colored yellow), hydrophobic (colored orange). Compounds owning field similarity more than 50% with field

of reference were selected for synthesis. Physical properties, total no of conformers, Molecular weight, total number of atoms, 2D structure similarities, SlogP, Topological Surface Area (TPSA), flexibility of bonds and rule of five (rof 5) with 3D similarity values are presented in Table 1. Most active antiproliferative agents, TP 17, TP24 and PTM6, PTM8 exhibited field similarity with reference compound Glaziovianin with Field similarity score TP17: 0.64; TP24: 0.61; PTM6: 0.64; PTM8: 0.65 (Figure 1).

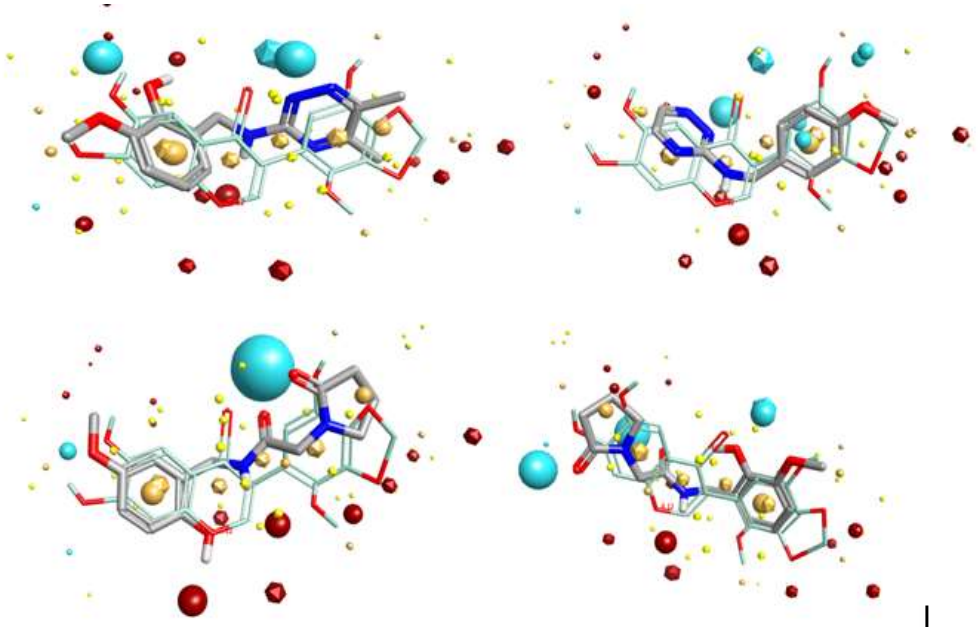


Figure 1: Field point alignment of 1,2,4-triazine and pyrrolidin-2-one derivatives (TP17, TP24 and PTM6, PTM8) on glaziovianin.
The size of the point indicates the potential strength of the interaction. Round-shaped field points are of test compounds. Diamond-shaped field points are of reference glaziovianin. Field similarity score, TP17: 0.64; TP24: 0.61, PTM6: 0.64, PTM8: 0.65

Table 1: Physical properties of designed compound and their similarity value with reference

Compound code	Conformers	Alignments	MW	Atoms	sLog P	TPSA	Flexibility	Rof5	Similarity
TP11	82	10	260.3	19	1.8	80.2	3.3	0	0.641
TP13	87	10	299.2	19	3.2	70.9	2.8	0	0.645
TP14	30	10	244.3	18	2.1	59.9	2.8	0	0.635
TP17	99	10	260.3	17	1.8	80.2	3.3	0	0.646
TP22	70	10	271.1	16	3.2	70.9	2.8	0	0.643
TP24	28	10	216.2	19	1.5	59.9	2.8	0	0.62
PTM1	200	10	317.2	20	2	69.6	5	0	0.65
PTM2	200	10	278.3	20	0.6	78.9	5.5	0	0.632
PTM5	200	10	262.3	19	0.9	59.6	5	0	0.649
PTM6	200	10	278.3	20	0.6	78.9	5.5	0	0.628
PTM8	200	10	278.3	20	0.6	78.9	5.5	0	0.624

Chemistry

The 1,2,4-triazin-3-amine reacts with respective aldehydes and forms corresponding Schiff base. The Schiff base is than reduced with sodium borohydride to obtain desired compounds. The spectral data of compounds are confirmed by the spectral datas. The numbers of carbons are calculated by dividing % abundance of M+1 peak by 1.1%. After this M/M+2 ratio was calculated to determine the presence of Cl, Br or sulphur groups. Cl and Br yield intensity ratios of about 31.9% and 97.2% respectively. The nitrogen rule states that when m/z for M has an even mass (even number of amu), the corresponding molecular formula has an even number of nitrogen atoms (0, 2, 4, etc.) and when m/z for M has an odd mass (odd number of amu), the corresponding molecular formula has an odd number of nitrogen atoms (1, 3, 5, etc.). The hydrogen rule states that for a molecule containing only hydrogen, carbon, oxygen, nitrogen, fluorine, chlorine, bromine, and iodine, the maximum number of monovalent atoms possible (max H) for a given number of Carbons (C) and Nitrogens (N) is given by formula:

As per hydrogen rule possible Max. of H atoms=2C+N+2

Further all the compounds are interpreted by spectroscopic data and found in full agreement with the proposed structures.

Antiproliferative activity

As per the protocol of NCI, eleven representative compounds, of the series, were selected and granted NSC D-codes viz; NSC D-782218 (TP11), NSC D-782219 (TP13), NSC D-782220 (TP14), NSC D-782221 (TP17), NSC D-783147 (TP22), NSC D-783148 (TP24), NSC D-783149 (PTM

1), NSC D-783150 (PTM 2), NSC D-783151 (PTM 5), NSC D-783152 (PTM 6) and NSC D-783153 (PTM 8) (Annexure 1). The compounds were evaluated at single concentration of 10^{-5}M towards the panel of approximately 60 cancer cell lines derived from nine different cancer types: Leukaemia, lung, colon, CNS, melanoma, ovarian, renal and prostate and breast cancers. Results of mean graph of inhibition of cancer cell lines are displayed in Table 1. The most efficient antiproliferative potential was exhibited by compounds PTM8, PTM6 and TP24.

As evident from one dose mean graph, compound PTM 8 showed highest growth inhibitory activity against Leukemia cell lines. It showed growth inhibition in CCRF-CEM Leukaemia cell line with 73.15% Inhibition of Growth (IG). Compound TP24 showed highest activity against leukemia. It showed good growth inhibition on MOLT4 and SR leukaemia cell lines with respectively 50.86% and 46.44% inhibition of growth (GI). It showed moderate inhibition on HL-60(TB) leukemia cell lines with GI, 30.13%. It also showed very week inhibition on K-562 cell lines (GI 4%) and no effect on CCRF-CEM and RPMI-8226 growth of leukemia cell lines. Compound PTM 6 exhibited highest activity on NCI-H522 non-small cell lung cancer cell line with GI 41.08% inhibition of growth of cell line. It also showed week to moderately inhibition of SNB-75 cancer cell line (GI 19.40%), M14 melanoma cell line (GI 14.00%) and BT-549 cell line (GI 13.0%).

The other submitted compound showed weakly to moderately inhibition of cell lines. Compound TP11, TP14 and TP17 showed growth inhibition on HOP-92 (Non-small cell lung cancer) with GI 19.37%, 17.80% and 24.17% respectively. Compound TP22, PTM1 and PTM2 showed growth inhibition on SNB 75 CNS Cancer cell line with GI 23.10%, 23.11% and 17.43 respectively. Compound TP13 exhibited moderate inhibition of growth of MCF7 breast cancer cell line with GI 27.34%. PTM5 showed sensible inhibition of growth of SR leukaemia cell line with GI 31.18% (Table 2) (Annexure 1).

Table 2: NCI developmental therapeutics program, *in vitro* testing results of selected compounds

S. No.	Compound code	60 cell line assay in one dose at 10^{-5} concentration		
		Number assigned by NCI	Most sensitive cell line	Growth of most sensitive cell line (%)
1	TP11	782218	HOP-92 (Non-Small Cell Lung Cancer)	-19.37
2	TP13	782219	MCF-7 (Breast Cancer)	-27.34
3	TP14	782220	HOP-92 (Non-Small Cell Lung Cancer)	-17.80
4	TP17	782221	HOP-92 (Non-Small Cell Lung Cancer)	-24.17
5	TP22	783147	SNB 75 (CNS Cancer)	-23.10
6	TP24	783148	MOLT4 (Leukaemia) SR (Leukaemia)	-50.86 -46.44
7	PTM1	783149	SNB 75 (CNS Cancer)	-23.11
8	PTM2	783150	SNB 75 (CNS Cancer)	-17.43
9	PTM5	783151	SR (Leukaemia)	-31.18
10	PTM6	783152	NCI-H522 (Non-Small Cell Lung Cancer)	-41.08
11	PTM8	783153	CCRF-CEM (Leukaemia)	-73.15

Molecular docking

Microtubules are cytoskeletal polymers of tubulin involved in many cellular functions. Tubulin is the target of numerous small molecule ligands that interfere with microtubule dynamics, several of which are of clinical use, in particular for cancer treatment. Most of them bind to one of three different sites: the colchicine, vinblastine and podophylltoxin sites. The molecular docking was performed into the podophyllotoxin binding site of tubulin proteins (Pdb: 1SA1) with the aim to predict tubulin binding property of the most active compounds of the study (compounds TP24, PTM6 and PTM8). The superimposition of the best scored docked conformers of TP24, PTM6 and PTM8 and X-ray structure bound podophyllotoxin showed the similarity values 5.7, 5.6, 5.7 respectively (Figure 2). The complexes were energy-minimized with a MMFF94 force field till the gradient convergence 0.01 kcal/mol was reached (Table 3). Reference glaziovianin showed it's binding in podophyllotoxin site of tubulin protein by interaction with ASN101 (-0.6627), ALA12 (-0.5184) through hydrogen bonding. It also showed polar interaction GLN15 (-0.3035), GLN11 (-1.8779), THR179 (-0.6698), ASN228 (-0.1227), SER140 (-0.5575) (Figure 3). Compound TP24 exhibited hydrophobic interactions; ALA 12 (-0.482 A); Hydrogen bonding with THR 145 (0.9942 A), SER 140 (-0.3947A), ASP 69 (0.0628) and polar interactions GLN 11 (-2.1823 A), THR 179 (0.3926), TYR 224 (0.3789) (Figure 3). Compound PTM6 exhibited hydrophobic interactions; ILE171 (-0.6357 A), ALA12 (-0.5193 A), PRO173 (-0.4702 A); hydrogen bonding with TYR224 (-2.1255 A), GLU183 (-0.1869 A) and polar interactions with THR179 (-0.3664 A) (Figure 4). Compound PTM8 displayed hydrophobic interactions with, ILE171 (-0.7464 A), PRO173 (-0.5895 A), ALA12 (-0.4468 A), ILE16 (-0.3386 A), ILE231 (-0.1217 A), hydrogen bonding with TYR224 (-1.8446 A), THR179 (-0.3527 A), GLU183 (-0.3353 A) and polar interactions with ASN206 (-0.6352 A), GLN15 (-0.4239 A) (Figure 5).

Table 3: Energy table of most active anti-proliferative compounds

Compound	Est. Free energy of binding (kcal/mol)	Est. Inhibition constant, Ki uM	vdW + H bond + dissolve energy (kcal/mol)	Electrostatic energy (kcal/mol)	Total intermolecular energy (kcal/mol)	Interact. Surface
TP24	-5.75	58.75	-6.44	-0.11	-6.54	858.93
PTM6	-6.12	32.13	-7.25	-0.03	-7.29	816.26
PTM8	-5.29	131.53	-6.89	+0.06	-6.84	819.42
Glaziovianin	-5.66	70.97	-6.58	-0.36	-6.94	1065.49

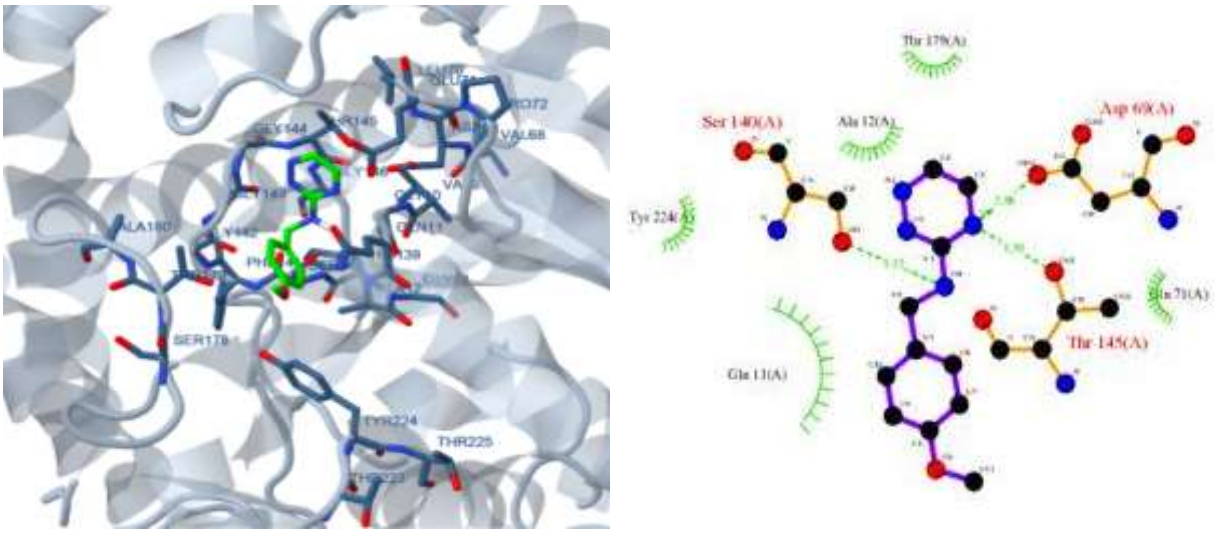


Figure 2: Molecular Docking of compound TP24 into the active site of tubulin protein pdb: 1SA1. Hydrogen bonding interactions are shown in green colour dashed lines

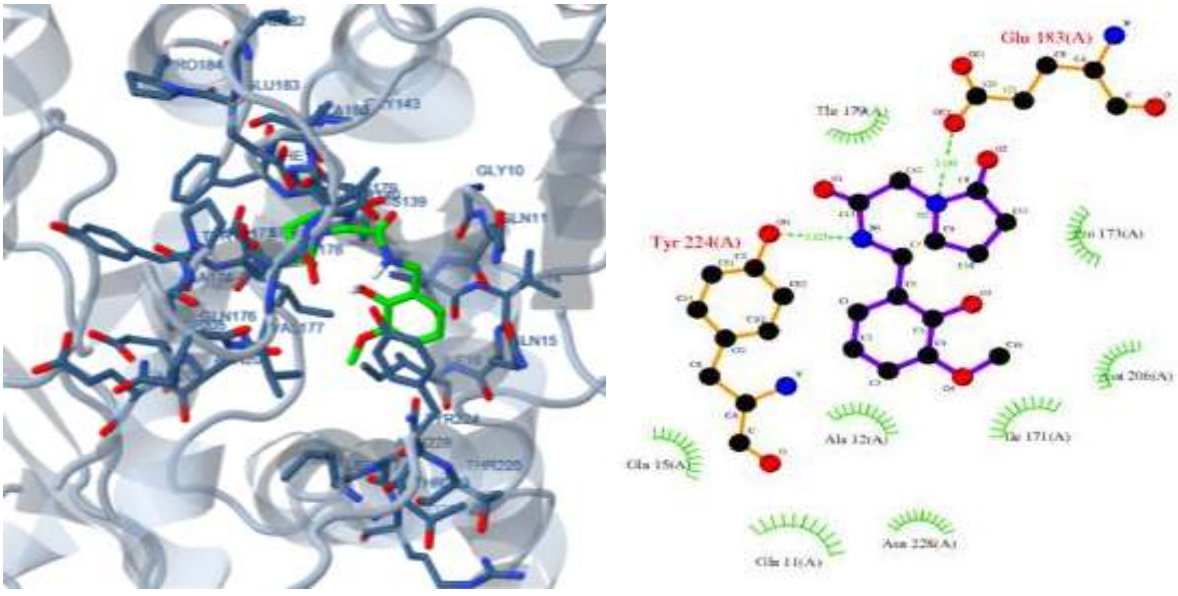


Figure 3: Molecular Docking of compound PTM6 into the active site of tubulin protein pdb: 1SA1. Hydrogen bonding interactions are shown in green colour dashed lines

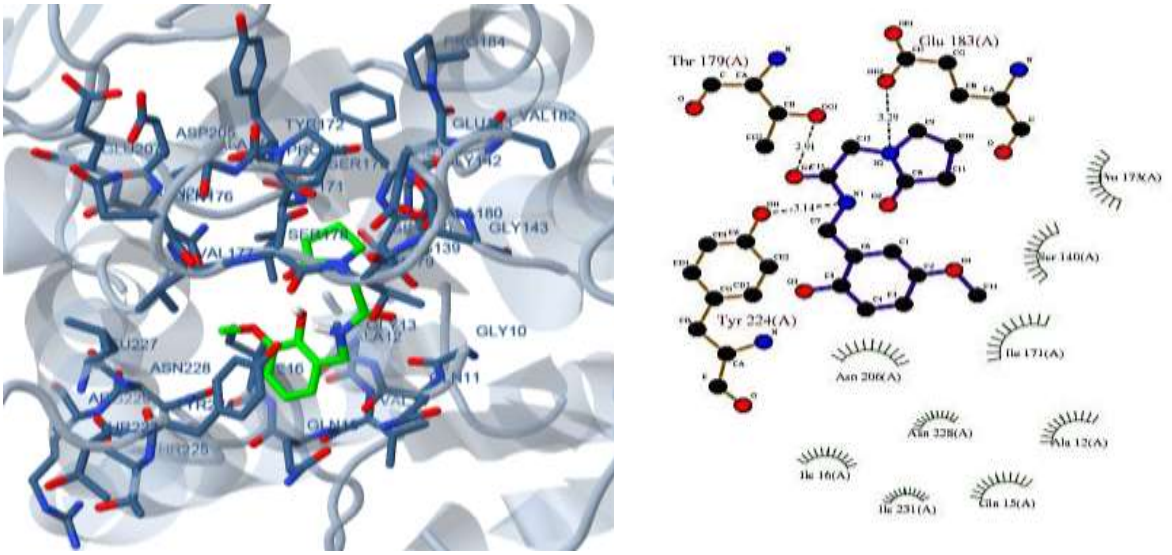


Figure 4: Molecular Docking of compound PTM8 into the active site of tubulin protein pdb: 1SA1 Podophyllotoxin binding pocket. Hydrogen bonding interactions are shown in black colour dashed lines

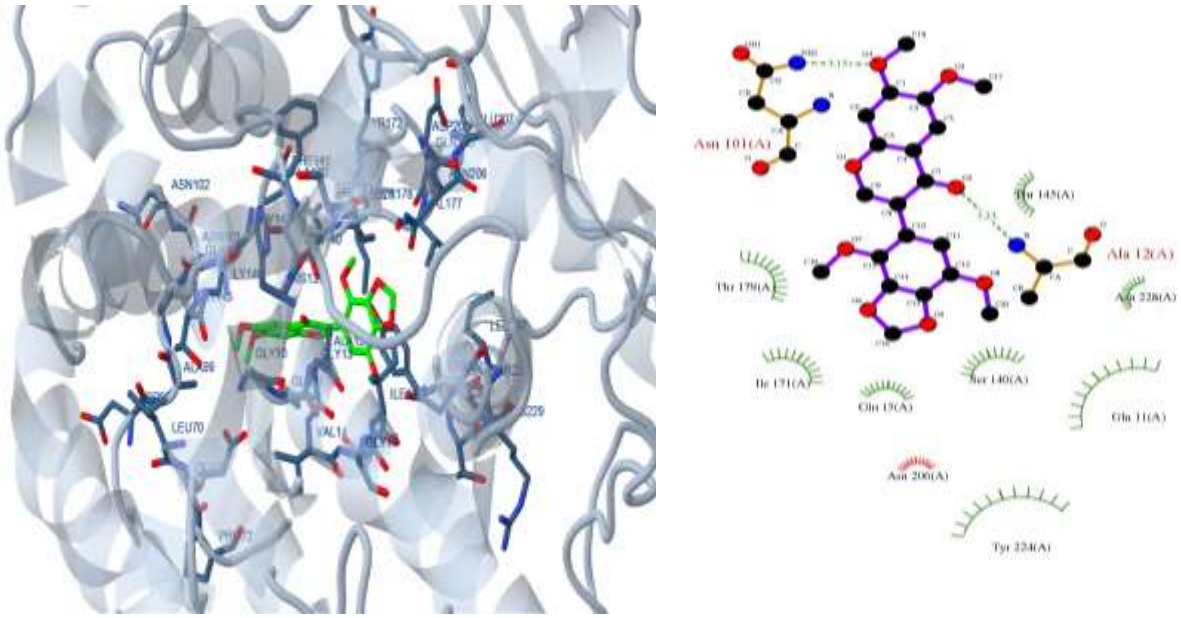


Figure 5: Molecular Docking of reference compound Glaziovianin to the active site of tubulin protein pdb: 1SA1 Podophyllotoxin binding pocket

CONCLUSION

To summarize, two novel series of heterocyclic compounds have been synthesized. In first series, Substituted 1,2,4-Triazine was allowed to react with substituted salicylaldehyde compounds to get desired Schiff bases. In second series, Pyrrolidin-2-one derivatives with 1,4 substituted salicylaldehyde were synthesized. All compounds were designed using DTP submission guidelines, Data of All designed compounds were submitted to NCI. The anti-proliferative activity of the selected compounds was performed by National Cancer Institute (NCI), USA against 60 human tumor cell lines derived from nine neoplastic diseases. Compounds exhibited anti-proliferative activity in varying ratios. Eleven compounds were tested and most of them displayed antitumor activity on CNS cancer, leukemia and Non-small cell lung cancer cell lines. The most efficient anticancer compounds were found to be active with selective influence PTM 8 on CCRF-CEM Leukemia cell line with 73.15% inhibition of growth of cell line and TP24 on MOLT4 and SR leukemia cell lines with respectively 50.86% and 46.44% inhibition of growth of cell line and PTM 6 on NCI-H522 Non-Small Cell Lung Cancer cell line with 41.08% inhibition of growth of cell line. Molecular docking revealed that all the most active compounds binds to tubulin protein Podophyllotoxin binding site. The obtained results prove the necessity for further investigations to clarify the features underlying the antitumor potential of tested Compounds.

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