THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

A. Anton Smith et al, The *Experiment, 2014, Vol. 21(1), 1439-1452*

IN-SILICO DESIGN, SYNTHESIS AND IN VITRO ANTICANCER EVALUATION OF SOME NOVEL 1, 2, 4 - TRIAZOLE DERIVATIVES

ABSTRACT

Objective: To design, synthesize and in vitro anticancer evaluation of some new 1,2,4–triazole derivatives.
Methods: Novel 1,2,4–triazole derivatives were designed by using various soft wares like ACD Lab Chemsketch, Molinspiration, Prediction of activity spectra for substances(PASS) and Schrodinger Glide XP (Grid based ligand docking with energetics). The designed molecules having required physico-chemical properties, drug likeness and obeying Lipinski's rule of five were selected for the synthesis. The synthesized compounds were subjected to TLC, melting point determination, FTIR and ^{1}HNMR spectroscopic studies. The in vitro anticancer activity of selected compounds was evaluated against SKMEL, MCF7 and Hep2 cell lines by MTT assay method.
Results: Three derivatives (MB-2, MB-6 and MB-9) were selected for the synthesis with the help of in-silico modeling. The selected derivatives were synthesized by conventional method. All the synthesized compounds showed characteristic peak in FTIR and ^{1}HNMR spectroscopic studies. Based on the Schrodinger GlideXP score, compound MB-6 and MB-9 were selected for the in vitro anticancer evaluation. The compound MB-6 showed significant anticancer activity against Hep2 and MCF7 cell lines.
Conclusion: These results are useful for further investigation in the future.

Key words: 1,2,4-triazole derivatives, conventional synthesis, spectral study, in vitro anticancer activity.

1. INTRODUCTION

The synthesis of high nitrogen containing heterocycles derived from 1,2,4-triazoles attracting increasing interest over the past decade due to their usefulness in different areas of biological activities and as industrial intermediates. 1,2,4-triazole moiety appears frequently in the structure of various natural products and the synthesis of compounds incorporating this moiety has attracted widespread attention of chemists as well as biologists mainly due to their diverse biological activities in pharmaceutical and agrochemical fields[1]. A large variety of 1,2,4-triazole derivatives possess antibacterial[2,3,4,5], antifungal[2,3,4,5,6,7,8], anti-tumor[9], antiviral[10], anti-inflammatory[5], anti-convulsant, anti-depressant, anti-tubercular[5], anti-hypertensive, analgesic, enzyme inhibitor, hypoglycemic, sedative, hypnotic, insecticide and plant growth activities [2].
Our research directed toward the design and synthesis of some new 1,2,4-triazole derivatives and the investigation of in vitro anticancer activity of newly synthesized compounds, an attempt to provide a direction for further research.

2. Materials and methods

2.1 In-silico molecular modification

In-silico molecular modification was the most important preliminary step in the rational drug designing of novel drugs. In the present study different proposed derivatives are screened for different physico-chemical properties by using different softwares. ACD Lab Chemsketch was used for 3-D drawing, optimizing and calculating various physicochemical descriptors of the proposed molecules. The Molinspiration software was used for calculating logP values, Lipinski's rule of five and drug likeness. The proposed molecules were screened for whether they obey the rule of five or not. The general biological activities of proposed molecules were predicted by using PASS (Prediction of activity spectra for substances) software. Schrodinger Glide XP (Grid based ligand docking with energetics) software was used for the molecular docking of proposed molecules. Three 1,2,4-triazole derivatives were selected for synthesis with the help of these selection parameters.

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They are

1) 2- [(diethyl amino) methyl] -5- (4-hydroxy phenyl) -4- {[(4-nitro phenyl) methylidene] amino}-1,2,4-triazolin-3-thione. (MB-2)
2) 2- (piperidin-1-ylmethyl) -5- (4-hydroxy phenyl) -4- {[(4-dimethyl amino) phenyl methylidene] amino}-1,2,4-triazolin-3-thione. (MB-6)
3) 2- (morpholin-4-ylmethyl) -5- (4-hydroxy phenyl) -4- {[(4-dimethyl amino) phenyl methylidene] amino}-1,2,4-triazolin-3-thione. (MB-9)

2.2 Synthesis of selected 1,2,4-triazole derivatives

The selected compounds were synthesized by conventional method through a series of four steps.

2.2.1 Synthesis of aromatic hydrazide from an aromatic ester and hydrazine hydrate.

Methyl 4- hydroxyl benzoate 1.52g (0.01mole) and hydrazine hydrate 0.97ml (0.02mole) were refluxed in absolute ethanol (50ml) for 18 h. The reaction mixture was concentrated and the ethanol was removed by distillation, condensation and then the reaction mixture was cooled in an ice bath with continuous stirring and kept in the room temperature for 3-4 h. The solid product thus separated out was filtered, dried and recrystallized from ethanol. Yield and melting point of product obtained were determined. A single spot on the TLC plate established the purity of the compound. The solvent system used was n-hexane : ethyl acetate (1:1).

2.2.2 Synthesis of 4-amino 5-aryl 1,2,4-triazolin-3-thione through the formation of potassium dithiocarbazinate from aromatic hydrazide.

13.7g (0.1mole) 4-hydroxybenzohydrazide was dissolved in 200ml of absolute alcohol containing 5.6g (0.1mole) of potassium hydroxide at room temperature. 12.5ml of carbon disulphide was added in parts and stirred for 16 h at room temperature. Then 100ml of diethyl ether was added and stirred for further 3 h. The resultant product was separated and dried. Yield, R_f value and melting point of the product were recorded.

10.3g hydrazine hydrate (0.1mole, 99%) was gradually added to Potassium dithiocarbazinate dissolved in 100ml of water with stirring and refluxed for 8 h during which hydrogen sulphide gas evolved and the colour of the reaction mixture was changed to deep green. It was then cooled to 0-5^{0}C and acidified with hydrochloric acid to pH 1. The resultant product was isolated by filtration and recrystallized from ethanol. Yield, melting point and R_f value of the product were recorded.

2.2.3 Synthesis of different Schiff's bases by reacting 4-amino -5- aryl 1,2,4-triazolin-3-thione with different aromatic aldehydes.

2-3 drops of concentrated sulphuric acid was added to the solution of 0.01mole 4-amino-5-(4-hydroxyphenyl)–1,2,4-triazolin-3-thione in 20ml of ethanol. 0.01mole of different benzaldehyde derivative was added and refluxed for 2-6 h. Then the reaction mixture was cooled to 0^{0}C and the precipitate obtained was filtered, dried and recrystallized from ethanol.

2.2.4 Synthesis of different Mannich bases (final compounds) by treating above Schiff's bases with various secondary amines in the presence of formaldehyde.

Various secondary amines (0.01mole) were gradually added to the solution of Schiff's base (0.01mole) in 12ml of dry ethanol. 38% formaldehyde solution (0.8ml, 0.015mole) was added to it. The pH of the solution was maintained in between 3-4 by adding hydrochloric acid. Then the reaction mixture was stirred for 1 h at room temperature and allowed to stand overnight at 0^{0}C. The precipitate obtained was filtered, dried and recrystallized from ethanol. Yield, melting point and R_f value of the product were recorded.

For the synthesis of compound MB-2, Para nitro benzaldehyde and diethyl amine were used in Step-3 and Step-4 respectively. For the synthesis of compound MB-6, Para dimethyl amino benzaldehyde and piperidine were used in Step-3 and Step-4 respectively. For the synthesis of compound MB-9, Para dimethyl amino benzaldehyde and morpholine were used in Step-3 and Step-4 respectively.

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2.3 Characterization of synthesized compound by spectral study

2.3.1 IR Spectrum

IR spectra were recorded by using KBr pellets in the range of 4000 – 500 cm-1 on Jasco FTIR Model 4100 Type A to elucidate the structure of the compounds.

2.3.2 ^{1}HNMR Spectrum

Proton NMR (300 MHZ) spectra were recorded in $CDCl_3$. Chemical shifts were recorded in parts per million downfield with reference to internal standard Tetra Methyl Silane (TMS) on Bruker ultrashield model 400.

2.4 In vitro anticancer activity

Anticancer activities of selected compounds were evaluated against SKMEL (Human malignant melanoma cell line), MCF7 (Breast cancer cell line) and Hep2 (He La derivative) cell lines by MTT assay method. The cell lines were procured from NCCS, (National centre for cell science) Pune, India.

2.4.1 Sub culturing and maintenance of cell line

The cell lines were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% heat inactivated foetal bovine serum (FBS) and incubated at 37^0C in a humidified atmosphere of 5% CO_2

2.4.2 Assessment of anticancer activity by MTT assay

Cells were transferred in to 96- well flat bottom plates at the concentration of 1×10^4 cells/ml and incubated at 37^0C in a humidified incubator (5% CO_2) for 24 h followed by exposure to various concentrations of tested compounds for 48 h. Then 20µl of MTT (3-(4,5-dimethyl thiazol-2yl)-2,5- diphenyl tetrazolium bromide) reagent dissolved in PBS (phosphate buffered saline, pH 7.4) was added to each well and mixed and incubated for an additional 4 h. Subsequently, the supernatant was removed, 150 µl DMSO (dimethyl sulphoxide) was added to each well for dissolving the MTT- formazan crystals. Finally absorbance was recorded at 570nm using a micro plate reader with DMSO as a blank for determining the cell growth inhibition which was calculated by using the following equation

Growth inhibition = 1- OD of treated cells / OD of control cells × 100

The concentration required to inhibit the growth by 50% (IC_{50}) were determined from absorbance value

3. Results

In the present study, in-silico molecular modifications of proposed derivatives were done by using different softwares. 3D- drawing, optimizing and calculating various descriptors of proposed derivatives were done by using ACD Lab Chemsketch software. The molinspiration software was used to study the LogP values, violation of Lipinski's rule of five and drug likeness by comparing with already existing standard drugs (Table 1, 2, 3 & 4.Figure 1). The PASS software was used to predict the general biological activities of proposed molecules. The result of prediction is presented as the list of activities with appropriate Pa (Probability to be active) and Pi (Probability to be inactive) sorted in descending order of the difference (Pa-Pi)>0. Pa and Pi are the estimates of probability for the compound to be active or inactive respectively for each type of activity from the biological activity spectrum. Their values vary from 0.000 to 1.000.

If Pa > 0.7, the compound is very likely to reveal this activity in experiments, but in this case the chance of being the analogue of the known pharmaceutical agents for this compound is also high.

If 0.5 < Pa < 0.7, the compound is likely to reveal its activity in experiments, but this probability is less, and the compound is not so similar to the known pharmaceutical agents.

THE EXPERIMENT
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RESEARCH ARTICLE

If Pa < 0.5, the compound is unlikely to reveal its activity in experiments, but if the presence of this activity is confirmed in the compound, it might be a new chemical entity (Table 5).

Schrodinger Glide XP software was used for predicting the protein-ligand binding modes. In this study, the compound having high (-) value is considered as the best one (Table 6 Figure 2). With the help of these selection parameters three analogues were selected for the synthesis. **.** They were named as MB-2, MB-6 and MB-9.

The selected compounds were synthesized by conventional method through a series of four steps. Purity of the synthesized compounds was ascertained by TLC and melting point determination by open capillary tube method (Table 7) and they were characterized by FTIR and ^{1}HNMR spectroscopic methods (Graph 1, 2 & 3). Based on the Schrodinger Glide XP score, MB-6 and MB-9 were selected for in vitro anticancer evaluation against SKMEL, MCF7 and Hep2 cell lines by MTT assay method. The tested derivatives showed cytotoxic activity against the three tested cancer cell lines. But the compound MB-6 showed significant anticancer activity in both Hep2 and MCF7 cell lines with IC_{50} value 125 µg/ml and 128 µg/ml respectively (Table 8).

4. DISCUSSION

The in-silico molecular modifications of proposed derivatives were done by using ACD Lab Chemsketch, Molinspiration, PASS and Schrodinger GlideXP. With the help of these parameters, three derivatives (MB-2, MB-6 and MB-9) were selected for the synthesis by conventional method. The synthesized compounds were subjected to TLC, melting point determination, FTIR and ^{1}HNMR spectroscopic studies. All these evaluation ensured the synthesized compounds. In the in vitro anticancer evaluation by MTT assay, the results showed that the compound MB-6 having significant anticancer activity against Hep2 and MCF7 cell lines. Of course this compound needs further studies such as toxicity and in vivo evaluation. Importantly previous reports indicated that a large variety of 1,2,4- triazole derivatives possess antibacterial, antifungal and anti-tubercular activities. The study of the synthesized compounds in this direction may give valuable results. So it is clear that further works needed to be done in the future for the development of clinically useful chemotherapeutic agents

5. CONCLUSION

In summary, the prime objective of the present work was to design, synthesize and biologically screen some of the 1, 2, 4-triazole derivatives. Based on that particular objective, various 1, 2, 4-triazole derivatives were designed by preliminary in silico methods using various softwares. According to the in silico study results, three derivatives were synthesized by conventional methods and the purity of the compounds thus synthesized were ascertained by consistency in melting point and R_f value and characterized by IR and ^{1}H NMR spectroscopy. In the in vitro anticancer evaluation of synthesized compounds MB-6 and MB-9, the compound MB-6 showed significant anticancer activity in both Hep2 and MCF7 cell lines. Hopefully, this study could discover a new specific lead to target the cancer cells.

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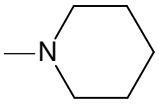
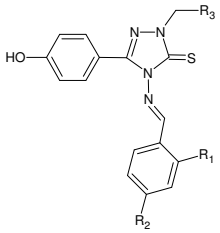
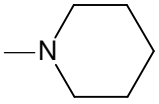
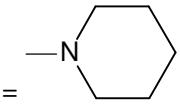
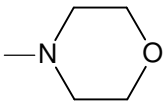
Table 1: SMILES and cLogP values of proposed 1,2,4-triazole derivatives

Structure	Code	Substitution	Smile Notation	MiLogP
	MB-1	$R_1 = R_2 =$ Cl $R_3 = N(C_2H_5)_2$	CCN(CN2N=C(N(/N=C/c1ccc(Cl)cc1Cl)C2=S)c3ccc(O)cc3)CC	4.638
	MB-2	R_1 = H, $R_2 = NO_2$ $R_3 = N(C_2H_5)_2$	CCN(CC)Cn3nc(c1ccc(O)cc1)n(N=Cc2ccc(N(=O)=O)cc2)c3=S	3.313
	MB-3	R_1 = H, $R_2 = N(CH_3)_2$ $R_3 = N(C_2H_5)_2$	CCN(CC)Cn3nc(c1ccc(O)cc1)n(N=Cc2ccc(N(C)C)cc2)c3=S	3.457
	MB-4	$R_1 = R_2 =$ Cl R_3 = 	Clc4ccc(/C=N/N2C(=S)N(CN1CCCCC1)N=C2c3ccc(O)cc3)c(Cl)c4	4.794
	MB-5	R_1 = H, $R_2 = NO_2$ R_3 = 	[O][N+](=O)c1ccc(cc1)/C=N/N3C(=S)N(CN2CCCCC2)N=C3c4ccc(O)cc4	3.469
	MB--6	R_1 = H, $R_2 = N(CH_3)_2$ R_3 = 	CN(C)c1ccc(cc1)/C=N/N3C(=S)N(CN2CCCCC2)N=C3c4ccc(O)cc4	3.613
	MB-7	$R_1 = R_2 =$ Cl R_3 = 	Clc4ccc(/C=N/N2C(=S)N(N=C2c1ccc(O)cc1)CN3CCOCC3)c(Cl)c4	3.732
	MB-8	R_1 = H, $R_2 = NO_2$ R_3 =	[O-][N+](=O)c1ccc(cc1)/C = N/N3C(=S)N(N=C3c2ccc	2.407

THE EXPERIMENT
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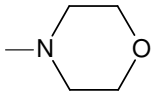
RESEARCH ARTICLE

A. Anton Smith et al, The *Experiment, 2014, Vol. 21(1), 1439-1452*



(O)cc2)CN4CCOCC4

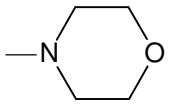
MB-9	R_1 = H, R_2=N(CH_3)$_2$ R_3 = 	CN(C)c1ccc(cc1)/C=N\N3C(=S)N(N=C3c2ccc(O)cc2)CN4CC OCC4	2.551

Table 2: Physico-chemical properties of the proposed 1,2,4-triazole derivatives

Comp.	Molar Refractivity (Cm^3)	Molar Volume (Cm^3)	Parachor (Cm^3)	Surface tension (dyne/cm)	Polari-zability (Cm^3) $^{-24}$	Mi log P
MB-1	122.05±0.5	333.4±7.0	881.1±8.0	48.7±7.0	48.38±0.5	4.638
MB-2	118.51±0.5	320.1±7.0	868.9±8.0	54.2±7.0	46.98±0.5	3.313
MB-3	125.66±0.5	356.0±7.0	919.8±8.0	44.5±7.0	49.81±0.5	3.457
MB-4	124.48±0.5	322.7±7.0	880.0±8.0	55.2±7.0	49.35±0.5	4.794
MB-5	120.94±0.5	309.4±7.0	867.7±8.0	61.8±7.0	47.94±0.5	3.469
MB-6	128.09±0.5	345.3±7.0	918.6±8.0	50.0±7.0	50.77±0.5	3.613
MB-7	121.26±0.5	313.3±7.0	860.5±8.0	57.0±7.0	48.07±0.5	3.732
MB-8	117.72±0.5	299.8±7.0	848.3±8.0	64.0±7.0	46.67±0.5	2.407
MB-9	124.87±0.5	335.7±7.0	899.2±8.0	51.4±7.0	49.5±0.5	2.551

comp. –compound

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Table 3: Lipinski's rule analysis of standard drugs and proposed 1,2,4-triazole derivatives

Comp	MiLog P	M .W	n.HDO	n.HAC	n.rotb	n.violation
4-Hydroxy tamoxifen	5.58	387.50	1	3	8	1
Mechloretha-mine	1.554	156.05	1	0	4	0
Cyclophosp-hamide	0.755	261.08	1	4	5	0
MB-1	4.638	450.395	1	6	7	0
MB-2	3.313	426.502	1	9	8	0
MB-3	3.457	424.574	1	7	8	0
MB-4	4.794	462.406	1	6	5	0
MB-5	3.469	438.513	1	9	6	0
MB-6	3.613	436.585	1	7	6	0
MB-7	3.732	464.378	1	7	5	0
MB-8	2.407	440.485	1	10	6	0
MB-9	2.551	438.557	1	8	6	0

M.W- molecular weight, nHDO- number of hydrogen bond donar, nHDA- number of hydrogen bond acceptor, n.rotb- number of rotatable bonds

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Table 4: Drug likeness analysis of standard drugs and proposed 1,2,4-triazole derivatives

Comp.	GPCR Ligand	Ion Channel Modulator	Kinase inhibitor	Nuclear receptor ligand
4-Hydroxy tamoxifen	0.33	0.05	0.03	0.66
Mechlorethamine	-3.18	-2.98	-2.78	-0.95
Cyclophosphamide	-0.65	-0.38	-0.59	-3.52
MB-1	-0.95	-1.08	- 0.99	- 1.20
MB-2	-1.02	-1.06	-1.05	-1.17
MB-3	- 0.88	-1.03	-0.89	-1.08
MB-4	-0.87	-0.99	-0.94	-1.13
MB-5	-0.94	-0.98	-0.99	-1.11
MB-6	-0.81	-0.95	-0.85	-1.02
MB-7	-0.94	-1.07	-0.91	-1.16
MB-8	-1.01	-1.06	-0.97	-1.14
MB-9	-0.88	-1.03	-0.82	-1.05

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

Table 5: PASS of proposed 1,2,4-triazole derivatives for anticancer activity

Compound	Pa	Pi	Pa –Pi
MB-1	0.000	0.000	0.000
MB-2	0.347	0.132	0.215
MB-3	0.309	0.157	0.152
MB-4	0.000	0.000	0.000
MB-5	0.276	0.182	0.094
MB-6	0.355	0.140	0.215
MB-7	0.215	0.162	0.053
MB-8	0.317	0.151	0.166
MB-9	0.377	0.116	0.261

Table 6: Schrodinger Glide XP scores of selected 1,2,4-triazole derivatives Protein used for docking: Tubulin, PDB ID: 3M89

Compound	Glide score
MB- 2	-6.47
MB- 6	**-7.64**
MB- 9	**-6.98**

THE EXPERIMENT
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A. Anton Smith et al, The *Experiment, 2014, Vol. 21(1), 1439-1452*

Table 7: Characterization data of synthesized 1,2,4-triazole derivatives

Compound Code	Molecular Weight (gm)	m.p (^{0}c)	R_f value
MB-2	426.502	260-262	0.4
MB-6	436.585	262-265	0.82
MB-9	438.557	265-267	0.9

m.p – melting point

Table 8: In vitro cytotoxicity of selected 1,2,4-triazole derivatives against three different cancer cell lines

Compound	IC_{50} (µg/ml)		
	SKMEL	**MCF 7**	**Hep2**
MB-6	180	128	125
MB-9	150	137	160

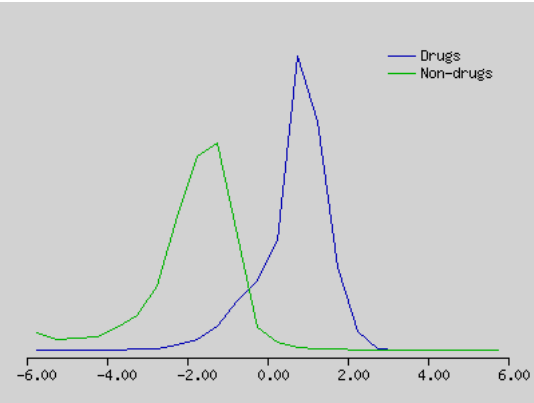
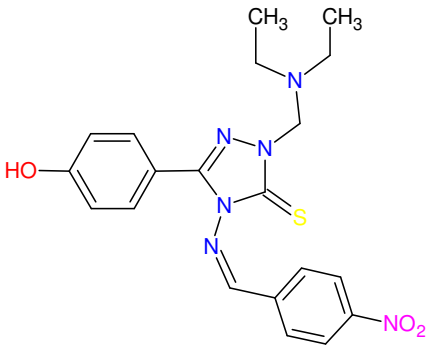


Figure1: Drug-likeness model score of compound MB2



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INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

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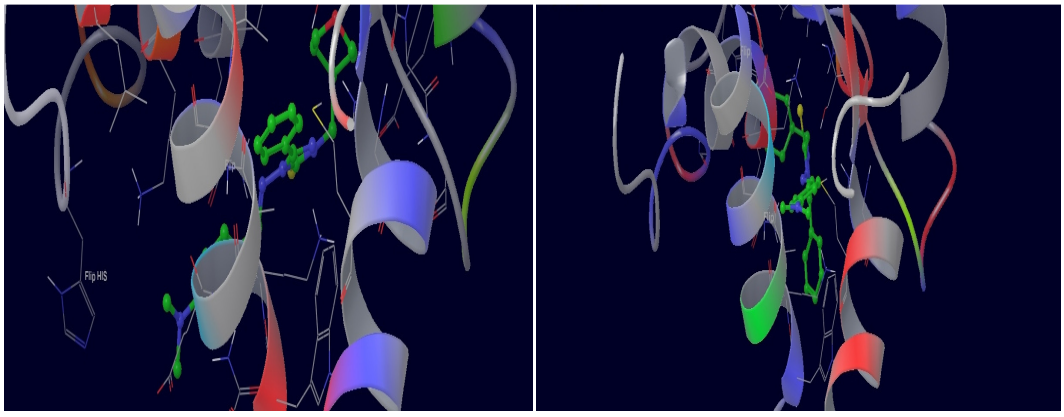
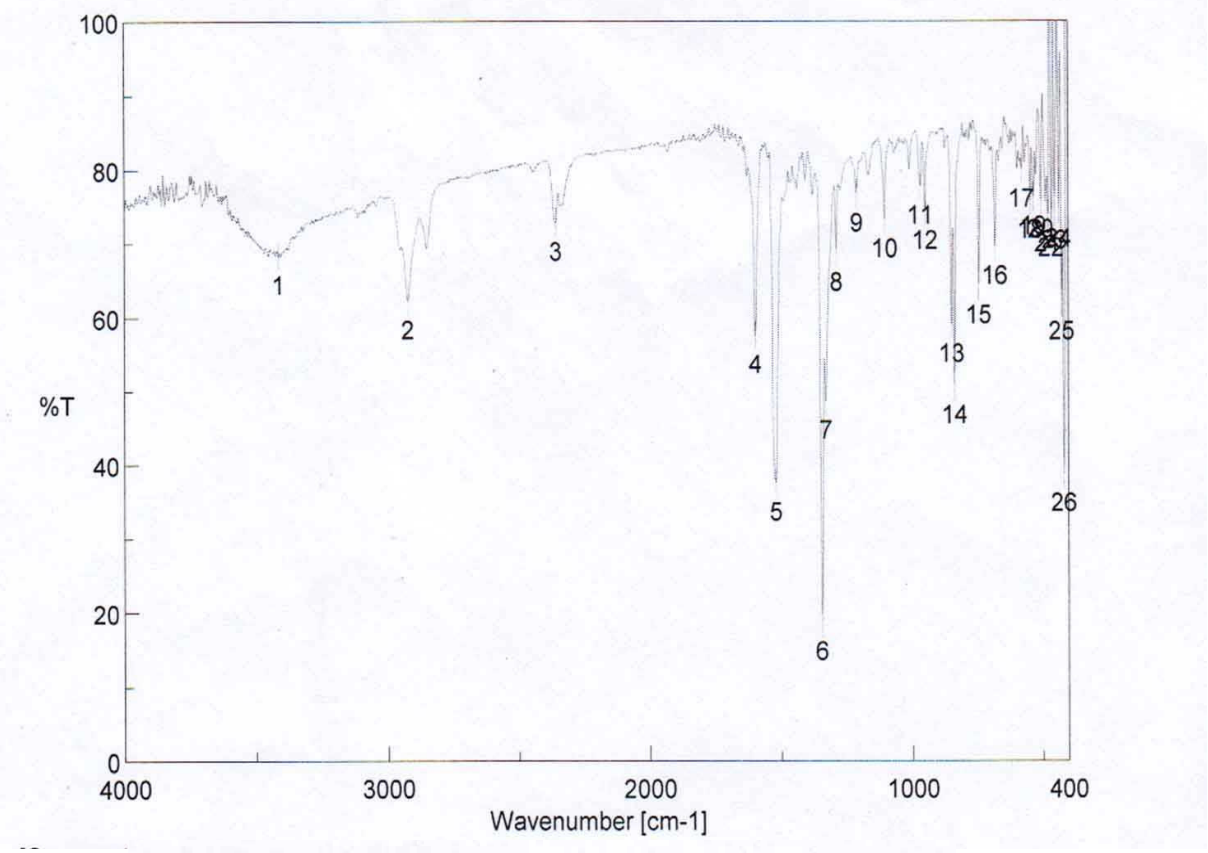
Figure 2: Docking image of (a) MB-6 and (b) MB-9 to Tubulin (3M89)



(a) **(b)**

Graph 1: FTIR report for the Schiff's base obtained in the synthesis of compound MB-2



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3416.28	68.2713	2	2924.52	62.1934
3	2358.52	72.7271	4	1596.77	57.5646
5	1519.63	37.4812	6	1346.07	18.6554

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

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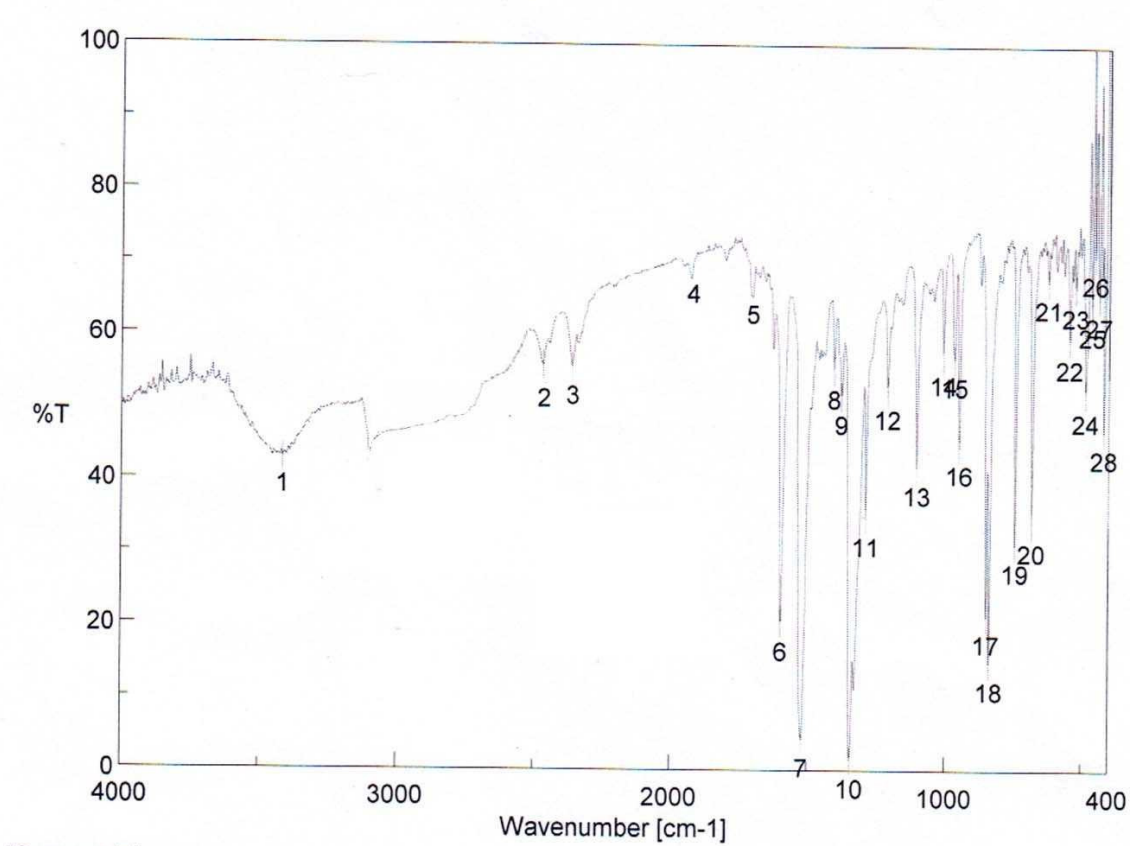
Graph 2: FTIR report for compound MB-2 (Mannich base)



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3415.31	42.9763	2	2468.44	55.6239
3	2362.37	5.3992	4	1902.193	39.631
5	1650.193	42.0263	6	1596.77	20.2588

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

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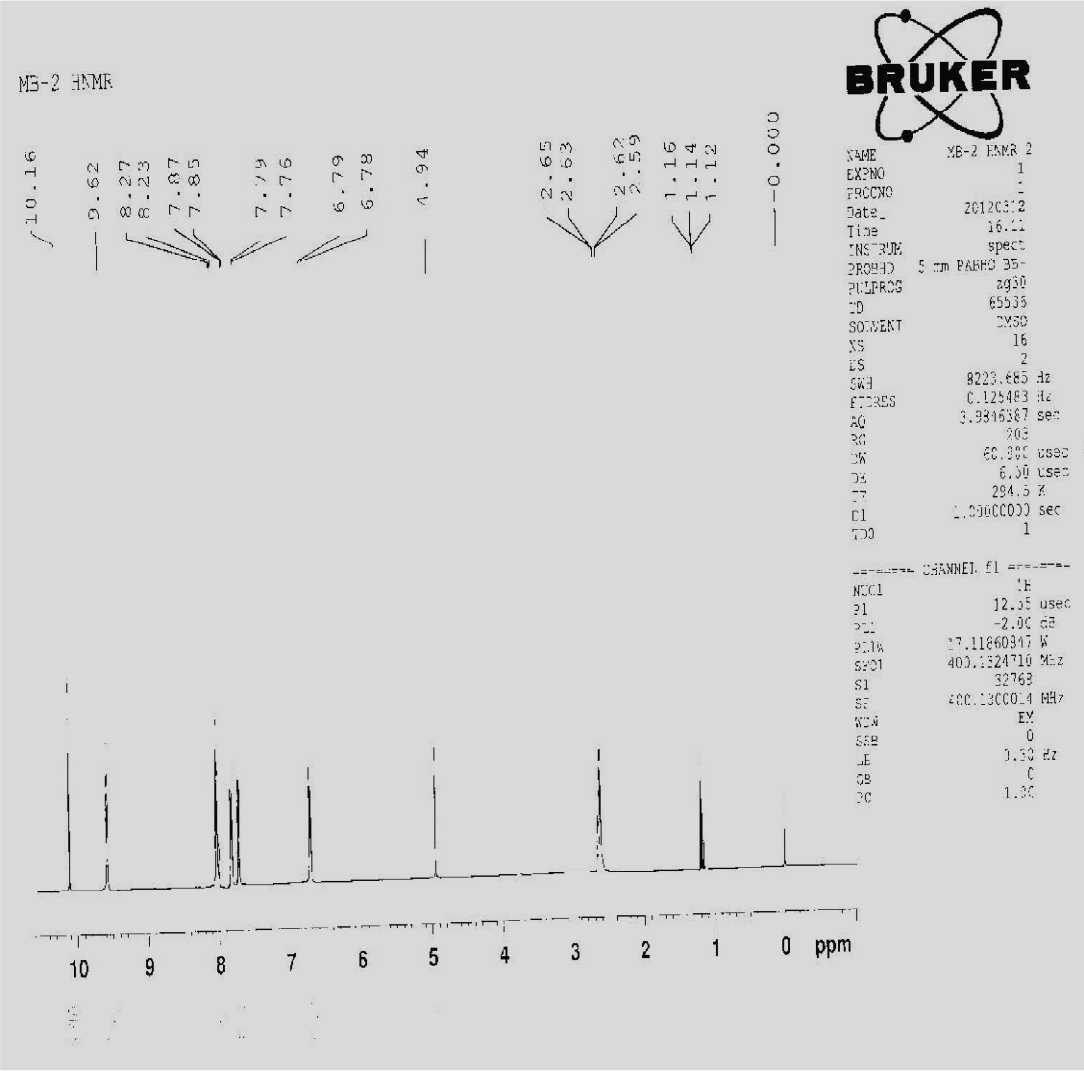
A. Anton Smith et al, The *Experiment, 2014, Vol. 21(1), 1439-1452*

Graph 3: [1]HNMR report for compound MB-



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