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Synthesis, characterization, dft calculation, docking studies, antioxidant and anticancer activities of some 3-(2-alkylaminothiazol-5-oyl)pyridines

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The 3-(2-alkylaminothiazol-5-oyl)pyridines were synthesized and characterized by different physicochemical techniques such as IR, 1H NMR, MS, electronic parameters etc. Geometrical and electronic properties of 3-(2-alkylaminothiazol-5-oyl)pyridines derivatives was computed theoretically using B3LYP /6-31G (d, p) basis sets. The energy gap between HOMO and LUMO explained the charge transfer within the molecule. The optimized structures of all the derived compound shows that in-plane and out of a plane in the molecule. All the compounds exhibited good docking scores against 4mmh liver cancer. The antioxidant study also evaluated excellent IC_{50} value. It shows the best inhibitory concentration against breast cancer. Among the 3-(2-alkylaminothiazol-5-oyl)pyridines, compound 6a was highly active on the MDA-MB-231 breast cancer cell line.

Keywords: B3LYP, DFT, Concentration, Decarboxylation, Electronic, Pyridinyl, Pyridines

Most of the bicyclic compounds are playing a vital role in drug discovery and design because of their different biological activities. Some of the heterocyclic compounds are a key role in the life of plants and animals[1]. Aza-heterocycles are more interesting because of this compound can be modifying the electron distribution. It is also used for the alternation of the physical and chemical properties of the compounds. Nitrogen-containing six-membered heterocylic compounds, like pyridine or piperidine is often found in naturally occurring bioactive compounds like alkaloids[2]. In the present life, antimicrobial drugs are a complex problem for influencing the health of people all over the world. Every year, more than 1 million people die from microbial infections and the number of deaths is increases[3]. Novelty must be strengthened in research activities related to effective antimicrobial and antifungal drugs[4]. The most common N-hetero aromatics like pyridine incorporated into the structure of various therapeutic agents. Naturally available and synthetic compounds containing pyridine scaffold possess interesting biological properties including anticancer, antimalarial, anticonvulsant, antibacterial, anti-inflammatory, antitumor, and antiviral activities[5-8]. Pyridines are very important, as in medicinal drugs and to make some herbicides, insecticides, and plant growth regulators. In organic synthesis, pharmaceutical and agricultural, acetylpyridine is used as a chemical intermediate and as an analytical reagent[9]. All the nitrogen- containing flavonoid derivatives should be highly active in tumours[10]. Anyhow nitrogen -containing pyridine molecule was a well-studied six-membered heterocyclic compound and displays various biological activities[11].

Experimental

General Procedure

Synthesis of 3-(2-alkylaminothiazol-5-oyl)pyridines (6a-6d): Ethyl Nicotinate treated with ethylacetate to form an intermediate compound, followed by decarboxylation occurs to form 3-acetylpyridine. The 3-acetylpyridine undergoes bromination to give 3-bromoacetylpyridine. The mixture of 3-bromoacetyl pyridine with different 1-alkyl-3-(N,N-dimethylimidoyl) thiourea in the presence of triethylamine as a reagent afforded an yellow crystalline solid. The yellow solid was crystallized using methanol-water (2:1) used as a solvent (Scheme 1).

DFT Calculation

In our study, all the synthesized compounds were executed with theoretical studies using Gaussian 09 program package employing density functional theory (DFT). Complete optimization of compounds was carried out by B3LYP/6-31G level of theories. Vibrational frequencies of the compounds are found real

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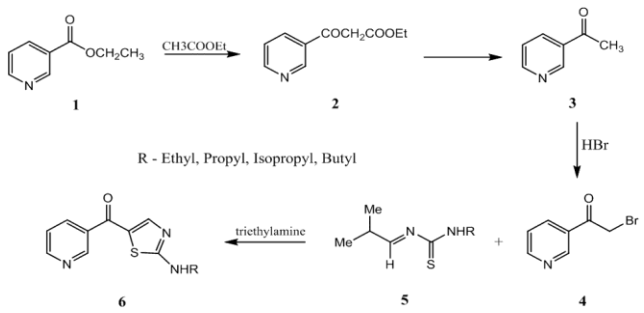


Scheme 1 — Synthetic route of 3-(2-alkylaminothiazol-5-oyl)pyridines

(positive) assures for the optimized geometries based on the true minimum in the potential energy surface[12]. Electronic properties like molecular orbital energy, electron affinity, ionization potential, hardness, softness, electronegativity were found[13]. The structural parameters like bond angles, bond distances, and dihedral angles were derived.

$$\mu = -\chi = -(\delta E/\delta N)\ V(r) \quad \ldots (1)$$

$$\chi = -\mu = (I+A)/2 \quad \ldots (2)$$

χKoopmans = (EHOMO+ELUMO)/2

$$\omega = \mu 2/2\eta \quad \ldots (3)$$

Hardness is defined as

η = ½ (δ2E/δN2) V (r) η= ½ (I – A) η = ½ (ELUMO-EHOMO)

By using the above three equations (1), (2) and (3) the chemical potential, hardness and electrophilicity index have been calculated.

Protein structure preparation

The suitable Protein was downloaded from Protein Data Bank (PDB: http://www.rcsb.org/pdb/home/home.do) 4 mmh is PDB id of the target protein HepC.

Preparation of ligands

Four synthesized compounds were selected as ligand considering their biological activities. Then the 2D structure of these compounds can be converted into 3D structures with the help of ACD\ChemSketch 1.1. It explores ways in which two molecules, such as drugs and an enzyme Hep-C (liver cancer cell line) receptor fit together and dock each other well. The molecules binding to a receptor, inhibit its function and thus act as a drug. Pyridinoyl thiazoles and receptors were selected *via* docking and their stabilities were estimated by molecular dynamics and their binding affinities by employing free energy imitations.

Validation of the docking protocol

The exactness of the docking procedure is calculated by redocking the co-crystallized ligand with the protein. The PyRx has reproduced the reported docking values and the experimentally determined binding sites of the ligand into 4 mmh.

Results and Discussion

3-(2-ethylaminothiazol-5-oyl)pyridine (6a): Yellow solid; yield 68 %; m.w.:233.29.: mp.:140-142°C; IR (KBr, ν_{max}, cm^{-1}): 3083 (C-H), 1686 (C=N), 1609 (C=O), 1H NMR (solvent:DMSO-d6): δ 9.17 (s, 1H, H-2 of pyridine), 9.4 (s, 1H, NH), 8.62 (d, 1H, H-6), 8.49 (m, 2H, pyridine), 8.21 (s, 1H, thiazole), 2.25 (q, 2H, CH2), 1.28 (t, 3H, CH3), m/z: 233.06 (100.0%), Elemental analysis of C11H11N3OS calcd. (found) %: C, 56.63 (56.61); H, 4.75 (4.45); N, 18.01 (17.98).

3-(2-propylaminothiazol-5-oyl)pyridine (6b): Yellow solid; yield 66%; m.w.:247.08.: mp.:135-147°C; IR (KBr, ν_{max}, cm^{-1}): 3099 (C-H), 1713 (C=N), 1680 (C=O), 1H NMR (solvent: DMSO-d6): δ 9.23 (s, 1H, H-2 of pyridine), 9.40 (s, 1H, NH), 8.72 (d, 1H, H-6), 8.28 (d, 1H, pyridine), 7.50 (t, 1H, pyridine), 8.05 (s, 1H, thiazole), 2.49 (t, 2H, CH2), 1.55 (m, 2H, CH2), 1.17 (t, 3H, CH3), m/z: 247.08(100.0%), Elemental analysis of C12H13N3OS calcd. (found) %: C, 58.28 (58.07); H, 5.30 (5.26); N, 16.99 (16.97); O, 6.47 (6.45); S, 12.96 (12.93).

3-(2-isopropylaminothiazol-5-oyl)pyridine (6c): Yellow solid; yield 65%; m.w.:247.08.: mp.:135-145°C; IR (KBr, ν_{max}, cm^{-1}): 3092 (C-H), 1686 (C=N), 1650 (C=O), 1H NMR (solvent: DMSO-d6): δ 9.17 (s, 1H, H-2 of pyridine), 9.07 (s, 1H, NH), 8.73 (d, 1H, H-6), 8.34 (d, 1H, pyridine), 8.74 (t, 1H, pyridine), 8.04 (s, 1H, thiazole), 2.28 (d, 1H, CH), 1.16 (m, 6H, 2CH3), m/z:247.08 (100.0%), Elemental analysis of C12H13N3OS calcd. (found) %: C, 58.28 (58.06); H, 5.30 (5.29); N, 16.99 (16.9); O,6.47 (6.46); S, 12.96 (12.95).

3-(2-butylaminothiazol-5-oyl)pyridine (6d): Yellow solid; yield 68%; m.w.:261.09.: mp.:140-143°C; IR (KBr, ν_{max}, cm^{-1}): 3215 (C-H), 2307 (C=N), 1760 (C=C), 1220 (C=N), 1682 (C=O), 1H NMR (solvent: DMSO-d6): δ 8.91 (s, 1H, H-2 of pyridine), 9.27 (s, 1H, NH), 8.74 (d, 1H, H6), 8.62 (d, 1H, pyridine), 7.63 (t, 1H, pyridine), 8.10 (s, 1H, thiazole), 2.503 (t, 2H, CH2), 1.56 (m, 2H, CH2), 1.34 (m, 2H, CH2), 0.91 (t, 3H, CH3) m/z:261.09 (100.0%), Elemental analysis of C13H15N3OS calcd. (found) %: C, 59.75 (59.72); H, 5.79 (5.72); N,16. 08 (16.03); O, 6.12 (6.11); S, 12.27 (12.25) (Fig. 1).

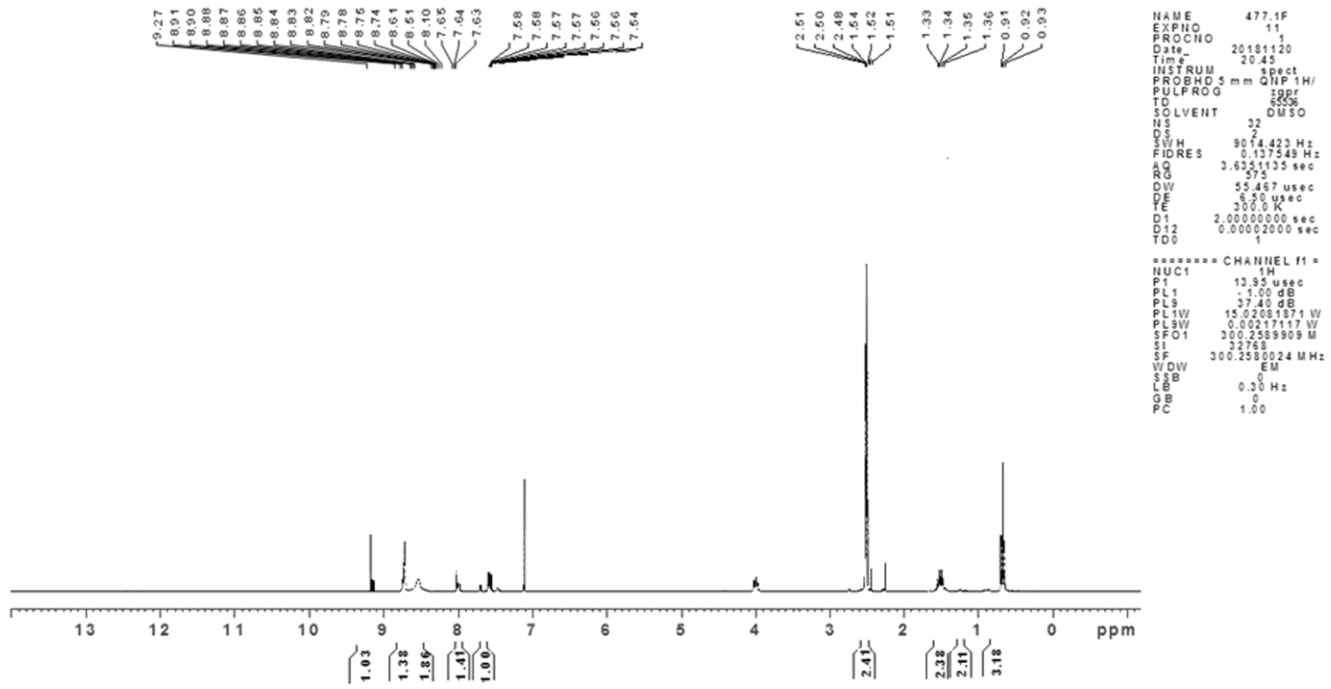


Fig. 1 — H1 NMR spectrum of 3-(2-butylaminothiazol-5-oyl)pyridine (6d)

Molecular Geometry

Molecular structure through the numbering scheme of atoms is acquired from Gaussian 09 package. Minimum energy obtained by DFT structure optimization using 6-31G (d, p) basis sets[14] for the synthesized compounds as –1294.22 amu, –1333.3 amu, –1333.53 amu and –1372.83 amu, respectively. And the structural parameters of the optimized compounds (bond length, bond angle, and dihedral angle) were calculated and tabulated. Then C - S bond length is greater than C - N, C - O and C - H because the size of the atom increases bond length also increases. For computational chemistry, we need to be more precise by using cartesian coordinates, bond lengths, and bond angles to find the optimal molecular geometry (Fig. 2 & Table 1).

Electronic Properties

In 3-(2-alkylaminothiazol-5-oyl)pyridines HOMO presented in carbonyl group and the LUMO is located in the thiazole ring. HOMO– LUMO energies divulge that the all HOMO orbitals of this series of derived compounds mainly from 2p orbitals of oxygen atoms present in the carbonyl moiety and LUMO orbitals are presented in 2p orbital of both nitrogen from thiazole moiety and an amino group (Fig. 3 & Table 2).

Docking study

The interaction energy between molecules and protein can be analyzed by using a very popular

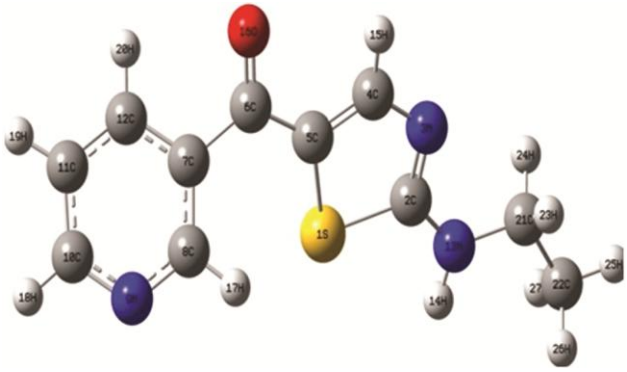


Fig. 2 — The optimized structure of 3-(2-ethylaminothiazol-5-oyl)pyridine (6a)

molecular docking technique. Researchers utilize docking in cancer research since it gives significant insight into ligand binding mechanisms, protein-ligand interactions, and knowledge of the optimal orientation of the ligand to its target[15]. In 3-(2-alkylaminothiazol-5-oyl)pyridines derivatives were selected for the docking studies with 4 mmh (PDB ID) using PyRx virtual screening tool (Fig. 4). Hydrogen bonding interaction and docking score of the pyridinoylthiazole derivatives were summarized in (Table 3). The above four synthesized compounds 6c is the most active ligand and the active site of this ligand containing one hydrogen bond interaction. Observed amino acid residue for 6c is ARG-248.

Antioxidant Activity

The simple, quick, and inexpensive method to assess the antioxidant extent of food involves the use of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) which is commonly used to test the ability of compounds to act as hydrogen donors and free radical scavengers and to estimate antioxidant activity. Hence, the DPPH radicals are broadly used to analyze the radical scavenging activity of compounds. Thus, the assessment of antioxidant ability was executed *in vitro* by DPPH scavenging assay. The free radical DPPH and the odd electron provide maximum absorption at 517 nm (purple colour). The radical is scavenged by the antioxidants, the absorbance decreases resulting in colour change from purple to pale yellow. The absorbance of DPPH at 517 nm was determined using ultraviolet spectra after 30 minutes. The DPPH concentration in the reaction solution was calculated from the calibration curve plotted at 517 nm at different concentrations and inhibition percentages. Butylated hydroxyanisole (BHA) was used as a standard in our study[16] (Fig. 5). Antioxidant study using effective free scavenging activity of the phycocynin as concentration dependent manner[17].

The antioxidant activity of 3-(2-alkylaminothiazol-5-oyl)pyridines shows good antioxidant activities. The compound 3-(2-ethylaminothiazol-5-oyl)pyridine 6a shows excellent antioxidant activity and 3-(2-

Table 1 — Data of 3-(2-alkylaminothiazol-5-oyl)pyridines

Bond length data					
Position	Parameter	6a	6b	6c	6d
Thiazole	C-N	1.3477	1.3475	1.3479	1.3479
Thiazole	C = N	1.3233	1.3231	1.3238	1.3231
Thiazole	C-S	1.8397	1.8403	1.8415	1.8405
Thiazole	C-H	1.0809	1.0809	1.0809	1.0809
Pyridine	C-C	1.4051	1.4051	1.4051	1.4051
Pyridine	C-N	1.3512	1.3512	1.3512	1.3512
Pyridine	C-H	1.0833	1.0833	1.0837	1.0837
Chain	C-C	1.5286	1.5332	1.5378	1.5332
Chain	C-N	1.3477	1.3475	1.3479	1.3479
Chain	N-H	1.0084	1.0085	1.0090	1.0088
Bond angle data					
Thiazole	S-C-N	114.38	114.38	114.22	114.34
Thiazole	C-N-C	111.96	111.96	112.06	111.98
Thiazole	N-C-C	118.57	118.58	118.60	118.59
Thiazole	C-C-C	124.54	124.54	124.47	124.53
Pyridine	C-C-C	122.45	122.45	122.48	122.46
Pyridine	C-C-N	123.23	123.23	123.23	123.23
Pyridine	C-N-C	118.07	118.07	118.07	118.07
Chain	N-C-H	108.79	-	-	-
Chain	C-C-C	-	111.89	-	112.28
Chain	N-C-C	110.19	110.45	110.45	110.45
Dihedral angle data					
Parameter		6a	6b	6c	6d
S–C-N-C		–179.67	–179.68	179.87	179.67
C-N-C-H		–178.96	–178.95	–179.95	–179.94
C-C-C-C		–172.15	–172.22	–172.38	–172.38
O-C-C-C		–150.24	–150.18	–150.18	–150.30
N-C-C-H		–178.94	–178.94	–179.93	–178.94
H-C-C-H		-	178.61	-	-

Table 2 — Electronic parameters of 3-(2-alkylaminothiazol-5-oyl)pyridines

Parameters	6a	6b	6c	6d
Total Energy	–1063.2	–1102.5	–1102.5	–1141.8
DipoleMoment	4.6467	4.7960	4.545	4.8308
E_{HOMO}	–0.2548	–0.2543	–0.2545	–0.2542
E_{LUMO}	–0.0367	–0.0361	–0.0377	–0.0361
ΔE	0.21805	0.21826	0.21685	0.21805
X	0.1457	0.1452	0.1461	0.14517
I	0.25481	0.25438	0.25455	0.25420
H	0.10902	0.10913	0.108425	0.10902
S	4.58610	4.5816	4.61148	4.58610

Electronegativity (χ), Ionisation Potential (I), Hardness (η), Softness (S)

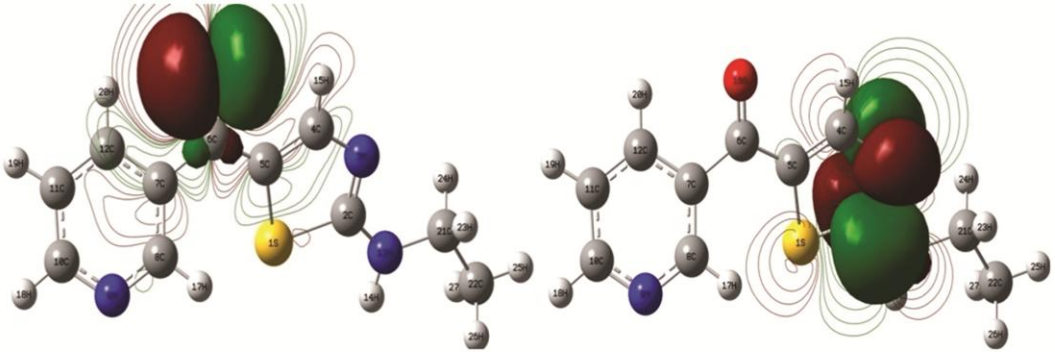


Fig. 3 — HOMO and LUMO of 3-(2-ethylaminothiazol-5-oyl)pyridine (6a)

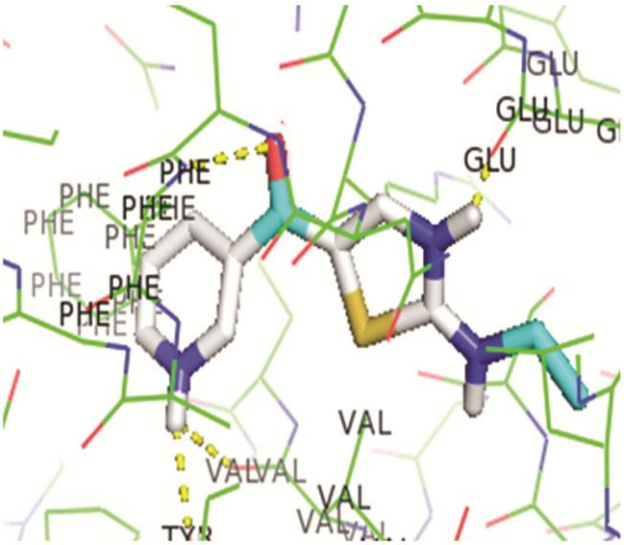


Fig. 4 — Docking image of 3-(2-ethylaminothiazol-5-oyl)pyridine (6a)

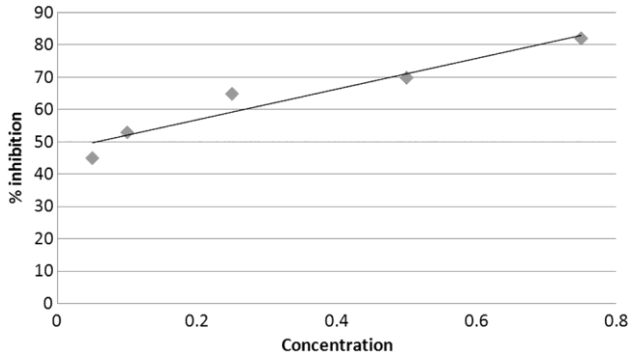


Fig. 5 — Plot of % inhibition *vs* concentration of 3-(2-ethylaminothiazol-5-oyl)pyridine (6a)

Table 3 — Docking Score and hydrogen bonding interactions of 3-(2-alkylaminothiazol-5-oyl)pyridine derivatives

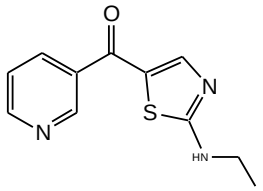
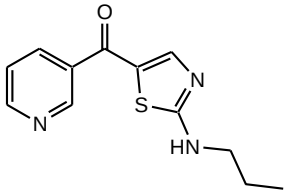
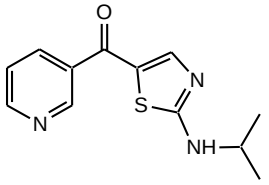
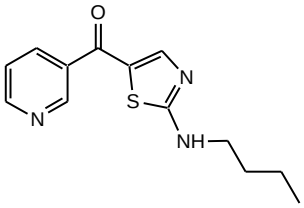
Compound	Docking Score (Kcal/mol)	Residue involved in Hydrogen bonding
6a	–7.5	PHE-251,GLU-245,VAL-196,PHE-251
6b	–7.3	GLN-247, ALA-246
6c	–8.0	ARG-248
6d	–7.8	GLN-247, VAL-196

propylaminothiazol-5-oyl)pyridine 6b, 3-(2-isopropyl aminothiazol-5-oyl)pyridine 6c have IC_{50} values 136 μM and 111 μM, respectively, and hence they exhibit show uncontrolled cell growth, good antioxidant activities (Table 4).

Anticancer Activity

Cancer is a group of cells that show uncontrolled cell growth, incursion, and occasionally metastasis. Cancer is the world's second leading cause of death and a major

Table 4 — Antioxidant activities of 3-(2-alkylaminothiazol-5-oyl)pyridines (6a-d)

Compound	IC_{50} Value (μM)
 6a	57
 6b	136
 6c	111
 6d	217
BHA(std)	624

health problem in developed countries. Advancement in treatment and awareness of the disease has led to a reduction in cancer deaths, but the number of new diagnoses continues to rise. Several anticancer drugs such as doxorubicin have been identified, which act by binding to DNA and DNA enzymes[18]. In advances, chemotherapy is one of the terms for cancer, which involves the use of one or more anti-cancer drugs, which can specifically kill cancer cells without making a significant impact on normal cells. Various types of compounds are used to fight cancer. Pyridine derivatives are used for the study of anticancer activity (Table 5).

The synthesized compounds with high antioxidant activities were examined for their anticancer activity against MDA-MB-231 cell line *in vitro* by MTT assay method.

Table 5 — Anticancer activity of 3-(2-ethylaminothiazol-5-oyl)pyridine 6a

Sample Concentration (µg/mL)	Percentage Viability
6.25	92.58
12.5	81.17
25	63.52
50	42.35
100	22.35
IC_{50}	42.23

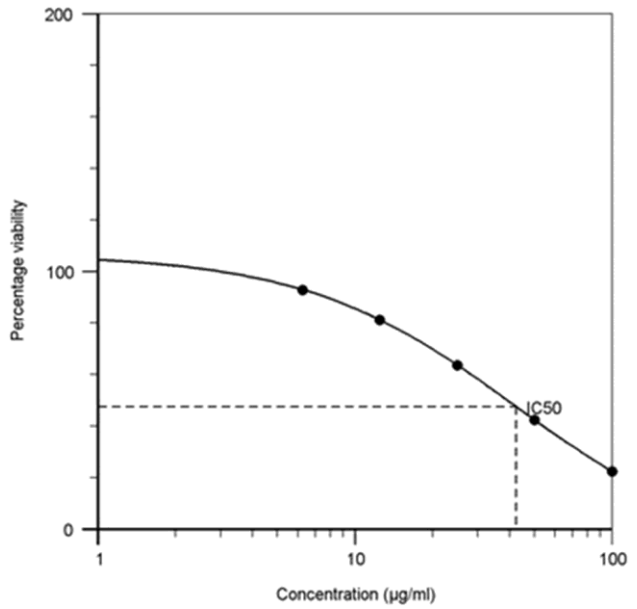


Fig. 6 — Plot of % viability *vs* concentration of 3-(2-ethylaminothiazol-5-oyl)pyridine (6a)

The percentage of growth inhibition was calculated using the formula (Fig. 6):

$$\%\ \text{of viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of Control}} \times 100$$

Conclusion

In summary, we have designed a viable route and suitable reaction conditions to synthesize 3-(2-alkylaminothiazol-5-oyl)pyridine derivatives. All the synthesized compounds were characterized by IR, 1H NMR, MS, and analytical data. The computational studies and electronic parameters were calculated using DFT method with B3LYP/6-31G (d, p) basis sets. All the compounds screened for better docking scores. All the compounds screened for better docking scores. The bond gap energy of all pyridinyl derivatives very small it shows highest biological activity. It confirmed the 3-(2-alkylaminothiazol-5-oyl)pyridine derivatives highly bioactive character in medicinal chemistry. On the basis of docking result, all the compounds were highly active so we conclude that it possess high agreements with the liver cancer cell line. The compound 6a possess excellent antioxidant activity, it also proved the compound posses very good anticancer activity against breast cancer cell line MDA-MB-231.

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Conflict of interest

All authors declare no conflict of interest.

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