DRUG DESIGN STUDIES

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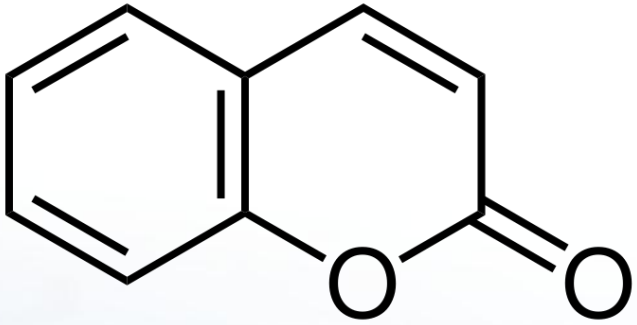
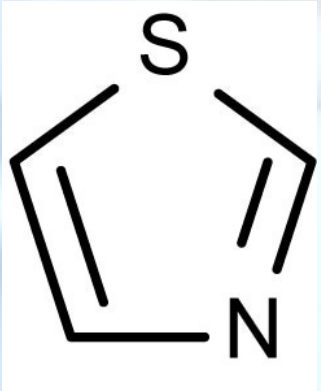
1. COUMARINE COMPOUNDS CONTAINING THIAZOLE SKELETON

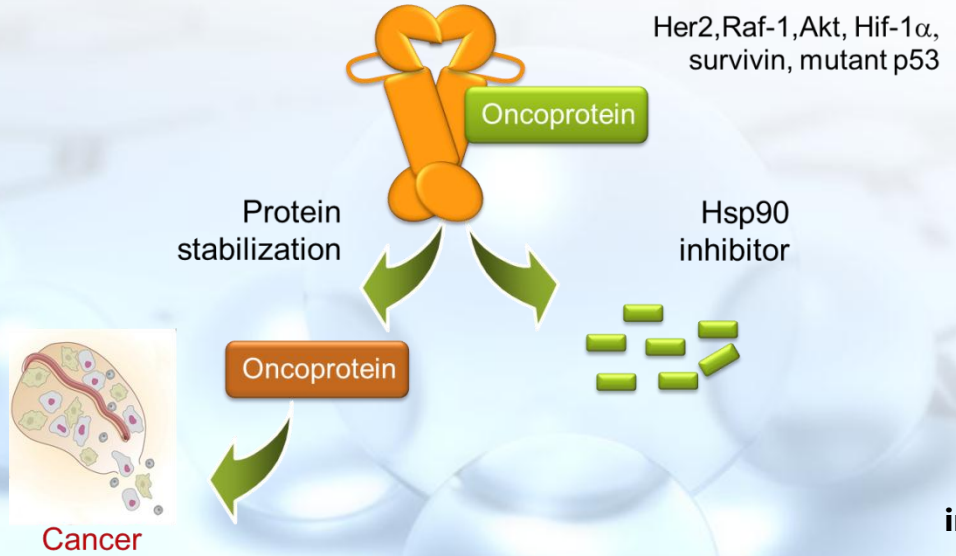
Coumarins (2*H*-1-benzopyran-2-ones) are classified as member of the benzopyrone family of compounds which possess a wide spectrum of biological activity as anticancer, antimicrobial, anti-inflammatory, and analgesic agent.



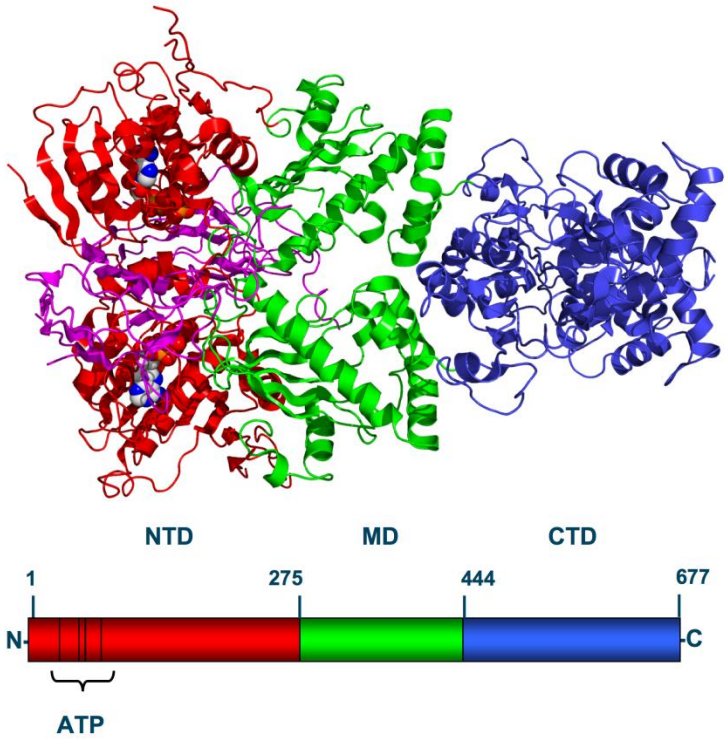
Thiazoles are important class of azoles heterocyclic compounds that include imidazoles and oxazoles. Thiazole and its derivatives were evaluated in drug design for the treatment of bacterial and fungal infections, tuberculosis, allergies, HIV infections, neurodegenerative diseases and recently for the treatment of cancer.



Recent studies have shown that coumarin derivative compounds (novobiocin, chlorobiocin, coumermycin A1 and KU135) can bind to CTD of Hsp90 and disrupt Hsp90 dimerization and degrade oncogenic client proteinsvia ubiquitination proteasome pathway.

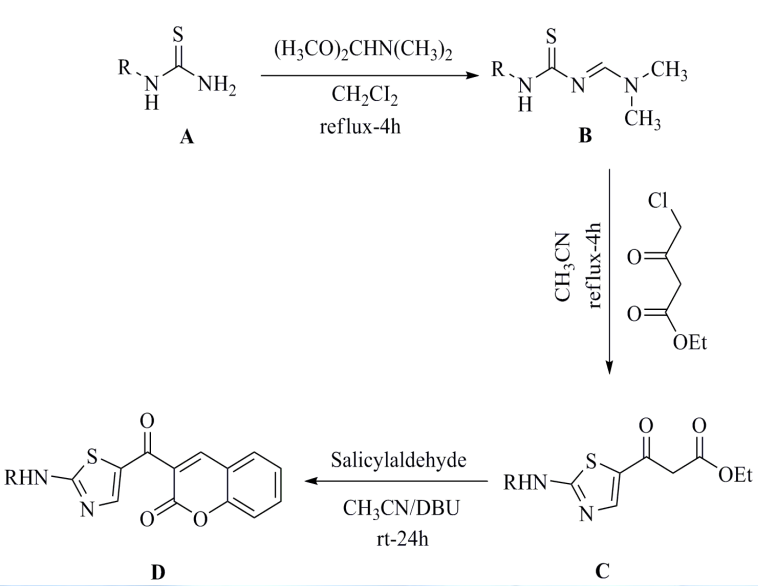


Heat shock protein 90 (Hsp90) is overexpressed in tumor tissues and is required for growth and survival of cancer cells. To date, more than two hundred Hsp90 client proteins were identified and some of the client proteins (cell cycle regulators, transcription factors, transmembrane tyrosine kinase, steroid receptors, mutated signaling proteins, chimeric signaling proteins) are associated with signaling pathways in tumor development.7,8,13,14 These client proteins (i.e. ErbB2, v-Src, p53, Raf-1, Akt, survivin, Bcr-Abl, Cdk4, telomerase, Her-2, Cdk6 and MET) play important roles in apoptosis, metastasis, angiogenesis and immunoregulation processes.Hsp90 inhibitors lead to the degradation of oncogenic client proteins and therefore inhibition of Hsp90 has emerged as an important therapeutic strategy which effects on multiple oncogenic pathways in tumors.

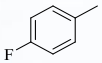
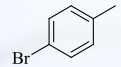
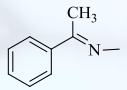
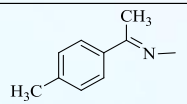
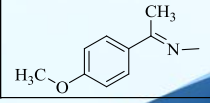


- **Heat shock protein 90 represent 1-2% of all cellular proteins**
- **Facilitate protein-folding and stabilization. Induced under stress, hypoxia and oxidative damage.**
- **Generally, the expression level of Hsp90 is increased at up to 2- to 10-fold in human cancer cells than in normal cells.**

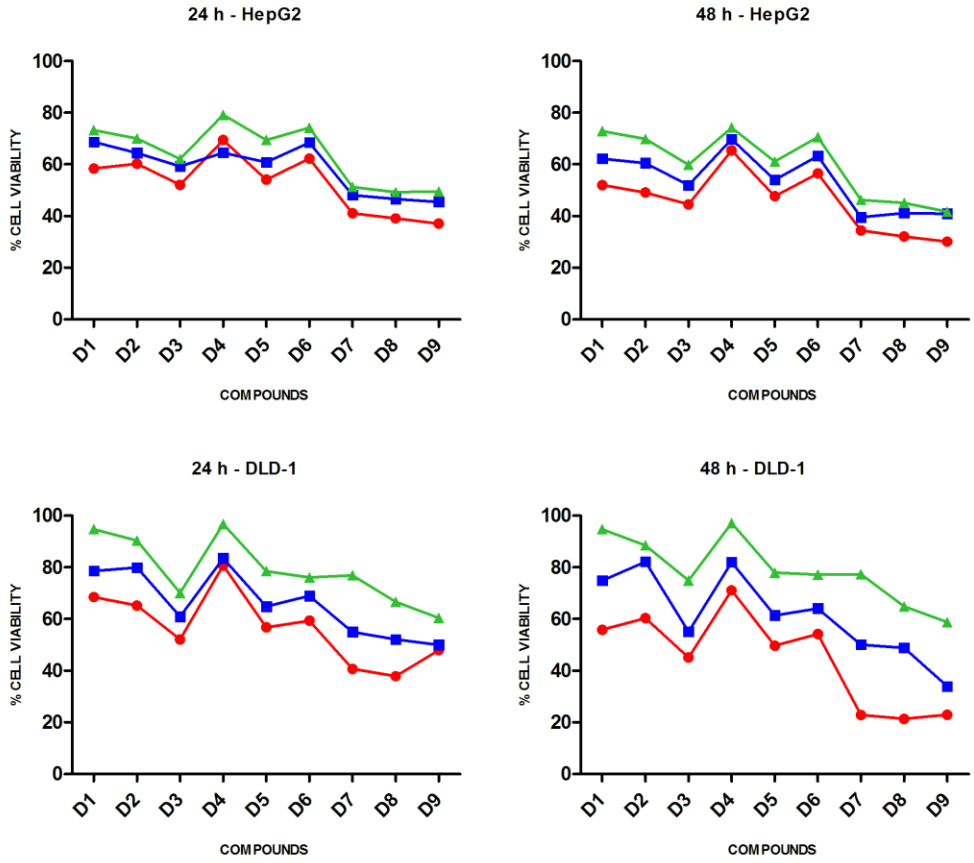
SYNTHESIS



R (A, B, C, D)
1, CH_3
2, CH_3CH_2
3, C_6H_5
4, p-F-C_6H_5
5, CH_2=$CHCH_2$
6, p-Br-C_6H_5
7, $C_6H_5C(CH_3)$=N
8, p-CH_3-$C_6H_5C(CH_3)$=N
9, p-CH_3O-$C_6H_5C(CH_3)$=N

Entry	R-	M_p (°C)	Mol. Formula (Mol. wt.)	Yield	Elemental Analyses	Calcd. %	Found
D1	H_3C—	269	$C_{14}H_{10}N_2O_3S$ 286.31	59	C H N S	58.73 3.52 9.78 11.20	58.50 3.54 10.09 11.48
D2	CH_3CH_2—	268	$C_{15}H_{12}N_2O_3S$ 300.33	56	C H N S	59.99 4.03 9.33 10.68	60.02 4.14 9.20 10.82
D3		329	$C_{19}H_{12}N_2O_3S$ 348.38	61	C H N S	65.50 3.47 8.04 9.20	65.95 3.31 8.48 8.62
D4		357	$C_{19}H_{11}FN_2O_3S$ 366.37	52	C H N S	62.29 3.03 7.65 8.75	62.66 2.94 7.21 8.32
D5	CH_2=$CHCH_2$—	246	$C_{16}H_{12}N_2O_3S$ 312.34	60	C H N S	61.53 3.87 8.97 10.27	61.05 3.96 8.72 9.71
D6		330	$C_{19}H_{11}BrN_2O_3S$ 427.27	64	C H N S	53.41 2.59 6.56 7.50	53.05 2.51 6.19 7.39
D7		317	$C_{21}H_{15}N_3O_3S$ 389.43	67	C H N S	64.77 3.88 10.79 8.23	64.68 3.82 10.61 8.15
D8		308	$C_{22}H_{17}N_3O_3S$ 403.45	43	C H N S	65.49 4.25 10.42 7.95	65.79 4.17 9.99 8.16
D9		298	$C_{22}H_{17}N_3O_4S$ 419.45	39	C H N S	63.00 4.09 10.02 7.64	63.26 4.04 9.80 7.63

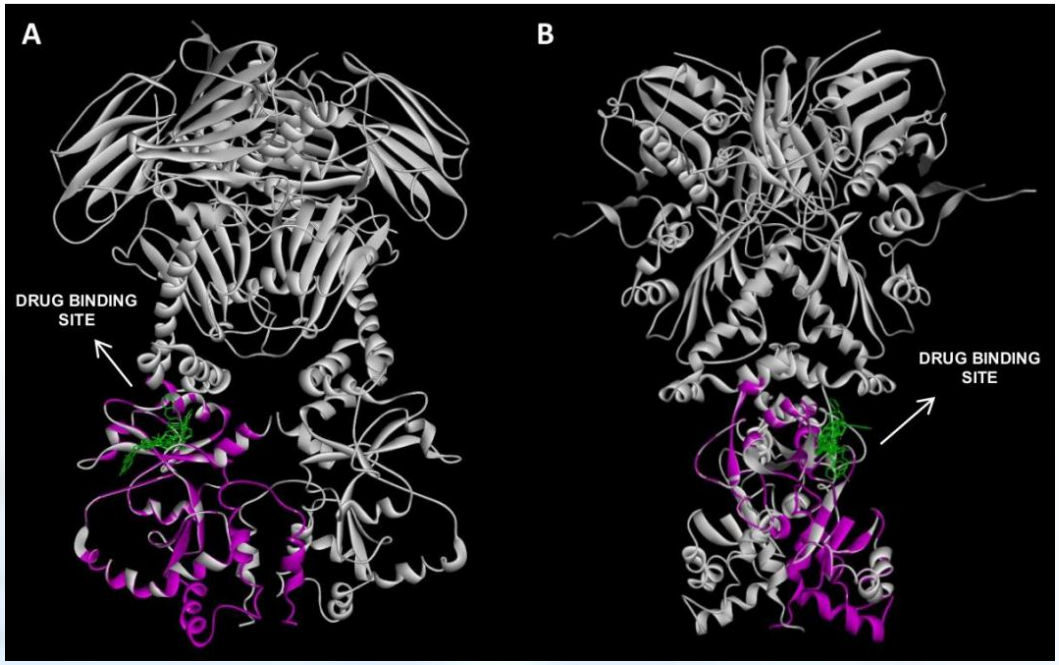
IN-VITRO STUDIES



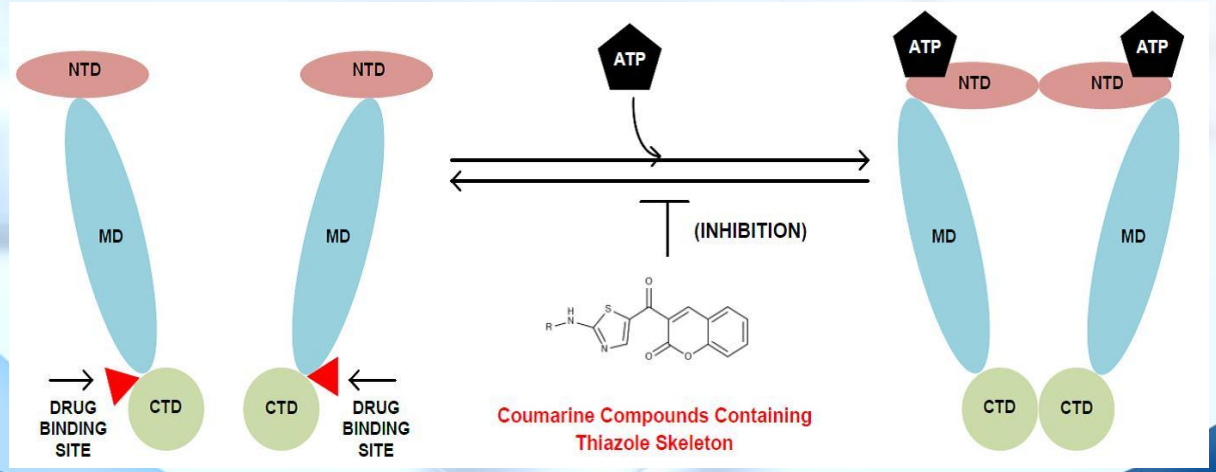
Antitumor properties of thiazolyl coumarine derivatives were tested *in vitro* against

human colon (DLD-1) and liver (HepG2) cancer cell lines

IN-SILICO STUDIES



Binding regions of compounds (D1-D9). A. Front view, B. Side view. C terminal domain was shown in magenta and ligands are in green color.



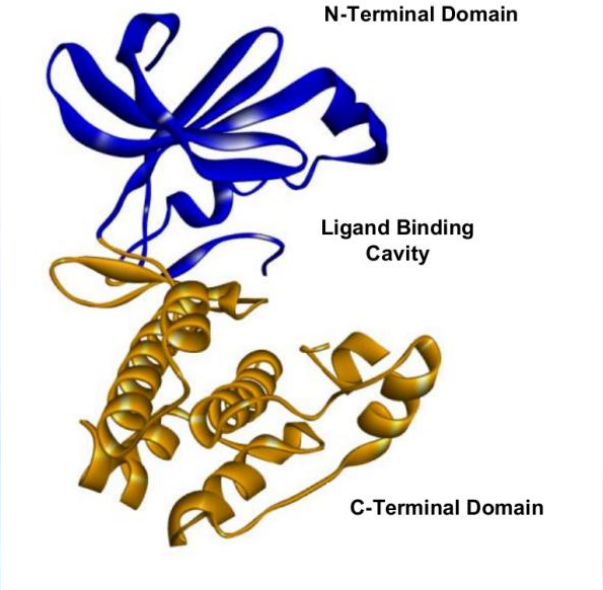
Drug binding to Hsp90; in the presence of ATP, Hsp90 forms dimer. The cavity between the monomers form a hydrophobic environment and help substrate proteins fold to their native conformation. In the presence of D1-D9, the drugs interfere with CTD conformational change and as a result Hsp90 cannot form dimer. Thus, substrate peptides may not fold properly.

2. PYRAZOLYL ACYL THIOUREAS

Acyl thiourea derivatives have been increasingly important with a wide diversity of applications in heterocyclic chemistry, metal complexes, molecular electronics and exhibit an array of biological activities. Some of them are employed as fungicidal, antiviral, antimicrobial, parasiticidal, antitumoral and pesticidal agent.

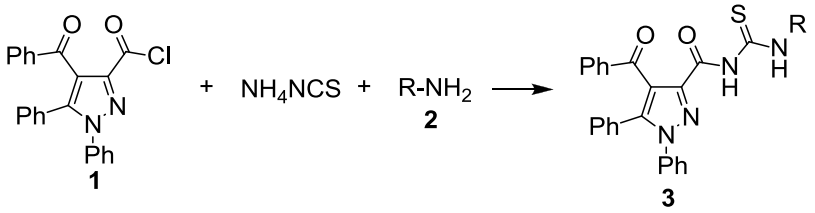
Pyrazole is five member ring heterocyclic compound and its anticancer, antimicrobial, anti-inflammatory, antiviral, antifungal, and analgesic properties are known for a long time. Particularly, the pyrazole nucleus is found to be very important in the development of anticancer agents [19,20]. Pyrazole derivatives show anticancer activities by inhibition of Aurora kinases and pyrazole derivate Aurora kinase inhibitors were reported in the literature.

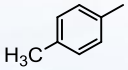
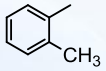
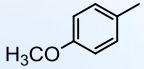
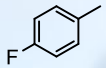
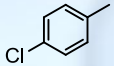
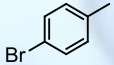
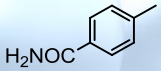
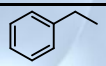
Aurora kinases play significant roles in cell cycle control and regulation of mitosis including centrosome duplication, mitotic spindle formation, chromosome alignment upon spindle, mitotic checkpoint activation, and cytokinesis. There are three mammalian Aurora kinases; Aurora-A, Aurora-B and Aurora-C, which are expressed in different stages of the cell cycle.



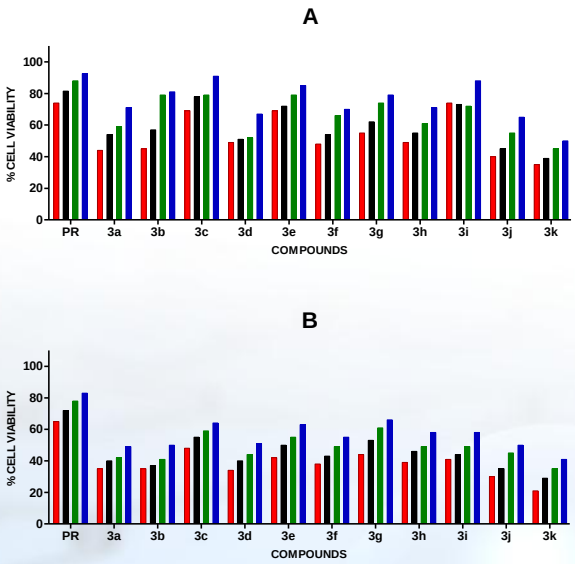
Aurora-A and Aurora-B are overexpressed in different human cancers. The aberrant expression of Aurora kinases causes genetic instability (aneuploidy) and deregulation of cell division which may trigger tumorigenesis.

SYNTHESIS

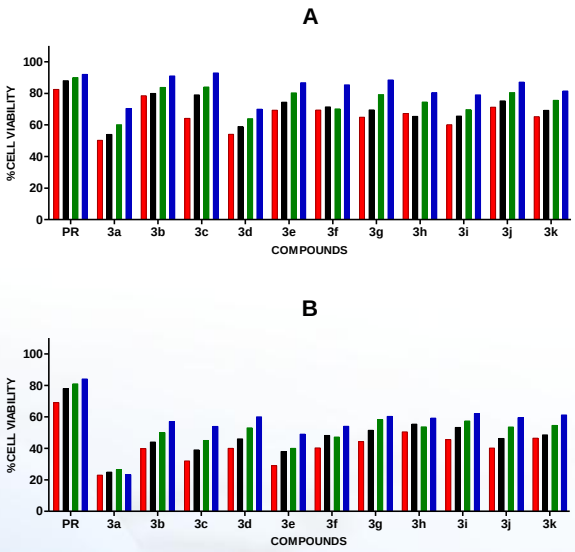


COMPOUND	-R	COMPOUND FORMULA	COMPOUND NAME	MOLECULAR WEIGHT (g/mol)
3a		$C_{30}H_{22}N_4O_2S$	4-benzoyl-1,5-diphenyl-N-(phenylcarbamothioyl)-1H-pyrazole-3-carboxamide	502.59
3b		$C_{31}H_{24}N_4O_2S$	4-benzoyl-1,5-diphenyl-N-(p-tolylcarbamothioyl)-1H-pyrazole-3-carboxamide	516.61
3c		$C_{31}H_{24}N_4O_2S$	4-benzoyl-1,5-diphenyl-N-(o-tolylcarbamothioyl)-1H-pyrazole-3-carboxamide	516.61
3d		$C_{31}H_{24}N_4O_3S$	4-benzoyl-N-(4-methoxyphenylcarbamothioyl)-1,5-diphenyl-1H-pyrazole-3-carboxamide	532.61
3e		$C_{30}H_{21}FN_4O_2S$	4-benzoyl-N-(4-fluorophenylcarbamothioyl)-1,5-diphenyl-1H-pyrazole-3-carboxamide	520.58
3f		$C_{30}H_{21}ClN_4O_2S$	4-benzoyl-N-(4-chlorophenylcarbamothioyl)-1,5-diphenyl-1H-pyrazole-3-carboxamide	537.03
3g		$C_{30}H_{21}BrN_4O_2S$	4-benzoyl-N-(4-bromophenylcarbamothioyl)-1,5-diphenyl-1H-pyrazole-3-carboxamide	581.48
3h		$C_{34}H_{24}N_4O_2S$	4-benzoyl-N-(naphthalen-1-ylcarbamothioyl)-1,5-diphenyl-1H-pyrazole-3-carboxamide	552.65
3i		$C_{31}H_{23}N_5O_3S$	4-benzoyl-N-(4-carbamoylphenylcarbamothioyl)-1,5-diphenyl-1H-pyrazole-3-carboxamide	545.61
3j		$C_{31}H_{24}N_4O_2S$	4-benzoyl-N-(benzylcarbamothioyl)-1,5-diphenyl-1H-pyrazole-3-carboxamide	516.61
3k		$C_{28}H_{20}N_6O_2S$	4-benzoyl-1,5-diphenyl-N-(pyrimidin-2-ylcarbamothioyl)-1H-pyrazole-3-carboxamide	504.56

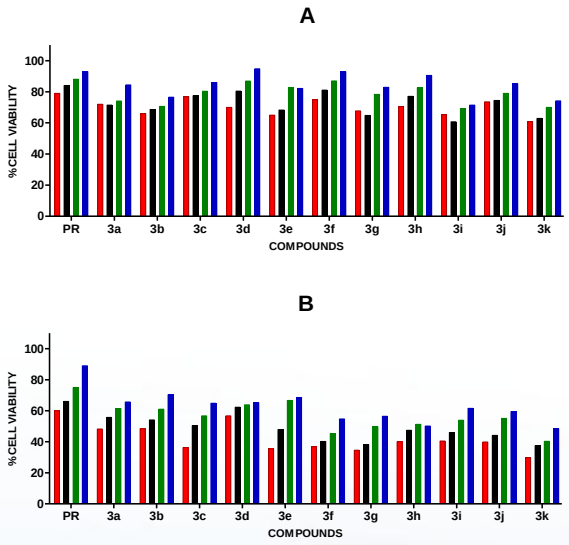
IN-VITRO STUDIES



MCF-7



MG-63

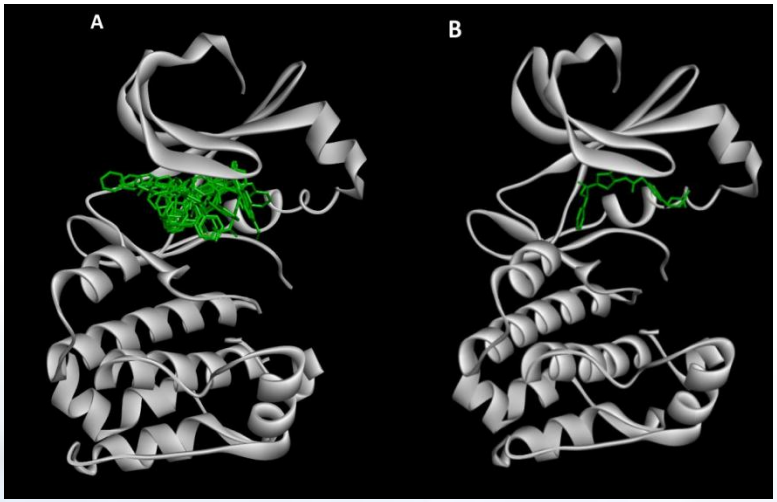


Saos-2

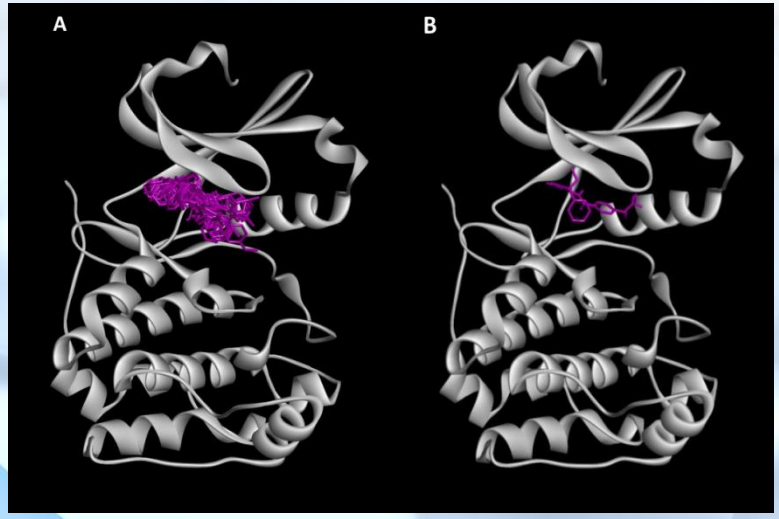
Cell proliferation assay of MCF-7, MG-63 and Saos-2 cell lines in the presence of the novel compounds and pyrazole root (PR) at 24 (A) and 48 (B) hours. (red: 10-6 M, black: 10-7 M, green: 10-8 M, blue: 10-9 M)

Figure-7

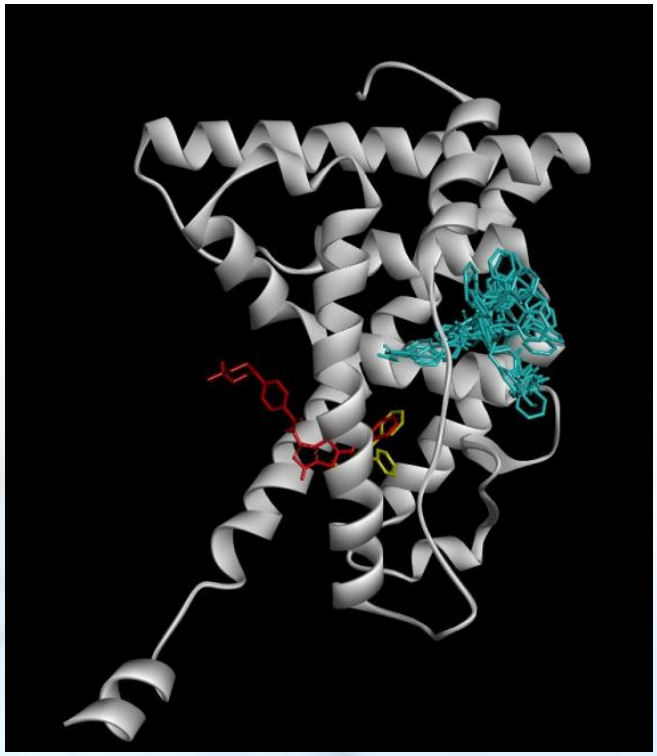
IN-SILICO STUDIES



Aurora kinase-A interaction with A. Novel inhibitors B. PHA-739358. The inhibitors bind to the same pocket as PHA-739358.



Aurora kinase-B interaction with A. Novel inhibitors B. PHA-739358. The inhibitors bind to the same pocket as PHA-739358.



Estrogen receptor α interaction with novel inhibitors (cyan), PHA-739358 (red-long chain) and pyrazole root (yellow-short chain). The inhibitors cannot bind to the same pocket as PHA-739358 and pyrazole root.

All novel compounds were docked to Aurora-A and Aurora-B and the compounds were fit into the ligand binding cavity in the same manner as commercial pyrazole based Aurora kinase inhibitor PHA-739358. However, the compounds may not effectively dock to ER-α ligand binding site as PHA-739358. The compounds bound out of ER-α ligand binding region as shown in Figure 8. It was proposed that addition of acyl thiourea group changed the binding characteristics-affinity of ER-α to the pyrazole ring. Structural modifications of the pyrazole ring prevent ER-α inhibition and this may help us to specifically inhibit the desired protein at related oncogenic pathway.

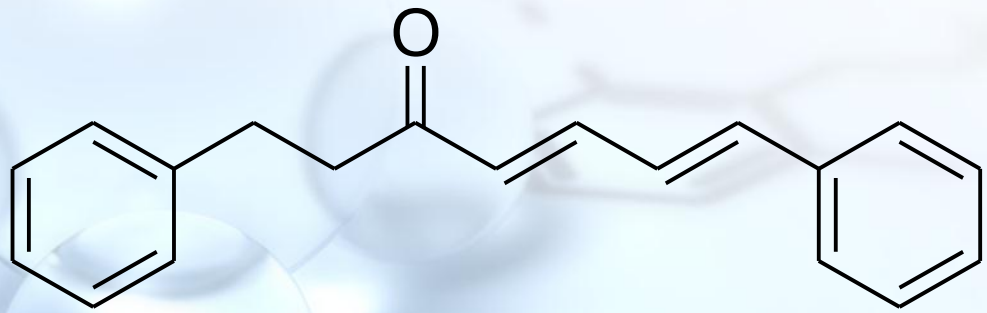
3. ALNUSTONE DERIVATIVE COMPOUNDS

The biological activities of alnustone as well as their synthesis have been the subject of numerous studies. Remarkable antihepatotoxic actions of alnustone amongst many natural diarylheptanoids were reported.

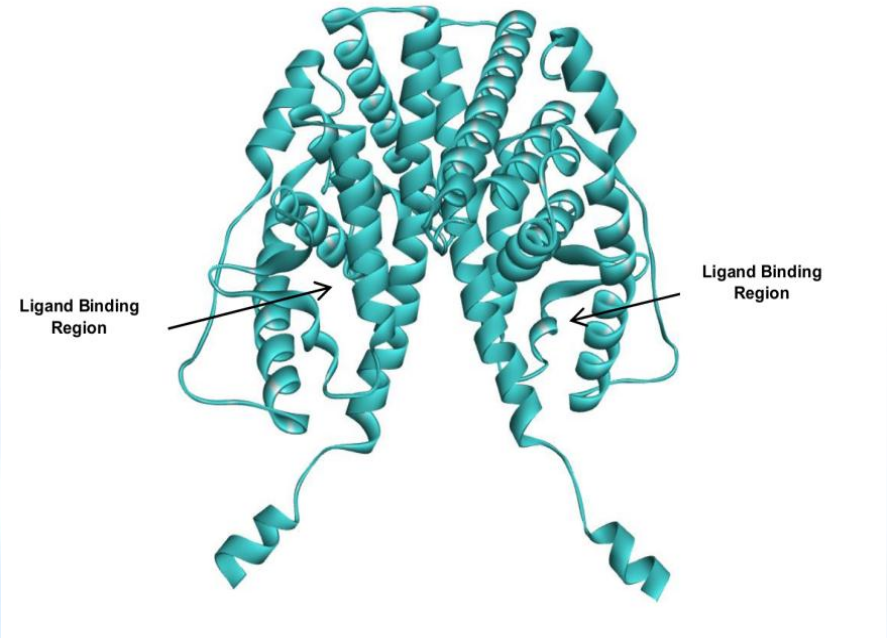
Anti-inflammatory activity of alnustone isolated from Curcuma xanthorrhizawas reported. Antibacterial activity of alnustone against well-known bacteria species were also reported. They also reported alnustone to have antiemetic activity. Also, weak estrogenic activity of isolated alnustone from rhizomes of Curcuma comosawas determined.

Alnustone shows neuraminidase inhibitory activity and it was concluded that the compoundmay be employed as antiviral agent.

Some alnustone derivatives compounds evaluated their antitumor activities in vitro.

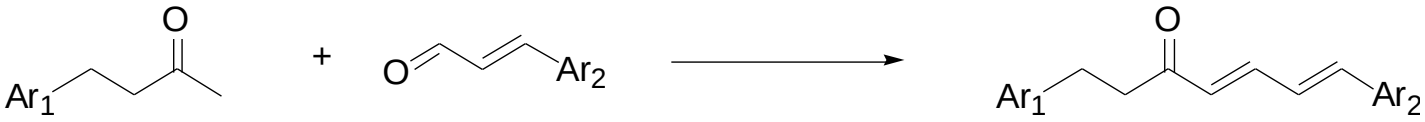


The alnustone compounds are potential drug candidates as Estrogen Receptor-α antagonist.

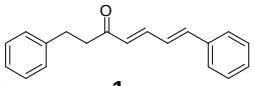


Prognosis of the breast cancer depends on genetic andlifestyle-related factors, aging and estrogen hormone. Estrogen stimulatesboth normal and malignant mammary tissues. ER-αis overexpressed around70% of the breast cancer cases (known as ER-α positive breast cancer), and binding of estrogen to the ER-αprotein triggersthe formation of the breast tumors. Therefore, many ER-α antagonists have been developed for blocking ER-α protein in the breast cancer treatment.

SYNTHESIS

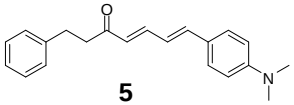


Synthesis of Alnustones



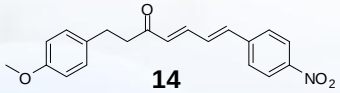
1

Chemical Formula: $C_{19}H_{18}O$
Elemental Analysis: C, 86.99; H, 6.92; O, 6.10



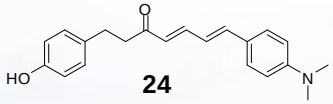
5

Chemical Formula: $C_{21}H_{23}NO$
Elemental Analysis: C, 82.58; H, 7.59; N, 4.59; O, 5.24



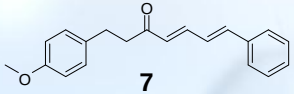
14

Chemical Formula: $C_{20}H_{19}NO_4$
Elemental Analysis: C, 71.20; H, 5.68; N, 4.15; O, 18.97



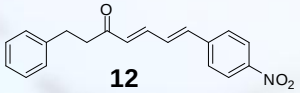
24

Chemical Formula: $C_{21}H_{23}NO_2$
Elemental Analysis: C, 78.47; H, 7.21; N, 4.36; O, 9.96



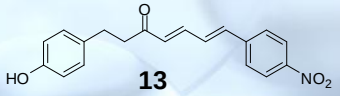
7

Chemical Formula: $C_{20}H_{20}O_2$
Elemental Analysis: C, 82.16; H, 6.89; O, 10.94



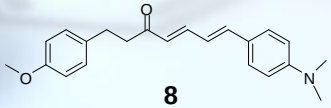
12

Chemical Formula: $C_{19}H_{17}NO_3$
Elemental Analysis: C, 74.25; H, 5.58; N, 4.56; O, 15.62



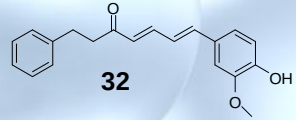
13

Chemical Formula: $C_{19}H_{17}NO_4$
Elemental Analysis: C, 70.58; H, 5.30; N, 4.33; O, 19.79



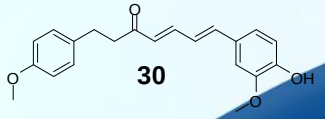
8

Chemical Formula: $C_{22}H_{25}NO_2$
Elemental Analysis: C, 78.77; H, 7.51; N, 4.18; O, 9.54



32

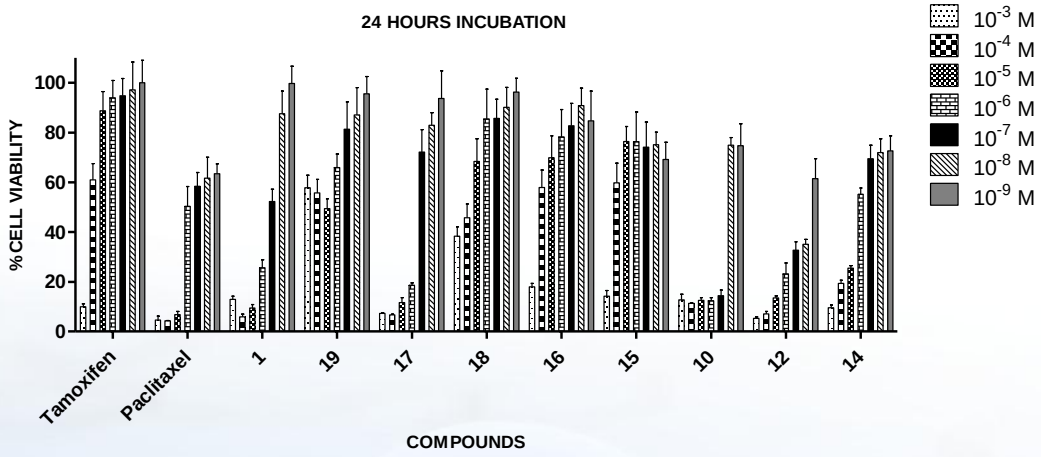
Chemical Formula: $C_{20}H_{20}O_3$
Elemental Analysis: C, 77.90; H, 6.54; O, 15.57

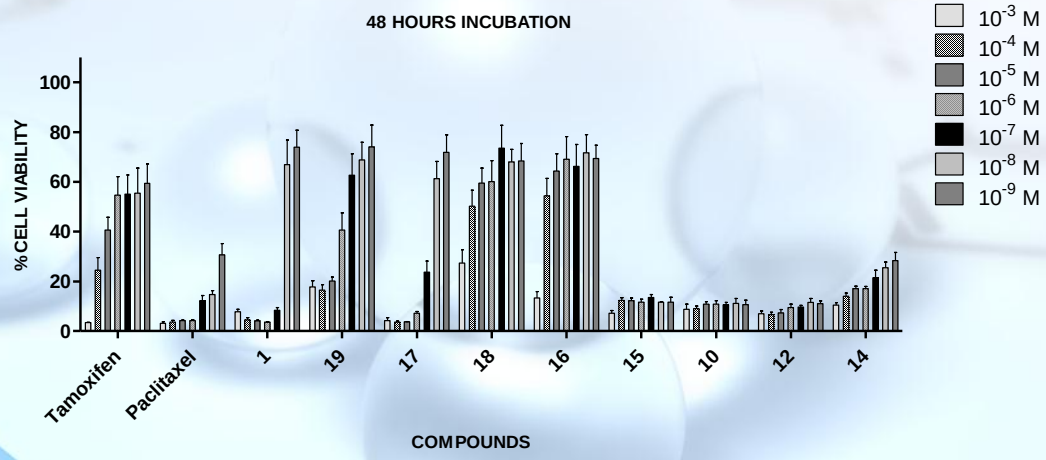


30

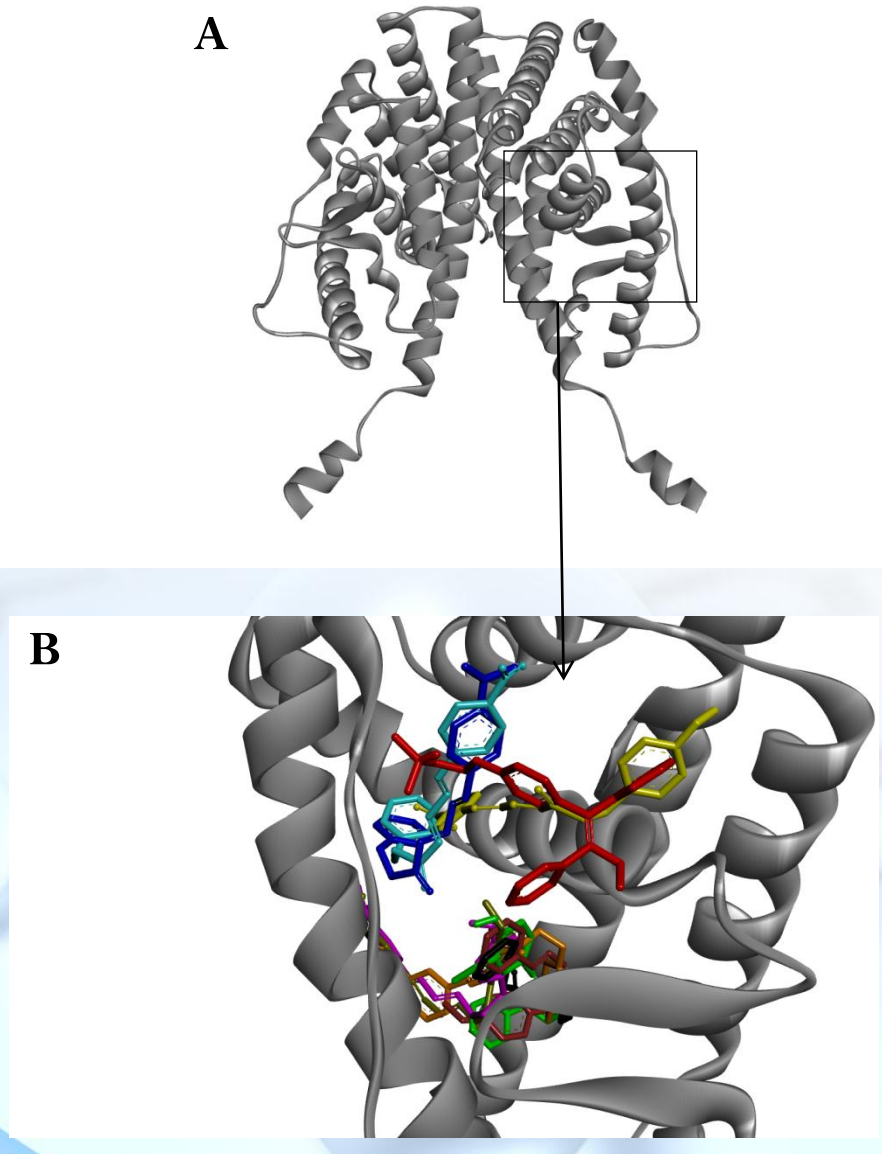
Chemical Formula: $C_{21}H_{22}O_4$
Elemental Analysis: C, 74.54; H, 6.55; O, 18.91

IN-VITRO STUDIES





Anticancer activities of tamoxifen, paclitaxel and ten alnustone-like compounds on MCF-7 cell line after 24 and 48 hours incubation. Compound 8 did not provide consistent statistical results and therefore, omitted from the scientific evaluation.



A) Structure of human estrogen receptor alpha (ER-α) B)Binding regions of tamoxifen and alnustone-like compounds (Tamoxifen: red, 1: black, 19: blue, 16: cyan, 15: yellow, 17: brown, 18: cement, 10: pink, 12: orange, 14: green).

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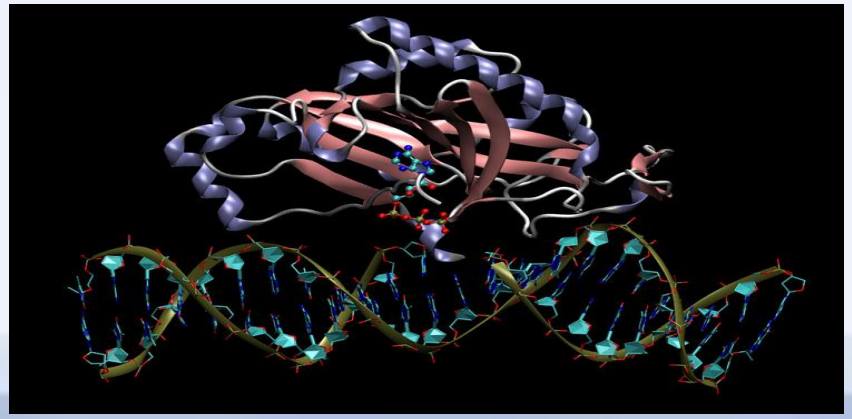

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