The Interactions between Heterocyclic Compounds and Target Proteins Involved with Cancer

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Abstract— *Cancer is the second leading cause of mortality globally. The World Health Organization forecasts that by 2030, there will be 22 million new cases of cancer globally. Extensive global research focuses on cancer prevention, diagnosis, and treatment procedures. The metabolic profile of cancer cells is distinct from that of normal cells, attributable to epigenetic and genetic abnormalities. Numerous anti-cancer drugs available commercially feature heterocycles as their main structural element. Furthermore, anticancer drugs approved by the FDA from 2010 to 2015 contain heterocyclic rings in their chemical structure. Their extensive cellular processes and mechanisms, along with their prevalence in nature, account for their inclusion in anti-cancer medications. This study elucidates several heterocyclic compounds exhibiting anticancer effects on various cell lines. These compounds feature rings composed of nitrogen, sulfur, and oxygen. The collection of information on heterocyclic rings may facilitate the discovery of novel compounds with potential anticancer properties in the future.*

Keywords— Heterocyclic compounds, Anticancer activity, Cell lines, Cytotoxicity, Natural product.

I. INTRODUCTION

Most research has concentrated on VEFG as an initiator; however, various cancer targets have been identified, including enzymes that deacetylate histones, tyrosine kinase, the growth factor TGF-α, fibroblast growth factor (FGF), phosphoglycerate geranylgeranyl transferase (PGF), epidermal growth factor (EGF), and phosphodiesterase types I and II. Despite numerous instances of disease progression post-therapy, inhibition of VEGF signaling has not demonstrated significant efficacy. Numerous heterocyclic anticancer agents, originating from both natural and synthetic sources, are currently utilized, and investigations for further compounds are ongoing. Figure 1 illustrates several instances. The therapeutic properties of heterocyclic compounds, which are cyclic structures containing carbon along with one or more nitrogen, oxygen, or sulfur atoms, have been studied for their potential in treating cancer and various other conditions. Druggable candidates are optimized for ADMET (Adsorption, Distribution, Metabolism, Excretion, and Toxicity) through the introduction of heteroatoms, enhancing their solubility, polarity, and hydrogen bonding capacities. Brevilin A, also referred to as 11, is a natural substance derived from Centipeda minimum. It is a heterocyclic sesquiterpene lactone with demonstrated anticancer properties. Research indicates that Brevilin A may inhibit cell growth, induce apoptosis, and reduce cell metastasis by diminishing the activity of tyrosine kinase, signal transducer, and activator of transcription 3 (STAT3). Lee, Chan, and colleagues synthesized analogues of Brevilin A for their investigation. It was found that compounds 13 and 14, synthesized from paraformaldehyde and 11 via an aldol reaction with sodium carbonate, exhibited greater anticancer efficacy than 11. Cancer therapies frequently encounter issues such as drug resistance, systemic toxicity from treatments, and ineffective medications. Identifying novel anticancer agents as potential drug leads is essential due to the challenges in discovering effective therapeutic agents for tumor treatment. These challenges arise from the inherent variability of cancer cells and the intricate nature of signaling networks. A potential solution to these issues is the

application of multi-target heterocyclic inhibitors in cancer treatment. Several new heterocyclic compounds demonstrate established anticancer effects, including Midostaurin (16), Vorinostat (17), and Sunitinib (15). Their capacity to concurrently regulate multiple growth factors, such as VEGFR, c-Kit from PDGFRA, and FLT-3, is notable. Figure 2 illustrates that numerous drugs exhibiting promising inhibitory potentials are currently undergoing Phase 3 clinical trials. This includes various HER1 and HER2 inhibitors such as sotagliflozin (21), lapatinib (20), erlotinib (19), and gefitinib (18).

II. LITERATURE REVIEW

Ledade (2022) Fused nitrogen heterocyclic molecules have gained attention for their therapeutic properties in recent years. N-Heterocyclic scaffolds, which are versatile and easily synthesized, have numerous potential applications in synthetic organic chemistry and the biological domain. These compounds offer broad-spectrum antibacterial and anticancer medicines with low toxicity levels, but cytotoxicity levels are higher than those of cisplatin, the gold standard anticancer drug. Numerous synthetic techniques have been developed to synthesize N-heterocycles and their derivatives, offering a range of structural flexibility for targeted biological uses.

Martins (2015) Heterocycle molecules and fragments are essential in medicinal chemistry due to their adaptability, physicochemical characteristics, and prevalence in medicines. They are being studied for their potential effectiveness against various types of cancer. Their unique flexibility and dynamic structure have been specifically used in anticancer research. However, these compounds have drawbacks, including potential limiting concerns. This summary discusses the key biological objectives, structure-activity relationships, biochemical processes of action, and intrinsic limiting concerns with heterocyclic compounds, focusing on those beneficial for cancer treatment. The article also discusses the potential of nano vectorization to enhance the pharmacokinetic and pharmacodynamic features of heterocycles, particularly with the introduction of nanotechnology for effective selective drug targeting.

Didehban (2018) Heterocyclic systems are essential building blocks in organic synthesis and are found in numerous compounds, including over 90% of new drug structures. Researchers are working on innovative one-pot methods to synthesize these organic molecules using basic, cheap, and easily accessible building blocks. One of the most intriguing and encouraging synthetic operations is the chemical fixation of carbon dioxide onto organic molecules. This approach has evolved over the last five years, allowing for the synthesis of several biologically significant heterocyclic systems. This brief overview focuses on recent developments in this field of chemistry, particularly in terms of reactions' mechanisms.

Naturalista (2024) Pyrazole derivatives are heterocyclic compounds with diverse biological and therapeutic applications. They have a broad range of effects, including antibacterial, anti-inflammatory, antioxidant, antiviral, antidiabetic, and neuroprotective effects. Contemporary techniques, such as solvent-free methods, microwave-assisted synthesis, green chemistry, catalytic methods, and multicomponent reactions, have replaced traditional methods in synthesis. These developments have made pyrazole-based compounds more efficient and selective, improving their use in the pharmaceutical industry. This article provides a comprehensive overview of the synthetic processes used to generate pyrazole derivatives and investigates the structure-activity relationship (SAR) of these compounds. It highlights the importance of pyrazole derivatives in developing novel medicinal medicines and provides guidance for future studies. The comprehensive analysis aims to highlight the potential of pyrazole derivatives as flexible candidates for creating new medications, contributing to continuous advancements in medicinal chemistry.

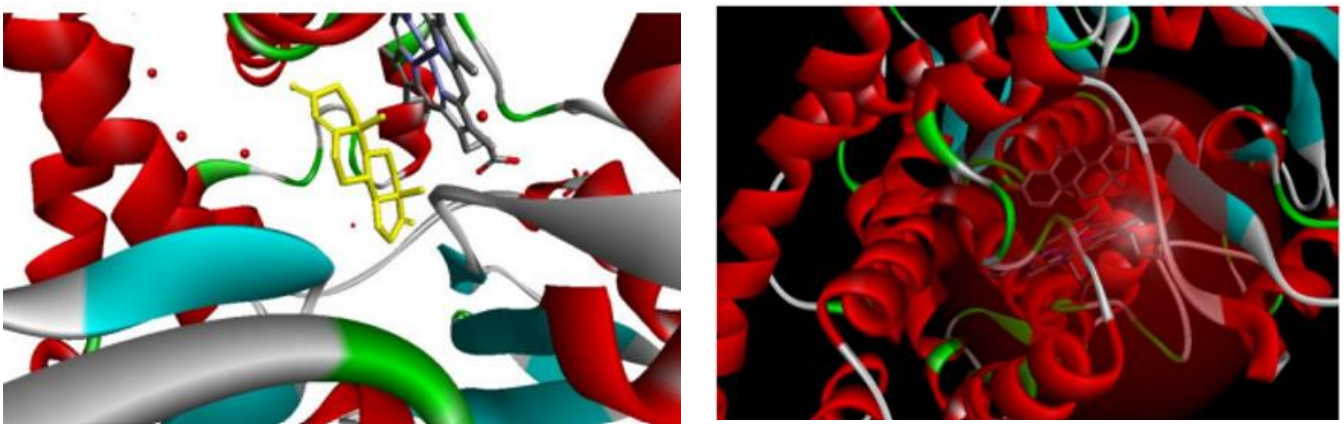
Mirza, Agha Zeeshan. (2019). Cancer is a major public health issue with millions of deaths annually. Chemotherapeutic medications, including synthetic versions of natural molecules, are effective in treating cancer. Nucleoside analogues, which have been used in antitumor chemotherapy, neoplasm treatment, and viral infection management, have become essential in cancer therapy. This review examines nucleosides and their potential utility in cancer treatment, including those pending FDA approval. The article also discusses the impact of substitution on nucleoside analogues and discusses the progress of computational chemistry in this field.

III. MATERIAL AND METHODS

3.1 Investigating Novel Non-Estradiol Chemo-Types as Aromatase Inhibitors in a Controlled Environment:

The urgent demand for innovative pharmaceutical discoveries requires the creation of new drug models, and in-silico trials offer an effective approach to achieve this goal. The activity of the aromatase enzyme has been elucidated, and advanced artificial intelligences have been developed, owing to in-silico studies conducted by various researchers utilizing state-of-the-art methodologies such as Structure-Guided Design, High-Throughput Docking, and pharmacophore-based modeling approaches. Advancements in understanding the structure and function of aromatase have been facilitated by sophisticated methods, including membrane-bound molecular dynamic simulations.

Results indicate that the charged amino acid sequence of aromatase, comprising alkyl and aryl amino acids, is essential for interaction with aromatase inhibitors. Heme porphyrin, located at the active site, stabilizes the transition state of the substrate through its oxidation state and functions as an electron donor. Park et al. demonstrated in their study on molecular modelling of the aromatase active site that the Aspartic acid 309 residue, located near the entrance of the active site, is essential for substrate access to the active site channel. The aromatase active site is frequently coordinated by the heme-porphyrin complex in the presence of sp2 nitrogen within the heterocyclic structure of aromatase inhibitors. Amino acid residues 133, 235, 395, 474, 302, 308, 309, Threonine, Serine 478, and 480 comprise sixteen distinct mutations in aromatase. Mutations significantly alter ligand binding affinity at the aromatase active site amino acid residues, either enhancing or diminishing it.



(A) **(B)**

FIGURE 1: The 3S7S and 3EQM aromatase proteins

3.2 Creating a QSAR prototype:

The IC50 activity and chemical diversity of the ligands in the aligned training set were employed to validate the model. The objective was to produce significant predictions. Four distinct PLS components were utilized to develop a series of models for regression through a partial least squares (PLS) approach. Table 1 indicates that the highest overall model significance and statistical significance were attained with PLS factor 4 (# Factor), chosen based on the robust correlation between the training set and Partial Least Square factors. The predictive power of the test set was assessed, yielding a Q2 of 0.7854, RMSE of 0.5284, and Pearson R of 0.9111. The measure of variance is denoted as F. Regressions exhibiting higher F values are deemed more statistically significant. P denotes the significance level of the variance ratio. Smaller P values indicated a higher level of certainty. The Q-squared value of the expected activities is the product of Q2 with itself. Pearson-R quantifies the extent of concordance between the anticipated and observed activity in the test set. The most effective 3D QSAR models for prediction were those that fulfilled all of these criteria concurrently.

TABLE 1
ASSESSMENT OF THE BEST PHARMACOPHORE HYPOTHESIS ARR.1 USING PARTIAL LEAST SQUARES

S. No.	# Factors	SD	R-Squared	F	P	Stability	RMSE	Q-Squared	Pearson-R
1	1	0.7851	0.5544	27.4	3.014e-005	0.6157	0.8218	0.4809	0.7716
2	2	0.5422	0.7972	41.3	5.3l3e-008	0.3104	0.6054	0.7183	0.8589
3	3	0.2807	0.9482	122.1	5.006e-013	0.1769	0.5096	0.8003	0.9302
4	4	0.1265	0.99	470.6	l.033e-018	0.094	0.5284	0.7854	0.9111

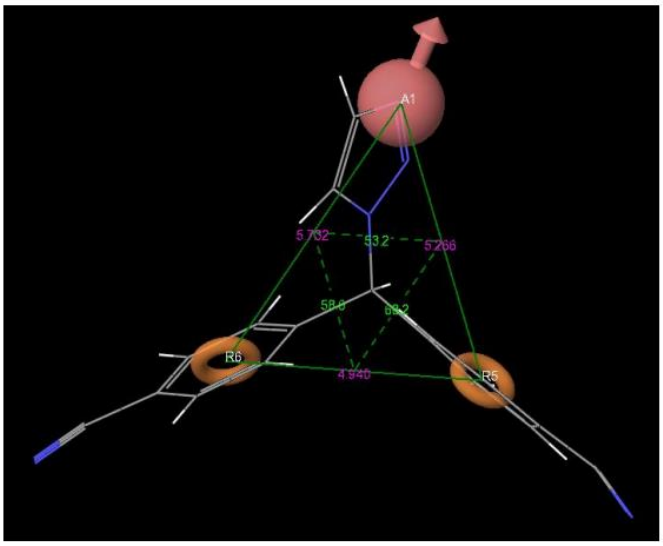
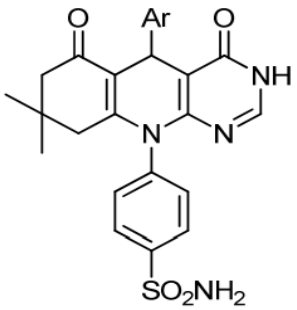


FIGURE 2: Potential structure of a pharmacophore

TABLE 2
ANALYSIS OF COMPOUNDS 6A AND 6B USING MICROSCOPY AND PHYSICAL DATA

Compd. No.	*Ar*	M,p, ('C)	Yield (%)	Mol. Formula(M. wt.)	Microanalysis	
					Calculated	Found
6a	**C_6H_4F-4**	**282-4**	*86*	**$C_{24}H_{23}FN_4O_3S$** (466.53)	C: 61.79 H:4.97 N:12.01	C: 61.91 H:4.73 N:11.80
6b	**C_6H_4Cl-4**	**280-2**	85	**$C_{24}H_{23}ClN_4O_3S$** (482.98)	C: 59.68 H:4.80 N:11.60	C: 59.92 H:5.00 N:11.33

5-[5-(4-fluorophenyl)-8,8-dimethyl-4,6-dioxo-3,4,6,7,8,9-hexahydro-pyrimido[4,5-b]quinolin-10(5H)-yl]`(7a) benzenesulfonamide and 4–[5-(4-chlorophenyl)-8,8-dimethyl-4,6-dioxo-3,4,6,7,8,9-hexahydropyrimido[4,5-b] quinolin-10(5H)-yl]isothiocyanate (7b)



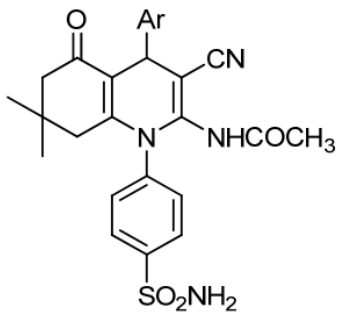
(7a & 7b)

After refluxing a solution of compound 6a or 6b (0.001 mol) in 20 ml of formic acid for 5 hours, cooling the mixture, and 7a and 7b were produced when the ensuing solid was crystallised from dioxane after it was ultimately poured into cold water.

TABLE 3
MICROANALYSIS AND PHYSICAL DATA OF MOLECULES 7a and 7b

Compd. No.	Ar.	M.p. (°C)	Yield (%)	Mol. Formula (M. wt.)	Microanalysis	
					Calculated	Found
7a	**C_6H_4F-4**	168-70	79	**$C_{24}H_{23}FN_4O_4S$** (494.54)	C: 60.72 H:4.69 N:11.33	C: 60.82 H:4.84 N:11.49
7b	**C_6H_4Cl-4**	162-4	78	**$C_{24}H_{23}ClN_4O_4S$** (510.99)	C: 58.76 H:4.54 N:10.96	C: 58.94 H:4.38 N:11.18

1,4,5,6,7,8-hexahydroquinolin-2-yl -[3-Cyano-4-(4-fluorophenyl)-7,7-dimethyl-5-oxo-1-(4-sulfamoyl-phenyl)] Eighthly, acetamide in addition to N-[4-(4-chlorophenyl)-3- cyano-7, 7-dimethyl-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl] 8-benzoic acid



(8a & 8b)

For the preparation of 8a and 8b, respectively, after a solution of compound 6a or 6b (0.001 mol) was refluxed in 20 ml of acetic anhydride for 5 hours, the reaction mixture was concentrated. The solid that had separated was then crystallised from ethanol.

TABLE 4
ANALYSIS OF COMPOUNDS 8A AND 8B USING MICROSCOPY AND PHYSICAL DATA

Compd. No.	Ar.	M.p. (°C)	Yield (%)	Mol. Formula (M. wt.)	Microanalysis	
					Calculated	Found
8a	**C_6H_4F-4**	150-2	97	**$C_{24}H_{23}FN_4O_4S$** (508.56)	C: 61.40 H:4.95 N:11.01	C: 61.68 H:5.11 N:10.83
8b	**C_6H_4Cl-4**	149-51	82	**$C_{24}H_{23}ClN_4O_4S$** (525.02)	C: 59.48 H:4.80 N:10.67	C: 59.31 H:5.05 N:10.49

IV. RESULTS

4.1 3D QSAR:

The ARR three-point pharmacophore model includes one hydrogen bond acceptor (A) and two aromatic rings (R). The 3D-QSAR model successfully predicted the performance of both the training and test sets, in accordance with the

pharmacophore-based alignment hypothesis. Analysis of the 3DQSAR model presented in the Workspace allows for the assessment of the influence of ligand properties on the expected activity, whether positive or negative. This 3D QSAR model depicts letrozole in its prototype form. The presence of oxygen and nitrogen in the blue cubes within the hydrogen bond acceptor region indicates an optimal environment for the binding of these atoms, subsequently enhancing biological activity. An unfavorable environment for the attachment of functional groups, indicated by the red cubes adjacent to the H-bond acceptor zone, negatively affects biological activity. Aromatic rings and acceptors are vector characteristics that significantly influence aligned structures, as evidenced by higher vector score values. The overlapping van der Waals models of non-hydrogen atoms assess the volume score for each pair of structures. To enhance the efficacy of the ligand, a hydrogen bond acceptor (A), which may be nitrogen or oxygen, is necessary.

TABLE 5

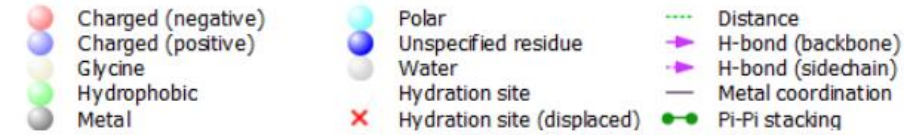
NOVEL CHEMOTYPES' 3D QSAR FINDINGS (COMPOUND 35-66)

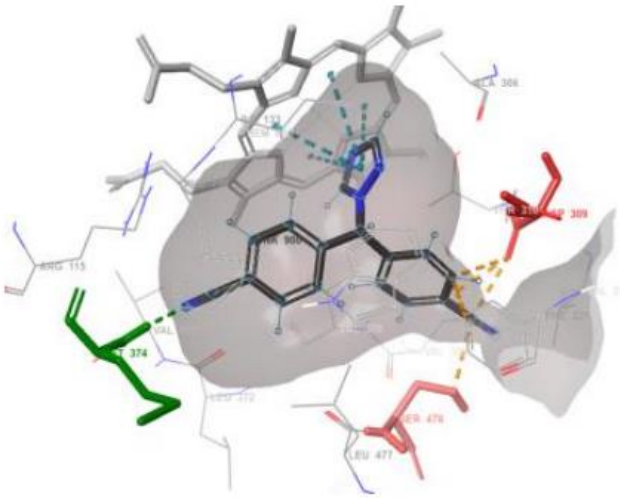
S.No.	Ligand	Predicted Activity	Align Score	Vector Score	Volume Score	Fitness
1	35	0.8697	0.043914	0.923434	0.553571	2.440411
2	36	-0.80296	0.256423	0.784339	0.442922	2.013576
3	37	-0.80296	0.2564	0.784376	0.454333	2.025042
4	38	-0.80296	0.256671	0.784165	0.457547	2.02782
5	39	-0.80296	0.256245	0.78448	0.456471	2.027413
6	40	0.737991	0.044625	0.923212	0.425	2.311024
7	41	0.852453	0.043946	0.923415	0.563636	2.450429
8	42	-0.73642	0.256497	0.784367	0.464115	2.034734
9	43	-0.73642	0.25638	0.784372	0.460808	2.03153
10	44	-0.70664	0.277472	0.772249	0.478673	2.019696
11	*4S*	0.800144	0.277472	0.712249	0.478673	2.0 19696
12	46	-0.7974	0.043947	0.92334	0.S70SS2	2.457269
13	47	-0.7974	0.25642	0.784365	0.468599	2.03928
14	48	-0.7974	0.256828	0.784019	0.454333	2.024329
15	49	-0.7670 1	*0.256095*	0.784621	0.465228	2.036436
16	*50*	0.965794	0.428475	0.805351	*0.5075*	l.955788
17	51	-0.47892	0.066766	0.949119	*0.566265*	2.459746
18	52	-0.81155	0.407089	0.839301	0.453488	1.953549
19	SJ	-0.69853	0.407735	0.839245	0.447005	1.946471
20	S4	-0.59219	0.40921	0.839001	0.45093.S	1.948927
21	*SS*	0.633341	0.406863	0.834213	0.44213	1.93729
22	S6	-0.66154	0.066775	0.9491	0.533851	2.477305
23	S7	*-0.654*	0.417373	0.760761	0.459135	l .872085
24	$8	--0.654	0.417764	0.839176	0.455399	1.95477 1
25	S9	-0.654	0.4-07765	0.839 1 27	0.46747	1.96()792
26	60	0.791436	0.4078	0.839162	0.472019	1 .971348
27	61	0.767623	0.044013	0.923331	0.561934	2.448587
28	62	-0.61032	0.066866	0.949051	0.582043	2.475373

4.2 Docking Results:

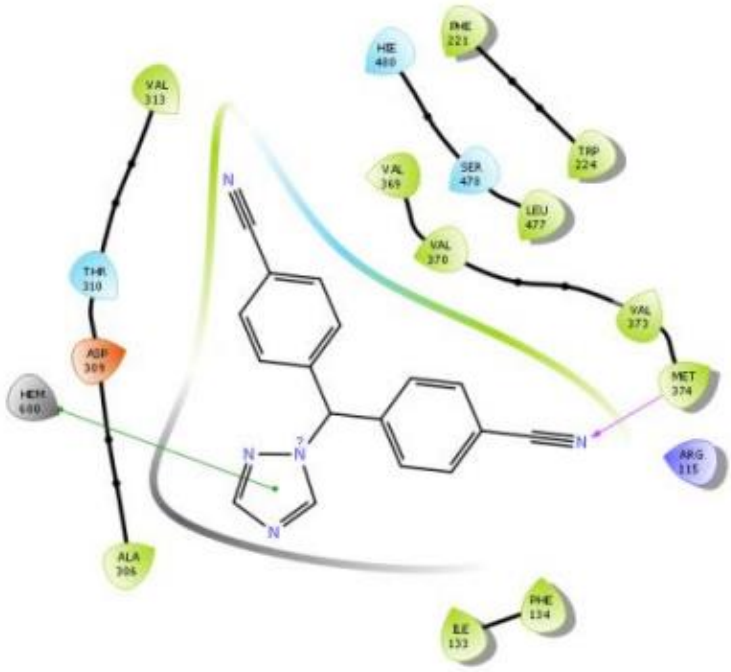
In order to enhance the interactions between ligands and receptors, the maestro workspace's Glide XP Visualiser was used to construct the active site surface mesh. Among the intriguing discoveries is the geometry of the aromatase receptor site. It resembles a standard iodine flask in appearance. The active cite is hydrophobic and has a conical entrance composed of L-phenylalanine 221 and L-Valine 313. The flat base of the flask, formed by the heme prosthatic group (grey), is essential for aromatisation because it provides electrons to the substrate.

Legend: Orange (charged-negative); Violet (charged-positive); Green (hydrophobic); Grey (metal); Sky blue (polar); (S) = Sinister configuration





Letrozole 3D docking



Letrozole 2D docking

4.3 Biological Activity:

4.3.1 In vitro anticancer screening:

The pharmacology department at Cairo University's National Cancer Institute conducted in vitro anticancer screening. This study utilized the MCF7 human breast tumor cell line. The cytotoxic activity of the newly synthesized compounds was assessed in vitro utilizing the Sulfo-Rhodamine-B stain (SRB) assay, as outlined by Skehan et al. The SRB test, established in 1990, is one of the most widely utilized methods for in vitro cytotoxic screening. This study evaluates SRB's binding

affinity for trichloroacetic acid (TCA)-fixed cell protein components on tissue-culture plates. Under mildly acidic conditions, the bright-pink aminoxanthene dye SRB interacts with basic amino acid residues; however, it dissociates in alkaline environments. The dye comprises two sulfonic groups.

Prior to treatment with the compounds under investigation, cells were permitted to adhere to the plate wall by being plated on a 96-well plate at a density of 10^4 cells per well for a duration of 24 hours. Three distinct wells were created for each concentration. The cells were incubated with the substance(s) for 48 hours at 37 ˚C in a 5% CO2 environment. After 48 hours, the cells were fixed, washed, and stained for 30 minutes using a solution of 0.4% (wt/vol) SRB in 1% acetic acid. Four washes with 1% acetic acid were required to remove the excess unbound color, followed by the application of Tris-EDTA buffer to restore the attached stain. The 570 nm wavelength was employed to measure color intensity in an ELISA reader.

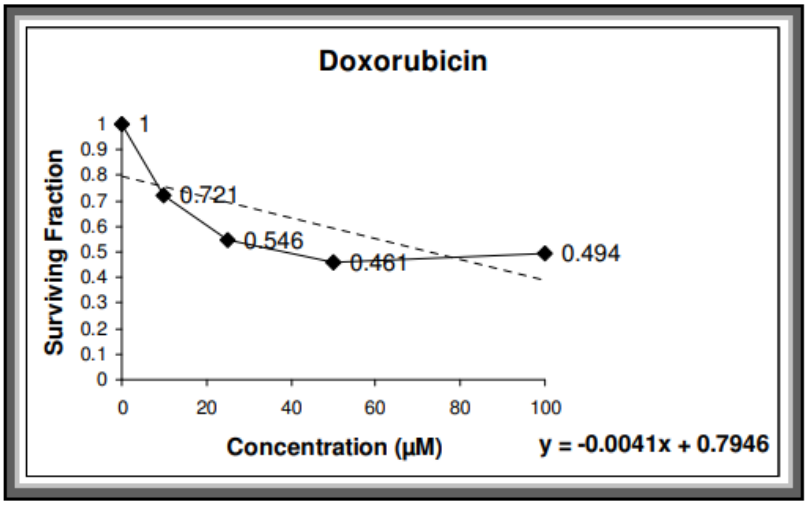


FIGURE 3: Time to death plot for doxorubicin

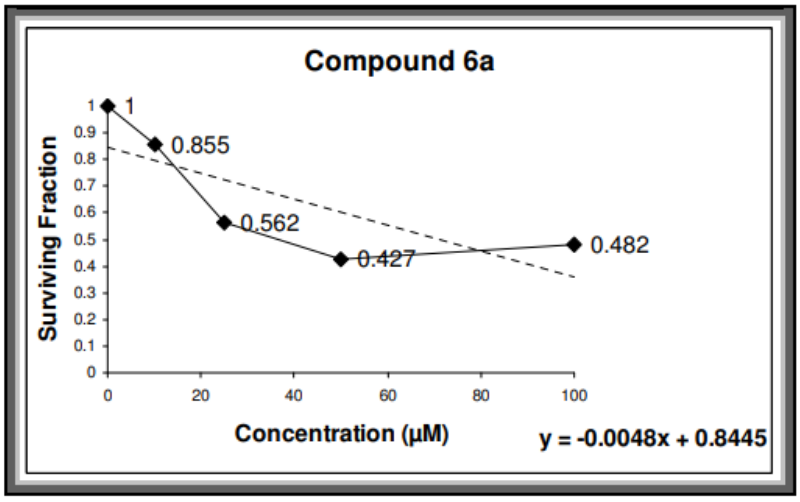


FIGURE 4: Time required for component 6a to degrade

V. CONCLUSION

This study focuses on recent advances in synthesizing anticancer derivatives that incorporate heterocyclic rings, emphasizing the development of targeted anticancer therapies. Anticancer medicines' pharmacokinetic and pharmacodynamic qualities are enhanced by the presence of heterocyclic moieties, which are found in the majority of medications. Approximately 30% of FDA-approved anticancer medications contain one or more heterocyclic rings that include oxygen, nitrogen, and sulfur. Heterocyclic moieties play a significant role in the metabolic reactions that are crucial for the survival of all living organisms. Approximately two-thirds of the anticancer medications approved by the FDA in the first half of the decade incorporated them, highlighting their pivotal role in cancer research and treatment efforts. Recent advancements in the

application of heterocyclic compounds as anticancer agents and innovative strategies for their development have been central to these efforts.

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