**Synthesis and anticancer properties of *‘azole’* based chemotherapeutics as emerging chemical moieties: A comprehensive review**

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**Table 1.** List of Abbreviations

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| **Abbreviation** | **Expanded form** |
| MEKK | mitogen-activated kinase kinase kinase |
| MEK | mitogen-activated kinase |
| ERK | extracellular signal-regulated kinase |
| MAPK | Mitogen-activated protein kinase. |
| CuAAC | Copper (I)-catalyzed alkyne-azide cycloaddition. |
| LLE | Ligand lipophilicity efficiency |
| VEGF | vascular endothelial growth factor |
| HUVEC | stimulated human umbilical vein endothelial cells |
| SAR | Structure activity relationship |
| TNF-α | Tumor necrosis factor alpha |
| NF-κB | Nuclear factor kappa-light-chain-enhancer of activated B cells |
| RANKL | Receptor activator of nuclear factor-kappa B |
| EDC | 1-ethyl-3- (3′-dimethyl amino) carbodiimide |
| TMSOTf | Trimethylsilyl trifluoromethanesulfonate |
| CDK/Cyclins | Cyclin-dependent kinases |
| *Tm* | Melting temperature |
| VEGF | Vascular endothelial growth factor |
| VEGFR-1 | Vascular endothelial growth factor receptor 1 |
| DNA | Deoxyribonucleic acid |
| RNA | Ribonucleic acid |
| IC50 | Half maximal inhibitory concentration |
| (RSK) | Ribosomal protein S6 kinase |

**Abstract**

Azole frameworks serve as privileged scaffolds in the contemporary drug design paradigm owing to their unique physicochemical profile that promotes the development of highly selective, physiological benevolent chemotherapeutics. Several azole nuclei function as bioisostere in medicinal chemistry and prompt the development of tailored therapeutics for targeting the desired biological entities. Besides, the azole scaffold forms an integral part in the advanced drug designing methodologies such as target template in-situ drug synthesis, that assist in a rapid identification of the hit molecules form a diverse pool of leads; and direct biomolecule-drug conjugation, along with bioorthogonal strategies that ensure localization, and a superior target specificity of the directed therapeutic. Lastly, the structural diversity of azole framework and high yielding click synthetic methods provide a comprehensive Structure Activity Relationship analysis for design optimization of the potential drug molecules by fine-tuning the placement of different substituents critical for the activity. This review provides a comprehensive analysis of the synthesis and anticancer potential of azole based chemotherapeutics.

**Keywords:** Azole, anticancer drugs, chemotherapeutics, molecular inhibitors, anticancer pathways, MAPK, TNF-α, NF-κB.

1. **Introduction**

The azole moiety provides a desirable framework for the current doctrine of pharmaceuticals development [1]. From tailored therapeutics to target template drug synthesis, the azole nucleus presents a versatile profile for therapeutics development including antifungal drugs [2], anti-inflammatory molecules [3], and anticancer chemotherapeutics [4]. In addition, the azole-based bioactive molecules constitute a substantial portion of the therapeutically advantageous natural product extracts [5, 6] for effectually managing several diseases and disorders. The simpler, high yielding chemical protocols for the synthesis of azoles [7] enable the generation of large compound libraries for comprehensively investigating the structure-activity relationship (SAR) analysis on azole derivatives [8] that inspect minute aspects of compound-biomolecule interactions for an optimum bioactivity [9]. The stereoselectivity of click reactions to generate azoles rings led to the identification of *in situ* kinetic target guided synthesis [10-13] of triazole containing molecular inhibitors from parent fragments within the active site cavity of the target enzyme that acts as reaction vessel [14-16]. The approach proved highly beneficial by supplementing the customary laborious approaches including library synthesis for the identification of ’hits’ and ‘leads’ [17]. The polar nature of azoles improves the solubility of rationally designed derivatives, hence imparting a superior drug bioavailability and pharmacology profile [18]. Notably, the azole nucleus serves as bioisostere of diverse functional groups that further improves the physiological stability of drugs. As such, triazoles that functions as amide bond bioisostere [19-21], provides the intracellular stability to therapeutically important peptide mimics by preventing their *in vivo* degradation by cellular proteases [22-24]. These properties mutually validate the robust candidature of azole moiety as a desirable pharmacophore for generating the pharmaceutically advantageous anticancer molecules and derivatives.

1. **Regulating Mitogen-activated protein kinase (MAPK) pathway by azole derivatives**

The MAPK pathway involves a chain of proteins inside the cells that play a vital cell-signaling role from the surface of the cell to DNA inside the nucleus. The signal begins when a signaling molecules bind to the receptors present on the cell surface, which eventually ends as protein expression by the DNA [25]. Cellular phosphorylation coupled with multiple signaling components including Ras/Raf/MEK/ERK cascades constitutes the mitogen-activated protein kinase pathway (MAPK) [26]. When activated, the MAPK pathway regulates cell growth, differentiation and proliferation, apoptosis, and senescence by complex extracellular signaling mechanisms [27]. The activation of MAPK pathway reportedly amends the translation of mRNA to proteins by phosphorylating the 40 ribosomal protein S6 kinase (RSK) [ 28]. The activation of the MAPK pathway regulates the transcription of genes responsible for optimal cellular processes, due to which the onset of functional or structural aberrations in the pathway manifests critical cellular tumorigenesis [29]. The p38α MAPK signaling pathway mediates the expression of pro-inflammatory cytokines [30, 31], which elevate in response to cellular morbidity and oncogenesis [32]. The activation of MAPK pathway by immune cells regulates the expression of proinflammatory cytokines that plays a critical role in triggering of the immune response in the host [33]. The representative pyridinyl-imidazole based ATP competitive inhibitors of the pathway failed to advance in the clinical trials owing to considerable toxicity, off-target selectivity and high lipophilicity [34]. The compounds **3** (Figure 1) based on the pyridinyl-triazole scaffold presented superior physicochemical profile including targeted delivery via incorporation into the cationic liposomes (SAINT-O-Somes). The test compounds obtained via CuAAC click reaction between the precursor alkyne **1** (Figure 1) with structurally diverse aromatic and aliphatic azides **2** (Figure 1). The radiometric IC50 profiling assay, ligand lipophilicity efficiency (LLE) analysis, and water-solubility (LogS) investigations suggested the high efficiency of imidazole-triazolyl compounds for the inhibition of p38α MAPK signaling pathway, compared to the isosteric isoxazole derivatives. The structure-activity relationship (SAR) analysis confirmed that the presence of lipophilic substituents including *N,N*-dimethylaniline, tetra-*O*-acetyl-protected 2 glucose, ethylene glycol ester, and diethyl phosphonate produced a trivial effect on the bioactivity of the test compounds. The triazoles with favorable physicochemical properties displayed high retention while formulation in liposomes (SAINT-O-Somes) for an *in vitro* downregulation of p38α MAPK signaling pathway [35]



**Figure 1.** Triazole based compounds as MAPK inhibitors

The MAPK pathway comprises a sequential phosphorylation of the successive kinases including the mitogen-activated kinase kinase kinase (MEKK), mitogen-activated kinase (MEK) and the extracellular signal-regulated kinase (ERK), which translocates in the subcellular compartments to effect modification of the optimal cellular functioning [36]. The phosphorylation cascade initiated by MEKK and terminating at ERK plays important role in cell growth, its transformation to malignant melanoma, and the development of drug resistance, hence presenting a desirable target for anticancer drug development [37]. The synthesis of benzimidazole derivatives **7**, **8**, and **9** (Figure 2) deliberated as MAPK inhibitors commence from the reactant **4** (Figure 2) containing 4-nitro substituent as a protective group that converts to amine in compound **5** (Figure 2) under optimized reaction conditions. Similarly, the 6-methoxy substituent in precursor molecule **4**, converts to the 6-alkoxy group in molecule **6** (Figure 2) via acidic demethylation followed by Mitsunobu coupling. Further benzylation of compound **5** yields the derivative **7**, in the presence of sodium triacetoxyborohydride, whereas the compound **6** yields the derivatives **8**, and **9** by Molander-Suzuki coupling reaction of triflated phenolic group in the reactant with benzylic trifluoroborate, and triphenylphosphine respectively. The test derivatives strongly inhibited the ERK phosphorylation and selectively inhibited the growth of MCF-7 cells in single dose survey. The western blot analysis confirmed that the test benzimidazole derivatives inhibited MEK5 mediated ERK5 phosphorylation in human embryonic kidney (HEK293) cells. The presence of isopropyl substituent on the N1-atom together with the functionalization of 4-amine and 6-carbon of benzimidazole potentiated the inhibitory potential of the test derivatives as confirmed by the SAR analysis [38].



**Figure 2.** Benzimidazoleconjugatesfor MAPK inhibition

The activation of the MAPK pathway occurs via the upregulation of vascular endothelial growth factor (VEGF), and its receptors VEGFR-1, 2 that mediate physiological and pathological angiogenesis, thereby making it a potential target for designing therapeutics that target the pathological angiogenesis [39]. Rationally designed pyrazolylureas and imidazopyrazolecarboxamides **13,** and **15** (Figure 3) demonstrated a marked inhibition of p38MAPK pathway and neutrophil chemotaxis in the endothelial cells during angiogenesis. The synthesis of compounds started from the parent molecules **10**, **11** (Figure 3) which reacted to yield the substituted pyrazolyl derivative **12** (Figure 3). Further treatment with phenylisocyanate converts **12** to substituted pyrazolylureas derivative **13** (Figure 3). The treatment of derivative **12** with concentrated H2SO4 followed by extraction using weak base results in cyclization reaction to yield compound **14** (Figure 3). The derivative **14**, in the presence of a cyclic base and a polar solvent subsequently converts to the substituted imidazopyrazolecarboxamide **15** (Figure 3). The test compounds prevented p38MAPK phosphorylation, in addition to the downregulation of key mediators of MAPK and PI3K signaling pathways, the intensity of which depends on the substitution pattern on compounds. The rigid imidapyrazole derivatives supported the p38MAPK phosphorylation compared to the flexible analogues thereby suggesting that the rigid structure of 7-substituted imidapyrazoles reduced their potency. While, the structurally flexible 6-, and 3-substituted imidapyrazoles demonstrated marked inhibition of p38MAPK phosphorylation. The wound healing analysis of the test compound on vascular endothelial growth factor (VEGF) stimulated human umbilical vein endothelial cells (HUVEC) revealed their inhibitory potential on the endothelial cell migration towards the injured site, hence confirming their anti-angiogenesis potency. Notably, the SAR analysis identified the role of carboxyethyl functionality in pyrazole and imidapyrazole moieties in downregulating MAPK signaling pathways [40].



**Figure 3.** Imidazopyrazolecarboxamides derivatives for MAPK inhibition

1. **Targeting Tumor necrosis factor alpha (TNF-α) by azole compounds**

The multifunctional pro-inflammatory cytokine TNF-α regulates diverse cellular events including their survival, differentiation, proliferation, and death [41]. TNF-α applies its functions by serially activating a cascade of signaling pathways and mediates inflammation-associated tumorigenesis [42] by promoting tumor cell proliferation and malignancy, its metastasis, and tumor angiogenesis [43]. The cancer cells demonstrate elevated TNF-α level that diminishes miraculously with chemotherapy, thereby suggesting its prominence as cancer biomarker [44]. Reportedly, the inflammatory cells secrete TNF that plays a critical role in the regulation of inflammation-mediated carcinogenesis [45]. The synthesis of TNF occurs as membrane-bound protein, referred as pro-TNF, which releases from the membrane by the catalytic cleavage in the presence of TNF-converting enzyme (TACE)-mediated cleavage [46-47]. The association of TNF-α in the development of malignant neoplasms marks it as an anticipated target in the incipient anticancer chemotherapy. Reportedly, TNF-α exerts marked applications as the first cytokine evaluated for cancer biotherapy [48]. Logically designed 1,5-disubstituted tetrazole derivatives **17** (Figure 4) obtained by one-pot synthesis from Baylis Hillman acetate **16** in the presence of reagents TMS azide and arylnitrile, presented a laudable profile against TNF-α. The bioactivity of the tetrazole derivatives arises from their resistance to biological degradation, and isosteric analogy as a substituent with diverse functional groups for the development of bioactive molecules. The derivatives delivered a sturdy candidature for anticancer therapeutics development for targeting TNF-α [49]. In addition to cancer metastasis, the expression of TNF-α manifests neurotoxicity, neural cell death by the production of nitric oxides and free radicals mediated oxidative stress during the exposure to chemotherapeutics [50]. Hence, capping of the TNF-α pathway provides imminent benefits during the anticancer chemotherapy [51]. The thiazole derivatives **21** (Figure 4) presented a similar profile. The synthesis commences with reactant alkylamine **18** (Figure 4) that converts to isothiocyanate **19** (Figure 4) in the presence of thiophosgene and aqueous calcium carbonate, which further generate thioester analogues **20** (Figure 4) in aq. ammonia solution. Further reaction of these thioester compounds with α-haloketones **X** (figure 4) yields the desired thiazole compounds **21**. The SAR analysis of the test compounds **21** suggested the relevance of bulky substituents including cyclopentyl, cyclooctyl, and 1-adamantyl for an optimal activity for the inhibition of lipopolysaccharide-induced TNF-α neurotoxicity and neural cell death. These compounds provided a distinctive profile as potential leads for the effective management of TNF-α induced malignant melanoma [52].



**Figure 4.** Tetrazole and thiazole based inhibitors of TNF-α

Compounds **26** (Figure 5) validated the potency of sulfonamide functionality appended on imidazopyridazine scaffold for the inhibition of lipopolysaccharide mediated TNF-α production. The synthesis of the compounds started with pyridazine reactant **22** (Figure 5) with chloroacetaldehyde in the presence of sodium acetate followed by reflux with 60 % EtOH (aq.) to generate chloroimidazopyridazine adduct **23** (Figure 5). Further, one pot reaction of **23** with chlorosulfonic acid and phosphorus oxychloride to yield the chlorosulfonated derivative **24** (Figure 5). The amination of this derivative in the presence of trimethylamine and ethylenedichloride at room temperature yielded the compounds **25** (Figure 5), which eventually undergoes Suzuki-Miyaura coupling reaction in the presence of boronic acid/ pinacol ester and catalytic amount of bis-(triphenylphosphine)palladium(II) dichloride to yield the desired compounds **26** (Figure 5). The test compounds exerted their activity due to the optimal accommodation and high conformational stability of the deliberated compounds via π-π stacking and hydrogen bonding interactions with the active site loop of TNF-α. The extraordinary stability of the compounds in the active site arises mainly due to the three crucial H-bonding interactions of the residues TyrB151, GlyA121 with pyridine ring; and TyrA151 with the amino functionality of sulfonamide moiety. The inhibition potential of the test compounds surpassed the standard drug rolipram, hence validating the candidature of Imidazopyridazine and sulfonamide moieties as suitable pharmacophores in the rational development of therapeutics that effectively target TNF-α expression for the management of morbid conditions [53].



**Figure 5.** Imidazopyridazine sulfonamide derivatives for TNF-α inhibition

Further developments in the identification of novel scaffolds for inhibiting TNF-α led to the screening of pyrazole pyrimidines **32** (Figure 6) with marked ulcerogenic liability. The synthesis of these compounds begins with phenyl hydrazine **27** (Figure 6) that refluxes with ethoxymethylene malononitrile **28** (Figure 6) in ethanol solvent to yield a 4-cyano-5-amino compound **29** (Figure 6). This cyano derivative when refluxed in formic acid converts to a keto derivative **30** (Figure 6), which on further treatment with phosphorus oxychloride, ethyl-4-aminobenzoate and subsequent hydrolysis yields the acid derivative **31** (Figure 6). The refluxing of compound **31** with amines in the presence of 1-ethyl-3- (3′-dimethyl amino) carbodiimide HCl salt (EDC), followed by treatment with 1-hydroxy benzotriazole (HOBt), and subsequently with diisopropylethylamine using THF-DMF solvent results in the synthesis of amide derivatives **32** (Figure 6). The test derivatives **32** presented a significant downregulation of TNF-α, and a marked reduction in the ulcerogenic activity *in vivo*. The SAR analysis of the compounds suggested that the compound with 4-chlorophenyl substituent at the amide linkage displayed maximum activity. The activity lowered on replacing this substituent with 4-methoxyphenyl and/ or 4-methylphenyl group, mainly due to their electron-donating properties; and with 4-fluorophenyl due to the smaller size, and high electronegativity of the ‘F’ atom [54].



**Figure 6.** Pyrazolopyrimidine scaffold for TNF-α inhibition

1. **Downregulating Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) by azoles**

The activation of NF-κB regulates composite tumorigenic cellular processes that include cellular proliferation [55], angiogenesis, invasion, metastasis, radioresistance and chemoresistance [56]. The NF-κB performs constitutive activity in the tumor cells due to inflammatory microenvironment and oncogenic mutations [57], and its downregulation inhibits the growth of morbid cells [58]. Besides promoting the tumor cell proliferation, NF-κB discourages apoptosis, promotes angiogenesis [59-60], and prompts epithelial-mesenchymal transition that eventually expedites distant metastasis [61]. NF-κB mediates the association between inflammation, and tumorigenesis. The tumor tissues demonstrated elevated levels of NF-κB thereby leading to hyperaccumulation of pro-inflammatory cytokines at the morbid site [62] These events result in the development of pro-tumorigenic microenvironment [63]. The activation of NF-κB occurs via signal-induced degradation of IkB kinase by proteasomes due to the phosphorylation of serine residues [64]. Further, the activation of NF-κB initiates remodeling of local cellular metabolism, which functionally incapacitates the innate immune system hence favoring the tumor growth [65, 66]. The suppression of NF-κB in myelogenous cells promotes tumor suppression, which suggests the NF-κB pathway as an essential target in cancer chemotherapy [67]. Compounds **35** (Figure 7) displayed a commendable pharmacophoric profile for the inhibition of NF-κB pathway. The synthesis of these compounds begins with reactants substituted benzene-1,2-diamine **33** (Figure 7) and multisubstituted 2-phenylacetic acids **34** (Figure 7), which react in the presence of the catalytic amount of boric acid in xylene solvent to yield the desired compounds **35** (Figure 7). SAR analysis revealed that the presence of spacer molecule connecting phenyl and benzimidazole moieties plays a crucial role in the bioactivity of the test compounds.

In addition, the mild electron-withdrawing groups and hydrophobic functionalities promote the inhibition of NF-κB. Importantly, the hydrophobic nature of chloro substituent on 4-position of the phenyl ring optimizes the bioactivity of the compounds against NF-κB, which further diminished with an additional ‘Cl’ atom in the dichloro substituted phenyl ring. The presence of hydrophobic hydrogen bonding acceptor -OCH3 substituent on the phenyl ring produced a marked amplification of NF-κB inhibition [68]. The optimization of benzylbenzimidazole framework in an efficient blocking of the NF-κB pathway led to the identification of compounds **38** (Figure 7). The refluxing of reactants 6-hydroxyl-benzene-1,2-diamine **36** (Figure 7) and 4/5-substituted phenylacetic acids **37** (Figure 7) in 6N HCl led to the synthesis of desired compounds **38** (Figure 7). The SAR analysis suggested that the 4/5-substitution of benzimidazole ring with bulky hydrophobic cyclohexylmethoxy functionality enhanced the inhibitory potential of the test compounds. Similarly, the presence of hydrophilic hydrogen bonding donor -OH group at 2/4-position of the phenyl ring favors NF-κB inhibition by the deliberated compounds. Notably, the presence of hydrophobic -OCH3, and -Cl substituents on the phenyl ring discouraged the bioactivity. Notably, the length of spacer molecule connecting benzimidazole moiety and phenyl ring decides the bioactivity of the resulting compounds. In the test compounds, methylene group demonstrates a profound effect on bioactivity compared to the higher analogues [69].



**Figure 7.** Benzylbenzimidazole framework for NF-κB downregulation

The coumarin-triazole derivatives **40**, **41** (Figure 8) obtained from the CuAAC click reaction of the allyl adduct **39** (Figure 8), with azides RN3, provided highly effective pharmacophores for the inhibition of NF-κB pathway. The test derivatives inhibited receptor activator of nuclear factor-kappa B (RANKL)-induced p65 phosphorylation of NF-κB in a dose-dependent manner. The SAR analysis confirmed the role of ‘Br’ substituted aryl triazole framework as the lead compound to inhibit osteoclast differentiation [70]. The RANKL-induced osteoclastogenesis mediated the differentiation of osteoclasts and osteoblasts, necessary for bone resorption, while its upregulation manifests bone metastasis in breast cancer [71]. Therefore, targeting of RANKL provides a desirable approach to downregulate the progression of bone metastases [72, 73]. The naturally occurring coumarins reportedly inhibit osteoclastogenesis, which screens them as scaffold for appending triazole moiety for displaying an optimal activity. The featured compounds inhibiting RANKL-induced osteoclastogenesis through NF-κB signaling pathway provide the vital pharmacophore framework in rational designing of the effectual chemotherapeutic leads.



**Figure 8.** Triazole-coumarin conjugation for NF-κB downregulation

1. **Nucleoside analogues based on azole nucleus**

The systemic appraisal of regulatory genes and pathways manifesting the development and progression of aggressive tumors resulted in a paradigm shift in the contemporary cancer chemotherapeutics doctrine [74-78]. The recent decades observed the transformation of anticancer medication from the representative cytotoxic agent-based therapy to the highly precise gene and molecular targeting approach [79, 80]. The sophisticated molecular medicine therapy ensures selective targeting of the morbid cells, without causing imminent damage to the peripheral healthy tissues in the tumor microenvironment [81, 82]. Anticancer nucleoside analogues present an advanced approach in the clinical oncotherapy. The featured compounds **44** (Figure 9) acting as nucleoside mimics displayed antitumor properties. The synthesis of these compounds starts from the D-ribose derivative **42** (Figure 9), which gives azidoribofuranoside **43** (Figure 9) in the presence of trimethylsilyl azide and stannic chloride in DCM solvent. Compound **43** undergoes CuAAC click reaction with alkynes in the presence of CuSO4 and sodium ascorbate to yield the final compounds **44** (Figure 9) after the removal of benzyl protecting group by using ethanolic solution of methylamine. These nucleoside analogues displayed marked bioactivity against a variety of tumor cell lines including human lung adenocarcinoma cell line (A549), human pulmonary carcinoma cell line (LAC), human hepatocellular liver carcinoma cell line (HepG2), and human cervical carcinoma cell line (Hela), with IC50 in the range 9.6 to 10.98 µM. The presence of triazole exerts a pronounced effect on the anticancer activity of the nucleoside analogues. However, the presence of methylene spacer between the nucleobase and triazole moiety interferes with the conjugation of the two ring systems reduces the bioactivity due to weaker binding with the biological target in the presence of spacer molecule [83].



**Figure 9.** Triazole-based nucleoside analogues with anticancer properties

The nucleoside mimics imitate the endogenous nucleosides and demonstrates cytotoxicity toward the morbid cells. The cytotoxicity appears due to the inhibition of key enzymes or by the replacement of endogenous nucleosides by analogous substrates, thereby damaging DNA or RNA and interfering with DNA methylation [84]. The optimal functioning of nucleoside mimics necessitates their specific entry at the morbid sites in a non-degraded form for an enhanced cellular uptake to ensure conversion to the active metabolites before hitting their molecular targets [85]. The nucleoside analogues **48** (Figure 10) presented antiproliferative activity by binding to the DNA minor groove. The synthesis of the featured compounds in figure 10 begins with precursor compound **45** (Figure 10). Subsequent deprotection and protection processes eventually yielded the triacetate adduct **46** (Figure 10), which undergoes Vorbruggen glycosylation reaction with different benzimidazole bases with the reagents N,O-bis(trimethylsilyl)acetamide (BSA) and trimethylsilyl trifluoromethanesulfonate (TMSOTf ) to yield β-nucleosides **47** (Figure 10).

Further deprotection of the acetate group followed by ring closing metathesis in the presence of Grubbs II generation catalyst, and dehydrogenation yields bicyclic nucleosides **48** (Figure 10). Principally, the *in silico* analysis confirmed the binding of nucleoside mimics in the minor groove region of DNA, with the hydroxyl groups interacting with guanine bases via hydrogen bonding. Similarly, the benzimidazole ring interacts with guanine base via T-shaped π-π interactions. Nevertheless, the benzimidazole ring exhibited anion-π interactions with the phosphate groups of the DNA, whereas the methyl substituent on benzimidazole actively involves in bonding with DNA base pairs. The test nucleoside mimics demonstrated marked antiproliferative potency against the breast cancer-cell lines, with IC50 in low micromolar concentration. Importantly, the absence of double bonds in test nucleoside mimics **48** (Figure 10) plays a distinct role in affording the DNA binding property for imparting antiproliferative activity, which further improves in the presence of ‘Cl’ substituent [86].



**Figure 10.** Benzimidazole-tethered nucleoside mimics with anticancer properties

Cyclin-dependent kinases (CDK/Cyclins) mediate the progression of the cell cycle, transcription, neural differentiation, and cell metabolism [87]. The abnormal functioning of these kinases manifests cancer cell proliferation, hence acting as biomarker and an attractive pharmacological target owing to their highly conserved structure and ATP-binding loop [88, 89]. The nucleoside mimics **52**, and **53** (Figure 11) based on pyrazole moiety displayed antiapoptotic activity by inhibiting CDK2/cyclin A2 enzyme, with significant cytotoxicity against four different human cancer cell lines including HepG2, MCF-7, A549 and Caco2. The synthesis of the analogues begins with reactant **49** (Figure 11) which yields the adduct **51** (Figure 11) via the intermediate **50** (Figure 11). The adduct **51** serves as the starting point to procure the nucleoside mimic **52**, and **53** (Figure 10) in the presence of arylamine/ trieythylorthoformate in DMSO to synthesize the former; whereas the refluxing in presence of aromatic aldehyde and ethyl acetate yields the latter set of derivatives. The pyrazole nucleus of the deliberated compounds occupied the adenine region of the ATP binding pocket, with its ‘N’ atoms, -C=O functionality, and –NH of the aminomethylene arm at 4-position demonstrating donor-acceptor H-bonding interactions. The substituted phenyl aminomethylene groups in derivatives **52,** and phenylfuranyl methylene group in derivatives **53** improved the hydrophilic profile of the test compounds. While, the phenyl amino substitution at 5-position of pyrazole ring occupied the hydrophobic pockets of CDK2/cyclin A2 enzyme. Flow cytometry cell cycle investigations revealed the anti-apoptotic activity of the test compounds suggesting that these compounds triggered the arrest of the cell cycle in the G0-G1 phase thereby fortifying apoptotic DNA fragmentation. Importantly, the compounds displayed a comparable anticancer profile as the standard drug doxorubicin. The *in silico* investigations of test compounds in the active site loop of target CDK2/cyclin A2 enzyme revealed hydrogen bonding interactions with the key residue LEU83. These properties presented the perspective profile of the candidate nucleoside mimic compounds as latent anticancer chemotherapeutics [90].



**Figure 11.** Pyrazole-template nucleoside mimics with anticancer properties

1. **Inhibition of DNA topoisomerase II by azole molecules**

Topoisomerase II regulates DNA replication and transcription, along with the chromosomal segregation that sustains the genomic integrity [91, 92]. Interfering with topoisomerase II activity induces the enzyme-mediated DNA damage, hence presenting an effective anticancer therapy [93-95]. The glycosyl-triazole hybrid compounds **56** (Figure 12) directed as topoisomerase II inhibitors displayed marked anticancer activity against four human cancer cell lines HeLa, A-549, Du145, and MCF-7. The synthesis of these derivatives starts from 4β-*O*-propargyl podophyllotoxin **55** (Figure 12) obtained by reaction with propargyl alcohol via single step synthesis in the presence of boron trifluoride etherate. The conjugates **55** further react with sugar azides to yield the derivatives **56** (Figure 12), which presented a superior anticancer profile compared to the standard drug etoposide. The *in silico* investigations confirmed hydrogen bonding, and alkyl-π interactions between the test compounds and the active site loop of topoisomerase. The glycosyl moiety forms hydrogen bonds with the residues Asp92, Thr184, Arg70, and Ser120, and Asn122, whereas the triazole ring system interacted with Arg70 mainly via alkyl-π interactions. Reportedly, the compounds with acetyl protected sugar moieties displayed better anticancer activity due to the high binding affinity of the acetyl group with the target enzyme [96].



**Figure 12.** Glycosyl triazole derivatives with anticancer properties

The featured compounds **60** (Figure 13), synthesized by refluxing a mixture of derivative **57** (Figure 13), benzothiazole **58** (Figure 13), and β-naphthol in methanol; displayed marked inhibition of topoisomerase II coupled with characteristic DNA binding potency, while arresting the G2/M phase of cell cycle. The topoisomerase mediates topological alterations in DNA by nicking the single-stranded DNA and joining with the phosphate backbone during a normal cell cycle. Either the representative inhibitors of topoisomerase function by directly binding to the DNA thereby altering its morphology, or they directly inhibit the enzyme itself. The test inhibitors adopt the former mechanism [97].



**Figure 13.** Pyrazole-benzothiazole derivatives with anticancer properties

The test compounds **64** (Figure 14) incorporate the DNA binding properties of 1,8-naphthalimide for an effective inhibition of topoisomerase. Synthesis of the test compounds occurs by refluxing a library of aromatic and aliphatic amines with the intermediate compound **63** (Figure 14), obtained by the Suzuki-Miyaura cross-coupling reaction between **61**, and **62** (Figure 14). The compounds demonstrated *in vitro* anti-proliferative activity against a variety of cancer cell lines. The investigations with fluorescence emission spectroscopy suggested DNA groove binding ability of the test compounds for displaying the inhibitory potential. The thermal denaturation analysis to appraise the binding interactions of test compounds with ct-DNA suggested negligible change in melting temperature *Tm* [98] The intercalation stabilizes DNA double-helical structure thereby raising the *Tm*, while the groove binding exerts no change in *Tm* [99]. Therefore, the thermal denaturation method revealed the groove-binding mode of test compounds over the DNA intercalation to display optimum activity. The *in silico* analysis suggested that the ‘O’ atoms of naphthalimide rings displayed hydrogen bonding interactions with the sugar backbone of DNA attached with cytosine base pair. The ‘N’ atoms in piperazine ring, display hydrogen bonding interactions with the thiamine associated phosphate backbone. In addition, the aromatic framework constituting naphthalimide and phenanthro [9,10-*d*]imidazole system offers the hydrophobic interactions with DNA. The above analysis validated the potential of naphthalimide-imidazole ring system in developing latent anticancer chemotherapy [100].



**Figure 14.** Phenanthro-imidazole based molecules with anticancer properties

1. **Conclusion**

Cancer claims a considerable morbidity and mortality rate globally with a history of rationally designed pharmaceuticals in the pipeline, and the therapeutics with clinical success. Several composite pathways manifest the onset and propagation of tumorigenesis. Successful anticancer chemotherapy must urgently counter these pathways to ensure the systemic obliteration of morbid cells, and to minimize the cancer relapse. Evading the overexpressed cancer manifesting pathways including MAPK, TNF-α, NF-κB, by rationally designed nucleoside analogues, topoisomerase inhibitors produced encouraging results. However, the development of evolved pathways necessitates the identification of novel pharmacophores and moieties for managing the cancer-related cellular morbidity. The systemic efforts identified ‘hit’ pharmacophores for the discovery of ‘lead’ molecules that efficiently countered the current intricacies associated with composite regulatory mechanisms adopted by cancer cells, including multidrug resistance. Azole framework provides diverse dimensions owing to its privileged biological profile, hence acting as an ideal scaffold for designing the anticancer drugs. The azole framework enables extensive SAR analysis of diverse substituents vital for the bioactivity of the compounds for effectively identifying the potent pharmaceuticals. In addition, the synthetic ease encourages the combinatorial chemistry presented by triazole nucleus for library synthesis of active compounds, and the appending of molecular therapeutics with biomacromolecules, including DNA, RNA, and enzymes via click triazoles present highly potent molecular therapeutics. The high yield synthetic ease by click reactions, with extraordinary physiological stability and tolerability emphasize on the application of azole nucleus in contemporary drug design paradigm. The azole based compounds deliberated as anticancer drugs effectively bind to the target proteins, and enzymes via non-covalent interactions including coordination bonding, van der Waals forces, hydrophobic interactions, hydrogen bonding, and electrostatic interactions. Several heterocyclic ring systems in azole family function as bioisosteres that further promotes their application as substrate mimics of targeted disease-causing overexpressed enzymes, and as receptor antagonists for managing the diseased conditions. The emerging role of azole nucleus in the imminent anticancer chemotherapy therefore obliges a rational consideration of azole nucleus and related analogues in the contemporary doctrine of oncology.

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2. **References:**
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