Bulletin of Pharmaceutical Research 2024;14(1):183

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

DOI: 10.21276/bpr.2024.14.1.3

REVIEW ARTICLE

EXPLORING UTILITY OF PYRAZINE-BASED HETEROCYCLIC COMPOUNDS IN ANTICANCER DRUG DEVELOPMENT

Praveen Rawat, Priyanshu Nema and Sushil Kumar Kashaw*

Integrated Drug Discovery Research Laboratory, Department of Pharmaceutical Sciences, Dr. Harisingh Gour University (A Central University), Sagar-470003, Madhya Pradesh, India

**E-mail*: sushilkashaw@dhsgsu.edu.in
Tel.: +91 9425655720.

Received: Jan 10, 2024 / Revised: Apr 22, 2024 / Accepted: Apr 23, 2024

Cancer is witnessing a rising global incidence, affecting a predominant proportion of the populace. In response, ongoing endeavors are underway to develop novel anticancer pharmaceuticals, with their safety profiles currently undergoing evaluation. Among these, pyrazine-based medications constitute a significant contribution, representing a pivotal pharmacophore prevalent in both synthetic and natural heterocyclic compounds. Characterized by a six-membered aromatic heterocycle with two nitrogen atoms, pyrazine exhibits versatile therapeutic applications in drug development, offering myriad prospects for the refinement of future anticancer agents through targeted interactions with pivotal receptors. Pyrazine compounds, through their inherent structural attributes, have demonstrated efficacy in inhibiting enzymes, receptors, and various other targets implicated in cancer pathogenesis. Contemporary research endeavors are centered on the synthesis of novel pyrazine derivatives tailored for cancer treatment, often in combination with other molecular moieties. Consequently, this review expounds upon the recent therapeutic advancements in pyrazine-based drugs, providing insights into their synthetic pathways, marketed drugs elucidating their primary targets, and a compendium of recently patented and published research papers. This collective information serves as a valuable resource for scientists engaged in the formulation of efficacious medications endowed with the requisite pharmacological activity.

Key words: Heterocyclic compounds, Cancer, Pyrazine-based drugs, Anticancer pharmaceuticals.

INTRODUCTION

Pyrazine is an azaheterocycle that is aromatic and contains two nitrogen atoms. Specifically, pyrazine is a diazine and is isomeric with pyrimidine and pyridazine. Pyrazine is an electron-deficient molecule because of the presence of two electronegative N-atoms which withdraws electron density from the aromatic ring. Pyrazine derivatives are well-known in natural products and are reported to have medicinal and biological significance. In the laboratory, it was first synthesized in 1876 [1]. Pyrazine is an important pharmacophore since it constitutes the integral scaffold in several types of drugs. Although literature is enriched with reports of cytotoxic potential of heterocyclic and other aromatic compounds [2, 3], but due to the wide bio-spectrum of pyrazine derivatives, this motif is present in anti-inflammatory, anticancer, antidiabetic, and diuretic drugs [4].

Furthermore, pyrazine derivatives are also reported to possess high antimicrobial activities [5]. In addition to the importance of pyrazine derivatives in the pharmaceutical sector, these molecules have immense commercial importance in flavor [6], fragrance [7], and food industries [8]. Pyrazine, a naturally occurring

compound in various living organisms, primarily serves as a flavor enhancer in raw foods. Beyond its natural occurrence, industries produce pyrazine and its derivatives in fragrances, flavors, and pharmaceuticals. This review delves into the historical evolution of pyrazine, from its discovery to recent synthetic methodologies, notably focusing on the synthesis of pyrazinium. Six primary synthetic approaches are explored: condensation reactions, ring closure, metal catalysis, green reactions, the Maillard reaction, and acid-catalyzed N-substitution. The initial five methods primarily target substitutions at 2nd, 3rd, 5th, and 6th positions in the pyrazine ring, while the last method specifically focuses on positions 1 and 4 in the pyrazine structure [9]. Pyrazine, a type of six-membered nitrogen-based heterocycle, is well-known for its application in rational drug development [10]. Its ring ligands have been intensively explored, and their p-donor properties are promising [11]. It is an aromatic heterocyclic compound having 1,4-substituted benzene nitrogen atoms used as tastes, fragrances, medicines, and chemical raw materials [12]. Many natural pyrazines have been discovered over the last 70 years [13, 14]. It has received much attention because of the diazine rings, a beautiful class of molecular structures found in both natural and synthesized molecules [15]. The bioactive properties of target molecules are significantly altered by structural modifications in the pyrazine moiety. As a result, ongoing attempts have been made to synthesize a wide class of pyrazine derivatives that may be effective in the battle against cancer cells [16]. Even pyrazine-containing compounds have an important role in everyday life, having applications in materials science [17] and pharmaceutical chemistry [18-23] (**Figure 1**).

General synthesis of Pyrazine

Since pyrazine is a therapeutically important moiety, its contribution is increasing widely in drug discovery. It can be synthesized in various ways; some of the reported synthesis schemes are mentioned below (**Schemes 1-4**).

Anti-tumor activity of pyrazine derivatives

BRAF and tubulin inhibitors

The B-Raf protein is produced by the BRAF gene in humans; the protein is called serine/ threonine-protein kinase B-Raf. The B homologs of the murine sarcoma viral oncogenes B-Raf and v-Raf are proto-oncogenes [30, 31]. The B-Raf protein is in charge of delivering signals that

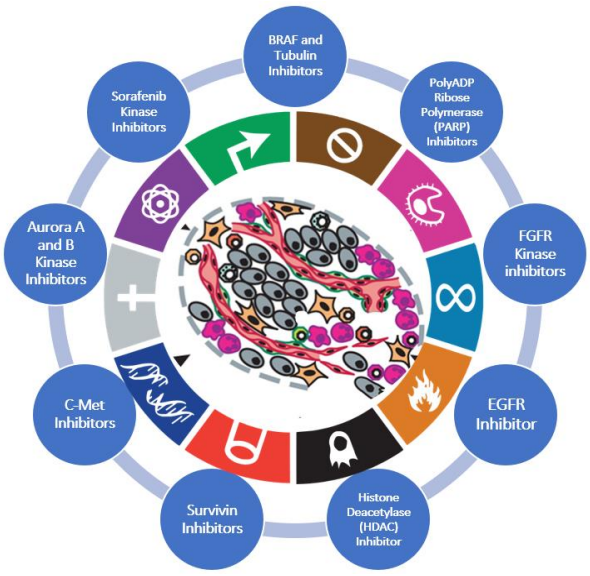


Fig. 1. Diverse activities of pyrazine derivatives

govern cell growth inside cells. It was discovered to be mutated in some human malignancies in 2002 [32]. Tubulin is a component in the cytoskeleton of the eukaryotic cells that plays a role in intracellular movement and transport, as well as cell signaling, mitosis, polarity, and cell shape maintenance [33]. They are also involved in the process of cell division by assisting in the attachment and movement of chromosomes throughout numerous phases of mitosis. As a result, microtubule dynamics is a key target for anti-tumor therapy development [34].

A series of compounds containing the pyrazino [1, 2-*a*] indol-1(2*H*)-one pharmacophore and evaluated their anticancer activity against a panel of cancer cell lines [35]. Compounds 1c and 1d were the most effective with inhibitory IC_{50} values of (1c, 2.4±1.2 and 1.0±0.2) and (1d, 1.7±0.5 and 0.1±0.07, 0.6±0.2 μM) against EGFR and BRAF, respectively. Similarly, the tubulin effect of 1c and 1d compounds were satisfactory at 930±242 and 650±227 (arbitrary units), respectively. These most potent compounds have methyl (1d) and hydrogen (1c) substitutions at the pyrrole ring of the indole moiety and chloro-substitutions at the aromatic ring, making them the best inhibitory and antiproliferative agents. Furthermore, the indole carboxylic amide moiety is crucial for the ROS inhibitory effect, and only these two compounds, 1c and 1d, had a substantial impact on tubulin assembly (**Figure 2**).

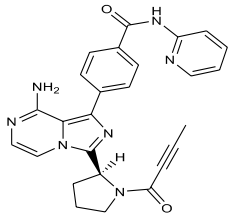
PolyADP ribose polymerase (PARP) inhibitors

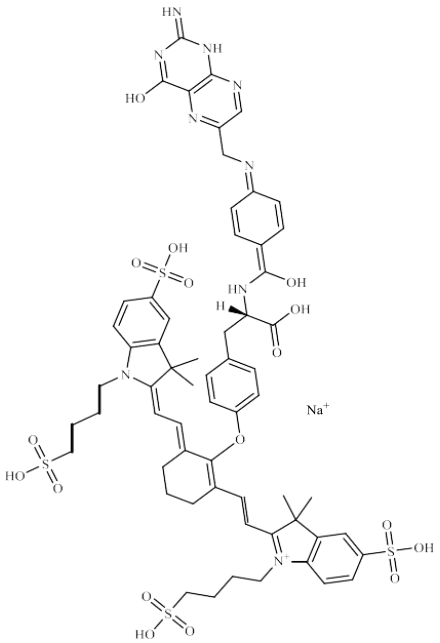
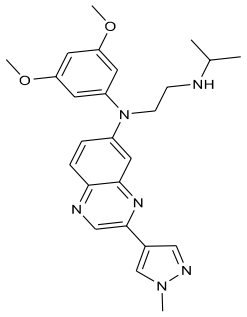
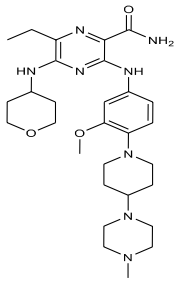
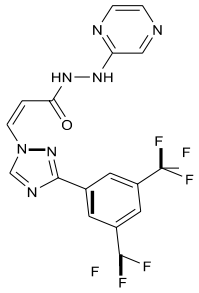
PARP inhibitors are medications that stop the enzyme poly ADP-ribose polymerase (PARP)

Sch. 1. Formation of pyrazine in the presence of zinc was reported by Sato (1978) [24]; **Sch. 2.** Synthesis of pyrazine *via* electrochemical dehydrogenative [4+2] annulation between ketones and diamines [25]; **Sch. 3.** The green approach in producing pyrazine was reported by Ghosh and Mandal (2012) [26, 27]; **Sch. 4.** Synthesis via interaction with ammonia, an aminoketone is formed, which is subsequently condensed and oxidized to pyrazine [28]

Further, a list of pyrazine-based drugs approved by the US FDA for cancer treatment with their primary purpose are listed in **Table 1**.

Table 1. A list of drugs approved by the US FDA for cancer treatment with their primary purpose [29]

S. No.	Drug	FDA approval	Primary target	Company and brand	Structure
1.	Acalabrutinib	Nov 21, 2019	Binds cysteine residue in the BTK	CALQUENCE, AstraZeneca	

2.	Pafolacianine	Nov 29, 2021	Folate receptor	CYTALUX, On Target Laboratories, LLC	
3.	Erdafitinib	Apr 12, 2019	FGFR phosphorylation and signaling	BALVERSA, Janssen Pharmaceutical Companies	
4.	Gilteritinib	Nov 28, 2018	Tyrosine kinases	XOSPATA, Astellas Pharma US Inc	
5.	Selinexor	Jun 22, 2020	Nuclear export of tumor suppressor proteins (tsps)	XPOVIO, Karyopharm Therapeutics	

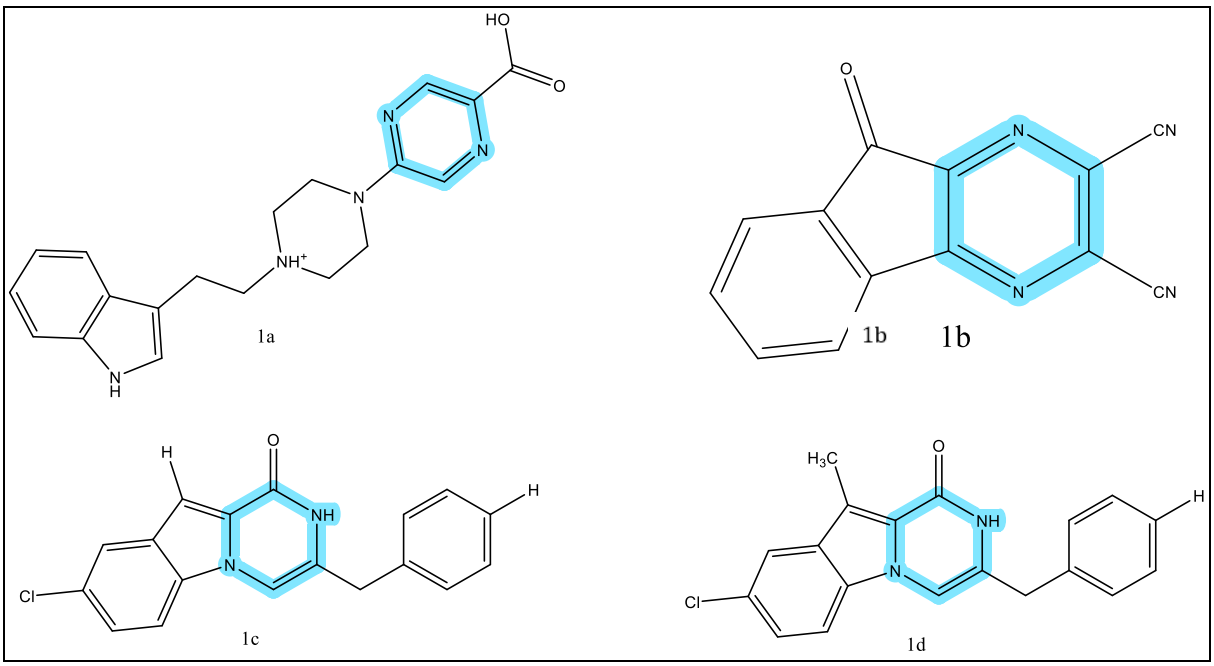


Fig. 2. Structure of compounds 1a-d

from working. They're being created for a variety of applications, including treatment of hereditary malignancies [36]. PARP (PARP1, PARP2, etc.) is more important in cancer cells as compared to normal cells, making it a very important target in cancer therapy [37, 38]. As indicated mostly by olaparib coupled to standard treatment, in women who have recurrent platinum-sensitive ovarian cancer, PARP inhibitors appear to improve progression-free survival [39].

The synthesis of 3,4- dihydropyrrolo[1,2-*a*] pyrazine derivatives via the [4+1+1] annulation approach is reported with evaluation of their anti-cancer properties [40]. The cytotoxic effect of compound 2b was the highest, with IC_{50} values of 1.18±0.05 μM and 1.95±0.04 μM against PC-3 and MCF-7 cell lines, respectively. Also, it significantly decreased cell migration in a dose-dependent manner and caused apoptosis in PC-3 and MCF-7 cells by activating caspase-3 and cleaving PARP. Furthermore, a phenacyl group is present at the nitrogen of the pyrazine ring in compound 2b, due to which it more potently decreases cell viability than other derivatives (**Figure 3**).

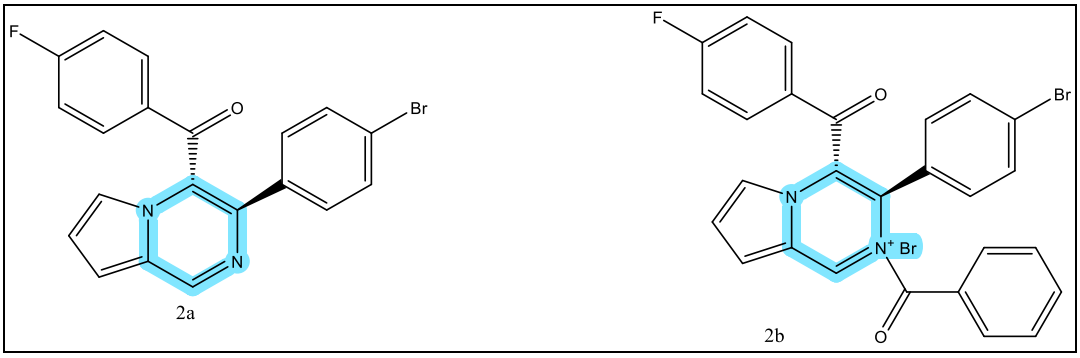


Fig. 3. Structure of compounds 2a and 2b

FGFR kinase inhibitors

Recently, the signaling pathways initiated by FGFs have been discovered to be very critical in the development and progression of various malignancies. The fibroblast growth factor receptors (FGFR) bind to members of the FGF family of proteins, as their name implies [41-44]. Pathological disorders involve some of these receptors. The small molecule inhibitors are divided into non-selective and selective FGFR inhibitors. They all target the ATP-binding site in the kinase domain [45]. A class of 5*H*-pyrrolo[2,3-*b*] pyrazine-based derivatives as strong FGFR inhibitors, the best of those had

sub-nanomolar enzymatic activity [46]. Among all, chemical 3d was found to be effective against tumors with fibroblast growth factor receptors (FGFR) genetic alterations, with IC_{50} values of 0.6±0.0 and 0.5±0.3 nmol/l against FGFR1 and KG1, respectively. Furthermore, compound 3d possesses a dimethoxybenzene ring that, by extending from methyl to ethyl or cyclist groups, drastically reduces the activity of inhibition. The fluorine group in the dimethoxybenzene ring slightly increased the IC_{50} activity to 0.4 nmol/l. These potentials of compound 3d make it more conducive to further development (**Figure 4**).

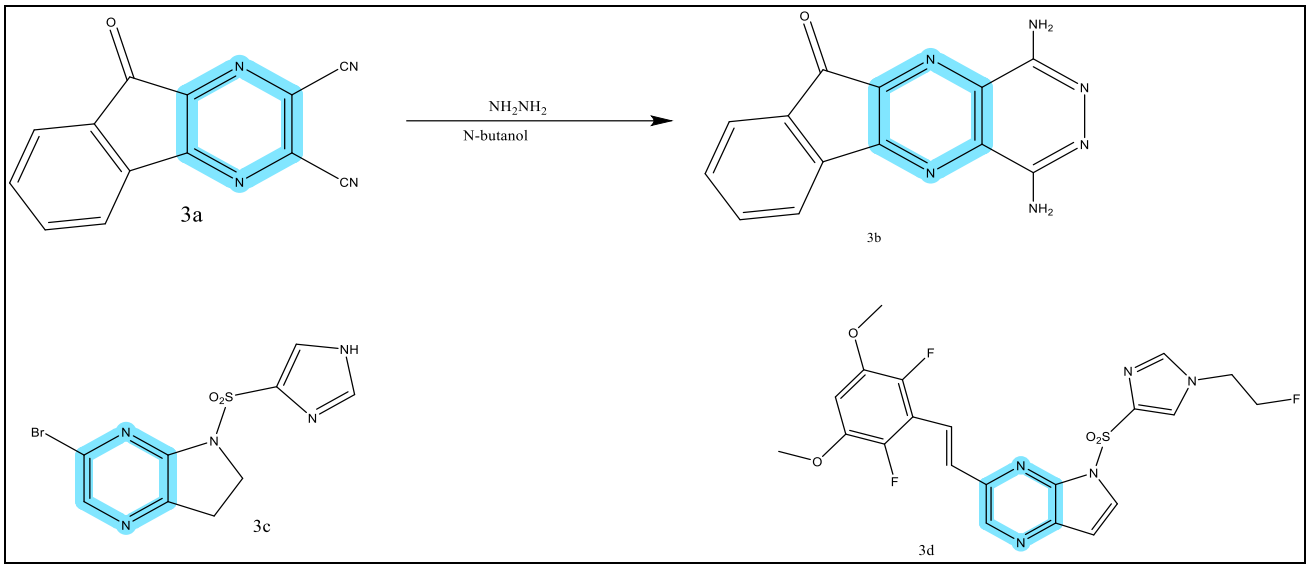


Fig. 4. Structure of compounds 3a-d

Numerous effective FGFR kinase inhibitors, including a series of 5*H*-pyrrole [2, 3-*b*] pyrazine derivatives that were rationally designed, synthesized, and biologically evaluated [47]. Compound 3e was a promising lead for further development due to its excellent selectivity and favorable metabolic characteristics, with an IC_{50} = 0.6±0.2 nM against FGFR1. Furthermore, compound 3e has an unsubstituted pyrazole ring, which showed much higher activity than others when isopropyl, carbonyl, or any other group was introduced to the pyrazole ring. Its activity decreased drastically against FGFR1. In addition, the docking study also showed that compound 3e has excellent binding affinity to the crystal structure of FGFR1 due to its imidazole, pyrazole, & pyrazine rings (**Figure 5**).

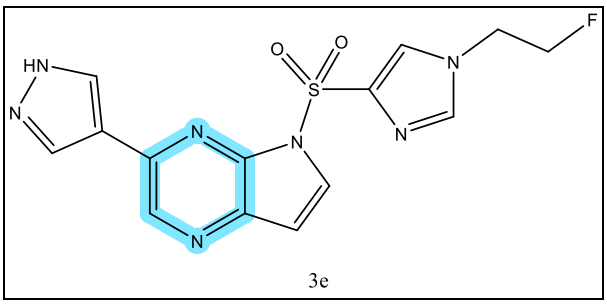


Fig. 5. Structure of compound 3e

EGFR Inhibitor

The epidermal-growth-factor receptor (EGFR/ HER1/ ErbB-1) is a transmembrane protein that serves as a receptor for extracellular proteins belonging to the EGF family [48]. The ErbB family of receptors comprises four closely associated tyrosine kinase receptors: HER2/neu (ErbB-2), ErbB-1, Her 4 (ErbB-4), and Her 3 (ErbB-3). Tumors can be caused by mutations that affect EGFR expression or function in a range of cancers [49] reported the synthesis of fused quinoxaline and pyrazine derivatives, which were cytotoxically tested against MCF-7 and A549 cell lines was reported [50]. A compound named 2b, obtained by reacting compound 2a with hydrazine hydrate in n-butanol, was found to be the most active compound, with IC_{50} values of 4.3 and 5.4 µM against A549 and MCF-7, respectively. A study on molecular docking also revealed that compound 2b exhibited a good binding affinity towards the EGFR receptor binding site (PDB ID: 1M17).

Histone deacetylase (HDAC) inhibitor

HDACs catalyze the removal of acetyl groups from histones, which leads to heterochromatin (condensed chromatin) and gene transcription

inhibition [51]. Based on sequence homology and domain organization, human HDACs are split into four groups. Class-I (HDACs 1, 2, 3, and 8) zinc-dependent deacetylases, class-II (HDACs 4, 5, 6, 7, 9, and 10) zinc-dependent deacetylases, and class-IV (HDAC11) NAD+-dependent deacetylases (sirtuins SIRT1-7) [52]. HDACs 1, 2, 3, and 8 are predominantly present in the nucleus and are involved in cell proliferation cell cycle progression, maintenance, and also in the formation of cancer cell phenotypes [53]. HDAC inhibitors (HDACi) have been created and explored as possible anticancer therapies in recent years since HDACs are critical for tumor formation and progression [54].

The synthesis of a family of class I selective HDAC inhibitors (HDACi) that have a zinc-binding group attached to a central (piperazin-1-yl) pyrazine scaffold [55]. Compounds 5a, 5b and 5c were shown to be the most active compounds against HDAC 1, 2, and 3, with IC_{50} values of (0.13, 0.28, 0.31 µM), (0.26, 2.47, 0 µM), and (0.07,0.26,6.1 µM), respectively. Furthermore, evaluation of these compounds showed that 1c is superior; the inhibitory action against HDAC is attributed to its 3-indolyl ring, and its activity is decreased by the replacement of the methylene that connects the indole and piperazine groups with ethylene. The docking study also revealed a good interaction of compound 5a with the crystal structure of HDAC (PDB IDs: 4BKX, 4LY1, and 4A69) (**Figure 6**).

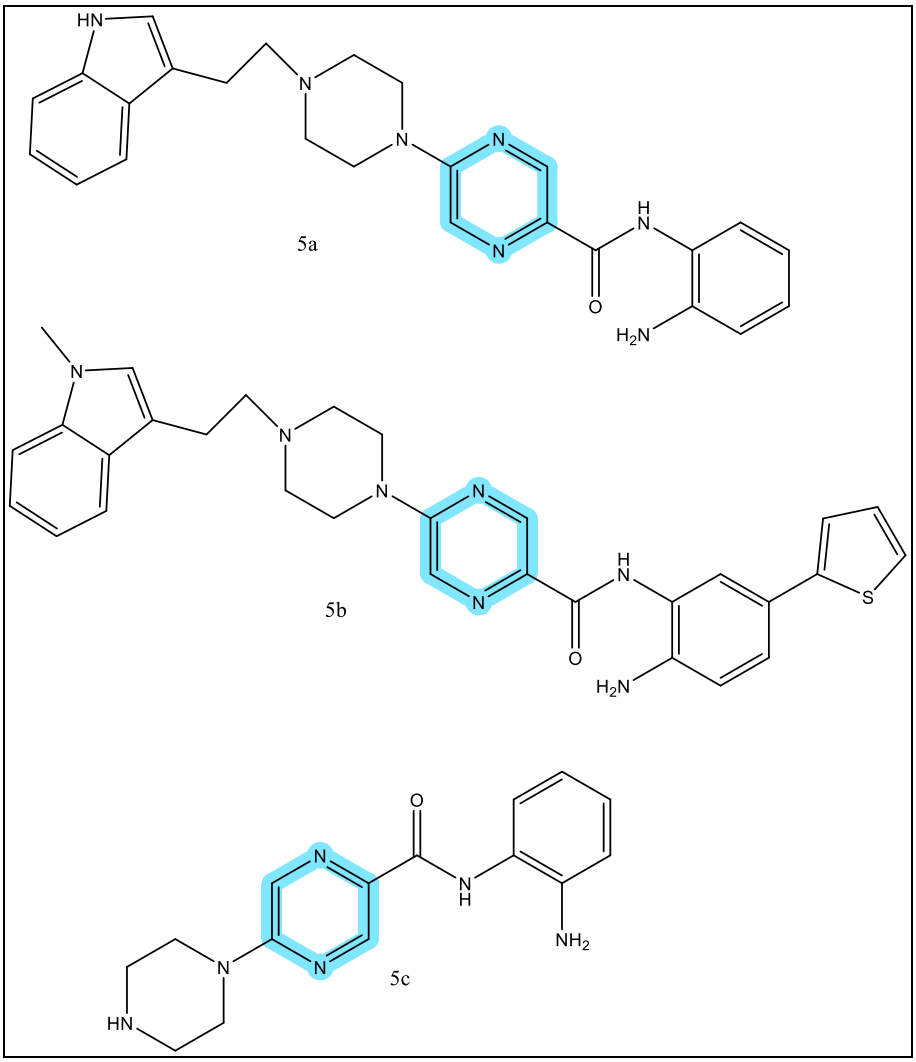


Fig. 6. Structure of compound 5a-c

Survivin inhibitors

Survivin is a protein that is also known as BIRC5 (baculoviral inhibitor of apoptosis repeat-containing 5), which is represented by the BIRC5 gene in humans [56, 57]. It belongs to the inhibitor of apoptosis (IAP) family, and its function has been conserved throughout evolution, as homologs of the protein have been found in both vertebrates and invertebrates [58]. In most human Tumors and foetal tissue,

the survivin protein is widely expressed but is completely absent in terminally differentiated cells [59]. A tetracyclic aromatic derivative (compound 6b) using a modified version of a previously published compound 11a and assessed its anti-tumor efficacy [60]. It has greatly enhanced potency, with IC_{50} values of 0.16 and 0.17 M against PC-3 and C4-2 cells, and causes prostate cancer cells to spontaneously apoptosis. It also more potently inhibits survivin dimerization and promotes survivin breakdown. The 1,2,5-oxadiazole moiety does not appear to be necessary as it interacts moderately with survivin; therefore, its replacement or removal changes the effectiveness. Furthermore, the docking simulation with a human survivin (PDB ID: 1F3H) similarly demonstrated the 6b's increased potency (**Figure 7**).

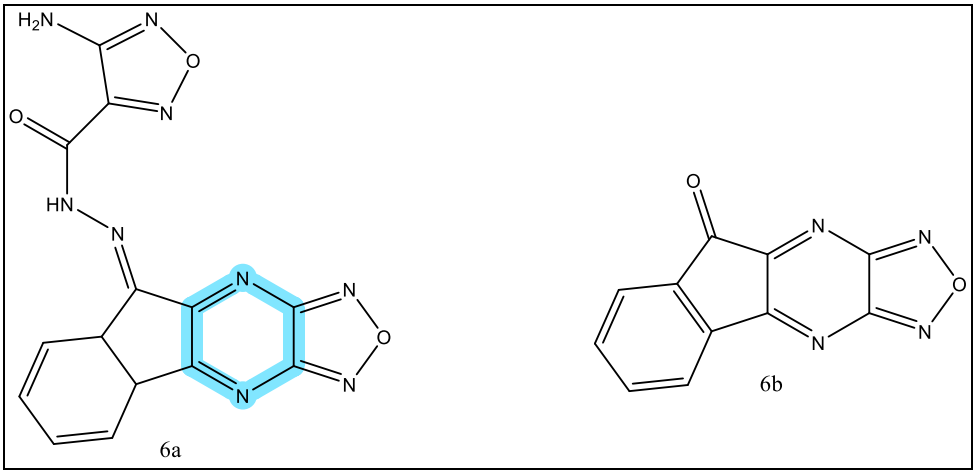


Fig. 7. Structure of compounds 6a and 6b

C-Met inhibitors

Tumor activity against the cell lines - Hela (oldest and commonly used human cell line), A549, and MCF-7 with IC_{50} values of 2.85±0.74, 0.83±0.07, and 0.15±0.08 µM, respectively. c-Met-kinase inhibition was also superior at the nanomolar level (IC_{50}=48 nM). Additionally, compound 7a showed a greater antitumor activity than other compounds due to the presence of a bigger electron-withdrawing group (trifluoromethyl group) on the phenyl ring. Furthermore, the docking investigation demonstrated its potential for binding to the crystal structure of c-Met (PDB ID: 3LQ8). The findings suggested that it could be a potential inhibitor (**Figure 8**).

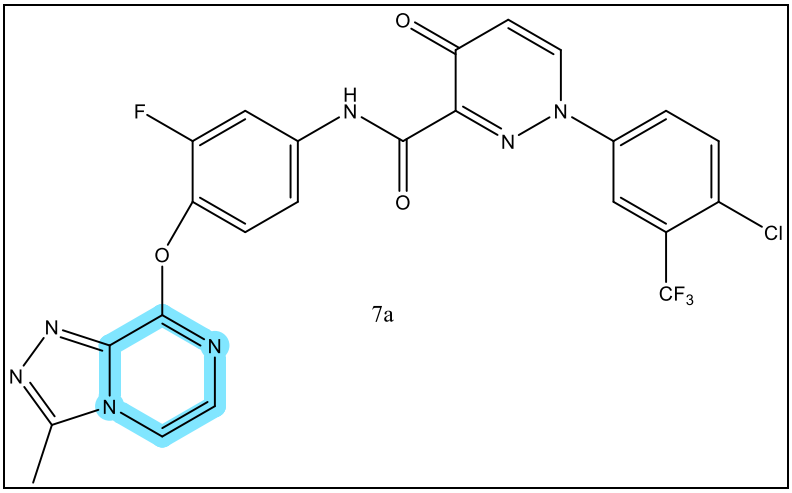


Fig. 8. Structure of compound 7a

In healthy people, the (HGFR), commonly known as C-Met, is involved in invasive growth during embryonic development, wound healing, and tissue repair [61-63]. Tumor development and metastasis are mediated by abnormal C-Met signaling. As a result, a variety of strategies have been used to reduce the aberrant c-Met axis as an anticancer therapy [64-66]. Small molecule

inhibitors have gotten a lot of attention among them [67, 68].

The c-Met kinase inhibitory and antiproliferative properties of several 5-methylpyrazolo[1,5-*a*]pyrimidine derivatives were reported [69]. The majority of the drugs inhibited c-Met kinase with remarkable potency and selectivity against the cancer cell lines. The compounds with the most active antiproliferative effect were compounds 7c and 7b, which had IC_{50} values of 7c (26.83±2.41 and 73.33±1.75) and 7b (93.73±4.24 and 20.20±2.04 µM) against MDA-MB-231 and A549, respectively. Accordingly, compound 7c showed to be the most effective c-Met inhibitor, with an IC_{50} value of 5.170.48 nM, while compound 7b showed to be the second most effective inhibitor, with an IC_{50} value of 5.620.78 nM. Furthermore, compound 7c includes a pyrazine moiety, which increases its potency. Similarly, compound 7b has 4-fluorophenyl, which likewise shows good cytotoxicity toward all cell types. These findings revealed that the addition of fluorophenyl and pyrazine rings was advantageous for broad-spectrum anticancer activity, even if other benzene ring substitutions, such as methyl, chloro, metamethoxy, and 3-chloro-4-methoxy, decreased cytotoxicity. Additionally, docking analysis revealed that substituting pyrazine might enhance the ability to inhibit c-Met kinase and the antiproliferative potential (PDB IDs: 3U6I and 4KNB) (**Figure 9**).

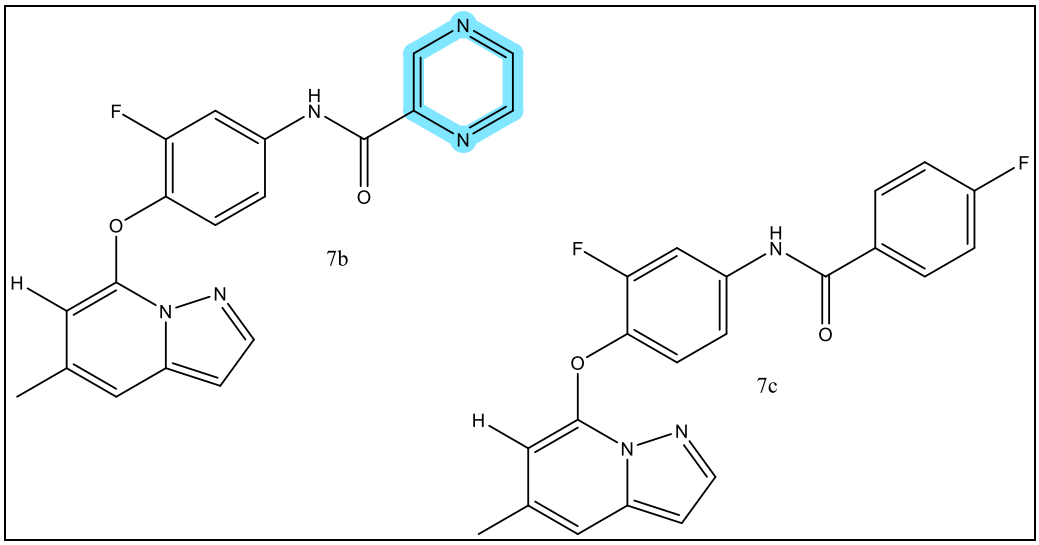


Fig. 9. Structure of compound 7b, 7c

A series of [1,2,4]-triazolo [4,3-*a*]-pyrazine compounds containing the 4-oxo-pyridazinone moiety and tested them against cancer cell lines as well as the c-Met kinase [70]. The most potent chemical, 10e, showed excellent anti-tumor activity against the cell lines Hela, A549, and MCF-7 with IC_{50} values of 2.85±0.74, 0.83±0.07, and 0.15±0.08 µM, respectively. c-Met-kinase inhibition was also superior at the nanomolar level (IC_{50}=48 nM). Additionally, compound 10e showed greater antitumor activity than other compounds due to the presence of a bigger electron-withdrawing group (trifluoromethyl group) on the phenyl ring. Furthermore, the docking investigation demonstrated its potential for binding to the crystal structure of c-Met. This suggested that it could be a potential inhibitor.

Aurora A and B kinase inhibitors

The aurora kinase family is a serine / threonine kinase subfamily that controls centrosome maturation, mitotic spindle formation [71], cytokinesis, and chromosomal segregation during mitosis [72]. Aurora A inhibition causes mitotic latency and the production of monopolar spindles, which leads to cell death [73], while aurora B is a transcription factor that controls chromatin remodeling, kineto- chore-spindle attachment, and cytokinesis [74]. Inhibition of Aurora B causes endoreduplication and cytokinesis to be arrested, resulting in apoptosis. When both aurora A and B are blocked, the inhibition phenotype of aurora B dominates [75]. The antiproliferation of 3,5-disubstituted-2-aminopyrazines as aurora kinase inhibitors was reported [76]. Among all, compound 8a had the highest antiproliferative activity against HepG2, LoVo, HeLa, and U38 cells, with IC_{50} values of 7.30±1.56, 1.64±0.48, 1.34±0.23, and 11.5±3.2 µM, also inhibited aurora-A and B with IC_{50}

values of 90 and 152 nM, respectively. The majority of the compounds with different substitutions at the pyrazine's C-3 position exhibited a range of antiproliferative effects, but compound 8a, which contained 3-chloroaniline in p-hydroxybenzoic chains, displayed the strongest inhibitory and antiproliferative activity compared to other compounds. The C-5 position of the pyrazine was changed to 3,5-dimethyl-isoxazole, which also increases the activity of the compound. Compound 8a appears to form stable hydrogen bonds with aurora-A and B (PDB IDs: 3H0Z and 4C2V), according to molecular docking studies. These findings suggested that 8a could be used to generate strong anticancer medicines (**Figure 10**).

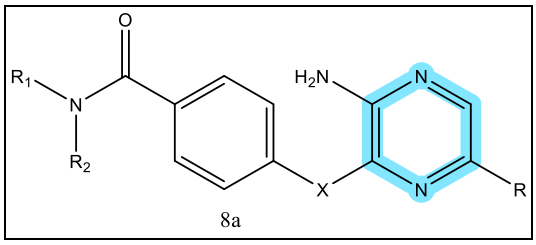


Fig. 10. Structure of compound 8a

Sorafenib kinase inhibitors

Sorafenib is a protein kinase inhibitor that inhibits a variety of kinases, including VEGFR, PDGFR, and RAF [77, 78]. It targets c-Raf rather than B-RAF. Among the RAF kinases [79], autophagy is induced by sorafenib treatment [80], which may slow tumor growth. It is also a robust soluble epoxide hydrolase inhibitor, thanks to its 1,3-disubstituted urea structure, which likely lessens the severity of its side effects [81].

A variety of sorafenib analogs and derivatives as tumor inhibitors were designed, synthesized, and evaluated [82]. As the lead molecule, sorafenib was employed, with bioisosteres and the alkyl principle being used to achieve changes. Among all, compounds 9a and 9b were shown to be the most powerful and potent with IC_{50} values of inhibition (3.78±0.21, 16.23±1.23 and 2.34±0.11), and (1.35±0.02, 13.58±1.67 and 2.67±0.12 µmol/l against A549, Hela and H1975 (human lung cancer), respectively. Numerous substituents on the aniline structure were altered during the structural modification of the lead molecule (sorafenib). The following substitutes were chosen and tested: the target compounds' physical and chemical characteristics (log P and pKa) improved with the addition of CH_3 at R1, pyrazine and pyridazine at R2, morpholine and pyrimidine at R3, and these substituents also produced high biological activity (**Figure 11**).

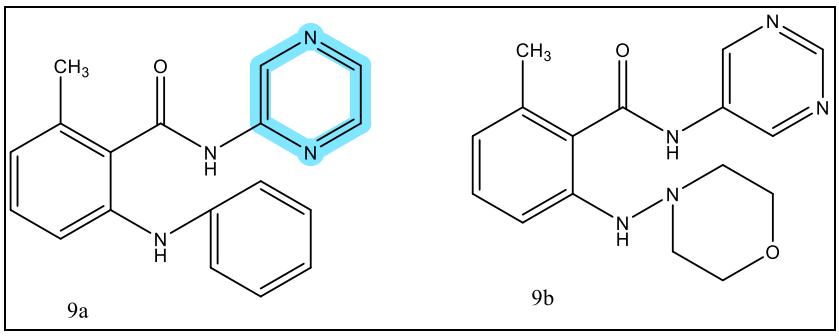


Fig. 11. Structure of compounds 9a and 9b

Miscellaneous

The synthesis of a variety of ligustrazine-chalcone hybrids and tested their anticancer activity [83]. Most of these compounds displayed considerable in vitro cytotoxicity against different cell lines. According to the findings, compounds 10a and 10b had the best overall features for antiproliferation effects, with IC_{50} values of 10a: 1.67±1.25, 1.54±0.30 and 10b:1.60± 0.21, 1.41± 0.23 µM against MDA-MB231, MCF-7 cell lines, respectively. Furthermore, the addition of the chalcone moiety to the backbone of ligustrazine may considerably increase its anticancer activity, and the mono substitution of the phenyl moiety at position C-4 in the benzamide segment of the chalcone portion makes it the most potent antiproliferative compound (**Figure 12**).

4-(4-methoxyphenyl)-2-(2- (1-(pyrazin-2yl) ethylidene) hydrazinyl) thiazole is being used to make iron (II), cobalt (III), and nickel (II) complexes (PyztH). $[Fe(Pyzt)_2]$ Br_2 (Compound 10c), $[Co(Pyzt)_2]$ PF6 (Compound 10d), and [Ni (Pyzt) (PyztH)] ClO_4 (Compound 10e), having an octahedral environment around the metal center and NNN donor atoms from the two

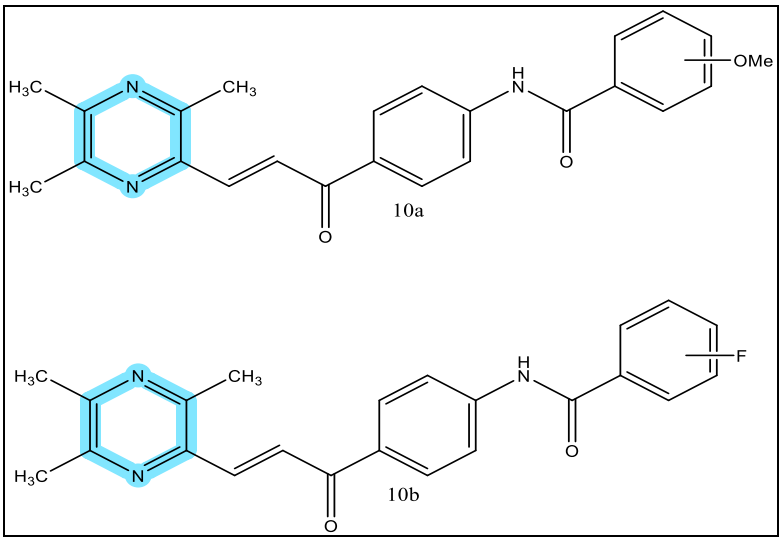


Fig. 12. Structure of compounds 10a and 10b

coordinating ligands [84]. The cytotoxicity of the complexes was tested in U-937 human monocytic cells, with IC_{50} values of 132 (for compound 10c), 45 (compound 10d), and 162 µM (for compound 10e). The docking study also displayed favorable binding of these derivatives with the crystal structure of focal adhesion kinase (FAK) (PDB ID: 6cb0) (**Figure 13**).

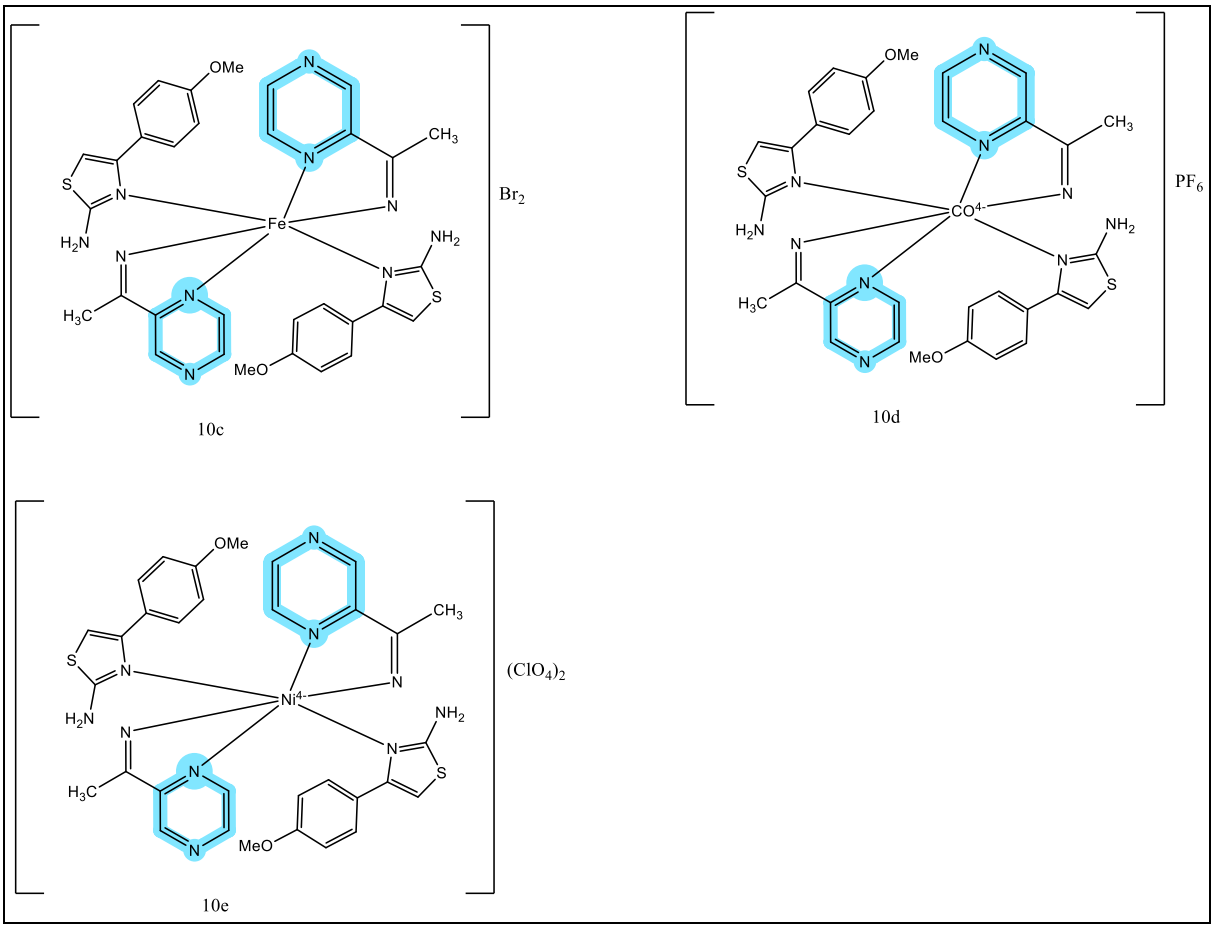


Fig. 13. Structure of compounds 10c-e

The usage of crucial keto-enol intermediates to synthesize two series of A-ring fused steroidal pyrazines from commercially available progesterone [85]. Additional mechanistic research revealed that the most active molecule, 10f (IC_{50}, 0.93 µM; SI 28.71), could potentially

cause cell cycle arrest in the G2/M phase and trigger cell death in PC-3 cells in a dose-dependent manner. It seems to fit the active sites of CYP17A1 (PDB ID: 6CIZ) well in a molecular docking analysis. Furthermore, the activities were found to vary significantly depending on where the phenyl ring was replaced; however, beta-phenylethyl substitution displayed the highest level of activity (**Figure 14**).

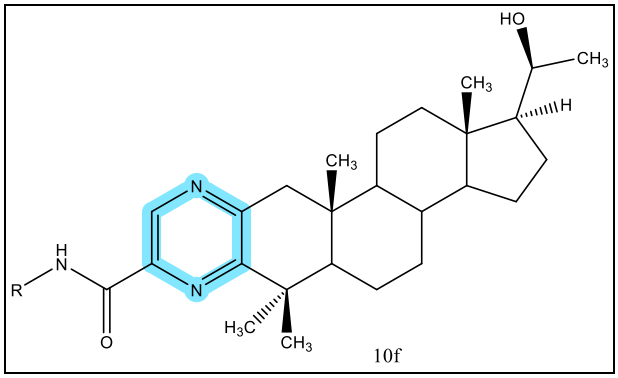


Fig. 14. Structure of compound 10f

Novel hederagenin-pyrazine derivatives, which were tested for *in vitro* cytotoxicity against tumor cell lines [86]. Among all, compound 10g was discovered to be the most powerful molecule, having IC_{50} values of 3.45±0.59, 8.73±1.49, 8.71±0.38, 14.11±0.04, and 16.69±0.12 µM against A549, MCF-7, MCF-7, MDCK, and H9c2, respectively. Furthermore, the methyl-substituted pyrazine group of compounds 10g had a significant impact on the anticancer activity; any change or replacement in the pyrazine moiety reduces its activity (**Figure 15**).

CONCLUSION

Following cardiovascular diseases, cancer stands as the second-leading cause of mortality, contributing to one-sixth of global deaths. The rapid developmental strides observed in emerging nations led to a projected 8.9 million cancer-related deaths in 2016, with an anticipated doubling of these figures by the conclusion of 2040.

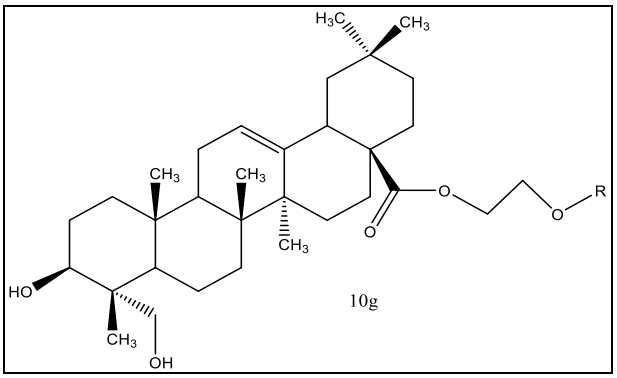


Fig. 15. Structure of compound 10g

Consequently, there is an imperative need for heightened research endeavors directed toward pioneering chemotherapeutic treatments characterized by reduced side effects and heightened efficacy.

This investigation furnishes an abundance of scientific insights aimed at advancing the development of innovative pyrazine analogs or derivatives. These compounds exhibit a diverse array of anticancer characteristics, positioning them within the lucrative and privileged domain of pharmaceutical science. The study delves into various targets associated with these analogs, shedding light on their potential as viable candidates for anticancer therapeutics.

Furthermore, it scrutinizes recently published scientific works across multiple publications, identifying them as prospective alternative treatment options. The comprehensive coverage includes detailed synthesis schemes, tabulated information on current clinical trial drugs, and an overview of recently patented and published research works. This amalgamation of data aims to empower scientists in the development of pharmaceuticals that are not only effective but also possess extraordinary medicinal value.

REFERENCES

1. Staedel W, Rügheimer L. Ueber die Einwirkung von Ammoniak auf Chloracetylbenzol. *Ber Dtsch Chem Ges*. 1876;9:563-4. doi:10.1002/cber.187600902218
2. Dahiya R, Dahiya S, Fuloria NK, Kumar S, Mourya R, Chennupati SV, et al. Natural bioactive thiazole-based peptides from marine resources: Structural and pharmacological aspects. *Mar Drugs*. 2020;18(6):329. doi:10.3390/md18060329
3. Senthil Kumar R, Vinoth Kumar S, Sudhakar P. Anticancer activity of methanolic leaf extract of Morinda tinctoria roxb. against Ehrlich ascites carcinoma in mice. *Bull Pharm Res*. 2017;7(2):146.
4. Dolezal M, Zitko J. Pyrazine derivatives: a patent review (June 2012–present). *Exp Opin Ther Pat*. 2015;25(1):33-47. doi:10.1517/13543776.2014.982533
5. Seitz LE, Suling WJ, Reynolds RC. Synthesis and antimycobacterial activity of pyrazine and quinoxaline derivatives. *J Med Chem*. 2002;45(25):5604-6. doi:10.1021/jm020310n
6. Czerny M, Grosch W. Potent odorants of raw Arabica coffee. Their changes during roasting. *J Agric Food Chem*. 2000;48(3):868-72. doi:10.1021/jf990609n
7. Marais J, Hunter JJ, Haasbroek PD. Effect of canopy microclimate season and region on Sauvignon blanc

grape composition and wine quality. *S Afr J Enol Vitic*. 1999;20(1):19-30. doi:10.21548/20-1-2223
8. Mortzfeld FB, Hashem C, Vranková K, Winkler M, Rudroff F. Pyrazines: Synthesis and industrial application of these valuable flavor and fragrance compounds. *Biotechnol J*. 2020;15(11):2000064. doi:10.1002/biot.202000064
9. Ong KT, Liu ZQ, Tay MG. Review on the synthesis of pyrazine and its derivatives. *Borneo J Res Sci Technol*. 2017;7(2):60-75. doi:10.33736/bjrst.591.2017
10. Podraza KF. Synthesis and thermal rearrangement of allylic 3,5,6-trimethyl-2-pyrazinylacetates: A heterocyclic carroll rearrangement. *J Heterocycl Chem*. 1986; 23(2):581-3. doi:10.1002/jhet.5570230254
11. Gao J, Luo X, Li Y, Gao R, Chen H, Ji D. Synthesis and biological evaluation of 2-oxo-pyrazine-3-carboxamide-yl nucleoside analogs and their epimers as inhibitors of influenza A viruses. *Chem Biol Drug Des*. 2015;85(3): 245-52. doi:10.1111/cbdd.12382
12. Zhao C, Cao H, Xiao J. Pyrazines in Food. In: Xiao J, Sarker SD, Asakawa Y. (eds) Handbook of Dietary Phytochemicals. Springer, Singapore; 2021. doi:10.1007/978-981-15-4148-3_44
13. Cheeseman GWH, Werstiuk ESG. Recent advances in pyrazine chemistry. *Adv Heterocycl Chem*. 1972;14:99-209. doi:10.1016/S0065-2725(08)60953-8
14. Kosuge T, Kamiya H. Discovery of a pyrazine in a natural product: Tetramethylpyrazine from cultures of a strain of *Bacillus subtilis*. *Nature*. 1962;193:776. doi:10.1038/193776a0
15. Liu W, Tang Y, Guo Y, Sun B, Zhu H, Xiao Y, et al. Synthesis, characterization and bioactivity determination of ferrocenyl urea derivatives. *Appl Organomet Chem*. 2012;26(4):189-93. doi:10.100 2/aoc.2837
16. Temple C Jr, Wheeler GP, Elliott RD, Rose JD, Kussner CL, Comber RN, et al. New anticancer agents: synthesis of 1,2-dihydropyrido[3,4-b]pyrazines (1-deaza-7,8-dihydropteridines). *J Med Chem*. 1982;25(9):1045-50. doi:10.1021/jm00351a008
17. Saito R, Matsumura Y, Suzuki S, Okazaki N. Intensely blue-fluorescent 2,5-bis(benzoimidazol-2-yl) pyrazine dyes with improved solubility: Their synthesis, fluorescent properties, and application as micro-environment polarity probes. *Tetrahedron*. 2010;66 (42):8273-79. doi:10.1016/j.tet.2010.08.036
18. Niculescu-Duvaz I, Roman E, Whittaker SR, Friedlos F, Kirk R, Scanlon IJ, et al. Novel inhibitors of the v-raf murine sarcoma viral oncogene homologue B1 (BRAF) based on a 2,6-disubstituted pyrazine scaffold. *J Med Chem*. 2008;51(11):3261-74. doi:10.1021/jm070776b
19. Staedel W, Rügheimer L. Ueber die einwirkung von ammoniak auf chloracetylbenzol. *Ber Dtsch Chem Ges*. 1876;9(2):1758-61. doi:10.1002/cber.187600902218
20. Gastaldi, G. Pyrazines synthesis. *Gazz Chim Ital*. 1921;51:233-55.
21. Lawrence SA. Amines: Synthesis, properties and applications; Cambridge University Press, 2004.
22. Masuda H, Tanaka M, Akiyama T, Shibamoto T. Preparation of 5- substituted 2,3-dimethylpyrazines from the reaction of 2,3-dimethyl-5,6-dihydropyrazine and aldehydes or ketones. *J Agric Food Chem*. 1980; 28(2):244-6. doi:10.1021/jf60228a058
23. Yang XJ, Grégoire S. Class II histone deacetylases: From sequence to function, regulation, and clinical implication. *Mol Cell Biol*. 2005;25(8):2873-84. doi:10.1128/MCB.25.8.2873-2884.2005
24. Sato K. US Patent No. 4,097,478. Washington, DC: US Patent and Trademark Office, 1978.
25. Borowski E, Bontemps-Gracz MM, Piwkowska A. Strategies for overcoming ABC-transporters-mediated multidrug resistance (MDR) of tumor cells. *Acta Biochim Pol*. 2005;52(3):609-27.
26. Ghosh P, Mandal A. Greener approach toward one-pot route to pyrazine synthesis. *Green Chem Lett Rev*. 2012;5:127-34. doi:10.1080/17518253.2011.585182
27. Ghosh R, Hansda S. Targeted and non-targeted effects of radiation in mammalian cells: An overview. *Arch Biotechnol Biomed.* 2021;5(1):13-9. doi:10.29328/journal.abb.1001023
28. Yahyapour R, Salajegheh A, Safari A, Amini P, Rezaeyan A, Amraee A, et al. Radiation-induced non-targeted effect and carcinogenesis; implications in clinical radiotherapy. *J Biomed Phys Eng*. 2018;8(4):435-46.
29. Oncology (Cancer) / hematologic malignancies approval notifications. Available from: https://www.fda.gov
30. Sithanandam G, Kolch W, Duh FM, Rapp UR. Complete coding sequence of a human B-raf cDNA and detection of B-raf protein kinase with isozyme specific antibodies. *Oncogene*. 1990;5(12):1775-80.
31. Sithanandam G, Druck T, Cannizzaro LA, Leuzzi G, Huebner K, Rapp UR. B-raf and a B-raf pseudogene are located on 7q in man. *Oncogene*. 1992;7(4):795-9.
32. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et. al. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417(6892):949-54. doi:10.1038/nature00766
33. Perez EA. Microtubule inhibitors: Differentiating tubulin-inhibiting agents based on mechanisms of action, clinical activity, and resistance. *Mol Cancer Ther*. 2009;8:2086-95. doi:10.1158/1535-7163.MCT-09-0366
34. Jordan MA. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. *Curr Med Chem Anticancer Agents*. 2002;2(1):1-17. doi:10.2174/1568011023354290
35. Youssif BGM, Abdelrahman MH, Abdelazeem AH, Abdelgawad MA, Ibrahim HM, Salem OIA, et al. Design, synthesis, mechanistic and histopathological studies of small-molecules of novel indole-2-carboxamides and pyrazino[1,2-a]indol-1(2H)-ones as potential anticancer agents effecting the reactive oxygen species production. *Eur J Med Chem*. 2018;146:260-73. doi:10.1016/j.ejmech.2018.01.042
36. Chen A. PARP inhibitors: Its role in treatment of cancer. *Chin J Cancer*. 2011;30(7):463-71. doi:10.5732/cjc.011.10111
37. Rose M, Burgess JT, O'Byrne K, Richard DJ, Bolderson E. PARP Inhibitors: Clinical Relevance, Mechanisms of Action and Tumor Resistance. *Front Cell Dev Biol*. 2020;8:564601. doi:10.3389/fcell.2020.564601
38. Patel A, Kaufmann SH. Development of PARP inhibitors: An unfinished story. *Oncology*. 2010;24(1):66, 68.
39. Furlan AD, Giraldo M, Baskwill A, Irvin E, Imamura M. Massage for low-back pain. *Cochrane Database Syst Rev*. 2015;2015(9):CD001929. doi:10.1002/14651858.CD001929.pub3
40. Seo Y, Lee JH, Park SH, Namkung W, Kim I. Expansion of chemical space based on a pyrrolo[1,2-a]pyrazine core: Synthesis and its anticancer activity in prostate cancer and breast cancer cells. *Eur J Med Chem*. 2020;188: 111988. doi:10.1016/j.ejmech.2019.111988
41. Babina IS, Turner NC. Advances and challenges in targeting FGFR signalling in cancer. *Nat Rev Cancer*. 2017;17(5):318-32. doi:10.1038/nrc.2017.8

42. Touat M, Ileana E, Postel-Vinay S, André F, Soria JC. Targeting FGFR Signaling in Cancer. *Clin Cancer Res*. 2015;21(12):2684-94. doi:10.1158/1078-0432.CCR-14-2329
43. Hallinan N, Finn S, Cuffe S, Rafee S, O'Byrne K, Gately K. Targeting the fibroblast growth factor receptor family in cancer. *Cancer Treat Rev*. 2016;46:51-62. doi:10.1016/j.ctrv.2016.03.015
44. Katoh M. Therapeutics targeting FGF signaling network in human diseases. *Trends Pharmacol Sci*. 2016;37(12):1081-96. doi:10.1016/j.tips.2016.10.003
45. Hierro C, Rodon J, Tabernero J. Fibroblast growth factor (FGF) receptor/FGF inhibitors: Novel targets and strategies for optimization of response of solid tumors. *Semin Oncol*. 2015;42(6):801-19. doi:10.1053/j.seminoncol.2015.09.027
46. Wei P, Liu B, Wang R, Gao Y, Li L, Ma Y, et al. Discovery of a series of dimethoxybenzene FGFR inhibitors with 5*H*-pyrrolo[2,3-*b*]pyrazine scaffold: structure-activity relationship, crystal structural characterization and *in vivo* study. *Acta Pharm Sin B*. 2019;9(2):351-68. doi:10.1016/j.apsb.2018.12.008
47. Jiang A, Liu Q, Wang R, Wei P, Dai Y, Wang X, et al. Structure-based discovery of a series of 5*H*-pyrrolo[2,3-b]pyrazine FGFR kinase inhibitors. *Molecules*. 2018;23(3):698. doi:10.3390/molecules23030698
48. Herbst RS. Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys*. 2004;59(2S):21-6. doi:10.1016/j.ijrobp.2003.11.041
49. Zhang H, Berezov A, Wang Q, Zhang G, Drebin J, Murali R, et al. ErbB receptors: From oncogenes to targeted cancer therapies. *J Clin Invest*. 2007;117(8):2051-8. doi:10.1172/JCI32278
50. Tantawy ES, Amer AM, Mohamed EK, Abd Alla MM, Nafie MS. Synthesis, characterization of some pyrazine derivatives as anti-cancer agents: In vitro and In silico approaches. *J Mol Struct*. 2020;1210:128013. doi:10.1016/j.molstruc.2020.128013
51. Fan J, Krautkramer KA, Feldman JL, Denu JM. Metabolic regulation of histone post-translational modifications. *ACS Chem Biol*. 2015;10(1):95-108.
52. Chadha S, Wang L, Hancock WW, Beier UH. Sirtuin-1 in immunotherapy: A Janus-headed target. *J Leukoc Biol*. 2019;106(2):337-43. doi:10.1002/JLB.2RU1118-422R
53. Pant K, Peixoto E, Richard S, Gradilone SA. Role of histone deacetylases in carcinogenesis: Potential role in cholangiocarcinoma. *Cells*. 2020;9(3):780. doi:10.3390/cells9030780
54. Gryder BE, Sodji QH, Oyelere AK. Targeted cancer therapy: giving histone deacetylase inhibitors all they need to succeed. *Future Med Chem*. 2012;4(4):505-24. doi:10.4155/fmc.12.3
55. Ibrahim HS, Abdelsalam M, Zeyn Y, Zessin M, Mustafa AM, Fischer MA, et. al. Synthesis, molecular docking and biological characterization of pyrazine linked 2-aminobenzamides as new class I selective histone deacetylase (HDAC) inhibitors with anti-leukemic activity. *Int J Mol Sci*. 2021;23(1):369. doi:10.3390/ijms23010369
56. Altieri DC. Molecular cloning of effector cell protease receptor-1, a novel cell surface receptor for the protease factor Xa. *J Biol Chem*. 1994;269(5):3139-42. doi:10.1016/S0021-9258(17)41838-2
57. Altieri DC. Splicing of effector cell protease receptor-1 mRNA is modulated by an unusual retained intron. *Biochemistry*. 1994;33(46):13848-55. doi:10.1021/bi00250a039
58. Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T, et al. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. *Cancer Res*. 1998;58(23):5315-20.
59. Sah NK, Khan Z, Khan GJ, Bisen PS. Structural, functional and therapeutic biology of survivin. *Cancer Lett*. 2006;244(2):164-71. doi:10.1016/j.canlet.2006.03.007
60. Peery R, Cui Q, Kyei-Baffour K, Josephraj S, Huang C, Dong Z, et al. A novel survivin dimerization inhibitor without a labile hydrazone linker induces spontaneous apoptosis and synergizes with docetaxel in prostate cancer cells. *Bioorg Med Chem*. 2022;65:116761. doi:10.1016/j.bmc.2022.116761
61. Liu X, Yao W, Newton RC, Scherle PA. Targeting the c-MET signaling pathway for cancer therapy. *Expert Opin Investig Drugs*. 2008;17(7):997-1011. doi:10.1517/13543784.17.7.997
62. Blumenschein GR Jr, Mills GB, Gonzalez-Angulo AM. Targeting the hepatocyte growth factor-cMET axis in cancer therapy. *J Clin Oncol*. 2012;30(26):3287-96. doi:10.1200/JCO.2011.40.3774
63. Sahu R, Mishra R, Kumar R, Salahuddin, Majee C, Mazumder A, et al. Pyridine moiety: An insight into recent advances in the treatment of cancer. *Mini Rev Med Chem*. 2022;22(2):248-72. doi:10.2174/1389557521666210614162031
64. Gherardi E, Birchmeier W, Birchmeier C, Vande Woude G. Targeting MET in cancer: rationale and progress. *Nat Rev Cancer*. 2012;12(2):89-103. doi:10.1038/nrc3205
65. Mishra R, Kumar N, Sachan N. Synthesis, biological evaluation, and docking analysis of novel tetrahydrobenzothiophene derivatives. *Lett Drug Des Discov*. 2022;19(6):530-40. doi:10.2174/1570180819666220117123958
66. Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. *Nat Rev Mol Cell Biol*. 2010;11(12):834-48. doi:10.1038/nrm3012
67. Cui J. Inhibitors targeting hepatocyte growth factor receptor and vascular endothelial growth factor receptor tyrosine kinases. *Expert Opin Ther Pat*. 2006;16(5):713-8. doi:10.1517/13543776.16.5.713
68. Parikh PK, Ghate MD. Recent advances in the discovery of small molecule c-Met Kinase inhibitors. *Eur J Med Chem*. 2018;143:1103-1138. doi:10.1016/j.ejmech.2017.08.044
69. Luo G, Ma Y, Liang X, Xie G, Luo Y, Zha D, et al. Design, synthesis and antitumor evaluation of novel 5-methylpyrazolo[1,5-a]pyrimidine derivatives as potential c-Met inhibitors. *Bioorg Chem*. 2020;104:104356. doi:10.1016/j.bioorg.2020.104356
70. Zhang B, Liu X, Xiong H, Zhang Q, Sun X, Yang Z, et al. Discovery of [1,2,4]triazolo[4,3-*a*]pyrazine derivatives bearing a 4-oxo-pyridazinone moiety as potential c-Met kinase inhibitors. *New J Chem*. 2020;44(21):9053-63. doi:10.1039/D0NJ00575D
71. Fancelli D, Moll J. Inhibitors of Aurora kinases for the treatment of cancer. *Exp Opin Therap Pat*. 2005;15(9):1169-82. doi:10.1517/13543776.15.9.1169
72. Barr AR, Gergely F. Aurora-A: the maker and breaker of spindle poles. *J Cell Sci*. 2007;120(Pt 17):2987-96. doi:10.1242/jcs.013136
73. Girdler F, Gascoigne KE, Eyers PA, Hartmuth S, Crafter C, Foote KM, Keen NJ, Taylor SS. Validating Aurora B as an anti-cancer drug target. *J Cell Sci*. 2006;119(Pt 17):3664-75. doi:10.1242/jcs.03145

74. Carmena M, Earnshaw WC. The cellular geography of aurora kinases. *Nat Rev Mol Cell Biol*. 2003;4:842-54.
75. Yang H, Burke T, Dempsey J, Diaz B, Collins E, Toth J, et al. Mitotic requirement for aurora A kinase is bypassed in the absence of aurora B kinase. *FEBS Lett*. 2005;579(16):3385-91.
76. Bo YX, Xiang R, Xu Y, Hao SY, Wang XR, Chen SW. Synthesis, biological evaluation and molecular modeling study of 2-amino-3,5-disubstituted-pyrazines as Aurora kinases inhibitors. *Bioorg Med Chem*. 2020;28(5): 115351. doi:10.1016/j.bmc.2020.115351
77. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol Cancer Ther*. 2008;7(10):3129-40. doi:10.1158/1535-7163.MCT-08-0013
78. Keating GM, Santoro A. Sorafenib: a review of its use in advanced hepatocellular carcinoma. *Drugs*. 2009;69(2):223-40. doi:10.2165/00003495-200969020-00006
79. Smalley KS, Xiao M, Villanueva J, Nguyen TK, Flaherty KT, Letrero R, et al. CRAF inhibition induces apoptosis in melanoma cells with non-V600E BRAF mutations. *Oncogene*. 2009;28(1):85-94.
80. Zhang Y, Xue D, Wang X, Lu M, Gao B, Qiao X. Screening of kinase inhibitors targeting BRAF for regulating autophagy based on kinase pathways. *Mol Med Rep*. 2014;9(1):83-90. doi:10.3892/mmr.2013.1781
81. Grune T, Klotz LO. Encyclopedia of molecular pharmacology. Springer International Publishing: 2020, 1-7.
82. Zhou S, Chen G. Design, synthesis, and bioactivity evaluation of antitumor sorafenib analogues. *RSC Adv*. 2018;8(66):37643-51. doi:10.1039/C8RA08246D
83. Luo Y, Wu W, Zha D, Zhou W, Wang C, Huang J, et al. Synthesis and biological evaluation of novel ligustrazine-chalcone derivatives as potential anti-triple negative breast cancer agents. *Bioorg Med Chem Lett*. 2021;47:128230. doi:10.1016/j.bmcl.2021.128230
84. Bera P, Aher A, Brandao P, Manna SK, Mondal G, Jana A, et al. Induced apoptosis against U937 cancer cells by Fe(II), Co(III) and Ni(II) complexes with a pyrazine-thiazole ligand: Synthesis, structure and biological evaluation. *Polyhedron*. 2020;182:114503. doi:10.1016/j.poly.2020.114503
85. Wang S, Yuan X, Qian H, Li N, Wang J. Design, synthesis, and bio- logical evaluation of two series of novel a-ring fused steroidal pyrazines as potential anticancer agents. *Int J Mol Sci*. 2020;21(5):1665. doi:10.3390/ijms21051665
86. Fang K, Zhang XH, Han YT, Wu GR, Cai DS, Xue NN, et al. Design, synthesis, and cytotoxic analysis of novel hederagenin- pyrazine derivatives based on partial least squares discriminant analysis. *Int J Mol Sci*. 2018; 19(10):2994. doi:10.3390/ijms19102994

*****