Targeting hematological malignancies with isoxazole derivatives

Monika Majirská [1], Martina Bago Pilátová [1,*], Zuzana Kudličková [2], Martin Vojtek [3,*], Carmen Diniz [3]

[1] Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia
[2] NMR Laboratory, Institute of Chemistry, Faculty of Science, Pavol Jozef Šafárik University in Košice, Slovakia
[3] LAQV/REQUIMTE, Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal

Compounds with a heterocyclic isoxazole ring are well known for their diverse biologic activities encompassing antimicrobial, antipsychotic, immunosuppressive, antidiabetic and anticancer effects. Recent studies on hematological malignancies have also shown that some of the isoxazole-derived compounds feature encouraging cancer selectivity, low toxicity to normal cells and ability to overcome cancer drug resistance of conventional treatments. These characteristics are particularly promising because patients with hematological malignancies face poor clinical outcomes caused by cancer drug resistance or relapse of the disease. This review summarizes the knowledge on isoxazole-derived compounds toward hematological malignancies and provides clues on their mechanism(s) of action (apoptosis, cell cycle arrest, ROS production) and putative pharmacological targets (c-Myc, BET, ATR, FLT3, HSP90, CARM1, tubulin, PD-1/PD-L1, HDACs) wherever known.

Keywords: isoxazole derivative; myeloma; leukemia; lymphoma; cancer; blood

Introduction

Cancer remains a growing global health problem and stands as the second highest contributor to mortality across the world, accounting for ~10 million deaths in 2020.(p1) Among the most common cancer types, hematological malignancies rank in the top 10 with nearly 1.2 million new cases globally each year.(p2) Hematological malignancies are a large, heterogeneous group of neoplasms of the lymphohematopoietic system with variable clinical presentations and outcomes(p3) affecting the blood, bone marrow, lymph nodes and other parts of the lymphatic system, and can be categorized (Table 1) into leukemia [chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL)], lymphomas [non-Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma (HL)] and myelomas [multiple myeloma (MM)]. The readers are encouraged to refer to Taylor and co-workers(p4) article, which provides an excellent overview on the types of hematological malignancies.

During the past 35 years, there has been a global rise in the incidence of hematological malignancies, which is influenced by factors such as type, age and geographic location, associated with different stages of socioeconomic development and lifestyle.(p5),(p6) The regions with highest incidence trends for NHL (~85% of all lymphoma cases), HL, MM and leukemia were Central Europe, Eastern Europe, East Asia and the Caribbean, respectively.(p5) ALL is the most common type of leukemia in children and has a lower incidence in adults, whereas CLL is rare under the age of 30 but the prevalence increases with age, being more common in the elderly (with gender differences, with more cases in men than in women).(p7) CML is prevalent between 40 and 60 years and more frequent in African Americans than in Caucasians.(p7)

* *Corresponding authors. Pilátová, M.B. (martina.pilatova@upjs.sk), Vojtek, M. (mvojtek@ff.up.pt).*

1359-6446/
https://doi.org/10.1016/j.drudis.2024.104059

TABLE 1

Overview of hematological malignancy types.

Malignancy	Type	Definition
Myeloma	Multiple myeloma (MM)	Malignant proliferation of clonal plasma cells in the bone marrow and typically accompanied by the secretion of monoclonal immunoglobulins (CRAB criteria – hypercalcemia, renal failure, anemia and lytic bone lesions)[p141]
Lymphoma	Hodgkin's lymphoma (HL)	Presence of pathologic Hodgkin Reed–Sternberg cells, accounted for 10% of lymphomas
	Non-Hodgkin's lymphoma (NHL)	(i) Mature B-cell neoplasms: Diffuse large B-cell lymphoma (DLBCL) – diffuse involvement by large lymphoid cells that stain positive for the B-cell marker CD20 Burkitt lymphoma – translocation of the notorious cell proliferation protooncogene, C-MYC (ii) Mature T-cell and natural killer (NK) cell neoplasms: T cell large granular lymphocytic leukemia Chronic lymphoproliferative disorder of NK cells[p142]
Leukemia	Acute lymphoblastic leukemia (ALL)	Chromosomal translocations and somatic mutations that lead to leukemogenesis[p143]
	Chronic lymphocytic leukemia (CLL)	Lymphoid malignancy characterized by the proliferation and accumulation of mature CD5+ B cells in the blood, bone marrow and lymphoid tissues[p144]
	Acute myeloid leukemia (AML)	Dominant in adults, representative genetic abnormalities are in fms-related tyrosine 3 kinase (FLT3), nucleophosmin 1 (NPM1), CCAAT/enhancer-binding protein alpha (CEBPA), runt-related transcription factor 1 (RUNX1)
	Chronic myeloid leukemia (CML)	Myeloproliferative neoplasm caused by a translocation between chromosomes 9 and 22, involving a fusion of the Abelson oncogene (ABL) from chromosome 9q34 with the breakpoint cluster region (BCR) gene on chromosome 22q11.2 leading to a chimeric gene product known as BCR-ABL[p145]

Leukemia, a cancer of the blood and bone marrow characterized by the overproduction of abnormal white blood cells including various types such as acute lymphoblastic leukemia, chronic lymphocytic leukemia, acute or chronic myeloid leukemia. Lymphoma, a cancer of lymphocytes in the lymphatic system, which includes the lymph nodes, spleen and other lymphoid tissues including Hodgkin's lymphoma and non-Hodgkin's lymphoma. Myeloma, a cancer of plasma cells including multiple myeloma.

Current therapeutic options for hematological malignancies

Global initiatives in prevention and treatment have successfully reduced the mortality rate attributed to hematological malignancies. The conventional approach to chemotherapy involves the administration of drugs designed to eradicate or inhibit the proliferation of rapidly dividing cancer cells but, owing to indiscriminatory effects, these drugs also impact healthy cells, leading to side effects such as nausea, hair loss and fatigue. Occurrences of such indiscriminatory effects resulting from the inhibition of entire enzyme–receptor families have prompted interest in developing anticancer agents with high selectivity toward targets specific to the cancerous cells. Currently, hematological malignancies are treated with a variety of drugs or combinations of drugs, including chemotherapy (conventional drugs), targeted therapies, immunotherapy, immune checkpoint inhibitors and chimeric antigen receptor T cells (CAR-T).[p8] Chemotherapeutics commonly used in therapy of hematological malignancies include alkylating agents (cyclophosphamide, melphalan, bendamustine, cisplatin, dacarbazine, procarbazine), antimetabolites (methotrexate, cytarabine, fludarabine), plant alkaloids (vincristine, vinblastine, etoposide) and antitumor antibiotics (daunorubicin, doxorubicin, mitoxantrone, bleomycin). Furthermore, proteasome inhibitors (bortezomib), monoclonal antibodies (rituximab), enzymes (L-asparaginase) and corticosteroids (prednisone) are also used.[p9] These drugs are frequently combined into specific regimens tailored to the type and stage of the hematological malignancy being treated and overall patient's health.[p9] The profound understanding of the molecular differences in biology of hematological and non-hematological malignancies has significant importance for a successful targeted therapy. Currently, several molecular-oriented studies have discriminated putative targets selective to hematological malignancies, providing a strong rationale for the design and development of targeted therapeutic approaches, acting on neoplastic cells with overexpression of specific proteins.[p10]

In hematological malignancies, several specific targets have already been identified, including phosphoinositide 3-kinase (PI3K), a crucial signaling molecule that can trigger protein kinase B (Akt), involved in cell differentiation and proliferation that can be controlled by upstream regulators like receptor tyrosine kinases and G-protein-coupled receptors (GPCRs).[p11] Additionally, β-arrestin 2, Janus kinase (JAK) and rat sarcoma protein (RAS) have also been identified as contributors to the regulation of PI3K.[p12] Epidermal growth factor receptor (EGFR) is another specific target identified owing to its overexpression in cancer. The EGFR pathway, when activated, leads to protein phosphorylation, a signal that is eventually recognized by cyclins (particularly cyclin D) and cyclin-dependent kinases (CDKs) in the nucleus, resulting in the activation of cell division. Moreover, the activation of EGFR triggers communication between the cell survival pathway (PI3K/Akt) and the mitogenic signaling pathway but can also lead to inhibition of apoptosis and promotion of invasion inducing cell division.[p10] CDKs, a class of Ser/Thr kinases that plays a vital part in regulating the cell cycle and promoting cell proliferation, are activated through their interaction with cyclin partner proteins and, therefore, can also be used as a specific target to combat hematological malignancies.[p10] Because intracellular cell division and transportation requires the involvement of microtubules (formed by tubulin protein polymerization into long chains or filaments), which serve as a structural framework for living cells, targeting tubulin by inhibiting microtube assembly typically triggers apoptosis or programmed cell death.[p13],[p14],[p15],[p16]

The first signal transduction inhibitor ever used in clinical practice, imatinib, a tyrosine kinase inhibitor, can be administered as a standalone treatment or in conjunction with conventional anticancer drugs to treat CML. By binding to the breakpoint cluster region/Abelson (BCR/ABL) kinase domain, imatinib is capable of preventing the phosphorylation of tyrosine kinases, thus avoiding the activation of constitutive tyrosine kinases. Consequently, the signal from the leukemic cell to the nucleus is obstructed, leading to the induction of apoptosis.[p17] The second-generation tyrosine kinase inhibitors such as dasatinib, nilotinib and bosutinib can be used as alternatives or particularly for patients with resistance or intolerance to imatinib, whereas ponatinib, a third-generation tyrosine kinase inhibitor, is used for CML patients with the T315I mutation (a mutation that confers resistance to other tyrosine kinase inhibitors).[p18] Other protein inhibitors are also in development: ibrutinib and acalabrutinib can inhibit Bruton's tyrosine kinase (BTK)[p19] and are used to treat CLL, mantle cell lymphoma (MCL) and other B cell malignancies, whereas the PI3K inhibitors idelalisib and duvelisib can be used to treat certain types of NHL in addition to CLL.[p20] JAK inhibitors (such as ruxolitinib) have demonstrated efficacy against myelofibrosis and polycythemia vera.[p19] Other inhibitors in clinical use include bortezomib, carfilzomib and ixazomib, which can inhibit proteasomes in cells, leading to apoptosis of myeloma cells, and these have been used to treat MM or resistant cancer.[p21]

Immunotherapy is also a therapeutic strategy employed in the treatment of hematological cancers, which focuses on manipulating the immune system to combat cancer, aiming at enhancing or suppressing overall immune responses to target and eliminate cancer cells by directly activating the innate and/or adaptative immune systems. The process of cancer immune reprogramming can be divided into the following steps: (i) triggering the innate and/or adaptive immune systems to remove cancer cells, (ii) allowing the survival of aberrant malignant cells capable of triggering immune reprogramming and (iii) establishing an immunosuppressive microenvironment and low-immunogenic tumors.[p22],[p23] The currently known immunotherapies for hematological malignancies include immune checkpoint inhibitors, monoclonal antibodies or CAR-T cell therapy, tumor vaccines, immunomodulatory drugs (IMiDs) and stem cell transplantation.[p24],[p25],[p26],[p27] The use of monoclonal antibodies such as rituximab, which targets the CD20 antigen on B cells, has proven to be a successful approach to effectively eliminate cancer cells in NHL and CLL. Daratumumab, which targets the CD38 antigen on plasma cells, is used to treat MM. Brentuximab, which targets the CD30 antigen on lymphoma cells, is effective in treating HL and certain types of NHL.[p28] Another immunotherapy approach is based on modulation of the immune system and induction of antiangiogenic effects, promoted by IMiDs, such as lenalidomide and pomalidomide, in MM and myelodysplastic syndromes.[p29] In addition, new strategies for precision therapy have emerged, such as bispecific antibodies and CAR-T cell therapy. The latter involves modifying a patient's T cells, empowering them to recognize and combat against cancer cells.[p30],[p31] They are used to treat certain types of lymphoma and ALL. However, the treatment of hematological malignancies remains a major challenge owing to relapsed or refractory neoplasms.[p32],[p33] In summary, targeted therapies include tyrosine kinase inhibitors such BTK inhibitors, monoclonal antibodies, proteasome inhibitors, PI3K inhibitors, JAK inhibitors, IMiDs and CAR-T cell therapy. It is important to emphasize that targeted therapeutics are relatively new approaches and further research is still required to fully understand their potential risk for secondary cancers, although some drugs like vemurafenib and dabrafenib have already been described as having increased risk of squamous cell carcinomas of the skin.[p34] In addition to targeted therapeutics, chemotherapy has also been associated with increased risk of development of secondary cancers, such as myelodysplastic syndrome (MDS), AML and ALL.[p35],[p36] Compared with radiation therapy, chemotherapy is considered a higher risk factor for causing leukemia. The risk of developing secondary cancer is higher with higher drug doses, longer treatment duration and higher dose intensity.[p34]

The treatment of hematological malignancies is, therefore, challenging owing to the occurrence of relapsed or refractory neoplasms and development of drug resistance. Cancer drug resistance is a common and major therapeutic drawback of all types of currently available treatments. Although CML and promyelocytic leukemia show the beneficial effects of targeted therapy, most other forms of leukemia and lymphoma (the main cause of recurrence and treatment failure) remain a major public health problem.[p24],[p32] Indeed, regardless of the type of treatment, malignant hematopoietic cells continuously evolve cellular strategies to adapt to and survive therapeutic agents. Such adaptations can involve different molecular and cellular mechanisms, including the acquisition of mutations. In addition, the modulation of the signaling pathways involved in the regulation of apoptosis, autophagy, proteostasis, proliferation, differentiation, metabolism, epigenetic modifications and oncogenes or tumor suppressors represent additional processes that can lead to therapy-induced resistance. Other potential mechanisms of resistance arise from the tumor stromal niche; for instance, through cytokine and growth factor production or exosome secretion. Therefore, new approaches are urgently needed to overcome resistance to a specific drug or combination of drugs after an initial successful therapy. A deep understanding of the underlying resistance mechanisms provides opportunities to design new therapeutic approaches with the view of improving clinical management to cure patients (including those with relapsed and refractory disease).[p33]

The isoxazole moiety is a five-membered heterocycle containing an oxygen and nitrogen atom in adjacent positions.[p37] Natural compounds with an isoxazole skeleton, such as cycloserine, acivicin or muscimol, were first discovered in bacteria, fungi, higher plants and marine sponges.[p38] Isoxazole-derived compounds show diverse biological activities spanning from antimicrobial,[p39] antipsychotic,[p40] anti-inflammatory, immunomodulatory,[p41] antidiabetic,[p42] antiviral[p43] to anticancer effects,[p44],[p45],[p46],[p47],[p48] as summarized in Figure 1. The isoxazole scaffold can increase efficacy, decrease toxicity and improve pharmacokinetic profiles of chemical compounds.[p37] Drugs containing an isoxazole ring have already been successfully introduced into clinical practice. The most important ones: the disease-modifying anti-rheumatic drug

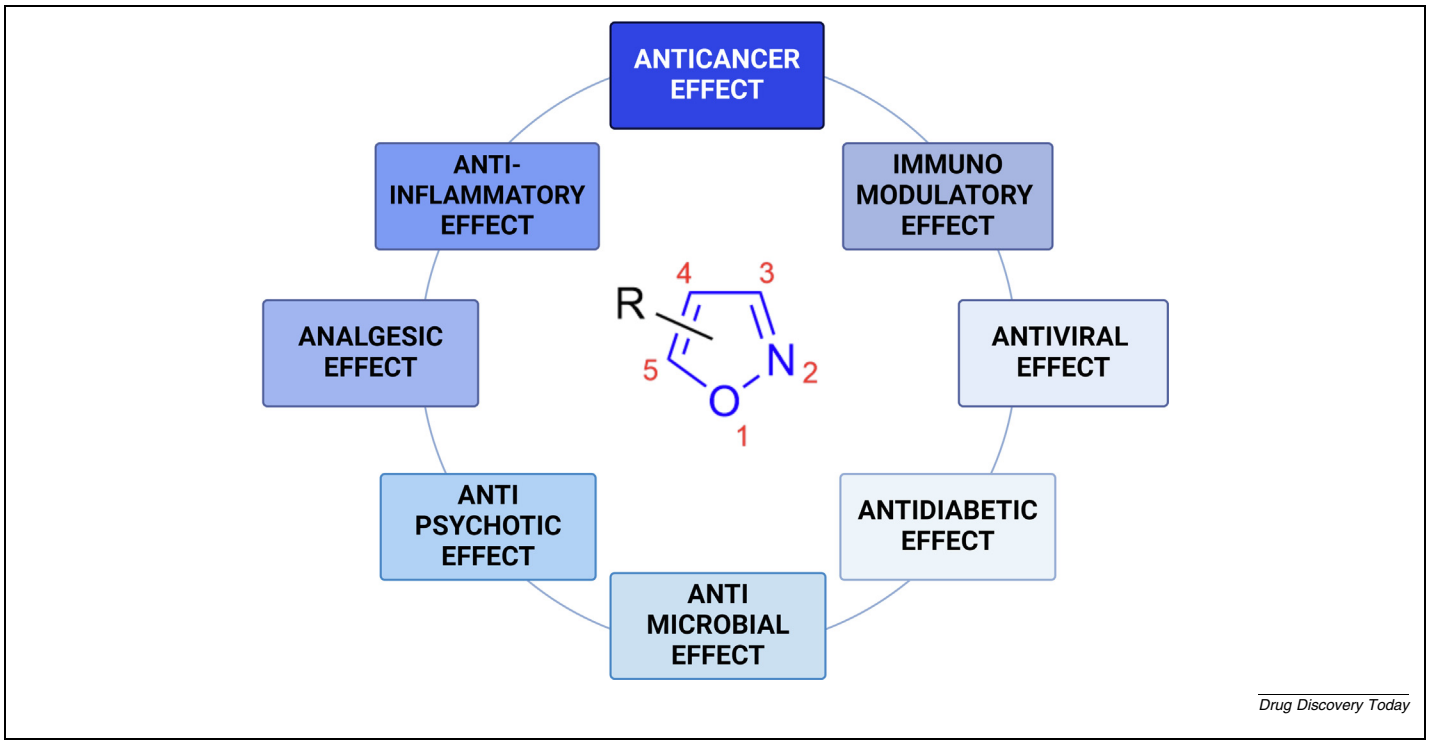


FIGURE 1

Several biological effects of isoxazole derivatives that have been previously reported by various articles, including anti-inflammatory, analgesic, antipsychotic, antimicrobial, antidiabetic, antiviral, immunomodulatory and anticancer activity. Isoxazole ring (blue color) is shown with numbers (red color) that mark possible substitutions in position 3, 4 or 5 with radicals or ligands (black color). Created with BioRender.com.

leflunomide,[p49] the neuroleptic drug risperidone, the antiepileptic drug zonisamide[p50] and bacteriostatic sulfamethoxazole,[p51] are examples of a successful implementation of isoxazole derivates into the therapeutic landscape. Furthermore, a reduced form of isoxazole, isoxazoline, is contained in the antituberculosis drug cycloserine.[p51] Notwithstanding, 33 patents have been reported in the literature (2016–2018) for treatment of different types of diseases, especially cancer.[p37] Indeed, some of the latest isoxazole-derived compounds have shown encouraging cancer selectivity, low toxicity to peripheral blood lymphocytes and normal tissues coupled to capability to overcome resistance of cancer cells to conventional treatments. This review intends to summarize the knowledge generated so far on isoxazole-derived compounds exerting their activity toward hematological malignancies and to emphasize putative targets, mechanisms of action and selectivity wherever known.

Isoxazole derivatives and their effects against hematological malignancies

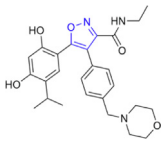
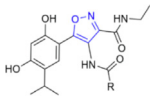
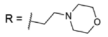
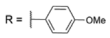
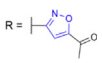
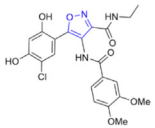
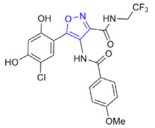
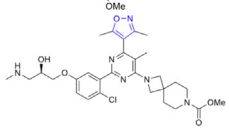
To the best of our knowledge, the present review is the first one to focus on the activity of isoxazole derivatives and their effects on hematological malignancies. We reviewed studies published between 2009 and 2023 reporting a total of 119 isoxazole compounds, which are summarized in Table 2. The compounds were divided into five groups according to the substituent binding position, including 3,4,5-trisubstituted isoxazole compounds, fused isoxazole compounds, 3,5-disubstituted isoxazole compounds, 3,5-disubstituted isoxazoline compounds and 4,5-disubstituted isoxazole compounds (Figure 2). The available knowledge on each of the compounds has also been summarized in Table 2. In particular, information on the level of evidence [target screening (no cells)/cell-based/animal study/human patient-derived cells or clinical trial], half-maximal inhibitory concentration IC_{50} values in hematological cell lines, targets and mechanism(s) of action, selectivity and toxicity to healthy cells has been provided whenever known. All compounds showed IC_{50} (growth inhibition) values between 0.56 nM and 184.4 μM. Orally bioavailable derivatives appear to be **3**, **4**, **5**, **9**, **19**, **25**, **28a**, **28b**, **31q**, **32** and also leflunomide (**35**) and quizartinib (**27**), which are already used in clinical practice. The descriptions of the mechanism(s) of action and targets ascribed to these compounds with activity against hematological malignancies are summarized in Figure 3. The most common mechanisms of action identified were apoptosis and cell-cycle arrest, which were not associated with any specific group. Low toxicity to healthy cells was also observed in each group of the identified isoxazole derivates constituting a promising feature for their further development.

Induction of apoptosis

The majority of isoxazole compounds induced apoptosis, namely derivatives **1**, **2a-h**, **4**, **5**, **6**, **7**, **10**, **15**, **16b**, **18**, **19**, **20a**, **20b**, **21a**, **21b**, **22a**, **22b**, **23a**, **23b**, **24a**, **28a**, **29s**, **31q**, **32** and **33**. Apoptosis is a programmed cell death that is an important anticancer target consisting of two main pathways: the extrinsic and the intrinsic, depending on the stimuli. The induction of the

TABLE 2

Overview of isoxazole derivatives with activity against hematological malignancies.

Compound number and name	Chemical structure	Level of evidence	Cell line/malignancy	IC_{50} (cell growth)	Mechanism of action	Target information	Toxicity	Selectivity	Year/Refs
3,4,5-TRISUBSTITUTED ISOXAZOLE COMPOUNDS									
1 Luminespib; **NVP AUY-922**; 5-(2,4-dihydroxy-5-propan-2-ylphenyl)-*N*-ethyl-4-[4-(morpholin-4-ylmethyl)phenyl]-1,2-oxazole-3-carboxamide		Cell based; human patient-derived cells; clinical trial	HL-60, NB4, CLL primary cells, AML primary cells, RRMM, NHL	LD_{50} 48 h: HL-60, NB4: 0.009 ± 0.0003 μM ref.: Cytarabine 0.42 ± 0.28 μM CLL primary cells: 0.18 ± 0.20 μM ref.: Fludarabine 1.16 ± 1.74 μM AML primary cells: 0.12 ± 0.28 μM ref.: Cytarabine 6.98 ± 7.24 μM	HSP 90 inhibition, apoptosis, modulating NF-κB, PI3K pathway via HSP90 inhibition, synergism with cytarabine and Fludarabine	HSP90 (molecular chaperone involved in signaling pathways for cell proliferation, survival, and cellular adaptation)	Reverse ocular toxicity, fatigue, diarrhea	Activity also on other cancer cell lines	2015/[p128] 2013/[p129] 2012/[p130] 2015/[p131]
General structure for 2a-f									
2a 5-(2,4-Dihydroxy-5-isopropylphenyl)-4-(3-morpholin-4-ylpropionylamino)isoxazole-3-carboxylic acid ethylamide		Cell based; animal study	MV4-11, K562	72 h: MV4-11: **2a** 0.40 ± 0.07 μM **22 and 50% complete response *in vivo*** 72 h/96 h: K562: **2a** 71.57 ± 4.89 nM **2b** 18.01 ± 0.69 nM **2c** 44.25 ± 10.90 nM **2d** 70.12 ± 5.80 nM **2e** 35.21 ± 6.20 nM **2f** 45.43 ± 13.10 nM **2g** 779.40 ± 151.00 nM **2h** 3.20 ± 1.10 μM	HSP90 inhibition, apoptosis	HSP90 (molecular chaperone involved in signalling pathways for cell proliferation, survival, and cellular adaptation)	**2a** well tolerated *in vivo*	Activity also on other tested cancer cell lines	2014/[p146] 2020/[p147] 2011/[p115]
2b 5-(2,4-Dihydroxy-5-isopropylphenyl)-4-(4-methoxybenzoylamino)isoxazole-3-carboxylic acid ethylamide									
2c 5-(2,4-Dihydroxy-5-isopropylphenyl)-4-(2,2-dimethylpropionylamino)isoxazole-3-carboxylic acid ethylamide									
2d 4-Acetylamino-5-(2,4-dihydroxy-5-isopropylphenyl)isoxazole-3-carboxylic acid ethylamide									
2e 5-(2,4-Dihydroxy-5-isopropylphenyl)-4-[(3-methylthiophene-2-carbonyl)amino] isoxazole-3-carboxylic acid ethylamide									
2f 4-(5-Acetylisoxazole-3-carbonyl)amino-5-(2,4-dihydroxy-5-isopropylphenyl) isoxazole-3- carboxylic acid ethylamide									
2g 5-(5-Chloro-2,4-dihydroxyphenyl)-4-(3,4-dimethoxybenzoylamino)isoxazole-3-carboxylic acid ethylamide									
2h 4-(4-Methoxybenzamido)-5-(5-chloro-2,4-dihydroxyphenyl)-*N*-(2,2,2-trifluoroethyl) isoxazole-3-carboxamide									
3 EZM 2302; (*R*)-2-[2-[2-Chloro-5-(2-hydroxy-3-methylaminopropoxy)-phenyl]-6-(3,5-dimethylisoxazol-4-yl)-5-methylpyrimidin-4-yl]-2,7-diaza-spiro[3.5]nonane-7-carboxylic acid methyl ester		Target screening (no cells); cell based; animal study	36 hematological cell lines, RPMI-8226 (*in vivo*)	14d: 0.015 to >10 μM 9 cell lines: <0.1 μM **tumor regression 45–63% *in vivo***	CARM1 inhibition	CARM1 – (methylationtranscription factor RUNX1, resulting in blocking myeloid differentiation in AML)	No signs of toxicity *in vivo*	Potential selectivity through 12 adherent cancer cell lines (breast, colon, prostate)	2017/[p82]

(continued on next page)

TABLE 2 (*CONTINUED*)

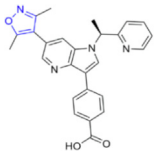
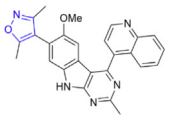
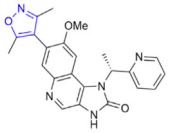
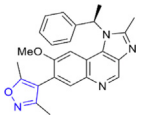
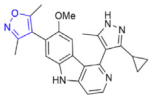
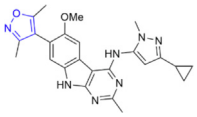
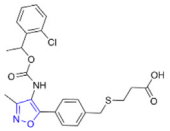
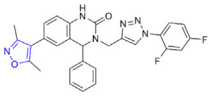
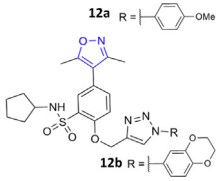
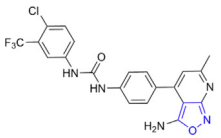
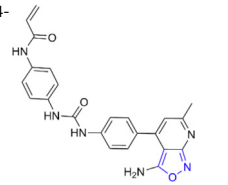
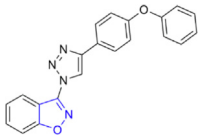
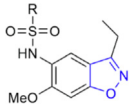
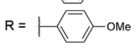
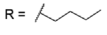
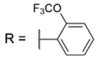
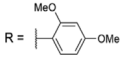
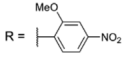
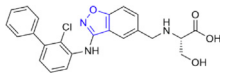
Compound number and name	Chemical structure	Level of evidence	Cell line/malignancy	IC_{50} (cell growth)	Mechanism of action	Target information	Toxicity	Selectivity	Year/Refs
4 PLX51107; (*S*)-4-(6-(3,5-dimethylisoxazol-4-yl)-1-(1-(pyridin-2-yl)ethyl)-1*H*-pyrrolo[3,2-*b*] pyridin-3-yl)benzoic acid		Cell based; human patient-derived cells; animal study; clinical trial	69 hematological cell lines, Eμ-Myc/TCL1 (*in vivo*), RR AML and MDS	0.02 – > 20 μM reduction of spleen size and leukemia cells (*in vivo*)	Selective BET inhibition, cell cycle arrest, apoptosis	BET subfamily of proteins involved in regulation of cancer genes (MYC, BCL6)	No toxicity –healthy T cells; neutropenia, pneumonia (clinical trial)	ND	2018/[p148] 2023/[p133]
5 CD161; 4-(6-Methoxy-2-methyl-4-(quinolin-4-yl) -9*H*-pyrimido[4,5-b]indol-7-yl)-3,5-dimethylisoxazole		Target screening (no cells); cell based; animal study	MV4-11, MOLM-13	96h: MV4-11: 26 ± 5 nM MOLM-13: 53 ± 11 nM	Selective BET inhibition, induction of cleavage PARP, upregulation of p21	BET subfamily of proteins (same as above)	No signs of toxicity *in vivo*	Activity also on other tested cancer cells MDA-MB-231 *in vivo*	2017/[p135]
6 GSK1210151A; **I-BET151**; (*R*)-7-(3,5-dimethylisoxazol-4-yl)-8-methoxy-1-(1-(pyridin-2-yl)ethyl)-1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-one		Cell based; animal study; human patient derived cells	MV4-11, RS4-11, MOLM13, NOMO1, HEL, K562, MEG01, HL-60, MLL-fusion leukemia (*in vivo*)	15 nM – >100 μM	Selective BET inhibition, apoptosis, cell cycle arrest, inhibition of BCL2, C-MYC, CDK6	BET subfamily of proteins (same as above)	ND	ND	2011/[p132]
7 (*R*)-4-(8-Methoxy-2-methyl-1-(1-phenylethyl)-1*H*-imidazo[4,5-c]quinolin-7-yl)-3,5-dimethylisoxazole		Target screening (no cells); cell based; animal study	MV4-11, MOLM-13, Jurkat, MM.1S, MV4-11 (*in vivo*)	**0.19 ± 0.12 μM** 0.43 ± 0.13 μM 2.08 ± 0.08 μM 0.53 ± 0.51 μM **55.11% and 70.40% tumor regression *in vivo***	Selective BET inhibition, cell cycle arrest, promoting apoptosis, inhibition of c-Myc, CDK6	BET subfamily of proteins (same as above)	ND	ND	2024/[p149]
8 RX-37; 4-(1-(3-Cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-8-methoxy-5*H*-pyrido[4,3-*b*]indol-7-yl)-3,5-dimethylisoxazole		Target screening (no cells); cell based	MV4-11, MOLM-13	20 nM 66 nM	Selective BET inhibition	BET subfamily of proteins (same as above)	ND	Specificity over K562 cell line	2015/[p134]
9 CF53; N-(3-Cyclopropyl-1-methyl-1*H*-pyrazol-5-yl)-7-(3,5-dimethylisoxazol-4-yl)-6-methoxy-2-methyl-9*H*-pyrimido[4,5-b]indol-4-amine		Target screening (no cells); cell based; animal study	MOLM-13, RS4-11 (*in vivo*)	96 h: 10.3 nM **49.3 - 72.3% tumor regression *in vivo***	Selective BET inhibition	BET subfamily of proteins (same as above)	No signs of toxicity *in vivo*	Activity also on other tested cancer cells	2018/[p137]
10 Ki16425;3-(4-[4-([1-(2-chlorophenyl) ethoxy]carbonylamino)-3-methyl-5-isoxazolyl]benzylsulfanyl)propanoic acid		Cell based; animal study	HuT-78, Jurkat J6, DL (*in vivo*)	–	LPA antagonist, apoptosis, immunoactivation…	LPA - small phospholipid acting as an extracellular lipid mediator	ND	ND	2022/[p150]
11 [3-((1-(2,4-difluorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-6-(3,5-dimethylisoxazol-4-yl)-4-phenyl-3,4-dihydroquinazolin-2(1*H*)-one		Target screening (no cells); cell based	HL-60, MV4-11, Raji	0.120 ± 0.06 μM 0.095 ± 0.03 μM 1.65 ± 0.14 μM	BET (BRD4) inhibition, inhibition of c-Myc	BET subfamily of proteins involved in regulation of cancer genes (MYC, BCL6)	ND	ND	2018/[p151]

TABLE 2 (*CONTINUED*)

Compound number and name	Chemical structure	Level of evidence	Cell line/malignancy	IC_{50} (cell growth)	Mechanism of action	Target information	Toxicity	Selectivity	Year/Refs
12a C26 *N*-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl)-2-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl) methoxy)benzenesulfonamide **12b C29** 2-((1-(benzo[*d*][1,3]dioxol-5-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)-*N*-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl) benzenesulfonamide		Target screening (no cells); cell based	HL-60, MV4-11	HL-60: **12a** >10 μM **12b** 2.85 ± 0.21 μM MV4-11: **12a** 1.47 ± 0.11 μM **12b** 0.86 ± 0.07 μM	BET (BRD4) inhibition	BET subfamily of proteins (same as above)	ND	ND	2021/[p152]
FUSED ISOXAZOLE COMPOUNDS									
13 Danatinib;1-(4-(3-Amino-6-methylisoxazolo[3,4-*b*]pyridin-4-yl) phenyl)-3-(4-chloro-3-(trifluoromethyl) phenyl)urea		Target screening (no cells); cell based; animal study	Screening on various non-hematological and hematological cell lines, MV4–11 *(in vivo)*	MV4-11 0.89 ± 0.03 nM MOLM-13 4.16 ± 0.13 nM ref. Linifanib: MV4-11 3.4 ± 1 nM MOLM-13 19 ± 3 nM	FLT3 inhibtion; inhibition of downstream signaling pathways, apoptosis, decrease in ROS	FLT3 - tyrosine kinase that plays pivotal role in the survival, proliferation, differentiation of hematopoietic cells	Acceptable safety profile *in vivo*	Potential selectivity through other tested cell lines and kinases	2023/[p70]
14 *N*-(4-(3-(4-(3-Amino-6-methylisoxazolo[3,4-*b*]pyridin-4-yl)phenyl)ureido)phenyl) acrylamide		Target screening; cell-based	MOLM-13, MV4-11	507 nM 325 nM	FLT inhibtion, cell cycle arrest, apoptosis, inhibtion od downstream mediators STAT5, Akt and ERK	FLT3 - tyrosine kinase (same as above)	ND	High selectivity through other tested cell line HL-60, A549, HepG2, K562, HUVEC (IC_{50} > 10 μM)	2022/[p138]
15 PTB; 3-(4-(4-phenoxyphenyl) -1*H*-1,2,3-triazol-1-yl)benzo[*d*]isoxazole		Cell-based	MV4-11, MOLM-13, MOLM-14	96 h: 1–2.5 μM	Apoptosis, cell cycle arrest, increase in acetylation Histone3 and tubulin	HDACs – enzymes deacetylating lysine residues present on histones, regulating gene transcription	No toxicity to normal bone marrow cells C57BL/6	ND	2015/[p104]
General structure for 16a-f									
16a *N*-(3-Ethyl-6-methoxybenzo[*d*]isoxazol-5-yl)-2-methoxybenzenesulfonamide		Cell-based	MV4-11	(EC_{50}) 120 h: **16a 0.78 μM** **16b 0.87 μM** **16c** 2.5 μM **16d** 5.53 μM **16e** 2.5 μM **16f** 3.79 μM	Selective BET inhibition **16b** inhibition of CDK6, c-Myc, cell cycle arrest, apoptosis	CDKs – serine-threonine kinases regulating cell cycle and cell proliferation	ND	ND	2022/[p153]
16b *N*-(3-Ethyl-6-methoxybenzo[*d*]isoxazol-5-yl)-4-methoxybenzenesulfonamide									
16c *N*-(3-Ethyl-6-methoxybenzo[*d*]isoxazol-5-yl)butane-1-sulfonamide									
16d *N*-(3-Ethyl-6-methoxybenzo[*d*]isoxazol-5-yl)-2-(trifluoromethoxy) benzenesulfonamide									
16e *N*-(3-Ethyl-6-methoxybenzo[*d*]isoxazol-5-yl)-2,4- dimethoxybenzenesulfonamide									
16f *N*-(3-Ethyl-6-methoxybenzo[*d*]isoxazol-5-yl)-2-methoxy-4-nitrobenzenesulfonamide									
17 P20; ((3-((2-chloro-[1,1'-biphenyl]-3-yl)amino) benzo[*d*]isoxazol-5-yl)methyl)-L-serine		Target screening (no cells)	Not applicable	IC_{50} (target): 26.8 nM	PD-1/PD-L1 inhibition	PD-1 – inhibition of immune responses, modulation of activity of T-cells; PD-L1 – co-inhibitory factor [p86]	ND	ND	2021/[p154]

(continued on next page)

POST-SCREEN (GREY)

TABLE 2 (*CONTINUED*)

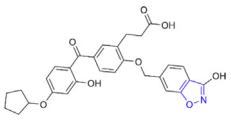
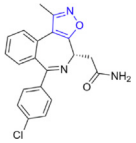
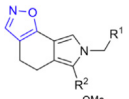
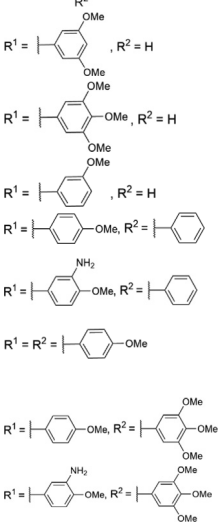
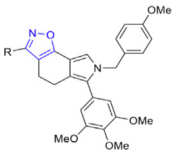
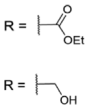
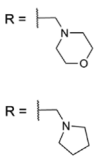
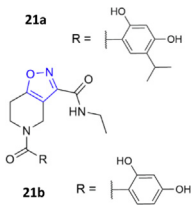
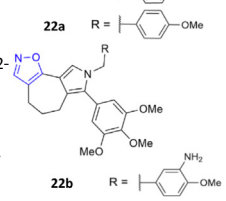
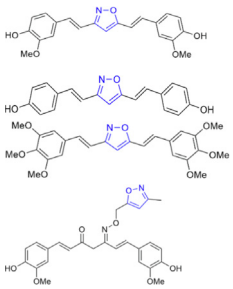
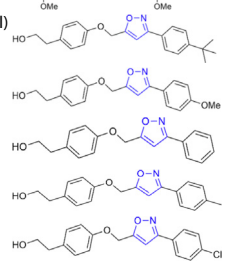
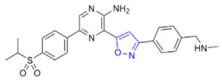
Compound number and name	Chemical structure	Level of evidence	Cell line/malignancy	IC_{50} (cell growth)	Mechanism of action	Target information	Toxicity	Selectivity	Year/Refs
18 T-5524;3-[5-(4-cyclopentyloxy-2-hydroxybenzoyl)-2-[(3-oxo-1,2-benzoxazol-6-yl)methoxy]phenyl] propanoic acid		Cell-based; human patient derived cells	ARP-1, H929, U266 and RPMI8226, MM primary cells	–	Selective AP-1 inhibitor; inhibition of IRF4/MYC leading to cell cycle arrest, apoptosis, synergism with bortezomib	AP-1 modulates the transcription of multiple cytokines and growth factors and is associated with proliferation, survival, differentiation and transformation of cells	Low toxicity to healthy PBMC cells	ND	2023/[p94]
19 Pelabresib; **CPI0610**; 2-((4*S*)-6-(4-chlorophenyl)-1-methyl-4*H*-benzo[*c*]isoxazolo[4,5-*e*]azepin-4-yl) acetamide		Target screening (no cells); Cell based; animal study, human patient derived cells; clinical trial	INA-6, RPMI, LR5, H929, MM1S, MM1R, U266, Dox40, ANBL6-WT, ANBL6-VR, MV4-11 *in vivo*, MM primary cells, RRMM, myelofybrosis	72 h cell lines: 0.2–0.9 μM **41–80% tumor growth inhibition *in vivo***	Selective BET inhibition, downregulation NF-κB signaling, cell cycle arrest, caspase-dependent apoptosis	BET subfamily of proteins (same as above)	Common set of toxicities	ND	2016/[p155] 2016/[p156] 2022/[p157] 2022/[p127]
General structure for 20a-h									
20a 7-(3,5-dimethoxybenzyl)-5,7-dihydro-4H-isoxazolo[5,4-*e*]isoindole **20b SIX2G** 7-(3,4,5-trimethoxybenzyl)-5,7-dihydro-4*H*-isoxazolo[5,4-*e*]isoindole **20c** 7-(3-Methoxybenzyl)-5,7-dihydro-4*H*-[1,2] oxazolo[5,4-*e*]isoindole **20d** 7-(4-Methoxybenzyl)-6-phenyl-5,7-dihydro-4*H*-[1,2]-oxazolo[5,4-*e*]isoindole **20e** 2-Methoxy-5-[(6-phenyl-4,5-dihydro-7*H*-[1,2]oxazolo-[5,4-*e*]isoindol-7-yl)methyl] aniline **20f** 7-(4-Methoxybenzyl)-6-(4-methoxyphenyl)-5,7-dihydro-4*H*-[1,2] oxazolo[5,4-*e*]isoindole **20g** 7-(4-Methoxybenzyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-[1,2] oxazolo[5,4-e]isoindole **20h** 2-methoxy-5-{[6-(3,4,5-trimethoxyphenyl)-4,5-dihydro-7*H*-[1,2] oxazolo[5,4-*e*]isoindol-7-yl]methyl}aniline		Cell based; human patient derived cells	**20a-l**: VL51, MINO, HBL1, SU-DHL-10, (lymphoma cell lines) **20b:** AMO-1, AMO-BZB, JJN-3, NCI-H929, MM.1S (MM cell lines)	72 h: **20a 0.02–0.04 μM** **20b 0.04–0.12 μM** **20c** 0.7–1 μM **20d** 0.23–0.28 μM **20e** 0.23–0.27 μM **20f** 1.1–1.7 μM **20g** 0.27–0.5 μM **20h** 0.07–0.1 μM **20i** 1.4–2.0 μM **20j** 1.4–1.8 μM **20k** 0.2–0.5 μM **20l** 0.15–0.7 μM	**20a, 20b, 20d, 20e, 20f, 20h, 20k** inhibited tubulin polymerization **20a, 20b** apoptosis, cell cycle arrest	Microtubules – cytoskeletal protein filaments involved in the cellular architecture maintenance, mitosis, cell signaling, motility and intracellular trafficking of organelles and macromolecules. Each microtubule is composed of two globular proteins, α- and β-tubulin	**20a, 20b, 20k, 20l** showed low toxicity to healthy PBMC cells (**20a, 20b** showed 15% max. increase of apoptosis in higher dose 0,5 μM)	Effective also on other tested cancer cell lines	2022/[p124] 2022/[p158]
General structure for 20i-l									
20i Ethyl 7-(4-methoxybenzyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4*H*-[1,2] oxazolo[5,4-*e*]isoindole-3-carboxylate **20j** [7-(4-methoxybenzyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4*H*-[1,2] oxazolo[5,4-e]isoindol-3-yl]methanol									

TABLE 2 (*CONTINUED*)

Compound number and name	Chemical structure	Level of evidence	Cell line/malignancy	IC_{50} (cell growth)	Mechanism of action	Target information	Toxicity	Selectivity	Year/Refs
20k 7-(4-Methoxybenzyl)-3-(morpholinomethyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole **20l** 7-(4-methoxybenzyl)-3-(pyrrolidin-1-ylmethyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole									
21a 5-(2,4-dihydroxy-5-isopropylbenzoyl)-*N*-ethyl-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine-3-carboxamide **21b** 5-(2,4-dihydroxybenzoyl)-*N*-ethyl-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine-3-carboxamid		Cell-based	K562	72 h/96 h: **21a** 627.30 ± 49.00 nM **21b** 68.30 ± 5.20 μM	Apoptosis		ND	Activity also on other tested cancer cell lines	2020/[p147]
22a 8-(4-Methoxybenzyl)-7-(3,4,5-trimethoxyphenyl)-4,5,6,8-tetrahydropyrrolo[3′,4′:3,4]cyclohepta [1,2-*d*] [1,2]oxazole **22b** 2-Methoxy-5-((7-(3,4,5-trimethoxyphenyl)-5,6-dihydropyrrolo [3′,4′:3,4]cyclohepta [1,2-*d*]isoxazol-8(4*H*)-yl)methyl)aniline		Cell-based; human patient derived cells	VL51, MINO, HBL1, SU-DHL-10, (lymphoma cell lines)	72 h: **22a** 0.4-–1.8 μM **22b** 0.1-–0.5 μM	Cell cycle arrest, apoptosis		IC_{50} > 10 μM on healthy PBL	Activity also on other tested cancer cell lines	2023/[p159]
3,5-DISUBSTITUTED ISOXAZOLE COMPOUNDS									
23a 4-[(*E*)-2-[3-[(*E*)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-1,2-oxazol-5-yl] ethenyl]-2-methoxyphenol **23b** 4-[(*E*)-2-[3-[(*E*)-2-(4-hydroxyphenyl) ethenyl]-1,2-oxazol-5-yl]ethenyl]phenol **23c** 3,5-bis[2-(3,4,5-trimethoxyphenyl) ethenyl]-1,2-oxazole **23d** (1*E*,5*Z*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-5-(((3-methylisoxazol-5-yl)methoxy)imino)hepta-1,6-dien-3-one		Cell-based	K562	72 h: **23a 0.5 ± 0.1 μM** **23b 0.5 ± 0.1 μM** **23c** 15.9 ± 0.9 μM **23d** 12.1 ± 0.5 μM ref.: Curcumin 17 ± 1 μM	**23a, 23b** cell cycle arrest, apoptosis **23b** overcome resistance to imatinib		ND	**23b** potent selectivity through other 6 tested cell lines	2023/[p160]
24a 2-(4-((3-(4-(*tert*-butyl)phenyl)isoxazol-5-yl) methoxy)phenyl)ethanol **24b** 2-(4-((3-(4-methoxyphenyl)isoxazol-5-yl) methoxy)phenyl)ethanol **24c** 2-(4-((3-phenylisoxazol-5-yl)methoxy) phenyl)ethanol **24d** 2-(4-((3-(p-tolyl)isoxazol-5-yl)methoxy) phenyl)ethanol **24e** 2-(4-((3-(4-chlorophenyl)isoxazol-5-yl) methoxy)phenyl)ethanol		Cell-based; human patient derived cells	K562, CML primary cells	72 h: K562: **24a 45 ± 2.8 μM** **24b** 55 ± 6.1 μM **24c** 81 ± 3 μM **24d** 58 ± 9.6 μM **24e** 54.5 ± 7.5 μM CML: **24a** 70 μM to 184.4 μM	**24a** apoptosis, ROS production, overcome resistance to imatinib		Low hemotoxicity 25–400 μg/ml (except **24a** 40% hemolysis at 400 μg/ml)	Activity also on other tested cancer cell line (U87)	2022/[p65] 2021/[p161]
25 Berzosertib; VX-970; M6620; VE-822;3-[3-[4-(methylaminomethyl)phenyl]-1,2-oxazol-5-yl]-5-(4-propan-2-ylsulfonylphenyl) pyrazin-2-amine		Cell-based; animal study	MyLa2000, SeAx, — Mac2a, MV4-11 (*in vivo*)	—	Selective ATR inhibition, sensitization lymphoma cells to UVA radiation	ATR — serine- threonine kinase, which phosphorylates CHK1 in response to stalled replication forks	No bone marrow dysmorphology	Activity also on solid tumors	2018/[p118] 2016/[p119]

(continued on next page)

POST-SCREEN (GREY)

TABLE 2 (*CONTINUED*)

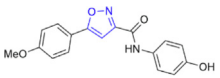
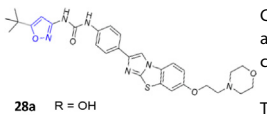
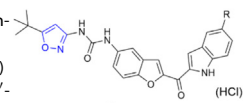
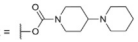
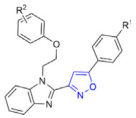
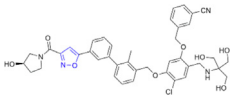
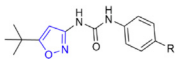
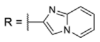
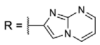
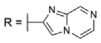
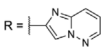
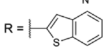
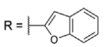
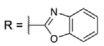
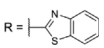
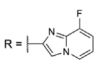
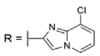
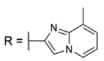
Compound number and name	Chemical structure	Level of evidence	Cell line/malignancy	IC_{50} (cell growth)	Mechanism of action	Target information	Toxicity	Selectivity	Year/Refs
26 N-(4-Hydroxyphenyl)-5-(4-methoxyphenyl) isoxazole-3-carboxamide		Cell-based	60 various cell lines	(IC_{50} not determined) 48 h growth inhibition at 10 μM: CCRF: 68.98% HL-60: 73.56% K-562: 70.79% MOLT-4: 80.79% RPMI-8226: 17.92%	ND		ND	Activity also on other tested cancer cell lines	2021/[p162]
27 Quizartinib; **AC220**; 1-(5-*tert*-butyl-1,2-oxazol-3-yl)-3-[4-[6-(2-morpholin-4-ylethoxy)imidazo[2,1-*b*][1,3] benzothiazol-2-yl]phenyl]urea		Cell-based; animal study; clinical trial	MV4-11, AML	72 h: MV4-11: 0.56 ± 0.3 nM **complete tumor regression *in vivo***	Selective FLT3 inhibition	FLT3 - tyrosine kinase that plays pivotal role in the survival, proliferation, differentiation of hematopoietic cells	Tolerated by patients	Selectivity tested on A375 > 10 000 nM	2009/[p163] 2009/[p164] 2019/[p165]
28a Marbotinib; 1-(5-(*tert*-butyl)isoxazol-3-yl)-3-(2-(5-hydroxy-1*H*-indole-2-carbonyl)benzofuran-5-yl)urea **28b** 2-(5-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido) benzofuran-2-carbonyl)-1*H*-indol-5-yl [1,4′-bipiperidine]-1′-carboxylate hydrochloride	**28a** R = OH  (HCl) **28b** R = 	Target screening (no cells); cell-based; animal study; human patient derived cells	19–27 hematological cell lines	96 h: **28a** < 0.001––3.424 μM **28b** 0.0001––5.416 μM	Selective FLT3 inhibition **28a** apoptosis, modulation of FLT3 downstream mediators	FLT3 - tyrosine kinase (same as above)	No signs of toxicity *in vivo*	ND	2020/[p166] 2022/[p74]
General structure for 29a-s									
29a 5-(4-methoxyphenyl)-3-(1-(2-phenoxyethyl)-1*H*-benzo[*d*]imidazol-2-yl) isoxazole	R^1 = OMe, R^2 = H	**29a-s**: cell based **29s**: animal study	RPMI-8226 (**29s** RPMI-8226 and NCI-H929)	48 h: **29a** 25.28 ± 6.60 μM **29b** 22.96 ± 3.89 μM **29c** 19.83 ± 2.72 μM **29d** 12.56 ± 0.85 μM **29e** 28.93 ± 2.79 μM **29f** 13.18 ± 0.57 μM **29g** 61.97 ± 34.52 μM **29h** 7.55 ± 0.85 μM **29i** 5.42 ± 1.41 μM **29j** 14.64 ± 2.45 μM **29k** 17.80 ± 1.56 μM **29l** 10.05 ± 1.93 μM **29m** 16.32 ± 0.92 μM **29n** 19.21 ± 5.54 μM **29o** 27.07 ± 5.28 μM **29p** 20.97 ± 6.82 μM **29q** 7.50 ± 0.37 μM **29r** 12.91 ± 1.43 μM **29s** 6.16 ± 0.59 μM	**29s** inhibition c-myc, apoptosis, modulation NF-κB signalling pathway, overcome resistance to bortezomib	c-Myc – proto-oncogene with role in cell cycle regulation, metabolism, apoptosis, differentiation, cell adhesion, tumorigenesisparticipates in regulating hematopoietic homeostasis [p89]	**29s** no weight loss *in vivo*	ND	2023/[p92] 2023/[p167]
29b 3-(1-(2-(3,5-dimethylphenoxy)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-5-(4-methoxyphenyl)isoxazole	R^1 = OMe, R^2 = 3,5-di-Me								
29c 3-(1-(2-(4-ethylphenoxy)ethyl)-1*H*-benzo [*d*]imidazol-2-yl)-5-(4-methoxyphenyl) isoxazole	R^1 = OMe, R^2 = 4-Et								
29d 3-(1-(2-(2,4-dimethylphenoxy)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-5-(4-methoxyphenyl)isoxazole	R^1 = OMe, R^2 = 2,4-di-Me								
29e 5-(4-methoxyphenyl)-3-(1-(2-(o-tolyloxy) ethyl)-1*H*-benzo[*d*]imidazol-2-yl)isoxazole	R^1 = OMe, R^2 = 2-Me								
29f 5-(4-methoxyphenyl)-3-(1-(2-(4-nitrophenoxy)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)isoxazole	R^1 = OMe, R^2 = 4-NO_2								
29g 3-(1-(2-(2-ethoxyphenoxy)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-5-(4-methoxyphenyl)isoxazole	R^1 = OMe, R^2 = 2-OEt								
29h 3-(1-(2-(4-methoxyphenoxy)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-5-(4-methoxyphenyl)isoxazole	R^1 = OMe, R^2 = 4-OMe								
29i methyl-4-(2-(2-(5-(4-methoxyphenyl) isoxazol-3-yl)-1*H*-benzo[*d*]imidazol-1-yl) ethoxy)benzoate	R^1 = OMe, R^2 = 4-COOMe								
29j 3-(1-(2-(4-fluorophenoxy)ethyl)-1*H*-benzo [*d*]imidazol-2-yl)-5-(4-methoxyphenyl) isoxazole	R^1 = OMe, R^2 = 4-F								
29k 5-(4-fluorophenyl)-3-(1-(2-phenoxyethyl)-1*H*-benzo[*d*]imidazol-2-yl)isoxazole	R^1 = 4-F, R^2 = H								

TABLE 2 (*CONTINUED*)

Compound number and name	Chemical structure	Level of evidence	Cell line/malignancy	IC_{50} (cell growth)	Mechanism of action	Target information	Toxicity	Selectivity	Year/Refs
29l 3-(1-(2-(4-ethylphenoxy)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-5-(4-fluorophenyl) isoxazole	R^1 = 4-F, R^2 = 4-Et								
29m 5-(4-fluorophenyl)-3-(1-(2-(o-tolyloxy) ethyl)-1*H*-benzo[*d*]imidazol-2-yl)isoxazole	R^1 = 4-F, R^2 = 2-Me								
29n 5-(4-fluorophenyl)-3-(1-(2-(4-nitrophenoxy)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)isoxazole	R^1 = 4-F, R^2 = 4-NO_2								
29o 3-(1-(2-(2-ethoxyphenoxy)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-5-(4-fluorophenyl) isoxazole	R^1 = 4-F, R^2 = 2-OEt								
29p 5-(4-fluorophenyl)-3-(1-(2-(4-methoxyphenoxy)ethyl)-1*H*-benzo[*d*] imidazol-2-yl)isoxazole	R^1 = 4-F, R^2 = 4-OMe								
29q Methyl-4-(2-(2-(5-(4-fluorophenyl) isoxazol-3-yl)-1*H*-benzo[*d*]imidazol-1-yl) ethoxy)benzoate	R^1 = 4-F, R^2 = 4-COOMe								
29r 3-(1-(2-(4-fluorophenoxy)ethyl)-1*H*-benzo[*d*]imidazol-2-yl)-5-(4-fluorophenyl) isoxazole	R^1 = R^2 = 4-F								
29s (**EP12 original name**) 3-(1-(2-(3,5-dimethylphenoxy)ethyl)-1*H*-benzo[*d*] imidazol-2-yl)-5-(4-fluorophenyl)isoxazole	R^1 = 4-F, R^2 = 3,5-di-Me								
30 (*R*)-3-((4-chloro-2-(((1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)amino) methyl)-5-((3′-(3-(3-hydroxypyrrolidine-1-carbonyl)isoxazol-5-yl)-2-methyl-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)methyl) benzonitrile		Target screening (no cells)	Not applicable	IC_{50} (target): 23 ± 2.1 nM	PD-1/PD-L1 inhibition	PD-1 – responsible for inhibition of immune responses, modulation of activity of T-cells; PD-L1 - co-inhibitory factor	ND	ND	2022/[p168]
General structure for 31a-ac									
31a *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*′-(4-(imidazo[1,2-*a*]pyridin-2-yl)phenyl)urea	R = 	Cell-based; animal study	MV4-11	72 h: **31a** 0.68 nM **31b** 3.09 nM **31c** 44.83 nM **31d** 59.05 nM **31e** > 900 nM **31f** 195.14 nM **31g** 82.57 nM **31h** 172.31 nM **31i** 12.21 nM **31j** 352.76 nM **31k** 248.42 nM **31l** > 900 nM **31m** 5.91 nM **31n** 9.59 nM **31o** 0.79 nM **31p** 3.46 nM **31q 2.84 nM complete tumor regression *in vivo*** **31r** 11.59 nM **31s** 29.39 nM **31t** 20.94 nM **31u** 24.75 nM **31v** 44.48 nM	FLT3 inhibition, **31q** apoptosis	FLT3 – tyrosine kinase that plays pivotal role in the survival, proliferation, differentiation of hematopoietic cells	**31q** no signs of toxicity *in vivo*	**31q** selectivity through other tested 11 cancer cell lines (leukemia, breast, lung, pancreas)	2015/[139]
31b *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*′-(4-(imidazo[1,2-*a*]pyrimidin-2-yl)phenyl)urea	R = 								
31c *N*-(5-(*tert*-butyl)Isoxazol-3-yl)-*N*′-(4-(imidazo[1,2-*a*]pyrazin-2-yl)phenyl)urea	R = 								
31d *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*′-(4-(imidazo[1,2-*b*]pyridazin-2-yl)phenyl)urea	R = 								
31e *N*-(4-(benzo[*b*]thiophen-2-yl)phenyl)-*N*′-(5-(*tert*-butyl) isoxazol-3-yl)urea	R = 								
31f *N*-(4-(benzofuran-2-yl)phenyl)-*N*′-(5-(*tert*-butyl)isoxazol-3-yl)urea	R = 								
31g *N*-(4-(benzo[*d*]oxazol-2-yl)phenyl)-*N*′-(5-(*tert*-butyl)isoxazol-3-yl)urea	R = 								
31h *N*-(4-(benzo[*d*]thiazol-2-yl)phenyl)-*N*′-(5-(*tert*-butyl) isoxazol-3-yl)urea	R = 								
31i *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*′-(4-(8-fluoroimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea	R = 								
31j *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*′-(4-(8-chloroimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea	R = 								
31k *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*′-(4-(8-methylimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea	R = 								

(continued on next page)

TABLE 2 (*CONTINUED*)

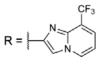
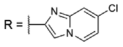
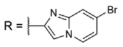
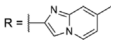
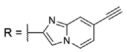
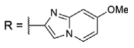
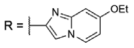
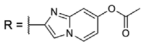
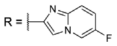
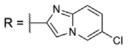
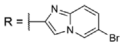
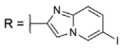
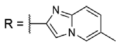
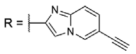
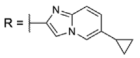
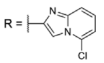
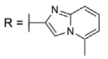
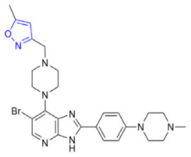
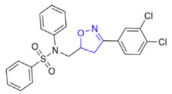
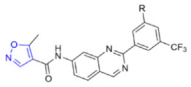
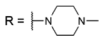
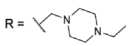
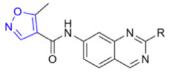
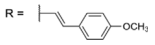
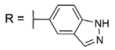
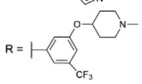
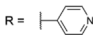
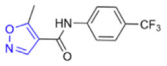
Compound number and name	Chemical structure	Level of evidence	Cell line/malignancy	IC_{50} (cell growth)	Mechanism of action	Target information	Toxicity	Selectivity	Year/Refs
31l *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(8-(trifluoromethyl)imidazo[1,2-*a*]pyridin-2-yl) phenyl)urea				**31w** 98.55 nM **31x** 4.08 nM **31y** 8.62 nM **31z** 15.83 nM **31ab** 56.08 nM **31ac** 20.24 nM					
31m *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(7-chloroimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea									
31n *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(7-bromoimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea									
31o *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(7-methylimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea									
31p *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(7-ethynylimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea									
31q *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(7-methoxyimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea									
31r *N*-(5-(*tert*-butyl)isoxazol-3-yl)-N'-(4-(7-ethoxyimidazo [1,2-*a*]pyridin-2-yl)phenyl) urea									
31s 2-(4-(3-(5-(*tert*-butyl)isoxazol-3-yl)ureido) phenyl)imidazo[1,2-*a*]pyridin-7-yl acetate									
31t *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(6-fluoroimidazo [1,2-*a*]pyridin-2-yl)phenyl) urea									
31u *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(6-chloroimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea									
31v *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(6-bromoimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea									
31w *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(6-iodoimidazo[1,2-*a*]pyridin-2-yl)phenyl)urea									
31x *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(6-methylimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea									
31y *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(6-ethynylimidazo[1,2-a]pyridin-2-yl)phenyl) urea									
31z *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(6-cyclopropylimidazo[1,2-*a*]pyridin-2-yl) phenyl)urea									
31ab *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(5-chloroimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea									
31ac *N*-(5-(*tert*-butyl)isoxazol-3-yl)-*N*'-(4-(5-methylimidazo[1,2-*a*]pyridin-2-yl)phenyl) urea									
32 CCT137690; 6-bromo-7-[4-[(5-methyl-3-isoxazolyl)methyl]-1-piperazinyl]-2-[4-(4-methyl-1-piperazinyl) phenyl]-3*H*-imidazo[4,5-*b*]pyridine;		Cell based; animal study	MOLM-13, MV4-11	0.023 and 0.062 μM **50% of mice – complete remission *in vivo***	Dual FLT3-Aurorainhibition, cell cycle arrest, apoptosis	Aurora – serine/threoninekinases regulating several stages of mitosis	No signs of toxicity *in vivo*	Activity also on other cancer cell lines	2012/[p169]

TABLE 2 (*CONTINUED*)

Compound number and name	Chemical structure	Level of evidence	Cell line/malignancy	IC_{50} (cell growth)	Mechanism of action	Target information	Toxicity	Selectivity	Year/Refs
3,5-DISUBSTITUTED ISOXAZOLINE COMPOUND									
33 *N*-[3-(3,4-Dichlorophenyl)-isoxazolin-5-ylmethyl]-*N*-phenylbenzenesulfonamide		Cell based	HL-60	48 h: 62 ± 2 μM	Apoptosis		ND	ND	2021/[p170]
4,5-DISUBSTITUTED ISOXAZOLE COMPOUNDS									
General structure for 34a-d									
34a 5-methyl-*N*-(2-(3-morpholino-5-(trifluoromethyl)phenyl)quinazolin-7-yl) isoxazole-4-carboxamide	R = 	Target screening (no cells)		IC_{50}(target): FLT3: **34a 3.98 μM** **34b** 1.58 μM **34c 0.106 μM** **34d** 1.03 μM **34e** 4.7 μM **34f** 0.79 μM **34g** 3.59 μM **34h** > 10 μM ref.: Staurosporine 1.13 nM FLT (ITD) : **34a 3.11 μM** **34b** 3.16 μM **34c 0.301 μM** **34d** 13.9 μM **34e** 5 μM **34f** 0.908 μM **34g** 4.96 μM **34h** > 10 μM Ref.: Staurosporine 0.88 nM	FLT3 inhibition	FLT3 - tyrosine kinase that plays pivotal role in the survival, proliferation, differentiation of hematopoietic cells	ND	**34a**, **34c** excelent selectivity profiles over 36 protein kinases	2020/[p140]
34b 5-methyl-*N*-(2-(3-(4-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)phenyl) quinazolin7-yl)isoxazole-4-carboxamide	R = 								
34c 5-methyl-*N*-(2-(3-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)phenyl)quinazolin-7-yl)isoxazole-4-carboxamide	R = 								
34d *N*-(2-(3-((4-ethylpiperazin-1-yl)methyl)-5-(trifluoromethyl)phenyl)quinazolin-7-yl)-5-methylisoxazole-4-carboxamide	R = 								
General structure for 34e-h									
34e (*E*)-*N*-(2-(4-methoxystyryl)quinazolin-7-yl)-5-methylisoxazole-4-carboxamide	R = 								
34f *N*-(2-(1*H*-indazol-5-yl)quinazolin-7-yl)-5-methylisoxazole-4-carboxamide	R = 								
34g 5-methyl-*N*-(2-(3-((1-methylpiperidin-4-yl)oxy)-5- (trifluoromethyl)phenyl) quinazolin-7-yl)isoxazole-4-carboxamide	R = 								
34h 5-methyl-*N*-(2-(pyridin-4-yl)quinazolin-7-yl)isoxazole-4-carboxamide	R = 								
35 Leflunomide; 5-methyl-*N*-[4-(trifluoromethyl)phenyl]-1,2-oxazole-4-carboxamide (name reaxys)		Cell based; animal study; human patient derived cells; clinical trial	RPMI-822, MM.1S *in vivo*, MM primary cells	RPMI-822: Metabolite Teriflunomide 99.87 μM MM primary cells: Metabolite Teriflunomide 110 μM	Inhibition of PIM kinases, c-Myc; benefit in combination with lenalidomid *in vivo*	PIM – serine/threonine kinases regulating cell proliferation, survival, metabolism, cellular trafficking and signaling[p171]	No dose-limiting toxicities (DLTs) in clinical trial	ND	2019/[p93] 2020/[p172] 2021/[p173]

For each compound, chemical structure, information on the level of evidence [target screening (no cells)/cell-based/animal study/human patient-derived cells or clinical trial], half-maximal inhibitory concentration IC_{50} values in hematological cell lines, targets/mechanism(s) of action, selectivity and toxicity to healthy cells has been summarized whenever known. Target information is provided for better understanding of various mechanisms of action associated with isoxazole derivatives. Abbreviations: AML, acute myelogenous leukemia; ATR, ataxia telangiectasia and rad3-related serine/threonine kinase; BET, bromodomain and extra-terminal proteins; CARM1, coactivator-associated arginine methyltransferase 1; CCRF, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; c-Myc, cellular myelocytomatosis oncogene; Eμ-Myc/TCL1, adoptive transfer model of high grade lymphoma analogous to RT (Richter's transformation); FLT (ITD), the FMS-like tyrosine kinase-3 internal tandem duplication; FLT3, FMS-like receptor tyrosine kinase; HBL1, AIDS-related non-Hodgkin's lymphoma; HDACs, histone deacetylases; HL-60, acute promyelocytic leukemia; HSP90, heat shock protein 90; K562, chronic myelogenous leukemia; LD_{50}, lethal dose 50; LPA, lysophosphatidic acid; CDK6, cyclin-dependent kinase 6; MDS, myelodysplasia; MINO, mantle cell lymphoma; MLL, mixed-lineage leukemia; MM, multiple myeloma; MM.1S, immunoglobulin A lambda myeloma; MOLM-13 and sister cell line of MOLM-14, acute myeloid leukemia AML FAB M5a; MOLT-4, acute lymphoblastic leukemia; MV-4-11, B-myelomonocytic leukemia; NCI-H929, plasmacytoma; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; p21, tumor suppressor protein with inhibitory effect on cell cycle progression; PBL, a peripheral blood lymphocyte; PBMC, a peripheral blood mononuclear cell; PD1/PDL1, programmed cell death 1 receptor/programmed cell death ligand 1; PI3K, phosphatidylinositol 3-kinase; PARP, poly(ADP-ribose) polymerase; PIM kinase, proto-oncogene serine/threonine-protein kinase; ROS, reactive oxygen species; RPMI-8226, plasmacytoma; RR AML, relapsed/refractory acute myelogenous leukemia; RRMM, relapsed/refractory multiple myeloma; RS4-11, acute lymphoblastic leukemia; SU-DHL-10, large cell lymphoma; VL51, splenic lymphoma with circulating villous lymphocytes.

intrinsic pathway was evidenced for compounds **15**, **22a** and **22b** through the cleavage of caspase 9. An important marker of apoptosis, induction of cleavage of poly(ADP-ribose) polymerase (PARP) was reported for compounds CD161 (**5**), **15**, **18**, CCT137690 (**32**) and **33**, which also induced cleavage of caspase 3. Compound **20b** is associated with the extrinsic pathway via an increase in cleaved caspase 8 and additionally induces immunogenic cell death. Apoptosis was also reported for heat shock protein 90 (HSP90) inhibitors luminespib (**1**) and its derivatives **2a-h,** bromodomain and extra-terminal (BET) inhibitors PLX51107 (**4**), CD161 (**5**), GSK1210151A (**6**), **7**, **16b** and FMS-like receptor tyrosine kinase (FLT3) inhibitors **28a**, **31q**, CCT137690 (**32**).

Cell-cycle arrest

Uncontrolled cell division is a hallmark of cancer cells associated with the dysregulation of CDKs and cyclins.[p52] Modulation of the cell cycle is one of the most common mechanisms of action of the studied isoxazole derivatives. In particular, compounds **15**, **20a**, **20b**, **22a**, **22b** and **23b** showed promising cell-cycle arrest in the G2/M phase. Accumulation of cells in subG0/G1, suggestive of cell death, was found already at **15**, **20a** and **20b**. Furthermore, G0/G1 phase arrest was reported for compounds **4**, **6**, **7, 13**, **14**, **16b**, **18** and pelabresib (**19**), whereas G1/S arrest was observed for compound **32**.

Production of ROS

Reactive oxygen species (ROS) generated by normal cellular metabolism can cause damage to DNA, proteins and lipids leading to cancer-promoting mutations if their levels become too high.[p53] Elevated ROS levels have already been observed in several types of hematological malignancies.[p54],[p55],[p56],[p57] ROS levels can affect the PI3K/Akt and mitogen-activated protein kinase (MAPK) signaling pathways required for hematopoietic stem cell (HSC) proliferation and therefore maintaining low ROS levels is essential for ensuring the self-renewal capacity of HSCs.[p58] Several isoxazole derivatives have been reported to have antioxidant activity that might suggest chemopreventative effects.[p51],[p59],[p60],[p61],[p62],[p63] By contrast, the acceleration of accumulative ROS disrupts redox homeostasis and causes severe damage in cancer cells.[p64] Derivative **24a** induced apoptosis in K562 cells via ROS accumulation related to dephosphorylation of MAPK, extracellular-signal-regulated kinases (ERK1/2) and Akt.[p65] Several authors have reported that increased ROS production has been shown to be an effective strategy to overcome drug resistance in leukemia[p66],[p67],[p68] and increased intracellular ROS levels in leukemic cells were associated with an elevated sensitivity to arsenic trioxide.[p69] Despite these findings, danatinib (**13**) reduces ROS levels to reduce mutation frequency in FMS-like tyrosine kinase-3 internal tandem duplication (FLT3-ITD) cells.[p70]

Inhibition of FLT3

FLT3 or CD135 is preferentially expressed on hematopoietic stem cells and plays an important part in the survival, proliferation and differentiation of stem cells. It is expressed on the surface of a high proportion of AML and B-lineage ALL.[p71] Inhibition of FLT3 and its downstream signaling mediators such as PI3K/Akt, MAPK/ERKs and signal transducer and activator of transcription 5A (STAT5) is a promising therapeutic target for AML therapy.[p72] FLT3 inhibition was detected in the group of fused isoxazole compounds **13** and **14**, 3,5-disubstituted compounds such as CCT137690 (**32**), *tert*-butyl-isoxazol-ureas quizartinib (**27**), **28a**, **28b** and **31a-ac**, and 4,5-disubstituted methyl-isoxazole-carboxamides **34a-h**. Quizartinib (**27**), a potent and specific FLT3 inhibitor, is already used in newly diagnosed FLT3-ITD-positive AML patients. The *tert*-butyl isoxazole core moiety of quizartinib fits within the specific hydrophobic backward-pocket present in FLT3.[p72],[p73] Its analog marbotinib (**28a**), with 5-hydroxyindole-2-carbonylbenzofurane moiety, suppressed the growth of AML cells with FLT3-ITD and leukemic cells carrying FLT3-ITD and mutations in the tyrosine kinase domain (FLT3-TKD) that confer resistance to clinically used FLT3 inhibitors.[p74] *N*-[5-(*tert*-butyl)isoxazol-3-yl]-*N*′-phenylurea analogs (**31a-ac**) and 5-methyl-*N*-(2-arylquinazolin-7-yl)isoxazole-4-carboxamide analogs (**34a-h**) showed highly selective inhibition of FLT3 in preclinical experiments.

Inhibition of CARM1

Epigenetic dysregulation plays an important part in the initiation and progression of cancer by inducing changes in histone modifications and subsequent changes in gene expression.[p75],[p76] Coactivator-associated arginine methyltransferase-1 (CARM1), also known as protein arginine methyltransferase 4 (PRMT4), catalyzes the methylation of protein arginine residues of histones and other chromatin-related proteins essential in the regulation of gene expression.[p77],[p78] This epigenetic modifying enzyme has little effect on normal hematopoietic function but is highly expressed in HL and AML, and its high expression is closely associated with poor prognosis in MM.[p77],[p79],[p80] CARM1 overexpression blocks myeloid differentiation of human stem or progenitor cells through methylation of the transcription factor RUNX1.[p81],[p82] EZM2302 (**3**) is a selective inhibitor of CARM1 containing a dimethylisoxazole group responsible for van der Waals contacts with the side chains in the complex of CARM1 with *S*-adenosyl-L-homocysteine.[p82]

Inhibition of BET

The BET protein family is associated with transcriptional elongation of genes such as cellular myelocytomatosis oncogene (c-MYC) and B cell lymphoma 2 (BCL-2); and is involved in the regulation of the cell cycle and apoptosis. BET includes four subtypes: bromodomain-containing protein 2 (BRD2), BRD3, BRD4 and the testis-and-ovary-specific BRDT form – BRD4 is related to the expression of the proto-oncogene MYC.[p83] Recent studies have reported an important role for BET inhibitors in hematological malignancies.[p75],[p83],[p84] Gehling and coworkers proposed that incorporating the isoxazole motif into an azepine scaffold could increase potency against BRD4.[p85] Trisubstituted 3,5-dimetyl-4-arylisoxazole derivatives **4-9**, **11**, **12a**, **12b** and fused isoxazoles **16a-f**, **19** showed potent BET inhibition with further determination in Table 2.

Inhibition of PD-1/PD-L1

One of the direct targets of BRD4 is the CD274 gene encoding programmed cell death ligand 1 (PD-L1), which binds to its

receptor programmed cell death 1 receptor (PD-1, CD279). The PD-1/PD-L1 axis represents one of the immune checkpoints involved in the suppression of antitumor immune responses and is essential for reducing T-cell activation.[p86] Patients suffering from hematological malignancies have an increased number of PD-1$^+$ T cells. Increased PD-1 expression in T cells is considered as an independent adverse risk factor for treatment response and survival in AML.[p87] Inhibition of this pathway could also be an effective approach to diffuse large B-cell lymphoma treatment.[p86] Compounds **17** and **30** were potent PD-1/PD-L1 inhibitors bearing a benzo[d]isoxazole or isoxazole biphenyl scaffold, respectively.

Inhibition of c-Myc

The proto-oncogene c-Myc is important for cell-cycle regulation, metabolism, apoptosis, differentiation, cell adhesion and participates in the regulation of hematopoietic homeostasis. Alterations in MYC expression are associated with hematological malignancies and poor prognosis.[p88],[p89] For instance, MYC rearrangements were reported in most cases of Burkitt lymphoma.[p90] BET inhibitors suppress c-Myc expression and have been shown to be promising and effective targets in c-Myc-dependent hematological malignancies.[p75] Isoxazole compounds **6**,**7**,**11** and **16b** which inhibited BET proteins also effectively inhibited c-Myc. It has been observed that c-Myc G4 (G-quadruplexes) controls 85–90% of the transcriptional activation of c-Myc.[p91],[p92] Benzimidazolyl isoxazole derivative EP12 (**29s**) inhibited the expression of c-Myc mRNA and c-Myc protein and stabilized c-Myc G4 in MM cells. Leflunomide (**35**) was also found to be potent inhibitor of c-Myc[p93] and T-5224 (**18**) regulated the interferon regulatory factor 4 (IRF4)/MYC axis in anti-myeloma synergy with bortezomib.[p94]

Inhibition of HDACs

Histone deacetylases (HDACs) are chromatin-modifying enzymes that regulate targets including tumor protein p53, transcription factor 1 (E2F), c-Myc, p300/CBP-associated factor (PCAF), myogenic differentiation 1, signal transducer and activator of transcription 3 (STAT3), nuclear factor (NF)-κB p65, HSP90, signal transduction molecules and α-tubulin.[p95],[p96] These epigenetic modulators can affect hematopoiesis, HSC proliferation and differentiation, and lineage commitment.[p95],[p97] Aberrant HDAC expression has been observed in various hematological malignancies.[p97],[p98] Several publications deal with a more detailed discussion of the evaluation of HDAC inhibitors in the treatment of hematological malignancies.[p99],[p100],[p101],[p102] Tapadar and colleagues observed selective and highly potent isoxazole inhibitors of HDAC6 and HDAC3.[p103] Inhibition of HDACs has also been observed with 1,2-benzisoxazole-tethered 1,2,3-triazoles such as compound **15** which has shown a high degree of conformational complementarity to the HDAC6 binding site, allowing the formation of multiple molecular interactions in the hydrophobic region and the formation of H-bonds to the phenolic side chain.[p104]

Inhibition of HSP90

HSP90 is an ATP-dependent molecular chaperone that facilitates protein maturation, activation and stability of various client proteins such as protein kinases, transcription factors and others or targets them for proteasomal degradation.[p105],[p106] Proliferation and survival of leukemia cells are regulated by HSP90, which is required for the stabilization of multiple oncogenic kinases such as BCR-ABL, FLT3-ITD or STAT3/5.[p107] The presence of isoxazole in the chemical structure of HSP90 inhibitors can improve their efficacy and pharmacokinetic profile and at the same time reduce their toxicity.[p108] Isoxazole derivatives can bind to the NH_2-terminal nucleotide-binding region of human HSP90 leading to inhibition of cell growth and subsequent apoptosis.[p109] The role of HSP90 in various hematological malignancies was reported by several authors.[p107],[p110],[p111],[p112] HSP90 can serve as a prognostic marker in some types of leukemias and lymphomas.[p113] Some HSP90 inhibitors can overcome the resistance to FLT3 inhibitors observed in AML. The HSP90 inhibitor NVP-AUY922 (**1**) demonstrated synergistic antileukemic activity with cytarabine *in vivo*.[p114] Compounds **2a–h** also showed effective HSP90 inhibition in MV4-11 and K562 cell lines.[p115]

Inhibition of ATR

Closely linked to the cell cycle is the ataxia telangiectasia and rad3-related (ATR) Ser/Thr kinase, which phosphorylates checkpoint kinase 1 (CHK1) in response to stalled replication forks. ATR/CHK1 suppresses replication initiation, particularly in cells expressing activated oncogenes and regulates cell-cycle checkpoints. Combination of ATR/CHK1 inhibitors with hydroxyurea or gemcitabine, two antimetabolites that inhibit ribonucleotide reductase and lead to inhibition of replication fork progression, seems to be advantageous.[p116],[p117],[p118] Among described isoxazole derivatives, berzosertib (**25**) was a selective ATR inhibitor that potentiated the cytotoxicity of gemcitabine in AML therapy *in vivo* and sensitized lymphoma cells to UVA radiation.[p119] The importance of ATR inhibition in various hematological malignancies has been emphasized in several articles.[p120],[p121]

Inhibition of tubulin dynamics

The successful introduction of microtubule-targeting drugs such as vincristine or paclitaxel underscores the importance of developing new anticancer drugs in this area. Microtubules, composed of α,β-tubulin heterodimers and microtubule-associated proteins, are the key components of the cytoskeleton. Agents that interfere with microtubules inhibit tumor cell division and induce G2/M phase arrest.[p122] A review by Barreca and colleagues highlights the importance of anti-tubulin agents in lymphoma therapy.[p123] Potential isoxazole candidates that inhibited tubulin polymerization *in vitro* were **20a**, **20b**, **20d**, **20e**, **20f**, **20h** and **20k**, of which compounds **20a** and **20b** were the most promising for the treatment of refractory lymphomas.[p124] By contrast, the build-up of acetylated tubulin stabilizes the microtubules and induces apoptosis, and was reported for the treatment of AML cells with compound **15**.[p104]

Overcoming the resistance of hematological cancers to conventional therapy with isoxazole derivatives

Drug resistance developed by cancer cells is a complex of mechanisms that can be divided into acquired (manifesting in the context of prolonged treatment) or intrinsic (pre-existing in the

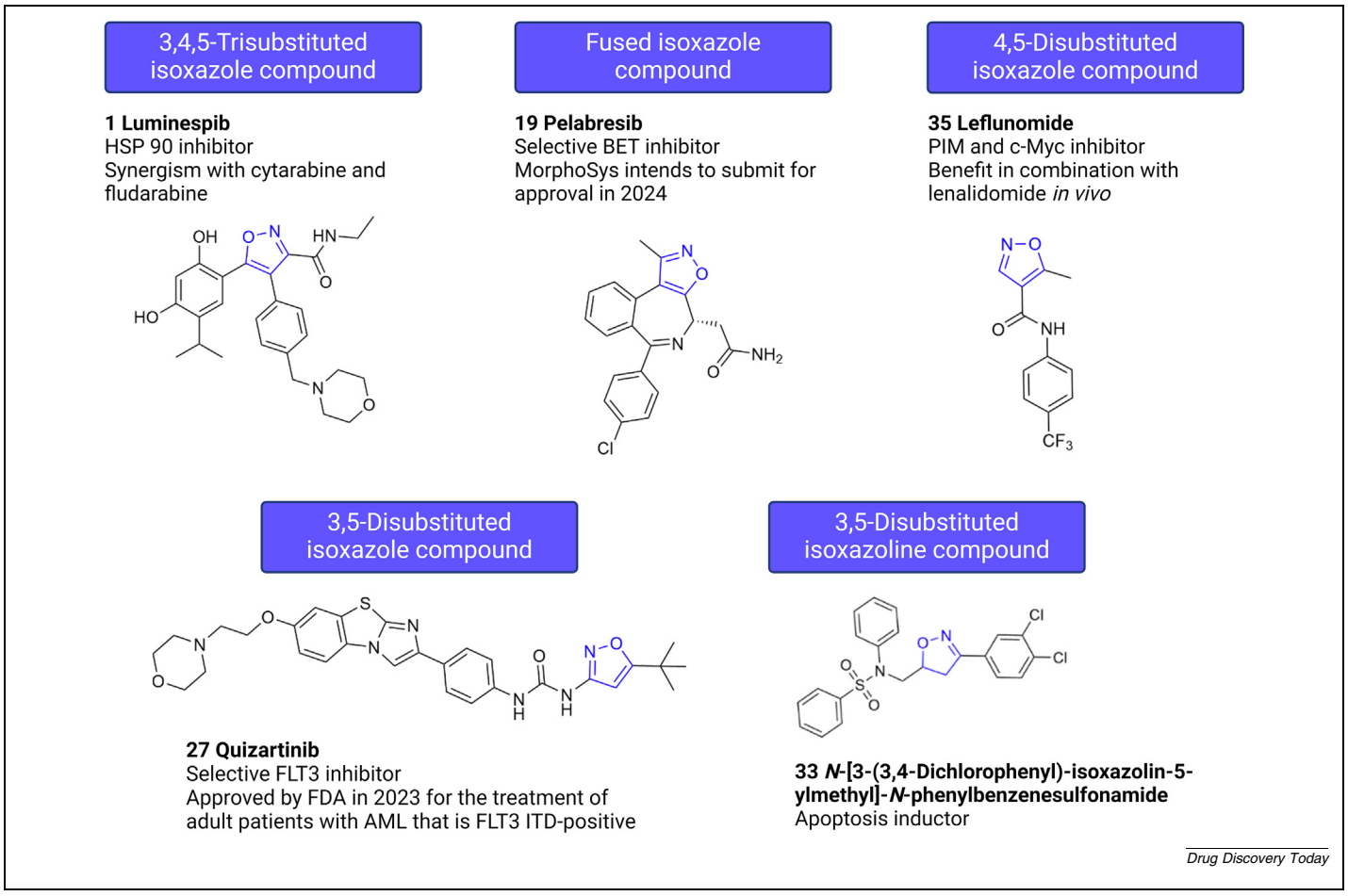


FIGURE 2

Selected chemical structures of important isoxazole derivatives targeting hematological malignancies classified into groups according to the substituent binding position. Luminespib with number **1**, which acts as a heat shock protein 90 (HSP90) inhibitor, belongs to group of 3,4,5-trisubstituted isoxazole compounds. Fused isoxazole compound pelabresib (**12**) selectively inhibits bromodomain and extra-terminal (BET) proteins. 3,5-Disubstituted quizartinib (**20**) as selective FMS-like receptor tyrosine kinase (FLT3) inhibitor and leflunomide (**28**) classified as 4,5-disubstituted isoxazole compound are also shown. Created with BioRender.com.

cancer cell population).[p125] The isoxazole curcumin analog **23b** was able to reverse drug resistance in K562 cells resistant to imatinib. The cytotoxicity of **23b** was associated with upregulation of cyclin-dependent kinase inhibitor 1A (CDKN1A) gene expression and checkpoint suppressor forkhead box N3 (FOXN3). Reduction of CDKN1A expression was consistent with a drug resistance mechanism and decreased level of FOXN3 gene is associated with dysregulation of the cell cycle. Although we can say that commercial leukemia cell lines do not accurately represent the full characteristics of the disease, it is also very important to look at the *in vivo* effect or, more importantly, the effect in patient samples. Related to this idea, another study found that resistance to imatinib is also overcome by the compound **24a** in primary human leukemia peripheral blood mononuclear cell (PBMC) samples. When **24a** was compared with the triazole derivative, the effect was also stronger in CML patient samples. Bortezomib is an anticancer drug commonly used to treat MM and nearly half of MM patients show no initial response to bortezomib therapy, indicating intrinsic resistance.[p125] Isoxazole derivative EP12 (**29s**) promoted apoptosis in bortezomib-resistant myeloma cells (RPMI-8226) by inducing genomic instability and DNA damage. T-5224 (**18**) reversed MM cell resistance to bortezomib. Compound CCT137690 (**32**), the dual FLT3–aurora-kinase inhibitor, overcame resistance to selective FLT3 inhibition *in vitro* and *in vivo*.

Isoxazole derivates with low toxicity to healthy cells

The therapeutic potential of various anticancer drugs used in clinical practice is limited by their off-target toxicity to healthy cells and tissues. Drug selectivity toward cancer cells is a highly desired property in the search for new, more-effective compounds. Derivatives **22a** and **22b** showed very high selectivity to cancer cells and low toxicity toward healthy peripheral blood lymphocytes. Derivatives **20a**, **20b**, **20k** and **20l** also showed minimal toxicity and apoptosis induction on healthy PBMCs, requiring tenfold higher doses, suggesting a favorable therapeutic index. Compound **4** demonstrated no antiproliferative effect in healthy T cells coupled to no disruption of cytokine production.

Many therapeutic compounds do not enter clinical trials owing to their high hemolytic activity. With this in mind, derivatives **24b**, **24c**, **24d** and **24e** have shown minimal induc-

tion of hemolysis (<10%, even at a higher concentration of 400 μg/ml). Compound **24a** was also minimally hemolytic-like up to a concentration of 200 μg/ml and showed 40% hemolysis at a concentration level of 400 μg/ml (~1.14 mM). Favorable systemic tolerability was successfully demonstrated for derivatives **3**, **5**, **28** and **31q** in animal studies. Additionally, compound **15** showed no cytotoxic effect on normal bone marrow (C57BL/6), whereas berzosertib (**25**) did not induce bone marrow dysmorphology. By contrast, some compounds show considerable toxicity to healthy cells: reverse ocular toxicity was reported for luminespib (**1**), which prevents its use in clinical practice and its liposomal administration is currently under investigation.[p126] Common adverse drug reactions such as reversible thrombocytopenia, nausea and fatigue were observed in a clinical trial focused on pelabresib (**19**), showing an acceptable risk–benefit profile with a recommendation to proceed to the next phase of clinical trials.[p127]

SAR of isoxazole-derived compounds against hematological malignancies

The variability in nature of the ligands bound to the isoxazole ring hindered a precise determination of SAR. Nevertheless, some observations could be drawn according to their distinct biological activities. The most potent molecule from the first 3,4,5-trisubstituted isoxazoles is luminespib (**1**), a HSP90 inhibitor.[p128],[p129],[p130],[p131] Its analogs **2a-f** have the identical 4-isopropylresorcinol group on the C-5 position and *N*-ethylamidic function on the C-3 position of the isoxazole. The addition of a polar alkyl or aryl amide group at the C-4 position (**2a-f**) resulted in the increased antiproliferative activity on K562 cells (IC_{50} = 18.01–71.57 nM) compared to luminespib (IC_{50} = 61 nM).[p115]

BET inhibition is the most frequently observed mechanism of action among the 3,4,5-trisubstituted compounds. The 3,5-

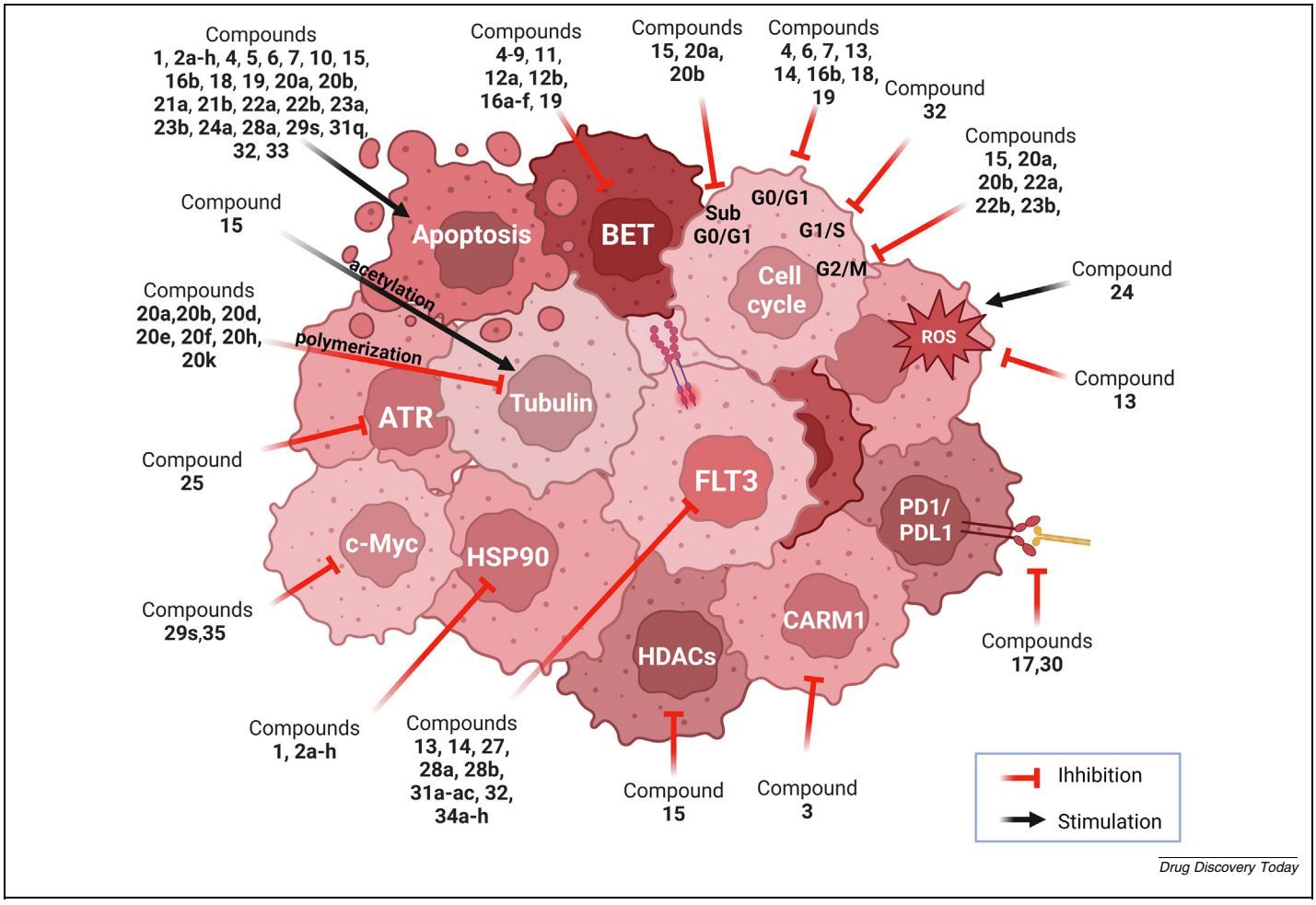


FIGURE 3

Isoxazole derivatives described in Table 2 and their mechanisms of action toward hematological malignancies including induction of apoptosis, cell-cycle arrest and reactive oxygen species (ROS) production, inhibition or stimulation of specific targets such as tubulin polymerization. Abbreviations: CARM1, coactivator-associated arginine methyltransferase 1; ATR, ataxia telangiectasia and rad3-related Ser/Thr kinase; BET, bromodomain and extra-terminal proteins; PD1/PDL1, programmed cell death 1 receptor/programmed cell death ligand 1; HDACs, histone deacetylases; C-Myc, cellular myelocytomatosis oncogene; FLT3, FMS-like receptor tyrosine kinase; HSP90, heat shock protein 90. Created with BioRender.com.

dimethyl isoxazole containing a bicyclic or tricyclic system in position 4 with at least two nitrogen atoms was disclosed as a preferred binding motif for bromodomains. The methoxy group is also presented on this fused ring in the position next to the isoxazole linkage. Compound I-BET151 (**6**) is the first dimethylisoxazole template with potent activity against cell lines harboring various MLL fusions (MV4-11, RS4-11, MOLM13, NOMO1, HEL, MEG01, HL-60) without affecting leukemia cells induced by tyrosine kinase activation.[p132] The activity of its 5*H*-pyrido and 9*H*-pyrimido[4,3-*b*]indole analogs on MV4-11 and MOLM-13 cells is summarized in Figure S1 (see supplementary material online). Its analog PLX51107 (**4**) with 4-azaindole cycle and polar benzoic scaffold is in clinical trials against solid tumors, lymphoma, AML and MDS.[p133] BET inhibition was also reported for 5*H*-pyrido and 9*H*-pyrimido[4,3-*b*]indole analog RX-37 (**8**)[p134] and similarly effective 9*H*-pyrimido[4,3-b]indole with a quinoline ring CD161 (**5**) showing good oral pharmacokinetics *in vivo*.[p135] Selective BET inhibitors pelabresib (**19**) and the *N*-(3-ethyl-6-methoxybenzo[d]isoxazol-5-yl)-4-benzenesulfonamides (**16a-f**) can be found among fused isoxazole compounds. Pelabresib (**19**, CPI-0610), containing a lipophilic chlorophenyl group and polar acetamide group, is currently being evaluated in clinical trials for myelofibrosis therapy.[p136] Compound CF53 (**9**) with 9*H*-pyrimido[4,3-*b*]indole scaffold and the amine group between two cycles binds to BRD4 BD1 protein with K_i values <1 nM and achieves the lowest nanomolar IC_{50} values of 11.7 nM in MOLM-13 cells.[p137]

Effective inhibition of FLT3 was reported for fused isoxazole compounds *N*,*N*′-diaryl-ureas **13** and **14** with a 3-amino-isoxazol[3,4-*b*]pyridine scaffold. The isoxazole could serve as a favorable pharmacophore (Figure S2, see supplementary material online) with an essential role in binding with the ATP-binding site of FLT3.[p174] Compound **14** containing the acrylamide Michael acceptor acts as an irreversible covalent FLT3 inhibitor with strong inhibitory activity against MOLM-13 cells (IC_{50} = 507-nM), as well as MV4-11 (IC_{50} = 325 nM) bearing a FLT3-ITD mutation.[p138] Danatinib (**13**) with a 4-chloro-3-trifluoromethyl group on a phenyl scaffold resulted in decreased IC_{50}s: 0.89 nM and 4.6 nM in MV4–11 and MOLM-13 cells, respectively.[p70]

The FLT3 inhibitors **27**, **28a**, **28b** and **31a-ac** with similar *N*, *N*'-diaryl-urea moiety belong to the 3,5-disubstituted isoxazole compounds. They connect the 5-*tert*-butylisoxazol-3-yl pharmacophore with various fused rings (Figure S3, see supplementary material online). Marbotinib (**28a**) shows high selectivity for FLT3 and alters signaling, reminiscent of the genetic elimination of FLT3-ITD. The clinical development of this compound is planned.[p74] Among the series of *N*-[5-(*tert*-butyl)isoxazol-3-yl]-*N*'-phenylurea derivatives of quizartinib **31a-ac** is an electron-rich fused ring: imidazo[1,2-*a*]pyridine at the phenyl most effective for the antiproliferative activity of MV4-11 cells and the introduction of substituents in C7 and C-6 positions were more tolerated than in C5 and C8 position.[p139] The last series of 4,5-disubstituted isoxazole compounds involve 4-arylamido 5-methylisoxazole derivatives with a quinazoline core **34a-h** and significant activity against FLT3 and FLT3-ITD. Compound **34c** (4-methylpiperazine substituted) showed the most potent inhibition of FLT3 and FLT3-ITD, and an excellent selectivity profile having 20% or less activity toward other kinases.[p140]

Concluding remarks

The current review article has examined the role of isoxazole derivatives in the treatment of hematological malignancies, where there is still an unmet medical need, and has provided guidance on the potential therapeutic strategies offered by these compounds. Through the analysis of the available evidence, the promising prospects of isoxazole derivatives as anticancer agents in the context of hematological malignancies have been summarized. Some of the isoxazole derivatives have shown the ability to target specific pathways in cancer cell proliferation and survival providing an exciting opportunity for the development of novel treatment strategies. In particular, isoxazole derivatives have shown high cancer selectivity coupled with the ability to overcome cancer drug resistance to conventional therapy while having minimal effects on healthy cells. Despite encouraging findings, further research is required to elucidate their efficacy and safety profiles, optimize dosing regimens and explore potential synergies with existing therapies. With continued investigation and translational efforts, compounds with an isoxazole skeleton in their structure could emerge as valuable additions to the therapeutic landscape for the treatment of hematological malignancies, offering improved outcomes and enhanced quality of life for patients.

Conflicts of interest

The authors report no conflicts of interest.

CRediT authorship contribution statement

Monika Majirská: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Martina Bago Pilátová:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Zuzana Kudličková:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Martin Vojtek:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Carmen Diniz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

No data was used for the research described in the article.

Acknowledgments

This work received support and help from Portuguese Foundation for Science and Technology (FCT)/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2021.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.drudis.2024.104059.

References

1. Cancer. Accessed February 13, 2024. https://www.who.int/news-room/fact-sheets/detail/cancer.
2. Krok-Schoen JL et al. Incidence and survival of hematological cancers among adults ages ≥75 years. *Cancer Med*. 2018;7:3425–3433. https://doi.org/10.1002/CAM4.1461.
3. Karagianni P, Giannouli S, Voulgarelis M. From the (epi)genome to metabolism and vice versa; examples from hematologic malignancy. *Int J Mol Sci*. 2021;22. https://doi.org/10.3390/IJMS22126321.
4. Taylor J, Xiao W, Abdel-Wahab O. Diagnosis and classification of hematologic malignancies on the basis of genetics. *Blood*. 2017;130:410–423. https://doi.org/10.1182/BLOOD-2017-02-734541.
5. Zhang N et al. Global burden of hematologic malignancies and evolution patterns over the past 30 years. *Blood Cancer J*. 2023;13. https://doi.org/10.1038/S41408-023-00853-3.
6. Hemminki K, Hemminki J, Försti A, Sud A. Survival trends in hematological malignancies in the Nordic countries through 50 years. *Blood Cancer J*. 2022;12:1–4. https://doi.org/10.1038/s41408-022-00728-z.
7. Klausner R. Leukemia, Lymphoma, and multiple myeloma: toward a new understanding – June 21, 2001. Published online 2001.
8. Nelson MH, Paulos CM. Novel immunotherapies for hematologic malignancies. *Immunol Rev*. 2015;263:90–105. https://doi.org/10.1111/IMR.12245.
9. Sochacka-ćwikła A, Mączyński M, Regiec A. FDA-approved drugs for hematological malignancies—the last decade review. *Cancers (Basel)*. 2022;14. https://doi.org/10.3390/CANCERS14010087.
10. Hawash M. Highlights on specific biological targets; cyclin-dependent kinases, epidermal growth factor receptors, ras protein, and cancer stem cells in anticancer drug development. *Drug Res*. 2019;69:471–478. https://doi.org/10.1055/A-0898-7347.
11. Yanamandra M, Mitra S, Giri A. Development and application of PI3K assays for novel drug discovery. *Expert Opin Drug Discov*. 2015;10:171–186. https://doi.org/10.1517/17460441.2015.997205.
12. Wang D, Zhou W, Chen J, Wei W. Upstream regulators of phosphoinositide 3-kinase and their role in diseases. *J Cell Physiol*. 2019;234:14460–14472. https://doi.org/10.1002/JCP.28215.
13. Ducki S. Antimitotic chalcones and related compounds as inhibitors of tubulin assembly. *Anticancer Agents Med Chem*. 2009;9:336–347. https://doi.org/10.2174/1871520610909030336.
14. Hamel E. Evaluation of antimitotic agents by quantitative comparisons of their effects on the polymerization of purified tubulin. *Cell Biochem Biophys*. 2003;38:1–21. https://doi.org/10.1385/CBB:38:1:1.
15. Flynn BL, Hamel E, Jung MK. One-pot synthesis of benzo[b]furan and indole inhibitors of tubulin polymerization. *J Med Chem*. 2002;45:2670–2673. https://doi.org/10.1021/JM020077T.
16. Hawash M. Recent Advances of tubulin inhibitors targeting the colchicine binding site for cancer therapy. *Biomolecules*. 2022;12:1843. https://doi.org/10.3390/BIOM12121843.
17. Sacha T. Imatinib in chronic myeloid leukemia: an overview. *Mediterr J Hematol Infect Dis*. 2014;6. https://doi.org/10.4084/MJHID.2014.007.
18. Pottier C, Fresnais M, Gilon M, Jérusalem G, Longuespée R, Sounni NE. Tyrosine kinase inhibitors in cancer: breakthrough and challenges of targeted therapy. *Cancers*. 2020;12:731. https://doi.org/10.3390/CANCERS12030731.
19. Min HY, Lee HY. Molecular targeted therapy for anticancer treatment. *Exp Mol Med*. 2022;54:1670–1694. https://doi.org/10.1038/s12276-022-00864-3.
20. Sirico M, D'Angelo A, Gianni C, Casadei C, Merloni F, De Giorgi U. Current state and future challenges for PI3K inhibitors in cancer therapy. *Cancers*. 2023;15:703. https://doi.org/10.3390/CANCERS15030703.
21. Leonardo-sousa C et al. Revisiting proteasome inhibitors: molecular underpinnings of their development, mechanisms of resistance and strategies to overcome anti-cancer drug resistance. *Molecules*. 2022;27:2201. https://doi.org/10.3390/MOLECULES27072201/S1.
22. Gribben JG, Riches JC. Immunotherapeutic strategies including transplantation: eradication of disease. *Hematology*. 2013;2013:151–157. https://doi.org/10.1182/ASHEDUCATION-2013.1.151.
23. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases — elimination, equilibrium and escape. *Curr Opin Immunol*. 2014;27:16–25. https://doi.org/10.1016/J.COI.2014.01.004.
24. Tang L, Huang Z, Mei H, Hu Y. Immunotherapy in hematologic malignancies: achievements, challenges and future prospects. *Signal Transduct Targeted Therapy*. 2023;8:1–39. https://doi.org/10.1038/s41392-023-01521-5.
25. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature*. 2011;480:480–489. https://doi.org/10.1038/nature10673.
26. Gupta SL, Basu S, Soni V, Jaiswal RK. Immunotherapy: an alternative promising therapeutic approach against cancers. *Mol Biol Rep*. 2022;49:9903–9913. https://doi.org/10.1007/s11033-022-07525-8.
27. Tran E, Robbins PF, Rosenberg SA. "Final common pathway" of human cancer immunotherapy: targeting random somatic mutations. *Nat Immunol*. 2017;18:255–262. https://doi.org/10.1038/ni.3682.
28. Podhorecka M, Markowicz J, Szymczyk A, Pawlowski J. Target therapy in hematological malignances: new monoclonal antibodies. *Int Sch Res Notices*. 2014;2014:1–16. https://doi.org/10.1155/2014/701493.
29. Guo H, Yang J, Wang H, Liu X, Liu Y, Zhou K. Reshaping the tumor microenvironment: the versatility of immunomodulatory drugs in B-cell neoplasms. *Front Immunol*. 2022;13:1017990. https://doi.org/10.3389/fimmu.2022.1017990.
30. Chen J, Jiang H. Current challenges and strategies for chimeric antigen receptor-T-cell therapy for solid tumors. *Crit Rev™ Immunol*. 2021;41:1–12. https://doi.org/10.1615/CRITREVIMMUNOL.2020036178.
31. Hauth F, Ho AY, Ferrone S, Duda DG. Radiotherapy to enhance chimeric antigen receptor T-cell therapeutic efficacy in solid tumors: a narrative review. *JAMA Oncol*. 2021;7:1051–1059. https://doi.org/10.1001/JAMAONCOL.2021.0168.
32. Auberger P, Tamburini-Bonnefoy J, Puissant A. Drug resistance in hematological malignancies. *Int J Mol Sci*. 2020;21:6091. https://doi.org/10.3390/IJMS21176091.
33. Friman TK et al. Cancer risk and mortality after solid organ transplantation: a population-based 30-year cohort study in Finland. *Int J Cancer*. 2022;150:1779–1791. https://doi.org/10.1002/IJC.33934.
34. Demoor-Goldschmidt C, De Vathaire F. Review of risk factors of secondary cancers among cancer survivors. *Br J Radiol*. 2019;92. https://doi.org/10.1259/BJR.20180390.
35. Zhang Q et al. Secondary cancer risk after radiation therapy for breast cancer with different radiotherapy techniques. *Sci Rep*. 2020;10:1–12. https://doi.org/10.1038/s41598-020-58134-z.
36. Dracham CB, Shankar A, Madan R. Radiation induced secondary malignancies: a review article. *Radiat Oncol J*. 2018;36:85–94. https://doi.org/10.3857/ROJ.2018.00290.
37. Zhu J, Mo J, Lin HZ, Chen Y, Sun HP. The recent progress of isoxazole in medicinal chemistry. *Bioorg Med Chem*. 2018;26:3065–3075. https://doi.org/10.1016/j.bmc.2018.05.013.
38. Rahbak L, Christophersen C. *Isoxazole Alkaloids*. 2001.
39. Saravanan G, Alagarsamy V, Dineshkumar P. Synthesis, analgesic, anti-inflammatory and in vitro antimicrobial activities of some novel isoxazole coupled quinazolin-4(3H)-one derivatives. *Arch Pharm Res*. 2021;44:1–11. https://doi.org/10.1007/s12272-013-0262-8.
40. Dupont S, Stefan H. Zonisamide in clinical practice. *Acta Neurol Scand*. 2012;126:29–35. https://doi.org/10.1111/ane.12017.
41. Maczynski M et al. Synthesis, immunosuppressive properties, and mechanism of action of a new isoxazole derivative. *Molecules*. 2018;23. https://doi.org/10.3390/molecules23071545.

42. Nie JP et al. Discovery and anti-diabetic effects of novel isoxazole based flavonoid derivatives. *Fitoterapia*. 2020;142. https://doi.org/10.1016/j.fitote.2020.104499.
43. Li F, Hu Y, Wang Y, Ma C, Wang J. Expeditious lead optimization of isoxazole-containing influenza A virus M2–S31N inhibitors using the suzuki-miyaura cross-coupling reaction. *J Med Chem*. 2017;60:1580–1590. https://doi.org/10.1021/acs.jmedchem.6b01852.
44. Arya GC, Kaur K, Jaitak V. Isoxazole derivatives as anticancer agent: a review on synthetic strategies, mechanism of action and SAR studies. *Eur J Med Chem*. 2021;221. https://doi.org/10.1016/j.ejmech.2021.113511.
45. Hawash M et al. Synthesis, chemo-informatics, and anticancer evaluation of fluorophenyl-isoxazole derivatives. *Open Chem*. 2021;19:855–863. https://doi.org/10.1515/chem-2021-0078.
46. Hawash M et al. Design, synthesis, and biological evaluation of phenyl-isoxazole-carboxamide derivatives as anticancer agents. *Heterocycl Comm*. 2021;27:133–141. https://doi.org/10.1515/hc-2020-0134.
47. Hawash M et al. Synthesis of novel isoxazole–carboxamide derivatives as promising agents for melanoma and targeted nano-emulgel conjugate for improved cellular permeability. *BMC Chem*. 2022;16:1–12. https://doi.org/10.1186/s13065-022-00839-5.
48. Hawash M, Kahraman DC, Ergun SG, Cetin-Atalay R, Baytas SN. Synthesis of novel indole-isoxazole hybrids and evaluation of their cytotoxic activities on hepatocellular carcinoma cell lines. *BMC Chem*. 2021;15:1–14. https://doi.org/10.1186/s13065-021-00793-8.
49. Alfaro-Lara R, Espinosa-Ortega HF, Arce-Salinas CA. Systematic review and meta-analysis of the efficacy and safety of leflunomide and methotrexate in the treatment of rheumatoid arthritis. *Reumatol Clin*. 2019;15:133–139. https://doi.org/10.1016/j.reuma.2017.07.020.
50. Uto Y. 1,2-Benzisoxazole: a privileged structure with a potential for polypharmacology. *Curr Pharm Des*. 2016;22:3201–3211. https://doi.org/10.2174/1381612822666160224142648.
51. Shaik A, Bhandare RR, Palleapati K, Nissankararao S, Kancharlapalli V, Shaik S. Antimicrobial, antioxidant, and anticancer activities of some novel isoxazole ring containing chalcone and dihydropyrazole derivatives. *Molecules*. 2020;25. https://doi.org/10.3390/molecules25051047.
52. Santo L, Siu KT, Raje N. Targeting cyclin-dependent kinases and cell cycle progression in human cancers. *Semin Oncol*. 2015;42:788–800. https://doi.org/10.1053/J.SEMINONCOL.2015.09.024.
53. Didier AJ, Stiene J, Fang L, Watkins D, Dworkin LD, Creeden JF. Antioxidant and anti-tumor effects of dietary vitamins A, C, and E. *Antioxidants*. 2023;12. https://doi.org/10.3390/ANTIOX12030632.
54. Devi GS, Prasad MH, Saraswathi I, Raghu D, Rao DN, Reddy PP. Free radicals antioxidant enzymes and lipid peroxidation in different types of leukemias. *Clinica Chimica Acta*. 2000;293:53–62. https://doi.org/10.1016/S0009-8981(99)00222-3.
55. Hole PS et al. Overproduction of NOX-derived ROS in AML promotes proliferation and is associated with defective oxidative stress signaling. *Blood*. 2013;122:3322–3330. https://doi.org/10.1182/BLOOD-2013-04-491944.
56. Sillar JR, Germon ZP, De Iuliis GN, Dun MD. The role of reactive oxygen species in acute myeloid leukaemia. *Int J Mol Sci*. 2019;20. https://doi.org/10.3390/IJMS20236003.
57. Trombetti S et al. Oxidative stress and ROS-mediated signaling in leukemia: novel promising perspectives to eradicate chemoresistant cells in myeloid leukemia. *Int J Mol Sci*. 2021;22:1–19. https://doi.org/10.3390/IJMS22052470.
58. Romo-González M, Ijurko C, Hernández-Hernández à. Reactive oxygen species and metabolism in leukemia: a dangerous liaison. *Front Immunol*. 2022;13:1. https://doi.org/10.3389/FIMMU.2022.889875.
59. Hawash M et al. In vitro and in vivo assessment of the antioxidant potential of isoxazole derivatives. *Sci Rep*. 2022;12:1–8. https://doi.org/10.1038/s41598-022-23050-x.
60. Fandakli S. Synthesis of some new isoxazole compounds and their biological tyrosinase and antioxidant activities. *Turk J Chem*. 2022;46:747–753. https://doi.org/10.55730/1300-0527.3364.
61. Shilpa M, Krishna B, Uma B, Hanmanthu G. Efficient synthesis, antioxidant activity evaluation, and molecular docking of novel isoxazole derivatives. *Russian J Org Chem*. 2023;59:1961–1967. https://doi.org/10.1134/S1070428023110143.
62. Padmaja A, Rajasekhar C, Muralikrishna A, Padmavathi V. Synthesis and antioxidant activity of oxazolyl/thiazolylsulfonylmethyl pyrazoles and isoxazoles. *Eur J Med Chem*. 2011;46:5034–5038. https://doi.org/10.1016/J.EJMECH.2011.08.010.
63. Eid AM et al. Synthesis and biological evaluation of novel isoxazole-amide analogues as anticancer and antioxidant agents. *Biomed Res Int*. 2021;2021. https://doi.org/10.1155/2021/6633297.
64. Kim SJ, Kim HS, Seo YR. Understanding of ROS-inducing strategy in anticancer therapy. *Oxid Med Cell Longev*. 2019;2019. https://doi.org/10.1155/2019/5381692.
65. Abdelkafi-Koubaa Z, Aissa I, Ben Jannet H, Srairi-Abid N, Marrakchi N, Menif S. Tyrosol derivatives, bearing 3,5-disubstituted isoxazole and 1,4-disubstituted triazole, as potential antileukemia agents by promoting apoptosis. *Molecules*. 2022;27. https://doi.org/10.3390/molecules27165086.
66. Tonino SH, Van Laar J, Van Oers MH, Wang JY, Eldering E, Kater AP. ROS-mediated upregulation of Noxa overcomes chemoresistance in chronic lymphocytic leukemia. *Oncogene*. 2011;30:701–713. https://doi.org/10.1038/ONC.2010.441.
67. Ruvolo VR et al. Role for PKC in fenretinide-mediated apoptosis in lymphoid leukemia cells. *J Signal Transduct*. 2010;2010:1–15. https://doi.org/10.1155/2010/584657.
68. Changchien JJ, Chen YJ, Huang CH, Cheng TL, Lin SR, Sen CL. Quinacrine induces apoptosis in human leukemia K562 cells via p38 MAPK-elicited BCL2 down-regulation and suppression of ERK/c-Jun-mediated BCL2L1 expression. *Toxicol Appl Pharmacol*. 2015;284:33–41. https://doi.org/10.1016/J.TAAP.2015.02.005.
69. Togashi K, Masuda H, Rankinen T, Tanaka S, Bouchard C, Kamiya H. The inherent cellular level of reactive oxygen species: one of the mechanisms determining apoptotic susceptibility of leukemic cells to arsenic trioxide. *Apoptosis*. 2002;7:209–215. https://doi.org/10.1023/A:1015331229263/METRICS.
70. Sun SL et al. Preclinical characterization of danatinib as a novel FLT3 inhibitor with excellent efficacy against resistant acute myeloid leukemia. *Biomed Pharmacotherapy*. 2023;169, 115905. https://doi.org/10.1016/J.BIOPHA.2023.115905.
71. Kiyoi H, Naoe T. FLT3 in human hematologic malignancies. *Leuk Lymphoma*. 2002;43:1541–1547. https://doi.org/10.1080/1042819021000002866.
72. Ezelarab HAA, Ali TFS, Abbas SH, Hassan HA, Beshr EAM. Indole-based FLT3 inhibitors and related scaffolds as potential therapeutic agents for acute myeloid leukemia. *BMC Chem*. 2023;17:1–31. https://doi.org/10.1186/S13065-023-00981-8.
73. Levis M. Quizartinib for the treatment of FLT3/ITD acute myeloid leukemia. *Future Oncology*. 2014;10:1571–1579. https://doi.org/10.2217/fon.14.105.
74. Beyer M et al. Identification of a highly efficient dual type I/II FMS-like tyrosine kinase inhibitor that disrupts the growth of leukemic cells. *Cell Chem Biol*. 2022;29:398–411.e4. https://doi.org/10.1016/J.CHEMBIOL.2021.10.011.
75. Abedin SM, Boddy CS, Munshi HG. BET inhibitors in the treatment of hematologic malignancies: current insights and future prospects. *Onco Targets Ther*. 2016;9:5943. https://doi.org/10.2147/OTT.S100515.
76. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet*. 2002;3:415–428. https://doi.org/10.1038/nrg816.
77. Yang L, Ma L, Gong Q, Chen JP, Huang Q. Inhibition of CARM1 suppresses proliferation of multiple myeloma cells through activation of p53 signaling pathway. *Mol Biol Rep*. 2023;50:7457–7469. https://doi.org/10.1007/S11033-023-08645-5.
78. Qiu Y et al. Systematic pan-cancer landscape identifies CARM1 as a potential prognostic and immunological biomarker. *BMC Genom Data*. 2022;23:1–21. https://doi.org/10.1186/S12863-021-01022-W.
79. Leonard S, Gordon N, Smith N, Rowe M, Murray PG, Woodman CB. Arginine methyltransferases are regulated by epstein-barr virus in B cells and are differentially expressed in Hodgkin's lymphoma. *Pathogens*. 2012;1:52–64. https://doi.org/10.3390/PATHOGENS1010052.
80. Vu LP et al. PRMT4 blocks myeloid differentiation by assembling a methyl-RUNX1-dependent repressor complex. *Cell Rep*. 2013;5:1625–1638. https://doi.org/10.1016/J.CELREP.2013.11.025.
81. Santos M, Hwang JW, Bedford MT. CARM1 arginine methyltransferase as a therapeutic target for cancer. *J Biol Chem*. 2023;299. https://doi.org/10.1016/J.JBC.2023.105124.
82. Drew AE et al. Identification of a CARM1 inhibitor with potent in vitro and in vivo activity in preclinical models of multiple myeloma. *Sci Rep*. 2017;7:1–13. https://doi.org/10.1038/s41598-017-18446-z.
83. Ma T et al. BET in hematologic tumors: immunity, pathogenesis, clinical trials and drug combinations. *Genes Dis*. 2023;10:2306–2319. https://doi.org/10.1016/J.GENDIS.2022.03.004.

84. Vakoc CR. BET bromodomain inhibition as a therapeutic approach in hematological malignancies. *Histone Recogn*. 2015;185–198. https://doi.org/10.1007/978-3-319-18102-8_9/FIGURES/2. Published online January 1, 2015.
85. Gehling VS et al. Discovery, design, and optimization of isoxazole azepine BET inhibitors. *ACS Med Chem Lett*. 2013;4:835–840. https://doi.org/10.1021/ML4001485.
86. Han Y, Liu D, Li L. PD-1/PD-L1 pathway: current researches in cancer. *Am J Cancer Res*.. 2020;10:727. Accessed February 4, 2024. /pmc/articles/PMC7136921/.
87. Zhong M et al. BET bromodomain inhibition rescues PD-1-mediated T-cell exhaustion in acute myeloid leukemia. *Cell Death Dis*. 2022;13:1–15. https://doi.org/10.1038/s41419-022-05123-x.
88. Ahmadi SE, Rahimi S, Zarandi B, Chegeni R, Safa M. MYC: a multipurpose oncogene with prognostic and therapeutic implications in blood malignancies. *J Hematol Oncol*. 2021;14:1–49. https://doi.org/10.1186/S13045-021-01111-4.
89. Hoffman B, Amanullah A, Shafarenko M, Liebermann DA. The proto-oncogene c-myc in hematopoietic development and leukemogenesis. *Oncogene*. 2002;21:3414–3421. https://doi.org/10.1038/SJ.ONC.1205400.
90. Jovanović KK, Roche-Lestienne C, Ghobrial IM, Facon T, Quesnel B, Manier S. Targeting MYC in multiple myeloma. *Leukemia*. 2018;32:1295–1306. https://doi.org/10.1038/s41375-018-0036-x.
91. Wang W et al. Human MYC G-quadruplex: from discovery to a cancer therapeutic target. *Biochim Biophys Acta (BBA) – Rev Cancer*. 2020;1874, 188410. https://doi.org/10.1016/J.BBCAN.2020.188410.
92. Geng X et al. Design, synthesis, and biological evaluation of novel benzimidazolyl isoxazole derivatives as potential c-Myc G4 stabilizers to suppress c-Myc transcription and myeloma growth. *J Mol Struct*. 2023;1275. https://doi.org/10.1016/j.molstruc.2022.134673.
93. Buettner R et al. Leflunomide regulates c-Myc expression in myeloma cells through PIM targeting. *Blood Adv*. 2019;3:1027. https://doi.org/10.1182/BLOODADVANCES.2018027227.
94. Tang S et al. The selective activator protein-1 inhibitor T-5224 regulates the IRF4/MYC axis and exerts cooperative antimyeloma activity with bortezomib. *Chem Biol Interact*. 2023;384, 110687. https://doi.org/10.1016/J.CBI.2023.110687.
95. Deng Y, Cheng Q, He J. HDAC inhibitors: promising agents for leukemia treatment. *Biochem Biophys Res Commun*. 2023;680:61–72. https://doi.org/10.1016/J.BBRC.2023.09.023.
96. Zhao MN et al. Iron-catalyzed cycloaddition of amides and 2,3-diaryl-2 H-azirines to access oxazoles via C-N bond cleavage. *J Org Chem*. 2021;86:2957–2964. https://doi.org/10.1021/acs.joc.0c02843.
97. Mina A, Pro B. T time: Emerging and new therapies for peripheral T-cell lymphoma. *Blood Rev*. 2022;52. https://doi.org/10.1016/J.BLRE.2021.100889.
98. Holbert MA, Marmorstein R. Structure and activity of enzymes that remove histone modifications. *Curr Opin Struct Biol*. 2005;15:673–680. https://doi.org/10.1016/J.SBI.2005.10.006.
99. José-Enériz ES, Gimenez-Camino N, Agirre X, Prosper F. HDAC inhibitors in acute myeloid leukemia. *Cancers (Basel)*. 2019;11. https://doi.org/10.3390/CANCERS11111794.
100. Stimson L, Wood V, Khan O, Fotheringham S, La Thangue NB. HDAC inhibitor-based therapies and haematological malignancy. *Ann Oncol*. 2009;20:1293–1302. https://doi.org/10.1093/ANNONC/MDN792.
101. Rangwala S, Zhang C, Duvic M. HDAC inhibitors for the treatment of cutaneous T-cell lymphomas. *Future Med Chem*. 2012;4:471–486. https://doi.org/10.4155/FMC.12.6.
102. Imai Y, Hirano M, Kobayashi M, Futami M, Tojo A. HDAC inhibitors exert anti-myeloma effects through multiple modes of action. *Cancers (Basel)*. 2019;11. https://doi.org/10.3390/CANCERS11040475.
103. Tapadar S, He R, Luchini DN, Billadeau DD, Kozikowski AP. Isoxazole moiety in the linker region of HDAC inhibitors adjacent to the Zn-chelating group: effects on HDAC biology and antiproliferative activity. *Bioorg Med Chem Lett*. 2009;19:3023–3026. https://doi.org/10.1016/J.BMCL.2009.04.058.
104. Ashwini N et al. Synthesis of 1,2-benzisoxazole tethered 1,2,3-triazoles that exhibit anticancer activity in acute myeloid leukemia cell lines by inhibiting histone deacetylases, and inducing p21 and tubulin acetylation. *Bioorg Med Chem*. 2015;23:6157–6165. https://doi.org/10.1016/J.BMC.2015.07.069.
105. Pearl LH, Prodromou C, Workman P. The Hsp90 molecular chaperone: an open and shut case for treatment. *Biochem J*. 2008;410:439–453. https://doi.org/10.1042/BJ20071640.
106. Birbo B et al. Role of HSP90 in cancer. *Int J Mol Sci*. 2021;22:10317. https://doi.org/10.3390/IJMS221910317.
107. Ho N, Li A, Li S, Zhang H. Heat shock protein 90 and role of its chemical inhibitors in treatment of hematologic malignancies. *Pharmaceuticals*. 2012;5:779–801. https://doi.org/10.3390/PH5080779.
108. Ardestani M, Khorsandi Z, Keshavarzipour F, Iravani S, Sadeghi-Aliabadi H, Varma RS. Heterocyclic compounds as Hsp90 inhibitors: a perspective on anticancer applications. *Pharmaceutics*. 2022;14:2220. https://doi.org/10.3390/PHARMACEUTICS14102220.
109. Sharp SY et al. Inhibition of the heat shock protein 90 molecular chaperone in vitro and in vivo by novel, synthetic, potent resorcinylic pyrazole/isoxazole amide analogues. *Mol Cancer Ther*. 2007;6:1198–1211. https://doi.org/10.1158/1535-7163.MCT-07-0149.
110. Cabaud-Gibouin V, Durand M, Quéré R, Girodon F, Garrido C, Jego G. Heat-shock proteins in leukemia and lymphoma: multitargets for innovative therapeutic approaches. *Cancers*. 2023;15:984. https://doi.org/10.3390/CANCERS15030984.
111. Flandrin P et al. Significance of heat-shock protein (HSP) 90 expression in acute myeloid leukemia cells. *Cell Stress Chaperones*. 2008;13:357–364. https://doi.org/10.1007/S12192-008-0035-3.
112. Mjahed H, Girodon F, Fontenay M, Garrido C. Heat shock proteins in hematopoietic malignancies. *Exp Cell Res*. 2012;318:1946–1958. https://doi.org/10.1016/J.YEXCR.2012.05.012.
113. Žáčková M, Moučková D, Lopotová T, Ondráčková Z, Klamová H, Moravcová J. Hsp90 — a potential prognostic marker in CML. *Blood Cells Mol Dis*. 2013;50:184–189. https://doi.org/10.1016/J.BCMD.2012.11.002.
114. Katayama K, Noguchi K, Sugimoto Y. Heat shock protein 90 inhibitors overcome the resistance to Fms-like tyrosine kinase 3 inhibitors in acute myeloid leukemia. *Oncotarget*. 2018;9:34240–34258. https://doi.org/10.18632/ONCOTARGET.26045.
115. Baruchello R et al. Novel 3,4-isoxazolediamides as potent inhibitors of chaperone heat shock protein 90. *J Med Chem*. 2011;54:8592–8604. https://doi.org/10.1021/JM201155E.
116. Qiu Z, Oleinick NL, Zhang J. ATR/CHK1 inhibitors and cancer therapy. *Radiother Oncol*. 2018;126:450. https://doi.org/10.1016/J.RADONC.2017.09.043.
117. Mei L, Zhang J, He K, Zhang J. Ataxia telangiectasia and Rad3-related inhibitors and cancer therapy: where we stand. *J Hematol Oncol*. 2019;12:1–8. https://doi.org/10.1186/S13045-019-0733-6/TABLES/1.
118. Fordham SE et al. Inhibition of ATR acutely sensitizes acute myeloid leukemia cells to nucleoside analogs that target ribonucleotide reductase. *Blood Adv*. 2018;2:1157–1169. https://doi.org/10.1182/bloodadvances.2017015214.
119. Biskup E, Naym DG, Gniadecki R. Small-molecule inhibitors of Ataxia Telangiectasia and Rad3 related kinase (ATR) sensitize lymphoma cells to UVA radiation. *J Dermatol Sci*. 2016;84:239–247. https://doi.org/10.1016/J.JDERMSCI.2016.09.010.
120. Takagi M. DNA damage response and hematological malignancy. *Int J Hematol*. 2017;106:345–356. https://doi.org/10.1007/S12185-017-2226-0.
121. De Mel S et al. Targeting the DNA damage response in hematological malignancies. *Front Oncol*. 2024;14. https://doi.org/10.3389/FONC.2024.1307839.
122. Song J et al. Discovery of novel coumarin-indole derivatives as tubulin polymerization inhibitors with potent anti-gastric cancer activities. *Eur J Med Chem*. 2022;238, 114467. https://doi.org/10.1016/J.EJMECH.2022.114467.
123. Barreca M, Stathis A, Barraja P, Bertoni F. An overview on anti-tubulin agents for the treatment of lymphoma patients. *Pharmacol Ther*. 2020;211, 107552. https://doi.org/10.1016/J.PHARMTHERA.2020.107552.
124. Barreca M et al. Development of [1,2]oxazoloisoindoles tubulin polymerization inhibitors: Further chemical modifications and potential therapeutic effects against lymphomas. *Eur J Med Chem*. 2022;243. https://doi.org/10.1016/j.ejmech.2022.114744.
125. Kelley ME et al. High-content microscopy reveals a morphological signature of bortezomib resistance. *Elife*. 2023;12:91362. https://doi.org/10.7554/ELIFE.91362.
126. Epp-Ducharme B et al. Heat-activated nanomedicine formulation improves the anticancer potential of the HSP90 inhibitor luminespib in vitro. *Sci Rep*. 2021;11. https://doi.org/10.1038/s41598-021-90585-w.
127. Blum KA et al. A phase I study of pelabresib (CPI-0610), a small-molecule inhibitor of BET proteins, in patients with relapsed or refractory lymphoma. *Cancer Res Commun*. 2022;2:795. https://doi.org/10.1158/2767-9764.CRC-22-0060.
128. Seggewiss-Bernhardt R et al. Phase 1/1B trial of the heat shock protein 90 inhibitor NVP-AUY922 as monotherapy or in combination with bortezomib in

patients with relapsed or refractory multiple myeloma. *Cancer*. 2015;121:2185–2192. https://doi.org/10.1002/CNCR.29339.
129. Walsby EJ, Lazenby M, Pepper CJ, Knapper S, Burnett AK. The HSP90 inhibitor NVP-AUY922-AG inhibits the PI3K and IKK signalling pathways and synergizes with cytarabine in acute myeloid leukaemia cells. *Br J Haematol*. 2013;161:57–67. https://doi.org/10.1111/BJH.12215.
130. Walsby EJ et al. The Hsp90 inhibitor NVP-AUY922-AG inhibits NF-κB signaling, overcomes microenvironmental cytoprotection and is highly synergistic with fludarabine in primary CLL cells. *Oncotarget*. 2012;3:525–534. https://doi.org/10.18632/ONCOTARGET.491.
131. Oki Y et al. Experience with HSP90 inhibitor AUY922 in patients with relapsed or refractory non-Hodgkin lymphoma. *Haematologica*. 2015;100:e272–e274. https://doi.org/10.3324/HAEMATOL.2015.126557.
132. Dawson MA et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature*. 2011;478:529–533. https://doi.org/10.1038/nature10509.
133. Senapati J et al. Phase I results of bromodomain and extra-terminal inhibitor PLX51107 in combination with azacitidine in patients with relapsed/refractory myeloid malignancies. *Clin Cancer Res*. 2023;29:4352–4360. https://doi.org/10.1158/1078-0432.CCR-23-1429/728468/.
134. Ran X et al. Structure-based design of γ-carboline analogues as potent and specific BET bromodomain inhibitors. *J Med Chem*. 2015;58:4927–4939. https://doi.org/10.1021/ACS.JMEDCHEM.5B00613.
135. Zhao Y et al. Structure-based discovery of 4-(6-methoxy-2-methyl-4-(quinolin-4-yl)-9H-pyrimido[4,5-b]indol-7-yl)-3,5-dimethylisoxazole (CD161) as a potent and orally bioavailable BET bromodomain inhibitor. *J Med Chem*. 2017;60:3887–3901. https://doi.org/10.1021/ACS.JMEDCHEM.7B00193.
136. Ferreira Gomes G, Harrison C. Pelabresib (CPI-0610): an exciting novel drug for the treatment of myelofibrosis. *Curr Hematol Malig Rep*. 2023;18:113–120. https://doi.org/10.1007/s11899-023-00696-6.
137. Zhao Y et al. Structure-based discovery of CF53 as a potent and orally bioavailable bromodomain and extra-terminal (BET) bromodomain inhibitor. *J Med Chem*. 2018;61:6110–6120. https://doi.org/10.1021/ACS.JMEDCHEM.8B00483.
138. Kang JB et al. Synthesis and biological evaluation of 4-(4-aminophenyl)-6-methylisoxazolo[3,4-b] pyridin-3-amine covalent inhibitors as potential agents for the treatment of acute myeloid leukemia. *Bioorg Med Chem*. 2022;70, 116937. https://doi.org/10.1016/J.BMC.2022.116937.
139. Xu Y et al. Discovery of novel N-(5-(tert-butyl)isoxazol-3-yl)-N′-phenylurea analogs as potent FLT3 inhibitors and evaluation of their activity against acute myeloid leukemia in vitro and in vivo. *Bioorg Med Chem*. 2015;23:4333–4343. https://doi.org/10.1016/j.bmc.2015.06.033.
140. Im D, Moon H, Kim J, Oh Y, Jang M, Hah JM. Discovery of 5-methyl-N-(2-arylquinazolin-7-yl)isoxazole-4-carboxamide analogues as highly selective FLT3 inhibitors. *J Enzyme Inhib Med Chem*. 2020;35:1110–1115. https://doi.org/10.1080/14756366.2020.1758689.
141. Röllig C, Knop S, Bornhäuser M. Multiple myeloma. *The Lancet*. 2015;385:2197–2208. https://doi.org/10.1016/S0140-6736(14)60493-1.
142. Mugnaini EN, Ghosh N. Lymphoma. *Primary Care: Clin Office Pract*. 2016;43:661–675. https://doi.org/10.1016/J.POP.2016.07.012.
143. DeAngelo DJ, Jabbour E, Advani A. Recent advances in managing acute lymphoblastic leukemia. *https://doi.org/101200/EDBK_280175*. 2020;40:330–342. https://doi.org/10.1200/EDBK_280175.
144. Delgado J, Nadeu F, Colomer D, Campo E. Chronic lymphocytic leukemia: from molecular pathogenesis to novel therapeutic strategies. *Haematologica*. 2020;105:2205. https://doi.org/10.3324/HAEMATOL.2019.236000.
145. Shimada A. Hematological malignancies and molecular targeting therapy. *Eur J Pharmacol*. 2019;862, 172641. https://doi.org/10.1016/J.EJPHAR.2019.172641.
146. Vesci L, Milazzo FM, Carolo V, Pace S, Gianini G. Preclinical antitumor activity of SST0116CL1: A novel heat shock protein 90 inhibitor. *Int J Oncol*. 2014;45:1421–1429. https://doi.org/10.3892/ijo.2014.2575.
147. Lampronti I et al. Pro-apoptotic activity of novel synthetic isoxazole derivatives exhibiting inhibitory activity against tumor cell growth in vitro. *Oncol Lett*. 2020;20. https://doi.org/10.3892/OL.2020.12002.
148. Ozer HG et al. BRD4 profiling identifies critical chronic lymphocytic leukemia oncogenic circuits and reveals sensitivity to PLX51107, a novel structurally distinct BET inhibitor. *Cancer Discov*. 2018;8:458–477. https://doi.org/10.1158/2159-8290.CD-17-0902.
149. Yu S et al. Discovery of (R)-4-(8-methoxy-2-methyl-1-(1-phenylethy)-1H-imidazo[4,5-c]quinnolin-7-yl)-3,5-dimethylisoxazole as a potent and selective BET inhibitor for treatment of acute myeloid leukemia (AML) guided by FEP calculation. *Eur J Med Chem*. 2024;263, 115924. https://doi.org/10.1016/J.EJMECH.2023.115924.
150. Gupta VK, Kumar A. Targeting lysophosphatidic acid receptor with Ki16425 impedes T cell lymphoma progression through apoptosis induction, glycolysis inhibition, and activation of antitumor immune response. *Apoptosis*. 2022;27:382–400. https://doi.org/10.1007/S10495-022-01723-2.
151. Li X, Zhang J, Zhao L, Yang Y, Zhang H, Zhou J. Design, synthesis, and in vitro biological evaluation of 3,5-dimethylisoxazole derivatives as BRD4 inhibitors. *ChemMedChem*. 2018;13:1363–1368. https://doi.org/10.1002/CMDC.201800074.
152. Feng Z et al. Design, synthesis, and biological activity evaluation of a series of novel sulfonamide derivatives as BRD4 inhibitors against acute myeloid leukemia. *Bioorg Chem*. 2021;111, 104849. https://doi.org/10.1016/J.BIOORG.2021.104849.
153. Zhang MF et al. Design, synthesis and pharmacological characterization of N-(3-ethylbenzo[d]isoxazol-5-yl) sulfonamide derivatives as BRD4 inhibitors against acute myeloid leukemia. *Acta Pharmacol Sin*. 2022;43:2735–2748. https://doi.org/10.1038/s41401-022-00881-y.
154. Huang X, Chen H, Dai X, Xu M, Wang K, Feng Z. Design, synthesis, and structure-activity relationship of PD-1/PD-L1 inhibitors with a benzo[d] isoxazole scaffold. *Bioorg Med Chem Lett*. 2021;52:960–1894. https://doi.org/10.1016/j.bmcl.2021.128403.
155. Albrecht BK et al. Identification of a benzoisoxazoloazepine inhibitor (CPI-0610) of the bromodomain and extra-terminal (BET) family as a candidate for human clinical trials. *J Med Chem*. 2016;59:1330–1339. https://doi.org/10.1021/acs.jmedchem.5b01882.
156. Siu KT et al. Preclinical activity of CPI-0610, a novel small-molecule bromodomain and extra-terminal protein inhibitor in the therapy of multiple myeloma. *Leukemia*. 2016;31:1760–1769. https://doi.org/10.1038/leu.2016.355.
157. Harrison CN et al. Phase III MANIFEST-2: pelabresib + ruxolitinib vs placebo + ruxolitinib in JAK inhibitor treatment-naive myelofibrosis. *Future Oncol*. 2022;18:2987–2997. https://doi.org/10.2217/FON-2022-0484.
158. Grillone K et al. The new microtubule-targeting agent SIX2G induces immunogenic cell death in multiple myeloma. *Int J Mol Sci*. 2022;23. https://doi.org/10.3390/IJMS231810222/S1.
159. Barreca M et al. Identification of pyrrolo[3′,4′:3,4]cyclohepta[1,2-d][1,2] oxazoles as promising new candidates for the treatment of lymphomas. *Eur J Med Chem*. 2023;254. https://doi.org/10.1016/j.ejmech.2023.115372.
160. Feriotto G et al. Cytotoxicity of isoxazole curcumin analogs on chronic myeloid leukemia-derived K562 cell lines sensitive and resistant to imatinib. *Int J Mol Sci*. 2023;24. https://doi.org/10.3390/IJMS24032356.
161. Aissa I et al. Glioblastoma-specific anticancer activity of newly synthetized 3,5-disubstituted isoxazole and 1,4-disubstituted triazole-linked tyrosol conjugates. *Bioorg Chem*. 2021;114, 105071. https://doi.org/10.1016/J.BIOORG.2021.105071.
162. Abou-Seri SM, Eissa AAM, Behery MGM, Omar FA. Synthesis, in vitro anticancer activity and in silico studies of certain isoxazole-based carboxamides, ureates, and hydrazones as potential inhibitors of VEGFR2. *Bioorg Chem*. 2021;116. https://doi.org/10.1016/j.bioorg.2021.105334.
163. Zarrinkar PP et al. AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML). *Blood*. 2009;114:2984. https://doi.org/10.1182/BLOOD-2009-05-222034.
164. Chao Q et al. Identification of N-(5-tert-butyl-isoxazol-3-yl)-N′-{4-[7-(2-morpholin-4-yl-ethoxy)imidazo[2,1-b][1,3]benzothiazol-2-yl]phenyl}urea dihydrochloride (AC220), a uniquely potent, selective, and efficacious FMS-like tyrosine kinase-3 (FLT3) inhibitor. *J Med Chem*. 2009;52:7808–7816. https://doi.org/10.1021/JM9007533.
165. Zhou F, Ge Z, Chen B. Quizartinib (AC220): a promising option for acute myeloid leukemia. *Drug Des Devel Ther*. 2019;13:1117. https://doi.org/10.2147/DDDT.S198950.
166. Sellmer A et al. A series of novel aryl-methanone derivatives as inhibitors of FMS-like tyrosine kinase 3 (FLT3) in FLT3-ITD-positive acute myeloid leukemia. *Published Online*. 2020. https://doi.org/10.1016/j.ejmech.2020.112232.
167. Yao R, Zhang Y, Zeng Y, Zhang Y, Liu L, Gao J. Novel c-Myc G4 stabilizer EP12 promotes myeloma cytotoxicity by disturbing NF-κB signaling. *Exp Cell Res*. 2023;431, 113759. https://doi.org/10.1016/j.yexcr.2023.113759.
168. Zhu P, Zhang J, Yang Y, Wang L, Zhou J, Zhang H. Design, synthesis and biological evaluation of isoxazole-containing biphenyl derivatives as small-

molecule inhibitors targeting the programmed cell death-1/ programmed cell death-ligand 1 immune checkpoint. *Mol Divers*. 2022;26:245–264. https://doi.org/10.1007/s11030-021-10208-4.

169. Moore AS et al. Selective FLT3 inhibition of FLT3-ITD+ acute myeloid leukaemia resulting in secondary D835Y mutation: a model for emerging clinical resistance patterns. *Leukemia*. 2012;26:1462. https://doi.org/10.1038/LEU.2012.52.
170. Talha A et al. Ultrasound-assisted one-pot three-component synthesis of new isoxazolines bearing sulfonamides and their evaluation against hematological malignancies. *Ultrason Sonochem*. 2021;78. https://doi.org/10.1016/j.ultsonch.2021.105748.
171. Bellon M, Nicot C. Targeting Pim kinases in hematological cancers: molecular and clinical review. *Mol Cancer*. 2023;22:1–25. https://doi.org/10.1186/S12943-023-01721-1.
172. Rosenzweig M et al. Repurposing leflunomide for relapsed/refractory multiple myeloma: a phase 1 study. *Leuk Lymphoma*. 2020;61:1669–1677. https://doi.org/10.1080/10428194.2020.1742900.
173. Adamczuk G, Humeniuk E, Iwan M, Natorska-Chomicka D, Adamczuk K, Korga-Plewko A. The mitochondria-independent cytotoxic effect of leflunomide on RPMI-8226 multiple myeloma cell line. *Molecules*. 2021;26:5653. https://doi.org/10.3390/MOLECULES26185653.
174. Shi ZH, Liu FT, Tian HZ, Zhang YM, Li NG, Lu T. Design, synthesis and structure-activity relationship of diaryl-ureas with novel isoxazol(3,4-b) pyridine-3-amino-structure as multi-target inhibitors against receptor tyrosine kinase. *Bioorg Med Chem*. 2018;26(16):4735–4744. https://doi.org/10.1016/j.bmc.2018.08.013.