Journal of Natural Science Review

Vol. 4, No.2, 2026
https://kujnsr.com
e-ISSN: 3006-7804

Synthesis of Oxindole conjugated Imidazo[2,1-b][1,3,4]thiadiazole as BCL-2 inhibitors with anticancer activity

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ABSTRACT

The anti-apoptotic protein BCL-2 is overexpressed in a wide range of human malignancies and plays a pivotal role in tumor progression, therapeutic resistance, and evasion of programmed cell death. Consequently, selective inhibition of BCL-2 has emerged as an attractive strategy in anticancer drug discovery. Despite the clinical success of currently available BCL-2 inhibitors, many compounds still suffer from limitations, including off-target toxicity, resistance development, and adverse hematological effects, underscoring the need for safer, more effective therapeutic alternatives. This study aimed to synthesize oxindole-conjugated imidazo[2,1-b][1,3,4] thiadiazoles inspired by the structural framework of Disarib, a selective BCL-2 inhibitor known for its unique BH1-domain binding mode and minimal platelet toxicity. A molecular hybridization strategy was employed to combine imidazothiadiazole and oxindole pharmacophores into a single scaffold. The target compounds were synthesized via a multistep synthetic route that involved construction of the 1,3,4-thiadiazole core, cyclization to the imidazothiadiazole framework, Vilsmeier–Haack formylation, and final condensation with substituted oxindoles. Structural characterization was accomplished using ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectrometry (HRMS). Three derivatives were successfully obtained in good yields (80–84%) and exhibited high structural stability. Spectroscopic analyses confirmed the successful formation of the desired molecular frameworks. Furthermore, the incorporation of chlorine, bromine, and methoxy substituents was expected to modulate electronic properties and enhance interactions with the BCL-2 binding site. These findings suggest that the synthesized compounds are promising candidates for the development of selective BCL-2-targeted anticancer agents and warrant further biological and pharmacological evaluation.

ARTICLE INFO

Article history:

Received: April20, 2026
Revised: May20, 2026
Accepted: June15, 2026
Published: June 30, 2026

Keywords:

Anticancer; BCL-2 inhibitor; Imidazothiadiazole; Oxindole; Toxicity

To cite this article: Nasiri, M.A., Ahmadi, I., & Fekrat, N. (2026). Synthesis of Oxindole conjugated Imidazo[2,1-b][1,3,4]thiadiazole as BCL-2 inhibitors with anticancer activity. *Journal of Natural Science Review*, 4 (2), 577-600. https://doi.org/10.62810/jnsr.v4i2.523

Link to this article: https://kujnsr.com/JNSR/article/view/523

INTRODUCTION

Cancer remains one of the most serious global health challenges and is characterized by uncontrolled cellular proliferation, resistance to apoptosis, invasion of surrounding tissues,

and metastasis. Despite remarkable advances in chemotherapy, radiotherapy, and targeted therapy, many cancers still exhibit resistance to treatment, leading to poor clinical outcomes and high mortality rates. One of the major molecular mechanisms responsible for this resistance is the dysregulation of apoptosis, particularly through the overexpression of anti-apoptotic proteins in the BCL-2 Family (Adams & Cory, 2007). Among these proteins, BCL-2 (B-cell lymphoma 2) plays a critical role in promoting cancer cell survival by preventing mitochondrial-mediated apoptosis. Elevated BCL-2 expression has been reported in several malignancies, including leukemia, lymphoma, breast cancer, and lung cancer, making it an attractive target for anticancer drug development.

Over the past decades, significant efforts have been devoted to the discovery of selective BCL-2 inhibitors. Although currently available inhibitors such as venetoclax (ABT-199) have shown promising therapeutic efficacy (Roberts et al., 2016), they are still associated with several limitations, including resistance development, hematological toxicity, gastrointestinal complications, and undesirable off-target effects. Furthermore, many early-generation BCL-2 inhibitors also inhibited BCL-XL, causing thrombocytopenia due to platelet toxicity. Therefore, the development of novel BCL-2 inhibitors with improved selectivity, enhanced anticancer efficacy, and reduced adverse effects remains an urgent need in medicinal chemistry and cancer therapeutics (Li et al, 2012).

Recently, Disarib, a novel small-molecule BCL-2 inhibitor, has attracted considerable scientific attention due to its unique mechanism of action. Unlike conventional BH_3 mimetics, Disarib predominantly binds to the BH1 domain of BCL-2 and selectively disrupts the BCL-2–BAK interaction, thereby inducing apoptosis with minimal platelet toxicity. These promising biological properties suggest that Disarib may serve as an excellent lead compound for further structural optimization and drug development (Campos et al, 1993).

In medicinal chemistry, heterocyclic compounds are recognized as important structural motifs in the design of biologically active molecules. Among them, imidazo[2,1-b][1,3,4]thiadiazole derivatives exhibit a wide range of pharmacological activities, particularly anticancer properties, owing to their favorable electronic and structural properties. Similarly, oxindole derivatives have gained considerable importance due to their antiproliferative and kinase-inhibitory activities (Rashdan et al., 2021), and several oxindole-based drugs are already used clinically in cancer therapy (Willis et al., 2007). Therefore, the molecular hybridization of imidazothiadiazole and oxindole scaffolds represents a rational strategy for designing novel compounds with enhanced biological activity and improved pharmacological profiles (Montero & Letai, 2018).

Based on these scientific considerations, the present study focuses on the design and synthesis of novel oxindole-conjugated imidazo[2,1-b][1,3,4]thiadiazole derivatives as potential BCL-2 inhibitors. The incorporation of electron-withdrawing halogens and electron-donating substituents was intended to investigate structure–activity relationships and improve target specificity. The synthesized compounds were fully characterized using modern spectroscopic techniques, including ^{1}H NMR, ^{13}C NMR, and HRMS.

The objectives of this scientific research are:

- To design and synthesize novel oxindole-conjugated imidazo[2,1-b][1,3,4]thiadiazole derivatives inspired by the structure of Disarib as potential BCL-2 inhibitors.
- To characterize the synthesized compounds using advanced spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR, and High-Resolution Mass Spectrometry (HRMS).
- To investigate the influence of different substituents, including halogens and methoxy groups, on the structural and potential biological properties of the synthesized derivatives.
- To evaluate the potential anticancer activity and BCL-2 inhibitory properties of the synthesized compounds for future development of safer and more effective targeted anticancer agents.

METHODS AND MATERIALS

This study employed an integrated experimental and literature-based approach to design, synthesize, and characterize novel oxindole-conjugated imidazo[2,1-b][1,3,4]thiadiazole derivatives as potential BCL-2 inhibitors. A systematic review of relevant scientific literature was conducted to support compound design and structure–activity relationship (SAR) investigations. The target compounds were synthesized using established organic synthetic procedures and purified by conventional laboratory techniques. Reaction progress and purity were monitored by thin-layer chromatography (TLC), while structural characterization was performed using melting point analysis, ^{1}H NMR, ^{13}C NMR, and High-Resolution Mass Spectrometry (HRMS). Experimental data, including reaction yields, physicochemical properties, and spectroscopic results, were analyzed qualitatively and comparatively to confirm molecular structures and assess the influence of different substituents on the synthesized derivatives. The reliability and validity of the findings were ensured through standardized experimental protocols and advanced analytical techniques. More details are provided below:

Research site

This research was conducted at two academic institutions. The theoretical and literature review components were carried out at the Faculty of Natural Sciences, Bamyan University, Afghanistan, and the University of Mysore, Karnataka (India). The experimental work, including chemical synthesis, purification, and preliminary characterization of the synthesized compounds, was performed in the Organic Chemistry Laboratory of the University of Mysore. Spectroscopic analyses, including ^{1}H NMR, ^{13}C NMR, and HRMS, were conducted using specialized instrumentation available in collaborative research laboratories associated with the University of Mysore.

Data Collection Instruments

Data collection in this study was carried out using a combination of laboratory equipment, analytical instruments, and scientific databases. For the theoretical component of the research, scientific literature was collected through comprehensive searches of major academic databases, including Scopus, PubMed, and Google Scholar. These databases were used to identify relevant publications concerning BCL-2 inhibitors, Disarib, oxindole derivatives, imidazo[2,1-b][1,3,4]thiadiazoles, anticancer activity, and apoptosis. For the experimental component, several laboratory instruments were employed for synthesis, purification, monitoring, and characterization of the synthesized compounds. Melting points were determined using a Thomas Hoover melting point apparatus. Reaction progress and compound purity were monitored by thin-layer chromatography (TLC) using silica gel 60F254 plates (Merck) and visualized under ultraviolet (UV) light. Synthetic reactions were carried out using standard organic chemistry equipment, including reflux apparatus, oil baths, magnetic stirrer-heaters, and a high-precision digital analytical balance. Structural characterization and confirmation of the synthesized compounds were performed using advanced spectroscopic instruments. Proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded using Fourier Transform NMR spectrometers operating at 300 and 400 MHz. High-resolution molecular mass determination was conducted using a High-Resolution Mass Spectrometry (HRMS) instrument. These analytical techniques provided essential data for structural verification, purity assessment, and confirmation of the molecular composition of the synthesized oxindole-imidazo[2,1-b][1,3,4]thiadiazole derivatives.

Data Collection Method

A systematic literature review was conducted following a structured screening process. Relevant articles published between 1993 and 2021 were identified through searches in Scopus, PubMed, and Google Scholar using predefined keywords related to BCL-2 inhibitors, oxindole, and imidazothiadiazole derivatives. After removing duplicate and irrelevant records, eligible studies were selected based on predefined inclusion criteria, and the extracted data were analyzed to compare reported biological activities and support structure-activity relationship (SAR) investigations. During the experimental phase, data were generated by synthesizing target compounds using established organic synthesis procedures. Experimental observations and measurements were recorded during reaction monitoring, purification, and characterization processes. Data collection included determination of reaction yields, melting point measurements, thin-layer chromatography (TLC) analysis, and structural characterization using spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR, and High-Resolution Mass Spectrometry (HRMS). The collected data were subsequently organized and used for qualitative comparison, structural verification, and structure-activity relationship (SAR) evaluation of the synthesized compounds.

Sampling Method

A purposive sampling method was employed in the theoretical component of this study. Scientific articles were initially identified through a systematic search of major academic databases using predefined keywords related to BCL-2 inhibitors, Disarib, oxindole derivatives, and imidazo[2,1-b][1,3,4]thiadiazoles. From the 68 articles initially retrieved, only studies containing relevant experimental data on compound synthesis and BCL-2 inhibitory activity were selected according to predefined inclusion criteria. As a result, 26 articles were chosen as the primary sources for literature review and analysis. In the experimental phase, target compounds were selected and synthesized using a rational drug design strategy derived from the structural features of Disarib and the intended structure-activity relationship (SAR) investigation.

Data Analysis

Data analysis was conducted using a qualitative and comparative approach. The synthesized compounds were evaluated based on key experimental parameters, including reaction yield, melting point, purity, and spectroscopic characteristics obtained from ^{1}H NMR, ^{13}C NMR, and HRMS analyses. Structural confirmation was achieved through interpretation of spectral data and comparison with the expected molecular structures. Furthermore, the influence of different electron-donating and electron-withdrawing substituents on the physicochemical and structural properties of the synthesized derivatives was assessed through structure–activity relationship (SAR) analysis. The findings were subsequently compared with previously reported data on Disarib and other BCL-2 inhibitors to assess the potential biological relevance and anticancer activity of the newly synthesized compounds.

Validity and Reliability

The validity and reliability of the experimental results were ensured through the use of standardized synthetic procedures and well-established analytical techniques. Structural characterization of the synthesized compounds was confirmed using ^{1}H NMR, ^{13}C NMR, and HRMS analyses, while compound purity was assessed through melting point determination and thin-layer chromatography (TLC). The consistency of the obtained data and comparison with previously reported literature further enhanced the reliability and accuracy of the study findings.

FINDING

In this study, three novel oxindole-imidazo[2,1-b][1,3,4]thiadiazole derivatives were designed and synthesized as potential inhibitors of the anti-apoptotic BCL-2 protein, inspired by the Disarib structure. The design strategy was based on molecular hybridization, combining the imidazothiadiazole core with the oxindole moiety to enhance selective binding to BCL-2 while reducing side effects.

The synthesis results demonstrated that the selected multistep synthetic pathway, including formation of the 1,3,4-thiadiazole core, cyclization to generate the

imidazothiadiazole ring system, Vilsmeier–Haack formylation, and final condensation with substituted oxindoles, was successfully achieved. The final compounds were obtained in satisfactory yields of 80%-84%. The synthesized compounds were isolated as yellow solids with relatively high melting points (185–198 °C), indicating good structural stability.

The ^{1}H NMR and ^{13}C NMR spectroscopic analyses confirmed the presence of all expected functional groups. In the proton NMR spectra, the NH proton signal observed around 10.7 ppm confirmed the oxindole structure. In addition, signals corresponding to the methoxy group in the methoxy-substituted derivative, along with multiple aromatic proton signals, indicated successful formation of the target molecules. The ^{13}C NMR spectra also showed all expected carbon signals, including aromatic, methoxy, and carbonyl carbons, within the appropriate chemical shift regions. Furthermore, High-Resolution Mass Spectrometry (HRMS) results showed excellent agreement between the calculated and experimental molecular masses, confirming the purity and structural accuracy of the synthesized compounds.

In this study, the effects of different substituents, including chlorine, bromine, and methoxy groups, on the electronic and structural properties of the compounds were investigated. The presence of halogen atoms such as chlorine and bromine, due to their electron-withdrawing nature, may enhance hydrophobic interactions and improve binding to the active site of the BCL-2 protein. On the other hand, the methoxy group, as an electron-donating substituent, may contribute to modulating electron distribution and stabilizing molecular interactions. These structural modifications may ultimately lead to improved anticancer activity and enhanced selectivity for BCL-2 inhibition.

Comparison of the synthesized compounds with previously reported BCL-2 inhibitors suggests that the newly designed molecular framework possesses promising characteristics for the development of targeted anticancer agents. Since Disarib binds to the BH1 domain of BCL-2 with minimal platelet toxicity, it is anticipated that the newly synthesized derivatives may retain this advantage while exhibiting enhanced anticancer efficacy.

Overall, the findings of this study demonstrated that:

1. Novel oxindole-imidazothiadiazole derivatives were successfully synthesized.
2. The synthetic method provided satisfactory yields and good reproducibility.
3. NMR and HRMS analyses confirmed the final structures of all synthesized compounds.
4. Halogen and methoxy substituents may improve the pharmacological properties of the compounds.
5. The synthesized compounds possess potential as novel selective BCL-2 inhibitors for the treatment of cancers associated with BCL-2 overexpression.

Based on these findings, further biological studies, including cytotoxicity assays, evaluation of BCL-2 inhibitory activity, platelet toxicity assessment, and in vivo

investigations, are necessary to fully determine the anticancer potential and safety profile of these compounds.

BCL-2 (B-cell lymphoma 2)

The BCL2 gene encodes BCL-2, a key member of the BCL-2 protein family that plays a central role in regulating programmed cell death (apoptosis). Members of this family can either promote or suppress apoptosis; however, BCL-2 itself primarily functions as an anti-apoptotic protein that enhances cell survival by preventing apoptotic signaling pathways (Youle & Strasser, 2008). The name BCL-2 originates from B-cell lymphoma 2, reflecting its initial identification in chromosomal translocations involving chromosomes 14 and 18 that were associated with follicular lymphoma. This discovery established BCL-2 as one of the first proteins linked to the regulation of apoptosis in cancer development (Petros et al, 2001).

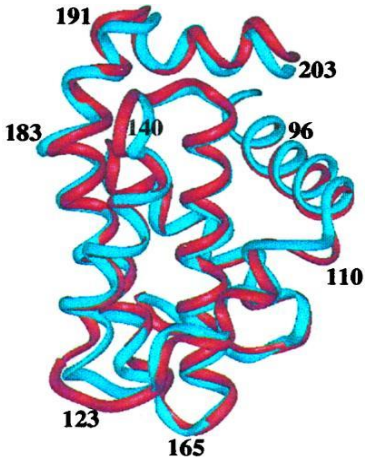


Fig 1. *Comparison of the backbone structures of BCL-2 isoforms 1 and 2. The backbone of BCL-2(1) is represented in red, whereas BCL-2(2) is depicted in blue (adapted from Petros et al, 2001)*

Isoforms

BCL-2 exists in at least two major isoforms, commonly referred to as Isoform 1 (1G5M) and Isoform 2 (1G5O/1GJH). Although both isoforms share a highly similar overall three dimensional architectures, variations in their interactions with pro-apoptotic proteins such as BAD and BAK, together with differences in the topology and electrostatic characteristics of their binding grooves, indicate that they may differ in their capacity to suppress apoptosis (Zitvogel et al, 2013).

Mitochondria play a central role in regulating both apoptotic and necrotic forms of cell death. Following exposure to many anticancer agents or other cellular stress signals, cytochrome c is released from the mitochondrial intermembrane space into the cytoplasm. Once released, cytochrome c associates with apoptotic protease-activating factor-1 (Apaf-1), leading to the activation of initiator and executioner caspases that ultimately drive the apoptotic cascade. Conversely, depletion of mitochondrial cytochrome c may impair electron transport chain function, resulting in excessive generation of reactive oxygen species (ROS), reduced ATP production, and eventual necrotic cell death (Kirkin et al, 2004).

Anti-apoptotic members of the BCL-2 family, particularly BCL-2 and BCL-XL, contribute to cell survival by preventing cytochrome c release from mitochondria and by interfering with caspase activation through interactions with Apaf-1. Furthermore, once activated, caspases can amplify mitochondrial dysfunction, creating a positive feedback mechanism that promotes additional cytochrome c release and accelerates the cell death process (Adams & Cory, 2007).

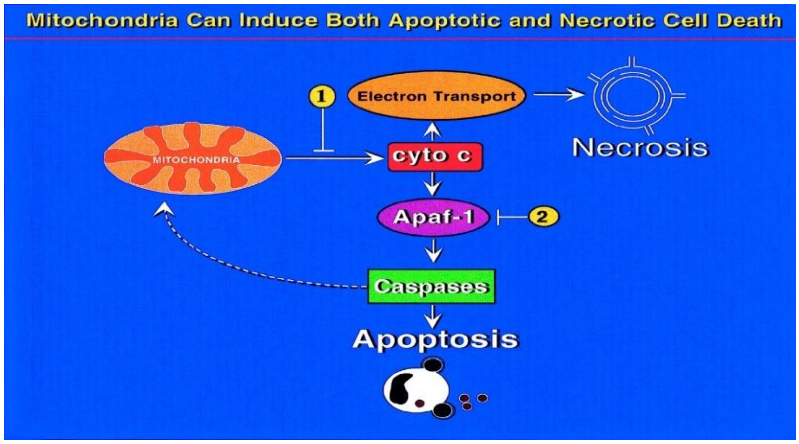


Fig 2. *Schematic representation of mitochondrial involvement in apoptotic and necrotic cell death pathways (adapted from Adams & Cory, 2007)*

Normal physiological function

BCL-2 is primarily located on the outer membrane of mitochondria, where it plays a crucial role in maintaining cell viability by counteracting apoptotic signaling pathways. Members of the pro-apoptotic BCL-2 family, particularly Bax and Bak, facilitate mitochondrial outer membrane permeabilization, leading to the release of cytochrome c and initiation of the apoptotic cascade. The activity of these proteins is regulated by BH3-only proteins, whereas anti-apoptotic proteins such as BCL-2 act to suppress their function and prevent cell death.

Beyond its established role in apoptosis regulation, BCL-2 has been implicated in several additional cellular processes. Emerging evidence suggests that this protein contributes to the regulation of mitochondrial dynamics by influencing fusion and fission events that are essential for mitochondrial homeostasis. Furthermore, BCL-2 and the related protein BCL-XL participate in the regulation of metabolic functions in pancreatic β-cells, including insulin secretion and energy metabolism. Experimental studies have shown that inhibition of BCL-2 can enhance metabolic activity, indicating that the protein may provide metabolic protection under conditions of increased cellular demand (Delbridge et al., 2016).

Role of BCL2 in cancer

BCL-2 is a key regulator of programmed cell death and contributes to the maintenance of cellular homeostasis by preventing unnecessary apoptosis. Under normal physiological conditions, this function supports tissue integrity and cell survival. However, the controlled

elimination of damaged, aged, or abnormal cells through apoptosis is essential for preventing the accumulation of potentially harmful cells (Cotter, 2009).

Dysregulation of apoptotic pathways is a hallmark of many cancers. When apoptosis is impaired, abnormal cells can evade cell death signals, continue proliferating, and ultimately contribute to tumor development. Because BCL-2 suppresses apoptotic mechanisms, its overexpression has been associated with enhanced survival of malignant cells. Consequently, inhibition of BCL-2 has emerged as an important therapeutic strategy for limiting cancer progression and restoring apoptotic responses in tumor cells (Souers et al., 2013).

Despite the therapeutic potential of BCL-2-targeted agents, the development of highly selective inhibitors remains challenging. Members of the BCL-2 protein family possess considerable structural homology, although they perform distinct biological functions. As a result, many inhibitors designed to target BCL-2 may also interact with related family members, leading to off-target effects and treatment-associated toxicities. One important concern is the unintended impact on platelet survival, which is regulated by anti-apoptotic proteins within the BCL-2 family. Such interactions may result in thrombocytopenia and other hematological complications (Reed et al., 1996).

An ideal BCL-2 inhibitor should therefore selectively induce apoptosis in cancer cells while minimizing toxicity to normal tissues and preserving platelet function. Venetoclax (ABT-199), the first selective BCL-2 inhibitor approved by the U.S. Food and Drug Administration (FDA), represents a major advance in this field because it effectively promotes apoptosis in malignant cells with reduced platelet toxicity. Nevertheless, clinical use of Venetoclax may still be associated with adverse effects, including neutropenia, anemia, gastrointestinal disturbances, and, in some patients, more severe complications such as pneumonia or tumor lysis syndrome (Ashkenazi et al., 2017).

Diagnostic use

Antibodies to BCL-2 can be used with immunohistochemistry to identify cells containing the antigen. In healthy tissue, these antibodies react with B-cells in the mantle zone, as well as some T-cells. However, positive cells increase considerably in follicular lymphoma, as well as many other forms of cancer. In some cases, the presence or absence of BCL-2 staining in biopsies may be significant for the patient's prognosis or likelihood of relapse (Reed, 1997).

Inside a cell, there is always a balance between proteins that promote cell death (apoptosis) and those that suppress it. Since the proteins (BAX and BAK) that promote cell death get bound to BCL2, normal cell death is suppressed, and cancer cells can live longer. Disarib disrupted the interaction between BCL2 and the apoptosis-promoting BAK protein, leading to the death of cancer cells (Hamilton et al., 2021).

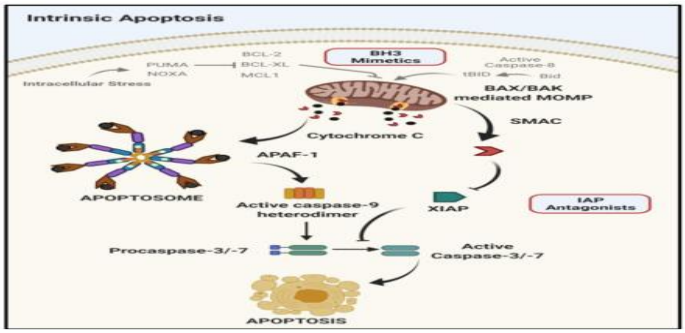


Fig 3*. Intrinsic pathway of apoptosis (Hamilton et al, 2021)*

The mitochondrial (intrinsic) pathway of apoptosis is controlled largely by members of the BCL-2 protein family. In response to intracellular stress signals, BH3-only proteins become activated and counteract the protective effects of anti-apoptotic proteins such as BCL-2. As a consequence, the pro-apoptotic effectors BAX and BAK undergo activation, promoting permeabilization of the mitochondrial outer membrane and facilitating the release of apoptogenic factors, including cytochrome c and SMAC, into the cytosol (Ferri & Kroemer, 2001).

Following its release, cytochrome c associates with apoptotic protease-activating factor-1 (APAF1), leading to apoptosome formation and subsequent activation of caspase-9. Activated caspase-9 initiates a proteolytic cascade by stimulating executioner caspases, particularly caspase-3 and caspase-7, which ultimately execute the apoptotic program. In parallel, SMAC promotes cell death by antagonizing X-linked inhibitor of apoptosis protein (XIAP), thereby relieving its inhibitory effect on caspases and enhancing apoptotic signaling (Hamilton et al., 2021).

Disarib as a BCL-2 inhibitor

Disarib is a recently developed small-molecule inhibitor that selectively targets the anti-apoptotic protein BCL-2. Because BCL-2 is frequently overexpressed in many malignant cells while being present at much lower levels in normal tissues, it represents an attractive target for cancer therapy. By interacting with BCL-2, Disarib interferes with its survival-promoting function and facilitates apoptotic cell death in tumor cells. Experimental studies have demonstrated that this compound exhibits significant antitumor activity with minimal effects on normal cells, highlighting its potential as a selective anticancer agent. The preferential targeting of BCL-2 by Disarib makes it a promising candidate for the development of safer and more effective therapies against BCL-2-dependent malignancies (Dandawate et al., 2020).

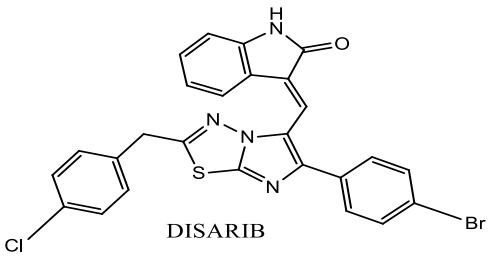


Fig 4*. Show Disarib (Dandawate et al., 2020)*

Disarib developed to target the anti-apoptotic protein BCL-2 and restore apoptotic signaling in cancer cells. Programmed cell death is controlled by a delicate balance between pro-apoptotic and anti-apoptotic members of the BCL-2 family. Because elevated BCL-2 expression is frequently observed in malignant cells, selective inhibition of this protein has emerged as an attractive therapeutic strategy with the potential to minimize toxicity to normal tissues (Iyer & Raghavan, 2013).

Unlike conventional BCL-2 inhibitors that primarily interact with the BH3-binding groove, Disarib exhibits a distinct binding profile by preferentially associating with the BH1 domain of BCL-2. This unique interaction selectively interferes with the BCL-2–BAK complex while having little effect on the association of BCL-2 with other pro-apoptotic proteins such as BAX, BIM, and PUMA. Biochemical analyses have demonstrated strong inhibition of the BCL-2–BAK interaction, supporting the ability of Disarib to promote apoptosis through a BAK-dependent mechanism (Iyer et al., 2016).

Further experimental evidence obtained from BAX- and BAK-deficient cell models confirmed that the apoptotic activity of Disarib relies predominantly on BAK activation. In addition, fluorescence resonance energy transfer (FRET) studies revealed disruption of the BCL-2–BAK interaction and subsequent activation of the intrinsic apoptotic pathway following treatment with Disarib. These findings suggest that the compound possesses a distinctive mechanism of action and may represent a promising alternative to currently available BCL-2-targeted therapies (Iyer et al., 2016).

The cytotoxic potential of Disarib has also been evaluated in several cancer cell lines exhibiting different levels of BCL-2 expression. Experimental results demonstrated that cells expressing higher amounts of BCL-2 were more sensitive to treatment, showing a substantial reduction in viability after exposure to the compound. In contrast, only limited toxicity was observed in non-malignant cells, indicating a degree of selectivity toward cancerous tissues. Moreover, the observed anticancer activity correlated strongly with BCL-2 expression levels, supporting the conclusion that the biological effects of Disarib are primarily mediated through BCL-2 inhibition rather than nonspecific chemical reactivity of its molecular scaffold (Dandawate et al., 2020).

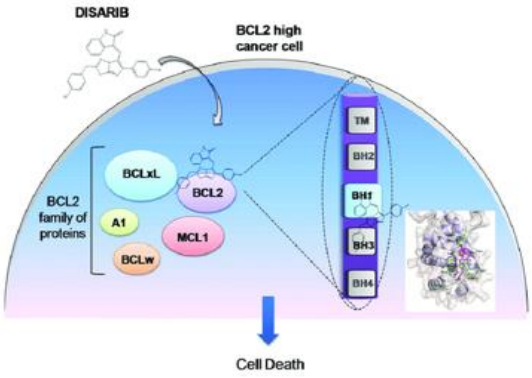


Fig 5. *Disarib selectively targets cancer cells with high BCL2 expression and inhibits BCL2 activity with limited impact on other anti-apoptotic proteins such as BCL-XL, MCL1, BCL-w, and BCL2A1. It is thought to bind primarily to the BH1 domain of BCL2, representing a unique inhibitory mechanism that leads to*

The most significant properties of disarib are: (Dandawate et al, 2020).

1. Disarib triggers cancer cell death independently of ROS generation and without causing cell cycle arrest; mechanistic studies using ex vivo assays in highly BCL2-expressing NALM6 cells (the most sensitive line tested) were used to evaluate its effects.
2. The compound causes loss of mitochondrial membrane potential, which contributes to the induction of apoptosis in treated cells.
3. It promotes programmed cell death primarily through activation of the intrinsic apoptotic signaling pathway.
4. In animal models, including both allograft and xenograft systems, Disarib leads to significant tumor regression.
5. Compared with the established BCL2 inhibitor ABT199, Disarib demonstrates superior efficacy in both in vivo and ex vivo experiments.
6. Toxicity studies indicate that Disarib has minimal adverse effects, with particularly low impact on platelet function.
7. In vivo findings further confirm that Disarib activates the intrinsic apoptosis pathway within tumor tissues.

Since disarib shows very good activity in cancer treatment, in our dissertation work we plan to synthesize its new derivatives to achieve an ideal BCL2 inhibitor with better efficacy and target specificity, with no/minimum side effects. It was envisaged that the introduction of halogens, amides, hydroxy groups, and heterocyclic compounds into the aromatic rings of disarib would generate novel molecular templates that are likely to exhibit interesting biological properties in animal models. The development of elegant synthetic methods for disarib and its derivatives is of prime importance because of their potential biological and pharmaceutical activities (Vartak et al., 2016).

Instrumentation

Analytical thin-layer chromatography (TLC) was carried out using E. Merck silica gel 60 F254 aluminum plates, with compound visualization performed under ultraviolet light. Melting points were determined using a Thomas Hoover capillary melting point apparatus and were not corrected. TLC analyses employed various solvent systems consisting of different proportions of hexane and ethyl acetate. Structural characterization of the synthesized compounds was performed using ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Spectra were recorded on Fourier transform NMR spectrometers operating at 300 and 400 MHz. Chemical shifts were expressed in parts per million (ppm) using $CDCl_3$ or DMSO-d_6 as solvents, with tetramethylsilane (TMS) serving as the internal reference standard. Signal multiplicities were described as app (apparent), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), comp (complex), and br (broad), while coupling constants were reported in hertz (Hz).

Chemicals required

- 6-Chloro oxindole

- Acetophenone and 4-Methoxyacetophenone
- Para-toluene sulphonic acid (p-TSA)
- Acetonitrile
- 4-Chlorophenylacetic acid
- Thiosemicarbazide
- Sulphuric acid
- Ethanol
- Ammonia
- $POCl_3$
- Dimethylformamide (DMF)
- Sodium Carbonate
- Sodium Bicarbonate
- Methanol
- Piperidine

All reagents and solvents listed above were of analytical grade and used as received from commercial suppliers without further purification.

General scheme for synthesis of novel oxindole-conjugated imidazo [2,1-b][1,3,4] thiadiazole

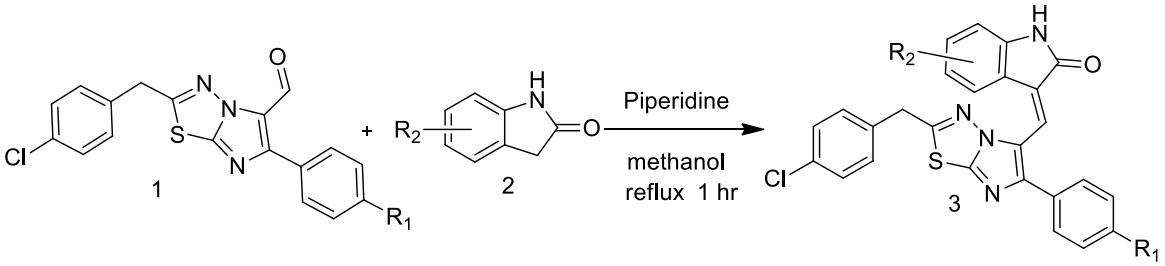


***Fig 6**. General scheme for synthesis of novel oxindole-conjugated imidazo [2,1-b][1,3,4] thiadiazole (Authors' findings)*

The substituted oxindole (1 eq) was dissolved in methanol and treated with the appropriate carbonyl compound (1 eq) and piperidine. The reaction mixture was refluxed for 1–5 h (as determined by TLC), cooled, and concentrated under reduced pressure. The resulting precipitate was collected by filtration, with a yield of 70–85%, and purified by recrystallization from ethanol.

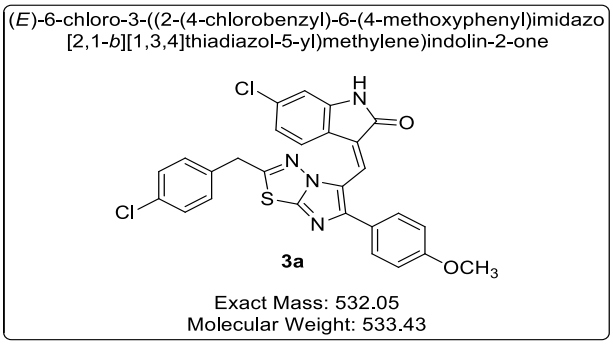
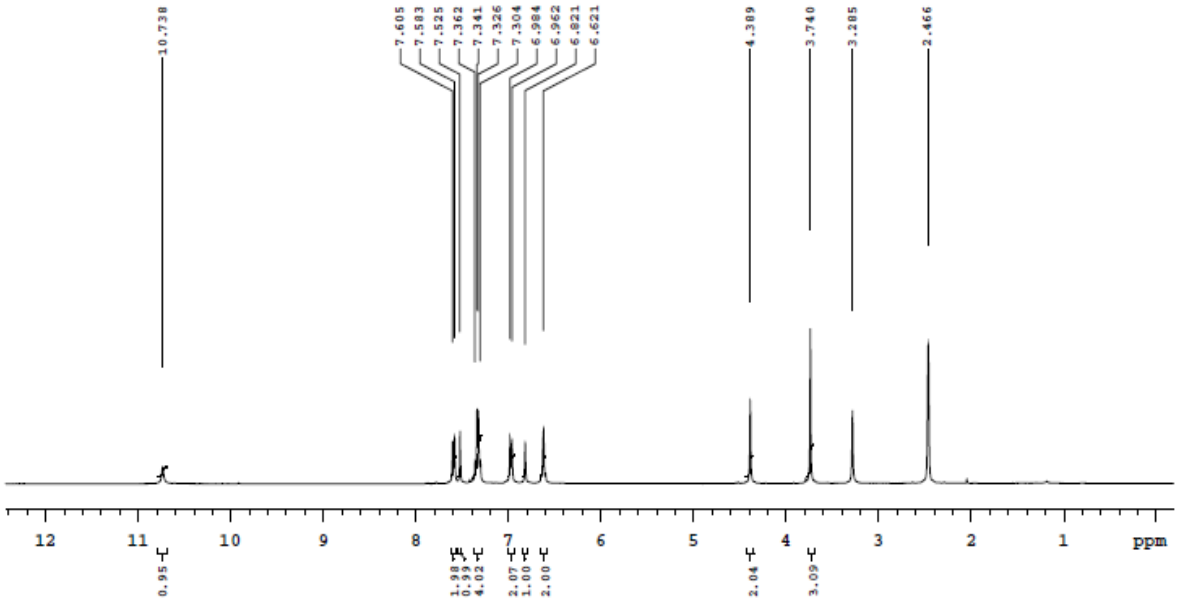


***Fig 7**. Show the compound of (E)-6-Chloro-3-((2-(4-chlorobenzyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)indolin-2-one (Authors' findings)*

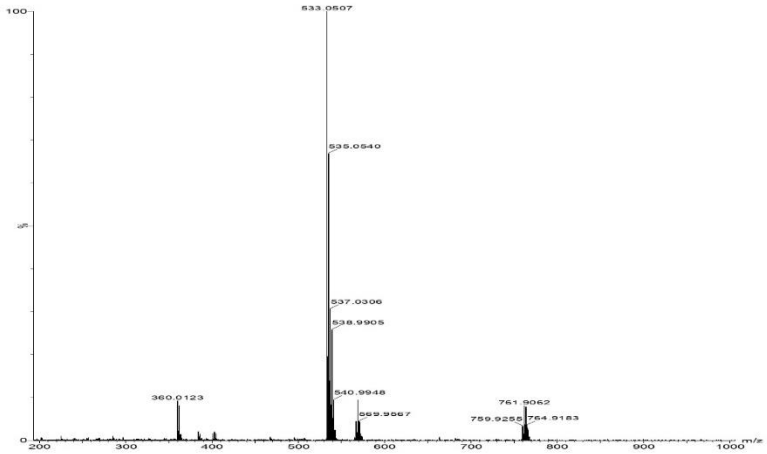
Yield 80 %; MP. 192-195 °C; yellow solid; ^{1}H-NMR (400 Hz, DMSO-d_6): δ ppm: 3.740 (s, 3H, -OCH_3), 4.389 (s, 2H, -CH_2-), 6.621 (s, 2H, Ar), 6.821 (s, 1H, Ar), 6.962-6.984 (d, 2H, Ar, 8.8 Hz), 7.304-7.362 (m, 4H, Ar), 7.525 (s, 1H, Ar), 7.583-7.605 (d, 2H, Ar, J=8.8 Hz), 10.738 (s, 1H, -NH-); ^{13}C- NMR (400 Hz, DMSO-d_6): δ ppm: 36.498, 55.674, 109.875, 114.759, 118.300, 119.127, 120.373, 121.044, 126.191, 126.531, 126.745, 129.080, 131.357, 132.573, 134.032, 135.307, 144.189, 147.633, 148.548, 159.989, 164.825, 169.096; HRMS-(ESI): m/z calculated for [$C_{27}H_{18}Cl_2N_4O_2S$ + H^+] = 533.0528: found = 533.0507.

^{1}H-NMR (400MHz-DMSO-d_6) of compound (3a)



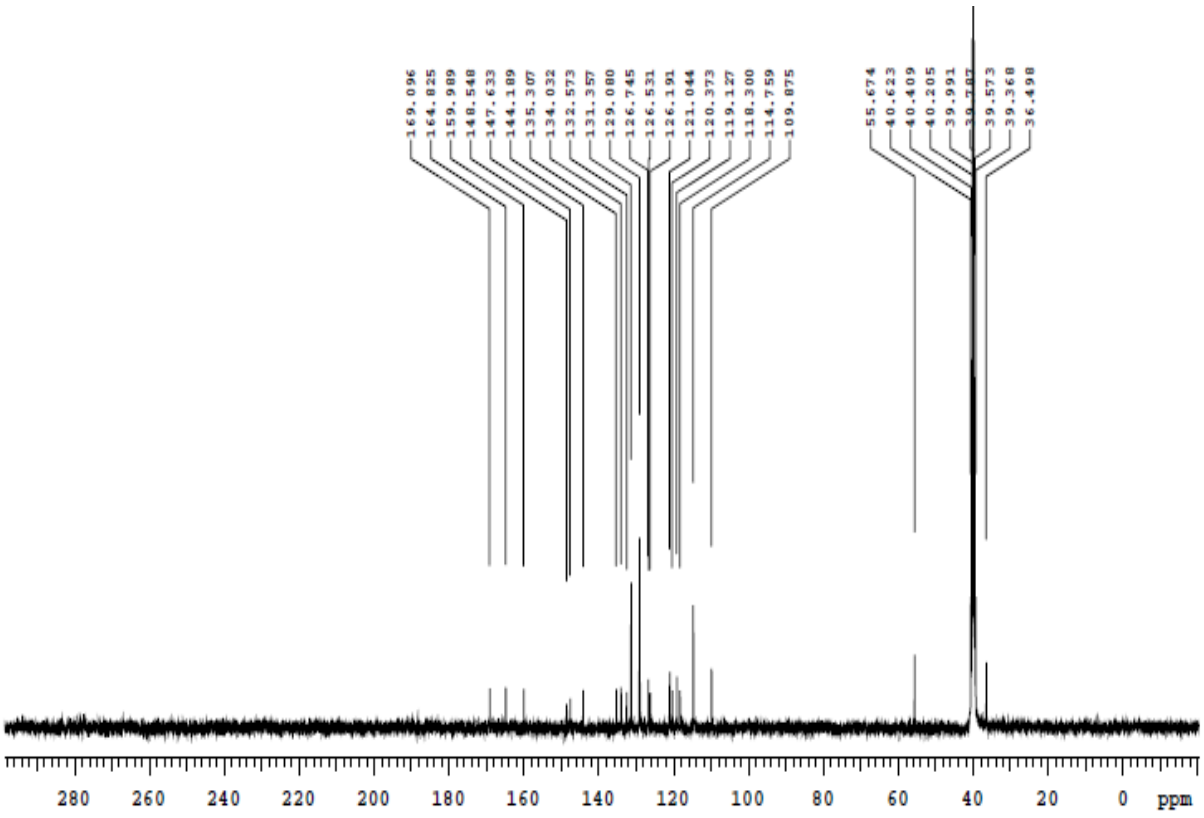
Graph.1. *The ^{1}H-NMR spectrum (400MHz-DMSO-d_6) of compound (3a) is shown above*

Mass spectra of compound (3a)



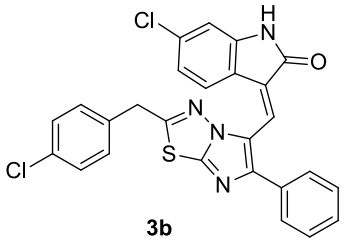
Graph 2. *The Mass spectra of compound (3a) is shown above*

^{13}C-NMR spectrum (400MHz-DMSO-d_6) of compound (3a)



Graph 3. *The ^{13}C-NMR spectrum (400MHz-DMSO-d_6) of compound (3a) is shown above*

(*E*)-6-chloro-3-((2-(4-chlorobenzyl)-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)methylene)indolin-2-one



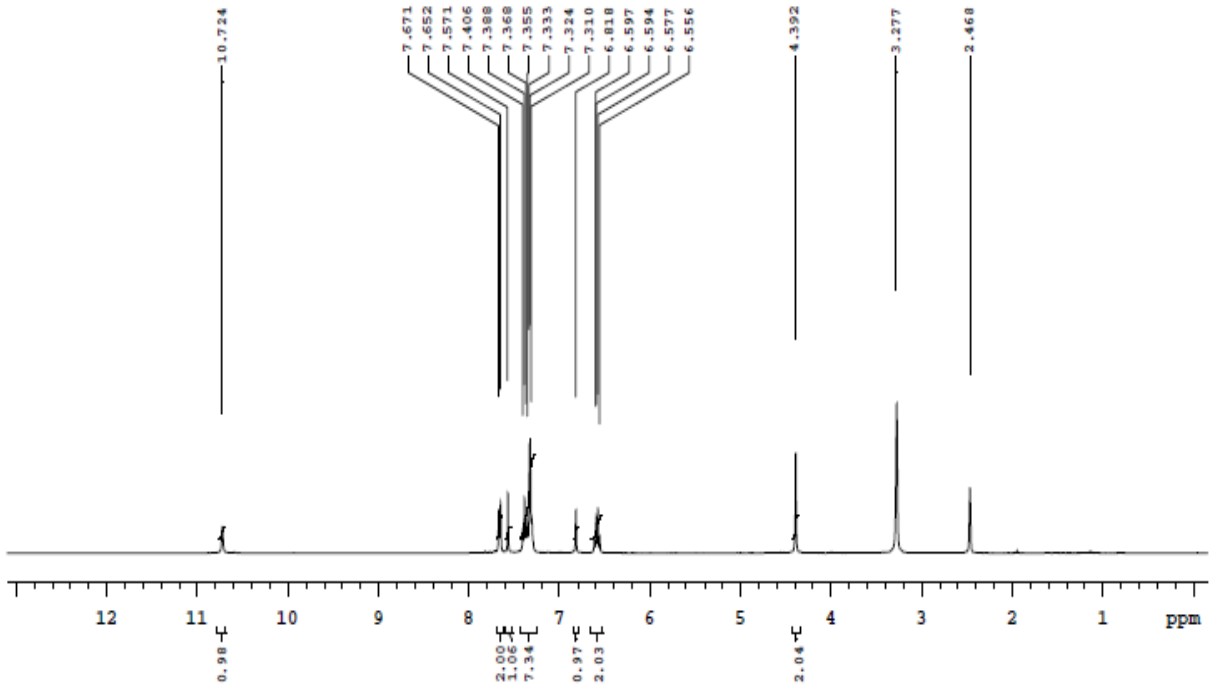
3b

Exact Mass: 502.04
Molecular Weight: 503.40

Fig 8*. Show the compound of (E)-6-Chloro-3-((2-(4-chlorobenzyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)indolin-2-one(Authors' findings)*

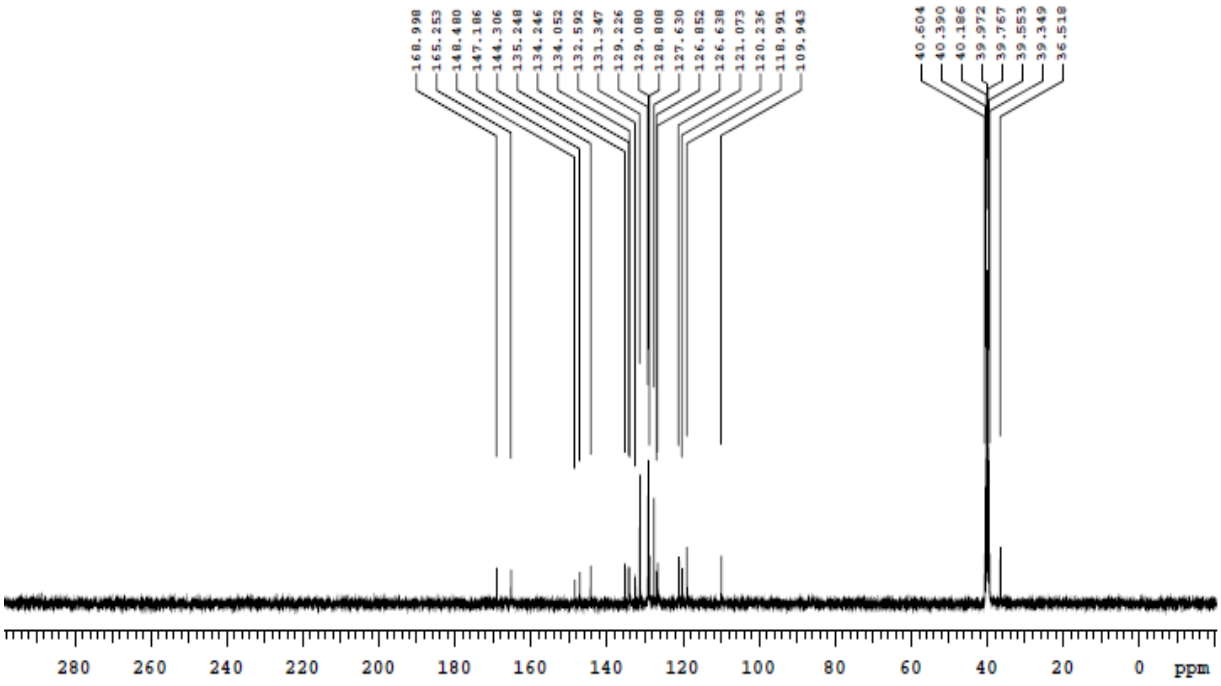
Yield 82 %; MP. 195-198 °C; yellow solid; ^{1}H-NMR (400 Hz, DMSO-d_6): δ ppm: 4.392 (s, 2H, -CH_2-), 6.556-6.597 (m, 2H, Ar), 6.818 (s, 1H, Ar), 7.310-7.406 (m, 7H, Ar), 7.571 (s, 1H, Ar), 7.652-7.671 (d, 2H, Ar, J=7.6 Hz), 10.724 (s, 1H, -NH-); ^{13}C- NMR (400 Hz, DMSO-d_6): δ ppm: 36.518, 109.943, 118.991, 120.236, 121.073, 126.638, 126.852, 127.630, 128.808, 129.080, 129.226, 131.347, 132.592, 134.052, 134.246, 135.248, 144.306, 147.186, 148.480, 165.253, 168.998; HRMS-(ESI): m/z calculated for [$C_{26}H_{16}Cl_2N_4OS + H^+$] = 503.0422: found = 502.9021.

^{1}H-NMR (400 MHz, DMSO-d_6) of compound (3b)



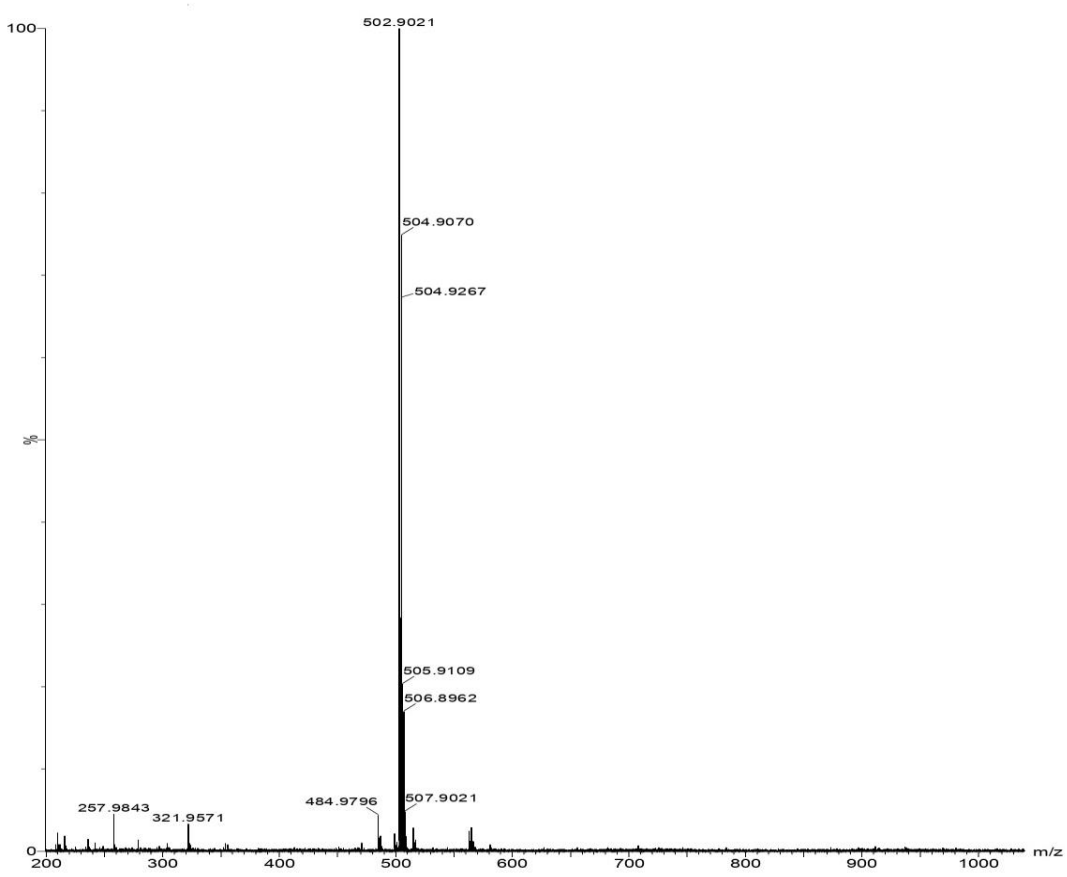
Graph 4. *The ^{1}H-NMR spectrum (400MHz-DMSO-d_6) of compound (3b) is shown above*

^{13}C-NMR (400 MHz, DMSO-d_6) of compound (3b)



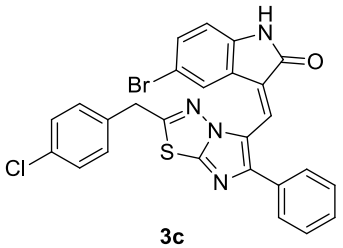
Graph 5. *The ^{13}C-NMR (400MHz-DMSO-d_6) of compound (3b) is shown above*

The Mass spectra of compound (3b)



Graph 6. *The Mass spectra of compound (3b) is shown above*

(*E*)-5-bromo-3-((2-(4-chlorobenzyl)-6-phenylimidazo[2,1-*b*][1,3,4] thiadiazol-5-yl)methylene)indolin-2-one



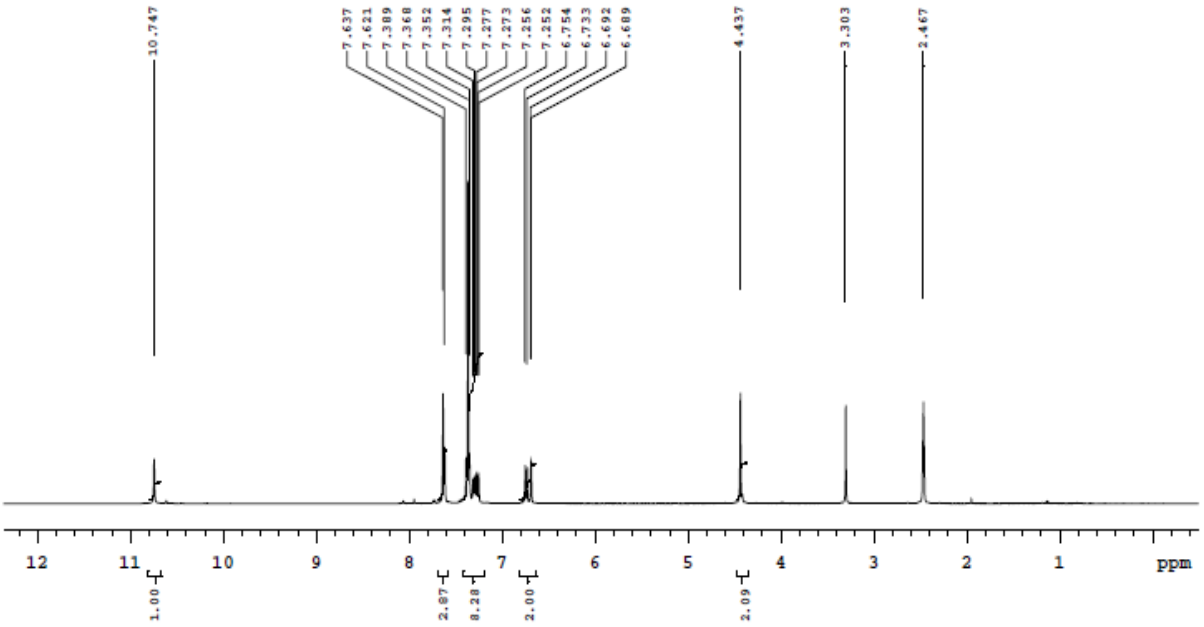
3c
Exact Mass: 545.99
Molecular Weight: 547.85

Fig 9*. Show the compound of (E)-5-Bromo-3-((2-(4-chlorobenzyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)indolin-2-one(Authors' findings)*

Yield 84 %; MP. 185-188 °C; yellow solid; ^{1}H-NMR (400 Hz, DMSO-d_6): δ ppm: 4.437 (s, 2H, -CH_2-), 6.689-6.754 (m, 2H, Ar), 7.252-7.389 (m, 8H, Ar), 7.621-7.637 (d, 2H, Ar, J=6.0 Hz), 10.747 (s, 1H, -NH-); ^{13}C- NMR (400 Hz, DMSO-d_6): δ ppm: 36.644, 111.646, 113.115, 118.952, 119.906, 123.175, 126.327, 127.514, 127.708, 128.895, 129.236, 131.366, 132.388, 132.690,

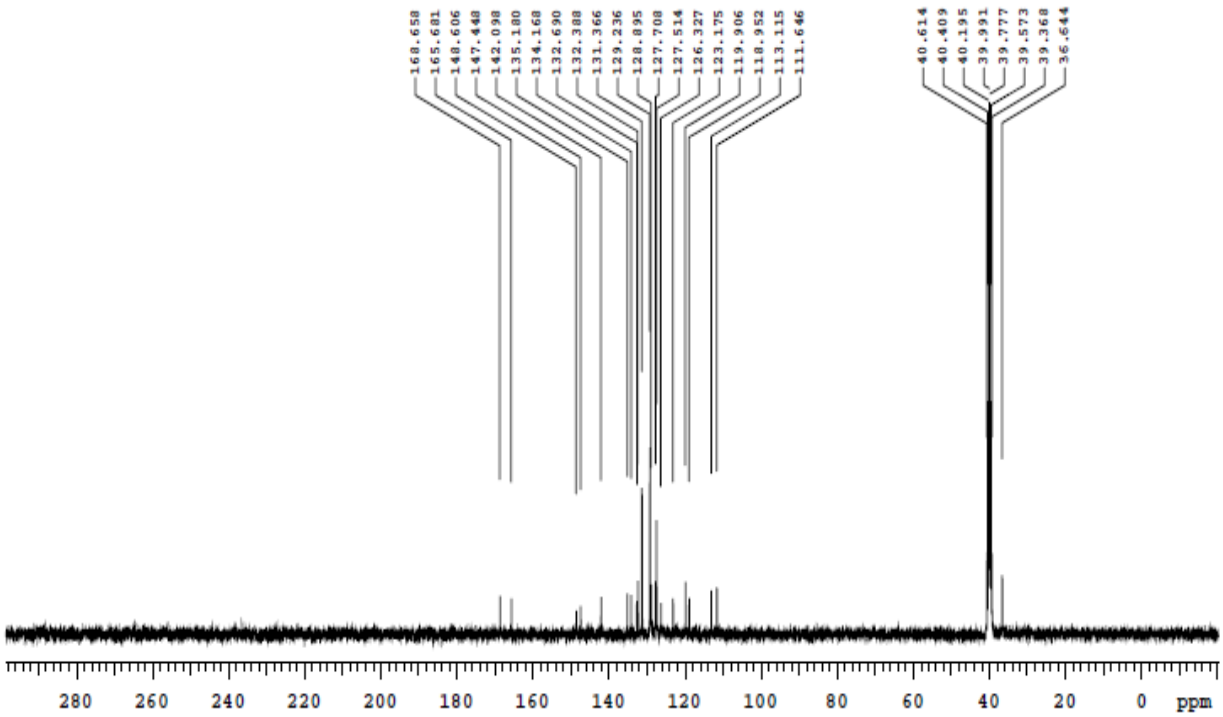
134.168, 135.180, 142.098, 147.448, 148.606, 165.681, 168.658; HRMS-(ESI): m/z calculated for [$C_{26}H_{16}BrClN_4OS + H^+$] = 546.9917: found =547.0298.

^{1}H-NMR (400MHz-DMSO-d_6) of compound (3c)



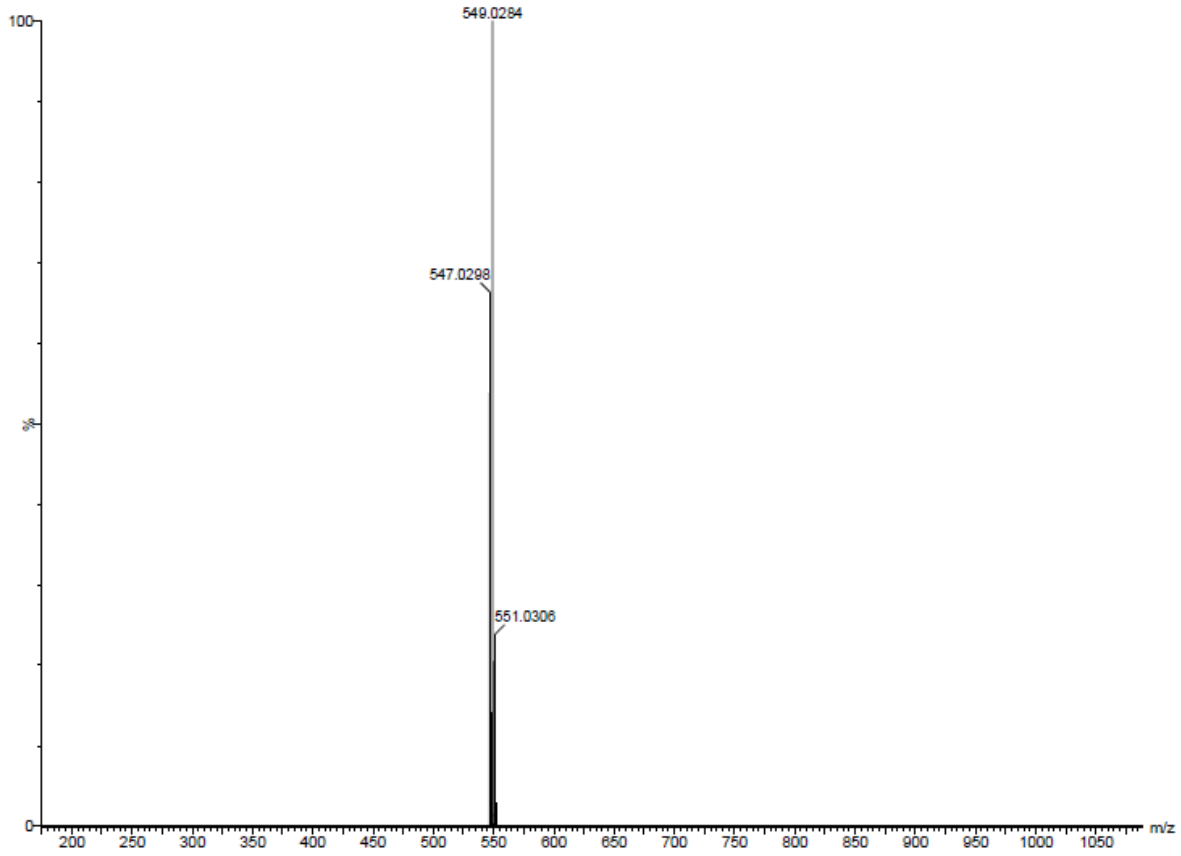
Graph 7. *^{1}H-NMR (400MHz-DMSO-d_6) of compound (3c)*

^{13}C-NMR (400MHz-DMSO-d_6) of compound (3c)



Graph 8. ^{13}C-NMR (400MHz-DMSO-d_6) of compound (3c)

Mass spectra of compound (3c)



Graph 9. *Mass spectra of compound (3c)*

DISCUSSION

The present study was conducted to design and synthesize novel oxindole–imidazo[2,1-b][1,3,4]thiadiazole derivatives as potential BCL-2 inhibitors. The results showed that the molecular hybridization strategy, inspired by the Disarib structure, successfully yielded three novel compounds with satisfactory yields and desirable structural stability. The spectroscopic analyses obtained from ^{1}H NMR, ^{13}C NMR, and HRMS fully confirmed the final structures of all synthesized compounds, indicating that the selected synthetic methodology was highly efficient, reliable, and reproducible. Furthermore, the synthetic approach employed relatively mild reaction conditions and a straightforward purification process, making it suitable for the preparation of structurally diverse analogues in future studies. The successful construction of these hybrid molecules demonstrates the feasibility of combining two biologically relevant pharmacophores within a single molecular framework without compromising structural integrity. This provides a robust chemical platform for subsequent biological evaluation and further optimization in the development of novel apoptosis-targeted anticancer agents.

The interpretation of the findings suggests that incorporating the imidazothiadiazole core and the oxindole moiety into a single molecular framework may significantly enhance

interactions with the BCL-2 protein. The rigid and planar nature of these fused heterocyclic systems is expected to facilitate favorable molecular recognition within the hydrophobic binding groove of BCL-2, while the oxindole carbonyl and NH groups may provide additional opportunities for hydrogen-bond formation with amino acid residues in the binding pocket. The presence of halogen substituents such as chlorine and bromine, owing to their electron-withdrawing properties, is likely to strengthen hydrophobic interactions and may also promote halogen bonding, thereby improving binding affinity and ligand stability. In contrast, the methoxy group, acting as an electron-donating substituent, may contribute to regulating electron distribution, modulating molecular polarity, and stabilizing ligand–protein interactions through favorable electronic effects. Collectively, these structural modifications suggest that careful tuning of the electronic properties of the Disarib scaffold could influence both binding characteristics and pharmacological behavior. Although biological evaluation was beyond the scope of the present study, the structural features of the synthesized compounds are consistent with current medicinal chemistry principles for the rational design of selective BCL-2 inhibitors. Therefore, these derivatives represent promising candidates for future molecular docking, in vitro cytotoxicity, and apoptosis studies to validate their potential anticancer activity.

increasing binding affinity. In contrast, the methoxy group, acting as an electron-donating substituent, may contribute to regulating electron distribution and stabilizing molecular interactions. Therefore, the findings of this study indicate that targeted electronic modifications of the Disarib scaffold may lead to derivatives with improved pharmacological properties.

The findings of the present study can be interpreted and discussed in comparison with several previous investigations that have explored BCL-2 inhibition, apoptosis regulation, and the design and development of anticancer agents.

The study by Iyer and colleagues in (2016) demonstrated that Disarib is a selective BCL-2 inhibitor that disrupts the BCL-2–BAK interaction through binding to the BH1 domain. While their work focused on its mechanism of action, the present study extends this approach by incorporating oxindole and imidazothiadiazole pharmacophores into the Disarib scaffold to develop novel analogs with potentially improved BCL-2 affinity and selectivity. Dandawate and colleagues in (2020) reported that Disarib induces apoptosis and tumor regression with minimal platelet toxicity, highlighting the importance of selective BCL-2 inhibition. In line with these findings, the present study aims to retain Disarib's favorable safety profile while potentially enhancing its anticancer activity through strategic structural modifications. Souers and colleagues in (2013) developed Venetoclax (ABT-199), the first clinically approved selective BCL-2 inhibitor.

Despite its success, resistance and hematological toxicities remain challenges. The present study addresses these limitations by exploring alternative oxindole–imidazothiadiazole-based scaffolds that may provide novel interactions with the BCL-2 binding site. The findings of Roberts and colleagues (2016) demonstrated the clinical

effectiveness of Venetoclax in chronic lymphocytic leukemia, confirming the therapeutic importance of BCL-2 inhibition. Similarly, the present study supports BCL-2 as a valuable anticancer target and introduces newly synthesized derivatives as potential therapeutic candidates. Rashdan and colleagues in (2021) reported significant anticancer activity for novel oxindole derivatives, highlighting oxindole as a privileged medicinal chemistry scaffold. Consistent with these findings, the present study combines oxindole with imidazothiadiazole to generate hybrid molecules with potentially enhanced pharmacological properties. Prakash and Raja (2012) emphasized the anticancer potential of indolinone (oxindole) derivatives and the importance of scaffold modification in influencing biological activity. Accordingly, halogen and methoxy substitutions were introduced in the present study to optimize the electronic and biological properties of the designed compounds. Montero and Letai (2018) suggested that future BCL-2-targeted therapies require improved selectivity, lower toxicity, and stronger apoptotic effects. The design strategy employed in the present study addresses these goals through rational hybridization and optimization of the Disarib scaffold. Kamal and colleagues (2014) demonstrated that imidazothiadiazole-containing compounds exhibit significant apoptosis-inducing and antiproliferative activities against cancer cells. These findings support the rationale of the present study and highlight the anticancer potential of imidazothiadiazole-based derivatives.

One of the major research questions of this study was whether molecular hybridization could be employed to design novel derivatives with potentially superior properties compared to Disarib. The results indicate that this objective was largely achieved, as the new compounds were successfully synthesized and characterized, exhibiting the expected structural features. Another important question concerned the influence of different substituents on the structural and electronic properties of the synthesized compounds. The results indicated that the incorporation of chlorine, bromine, and methoxy groups produced noticeable changes in the spectroscopic characteristics and potentially in the biological behavior of the compounds. This observation may hold considerable significance for future structure–activity relationship (SAR) investigations.

Despite promising outcomes, the study was limited by several factors. The most important limitation was the lack of biological and cellular evaluations to assess anticancer activity and BCL-2 inhibitory potential directly. Due to limited laboratory facilities and the high cost of biological experiments, in vitro and in vivo studies were not performed at this stage. In addition, molecular docking and computational analyses investigating the interactions between the synthesized compounds and the BCL-2 protein were not conducted, although such studies could have provided deeper insights into binding modes and inhibitory potency. Another limitation was the relatively small number of synthesized derivatives, which restricted broader exploration of structure-activity relationships.

Based on the findings and existing limitations, it is recommended that future studies include comprehensive biological investigations such as cytotoxicity assays against various cancer cell lines, evaluation of BCL-2 inhibition, platelet toxicity studies, molecular docking

analyses, and molecular dynamics simulations. Furthermore, animal studies and xenograft models would play a crucial role in determining the actual efficacy and safety profile of these derivatives. The synthesis of additional derivatives containing diverse electron-donating and electron-withdrawing substituents may also contribute to a deeper understanding of structure-activity relationships and facilitate the design of more selective and potent inhibitors.

CONCLUSION

Selective inhibition of the anti-apoptotic BCL-2 protein remains a promising strategy for anticancer therapy, despite limitations associated with current drugs like venetoclax. Disarib, a novel BCL-2 inhibitor targeting the BH1 domain with minimal platelet toxicity, provides an attractive lead for optimization. In this study, three novel disarib derivatives were rationally designed by hybridizing an imidazo[2,1-b][1,3,4]thiadiazole core with halogen-substituted oxindole moieties to explore structure-activity relationships and enhance BCL-2 binding.

The target compounds were successfully synthesized via a multi-step sequence involving thiadiazole formation, imidazothiadiazole construction, Vilsmeier-Haack formylation, and piperidine-catalyzed condensation. All three conjugates were fully characterized using ^{1}H NMR, ^{13}C NMR, and HRMS, confirming their structural integrity. This synthetic approach successfully produced novel molecular frameworks distinct from the parent disarib molecule.

We anticipate that these compounds will demonstrate superior BCL-2 inhibition and improved target specificity, driven by favorable halogen-mediated interactions. If validated experimentally, these disarib-inspired hybrids could offer a promising therapeutic option for BCL-2-overexpressing cancers with a favorable safety profile. Future studies will include cytotoxicity profiling, binding assays, platelet toxicity evaluation, and in vivo xenograft studies.

Author's Contributions

- The study was conceived and overseen by Mohammad Ali Nasiri, who provided overall supervision throughout the research process.
- Raw data collection was carried out by Ismael Ahmadi and Neamatullah Fekrat.
- Data analysis and manuscript preparation were performed by Mohammad Ali Nasiri, with contributions and feedback from all co-authors.
- The final manuscript was critically reviewed and approved by all authors prior to submission.

Acknowledgements

We would like to thank all our dear friends and colleagues, especially the Honorable Abdulrauf Rashid and the Honorable Hussain Bakhsh Nazari, for their cooperation and participation in the research process.

Funding Information

No funding is available for the manuscript.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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