Synthesis and antitumor evaluation of some new N\textsuperscript{4} substituted sulfapyridine derivatives with studying the synergistic effect of γ-irradiation

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تحضير بعض مشتقات $N^4$ السلفابيردين لتقييمها كمضادات للأورام السرطانية مع دراسة التأثير التحفيزى للتشعيع الجامى

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بكالوريوس العلوم الصيدلية
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"قالوا سبحانك لا علم لنا إلا ما علمتنا إفك أنت العلي الحكيم

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<td>Analysis of variance</td>
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<td>Cyclin dependent kinase</td>
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**Synthesis and antitumor evaluation of some new \( N^4 \) substituted sulfapyridine derivatives with studying the synergistic effect of \( \gamma \)-irradiation**

The aim of the present investigation is to synthesize a new class of \( N^4 \) substituted sulfapyridine derivatives with anticipated cytotoxic activity. All the newly synthesized compounds were screened for their *in-vitro* cytotoxic activity against breast cancer cell line (MCF-7) compared to the reference drug Doxorubicin. Also the synergism of the most potent synthesized compounds with \( \gamma \)-radiation was studied. Moreover, a molecular docking study was carried out by docking the most active synthesized compounds in the active site of Cyclin Dependent Kinase 2 receptor to assess their inhibitory effect upon this enzyme as this may have a role in their anticancer activity.

**Keywords:** sulfapyridine, cytotoxic activity, \( \gamma \)-radiation, doxorubicin, Cyclin Dependent Kinase 2.

**The thesis includes the following parts:**

**Introduction:**

This part comprises a brief literature review about principles of cancer chemotherapy and radiotherapy as well as the rationale for combining chemotherapy and radiotherapy. A survey on the anticancer activity of sulfonamides regarding their mechanisms of action is also mentioned. Furthermore, the different methods for the synthesis of pyrroles and thiazoles are discussed.
Abstract

**Aim of the work:**

This part illustrates the basis on which the synthesized compounds were chosen in order to explore the potential cytotoxic activity of these compounds. The classes to which the synthesized compounds belong were briefly mentioned.

**Theoretical Discussion:**

This part deals with the discussion of the experimental methods adopted for the synthesis of the designed compounds, identification and verification of structures of the newly synthesized compounds by elemental analyses and spectroscopic methods. Schemes (1–6) explaining the synthetic pathways adopted in the preparation of the designed compounds are given.

**Experimental:**

This part describes the practical procedures used for the synthesis of 24 new compounds, 2 reported intermediates and 2 reported final products, with their elemental analyses and spectral data (IR, $^1$H-NMR and mass spectroscopy).

**Reported intermediates:**

- 2-Chloro-N-(4-(N-pyridin-2-ylsulfamoyl)phenyl)acetamide (2)
- 4-(5-Amino-4-cyano-2-oxo-2,3-dihydro-1H-pyrrol-1-yl)-N-(pyridin-2-yl)benzenesulfonamide (11)

**Final products:**

**Reported compounds:**

- 2-(Piperidin-1-yl)-N-(4-(N-pyridin-2-yl sulfamoyl)phenyl) acetamide (8)
- 2-(Morpholino)-N-(4-(N-pyridin-2-yl sulfamoyl)phenyl) acetamide (9)

**Newly synthesized compounds:**
• 4-(4-Hydroxythiazol-2-ylamino)-N-(pyridin-2-yl)benzenesulfonamide (3)
• 4-(4-Chlorothiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (4)
• 2-{2-Oxo-2-[4-(N-pyridin-2-yl sulfamoyl) phenylamino] ethylamino} acetic acid (5a)
• 2-{2-Oxo-2-[4-(N-pyridin-2-yl sulfamoyl) phenylamino] ethylamino} propanoic acid (5b)
• 2-{2-Oxo-2-[4-(N-pyridin-2-yl sulfamoyl) phenylamino] ethylamino} 3-methylpentanoic acid (5c)
• 2-{2-Oxo-2-[4-(N-pyridin-2-yl sulfamoyl) phenylamino] ethylamino} succinic acid (5d)
• 2-{2-Oxo-2-[4-(N-pyridin-2-yl sulfamoyl) phenylamino] ethylamino} 3-phenylpropanoic acid (5e)
• 2-{2-Oxo-2-[4-(N-pyridin-2-yl sulfamoyl) phenylamino] ethylamino} 3-(4-hydroxyphenyl) propanoic acid (5f)
• 2-(Diethylamino)-N-(4-(N-pyridin-2-yl sulfamoyl)phenyl) acetamide (6)
• 2- (3-Hydroxypropylamino)-N- (4- (N-pyridin-2-yl sulfamoyl) phenyl) acetamide (7)
• 2- (4-Chlorophenylamino)-N- (4- (N-pyridin-2-yl sulfamoyl) phenyl) acetamide (10)
• N-(4-Cyano-2-oxo-1-(4-(N-pyridin-2-ylsulfamoyl)phenyl)-2,3-dihydro-1H-pyrrol-2-yl) acetamide (12)
• N-acetyl-N-(4-cyano-2-oxo-1-(4-(N-pyridin-2-ylsulfamoyl) phenyl)-2,3-dihydro-1H-pyrrol-2-yl) acetamide (13)
• 4-(4-Cyano-5-(3-ethylthioureido)-2-oxo-2,3-dihydro-1H-pyrrol-1-yl)-N-(pyridin-2-yl) benzenesulfonamide (14)
Abstract

- 4-(4-Mercaptothiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (15)
- 4- (4- (Methylthio) thiazol-2-yl amino)-N- (pyridin-2-yl) benzenesulfonamide (16)
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- 4-(4-(2-(2,4-Dinitrophenyl) hydrazinyl) thiazol-2-yl amino)-N-(pyridin-2-yl) benzenesulfonamide (21)
- 4-[4-( Isothiocyanato)thiazol-2-yl amino)-N-(pyridin-2-yl) benzenesulfonamide (22)
- 4-(4-(4-Chlorophenylamino) thiazol-2-yl amino)-N-(pyridin-2-yl) benzenesulfonamide (23)
- 2- (2- (4-(N-Pyridin-2-yl sulfamoyl) phenylamino) thiazol-4-yl amino)-benzoic acid (24)

**Biological Activity:**

In this part the procedures and the results of screening of 24 newly synthesized and 3 reported compounds for their in-vitro cytotoxic activity against human breast cancer cell line (MCF-7) were mentioned. The most potent six compounds were further tested in combination with γ- radiation to
evaluate the possible synergism resulted from this combination. The results are presented and discussed.

**Molecular Docking:**

This part includes the docking of the most active synthesized compounds in the active site of the Cyclin Dependent Kinase 2 receptor to give an idea if these compounds could act as enzyme inhibitors as this may have a role in their anticancer activity.

**References:**

This part includes 129 References.

**Arabic summary:**
Introduction

1.1 Modalities of Cancer Treatment

Four primary modalities are employed in the approach to cancer treatment: surgery, radiation, chemotherapy, and biological therapy [1].

1.1.1 Surgery

The oldest of these is surgery, which plays a major role in the diagnosis and treatment of cancer. Surgery remains the treatment of choice for most solid tumors diagnosed in the early stages. Cancer surgery attempts to remove localized, well defined tumors, or precancerous conditions [1].

1.1.2 Radiation

Radiation therapy uses high-energy radiation to shrink tumors and kill cancer cells. X-rays, gamma rays, and charged particles are types of radiation used for cancer treatment. Radiation therapy can either damage DNA directly or create charged particles (free radicals) within the cells that can in turn damage the DNA. Cancer cells whose DNA is damaged beyond repair stop dividing or die [2].

Radiation therapy is sometimes given with curative intent (that is, with the hope that the treatment will cure a cancer, either by eliminating a tumor, preventing cancer recurrence, or both) [2]. In such cases, radiation therapy may be used alone or in combination with surgery, chemotherapy, or both. Radiation therapy may also be given with palliative intent. Palliative treatments are not intended to cure. Instead, they relieve symptoms and reduce the suffering caused by cancer such as radiation given to shrink a tumor that is pressing on the spine or growing within a bone, which can cause pain [2].
A patient may receive radiation therapy before (pre-operative or neoadjuvant radiation), during (intraoperative radiation therapy (IORT), or after surgery (post-operative or adjuvant radiation therapy). Some patients may receive radiation therapy alone, without surgery or other treatments. Some patients may receive radiation therapy and chemotherapy at the same time. The time of radiation therapy depends on the type of cancer being treated and the goal of treatment (cure or palliation) [2].

Although very effective for treating many types of cancer, surgery and radiation are local treatments. These modalities are likely to produce a cure in patients with truly localized disease. But because most patients with cancer have metastatic disease at diagnosis, localized therapies often fail to completely eliminate the cancer [1].

1.1.3 **Chemotherapy**

The goals of cancer treatment include cure, prolongation of life, and relief of symptoms. Surgery and radiation therapy provide the best chance of cure for patients with localized cancers, but systemic treatment methods are required for disseminated cancers.

Most tumors rapidly develop resistance to single agents given on their own. For this reason the principle of combined chemotherapy was developed. Several drugs are combined together, chosen on the basis of differing mechanisms of action and non-overlapping toxicities. These drugs are given over a period of a few days followed by a rest of a few weeks, during which time the normal tissues have the opportunity for regrowth [3].

1.1.4 **Biological therapies**

Biological therapies are currently considered in the broader sense of immunotherapy or targeted therapies. Immunotherapy, the earliest important form of biological therapy, usually involves stimulating the host’s immune
system to fight the cancer. The agents used in immunotherapy are usually naturally occurring cytokines, which have been produced with recombinant DNA technology. Examples of agents used in immunotherapy include interferons and interleukins (ILs). Targeted therapies include monoclonal antibodies, tyrosine kinase inhibitors, proteosome inhibitors, and others [1].

1.1.5 Adjuvant therapy

Adjuvant therapy is systemic therapy that is administered to eradicat e any existing micrometastases remaining after surgical excision of localized disease or radiation or both. The goal of adjuvant therapy given in this setting is to reduce subsequent recurrence rates and prolong long-term survival. Thus, adjuvant therapy is given to patients with potentially curable malignancies who have no clinically detectable disease after surgery or radiation. Because adjuvant therapy is given at a time that the cancer is undetectable (i.e., no measurable disease), the value of adjuvant therapy is best established in colorectal and breast cancers [1].

The management of most types of cancer involves the use of combined modalities. Early stage breast cancer is a good example of the use of a combined-modality approach. The primary tumor is removed surgically, and radiation therapy is delivered to the remaining breast (after lumpectomy) or to the axilla (if there is marked lymph node involvement). Adjuvant therapy (chemotherapy, targeted therapy, and/or hormonal therapy) is then administered to eradicate any micrometastatic disease [1].
1.2 Chemoradiotherapy (CRT)

The combination of chemotherapy and radiation therapy given at the same time is sometimes called chemoradiation or radiochemotherapy. For some types of cancer, the combination of chemotherapy and radiation therapy may kill more cancer cells (increasing the likelihood of a cure) [2],[4].

1.2.1 Rationale for combining chemotherapy (CT) and radiotherapy (RT)

The rationale for combining CT and RT is mainly based on two ideas, one being spatial cooperation, and the other is the enhancement of radiation effects [5]-[7]. Spatial cooperation is effective if CT is sufficiently active to eradicate subclinical metastases and if the primary local tumor is effectively treated by RT. In this regard, no interaction between RT and CT is required, but differing toxicities are needed so that both modalities can be used at effective dosages.

To decrease the local failure rate, the enhancement of RT effects is necessary. In the presence of chemotherapeutic drugs, an increased response such as enhancement occurs within the irradiated volume. However, virtually all chemotherapeutic agents enhance radiation damage to normal tissues as well. Consequently, a therapeutic benefit is only achieved if enhancement of the tumor response is greater than that for normal tissues.

Among the many chemotherapeutic agents used, cisplatin is one of the best agents for yielding a therapeutic benefit. An enhancing effect by the additional use of daily cisplatin before each RT fraction was observed in an in-vivo animal study [8].
1.2.2 **Therapeutic ratio**

In general, tumor response and normal tissue damage are positively correlated with the dose of radiation, and this relationship is commonly described by a sigmoid curve as shown in Figure 1.1. The therapeutic ratio is defined as the ratio of the dose that produces a given probability (50% is most commonly used in experimental studies) of normal tissue damage and the dose that produces the same probability of tumor control. When CT is combined with RT the tumor control curve shifts to the left, along with the response curve for normal tissue damage. The goal of combining CT and RT is to obtain a positive therapeutic ratio, and thus to enhance the antitumor effect while minimizing toxicity to critical normal tissues [9].

![Cisplatin](image)

**Cisplatin**

Figure 1.1 – Dose-response curves for tumor control and normal tissue damage.

Figure 1.1 shows that when chemotherapy (CT) is combined with radiotherapy (RT), the tumor control curve shifts to the left (long arrow), and the response curve for normal tissue damage also shifts in the same direction, as indicated by the short arrow.
1.2.3 Mechanisms responsible for CT-RT interactions

Recent clinical trials, including metaanalyses, have shown that CT given concurrently with RT results in improved local control and survival, [10]-[14]. Five major mechanisms responsible for CT-RT interactions are discussed.

1.2.3.1 Initial radiation damage

The first mechanism responsible for CT-RT interaction is the direct enhancement of the initial radiation damage, resulting from the incorporation of the chemotherapeutic drugs into DNA. The primary target for radiation injury is DNA, where halogenated pyrimidines such as 5-fluorouracil (5-FU) are incorporated, making the DNA more susceptible to RT.

![5-Flurouracil](attachment:5-flurouracil.png)

Cisplatin interacts with nucleophilic sites on DNA or RNA to form intra- and interstrand cross-links. When cisplatin-DNA cross-links are formed during RT, radioenhancement by cisplatin may occur. This has been observed in both hypoxic and oxygenated cells [7].

1.2.3.2 Inhibition of radiation damage repair

Secondly, the inhibition of cellular repair increases radiation damage. Cells have the ability to repair sublethal and potentially lethal radiation damage [5]. This inhibition of cellular repair can be effective when drugs are administered following fractionated RT. In general, nucleoside analogs such as fludarabine and gemcitabine are potent radiosensitizers [9].
In animal experiments, the effect of fludarabine on radiocurability was greater when fludarabine was combined with fractionated RT than when it was combined with single dose RT [15].

1.2.3.3 Cell-cycle effects

The third mechanism focuses on a cell-cycle effect. The cytotoxicity of most chemotherapeutic agents and that of radiation is highly dependent on the phase of the cell cycle. Both chemotherapeutic agents and radiation are more effective against proliferating cells than against nonproliferating cells as shown in Figure 1.2 which describes the cell cycle.

Among proliferating cells, cells in the G2 and M phases are the most radiosensitive, and the cells in the S phase are the most radioresistant [5]. Based
on this variation in radiosensitivity over the cell cycle, there exist two strategies for CRT, the use of chemotherapeutic agents that accumulate cells in a radiosensitive phase or those that eliminate radioresistant S-phase cells. The latter strategy is related to the mode of action of nucleoside analogs. Fludarabine and gemcitabine are incorporated into radioresistant S-phase cells, many of which die by apoptosis. This preferential removal of S-phase cells therefore contributes to the radioenhancement effects. Taxanes such as paclitaxel and docetaxel inhibit tubulin depolymerarization and promote microtubule assembly and stability, which leads to cell-cycle arrest in the radiosensitive G2 and M phases. The ability of taxanes to block cells in the G2/M phases is the biological rationale for combining these agents with radiation [9].

1.2.3.4 Hypoxic cells

Hypoxic cells are 2.5–3.0 times less sensitive to radiation than well-oxygenated cells [5], [6]. Tumors often include hypoxic areas, which is a cause of radioresistance. Chemotherapeutic agents can improve the RT effect by: selectively eliminating hypoxic cells, or sensitizing the hypoxic cells to radiation.

1.2.3.5 Repopulation of tumor cells

The importance of the overall treatment time (OTT) for local tumor control by RT has been documented in a number of studies of head and neck cancers, uterine cervical cancer, and esophageal cancer [16], [17]. Withers and colleagues [18] found that the TCD50 (the radiation dose which yields local control in 50% of tumors) progressively increased over time if the OTT was prolonged beyond 30 days. Their analysis of esophageal and laryngeal squamous cell carcinomas treated by RT alone showed that the prolongation of OTT significantly reduced the local control rate [16], [17]. One mechanism
responsible for this may be the accelerated repopulation of tumor cells during fractionated RT.

Any approach that reduces or eliminates the accelerated repopulation of tumor cells improves the efficacy of RT. This is likely to be one of the major mechanisms by which CT improves local tumor control when given concurrently with RT. Even a small decrease in repopulation between radiation fractions can significantly improve the tumor response to fractionated RT. However, most chemotherapeutic drugs inhibit repopulation not only in the tumor, but also in the compensatory cell regeneration of normal tissues that occurs during fractionated RT. Thus, a therapeutic benefit is expected if drugs are tumor selective or if repopulation is faster in the tumor.
1.3 Sulfonamides

Sulfonamides have been found to be associated with broad and wide spectrum of biological activities including antibacterial [19], hypoglycemic [20], diuretic [21], [22], antithyroid [23], and anti-human immunodeficiency virus (HIV) [24].

Recently, A large number of structurally novel sulfonamide derivatives have ultimately been reported to show substantial antitumor activity in-vitro and in-vivo [25]-[28], E7010 and ER-34410 are examples of sulfonamides in advanced clinical trials. E7070 (Indisulam) is now clinically used for its antitumor activity.

There are a variety of mechanisms of sulfonamides antitumor action, such as Cell cycle arrest in the G1 phase, Carbonic anhydrase inhibition, Histone deacetylases (HDACs) inhibition, Methionine aminopeptidases (MetAPs) inhibition, Matrix metalloproteinase (MMPs) inhibition, Nicotinamide Adenine...
Dinucleotide (NADH) oxidase inhibition, Cyclin-Dependent Kinase (CDK) inhibition, binding to β-Tubulin, and disruption of microtubule assembly.

1.3.1.1 **Cell cycle arrest:**

G1 phase of the cell cycle is an important period where various complex signals interact to decide a cell’s fate: proliferation, quiescence, differentiation, or apoptosis. It is now well-recognized that malfunctioning of cell cycle control in G1 phase is among the most critical molecular bases for tumorigenesis and tumor progression [29].

Indisulam, N-(3-chloro-7-indolyl)-1, 4-benzenedisulfonamide, (E7070) is a novel sulfonamide anticancer agent for the treatment of solid tumours [30]. Its mechanism of action is multifactorial, as arresting cell cycle in the G1 phase, and blocking the entry of human Non-small cell lung cancer (NSCLC A549 cells) into the S phase, leading to the accumulation of cells in the late G1 phase [31]-[33].

Chloroquinoxaline sulfonamide (CQS) I, possessed efficient antitumor properties against breast, lung, melanoma and ovarian carcinomas by causing cell cycle arrest at the G1 phase [34], [35].
1.3.1.2 **Sulfonamides acting as carbonic anhydrase (CA) inhibitors**

Carbonic anhydrases are able to catalyze the hydration of CO$_2$ to bicarbonate at physiological pH. This chemical interconversion is crucial since bicarbonate is the substrate for several carboxylation steps in a number of fundamental metabolic pathways such as gluconeogenesis, biosynthesis of several amino acids, lipogenesis, ureagenesis and pyrimidine synthesis [36].

The role of CAs in cancer can be explained in light of the metabolic processes required by growing cancer cells that develop with a higher rate of replication than normal cells. Such a circumstance requires a high flux of bicarbonate into the cell in order to provide substrate for the synthesis of either nutritionally essential components (nucleotides) or cell structural components (membrane lipids) [36]. Sulfonamides are known to possess high affinity for CAs as such compounds possess a zinc binding group (ZBG) by which they interact with the metal ion in the active site of the enzyme and the residues Thr 199 and Glu 106 in its neighborhood [37].

Clinically used sulfonamides acting as CA inhibitors such as acetazolamide II, methazolamide III, or ethoxzolamide IV were found to inhibit the growth of human lymphoma cells [38]. Additionally, E7070 strongly inhibits carbonic anhydrase [25].

![Chemical structures of II, III, and IV](image)

The N, N-dialkylthiocarbanylsulfenamido-sulfonamides V behaved as strong CA inhibitors and were also tested as inhibitors of tumor cell growth *in-vitro*, showing potent inhibition of growth against several leukemia, lung,
ovarian, melanoma, colon, CNS, renal, prostate and breast cancer cell lines [39], [40].

\[ \text{R= Me, Et} \]
\[ n = 0-2 \]

1.3.1.3 **Inhibition of histone deacetylases (HDACs)**

Inhibition of histone deacetylases (HDACs) is emerging as a new strategy in human cancer therapy. HDAC inhibitors have been shown to have antiproliferative effects on tumor cells *in-vitro* through a number of mechanisms including cell cycle arrest, apoptosis, and expression of genes related to the malignant phenotype in a variety of ovarian cancer cell lines [41].

In eukaryotic cells, histone acetylation/deacetylation, which is co-regulated by enzymes called histone acetyltransferases (HATs) and histone deacetylases (HDACs), is essential for chromatin remodeling and the functional regulation of gene transcription. HDACs, as transcription co-repressors, remove the acetyl groups from the acetylated lysines in histones. Deregulation of HDAC activity can cause malignant diseases in humans.

Compounds having a hydroxamic acid moiety are highly potent HDAC inhibitors, such as the natural product trichostatin A (TSA) and its analogues, suberoylanilide hydroxamic acid (SAHA) and the eneyne oxamflatin. In
addition, PXD101 was reported to act as HDAC inhibitor and in clinical trials [42].

Novel nonhydroxamate sulfonamide anilides were synthesized that can inhibit human HDAC enzymes and can induce hyperacetylation of histones in human cancer cells. These compounds selectively inhibit proliferation and cause cell cycle blocks in various human cancer cells but not in normal cells [41]. One of these compounds, N-2-aminophenyl-3-[4-(4-methyl benzene sulfonlamino)-phenyl]-2 propenamide VI which significantly reduces tumor growth of implanted human colon tumors in mice, and available for clinical and research purposes [41]. KD5170 was identified as potent histone deacetylase inhibitor. It demonstrated a broad spectrum cytotoxic activity against a wide range of human tumor cell lines [43].
1.3.1.4 **Methionine aminopeptidases (MetAPs) inhibition:**

Synthesis of cellular proteins in eukaryotic cells starts with an N-terminal methionine, when the polypeptide chain is synthesized, post-translational modifications such as acetylation and myristoylation are required for proper localization and stability of the protein. Removal of the N-terminal initiator methionines from nascent polypeptide chains is an important co-translational modification step required for a protein to undergo post-translational modifications. The hydrolytic removal of this residue is catalyzed by a family of metalloprotease enzymes known as methionine aminopeptidases (MetAPs) [44]. There are three known mammalian methionine aminopeptidases, MetAP1, MetAP2, and the recently reported MetAP1D [45], [46]. Methionine aminopeptidase 2 (MetAP2) plays a critical role in the regulation of post-translational processing, protein synthesis, cell proliferation and the development of different types of cancer. Recently, a high expression of MetAP2 in human colorectal cancer tissues and colon cancer cell lines was observed [47]-[49].
Sulfonamide derivatives VII which act as a potent methionine amino-peptidase type II inhibitors with antiproliferative properties have been developed as a novel approach toward antiangiogenesis and anticancer therapy [50].

![Chemical structure](attachment:chemical_structure.png)

VII

\[ X = H; R = H \quad X = F; R = CH_3 \]

### 1.3.1.5 Matrix metalloproteinase inhibition (MMPIs):

Matrix metalloproteinase are endoproteinases which play an important role in the invasiveness and metastasis of cancer cells, they are extremely destructive enzymes involved in the normal turnover and remodeling of the extracellular matrix (ECM) that forms the connective material between cells and around tissues or connective tissue. This process is usually tightly controlled by natural protein inhibitors. However, excessive activity can result in chronic degenerative diseases, inflammation, and tumor invasiveness. (MMP-2, MMP - 9) are implicated in tumor growth, invasion, metastasis, and angiogenesis; therefore inhibition of these enzymes has been considered an effective therapeutic approach [51].

Matrix metalloproteinase inhibitors (MMPIs) can be used to inhibit the breakdown of the extracellular matrix, to make it more difficult for cancer cells to escape and metastasize. They can also be used to inhibit angiogenesis by
blocking the release of vascular endothelial growth factor (VEGF) from storage depots in the extracellular matrix [51], [52].

Sulfonylated amino acid hydroxamates were discovered to act as efficient MMPIs [53]. The first compounds from this class to be developed for clinical trials are (CGS 27023A) and (CGS 25966) [54]. In addition, arylsulfonyl hydroxamates VIII derived from glycine, L-alanine, L-valine and L-leucine possessing N-benzyl moieties were reported as MMPIs [55].

![Chemical structures](attachment:image.png)

**CGS 27023A, Y = N  
CGS 25966, Y = CH**

1.3.1.6 **Nicotinamide adenine dinucleotide (NADH) oxidase inhibition:**

NADH oxidase is a membrane-bound enzyme complex. Several studies have produced evidence for the involvement of this hormone and growth factor stimulated NADH oxidase in the control of cell proliferation [56]. NADH oxidase of the mammalian plasma membrane constitutively activated in transformed cells [56]. A series of benzo[b]-thiophene-sulfonamide 1, 1 -dioxide derivatives (BTS) IX and X were reported as a new class of potential antineoplastic agents [57]. These compounds showed a clear correlation
between their cytotoxic activities and their ability to inhibit a specific NADH oxidase located at the membrane of leukaemia cells [58].

Unsubstituted BTS induced an apoptic process in the membrane of leukaemia cells that is mediated by the accumulation and overexpression of reactive oxygen species (ROS), and includes typical apoptic features such as cell shrinkage, mitochondrial dysfunction, chromatin condensation and internucleosomal DNA degradation [59]. Thus these compounds may exert their effect by affecting the activity of enzymes related with the control of cellular ROS levels.

The BTS derivative X with lipophilic substituents on the sulfonamide group having stronger antineoplastic activity than the lead compound IX. It was also active at the same level of Doxorubicin against cultered normal human lung fibroblasts [60].

1.3.1.7 **Cyclin dependent kinase (CDK) inhibition:**

Cyclin-dependent kinases (CDKs) are a family of protein kinases (Protein kinases belong to the enzyme family of transferases that catalyze the transfer of a phosphoryl group from a donor to an acceptor). These protein kinases are generally categorized into G1, S, and G2 phase regulators because they are present at various checkpoints in the cell cycle. They are involved in regulating the cell cycle, regulating transcription, mRNA processing, and the differentiation of nerve cells [61]. Cyclin-dependent kinases (CDKs) are
serine/threonine kinases that require association with a cyclin regulatory protein for activation. They are responsible for regulating progression through the cell division cycle. CDKs are required for the correct timing and order of events of cell division [62]. CDK2, a crucial component of the CDK complex, is responsible for the G1/S phase transition [62]. Mutations in CDKs and/or their inhibitors are associated with overexpression and amplification, leading to uncontrolled cell growth; tumors [62].

CDK2 is regulated by the A and E forms of cyclin. Cyclin E binds to CDK2 for progression from the G1 phase to the S phase and Cyclin A is critical for progression through the S phase of the cell division cycle. Cyclin E is found in high concentrations in most tumors and almost all aggressive cancers. This fact further stresses the importance of finding an inhibitor to act as an antitumor agent, which will selectively bind CDK2 and prevent binding with Cyclin E [62], [63].

Two classes of synthetic inhibitors to CDK2 were known: Thiazolidinone and thiazole inhibitors having sulfonate group or aminopyrimidine inhibitors [62]. Compounds XI showed significant tumour growth delay in mouse tumour xenograft models and some examples were reported to have > 100-fold selectivity for CDK4, as well as other examples were equipotent against CDK1, CDK2, and CDK4 [64]. Compound XII showed potential both in-vitro and in-vivo as CDK2 inhibitors [62]. Huang S. et. al. [65], developed some sulfonamide derivatives XIII as CDK inhibitors and hence anticancer agents.


Introduction

1.3.1.8 Sulfonamides targeting β-Tubulin and causing disruption of microtubules

Several widely used antitumor drugs (such as the vinca alkaloids, vincristine and vinblastine; colchicine and paclitaxel) exert their action by binding to tubulin, the key component of microtubules and thus of the cytoskeleton, sustaining the cell shape and facilitating the movement and deposition of protein complexes, organelles and membrane vesicles [66]-[68]. Tubulins also play a crucial role in cell division, being among the major constituents of mitotic spindles, which are essential for chromosomal separation during mitosis.

E7010 is a novel sulfonamide antimitotic derivative binds to the colchicine binding site on β-tubulin to inhibit microtubule polymerization that leads to a block in the cell cycle at the G2M phase, resulting in cellular apoptosis and is distinct from the current clinically approved tubulin binding agents, the Vinca alkaloids and the taxanes. E7010 is active against cell lines resistant to vincristine, doxorubicin, and cisplatin [69].
Introduction

The effects of E7010 on microtubule structure in colon cells were examined, and were shown to cause the disappearance of cytoplasmic microtubules and mitotic spindles. The experiments clearly demonstrated that the growth-inhibitory activity of E7010 is caused by the inhibition of microtubule assembly [70].

![E7010](image)

1.3.1.9 Other sulfonamides possessing antitumor activity:

Randomized clinical trials have confirmed that the selective cyclooxygenase (COX)-2 inhibitor, Celecoxib effectively inhibit the growth of adenomatous polyps and cause regression of existing polyps in patients with the unusual hereditary condition familial adenomatous polyposis (FAP), so Celecoxib has a promise as anticancer agent [71].

![Celecoxib](image)
1.4 Synthesis of pyrroles

1.4.1 From 1, 4-dicarbonyl compounds and primary amines

Paal Knorr reaction is one of the most common approaches in which γ-diketone are converted to pyrroles from the reaction with primary amines (or ammonia) in the presence of various promoting agents. 2, 5-Dimethylpyrrole XV was prepared from reaction of acetonilacetone XIV with ammonia in benzene [72].

Reaction of acetonilacetone XIV with aniline in the presence of a catalytic amount of metal triflates Sc(OTf)$_3$ without any solvent yielded the corresponding pyrrole derivative XVI [73].
1.4.2 From α-aminocarbonyl compounds and activated ketones

Knorr synthesis utilizes two components: α-aminocarbonyl component XVII and a second compound that possesses a methylene group α to carbonyl XVIII in preparation of the pyrrole derivative XIX [72].

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{O} \\
\text{NH}_2 & \quad \text{O} \\
\text{C} & \quad \text{O} \\
\text{COOC}_2\text{H}_5 & \quad \text{COOCH}_3
\end{align*}
\]

\[
\xrightarrow{\text{aq. KOH, rt}}
\]

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{COOEt} \\
\text{H} & \quad \text{COOH}
\end{align*}
\]

XVII  XVIII  XIX

1.4.3 From 1,3 dicarbonyl compounds and glycine esters

1, 3 Dicarbonyl compounds XX react with glycine esters XXI to give pyrrole-2-esters XXII.

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{H} & \quad \text{N} & \quad \text{COOR}
\end{align*}
\]

\[
\xrightarrow{-2 \text{H}_2\text{O}}
\]

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{COOR} \\
\text{H} & \quad \text{COOR}
\end{align*}
\]

XX  XXI  XXII

Condensation between 2-oxocyclopentanecarbaldehyde XXIII and glycine ethyl ester HCl XXIV using TEA as a base produced the intermediate enamino-ketone XXV which was cyclized to yield the corresponding pyrrole 2-ester derivative XXVI [72].

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{O} \\
\text{N} & \quad \text{COOEt}
\end{align*}
\]

\[
\xrightarrow{\text{Et}_3\text{N, EtOH, rt}}
\]

\[
\xrightarrow{\text{EtONa, EtOH, reflux}}
\]

XXIII  XXIV  XXV  XXVI
1.4.4 From dialkyl acetylenedicarboxylate and isocyanide

Reaction of tosylmethyl isocyanide (TOSMIC) XXVII, and a dialkyl acetylene-dicarboxylates XXVIII, in the presence of a catalytic amount of 1-methylimidazolium undergo a smooth addition reaction in anhydrous CH$_2$Cl$_2$ at ambient temperature to afford 2, 3, 4-trisubstituted pyrrole derivatives XXIX [74].

\[
\begin{align*}
\text{XXXVII} & \quad + \quad \text{XXXVIII} \\
\text{XXVII} & \quad \text{cat., rt, 2h} \quad \text{XXXIX}
\end{align*}
\]

1.4.5 From 2, 5-dimethoxytetrahydrofuran

Reaction of 2, 5-dimethoxytetrahydrofuran XXX and different amines using microwave induced bismuth nitrate catalyzed reaction without solvent resulted in the formation of N-substituted pyroles XXXI with excellent yield [75].

\[
\begin{align*}
\text{XXX} & \quad \text{MWI} \quad \left[\begin{array}{c}
\text{H-O} \\
\text{O-H}
\end{array}\right] \quad \text{cat.} \quad \text{R-NH}_2 \quad \text{XXXI}
\end{align*}
\]

1.4.6 From amines and malononitrile derivatives

The synthesis of 4-(2-amino-3-cyano-4-phenyl-pyrrol-1-yl) benzenesulfonamide XXXIV was achieved via the reaction of sulfanilamide XXXII with phenacyl bromide in refluxing ethanol and subsequent reaction of the product XXXIII with malononitrile in presence of sodium ethoxide [26].
Condensation of 2-(ethoxymethylene) malononitrile XXXV with diethyl 2-aminomalonate XXXVI yielded 3-amino-4-cyano-1H-pyrrole-2-carboxylate XXXVII [76].

1.4.7 From acetonitrile derivatives

The pyrrole derivative XXXIX was synthesized from m-methoxy phenyl acetonitrile XXXVIII by three stepwise reactions with ethyl formate, 2-aminoacetonitrile and ethyl chloroformate [77].
1.5 Synthesis of Thiazoles and Thiazolidinones

1.5.1 Synthesis of thiazoles

1.5.1.1 From thiosemicarbazones:

The 2, 4- disubstituted thiazole derivatives XLI were prepared by reaction of thiosemicarbazones XL with suitable 2-haloacetophenones [78].

1.5.1.2 From thioamides:

Ethyl 2-(N-tert-butoxycarbonyl-2,4-pyrrolidinyl) thiazole-4-carboxylate XLIII was prepared by reaction of N- Boc (t-butoxycarbonyl) protected thioprolinamide XLII with ethyl bromopyruvate, via a modified Hantzsch reaction in presence of KHCO$_3$ and DME, followed by deprotection of the resulting carboxylic ester XLIII by HCl in 1, 4-dioxane to give XLIV [79].
The thiazole derivatives XLVII were obtained by stirring phenacyl bromide XLV with thioacetamide or thiourea XLVI in presence of ammonium salt of a heteropoly acid (AMP) as a catalyst at room temperature [80].

\[
\text{BrCH}_2\text{CO}_2\text{R} + \text{H}_2\text{N}\text{SR} \xrightarrow{\text{AMP, MeOH, r.t., 20 min}} \text{XLVII}
\]

Thiazole 5-carboxylic acid derivatives L were obtained in good yields by condensation of α-chloro-β-ketoester XLVIII with 2-(2,6-dichloro-phenyl) ethanethioamide XLIX, followed by a basic hydrolysis of the resulting esters [81].

\[
\text{XLVIII} + \text{XLIX} \xrightarrow{\text{EtOH/Pyridine, NaOH, MeOH/THF}} \text{L}
\]

1.5.1.3 From thionoester:

The 2,4- diphenyl-5-acetoxythiazole LII was obtained by the reaction of thionoester LI with phenylglycine [82].
1.5.1.4 **From isothiocyanate:**

4-Amino-3-substituted-2-thioxo-2, 3-dihydro-thiazole-5-carbonitrile derivatives LIV were obtained by stirring the isothiocyanates LIII, malononitrile and sulfur powder in DMF containing TEA [83].

\[
\begin{align*}
R\text{-NCS} + \text{CN-CN} + S & \xrightarrow{\text{TEA}} R\text{-N}_{2}\text{H} \\
\text{LIII} & \xrightarrow{\text{LIV}} \text{LIV}
\end{align*}
\]

1.5.1.5 **From cyanamide:**

The substituted thiazole derivative LVII, was obtained by reaction of cyanamide LV, CS₂ with CH₃I in presence of KOH in a one-pot three step reaction that afforded dimethyl cyanocarbonimidodithioate LVI, which was reacted successively with sodium sulfide, chloroacetonitrile, and potassium carbonate to obtain the substituted thiazole derivative LVII [84].

\[
\begin{align*}
\text{NH}_2\text{CN} & \xrightarrow{\text{CS}_2, \text{CH}_3\text{I, KOH}} \text{H}_3\text{C-S} \xrightarrow{\text{Na}_2\text{S, K}_2\text{CO}_3} \text{H}_3\text{C-S} \\
\text{LV} & \xrightarrow{\text{LVI}} \text{LVII}
\end{align*}
\]
1.5.1.6 From aromatic nitriles:

A convenient, green, short reaction time and high yield method for the synthesis of aromatic 2-thiazolines LX was adopted by reaction of aromatic nitriles LVIII and 2-aminoethanethiol LIX under solvent-free conditions using tungstophosphoric acid (TPA) as catalyst [85].

\[
\text{Ar-CN} + \text{H}_2\text{N-SH} \xrightarrow{\text{TPA}} \text{Ar-S-N} \quad \text{LVIII} \quad \text{LIX} \quad \text{LX}
\]

\[
\text{Ar} = \begin{array}{c}
\text{Ph} \\
\text{thiophene} \\
\text{pyridine}
\end{array}
\]

Benzonitrile LXI was stirred with L-cysteine LXII in MeOH/pH 6.4 phosphate buffer solution to yield the thiazole derivative LXIII [86].

\[
\text{H}_2\text{N-SH-COOH} \xrightarrow{\text{MeOH/pH 7 days}} \text{LXIII}
\]
1.5.2 Synthesis of thiazolidinones

Several protocols for the synthesis of 4-thiazolidinones are available in the literature.

1.5.2.1 From Schiff’s bases and mercaptoacid derivatives:

Three component condensation involving an amine, a carbonyl compound, and a mercaptoacid derivatives led to the formation of 4-thiazolidinone derivatives LXIV using N, N-dicyclohexyl carbodiimide (DCC) or 2-(1H-benzotriazo-1-yl)-1,1,3,3-tetramethyl uranium hexafluorophosphate (HBTU) as a dehydrating agent to accelerate the intramolecular cyclization resulting in faster reaction and improved yields [87], [88].

\[
R_1\text{NH}_2 + O\equiv R_2 R_3 \xrightarrow{\text{Step 1}} R_1\text{N} = C\equiv R_2 R_3 \\
\xrightarrow{\text{Step 2}} \xrightarrow{\text{Step 3}} \xrightarrow{-H_2O} \\
\]

A series of 2-(2, 6-dibromophenyl)-3-heteroaryl-1, 3-thiazolidin-4-ones LXV were synthesized by refluxing a suitably substituted heteroaromatic amine.
with 2, 6- dibromobenzaldehyde in the presence of an excess of mercaptoacetic acid in toluene [89], [90].

\[
\text{Ar-NH}_2 + \text{Br-CHO Br} + \text{HS-COOH} \rightarrow \text{Toluene Reflux} \quad 24 \text{h}
\]

LXV

The synthesis of a series of thiazolidinone LXVII was reported by addition of 3-benzylidene-N, N-dimethylpropyl-amine LXVI to mercapto-acetic acid in refluxing dry benzene [91].

\[
\text{H=NNCH}_3 + \text{HS-COOH} \rightarrow \text{Dry benzene, } \Delta
\]

LXVI

LXVII

Cyclocondensation of Schiff’s bases LXVIII with mercaptoacetic acid in dry dioxane, afforded the corresponding thiazolidinones LXIX [92].

\[
\text{O=S=O} + \text{HS-COOH} \rightarrow \text{Dioxane, r.t}
\]

LXVIII

LXIX
Schiff’s base LXX was refluxed with mercaptoacetic acid in dry benzene to obtain the corresponding thiazolidinone derivative LXXI [93].

![Schiff’s base and thiazolidinone derivative](image)

Dandia et al. [94] have reported a microwave-assisted three-component, regioselective one-pot cyclocondensation method for the synthesis of a series of novel spiro [indole-thiazolidinones] LXXIV by reaction of indoline-2,3-dione derivatives LXXII, 1H-benzo[d] imidazol-2-amine LXXIII and mercapto- acid derivatives. This rapid method produced pure products in high yields within few minutes, in comparison to a conventional two-step procedure.

![Microwave-assisted cyclocondensation](image)

A series of 7-(2-substituted phenylthiazolidinyl)-benzopyran-2-one derivatives LXXVII have been synthesized by reaction of an appropriate substituted aldehydes with 7-amino-4-methyl-benzopyran-2-one LXXV to obtain various Schiff’s bases LXXVI which on treatment with mercaptoacetic acid
acid afforded the corresponding thiazolidinyl coumarin derivatives LXXVII [95].

\[ \text{LXXV} \quad \rightarrow \quad \text{LXXVI} \]

\[ \text{LXXVII} \]

1.5.2.2 From ketimine and chlorocarboxylsulfenyl chloride

The reaction of ketones with amines yielded the key intermediates N-(1-phenyl ethylidene) benzenamines LXXVIII in a good yield. Reaction of the ketimine derivatives LXXVIII with chlorocarboxylsulfenyl chloride in presence of pyridine furnished the corresponding thiazolidinone derivatives LXXIX [96].

\[ \text{LXXVIII} \quad \rightarrow \quad \text{LXXIX} \]
1.5.2.3 From chloroacetamido derivatives and Ammonium thiocyanate

Thiazolidinone derivative LXXXI was produced by stirring 2-aminothiazole with chloroacetyl chloride in DMF at room temperature, then the chloroacetamido derivative LXXX was refluxed with ammonium thiocyanate in ethanol [97].

![Chemical Reaction Diagram](https://via.placeholder.com/150)

LXXX \[\xrightarrow{\text{DMF, rt, 2h}}\] LXXXI

1.5.2.4 From thiourea derivatives:

Ottana et al. [98] reported the synthesis of 4-thiazolidinones LXXXIII, LXXXIV by reaction of N-propyl-N-phenylthiourea LXXXII with chloroacetyl chloride in CHCl₃ in the presence of TEA.

![Chemical Reaction Diagram](https://via.placeholder.com/150)

LXXXII \[\xrightarrow{\text{Et}_3\text{N, CHCl₃, rt, 12 h}}\] LXXXIII \[\text{LXXXIV} \]

Thiazolidinone derivatives LXXXVI can also be prepared from arylthioureas LXXXV with ethyl bromoacetate [99].

![Chemical Reaction Diagram](https://via.placeholder.com/150)

LXXXV \[\xrightarrow{\text{EtOH, 60 °C}}\] LXXXVI
1.5.2.5 **From isothiocyanates:**

Cyclocondensation of isothiocyanates LXXXVII with mercaptoacetic acid in dioxane yielded the corresponding 2-thioxo-4-thiazolidinones LXXXVIII [100].

![Chemical Reaction Diagram]

LXXXVII  
\[
\begin{align*}
\text{N} & \equiv \text{C} = \text{S} \\
\text{O} & \equiv \text{S} \\
\text{N} & \equiv \text{H} \\
\text{R} & = \text{H, Pyrimidine}
\end{align*}
\]

LXXXVIII

Reaction of 2-(2-cyano-acetylamino)- 4, 5, 6, 7-tetrahydro-benzo[b] thiophene-3-carboxamide LXXXIX with phenyl isothiocyanate in DMF in presence of potassium hydroxide gave XC. Reaction of XC with chloroacetyl chloride gave the thiazolidinone derivative XCI [101].

![Chemical Reaction Diagram]

LXXXIX  
\[
\begin{align*}
\text{H}_2\text{N} & \equiv \text{O} \\
\text{N} & \equiv \text{C} = \text{S} \\
\text{PhHN} & \equiv \text{S} \text{K}^+
\end{align*}
\]

XC  
\[
\begin{align*}
\text{H}_2\text{N} & \equiv \text{O} \\
\text{N} & \equiv \text{C} = \text{S} \\
\text{PhHN} & \equiv \text{S} \text{K}^+
\end{align*}
\]

XCI
1.5.2.6 From thiosemicarbazones:

2-hydrazolyl-4-thiazolidinones XCIV were prepared by reaction of thiosemicarbazones XCII with maleic anhydride XCIII [102].

\[
\text{R-N-NH}_2 + \text{PhMe/DMF (25:1), reflux} \rightarrow \text{R-N-NH}_2 \text{S-NHR}_1 \text{O} \text{O} \text{S-NHR}_1 \text{O} \text{OH} \text{O} \text{N-NR}_1
\]

XCII  XCIII  XCIV

A 3-component, one pot reaction was adopted for preparation of the 2-hydrazolyl-4-thiazolidinones XCVI using different aldehydes, thiosemicarbazones XCV, and maleic anhydride XCIII in toluene/DMF (1:1) as a solvent, with a catalytic amount of p-TsOH and microwave irradiation [103].

\[
\text{R-CN} + \text{H}_2\text{N-NH}_2 + \text{PhMe/DMF} \text{MW 100-120°C} \text{p-TsOH (cat.)} \rightarrow \text{R-N-NH}_2 \text{S-NHR}_1 \text{O} \text{O} \text{S-NHR}_1 \text{O} \text{OH} \text{O} \text{N-NR}_1
\]

XCV  XCIII  XCVI
Aim of the Work

Sulfonamides are among a growing list of compounds with promising anticancer activity [25]-[28]. Some structurally novel sulfonamide derivatives present useful applications as antitumor agents in-vitro and/or in-vivo. E7010 and ER-34410 are examples of sulfonamides in advanced clinical trials [28], [43]. E7070 (Indisulam) is now clinically used for its antitumor activity.

In addition, sulfapyridine derivatives in which the 4-amino group is incorporated into a heterocyclic system or substituted showed cytotoxic or anticancer activity, for example: recently, the sulfapyridine derivatives LXIX and XCVII showed cytotoxic activity against breast carcinoma cell line (MCF-7) and cervix carcinoma cell line (HELA) in comparison with 5-Flourouracil and doxorubicin [92]. Moreover, the sulfapyridine derivative XCVIII showed good activities against several human tumor cell lines, such as Molt-3 (T-cell leukemia), Bel-7402 (hepatoma), MCF-7 (breast cancer), DND-1(melanoma), DU-145 and PC-3 (prostate cancer) [104]. Compound XCIX was found to be nearly as active as doxorubicin against liver cell line (HEPG2) [105].
Aim of the Work

On the other hand, bioisosteres of sulfapyridine in which the pyridine ring was replaced by other heterocyclic rings such as compound C demonstrated high antitumor activity and was more potent and safer than chlorambucil CBL [106].

Similarly, several bioisosteres of sulfapyridine having N⁴ substituents or in which N⁴ is incorporated into a heterocyclic system showed anticancer activity, such as N⁴-di-2-chloro-n-propylsulphadiazine, CI, which demonstrated high activity as an inhibitor of the growth of the Yoshida tumor and of the Walker rat carcinoma [107]. In addition, compound CII bearing sulfa-methoxazole moiety have been reported to possess in-vitro anticancer activity [108], and the sulfathiazole derivative CIII was found to have cytotoxic activity against liver cell line (HEPG2) [105].
Aim of the Work

Other sulfonamides having the 4-amino group substituted or incorporated into a heterocyclic system were found to possess cytotoxic or anticancer activity, such as XIII were reported as Cyclin-Dependent Kinase inhibitors and hence anticancer agents [63], also CIV was found to have a promising antitumor activity [109]. Celecoxib effectively inhibited the growth of adenomatous polyps and cause regression of existing polyps in patients with the unusual hereditary condition familial adenomatous polyposis (FAP), so Celecoxib has a promise as anticancer agent [71].

![Chemical Structures XIII and CIV](image)

\[ R = \text{CH}_2\text{NH}_2, \text{CH}_2\text{CH}_2(\text{O})\text{COCH}_2\text{CH}_3 \]

On the other hand, the sulfonamide derivative CV bearing an amino acid moiety showed inhibitory activity \textit{in-vitro} against tumor cell line (HOP-62, cell lung tumor) [110].
Consequently, as a continuation of the previous work in the field of sulfonamides as antitumor agents [105], [108], [111]-[114], the present work aims to design and synthesize other novel compounds characterized by $N^1$ substituted sulfonamide moiety (sulfapyridine), in which the 4-amino group is substituted or incorporated into a heterocyclic system.

The \textit{in-vitro} cytotoxic activity is measured for all the synthesized compounds against human breast cancer (MCF-7) cell line using the Sulfo-Rhodamine-B stain (SRB) assay in comparison with doxorubicin. The cell killing effect of $\gamma$-irradiation is studied for its synergistic effect with the most active compounds.

Molecular Docking study of the most active synthesized compounds will be done on the Cyclin-Dependent Kinase 2 receptor in order to give an idea if these compounds may act as Cyclin-Dependent Kinase (CDK) inhibitors.

\textbf{The synthesized compounds belong to the following classes:}
i. 2-(2-Oxo-2-(4-(N-pyridin-2-ylsulfamoyl)phenylamino)ethylamino)-alkanoic acids (5 a-f).

\[ \text{5a, } R_1 = \text{H} \]
\[ \text{b, } R_1 = \text{CH}_3 \]
\[ \text{c, } R_1 = \text{HC}_2\text{H}_5 \]
\[ \text{d, } R_1 = \text{CH}_2\text{COOH} \]
\[ \text{e, } R_1 = \text{H}_2\text{C} \]
\[ \text{f, } R_1 = \text{H}_2\text{C} \]

ii. 2-(Substituted-amino)-N-(4-(N-pyridin-2-ylsulfamoyl)phenyl)-acetamides (6-10).

\[ \text{6, } R_1 = -\text{N} \]
\[ \text{7, } R_1 = -\text{HN} \]
\[ \text{8, } R_1 = -\text{N} \]
\[ \text{9, } R_1 = -\text{N} \]
\[ \text{10, } R_1 = -\text{HN} \]
iii. 4-(5-Substituted-4-cyano-2-oxo-2,3-dihydro-1H-pyrrol-1-yl)-N-(pyridin-2-yl)benzenesulfonamides (11-14).

![Chemical structure](image)

- 11, $R_1 = NH_2$
- 12, $R_1 = NHCOCH_3$
- 13, $R_1 = N(COCH_3)_2$
- 14, $R_1 = NHCSNH_2H_5$

iv. 4-(4-Substituted-thiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamides (3, 4, 15-24).

![Chemical structure](image)

- 3, $R_1 = OH$
- 4, $R_1 = Cl$
- 15, $R_1 = SH$
- 16, $R_1 = SCH_3$
- 17, $R_1 = NHNH_2$
- 20, $R_1 = NHNH-C_6H_5$
- 21, $R_1 = NHNH-C_6H_3(NO_2)_2-2,4$
- 22, $R_1 = NCS$
- 23, $R_1 = NH-C_6H_3-Cl-4$
- 24, $R_1 = NH-C_6H_4-COOH-2$
v. 4-(5-Amino-7-(4-chlorophenyl)-6-cyano-7H-pyrano[2,3-d]thiazol-2-ylamino)-N-(pyridin-2-yl)benzenesulfonamide (18), 4-[3H-5-(4-chlorophenyl)thiazolo[4,5-b]pyrano[2,3-d]pyrimidin-4-one]-N-(pyridin-2-yl)benzenesulfonamide (19).
Theoretical Discussion

Schemes (1-6) illustrate the pathways for the synthesis of the target compounds. Scheme 1 showed the routes adopted for the synthesis of the key intermediates 2, 3, 4 from sulfapyridine.

\[
\begin{align*}
(1) & : \text{NH}_2 \begin{array}{c}
\text{O=S=O} \\
\text{NHR}
\end{array} \\
(2) & : \text{NH} \begin{array}{c}
\text{O=S=O} \\
\text{NHR}
\end{array} \xrightarrow{\text{Cl \(\text{CH}_2\text{CH}_2\text{Cl}\)}} \xrightarrow{\text{DMF / r.t.}} \text{NH} \begin{array}{c}
\text{O=S=O} \\
\text{NHR}
\end{array} \\
(4) & : \text{HN} \begin{array}{c}
\text{N} \text{Cl} \\
\text{SOCl}_2
\end{array} \xrightarrow{\Delta} \text{HN} \begin{array}{c}
\text{N} \text{Cl} \\
\text{SOCl}_2
\end{array} \\
(3) & : \text{HN} \begin{array}{c}
\text{N} \text{OH} \\
\text{SOCl}_2
\end{array} \xrightarrow{\text{NH}_4\text{SCN \ EtOH}} \text{HN} \begin{array}{c}
\text{N} \text{OH} \\
\text{SOCl}_2
\end{array}
\end{align*}
\]

Scheme 1
Theoretical Discussion

Scheme 2

(2) + H$_2$N-C-COOH + Na$_2$CO$_3$ \[\text{stir.} \& \text{reflux 5h.}\] \[\text{R}_1\] = $\begin{cases} 5a &= H \\ 5b &= \text{CH}_3 \\ 5c &= \text{HC}_2\text{H}_5 \\ 5d &= \text{CH}_2\text{COOH} \\ 5e &= \text{H}_2\text{C} \\ 5f &= \text{H}_2\text{C-OH} \end{cases}$
Scheme 3
Scheme 4
Scheme 5
Scheme 6
2-Chloro-N-(4-(N-pyridin-2-ylsulfamoyl) phenyl) acetamide (2): [115], [97]

Compound 2 was previously prepared by dropwise slow addition of chloroacetyl chloride to a stirred solution of 4-amino-N-pyridin-2-ylbenzenesulfonamide in sodium hydroxide [115]. However, in the present study, stirring a mixture of 4-amino-N-pyridin-2-ylbenzenesulfonamide 1 and chloroacetyl chloride in DMF for 2 h. at room temperature as adopted procedure [97], afforded 2 in good yield 96%.

\[
\begin{align*}
\text{(1)} & \quad \text{NH}_2 \quad \text{O=S=O} \quad \text{NHR} \\
\text{(2)} & \quad \text{Cl} \quad \text{C} \quad \text{-HCl} \\
\end{align*}
\]

The formation of compound 2 was supported from its microanalytical and spectral data. IR spectrum showed a band at 1680 cm\(^{-1}\) (C=O). \(^1\)H-NMR spectrum revealed a singlet at 4.2 ppm (CH\(_2\)), multiplets at 6.8-8.0 ppm (Ar-H + NH, D\(_2\)O exchangeable), and singlet at 10.6 ppm (SO\(_2\)NH, D\(_2\)O exchangeable).

4-(4-Hydroxythiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (3):

Treatment of the N\(^4\)-chloroacetamide derivative 2 with ammonium thiocyanate in ethanol as adopted procedure [97], yielded the corresponding thiazolidinone derivative 3, via intramolecular cyclization. Figure 3.1 showes the proposed mechanism of heterocyclization.
The formation of compound 3 was proved on the basis of its microanalytical and spectral data. IR spectrum of compound 3 showed a broad band at 3448 cm\(^{-1}\) (OH) and band at 1602 cm\(^{-1}\) (C=N). \(^1\)H-NMR spectrum showed a singlet at 6.84 ppm (CH thiazole), multiplets at 6.86-8.0 ppm (Ar-H + NH, D\(_2\)O exchangeable), singlet at 10.9 ppm (SO\(_2\)NH, D\(_2\)O exchangeable), and singlet at 11.7 ppm (OH, D\(_2\)O exchangeable). Finally, mass spectrum of compound 3 exhibited a molecular ion peak at m/z 348 (M\(^+\), 0.7%), 349 (M+1, 0.3%) with a base peak at m/z 184.
4-(4-Chlorothiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (4):

A mixture of 4-hydroxy thiazole derivative 3 and thionyl chloride was refluxed for 2 h. as adopted procedure [26] to afford the corresponding 4-chloro-thiazole derivative 4.

\[
\text{HN} \quad \text{N} \quad \text{OH} \\
\text{O=S=O} \quad \text{NHR} \\
\text{SOCl}_2 \\
\triangle \\
(3) \\
\]

IR spectrum of compound 4 revealed the absence of the broad band corresponding to the 4-hydroxy group and appearance of a band at 684 cm\(^{-1}\) (C-Cl). In addition, \(^1\)H-NMR spectrum of compound 4 showed a singlet at 6.6 ppm (CH thiazole) and absence of signal at 11.7 ppm (OH), its mass spectrum revealed a molecular ion peak at m/z 366 (M\(^+\), 3.46 %), 368 (M+2, 1.08 %) with a base peak at m/z 78.

2-{2-Oxo-2-[4-(N-pyridin-2-ylsulfamoyl)phenylamino]ethylamino} alkanoic acid derivatives 5 a-f:

Refluxing and stirring chloroacetamide derivative 2 with the solution of sodium salt of various amino acids namely; glycin, alanine, isoleucine, aspartic acid, phenylalanine, tyrosine in water for 5 h. followed by acidification with formic acid as adopted procedure [116], afforded the corresponding alkanoic acid derivatives 5 a-f.
Theoretical Discussion

The IR spectra of 5a-f showed a broad absorption band at 3463-3432 cm\(^{-1}\) (OH), bands at 3394-3103 (3NH) and bands at 1707-1628 cm\(^{-1}\) (2 \(\text{C=O}\)) for 5a-c, e, f and (3 \(\text{C=O}\)) for 5d.

\(^{1}\)H-NMR spectra of compounds 5a-e showed singlets at 5.0 ppm (\(\text{COCH}_2\)), singlets at 4.3 - 5.9 ppm (\(\text{COCH}_2\text{NH}, \text{D}_2\text{O exchangeable}\)) and singlets at 10.1 - 11.3 ppm (OH, \(\text{D}_2\text{O exchangeable}\)).

Moreover, \(^{1}\)H-NMR spectrum of Compound 5a revealed a singlet at 3.3 ppm (\(\text{NHCH}_2\)), Compound 5b revealed a doublet at 1.2 ppm (\(\text{CH}_3\)) and quartet at 3.2 ppm (CH), while Compound 5c showed a triplet at 0.8 ppm (\(\text{CH}_2\text{CH}_3\)), doublet at 1.4 ppm (\(\text{CHCH}_2\)), multiplets at 1.6-1.8 ppm (\(\text{CH}_2\text{CH}_3\)) and 3.0-3.2 ppm (\(\text{CH(CH}_3)\text{CH}_2\)), and doublet at 3.3 ppm (\(\text{CHCOOH}\)). Compounds 5d showed a doublet at 2.5 ppm (\(\text{CH}_2\text{COOH}\)) and triplet at 3.3 ppm (CH). Compound 5e showed a doublet at 2.2 ppm (\(\text{CH}_2\text{Ph}\)) and triplet at 2.8 ppm (CH).
Mass spectrum of compound 5b showed a molecular ion peak at m/z 378 (M+, 4.57%) with a base peak at m/z 61, compound 5e showed a molecular ion peak at m/z 454 (M+, 14.06%) with a base peak at m/z 184. Finally, compound 5f showed a molecular ion peak at m/z 470 (M+, 6.19%) with a base peak at m/z 107.

2-(Substituted-amino)-N-(4-(N-pyridin-2-ylsulfamoyl) phenyl) acetamides (6-10):

When chloroacetamide derivative 2 was refluxed with aliphatic or aromatic amines namely; diethylamine, 3-aminopropanol, piperidine, morpholine, and 4-chloroaniline in ethanol as adopted procedure [117], compounds 6-10 were obtained, respectively.

\[
\begin{align*}
\text{HN} & \quad \text{Cl} \\
\text{HN} & \quad \text{R} \\
\text{O} & \quad \text{SO}_2 \\
\text{NHR} &
\end{align*}
\]

(2)

\[
\begin{align*}
\text{HN} & \quad \text{R}_1 \\
\text{HN} & \quad \\
\text{O} & \quad \text{SO}_2 \\
\text{NHR} &
\end{align*}
\]

(6), \( R_1 = \text{N} \)

(7), \( R_1 = \text{HN-\text{CH}_2-\text{OH}} \)

(8), \( R_1 = \text{N} \)

(9), \( R_1 = \text{N} \)

(10), \( R_1 = \text{HN-\text{CH}_2-\text{Cl}} \)

\(^1\)H-NMR spectrum of compound 6 showed the presence of significant triplet at 1.1 ppm (2CH\text{$_2$}CH$_3$), quartet at 2.9 ppm (2CH\text{$_2$}CH$_3$) and singlet at 5.0 ppm (COCH\text{$_2$}).
IR spectrum of compound 7 was characterized by the presence of a band at 3403 cm\(^{-1}\) (OH). \(^1\)H-NMR spectrum of 7 showed multiplet at 1.5-1.6 ppm (CH\(_2\)CH\(_2\)OH), triplet at 2.5 ppm (NHCH\(_2\)), triplet at 2.7 ppm (CH\(_2\)OH), singlet at 5.0 ppm (COCH\(_2\)) and singlet at 11.3 ppm (OH, D\(_2\)O exchangeable).

Compounds 8 and 9 were previously obtained by refluxing the chloroacetamide derivative 2 dissolved in benzene - toluene mixture with piperidine or morpholine, respectively for 10-15 h. in a yield of 70 % and 61 %, respectively [118]. In the present study, the chloroacetamide derivative 2 was refluxed with piperidine or morpholine, respectively in ethanol for 2 h. as adopted procedure [117] to give better yields 88 %, and 79 %, respectively. Mass spectrum of compound 8 exhibited molecular ion peak at m/z 374 (M\(^+\), 1.19 %) with a base peak at m/z 98. While \(^1\)H-NMR spectrum of compound 9 showed two triplets one at 3.0 ppm (2 CH\(_2\)N) and the other 3.7 ppm (2CH\(_2\)O).

\(^1\)H-NMR spectrum of compound 10 revealed a singlet at 3.3 ppm (CH\(_2\)NH, D\(_2\)O exchangeable) and another singlet at 3.8 ppm (COCH\(_2\)). Moreover, Mass spectrum of compound 10 showed a molecular ion peak at m/z 416 (M\(^+\), 15.99 %), 418 (M+2, 5.36 %) with a base peak at m/z 63.

\textbf{4-(5-Amino-4-cyano-2-oxo-2,3-dihydro-1H-pyrrol-1-yl)-N-(pyridin-2-yl)benzenesulfonamide (11):} [119]

Compound 11 was obtained by refluxing compound 2 with malononitrile in dioxane containing a catalytic amount of TEA for 24 h. as adopted procedure [119]. In the present study, the chloroacetamide derivative 2 was refluxed with malononitrile in DMF containing a catalytic amount of piperidine for 2 h. as this method needs shorter time.

The formation of compound 11 is assumed to proceed via alkylation of malononitrile, followed by intramolecular cyclization.
IR spectrum of compound \(11\) was characterized by the presence of bands at 3250, 3198, 3115 cm\(^{-1}\) (NH, NH\(_2\)) and a strong band at 2201 cm\(^{-1}\) (C≡N). \(^1\)H-NMR spectrum of compound \(11\) revealed a singlet at 2.7 ppm (CH\(_2\) pyrrole) and a singlet at 4.7 ppm (NH\(_2\), D\(_2\)O exchangeable).

\(4\)-(5-Substituted-4-cyano-2-oxo-2,3-dihydro-1H-pyrrol-1-yl)-N-(pyridin-2-yl)benzenesulfonamides (12), (13):

When compound \(11\) was refluxed in excess acetic anhydride for 2 h., the corresponding monoacetyl derivative \(12\) was obtained, while refluxing for a longer time (15 h.) the corresponding diacetyl derivative \(13\) was produced instead of the expected fused pyrrolo [2,3-d] pyrimidine systems \(13''\) as reported in a similar reaction [120].
IR spectrum of compound 12 showed a band at 2215 cm\(^{-1}\) (C≡N) which indicates that no cyclization occurred, and two bands at 1716 cm\(^{-1}\) and 1639 cm\(^{-1}\), (2C=O) of the acetyl group and the 5-oxo group. \(^1\)H-NMR spectrum of compound 12 revealed the acetyl protons as a singlet at 2.0 ppm (COCH\(_3\)) and another singlet at 2.7 ppm (CH\(_2\) pyrrole). Moreover, mass spectrum of
compound 12 revealed a molecular ion peak at 397 m/z \((M^+, 0.61\%)\) with a base peak at m/z 92.

IR spectrum of compound 13 showed a band at 2219 cm\(^{-1}\) (C≡N), and two bands at 1710 cm\(^{-1}\) and 1625 cm\(^{-1}\) (C=O). \(^1\)H-NMR spectrum of 13 revealed the acetyl protons as a singlet at 1.9 ppm \((2\text{COCH}_3)\) and a singlet at 2.4 ppm \((\text{CH}_2\text{pyrrole})\). Mass spectrum of 13 revealed a molecular ion peak at 439 m/z \((M^+, 2.10\%)\) with a base peak at m/z 62.

\(4-(4\text{-Cyano-5-(3-ethylthioureido)-2-oxo-2,3-dihydro-1H-pyrrol-1-yl})-N-(\text{pyridin-2-yl})\text{benzenesulfonamide (14)}:\)

Refluxing compound 11 with ethyl isothiocyanate in DMF in the presence of a catalytic amount of TEA for 10 h., yielded the thioureido derivative 14.

This was confirmed by IR spectrum which revealed the presence of a band at 2192 cm\(^{-1}\) (C≡N) and 3471, 3336, 3298 cm\(^{-1}\) (3NH). \(^1\)H-NMR spectrum of compound 14 showed the presence of triplet at 1.1 ppm \((\text{CH}_3\text{CH}_2)\), quartet at 4.4 ppm \((\text{CH}_3\text{CH}_2)\) and disappearance of \(\text{NH}_2\) signal at 4.7 ppm. Additionally,
mass spectrum of compound 14 revealed a molecular ion peak at 442 m/z (M⁺, 2.84 %) with a base peak at m/z 65.

4-(4-Mercaptothiazol-2-ylamino)-N-(pyridin-2-yl)benzenesulfonamide (15):

Reaction of the 4-hydroxy derivative 3 with phosphorus pentasulfide in pyridine for 8 h. as adopted procedure [121], afforded the 4-mercaptothiazole derivative 15.

\[
\begin{align*}
HN\hspace{1cm}S\hspace{1cm}N\hspace{1cm}R
\end{align*}
\]

\[
\begin{align*}
O=S=O
\end{align*}
\]

IR spectrum of compound 15 revealed the absence of a band at 3448 cm⁻¹ (OH).¹H-NMR spectrum of compound 15 showed a singlet at 6.86 ppm (CH thiazole), a singlet at 13.8 ppm (SH, D₂O exchangeable) and disappearance of OH signal at 11.7 ppm. In addition, mass spectrum of compound 15 revealed a molecular ion peak at m/z 364 (M⁺, 1.60%) with a base peak at m/z 63.

4-(4-(Methylthio) thiazol-2-yl amino)-N-(pyridin-2-yl) benzenesulfonamide (16):

Reaction of compound 15 with methyl iodide in methanol at ambient temperature for 48 h., then heating under reflux for 6 h. as adopted procedure [122], afforded the methylthio- thiazole derivative 16.
IR spectrum of compound 16 showed bands at 2928, 2860 (CH aliph.). $^1$H-NMR spectrum revealed a singlet at 2.4 ppm (SCH$_3$) and a singlet at 6.8 ppm (CH thiazole).

4-(4-Hydrazinylthiazol-2-ylamino)-N-(pyridin-2-yl)benzenesulfonamide (17):

Compound 17 was obtained by two different procedures either by refluxing compound 16 and hydrazine hydrate in methanol for 24 h. as adopted procedure [123] to give 17 in 20% yield or by refluxing the 4-chloro derivative 4 with hydrazine hydrate in ethanol for 5h. as adopted procedure [26] to give 17 in 58% yield.
TLC for the two products, melting point and the mixed melting point indicate that the two products were the same.

IR spectrum of compound 17 showed the appearance of a new forked band at 3215, 3113 cm\(^{-1}\) (NH\(_2\)). Moreover, \(^1\)H-NMR spectrum of compound 17 showed a singlet at 2.4 ppm (NH\(_2\), D\(_2\)O exchangeable), another singlet at 4.7 ppm (NHNH\(_2\), D\(_2\)O exchangeable).

4-(5-Amino-7-(4-chlorophenyl)-6-cyano-7H-pyran[2,3-d]thiazol-2-yl amino)-N-(pyridin-2-yl) benzenesulfonamide (18):

Treatment of compound 3 with 2-(4-chlorobenzylidene) malononitrile in ethanol in presence of a catalytic amount of TEA, as a basic catalyst as adopted procedure [124], yielded the corresponding pyranothiazole derivative 18, via intramolecular cyclization. The 2-(4-chlorobenzylidene) malononitrile was prepared by stirring the 4-chlorobenzaldehyde with malononitrile for about 20 minute in ethanol containing few drops of TEA at room temperature.

The basic catalyst, TEA; was required to generate the anion of derivative 3, thus facilitating the addition to the unsaturated nitrile.
The theoretical discussion of compound 18 was established on the basis of elemental analysis and spectral data. IR spectrum of compound 18 showed appearance of a forked band at 3250, 3191 cm\(^{-1}\) (NH\(_2\)), sharp band at 2219 cm\(^{-1}\) (C≡N) and band at 780 cm\(^{-1}\) (C-Cl). \(^1\)H-NMR spectrum of compound 18 showed the presence of a singlet at 3.8 ppm (NH\(_2\), D\(_2\)O exchangeable) and a singlet at 4.1 ppm (CH pyran) and multiplets at 6.6-8.0 ppm (13H, Ar-H + NH, D\(_2\)O exchangeable). Mass spectrum of compound 18 exhibited molecular ion peak at m/z 536 (M\(^+\), 1.00 %), 538 (M+2, 0.35%) with a base peak at m/z 168.

4-[1H -8-(4-Chlorophenyl)-thiazolo [4,5-b] pyrano [2,3-d] pyrimidin-9-one-6-vamino]-N-(pyridin-2-yl) benzenesulfonamide (19):

The thiazolo[4,5-b]pyrano[2,3-d]pyrimidin-9-one-6-vamino]-N-(pyridin-2-yl) benzenesulfonamide derivative 19 was obtained by refluxing compound 18 in formic acid. This reaction was proceeded via condensation and
elimination of two moles of water followed by cyclization to give the thiazolo [4,5-b]-pyrano [2,3-d] pyrimidine derivative 19 as adopted procedure [121].

IR spectrum of compound 19 revealed the absence of the (C≡N) band at 2219 cm⁻¹ and the presence of band at 1713 cm⁻¹ (C=O). ¹H-NMR spectrum of compound 19 showed the presence of a singlet at 4.1 ppm (CH pyran), multiplets at 6.8-7.9 ppm (14H, Ar-H + 2NH, D₂O exchangeable), and singlet at 8.0 ppm (CH pyrimidine). Mass spectrum of compound 19 exhibited a molecular ion peak at m/z 564 (M⁺, 6.19 %), 566 (M+2, 2.1 %) with a base peak at m/z 157.
4-(4-(2-Substituted-phenylhydrazinyl) thiazol-2-yl amino) -N-(pyridin-2-yl) benzenesulfonamide (20), (21):

When compound 4 was refluxed with phenylhydrazine or 2, 4 dinitro-phenylhydrazine in ethanol for 5 h. as adopted procedure [26], compounds 20 or 21 were afforded, respectively.

![Chemical structures](image)

Compound 20 was characterized by the presence of a singlet at 3.5 ppm (NHNH, D₂O exchangeable) and multiplets at 6.9-8.0 ppm (14 H, Ar-H + NH, D₂O exchangeable) in its ¹H-NMR spectrum. Additionally, mass spectrum of compound 20 showed a molecular ion peak at m/z 438 (M⁺, 0.74%) with a base peak at 61.

IR spectrum of compound 21 showed bands at 1520, 1387 cm⁻¹ (NO₂). ¹H-NMR spectrum of 21 was characterized by the presence of a singlet at 3.7 ppm (NHNH, D₂O exchangeable), and multiplets at 6.9-8.2 ppm (12H, Ar-H + NH, D₂O exchangeable). Mass spectrum of compound 21 showed a molecular ion peak at m/z 528 (M⁺, 1.71%) with a base peak at 61.
4-(4-(Isothiocyanato)thiazol-2-ylamino)-N-(pyridin-2-yl)benzenesulfonamide (22):

The titled compound 22 was obtained by refluxing 4-chlorothiazolo derivative 4 with ammonium thiocyanate in dry acetone as adopted procedure [26].

IR spectrum of compound 22 was characterized by the presence of a strong band at 2051 cm\(^{-1}\) (N=C=S). Additionally, \(^1\)H-NMR spectrum of compound 22 showed a singlet at 6.8 ppm (CH thiazole). Mass spectrum of compound 22 revealed a molecular ion peak at m/z 389 (M\(^+\), 0.9%) with a base peak at 64.

4-(4-(4-Chlorophenylamino) thiazol-2-ylamino)-N-(pyridin-2-yl) benzene-sulfonamide (23):

Interaction of compound 4 with 4-chloroaniline in DMF under reflux for 4 h. gave the corresponding 4-chlorophenylamino derivative 23.
The structure of compound 23 was proved by its microanalytical and spectral data. IR spectrum of compound 23 showed band at 3386, 3345, 3264 cm\(^{-1}\) (3NH) and a band at 620 cm\(^{-1}\) (C-Cl). Moreover, mass spectrum of compound 23 showed a molecular ion peak at m/z 457 (M\(^+\), 0.81\%), 459 (M+2, 0.27\%) with a base peak at 54.

\[ \text{2-}(2-(4-(\text{N-pyridin-2-ylsulfamoyl}) \text{ phenylamino}) \text{ thiazol-4-ylamino}) \text{ benzoic-acid} \ (24): \]

The reaction of compound 4 with anthranilic acid in n-butanol under reflux for 5 h. as adopted procedure [26], yielded the corresponding aminobenzoic acid derivative 24.
IR spectrum of compound 24 showed the presence of bands at 3424 cm\(^{-1}\) (OH), 3347, 3286, 3249 cm\(^{-1}\) (NH) and 1681 cm\(^{-1}\) (C=O). \(^1\)H-NMR spectrum of compound 24 showed a singlet at 6.84 ppm (CH thiazole), singlet at 10.9 ppm (SO\(_2\)NH, D\(_2\)O exchangeable), and singlet at 11.8 ppm (OH, D\(_2\)O exchangeable). Mass spectrum of compound 24 showed a molecular ion peak at m/z 467 (M\(^+\), 2.7\%) with a base peak at 184.
Experimental

- All melting points are uncorrected and were determined on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK).
- Elemental analyses (C, H, N) were performed on Perkin-Elmer 2400 analyser (Perkin-Elmer, Norwalk, CT, USA) at the Microanalytical Laboratories of the Faculty of Science, Cairo University.
- Infrared spectra (KBr) were determined using Shimadzu IR-110 spectrophotometer (Shimadzu, Koyoto, Japan).
- $^1$H-NMR spectra were carried out using BRUCKER proton NMR-Avance 300 (300, MHz), in DMSO-$d_6$ as a solvent, using tetramethysilane (TMS) as internal standard.
- Mass spectra were run on JEOL JMS AX-500 mass spectrometer (JEOL JMS, Tokyo, Japan).
- Nomenclature was done using ChemBioDraw Ultra 10.
- All reactions were monitored by thin layer chromatograph (TLC) using precoated Aluminium sheets Silica gel Merck 60 F254. Ethyl acetate/cyclohexane (2.5:7.5 mL) mixture was used as eluting solvent and TLC sheets were visualized by UV lamp (Merck, Darmstadt, Germany).
- $\gamma$ – Irradiation was performed in the National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt, using Gamma cell-40 ($^{137}$Cs) source at a dose level of 8 Gy with a dose rate of 2Gy/min.
2-Chloro-N-(4-(N-pyridin-2-ylsulfamoyl) phenyl) acetamide (2): [115], [97]

![Chemical Structure of 2-Chloro-N-(4-(N-pyridin-2-ylsulfamoyl) phenyl) acetamide (2)]

A mixture of 4-amino-N-pyridin-2-ylbenzenesulfonamide 1 (0.249g, 0.001 mol) and chloroacetyl chloride (0.08 ml, 0.001 mol) in DMF (20 ml) was stirred for 2 h. at room temperature, the reaction mixture was cooled then poured onto ice – water. The obtained solid was filtered off and crystallized from dioxane to give 2. Yield: 96%, m.p. 192- 193 °C, as reported. Anal. Calcd. for C_{13}H_{12}ClN_{3}O_{3}S (325.77): C, 47.93; H, 3.71; N, 12.92. Found: C, 48.00; H, 3.69; N, 12.72.

**Spectral data for compound 2:**

**IR (KBr, cm⁻¹):**  3318, 3296 (NH), 3053(CH arom.), 2945, 2838 (CH aliph.), 1680 (C=O), 1589 (C=N), 1392, 1145 (SO₂), 680 (C-Cl).

**¹H-NMR (DMSO-δ₆) δ:** 4.2 [s, 2H, CH₂], 6.8-8.0 [m, 9H, Ar-H, + NH, D₂O exchangeable], 10.6 [s, 1H, SO₂NH, D₂O exchangeable].
4-(4-Hydroxythiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (3):

A mixture of compound 2 (0.325g, 0.001 mol) and ammonium thiocyanate (0.076g, 0.001 mol) in ethanol (20 ml) was refluxed for 1 h. The reaction mixture was filtered while hot and the obtained solid was crystallized from dioxane to give 3. Yield 85 %, m.p. 240-242°C. Anal. Calcd. for C_{14}H_{12}N_{4}O_{3}S_{2} (348.40): C, 48.27; H, 3.44; N, 16.09. Found: C, 48.59; H, 3.26; N, 16.32.

Spectral data for compound 3:

IR (KBr, cm\(^{-1}\)): 3448 (OH), 3366, 3191 (NH), 3047(CH arom.), 1602 (C=N), 1397, 1137 (SO\(_2\)).

\(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\): 6.84 [s, 1H, CH, thiazole], 6.86-8.0 [m, 9H, Ar-H + NH, D\(_2\)O exchangeable], 10.9 [s, 1H, SO\(_2\)NH, D\(_2\)O exchangeable], 11.7 [s, 1H, OH, D\(_2\)O exchangeable].

EI/MS (m/z) (%): 348 [M\(^+\)] (0.7), 349 [M+1] (0.03), 184 (100).
4-(4-Chlorothiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (4):

A solution of compound 3 (0.348g, 0.001 mol) in thionyl chloride (10 ml) was refluxed for 2 h., thionyl chloride was then removed by distillation and the residual solid was washed twice with benzene and crystallized from dioxane to give 4. Yield 95%, m.p. 198-200°C. Anal. Calcd. for C_{14}H_{11}ClN_{4}O_{2}S_{2} (366.85): C, 45.90; H, 3.00; N, 15.30. Found: C, 45.63; H, 3.36; N, 15.51.

Spectral data for compound 4:

**IR (KBr, cm^{-1}):** 3192, 3157 (NH), 3040 (CH arom.), 1616 (C=N), 1390, 1165 (SO_{2}), 684 (C-Cl).

**^{1}H-NMR (DMSO-d_{6}) δ:** 6.6 [s, 1H, CH, thiazole], 6.8-8.0 [m, 9H, Ar-H + NH, D_{2}O exchangeable], 10.9 [s, 1H, SO_{2}NH, D_{2}O exchangeable].

**EI/MS (m/z) (%):** 366 [M^{+}] (3.46), 368 [M+2] (1.08), 78 (100).
2-{2-Oxo-2-[4-(N-pyridin-2-ylsulfamoyl) phenylamino]ethylamino} acetic acid (5a), propanoic acid (5b), 3-methylpentanoic acid (5c), succinic acid (5d), 3-phenylpropanoic acid (5e), 3-(4-hydroxyphenyl) propanoic acid (5f):

\[
\text{NH} \quad \text{R}_1 \quad \text{NH} \\
\text{O=S=O} \\
\text{NH} \\
\text{O=O} \\
\text{NH}
\]

5a, R₁ = H
5b, R₁ = CH₃
5c, R₁ = CH₂COOH
5e, R₁ = H₂C
5f, R₁ = H₂C

The appropriate amino acids (0.0096 mol) and sodium carbonate (0.57 g, 0.0054 mol) were dissolved in water (10 mL). The chloro compound 2 (1.56 g, 0.0048 mol) was then added, and the mixture was stirred at 100 °C for 5h. The reaction mixture was left overnight at room temperature and then treated with formic acid. The solid product obtained was filtered off, washed with water, and crystallized from dioxane to give compounds 5 a-f. (Tables 1, 2) showed the physical and spectral data for compounds 5 a-f.
Table 1 – Physical and analytical data for compounds 5a-f.

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>M.p. (°C)</th>
<th>Yield (%)</th>
<th>Mol. Formula (M. wt.)</th>
<th>Microanalysis %</th>
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<tr>
<td>5a</td>
<td>176-178</td>
<td>58</td>
<td>C_{13}H_{16}N_{4}O_{2}S (364.38)</td>
<td>C: 49.45</td>
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<td>C_{17}H_{18}N_{4}O_{2}S (422.41)</td>
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<td>219-221</td>
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<td>C_{22}H_{22}N_{4}O_{6}S (470.50)</td>
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<td>C: 56.39</td>
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Table 2 – Spectral data for compounds 5a-f.

<table>
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<tr>
<th>Compd. No.</th>
<th>IR (KBr, cm(^{-1}))</th>
<th>(^1)H-NMR (DMSO-d(_6))</th>
</tr>
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<tr>
<td>5a</td>
<td>3463 (OH), 3253, 3108 (NH), 3056 (CH arom.), 2935, 2829 (CH aliph.), 1697, 1630 (2 C=O), 1582 (C=N), 1368, 1135 (SO(_2)).</td>
<td>3.3 [s, 2H, NHCH(_2)], 5.0 [s, 2H, COCH(_2)], 5.9 [s, 1H, CH(_2)NH, D(_2)O exchangeable], 6.8-8.0 [m, 9H, Ar-H + NHCO, D(_2)O exchangeable], 10.7 [s, 1H, SO(_2)NH, D(_2)O exchangeable], 10.8 [s, 1H, OH, D(_2)O exchangeable].</td>
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</table>
| 5b | **IR (KBr, cm⁻¹)** | 3453(OH), 3246, 3103 (NH), 3056 (CH arom.), 2957, 2831 (CH aliph.), 1698, 1632 (2 C=O), 1584 (C=N), 1391, 1135 (SO₂).
 | **¹H-NMR (DMSO-d₆)** | 1.2 [d, 3H, CH₃, J = 7.5 Hz], 3.2 [q, 1H, CH], 5.0 [s, 2H, COCH₂], 5.9 [s, 1H, CH₂NH, D₂O exchangeable], 6.7-8.0 [m, 9H, Ar-H + NHCO, D₂O exchangeable], 10.8 [s, 1H, SO₂NH, D₂O exchangeable].
 | **EI/MS (m/z) (%)** | 378 [M⁺] (4.57), 61 (100).

| 5c | **IR (KBr, cm⁻¹)** | 3446(OH), 3274, 3190 (NH), 3053 (CH arom.), 2956, 2865 (CH aliph.), 1707, 1629 (2 C=O), 1600 (C=N), 1360, 1135 (SO₂).
 | **¹H-NMR (DMSO-d₆)** | 0.8 [t, 3H, CH₂CH₃], 1.4 [d, 3H, CHCH₂-J = 6.9 Hz], 1.6-1.8 [m, 2H, CH₂CH₃], 3.0-3.2 [m, 1H, CH(CH₃)CH₂], 3.3 [d, 1H, CHCOOH, J = 6.9 Hz], 4.3 [s, 1H, CH₂NH, D₂O exchangeable], 5.0 [s, 2H, COCH₂], 6.8-8.3 [m, 9H, Ar-H + NHCO, D₂O exchangeable], 10.4 [s, 1H, SO₂NH, D₂O exchangeable], 10.9 [s, 1H, OH, D₂O exchangeable].

| 5d | **IR (KBr, cm⁻¹)** | 3449(OH), 3268, 3242 (NH), 3058 (CH arom.), 2958, 2837 (CH aliph.), 1696, 1628 (3C=O), 1580 (C=N), 1370, 1135 (SO₂).
 | **¹H-NMR (DMSO-d₆)** | 2.5 [d, 2H, CH₂COOH, J = 7.8 Hz], 3.3 [t, 1H, CH], 5.0 [s, 2H, COCH₂], 5.9 [s, 1H, CH₂NH, D₂O exchangeable], 6.8-8.0 [m, 9H, Ar-H + NHCO, D₂O exchangeable], 10.3 [s, 1H, SO₂NH, D₂O exchangeable], 10.8 [s, 2H, 20H, D₂O exchangeable].

| 5e | **IR (KBr, cm⁻¹)** | 3432(OH), 3394, 3253 (NH), 3053 (CH arom.), 2943, 2826(CH aliph.), 1699, 1628 (2 C=O), 1599 (C=N), 1390, 1136 (SO₂).
 | **¹H-NMR (DMSO-d₆)** | 2.2 [d, 2H, CH₂Ph, J = 7.2 Hz], 2.8 [t, 1H, CH], 5.0 [s, 2H, COCH₂], 5.9 [s, 1H, CH₂NH, D₂O exchangeable], 6.4-8.3 [m, 14H, Ar-H + NHCO, D₂O exchangeable], 10.0 [s, 1H, SO₂NH, D₂O exchangeable], 10.1 [s, 1H, OH, D₂O exchangeable].

74
<table>
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<tr>
<th>5f</th>
<th><strong>EI/MS (m/z) (%)</strong></th>
<th><strong>IR (KBr, cm(^{-1}))</strong></th>
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<tbody>
<tr>
<td></td>
<td>454 [M(^+)] (14.06), 184 (100).</td>
<td>3433 (OH), 3207, 3118 (NH), 3017 (CH arom.), 2957, 2829(CH aliph.), 1696, 1628 (2 C=O), 1595 (C=N), 1366, 1136 (SO(_2)).</td>
</tr>
<tr>
<td></td>
<td>470 [M(^+)] (6.19), 107 (100).</td>
<td><strong>EI/MS (m/z) (%)</strong></td>
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</table>
2-(Diethylamino)-N-(4-(N-pyridin-2-ylsulfamoyl) phenyl) acetamide (6), 2-(3-hydroxypropylamino)-N-(4-(N-pyridin-2-ylsulfamoyl)phenyl) acetamide (7), 2-(piperidin-1-yl)-N-(4-(N-pyridin-2-ylsulfamoyl) phenyl) acetamide (8) [118], 2-(morpholino)-N-(4-(N-pyridin-2-ylsulfamoyl) phenyl) acetamide (9) [118], 2-(4-chlorophenylamino)-N-(4-(N-pyridin-2-ylsulfamoyl)phenyl) acetamide (10):

![Chemical Structure](image)

A mixture of 2 (0.65g, 0.002 mol) and the appropriate amines (0.0023 mol) in ethanol (20 ml) was refluxed for 2 h. The reaction mixture was distilled under reduced pressure and poured onto ice – water; the obtained solid was filtered, washed with water and crystallized from acetonitrile to give 6-10, respectively. (Tables 3, 4) showed the physical and spectral data for compounds 6-10.
### Table 3 – Physical and analytical data for compounds 6-10.

| Compd. No. | M.p. (°C) | Yield (%) | Mol. Formula (M. wt.) | Microanalysis %
<table>
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<td>Calculated</td>
</tr>
<tr>
<td>6</td>
<td>&gt;290</td>
<td>41</td>
<td>C_{17}H_{22}N_{4}O_{3}S (362.45)</td>
<td>C: 56.35 H: 6.07 N: 15.46</td>
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<tr>
<td>7</td>
<td>&gt;290</td>
<td>59</td>
<td>C_{16}H_{20}N_{4}O_{4}S (364.42)</td>
<td>C: 52.74 H: 5.49 N: 15.38</td>
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<tr>
<td>8</td>
<td>223, as reported</td>
<td>88</td>
<td>C_{16}H_{22}N_{4}O_{3}S (374.46)</td>
<td>C: 57.75 H: 5.88 N: 14.97</td>
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<td>9</td>
<td>228-230, as reported</td>
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<td>C_{17}H_{20}N_{4}O_{4}S (376.43)</td>
<td>C: 54.25 H: 5.31 N: 14.89</td>
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<td>10</td>
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<td>C_{19}H_{17}ClN_{4}O_{3}S (416.88)</td>
<td>C: 54.80 H: 4.08 N: 13.46</td>
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### Table 4 – Spectral data for compounds 6-10.

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<th>¹H-NMR (DMSO-d(_6))</th>
<th>EI/MS</th>
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<td>3405, 3328 (NH), 3054 (CH arom.), 2999, 2811 (CH aliph.), 1693 (C=O), 1587 (C=N), 1373, 1136 (SO(_2)).</td>
<td>1.1 [t, 6H, 2CH(_3)], 2.9 [q, 4H, 2CH(_2)], 5.0 [s, 2H, COCH(_2)], 6.7 – 8.0 [m, 9H, Ar-H + NHCO, D(_2)O exchangeable], 10.2 [s, 1H, SO(_2)NH, D(_2)O exchangeable].</td>
<td>3257(NH), 3051 (CH arom.), 2934, 2848 (CH aliph.), 1693 (C=O), 1593 (C=N), 1393, 1141 (SO(_2)).</td>
</tr>
<tr>
<td>7</td>
<td>3403 (OH), 3326, 3188 (NH), 3056 (CH arom.), 2947, 2882 (CH aliph.), 1692 (C=O), 1598 (C=N), 1371, 1133(SO(_2)).</td>
<td>1.5-1.6 [m, 2H, CH(_2)CH(_2)OH], 2.5 [t, 2H, NHCH(_3)], 2.7 [t, 2H, CH(_2)OH], 3.7 [s, 1H, CH(_2)NH, D(_2)O exchangeable], 5.0 [s, 2H, COCH(_2)], 6.7 - 8.0 [m, 9H, Ar-H + NHCO, D(_2)O exchangeable], 10.3 [s, 1H, SO(_2)NH, D(_2)O exchangeable], 11.3 [s, 1H, OH, D(_2)O exchangeable].</td>
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<tr>
<td></td>
<td><strong>(m/z) (%)</strong></td>
<td><strong>374 [M⁺] (1.19), 98 (100).</strong></td>
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<td><strong>9</strong></td>
<td><strong>IR (KBr, cm⁻¹)</strong></td>
<td>3273(NH), 3033 (CH arom.), 2925, 2831 (CH aliph.), 1692 (C=O), 1601 (C=N), 1391, 1142 (SO₂).</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>¹H-NMR (DMSO-d₆)</strong></td>
<td>3.0 [t, 4H, 2CH₂N], 3.7 [t, 4H, 2CH₂O], 5.0 [s, 2H, COCH₂], 6.8-8.0 [m, 9H, Ar-H + NHCO, D₂O exchangeable], 10.0 [s, 1H, SO₂NH, D₂O exchangeable].</td>
<td></td>
</tr>
<tr>
<td><strong>10</strong></td>
<td><strong>IR (KBr, cm⁻¹)</strong></td>
<td>3369, 3101(NH), 3054 (CH arom.), 2933, 2825 (CH aliph.), 1691 (C=O), 1583 (C=N), 1391, 1137(SO₂), 695 (C-Cl).</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>¹H-NMR (DMSO-d₆)</strong></td>
<td>3.3 [s, 1H, CH₂NH, D₂O exchangeable], 3.8 [s, 2H, COCH₂], 6.7 - 8.0 [m, 13H, Ar-H+ NHCO, D₂O exchangeable], 10.3 [s, 1H, SO₂NH, D₂O exchangeable].</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>EI/MS (m/z) (%)</strong></td>
<td>416 [M⁺] (15.99), 418 [M+2] (5.36), 63 (100).</td>
<td></td>
</tr>
</tbody>
</table>
4-(5-Amino-4-cyano-2-oxo-2,3-dihydro-1H-pyrrol-1-yl)-N-(pyridin-2-yl)benzenesulfonamide (11): [119]

A mixture of compound 2 (0.325g, 0.001 mol) and malononitrile (0.066 g, 0.001 mol) in DMF (10 ml) containing a catalytic amount of piperidine was refluxed for 2 h. The reaction mixture was cooled and then poured onto ice – water, and the precipitated solid was crystallized from ethanol to give 11. Yield: 70 %, as reported. m.p. 140-142°C, as reported. Anal. Calcd. for C₁₆H₁₃N₅O₃S (355.37): C, 54.08; H, 3.66; N, 19.71. Found: C, 54.37; H, 3.89; N, 20.03.

Spectral data for compound 11:

IR (KBr, cm⁻¹): 3250, 3198, 3115 (NH, NH₂), 3052 (CH arom.), 2937, 2823 (CH aliph.), 2201 (C≡N), 1679 (C=O), 1589 (C=N), 1386, 1139 (SO₂).

¹H-NMR (DMSO-d₆) δ: 2.7 [s, 2H, CH₂, pyrrole], 4.7 [s, 2H, NH₂, D₂O exchangeable], 6.7-8.1 [m, 8H, Ar-H], 10.5 [s, 1H, SO₂NH, D₂O exchangeable].
N-(4-Cyano-2-oxo-1-(4-(N-pyridin-2-ylsulfamoyl)phenyl)-2,3-dihydro-1H-pyrrol-2-yl) acetamide (12), N-acetyl-N-(4-cyano-2-oxo-1-(4-(N-pyridin-2-ylsulfamoyl) phenyl)-2,3-dihydro-1H-pyrrol-2-yl) acetamide (13)

A solution of compound 11 (0.355 g, 0.001 mol) in excess acetic anhydride (20 ml) was refluxed for 2 h., the reaction mixture was evaporated to give a sticky residue which was triturated by ethanol. The obtained solid was collected by filtration and crystallized from dioxane to give 12. Compound 13 was obtained similarly but by refluxing for 15 h.

**Microanalytical and spectral data of 12:**

Yield 65%, m.p. 160-162 °C, anal. Calcd. for C\textsubscript{18}H\textsubscript{15}N\textsubscript{5}O\textsubscript{4}S (397.41), C, 54.40; H, 3.77; N, 17.63. Found: C, 54.66; H, 3.94; N, 17.37.

**IR (KBr, cm\textsuperscript{-1}):** 3446, 3351 (NH), 3001 (CH arom.), 2933, 2856 (CH aliph.), 2215 (C≡N), 1716, 1639 (2C=O), 1607 (C=N), 1369, 1174(SO\textsubscript{2}).

**\textsuperscript{1}H-NMR (DMSO-\textit{d}_6) \delta:** 2.0 [s, 3H, COCH\textsubscript{3}], 2.7 [s, 2H, CH\textsubscript{2}, pyrrole], 6.7- 8.1 [m, 9H, Ar-H + NHCO, D\textsubscript{2}O exchangeable], 10.7 [s, 1H, SO\textsubscript{2}NH, D\textsubscript{2}O exchangeable].
**EI/MS (m/z) (%):** 397 [M⁺] (0.61), 92 (100).

**Microanalytical and spectral data of 13:**


**IR (KBr, cm⁻¹):** 3455 (NH), 3035 (CH arom.), 2929, 2858 (CH aliph.), 2219 (C≡N), 1710, 1625 (3 C=O), 1603 (C=N), 1371, 1172(SO₂).

**¹H-NMR (DMSO-d₆) δ:** 1.9 [s, 6H, 2COCH₃], 2.4 [s, 2H, CH₂, pyrrole], 6.8-8.2 [m, 9H, Ar-H + NHCO, D₂O exchangeable], 10.2 [s, 1H, SO₂NH, D₂O exchangeable].

**EI/MS (m/z) (%):** 439 [M⁺] (2.10), 62 (100).
Experimental

4-(4-Cyano-5-(3-ethylthioureido)-2-oxo-2,3-dihydro-1H-pyrrol-1-yl)-N-(pyridin-2-yl) benzenesulfonamide (14):

A mixture of compound 11 (0.355 g, 0.001 mol) and ethyl isothiocyanate (0.087 g, 0.001 mol) in DMF (20 ml), containing a catalytic amount of of TEA, was refluxed for 10 h. The reaction mixture was cooled and then poured onto ice – water, and the precipitated solid was crystallized from ethanol to give 14. Yield 54 %, m.p. 173-175 °C, anal. Calcd. for C_{19}H_{18}N_{6}O_{3}S_{2} (442.51), C, 51.58; H, 4.07; N, 19.00. Found: C, 51.35; H, 4.42; N, 18.65.

Spectral data for compound 14:

**IR (KBr, cm⁻¹):** 3471, 3336, 3298 (NH), 3070 (CH arom.), 2927, 2863 (CH aliph.), 2192 (C≡N), 1646 (C=O), 1592 (C=N), 1257 (C=S), 1384, 1135(SO₂).

**¹H-NMR (DMSO- d₆) δ:** 1.1 [t, 3H, CH₃], 2.7 [s, 2H, CH₂, pyrrole], 4.4 [q, 2H, CH₂], 6.8- 8.2 [m, 10H, Ar-H + 2 NH, D₂O exchangeable], 10.7 [s, 1H, SO₂NH, D₂O exchangeable].

**EI/MS (m/z) (%):** 442 [M⁺] (2.84), 65 (100).
4-(4-Mercaptothiazol-2-ylamino)-N-(pyridin-2-yl)benzenesulfonamide (15):

A mixture of compound 3 (0.348 g, 0.001 mol) and phosphorus pentasulfide (0.22 g, 0.001 mol) in pyridine (20 ml) was refluxed for 8 h., the reaction mixture was cooled and then poured onto ice – water and acidified with dil HCl. The solid obtained was crystallized from ethanol to give 15. Yield 47%, m.p. 250-251°C. Anal. Calcd. for C_{14}H_{12}N_4O_2S_3 (364.47): C, 46.15; H, 3.29; N, 15.38. Found: C, 46.48; H, 2.99; N, 15.64.

Spectral data for compound 15:

IR (KBr, cm^{-1}): 3445, 3233(NH), 3056 (CH arom.), 2553 (SH), 1590 (C=N), 1388, 1138 (SO_2).

^{1}H-NMR (DMSO-d_6) δ: 6.86 [s, 1H, CH, thiazole], 6.88-8.0 [m, 9H, Ar-H + NH, D_2O exchangeable], 10.9 [s, 1H, SO_2NH, D_2O exchangeable], 13.8 [s, 1H, SH, D_2O exchangeable].

EI/MS (m/z) (%): 364 [M^+] (1.60), 63 (100).
4-(4-(Methylthio)thiazol-2-yl amino)-N-(pyridin-2-yl) benzenesulfonamide (16):

![Chemical structure of compound 16]

A mixture of compound 15 (0.364g, 0.001 mol) and methyl iodide (0.152 ml, 0.142 g, 0.001 mol) in methanol (20 ml) was left at ambient temperature for 48 h. then heated under reflux for 6 h. The solvent was removed by evaporation and the oily residue was triturated with diethyl ether. The crude solid was collected, washed with cold ethanol and finally crystallized from 2- propanol to give 16. Yield 55%, m.p. 194-196°C. Anal. Calcd. for C_{15}H_{14}N_{4}O_{2}S_{3} (378.49): C, 47.61; H, 3.70; N, 14.81. Found: C, 47.34; H, 4.06; N, 14.64.

**Spectral data for compound 16:**

**IR (KBr, cm⁻¹):** 3440, 3239 (NH), 3057 (CH arom.), 2928, 2860 (CH aliph), 1540 (C=N), 1390, 1140 (SO₂).

**¹H-NMR (DMSO-d₆) δ:** 2.4 [s, 3H, SCH₃], 6.8 [s, 1H, CH, thiazole], 6.9-8.2 [m, 9H, Ar-H + NH, D₂O exchangeable], 10.6 [s, 1H, SO₂NH, D₂O exchangeable].
Experimental

4-(4-Hydrazinylthiazol-2-ylamino)-N-(pyridin-2-yl) benzenesulfonamide (17):

\[
\begin{array}{c}
\text{HN} \\
\text{O=S=O} \\
\text{NH} \\
\text{N} \\
\text{HNNH}_2 \\
\text{N} \\
\text{S} \\
\text{H} \\
\end{array}
\]

(17)

**Method A:**

A mixture of compound 16 (0.378 g, 0.001 mol) and hydrazine hydrate (0.0012 mol) was refluxed in methanol for 24 h. The reaction mixture was cooled and poured onto ice – water. The solid obtained was crystallized from dioxane to give 17 in 20% yield, m.p. 210-212 °C.

**Method B:**

A mixture of compound 4 (0.366 g, 0.001 mol) and hydrazine hydrate (0.0012 mol) was refluxed in ethanol (20 ml) for 5 h. The reaction mixture was cooled and poured onto ice – water and acidified with dilute hydrochloric acid. The solid obtained was collected by filtration and crystallized from dioxane to give 17 in 58% yield, m.p. 210-212 °C.

Anal. Calcd. for C_{14}H_{14}N_{6}O_{2}S_{2} (362.43): C, 46.40; H, 3.86; N, 23.20. Found: C, 46.67; H, 4.10; N, 23.38.
Spectral data for compound 17:

**IR (KBr, cm⁻¹):** 3357, 3280, 3235, 3215, 3113, (NH, NH₂), 3098 (CH arom.), 1600 (C=N), 1389, 1138 (SO₂).

**¹H-NMR (DMSO-\(d_6\)) \(\delta\):** 2.4 [s, 2H, NH₂, D₂O exchangeable], 4.7 [s, 1H, NHNH₂, D₂O exchangeable], 6.7 [s, 1H, CH thiazole], 6.9-7.9 [m, 9H, Ar-H + NH, D₂O exchangeable], 10.2 [s, 1H, SO₂NH, D₂O exchangeable].
Experimental

4-(5-Amino-7-(4-chlorophenyl)-6-cyano-7H-pyran[2,3-d]thiazol-2-yl amino)-N-(pyridin-2-yl) benzenesulfonamide (18):

A mixture of compound 3 (0.348g, 0.001 mol) and 2-(4-chlorobenzylidene)malononitrile (0.188g, 0.001 mol) in ethanol (20 ml), containing a catalytic amount of TEA, was refluxed for 5 h. The reaction mixture was cooled, poured onto ice – water and acidified with dil. HCl. The obtained solid was crystallized from dioxane to give 18. Yield 86%, m.p. 270-272°C. Anal. Calcd. for C_{24}H_{17}ClN_{6}O_{3}S_{2} (536.05): C, 53.73; H, 3.17; N, 15.67. Found: C, 53.39; H, 3.51; N, 15.45.

Spectral data for compound 18:

IR (KBr, cm\(^{-1}\)): 3250, 3191, 3113 (NH, NH\(_2\)), 3046 (CH arom.), 2939, 2825 (CH aliph.), 2219 (C=\(\equiv\)N), 1603 (C=\(\equiv\)N), 1391, 1138 (SO\(_2\)), 780 (C-Cl).

\(^1\)H-NMR (DMSO-\(d_6\) \(\delta\)): 3.8 [s, 2H, NH\(_2\), D\(_2\)O exchangeable], 4.1 [s, 1H, CH, pyran], 6.6-8.0 [m, 13H, Ar-H + NH, D\(_2\)O exchangeable], 8.9 [s, 1H, SO\(_2\)NH, D\(_2\)O exchangeable].

EI/MS (m/z) (%): 536 [M\(^+\)] (1.00), 538 [M+2] (0.35), 168 (100).
4-[1H-8-(4-Chlorophenyl) thiazolo [4,5-b]pyrano [2,3-d] pyrimidin-9-one-6-ylamino]-N-(pyridin-2-yl) benzenesulfonamide (19):

![Chemical Structure](image)

A solution of compound 18 (0.536 g, 0.001 mol) in formic acid (20 ml) was refluxed for 5 h. The reaction mixture was cooled and then poured onto ice – water and the obtained solid was crystallized from ethanol to give 19. Yield: 67%, m.p. > 290°C. Anal. Calcd. for C_{25}H_{17}ClN_{6}O_{4}S_{2} (564.04): C, 53.19; H, 3.01; N, 14.89. Found: C, 52.91; H, 2.78; N, 14.69.

Spectral data for compound 19:

**IR (KBr, cm⁻¹):** 3273, 3198, 3120 (NH), 3046 (CH arom.), 2936, 2822 (CH aliph.), 1713 (C=O), 1589 (C=N), 1371, 1138 (SO₂), 778 (C-Cl).

**¹H-NMR (DMSO-ｄ₆)** δ: 4.1 [s, 1H, CH, pyran], 6.8-7.9 [m, 14H, Ar-H + 2NH, D₂O exchangeable], 8.0 [s, 1H, CH, pyrimidine], 10.9 [s, 1H, SO₂NH, D₂O exchangeable].

**EI/MS (m/z) (%)**: 564 [M⁺] (6.19), 566 [M+2] (2.1), 157 (100).
4-(4-(2-Phenylhydrazinyl) thiazol-2-yl amino)-N-(pyridin-2-yl) benzenesulfonamide (20), 4-(4-(2,4-dinitrophenvyl) hydrazinyl) thiazol-2-yl amino)-N-(pyridin-2-yl) benzenesulfonamide (21):

A mixture of compound 4 (0.366 g, 0.001 mol) and phenylhydrazine (0.0012 mol) or 2,4-dinitrophenylhydrazine (0.237 g, 0.001 mol) in ethanol (20 ml) was refluxed for 5 h. The reaction mixture was cooled and poured onto ice – water. The solid obtained was crystallized from dioxane to give 20 and 21, respectively.

Microanalytical and spectral data of 20:
Yield: 72%, m.p.130-132°C. Anal. Calcd. for C_{20}H_{18}N_{6}O_{2}S_{2} (438.53): C, 54.79; H, 4.10; N 19.17. Found: C, 54.55; H, 4.39; N, 18.98.

IR (KBr, cm^{-1}): 3364, 3313, 3287, 3225 (NH), 3057 (CH arom.), 1582 (C=N), 1389, 1143 (SO_{2}).
Experimental

\(^1\text{H-NMR (DMSO-\text{d}_6, \delta \text{ ppm})}:\) 3.5 [s, 2H, NHNH, \text{D}_2\text{O exchangeable}], 6.8 [s, 1H, CH, thiazole], 6.9-8.0 [m, 14 H, Ar-H + NH, \text{D}_2\text{O exchangeable}], 10.1 [s, 1H, SO\textsubscript{2}NH, \text{D}_2\text{O exchangeable}].

\text{EI/MS (m/z) (%):} 438 [M'] (0.74), 61 (100).

\textbf{Microanalytical and spectral data of 21:}

Yield: 73%, m.p. 168-170°C. Anal. Calcd. for C\textsubscript{20}H\textsubscript{16}N\textsubscript{8}O\textsubscript{6}S\textsubscript{2} (528.52): C, 45.45; H, 3.03; N, 21.21. Found: C, 45.80; H, 3.33; N, 21.53.

\textbf{IR (KBr, cm\textsuperscript{-1})}: 3421, 3347 (NH), 3068 (CH arom.), 1593 (C=N), 1520, 1387 (NO\textsubscript{2}), 1335, 1135 (SO\textsubscript{2}).

\(^1\text{H-NMR (DMSO-\text{d}_6, \delta \text{ ppm})}:\) 3.7 [s, 2H, NHNH, \text{D}_2\text{O exchangeable}], 6.8 [s, 1H, CH, thiazole], 6.9-8.2 [m, 12H, Ar-H + NH, \text{D}_2\text{O exchangeable}], 9.7 [s, 1H, SO\textsubscript{2}NH, \text{D}_2\text{O exchangeable}].

\text{EI/MS (m/z) (%):} 528 [M'] (1.71), 61(100).
4-[4-(Isothiocyanato)thiazol-2-ylamino)-N-(pyridin-2-yl)benzenesulfonamide (22):

A mixture of compound 4 (0.366 g, 0.001 mol) and ammonium thiocyanate (0.091 g, 0.0012 mol) was refluxed in dry acetone (20 ml) for 1 h. The reaction mixture was cooled and poured onto ice – water. The solid obtained was crystallized from ethanol to give 22. Yield 69%, m.p. 215-217°C. Anal. Calcd. for C_{15}H_{11}N_{5}O_{2}S_{3} (389.48): C, 46.27; H, 2.82; N, 17.99. Found: C, 45.98; H, 3.04; N, 17.62.

**Spectral data for compound 22:**

**IR (KBr, cm^{-1}):** 3392, 3128 (NH), 3051 (CH arom.), 2051 (N=C=S), 1587 (C=N), 1393, 1129 (SO_{2}).

**\(^1\)H-NMR (DMSO-d_{6}, \delta ppm):** 6.8 [s, 1H, CH, thiazole], 6.9 -8.0 [m, 9H, Ar-H + NH, D_{2}O exchangeable], 10.8 [s, 1H, SO_{2}NH, D_{2}O exchangeable].

**EI/MS (m/z) (%) :** 389 [M^{+}] (0.9), 64 (100).
4-(4-(4-Chlorophenylamino)thiazol-2-ylamino)-N-(pyridin-2-yl)benzene-sulfonamide (23):

A mixture of compound 4 (0.366 g, 0.001 mol) and 4-chloroaniline (0.152 g, 0.0012 mol) was refluxed in DMF (20 ml) for 4 h. The reaction mixture was cooled and poured onto ice – water. The solid obtained was crystallized from ethanol to give 23. Yield 64%, m.p. > 290°C. Anal. Calcd. for C$_{20}$H$_{16}$ClN$_5$O$_2$S$_2$ (457.96): C, 52.51; H, 3.50; N, 15.31. Found: C, 52.19; H, 3.68; N, 15.54.

Spectral data for compound 23:

**IR (KBr, cm$^{-1}$):** 3386, 3345, 3264 (NH), 3076 (CH arom.), 1602 (C=N), 1397, 1130 (SO$_2$), 620 (C-Cl).

**EI/MS (m/z) (%):** 457 [M$^+$] (0.81), 459 [M+2] (0.27), 54 (100).
2-(2-(4-(N-Pyridin-2-ylsulfamoyl) phenylamino) thiazol-4-ylamino)benzoic-acid (24):

A mixture of compound 4 (0.366 g, 0.001 mol) and anthranilic acid (0.137g, 0.001 mol) was refluxed in n- butanol (20 ml) for 5 h. The reaction mixture was cooled and poured onto ice – water. The solid obtained was crystallized from ethanol to give 24. Yield 69%, m.p. > 290°C. Anal. Calcd. for C_{21}H_{17}N_{5}O_{4}S_{2} (467.52): C, 53.96; H, 3.64; N, 14.98. Found: C, 54.27; H, 3.45; N, 14.61.

**Spectral data for compound 24:**

**IR (KBr, cm^{-1}):** 3424 (OH), 3347, 3286, 3249 (NH), 3069 (CH arom.), 1681 (C=O), 1574 (C=N), 1390, 1142 (SO_{2}).

**^1H-NMR (DMSO-d_{6}, δ ppm):** 6.84 [s, 1H, CH, thiazole], 6.85-7.9 [m, 14 H, Ar-H + 2NH, D_{2}O exchangeable] 10.9 [s, 1H, SO_{2}NH, D_{2}O exchangeable], 11.8 [s, 1H, OH, D_{2}O exchangeable].

**EI/MS (m/z) (%):** 467 [M^{+}] (2.7), 184 (100).
Biological Activity

5.1 *In-vitro* anticancer screening

The *in-vitro* anticancer screening was done by the pharmacology unit at the National Cancer Institute, Cairo University.

In the present study, the cytotoxic activity of twenty seven synthesized compounds was measured *in-vitro* on human tumor breast cell line (MCF-7), using the Sulfo-Rhodamine-B stain (SRB) assay method as described by Skehan et al. [125]. Doxorubicin, the clinically used drug, was used as a standard in this study.

The SRB assay, which was developed in 1990, remains one of the most widely used methods for *in-vitro* cytotoxic screening [125]. The assay relies on the ability of SRB to bind to protein components of cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA). SRB is a bright-pink aminoxanthene dye with two sulfonic groups that bind to basic amino acid residues under mild acidic conditions, and dissociate under basic conditions. As the binding of SRB is stoichiometric, the amount of dye extracted from stained cells is directly proportional to the cell mass.

The strong intensity of SRB staining allows the assay to be carried out in a 96-well format. Skehan et al. [125] showed that the assay can detect densities as low as 1,000 - 2,000 cells per well, and with a signal-to-noise ratio of 4.83 at a density of 5,000 cells per well.
5.1.1 **Procedure:**

- Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h. before treatment with the tested compound(s) to allow attachment of cell to the wall of the plate.

- For each tested compound, a solution of the compound was prepared by dissolving 2 µmol of each compound in 1 mL dimethyl sulfoxide. 1, 2.5, 5 and 10 µL of the prepared solution were added to the wells, and the final volume in each well was completed to 200 µL, to obtain a concentration of 10, 25, 50, and 100 µM for each tested compound, respectively. Triplicate wells were prepared for each individual concentration.

- Monolayer Cells were incubated with the compound(s) for 48 h. at 37 °C and in atmosphere of 5% CO₂.

- After 48 h., cells were fixed, washed and stained for 30 minutes with 0.4% (wt/vol) SRB dissolved in 1% acetic acid.

- Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris–EDTA buffer.

- Color intensity was measured in an ELISA reader at a wave length of 570 nm.

- The relation between surviving fraction and drug concentration (µM /L) was plotted using the Microsoft Office Excel 2003 program. A third order polynomial equation was used to get the best fitting survival curve for breast tumor cell line after the specified time for each compound (Figure 5.1- 5.28).

- The molar concentration required for 50% inhibition of cell viability (IC₅₀) was calculated by solving the third order polynomial equation using simple designed software written on
Biological Activity

‘Matlab R2008a’ program and compared with the reference drug Doxorubicin. The surviving fractions were expressed as mean value ± standard error. The results are given in Table 5.

Figure 5.1 – Survival curve of (MCF-7) for **Doxorubicin**, 3<sup>rd</sup> order approximation

Figure 5.2 – Survival curve of (MCF-7) for compound 3, 3<sup>rd</sup> order approximation
Figure 5.3 – Survival curve of (MCF-7) for compound 4, 3rd order approximation

Figure 5.4 – Survival curve of (MCF-7) for compound 5a, 3rd order approximation

Figure 5.5 – Survival curve of (MCF-7) for compound 5b, 3rd order approximation
Figure 5.6 – Survival curve of (MCF-7) for compound 5c, 3rd order approximation

Figure 5.7 – Survival curve of (MCF-7) for compound 5d, 3rd order approximation

Figure 5.8 – Survival curve of (MCF-7) for compound 5e, 3rd order approximation
Figure 5.9 – Survival curve of (MCF-7) for compound $5f$, 3$^{rd}$ order approximation

Figure 5.10 – Survival curve of (MCF-7) for compound $6$, 3$^{rd}$ order approximation

Figure 5.11 – Survival curve of (MCF-7) for compound $7$, 3$^{rd}$ order approximation
Figure 5.12 – Survival curve of (MCF-7) for compound 8, 3\textsuperscript{rd} order approximation

Figure 5.13 – Survival curve of (MCF-7) for compound 9, 3\textsuperscript{rd} order approximation

Figure 5.14 – Survival curve of (MCF-7) for compound 10, 3\textsuperscript{rd} order approximation
Figure 5.15 – Survival curve of (MCF-7) for compound 11, 3rd order approximation

Figure 5.16 – Survival curve of (MCF-7) for compound 12, 3rd order approximation

Figure 5.17 – Survival curve of (MCF-7) for compound 13, 3rd order approximation
Biological Activity

Figure 5.18 – Survival curve of (MCF-7) for compound 14, 3rd order approximation

Figure 5.19 – Survival curve of (MCF-7) for compound 15, 3rd order approximation

Figure 5.20 – Survival curve of (MCF-7) for compound 16, 3rd order approximation
Figure 5.21 – Survival curve of (MCF-7) for compound 17, 3\textsuperscript{rd} order approximation

Figure 5.22 – Survival curve of (MCF-7) for compound 18, 3\textsuperscript{rd} order approximation

Figure 5.23 – Survival curve of (MCF-7) for compound 19, 3\textsuperscript{rd} order approximation
Figure 5.24 – Survival curve of (MCF-7) for compound 20, 3rd order approximation

Figure 5.25 – Survival curve of (MCF-7) for compound 21, 3rd order approximation

Figure 5.26 – Survival curve of (MCF-7) for compound 22, 3rd order approximation
Figure 5.27 – Survival curve of (MCF-7) for compound 23, 3\textsuperscript{rd} order approximation

Figure 5.28 – Survival curve of (MCF-7) for compound 24, 3\textsuperscript{rd} order approximation
Table 5 – *In-vitro* cytotoxic screening of the synthesized compounds against human breast cancer cell line (MCF-7).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Compound Concentration (µM)</th>
<th></th>
<th></th>
<th></th>
<th>IC₅₀ (µM) 3rd order</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>25</td>
<td>50</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Surviving Fraction (Means ± SE)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.721±0.02</td>
<td>0.546±0.02</td>
<td>0.461±0.01</td>
<td>0.494±0.03</td>
<td>26.13</td>
</tr>
<tr>
<td>3</td>
<td>0.9294±0.11</td>
<td>0.6825±0.02</td>
<td>0.2583±0.04</td>
<td>0.3387±0.01</td>
<td>34.53</td>
</tr>
<tr>
<td>4</td>
<td>0.9032±0.08</td>
<td>0.5566±0.03</td>
<td>0.2540±0.07</td>
<td>0.3272±0.03</td>
<td>31.65</td>
</tr>
<tr>
<td>5a</td>
<td>0.7495±0.05</td>
<td>0.6356±0.04</td>
<td>0.4500±0.12</td>
<td>0.1993±0.04</td>
<td>33.85</td>
</tr>
<tr>
<td>5b</td>
<td>0.7793±0.04</td>
<td>0.5216±0.21</td>
<td>0.2649±0.01</td>
<td>0.2565±0.05</td>
<td>26.10</td>
</tr>
<tr>
<td>5c</td>
<td>0.9128±0.11</td>
<td>0.6316±0.22</td>
<td>0.3658±0.05</td>
<td>0.2245±0.04</td>
<td>37.81</td>
</tr>
<tr>
<td>5d</td>
<td>0.6985±0.09</td>
<td>0.6209±0.17</td>
<td>0.3675±0.07</td>
<td>0.1967±0.02</td>
<td>37.77</td>
</tr>
<tr>
<td>5e</td>
<td>0.9710±0.07</td>
<td>0.7702±0.06</td>
<td>0.1278±0.02</td>
<td>0.2609±0.004</td>
<td>37.49</td>
</tr>
<tr>
<td>5f</td>
<td>0.9407±0.11</td>
<td>0.6955±0.14</td>
<td>0.3813±0.03</td>
<td>0.2405±0.01</td>
<td>38.70</td>
</tr>
<tr>
<td>6</td>
<td>0.9218±0.07</td>
<td>0.7146±0.10</td>
<td>0.3341±0.01</td>
<td>0.2263±0.06</td>
<td>38.12</td>
</tr>
<tr>
<td>7</td>
<td>0.8666±0.01</td>
<td>0.6875±0.01</td>
<td>0.3502±0.09</td>
<td>0.2654±0.03</td>
<td>45.28</td>
</tr>
<tr>
<td>8</td>
<td>0.8425±0.06</td>
<td>0.5324±0.09</td>
<td>0.2731±0.03</td>
<td>0.2284±0.05</td>
<td>27.50</td>
</tr>
<tr>
<td>9</td>
<td>0.8890±0.04</td>
<td>0.7121±0.09</td>
<td>0.4164±0.08</td>
<td>0.1958±0.02</td>
<td>42.33</td>
</tr>
<tr>
<td>10</td>
<td>0.8334±0.09</td>
<td>0.6153±0.08</td>
<td>0.1349±0.04</td>
<td>0.1502±0.03</td>
<td>29.00</td>
</tr>
<tr>
<td>11</td>
<td>0.9023±0.01</td>
<td>0.5222±0.09</td>
<td>0.2863±0.04</td>
<td>0.2259±0.004</td>
<td>30.31</td>
</tr>
<tr>
<td>12</td>
<td>0.7656±0.07</td>
<td>0.4462±0.07</td>
<td>0.3136±0.06</td>
<td>0.1544±0.02</td>
<td>22.42</td>
</tr>
<tr>
<td>13</td>
<td>0.8169±0.12</td>
<td>0.5012±0.07</td>
<td>0.2891±0.01</td>
<td>0.1933±0.02</td>
<td>29.48</td>
</tr>
<tr>
<td>14</td>
<td>0.7474±0.03</td>
<td>0.4813±0.02</td>
<td>0.1916±0.08</td>
<td>0.0992±0.04</td>
<td>22.16</td>
</tr>
<tr>
<td>15</td>
<td>0.8624±0.09</td>
<td>0.5843±0.08</td>
<td>0.3262±0.07</td>
<td>0.1474±0.02</td>
<td>32.94</td>
</tr>
<tr>
<td>16</td>
<td>0.7797±0.04</td>
<td>0.4775±0.08</td>
<td>0.1452±0.01</td>
<td>0.1448±0.01</td>
<td>23.24</td>
</tr>
<tr>
<td>17</td>
<td>0.8047±0.02</td>
<td>0.4019±0.04</td>
<td>0.1122±0.02</td>
<td>0.1521±0.02</td>
<td>21.70</td>
</tr>
<tr>
<td>18</td>
<td>0.8502±0.10</td>
<td>0.6243±0.03</td>
<td>0.3514±0.03</td>
<td>0.2735±0.01</td>
<td>35.51</td>
</tr>
</tbody>
</table>
### Biological Activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Compound Concentration (µM)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Surviving Fraction (Means ± SE)注</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>19</td>
<td>0.4921±0.03</td>
<td>0.2722±0.06</td>
<td>0.1659±0.03</td>
</tr>
<tr>
<td>20</td>
<td>0.7347±0.05</td>
<td>0.4925±0.06</td>
<td>0.2606±0.04</td>
</tr>
<tr>
<td>21</td>
<td>0.6666±0.10</td>
<td>0.4294±0.03</td>
<td>0.2076±0.04</td>
</tr>
<tr>
<td>22</td>
<td>0.8940±0.06</td>
<td>0.6655±0.07</td>
<td>0.3155±0.09</td>
</tr>
<tr>
<td>23</td>
<td>0.7831±0.24</td>
<td>0.6426±0.08</td>
<td>0.4252±0.03</td>
</tr>
<tr>
<td>24</td>
<td>0.7510±0.07</td>
<td>0.5662±0.07</td>
<td>0.2576±0.02</td>
</tr>
</tbody>
</table>

#: Each value is the mean of three values ± Standard Error.

IC<sub>50</sub>: Compound concentration required to inhibit tumor cell proliferation by 50%.

#### 5.1.2 Results and Discussion

All of the tested compounds showed *in-vitro* cytotoxic activity against (MCF-7) but differs in potency comparable to doxorubicin. From the results in Table 5 we can conclude the following:

Eight compounds belong to different synthesized series 19, 21, 17, 14, 12, 20, 16, and 24 exhibited higher cytotoxic activity, and one compound 5b showed nearly comparable activity when compared with doxorubicin as the reference drug (IC<sub>50</sub> = 26.13 µM). Five of these compounds 16, 17, 20, 21, and 24 belong to the thiazole series, one compound 19 belong to the thiazolopyranopyrimidines, two compounds belong to the pyrrole series and one to the amino acid derivatives. The most active compound of all the synthesized compounds was the thiazolo[4,5-b]pyrano[2,3-d]pyrimidine derivative 19 (Figure 5.29).
Figure 5.29 – Compounds which showed cytotoxic activity higher than or comparable to doxorubicin.
Although, the relation between the structure of the tested compounds and their in-vitro cytotoxic activity results is not very clear, the following points can still be concluded:

- Substitutions of N^4 of sulfapyridine with aromatic substituents are more active than the aliphatic substituents.

- Concerning the amino acid derivatives 5 a-f, all the derivatives showed cytotoxic activity lower than the reference drug (higher IC_{50}) except the alanine derivative 5b (IC_{50}= 26.10 µM) which is nearly as active as doxorubicin (IC_{50}= 26.13 µM).

- Concerning the acetamide derivatives (6-10), the most promising compounds in this class were the piperidine derivative 8 (IC_{50}= 27.50 µM) and the 4-chloroaniline derivative 10 (IC_{50}= 29.00 µM) but they have cytotoxic activity lower than doxorubicin.

- Incorporation of N^4 into pyrrole ring (11-14), generally increased the cytotoxic activity. The most active two compounds in this class were the monoacetyl derivative 12 and the thioureido derivative 14.

- Concerning the thiazole derivatives (3, 4, 15-17, 20-24). Five compounds (21, 17, 20, 16, and 24) showed cytotoxic activity higher than doxorubicin. In addition, it was noticed that the 4-hydrazinyl-thiazolo derivatives (17, 20, and 21) showed better in-vitro cytotoxic activity than the other 4-substituent, which may indicate that the hydrazinyl group had a remarkable effect on the cytotoxic activity.

- The thiazolo[4,5-b]pyrano[2,3-d]pyrimidine derivative 19 was the most active compound which showed a remarkable increase in the cytotoxic activity and had the lowest IC_{50} of all the synthesized compounds, it exhibited higher cytotoxic activity than doxorubicin.
5.2 Radiosensitizing evaluation

This study was conducted to evaluate the ability of the cell killing effect of $\gamma$-radiation to synergize the in-vitro cytotoxic activity of the most active compounds.

5.2.1 Procedure:

Irradiation was performed in the National Center for Radiation Research and Technology, Atomic Energy Authority, using Gamma cell-40 ($^{137}$Cs) source. The most six active compounds $5b$, $12$, $14$, $17$, $19$ and $21$ were selected to be reevaluated again for their in-vitro cytotoxic activity in combination with $\gamma$-irradiation.

- Cells were plated in 96-multiwell plate ($10^4$cells/well) for 24 h. before $\gamma$-irradiation with a single dose of 8 Gy.
- Cells were incubated for 48 h. at 37 °C in atmosphere of 5% CO$_2$. After 48 h., cells were fixed, washed and stained with 0.4% (wt/vol) SRB dissolved in 1% acetic acid, for 30 minutes.
- Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader at a wave length of 570 nm.
- In another multiwell plate, cells were incubated with the previously mentioned selected compounds; compounds $5b$, $12$, $14$, $17$, $19$ and $21$ in molar concentrations of 10, 25, 50, 100 $\mu$M.
- After 2 h., cells were subjected to a single dose of $\gamma$-radiation at a dose level of 8 Gy with a dose rate of 2 Gy/min then the cytotoxicity was measured 48h. after irradiation [126].
Biological Activity

- The surviving fractions were measured using the above mentioned procedures by ELISA reader. The surviving fractions were expressed as mean values ± standard error. The results were analyzed using 1-way ANOVA test and given in Table 6.

Table 6 – *In-vitro* cytotoxic screening of compounds 5b, 12, 14, 17, 19 and 21 against human breast cancer cell line (MCF-7) in combination with γ-radiation.

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Control</th>
<th>Irradiated (8 Gy)</th>
<th>Compound Concentration (µM) + Irradiation (8 Gy)</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>5b</td>
<td>1.000</td>
<td>0.927 ± 0.02*</td>
<td>0.5885 ± 0.03*</td>
<td>0.5223 ± 0.08*</td>
</tr>
<tr>
<td>12</td>
<td>1.000</td>
<td>0.927 ± 0.02*</td>
<td>0.5518 ± 0.08*</td>
<td>0.4817 ± 0.02*</td>
</tr>
<tr>
<td>14</td>
<td>1.000</td>
<td>0.927 ± 0.02*</td>
<td>0.4777 ± 0.13*</td>
<td>0.2128 ± 0.05*</td>
</tr>
<tr>
<td>17</td>
<td>1.000</td>
<td>0.927 ± 0.02*</td>
<td>0.5256 ± 0.16*</td>
<td>0.1543 ± 0.04*</td>
</tr>
<tr>
<td>19</td>
<td>1.000</td>
<td>0.927 ± 0.02*</td>
<td>0.5751 ± 0.18*</td>
<td>0.2846 ± 0.04*</td>
</tr>
<tr>
<td>21</td>
<td>1.000</td>
<td>0.927 ± 0.02*</td>
<td>0.4286 ± 0.06*</td>
<td>0.3089 ± 0.05*</td>
</tr>
</tbody>
</table>

#: Each value is the mean of three values ± Standard Error.
*: Significant difference from control group at p<0.001

Table 7 – *In-vitro* cytotoxic screening of compounds 5b, 12, 14, 17, 19 and 21 against (MCF-7) before and after radiation.

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>IC₅₀ (µM) Before radiation</th>
<th>IC₅₀ (µM) After radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5b</td>
<td>26.10</td>
<td>18.11</td>
</tr>
<tr>
<td>12</td>
<td>22.42</td>
<td>20.38</td>
</tr>
<tr>
<td>14</td>
<td>22.16</td>
<td>10.86</td>
</tr>
<tr>
<td>17</td>
<td>21.70</td>
<td>11.93</td>
</tr>
<tr>
<td>19</td>
<td>12.00</td>
<td>11.79</td>
</tr>
<tr>
<td>21</td>
<td>20.00</td>
<td>11.67</td>
</tr>
</tbody>
</table>
5.2.2 Results and discussion

The results in (Tables 6, 7) showed that the IC$_{50}$ values for compounds 5b, 12, 14, 17, 19 and 21 were synergistically decreased to 18.11, 20.38, 10.86, 11.93, 11.79 and 11.67 µM, respectively when cells were irradiated with a single dose of 8 Gy after treatment with the synthesized drugs (Figure 5.30-5.35).

Figure 5.30 – Survival curve of (MCF-7) for compound 5b alone and in combination with γ-irradiation (8 Gy)

Figure 5.31 – Survival curve of (MCF-7) for compound 12 alone and in combination with γ-irradiation (8 Gy)
Figure 5.32 – Survival curve of (MCF-7) for compound 14 alone and in combination with γ-irradiation (8 Gy)

Figure 5.33 – Survival curve of (MCF-7) for compound 17 alone and in combination with γ-irradiation (8 Gy)

Figure 5.34 – Survival curve of (MCF-7) for compound 19 alone and in combination with γ-irradiation (8 Gy)
Figure 5.35 – Survival curve of (MCF-7) for compound 21 alone and in combination with γ-irradiation (8 Gy).
Molecular Docking

6.1 Introduction

Cyclin-dependant kinase 2 enzyme (CDK2) is one of the most important enzymes in protein kinases family. CDK2 is a monomer composed of 298 amino acids made up of α-helix elements and a β-sheet [127]. It is responsible for G1/S phase in the cell cycle [62], where it acts by transferring phosphoryl group from a donor to an acceptor thus activating a cyclin protein and regulating cell division [62]. CDK2 interacts with Cylin A and E only to drive the cell from G1 phase to S phase [128].

Understanding the binding mode of cyclin A and E to CDK2 was of great help in the designing of CDK2 inhibitors as antitumor agents. The CDK2/cyclin interface area is 3252 Å² in Cyclin E and 2839 Å² in Cyclin A [63], [128].

Two classes of synthetic inhibitors to CDK2 were studied to understand the binding mode to the active site of CDK2 in order to recognize the key amino acids in this site. Thiazolidinone inhibitors bind by two hydrogen bonds with Glu 81 and Leu 83 and the sulfonate group of these inhibitors interacts with Asp 86 and leu 10 of the backbone [129]. On the other hand aminopyrimidine inhibitors interact with Asp 86 and Ile10 [62].

A protein data bank file with the code 1FVV was selected for this purpose. The file contains CDK2 enzyme co-crystallized with a sulphone ligand CVI by Davis et al [130]. All docking procedures were achieved by MOE (Molecular Operating Enviroment) software 10.2008 provided by chemical computing group, Canada.
6.2 Preparation for docking

In order to perform docking some procedures should be taken:

1- Acting on only one chain of amino acids containing one molecule of the inhibitor.

2- 3D protonation for the amino acid side chain and the ligand.

3- Deleting all water of crystallization away from the active site.

4- Isolation of the active site and recognition of the amino acids.

5- Studying the interaction of the ligand with the amino acids of the active site.

All the above procedures were taken and the 2D interactions of ligand with the amino acids of the active site are shown Figure 6.1.

Figure 6.1 – Interactions of the ligand on the active site of CDK2.

From the above figure the sulfone ligand interacts with the active site of CDK2 by four interactions:

- The sulfonate group interacts with Lys 89 with a hydrogen bond of 3.13 Å & Asp 86 with a hydrogen bond of 3.22 Å
• The carbonyl group of pyrrolone interacts with Leu 83 with a hydrogen bond of 2.96 Å.

• The NH group of pyrrolone interacts with Glu 81 with a hydrogen bond of 1.98 Å.

In order to visualize these interactions in a better manner 3D interactions were illustrated in Figure 6.2.

![3D interactions of the ligand on the active site of CDK2.](image)

The docking of the most active 12 synthesized compounds in the active site of CDK2 enzyme was done to try to explain the cytotoxic activity of these agents.

### 6.3 Validation of docking procedure

To perform accurate validation of the docking protocol docking of the co-crystallized ligand should be carried out to study the scoring energy (S), root mean standard deviation (rmsd) and amino acid interactions.
Docking was performed using London dG force and refinement of the results was done using Force field energy. Figure 6.3 illustrates docking of co-crystallized ligand on the active site of CDK2 where the compound in green colour, representing the original co-crystallized ligand and the compound in the red colour representing the docked ligand.

![Docking illustration](image)

Figure 6.3 – Validation of docking protocol on the active site of CDK2.

Validation of the docking protocol indicates that the ligand is fitted in the active site pocket with \( S = -18.1741 \text{ Kcal/mol} \) and \( \text{rsmd}=0.7723 \).

### 6.4 Preparing compounds for docking

Preparation of the synthesized compounds for docking was achieved via their 3D structure built by MOE. Certain procedures should be taken before docking which includes:

1. 3D protonation of the structures.
2. Running conformational analysis using systemic search.
3. Selecting the least energetic conformer.
4. Applying the same docking protocol used with the ligand.
Molecular Docking

The previous measures were taken and docking for 12 compounds of the synthesized compounds was applied. Energy scoring (S) and amino acids interactions and the hydrogen bond lengths were measured and illustrated in Table 8.

Table 8 –The docking results based on the conformational energy, the distance of hydrogen bonds between the compounds and amino acids in CDK2.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>S</th>
<th>Amino acids</th>
<th>Groups interacted</th>
<th>Length of H bonds (A)</th>
</tr>
</thead>
</table>
| 5b           | -13.1402 | Asp 86  
              | Asp 145  
              | Asp 145  
              | Lys 33  
              | C=O  
              | O=S=O  
              | COO  
              | 2.81  
              | 2.46  
              | 2.52  |
| 8            | -18.3068 | Lys 89  
              | O=S=O  
              | 2.95  |
| 10           | -19.3313 | Leu 83  
              | Lys 89  
              | NH  
              | O=S=O  
              | 2.03  
              | 2.82  |
| 12           | -16.8083 | Asp 86  
              | O=S=O  
              | 2.76  |
| 13           | -14.8403 | Lys 89  
              | Lys 88  
              | O=S=O  
              | C=O  
              | 2.89  
              | 2.6   |
| 14           | -16.2474 | Lys 89  
              | Gln 131  
              | O=S=O  
              | C=N  
              | 2.56  
              | 2.81  |
| 16           | -15.9589 | Lys 89  
              | O=S=O  
              | 3.16  |
| 17           | -14.7205 | Asp 86  
              | Glu 51  
              | O=S=O  
              | NH-NH₂  
              | 2.81  
              | 1.58  |
| 19           | -10.7351 | Lys 89  
              | Gln 131  
              | O=S=O  
              | N (pyridine)  
              | 2.59  
              | 2.85  |
| 20           | -21.8927 | Lys 89  
              | Asp 86  
              | O=S=O  
              | NH (thiazole)  
              | 2.77  
              | 1.72  |
| 21           | -17.5159 | Lys 89  
              | Asp 86  
              | O=S=O  
              | NH (thiazole)  
              | 2.65  
              | 2.29  |
| 24           | -16.3041 | Lys 89  
              | Lys 33  
              | O=S=O  
              | NH  
              | COO  
              | 2.74  
              | 1.68  
              | 2.79  |
6.5 Results

The docking results of the most active 12 compounds in the biological screening showed that all the docked compounds exhibit similar interactions as those previously reported with the ligand except 5b.

- Compounds 8, 10, 13, 14, 16, 19, 20, 21 and 24 bind to Lys 89 by hydrogen bond through SO$_2$ group.
- Compounds 12 and 17 bind to Asp 86 by hydrogen bond through SO$_2$ group.
- Compounds 10, 20, 21 and 24 bind by 2 hydrogen bonds to the active site of the enzyme.

Figures (6.4 – 6.10) represent the interaction maps in the active site of CDK2.

![Figure 6.4 – Interaction map of compound 8 in the active site of CDK2.](image-url)
Figure 6.5 – Interaction map of compound 10 in the active site of CDK2.

Figure 6.6 – Interaction map of compound 12 in the active site of CDK2.

Figure 6.7 – Interaction map of compound 14 in the active site of CDK2.
Figure 6.8 – Interaction map of compound 19 in the active site of CDK2.

Figure 6.9 – Interaction map of compound 20 in the active site of CDK2.

Figure 6.10 – Interaction map of compound 24 in the active site of CDK2.
Conclusion

- Some of the synthesized compounds showed significant activity against breast human tumor cell line (MCF7) and were found to be equivalent or even more potent than doxorubicin.

- Concerning the measurement of the synergism with radiation, all the tested six compounds showed a significant decrease in the IC\textsubscript{50} when tested on breast human tumor cell line after subjected to radiation and this proves the efficacy of combining chemotherapy with radiotherapy in the treatment of patients with cancer.

- Additionally, it can be seen from the docking study that some of the docked compounds exhibited similar binding interaction as those previously reported by the ligand when docked into the active site of CDK, as all the docked compounds bind to either Lys 89 or Asp 86 through SO\textsubscript{2} by one hydrogen bond and these results indicate that these compounds might possibly act as CDK2 inhibitors.

- Compound 20 showed best energy score \((S) = -21.8927 \text{ Kcal/ mol}\) with two hydrogen bond interactions with Asp 86 through NH group of its thiazole ring and with Lys 89 through its SO\textsubscript{2} group suggesting CDK2 inhibition mechanism as cytotoxic agent which was supported by its IC\textsubscript{50} value = 22.83 \(\mu \text{M}\) which was better than Doxorubicin.

- For compounds showing potent cytotoxic activity and at the same time doesn’t fulfill the requirements for fitting in the active site of CDK2, it may act as cytotoxic agent via other mechanism than inhibition of CDK2. Such as compound 19 showed poor energy score \((S) = -10.7351\) in spite of its IC\textsubscript{50} value = 12.0 \(\mu \text{M}\).
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تحضير بعض مشتقات N⁴ السلفابيردين لتقييمها كمضادات للأورام السرطانية مع دراسة التأثير التحفيزي للتشعيع الجاملي

يتناول هذا البحث تحضير وتشبيه مركبات جديدة من مشتقات N⁴ السلفابيردين المتوقع لها فاعلية كمضادات للأورام السرطانية، وقد تم استبда مجموعة 4-أمينو الجلدة بمجموعات الأحماض الأمينية، الاستاميد، أو الثيازول أو إدراجها في حلقة البيروال. وقد تم اختبار فعالية هذه المركبات الجديدة على خلايا السرطانية الأدمية للثدي (MCF-7) مقارنة بعقار الدوكسوروبسين، كما تم أيضا دراسة التأثير التحفيزي لأشعة جاما مع المركبات الأكثر فاعلية، وقد تم إرساء هذه المركبات في الموقع النشط لإيزيك السليتين ديندانت كينيزي 2.

الكلمات الدالة: السلفابيردين، التأثير النمو تخلية السرطانية، الإشعاع الجاملي، الدوكسوروبسين، الإرساء الجيني على إنزيم السليتين ديندانت كينيزي 2.

1. المقدمة:

تتضمن المقدمة سردا مختصرًا عن فاعلية المركبات التي تحتوي على السلفابيردين؛ وآليّة عمل هذه المركبات كمضادات للأورام السرطانية، وكذلك أيضاً أسباب الجمع بين العقاقير المضادة للسرطان والإشعاع في علاج أمراض الأورام السرطانية، وقد تم في هذا الجزء أيضاً استعراض بعض الطرق المستخدمة في تشييده تحضير مشتقات البيروال والثيازول.

2. الغرض من البحث:

يتناول هذا الجزء الحقائق والأسس العلمية التي استند عليها لتحضير المركبات الجديدة من مشتقات السلفابيردين لإختبار فاعليتها كمضادات للخلايا السرطانية، ودراسة قدرة بعض هذه المركبات على زيادة حساسية الخلايا السرطانية تجاه التأثير المضاد للسرطان لأشعة جاما، ومحاولة توقع آلية عمل هذه المركبات عن طريق الإرساء الجيني.

3. مناقشة الجزء العلمي:

يناقش هذا الجزء الطرق المستخدمة في تشييده المركبات الجديدة، وكذلك مناقشة طرق التأكد من التركيب البنائي الكيميائي للمركبات الجديدة، كما يحتوي هذا الجزء على الطرق المتبقية في تحضير المركبات المستهدفة.
الجزء العملي:

يتضمن هذا الجزء وصفاً تفصيلاً للطرق العملية التي اتبعت لتحضير 26 مركباً نهائياً ومركبين وسيطين معروفين، وقد تم التحقق من التركيب البنائي لهذه المركبات وذلك عن طريق التحليل الدقيق للعناصر المكونة لها، الأشعة تحت الحمراء، الرنين النووي المغناطيسي وكذلك مطياف الكتلة.

تتضمن الرسالة تحضير المركبات الآتية:

أ. المركبات الوسيطة المعروفة:

- 2-كلورو-4N-(4-伯二烯-2- يل سلفامول) فنين (أ)
- 4- (5-أمينو-4-سيانو-2-أوكسوب-3-ديايبيدرو-1-برول-1- يل) (伯二烯-2- يل)
- بنزين سلفوناميد (11) 

ب. المركبات النهائية:

- المركبات النهائية المعروفة:

  - 2-(伯二烯-1- يل) -4N-(4-伯二烯-2- يل سلفامول) فنين (أ)
  - 2-(موريولينو)-4N-(4-伯二烯-2- يل سلفامول) فنين (أ)

- المركبات الجديدة النهائية:

  - 4- (4-伯二烯-2- يل أمينو)(-伯二烯-2- يل) بنزين سلفوناميد (3)
  - 4- (4- كلوروبانيزول-2- يل أمينو)(-伯二烯-2- يل) بنزين سلفوناميد (4)
  - 2- (2-أوكسوب-2-4N-(4-伯二烯-2- يل سلفامول) فنين أمينو) ايثيل أمينو} اسبتاك اسيد (5 أ)
  - 2- (2-أوكسوب-2-4N-(4-伯二烯-2- يل سلفامول) فنين أمينو) ايثيل أمينو} اسبتاك اصيد (5 ب)
  - 2- (2-أوكسوب-2-4N-(4-伯二烯-2- يل سلفامول) فنين أمينو) ايثيل أمينو} اسبتاك اصيد (5 ج)
  - 2- (2-أوكسوب-2-4N-(4-伯二烯-2- يل سلفامول) فنين أمينو) ايثيل أمينو} بنزانوك اصيد (5 د)
- 2-أوكسو-2-(N-2-بريردين-2-يل سلفاموغل) فينيل امينو) [إيثيل امينو] سكينك
  
  - 2-أوكسو-2-[4-(N-2-بريردين-2-يل سلفاموغل) فينيل امينو] [إيثيل امينو] فينيل

بروبانوك اسيد (5 و 6)

- 2-أوكسو-2-(N-2-بريردين-2-يل سلفاموغل) فينيل امينو) [إيثيل امينو] فينيل

هيدروكسي فينيل بروبانوك اسيد (5 و 6)

- 2-(داي إيثيل امينو) -N-(4-N-بريردين -2-يل سلفاموغل) فينيل) استياميد (7)

- 2-(4-كلورو فينيل امينو) -N-(4-N-بريردين -2-يل سلفاموغل) فينيل) استياميد (8)

- 4-(3-إيثيل ثايووريدو)-2-أوكسو-2-(N-2-بريردين-2-يل سلفاموغل) فينيل) -3،2-دايبيرول

- 2-برورول-2-يل) استياميد (9)

- 3،2-دايبيرول-1-H1

- 2-برورول-2-يل) استياميد (10)

- 4-(3،2-دايبيرول-2-أوكسو-2-(4-N-بريردين-2-يل بنزين سلفوناميد) بنزين سلفوناميد (11)

- 4-(3،2-دايبيرول-2-أوكسو-2-(4-N-بريردين-2-يل) بنزين سلفوناميد (12)

- 4-(3،2-دايبيرول-2-أوكسو-2-(4-N-بريردين-2-يل) بنزين سلفوناميد (13)

- 4-(3،2-دايبيرول-2-أوكسو-2-(4-N-بريردين-2-يل) بنزين سلفوناميد (14)

- 4-(3،2-دايبيرول-2-أوكسو-2-(4-N-بريردين-2-يل) بنزين سلفوناميد (15)

- 4-(3،2-دايبيرول-2-أوكسو-2-(4-N-بريردين-2-يل) بنزين سلفوناميد (16)

- 4-(3،2-دايبيرول-2-أوكسو-2-(4-N-بريردين-2-يل) بنزين سلفوناميد (17)

- 4-(3،2-دايبيرول-2-أوكسو-2-(4-N-بريردين-2-يل) بنزين سلفوناميد (18)

- 4-(3،2-دايبيرول-2-أوكسو-2-(4-N-بريردين-2-يل) بنزين سلفوناميد (19)

- 4-(3،2-دايبيرول-2-أوكسو-2-(4-N-بريردين-2-يل) بنزين سلفوناميد (20)
4. (4-2(2ر-4،2 - داي نيتروفينيل) هيدرازينيل) ثيازول-2،1- أمينو)(-بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بيريدين-2،1-(بي