ISSN 2582 – 6050

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2023; 14(3):108-118

RESEARCH ARTICLE

Synthesis of MDM2 Inhibitors to Reactivate p53 Function

Chirag J. Gohil[1], Malleshappa N. Noolvi[2]

[1]*Discipline of Pharmacy, Gujarat Technological University, Ahmedabad, Gujarat, India,* [2]*Department of Pharmaceutical Chemistry, Shree Dhanvantary Pharmacy College, Surat, Gujarat, India*

Received: 26 April 2023; Revised: 15 May 2023; Accepted: 10 July 2023

ABSTRACT

Cancer is arise by inhibition of cell killing apoptosis process. Specific protein–protein interaction is responsible for the inhibition of apoptosis in the body. In this study, novel heterocyclic compounds (1,3,4-thiadiazole based) were synthesized. Synthesized compounds were successfully characterized by physical and spectral methods. And biological activity of the synthesized compounds was evaluated by cell-based assays. MTT assay was performed to determine the anti-neoplastic activity of the synthesized compounds. Moreover, a potency of the synthesized compounds was derived by IC_{50} value. In an assay (*in vitro*), the synthesized compound induced the apoptosis in the cancer cell line. Hence, it will be proven as a potential anticancer agent.

Keywords: 1,3,4-thiadiazole, apoptosis, cancer, IC_{50}, MDM2 Inhibitors, MTT assay, p53 protein

INTRODUCTION

Cancer is a collective pathological condition which contains the uncontrollable cell division and growth.[1] This manner of unorganized cell division and growth leads to the malignancies or tumor genesis. There are certain types of the cancers which are arise because of the internal stimuli (genetic) or by the external factors.

Normally, cell division and cell growth are tightly regulated in the human body. At cellular level, various control mechanisms govern this process. Autophagy and apoptosis are these kinds of the processes, which controls and regulated the total cellular turnover in the body.[2] Autophagy and Apoptosis both are cell-kill processes in nature but apoptosis is more vital in the body. Apoptosis is governed by various genes and proteins, among them p53 protein is the master regulator of the apoptosis.

The scope of p53 protein function is DNA damage response, cell cycle arrest, cell death, and autophagy. In an unstressed condition cellular level of the p53 protein is very low. In stressed conditions or response to external stimuli induces the p53 expression, so p53 level increases in the cell.[3]

Activation of the p53 protein will result in the following modifications: inhibition of glycolysis, induction of autophagy, promotion of oxidative phosphorylation, and induction of apoptosis. p53 response varies with cell condition. If DNA damage is repairable then p53 will harness the cell cycle and starts the DNA repair. However, if the DNA damage is irreparable then p53 will end up in the cell by means of the apoptosis.

p53 has been regulated by the specific protein–protein interaction, i.e., p53-MDM2 interaction.[4] In general, in a normal human body p53 function is well preserved. However, in certain conditions (genetic or external factor), cell override the p53 regulation and inhibits the p53 function. Hence, inhibition of the p53 function is responsible for the cancer occurrence.

It is found that most types of cancers have an inhibited function of p53 protein.[5] Technically, p53 is controlled by the protein–protein interaction, i.e., p53-MDM2 PPI [Figure 1].

***Corresponding Author:**
Chirag J. Gohil,
E-mail: gohil2711@gmail.com

MDM2 Inhibitors

The protein p53 is a tumor suppressor. Therefore, the activation of p53 in cancer cells would result in cancer cell apoptosis.[6] The suppression of the p53/MDM2 interaction is the most promising and researched.

Experiments in the clinic demonstrate that activation of p53 by MDM2 inhibitors produces p53-dependent cell cycle arrest and cell death in cancer cell lines[7,8] (in cells with p53 of wild type, but not in cells with deleted or altered p53). In normal cells, MDM2 inhibitor-induced p53 activation results in cell cycle arrest, but not cell death. Thus, the activation of p53 by MDM2 inhibitors may specifically target cancer cells.

The crystal structure of MDM2 linked to the transactivation domain of p53 reveals that MDM2 possesses a deep hydrophobic pocket that is filled by three side chains of the helical region of p53.[9] Therefore, the existence of this type of hydrophobic pocket on MDM2 has led to the hypothesis that this interaction can be suppressed by employing small compounds that resemble the three side chains of the p53 helix and can bind to the three pockets on MDM2.[10,11]

If we design a small molecule (non-peptidic) that can engage with p53 binding pockets of MDM2 (3 pocket binding), we can suppress the interaction between p53 and MDM2.[12] Therefore, p53 will be released, and p53 will be activated. Eventually induces apoptosis in cancer cells.

MDM2 inhibitors (p53-MDM2 interaction inhibitors) are an emerging class of anti-cancer medicines with the ability to selectively target cancer cells over normal ones.[13] Numerous possible MDM2 inhibitors have been produced by various researchers, and they have proven to be highly effective in the non-genotoxic activation of p53 protein activity in cancer cells (apoptosis).[14] While some MDM2 inhibitors mirror the p53 structure, others do not.

MDM2 inhibitors exhibit potent antitumor action *in vitro* and *in vivo*. However, mutant p53 cannot be targeted and impacted by this method.[15]

In 2004, Vassilev and co-workers at Roche Pharmaceutical revealed "Nutlin," the first powerful MDM2 inhibitor that reactivates p53 by inhibiting MDM2.[16,17] Nutlin is an analog of cis-imidazoline with an IC_{50} value between 100 and 300 nM12.

Nutlin is a class of chemicals [Figure 2] that includes nutlin-1, nutlin-2, and nutlin-3. They showed varying MDM2 inhibition potencies.[18] Nutlin 3, like nutlin 1 and 2, is a stereoactive chemical. Among the enantiomers of nutlin 3, nutlin 3a is 150 times more active than 3b. The family of chemicals known as nutlins may efficiently imitate the p53 side chain residue, and it has three-pocket binding.[19]

Nutlin 1, 2, and 3 are all part of the same series of compounds, but each one has a distinct capacity to inhibit MDM2.[20] The nutlin 3a compound has the highest activity of all the members of the class. Furthermore, the activity of 3a is 150 times higher than that of its enantiomer, 3b. Nutlins are a family of chemicals that have the ability to successfully imitate the residue found

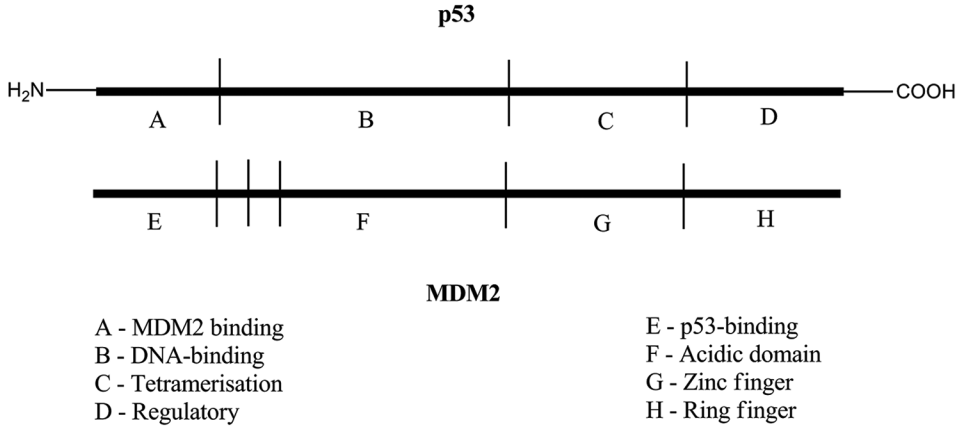


Figure 1: Domain composition of p53 and MDM2 protein

on the p53 side chain, and it has three binding pockets [Figure 3].[21]

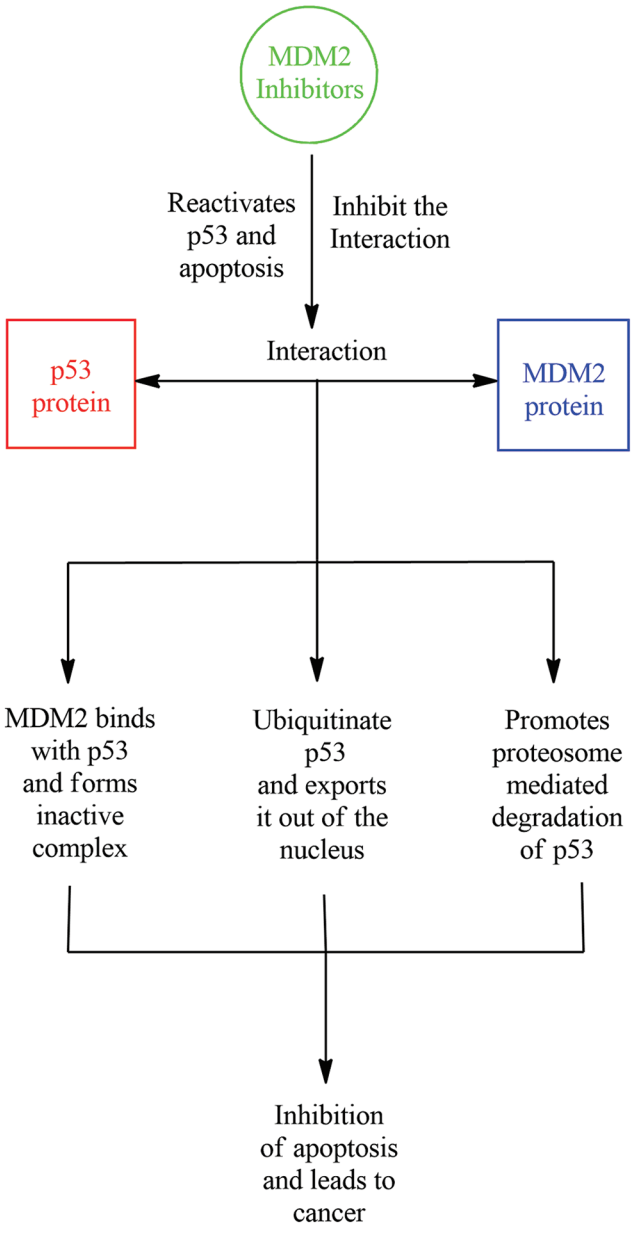


Figure 2: p53-MDM2 interaction

Clinical Application of MDM2 Inhibitors in Cancer Therapy

Chemoprotective effect

Conventional antineoplastic drugs have lack of the selectivity for the cancer cell over normal cells. Therefore, it causes lethal side effect to the normal cells.

Drugs that protect normal cells from the anticancer drugs by shielding them is called chemoprotective agents and therapy is known as cyclotherapy.[22] Most of the anticancer drug targets the dividing cells. Hence, if we can pause the cell division in the tissue using chemoprotective drugs, we can spare the non-cancerous normal cells. Then subsequent treatment with anticancer drugs will eradicate the cancer cells which are in S or M phase of the cells division.

MDM2 inhibitors induced the cell cycle arrest at G1/S and G2/M phases, so it can inhibit the activity of S-phase and M-phase specific chemotherapeutic drugs. Clinically, it is evident that nutlin-3 has been shown to have a mild, reversible cytostatic effect on normal cells.[23]

Nutlin-like MDM2 inhibitors do not require the phosphorylation for the activation, so they are non-genotoxic activators of the p53 pathway. They bind directly to the MDM2 protein and stabilise it, so it will impair the MDM2 down-regulating activity on p53.[24] Various studies indicate that MDM2 inhibitors protect the normal cell with the anticancer drugs which target the mitotic cells.[25] This suggests that MDM2 inhibitors could be used as chemoprotection purposes.

Use in blood malignancies

Various research findings indicating that MDM2 inhibitors are effective in the cancer cell which

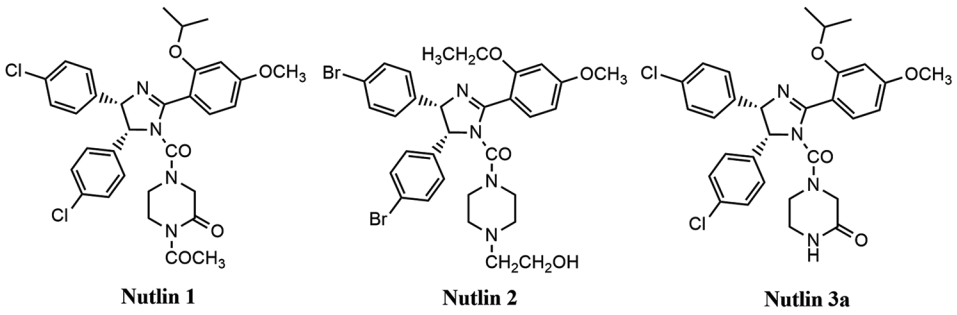


Figure 3: Structures of nutlin-1, nutlin-2 and nutlin-3

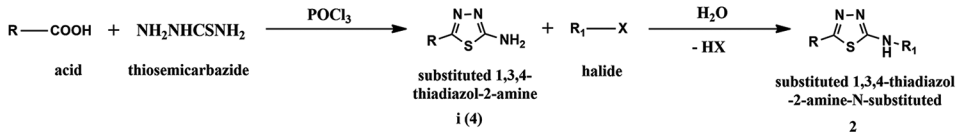


Figure 4: Scheme for the synthesis of substituted "1,3,4-thiadiazole"

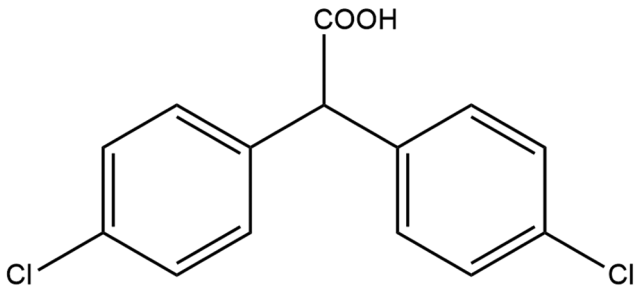


Figure 5: Structure of 2,2-bis(4-chlorophenyl)acetic acid

are having the wild-type p53. That means MDM2 inhibitors can be most effective in blood malignancies such as acute myeloid leukemia, B-chronic lymphocytic leukemia, and multiple myeloma. Because all these malignant cells retain the wild-type p53.[26]

Treating the blood malignancy patients with MDM2 inhibitors like Nutlin-3 and MI-63, indeed shown that they effectively trigger apoptosis. In leukemia, MDM2 inhibitors synergize the effect of the conventional anticancer drugs such as doxorubicin, cytosine arabinoside, and chlorambucil.[27]

Importantly, both the single agent and combination of the Nutlin-3 with the other anticancer drugs will spare the normal cell over the cancer cells and do not produce toxicity to peripheral blood mononuclear cells and bone marrow stromal epithelium cells.[28] Hence, MDM2 inhibitors prove to be more effective in the blood malignancies.

Effect of MDMX expression

MDMX or MDM4 (in mice) is a very homologous protein with the MDM2. MDMX has the similar binding site with p53 protein as of the MDM2 protein.[29] MDMX bind with N-terminus domain of p53 protein in a similar manner as of the MDM2 protein.

The basic features of the MDM2-p53 and MDMX-p53 interactions are similar. But unlike MDM2 protein, MDMX does not cause p53 degradation.[30] MDMX is not a transcriptional target of p53 protein. MDMX binds with MDM2 protein via C-terminus RING domains and forms a dimer protein. Moreover, this dimer-protein will bind with p53 and forms the complex, which regulates p53 function.

Amino acid chain and protein structure of the MDMX is similar to MDM2 protein. Hence, it is though that MDM2 inhibitors will also have an inhibitory effect on the MDMX protein. However, crystallographic study shows that MDMX has a different amino acid residue in its p53 binding pocket.[31]

MDM2 inhibitors such as Nutlin-3 and MI-219 specifically bind and interact with the MDM2 protein only. After treating with MDM2 inhibitors, they can spare the p53 protein but MDMX expression will decrease the activity of MDM2 inhibitors to activate the transcriptional function of p53.[32] Hence, MDM2 inhibitors were ineffective in cells transformed with MDMX and failed to induce apoptosis in various cancer cell lines.

To overcome this, MDM2 inhibitors can be combined with chemotherapeutic drugs which induce MDMX degradation.[33] Another approach is to design MDM2-MDMX dual inhibitors for the complete reactivation of p53 function.

Toxicity to normal tissues

MDM2 inhibitors concentration was found to be similar in the spleen and plasma to that of the cancer cells. But its concentration was much lower in the lung, liver, kidney, and other normal tissue. Therefore, it can pose low or minimum toxicity to normal cells and tissue at therapeutically efficacious doses.

In addition, MDM2 inhibitors did not cause apoptosis or toxicity in either radiosensitive or radioresistant normal mouse tissues.[34] Whereas conventional chemotherapeutic drugs and γ-radiation induce apoptosis in normal cells and

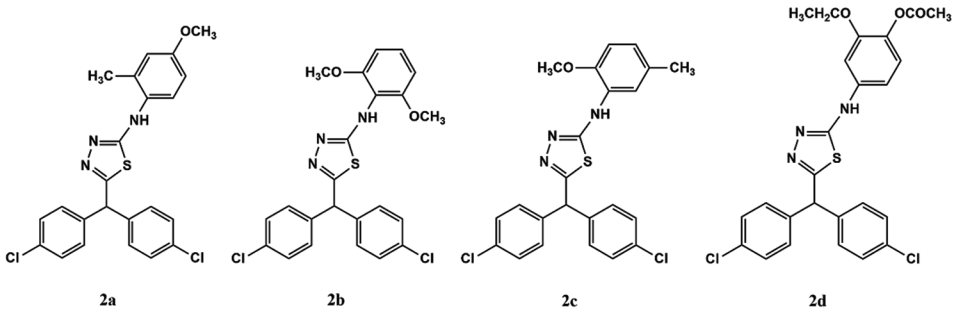


Figure 6: Structures of the compounds from series-1

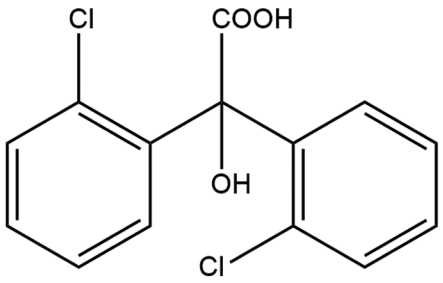


Figure 7: Structure of 2,2-bis (2-chlorophenyl)-2-hydroxyacetamide

Table 1: Physical characteristics of the compounds from series-2

Compound	Molecular weight	% Yield	M.P	Rf
2A	472.39	63	184-186	0.84
2B	488.39	67	193-196	0.81
2C	472.39	71	186-189	0.86
2D	530.42	69	214-216	0.91

tissue (small-intestine crypts and thymus). MDM2 inhibitors have a minimal p53 accumulation in normal tissues. Hence, we can say they are nontoxic to normal cells.

MATERIALS AND METHODS

Experimental

Each of the reagents, starting materials, and solvents was obtained from reputable commercial suppliers and was of a very high quality. Without additional purification, the chemicals and reagents/solvents were utilized. All melting points were obtained in open-ended capillary tubes immersed in liquid paraffin, without correction. The 1H spectra were attained in DMSO using a Bruker 400 MHz NMR Spectrometer Avance III, with no further purification of the solvents and reagents.

Procedure of Synthesis (Scheme-2)

Step 1 "(2-amino-5-ethyl-1,3,4-thiadiazole)"[35]

A mixture is prepared of 92.6 parts of acid, 215 parts of commercial polyphosphoric acid, and 1 part of 50% aqueous hypophosphorous acid, to which is added 91 parts of thiosemicarbazide. The reaction mixture is heated with stirring to between 102°C and 111°C for 1.5 h at which time the reaction is substantially complete. The reaction mixture is drowned in 500 parts of water and neutralized with ammonium hydroxide. The reaction mixture is filtered at rt, and the product of 2-amino-1,3,4-thiadiazole (i, **4**) washed with water and dried [Figure 4].

Step 2[36]

2-amino-1,3,4-thiadiazole (i,**4**, 5 mmol), sodium hydrogencarbonate (6 mmol), and sodium dodecyl sulfate (20 mg) were taken up in water or ethanol (20 mL) and heated for 5 min at 80°C. The reaction mixture received an addition of alkyl halide (6 mmol) and the reaction mixture was heated for 6–7 h. Monitor the completion of the reaction by thin-layer chromatography (TLC). The final product was formed by cooling the reaction mix and extracting the material with ethyl acetate. The final product was produced by cooling the reaction mixture and extracting material with ethyl acetate (2).

Starting Material for Series-1 [Figure 5]

Compound details of series-1

2a: 5-(bis(4-chlorophenyl)methyl)-N-(4-methoxy-2-methylphenyl)-1,3,4-thiadiazol-2-amine

2b: 5-(bis(4-chlorophenyl)methyl)-N-(2,6-dimethoxyphenyl)-1,3,4-thiadiazol-2-amine

2c: 5-(bis(4-chlorophenyl)methyl)-N-(2-methoxy-

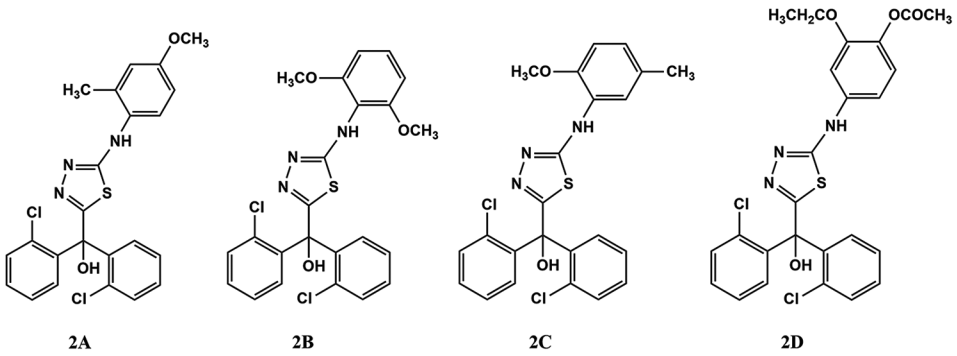


Figure 8: Structures of the compounds from series-2

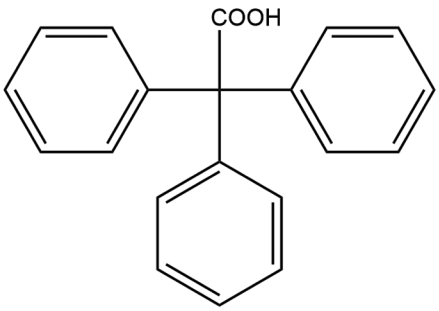


Figure 9: Structure of 2,2,2-triphenylacetic acid

Table 2: Physical characteristics of the compounds from series-3

Compound	Molecular Weight	% Yield	M.P	Rf
2aa	463.59	56	171-173	0.85
2bb	479.59	52	190-193	0.93
2cc	463.59	59	176-178	0.88
2dd	521.63	53	210-213	0.90

5-methylphenyl)-1,3,4-thiadiazol-2-amine
2d: 4-((5-(bis(4-chlorophenyl)methyl)-1,3,4-thiadiazol-2-yl)amino)-2-ethoxyphenyl acetate

Compound structures of series-1
Figure 6.

Compound characterization of series-1
Starting material
Crystalline white. IR (KBr): 3018.70 (Ar, C-H), 1699.34 (COOH, C=O), 1494.88 (Ar, C=C), 698.25 (C-Cl) cm^{-1}

Compound 2a
Pale yellow. 73 % yield, m.p. 182–185°C (lit), TLC (eluent: chloroform/methanol. 90: 10 v/v). Rf: 0.81, IR (KBr): 2833.52 (CH3, C-H), 1681.98 (N-H), 1247.99 (Ar, C=N), 1176.62 (OCH_3, C-O), 1026.16 (Ar, C-S), 810.13 (C-Cl) cm^{-1}, ^1H NMR (DMSO, 400 MHz,) δ: 2.353 (03H, CH_3), 3.753 (03H, OCH_3), 5.338 (01H, CH), 5.738-5.765 (01H, NH), 6.550-7.196 (11H, Ar-H), MS (EI, 70 eV) m/z: 459.2 [M+3]+, 457.3 [M+1]+, 359.4, 341.4, 331.4, 313.5.

Compound 2b
Yellow. 66 % yield, m.p. 188–190°C (lit), TLC (eluent: chloroform/methanol. 90: 10 v/v). Rf: 0.87, IR (KBr): 1510.31 (N-H), 1334.78 (Ar, C=N), 1145.75 (Ar, C-S), 1024.24 (OCH_3, C-O) cm-1, 1H NMR (400 MHz, DMSO) δ: 2.594 (06H, OCH3), 3.376 (01H, NH), 5.680 (01H, CH), 7.011 (03H, Ar-H), 7.011 (01H, Ar-H), 7.264-7.753 (07H, Ar-H), MS (EI, 70 eV) m/z: 472.06 [M]+, 359.17, 321.00, 240.19, 193.15.

Compound 2c
Light brown. 59 % yield, m.p. 191–193°C (lit), TLC (eluent: chloroform/methanol. 90: 10 v/v). Rf: 0.90, IR (KBr): 3051.49 (CH_3, C-H), 1685.84 (N-H), 1352.14 (Ar, C=N), 1118.75 (Ar, C-S) cm-1, ^1H NMR (400 MHz, $CDCl_3$) δ: 2.457 (03H, CH_3), 3.389 (03H, OCH_3), 3.624 (01H, NH), 5.618 (01H, CH), 6.774-6.801 (03H, Ar-H), 7.153-7.738(08H, Ar-H), MS (EI, 70 eV) m/z: 457.01 [M+1]+, 456.06 [M]+, 330.04, 279.18, 244.00.

Compound 2d
Light brown. 64 % yield, m.p. 202–205°C (lit), TLC (chloroform/methanol. 90: 10 v/v). Rf: 0.84, IR (KBr): 1616.40 ($OCOCH_3$, C=O), 1572.04 (N-

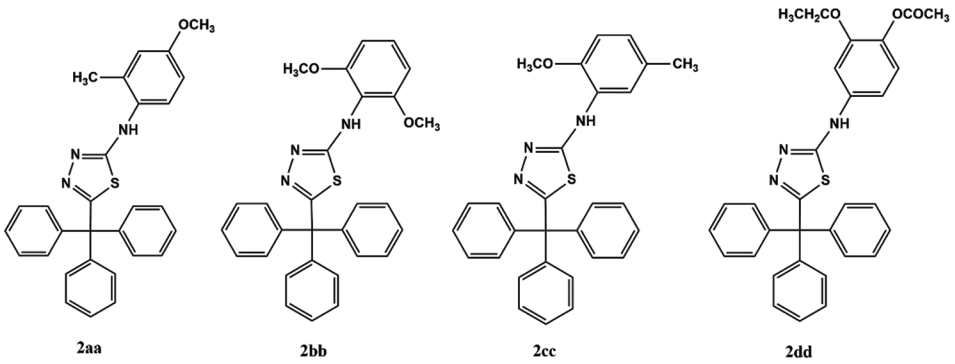


Figure 10: Structures of the compounds from series-3

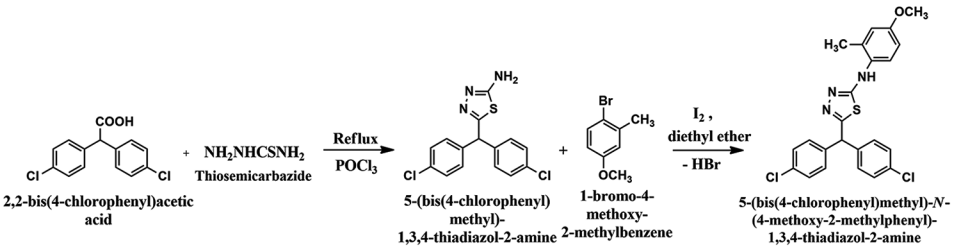


Figure 11: Scheme for the synthesis of lead compound 2a

H), 1321.28 (Ar, C=N), 1112.96 (Ar, C-S) cm-1, ^{1}H NMR (DMSO, 400 MHz) δ: 1.277 (03H, CH_3), 2.416 (03H, CH_3), 3.452 (02H, CH_2), 3.452 (01H, NH), 5.794 (01H, CH), 7.093–7.117 (03H, Ar-H), 7.264–7.973 (05H, Ar-H), 8.189–8.259 (03H, Ar-H), MS (EI, 70 eV) m/z: 513.02 [M-1]+, 460.11, 386.05, 166.09.

Starting Material for Series-2

Figure 7.

Compound details of series-2

2A: bis(2-chlorophenyl)(5-((4-methoxy-2-methylphenyl)amino)-1,3,4-thiadiazol-2-yl) methanol

2B: bis(2-chlorophenyl)(5-((2,6-dimethoxyphenyl) amino)-1,3,4-thiadiazol-2-yl)methanol

2C: bis(2-chlorophenyl)(5-((2-methoxy-5-methylphenyl)amino)-1,3,4-thiadiazol-2-yl) methanol

2D: 4-((5-(bis(2-chlorophenyl)(hydroxy) methyl)-1,3,4-thiadiazol-2-yl)amino)-2-ethoxyphenyl acetate.

Compound structures of series-2

Figure 8.

Compound characterization of series-2

Table 1.

Starting Material for Series-3

Figure 9.

Compound details of series-3

2aa: N-(4-methoxy-2-methylphenyl)-5-trityl-1,3,4-thiadiazol-2-amine

2bb: N-(2,6-dimethoxyphenyl)-5-trityl-1,3,4-thiadiazol-2-amine

2cc: N-(2-methoxy-5-methylphenyl)-5-trityl-1,3,4-thiadiazol-2-amine

2dd: 2-ethoxy-4-((5-trityl-1,3,4-thiadiazol-2-yl) amino)phenyl acetate.

Compound structures of series-3

Figure 10.

Compound characterization of series-3

Table 2.

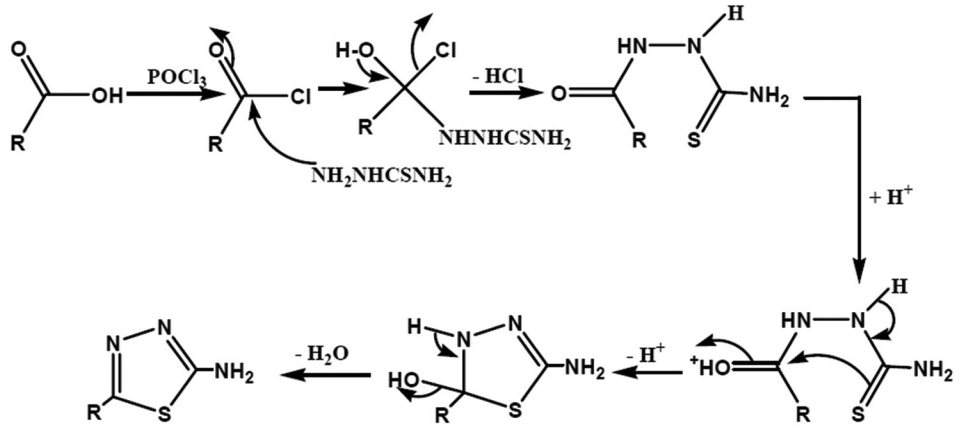


Figure 12: Mechanism of the 1,3,4-thiadiazole formation[40]

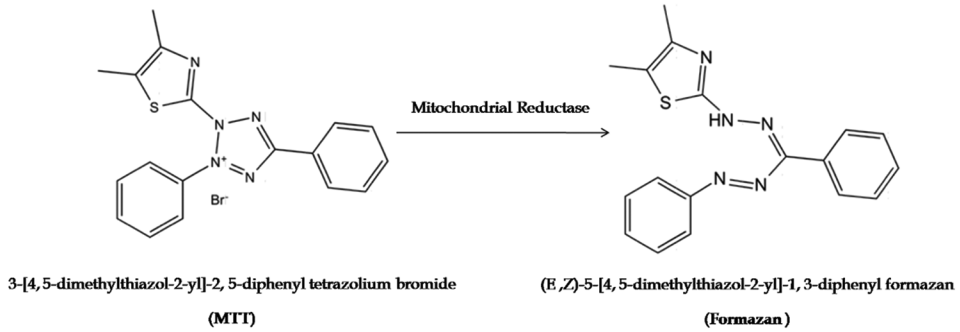


Figure 13: Principle of MTT assay test

RESULTS AND DISCUSSION

Our research focuses on the synthesis of novel MDM2 inhibitors, which are organic small molecules made up of distinct chemical building blocks including imidazothiadiazole and 1,3,4-thiadiazole. The aforementioned approach has been used to synthesize a total of four new chemicals.

Route of Synthesis for Lead Compound 2a

Preparation of the final compounds (Scheme-2) is simply two steps process. In the course of the reaction, acid reacted with thiosemicarbazide in the presence of $POCl_3$ to give 1,3,4-thiadiazole derivatives. Later on, the reaction of substituted benzyl halide with 1,3,4-thiadiazole forms the final compound.

In the study, lead compound (2a) was found as highly active molecule and it is tested for biological activity [Figure 11].

Mechanism

The reaction of the acid with $POCl_3$ generates the acetyl chloride derivative as reaction intermediate. Thiosemicarbazide bearing a nitrogen which has a lone pair of the electrons, that's why it acts as a negative centre.[37] In the next step, thiosemicarbazide as a nucleophile and attack on the positive center of the acetyl chloride derivative. This nucleophilic attack on positive center of acetyl chloride derivative causes the internal electron shifting.[38,39] The process of internal electron shifting produces the various intermediates. Finally, the reaction is completed by internal re-arrangement and cyclization, which gives the 1,3,4-thiadiazole derivative as product [Figure 12].

Cytotoxic Study

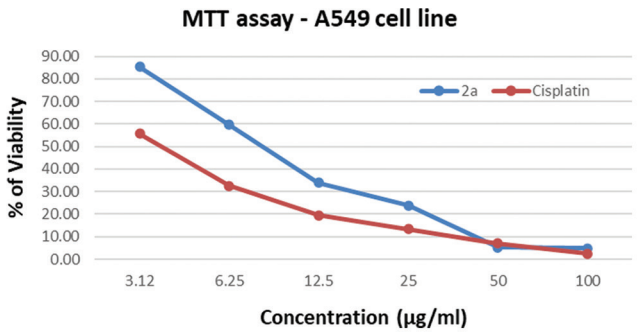
The vital dye staining viability technique has been used often in drug development research to determine the toxicity of substances on viable cells (MTT assay).

It is a traditional standard because the method based on the redox reaction of MTT dye proportionate to cellular activity is accurate by producing repeatable results, inexpensive, and simple to use.[41]
The quantity of formazan produced changes in direct proportion to the number of cells present, providing a direct indication of the cytotoxicity of the test substance [Figure 13].

Procedure[42]

Trypsinization of the monolayer cell culture was performed using a corresponding medium containing 10% FBS, and the density of cell was then raised to 1×10^5 cells/mL. A 100 µL portion of the cell suspension (diluted) containing 50 thousand cells per well was added further to individual well of the 96-well microtiter plate. When a partial monolayer had developed after 24 h, the supernatant was decanted, given one medium wash, and 100 µL of test substance or drug were added. The plate was kept incubated for 24 h at 37°C in a 5% CO_2 environment. Following incubation, the solutions (test) in the wells were removed, and each well received 100 µl of MTT (10 mL or 5 mg of MTT in PBS). The plate was kept in an environment of 5% CO_2 for 4 h at 37°C. To dissolve the formazan that had formed, DMSO (100 µL) was added after the supernatant was drained from the plate. Using a microplate reader, the absorbance at 570 nm was measured. The formula below was used to compute the percentage growth inhibition, and the dose-response curve for every cell line was used to create the 50% inhibitory concentration values.

Compound 2a



The 50% inhibitory concentration (IC_{50}) of given sample (compound **2a**) and standard (Cisplatin) was found to be 4.96 µg (10.868 nM) and 2.45 µg, respectively. The cell death was recorded due to apoptosis.

Proposed Mechanism of Action (p53 Dependent Apoptosis)

p53 is a powerful anti-tumor protein. p53 induces the apoptosis, cell cycle arrest, and senescence depending upon the cellular stress (DNA damage, oncogenic activity, ribosomal stress, hypoxia, and metabolic stress) Restoration of wild-type p53 protein function by MDM2 inhibition is extremely helpful for eradicating established tumors and it does not damage non-transformed cells (specific for tumor cells).[43]
MDM2 inhibition leads to re-activation of p53 function. Hence, p53 can activate its target genes followed by the induction of growth arrest or apoptosis and tumor suppression *in-vitro*.

Method

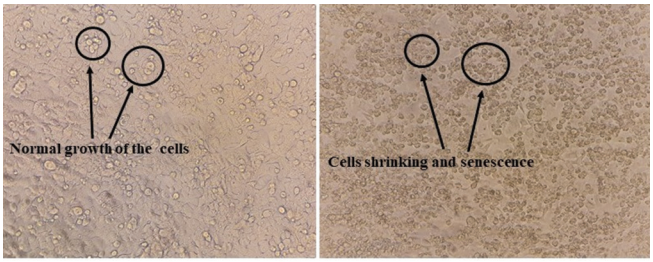
Among all *in vitro* anti-cancer assays/methods, MTT and Sulforhodamine-B assay are the most popular for estimating the anticancer activity of the compound. Here, we primarily used the MTT assay for the study of cellular stress and cellular damage of the tumor cells.

Marker of the apoptosis

Apoptosis can be characterized by various cellular processes such as cell shrinkage, membrane blebbing, chromosome condensation, nuclear fragmentation, DNA laddering, and the eventual engulfment of the cell by phagosomes.

Observation

Compound 2a



Control sample
(confluent growth of the cells)

Compound 2a
(blebbing and apoptosis of the cells)

1. Control sample: Shows the confluent growth of the cells
2. Test sample (compounds): As the concentration of the sample increased from 3.125 µg to 100 µg, cell experiences a stress, and cell death was recorded in the form of blebbing and apoptosis. The cells also lose the cell-cell contact and resultant in cell senescence, recorded as the concentration increases.

CONCLUSION

Here, we discussed the synthesis and biological evaluation of the novel heterocyclic compounds. The proposed mechanism for the formation of 1,3,4-thiadiazole was explored. We included the characteristics and structures of the substances produced

In conclusion to the synthetic aspect, Synthesized compounds were characterized and further evaluated for their biological efficacy. MTT assay indicates the good cytotoxic property of the synthesized compound. Further, the MDM2 inhibitory activity of the synthesized compounds can be confirmed by fluorescence polarization assay.

FUNDING

This research did not receive any outside financial support.

ACKNOWLEDGMENTS

Chirag J. Gohil, the first author, would like to express his gratitude to Dr. C.N. Patel and Dr. D. J. Sen for the support they provided as a part of my Doctoral Progress Committee.

In addition, the first author would like to express his appreciation to Dr. M. N. Noolvi, who served as both a supervisor and a mentor, for his assistance and support.

CONFLICTS OF INTEREST

The authors claim they have no competing interests.

REFERENCES

1. Elmore S. Apoptosis: A review of programmed cell death. Toxicol Pathol 2007;35:495-516.
2. Chavez-Valdez R, Martin LJ, Northington FJ**.** Programmed necrosis: A prominent mechanism of cell death following neonatal brain injury. Neurol Res Int 2012;2012:257563.
3. Roy AG, Sarkar BN, Roy R, Rao VR, Bandyopadhyay AR. Absence of P53 gene mutations in exons 5-7 among breast cancer patients of bengalee hindu caste females, West Bengal, India. Asian Pac J Cancer Prev 2012;13:4477-9.
4. Appella E, Anderson CW. Post-translational modifications and activation of p53 by genotoxic stresses. Eur J Biochem 2001;268:2764-72.
5. Gohil CJ, Noolvi MN. Types of p53/MDM2 interaction inhibitors. Eur J Bio Med Pharm Sci 2018;5:143-7.
6. Pettersson M, Quant M, Min J, Iconaru L, Kriwacki RW, Waddel MB, *et al.* Design, synthesis and evaluation of 2,5-diketopiperazines as inhibitors of the MDM2-p53 interaction. PLoS One 2015;10:e0137867.
7. Gohil CJ, Noolvi MN. p53 protein: Master regulator of apoptosis and its application in cancer therapy. Int J Pharm Biol Arch 2019;10:154-64.
8. Shadfan M, Lopez-Pajares V, Yuan ZM. MDM2 and MDMX: Alone and together in regulation of p53. Transl Cancer Res 2012;1:88-9.
9. Gohil CJ, Noolvi MN. Non peptidic small molecular inhibitors of the p53-MDM2 interaction. Int J Pharm Chem Anal 2019;6:104-9.
10. Momand J, Wu HH, Dasgupta G**.** MDM2--master regulator of the p53 tumor suppressor protein. Gene 2000;242:15-29.
11. Maya R, Balass M, Kim ST, Shkedy D, Leal JF, Shifman O, *et al*. ATM-dependent phosphorylation of Mdm2 on serine 395: Role in p53 activation by DNA damage. Genes Dev 2001;15:1067-77.
12. Gohil CJ, Noolvi MN. Design and *in silico* study of the novel small molecular MDM2 inhibitors. Biointerface Res Appl Chem 2021;11:8052-64.
13. Harris SL, Levine AJ**.** The p53 pathway: Positive and negative feedback loops. Oncogene 2005;24:2899-908.
14. Bliman D, Pettersson M, Bood M, Grotli M. 8-Bromination of 2,6,9-trisubstituted purines with pyridinium tribromide. Tetrahedron Lett 2014;55:2929-31.
15. Gohil CJ, Noolvi MN. Solvent-free synthesis of amide: A novel technique of green chemistry. Asian J Pharm Clin Res 2021;14:99-102.
16. Haupt Y, Maya R, Kazaz A, Oren M**.** Mdm2 promotes the rapid degradation of p53. Nature 1997;387:296-9.
17. Pettersson M, Bliman D, Jacobsson J, Nilsson JR, Min J, Iconaru L, *et al.* 8-Triazolylpurines: Towards fluorescent inhibitors of the MDM2/p53 interaction. PLoS One 2015;10:e0124423.
18. Gohil CJ, Noolvi MN, Patel CN, Sen DJ. Review of design approaches and clinical progress of MDM2

inhibitors. Int Res J Pharm 2021;12:1-7.
19. Carter S, Bischof O, Dejean A, Vousden KH. C-terminal modifications regulate MDM2 dissociation and nuclear export of p53. Nat Cell Biol 2007;9:428-35.
20. Elhag MA, Gabra NM, Baseer MA. Design and development of potent drug inhibitor to MDM2 protein in cancer through molecular docking studies. Indian J Appl Res 2013;3:28-30.
21. Pettersson M. Inhibition of the MDM2/p53 Interaction (Design, Synthesis and Evaluation of MDM2 Inhibitors. Ph. D Thesis, University of Gothenburg; 2015.
22. Blagosklonny MV, Darzynkiewicz Z**.** Cyclotherapy: Protection of normal cells and unshielding of cancer cells. Cell Cycle 2002;1:375-82.
23. Carvajal D, Tovar C, Yang H, Vu BT, Heimbrook DC, Vassilev LT**.** Activation of p53 by MDM2 antagonists can protect proliferating cells from mitotic inhibitors. Cancer Res 2005;65:1918-24.
24. Wallace M, Worrall E, Pettersson S, Hupp TR, Ball KL. Dual-site regulation of MDM2 E3-ubiquitin ligase activity. Mol Cell 2006;23:251-63.
25. van Leeuwen IM, Higgins M, Campbell J, Brown CJ, McCarthy AR, Pirrie L, *et al.* Mechanism-specific signatures for small-molecule p53 activators. Cell Cycle 2011;10:1590-8.
26. Secchiero P, Barbarotto E, Tiribelli M, Zerbinati C, di Iasio MG, Gonelli A, *et al.* Functional integrity of the p53-mediated apoptotic pathway induced by the nongenotoxic agent nutlin-3a in B-cell chronic lymphocytic leukemia (B-CLL). Blood 2006;107:4122-9.
27. Coll-Mulet L, Iglesias-Serret D, Santidrián AF, Cosialls AM, de Frias M, Castaño E, *et al.* MDM2 antagonists activate p53 and synergize with genotoxic drugs in B-cell chronic lymphocytic leukemia cells. Blood 2006;107:4109-14.
28. Kojima K, Konopleva M, Samudio IJ, Shikami M, Cabreira-Hansen M, McQueen T, *et al.* MDM2 antagonists induce p53-dependent apoptosis in AML: Implications for leukemia therapy. Blood 2005;106:3150-9.
29. Bottger V, Bottger A, Garcia-Echeverria C, Ramos YF, Van Der Eb AJ, Jochemsen AG, *et al.* Comparative study of the p53-mdm2 and p53-MDMX interfaces. Oncogene 1999;18:189-99.
30. Shvarts A, Steegenga WT, Riteco N, van Laar T, Dekker P, Bazuine M, *et al.* MDMX: A novel p53-binding protein with some functional properties of MDM2. EMBO J 1996;15:5349-57.
31. Jackson MW, Berberich SJ. MdmX protects p53 from Mdm2-mediated degradation. Mol Cell Biol 2000;20:1001-7.
32. Popowicz GM, Czarna A, Rothweiler U, Szwagierczak A, Krajewski M, Weber L, *et al.* Molecular basis for the inhibition of p53 by Mdmx. Cell Cycle 2007;6:2386-92.
33. Hu B, Gilkes DM, Farooqi B, Sebti SM, Chen J**.** MDMX overexpression prevents p53 activation by the MDM2 inhibitor Nutlin. J Biol Chem 2006;281:33030-5.
34. Shangary S, Qin D, McEachern D, Liu M, Miller RS, Qiu S, *et al.* Temporal activation of p53 by a specific MDM2 inhibitor is selectively toxic to tumors and leads to complete tumor growth inhibition. Proc Natl Acad Sci U S A 2008;105:3933-8.
35. Li WY, Song Y, Chen HB, Yang WL. Synthesis of 2-amino-5-mercapto-1,3,4-thiadiazole derivatives. Heterocycl Commun 2014;20:33-6.
36. Singh CB, Kavala K, Samal AK, Patel BK. Aqueous-mediated N-alkylation of amines. Eur J Org Chem 2007;1369-77.
37. Pellizzari Reaction, Big Chemical Encyclopedia. Available from: https://chempedia.info/info/pellizzari_reaction [Last accessed on 2019 Sep 01].
38. Bird CW, Wong CK. Einhorn-brunner reaction. Tetrahedron Lett 1974;15:1251-2**.**
39. Ranasinghe N, Mongeau E, Yuan G, Minden Z, Waldron S, Patrick Jr. C, *et al*. Flow and microwave induced pellizzari reactions: synthesis of heterocyclic analogues of the benzoxazepine antipsychotic agents loxapine and JL-13. Adv Chem 2017;8147421.
40. Al-Gwady MS. Synthesis of 2-amino-5-substituted-1,3,4-thiadiazoles (ATDA) and their derivatives using conventional and microwave techniques. J Raf Sci 2009;20:1-7.
41. Memariani T, Hosseini T, Kamali H, Mohammadi A, Ghorbani M, Shakeri A, *et al.* Evaluation of the cytotoxic effects of *Cyperus longus* extract, fractions and its essential oil on the PC3 and MCF7 cancer cell lines. Oncol Lett 2016;11:1353-60.
42. Gonzalez RJ, Tarloff JB. Evaluation of hepatic subcellular fractions for Alamar blue and MTT reductase activity. Toxicol *In vitro* 2001**;**15**:**257-9.
43. Abdul Rahman SN, Wahab NA, Abd Malek SN. *In vitro* morphological assessment of apoptosis induced by antiproliferative constituents from the rhizomes of *Curcuma zedoaria*. Evid Based Complement Alternat Med 2013;2013:257108.