A Platinum Open Access Journal for Organic Chemistry

Paper

Free to Authors and Readers | DOAJ Seal | *Arkivoc* **2024** (vii) 202412189

Synthesis, *In vitro* anticancer activity and molecular docking studies on some new phenylmorpholine linked aminotetrazoles and aryl tetrazoles

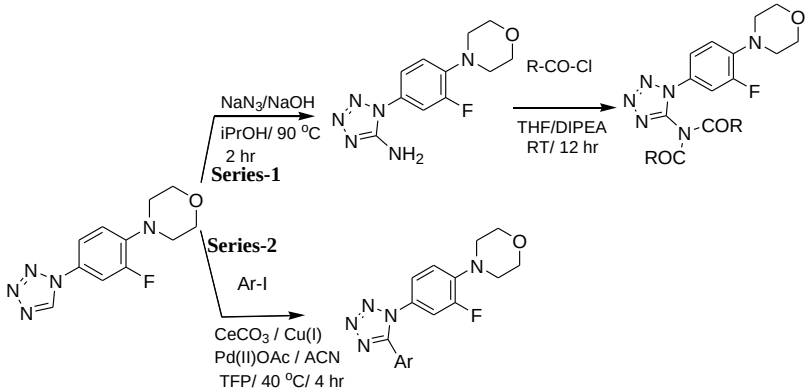
Suri Babu Patchipala,[a] Saphy Sharda,[b,c] Sunil Kumar Manna,[b] Ramya Krishna Pallapati,[a] J N Kolla,[d] Ravi Varala,[e] Mokesh Rayalu Golkonda,[f] and Hari Babu Bollikolla[a,g]*

[a]*Department of Chemistry, Acharya Nagarjuna University, NNagar, Guntur 522510, AP., India*
[b]*Centre for DNA Fingerprinting & Diagnostics (CDFD), Hyderabad, Telangana 500 039, India*
[c]*Graduate Studies, Regional Centre for Biotechnology, Faridabad, Haryana 121001, India*
[d]*Institute of Molecular Genetics, Vídeňská 1083, 142 20 Prague, Czech Republic*
[e]*Scrips Pharma, Mallapur 500 076, Hyderabad, Telangana, India & Research Fellow, INTI International University, Nilai 71800, Malaysia*
[f]*Department of Mathematics, School of Advanced Sciences,* Vellore Institute of Technology, Vellore -632 014
[g]*Department of Chemistry, Andhra Kesari University 523001, Ongole, AP., India*
Email: dr.b.haribabu@gmail.com

Received 02-25-2024 **Accepted** 05-20-2024 **Published on line** 06-09-2024

Abstract

This article presents two new series of tetrazole and morpholine hybrid derivatives that were produced using simple processes and good yields from 2-difluoro-4-nitrobenzene, morpholine, acid chlorides/(or) aryl iodide. By using IR, NMR, and HRMS spectrometry, all compounds were identified. The synthesized compounds were tested for anticancer efficacy against histiocytic lymphoma (U-937) cells. The route of action was investigated using molecular docking against tubulin, and **8h** and **10k** showed the best interactions with tubulin of all the drugs tested.



Keywords: Tetrazole, Morpholine, Hybrid molecules, Synthesis, Analysis, Anticancer efficacy, Molecular docking

Cite as *Arkivoc* **2024** (7) 202412189
DOI: https://doi.org/10.24820/ark.5550190.p012.189

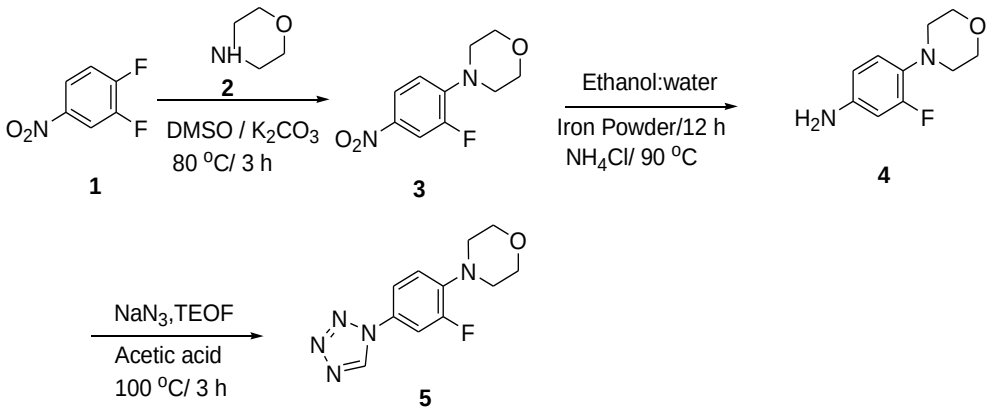
Introduction

A significant family of N-heterocyclic molecules with several applications were tetrazole compounds.[1] Tetrazoles were widely used in many different industries, for example, as stabilizers in photography and photo imaging,[2] chelating agents in coordination chemistry,[3] explosives in rocket propellants,[4] anti-wear and frictional agents in lubricants,[5] plant growth regulators, herbicides, and fungicides in agriculture. Tetrazole substances may also be utilized as bio-isosteres for carboxylic acids.[6] The bioavailability, metabolic stability, and cell permeability of a pharmacological molecule are all significantly increased when a carboxyl group is substituted with a tetrazole moiety Tetrazole compounds were consequently readily available as a variety of well-known drugs like Candesartan, Zolarsartan,[8] Losartan,[9] and Valsartan.[10] In addition, tetrazole compounds showed varied biological activities such as antibacterial,[11] antihypertensive,[12] anticonvulsant,[13] antifungal,[14] anticancer,[15] anti-inflammatory,[16] antitubercular,[17] antiallergic,[18] antineoplastic,[19] and antiviral[20] especially anti-HIV[21] activities.

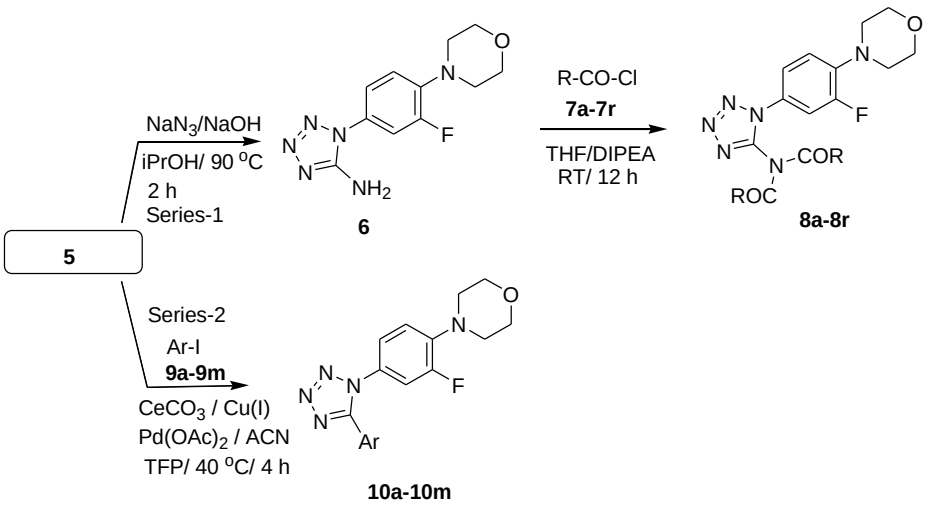
Morpholine key motif is present in many pharmaceutical drugs including Phenadoxone, Timolol, Linezolld[22], Reboxetine[23], Moclobemide[24], Rivaroxaban[25] etc. Morpholine derivatives have a variety of biological activities, including those that are anti-cancer, anti-bacterial, anti-fungal, and antiviral.[26] In this article, we present the synthesis of two series of novel tetrazole and morpholine hybrid derivatives as depicted in Schemes 1 and 2.

Results and Discussion

As illustrated in Scheme-1, the key intermediate (**5**) has been synthesized for generating the two series of 4-(2-fluoro-4-(1*H*-tetrazol-1-yl)phenyl) morpholine derivatives. Initially, the commercially easily accessible starting compounds 1,2-difluoro-4-nitrobenzene **1** was condensed with morpholine **2** in the presence of K_2CO_3 as a base in DMSO as solvent at 80 °C for 3 hr to form 4-(2-fluoro-4-nitrophenyl)morpholine **3** with 84% yield. Then, compound **3** underwent reduction with iron powder and ammonium chloride in a mixture of ethanol and water as solvent at 90 °C for 12 h to give 3-fluoro-4-morpholinoaniline **4** with 86% yield. Further, compound **4** was treated with sodium azide in acetic acid in the presence of triethyl orthoformate (TEOF) at 100 °C for 3 h to obtain 4-(2-fluoro-4-(1*H*-tetrazol-1-yl)phenyl)morpholine **5** with 87% yield.



Scheme 1. Synthesis of key intermediate **5**.



Scheme 2. Synthesis of 4-(2-fluoro-4-(1*H*-tetrazol-1-yl)phenyl)morpholine derivatives.

The resulting key intermediate **5** has been used for the synthesis of two series of new tetrazole derivatives. As illustrated in Scheme 2, compound **5** undergoes amination with the help of NaN_3, NaOH and Et_3N in *i*-PrOH followed by treatment with DMSO in acetic acid at 90 °C for 2 h to give 1-(3-fluoro-4-morpholinophenyl)-1*H*-tetrazol-5-amine **6** with 83% yield. Then, compound **6** underwent acetylation with different acid chlorides (**7**) in the presence of DIPEA as a base and THF as solvent at RT for 12 h to form **series-1** of tetrazole derivatives [diacylated tetrazoles (using 3 equiv. of acetyl chloride) & mono acetylation tetrazole compounds (using 1.1 equiv. of acetyl chloride). On the other hand, compound **5** underwent a coupling reaction with aryliodide in the presence of cesium carbonate, copper(I) iodide, palladium(II) acetate, and tris(2-furyl)-phosphine in dry acetonitrile at 40 °C in argon atmosphere for 4 h to form **series-2** of tetrazole derivatives (aryl tetrazole compounds). The structures of the synthesized compounds were ascertained by IR, NMR and mass analysis.

In the scenario, we have synthesized two classes of 1*H*-tetrazol-1-yl)phenyl)morpholine amide derivatives by monoacetylation and di-acetylation. The yields of the compounds were obtained in the range of 64-94%. The representative compound cyclopropanediamide **8d** obtained the highest yield (94%) and the di(trifluoromethyl)benzamide **8h** obtained with lowest yield (64%). All the tetrazolyl phenyl morpholine derivatives **(8a-8r)** were characterized by spectral data. The IR spectral data revealed that compounds (**8a-8j**) showed a strong C=O stretching band at around 1705-1744 cm^{-1} and ^{1}H-NMR chemical shift value of **8a** observed in the range of δ 2.30 as singlet for 6 H's revealed that the presence of diacetylated product on amine. Further, the absence of -NH_2 bands in IR spectra (**6**) at 3340, 3154 cm^{-1} also confirmed that smooth di-acetylation underwent on **6** and achieved **8a-8j** in very good yields. Furthermore, the monoacetylated compounds (**8k-8r**) also characterized by spectral data. The IR spectral data revealed that the aforementioned compounds showed a strong C=O stretching band at about 1677-1728 cm^{-1} and a strong -NH absorption band at 3181-3405 cm^{-1}. ^{13}C NMR and high-resolution mass spectral data (HRMS) also corroborated the structures of the all the morpholine amide derivatives (**8a-8r**).

Additionally, we have synthesized arylated tetrazol-morpholine derivatives (**10a-10m**) by adopting a coupling reaction[27,28] of compound **5** with various aryl iodides (**9a-9m**) in the presence of palladium(II) acetate and tris(2-furyl)-phosphine with good yields (78-87%). Additional ^{1}H-NMR chemical shift values at δ at 7.57–7.41 as multiplet for 5 H's of representative compound **10a** and its mass spectra showed *m/z* at 326 as $[M+H]^+$ also revealed that arylation takes place on tetrazole core of compound **5**. ^{1}H singlet peak at chemical shift

value at 8.90 in ^{1}H NMR is characteristic of hydrogen attached to carbon in tetrazole moiety present in compound **5**, the absence of this peak also affirmed the structures of compounds **10a-10m**.

Table 1. Yield and Melting range details of compounds obtained in **8(a-r)** series

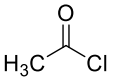
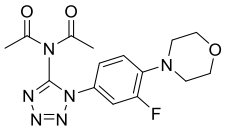
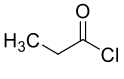
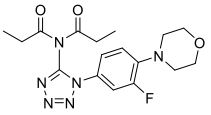
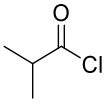
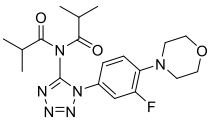
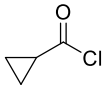
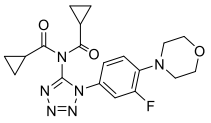
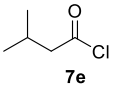
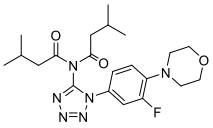
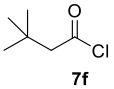
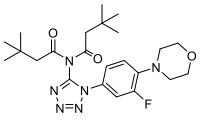
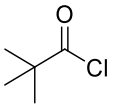
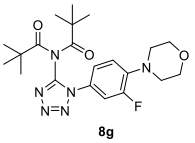
Entry	Acid chloride	Product	Mp Range (°C)	Yield (%)
1	 **7a**	 **8a**	274-276	91
2	 **7b**	 **8b**	158-160	90
3	 **7c**	 **8c**	191-193	82
4	 **7d**	 **8d**	103-105	94
5	 **7e**	 **8e**	--	91
6	 **7f**	 **8f**	138-140	89
7	 **7g**	 **8g**	--	82

Table 1. Continued

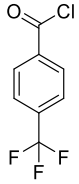
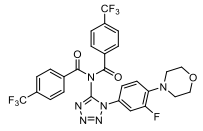
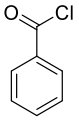
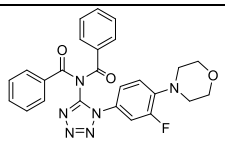
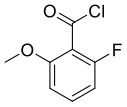
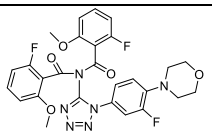
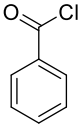
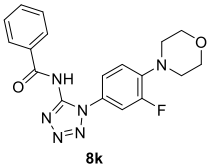
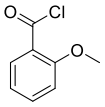
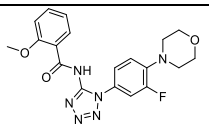
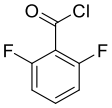
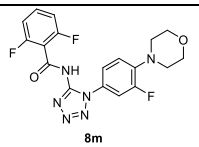
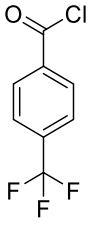
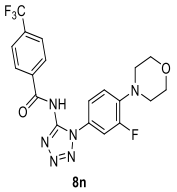
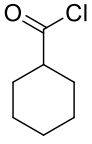
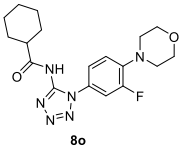
Entry	Acid chloride	Product	Mp Range (°C)	Yield (%)
8	 **7h**	 **8h**	138-141	64
9	 **7i**	 **8i**	101-104	77
10	 **7j**	 **8j**	239-241	87
11	 **7k**	 **8k**	257-259	85
12	 **7l**	 **8l**	196-198	92
13	 **7m**	 **8m**	214-216	90
14	 **7n**	 **8n**	202-204	87
15	 **7o**	 **8o**	201-205	85

Table 1. Continued

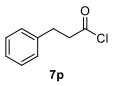
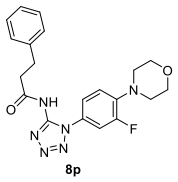
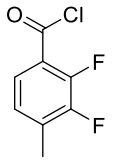
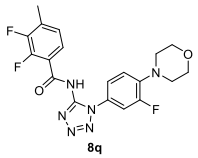
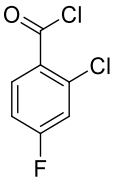
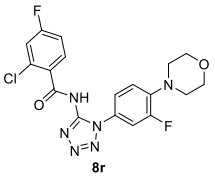
Entry	Acid chloride	Product	Mp Range (°C)	Yield (%)
16	 **7p**	 **8p**	180-183	92
17	 **7q**	 **8q**	112-115	91
18	 **7r**	 **8r**	172-175	88

Table 2. Yield and Melting point details of compounds obtained in **10(a-m)**series

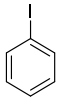
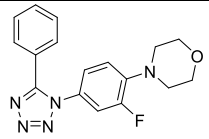
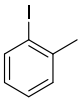
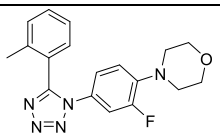
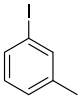
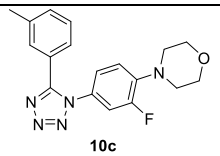
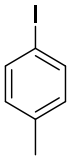
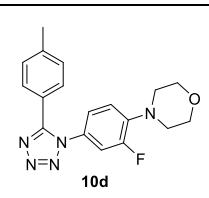
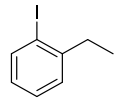
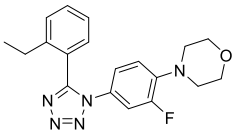
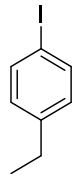
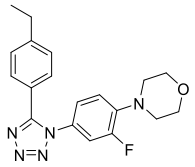
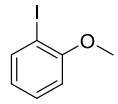
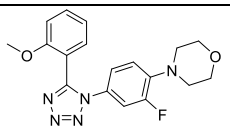
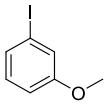
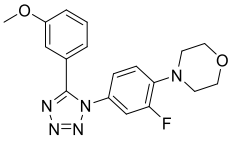
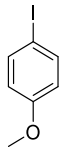
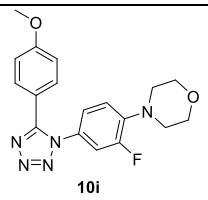
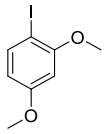
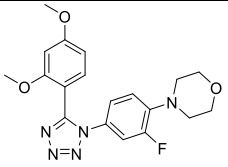
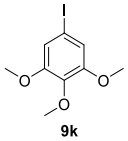
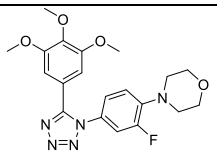
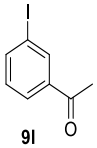
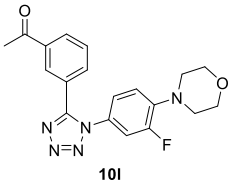
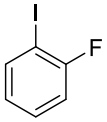
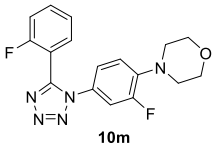
Entry	Aryl Iodide	Product	Melting range (°C)	Yield (%)
1	 **9a**	 **10a**	148-151	87
2	 **9b**	 **10b**	98-101	86
3	 **9c**	 **10c**	101-104	83
4	 **9d**	 **10d**	149-151	84

Table 2. Continued

Entry	Aryl Iodide	Product	Melting range (°C)	Yield (%)
5	 **9e**	 **10e**	--	81
6	 **9f**	 **10f**	118-120	78
7	 **9g**	 **10g**	123-125	80
8	 **9h**	 **10h**	110-114	84
9	 **9i**	 **10i**	154-156	81
10	 **9j**	 **10j**	104-106	86
11	 **9k**	 **10k**	130-132	85
12	 **9l**	 **10l**	157-159	85
13	 **9m**	 **10m**	124-126	86

Study of anticancer activity

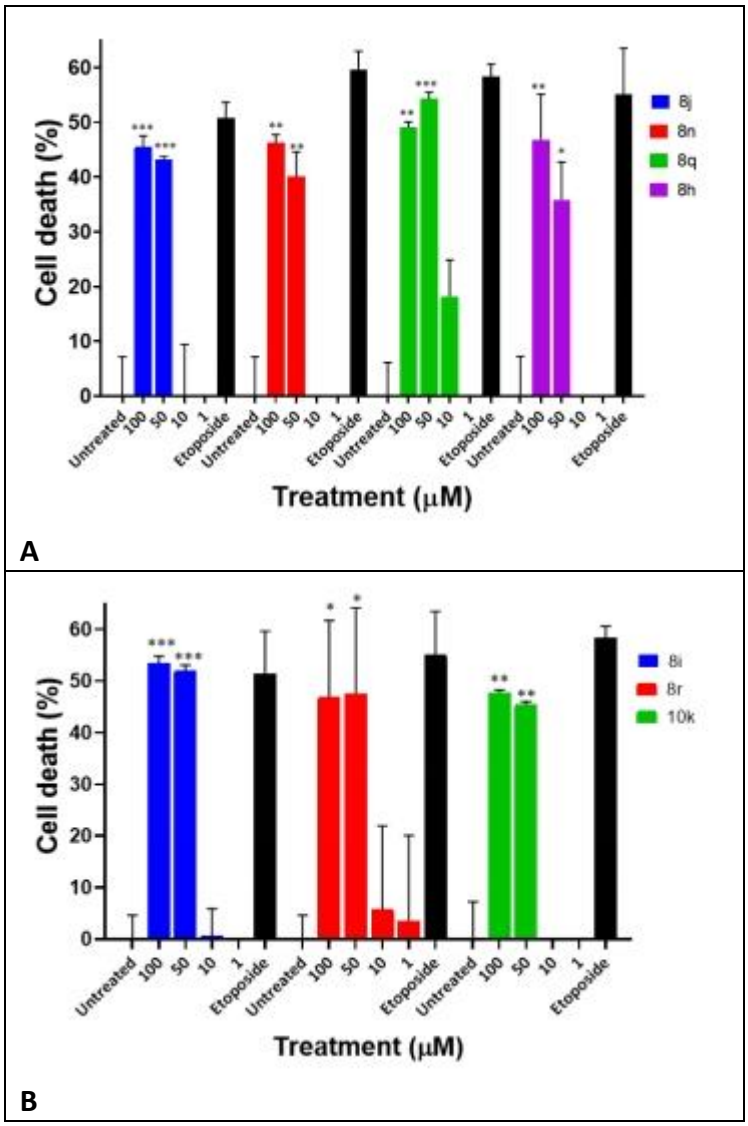


Figure 1. *In vitro* anticancer activity by MTT cell proliferation assay A) cytotoxicity of **8j, 8n, 8h** and **8q**. B) cytotoxicity of **8i, 8r** and **10k**. Etoposide (10 µM) used as assay control. The data presented are the average of triplicate experiments and ± SD (n = 3), statistical analysis performed two-tailed student's t test; * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$.

Cytotoxicity. U-937 cells were used to study the cell death of novel synthesized compounds and the compounds **8j, 8n, 8h, 8i, 8q, 8r** from Scheme I and **10k** from Scheme II have shown around 50% cell death at 50 µM concentration of each compound. The other compounds didn't show any significant cell death on U-937 cells. The cells treated with etoposide have shown around 50% of cell death at 10 µM. Among the effective compounds, **8j**, **8q** and **8i** were exhibited more significant anti-proliferative activity followed by **8n** and **10k** (Figure 1 A&B). The compounds **8h** and **8r** were slightly significant due to their experimental variations of % cell death. Popova *et.al.*[29] stated that tetrazole cycle is a capable pharmacophore widely used in the development of novel drugs. Over the decade, various isomeric forms of tetrazole have been advanced successfully for anticancer agents especially 5-oxo and 5- thiotetrazoles. In the present study, **8j, 8n, 8h, 8i, 8q, 8r** and **10k** possessed a variety of substitutes on tetrazole cycle such as N-di(*o*-methoxy, *o*-fluoro), N-(*p*-

triflurobenzyloxy), N-di(p-triflorobenzyloxy), N-dibenzoyl, *o*,*m*-difluoro, *p*-methylbenzyloxy, N-(*o*-chloro, *p*-fluorobenzoyl) and *m,m,p*-trimethoxyphenyl respectively. Furthermore, the compounds **8j, 8n, 8h, 8i, 8q, 8r** and **10k** characterizing towards anti-cancer mode of action.

Molecular docking study

Compound **1h** and **2k** were found to show better docking score among all the compound 1 and 2 series compounds when compared to control molecule Colchicine. Compounds **8h, 10k**, and Colchicine had shown the docking score -157.142, -140.827, and -123.11 based on the free energy calculations used in the Molegro Virtual Docker. The compound **8h** in the tubulin active site (Figure 2) formed two hydrogen bonds with the ALA250 while **10k** (Figure 3) formed no hydrogen bonds. The compound **8h** and **10k** formed better interaction with the tubulin active site. Although Colchicine is known tubulin inhibitor, its docking score was comparatively less when compared to compound **8h** and **10k**. Both the compounds found to show better interaction with the tubulin than Colchicine. Among all the derivatives of 1 and 2 series, compound **8h** has shown highest binding interaction efficiency. The compound **8h** was found to be best interacting molecule in the tubulin active site among all the compound 1 and 2 derivatives.

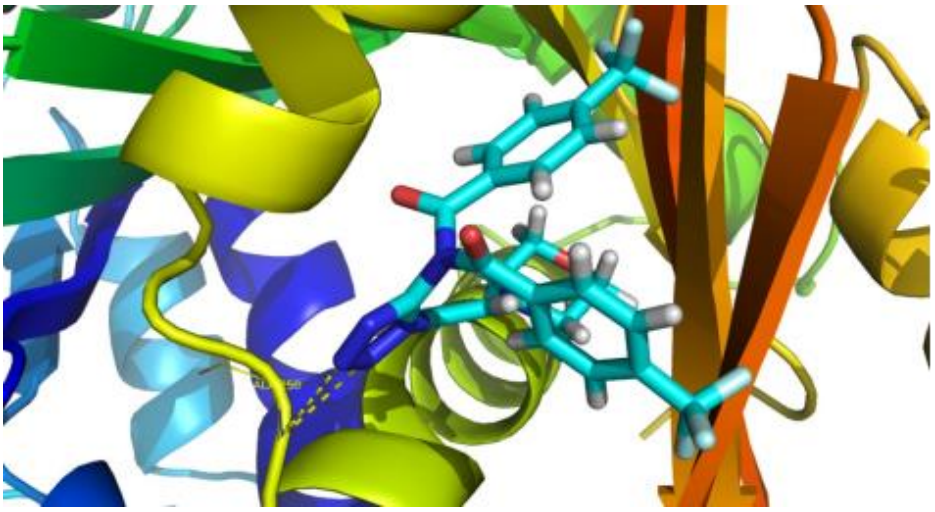


Figure 2. **8h** *In tubulin* binding site.

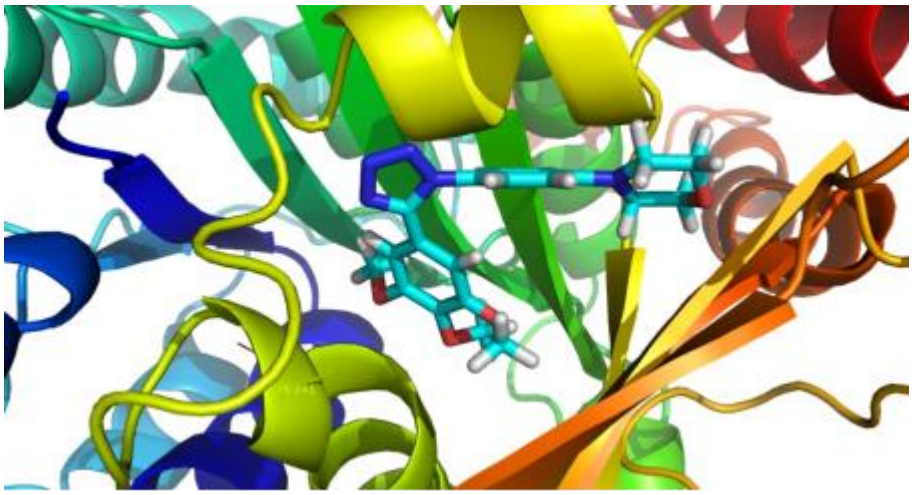


Figure 3. **10k** *In tubulin* binding site.

Conclusions

In conclusion, 3,4-difluoronitrobenzene was used as the starting material to generate tetrazole-morpholine hybrid derivatives. The crucial intermediate 4-(2-fluoro-4-(1*H*-tetrazol-1-yl)phenyl)morpholine (**5**) was developed, and it was then converted into two distinct tetrazole-morpholine linked hybrid series. To investigate the impact of various functional substitutions, including electron donating and withdrawing groups, a total of 31 compounds were synthesized. The anticancer activity of the synthesized compounds was evaluated in experiments using U-937 histiocytic lymphoma cells. Among all the drugs studied, **8h** and **10k** demonstrated the best interactions with tubulin when the mechanism of action was examined utilizing molecular docking against tubulin.

Experimental Section

General. Melting points were determined in open capillary tubes in sulphuric acid bath. FT-IR spectra were recorded on a VERTEX 70 Brucker by using KBr. A Bruker spectrometer 400 and 100 MHz was employed for recording ^{1}H NMR and ^{13}C NMR spectra respectively and $CDCl_3$ and DMSO-d_6 were used as solvent and TMS as an internal standard. Mass spectra were recorded on Agilent-LCMS instrument.

Procedure for synthesis of 4-(2-fluoro-4-nitrophenyl)morpholine (3). To a stirred solution of 3,4-difluoronitrobenzene **1** (40 g, 251.4 mmol) in DMSO (400 mL) was added K_2CO_3 (44.4 g, 321.6 mmol) and morpholine **2** (24 g, 276.6 mmol) and heated to 80 °C for 3 h. The reaction mixture was cooled to room temperature and diluted with water (400 mL) and extracted with ethyl acetate (3x400 mL). The organic layer was separated and washed with brine solution (300 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 4-(2-fluoro-4-nitrophenyl)morpholine **3** (48 g, Yield: 84%) as off white solid; mp: 112-114 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 8.01-7.93(m, 1H), 7.92 (d, *J* 2.8 Hz, 1H), 6.92 (t, *J* 8.8 Hz, 1H), 4.01 (t, *J* 4.8 Hz, 4H), 3.20 (t, *J* 4.8 Hz, 4H) ppm.^{13}C NMR (100 MHz, $CDCl_3$) δ= 154.3, 151.8, 145.4, 120.9, 116.8, 112.6, 66.5 (2C), 49.8 (2C) ppm. IR (KBr): 3432, 2925, 1739, 1604, 1242, 1050 cm^{-1}. HRMS (ESI): calcd for $C_{10}H_{12}N_2O_3F$ $(M+H)^+$: 227.0832 found 227.0838.

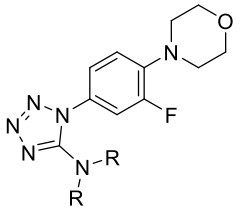
Procedure for synthesis of 3-fluoro-4-morpholinoaniline (4). To a stirred solution of 4-(2-fluoro-4-nitrophenyl)morpholine **3** (40 g, 177 mmol) in ethanol (360 mL) and water (40 mL) was added iron powder (94.16 g, 1681.47 mmol) and ammonium chloride (4.74 g, 88.48 mmol) and heated to 90 °C for 12 hours. The reaction mixture was cooled to room temperature and filtered through celite bed and washed with ethyl acetate, the organic layer was washed with water (400 mL) followed by brine (400 mL) solution. The organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo* to afford 3-fluoro-4-morpholinoaniline **4** as a pale brown solid (29.88g, Yield: 86%); mp: 125-127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 6.81 (t, *J* 8.4 Hz, 1H), 6.45-6.39 (m, 2H), 3.85 (t, *J* 4.8 Hz, 4H), 3.54 (brs, 2H), 2.96 (t, *J* 4.8 Hz, 4H) ppm.^{13}C NMR (100 MHz, DMSO) δ = 157.47, 145.52, 129.03, 120.49, 109.52, 66.38, 51.60, 66.05, 50.20 ppm. HRMS (ESI): calcd for $C_{10}H_{14}N_2OF$ $(M+H)^+$: 197.1090 found 197.1096.

Procedure for synthesis of 4-(2-fluoro-4-(1*H*-tetrazol-1-yl)phenyl)morpholine (5). To a stirred solution of 3-fluoro-4-morpholinoaniline **4** (20 g, 102.04 mmol) in acetic acid (100 mL) was added triethylorthoformate (24 g, 163.26 mmol) and NaN_3 (9.8 g, 153.06 mmol) and heated to 100 °C for 3 hours. The reaction mixture was cooled to room temperature and diluted with water (200 mL) and extracted with ethyl acetate (3x200 mL). The organic layer was washed with water(200 mL) followed by brine solution (150 mL), separated and dried

over Na_2SO_4 filtered and concentrated *in* vacuo to afford 4-(2-fluoro-4-(1*H*-tetrazol-1-yl)phenyl)morpholine **5** as off white solid (22.2 g, Yield: 87%); mp: 161-163 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 8.90 (s, 1H), 7.47-7.39 (m, 2H), 7.07 (m, 1H), 3.90 (t, *J* 6.4 Hz, 4H), 3.17 (t, *J* 6.4 Hz, 4H) ppm.^{13}C NMR (100 MHz, $CDCl_3$) δ= 155.4, 142.1, 140.6, 127.5, 119.7, 117.6, 110.0, 65.9,50.1 ppm. HRMS (ESI): calcd for $C_{11}H_{13}N_5OF$ $(M+H)^+$: 250.1104 found 250.1105.

Procedure for synthesis of 1-(3-fluoro-4-morpholinophenyl)-1*H*-tetrazol-5-amine (6). A stirred mixture of 4-(2-fluoro-4-(1*H*-tetrazol-1-yl)phenyl)morpholine **5** (16 g, 64.24 mmol), NaN_3 (6.2 g, 96.38 mmol), NaOH (3.8 g, 96.38 mmol) and Et_3N (12.8 g, 128.5 mmol) in *i*-PrOH (30 mL) was treated with DMSO (70 mL). The reaction mixture was stirred at room temperature until the gas evolution ceased (2 hours) and then was treated with glacial AcOH (11.4 g, 193.2 mmol). The resulting suspension was stirred at 90 °C for 2 hours. Cooled and diluted with water (200 mL). The precipitate was separated by filtration, washed with water and dried *in vacuo* at 50 °C to afford 1-(3-fluoro-4-morpholinophenyl)-1*H*-tetrazol-5-amine **6** as a white solid (14.0 g, Yield: 83%); mp: 207-209 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.27-7.24 (m, 2H), 7.07 (t, *J* 8.8 Hz, 1H), 4.80 (brs, 2H), 3.89 (t, *J* 4.8 Hz, 4H), 3.17 (t, *J* 4.8 Hz, 4H) ppm.^{13}C NMR (100 MHz, $CDCl_3$) δ = 157.4, 155.0, 145.4, 129.0, 120.4, 109.5, 66.3 (2H), 51.5 (2H) ppm. IR (KBr): 3340, 3154, 2836, 1664, 1522, 1233, 1120cm^{-1}. HRMS (ESI): calcd for $C_{11}H_{14}N_6OF$ $(M+H)^+$: 265.1213 found 265.1218.

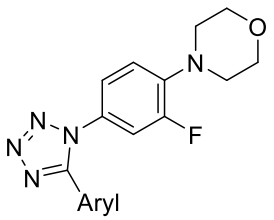
General procedure for compounds 8a-8j (diacetylationtetrazole compounds)



1-(3-Fluoro-4-morpholinophenyl)-1*H*-tetrazol-5-amine **6** (150 mg, 0.56 mmol) was dissolved in THF (10 mL), cooled to 0 °C and added DIPEA (20 mg, 1.7 mmol) followed by acid chloride (**7a-7j**) (3 equiv., 1.7 mmol). The reaction mixture was stirred at room temperature for 12 h and quenched with water and extracted with ethyl acetate (3x10 mL). The combined organic layer was washed with brine solution (10 mL) and dried over Na_2SO_4 and concentration *in vacuo* to afford respective amide derivatives **8a-8j.** The analytical data of all compounds are given in the supplementary material.

General procedure for compound 8k-8r (Mono acetylation tetrazole compounds). To a solution of 1-(3-fluoro-4-morpholinophenyl)-1*H*-tetrazol-5-amine **6** (150 mg, 0.56 mmol) was dissolved in THF (10 mL), cooled to 0 °C and added DIPEA (20 mg, 1.7 mmol) followed by acid chloride (**7k-7r**) (1.1 equiv., 0.616 mmol). The reaction mixture was stirred at room temperature for 2-6 hr and quenched with water and extracted with ethyl acetate (3x10 mL). The combined organic layer was washed with brine solution (10 mL) and dried over Na_2SO_4 and concentration *in vacuo* to afford respective amide derivatives **8k-8r.** The analytical data of all compounds are given in the supplementary material.

General procedure for compounds 10a-10m (Aryl tetrazole compounds)



A suspension of tetrazole **5** (300 mg, 1.00 mmol), aryl iodide/Iodo benzene **9a-9m**(1.00 mmol), cesium carbonate (1.10 mmol), copper(I) iodide (1.00 mmol), palladium(II) acetate $Pd(OAc)_2$ (0.05 mmol), and tris(2-furyl)-phosphine (0.10 mmol) in dry acetonitrile (6 mL) was heated at 40 °C under argon atmosphere for 4 h. The resulting mixture was diluted with ethyl acetate (40 mL) and filtered quickly through celite, and then the solvents were removed under reduced pressure. The residue was purified by column chromatography in hexane and ethyl acetate to afford tetrazole derivatives (**10a-10m**) (392 mg, 87%). The analytical data of all compounds are given in the supplementary material.

Study of anticancer activity

Cytotoxicity assay. To reveal anticancer activities of compounds under *in vitro* conditions, MTT- cell proliferation assay was performed[30]. U-937 cells (5 X 10^3 /well) seeded in 96-well plate were incubated at 37 ^oC with 5% CO_2 for 45 min prior to synthesised compound treatment. Then, cells were treated with compound for 48 hours in triplicates and cells treated with 10 µM etoposide were taken as positive control for the assay. Following the incubation, 10 µl of MTT dye [(3-(4,5-Dimethylthiazol-2-yl) −2,5-diphenyltetrazolium bromide), stock 4mg/ml in PBS] was added and cells were incubated at 37 ^OC with 5% CO_2 for 3 hours in dark. Then, MTT lysis buffer containing 20% SDS and 50% DMF in 1:1 ratio was added to each well and kept overnight on orbital shaker at room temperature in dark. The following day, the absorbance of each well was recorded at 595 nm in BIO-RAD iMark Microplate Reader and data was visualized using Microplate Analyst software. The average cell death was calculated and standard deviation was shown as error bar. The experiments were performed in triplicates.

Statistical analysis. For statistical analysis, a two-tailed t test (paired) was performed. Between the treated and untreated sets of experiments. A p value of < 0.05 is reflected to considered slightly significant and specified with an asterisk (*), a p value < 0.01 is significant witha double asterisk (**), and a p value < 0.001 is considered as more significant and marked with triple asterisk (***).

Methods. Docking study

The X-ray crystal structure 1SA0 of the tubulin was retrieved from the protein structural database protein data bank (PDB). Derivatives of compound 1 and 2 were sketched in marvinsketch. Molecular docking was performed between tubulin and compound 1 and 2 derivatives to validate the binding efficiency based on the molecular docking scores. The synthesized compounds were docked on to the tubulin protein active site with Molegro Virtual Docker 6.0 tool. The known tubulin inhibitor Colchicine was used as reference molecule for docking score comparison. Active site coordinates 116.99, 90.54, and 5.65 with 15 Å radius was considered for docking. Docked ligand poses in active site were analyzed in Pymol protein visualization tool.

Acknowledgements

The authors are thankful to Acharya Nagarjuna University, AP, India for constant support and encouragement. The author **5** is also sincerely thankful to INTI International University, Nilai, Malaysia for the award of Research Fellow.

Supplementary Material

Spectra of compounds **3, 4, 5, 6, 8a-8r, 10a-m**

References

1. Ostrovskii, V. A.; Koldobskii, G. I.; Trifonov, R. E. In *Comprehensive Heterocyclic Chemistry III*, vol. 6; Katritzky, A. R.; Ramsden, C. A.; Scriven, E. F. V.; Taylor, R. J. K., eds.; Elsevier: Oxford, 2008, p. 257. https://doi.org/10.1016/B978-008044992-0.00517-4
2. Jursic, B. S.; Leblanc, B. W. *J. Heterocycl. Chem.* **1998**, *35*, 405 and references cited therein https://doi.org/10.1002/jhet.5570350224
3. Mukhopadhyay, S.; Lasri, J.; Guedes da Silva, M. F. C.; Januário Charmier, M. A.; Pombeiro, A. J. L. *Polyhedron* **2008**, *27*, 2883. https://doi.org/10.1016/j.poly.2008.06.031 .
4. Kharaghiosoff, K.; Klapotke, T. M.; Mayer, P.; Piotrowski, H.; Polborn, K.; Willer, R. L.; Weigand, J. J. *J. Org. Chem.* **2006**, 71, 1295. https://doi.org/10.1021/jo0513820
5. Li, J.; Ren, T.; Liu, H.; Wang, D.; Liu, W. *Wear* **2000**, *246*, 130. https://doi.org/10.1016/S0043-1648(00)00500-7
6. Meanwell, N. A. *J. Med. Chem.* **2011**, *54*, 2529. https://doi.org/10.1021/jm1013693
7. Marvi, O.; Alizadeh, A.; Zarrabi, S. *Bull. Korean Chem. Soc.* **2011**, *32*, 4001. https://doi.org/10.5012/bkcs.2011.32.11.4001
8. Alonen, A.; Finel, M.; Kostiainen, R. *Biochem. Pharmacol.* **2008**, *76*, 763. https://doi.org/10.1016/j.bcp.2008.07.006
9. Alonen, A.; Jansson, J.; Kallonen, S.; Kiriazis, A.; Aitio, O.; Finel, M.; Kostiainen, R. *Bioorg. Chem.* **2008**, *36*, 148. https://doi.org/10.1016/j.bioorg.2008.02.004
10. Aureggi, V.; Sedelmeier, G. *Angew. Chem., Int. Ed.* **2007**, *46*, 8440. https://doi.org/10.1002/anie.200701045
11. Narasaiah, T.; SubbaRao, D.; Rasheed, S.; Madhava, G.; Srinivasulu, D.; Brahma, P.; Naga, C. *Der Pharm. Lett.* **2012**, *4*, 854.
12. Lusina, M.; Cindrić, T.; Tamaić, J.; Peko, M.; Pozaić, L.; Musulin, N. *Int. J. Pharm.* **2005**, *291*, 127. https://doi.org/10.1016/j.ijpharm.2004.07.050
13. Upadhayaya, R. S.; Jain, S.; Sinha, N.; Kishore, N.; Chandra, R.; Arora, S. K. *Eur. J. Med. Chem.* **2004**, *39*, 579. https://doi.org/10.1016/j.ejmech.2004.03.004
14. Rajasekaran, A.; Sankaranarayanan, M.; Rajagopal, K. A. *Arch. Pharm. Res.* **2006**, *29*, 535. https://doi.org/10.1007/BF02969261
15. De Souza, A. O.; Pedrosa, M. T.; Alderete, J. B.; Cruz, A. F.; Prado, M. A.; Alves, R. B.; Silva, C. L. *Pharmazie* **2005**, *60*, 396.
16. Mohite, P. B.; Pandhare, R. B.; Khanage, S. G.; Bhaskar, V. H. *J. Pharm. Res.* **2010**, *3*, 43.
17. Adamec, J.; Waisser, K.; Kunes, J.; Kaustova, J. *Arch. Pharm.* **2005**, *338*, 385. https://doi.org/10.1002/ardp.200400967
18. Peet, N. P.; Baugh, L. E.; Sunder, S.; Lewis, J. E.; Matthews, E. H.; Olberding, E. L.; Shah, D. *J. Med. Chem.* **1986**, *29*, 2403. https://doi.org/10.1021/jm00161a045
19. Akimoto, H.; Ootsu, K.; Itoh, F.; *Eur. Patent EP 530,537* **1993** *(CA 119:226417)*.

20. Vieira, E.; Huwyler, S.; Jolidon, S.; Knoflach, F.; Mutel, V.; Wichmann, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4628. https://doi.org/10.1016/j.bmcl.2005.05.135
21. Gagnon, A.; Landry, S.; Coulombe, R.; Jakalian, A.; Guse, I.; Thavonekham, B.; Bonneau, P. R.; Yoakim, C.; Simoneau, B. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1199. https://doi.org/10.1016/j.bmcl.2008.12.074
22. Ohui, K.; Afanasenko, E.; Bacher, F.; Ting, R. L. X.; Zafar, A.; Balnco-cabra, N.; Torrents, E.; Domotor, O.; May, N. V.; Darvasiova, D.; Enyedy, E. A.; Popovic-Bijelic, A.; Reynisson, J.; Rapta, P.; Babak, M. V.; Pastorin, G.; Arion, V. B. *J. Med. Chem.* **2019***, 62***,** 512. https://doi.org/10.1021/acs.jmedchem.8b01031
23. Arias, H. R.; Fedorov, N. B.; Benson, L. C.; Lippiello, P. M.; Gatto, G. J.; Feuerbach, D.; Ortells, M. O. *J. Pharmacol. Exp. Ther.* **2013**, *344*, 113. https://doi.org/10.1124/jpet.112.197905
24. Mayersohn, M.; Guentert, T. W. *Clin. Pharmacokinet.* **1995**, *29*, 292-332. https://doi.org/10.2165/00003088-199529050-00002
25. Perzborn, E.; Roehrig, S.; Straub, A.; Kubitza, D. Misselwitz, F. *Nat. Rev. Drug. Discov*. **2011**, *10*, 61-75. https://doi.org/10.1038/nrd3185
26. Angeliki, P. K. ; Dimitrios, X.; Ariadni, T., *Med. Res. Rev.,* **2020**, *40(2)*, 709-752. https://onlinelibrary.wiley.com/doi/10.1002/med.21634
27. Spulak, M.; Lubojacky, R.; Senel, P.; Kunes, J.; Pour, M. *J. Org. Chem.,* **2010**, *75*, 241-244. https://doi.org/10.1021/jo902180u
28. Harvill, E. K.; Herbst, R. M.; Schreiner, E. C.; Roberts, C. W. *J. Org. Chem*., **1950**, *15*, 662-670. https://doi.org/10.1021/jo01149a035
29. Popova, E. A.; Protas, A. V.; Trifonov, R. E. *Med. Chem.* **2018**, *17(14)*, 1856-1868. https://doi.org/10.2174/1871520617666170327143148
30. Ganot, N.; Meker, S.; Reytman, L.; Tzubery, A.; Tshuva, E. Y. *J. Vis. Exp.* **2013**, *81*, e50767. https://doi.org/10.3791/50767