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SYNTHESIS, CHARACTERIZATION AND ANTI-CANCER STUDIES OF 6-PHENYL-3-(2,2,6,6-TETRAMETHYLPIPERIDIN-4-YL)-3*H*-[1,2,3]TRIAZOLO[4,5-C]PYRIDAZINE DERIVATIVES

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ABSTRACT

With a remarkable selectivity across a panel of 208 kinases, N-linked triazolopyridazine derivatives exhibit nanomolar suppression of c-Met kinase activity and anti-proliferative effects, so based on the biological importance of these compounds, we described the synthesis of certain tetramethyl-piperidine-trazolopyridazine from trichloropyridazine and sodium benzenesulfinate in six steps with moderate to good yields and evaluated its anti-cancer activity. All the compounds showed good inhibitory activity against HepG2 cell lines. We synthesized all the intermediates and final compounds on a gram scale, making this process very practical for bulk-scale manufacture of tetramethyl piperidine trazolopyridazine derivatives for better therapeutic screening. All the intermediates and final compounds are characterized by using 1H NMR, 13C NMR, LC-MS & HRMS analysis.

Keywords: Trichloro Pyridazine, C-Met Kinases, HepG2 Cells, Anti-Cancer Studies.

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INTRODUCTION

Triazoles stood out among nitrogen-containing heterocyclic compounds for their exceptional pharmacological uses. Because of their structural properties,1,2,4-triazoles and 1,2,3-triazoles can accommodate a wide variety of substituents on their core structures, paving the way for the development of new bioactive compounds. Triazoles and their derivatives have substantial biological effects, including antitubercular, antibacterial, anti-inflammatory, antiviral, analgesic, anticancer, and antidepressant action.[1] The discovery of azole motifs in 1944 led to the development of triazole-containing antifungal medications, such as itraconazole, fluconazole, voriconazole, efinaconazole, andposaconazole, etc[2-3] are already in the market. Pyridazines are rare in nature due to a paucity of hydrazines. As a result, scientists have produced a huge number of heterocyclic compounds containing the pyridazine moiety. Some of these pyridazine compounds are utilized as pharmaceuticals, such as cefozopran, a fourth-generation cephalosporin that functions as an antibiotic.[4] Furthermore, pyridazine compounds have been demonstrated to have a variety of biological and pharmacological properties, including analgesic action, cardiovascular treatment, anticonvulsant, antiepileptic, antibacterial, antihypertensive, antitubercular, anti-secretory, anti-ulcer, and antihistamine properties.[4] Triazolopyridazines are the derivatives of triazole and pyridazine; hence, these molecules will exhibit distinct biological actions.[5] The imidazo pyridazines are the most selective positive allosteric modulators (PAMs) of GABAa[6-7], and triazolo pyridazines are majorly inhibitors of c-met kinases.[8-9] The compounds of 1,2,4-triazolo-pyridazine are used as an anticonvulsant and sedative because they decrease blood pressure without changing heart rate.[7,10-11] The triazolopyridazine derivative demonstrated notable selectivity across a panel of 208 kinases, exhibiting strong antiproliferative activity against A549 cells (Ki = 0.006 mM) and potent inhibition of c-Met kinase (Ki = 0.0103 mM; IC_{50} = 10 nM).[9,12-13] It was found that *N*-linked triazolopyridazine analogues have improved pharmacokinetic profiles and nanomolar inhibitory activity in contradiction to c-Met kinase.[14] Numerous c-Met kinase inhibitors have been previously identified, and a few of them are currently in

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phase 4 clinical studies. Amongst c-Met inhibitors, triazole-based bicyclic compounds had superior c-Met inhibitory activity and exceptional selectivity against other kinases.[15-16] The substitution of the bicyclic triazolopyridazine framework with a methylisoquinolinone scaffold resulted in a notable enhancement of c-Met kinase inhibition, with an IC_{50} value of 0.003 mM, indicating significant interactions with the deposits in the c-Met hinge region, and Triazolopyridazines with a methoxy functional group will operate as PDE-4 inhibitors.[17-18] Some researchers reported the synthesis of triazolopyridazine derivatives using various starting materials. Trudell *et. al* synthesized from an alkyne derivative and diamino pyridazine[19], Lee *et al*., prepared from 2-bromo phenyl acetic acid[20], Skoumbourdis *et al*., from di methoxy benzoic acid[18], Boezio *et.al* from the coupling of Boc-glycine and 3-chloro-6-hydrazinopyridazine[14], EM Ahmed *et al.,* from dichloropyridazine.[8] Based on the above interesting factors, we prepared the tetramethyl-piperidine-trazolopyridazine derivatives from trichloropyridazine and sodium benzenesulfinate in 6 steps with good yields.

EXPERIMENTAL

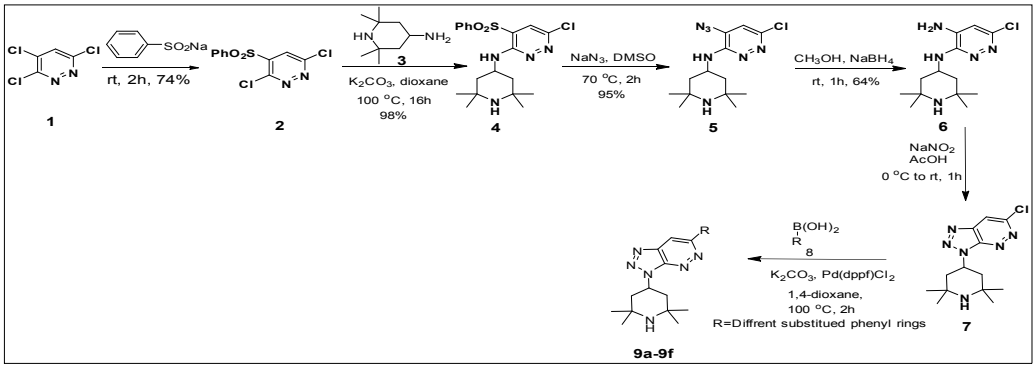
Standard Operating Procedure for Compounds 9a–9f Synthesis

Compound 7 (1.0 eq), phenylboronic acid (1.2 eq), Cs_2CO_3 (2.5 eq), water (4 v), and 1,4-dioxane (10 v) were introduced into a 100 mL round-bottom flask under an argon atmosphere and stirred for 5 minutes. Subsequently, $Pd(dppf)_2Cl_2$•DCM (0.15 eq) was mixed with the reaction mass at 100°C, continue the reaction was continued for 16 h, which was continuously monitored by TLC. After the reaction concluded, we introduced ice water and carried out the extraction using ethyl acetate. After removing the excess solvent (ethyl acetate) under vacuum, the resulting filtrate was purified by column chromatography on silica gel using a methanol/dichloromethane ($MeOH/CH_2Cl_2$) gradient ranging from 0% to 15% methanol. As a result, the derivatives 9a–9f showed up as brownish solids.

RESULTS AND DISCUSSION

Chemistry

Scheme-1 illustrates the synthetic method employed to synthesize the tetramethyl-piperidine-trazolopyridazine derivatives. We prepared Key Intermediate 7 according to our established procedure[21]. In conclusion, we synthesize compound 2 by treating trichloropyridazine **1** with sodium benzenesulfinate. Subsequently, we react the resulting compound **2** with tetramethylpiperidine **3** in the presence of a base, yielding substituted phenylsulfonyl tetramethylpiperidin-pyridazin-3-amine **4**. The resulting compound **4** underwent a reaction with NaN_3, resulting in the formation of the azide-substituted compound **5**. The azide compound **5** underwent reduction with $NaBH_4$, resulting in the formation of compound **6**. The resulting compound 6 was treated with $NaNO_2$ in acetic acid (CH_3COOH) to produce the triazole-fused pyridazine derivative, referred to as compound 7. This intermediate was then reacted with a series of boronic acids to obtain the triazolopyridazine derivatives 9a–9f, with overall yields ranging from 40% to 60%.



Scheme-1: Synthesis of Triazolopyridazine Derivatives 9a-9f.

Anti-Cancer Studies

By employing the MTT assay to screen for inhibitory activity against HepG2 cell lines at various concentrations (5, 10, 25, 50, and 100µg), all of the pyridazine derivatives demonstrated superior

inhibition action (IC_{50} values are displayed in Table-1). We conducted the activity studies in comparison with the standard drug, cisplatin.

Table-1: IC_{50} Values of Screened Compounds

S. No.	Sample Name	IC_{50} (µg/ml)
		$HepG_2$
1	9a	69.12
2	9b	50.42
3	9c	53.84
4	9d	39.17
5	9e	48.39
6	9f	45.53
7	Cisplatin (µM)	14.31

Chemicals

All glassware underwent oven drying, was assembled while warm, and was subsequently air-conditioned under a flow of dry N_2 to maintain an inert environment. Reactions with air-sensitive reagents were accomplished using established techniques appropriate for managing these materials. Solvents and reagents were obtained from local distributors, including Sigma-Aldrich, Alfa Aesar, and Tokyo Chemical Industry (TCI). Proton (^1H) nuclear magnetic resonance spectra were recorded at 400 MHz using DMSO-d_6 or $CDCl_3$ as solvents at room temperature. Chemical shifts are expressed in parts per million (ppm), referenced to Tetramethylsilane (TMS) set at $\delta = 0$ ppm. Carbon-13 NMR (^{13}C NMR) spectra of the synthesized compounds were collected at 100 MHz with DMSO-d_6 as the solvent. Merck precoated silica gel 60 F254 plates were used for Thin Layer Chromatography (TLC), and Merck silica gel (100–200 mesh) was used for column chromatography at atmospheric pressure.

MTT Assay

Dulbecco's Modified Eagle Medium (DMEM), trypsin, phosphate-buffered saline (PBS), EDTA, and the MTT reagent were obtained from Sigma-Aldrich Chemicals. Fetal Bovine Serum (FBS) was procured from Gibco, and the cell culture flasks (25 cm^2 and 75 cm^2), along with 96-well plates, were sourced from Eppendorf India. Cell viability studies were assessed with the help of the MTT assay. Each compound test was evaluated at five different concentrations, with experiments performed in triplicate. Cells were initially trypsinized, and viability studies were determined by means of the trypan blue exclusion assay. Cells were counted using a hemocytometer, and 5×10^3 viable cells were seeded into each well of a 96-well plate comprising 100 µL of culture medium. The plates were then incubated overnight at 37 °C. Following the incubation period, the medium was taken out and replaced with 100 µL of brand-new media that contained the test chemicals at the designated concentrations. Following 48 hours of exposure, this medium was discarded and replaced with new medium supplemented with MTT at a final concentration of 0.5 mg/mL. The plates were then incubated for an additional 3 h at 37°C to enable viable cells to convert MTT into formazan crystals. Subsequently, the formazan crystals produced by metabolically active mitochondria were dissolved in solvent, i.e., DMSO. A microplate reader was used to measure absorbance at 570 nm, and the percentage of cell growth inhibition was then calculated.

$$\text{Percentage Inhibition} = [100(\text{Control-Treatment})]/\ \text{Control}$$

To determine the IC_{50} value, a linear regression analysis was conducted using the equation $y = mx + c$, where y is fixed at 50, and m and c were derived from the plotted cell viability data.

CONCLUSION

In summary, we synthesized the tetramethyl-piperidine-trazolopyridazine derivatives on a gram scale using trichloropyridazine and sodium benzenesulfinate in six steps. All the pyridazine derivatives were screened for their anti-cancer activity; all the compounds showed significant inhibition against HepG2

cells. All derivatives and intermediates are synthesized on a gram scale, making it very feasible for the industrial production of tetramethyl piperidine trazolopyridazine derivatives.

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CONFLICT OF INTERESTS

The authors attest that they have no disclosed conflicts of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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