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Research Article

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Synthesis and antiviral activity of 3-substituted phenyl-6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazin

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Abstract A series of 3-substituted phenyl -6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine has been synthesized. An appropriate aromatic hydrocarbon reacts with succinic anhydride in presence of $AlCl_3$ to yield β-aroyl propionic acid. The corresponding acid was cyclised with hydrazine hydrate to give 6-(substituted aryl)-2, 3, 4, 5-tetrahydro-3-pyridazinone, which was heated on steam bath with phosphorous oxy chloride to yield 3-chloro 6-substituted phenyl pyradazine. This intermediate after reaction with hydrazine hydrate was converted into 3-hydrazino-6-substituted phenyl pyradazine. The resulting product was converted into3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine by reacting with substituted aroyl chloride. Spectral data (IR, NMR, mass spectra) confirmed the structures of the synthesized compounds. The synthesized compounds were investigated for their antiviral activity. The results indicated that the synthesized compounds have mild to potent activity with reference to their appropriate reference standards.

Keywords Pyridazin**,** Triazole, antiviral

Introduction

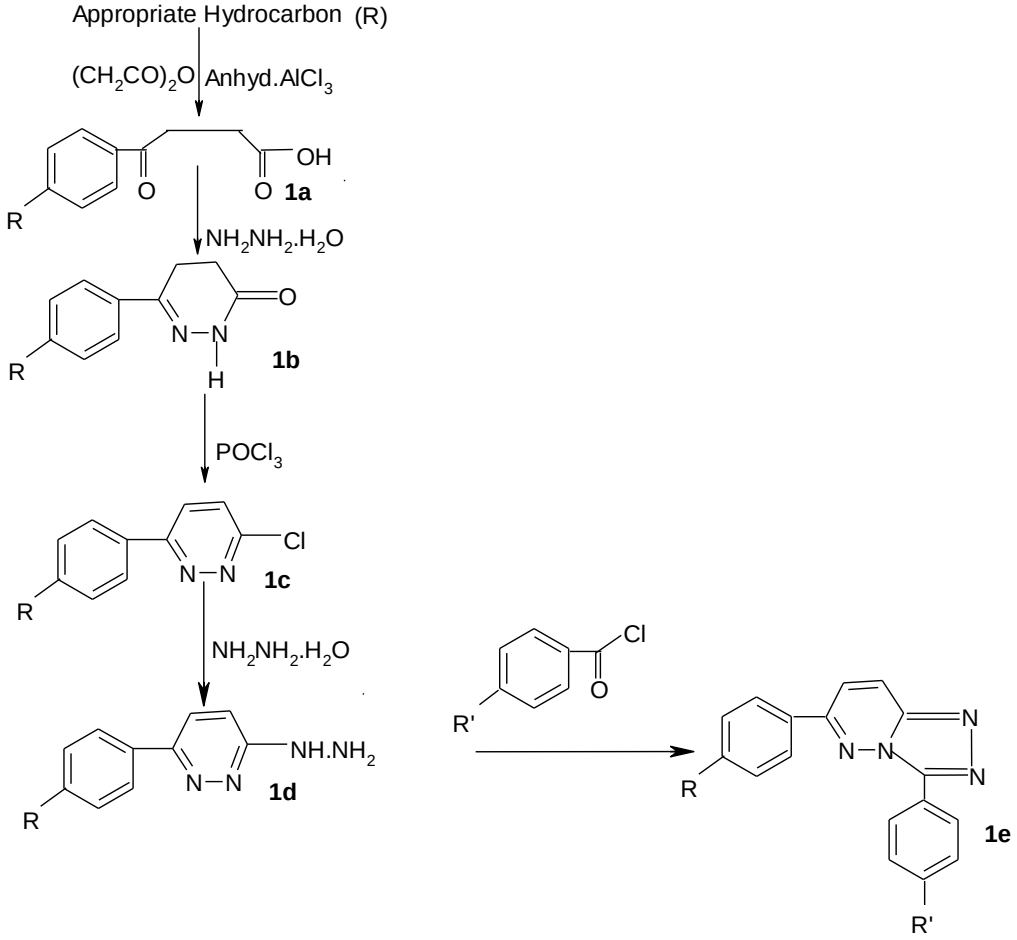
Triazole and it's derivatives are noteworthy for their physiological and biological importance. They paved the attention of medicinal chemist due to their wide range of biological activities like anti-inflammatory (1-5), antibacterial (6-8), anticonvulsant (9), antifungal (10-14) anticancer (15) and antiviral (20-21) etc. In view of above facts and inspired by the research going on triazole and its derivatives, particularly in relation to microbial infections, 3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine has been synthesized. The final compounds was synthesized as per reaction sequence is outlined in Scheme-I. Friedal-crafts acylation of appropriate hydrocarbons with succinic anhydride, in presence of anhydrous $AlCl_3$ yield β-aroyl propionic acid followed by hydrazinolysis with hydrazine hydrate to get substituted pyridazinones, which reacted with phosphorous oxy chloride to give 3-chloro 6-substituted phenyl pyradazine. This intermediate after reaction with hydrazine hydrate was converted into 3-hydrazino-6-substituted phenyl pyradazine. This product was reacted with substituted aroyl chloride to yield 3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine. All the final compounds was structurally elucidated on the basis of NMR, IR and mass spectral data. The final synthesized compounds were evaluated for antiviral activity. The physiochemical data of the final synthesized compounds are mentioned in Table-1.

Experimental Work

The melting points were determined on a X-4 microscope melting point apparatus and are uncorrected. The NMR spectra were recorded in $CDCl_3$ as solvent (using TMS as an internal standard). The NMR and mass spectra were

recorded on Jeol FX-100FT-NMR and Jeol BX 102/DA-6000 mass spectrometer respectively. The infrared spectra in KBr were recorded, on Buck Scientific M-500 Infrared Spectrophotometer. Solvent system used throughout the experimental work for running TLC plates Toluene, Ethyl formate, and Formic acid in the ratio of 5:4:1.

The triazole derivatives was synthesized as per Scheme-I. The overall reaction involves five steps for synthesis and characterization of compound It is illustrated with the synthesis of compound **1e.**



Scheme I: Synthesis of 3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine

Synthesis of β-Benzoyl propionic acid (1a)

After suspending anhydrous aluminum chloride (0.15 mol) in dry benzene (50ml) under anhydrous conditions, the contents was refluxed on a water bath. Succinic anhydride (0.10 mol) was then added to the reaction mixture in small portions with continuous stirring. Stirring and heating was continued for 6 h and the contents after leaving overnight at room temperature, ice cold solution of concentrated hydrochloric acid (2.5% v/v) was then added to the reaction mixture and the contents was concentrated to a small volume by heating on a water bath. The solid compound which separated out, was filtered. It was purified by dissolving in 5% w/v sodium bicarbonate solution, followed by extraction with ether. The aqueous layer on acidification with dilute hydrochloric acid gave benzoyl propionic acid, crystallized from aqueous ethanol to give a colourless compound.m.p.125^{o}C; R_f 0.25; %yield 73; ^{1}H-NMR(δ) 2.59 (t, 2H, CH_2), 3.23 (t, 2H, CH_2), 7.53-7.62 (m, 3H, H-3'-H-5'), 7.97 (d, 2H, H-2', H-6'), 12.17 (s, 1H, COOH).

All the remaining acids were synthesized by analogous procedure with minor modification in temperature of reaction and use of nitrobenzene as solvent.

Synthesis of 6-Phenyl 2,3,4,5-Tetrahydro pyridazin-3-one (1b)

To a solution of β-benzoyl propionic acid **(1a)** (0.1 mol) in methanol (30ml), hydrazine hydrate (1ml) and sodium acetate (0.5g) was added and the contents refluxed for 6 h. After completion of the reaction, methanol was distilled off and the content was poured into cold water. The solid that separated out, was filtered and crystallized from methanol, m.p.250 ^{o}C; R_f 0.45; % yield 72; IR (cm^{-1}) 3306(NH), 1678(C=O); ^{1}H-NMR (δ) 2.45 (t, 2H, CH_2), 2.93 (t, 2H, CH_2), 7.41(m, 3H, H-3'-H-5'), 7.74 (d, 2H, H-2', H-6'), 10.94 (s, 1H, CONH); Ms (m/z) 174,159, 147, 130, 115, 109.

Synthesis of 3-chloro 6- phenyl pyradazine (1c)

A mixture of 6-Phenyl 2,3,4,5-Tetrahydropyradazin-3-one (0.01 mol) and phosphorous oxy chloride ($POCl_3$) was heated on a steam bath for 6 h. After heating, the mixture was carefully poured on crushed ice. The water was rendered alkaline by sodium bicarbonate. Crude 3-chloro pyradazine was collected by filtration. m.p.135^{o}C, R_f 0.70, % yield 76; IR(cm^{-1}) 1630(C=N), 1590(C=C), 940, 702 (C-Cl); ^{1}H-NMR(δ) 7.42−7.51 ((m,7H,Ar-H).

Synthesis of 3-hydrazino-6- phenyl pyradazine (1d)

To an ethanolic solution of 3-chloro pyradazine (0.01 mol) in hydrazine hydrate (99 %) was added. The resulting reaction mixture was refluxed on steam bath for 16 h. The contents were concentrated, cooled and poured into crushed ice. The resulting solid which separated out is filtered, washed with water, dried and recrystallized from alcohol. m.p. 168 ^{o}C, R_f 0.75, %yield 79; IR (cm^{-1}) 3440(NH), 961(CH); 1638(C=N), 1582 (C=C), 938, 680; ^{1}H-NMR (δ) 2.5 (s, 2H, NH_2), 7.17-8.05 (m, 7H, Ar-H), 8.15 (m, 1H, Ar-NH);

Synthesis of 3,6-di-phenyl (1,2,4) triazolo (4,3-b) pyridazine (1e)

Compound 1d dissolve in xylene (20 ml) were added triethylamine (0.67 gm, 6.7 mol) and benzoyl chloride (0.58 gm, 4.4 m mol). The mixture was stirred at room temp. for 1 hr. and then heated at reflux for 16 hrs. The solution was cooled to room temp., the solvent evaporated under reduced pressure and the residue partitioned between CH_2Cl_2 (150 ml) and water (30 ml). The aqueous phase was separated and extracted further with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and evaporated and the residue was chromatographed on silica gel, eluted with ethyl acetoacetate to get the final compound, recrystallised with alcohol; m.p.219 ^{o}C, R_f 0..76, % yield 95. IR (cm^{-1}) 3004 (CH), 1686 (C=C), 1291, 907 , 931, 805, ^{1}H-NMR (δ) 7.22-8.45 (m, 12H, Ar-H); Ms (m/z) 272 (M^{+}), 257, 242, 230, 212, 168,104.

The remaining compounds was synthesized with analogous procedure and their m.p., %ge yield and molecular formula are mentioned in table 1.

Synthesis of Synthesis of 3-(4'-tolyl)-6-phenyl (1,2,4) triazolo (4,3-b) pyridazine (1f)

IR (cm^{-1}) 3002(CH), 1682(C=C), 1632(C=N), 980, 932, 810; ^{1}H-NMR (δ) 3.26(t, 3H, CH_3), 7.27-8.38 (m, 11H, Ar-H);

Synthesis of 3-phenyl 6-(4'-tolyl) (1,2,4) triazolo (4,3-b) pyridazine (2a)

IR (cm^{-1}) 1023, 3066 (CH), 1686(C=C), 1451, 1422, 1023, 917; ^{1}H-NMR (δ) 2.34(s, 3H, CH_3), 7.19-8.16 (m, 6H, Ar-H); Ms (m/z) 288(M^{+}+2), 265, 233, 146, 115,88;

Synthesis of 3,6-di(4'-tolyl) (1,2,4) triazolo (4,3-b) pyridazine (2b)

IR (cm^{-1}) 2992(CH), 1631(C=N), 1602(C=C), 936, 798; ^{1}H-NMR (δ) 2.3(s, 6H, 2xCH_3), 6.98-8.01 (m, 10H, Ar-H);

Synthesis of 3-phenyl 6-(2',4'-dimethyl phenyl) (1,2,4) triazolo (4,3-b) pyridazine (3a)

IR (cm^{-1}) 2963(CH), 1687(C=C), 1492, 1290, 1023, 930; ^{1}H-NMR (δ) 2.5(s, 6H, 2xCH_3), 7.29-8.15 (m, 10H, Ar-H);

Synthesis of 3-(4'-tolyl)-6-(2',4'-dimethyl phenyl) (1,2,4) triazolo (4,3-b) pyridazine (3b)

IR (cm^{-1}) 2971(CH), 1674(C=C), 1283, 752; ^{1}H-NMR (δ) 2.4(s, 9H, 3xCH_3), 7.19 (m, 9H, Ar-H);
Ms (m/z) 314(M^{+}+2), 289, 273, 146, 115,106;

Synthesis of 3-phenyl 6-(4'-phenoxy phenyl) (1,2,4) triazolo (4,3-b) pyridazine (4a)

IR (cm^{-1}) 2962(CH), 1632(C=N), 1584(C=C), 932, 672; ^{1}H-NMR (δ) 7.24-8.14 (m, 16H, Ar-H);

Synthesis of 3-(4'-tolyl)-6-(4'-phenoxy phenyl) (1,2,4) triazolo (4,3-b) pyridazine (4b)

IR (cm^{-1}) 2965(CH), 1630(C=N), 1584(C=C), 930, 671; ^{1}H-NMR (δ) 2.38(s, 3H, CH_3), 6.97-8.01 (m, 18H, Ar-H);

Synthesis of 3-phenyl 6-(4'-chloro phenyl) (1,2,4) triazolo (4,3-b) pyridazine (5a)

IR (cm^{-1}) 2980(CH), 1636(C=N), 1608(C=C), 1220, 1173, 1036, 754; ^{1}H-NMR (δ) 7.16-8.33 (m, 11H, Ar-H);

Synthesis of 3-(4'-tolyl)-6-(4'-chloro phenyl) (1,2,4) triazolo (4,3-b) pyridazine (5b)

IR (cm^{-1}) 2916(CH), 1605(C=C), 1223, 1170, 1038, 749; ^{1}H-NMR (δ) 2.4(s, 3H, CH_3), 7.25-8.0 (dd, 10H, Ar-H); Ms (m/z) 321\320.5(M^+), 221, 211;

Synthesis of 3-phenyl 6-(4'-bromo phenyl) (1,2,4) triazolo (4,3-b) pyridazine (6a)

IR (cm^{-1}) 2989(CH), 1632(C=N), 1605(C=C),1180, 976, 758; ^{1}H-NMR (δ) 7.37-8.14 (m, 11H, Ar-H);
Ms (m/z) 352(M-1), 331, 287, 221,211, 167, 91;

Synthesis of 3-(4'-tolyl)-6-(4'-bromo phenyl) (1,2,4) triazolo (4,3-b) pyridazine (6b)

IR (cm^{-1}) 2987(CH), 1631(C=N), 1607(C=C), 1175, 968, 678; ^{1}H-NMR (δ) 3.2(s, 3H, CH_3), 7.32-8.09 (m, 10H, Ar-H);

Synthesis of 3-phenyl 6-(4'-bi phenyl) (1,2,4) triazolo (4,3-b) pyridazine (7a)

IR (cm^{-1}) 2985(CH), 1627(C=N), 1603(C=C), 1085, 958, 718; ^{1}H-NMR (δ) 7.23-8.13 (m, 16H, Ar-H);

Synthesis of 3-(4'-tolyl)-6-(4'-bi phenyl) (1,2,4) triazolo (4,3-b) pyridazine (7b)

IR (cm^{-1}) 2989(CH), 1631(C=N), 1609(C=C); ^{1}H-NMR (δ) 2.38(s, 3H, CH_3), 6.99-8.01 (m, 15 H, Ar-H);

Table 1: Physicochemical data of 3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine

Compound	R	R'	M.P.(ºC)	Mol. Formula	% yield
1e	H	H	219	$C_{17}H_{12}N_4$	76
1f	H	$-CH_3$	232	$C_{18}H_{14}N_4$	97
2a	$-CH_3$	H	243	$C_{18}H_{14}N_4$	99
2b	$-CH_3$	$-CH_3$	236	$C_{19}H_{16}N_4$	97
3a	2,4-$(CH_3)_2$-	H	276	$C_{19}H_{16}N_4$	93
3b	2,4-$(CH_3)_2$-	$-CH_3$	246	$C_{20}H_{18}N_4$	98
4a	$-OC_6H_5$	H	296	$C_{23}H_{16}N_4O$	93
4b	$-OC_6H_5$	$-CH_3$	278	$C_{24}H_{18}N_4O$	98
5a	4-Cl	H	274	$C_{17}H_{11}N_4Cl$	90
5b	4-Cl	$-CH_3$	298	$C_{18}H_{13}N_4Cl$	79
6a	4-Br	H	310	$C_{17}H_{11}N_4Br$	79
6b	4-Br	$-CH_3$	298	$C_{18}H_{13}N_4Br$	81
7a	$-C_6H_5$	H	246	$C_{23}H_{16}N_4$	83
7b	$-C_6H_5$	$-CH_3$	276	$C_{24}H_{18}N_4$	78

Biological Evaluation

The synthesized compounds were evaluated for their antiviral activity.

Cytotoxicity Assay: Each synthesized compound was separately dissolved in 1 mL of distilled dimethyl sulphoxide (DMSO) and the volume was brought up to 10 mL with maintenance medium to obtain a stock solution of 1 mg/mL concentration. It was sterilized by filtration and further dilutions were made from the stock. The cytotoxicity assay was carried out using 0.1 mL of the cell suspension, containing 10000 cells seeded in each well of a 96-well microtitre plate. Fresh medium containing different concentrations of the test sample was added 24 h after the seeding. Control cells were incubated without the test sample and with DMSO.

The little percentage of DMSO present in the wells (maximal 0.2%) was found not to affect the experiment. The microtitre plates were incubated at 378C for a period of 72 h. 16 wells were used for each concentration of the test sample. The morphology of the cells was inspected daily and observed for microscopically detectable alterations, i. e., loss of monolayer, granulation, and vacuolization in the cytoplasm. The CTC50 (the minimum concentration of test drug required to kill 50% of exposed cell population) of each test drug were determined by the standard MTT assay.

Antiviral assay (MIC or EC50) (16-19): Confluent cell cultures in microtiter 96-well plates were inoculated with 100 CCID50 of virus, 1 CCID 50 being the virus dose required to infect 50 % of the cell cultures. After 1-2 h of virus adsorption period, residual virus was removed, and the cell cultures were incubated at 37∞C in the presence of varying concentrations of the test compounds (dilutions were made based upon CTC50). Viral cytopathogenicity was recorded as soon as it reached completion in the control virus infected cell cultures that were not treated with the test compounds after 7-8 days of post infection, microscopically. The antiviral activity of the compounds was expressed as the effective concentration required for inhibiting the viral cytopathic effect by 50% (MIC or EC50). The CTC 50 and MIC of the test compounds were compared with the standard drugs Brivudin (BVDU) and Ribavirin under similar conditions. By adopting the above procedure, the MIC or EC50 for all the synthesized compounds were determined.

Results and Discussion

The results of cytotoxicity assay indicate that all the compounds shown varying degree of cytotoxicity (i.e. from 16 to 240 µg/mL). Based on the CTC 50 nontoxic concentrations, all the synthesized compounds were subjected for antiviral activity determination against different viral strains. The results of antiviral activity (**Table 2**) indicate that triazoles with Cl substitution (compound no **5a**, and **5b**) were excellent in their action. Next in the order, triazoles with 4-(OC_6H_5)-C_6H_5 substitutions exhibited comparable activity with the reference drugs brivudin and ribavirin. The rest of the synthesized compounds exhibited mild activity.

Table 2: Cytotoxicity and antiviral activity of 3-substituted phenyl 6-substituted phenyl (1,2,4) triazolo (4,3-b) pyridazine in E6SM cell cultures

Compound	Minimum Cytotoxic Concentration (µg/mL)[b]	Minimum Inhibitory Concentration (mg/mL)[a]				
		Herpes Simplex Virus-1 (KOS)	Herpes Simplex Virus-2 (G)	Vaccinia Virus	Vesicular Stomatitis Virus	Herpes Simplex Virus-1 TK KOS ACV
1e	> 80	> 16	> 80	> 16	> 80	> 16
1f	≥ 110	> 16	240	> 16	> 16	> 16
2a	> 16	> 3.2	> 16	> 80	> 16	> 80
2b	240	> 16	> 80	> 80	> 16	> 80
3a	60	> 80	> 80	> 80	> 80	> 80
3b	80	> 80	> 6.5	> 16 (48)	> 16	240
4a	190	> 5.8	> 16	> 3.2	> 16	> 16
4b	> 140	> 16	> 16	> 16	> 16	> 16
5a	>240	> 16	> 80	> 16	80	> 80
5b	> 200	> 80	> 80	> 80	> 80	> 80
6a	> 80	> 80	240	80	> 16	> 16
6b	80	> 16	> 16	> 16	80	> 16
7a	16	9.6	48	9.6	240	9.6
7b	> 20	> 80	> 16	> 16	> 80	> 80
Brivudine	> 400	0.0768	240	1.92	> 400	80
Ribavirin	> 400	240	240	48	240	240

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus- induced cytopathogenicity by 50%.

Conclusion

From the above result, it concluded that compound **5a** and **5b** are potent antiviral drugs of synthesized traizole series.

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