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Microwave assisted nano ($ZnO–TiO_2$) catalyzed synthesis of some new 4,5,6,7-tetrahydro-6-((5-substituted-1,3,4-oxadiazol-2-yl)methyl)thieno[2,3-*c*]pyridine as antimicrobial agents

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ABSTRACT

Combined nano zinc oxide and titanium dioxide [nano ($ZnO–TiO_2$)] has been reported first time for the synthesis of novel series of 4,5,6,7-tetrahydro-6-((5-substituted-1,3,4-oxadiazol-2-yl)methyl)thieno[2,3-*c*]pyridine. All the synthesized compounds (**7a–7m**) are novel and were screened for their antimicrobial activity against four different strains like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis* and antifungal activity was determined against two strains *Candida albicans* and *Aspergillus niger*. SAR for the newly synthesised derivatives has been developed by comparing their MIC values with ampicillin, ciprofloxacin and miconazole for antibacterial and antifungal activities, respectively. Among the synthesized compounds, 2,6 dichlorophenyl analogue (**7f**), 4 fluorophenyl analogue (**7k**) and 2,6 dichlorophenyl analogue (**7l**) shows promising antibacterial as well as antifungal activity whereas thiophene substituted compound (**7j**) shows promising antibacterial activity.

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1,3,4-Oxadiazole and their analogues acts as an important bioactive class of heterocycles.[1] A variety of biological activities of substituted 1,3,4-oxadiazoles and 1,3,4-oxadiazole derivatives have been reported in the literature such as anti-inflammatory,[2] antimicrobial,[3] anti-convulsant and hypoglycemic activities,[4] antiviral,[5] antimalerial,[6] analgesic,[7] anticancer,[8–10] hypnotic and sedative.[9] 4,5,6,7-tetrahydrothienopyridine and its derivatives are important heterocycle with different activities like antibacterial,[11] antifungal[12] and antimicrobial.[13]

ZnO is a cheap heterogeneous catalyst with high catalytic activity; it is non-toxic, insoluble in polar as well as non-polar solvents. A wide range of organic reactions such as N-benzylation of amines with alkyl halides,[14] Friedel–Craft's acylation,[15] dehydration of aldoximes into nitriles,[16] nucleophilic ring opening of epoxides by amines to synthesize β-amino alcohols,[17] microwave assisted preparation of cyclic urea,[18] N-formylation of amines[19] and other organic transformation[20] are efficiently catalyzed by ZnO. Reduction of the ZnO particles to nanometer size leads to increased surface area with enhanced catalytic activity of nano ZnO.[21] Recently, zinc oxide nanoparticles have attracted the attention due to the potentially wide-ranging therapeutic applications.[22]

Nanocrystalline TiO_2 has been used as a solid acid catalyst in organic reactions like chemoselective trimethylsilylation of alcohols and phenols,[23] deprotection of silyl ethers,[24] Friedel–Crafts alkylation of indoles with epoxides,[25] synthesis of bis (indolyl) methanes,[26] Mannich synthesis of β-aminocarbonyls[27] and esterification of free fatty acids.[28]

Nano ZnO and nano TiO_2 both are important reagents in organic transformation. In the present work we have reported first time the use of combined nano ($ZnO–TiO_2$) as a catalyst in synthesis of some novel 1,3,4 oxadiazole analogues using microwave and conventional method.

Both 1,3,4-oxadiazole and 4,5,6,7-tetrahydrothienopyridine moieties have potential antimicrobial activities. Hence, it was thought worthwhile to synthesize coupled heterocyclic system containing 1,3,4-oxadiazole and 4,5,6,7-tetrahydrothienopyridine ring with the hope to have enhanced antimicrobial activities. Considering the above facts we report the synthesis of a novel series of 1,3,4-oxadiazole by one pot reaction of hydrazide, aromatic aldehyde in ethanol using combined nano ($ZnO–TiO_2$) as a catalyst. The synthesized compounds are novel and evaluated for in vitro antimicrobial activity.

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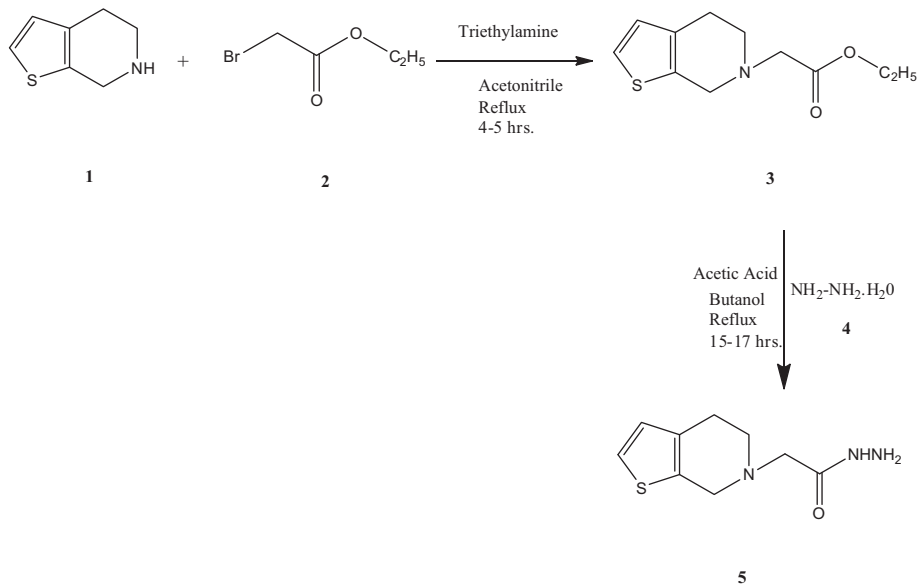
The ester compound **3** was prepared from commercially available 4,5,6,7-tetrahydrothienopyridine and ethyl bromo acetate **2** in presence of triethylamine described in reported method[29] (Scheme 1). Starting hydrazide compound **5** has been prepared from ester compound **3** using hydrazine hydrate **4**, glacial acetic acid in *n*-butanol (Scheme 1). Hydrazide compound **5** and different aromatic aldehydes were subjected to reflux in ethanol using combined nano (ZnO–TiO_2) (1 mmol each) as a catalyst to get the target compounds **7a–7m** as shown in (Scheme 2).

The formation of oxadiazole proceeds via cyclodehydro condensation reaction with the loss of water molecule, to form a cyclic intermediate which upon aromatic stabilization leads to formation of oxadiazole. Catalysts can be easily removed from the reaction mixture by simple filtration. The reaction was initially carried out using ZnO, nano ZnO and nano TiO_2 and combined nano (ZnO–TiO_2). The effect of these catalysts on the yield of the product (**7a**) was studied and the data is presented in Table 1. Among the results, the good yields are obtained by the use of catalyst in nano form. Also it is observed that combined nano (ZnO–TiO_2) gives better yield in short time as compared with other catalysts (96%; 12 min, and 91%; 6 h using microwave and conventional method, respectively). The synthetic procedure was extended for synthesis of all the compounds **7a–7m** using hydrazides and aromatic aldehydes. Results are summarized in Table 2. The yields were obtained in the range of 91–96% and 87–91% for microwave assisted and conventional method, respectively. All synthesized derivatives were characterized using MASS, ^{1}H NMR and ^{13}C NMR. Nano ZnO and Nano TiO_2 has been synthesized and characterized using reported methods[34].

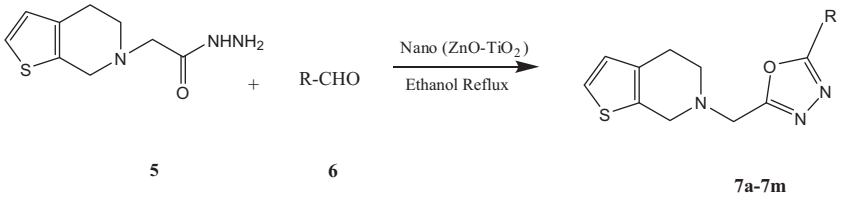
All the synthesized compounds were screened for in vitro antibacterial and antifungal activity. The antibacterial activity was evaluated against four different bacterial strains such as *Escherichia coli* (NCIM-2256), *Pseudomonas aeruginosa* (NCIM-2036), *Staphylococcus aureus* (NCIM-2901) and *Bacillus subtilis* (NCIM-2063). The antifungal activity was evaluated against two fungal strains *Candida albicans* (NCIM-3471) and *Aspergillus niger* (NCIM-1196). Minimum inhibitory concentration (MIC) values were determined using standard agar method.[30–33] Ciprofloxacin and Ampicillin were used as a standard for the comparison of antibacterial activity and Miconazole was used as a standard for the comparison of antifungal activity. Dimethyl sulfoxide was used as solvent control. MIC values of the tested compounds are presented in Table 3.

Many of the newly synthesized compounds were found to show moderate to good antibacterial and antifungal activity.

From the antibacterial activity data (Table 3), scaffold containing 1,3,4-oxadiazole and 4,5,6,7-tetrahydrothienopyridine shows considerable antibacterial activity. Also it was observed that compound **7a**, **7f, 7k and 7l** are the most active against most of the tested organisms. Unsubstituted phenyl analogue (**7b**) shows significant activity against *S. aureus* and *B. subtilis* than activity against *E. coli* and *P. aeruginosa*. Substituted phenyl analogues are more active than unsubstituted phenyl analogues against almost all of the tested organisms except for the compound (**7g**) and (**7h**) where the activity has reduced due to substitution. Introduction of *–Cl* at *para* position of phenyl (**7a**) increases the antibacterial activity against all of the tested organisms. Replacement of *–Cl* with *–OCH_3* at *para* position of phenyl (**7c**) decreases the activity. Introduction of *–OH* at 3 and 4 position of phenyl (**7d**) shows decrease in activity against *P. aeruginosa* and *B. subtilis* compared to unsubstituted phenyl analogues. Introduction of *–Cl* at 2 position of phenyl (**7e**) increases the activity compared to unsubstituted phenyl analogues but the activity is decreases as compared to 4-*Cl* substituted phenyl analogues except against *E. coli*. Addition of one more *–Cl* at 6 position of phenyl (**7f**) enhances the activity compared to the mono chloro (**7a**) substituted analogues. This compound shows significant activity against all tested organisms compared with standard. 2,4-Dimethoxy substitution on phenyl (**7g**) is not favorable against mono substituted analogue. Introduction of *–OH* at 4 position of phenyl (**7h**) in general decreases the activity compared to unsubstituted phenyl analogues. The di-chloro substitution on 2 and 4 position of phenyl (**7l**) gives comparatively more active compound compared with its 4-*Cl* substituted phenyl analogues (**7a**). Introduction of *–F* at 4 position of phenyl (**7k**) gives potent compound against *E. coli*, (MIC-25), *S. aureus* (MIC-25) and *B. subtilis* (MIC-50) compared to ciprofloxacin. Replacement of phenyl ring by other hetero rings like pyrrole (**7i**), thiophene (**7g**), and pyridine (**7m**) enhances antibacterial activity against all tested organisms. Among these analogues (**7j**) is most active compound against *E. coli* and *B. subtilis* compared to standard. Thus, compound **7f, 7k,** and **7j** are the most active antibacterial compound from the present series.



Scheme 1. Synthetic route of the intermediate 2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)acetohydrazide (**5**).

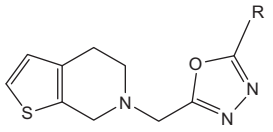


Scheme 2. Synthesis of 4,5,6,7-tetrahydro-5-((5-substituted-1,3,4-oxadiazol-2-yl) methyl) thieno [3,2-c]pyridine (**7**).

Table 1
Effect of the individual catalysts and in combined form on yield and reaction time for **7a**

Catalyst	Mol of	Microwave		Conventional	
		Reaction time (min)	Yield (%)	Reaction time (h)	Yield (%)
No catalyst	–	60	12	24	–
ZnO	1 mmol	22	72	15	68
Nano ZnO	1 mmol	16	88	8	81
TiO_2	1 mmol	24	70	16	65
Nano TiO_2	1 mmol	15	90	9	84
Nano (ZnO–TiO_2)	1 mmol each	12	96	6	91

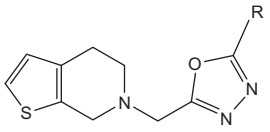
Table 2
Experimental data of the synthesized compounds **7a**–**7m**



Compound	R	Microwave		Conventional		Molecular formula/molecular weight
		Time in min	Yield	Time in h	Yield	
7a	Ph-4-Cl	12	96	06	91	$C_{16}H_{14}ClN_3OS$ 311.5
7b	Ph	12	91	06	88	$C_{16}H_{15}N_3OS$ 297
7c	Ph-4-OCH_3	13	95	6.5	91	$C_{17}H_{17}N_3O_2S$ 327
7d	Ph-3,4-di OH	14	91	7	87	$C_{16}H_{15}N_3O_3S$ 329
7e	Ph-2-Cl	12	95	06	91	$C_{16}H_{14}ClN_3OS$ 311.5
7f	Ph-2,6-di Cl	12	91	6.5	88	$C_{16}H_{13}Cl_2N_3OS$ 366
7g	Ph-2,4-di OCH_3	14	94	7	90	$C_{18}H_{19}N_3O_3S$ 357
7h	Ph-4-OH	13	94	6.5	89	$C_{16}H_{15}N_3O_2S$ 313
7i	C_4H_3N	15	95	7	87	$C_{14}H_{14}N_4OS$ 286
7j	C_4H_3S	14	94	6.5	88	$C_{14}H_{13}N_3OS_2$ 303
7k	Ph-4-F	13	94	6	90	$C_{16}H_{14}FN_3OS$ 315
7l	Ph-2,4-di Cl	12	92	7	89	$C_{16}H_{13}Cl_2N_3OS$ 366
7m	C_5H_4N	15	95	6.5	87	$C_{15}H_{14}N_4OS$ 298

From the antifungal activity data Table 3**,** it is observed that, some of the compounds like **7f, 7k,** and **7l** shows significant activity against *C. albicans*. No significant activity was observed against *A. niger*. Unsubstituted phenyl analogues (**7b**) not showing any considerable antifungal activity. Introduction of *–Cl* at *para* position (**7a**) enhance activity against *C. albicans* and *A. niger*. Replacement of *–Cl* by *–OCH_3* (**7c**) reduces activity compared with (**7a**). 3,4-Dihydroxy phenyl analogue (**7d**) shows considerable rise in antifungal activity against *C. albicans*. 2-*Cl* substitution on phenyl (**7e**) does not affect activity compared to unsubstituted analogue where as 2,6-dichloro analogue (**7f**) significantly enhance antifungal activity giving the potent compound against *C. albicans*. 2,4-Dichloro and 4-*F* substitution (**7l**) and (**7k**), respectively also leads to a potent compound against *C. albicans* in the series. 2,4-Dimethoxy substitution (**7g**) leads to decrease in activity compared to 4-OCH_3 substitution. Introduction of 4-*OH* on phenyl (**7h**) reduces the activity compared to its 3,4-dihydroxy substituted analogue. Replacement of phenyl by other hetero nuclei like thio-

Table 3
Antimicrobial activity of the synthesized compounds



Compound	R	MIC values in μg/mL[a] Antibacterial activity				MIC values in μg/mL[a] Antifungal activity	
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. niger*
7a	Ph-4-*Cl*	100 ± 2.11	250 ± 2.89	75 ± 1.83	100 ± 2.11	60 ± 1.52	100 ± 2.08
7b	Ph	150 ± 2.43	300 ± 2.57	125 ± 0.55	150 ± 1.44	80 ± 1.12	160 ± 2.01
7c	Ph-4-OCH_3	125 ± 1.25	175 ± 1.07	100 ± 2.34	125 ± 2.12	80 ± 0.48	140 ± 1.15
7d	Ph-3,4-di OH	125 ± 0.65	*	100 ± 1.37	*	40 ± 0.32	100 ± 1.68
7e	Ph-2-*Cl*	125 ± 0.78	200 ± 1.97	100 ± 2.00	100 ± 1.54	80 ± 1.12	140 ± 1.87
7f	Ph-2,6-di *Cl*	50 ± 1.09	100 ± 0.98	50 ± 0.17	50 ± 1.89	20 ± 0.15	120 ± 1.75
7g	Ph-2,4-di OCH_3	150 ± 2.02	150 ± 1.47	150 ± 1.22	150 ± 1.42	120 ± 0.64	180 ± 2.55
7h	Ph-4-OH	175 ± 1.67	*	175 ± 2.05	*	60 ± 0.34	140 ± 2.96
7i	C_4H_3N	50 ± 0.53	125 ± 1.46	75 ± 1.76	100 ± 2.01	60 ± 0.23	120 ± 0.48
7j	C_4H_3S	25 ± 1.45	150 ± 2.90	75 ± 1.51	50 ± 1.15	40 ± 0.98	80 ± 1.31
7k	Ph-4-*F*	25 ± 0.57	100 ± 1.47	25 ± 0.52	50 ± 1.12	20 ± 0.73	60 ± 1.14
7l	Ph-2,4-di *Cl*	50 ± 1.17	125 ± 2.49	75 ± 1.77	75 ± 0.56	20 ± 2.57	60 ± 1.92
7m	C_5H_4N	50 ± 0.43	150 ± 1.86	50 ± 0.76	100 ± 1.35	40 ± 1.04	60 ± 0.87
Standard	Ampicillin	100 ± 1.24	100 ± 2.14	250 ± 2.99	250 ± 0.88		
Standard	Ciprofloxacin	25 ± 1.00	25 ± 1.15	50 ± 1.44	50 ± 0.96		
Standard	Miconazole					25 ± 1.24	12.5 ± 1.17

* No activity was observed up to 400 μg/mL.
[a] Values are the average of three readings ± standard deviation.

phene (**7i**), pyrrole (**7j**) and pyridine (**7m**) ring seems to be advantageous in terms of antifungal activity against both *C. albicans and A. niger*. Thus, compound **7f, 7k** and **7l** are the most potent antifungal compound from the series.

In conclusion, we have reported for the first time use of combined nano ($ZnO–TiO_2$) for the synthesis of substituted 1,3,4-oxadiazole from hydrazide and aromatic aldehydes using conventional as well as microwave assisted method in good yields. All the synthesized compounds are novel and were tested for antibacterial and antifungal activity. Based on the activity data, SAR for the series has been developed. From the series **7f, 7k** and **7j** serve as an important pharmacophore for the design and development of new lead as antibacterial agent where as compound **7f, 7k** and **7l** shows promising antifungal activity indicating the future scope for optimization.

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