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International Letters of Chemistry, Physics and Astronomy

15 (2014) 48-54 ISSN 2299-3843

Synthesis, characterization and biological evaluation of 2,5-di-substituted 1,3,4-oxadiazole derivatives

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ABSTRACT

We have reported some novel 1,3,4-oxadiazole synthesized by conventional method .The reaction of 5-bromothiophene-2-carbohydrazide and different benzoic acid derivatives reflux in toluene using phosphorus oxychloride as a catalyst, yielded a series of 2,5-di-substituted 1,3,4-oxadiazole HM-2a to HM-2t. The newly synthesized 2,5-di-substituted 1,3,4-oxadiazole were purified by column chromatography and characterized by IR, Mass, 1H NMR, 13C NMR spectroscopy and elemental analysis. All synthesized compounds were screened for antimicrobial activity using cup plate method. All the compounds showed moderate to good antimicrobial activity and anti fungal activity.

Keywords: Substituted 1,3,4-oxadiazoles; phosphoric anhydride; antimicrobial activity

1. INTRODUCTION

In the field of synthetic organic chemistry, major challenges are to develop the new method for the synthesis of five member heterocyclic compounds. Literature survey reveals five member 1,3,4-oxadiazole derivatives, which belong to an important group of heterocyclic compounds, have been the issue of wide-ranging study in the recent time. Various reports have displayed their chemistry and use [1-3]. A Wide variety of substituted 1,3,4-oxadiazoles have attracted considerable attention in the field of drug discovery because of their wide range of pharmacological activities, including antiproliferative [4], antifungal [5], antibacterial [6,7], anticancer [8], anti-tubercular [9], GABAA receptor agonists [10], anti-inflammatory [11], anti HIV [12]. Different methods have been reported for the synthesis of 1,3,4-oxadiazoles involving cyclization of 1,4-di-substituted thiosemicarbazide in the presence dicyclohexylcarbodiimide (DCC) [13]. Several cyclodehydrating agents such as Et_2O-BF_3, triflic anhydride, thionyl chloride, polyphosphoric acid, 1,1,1,3,3,3-hexamethyldisilazane, sulfuric acid and phosphorus oxychloride [14] have been used. However, use of phosporic anhydried is better than phosphorous oxychloride [15].

2. EXPERIMENTAL

All chemicals and solvents were purchased from Spectrochem Pvt Ltd., Mumbai of AR grade and were used without further purification. Melting points were taken in open capillary method and are uncorrected. IR spectra were recorded on FTIR-8400 spectrophotometer (Shimadzu, Kyoto, Japan), using DRS probe KBr pallet. ^{1}H-NMR spectra of the synthesized compounds were recorded on a Bruker-Avance-II (400 MHz) DMSO-*d*6 solvent. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu, Kyoto, Japan). Physical constants of the synthesized compounds are shown in Table 1.

2. 1. General synthesis of methyl 5-bromothiophene-2-carboxylate (Int 1)

To a well stirred 5-bromothiophene-2-carboxylic acid (0.01 mol) in methanol (25 ml), add concentrated sulphuric acid (0.01 mol) at 0-15 °C and then reflux the reaction mass at 65-70 °C for 6-7 hours. Then after completion of the reaction cool the reaction mixture at 15 °C and add cold water and stir for 3 hours at room temperature. Separated solid washed with water and collected by filtration.

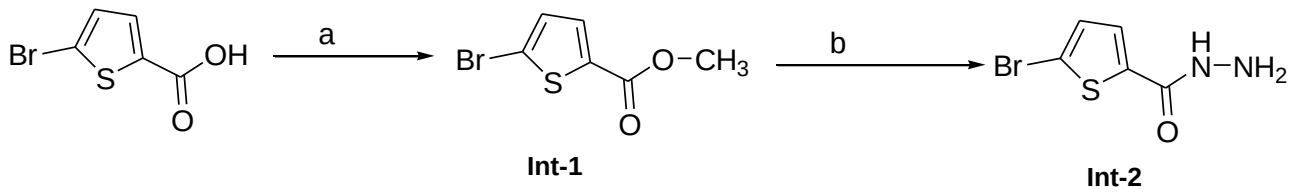
2. 2. General synthesis of 5-bromothiophene-2-carbohydrazide (Int 2)

Methyl 5-bromothiophene-2-carboxylate (0.01 mol) in methanol was refluxed with hydrazine hydrate (0.1 mol) for 5 to 6 hours. After completion of the reaction checked by TLC, the reaction mixture was cooled to room temperature. The separated solid was filtered, was with cold methanol and crystallized from methanol.

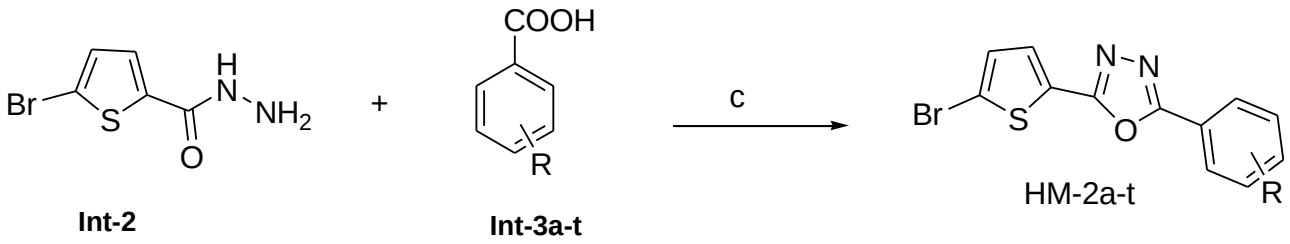
2. 3. General synthesis of Oxadiazole (Int HM-2a-t)

A mixture of 5-bromothiophene-2-carbohydrazide (2.17 g, 0.01 mol) and different aryl acids (0.01 mol) in phosphorous oxychloride (10 ml), was refluxed with continuous stirring. After completion the reaction (6-7 hour monitoring by TLC), the content was cooled to room temperature then add ice cooled water and neutralized with sodium

3. REACTION SCHEME



Scheme 1. (a) Methanol, sulfuric acid, 65 °C (b) Hydrazine Hydrate, 60 °C (c) $POCl_3$, Reflu.



Scheme 1(continue). (a) Methanol, sulfuric acid, 65 °C (b) Hydrazine Hydrate, 60 °C (c) $POCl_3$, Reflu.

Table 1. Synthesis of oxadiazoles.

Entry	R	Time h	Yield (%)	mp. °C
HM-2a	-C_6H_5	6.5	81	182-184
HM-2b	5-Cl C_4H_2	5.5	79	165-167
HM-2c	5-Br C_4H_2	6	87	172-174
HM-2d	CH_2-(4-OMe C_6H_4)	7	65	194-196
HM-2e	4-F C_6H_4	6.5	72	218-220
HM-2f	CH_2-(2,6-di-Cl C_6H_3)	6	77	206-208
HM-2g	4-OMe C_6H_4	6	84	166-168
HM-2h	3-Cl C_6H_4	6.5	81	178-180
HM-2i	3-NO_2 C_6H_4	7	76	192-194
HM-2j	2-Cl C_6H_4	6.5	78	156-158
HM-2k	4-NO_2 C_6H_4	6	85	202-204
HM-2l	2-OH C_6H_4	7	88	214-216
HM-2m	4-NH_2 C_6H_4	5.5	87	232-234
HM-2n	2-Cl-4-NO_2 C_6H_3	6	75	196-198
HM-2o	2-OEt C_6H_4	6	71	208-210
HM-2p	2,4-di-Cl C_6H_3	7	83	188-190
HM-2q	4-OH C_6H_4	6.5	87	196-198
HM-2r	3,5-di-NO_2 C_6H_3	5.5	89	220-222
HM-2s	2,5-di-NO_2 C_6H_3	6	77	238-240
HM-2t	2-Me C_6H_4	6.5	81	168-170

4. SPECTRAL DATA OF THE SYNTHESIZED COMPOUNDS

2-(5-bromothiophen-2-yl)-5-phenyl-1,3,4-oxadiazole (HM-2a):
Yellow solid; mp 182-182 °C; R_f 0.52 (4:6 EtOAc-toluene, acetic acid 1 drop); IR (KBr): 3093, 3063, 2906, 2349, 1799, 1697, 1641, 1593, 1548, 1489, 1425, 1274, 1209, 1066, 1033, 968. 819, 771, 688, 563 cm^{-1}; ^1H NMR: δ 7.096-7.106 (d, 1H, -CH thiophene ring, *j* = 4 *Hz*), 7.476-7.507 (m, 3H, Ar-H phenyl ring), 7.513-7.523 (d, -CH, Ar-H thiophene ring, *j* = 4 *Hz*), 8.027-8.051 (dd, 2H, Ar-H, phenyl ring, *j* = 1.6, 7.6 *Hz*). MS (*m/z*): 307 (M^+); Anal. Calcd for $C_{12}H_7BrN_2OS$: C, 44.59; H, 2.18; N, 8.67; Found: C, 43.12; H, 3.21; N, 9.31.

2-(5-bromothiophen-2-yl)-5-(5-chlorothiophen-2-yl)-1,3,4-thiadiazole (HM-2b):
Yellow solid; mp 165-167° C; R_f 0.46 (4:6 EtOAc-toluene, acetic acid 1 drop); IR (KBr): 3269, 3095, 3014, 2924, 2364, 1805, 1647, 1587, 1496, 1431, 1309, 1211, 1111, 1049, 1030, 968, 937, 802, 719, 663, 559 cm^{-1}; ^1H NMR: δ 6.950-6.960 (d, 1H, -CH, Ar-H, *j* = 4 *Hz*), 7.087-7.096(d, 1H, Ar-H, *j* = 3.6 *Hz*), 7.478-7.488 (d, 1H, Ar-H, *j* = 4 *Hz*), 7.512-7.522 (d, 1H, Ar-H, *j* = 4 *Hz*). MS (*m/z*): 347 (M^+); Anal. Calcd for $C_{10}H_4BrClN_2OS_2$: C, 34.55; H, 1.16; N, 8.06; Found: C, 36.27; H, 2.23; N, 7.37.

2,5-bis(5-bromothiophen-2-yl)-1,3,4-oxadiazole (HM-2c):
Yellow solid; mp 172-174 °C; R_f 0.51 (4:6 EtOAc-toluene, acetic acid 1 drop); IR (KBr): 3211, 3061, 2981, 2328, 1812, 1634, 1574, 1482, 1427, 1334, 1247, 1141, 1024, 1065, 936, 883, 752, 675, 567 cm^{-1}; MS (*m/z*): 392 (M^+); Anal. Calcd for $C_{10}H_4Br_2N_2OS_2$: C, 30.63; H, 1.03; N, 7.14; Found: C, 32.18; H, 2.24; N, 6.76.

2-(5-bromothiophen-2-yl)-5-(4-methoxybenzyl)-1,3,4-oxadiazole (HM-2d):
Yellow solid; mp 194-196 °C; R_f 0.42 (4:6 EtOAc-toluene, acetic acid 1 drop); IR (KBr): 3298, 3087, 2976, 2347, 1867, 1678, 1597, 1443, 1401, 1318, 1279, 1132, 1037, 1008, 967, 864, 789, 668, 587 cm^{-1}; MS (*m/z*): 351 (M^+); Anal. Calcd for $C_{10}H_4Br_2N_2OS_2$: C, 47.88; H, 3.16; N, 7.98; Found: C, 45.24; H, 2.47; N, 8.24.

2-(5-bromothiophen-2-yl)-5-(4-fluorophenyl)-1,3,4-oxadiazole (HM-2e): Yellow solid; mp 218-220 °C; R_f 0.57 (4:6 EtOAc-toluene, acetic acid 1 drop); IR (KBr): 3284, 3067, 3021, 2931, 2355, 1812, 1652, 1591, 1483, 1425, 1311, 1249, 1171, 1064, 1014, 991, 941, 845, 762, 647, 587 cm-1; MS (m/z): 325 (M^+); Anal. Calcd for $C_{10}H_4BrClN_2OS_2$: C, 44.33; H, 1.86; N, 8.62; Found: C, 47.24; H, 2.78; N, 7.86.

2-(5-bromothiophen-2-yl)-5-(2,6-dichlorobenzyl)-1,3,4-oxadiazole (HM-2f):
Yellow solid; mp 182-182 °C; R_f 0.52 (4:6 EtOAc-toluene, acetic acid 1 drop); IR (KBr): 3107, 3078, 2925, 2384, 1764, 1679, 1627, 1568, 1555, 1447, 1407, 1292, 1224, 1078, 1039, 982. 832, 786, 674, 587 cm^{-1}; MS (*m/z*): 390 (M^+); Anal. Calcd for $C_{13}H_7BrCl_2N_2OS$: C, 40.03; H, 1.81; N, 7.18; Found: C, 42.21; H, 3.04; N, 8.42.

2-(5-bromothiophen-2-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (HM-2g):
Yellow solid; mp 166-168 °C; R_f 0.53 (4:6 EtOAc-toluene, acetic acid 1 drop); IR (KBr): 3045, 3081, 2934, 2375, 1776, 1683, 1645, 1579, 1564, 1434, 1431, 1279, 1231, 1067, 1021, 974. 843, 778, 681, 563 cm^{-1}; MS (*m/z*): 337 (M^+); Anal. Calcd for $C_{13}H_9BrN_2O_2S$: C, 46.31; H, 2.69; N, 8.31; Found: C, 48.24; H, 3.71; N, 7.14.

5. BIOLOGICAL ACTIVITY

5. 1. Antimicrobial Sensitivity Testing

5. 1. 1. Well Diffusion/Agar Cup Method

In vitro affectivity of antimicrobial agents can be demonstrated by observing their capacity to inhibit bacterial growth on suitable media. The production of a zone depends on two factors namely bacterial growth and concentration of antimicrobial agent. The hole/well punch method was first used by Bennett. This diffusion method has proved more effective than many other methods. According to Lt. General Raghunath the well technique is 5-6 times more sensitive than using disk method.

5. 1. 2. Principle

When antimicrobial substance is added in agar cup (made in a medium previously inoculated with test organism) the redial diffusion of an antimicrobial agent through the agar, produces a concentration gradient. The test organism is inhibited at the minimum inhibitory concentration (MIC), giving rise to a clear zone of inhibition.

Table 2. Antimicrobial Sensitivity Assay.

Sr. No.	Code no.	MIC (µg/mL)						
		antibacterial activity				antifungal activity		
		E. coli	*P. aeruginosa*	*S. aureus*	*S. pyogenus*	*C. albicans*	*A. niger*	*A. clavatus*
1	HM-2a	250	250	500	500	500	250	>1000
2	HM-2b	500	500	250	500	250	500	250
3	HM-2c	250	500	250	500	500	250	1000
4	HM-2d	100	500	250	500	500	250	>1000
5	HM-2e	250	500	250	500	500	500	500
6	HM-2f	500	200	500	250	250	500	>1000
7	HM-2g	250	500	250	500	500	1000	500
8	HM-2h	100	250	200	125	200	250	250
9	HM-2i	500	500	250	250	500	250	500
10	HM-2j	250	250	500	250	100	100	100
11	HM-2k	500	250	250	500	200	500	500
12	HM-2l	250	250	500	250	250	100	250
13	HM-2m	100	200	500	125	100	250	250

14	HM -2n	250	200	500	250	500	250	500
15	HM -2o	250	500	250	500	1000	500	500
16	HM -2p	500	250	500	250	500	250	500
17	HM -2q	250	500	500	200	250	1000	>1000
18	HM -2r	100	200	250	125	100	250	250
19	HM -2s	500	250	500	250	250	500	1000
20	HM -2t	100	250	200	125	100	250	250
Gentamycin		0.05	1	0.25	0.5	-	-	-
Ampicilin		100	100	250	100	-	-	-
Chloramphenicol		50	50	50	50	-	-	-
Ciprofloxacin		25	25	50	50	-	-	-
Norfloxacin		10	10	10	10	-	-	-
Nystatin		-	-	-	-	100	100	100
Greseofulvin		-	-	-	-	500	100	100

6. CONCLUSION

We have developed a novel 2,5-disubstituted-oxadiazole by the reaction of different aromatic acid and hydrazide in high yield and purity. The reaction of various aromatic acid and 5-bromothiophene-2-carbohydrazide was carried out by refluxing in $POCl_3$. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antibacterial and antifungal screening data revealed that, the compounds HM-2h, HM-2m, HM-2r, and HM-2t shows good activity against bacterial stain, while HM-2h, HM-2j, HM-2k, HM-2r, and HM-2t shows comparatively good activity against fungal stain.

Acknowledgements

Authors are thankful to UGC, New Delhi for the financial support.

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(Received 15 May 2014; accepted 24 May 2014)