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Research Article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL5-(8-BROMONAPHTHO[2,1-*B*]FURAN-2-YL)-N-ALKYL/ARYL-1,3,4-THIADIAZOLE-2-AMINES AND 5-(8-BROMONAPHTHO[2,1-*B*]FURAN-2-YL-4-SUSTITUTED -4*H*-1,2,4-TRIAZOLE-3-THIOLS

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ABSTRACT

The key starting material 8-bromonaphtho [2,1-*b*]furan-2-carboxyhydrazide **1** was utilized for the synthesis of the title compounds. The carbohydrazide**1** was converted into thiosemicarbazide **2a-e** by the condensation of appropriate alkyl/arylisothiocyanates These thiosemicarbazides were cyclized using anhydrous phosphoric acid to 5-(8-bromonaphtho[2,1-*b*]furan-2-yl)-N-alkyl/aryl-1,3,4-thiadiazole-2-amines (**3a-e).**The thiosemicarbazides **2a-e** were cyclized using aqueous sodium hydroxide to 5-(8-bromonaphtho[2,1-*b*]furan-2-yl-4-sustituted-4*H*-1,2,4-triazole-3-thiols **3a-e.** The structures of newly synthesized compounds have been established by analytical and spectral studies. All the compounds in the series have been screened for their antibacterial and antifungal activities.

Keywords: Naphtho[2,1-*b*]furo-1,3,4-thiodiazole-2-amine, antibacterial activity, antifungal activity.

INTRODUCTION

The biheterocyclic compounds comprising triazoles, and thiadiazoles obtained from thiosemicarbazides have been produced as antimicrobial agents[1-4].

The [1,3,4] thiadiazoles have revealed antifungal[5], anti-inflammatory[6],antiparasitic[7,] analgesic[8,9], antiproteolytic[10], muscle relaxant[11] and various activities[12]possibly due to the presence of =N-C-S moiety. [1,3,4] thiadiazole derivatives of thiosemicarbazides and screened them for antimicrobial activites[13].

Triazoles and their derivatives represent an interesting class of compounds possessing a wide spectrum of biological activities. 1,2,4-Triazole system is a structural element of many drugs that have antimycotic activity such as fluconazol, itraconazol, voriconazol[14,15]. During the last few decades, a considerable attention has been devoted to the synthesis of 1,2,4-triazole derivatives possessing diverse pharmacological properties such as antimicrobial[13,16-19], antiinflammatory[20], analgesic[21], antitumorial[22], antihypertensive[23], anticonvulsant and antiviral activities[24]. Aseries of 3-mercapto-1,2,4-triazoles mono or disubstituted at 2,3- or 4 –positions and evaluated as antifungal agents[25].Here, we have focused mainly on8-bromo naphtho[2,1-*b*]furan as our basic structure and coupled it with 1,3,4-thiadiazole, 1,2,4-triazoleheterocycles.

MATERIALS AND METHODS

Melting points were determined by an open capillary method and are uncorrected. The IR spectra(in KBr pellets) were recorded on Shimadzu FTIR spectrum 8000 Spectrometer. The

NMR spectra (^1H and ^{13}C) were recorded on Bruker-400 MHz Spectrometer, using $CDCl_3$ and DMSO-d_6 as solvent and TMS as internal standard reference. Chemical shifts are expressed as δ values [in ppm]. The mass spectra were recorded on Brucker Apex-II mass Spectrophotometer and Shimadzu LC-MS Instrument. Purity of compounds was checked by thin layer chromatography (TLC) on a silica gel plate and visualizing the spots under iodine vapour.

EXPERIMENTA

Synthesis of8-bromonaphtho[2,1-*b*]furan-2-carboxyhydrazide 1

An aqueous solution of hydrazine hydrate (15 ml, 99%) was added to a solution of ethyl 8-bromonaphtho[2,1-*b*]furan-2-carboxylate (3.2g, 0.01 mol) in ethanol (30 ml). The reaction mixture was heated under reflux for 2 h and cooled to room temp. The hydrazide**1** that separated, as a solid, was collected and recrystallized from ethanol.

Synthesis of 2-[(8-bromonaphtho[2,1-*b*]furan-2yl)carbonyl]-N-arylhydrazine carbothioamide 2a-e

A mixture of equimolar amounts of 8-bromonaphtho[2,1-*b*]furan-2-carboxyhydrazide **1** (3.01 g, 0.01 mol) and the appropriate aromatic isothiocyanates (0.01 mol) in ethonol (20 mL) was refluxed for 3 hrs. The solution was poured on to crushed ice, the separated solid was filtered, washed with ethanol, dried and recrystallised from ethanol .

Cyclisation of arylhydrazinecarbothioamide 2a-e into 5-(8-bromonaphtho [2,1-*b*] furan-2-yl)-N-aryl-1,3,4-thiadiazole-2-amines3a-e

To the cold anhydrous orthophosphoric acid (6 mL), suitable thiosemicarbazide**2a-e**(0.01 mol) was added gradually with constant stirring during 20 min. The mixture was heated in an oil-bath at 150ºC for 0.5 hr. After the addition, the solution was left over night at room temperature. The syrupy solution was poured into crushed ice. The precipitate formed was filtered, washed thoroughly with water, dried and recrystallised from ethanol to obtain **3a-e**.

Cyclisation of arylhydrazinecarbothioamide 2a-e into 5-(8-Bromonaphtho[2,1-*b*]furan-2-yl)-4-aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones 4a-e

The appropriate thiosemicarbazide**2a-e**(0.001 mol) was suspended in aqueous NaOH (10 mL, 4%) and heated gently under reflux for 2 hrs. The solution was treated with charcoal and filtered. The filtrate was cooled and acidified carefully with dilute acetic acid (10%). The precipitate thus formed was filtered, washed with water and recrystallized from ethanol to get **4a-e.**

The physical data of newly synthesized compounds are tabulated in **Table – 1**.

Evaluation of Biological activities

The compounds encompassing naphthofuran, and thiadiazoles, triazoles are known to exhibit wide spectrum of biological activities. Hence, it was contemplated to evaluate newly synthesized compounds for antimicrobial activities by adopting literature procedure.

Antimicrobial activity

The newly synthesized compounds were screened for their antibacterial activity against *Staphyloccoccuspyogens, Staphylococcus aureus and* antifungal activity against *Aspergillusflavus, Candidaalbicans* according to cup plate method[26] at a concentration of 0.005 mol/ml against all the organisms. Chloramphenicol and Fluconazole were used as standard drugs for antibacterial and antifungal activity respectively. The zone of inhibition was compared with the standard drug after 24 h of incubation at 25^0 C for antibacterial activity and after 48 h at 30^0 C for antifungal activity.The results of such studies are given in **Table 2.**

RESULT AND DISCUSSION

The construction of thiadiazole ring was achieved in two steps. In the first step, the key starting material carboxyhydrazide 1 is converted into various 2-[(8-bromonaphtho[2,1-*b*]furan-2yl)carbonyl]-N- arylhydrazinecarbothioamide**2a-e** by the reaction between **1** and appropriate isothiocyanateswhich were synthesized in our laboratory by using well known procedure[27]. The logical approach for the selection of isothiocyanates is once again based on electron donating and electron withdrawing substituents, which would enable us to draw certain conclusions regarding impact of these substituents on various biological activities.The IR spectrum of **2a** exhibited the absorption band at 1677 cm^{-1} due to -C=O and 3072-3224 cm^{-1} due to –NH stretching frequency. The ^1H NMR spectrum exhibited the aromatic protons as a multiplet integrating for eleven aromatic protons between δ 7.1-8.5, three singlets at δ 9.81, 9.88, 10.89 integrating for three NH (D_2O exchangeable) protons. The IR and ^1H NMR spectral data were tabulated in **Table - 3.**

These thiosemicarbazides were cyclized using anhydrous phosphoric acid to 5-(8-bromonaphtho[2,1-*b*]furan-2-yl)-N-alkyl/aryl-1,3,4-thiadiazole-2-amines (**3a-e)** in good yield **Scheme-1**.

The IR spectrum of **3a** exhibited the absorption band at 1647 cm^{-1} due to C=N and 3197 cm^{-1} due to –NH stretching frequency. In ^1H NMR spectrum, the aromatic protons were observed as a multiplet integrating for eleven protons between δ 7.2-8.3 and only one singlet at δ 9.6 integrating for one proton of –NH (D_2O exchangeable) indicating the formation of thiadiazole ring. Further, the structure of **3a** was confirmed by mass spectral analysis, it exhibited a molecular ion peak at m/z 422 corresponding to its molecular weight.The **Table – 4** gives the spectral data of synthesized compounds.

The synthesis of triazoles coupled with naphtho[2,1-*b*]furan was accomplished by the cyclization of **2a-e** using aqueous sodium hydroxide to 5-(8-bromonaphtho[2,1-*b*]furan-2-yl-4-sustituted-4*H*-1,2,4-triazole-3-thiols **4a-e**. The IR spectrum of **4a** exhibited the absorption band at 1649 cm^{-1} due to C=N and 3165 cm^{-1} due to –NH group. In ^1H NMR spectrum, the aromatic protons were observed as a multiplet integrating for eleven aromatic protons at δ 7.2 - 8.2 and only one singlet at δ 9.5 integrating for one proton of –NH (D_2O exchangeable) indicated the formation of triazole ring. Further, the structure of **4a** was confirmed by mass spectral analysis, it exhibited a molecular ion peak at m/z 422 corresponding to its molecular weight. The peaks appearing at m/z 390, 343, 168, 177, 155, 136, 120, and 105 were in accordance with the fragmentation pattern. The spectral data of the synthesized compounds is given in **Table – 5.**

The compounds encompassing naphthofuran, thiadiazole ring systems are known to exhibit wide spectrum of biological activities. Hence, it was contemplated to evaluate newly synthesized compounds for antimicrobial activities by adopting literature procedure. The compounds **2a, 2e, 2d, 3b, 3c-d, 4d,** exhibited more antibacterial activity where as compounds **3a, 3d, 4b, 4c**exhibited moderate antibacterial activity against *S. pyogens.*The compounds **3a, 3c,** possessesmore antibacterial activity and compounds **2a-b, 3b, 3e, 3d**exhibited moderate antibacterial activity against *S. aureus.* The compounds**2b, 3d-e, 4d** possesses more antifungal activity andcompounds**2a, 2c, 3b, 4a, 4e** possesses moderate antifungalactivity against *A. flavus.*

The compounds **3c-d** exhibited more antifungal activity and the compounds **2d, 3a, 4d** possesses moderate antifungal activity against *C.albicans.* The remaining compounds were found to have moderate or slight active against tested organism and some of the compounds were found to be inactive.

CONCLUSION

The synthesis of novel biheterocycles comprising naphtho[2,1-*b*]furan and 1,3,4-thiadiazoles ring systems, containing bromine atom at position 8 of naphthofuran nucleus and various substituted 1,3,4-thiadiazole and 1,2,4 triazole nucleus has been carried out successfully. Overall conclusion is that introduction of bromine atom in position 8 of naphthofuran ring enhanced antimicrobial to certain extent. In general, electron donating groups on thiadiazole and triazole ring were found to exhibit more antimicrobial activity.

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Scheme - 1

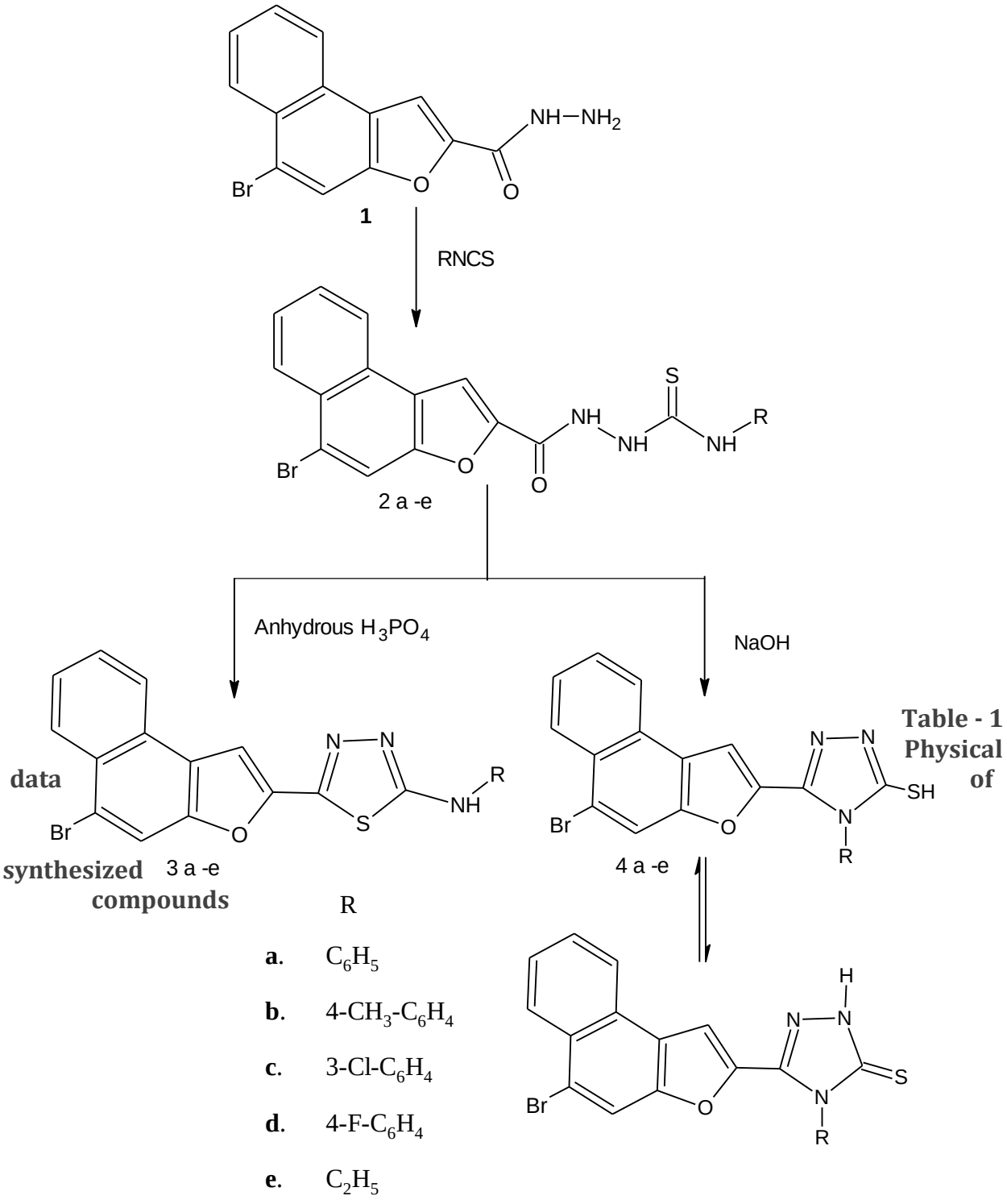

Table - 1 Physical data of synthesized compounds

	R
a.	C_6H_5
b.	4-CH_3-C_6H_4
c.	3-Cl-C_6H_4
d.	4-F-C_6H_4
e.	C_2H_5

Comp.	R	M.P.^{0}C	Yield (%)	Mol. Formula	Found (Calcd) %		
					C	H	N
3a	C_6H_5	190	76	$C_{20}H_{12}ON_3SBr$	67.06 (67.18)	4.34 (4.56)	11.02 (11.22)
3b	4-CH_3-C_6H_4	202	78	$C_{21}H_{14}ON_3SBr$	59.02 (59.11)	3.39 (3.47)	13.40 (13.79)
3c	3-Cl-C_6H_4	255	70	$C_{20}H_{11}ON_3SClBr$	69.18 (69.95)	3.51 (3.82)	11.98 (12.24)
3d	4-F-C_6H_4	254	57	$C_{20}H_{11}ON_3SFBr$	63.45 (63.57)	3.09 (3.20)	11.00 (11.14)
3e	C_2H_5	198	80	$C_{16}H_{12}ON_3SBr$	59.06 (59.11)	3.34 (3.47)	13.72 (13.79)
4a	C_6H_5	237	66	$C_{20}H_{12}ON_3SBr$	63.46 (63.57)	3.11 (3.20)	11.18 (11.14)
4b	4-CH_3-C_6H_4	233	60	$C_{21}H_{14}ON_3SBr$	67.29 (67.54)	4.01 (4.05)	11.06 (11.26)
4c	3-Cl-C_6H_4	236	61	$C_{20}H_{11}ON_3SClBr$	70.13 (70.57)	4.12 (4.23)	11.40 (11.76)
4d	4-F-C_6H_4	249	59	$C_{20}H_{11}ON_3SFBr$	61.53 (61.85)	3.02 (3.11)	13.98 (14.43)
4e	C_2H_5	248	67	$C_{16}H_{12}ON_3SBr$	67.21 (67.54)	4.04 (4.05)	11.00 (11.26)

Table 2: Results of Antimicrobial activity of compounds 2a-e, 3a-e and 4a-e

Comp.	R	Zone of inhibition in mm			
		Antibacterial activity		Antifungal activity	
		S. pyrogens	*S. aureus*	*A. flavus*	*C. albicans*
2a	C_6H_5	22	14	17	10
2b	4-CH_3-C_6H_4	21	15	18	-
2c	3-Cl-C_6H_4	-	12	17	-
2d	4-F-C_6H_4	21	10	-	12
2e	C_2H_5	20	-	14	-
3a	C_6H_5	17	19	10	13
3b	4-CH_3-C_6H_4	21	16	13	-
3c	3-Cl-C_6H_4	22	21	19	22
3d	4-F-C_6H_4	20	17	23	21
3e	C_2H_5	17	17	22	9
4a	C_6H_5	15	-	15	-
4b	4-CH_3-C_6H_4	18	-	-	11
4c	3-Cl-C_6H_4	17	21	10	09
4d	4-F-C_6H_4	22	12	24	12
4e	C_2H_5	12	11	15	-
(Standard) Chloamphenicol		24	26	-	-
Flucanazole		-	-	24	26

Zone of inhibition in millimeters.

Table 3: IR and 1H NMR Spectral data of 2-[(8-Bromonaphtho[2,1-*b*]furan-2yl) carbonyl]-N- arylhydrazinecarbothioamide.2a-e

Comp.	R	IR (KBr) cm^{-1}		^{1}H NMR($CDCl_3$)
		N-H	*C=O*	
10a	C_6H_5	3072-3224	1677	δ 7.1-8.5 (m, 11H, ArH), δ 9.81, 9.88, 10.89 (s, 3H, 3NH)
10b	4-CH_3-C_6H_5	3154-3165	1694	δ 1.7 (s, 3H, CH_3), δ 7.1-8.2 (m, 10H, ArH), δ 9.52, 9.56, 11.2 (s, 3H, 3NH)
10c	3-Cl-C_6H_5	3075-3100	1678	7.2-8.4 (m, 10H, ArH), 9.5, 9.8, 11.8 (s, 3H, 3NH)
10d	4-F-C_6H_5	3125-3225	1685	7.2 -8.5 (m, 10H, ArH), 8.8, 9.4, 11.8 (s, 3H, 3NH)
10e	C_2H_5	3100-3140	1686	δ 1.03-1.08 (t, 3H,CH_3), δ 3.41-3.48 (q, 2H, CH_2),δ 7.3-8.5 (m, 6H, ArH), 8.9, 9.4, 12.2 (s, 3H, 3NH)

Table 4: IR and 1H NMR Spectral data of 5-(8-bromonaphtho[2,1-b] furan-2-yl)-N-alkyl/aryl-1,3,4-thiadiazole-2-amines 3 a-e

Comp.	R	IR (KBr) cm^{-1}		^{1}H NMR($CDCl_3$)
		N-H	C=N	
3a	C_6H_5	3197	1647	δ 7.2-8.3 (m, 11H, ArH), 9.6 (s, 1H, NH)
3b	4-CH_3-C_6H_4	3120	1640	δ 1.5 (s, 3H, CH_3), δ 7.1-8.5 (m, 10H, ArH), 9.1 (s, 1H, NH)
3c	3-Cl-C_6H_4	3157	1601	7.2-8.3 (m, 10H, ArH), 9.3 (s, 1H, NH)
3d	4-F-C_6H_4	3087	1589	7.3-8.2 (m, 10H, ArH), 8.7 (s, 1H, NH)
3e	C_2H_5	3235	1625	δ 0.92-1.12 (t, 3H,CH_3), δ 3.5-3.62 (q, 2H, CH_2), δ 7.3-8.5 (m, 6H, ArH), 8.5 (s, 1H, NH),

Table 5: IR and ^{1}H NMR Spectral data of 5-(8-bromonaphtho[2,1-*b*]furan-2-yl-4-sustituted-4*H*-1,2,4-triazole-3-thiols 4a-e

Comp.	R	IR (KBr) cm^{-1}		^{1}H NMR($CDCl_3$)
		N-H	C=N	
4a	C_6H_5	3165	1649	δ 7.2-8.2 (m, 11H, ArH), 9.5 (s, 1H, NH)
4b	4-CH_3-C_6H_4	3112	1589	δ 1.6 (s, 3H, CH_3), δ 7.2-8.4(m, 10H, ArH), 9.1 (s, 1H, NH)
4c	3-Cl-C_6H_4	3078	1620	7.2-8.2 (m, 10H, ArH), 9.3 (s, 1H, NH)
4d	4-F-C_6H_4	3130	1599	7.3-8.2 (m, 10H, ArH), 8.7 (s, 1H, NH)
4e	C_2H_5	3135	1613	δ 1.02-1.12 (t, 3H,CH_3), δ 3.54-3.6 (q, 2H, CH_2), δ 7.2-8.3 (m, 6H, ArH), 8.5 (s, 1H, NH)

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