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Synthesis and Characterization of Novel Antibacterial Agents Enclosing Naphtho[2,1-b]Furan, Triazole, Azetidinone and Pyrrole Ring Systems

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ABSTRACT

Synthesis of novel heterocyclic system encompassing four different heterocycles viz., naphtho[2,1-b]furan, 1,2,4-triazole, azetidinone and pyrrole has been carried out to obtain novel antibacterial agents. Ethyl 3-(2,5-dimethylpyrrol)naphtho[2,1-b]furan-2-carboxylate **2,** synthesized by the reaction between ethyl 3-aminonaphtho[2,1-b]furan-2-carboxylate **1** and acetonyl acetone, was converted in to corresponding hydrazide **3.** The hydrazide **3** on treatment with carbon disulphide and hydrazine hydrate in presence of alkali produced 3-(2,5-dimethylpyrrol)-4-aminonaphtho[2,1-b]furan-2-yl-4H-1,2,4-triazole-3-thiol **4.** The reaction of triazole **4** with various substituted aromatic aldehydes resulted in the formation of respective Schiff bases **5a-e**. Synthesis of the desired compounds i.e. 3-(2,5-dimethylpyrrol)-4-{(4-aryl)methylene}amino-5-naphtho[2,1-b]-2-yl-4H-1,2,4-triazole-4-yl)-4-arylazetidin-2-ones **6a-e** was accomplished by reacting Schiff bases **5a-e** with chloroacetyl chloride. The structures of the newly synthesized compounds were established by IR, ^{1}H NMR and ^{13}C NMR spectral studies. The antibacterial activity of the synthesized compounds, at the concentration of 100 µg/ml, has been evaluated against four bacterial strains using Gentamycin and Amphicillin as standard drugs. Some of the compounds showed promising antibacterial activity.

Keywords: Naphthofuran; Triazole; Azetidinones; Pyrrole; Antibacterial Activity.

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INTRODUCTION

Pyrrole and its derivatives, which are found as a core structure of natural products [1], have been reported to possess wide range of pharmacological and biological activities such as anti-inflammatory [2], antimicrobial [3], HIV inhibitors [4-6], anticoagulant [7], antitumor [8], analgesic [9], antiallergic [10], and antitubarcular [11]. Triazoles, especially 1,2,4-triazoles are known to exhibit broad spectrum of activities like antimicrobial[12-15], anti-inflammatory[16], analgesic[17], antitmour[18], antihypertensive[19] and antiviral[20]. Similarly, the compounds encompassing azetidinones ring system also known to display many important pharmacological activities[21,22].

Synthesis of various derivatives of naphtho[2,1-b]furan with the objective of obtaining more potent molecules has been major research programme in our laboratory [23-27]. Thus, encouraged by the importance of triazole, azetidinones and pyrrole and in continuation of our research work on naphtho[2,1-b]furan we report in this paper synthesis of novel heterocyclic system encompassing all the four heterocyclic systems in a single molecular frame work.

MATERIALS AND METHODS

All the reagents were of A. R. grade and used without further purification. Melting points were determined with the open capillary and are uncorrected. IR spectra were recorded in KBr pellets by using SHIMADZU FT-IR - 8400 Spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d6 on Bruker Supercon FT-NMR 400 MHz instrument. Chemical shifts are reported in δ (ppm) relative to TMS as an internal standard. Compounds were analyzed for elemental analysis and all compounds showed satisfactory elemental analysis. The purity of the compounds was checked by TLC using Silica gel-G.

Synthesis of ethyl 3-aminonaphtho[2,1-b]furan-2-carboxylate, 1 [29]:

A mixture of 2-hydroxy-1-naphthaldoxime (0.93 g, 0.05 mol), ethyl chloro acetate (6.13 g, 0.05 mol) and anhydrous potassium carbonate (4.9 g, 0.05 mol)was heated under reflux in anhydrous dimethyl formamide (60 ml) for 12 hrs. The reaction mixture was cooled, potassium salts were fitered off and the filtrate was poured on to crushed ice to obtain the product as light brown coloured solid. It was collected by filtration and recrystallized from aqueous ethanol (Yield 7.5 g, 75%).

Synthesis of ethyl 3-(2,5-dimethylpyrrol)naphtho[2,1-b]furan-2-carboxylate, 2:

To a solution of ethyl 3-aminonaphtho[2,1-b]furan-2-carboxylate **1** (25.5 g, 0.1 mol) in glacial acetic acid (100 ml) , acetonyl acetone (13.69 g, 0.12 mol) was added and the reaction mixture was heated under reflux for 30 mins. After removal of the solvent the product that obtained was recrystallized from ethanol (Yield 26.6 g, 80%).

Synthesis of 3-(2,5dimethylpyrrol)naphtho[2,1-b]furan-2-hydrazide, 3:

A mixture of ethyl 3-(2,5-dimethylpyrrol)naphtho[2,1-b]furan-2-carboxylate **2** (3.33 g, 0.01 mol) and hydrazine hydrate (2.5 ml, 99%) in ethanol (10 ml) was heated under reflux for 5 hrs, cooled to room temperature and the solid thus separated was filtered, washed with ethanol and recrystallized from aqueous DMF to obtain the product as solid (Yield 2.6 g, 85%)

Synthesis of 3-(2,5-dimethylpyrrol-4-amino-5-naphtho[2,1-b]furan-2-yl-4H-1,2,4-triazole-3-thiol 4:

3-(2,5-Dimethylpyrrol)naphtho[2,1-b]furan-2-hydrazide **3** (3.03 g, 0.01 mol) was dissolved in ethanol (20 ml) and a solution of potassium hydroxide in ethanol (10%, 20 ml) and carbon disulphide (0.02 mol), were added The reaction mixture was stirred at room temperature for 12 hrs. The salt obtained was filtered and washed with dry ether. It was then refluxed with hydrazine hydrate (0.03 mole) in ethanol (50 ml) on a water bath until the evolution of hydrogen sulphide ceased (about 8 hrs).The mixture was then poured into ice cold water, acidified with glacial acetic acid and the poduct that separated as a solid was collected and purified by recrystallization from aqueous ethanol (Yield 2.4 g, 70%).

Synthesis of 3-[2,5- dimethylprrrol]-4-{(aryl)methylene]amino}-5-naphtho[2,1-b] furan-2-yl-4H-1,2,4-triazole -3-thiol, 5a-e:

To a solution of 3-(2,5-dimethylpyrrol-4-amino-5-naphtho[2,1-b]furan-2-yl-4H-1,2,4-triazole-3-thiol **4** (3.75g ,0.01 mol) in dioxane (30 ml), 4-methoxy benzaldehyde (1.81 g, 0.01 5 mol) were added, the mixture was refluxed on water bath for 5 hrs and then poured into ice cold water. The product that separated was filtered, dried and recrystallized from aqueous DMF. The compounds **5b-e** were synthesized by the same method described above by using appropriately substituted benzaldehydes.

Synthesis of 3-(2,5-dimethylpyrrol-4-{[4-(aryl)methylene]amino}-5-naphtho[2,1-b]furan-2-yl-4H-1,2,4triazole-4-yl)-4-aryl-azetidin-2-ones, 6a-e:

A solution of chloro acetyl chloride (0.6 ml, 0.0055 mol) in dioxane (20 ml), was cooled to -10^0 C using ice-salt bath and kept for stirring .To this, triethyl amine (0.5 g, 0.005 mol)was added drop wise maintaining the temperature below 0^0C, while white solid separated out. To this reaction mixture, solution of 3-[2,5 -dimethylprrrol]-4-{(aryl) methylene]amino}-5-naphtho[2,1-b]furan-2-yl-4H-1,2,4-triazole -3-thiol **5a**(1.23 g, 0.0025 mol) in dioxane (10 ml) was added drop wise regulating the temperature to less than 0^0C wit5h stirring. After the addition was over, the reaction mixture was refluxed for 16 hrs. The reaction mixture was poured into ice cold water to obtain 6a as solid which was collected, dried and recrystallized from dioxane. The compounds **6b-g** were synthesized from **5b**-g by the similar method. The sequence of reactions is presented in the scheme-1

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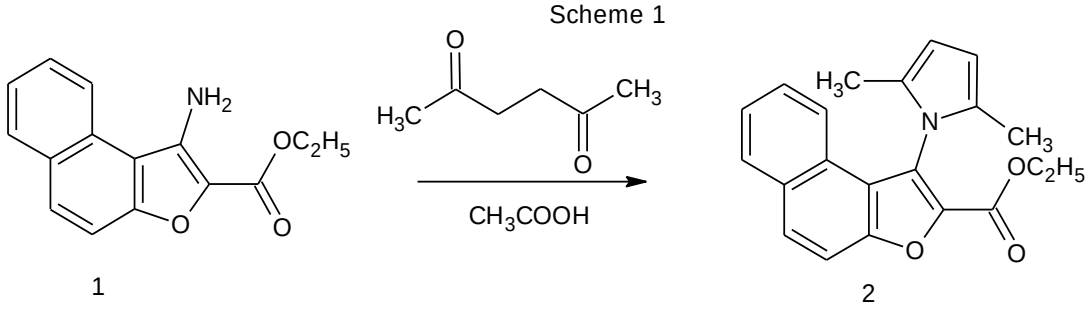
Scheme 1



R

a = 4 - OCH_3 -C_6H_4

b = C_6H_5

c = 3 - NO_2 -C_6H_4

d = 2 - Cl -C_6H_4

e = 4 - Cl -C_6H_4

The analytical and physical data of newly synthesized compounds are presented in Table-1

ISSN: 0975-8585

Table-1-Physical and Analytical Data of Newly Synthesized Compounds

Compound	R	Molecular formula	m.p. ^{0}C	Yield (%)	Found(Cald.) %		
					C	H	N
5a	4-OCH_3-C_6H_4	$C_{28}H_{23}N_5OS$	190	62	68.01 (68.15)	4.58 (4.66)	14.05 (14.19)
5b	C_6H_5	$C_{27}H_{21}N_5OS$	178	85	69.85 (69.97)	4.49 (4.53)	15.08 (15.11)
5c	3-NO_2-C_6H_4	$C_{27}H_{20}N_6O_3S$	185	90	63.69 (63.77)	3.88 (3.93)	16.47 (16.53)
5d	2-Cl-C_6H_4	$C_{27}H_{20}N_5OSCl$	162	59	65.10 (65.12)	4.00 (4.02)	14.02 (14.07)
5e	4-Cl-C_6H_4	$C_{27}H_{20}N_5OSCl$	221	72	65.09 (65.12)	4.01 (4.02)	14.04 (14.07)
6a	4-OCH_3-C_6H_4	$C_{30}H_{24}N_5O_3$	210	76	71.68 (71.71)	4.69 (4.78)	13.87 (13.94)
6b	C_6H_5	$C_{29}H_{22}N_5O_2$	189	65	73.63 (73.72)	4.61 (4.66)	14.33 (14.83)
6c	3-NO_2-C_6H_4	$C_{29}H_{21}N_6O_4$	201	88	67.29 (67.31)	4.02 (4.06)	16.18 (16.24)
6d	2-Cl-C_6H_4	$C_{29}H_{21}N_5O_2Cl$	170	79	68.68 (68.70)	4.11 (4.14)	13.79 (183.82)
6e	4-Cl-C_6H_4	$C_{29}H_{21}N_5O_2Cl$	230	60	68.67 (68.70)	4.12 (4.14)	13.78 (13.82)

Table-2 Antibacterial activity of the synthesized compounds

Compd	R	Zone of Inhibition in mm			
		B. subtilis	***E .coli***	***Pseudomonas spp.***	***S .aureus***
5a	4-OCH_3 C_6H_4	10	10	5	5
5b	-C_6H_5	-	5	10	-
6a	4-OCH_3 C_6H_4	15	5	15	10
6b	-C_6H_5	15	10	20	15
6c	3-NO_2- C_6H_4	15	10	10	10
6d	2-Cl- C_6H_4	5	5	10	-
6e	4-Cl-C_6H_4	5	10	-	5
Standard	Gentamycin	12	-	-	-
Standard	Amphicilin	-	20	15	16
Control	DMF	-	-	-	-

Antimicrobial Activity

The *in vitro* antimicrobial activity was carried out against 24 h old cultures of four bacteria by cup-plate method [28]. The compounds **5a-b** and **6a-e** have been investigated for their antibacterial activity against *Bacillus subtilis, Escherichia coli, Pseudomonas spp* and *Staphylococcus aureus.* Gentamycin and Amphicillin were used as standards for comparison for antibacterial activity. The compounds were tested at a concentration of 100 µg/ml in DMF

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against all organisms. The zone of inhibition was compared with the standard drug after 24 h of incubation at 25 ^{0}C for antibacterial activity. The results are presented in Table 2.

RESULTS AND DISCUSSION

In order to connect pyrrole ring system to naphtho[2,1-b]furan moiety, ethyl 3-aminonaphtho[2,1-b]fruan-2-carboxyalate **1** was thought to be an excellent starting material, which was synthesized by well established procedure in our laboratory [29]. Thus the amino ester **1** was reacted with acetonyl acetone in acetic acid to obtain ethyl 3-(2, 5-dimehtylpyrrol)naphtho[2,b]furan-2-caroxyalate **2** in good yield. The conspicuous absence of predominant absorption band at 3413 cm^{-1} in the IR spectrum of **2** indicated the involvement of amino group of **1** in the reaction. Its ^{1}H NMR spectrum showed peaks as triplet at δ 1.1, quartet at δ 4.1 due ester group protons, two singlets at δ 4.94 and δ 5.03 due to methyl protons, two more singlets at δ 4.76 and δ 4.98 due to the protons of pyrrole ring and a multiplet at δ 7.5-8-9 due to six aromatic protons. To introduce 1,2,4-triazole heterocyclic system, the ester **2** was first treated with hydrazine hydrate to get corresponding hydrazide **3**. The IR spectrum of the compound **3** exhibited characteristic absorption bands at 3302 cm^{-1}, 3247 cm^{-1} , and 1667 cm^{-1} due to –NH, -NH_2 and C=O groups respectively. Its ^{1}H NMR showed the absence of triplet and quartet due to –CH_2CH_3 protons of ester group. The hydrazide **3** on subsequent reaction with carbon disulphide and potassium hydroxide resulted in the formation of 5-[3-(2,5-dimethylpyrrol) naphtho[2-b]furan-2-yl]-4-amino-1,2,4-triazole-3-thiol **4**. The absorption bands due to -NH_2, -SH and C=N groups appeared at 3328 cm^{-1}, 2922 cm^{-1}and 1621 cm^{-1} in the IR spectrum of the compound **4.** The ^{1}H NMR spectrum of the compound **4** exhibited peaks as a singlet at δ 6.0, as multiplet at δ 7.6 and another singlet δ 14.1 integrating for two protons of -NH_2 group, six aromatic protons and one proton of –SH group. The triazole **4** on treatment with various substituted aromatic aldehydes underwent condensation and produced corresponding Schiff bases **5a-f** in good yield. The structures assigned to these Schiff bases were established by spectral studies. Thus, the IR spectrum of the compound **5a** showed absorption bands at 1667 cm^{-1} and 1610 cm^{-1} due to the stretching frequencies of C=N groups. The synthesis of desired compounds i.e. 3-(2,3-dimethylpyrrol-4{[4-(aryl)methylene]amino}-5-naphtho[2,1-b]furan-2-yl-(4H-1,2,4 azetidin-2-ones **6a-f** was achieved by the base catalyzed reaction of Schiff bases **5a-f** with chloro actyl chloride. The structures assigned to the compounds have been confirmed by IR and ^{1}H NMR spectral data which is presented in Table-3.

Table 3- Spectral data of synthesized compounds 6 a-e

Comp.	R	IR (KBr) cm^{-1}		^{1}H NMR in ppm
		C=O, amide	C=O, Keto	
6a	4-OCH_3 C_6H_4	1600	1715	d 3.9 (s, 3H, OCH3),d 4.1 (s, 1H, SH), δ 6.9-8.6 (m, 14H, 12ArH+CHPh+CHCl),
6b	-C_6H_5	1585	1723	δ 4.3 (s, 1H, SH), δ 7.4-8.6 (m, 15H, 13ArH+CHPh+CHCl),
6c	3-NO_2 C_6H_4	1598	1716	δ 4.2 (s, 1H, SH),δ 7.1-8.5 (m, 14H, 12ArH+CHPh+CHCl),
6d	2-Cl C_6H_4	1606	1720	δ 4.5 (s, 1H, SH) δ 7.4-8.4 (m, 14H, 12ArH+CHPh+CHCl),
6e	4-Cl C_6H_4	1610	1710	δ 4.1(s, 1H, SH) δ 7.2-8.6 (m, 14H, 12ArH+CHPh+CHCl),

The derivatives of 2,5-dimethylpyrroles were also known to exhibit wide spectrum of biological and pharmacological activities. Hence, it was intrigued to evaluate newly synthesized compounds for antimicrobial activity by adopting literature procedure. The newly synthesized compounds were evaluated for antimicrobial activity by cup-plate method. Antibacterial activity was carried out against *Bacillus Subtilis, Escherichia coli, Pseudomonas spp* and *Staphylococcus aureus*. Gentamycin and Amphicillin were used as standards. Zone of inhibition was measured in mm and results are presented in Table 2. The compound **6a, 6b, 6c** showed excellent activity against *Bacillus subtilis.* The compound **6b** showed moderate activity against *Escherichia coli* and the compound **6b** exhibited promising activity against *Staphylococcus aureus.* The title compounds **6 a-e** were synthesized and characterized by analytical and spectral studies. The newly synthesized compounds were evaluated for antibacterial activity. The biological profile of the compounds revealed that the coupling of four different heterocycles naphtho[2,1-b]furan, pyrrole, 1,2,4-triazole and azetidinones produced novel heterocyclic compounds which enhanced the activity to a considerable extent. The presence of electron donating and electron withdrawing groups has marked influence in enhancing activity. Hence, there is plenty of scope for systematic study to evaluate the compounds for other biological and pharmacological activities such as anti-fungal, antiviral, anti-inflammatory and analgesic activities. The substitutions can be varied to obtain more potent compounds.

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