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Note

Synthesis, characterization and pharmacological studies on some triazolothiadiazines and triazolothiadiazoles containing naphtho[2,*b*]furan

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The naphtho[2,1-*b*]furan-2-carbohydrazide **1,** on reaction with carbon disulphide and ethanolic potassium hydroxide followed by treatment with hydrazine hydrate/phenyl hydrazine give 4-amino/anilino-5-naphtho[2,1-*b*]furan-2-yl-4*H*-1,2,4-triazole-3-thiols **2a**,**b**. The compounds **2a,b** when treated with chloroacetic acid undergo cycloaddition to produce 3-naphtho[2,1-*b*]furan-2-yl-5-*H*/aryl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6(7*H*)-ones **3a**,**b**. Similar type of cycloaddition occurs on refluxing **2a**,**b** with carbon disulphide in ethanolic potassium hydroxide. The resulting biheterocyclic compounds are identified as 3-naphtho[2,1-*b*]furan-2-yl-5-*H*/aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-6(5*H*)-thiones **4a**,**b**. 3-Naphtho[2,1-*b*]furan-2-yl-6-aryl/5,6-diaryl-5,6-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles **5a-p** and 3-naphtho[2,1-*b*]furan-2-yl-6-aryl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles **6a-d** are obtained in good yield on reacting **2a**,**b**, with appropriate aldehydes and with carboxylic acids separately. The newly synthesized compounds have been characterized by elemental analysis and spectral studies. The few selected compounds have been evaluated foe antimicrobial and analgesic activity.

Keywords: Naphtho[2,1-*b*]furan, triazole, triazolothiadiazines, triazolothiadiazole, antimicrobial activity, analgesic activity

The heterocyclic systems encompassing 1,2,4-triazole, 1,3,4-thiadiazole and thiadiazines are explored to the maximum extent owing to their wide spectrum of pharmacological activities, such as fungicidal, insecticidal, bactericidal, herbicidal[1], anti-tumor[2], anti-inflammatory[3], antiviral[4] and CNS stimulant properties[5]. Similarly, various derivatives of naphtho[2,1-*b*]furan, synthesized in this laboratory have been found to exhibit antimicrobial, antiinflammatory, analgesic and anthelmintic activities[6-12]. It is a common observation that combination of two or more biologically active heterocyclic rings, either in condensed form or coupled form, results in enhancement of biological profile of such compounds by many folds. Guided by this fact, it was contemplated to synthesize new heterocyclic systems involving naphtho[2,1-*b*]furan, 1,2,4-triazole and 1,3,4-thiadiazole, and evaluate them for pharmacological activity.

Results and Discussion

o-Aminothiol functionality was thought to be more appropriate for synthesizing the title compounds. Thus, the required 4-amino/anilino-5-naphtho[2,1-*b*]furan-2-yl-4*H*-1,2,4-triazole-3-thiols **2a**,**b** were synthesized by the reaction of naphtho[2,1-*b*]furan-2-carboxyhydrazide **1** with carbon disulphide and hydrazine hydrate/phenylhydrazine in presence of ethalolic potassium hydroxide. Formation of **2a**,**b** was evident from the absence of carbonyl absorption frequency in their IR spectra. Three different synthetic strategies were adopted to construct bridgehead thiadiazole moiety on triazole ring system. In the first method, compounds **2a**,**b** were reacted with carbon disulphide in presence of ethanolic potassium hydroxide and the product obtained were identified as 3-naphtho[2,1-*b*]furan-2-yl-5-*H*/aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-6(5*H*)-thiones **4a**,**b** on the basis of spectral data. As expected ^{1}H NMR spectrum of **4a** exhibited only two signals, one as a multiplet between δ 7.4 and 8.4 due to seven aromatic protons and another as a broad singlet at δ 10.1 due to –NH proton. The spectrum was conspicuous by the absence of D_2O exchangeable broad singlet at δ 6.05 due to two –NH_2 protons, which was observed in its precursor **2a**. IR and mass spectral data of **4a** was consistent with the assigned structure. Similarly ^{1}H NMR spectrum of **4b** showed only a multiplet at δ 6.74-8.49 attributed to twelve aromatic protons. Primary molecular ion peak was observed at *m/z* 400 corresponding to its molecular weight in its mass spectrum. Fragmentation pattern was also in accordance with theoretical expectation.

In the second approach, the compounds **2a,b** were heated with appropriate aldehydes in presence of DMF, which resulted in the formation of a series of compounds, *i.e.,* 3-naphtho[2,1-*b*]furan-2-yl-6-aryl/5,6-diaryl-5,6-dihydro[1,2,4]tri azolo[3,4-*b*][1,3,4]thiadiazoles **5a-p**. ^{1}H NMR spectra of representative compounds **5c**, **5f**, **5h**, **5k**, **5n** and **5p** were recorded and found to support the assigned structure. The compound **5c** exhibited three signals in its ^{1}H NMR spectrum, one at δ 5.51 as singlet for –CH proton, another between δ 7.4-8.38 as multiplet for eleven aromatic protons and a broad singlet at δ 12.6 of –NH proton. The primary molecular ion peak at *m/z* 405 is consistent with the molecular weight. The structure assigned was further confirmed by recording its ^{13}C NMR spectrum.

The third synthetic strategy involved the reaction between **2a** and different aromatic carboxylic acids in presence of phosphorous oxychloride, to obtain 3-naphtho[2,1-*b*]furan-2-yl-6-aryl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles **6a-d** in good yield.

In the ^{1}H NMR spectrum of **6a**, only a multiplet at δ 7.33-8.35 integrating for twelve aromatic protons was observed. Molecular ion peak at *m/z* 368 and corresponding fragment ion peaks appeared in its mass spectrum, which substantiated the assigned structure. Similarly, ^{1}H NMR of **6b** exhibited two signals, one as D_2O exchangeable broad signlet at δ 6.41 due to two protons of $-NH_2$ group and another one as multiplet at δ 7.59-8.43 due to eleven aromatic protons. The mass spectrum of **6b** showed primary molecular ion peak at *m/z* 384 corresponding to its molecular weight and other fragment peaks at *m/z* 351 (-S), 248 ($-C_6H_4-NH_2$), 193 ($-N_2$, HCN), 143 (-CN, -C_2H_2) and 105 (-CO, -CH), which were in accordance with the expected fragmentation pattern.

To demonstrate the synthetic utility of **2a**,**b** in the synthesis of various bridgehead heterocycles, these compounds were refluxed with chloroacetic acid and fused sodium acetate in ethanol. This reaction yielded 3-naphtho[2,1-*b*]furan-2-yl-5*H*-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6(7*H*)-ones **3a**,**b**. The appearance of absorption band at 1673 cm^{-1} due to ring carbonyl in the IR spectrum of **3a**, three signals in its ^{1}H NMR spectrum and molecular ion peak at *m/z* 322 proved the assigned structure (**Scheme I**). The characterization data for all the compounds are tabulated in **Table I**.

Antimicrobial activity

The antimicrobial activity of some selected compounds were determined using cup plate method[13]. The *in vitro* antibacterial activity was carried out against 24 hr old culture of *Escherichia coli*, *Micrococus luteus* and *Staphylococcus aureus*. The fungi used were *Aspergellius flavus*, *Aspergillus niger* and *Curvuliaria lunata*. The compounds were tested at a concentration of 0.001 mole/mL in DMF using Chloramphenicol and Fluconazole as standard for antibacterial and antifungal activity respectively.

The results revealed that all the newly synthesized compounds exhibited promising antibacterial activity against all the tested organism. The compound **4a** showed equipotent activity against all the three fungal strains, when compared with the standard drug. The results are presented in **Table II**, which indicate that the activity is independent of nature of substituent in the compounds.

Analgesic activity

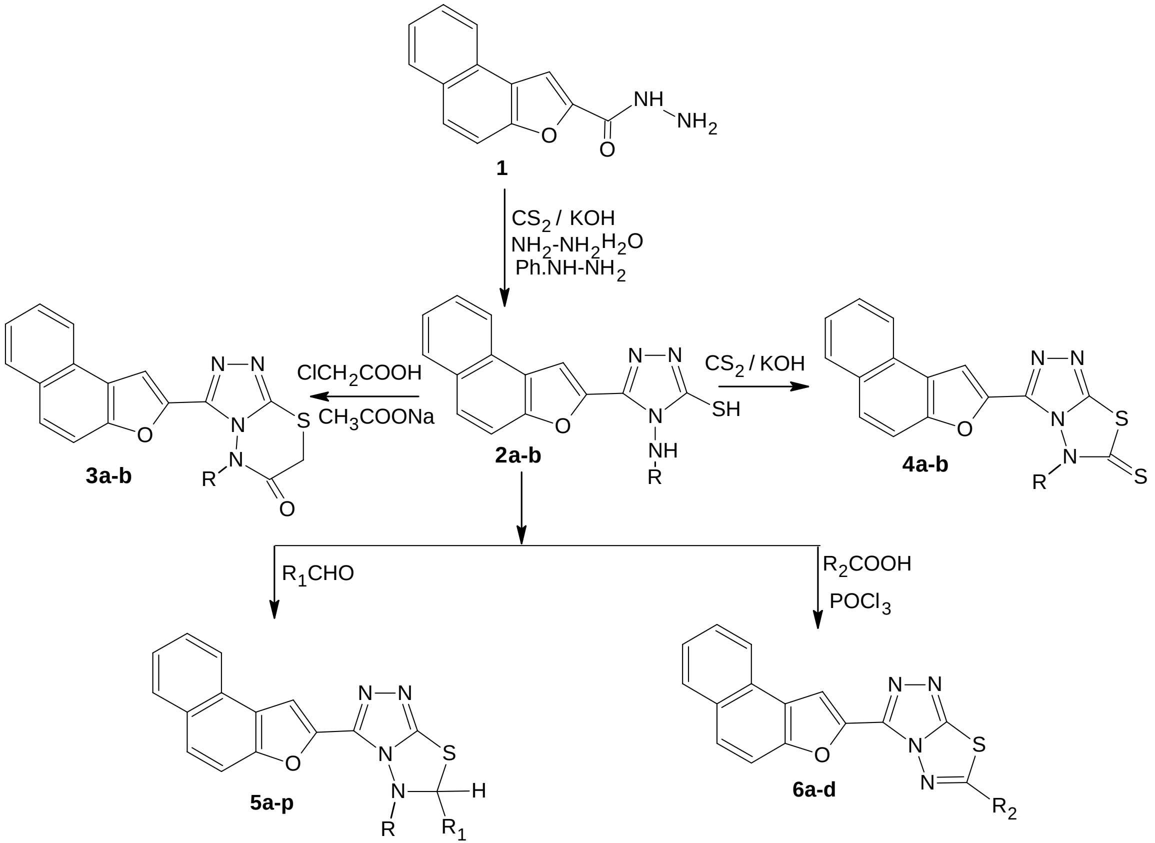
Colony bred albino mice (Swiss strain) of either sex weighing 22-35 g were used to evaluate analgesic activity. It was determined as described by the method based on acetic acid induced writhing response in mice[14].

For this experiment 42 mice were used and they were divided into 7 groups containing 6 animals each. All the animals received 0.6% v/v of 10 mL/kg body weight of acetic acid intraperitoneally and number of writhings was recorded after 10 min upto next 15 min. The same groups of animals were used next day for evaluating analgesic activity.

Group I received 0.5 mL Tween-80 (0.1%) and served as control, group II received 100 mg/kg body weight of acetyl salicylic acid (aspirin) orally and served as standard. The remaining 5 groups received various test compounds at a dose of 100 mg/kg body weight orally in the form of suspension in 0.1% Tween-80. After 1 hr, all the animals received 0.6% of 10 mL/kg body weight of acetic acid intraperitoneally. The writhings were counted similarly as in the previous day. The results are presented in **Table III**.

Experimental Section

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr pellets on FT-IR Research spectrophotometer series and Perkin-Elmer FT-IR (spectrum 1000); ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX (500 MHz) spectrometer using DMSO-d_6 or $CDCl_3$ as solvent and TMS as an internal standard (chemical shifts in δ) and mass spectra were recorded on a GC-MS instrument. Compounds were checked



	R
a	H
b	Ph

5	R$_1$	6	R$_2$
a,**i**	C_6H_5	**a**	C_6H_5
b,**j**	3-Cl-C_6H_4	**b**	4-NO_2-C_6H_4
c,**k**	4-Cl-C_6H_4	**c**	4-Cl-C_6H_4
d,**l**	3-OH-C_6H_4	**d**	4-NH_2-C_6H_4
e , **m**	4-OH-C_6H_4		
f , **n**	4-OCH_3-C_6H_4		
g , **o**	3-NO_2-C_6H_4		
h , **p**	4-NO_2-C_6H_4		

Scheme I

for their homogeneity by TLC on silica gel G plates and spots were visualized in iodine vapour. Satisfactory C, H, N analysis were obtained for all the compounds.

4-Amino/anilino-5-naphtho[2,1-*b*]furan-2-yl-4*H*-1,2,4-triazole-3-thiols, 2a,b

An ice-cold solution of potassium hydroxide (0.015 mole) in ethanol (50 mL) was treated with naphtho[2,1-*b*]furan-2-carboxyhydrazide **1** (0.01 mole) and carbon disulphide (0.02 mole) with constant stirring. The reaction-mixture was stirred further at RT for 12 hr. The product that separated as solid was filtered and washed with dry ether. It was then refluxed with hydrazine hydrate/phenyl hydrazine (0.03 mole), in ethanol (50 mL) on water bath until the evolution of hydrogen sulphide ceased (about 8 hr). The reaction-mixture was then poured into ice-cold water, acidified with glacial acetic acid and purified by recrystallization from aqueous ethanol to obtain **2a**,**b**.

3-Naphtho[2,1-*b*]furan-2-yl-5-*H*/aryl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6(7*H*)-ones, 3a,b

A mixture of **2a** (0.01 mole), chloroacetic acid (0.01 mole) and fused sodium acetate (0.01 mole) in

Table I — Characterization data of synthesized compounds

Compd	R	R_1	Mol. formula	m.p. °C	Yield (%)	Found % (Calcd)		
						C	H	N
2a	H	-	$C_{14}H_{10}N_4OS$	210	83	59.45	3.51	19.64
						(59.56	3.57	19.85)
2b	C_6H_5	-	$C_{20}H_{14}N_4OS$	208	78	67.00	3.88	15.29
						(67.02	3.94	15.67)
3a	H	-	$C_{16}H_{10}N_4O_2S$	245	70	59.49	3.05	17.25
						(59.62	3.13	17.38)
3b	C_6H_5	-	$C_{22}H_{14}N_4O_2S$	251	61	66.21	3.42	14.01
						(66.32	3.54	14.02)
4a	H	-	$C_{15}H_8N_4OS_2$	238	69	55.32	2.34	17.12
						(55.54	2.49	17.27)
4b	C_6H_5	-	$C_{21}H_{12}N_4OS_2$	247	72	62.76	3.00	13.84
						(62.98	3.02	13.99)
5a	H	C_6H_5	$C_{21}H_{14}N_4OS$	234	75	68.02	3.79	15.04
						(68.09	3.81	15.12)
5b	H	3-Cl C_6H_4	$C_{21}H_{13}N_4OSCl$	242	69	62.16	3.14	13.63
						(62.30	3.24	13.84)
5c	H	4-Cl C_6H_4	$C_{21}H_{13}N_4OSCl$	234	54	62.18	3.21	13.29
						(62.30	3.24	13.84)
5d	H	3-OH C_6H_4	$C_{21}H_{14}N_4O_2S$	246	71	65.21	3.54	14.39
						(65.27	3.65	14.50)
5e	H	4-OH C_6H_4	$C_{21}H_{14}N_4O_2S$	249	62	65.13	3.52	14.39
						(65.27	3.65	14.50)
5f	H	4-OCH_3 C_6H_4	$C_{22}H_{16}N_4O_2S$	232	74	65.79	4.01	13.76
						(65.98	4.03	13.99)
5g	H	3-NO_2 C_6H_4	$C_{21}H_{13}N_5O_3S$	223	66	60.45	3.09	16.68
						(60.71	3.15	16.86)
5h	H	4-NO_2 C_6H_4	$C_{21}H_{13}N_5O_3S$	249	59	60.45	3.09	16.68
						(60.71	3.15	16.86)
5i	C_6H_5	C_6H_5	$C_{27}H_{18}N_4OS$	243	73	72.53	3.99	12.42
						(72.63	4.02	12.55)
5j	C_6H_5	3-Cl C_6H_4	$C_{27}H_{17}N_4OSCl$	236	64	67.29	3.28	11.39
						(67.42	3.56	11.65)
5k	C_6H_5	4-Cl C_6H_4	$C_{27}H_{17}N_4OSCl$	223	69	67.33	3.38	11.57
						(67.42	3.56	11.65)
5i	C_6H_5	3-OH C_6H_4	$C_{27}H_{18}N_4O_2S$	238	72	70.02	3.77	12.05
						(70.11	3.92	12.11)
5m	C_6H_5	4-OH C_6H_4	$C_{27}H_{18}N_4O_2S$	236	65	70.10	3.56	12.04
						(70.11	3.92	12.11)
5n	C_6H_5	4-OCH_3 C_6H_4	$C_{28}H_{20}N_4O_2S$	225	81	70.49	4.22	11.64
						(70.57	4.23	11.76)
5o	C_6H_5	3-NO_2 C_6H_4	$C_{27}H_{17}N_5O_3S$	237	74	65.91	3.43	14.20
						(65.98	3.49	14.25)
5p	C_6H_5	4-NO_2 C_6H_4	$C_{27}H_{17}N_5O_3S$	235	71	65.87	3.33	14.19
						(65.98	3.49	14.25)
6a	-	C_6H_5	$C_{21}H_{12}N_4OS$	241	83	68.39	3.18	15.09
						(68.46	3.28	15.21)
6b	-	4-NH_2.C_6H_4	$C_{21}H_{13}N_5OS$	251	65	65.69	3.27	18.16
						(65.78	3.42	18.27)
6c	-	4-Cl.C_6H_4	$C_{21}H_{11}N_4OSCl$	245	73	62.49	2.61	13.76
						(62.61	2.75	13.91)
6d	-	4-NO_2.C_6H_4	$C_{21}H_{11}N_5O_3S$	229	76	61.00	2.57	16.87
						(61.01	2.68	16.94)

Table II — Antimicrobial activity of the some selected compounds

Compd	Antibacterial activity Zone of Inhibition in mm			Antifungal activity Zone of Inhibition in mm		
	E.coli	*M.luteus*	*S.aureus*	*A.flaves*	*A.niger*	*C.lunata*
2a	18	11	10	13	10	12
2b	23	25	23	12	15	15
3a	18	15	14	14	11	12
3b	29	27	26	15	14	09
4a	18	10	09	22	21	22
4b	23	19	12	12	08	10
5c	12	12	20	11	10	12
5e	22	20	22	11	12	11
5f	12	09	11	13	14	08
5k	30	36	35	17	12	15
5n	23	26	24	09	11	15
5p	08	16	12	12	06	10
6b	27	24	23	15	09	12
6d	20	22	20	10	12	09
Standard	38	37	44	16	19	16
DMF	+ve	+ve	+ve	+ve	+ve	+ve

+ ve indicates growth of microbes
Control: DMF (0.01% solution in distilled water)
Standard for antibacterial: Chloramphenicol (0.001 mole/mL)
Standard for antifungal: Fluconazole (0.001 mole/mL)

Table III — Results of analgesic activity of the selected compounds

Compd	Mean no. of writhing ± S.E.M	% Protection
Control	42.15 ± 3.16	-
Aspirin	10.00 ± 0.22	80.00
2a	15.31 ± 1.96	63.67
3a	17.84 ± 2.14	57.67
4a	18.23 ± 2.18	56.74
5c	16.43 ± 2.02	61.07
5e	14.78 ± 1.83	64.93

Index for analgesic activity
Method: Acetic acid induced writhing method
Animals: Albino mice
No. of animals per group: 6
Route of administration: ip
Standard: Acetyl salicylic acid (Aspirin)

absolute ethanol (50 mL) was heated under reflux for 6 hr and cooled in ice. The solid thus separated was filtered, washed thoroughly with water and purified by recrystallization from ethanol to yield **3a**. Similarly **3b** was synthesized starting from **2b**.

3-Naphtho[2,1-*b*]furan-2-yl-5-*H*/aryl-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole-6(5*H*)-thiones, 4a,b

Carbon disulphide (0.015 mole) was added dropwise with constant stirring to a solution of **2a** (0.01 mole) in ethanolic potassium hydroxide solution (0.01 mole in 50 mL). The reaction-mixture was heated on a steam bath for about 12 hr until the evolution of hydrogen sulphide ceased. The reaction-mixture was concentrated to one fourth of its volume and poured into ice and acidified with dilute hydrochloric acid. The precipitate thus obtained was filtered, washed with water and purified by recrystallization from pet ether:chloroform (6:4) to get **4a**. Similar reaction of **2b** yielded the product **4b**.

3-Naphtho[2,1-*b*]furan-2-yl-6-aryl/5,6-diaryl-5,6-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles, 5a-p

A mixture of **2a** (0.01 mole) and benzaldehyde (0.01 mole) in DMF (20 mL) was refluxed for 10 hr. The reaction-mixture was then poured into ice-cold water, the solid separated was filtered and purified by recrystallization from absolute ethanol to obtain **5a**. Similarly, **5b-p** were synthesized from **2a** and **2b**.

5f: IR (KBr): 803 (CH), 3224 (NH) cm^{-1}, ^{1}H NMR ($CDCl_3$): δ 3.9 (s, 3H, OCH_3), 7.08-8.6 (m, 12H, ArH and CH), 11.8 (b, 1H, NH); MS: *m/z* 400(M^+). **5h**: IR (KBr): 809 (CH), 3124 cm^{-1} (NH), ^{1}H NMR (DMSO-d_6): δ 7.6-8.7 (m, 12H, ArH and CH), 12.8 (b, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 30.12 (C_2), 112.04-150.16, MS: *m/z* 415(M^+), **5k**: IR (KBr): 1625 cm^{-1} (C=N). ^{1}H NMR ($CDCl_3$): δ 6.77-8.57 (m, 16H, ArH and CH), MS: *m/z* 481(M^+), **5n**: IR (KBr): 804 cm^{-1} (CH), 1618 (C=N). ^{1}H NMR ($CDCl_3$): δ 3.87 (s, 3H, OCH_3), 7.15-8.52 (m, 12H, ArH and CH). ^{13}C NMR (DMSO-d_6): δ 30.12 (C_2), 54.56 (OCH_3), 112.21-152.18, MS: *m/z* 476 (M^+). **5p**: IR (KBr): 819 cm^{-1} (CH), 1629 cm^{-1} (C=N). ^{1}H NMR ($CDCl_3$): δ 7.24-8.43 (m, 12H, ArH and CH). ^{13}C NMR (DMSO-d_6): δ 30.12 (C_2), 110.30-153.08, MS: *m/z* 491 (M^+).

3-Naphtho[2,1-*b*]furan-2-yl-6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles, 6a-d

A mixture of **2a** (0.01 mole) and benzoic acid (0.01 mole) in phosphorous oxychloride (10 mL) was heated in an oil bath at 120°C for 1 hr. The reaction-mixture was then cooled, poured into ice and neutralized with aqueous potassium carbonate solution. The solid thus separated was filtered, washed thoroughly with water and purified by recrystallization from ethanol to yield **6a**. Similarly **6b-d** were prepared from **2a**.

6c: IR(KBr): 1641 cm^{-1} (C=N), ^{1}H NMR ($CDCl_3$): δ 7.31-8.39 (m, 11H, ArH). MS: *m/z* 403 (M^+). **6d**: IR(KBr): 1637 cm^{-1} (C=N). ^{1}H NMR ($CDCl_3$): δ 7.40-8.47 (m, 11H, ArH), ^{13}C NMR ($CDCl_3$): δ 158.87 (C_8), 110.30-153.08, MS: *m/z*, 413 (M^+).

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