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Studies on nitrophenylfuran derivatives Part XII. Synthesis, characterization, antibacterial and antiviral activities of some nitrophenylfurfurylidene-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines☆

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Abstract

Synthesis of four 1-aryl-3-[5-(*p*-nitrophenyl)-2-furyl]-2-propen-1-ones starting from substituted acetophenones and *p*-nitrophenylfurfuraldehyde is described. These propenones were then converted into corresponding dibromo derivatives which on dehydrobromination afforded α-bromopropenones rather than acetylenic ketones. Condensation of these dibromopropanones with 4-amino-5-mercapto-1,2,4-triazoles yielded a new class of nitrophenylfurfurylidene-1,2,4-triazolothiadiazines. The structures of nitrophenylfurfurylidene-1,2,4-triazolothiadiazines were established on the basis of analytical, IR, NMR and mass spectral studies. The formation of 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines rather than 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazepines in the above condensation was unambiguously confirmed by X-ray crystallographic analysis of one of them. A possible mechanism is proposed to account for the formation of nitrophenylfurfurylidene-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines. Some of the newly synthesized triazolothiadiazines were screened for their antibacterial and antiviral properties.

Keywords: Nitrofurans; Nitrophenylfurans; Triazolothiadiazines; Antibacterial activity

1. Introduction

Antibacterial nitrofurans are a class of synthetic compounds characterized by the presence of a 5-nitro-2-furanyl group. The antimicrobial activity of nitrofurans was reported by Stillman et al. [1] and Dann et al. [2] independently. These initial reports, thereafter stimulated research on the synthesis of a large number of compounds belonging to this class and led to the exploration of nitrofurans as potential chemotherapeutic agents. A number of nitrofurans have attained commercial utility as antibacterial agents in human and veterinary medicine because of their broad spectrum antibacterial activity, relatively mild toxicity and low tendency to develop resistance towards bacterial strains [3].

As a part of structural modification of nitrofurans, with a view to reduce the toxicity of nitrofuran derivatives, a nitrophenylfuran moiety is introduced in place of nitrofuran in the compounds to be synthesized. Several nitrophenylfuran derivatives have been synthesized and screened for their antibacterial [4], tuberculostatic [5] and fungistatic [6] properties in recent years. A few arylfuran derivatives including the well known drug Dantrolene possess muscle relaxant and CNS depressant activities [7–15].

Triazoles fused with six-membered ring systems are found to possess diverse applications in the field of medicine, agriculture and industry. The commonly known systems are triazoles fused with pyridines, pyridazines, pyrimidines, pyrazines and triazines. The literature survey reveals that there are not many examples of triazoles fused with thiadiazines. Moreover, a large number of triazolothiadiazines has been shown to exhibit antidepressant [16], central nervous depressant

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[17], bactericidal [18], fungicidal [18], and diuretic activities [19] and as photographic couplers [20].

Further, nitrofuran containing triazolothiadiazines have been prepared [21] and many such derivatives are found to possess antibacterial activities. Interestingly, one such derivative was found to possess anticancer activity too. So, in order to study the structure–activity relationship, it was contemplated to synthesize triazolothiadiazines carrying arylfuran substituents.

Prompted by the varied biological properties of arylfuran derivatives and as a part of our general search for chemotherapeutically active N-bridged heterocycles [22–26], a project aimed at the synthesis of 1-aryl-3-[5-(*p*-nitrophenyl)-2-furyl]-2-propen-1-ones and their derivatives was undertaken. It was also contemplated to employ them in the synthesis of chemotherapeutically important N-bridged heterocycles. The results of such studies are described in this paper.

2. Chemistry

For the present work, four 1-aryl-3-[5-(*p*-nitrophenyl)-2-furyl]-2-propen-1-ones (**3**) were prepared by condensing 5-(*p*-nitrophenyl)-2-furfuraldehyde with acetophenone, *p*-chloroacetophenone, *p*-methyl acetophenone and *p*-methoxyacetophenone in the presence of aqueous sodium hydroxide. These propenones (**3**) were then brominated with bromine in glacial acetic acid to afford 2,3-dibromo-1-aryl-3-[5-(*p*-nitrophenyl)-2-furyl]-propan-1-ones (**4**). Dibromopropanones were then dehydrobrominated in the presence of triethylamine and were then condensed in situ with 3-substituted-4-amino-5-mercapto-1,2,4-triazoles (**7**) in ethanol medium. The condensation products are now identified as 6-aryl-7-[5-(*p*-nitrophenyl)-2-furfurylidene]-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**8**) based on the analytical and spectral data. The structure of one of the condensation products (**8b**) is unambiguously proved by X-ray diffraction studies [27].

An alternate regioisomeric structure for the condensation product in the above reaction is 6-aryl-8-(5-aryl-2-furyl)-1.2,4-triazolo[3,4-*b*]-1,3,4-thiadiazepine (**9**). Hence, it was considered worthwhile to investigate the reaction mechanism of the formation of the compound. In one such approach, it was decided to isolate the intermediate formed during the reaction. Thus, dehydrobromination of 2,3-dibromo-1-aryl-3-(5-aryl-2-furyl)-2 propanones (**4**) employing triethylamine in dry benzene was investigated. One such reaction afforded chemoselectively α-bromo-1-aryl-3-(5-aryl-2-furyl)-2-propenone (**5**) and not the acetylenic ketone (**6**), thus suggesting the loss of only one molecule of HBr during dehydrobromination. This α-bromo-1-aryl-3-(5-aryl-2-furyl)-2-propenone (**5**) was then condensed with 3-substituted-4-amino-5-mercapto-1,2,4-triazoles (**7**) in the presence of ethanolic potassium hydroxide to give regioselectively the 3-substituted-6-aryl-7-(5-aryl-2-furfurylidene)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**8**).

The IR spectrum of the condensation product (**8a**) showed an absorption band at 2926 cm^{-1}, characteristic of the exocyclic vinyl C–H stretching frequency The absorption around 1599 cm^{-1} was assigned to the C=N and C=C groupings. The asymmetric and symmetric stretching frequencies of the nitro group were found at 1509 and 1329 cm^{-1}, respectively. IR spectral data of a few other condensation products were also in conformity with the assigned structure (**8**) (Tables 1, 2 and 4).

The examination of the ^1H NMR spectra of some of the condensation products further confirmed the formation of triazolothiadiazines (**8**). The 270 MHz ^1H NMR spectrum of triazolothiadiazine (**8b**) showed a triplet at δ 1.3 ($J = 7.5$ Hz) and a quartet at δ 2.8 ($J = 7.5$ Hz) confirmed the presence of ethyl group in (**8b**). A sharp singlet at δ 6.9 seen in this ^1H NMR spectrum is assigned to the exocyclic vinyl proton. Two doublets($J = 3.8$ Hz) appearing at δ 7.2 and 7.5 are characteristic of the β-protons of the furan ring. The aromatic protons of the *p*-nitrophenyl ring resonated as two doublets at δ 8.1 and 8.4; while the signals of the

Table 1
Characterization data of 1-aryl-3-[5-(*p*-nitrophenyl)-2-furyl]-2-propen-1-ones (**3**)

Comp.	R	M.p. (°C)	Yield (%)	Molecular formula	Anal. (%), Found (calc.)		
					C	H	N
3a	H	170 [a]	93	$C_{19}H_{13}NO_4$	71.75 (71.47)	4.39 (4.07)	4.64 (4.38)
3b	Cl	212	92	$C_{19}H_{12}ClNO_4$	64.81 (64.49)	3.65 (3.39)	4.28 (3.96)
3c	CH_3	168	95	$C_{20}H_{15}NO_4$	72.34 (72.07)	4.81 (4.50)	4.38 (4.20)
3d	OCH_3	180	89	$C_{20}H_{15}NO_5$	68.93 (68.76)	4.17 (4.29)	4.15 (4.01)

UV (DMF): **3c**, λ_{max} 270 ($\varepsilon = 950$) and 400 nm ($\varepsilon = 4000$). IR (ν, cm^{-1}): **3a**, 1649 (C=O str.), 1585 (C=C str.), 1572 (NO_2 asym.), 1338 (NO_2 sym.); **3c**, 1668 (C=O), 1599 (C=C); **3d**, 1656 (C=O), 1558 and 1331 (NO_2 asym. and sym.). ^1H NMR (90 MHz): **3c**, δ 2.3 (s, 3H, tolyl-CH_3), 6.8 (d, 1H, furan 3H, $J = 3.3$ Hz), 7.1 (d, 1H, furan 4H, $J = 3.3$ Hz), 7.2–7.3 (d, 1H, ethylenic, $J = 15$ Hz), 7.4–7.9 (m, 5H, ethylenic and aromatic protons), 7.9–8.0 (d, 2H, *p*-nitrophenyl, $J = 8$ Hz), 8.1–8.2 (d, 1H, *p*-nitrophenyl, $J = 8$ Hz). Mass, m/z (% abundance): **3a**, 319 (M^+, 100), 273 (M–NO_2, 10), 242 (M–Ph, 18), 197 (M–nitrophenyl, 20) and 105 (Ph–CO^+, 28); **3d**, m/z 349 (M^+, 100).

[a] Although an earlier report [30] gave the m.p. of this compound as 192 °C, we did not observe an m.p. higher than 170 °C.

Table 2
Characterization data of 2,3-dibromo-1-aryl-3-[5-(*p*-nitrophenyl)-2-furyl]-propan-1-ones (**4**)

Comp.	R	M.p. (°C)	Yield (%)	Mol. formula	Colour and crystal form	Anal. (%), Found (calc.)		
						C	H	N
4a	H	265	83	$C_{19}H_{13}Br_2NO_4$	yellow microneedles	48.12 (47.59)	3.34 (2.71)	3.28 (2.92)
4b	Cl	238	84	$C_{19}H_{12}Br_2ClNO_4$	yellow microneedles	44.93 (44.40)	3.12 (2.33)	2.98 (2.72)
4c	CH_3	211–213	97	$C_{20}H_{15}Br_2NO_4$	brown shining microneedles	48.87 (48.41)	3.05 (3.11)	2.85 (2.75)
4d	OCH_3	120–121	87	$C_{20}H_{15}Br_2NO_5$	yellow needles	47.33 (47.16)	2.95 (2.88)	2.76 (2.87)

Mass: **4c**, *m*/*z*, 491,1493/495 (M^+, 2.5%/1.3%/1%), 303 (M–*p*-nitrophenylfuryl cation, 1.3%), 411 (M^+–HBr, 34.2%), 332 (411-Br, 59.4%), 119 (*p*-methyl benzoyl cation, 100%); **4d**, *m*/*z* 507/509/511 (M^+, 25.2%/13.8%/3.4%), 427 (M–HBr, 18.5%), 348 (427-Br, 76.3%), 361/363 (M–NO_2, 2.5%/6.1%), 319 (M–*p*-nitrophenylfuryl cation, 2.6%).

Table 3
Characterization data of α-bromo-1-aryl-3-[5-(*p*-nitrophenyl)-2-furyl]-2-propen-1-ones (**5**)

Comp.	R	M.p. (°C)	Yield (%)	Mol. formula	Anal. (%), Found (calc.)		
					C	H	N
5a	H	172	74	$C_{19}H_{12}BrNO_4$	57.84 (57.28)	3.66 (3.01)	3.87 (3.51)
5b	CH_3	120–122	63	$C_{20}H_{14}BrNO_4$	58.31 (58.39)	3.43 (3.40)	3.41 (3.40)
5c	OCH_3	168–170	44	$C_{20}H_{14}BrNO_5$	56.24 (56.20)	3.29 (3.27)	3.25 (3.27)

IR (cm^{-1}): **5a**, 1650 (C=C str.), 1602 (C=C str.). ^{1}H NMR (400 MHz, DMSO-d_6): **5b**, δ 2.08 (s, 3H, *p*-tolyl-CH_3), 7.27 (s, 1H, olefinic), 7.57–7.58 (d, 1H, furan 3H, $J = 3.4$ Hz), 7.65–766 (d, 1H, furan 4H, $J = 3.91$ Hz), 7.45–7.47 (d, 2H, *p*-tolyl, $J = 7.81$ Hz), 8.03–8.05 (d, 2H, *p*-tolyl, $J = 7.81$ Hz), 8.1–8.12 (d, 2H, *p*-nitrophenyl, $J = 8.79$ Hz), 8.32–8.34 (d, 2H, *p*-nitrophenyl, $J = 8.$ 79 Hz). Mass: **5a**, *m*/*z*, 397/399 (M^+/M+2, 13%/17%), 313 (M–Br, 40%), 105 (Ph–C=O, 100%), 77 (Ph, 43%); **5b**, *m*/*z* 411/413 (M^+/M+2, 5, 4%/4.4%), 332 (M–Br, 100%), 119 (*p*-methylbenzoyl cation, 65%), 91 (*p*-methylphenyl cation, 57%).

remaining aromatic protons appeared as a multiplet centered at δ 7. 6. The NMR spectral data of other triazolothiadiazines are given in Table 4. Due to poor solubility of the condensation products in the solvents commonly used in NMR spectral measurements, the spectra of all the products could not be measured.

The mass spectrum of the compound **8b** showed the expected molecular ion peak at *m*/*z* 443, consistent with the molecular formula $C_{23}H_{17}N_5O_3S$. The base peak was observed at *m*/*z* 29 corresponding to $C_2H_5^+$ ion. The peaks at *m*/*z* 413 and 397 are indicative of the loss of NO and NO_2 radicals from the molecular ion; which is typical of nitrophenyl derivatives. The mass spectrum of compound **8g** showed a very weak molecular ion peak at *m*/*z* 477, corresponding to the molecular formula, $C_{23}H_{16}ClN_5O_3S$. However, a peak at *m*/*z* 431 was observed which can be assigned to a fragment ion (M-46) obtained by the loss of an NO_2 radical from the molecular ion.

To explore the mechanistic pathways of such condensation reactions, the probable intermediate α-bromo-1-phenyl-3-[5-(*p*-nitrophenyl)-2-furyl]-2-propen-l-ones (**5**, R=H, Cl, CH_3 and OCH_3) were prepared starting from dibromopropanones (**4**), employing triethylamine in benzene medium. This α-bromopropenone (**5a**) was then condensed with 3-substituted-4-amino-5-mercapto-1,2,4-triazole (**7**, R^1-*p*-chlorophenyl) and the condensation product after usual work up was identified as 3-*p*-chlorophenyl-6-phenyl-7-[5-(*p*-nitrophenyl)-2-furylidene]-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (**8e**). The samples of **8e** obtained under two different experimental conditions were found to be identical.

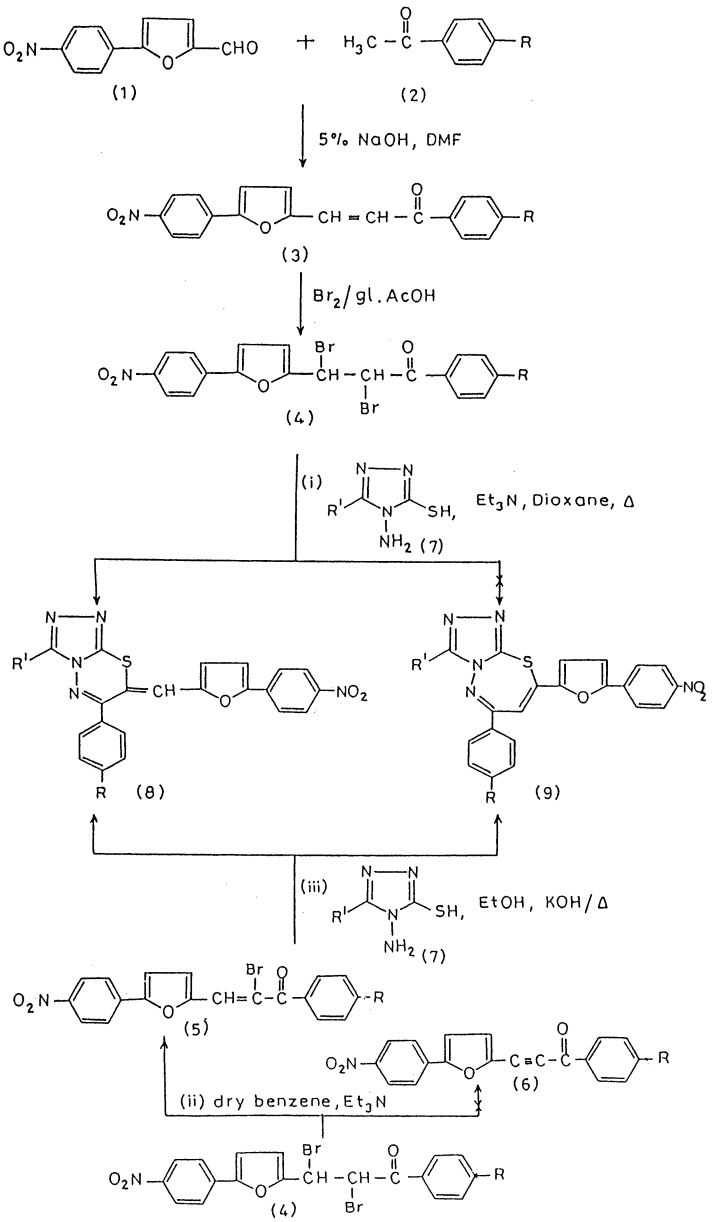
The structures of α-bromopropenones (**5a**,**c**) were confirmed by recording their mass spectra. The molecular ion of (**5c**) appeared at *m*/*z* 411/413 corresponding to the molecular formula $C_{20}H_{14}BrNO_4$ consistent with the structure assigned to it. The M + 2 peak was also observed at *m*/*z* 413 typical of bromine containing compounds. The peak at *m*/*z* 332 was attributed to an ion obtained by the loss of a bromine radical from the molecular ion (M-79). Similarly, the mass spectrum of α-bromopropenone (**5a**) showed a molecular ion at *m*/*z* 397 corresponding to the molecular formula $C_{19}H_{12}BrNO_4$ consistent with the structure assigned to it. The M + 2 peak was observed at *m*/*z* 399 typical of bromine containing compounds. This gave a peak at *m*/*z* 318 by losing a bromine radical from the molecular ion (M-79). The characterization data of these α-bromopropenones are given in Table 3. The structures of α-bromopropenones were also confirmed by recording their IR spectra. The strong absorption band appeared at 1650 cm^{-1} in the IR spectrum of **5** suggested the presence of a C=O group. The absorption at 1602 cm^{-1} could be assigned to the C=C bond. The absence of an absorption band around 2200 cm^{-1} ruled out the possibility of acetylenic ketone formation during dehydrobromination.

The formation of triazolothiadiazines (**8**) and not triazolothiadiazepines (**9**) was also proved by an alternate chemical synthesis of 3-*p*-chlorophenyl-6-phenyl-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (**11**) by condensing 4-amino-5-(*p*-chlorophenyl)-3-mercapto-1,2,4-triazole (**7**) with phenacyl bromide (Schemes 1 and 2).

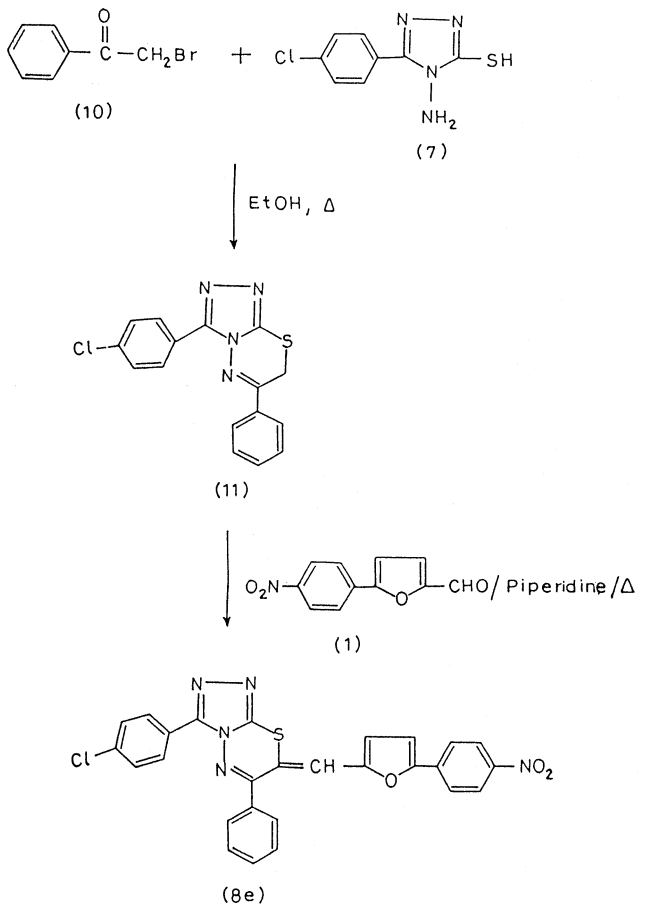
Condensation of triazolo[3,4-*b*]-1,3,4-thiadiazine (**11**) with 5-(*p*-nitrophenyl)-2-furfuraldehyde in the presence of piperidine afforded (**8e**). The IR spectra of samples of **8e** obtained by both methods were found to be superimposable, thus confirming the assigned structure **8** for the condensation product. The structure of **8e** was confirmed by elemental analysis and mass spectral data. The mass spectrum of the compound **8e** showed a molecular ion peak at m/z 525 corresponding to the molecular formula $C_{27}H_{16}ClN_5O_3S$ consistent with the assigned structure. The UV and IR spectra of this compound were also found to be identical with the compound obtained from the one-pot synthesis of **8e** employing chalcone dibromide (**4**) and the corresponding triazole (**7**).

The structures of the compounds (**8**) were unambiguously proved by recording the X-ray crystal structure [27] of the typical compound, 3-ethyl-6-phenyl-7-[5-(*p*-nitrophenyl)-2-furfurylidene]-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (**8b**).

A possible mechanism to account for the formation of 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines rather than



Scheme 1.



Scheme 2.

1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazepines (**9**) in the present investigation can be rationalized by a mechanism involving the initial formation of a Schiff base (**12**) and its subsequent cyclization and dehydrobromination via path (a) as shown in Scheme 3. In the initial stages of the investigation, it was believed that the condensation products were triazolothiadiazepines (**9**). However, X-ray crystallographic analysis of **8b** clearly confirmed that the condensation products are actually triazolothiadiazines (**8**) and not triazolothiadiazepines (**9**). In the present investigation, the formation of triazolothiadiazines (**8**) rather than triazolothiadiazepines (**9**) could also be attributed to the greater degree of inductive and mesomeric effect of *p*-nitrophenylfuran substituent as compared to the aroyl substituent of the α-bromopropenones (**5**).

3. Experimental

Melting points (m.p.s) of the newly synthesized compounds were determined by capillary method and are uncorrected. The UV spectra were recorded in DMF on a Beckman model-24 spectrophotometer. The IR spectra were recorded on a Perkin–Elmer model 529 in nujol mull. ^{1}H NMR spectra of a few selected compounds were recorded on a 270 MHz Bruker WH-200 pulsed FT NMR spectrometer using DMSO-d_6 as solvent and tetramethylsilane as an internal standard. All chemical shift values are expressed in δ scale. Mass spectra of some of the selected compounds were recorded on a JEOL JMS-D 300 mass spectrometer operating at 70 eV.

*3.1. General procedure for the synthesis of 1-aryl-3-[5-(p-nitrophenyl)-2-furyl]-2-propen-1-ones (**3**)*

To a solution of appropriate acetophenone in ethanol, an aqueous solution of sodium hydroxide (5%, 10 ml) was added. The resulting solution was heated to 80 °C and *p*-nitrophenylfurfuraldehyde (2.1 g, 10 mmol) was added with constant stirring. The reaction mixture was kept stirring at this temperature for 3–4 h, cooled to room temperature (r.t.) and was allowed to stand overnight. The solid product separated was collected by filtration, dried and recrystallized from a mixture of dimethylformamide and ethanol to yield the required propenones.

*3.2. General procedure for the synthesis of 2,3-dibromo-1-aryl-3-[5-(p-nitrophenyl)-2-furyl]-propan-1-ones (**4**)*

To a solution of 1-aryl-3-[5-(*p*-nitrophenyl)-2-furyl]-2-propen-1-one (**3**, 10 mmol) in glacial acetic acid, a solution of bromine in glacial acetic acid (10%, 18 ml) was added with constant stirring. The colour of the solution turned orange–red. The stirring was continued at r.t. for 24 h. The separated solid was collected by filtration, washed with ethanol, dried and recrystallized from glacial acetic acid to afford the dibromopropanones (**4**).

*3.3. General procedure for the synthesis of α-bromo-1-aryl-3-[(5-p-nitrophenyl)-2-furyl]-2-propen-1-ones (**5**)*

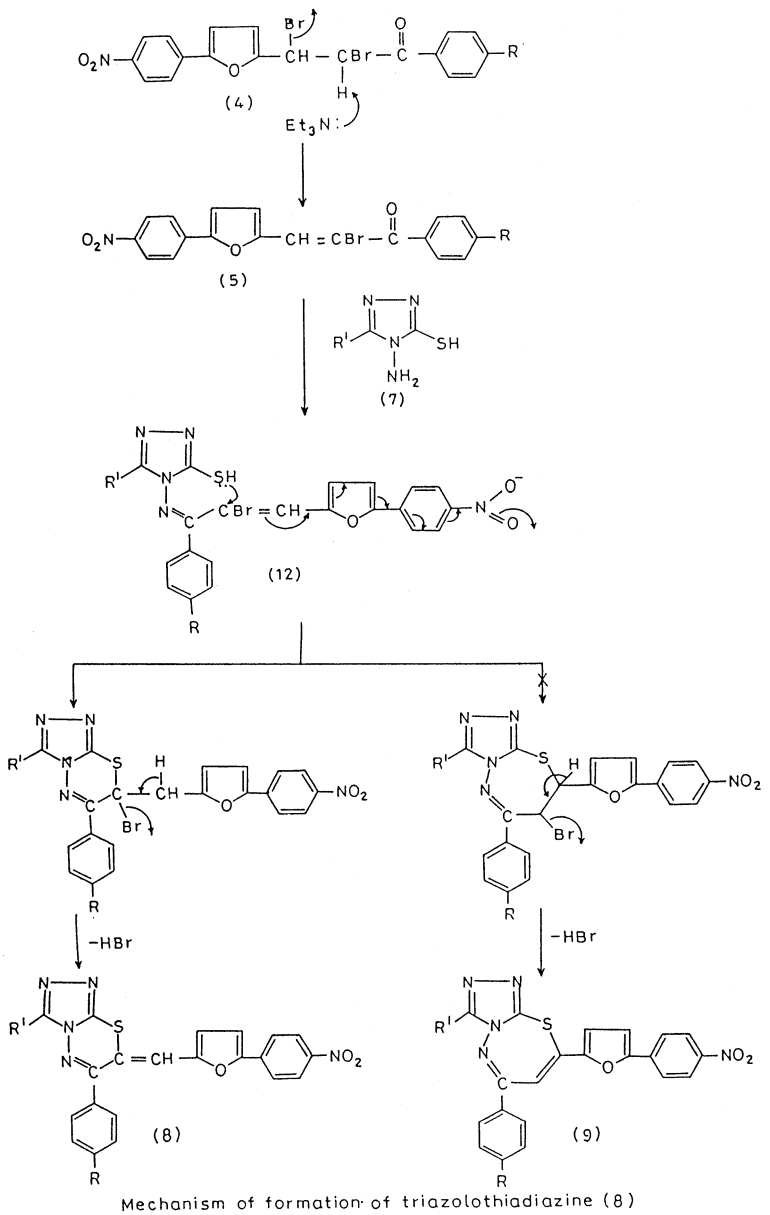
A solution of triethylamine (40 mmol) in dry benzene (30 ml) was added to a solution of 2,3-dibromo-1-phenyl-3-(5-aryl-2-furyl)-propan-1-one (5 g, 10 mmol) in dry benzene (100 ml) with stirring. The reaction mixture was stirred at r.t. for 24 h. After removal of the separated triethylamine hydrobromide, the filtrate was concentrated under reduced pressure to give α-bromo-1-aryl-3-[(5-*p*-nitrophenyl)-2-furyl]-2 propen-1-one (**5**) which was recrystallized from ethanol or a mixture of dimethyl formamide and ethanol. The characterization data of these compounds are given in Table 3.

3.4. Synthesis of 3-substituted-6-aryl-7-[5-(p-nitrophenyl)-2-furfurylidene]-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (8)

A suspension of 2,3-dibromo-1-aryl-3-[5-(*p*-nitrophenyl)-2 furyl]-propan-1-one (**4**, 20 mmol) in ethanol was treated with triethylamine(1 ml) for 2–3 h. Suitably substituted 4-amino-5-mercapto-1,2,4-triazole(**7**, 20 mmol) was then added to the reaction mixture and the contents were refluxed for 6–8 h. On cooling the reaction mixture, yellow to orange–red solid product separated out. It was collected by filtration, washed with water and dried. The product on recrystallization from dimethyl formamide or aqueous dimethyl formamide afforded the title compounds. The characterization data of 1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazines (**8**) prepared according to this general method are given in Table 4.

3.5. Synthesis of 3-substituted-6-aryl-7-[5-(p-nitrophenyl)-2-furfurylidene]-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (8)

A mixture of aminomercaptotriazoles (**7**, 10 mmol), α-bromo-1-phenyl-3-[5-(*p*-nitrophenyl)-2-furyl]-2-propen-1-ones (**5**, 10 mmol) and ethanolic potassium hydroxide (5%, 10 ml) were refluxed in ethanol medium for 6 h and cooled. The solid product thus separated was filtered, washed, dried and recrystallized from a



Scheme 3.

Table 4
Characterization data of 3-substituted-6-aryl-[5-(*p*-nitrophenyl)-2-furfurylidene]-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine (**5**)

Comp.	R^1	R	M.p. (°C)	Yield (%)	Mol. formula	Anal. (%), Found (calc.)		
						C	H	N
8a	Me	H	268	78	$C_{22}H_{17}N_5O_3S$	61.47 (61.25)	3.84 (3.94)	16.31 (16.24)
8b	Et	H	265	80	$C_{23}H_{19}N_5O_3S$	62.21 (62.02)	4.18 (4.26)	15.64 (15.73)
8c	Pr	H	270	75	$C_{24}H_{21}N_5O_3S$	62.96 (62.75)	4.42 (4.58)	15.32 (15.25)
8d	Ph	H	260	76	$C_{27}H_{19}N_5O_3S$	65.87 (65.72)	3.96 (3.85)	14.35 (14.19)
8e	*p*-ClC_6H_4	H	335	81	$C_{27}H_{18}ClN_5O_3S$	61.78 (61.48)	3.33 (3.41)	13.35 (13.28)
8f	Me	Cl	278	84	$C_{22}H_{16}ClN_5O_3S$	56.99 (56.77)	3.49 (3.44)	15.11 (15.05)
8g	Et	Cl	234	76	$C_{23}H_{18}ClN_5O_3S$	57.81 (57.62)	3.76 (3.75)	14.69 (14.61)
8h	Pr	Cl	194	75	$C_{24}H_{20}ClN_5O_3S$	58.61 (58.41)	3.99 (4.05)	14.23 (14.19)
8i	Ph	Cl	326	85	$C_{27}H_{18}ClN_5O_3S$	61.89 (61.48)	3.41 (3.41)	13.35 (13.28)
8j	*p*-ClC_6H_4	Cl	331	87	$C_{27}H_{17}Cl_2N_5O_3S$	57.85 (57.75)	2.96 (3.03)	12.66 (12.47)
8k	Me	Me	122–124	84	$C_{23}H_{17}N_5O_3S$	62.33 (62.30)	3.81 (3.83)	15.78 (15.80)
8l	Et	Me	132–134	88	$C_{24}H_{19}N_5O_3S$	63.04 (62.88)	4.12 (4.15)	15.29 (15.28)
8m	Pr	Me	135–137	87	$C_{25}H_{21}N_5O_3S$	63.71 (63.55)	4.42 (4.45)	14.89 (14.83)
8n	Ph	Me	134–136	81	$C_{28}H_{19}N_5O_3S$	66.49 (66.53)	3.74 (3.76)	13.83 (13.86)
8o	Me	OMe	195–197	74	$C_{23}H_{17}N_5O_4S$	60.09 (60.13)	3.72 (3.70)	15.28 (15.25)
8p	Et	OMe	155–157	78	$C_{24}H_{19}N_5O_4S$	60.83 (60.88)	4.03 (4.01)	14.75 (14.79)
8q	Pr	OMe	163–164	77	$C_{25}H_{21}N_5O_4S$	61.57 (61.60)	4.33 (4.31)	14.36 (14.37)
8r	Ph	OMe	140–141	95	$C_{28}H_{19}N_5O_4S$	64.53 (64.49)	3.62 (3.64)	13.46 (13.43)

UV (λ_{max}): **8c**, 270 nm ($\varepsilon = 1736$), 330 nm ($\varepsilon = 2102$) and 400 nm; **8b**, 2926 (exocyclic vinyl CH),1599 (C=N and C=C), 1509 and CH str.),1602 (C=N and C=C), 1506 and 1335 (NO_2 asym. and sym. str.). ^{1}H NMR (270 MHz): **8b**, δ 1.3 (t, 3H, $J = 7.5$ Hz), 2.8 (q, 2H, CH_2, $J = 7.5$ Hz), 6.9 (s, 1H, exocyclic vinyl proton), 7.2 (d, 1H, furan 3H, $J = 3.8$ Hz), 7.5 (d, 1H, furan 4H, $J = 3.8$ Hz), 8.1 (d, 2H, *p*-nitrophenyl, $J = 8$ Hz), 8.4 (d, 2H, *p*-nitrophenyl, $J = 8$ Hz), 7.6–8 (m, 5H, aromatic protons). ^{1}H NMR (90 MHz): **8e**, δ 7.4 (s, 1H, exocyclic vinyl CH), 7.2–8.4 (m, 11H, furyl and aromatic protons).
^{1}H NMR (90 MHz): **8g**, δ 1.3 (t, 3H, CH_3), 2.8 (q, 2H, CH_2), 6.9 (s, 1H, exocyclic vinyl proton), 7.3–8.6 (m, 10H, furyl and aromatic protons). ^{1}H NMR (400 MHz, DMSO-d_6): **8l**, δ 0.93–0.94 (t, 3H, CH_3, $J = 6.83$ Hz), 1.17–1.19 (q, 2H, CH_2, $J = 7.32$ Hz), 2.09 (s, 3H, *p*-tolyl-CH_3), 7.18 (s, 1H, exocyclic vinyl CH), 7.39–7.41 (d, 2H, *p*-tolyl, $J = 7.81$ Hz), 7.8–7.82 (d, 2H, *p*-tolyl, $J = 8.3$ Hz), 8.16–8.18 (d, 2H, *p*-nitrophenyl, $J = 8.31$ Hz), 8.29–8.32 (d, 2H, *p*-nitrophenyl, $J = 8.3$ Hz), 7.67–7.69 (d, 1H, furan 3H, $J = 3.3$ Hz), 7.72–7.73 (d, 1H, furan 4H, $J = 3.3$ Hz). ^{1}H NMR (400 MHz, DMSO-d_6): **8m**, δ 0.92–0.94 (t, 3H, CH_3, $J = 7.33$ Hz), 1.19–1.21 (t, 2H, CH_2, $J = 7.33$ Hz), 1.75 (m, 2H, CH_2), 6.96 (s, 1H, exocyclic vinyl CH), 2.09 (s, 3H, *p*-tolyl-CH_3), 7.26–7.27 (d, 1H, furan 3H, $J = 3.42$ Hz), 7.5–7.51 (d, 1H, furan 4H, $J = 3.42$ Hz), 7.39–7.41 (d, 2H, *p*-tolyl, $J = 7.81$ Hz), 8.07–8.09 (d, 2H, *p*-tolyl, 7.82 Hz), 8.17–8.19 (d, 2H, *p*-nitrophenyl, $J = 8.79$ Hz), 8.3–8.32 (d, 2H, *p*-nitrophenyl, $J = 8.79$ Hz).
^{1}H NMR (400 MHz, DMSO-d_6): **8p**, δ 1.02–1.07 (t, 3H, CH_3, $J = 6.84$ Hz), 3.5–3.55 (q, 2H, CH_2, $J = 3.9$ Hz), 3.91 (s, 3H, OCH_3), 6.83–6.84 (d, 1H, furan 3H, $J = 3.41$ Hz), 6.98–6.99 (d, 1H, furan 4H, $J = 3.4$ Hz), 7.01–7.03 (d, 2H, aromatic, $J = 8.6$ Hz), 7.88–7.91 (d, 2H, aromatic, $J = 8$ Hz), 7.26 (s, 1H, exocyclic vinyl CH), 8.06–8.09 (d, 2H, *p*-nitrophenyl, $J = 8.79$ Hz), 8.27–8.31 (d, 2H, *p*-nitrophenyl, $J = 8.79$ Hz). Mass: **8o**, m/z 459 (M^+, 19.8%), 428 (M–OCH_3, 2.5%), 133 (*p*-methoxy-benzonitrile, 3.6%), 214 (*p*-nitrophenyl furonitrile, 10.5%); **8r**, m/z 521 (M^+, 0.9%), 77 (phenyl cation, 42.4%); **8b**, 443 (M^+, 50%), 29 (Et^+, 100%), 413 (M–NO_2, 15%), 397 (M–NO_2, 12%), 256 (M–*p*-nitrophenyl-furan, 10.1%); **8g**, m/z 477 (M^+, 2%), 137 (*p*-chlorophenylnitrile, 100%); **8j**, m/z 559/561/563 ($M^+/M+2/M+4$, 50%/49%/30%), 137 (*p*-chlorophenylnitrile, 50%); **8d**, m/z 491 (M^+, 34%).

mixture of dimethylformamide and ethanol to yield triazolothiadiazines (**8**).

*3.6. Authentication of 3-(p-chlorophenyl)-6-phenyl-7-[5-(p-nitrophenyl)-2-furfurylidene]-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (**8e**)*

*3.6.1. Step 1: Synthesis of 3-(p-chlorophenyl)-6-phenyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**11**)*

To a solution of 3-(*p*-chlorophenyl)-5-mercapto-1,2,4-triazole (0.23 g, 10 mmol) in ethanol, phenacyl bromide(0.2 g, 10 mmol) was added and refluxed for 3 h to yield the title compound **11**, m.p. 256 °C, yield, 80%. *Anal.* Calc.: C, 58.81; H, 3.37; N, 17.15; Found: C, 59.12; H, 3.65; N, 17.45%

*3.6.2. Step 2: Synthesis of 3-(p-chlorophenyl)-6-phenyl-7-[5-(p-nitrophenyl)-2-furfurylidene]-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**8e**)*

The compound **11** (0.33 g, 10 mmol) was treated with *p*-nitrophenylfurfural (0.2 g, 10 mmol) in the presence of piperidine (0.1 ml) in ethanol and refluxed for 6 h. The solid separated out was filtered, dried and recrystallized from a mixture of dimethylformamide and ethanol to yield the title compound **8e**.

It was found to be identical with the sample of **8e** prepared by direct condensation of 3-(*p*-chlorophenyl)-4-amino-5-mercapto-1,2,4-triazole (0.22 g, 1 mmol) with α-bromo-1-phenyl-3-[5-(4-nitrophenyl)-2-furyl]-2-propen-1-one (0.4 g, 1 mmol) in ethanol, m.p. 335 °C (mixed m.p. 335 °C). The IR spectra of the 12 samples

obtained were superimposable. Similarly, UV spectra were also found to be identical.

4. Biological activity studies

4.1.1. Antibacterial activity

Some of the newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, according to the serial dilution method [28]. Their minimal inhibitory concentrations (MIC values) were determined. Solutions of the test compounds were prepared in dimethylformamide. Furacin was used as a standard drug for comparison and the solvent control was kept. Results of the screening studies are given in Table 5.

4.1.2. Results and discussion

Among the compounds tested, compound **8f** carrying *p*-nitrophenyl and a methyl group showed excellent antibacterial activities against all the bacteria tested. Compounds **8d**,**e**,**i** also showed good activity against all these four bacteria. Compound **8b** possessed a greater degree of antibacterial activity against *E. coli*, *S. aureus* and *P. aeruginosa* compared to Furacin. Compound **8p** showed good activity against *P. aeruginosa*. However, the antibacterial activities of the remaining compounds were comparable to that of Furacin. Hence, it is worth pursuing these compounds for other biological activities.

Table 5
Antibacterial activity data of 6-aryl-7-[5-(*p*-nitrophenyl)-2-furfurylidene]-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**8**)

Comp.	Minimum inhibitory concentration (μg/ml)			
	E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*
8b	4.4	10.4	11.7	14.1
8d	4.5	9.0	9.1	9.3
8e	4.0	9.0	9.0	9.8
8f	3.3	7.7	7.6	7.8
8i	3.9	8.8	9.2	9.8
8o	12.5	12.5	12.5	12.5
8p	6.0	12.5	6.0	12.5
8q	12.5	12.5	12.5	12.5
8r	12.5	12.5	12.5	12.5
Furacin (standard)	6.0	12.5	12.5	12.5

Table 6
Antiviral activity data of 3-(substituted)-6-[5-(*p*-nitrophenyl)-2-furyl]-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**8**)

Comp.	Effective molar concentration	Percent of control of CEM-IW cells infected with HIV
8a	5.89×10^{-9}	12.02
8e	5.45×10^{-8}	10.15
8o	3.54×10^{-6}	15.99

4.2. Antiviral activity

Some of the selected newly synthesized compounds in the present investigation were screened in vitro for their antiviral properties against human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS). The screening of the test compounds were carried out at eight different molar concentrations [29]. Solutions of the test compounds were injected into culture media containing CEM-IW cells infected with HIV and the percentage of protected cells was measured. The concentrations of the test compounds at which maximum protection of cells occurred and the percentage of protected cells are given in Table 6.

4.2.1. Results and discussion

The antiviral activity of the test compounds, however, gradually decreased at higher concentrations due to their toxicity; hence were inactive as drugs for AIDS.

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