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# Synthesis, spectral characterization and antihaemostatic activity of 1,2,4-triazoles incorporating 1,2,4-triazine rings

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**Abstract.** A simple and high yielding method for the integration of a 1,2,4-triazole ring with 1,2,4-triazine-5-one (**4a–j**) has been developed starting from 3-arylsydnones (**1a–d**). The structures were proved by their spectral data and screened for antihaemostatic activity.

**Keywords.** Sydnones; 1,3-dipolar cycloaddition; cyclocondensation; N-N-biheterocycles; antihaemostatic activity.

## 1. Introduction

Sydnones are some of the few heterocycles that have gained importance recently as they readily undergo ring transformation to various heterocycles by 1,3-dipolar cycloaddition reaction. The 3-arylsydnones not only offer interesting chemistry but their derivatives possess diverse chemotherapeutic properties.[1,2] A number of heterocyclic compounds have been synthesized from 3-arylsydnones.[3–5]

The aim of our present work is to extend the 1,3-dipolar cycloaddition to synthesise *bis*heterocycles containing 1,2,4-triazole and 1,2,4-triazine ring systems. 1,2,4-Triazoles have been reported as potential biologically active agents.[6–10] Triazines find brightening and fibre finishing uses in the textile industry. Triazine derivatives have also been used as chain lengthening agents in polyurethane polymerisation, azodyes, paints, plastic, rubber and also used as fungicides and insecticides.[11] Zhang *et al*[12] have reported the synthesis and antibacterial activity of 4-aryl-3-(1-*p*-chloro-phenyl-5-methyl-1,2,3-triazol-4-yl)-1,2,4-triazolin-5-thiones.

Prompted by these observations, the N-N biheterocycles, viz. 6-methyl-4-(3′-methyl-5′-oxo-1′-aryl-1,5-dihydro-1,2,4-triazol-4′-yl)-3-oxo/thioxo-3,4-dihydro-2*H*-1,2,4-triazin-5-ones (**4a–j**) have been synthesized.

Normally the haemostatic process plays a delicate balance between keeping blood in the fluid state to maintain flow and rapidly forming an occluding plug following vessel injury. Thrombosis occurs because of alteration in this balance. The recent advances in understanding of the haemostatic process have led to design novel antihaemostatic drugs. In the light of this observation, the title compounds were subjected to preliminary antihaemostatic activity.

## 2. Experimental

### 2.1 *Materials, methods and instruments*

Melting points were determined in open capillaries and are uncorrected. IR (KBr) spectra were recorded on Nicolet Impact-410 FT-IR spectrophotometer, NMR spectra ($CDCl_3$) on Varian-300 MHz FT–NMR spectrometer with TMS as internal standard. Purity of the compounds was checked by TLC. Compounds **1a–d** were prepared according to the literature method.[2]

2.2a *6-Methyl-4-(3'-methyl-5'-oxo-1'-phenyl-1,5-dihydro-1,2,4-triazol-4'-yl)-3-oxo-3,4-dihydro-2H-1,2,4-triazin-5-one* (**4a**): A mixture of **2a** (10 mmol) and semicarbohydrazide (**3k**, 10 mmol) in ethanol (10 ml) was refluxed for about 5 h. Pyruvic acid (10 mmol) and glacial acetic acid (20 ml) fused with sodium acetate (2 g) were added and further refluxed for 5 h. The reaction mixture was cooled and poured into ice. The solid obtained was filtered off and recrystallized from ethanol gave colourless needles (2·25 g, 75%) of **4a** (cf. table 1).

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**Table 1.** Characterization data of title compounds **4a–j**.

| Compound | Molecular formula | Yield (%) | m.p. (°C) | Calculated (found) | | |
|---|---|---|---|---|---|---|
| | | | | C | H | N |
| **4a** | $C_{13}H_{12}N_6O_3$ | 75 | 174–5 | 52·00 (51.96) | 4·03 (4·00) | 27·99 (28·01) |
| **4b** | $C_{14}H_{14}N_6O_3$ | 77 | 143–4 | 53·50 (53.49) | 4·49 (4·52) | 26·74 (26·75) |
| **4c** | $C_{14}H_{14}N_6O_4$ | 84 | 201–2 | 50·91 (50.93) | 4·27 (4·25) | 25·44 (25·42) |
| **4d** | $C_{13}H_{11}N_6ClO_3$ | 80 | 165–5 | 46·65 (46.61) | 3·31 (3·32) | 25·11 (25·08) |
| **4e** | $C_{13}H_{11}N_6BrO_3$ | 81 | 138–9 | 41·18 (41.21) | 2·92 (2·89) | 22·16 (22·18) |
| **4f** | $C_{13}H_{12}N_6O_2S$ | 79 | 192–3 | 49·36 (49.34) | 3·82 (3·79) | 26·57 (26·60) |
| **4g** | $C_{14}H_{14}N_6O_2S$ | 85 | 154–5 | 50·90 (50.87) | 4·27 (4·23) | 25·44 (25·41) |
| **4h** | $C_{14}H_{14}N_6O_3S$ | 88 | 226–7 | 48·55 (48.59) | 4·07 (4·02) | 24·26 (24·28) |
| **4i** | $C_{13}H_{11}N_6ClO_2S$ | 82 | 134–5 | 44·51 (44.48) | 3·16 (3·14) | 23·96 (23·95) |
| **4j** | $C_{13}H_{11}N_6BrO_2S$ | 80 | 218–9 | 39·51 (39.50) | 2·81 (2·83) | 21·26 (21·24) |

IR $n_{max}$ (KBr): 1559, 1585, 1654, 1673, 3196 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 1·98 (3H, *s*, $C_6$-$CH_3$), 2·04 (3H, *s*, $C_{3'}$-$CH_3$), 6·50–6·75 (5H, *m*, ArH), 9·0 (1H, *bs*, NH).

$^{13}$C NMR ($CDCl_3$): *d* 16·4 ($C_6$-$\underline{C}H_3$), 18·0 ($C_{3'}$-$\underline{C}H_3$), 119·5–135·6 (Ar$\underline{C}$), 151·0 ($C_{5'}$), 153·0 ($C_6$), 155·0 ($C_{3'}$), 157·0 ($C_3$), 160·0 ($C_5$).

2.2b *6-Methyl-4-(3'-methyl-5'-oxo-1'-p-tolyl-1,5-dihydro-1,2,4-triazol-4'-yl)-3-oxo-3,4-dihydro-2H-1,2,4 triazin-5-one* (**4b**): IR $n_{max}$ (KBr): 1544, 1585, 1640, 1668, 3204 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 2·12 (3H, *s*, $C_6$-$CH_3$), 2·20 (3H, *s*, $C_{3'}$-$CH_3$), 2·28 (3H, *s*, Ar$CH_3$), 6·50 (2H, *d*, *J* = 8·6 Hz, ArH), 6·74 (2H, *d*, *J* = 8·6 Hz, ArH), 10·05 (*bs*, 1H, NH).

$^{13}$C NMR ($CDCl_3$): *d* 15·9 ($C_6$-$\underline{C}H_3$), 17·2 ($C_{3'}$-$\underline{C}H_3$), 117·4–140.6 (ArC), 147·0 ($C_{5'}$), 149·0 ($C_6$), 152·0 ($C_{3'}$), 155·0 ($C_3$), 162·0 ($C_5$).

2.2c *6-Methyl-4-(3'-methyl-5'-oxo-1'-p-anisyl-1,5-dihydro-1,2,4-triazol-4'-yl)-3-oxo-3,4-dihydro-2H-1,2,4 triazin-5-one* (**4c**): IR $n_{max}$ (KBr): 1560, 1595, 1625, 1646, 3370 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 2·10 (3H, *s*, $C_6$-$CH_3$), 2·15 (3H, *s*, $C_{3'}$-$CH_3$), 3·50 (3H, *s*, $OCH_3$), 6·77 (2H, *d*, *J* = 5·9 Hz, ArH), 6·95 (2H, *d*, *J* = 5·9 Hz, ArH), 9·8 (*bs*, 1H, NH).

$^{13}$C NMR ($CDCl_3$): *d* 15·9 ($C_6$-$\underline{C}H_3$), 17·2.0 ($C_{3'}$-$\underline{C}H_3$), 50·7 (O$\underline{C}H_3$), 117·4–140·6 (ArC), 147·0 ($C_{5'}$), 149·0 ($C_6$), 152·0 ($C_{3'}$), 155·0 ($C_3$), 162·0 ($C_5$).

2.2d *6-Methyl-4-(3'-methyl-5'-oxo-1'-p-chlorophenyl-1,5-dihydro-1,2,4-triazol-4'-yl)- 3-oxo-3,4-dihydro-2H-1,2,4 triazin-5-one* (**4d**): IR $n_{max}$ (KBr): 1560, 1600, 1610, 1667, 3185 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 2·25 (3H, *s*, $C_6$-$CH_3$), 2·34 (3H, *s*, $C_{3'}$-$CH_3$), 6·85 (2H, *d*, *J* = 9·1 Hz, ArH), 7·01 (2H, *d*, *J* = 9·1 Hz, ArH), 11·2 (*bs*, 1H, NH).

$^{13}$C NMR ($CDCl_3$): *d* 16·0 ($C_6$-$\underline{C}H_3$), 17·8 ($C_{3'}$-$\underline{C}H_3$), 119·2–139·5 (ArC), 150·0 ($C_{5'}$), 151·9 ($C_6$), 154·0 ($C_{3'}$), 158·0 ($C_3$), 165·0 ($C_5$).

2.2e *6-Methyl-4-(3'-methyl-5'-oxo-1'-p-bromophenyl-1,5-dihydro-1,2,4-triazol-4'-yl)- 3-oxo-3,4-dihydro-2H-1,2,4 triazin-5-one* (**4e**): IR $n_{max}$ (KBr): 1574, 1598, 1625, 1680, 3320 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 2·40 (3H, *s*, $C_6$-$CH_3$), 2·58 (3H, *s*, $C_{3'}$-$CH_3$), 7·12 (2H, *d*, *J* = 6·8 Hz, ArH), 7·25 (2H, *d*, *J* = 6·9 Hz, ArH), 11·0 (*bs*, 1H, NH).

$^{13}$C NMR ($CDCl_3$): *d* 17·5 ($C_6$-$\underline{C}H_3$), 19·5 ($C_{3'}$-$\underline{C}H_3$), 118·7–137·4 (ArC), 152·0 ($C_{5'}$), 154 ($C_6$), 155·0 ($C_{3'}$), 157·0 ($C_3$), 168·0 ($C_5$).

2.3f *6-Methyl-4-(3'-methyl-5'-oxo-1'-phenyl-1,5-dihydro-1,2,4-triazol-4'-yl)-3-thioxo-3,4-dihydro-2H-1,2,4-triazin-5-one* (**4f**): The compound **4f** was prepared by the reaction of **2a** with thiosemicarbohydrazides **3l** followed by the reaction with pyruvic acid as explained in the experimental for **4a** (cf. table 1).

IR $n_{max}$ (KBr): 1310, 1565, 1591, 1620, 3295 $cm^{-1}$.

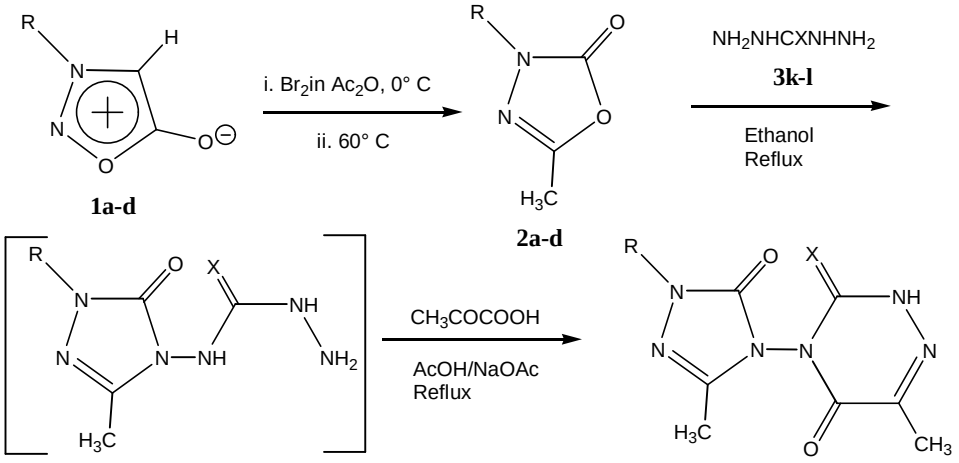
$^1$H NMR ($CDCl_3$): *d* 1·95 (3H, *s*, $C_6$-$CH_3$), 2·04 (3H, *s*, $C_{3'}$-$CH_3$), 7·00–7·64 (5H, *m*, ArH), 11·70 (1H, *bs*, NH).

$^{13}$C NMR ($CDCl_3$): *d* 15·8 ($C_6$-$\underline{C}H_3$), 17·4 ($C_{3'}$-$\underline{C}H_3$), 120·4–138·2 (ArC), 150·0 ($C_{5'}$), 152·0 ($C_6$), 155·0 ($C_{3'}$), 163·0 ($C_5$), 183·0 ($C_3$).

2.3g *6-Methyl-4-(3'-methyl-5'-oxo-1'-p-tolyl-1,5-dihydro-1,2,4-triazol-4'-yl)-3-thioxo-3,4-dihydro-2H-1,2,4-triazin-5-one* (**4g**): IR $n_{max}$ (KBr): 1315, 1570, 1615, 1667, 3250 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 2·00 (3H, *s*, $C_6$-$CH_3$), 2·10 (3H, *s*, $C_{3'}$-$CH_3$), 2·35 (3H, *s*, Ar$CH_3$), 7·04 (2H, *d*, *J* = 6·9 Hz, ArH), 7·15 (2H, *d*, *J* = 6·9 Hz, ArH), 9·7 (1H, *bs*, NH).

$^{13}$C NMR ($CDCl_3$): *d* 15·8 ($C_6$-$\underline{C}H_3$), 17·4 ($C_{3'}$-$\underline{C}H_3$), 120·4–138·2 (ArC), 150·0 ($C_{5'}$), 152·0 ($C_6$), 155·0 ($C_{3'}$), 163·0 ($C_5$), 183·0 ($C_3$).




**1a-d** **2a-d** **4a-j**

**a**; R = $C_6H_5$, X = O, **b**; R = *p*-$CH_3C_6H_4$, X = O, **c**; R = *p*-$OCH_3C_6H_4$, X = O, **d**; R = *p*-$ClC_6H_4$, X = O, **e**; R = *p*-$BrC_6H_4$, X = O, **f**; R = $C_6H_5$, X = S, **g**; R = *p*-$CH_3C_6H_4$, X = S, **h**; R = *p*-$OCH_3C_6H_4$, X = S, **i**; R = *p*-$ClC_6H_4$, X = S, **j**; R = *p*-$BrC_6H_4$, X = S, **k**; X = O, **l** ; X = S.

**Scheme 1.**

2.3h *6-Methyl-4-(3'-methyl-5'-oxo-1'-p-anisyl-1,5-dihydro-1,2,4-triazol-4'-yl)-3-thioxo-3,4-dihydro-2H-1,2,4-triazin-5-one* (**4h**): IR $n_{max}$ (KBr): 1318, 1550, 1605, 1631, 3390 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 2·01 (3H, *s*, $C_6$-$CH_3$), 2·12 (3H, *s*, $C_{3'}$-$CH_3$), 3·73 (3H, *s*, $OCH_3$), 6·75 (2H, *d*, *J* = 7·4 Hz, ArH), 7·03 (2H, *d*, *J* = 7·4 Hz, ArH), 12·0 (1H, *bs*, NH).

$^{13}$C NMR ($CDCl_3$): *d* 16·0 ($C_6$-$\underline{C}H_3$), 19·0 ($C_{3'}$-$\underline{C}H_3$), 56·0 (O$\underline{C}H_3$), 114·3–140·6 (ArC), 144·0 ($C_{5'}$), 149·0 ($C_6$), 151·0 ($C_{3'}$), 160·0 ($C_5$), 181·0 ($C_3$).

2.3i *6-Methyl-4-(3'-methyl-5'-oxo-1'-p-chlorophenyl-1,5-dihydro-1,2,4-triazol-4'-yl)-3-thioxo-3,4-dihydro-2H-1,2,4-triazin-5-one* (**4i**): IR $n_{max}$ (KBr): 1305, 1572, 1600, 1645, 3199 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 2·12 (3H, *s*, $C_6$-$CH_3$), 2·34 (3H, *s*, $C_{3'}$-$CH_3$), 7·31 (2H, *d*, *J* = 5·2 Hz, ArH), 7·49 (2H, *d*, *J* = 5·2 Hz, ArH), 11·00 (1H, *bs*, NH).

$^{13}$C NMR ($CDCl_3$): *d* 16·9 ($C_6$-$\underline{C}H_3$), 20·5 ($C_{3'}$-$\underline{C}H_3$), 121·8–136·3 (ArC), 151·0 ($C_{5'}$), 153·0 ($C_6$), 157·0 ($C_{3'}$), 164·0 ($C_5$), 186·0 ($C_3$).

2.3j *6-Methyl-4-(3'-methyl-5'-oxo-1'-p-bromophenyl-1,5-dihydro-1,2,4-triazol-4'-yl)-3-thioxo-3,4-dihydro-2H-1,2,4-triazin-5-one* (**4j**): IR $n_{max}$ (KBr): 1275, 1580, 1612, 1640, 3401 $cm^{-1}$.

$^1$H NMR ($CDCl_3$): *d* 2·19 (3H, *s*, $C_6$-$CH_3$), 2·35 (3H, *s*, $C_{3'}$-$CH_3$), 7·26 (2H, *d*, ArH, *J* = 8·0 Hz), 7·40 (2H, *d*, *J* = 8·0 Hz, ArH), 11·75 (1H, *bs*, NH).

$^{13}$C NMR ($CDCl_3$): *d* 19·5 ($C_6$-$\underline{C}H_3$), 21·0 ($C_{3'}$-$\underline{C}H_3$), 124·7–140·3 (ArC), 154·0 ($C_{5'}$), 156·0 ($C_6$), 160·0 ($C_{3'}$), 165·0 ($C_5$), 188·0 ($C_3$).

### 2.3 *Antihaemostatic screening*

Tail bleeding time in conscious mice was used to determine antihaemostatic activity of title compounds.[13] Mice of either sex weighing 20–25 g were divided into seven groups comprising ten mice in each group. Control group received 0·4 ml of 2% gum acacia. Test compounds were administered in 2% gum acacia to the remaining six groups. Thirty minutes after administration of the test compounds tail-bleeding time was measured and the results are shown in table 1.

## 3. Results and discussion

### 3.1 *Preparation of title compounds*

Synthesis of N–N biheterocycles, which is effected sequentially, requires bifunctional precursors, which are not always readily accessible. the 3-arylsydnone **1a–d** served as important synthetic precursor for the synthesis of title compounds.[2] Compound **1a–d** was subjected to bromination at 0°C followed by heating at 60°C underwent 1,3-dipolar cycloaddition with acetic anhydride to form 3-aryl-5-methyl-2-oxo-$\Delta^4$-

1,3,4-oxadiazole (**2a–d**). When the compound **2a–d** was refluxed with **3k** for about 5 h whereby transient ring opening, insertion of terminal nitrogen gave intermediates which underwent cyclisation *in-situ* readily with pyruvic acid in presence of acetic acid fused with sodium acetate to 6-methyl-4-(3′-methyl-5′-oxo-1′-aryl-1,5-dihydro-1,2,4-triazol-4′-yl)-3-oxo-3,4-dihydro-2*h*-1,2,4-triazin-5-one (**4a–e**). Similarly, the compound **4f–j** was prepared using **3l** (scheme 1).

The mechanism of the reaction involves the nucleophillic attack of **3** to carbonyl carbon atom of **2**, which proceeds to the formation of a condensed product that in turn eliminates a molecule of water. The other end of the **3** reacts with pyruvic acid followed by intramolecular cyclocondensation gave N-N bicyclic product (**4a–j**).

The IR, $^1$H and $^{13}$C NMR spectra of the title compounds were in complete agreement with the assigned structure. In case of IR spectral analyses a broad band due to NH group was appeared in all the title compounds between 3185 and 3401 cm$^{-1}$. The amide carbonyl functions appeared as sharp band in the range 1591–1680 cm$^{-1}$. Another band around 1275–1315 cm$^{-1}$ was appeared in the compounds **4f–j** due to C=S group. $^1$H NMR spectral analyses of the title compounds exhibited two singlets at *d* 1·95–2·58 ppm assigned to two methyl groups on $C_6$ and $C_{3'}$ carbons. All the title compounds have shown a broad singlet in the range *d* 9·0–12·0 ppm due to NH. Aromatic protons appeared in the expected range as two doublets (AA′BB′). $^{13}$C NMR spectral analyses showed a number of signals that are consistent with the number of carbons in the molecule.

**Table 2.** Results of antihaemostatic activity of the title compounds **4a–j**.

| Compound | Dose (mg kg$^{-1}$) | Average bleeding time (in seconds ± SEM) |
|---|---|---|
| **4a** | 100 | 158·0 ± 36·5 |
| **4b** | 100 | 144·5 ± 29·6 |
| **4c** | 100 | 170·3 ± 30·5 |
| **4d** | 100 | 180·3 ± 10·3 |
| **4e** | 100 | 370·5 ± 14·6 |
| **4f** | 100 | 359·8 ± 14·3 |
| **4g** | 100 | 403·8 ± 15·6 |
| **4h** | 100 | 400·0 ± 14·3 |
| **4i** | 100 | 445·7 ± 20·5 |
| **4j** | 100 | 470·5 ± 30·2 |
| 2% Gum acacia (control) | 0·4 ml | 89·2 ± 12·8 |
| Indomethacin (standard) | 100 | 491·1 ± 49·5 |

### 3.2 *Antihaemostatic activity*

Antihaemostasis results (table 2) reveal that the final compounds do not show activity more than or equal to standard drug indomethacin. Only compounds containing the C=S group with *p*-bromophenyl substituent **4j** (470·5) and *p*-chlorophenyl substituent **7p** (445·7) show considerable antihaemostatic activity. The oxo derivative **4a–e** exhibits poor antihaemostasis.

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## References

1. Earl J C and Mckney A W 1935 *J. Chem. Soc.* 899
2. Mallur S G and Badami B V 2000 *Farmaco* **55** 65
3. Hiremath U S, Yelamaggad C V and Badami B V 1995 *Indian J. Heterocycl. Chem.* **5** 19
4. Havanur S B and Puranik G S 1985 *Indian J. Chem.* **B24** 864
5. Badami B V and Puranik G S 1974 *Indian J. Chem.* **B12** 671
6. Hosur M C, Talwar M B, Laddi U V, Bennur R S and Bennur S C 1995 *Indian J. Chem.* **B34** 707
7. Hussain M I and Amin M 1986 *J. Indian Chem. Soc.* **63** 317
8. Gokce M, Cakir B, Erol K and Sahin M F 2001 *Arch. Pharm.* **334** 279
9. Duran A, Dogan H N and Rollas S 2002 *Farmaco* **57** 559
10. Xin-Zhi L I and Zong-Xing S I 2002 *Chinese J. Org. Chem.* **22** 178
11. Deohate P P and Berad B N 2005 *Indian J. Chem.* **B44** 638
12. Sun X W, Liang H T and Zhang Z Y 1999 *Indian J. Chem*. **B38** 679
13. Hornstra G, Christ-Hazelholt E, Haddeman E, Ten H F and Nagsteren D H 1981 *Prostaglandins* **21** 727