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Novel synthesis of [1]-benzothiepino[5,4-*b*]pyridine-3-carbonitriles and their anti-inflammatory properties

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Abstract—Reaction of 4-arylmethylene-3,4-dihydro-[1]-benzothiepin-5(2*H*)-ones **1** with malononitrile in the appropriate alcohol in the presence of sodium afforded the 2-alkoxy-4-aryl-5,6-dihydro-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitriles **2** and not the isomeric forms [1]-benzothiepino[4,5-*c*]pyridine-1-carbonitriles **3** in high regioselective manner. The assumed structure of **2** was inferred through independent synthetic reaction of 3,4-dihydro-[1]-benzothiepin-5(2*H*)-one (**4**) with ylidenemalononitriles **5** under the same applied reaction conditions and confirmed by single crystal X-ray diffraction studies. However, reaction of **4** with arylidenecyanothioacetamides **6** in refluxing ethanol in the presence of basic catalyst (piperidine or morpholine) does not afford the expected 4-aryl-3-cyano-5,6-dihydro-[1]-benzothiepino[5,4-*b*]pyridine-2(1*H*)-thiones **7** and instead 4-aryl-3,5-dicyano-6-thioxo-2(1*H*)-pyridinethiolate monohydrates were isolated as piperidinium or morpholinium salts **8**. On the other hand, reaction of **6** with cyanothioacetamide in the presence of a sufficient amount of basic catalyst yielded exclusively 2-amino-4-aryl-3,5-dicyano-2-pyridinethiolates as piperidinium or morpholinium salts **9**. Meanwhile, **7** were prepared through the reaction of **1** with cyanothioacetamide in refluxing ethanol in the presence of a catalytic amount of piperidine. Anti-inflammatory activity screening of the prepared compounds using in vivo acute carrageenan-induced paw oedema in rats exhibited that all the tested compounds possess considerable activity. In addition, few synthesized derivatives reveal remarkable anti-inflammatory properties (**2d**, **k**, **l**) comparable with indomethacin which was used as a reference standard during the pharmacological activity screening studies.

1. Introduction

Inflammation is a normal, essential, protective response to any noxious stimulus that may threaten the host and may vary from a localized reaction to a complex response involving the whole organism. An ideal anti-inflammatory drug should affect only aberrant, uncontrolled inflammation and not interfere with the normal inflammatory response, which is a part of the body's vital defence mechanism to invading microorganisms and other environmental insults.[1] Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for treatment of pain, pyrexia, inflammation, rheumatoid arthritis and osteoarthritis. NSAIDs block biosynthesis of prostaglandins by inhibiting enzyme prostaglandin H_2 endoperoxide synthase or cyclooxygenase (COX).[2–6] Recently, two different cyclooxygenase isoforms have been characterized COX-1 and COX-2. Inhibition of the COX-2 enzyme system results in anti-inflammatory action. Meanwhile, COX-1 is a constitutive enzyme and produces 'housekeeping' prostaglandins critical to the maintenance of normal renal function, gastric mucosal integrity, vascular hemostasis and the autocrine response to circulating hormones. This explains the observed side effects of COX-1-inhibitory anti-inflammatory active drugs as gastrointestinal ulcer and renal dysfunction.[7–16] Recent studies revealed that, in addition to arthritis and pain, cancer as well as neurodegenerative diseases like Alzheimer's disease could be potentially treated with COX-2 inhibitors.[12,17–22] On the bases of these findings, many publications intensively appeared searching for novel selective COX-2 inhibitors.

In the present work, it is intended not only to investigate synthesis of [1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile analogues but also to evaluate their anti-inflammatory properties. The interest in this study is originated by the fact that limited number of publications reported

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synthesis of the target heterocyclic nucleus which prompted the present investigation for establishing not only a facile synthetic approach but also a regioselective one. In addition, previous reports described valuable pharmacological importance of many other benzothiepinopyridine isomeric forms such as farnesyl protein transferase inhibitors,[23–25] PAF- and histamine-induced bronchospasm[26,27] in addition to anti-inflammatory as well as analgesic activities.[28–32] In a recent publication, descriptions for synthesis of 2-amino-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile-7,7-dioxides were reported through reaction of malononitrile with 4-arylidene-3,4-dihydro-[1]-benzothiepin-5(2*H*)-one-1,1-dioxide derivatives in presence of ammonium acetate which were found to be anticancer as well as anti-HIV active agents.[33]

2. Results and discussion

2.1. Chemistry

Reaction of 4-arylmethylene-3,4-dihydro-[1]-benzothiepin-5(2*H*)-ones **1** with malononitrile in the appropriate alcohol in the presence of sodium afforded colourless products. The structures of which were established to be 2-alkoxy-4-aryl-5,6-dihydro-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitriles **2** or their isomeric forms 2-alkoxy-4-aryl-5,6-dihydro-[1]-benzothiepino[4,5-*c*]pyridine-1-carbonitriles **3** based on spectroscopic and elemental analyses data. The IR spectra reveal the absence of any band assignable for carbonyl function. However, a strong nitrile stretching vibration band at ν = 2229–2219 cm^{-1} is observed. ^{1}H NMR spectra exhibit the alkoxide residue confirming the involvement of either methoxide (singlet at δ = 4.05–4.15) or ethoxide (triplet at δ = 1.39–1.50 and quartet at δ = 4.50–4.61) functions derived from the corresponding alcohol used in the reaction. In addition, each of the methylene protons at C-5 and C-6 appears as triplet signals at δ = 2.42–2.64, 3.11–3.50 regions, respectively, with a mutual coupling constant value J = 6.3–6.6 Hz.

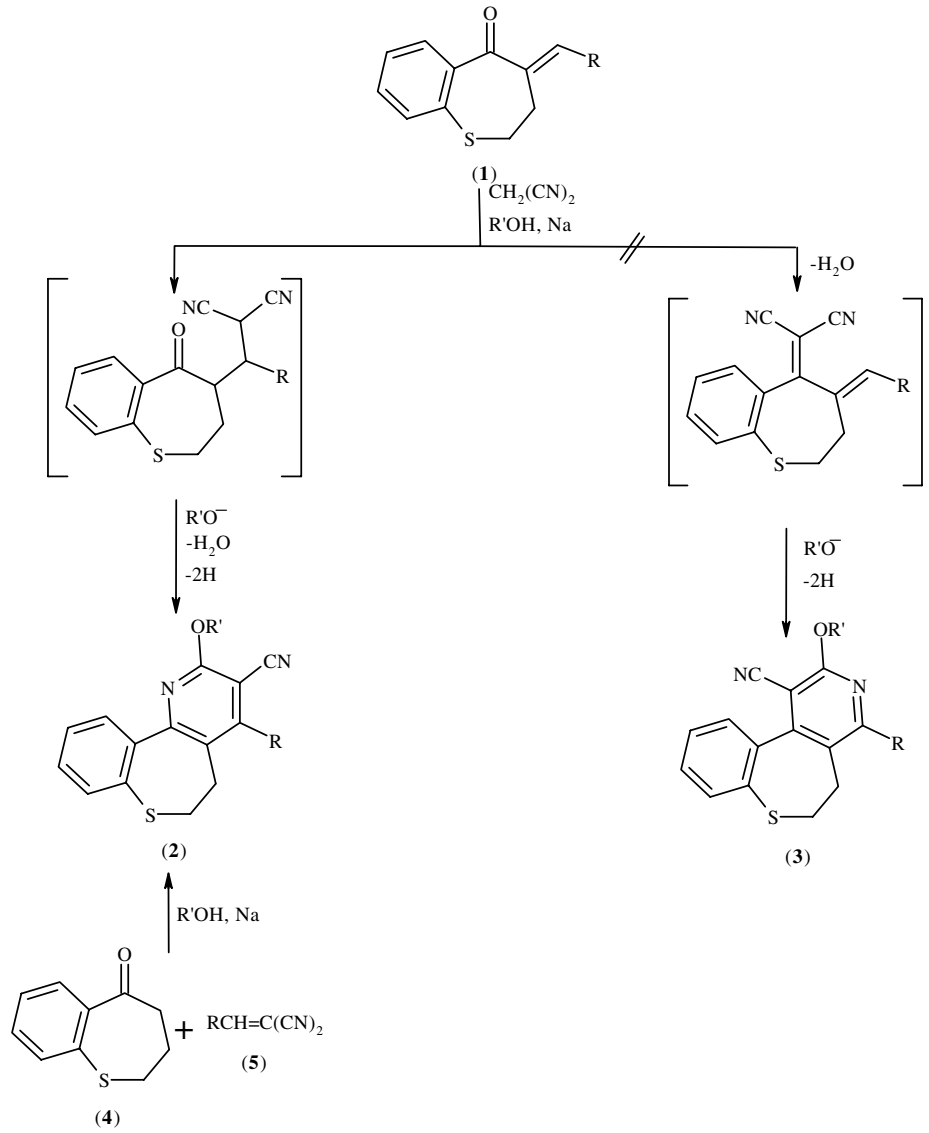
The reaction was assumed to take place via active methylene malononitrile Michael addition to the β-carbon of unsaturated system of **1**. Then, through alkoxide nucleophilic attack at one of Michael adduct intermediate nitrile groups, followed by dehydration and subsequent dehydrogenation, afforded finally the corresponding [1]-benzothiepino[5,4-*b*]pyridine-3-carbonitriles **2**. However, [1]-benzothiepino[4,5-*c*]pyridine-1-carbonitriles **3** could be produced through Knoevenagel condensation of the active methylene malononitrile with the ketonic function of **1** followed by alkoxide nucleophilic attack at one of the nitrile groups with subsequent cyclization and dehydrogenation (Scheme 1).

The isolated products were established to be **2** rather than **3** based on their independent synthesis through the reaction of 3,4-dihydro-[1]-benzothiepin-5(2*H*)-one (**4**) with ylidenemalononitriles **5a**–**h** under the same used reaction conditions. In other words, the reaction obeys Michael addition pathway rather than Knoevenagel condensation route exhibiting high regioselective reaction manner.

Single crystal X-ray diffraction studies of **2a**,**b** (Figs. 1 and 2) support the established structure exhibiting that the thiepine nucleus takes a distorted boat form configuration. ^{13}C NMR spectra 'on-resonance and APT' of **2a**,**b** add conclusive evidences for the assumed structure. Where the alkoxide (methoxide at δ = 54.46 in case of **2a** and ethoxide at δ = 14.45, 63.10 in case of **2b**) beside the nitrile (at δ = 114.81–114.87) carbons is well recognized. The methylene carbons at C-5 and C-6 are observed at δ = 27.21–27.31, 40.89–40.99, respectively, in addition to the characteristic C-3 at δ = 95.61–95.67.

On the other hand, reaction of 3,4-dihydro-[1]-benzothiepin-5(2*H*)-one (**4**) with arylidenecyanothioacetamides **6a**,**b** in refluxing ethanol in the presence of either piperidine or morpholine as a basic catalyst does not afford the expected 4-aryl-3-cyano-5,6-dihydro-[1]-benzothiepino[5,4-*b*]pyridine-2(1*H*)-thiones **7** and instead the corresponding 4-aryl-3,5-dicyano-6-thioxo-2(1*H*)-pyridinethiolate monohydrates were isolated as piperidinium or morpholinium salts **8a**–**d**. The reaction was assumed to take place via retro-Aldol reaction of **6** under the used basic reaction conditions, liberating cyanothioacetamide in the reaction medium. The latter in turn attacks another molecule of **6** followed by cyclization due to ammonia loses with subsequent dehydrogenation giving eventually **8**. The structure of **8** was inferred through spectroscopic (IR, ^{1}H NMR, MS) and elemental analyses data. The IR spectra of **8a**–**d** exhibit the nitrile stretching vibration band at ν = 2219–2196 cm^{-1}. In addition, ^{1}H NMR spectra reveal a well-distinguished piperidinyl (in case of **8a**,**c** at δ = 1.54–1.68, 2.99–3.03) or morpholinyl (in case of **8b**,**d** at δ = 3.09–3.12, 3.74–3.77) moieties. It has been noticed that the mass spectra (EI) of **8a**–**d** show the corresponding molecular weights after loses of the attached secondary amine moiety (piperidine or morpholine residue) accompanied with water molecule. Single crystal X-ray diffraction studies of **8b** (Fig. 3) add a conclusive support for the established structure revealing the morpholinium function as a distorted chair form configuration.

In an attempt for authentic preparation of **8**, the arylidenecyanothioacetamide **6b** was allowed to react with cyanothioacetamide in refluxing ethanol in the presence of equimolar amounts of either piperidine or morpholine. Unfortunately, the 2-amino-4-aryl-3,5-dicyano-2-pyridinethiolates were produced as piperidinium or morpholinium salts **9a**,**b** instead of the desired **8**, whose structures were deduced through spectroscopic and elemental analyses data. The reaction was assumed to take place through cyanothioacetamide active methylene attack at the β-carbon of unsaturated system of **6** followed by cyclization due to hydrogen sulfide elimination with subsequent dehydrogenation giving finally **9**. Similarly, refluxing **6b** in absolute ethanol in the presence of a sufficient amount of piperidine yielded **9a** via a reaction mechanistic pathway related to the aforementioned one. Where the cyanothioacetamide



1a, R = 4-ClC_6H_4
1b, R = 4-BrC_6H_4
1c, R = 4-$H_3CC_6H_4$
1d, R = 4-$H_3COC_6H_4$
1e, R = 2-thienyl

5a, R = C_6H_5
5b, R = 4-ClC_6H_4
5c, R = 4-BrC_6H_4
5d, R = 4-FC_6H_4
5e, R = 4-$H_3CC_6H_4$
5f, R = 4-$H_3COC_6H_4$
5g, R = 2-thienyl
5h, R = 2-furanyl

2a, R = C_6H_5, R' = CH_3
2b, R = C_6H_5, R' = C_2H_5
2c, R = 4-ClC_6H_4, R' = CH_3
2d, R = 4-ClC_6H_4, R' = C_2H_5
2e, R = 4-BrC_6H_4, R' = CH_3
2f, R = 4-BrC_6H_4, R' = C_2H_5
2g, R = 4-FC_6H_4, R' = CH_3
2h, R = 4-FC_6H_4, R' = C_2H_5
2i, R = 4-$H_3CC_6H_4$, R' = CH_3
2j, R = 4-$H_3CC_6H_4$, R' = C_2H_5
2k, R = 4-$H_3COC_6H_4$, R' = CH_3
2l, R = 4-$H_3COC_6H_4$, R' = C_2H_5
2m, R = 2-thienyl, R' = CH_3
2n, R = 2-furanyl, R' = CH_3

Scheme 1.

molecule, liberated due to retro-Aldol reaction of **6** under the effect of basic reaction conditions, facilitates the mechanistic reaction sequence (Scheme 2).

However, 4-aryl-3-cyano-5,6-dihydro-[1]-benzothiepino [5,4-*b*]pyridine-2(1*H*)-thiones **7** were isolated through the reaction of the appropriate 4-arylmethylene-3,4-

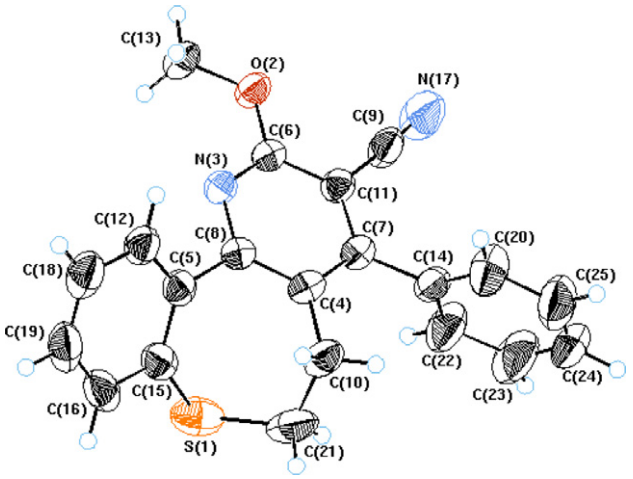


Figure 1. ORTEP projection of single crystal X-ray diffraction of **2a**. Selected intramolecular bond lengths (Å) and bond angles (°) of **2a**. S(1)–C(15) = 1.765(2), S(1)–C(21) = 1.810(3), O(2)–C(6) = 1.342(2), O(2)–C(13) = 1.429(2), N(3)–C(6) = 1.318(2), N(3)–C(8) = 1.349(2), C(4)–C(7) = 1.395(3), C(4)–C(8) = 1.402(2), C(4)–C(10) = 1.514(3), C(5)–C(8) = 1.486(3), C(5)–C(12) = 1.389(3), C(5)–C(15) = 1.401(3), C(6)–C(11) = 1.404(2), C(7)–C(11) = 1.399 (2), C(7)–C(14) = 1.495(2), C(9)–C(11) = 1.429(3), C(9)–N(17) = 1.147(3), C(10)–C(21) = 1.520(3), C(12)–C(18) = 1.377(3), C(14)–C(20) = 1.374(3), C(14)–C(22) = 1.372(3), C(15)–C(16) = 1.395(3), C(16)–C(19) = 1.389(4), C(18)–C(19) = 1.369(4), C(20)–C(25) = 1.388(3), C(22)–C(23) = 1.381(3), C(23)–C(24) = 1.360(4), C(24)–C(25) = 1.352(4), C(15)–S(1)–C(21) = 102.82(10), C(6)–O(2)–C(13) = 117.00(13), C(6)–N(3)–C(8) = 117.86(14), C(7)–C(4)–C(8) = 118.2(2), C(7)–C(4)–C(10) = 122.4(2), C(8)–C(4)–C(10) = 119.3(2), C(8)–C(5)–C(12) = 119.3(2), C(8)–C(5)–C(15) = 121.3(2), C(12)–C(5)–C(15) = 119.4(2), O(2)–C(6)–N(3) = 119.69(14), O(2)–C(6)–C(11) = 116.92(14), N(3)–C(6)–C(11) = 123.4(2), C(4)–C(7)–C(11) = 118.3(2), C(4)–C(7)–C(14) = 121.5(2), C(11)–C(7)–C(14) = 120.18(15), N(3)–C(8)–C(4) = 123.3(2), N(3)–C(8)–C(5) = 114.63(15), C(4)–C(8)–C(5) = 122.0(2), C(11)–C(9)–N(17) = 178.0(2), C(4)–C(10)–C(21) = 113.5(2), C(6)–C(11)–C(7) = 118.76(15), C(6)–C(11)–C(9) = 119.0(2), C(7)–C(11)–C(9) = 122.3(2), C(5)–C(12)–C(18) = 120.8(2), C(7)–C(14)–C(20) = 120.6(2), C(7)–C(14)–C(22) = 120.9(2), C(20)–C(14)–C(22) = 118.5(2), S(1)–C(15)–C(5) = 122.21(15), S(1)–C(15)–C(16) = 118.6(2), C(5)–C(15)–C(16) = 118.9(2), C(15)–C(16)–C(19) = 120.7(2), C(12)–C(18)–C(19) = 120.3(2), C(16)–C(19)–C(18) = 119.9(2), C(14)–C(20)–C(25) = 120.3(2), S(1)–C(21)–C(10) = 114.17(15), C(14)–C(22)–C(23) = 120.0(2), C(22)–C(23)–C(24) = 121.5(2), C(23)–C(24)–C(25) = 118.6(2), C(20)–C(25)–C(24) = 121.0(3).

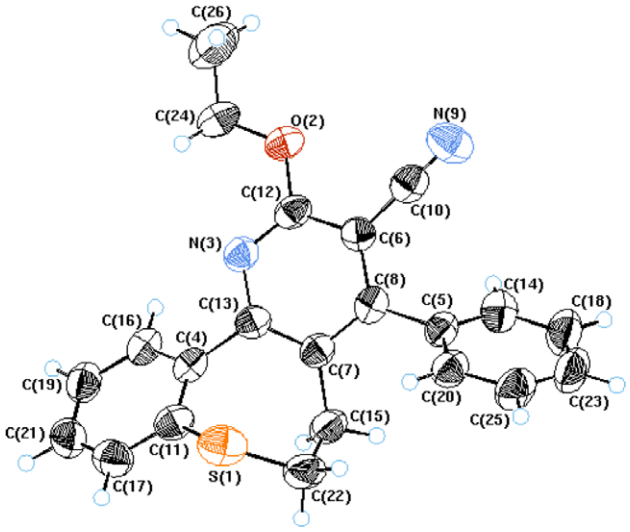


Figure 2. ORTEP projection of single crystal X-ray diffraction of **2b**. Selected intramolecular bond lengths (Å) and bond angles (°) of **2b**. S(1)–C(11) = 1.769(2), S(1)–C(22) = 1.813(2), O(2)–C(12) = 1.346(2), O(2)–C(24) = 1.444(2), N(3)–C(12) = 1.313(2), N(3)–C(13) = 1.342(2), C(4)–C(11) = 1.398(2), C(4)–C(13) = 1.490(2), C(4)–C(16) = 1.403(2), C(5)–C(8) = 1.493(2), C(5)–C(14) = 1.376(2), C(5)–C(20) = 1.382(2), C(6)–C(8) = 1.397(2), C(6)–C(10) = 1.440(2), C(6)–C(12) = 1.401(2), C(7)–C(8) = 1.406(2), C(7)–C(13) = 1.392(2), C(7)–C(15) = 1.502(2), N(9)–C(10) = 1.152(2), C(11)–C(17) = 1.400(2), C(14)–C(18) = 1.381(3), C(15)–C(22) = 1.510(2), C(16)–C(19) = 1.376(2), C(17)–C(21) = 1.366(2), C(18)–C(23) = 1.353(3), C(19)–C(21) = 1.365(2), C(20)–C(25) = 1.384(2), C(23)–C(25) = 1.366(3), C(24)–C(26) = 1.455(3), C(11)–S(1)–C(22) = 102.54(8), C(12)–O(2)–C(24) = 116.94(12), C(12)–N(3)–C(13) = 117.53(13), C(11)–C(4)–C(13) = 122.81(13), C(11)–C(4)–C(16) = 118.02(14), C(13)–C(4)–C(16) = 119.11(13), C(8)–C(5)–C(14) = 120.94(14), C(8)–C(5)–C(20) = 120.40(14), C(14)–C(5)–C(20) = 118.62(15), C(8)–C(6)–C(10) = 121.58(14), C(8)–C(6)–C(12) = 118.78(14), C(10)–C(6)–C(12) = 119.62(14), C(8)–C(7)–C(13) = 117.75(14), C(8)–C(7)–C(15) = 122.30(13), C(13)–C(7)–C(15) = 119.71(13), C(5)–C(8)–C(6) = 119.56(14), C(5)–C(8)–C(7) = 122.56(14), C(6)–C(8)–C(7) = 117.88(13), C(6)–C(10)–N(9) = 179.4(2), S(1)–C(11)–C(4) = 122.58(12), S(1)–C(11)–C(17) = 117.96(13), C(4)–C(11)–C(17) = 119.08(15), O(2)–C(12)–N(3) = 120.40(13), O(2)–C(12)–C(6) = 115.99(14), N(3)–C(12)–C(6) = 123.54(14), N(3)–C(13)–C(4) = 113.41(13), N(3)–C(13)–C(7) = 124.10(14), C(4)–C(13)–C(7) = 122.48(13), C(5)–C(14)–C(18) = 120.8(2), C(7)–C(15)–C(22) = 116.16(14), C(4)–C(16)–C(19) = 121.5(2), C(11)–C(17)–C(21) = 121.3(2), C(14)–C(18)–C(23) = 120.1(2), C(16)–C(19)–C(21) = 119.9(2), C(5)–C(20)–C(25) = 120.1(2), C(17)–C(21)–C(19) = 120.1(2), S(1)–C(22)–C(15) = 114.14(12), C(18)–C(23)–C(25) = 120.3(2), O(2)–C(24)–C(26) = 108.2(2), C(20)–C(25)–C(23) = 120.2(2).

dihydro-[1]-benzothiepin-5(2*H*)-ones **1** with cyanothioacetamide in refluxing ethanol in the presence of a catalytic amount of piperidine. The structure of **7** was established through spectroscopic (IR, ^{1}H NMR, MS) and elemental analyses data. The IR spectra of **7a**,**b** exhibit the nitrile stretching vibration band at ν = 2228–2227 cm^{-1} in addition to the imino function at ν = 3446–3433 cm^{-1}. ^{1}H NMR spectra of **7a**,**b** reveal the methylene protons at C-4 and, C-5 as broad signals at δ = 1.97–3.34 region. In addition, mass spectra (EI) of **7a**,**b** show the corresponding molecular weights as base peaks.

2.2. Anti-inflammatory activity

The anti-inflammatory activity of the prepared compounds **2a**,**c**,**d**,**k**–**n**, **7a**,**b** and **8c**,**d** (in a dose of 50 mg/kg body weight) was determined in vivo by the acute carrageenan-induced paw oedema standard method in rats.[34–37] The anti-inflammatory properties were recorded at successive time intervals (1, 2, 3 and 4 h) and compared with that of indomethacin which was used as a reference standard. From the obtained results (Table 1, Fig. 4), it has been noticed that all the tested compounds show considerable pharmacological properties. It has also been observed that the 2-alkoxy-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitriles **2** seem to be more active anti-inflammatory agents than the corresponding 2-thione analogues **7** 'as indicated by the pharmacological results of 4-(4-methoxyphenyl)- derivatives **2k**,**l** and **7b** at all the successive time intervals'. Similarly, 2-alkoxy-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitr-

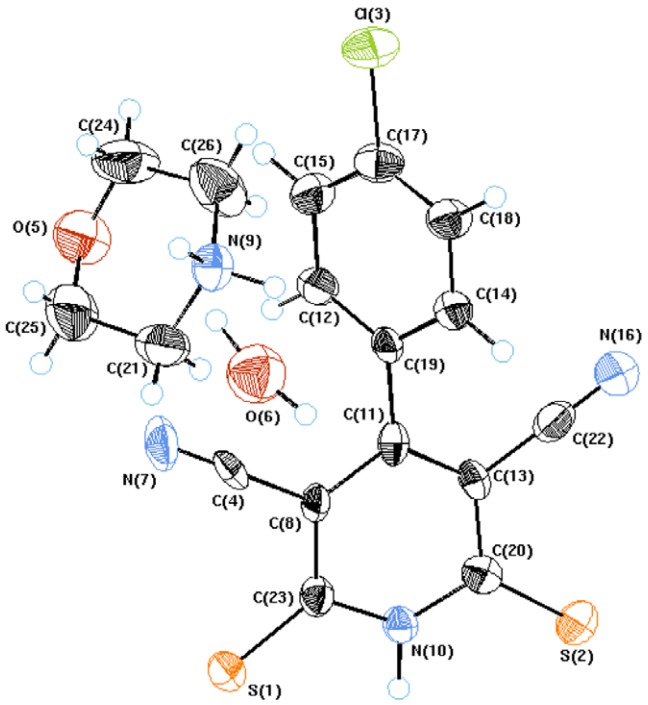


Figure 3. ORTEP projection of single crystal X-ray diffraction of **8b**. Selected intramolecular bond lengths (Å) and bond angles (°) of **8b**. S(1)–C(23) = 1.672(2), S(2)–C(20) = 1.634(2), Cl(3)–C(17) = 1.743(2), C(4)–N(7) = 1.158(3), C(4)–C(8) = 1.418(3), O(5)–C(24) = 1.393(2), O(5)–C(25) = 1.398(3), C(8)–C(11) = 1.400(3), C(8)–C(23) = 1.412(2), N(9)–C(21) = 1.465(3), N(9)–C(26) = 1.476(3), N(10)–C(20) = 1.374(2), N(10)–C(23) = 1.354(2), C(11)–C(13) = 1.375(3), C(11)–C(19) = 1.486(2), C(12)–C(15) = 1.390(2), C(12)–C(19) = 1.378(3), C(13)–C(20) = 1.430(3), C(13)–C(22) = 1.425(3), C(14)–C(18) = 1.377(2), C(14)–C(19) = 1.381(2), C(15)–C(17) = 1.373(3), N(16)–C(22) = 1.138(3), C(17)–C(18) = 1.366(3), C(21)–C(25) = 1.495(3), C(24)–C(26) = 1.468(3), O(6)–H(6) = 0.9599(4), O(6)–H(6′) = 0.9599(4), N(9)–H(9A) = 0.960(2), N(9)–H(9B) = 0.960(2), N(7)–C(4)–C(8) = 179.3(2), C(24)–O(5)–C(25) = 110.7(2), C(4)–C(8)–C(11) = 120.0(2), C(4)–C(8)–C(23) = 117.3(2), C(11)–C(8)–C(23) = 122.7(2), C(21)–N(9)–C(26) = 110.0(2), C(20)–N(10)–C(23) = 128.5(2), C(8)–C(11)–C(13) = 117.5(2), C(8)–C(11)–C(19) = 120.5(2), C(13)–C(11)–C(19) = 122.0(2), C(15)–C(12)–C(19) = 120.4(2), C(11)–C(13)–C(20) = 123.1(2), C(11)–C(13)–C(22) = 120.3(2), C(20)–C(13)–C(22) = 116.5(2), C(18)–C(14)–C(19) = 121.2(2), C(12)–C(15)–C(17) = 118.5(2), Cl(3)–C(17)–C(15) = 118.2(2), Cl(3)–C(17)–C(18) = 119.62(15), C(15)–C(17)–C(18) = 122.2(2), C(14)–C(18)–C(17) = 118.5(2), C(11)–C(19)–C(12) = 121.0(2), C(11)–C(19)–C(14) = 119.9(2), C(12)–C(19)–C(14) = 119.2(2), S(2)–C(20)–N(10) = 120.96(15), S(2)–C(20)–C(13) = 125.5(2), N(10)–C(20)–C(13) = 113.6(2), N(9)–C(21)–C(25) = 110.5(2), C(13)–C(22)–N(16) = 178.8(2), S(1)–C(23)–C(8) = 126.2(2), S(1)–C(23)–N(10) = 119.20(14), C(8)–C(23)–N(10) = 114.5(2), O(5)–C(24)–C(26) = 111.9(2), O(5)–C(25)–C(21) = 113.4(2), N(9)–C(26)–C(24) = 111.9(2), H(6)–O(6)–H(6′) = 160.3(3).

iles **2** reveal better anti-inflammatory properties than the corresponding 3,5-dicyano-6-thioxo-2(1*H*)-pyridinethiolates **8** 'as indicated by comparing the pharmacological results of **2k**,**l** with the corresponding analogues **8c**,**d** at all the tested time intervals'.

From the obtained data it has been also noticed that substitution of [1]-benzothiepino[5,4-*b*]pyridine-3-carbonitriles **2** with an ethoxy function at the 2-position improves the observed anti-inflammatory activities compared to the case of using a methoxy residue 'as observed in compounds **2c**,**d** and **2k**,**l**' which explains the role of alkoxy-chain length in affecting the pharmacological properties. It has been also observed that substitution of 2-alkoxy-[1]benzothiepino[5,4-*b*]pyridine-3-carbonitriles **2** with an (un)substituted phenyl at the 4-position 'as indicated in cases **2a**,**c**,**k**' enhances the anti-inflammatory properties compared to the case of using a heteroaryl residue 'as in compounds **2m**,**n**'. Concerning substitution of the phenyl nucleus, it has been found that the methoxy function (electron-donating group) plays an important role in enhancing the total observed pharmacological properties 'as in compounds **2k**,**l**' than the case of using chloro-substitution (deactivating function) 'compounds **2c**,**d**'.

Toxicological studies of the most promising prepared anti-inflammatory active agents (**2d**,**k**,**l**) were performed using LD_{50} standard method in mice[38] in 500, 750 and 1000 mg/kg (body weight) 'that is, 10- to 20-fold of the used anti-inflammatory effective dose'. However, no toxic symptoms or mortality rates were observed after 24 h postadministrations explaining the safe behaviour of the used doses.

Generally, it could be concluded that compounds **2d**,**k**,**l** exhibit better anti-inflammatory properties (50 mg/kg) than indomethacin itself, which was used as a reference standard (5 mg/kg) explaining the possibility of developing a novel promising anti-inflammatory active agent that could be a hint in a drug discovery programme.

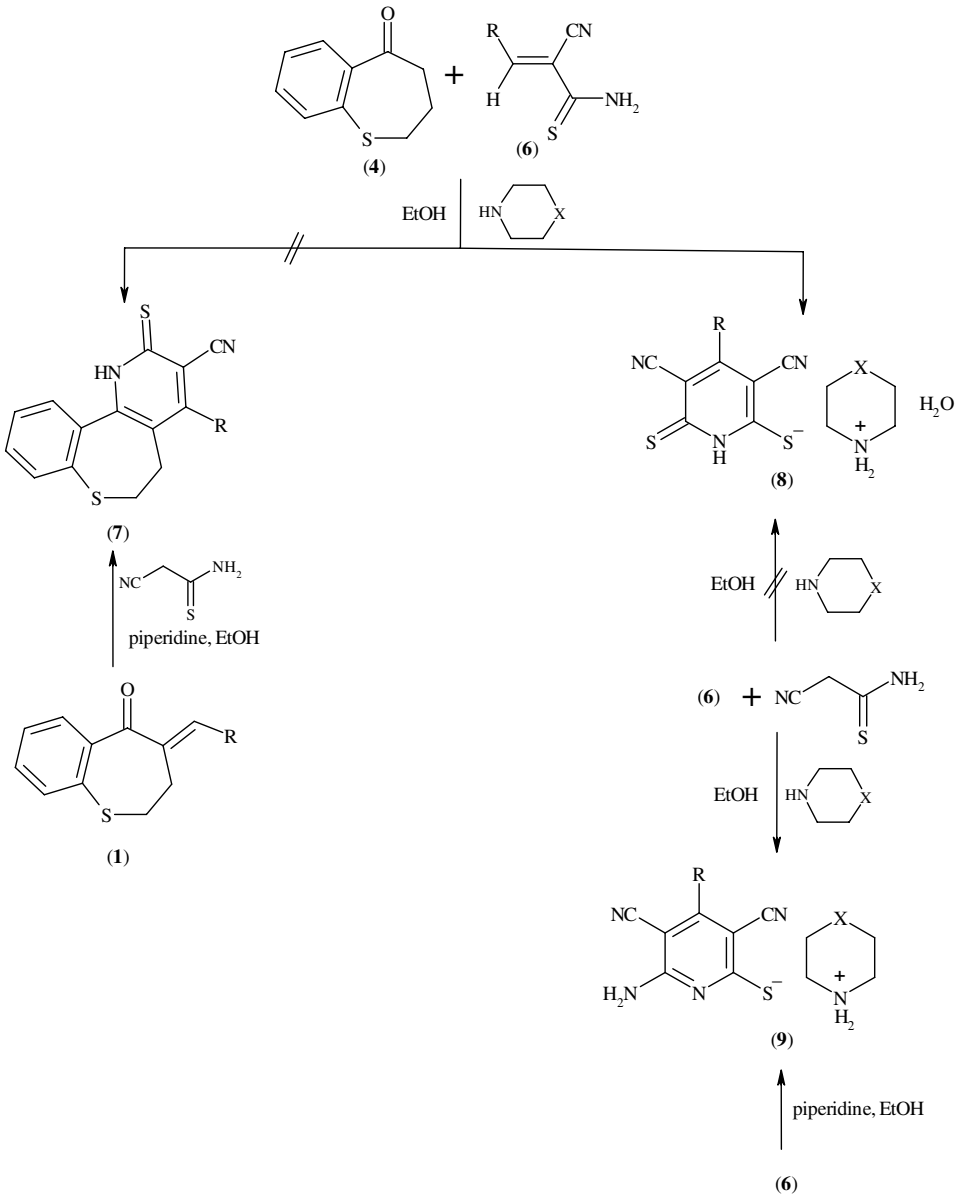
3. Experimental

Melting points are uncorrected and recorded on an Electrothermal 9100 digital melting point apparatus. IR spectra (KBr) were recorded on a Nexus 670 FT-IR spectrophotometer. NMR spectra were recorded on a Varian MERCURY spectrometer (^{1}H: 300, ^{13}C: 75 MHz). Mass spectra were recorded on Schimadzu GCMS-QP 1000 EX and Finnigan SSQ 7000 spectrometers (EI, 70 eV). The starting compounds **1a**–**e**,[39–41] **4**,[42] **5a**–**h**[43–48] and **6a**,**b**[49] were prepared according to the previously reported procedures.

3.1. Synthesis of 2-alkoxy-4-aryl-5,6-dihydro-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitriles 2a–n

3.1.1. Method 'A'. A mixture of equimolar amounts of **1** and malononitrile (5 mmol) in the appropriate alcohol (25 ml) containing sodium (0.5 g) was stirred at room temperature (20–25 °C) for the proper time. The separated solid was collected, washed with water and crystallized from a suitable solvent affording the corresponding **2** 'except in case of **2l** where the product was purified on silica gel TLC with a fluorescence indicator (F_{254})'.

3.1.2. Method 'B'. A mixture of equimolar amounts of **5a**–**h** and **4** (10 mmol) in the appropriate alcohol (25 ml) containing sodium (0.5 g) was stirred at room temperature (20–25 °C) for the proper time. The separated solid was collected, washed with water and crystallized from a suitable solvent affording **2a**–**n** 'except in



6a, R = 4-ClC_6H_4
6b, R = 4-$H_3COC_6H_4$

7a, R = 4-BrC_6H_4
7b, R = 4-$H_3COC_6H_4$

8a, R = 4-ClC_6H_4, X = CH_2
8b, R = 4-ClC_6H_4, X = O
8c, R = 4-$H_3COC_6H_4$, X = CH_2
8d, R = 4-$H_3COC_6H_4$, X = O

9a, R = 4-$H_3COC_6H_4$, X = CH_2
9b, R = 4-$H_3COC_6H_4$, X = O

Scheme 2.

case of **2l** where the product was purified on silica gel TLC with a fluorescence indicator (F_{254})'.

3.1.3. 5,6-Dihydro-2-methoxy-4-phenyl-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2a). Reaction time 48 h (method B), colourless crystals from ethanol, mp 202–203 °C, yield 64%. IR: ν_{max}/cm^{-1} 2220 (C≡N), 1553, 1496 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 2.44 (t, 2H, CH_2CH_2S, J = 6.6 Hz), 3.14 (t, 2H, CH_2S, J = 6.6 Hz), 4.06 (s, 3H, OCH_3), 7.18–7.73 (m, 9H, arom. H). ^{13}C NMR 'on-resonance and APT' ($CDCl_3$): δ 27.31 (SCH_2CH_2), 40.99 (SCH_2), 54.46 (OCH_3), 95.67 (C-3), 114.87 (C≡N), 128.14, 128.76, 128.99, 129.18, 130.04, 130.55, 134.75 (arom. CH), 126.20, 131.64, 135.44, 144.53, 156.00, 160.00, 162.58 (arom. quaternary C). MS:, *m*/*z* (%) 344 (M, 100), 343 (57), 329 (8), 316 (41), 315 (28). Anal. Calcd for $C_{21}H_{16}N_2OS$ (344.424): C, 73.23; H, 4.68; N, 8.14. Found: C, 73.35; H, 4.76; N, 8.21.

3.1.4. 5,6-Dihydro-2-ethoxy-4-phenyl-[1]-benzothiepino-[5,4-*b*]pyridine-3-carbonitrile (2b). Reaction time 72 h (method B), colourless crystals from methanol, mp

Table 1. Anti-inflammatory activity of the tested compounds using carrageenan-induced paw oedema in rats

Compound	Mean swelling volume 'ml' (% inhibition of oedema)			
	1 h	2 h	3 h	4 h
Control	0.605 ± 0.031 (00.0)	1.120 ± 0.073 (00.0)	1.368 ± 0.179 (00.0)	1.600 ± 0.203 (00.0)
Indomethacin	0.175 ± 0.047* (71.1)	0.195 ± 0.050* (82.6)	0.460 ± 0.058* (66.4)	0.278 ± 0.057* (82.6)
2a	0.278 ± 0.061* (54.0)	0.437 ± 0.091* (61.0)	0.558 ± 0.103* (59.2)	0.305 ± 0.103* (80.9)
2c	0.223 ± 0.033* (63.1)	0.283 ± 0.046* (74.7)	0.277 ± 0.061* (79.8)	0.396 ± 0.068* (75.3)
2d	0.113 ± 0.014* (81.3)	0.138 ± 0.042* (87.7)	0.158 ± 0.033* (88.5)	0.212 ± 0.058* (86.8)
2k	0.250 ± 0.026* (58.7)	0.203 ± 0.053* (81.9)	0.178 ± 0.047* (87.0)	0.238 ± 0.067* (85.1)
2l	0.230 ± 0.028* (62.0)	0.230 ± 0.047* (79.5)	0.128 ± 0.026* (90.6)	0.200 ± 0.021* (87.5)
2m	0.270 ± 0.066* (55.4)	0.447 ± 0.109* (60.1)	0.600 ± 0.149* (56.1)	0.695 ± 0.194* (56.6)
2n	0.124 ± 0.027* (79.5)	0.548 ± 0.132* (51.1)	0.572 ± 0.082* (58.2)	0.508 ± 0.099* (68.3)
7a	0.375 ± 0.041* (38.0)	0.485 ± 0.027* (56.7)	0.495 ± 0.053* (63.8)	0.485 ± 0.044* (69.7)
7b	0.275 ± 0.056* (54.5)	0.355 ± 0.050* (68.3)	0.500 ± 0.046* (63.5)	0.700 ± 0.106* (56.3)
8c	0.548 ± 0.045 (9.4)	0.594 ± 0.094* (47.0)	0.588 ± 0.063* (57.0)	0.658 ± 0.053* (58.9)
8d	0.420 ± 0.043* (30.6)	0.498 ± 0.037* (55.5)	0.440 ± 0.104* (67.8)	0.536 ± 0.044* (66.5)

* Significantly different from the control value at $p < 0.05$.

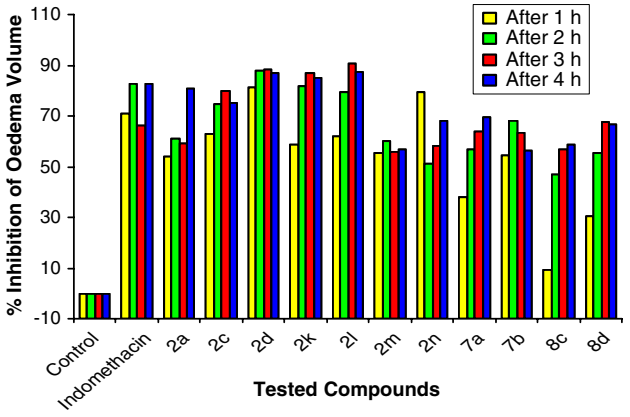


Figure 4. % inhibition of oedema for the tested compounds at successive time intervals.

163–165 °C, yield 67%. IR: ν_{max}/cm^{-1} 2219 (C≡N), 1553, 1493 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 1.50 (t, 3H, CH_3, $J = 7.2$ Hz), 2.52 (t, 2H, CH_2CH_2S, $J = 6.6$ Hz), 3.22 (t, 2H, CH_2S, $J = 6.6$ Hz), 4.61 (q, 2H, OCH_2, $J = 7.2$ Hz), 7.27–7.79 (m, 9H, arom. H). ^{13}C NMR 'on-resonance and APT' ($CDCl_3$): δ 14.45 (CH_3), 27.21 (SCH_2CH_2), 40.89 (SCH_2), 63.10 (OCH_2), 95.61 (C-3), 114.81 (C≡N), 128.04, 128.65, 128.87, 129.04, 129.88, 130.43, 134.64 (arom. CH), 125.84, 131.53, 135.44, 144.51, 155.88, 159.88, 162.24 (arom. quaternary C). MS: *m/z* (%) 358 (M, 78), 357 (100), 343 (37), 329 (66), 315 (10), 314 (13). Anal. Calcd for $C_{22}H_{18}N_2OS$ (358.444): C, 73.71; H, 5.06; N, 7.82. Found: C, 73.80; H, 5.10; N, 7.98.

3.1.5. 4-(4-Chlorophenyl)-5,6-dihydro-2-methoxy-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2c). Reaction time 24 h (methods A and B), colourless crystals from, *n*-butanol, mp 221–223 °C, yield 58%, 63% (methods A and B, respectively). IR: ν_{max}/cm^{-1} 2223 (C≡N), 1562, 1494 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 2.52 (t, 2H, CH_2CH_2S, $J = 6.6$ Hz), 3.21 (t, 2H, CH_2S, $J = 6.6$ Hz), 4.15 (s, 3H, OCH_3), 7.27–7.81 (m, 8H, arom. H). MS: *m/z* (%) 378 (M, 100), 377 (55), 363 (7), 350 (34), 349 (19). Anal. Calcd for $C_{21}H_{15}ClN_2OS$ (378.867): C, 66.57; H, 3.99; N, 7.40. Found: C, 66.71; H, 4.05; N, 7.29.

3.1.6. 4-(4-Chlorophenyl)-5,6-dihydro-2-ethoxy-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2d). Reaction time 48 h (methods A and B), colourless crystals from methanol, mp 174–176 °C, yield 46%, 56% (methods A and B, respectively). IR: ν_{max}/cm^{-1} 2222 (C≡N), 1560, 1494 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 1.40 (t, 3H, CH_3, $J = 7.2$ Hz), 2.42 (t, 2H, CH_2CH_2S, $J = 6.6$ Hz), 3.11 (t, 2H, CH_2S, $J = 6.6$ Hz), 4.52 (q, 2H, OCH_2, $J = 7.2$ Hz), 7.16–7.69 (m, 8H, arom. H). MS: *m/z* (%) 392 (M, 100), 391 (35), 377 (33), 363 (46), 349 (7), 348 (5). Anal. Calcd for $C_{22}H_{17}ClN_2OS$ (392.897): C, 67.25; H, 4.36; N, 7.13. Found: C, 67.14; H, 4.29; N, 6.99.

3.1.7. 4-(4-Bromophenyl)-5,6-dihydro-2-methoxy-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2e). Reaction time 24 h (method B), colourless crystals from *n*-butanol, mp 228–230 °C, yield 52%. IR: ν_{max}/cm^{-1} 2222 (C≡N), 1564, 1490 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 2.51 (t, 2H, CH_2CH_2S, $J = 6.6$ Hz), 3.20 (t, 2H, CH_2S, $J = 6.6$ Hz), 4.15 (s, 3H, OCH_3), 7.23–7.80 (m, 8H, arom. H). Anal. Calcd for $C_{21}H_{15}BrN_2OS$ (423.323): C, 59.58; H, 3.57; N, 6.62. Found: C, 59.45; H, 3.53; N, 6.52.

3.1.8. 4-(4-Bromophenyl)-5,6-dihydro-2-ethoxy-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2f). Reaction time 48 h (method B), colourless crystals from *n*-butanol, mp 179–181 °C, yield 68%. IR: ν_{max}/cm^{-1} 2221 (C≡N), 1562, 1489 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 1.49 (t, 3H, CH_3, $J = 7.2$ Hz), 2.50 (t, 2H, CH_2CH_2S, $J = 6.6$ Hz), 3.20 (t, 2H, CH_2S, $J = 6.6$ Hz), 4.60 (q, 2H, OCH_2, $J = 7.2$ Hz), 7.23–7.77 (m, 8H, arom. H). Anal. Calcd for $C_{22}H_{17}BrN_2OS$ (437.353): C, 60.41; H, 3.20; N, 6.41. Found: C, 60.49; H, 3.25; N, 6.28.

3.1.9. 5,6-Dihydro-4-(4-fluorophenyl)-2-methoxy-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2g). Reaction time 48 h (method B), colourless crystals from ethanol, mp 171–173 °C, yield 66%. IR: ν_{max}/cm^{-1} 2226 (C≡N), 1546, 1512 (C=N, C=C). ^1H NMR ($CDCl_3$):

δ 2.43 (t, 2H, CH_2CH_2S, J = 6.6 Hz), 3.13 (t, 2H, CH_2S, J = 6.6 Hz), 4.06 (s, 3H, OCH_3), 7.21–7.81 (m, 8H, arom. H). Anal. Calcd for $C_{21}H_{15}FN_2OS$ (362.414): C, 69.59; H, 4.17; N, 7.73. Found: C, 69.70; H, 4.26; N, 7.57.

3.1.10. 5,6-Dihydro-2-ethoxy-4-(4-fluorophenyl)-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2h). Reaction time 48 h (method B), colourless crystals from methanol, mp 143–145 °C, yield 48%. IR: ν_{max}/cm^{-1} 2222 (C≡N), 1557, 1509 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 1.49 (t, 3H, CH_3, J = 7.2 Hz), 2.51 (t, 2H, CH_2CH_2S, J = 6.6 Hz), 3.21 (t, 2H, CH_2S, J = 6.6 Hz), 4.61 (q, 2H, OCH_2, J = 7.2 Hz), 7.20–7.78 (m, 8H, arom. H). Anal. Calcd for $C_{22}H_{17}FN_2OS$ (376.444): C, 70.19; H, 4.55; N, 7.44. Found: C, 70.13; H, 4.51; N, 7.32.

3.1.11. 5,6-Dihydro-2-methoxy-4-(4-methylphenyl)-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2i). Reaction time 48 h (methods A and B), colourless crystals from ethanol, mp 207–209 °C, yield 50%, 61% (method A and B, respectively). IR: ν_{max}/cm^{-1} 2222 (C≡N), 1562, 1514 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 3H, $ArCH_3$), 2.45 (t, 2 H, CH_2CH_2S, J = 6.6 Hz), 3.14 (t, 2H, CH_2S, J = 6.6 Hz), 4.05 (s, 3H, OCH_3), 7.14–7.72 (m, 8H, arom. H). Anal. Calcd for $C_{22}H_{18}N_2OS$ (358.444): C, 73.71; H, 5.06; N, 7.82. Found: C, 73.58; H, 4.96; N, 7.96.

3.1.12. 5,6-Dihydro-2-ethoxy-4-(4-methylphenyl)-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2j). Reaction time 48 h (methods A and B), colourless crystals from methanol, mp 202–204 °C, yield 49%, 59% (methods A and B, respectively). IR: ν_{max}/cm^{-1} 2223 (C≡N), 1556, 1514 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 1.40 (t, 3H, CH_3, J = 7.2 Hz), 2.35 (s, 3H, $ArCH_3$), 2.44 (t, 2H, CH_2CH_2S, J = 6.6 Hz), 3.14 (t, 2H, CH_2S, J = 6.6 Hz), 4.51 (q, 2H, OCH_2, J = 7.2 Hz), 7.14–7.69 (m, 8H, arom. H). Anal. Calcd for $C_{23}H_{20}N_2OS$ (372.474): C, 74.16; H, 5.41; N, 7.52. Found: C, 74.08; H, 5.35; N, 7.63.

3.1.13. 5,6-Dihydro-2-methoxy-4-(4-methoxyphenyl)-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2k). Reaction time 24, 48 h (methods A and B, respectively), almost colourless crystals from *n*-butanol, mp 178–180 °C, yield 64%, 70% (methods A and B, respectively). IR: ν_{max}/cm^{-1} 2229 (C≡N), 1548, 1518 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 2.55 (t, 2H, CH_2CH_2S, J = 6.6 Hz), 3.25 (t, 2H, CH_2S, J = 6.6 Hz), 3.89 (s, 3H, OCH_3), 4.14 (s, 3H, OCH_3), 7.03–7.81 (m, 8H, arom. H). MS: *m*/*z* (%) 374 (M, 100), 373 (50), 359 (12), 346 (53), 345 (28). Anal. Calcd for $C_{22}H_{18}N_2O_2S$ (374.444): C, 70.56; H, 4.84; N, 7.48. Found: C, 70.62; H, 4.89; N, 7.57.

3.1.14. 5,6-Dihydro-2-ethoxy-4-(4-methoxyphenyl)-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2l). Reaction time 48 h (methods A and B), colourless crystals purified by silica gel TLC with fluorescence indicator (F_{254}) using chloroform—light petroleum (60–80 °C) mixture as 1:1 v/v for elution, mp 124–126 °C, yield 62%, 57% (methods A and B, respectively). IR: ν_{max}/cm^{-1} 2229 (C≡N), 1548, 1514 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 1.39 (t, 3H, CH_3, J = 7.2 Hz), 2.44 (t, 2H, CH_2CH_2S, J = 6.6 Hz), 3.14 (t, 2H, CH_2S, J = 6.6 Hz), 3.79 (s, 3H, OCH_3), 4.50 (q, 2H, OCH_2, J = 7.2 Hz), 6.93–7.68 (m, 8H, arom. H). MS: *m*/*z* (%) 388 (M, 100), 387 (33), 373 (34), 359 (37), 345 (10), 344 (5). Anal. Calcd for $C_{23}H_{20}N_2O_2S$ (388.474): C, 71.11; H, 5.19; N, 7.21. Found: C, 71.28; H, 5.29; N, 7.34.

3.1.15. 5,6-Dihydro-2-methoxy-4-(2-thienyl)-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2m). Reaction time 48, 24 h (methods A and B, respectively), colourless crystals from ethanol, mp 142–144 °C, yield 46%, 57% (methods A and B, respectively). IR: ν_{max}/cm^{-1} 2225 (C≡N), 1548, 1457 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 2.57 (t, 2H, CH_2CH_2S, J = 6.3 Hz), 3.28 (t, 2H, CH_2S, J = 6.3 Hz), 4.06 (s, 3H, OCH_3), 7.10–7.71 (m, 7H, arom. H). MS: *m*/*z* (%) 350 (M, 100), 349 (37), 335 (13), 322 (45), 321 (30). Anal. Calcd for $C_{19}H_{14}N_2OS_2$ (350.44): C, 65.12; H, 4.03; N, 8.00. Found: C, 65.22; H, 4.10; N, 7.86.

3.1.16. 5,6-Dihydro-4-(2-furanyl)-2-methoxy-[1]-benzothiepino[5,4-*b*]pyridine-3-carbonitrile (2n). Reaction time 48 h (method B), colourless crystals from ethanol, mp 142–144 °C, yield 54%. IR: ν_{max}/cm^{-1} 2222 (C≡N), 1556, 1462 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 2.64 (t, 2H, CH_2CH_2S, J = 6.3 Hz), 3.50 (t, 2H, CH_2S, J = 6.3 Hz), 4.05 (s, 3H, OCH_3), 6.54–7.72 (m, 7H, arom. H). Anal. Calcd for $C_{19}H_{14}N_2O_2S$ (334.384): C, 68.24; H, 4.22; N, 8.38. Found: C, 68.06; H, 4.13; N, 8.49.

3.2. Synthesis of 4-aryl-3-cyano-5,6-dihydro-[1]-benzothiepino[5,4-*b*]pyridine-2(1*H*)-thiones 7a,b

A mixture of equimolar amounts of **1** and cyanothioacetamide (5 mmol) in absolute ethanol (25 ml) containing piperidine (2–3 drops) was boiled under reflux for the appropriate time. The separated solid while refluxing was collected and crystallized from a suitable solvent affording **7a**,**b**.

3.2.1. 4-(4-Bromophenyl)-3-cyano-5,6-dihydro-[1]-benzothiepino[5,4-*b*]pyridine-2(1*H*)-thione (7a). Reaction time 10 h, yellow crystals from *N*,*N*-dimethylformamide (75%), mp 311–313 °C, yield 42%. IR: ν_{max}/cm^{-1} 3446 (br, NH), 2228 (C≡N), 1587, 1555, 1487 (C=C, C=S). ^{1}H NMR (DMSO-d_6): δ 1.97 (br s, 1H, upfield H of CH_2CH_2S), 2.41 (br s, 1H, downfield H of CH_2CH_2S), 2.97 (br s, 1H, upfield H of CH_2S), 3.34 (br s, 1H, downfield H of CH_2S), 7.33–7.81 (m, 9H, 8 arom. H + NH). MS: *m*/*z* (%) 425 [(M+1), 85], 424 (M, 100), 423 (71), 391 (6), 365 (1). Anal. Calcd for $C_{20}H_{13}BrN_2S_2$ (425.349): C, 56.47; H, 3.08; N, 6.59. Found: C, 56.56; H, 3.14; N, 6.70.

3.2.2. 3-Cyano-5,6-dihydro-4-(4-methoxyphenyl)-[1]-benzothiepino[5,4-*b*]pyridine-2(1*H*)-thione (7b). Reaction time 18 h, yellow crystals from *N*,*N*-dimethylformamide (80%), mp 288–290 °C, yield 53%. IR: ν_{max}/cm^{-1} 3433 (br, NH), 2227 (C≡N), 1606, 1557, 1512 (C=C, C=S). ^{1}H NMR (DMSO-d_6): δ 1.97 (br s, 1H, upfield

H of CH_2CH_2S), 2.44 (br s, 1H, downfield H of CH_2CH_2S), 2.97 (br s, 1H, upfield H of CH_2S), 3.32 (br s, 1H, downfield H of CH_2S), 3.83 (s, 3H, OCH_3), 7.09–7.69 (m, 8H, arom. H), 14.35 (br s, 1H, NH). MS: *m/z* (%) 377 [(M+1), 37], 376 (M, 100), 375 (71), 343 (14), 317 (11). Anal. Calcd for $C_{21}H_{16}N_2OS_2$ (376.48): C, 66.99; H, 4.28; N, 7.44. Found: C, 67.13; H, 4.39; N, 7.37.

3.3. Reaction of 4 with 6

A mixture of equimolar amounts of 3,4-dihydro-[1]-benzothiepin-5(2*H*)-one (**4**) and the corresponding arylidenecyanothioacetamide **6a**,**b** (10 mmol) in absolute ethanol (25 ml) containing the appropriate basic catalyst (piperidine or morpholine, 0.2 ml) was boiled under reflux for the suitable time. The separated solid while refluxing was collected (except in case of **8a**, where the reaction mixture was cooled at 4 °C overnight. So, the separated solid was collected) and crystallized from water affording **8a**–**d**.

3.3.1. Piperidinium 4-(4-chlorophenyl)-3,5-dicyano-6-thioxo-2(1*H*)-pyridinethiolate monohydrate (8a). Reaction time 16 h, yellow crystals, mp 306–308 °C, yield 73%. IR: ν_{max}/cm^{-1} 3450 (br, NH, NH_2), 2218 (C≡N), 1519, 1477 (C=C, C=S). ^1H NMR (DMSO-d_6): δ 1.54–1.68 (m, 6H, piperidinyl 3 CH_2), 2.99–3.03 (m, 4H, piperidinyl 2 NCH_2), 7.45 (d, 2H, arom. H, $J = 8.4$ Hz), 7.56 (d, 2H, arom. H, $J = 8.7$ Hz), 8.35 (br s, 2H, NH_2), 12.40 (br s, 1H, NH). MS: *m/z* (%) 304 (47), 303 {[M−($C_5H_{13}NO$)], 80}, 302 (93), 270 (45), 269 (52). Anal. Calcd for $C_{18}H_{19}ClN_4OS_2$ (406.933): C, 53.12; H, 4.71; N, 13.77. Found: C, 53.30; H, 4.81; N, 13.85.

3.3.2. Morpholinium 4-(4-chlorophenyl)-3,5-dicyano-6-thioxo-2(1*H*)-pyridinethiolate monohydrate (8b). Reaction time 20 h, yellow crystals, mp 296–298 °C, yield 53%. IR: ν_{max}/cm^{-1} 3582, 3508 (NH, NH_2), 2219 (C≡N), 1527, 1473 (C=C, C=S). ^1H NMR (DMSO-d_6): δ 3.09–3.12 (m, 4H, morpholinyl 2 NCH_2), 3.74–3.77 (m, 4H, morpholinyl 2 OCH_2), 7.44 (d, 2H, arom. H, $J = 8.7$ Hz), 7.55 (d, 2H, arom. H, $J = 8.7$ Hz), 8.66 (br s, 2H, NH_2), 12.81 (br s, 1H, NH). MS: *m/z* (%) 304 (38), 303 {[M−($C_4H_{11}NO_2$)], 76}, 302 (81), 270 (43), 269 (54). Anal. Calcd for $C_{17}H_{17}ClN_4O_2S_2$ (408.913): C, 49.93; H, 4.19; N, 13.70. Found: C, 49.74; H, 4.05; N, 13.76.

3.3.3. Piperidinium 3,5-dicyano-4-(4-methoxyphenyl)-6-thioxo-2(1*H*)-pyridinethiolate monohydrate (8c). Reaction time 23 h, yellow crystals, mp 271–273 °C, yield 86%. IR: ν_{max}/cm^{-1} 3431 (br, NH, NH_2), 2203 (C≡N), 1516, 1483 (C=C, C=S). ^1H NMR (DMSO-d_6): δ 1.55–1.65 (m, 6H, piperidinyl 3 CH_2), 3.01 (br s, 4H, piperidinyl 2 NCH_2), 3.82 (s, 3H, OCH_3) 7.02 (d, 2H, arom. H, $J = 7.5$ Hz), 7.34 (d, 2H, arom. H, $J = 7.8$ Hz), 8.21 (br s, 2H, NH_2), 12.60 (br s, 1H, NH). MS: *m/z* (%) 300 (9), 299 {[M−($C_5H_{13}NO$)], 45}, 298 (12), 266 (25), 265 (2). Anal. Calcd for $C_{19}H_{22}N_4O_2S_2$ (402.52): C, 56.69; H, 5.51; N, 13.92. Found: C, 56.63; H, 5.46; N, 14.04.

3.3.4. Morpholinium 3,5-dicyano-4-(4-methoxyphenyl)-6-thioxo-2(1*H*)-pyridinethiolate monohydrate (8d). Reaction time 14 h, yellow crystals, mp 278–280 °C, yield 65%. IR: ν_{max}/cm^{-1} 3431 (br., NH, NH_2), 2196 (C≡N), 1515, 1488 (C=C, C=S). ^1H NMR (DMSO-d_6): δ 3.09–3.12 (m, 4H, morpholinyl 2 NCH_2), 3.74–3.77 (m, 4H, morpholinyl 2 OCH_2), 3.82 (s, 3H, OCH_3), 7.02 (d, 2H, arom. H, $J = 8.7$ Hz), 7.35 (d, 2H, arom. H, $J = 8.7$ Hz), 8.40 (br s, 2H, NH_2), 12.60 (br s, 1H, NH). MS: *m/z* (%) 300 (23), 299 {[M−($C_4H_{11}NO_2$)], 95}, 298 (100), 266 (39), 265 (47). Anal. Calcd for $C_{18}H_{20}N_4O_3S_2$ (404.49): C, 53.45; H, 4.98; N, 13.85. Found: C, 53.34; H, 4.88; N, 13.69.

3.4. Synthesis of 6-amino-4-aryl-3,5-dicyano-2-pyridinethiolates 9a,b

3.4.1. Method 'A'. A mixture of equimolar amounts of **6b** and cyanothioacetamide (10 mmol) in absolute ethanol (25 ml) containing a quantitative amount of the corresponding basic catalyst (piperidine or morpholine) was boiled under reflux for the suitable time. The separated solid while refluxing was collected and crystallized from water affording **9a**,**b**.

3.4.2. Method 'B'. A solution of **6b** (10 mmol) in absolute ethanol (15 ml) containing piperidine (5 mmol) was boiled under reflux for the appropriate time. The separated solid while refluxing was collected and crystallized from water giving **9a**.

3.4.3. Piperidinium 6-amino-3,5-dicyano-4-(4-methoxyphenyl)-2-pyridinethiolate (9a). Reaction time 11, 23 h (methods A and B, respectively), yellow crystals, mp 248–250 °C, yield 54%, 33% (methods A and B, respectively). IR: ν_{max}/cm^{-1} 3450, 3345, 3230 (NH_2), 2204 (C≡N), 1634, 1532 (C=N, C=C). ^1H NMR (DMSO-d_6): δ 1.55–1.65 (m, 6H, piperidinyl 3 CH_2), 3.01–3.05 (m, 4H, piperidinyl 2 NCH_2), 3.60 (br s, 4H, 2 NH_2), 3.81 (s, 3H, OCH_3) 7.03 (d, 2H, arom. H, $J = 8.7$ Hz), 7.33 (d, 2H, arom. H, $J = 8.7$ Hz). MS: *m/z* (%) 282 {[M−($C_5H_{11}N$)], 100}, 281 (59), 267 (38), 251 (39), 238 (12). Anal. Calcd for $C_{19}H_{21}N_5OS$ (367.464): C, 62.10; H, 5.76; N, 19.06. Found: C, 62.28; H, 5.85; N, 19.17.

3.4.4. Morpholinium 6-amino-3,5-dicyano-4-(4-methoxyphenyl)-2-pyridinethiolate (9b). Reaction time 12 h (method A), yellow crystals, mp 214–216 °C, yield 51%. IR: ν_{max}/cm^{-1} 3394 (NH_2), 2203 (C≡N), 1604, 1542 (C=N, C=C). ^1H NMR (DMSO-d_6): δ 3.10–3.14 (m, 4H, morpholinyl 2 NCH_2), 3.50 (br s, 4H, 2 NH_2), 3.75–3.78 (m, 4H, morpholinyl 2 OCH_2), 3.83 (s, 3H, OCH_3) 7.04 (d, 2H, arom. H, $J = 8.7$ Hz), 7.38 (d, 2H, arom. H, $J = 8.7$ Hz). MS: *m/z* (%) 282 {[M−(C_4H_9NO)], 100}, 281 (39), 267 (26), 251 (34), 238 (13). Anal. Calcd for $C_{18}H_{19}N_5O_2S$ (369.434): C, 58.52; H, 5.18; N, 18.96. Found: C, 58.68; H, 5.29; N, 18.88.

3.5. Single crystal X-ray crystallographic data of 2a,b, 8b

The crystallographic data were collected at $T = 298$ K on a Kappa CCD Enraf Nonius FR 590 diffractometer using a graphite monochromator with Mo–Kα radiation

(λ = 0.71073 Å). The crystal structures were determined by SIR92[50] and refined by maXus[51] (Bruker Nonius, Delft and MacScience, Japan). Full crystallographic details, excluding structure factors, have been deposited at Cambridge Crystallographic Data Centre (CCDC) as supplementary publication numbers CCDC 633520, 633521 and 633522 for compounds **2a**, **2b** and **8b**, respectively.

3.5.1. Compound 2a. For X-ray crystallographic studies, compound **2a** was recrystallized as prismatic colourless crystals from ethanol. Chemical formula $C_{21}H_{16}N_2OS$, M_r = 344.436, monoclinic, crystallizes in space group $P2_1/c$, cell lengths 'a = 10.9231(3), b = 19.7190(5), c = 8.4935(2) Å', cell angles 'α = 90.00, β = 105.4428(11), γ = 90.00°', V = 1763.39(8) Å^3, Z = 4, D_c = 1.297 mg m^{-3},θ values 2.910–26.373°, absorption coefficient μ (Mo–Kα) = 0.19 mm^{-1}, $F(000)$ = 720. The unique reflections measured 5612 of which 2090 reflections with threshold expression $I > 3\sigma(I)$ were used in the structural analysis. Convergence for 226 variable parameters by least-squares refinement on F^2 with $w = 1/[\sigma^2(F_o^2) + 0.10000F_o^2]$. The final agreement factors were R = 0.045 and wR = 0.078 with a goodness-of-fit of 1.557.

3.5.2. Compound 2b. For X-ray crystallographic studies, compound **2b** was recrystallized as colourless needle crystals from methanol. Chemical formula $C_{22}H_{18}N_2OS$, M_r = 358.463, orthorhombic, crystallizes in space group $Pbca$, cell lengths 'a = 15.7880(7), b = 12.3536(6), c = 19.0546(12) Å', cell angles 'α = 90.00, β = 90.00, γ = 90.00°', V = 3716.4(3) Å^3, Z = 8, D_c = 1.281 mg m^{-3}, θ values 2.910–22.465°, absorption coefficient μ (Mo–Kα) = 0.19 mm^{-1}, $F(000)$ = 1504. The unique reflections measured 4812 of which 1508 reflections with threshold expression $I > 3\sigma(I)$ were used in the structural analysis. Convergence for 235 variable parameters by least-squares refinement on F^2 with $w = 1/[\sigma^2(F_o^2)+ 0.10000F_o^2]$. The final agreement factors were R = 0.041 and wR = 0.082 with a goodness-of-fit of 2.091.

3.5.3. Compound 8b. For X-ray crystallographic studies, compound **8b** was recrystallized as platelet yellow crystals from water. Chemical formula $C_{17}H_{17}ClN_4O_2S_2$, M_r = 408.930, monoclinic, crystallizes in space group C-2/c , cell lengths 'a = 23.555(2), b = 7.4063(5), c = 20.912(2) Å', cell angles 'α = 90.00, β = 96.911(3), γ = 90.00°', V = 3621.8(5) Å^3, Z = 8, D_c = 1.500 mg m^{-3}, θ values 2.910–25.028°, absorption coefficient μ (Mo–Kα) = 0.46 mm^{-1}, $F(000)$ = 1696. The unique reflections measured 4064 of which 1421 reflections with threshold expression $I > 3\sigma(I)$ were used in the structural analysis. Convergence for 228 variable parameters by least-squares refinement on F^2 with $w = 1/[\sigma^2(F_o^2)+ 0.10000F_o^2]$. The final agreement factors were R = 0.061 and wR = 0.145 with a goodness-of-fit of 3.863.

3.6. Anti-inflammatory activity screening

The anti-inflammatory activity screening for the prepared compounds was performed in vivo by the acute carrageenan-induced paw oedema standard method in rats.[34–37] Wister albino rats of either sex (pregnant female animals were excluded) weighing 160–190 g were divided into 13 groups of 6 animals each. Administration of indomethacin (reference standard in a dose of 5 mg/kg body weight) and the tested compounds (**2a**,**c**,**d**,**k**–**n**, **7a**,**b** and **8c**,**d**) dissolved in DMSO, in a dose of 50 mg/kg (body weight), was given intraperitoneally 1 h before induction of inflammation. The control group was given DMSO only. Carrageenan paw oedema was induced by subcutaneous injection of 1% solution of carrageenan in saline (0.1 ml/rat) into the right hind paw of rats. Paw volumes were measured volumetrically after successive time intervals (1, 2, 3 and 4 h) with plethysmometer 7150 (UGO BASILE, Italy) and compared with the initial hind paw volume of each rat for determining the oedema volume. Data were collected, checked, revised and analyzed. Quantitative variables from normal distribution were expressed as means ± SE 'standard error'. The significant difference between groups was tested by using one-way ANOVA followed by LSD test at $p < 0.05$.

The anti-inflammatory activity was expressed as percentage inhibition of oedema volume in treated animals in comparison with the control group (Table 1, Fig. 4).

$$\%\ \text{inhibition of oedema} = \frac{V_c - V_t}{V_c} \times 100$$

where V_c and V_t are the volumes of oedema for the control and drug-treated animal groups, respectively.

3.7. LD_{50} determination

The toxicological studies of the most promising prepared anti-inflammatory active agents (**2d**,**k**,**l**) were determined using standard known LD_{50} method in mice.[38] Albino mice weighing 20–25 g were divided in 12 groups of 6 mice each. Administrations of the tested compounds (**2d**,**k**,**l**) dissolved in DMSO in 500, 750 and 1000 mg/kg (body weight) were given intraperitoneally. The control groups were given DMSO only. The toxic symptoms, mortality rates and postmortem findings in each group were recorded 24 h postadministration.

LD_{50} of the tested compounds was calculated according to the following formula:

$$LD_{50} = D_m - \frac{\sum(z \times d)}{n}$$

where D_m = the largest dose which killed all animals, z = mean of dead animals between two successive groups, d = the constant factor between two successive doses, n = number of animals in each group, $\sum$ = the sum of ($z \times d$).

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