Synthetic Approaches to Bridgehead Nitrogen Methanesulfonamide Derivatives of 3-Amino-2-thioxo-2,3-dihydrothieno[2,3-*d*]pyrimidin-4(1*H*)-ones, Potential COX-2 Selective Inhibitors

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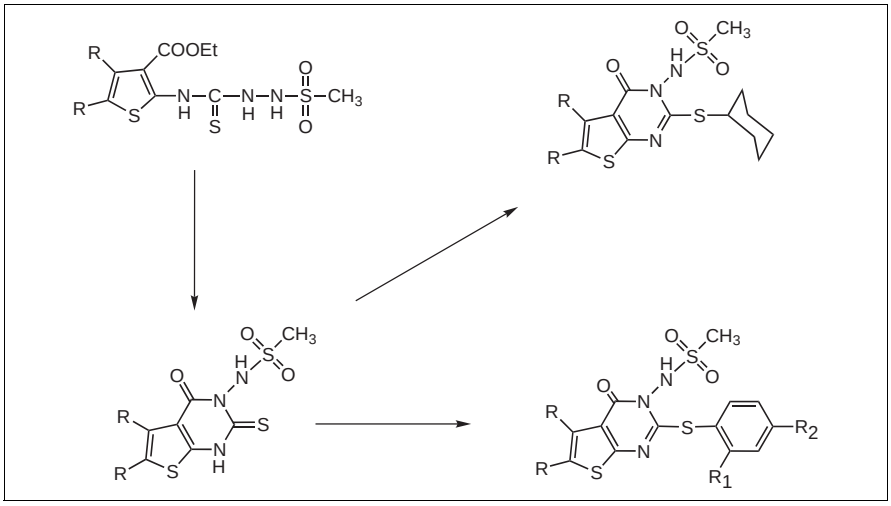

Methane sulfonamide derivatives of 3-amino-2-thioxo-2,3-dihydrothieno[2,3-*d*]pyimidin-4(1*H*)-one, potential selective COX-2 inhibitors, were synthesized and their structural elucidation is here reported. Some derivatives, at 10 μM concentration, showed a significant percentage of inhibition in some *in vitro* experiments.

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Introduction.

For many years we have focused our interests in the synthesis of thienopyrimidine derivatives in order to find compounds endowed with anti-inflammatory and analgesic activity and no or low ulcerogenic activity [1,2]. On the basis of recent developments in the research of non-steroidal anti-inflammatory drugs (NSAIDs) without ulcerogenic effects, acting as selective inhibitors of enzyme COX-2 [3,4], we have synthesized new derivatives of tetrahydro[1]benzo and 5,6-dimethyl-3-amino-2-thioxo-2,3-dihydrothieno[2,3-*d*]pyrimidin-4(1*H*)-one. These derivatives with functional groups and substituents, present in prototypical leads of above selective inhibitors of COX-2 [3,4], have been obtained through the methods of cyclization reported by Wamhoff [5,6], starting from heteroaromatic β-enamino esters. Their COX-2 inhibition activity have been assessed in intact cultured cells [7,8].

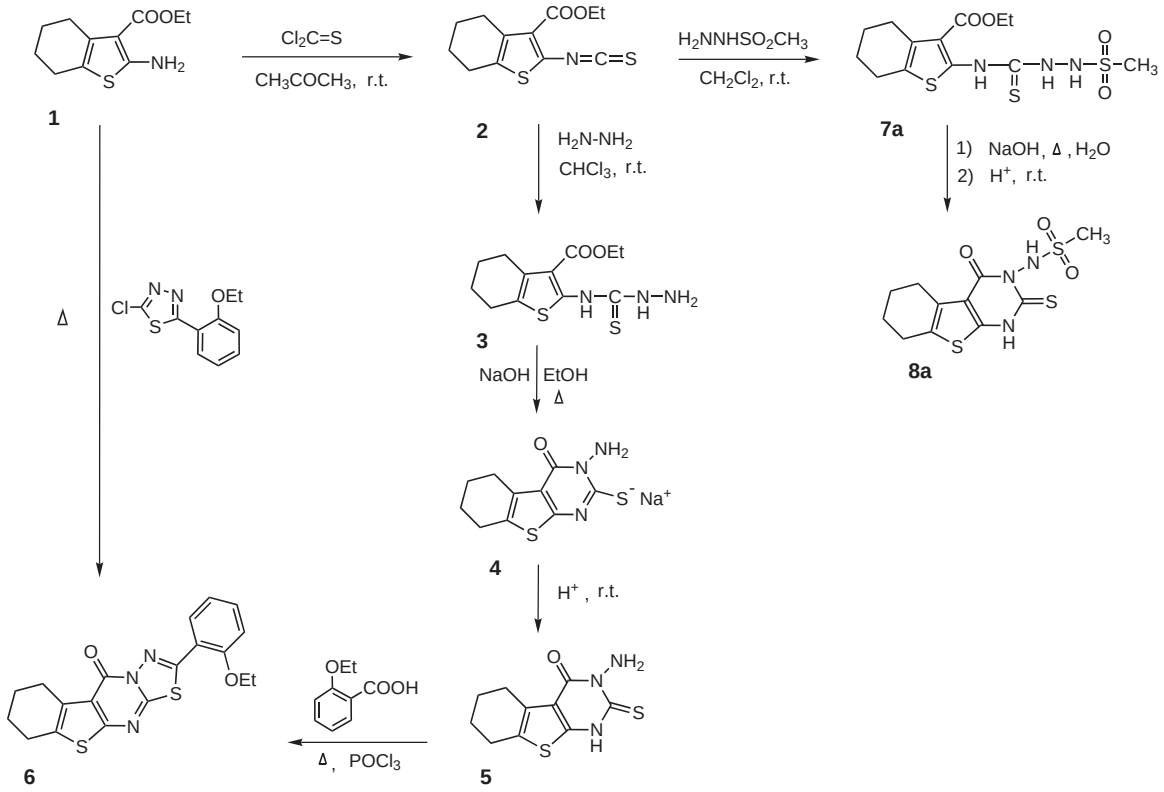
Results and Discussion.

The starting key compound in the synthesis of hexahydro[1]benzothieno[2,3-*d*]pyrimidin-4(1*H*)-one derivatives was 2-isothiocyanato-thiophene **2** prepared in acetone at room temperature by the reaction of β-enamino ester **1** with thiophosgene (Scheme 1).

The reaction at room temperature of isothiocyanate **2** with hydrazine or mesylhydrazide gave the hydrazine derivatives **3** and **7a**, respectively; the treatment of these hydrazines, according to the methods reporting by Wanhoff [5,6], *i.e.*, in refluxing solutions of alkaline hydroxides and subsequent acidification afforded amino-thioxo **4** and **5** and sulphonamide **8a** derivatives of the above heterocyclic system. The structure of these derivatives was confirmed by independent preparations. The tetracycle **6** obtained from the condensation of amino-ester **1** with an appropriate 2-chloro-1,3,4-thiadiazole was identical to the one prepared by the reaction of amino-thioxo derivative **5** and 2-ethoxy-benzoic acid (Scheme 1).

The thio-methyl derivative **11a**,**b**, being identical to the product obtained through alkaline hydrolysis of the disulfonated derivative **10a**,**b**, confirmed the proposed sulphonamide structure of **8a,b** and also that sulphur was more reactive than nitrogen adjacent to sulfonic group (Scheme 2).

Scheme 1

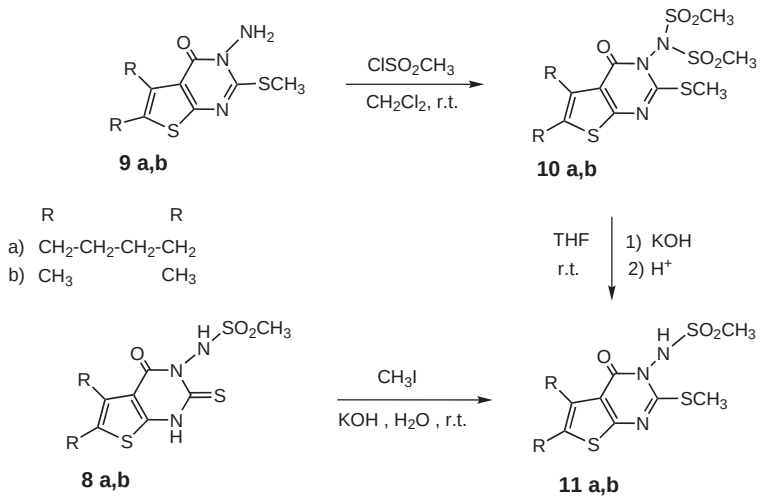


Analytical and spectral data of all above derivatives were in agreement with the proposed structures.

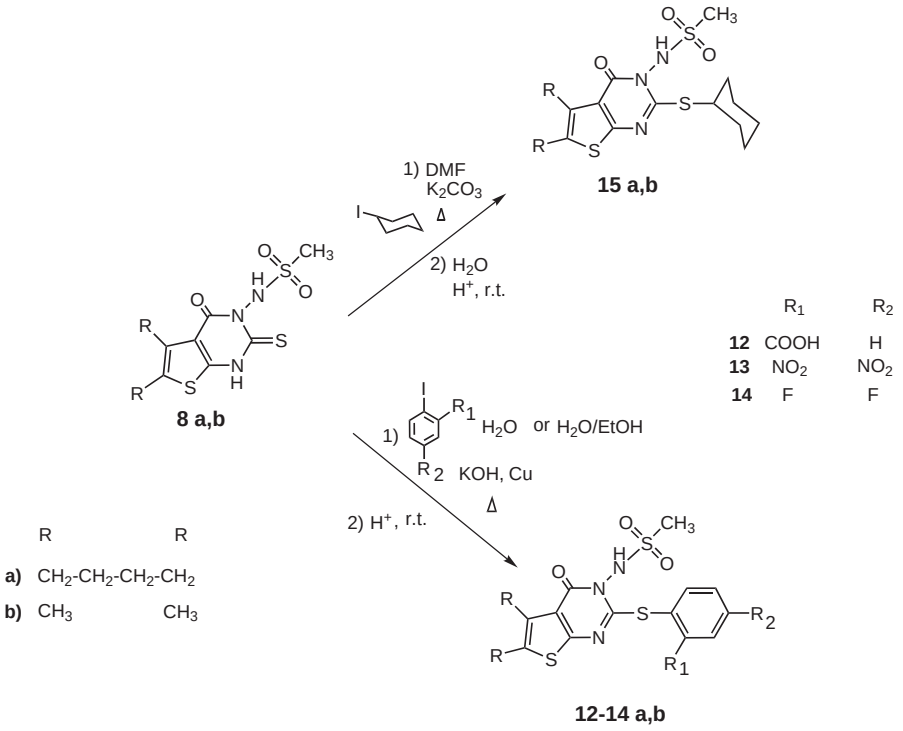
Heating of methanesulphonamide-thioxo derivative **8 a**,**b** with suitable aryl iodide in water and ethanol, under basic conditions and with catalytic powdery copper, gave thio-aryl derivatives **12-14 a,b** (Scheme 3). Alternatively, the thio-cycloesyl derivative **15a,b** was obtained in dimethylformamide at 80 °C in presence of potassium carbonate.

The proposed structures were confirmed by elemental analysis and IR, ^{1}H and ^{13}C nmr spectra. Specifically, ^{1}H nmr spectra in the region of 11.0-11.6 ppm showed a typical singlet attributable to NH of methanesulfonamide group; the nmr spectra of thio-aryl derivatives exhibited

Scheme 2



Scheme 3



the chemical shift of multiplet aromatic signals and ^{1}H nmr spectrum of cycloesyl derivative **15 a,b** showed at 1.22-1.99 ppm the multiplet due to ten methylenes and at 3.4-3.8 ppm the multiplet due to proton bonded to carbon adjacent to sulphur at position 2.

Derivatives **12-15 a**,**b** were assessed as COX-2 inhibitors at 10 μM in a test on culture cells [7,8] that allows the evaluation of the effects of a drug on COX-2 activity after its induction by lipopolysaccharide (LPS) by measuring the production of prostaglandin E_2 (PGE_2). Our preliminary results, showing that compounds **12-15a** and **13b** have an interesting inhibitory activity (Table 1), prompt us to synthesize other derivatives of above heterocyclic system as potential COX-2 inhibitors.

Table 1

Compound	% inhibition of PGE_2 generation (COX-2) at 10μM in Cell Culture
12 a	**80**
13 a	**97**
14 a	**77**
15 a	**95**
12 b	**75**
13 b	**81**
14 b	**51**
15 b	**65**

Assays were performed in duplicate and standard errors are within ±10. Indomethacine 100% of inhibition at 1μM and 10 μM

EXPERIMENTAL

Melting points are uncorrected and were determined in open capillary tubes on a SPM1 apparatus (Stuart Scientific, Staffordshire). IR spectra were recorded on a Perkin Elmer 1600 series FT-IR in potassium bromide disks. Elemental analyses for C, H, N and S were obtained on an EA 1108 elemental analyzer Fisons-Carlo Erba instrument. ^{1}H and ^{13}C nmr spectra were recorded at 200 MHz on a Varian Gemini 200 spectrometer; chemical shifts (δ) are reported in ppm from tetramethylsilane as internal standard; coupling constants (J) are in Hertz (Hz). The mp's of all crude compounds were within -3 °C, if compared with the pure product, therefore as synthetic intermediates they could be used without further purification. The purity of compounds was checked by thin layer chromatography on Merck silica gel 60 F-254 plates. All commercial chemicals were purchased from Aldrich, Fluka, Merck and Carlo Erba and were used without further purification.

Ethyl 2-isothiocyanato-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (**2**).

A solution of amino-ester **1** [9] (3.0 g, 13.3 mmoles in acetone (30 ml) was added slowly drop-wise at room temperature to a stirred solution of thiophosgene (1.0 ml, 97%, δ= 1.51, 12.7 mmoles) in acetone (15 ml). After 20 minutes of stirring water was added (150 ml); the solid separated was collected, washed first with 5% sodium hydroxide and then with water, dried and crystallized from *n*-hexane to give the isothiocyanate **2** as pale yellow microcrystals; yield 80%, mp 46-47°. The isothiocyanate **2** was identical to the sample obtained according to previous paper [10]. The unreported

spectral data are now reported; ir: 2125 (NCS), 1698 (C=O) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): δ 1.30 (t, J=7.0 Hz, 3H, CH_3), 1.71 (m, 4H, 2xCH_2), 2.64 (m, 2H, CH_2), 2.66 (m, 2H, CH_2), 4.25 (q, J=7.0 Hz, 2H, CH_2); ^{13}C nmr (dimethylsulfoxide-d_6): δ 14.29, 22.01, 23.13, 26.78, 27.01, 59.78, 100.24, 128.37, 129.88, 134.78, 163.30, 164.28.

Ethyl 2-[(hydrazinocarbonothioyl)amino]-4,5,6,7-tetrahydro-1-benzo-thiophene-3-carboxylate (**3**).

To a stirred solution of hydrazine hydrate (0.20 ml, 4.0 mmoles) in chloroform (15 ml) isothiocyanate **2** (1.0 g , 3.7 mmoles) in chloroform (10 ml) was added drop-wise at room temperature. After the addition was complete, the mixture was stirred at room temperature for 1 hour. The resulting solid was collected, washed with chloroform, dried and crystallized from dioxane/ethanol to give **3** as colourless crystals; yield 45%; mp 200-02° dec. The analytical and spectral data of compound **3** were identical to those of a sample obtained according to previous paper [11].

3-Amino-2-thioxo-2,3,5,6,7,8-hexahydro[1]benzothieno[2,3-*d*]-pyrimidin-4(1*H*)-one (**5**) from its Sodium Salt (**4**).

A suspension of derivative **3** (0.75 g, 2.5 mmoles) in a solution of sodium hydroxide (0.100 g, 2.5 mmoles) was refluxed under stirring for 1 hour; the resulting solid was colleted while hot, washed with warm dioxane and dried to give **4** as a white powder; yield 87%; mp>320°; ir: 3238 (NH_2); 1635 (C=O)cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): 1.72 (m, 4H, 2xCH_2), 2.54 (m, H, CH_2), 2.76 (m, 2H, CH_2), 6.33 (s, 2H, NH_2); ^{13}C nmr (dimethylsulfoxide-d_6): δ 21.40, 22.31, 24.06, 24.80, 115.32, 129.45, 130.90, 154.87, 161.85, 168.80.

Anal. Calcd. for $C_{10}H_{10}N_3NaOS_2$, C, 43.63; H, 3.63; N, 15.27; S, 23.27. Found: C, 43.50; H, 3.80; N, 15.00; S, 22.95.

To a suspension of sodium salt **4** (0.500 g, 1.8 mmoles) in water (100 ml) concentrated hydrochloride acid was added drop-wise under stirring to obtain pH 3-4; the mixture was stirred at room temperature for 1 hour; the resulting solid was collected, washed with water, dried and crystallized from dioxane to give **5** as colourless microcrystals, yield 50%; mp 264-66° dec. The analytical and spectral data were identical to those of a sample obtained according to previous paper [11].

2-(2-Ethoxyphenyl)-6,7,8,9-tetrahydro-10*H*-[1]benzothieno[2,3-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-10-one (**6**).

A mixture of amino-thioxo **5** (0.13 g, 0.5 mmoles), 2-ethoxybenzoic acid (0.20 g, 1.3 mmoles) and $POCl_3$ (1 ml) was heated at 100° for 1 hour. After cooling to room temperature, the reaction mixture was treated with ice/water and 5% sodium hydroxide The resulting solid was treated with warm ethanol, collected, washed with water, dried and crystallized from dimethylformamide to give **6** as a yellow powder ; yield 55%; mp 272-74°. The analytical and spectral data of 2-ethoxyphenyl derivative **6** were identical to those of a sample obtained according to Russo *et al*. [12].

Ethyl 2-({[2-(methylsulfonyl)hydrazino]carbonothioyl}amino)-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (**7a**).

To a stirred solution of methanesulphonyl hydrazide (0.48 g, 98%, 4.9 mmoles) in dichloromethane (20 ml) isothiocyanate **2** (1.1 g, 4.1 mmoles) in dichloromethane (10 ml) was added drop-wise; the mixture was stirred at room temperature for 2 hours. The resulting solid was collected, washed with dichloromethane and crystallized from ethanol to give **7a** as white micro-needles; yield 77%; mp 223-25 °C dec.; ir: 3294 and 3243 (NH), 1657 (C=O), 1324 and 1146 (SO_2N) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): δ 1.26 (t, J=7.0 Hz, 3H, CH_3), 1.70 (m, 4H, 2xCH_2), 2.59(m, 2H, CH_2), 2.71(m, 2H, CH_2), 3.08(s, 3H, CH_3), 4.30(q, J=7.0 Hz, 2H, CH_2), δ 9.95, 10.67 and 12.55 (s, 1H, NH); ^{13}C nmr (dimethylsulfoxide-d_6) : δ 14.02, 21.37, 22.48, 24.35, 25.62, 40.92, 60.77, 125.35, 134.67, 140.34, 150.29, 163.34, 177.99 .

Anal. Calcd. For $C_{13}H_{19}N_3O_4S_3$: C, 41.35; H, 5.03 ; N, 11.14; S,25.46. Found: C, 41.10 ;H, 5.10; N, 11.20; S, 25.15.

Ethyl 4,5-dimethyl-2-({[2-(methylsulfonyl)hydrazino]carbono-thioyl}aminothiophene-3-carboxylate (**7b**).

Same procedure adopted for compound **7a**. From dimethyl-sothiocyanate [10] (1.0 g, 4.1 mmoles); crystallized from ethanol/water to give **7b** as pale yellow powder; yield 60 %; mp 205-7° dec.; ir: 3289 and 3222 (NH), 1651 (C=O), 1307 and 1143 (SO_2N) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): δ 1.26 (t, J=7.0 Hz, 3H, CH_3), 2.19 (s, 3H, CH_3), 2.21 (s, 3H, CH_3) 3.08 (s, 3H, CH_3), 4.32 (q, J=7.0 Hz, 2H, CH_2), 9.95, 10.676 and 12.50 (s, 1H, NH); ^{13}C nmr (dimethylsulfoxide-d_6): δ 12.55, 12.72, 14.27, 43.55, 60.01, 122.34, 133.27, 135.62, 151.36, 161.27, 177.14 .

Anal. Calcd. for $C_{11}H_{17}N_3O_4S_3$: C, 37.60; H, 4.84; N, 11.96; S, 27.35. Found: C, 37.40; H, 4.60; N, 11.90; S, 27.10.

N-(4-Oxo-2-thioxo-1,4,5,6,7,8-hexahydro[1]benzothieno[2,3-*d*]-pyrimidin-3(2*H*)-yl)methanesulfonamide (**8a**).

A solution of mesylthiosemicarbazide **7a** (1.0 g, 2.65 mmoles) and sodium hydroxide (0.24 g, 6.0 mmol) in water (40 ml) was refluxed under stirring for 3 hours; the solution was filtered, quenched and acidified with concentrated hydrochloric acid to pH 3-4; the resulting solid was collected, washed with water, dried and crystallized from dimethylformamide/water to give **8a** as a white powder; yield 70%; mp 268-70° dec.; ir: 3212 (NH), 1681(C=O), 1345 and 1158 (SO_2-N) cm^{-1}; ^1H nmr (dimethyl-sulfoxide-d_6): δ 1.73 (m, 4H, 2xCH_2), 2.60 (m, 2H, CH_2), 2.65 (m, 2H, CH_2), 3.26 (s, 3H, CH_3), 10.28 (s,1H, NH), 13.82 (br s, 1H, NH) ; ^{13}C nmr (dimethylsulfoxide-d_6) : δ 21.46, 22.40, 23.97, 24.76, 44.51, 115.28, 129.38, 131.10, 149.00, 155.50, 174.53.

Anal. Calcd. for $C_{11}H_{13}N_3O_3S_3$: C, 39.86; H, 3.92; N, 12.67; S, 29.02. Found: C, 39.80; H, 3.80; N, 12.55; S, 28.65.

N-(5,6-Dimethyl-4-oxo-2-thioxo-1,4-dihydrothieno[2,3-*d*]-pyrimidin-3(2*H*)-yl)methanesulfonamide (**8b**).

Same procedure adopted for compound **8a**. From mesylthio-semicarbazide **7b** (0.930 g, 2.65 mmoles); crystallized from dimethylformamide/water to give **8b** as pale yellow powder yield; 60%; mp 265-67° dec.°C; ir: 3274 (NH), 1705 (C=O), 1326 and 1148 (SO_2N) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): δ 2.25 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.26 (s, 3H, CH_3), 10.30 (s, 1H, NH), 13.80 (br s, 1H, NH); ^{13}C nmr (dimethylsulfoxide-d_6): δ 12.08, 12.47, 44.50, 116.06, 126.39, 128.96, 148.24, 155.79, 174.44.

*Anal.*Calcd. for $C_9H_{11}N_3O_3S_3$: C, 35.40; H, 3.60; N, 13.70; S,31.47. Found : C, 35.60; H, 3.54; N, 13.61; S, 31.10.

N-(Methylsulfonyl)-*N*-[2-(methylthio)-4-oxo-5,6,7,8-tetrahydro[1]-benzothieno[2,3-*d*]pyrimidin-3(4*H*)-yl]methanesulfonamide (**10a**).

To a stirred solution of methyl derivative **9a** [13] (0.230 g, 0.86 mmoles) and triethylamine (1.0 ml) in dichloromethane (30 ml) a solution of methanesulfonyl chloride (0.22 ml, 2.85 mmoles, 99.5%, d=1.480) in dichloromethane (10 ml) was

added slowly drop-wise; the mixture was stirred at room temperature for 3 hours; the organic phase was washed with water, dried on sodium sulphate and concentrated under vacuum to give a solid that was collected, washed with diethyl ether and dried to give dimesyl derivative **10a** as an orange powder, resulted pure on thin layer chromatography; yield 20%; mp 204-07° dec.; ir : 1712 (C=O), 1376 and 1162 (SO_2-N) cm^{-1} .

*Anal.*Calcd. For : $C_{13}H_{17}N_3O_5S_4$: C, 36.87; H, 4.01; N, 9.92; S, 30.26. Found : C, 36.50; H, 3.90; N, 10.10; S, 29.95.

N-[5,6-Dimethyl-2-(methylthio)-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl]-N(methylsulfonyl)methanesulfonamide (**10b**).

Same procedure adopted for compound **10a**. From derivative **9b** [14] (0.200 g, 0.865 mmol) to obtain **10b** as a yellow powder; yield 30 % mp 228-31°dec. ; ir : 1711 (C=O), 1376 and 1163 N-SO_2) cm^{-1}.

Anal. Calcd. for: $C_{11}H_{15}N_3O_5S_4$: C, 33.25; H, 3.77; N, 10.57; S, 32.24. Found: C, 33.50; H, 3.60; N, 10.25; S, 32.00.

N-[2-(Methylthio)-4-oxo-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]-pyrimidin-3(4*H*)-yl]methanesulfonamide (**11a**)

A mixture of dimesyl derivative **10a** (0.100 g, 2.35 mmoles) in a solution of potassium hydroxide (1 *M*) in water (10 ml) and tetrahydrofuran (90 ml) was stirred at room temperature for 1 hour; the resulting solution was acidified with concentrated hydrochloride acid to pH 4-5, and then extracted with ethyl acetate; the extracts were concentrated under vacuum to give a residue that was collected and dissolved in 10% sodium hydroxide; the resulting solution was filtered and acidified with concentrated hydrochloride acid to pH 4-5, and the solid separated was collected, washed with water and dried to give the monosulfonyl derivative **11a** as an amorphous white powder, resulted pure on thin layer chromatography; yield 25%; mp 218-20 °C; ir : 3209 (NH), 1691 (C=O), 1325 and 1150 (N-SO_2) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.76 (s, 4H, 2XCH_2), 2.43 (s, 3H, CH_3), 2.50 (s, 2H, CH_2), 2.71 (s, 2H, CH_2), 3.29 (s, 3H, CH_3), 11.13 (s, 1H, NH); ^{13}C nmr (dimethylsulfoxide-d_6) : δ 14.01, 20.63, 21.98, 23.35, 24.97, 45.22, 115.23, 127.66, 134.15, 155.73, 158.33, 163.21 .

Anal. Calcd. for $C_{12}H_{15}N_3O_3S_3$: C,41.73; H, 4.34; N, 12.17; S, 27.82 . Found : C, 42.00; H, 4.20; N, 12.15; S, 27.60.

From monomesyl derivative **8a**. To a solution of thioxo-monomesyl derivative **8a** (0.122 g, 0.37 mmoles), dissolved in a solution of potassium hydroxide (42 mg, 0.73 mmoles) in water (20 ml), methyl iodide (0.3 ml),99 %, d=2.27) was added and the mixture was stirred at room temperature for 1.5 hours; the mixture was then filtered and the filtrate was acidified with concentrated hydrochloride acid to pH 4-5: the solid separated was collected, washed with water and dried to give **11a** as a white amorphous powder, resulted pure at thin layer chromatography; yield 75 %. This compound was identical to that obtained from hydrolysis of dimesyl derivative **10a**.

N-[5,6-Dimethyl-2-(methylthio)-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl]methanesulfonamide (**11b**).

Same procedure adopted for compound **11a**. From derivative **10b** (90 mg, 2.25 mmoles) to obtain **11b** as a white powder; yield 35%; mp 260-63°; ir : 3115 (NH), 1652 (C=O), 1355 and 1161 (SO_2-N) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): 2.34 (s, 6H, 2xCH_3), 2.42 (s, 3H,CH_3), 3.29 (s, 3H, CH_3), 11.10 (s, 1H,NH); ^{13}C nmr (dimethylsulfoxide-d_6): δ 12.10, 12.45, 14.12, 44.50, 114.68, 125.32, 136.23, 154.21, 157.62, 165.32

Anal. Calcd. for: $C_{10}H_{13}N_3O_3S_3$: C, 37.61; H, 4.07; N, 13.16; S, 30.09. Found: C, 37.75; H, 3.90; N, 13.40; S, 30.25.

From monomesyl derivative **8b**. The same procedure used from **8a**. The product **11b** was obtained as white amorphous powder and was identical to that obtained from hydrolysis of dimesyl derivative **10b**; yield 85 %.

2-({3-[(Methylsulfonyl)amino]-4-oxo-3,4,5,6,7,8-hexahydro[1]-benzothieno[2,3-*d*]pyrimidin-2-yl}thio)benzoic acid (**12a**).

To a solution of methane sulphonamide-thioxo derivative **8a** (0.34 g, 0.90 mmoles) and potassium hydroxide (100 mg, 1.8 mmoles) in water (40 ml) 2-iodobenzoic acid (0.240 g, 98%, 0.95 mmoles), dissolved in a small amount of 10 % sodium hydroxide and powdery copper (50 mg) were added. The mixture was heated at 100 °C under stirring for 5 hours. The mixture was filtered while hot and after cooling to room temperature the solution was acidified to pH 3-4: the resulting solid was collected, washed with water, dried and crystallized from dioxane/water to give **12a** as a white powder; yield 55 %; mp 236-38 °C; ir : 3304 (NH), 1695 (C=O), 1340 and 1151 (N-SO_2) cm^{-1}; ^{1}H nmr dimethtylsulfoxide-d_6): δ 1.75 (m, 4H, 2xCH_2), 2.67 (m, 2H, CH_2), 2.81 (m, 2H, CH_2), 3.16 (s, 3H, CH_3), 7.54-7.91 (m, 4H, Ar-H), 11.32 (s, 1H, NH), 13.07 (br s, 1H, COOH); ^{13}C nmr (dimethylsulfoxide-d_6): δ 21.01, 23.71, 24.88, 25.77, 44.12, 115.12, 125.16, 128.33, 130.01, 132.01, 133.44, 135.01, 135.66, 140.01, 158.22, 163.27, 168.01, 169.79 .

Anal. Calcd. for $C_{18}H_{17}N_3O_5S_3$: C, 47.89; H, 3.76; N, 9.31; S, 21.28.Found: C, 47.55; H, 4.00; N, 9.30; S, 21.00.

2-({5,6-Dimethyl-3-[(methylsulfonyl)amino]-4-oxo-3,4-dihydro-thieno[2,3-*d*]pyrimidin-2-yl}thio)benzoic acid (**12b**).

Same procedure adopted for compound **12a**. From sulphonamide **8b** (0.32 g, 0.9 mmol crystallized from water/ethanol to give **12b** as a white powder; yield 50%; mp 237-40°; Ir: 3306 (NH), 1695 (C=O), 1343 and 1153 (N-SO_2) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): δ 2.29 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.17 (s, 3H, CH_3), 7.53-7.91 (m, 4H, Ar-H), 11.29 (s, 1H, NH), 13.04 (br s, 1H, COOH); ^{13}C nmr (dimethylsulfoxide-d_6): δ 13.61, 14.67, 44.12, 114.67, 125.01, 125.67, 129.55, 131.98, 134.67, 135.01, 139.03, 140.66, 156.01, 164.07, 168.23, 170.01.

Anal. Calcd. for $C_{16}H_{15}N_3O_5S_3$: C,45.17; H, 3.52; N, 9.88; S, 22.58. Found: C, 44.85; H, 3.50; N, 9.81; S, 22.20.

N-[2-[(2,4-Dinitrophenyl)thio]-4-oxo-5,6,7,8-tetrahydro[1]benzo-thieno[2,3-*d*]pyrimidin-3(4*H*)-yl]methanesulfonamide (**13a**).

To a solution of methylsulfonylamino-thioxo derivative **8a** (0.14 g, 0.43 mmoles), and potassium hydroxide (50 mg, 0.85 mmoles) in ethanol /water 1:1 (40 ml) 2,4-dinitroiodobenzene (0.13 g, 97%, 0.43 mmoles) and powdery copper (50 mg) were added; the mixture was heated at 100 °C under stirring; after 4 hours the mixture was filtered while hot and the filtrate, cooled to room temperature, was acidified with concentrated hydrochloric acid to pH 3-4: the resulting red solid was collected, washed with water, dried and crystallized from ethanol/water to give **13a** as an orange powder; yield 69 %; mp 123-26° dec.; ir: 3010 br (NH), 1697 (C=O), 1343 and 1153 (N-SO_2) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.75 (s, 4H, 2xCH_2), 2.62(s, 2H, CH_2), 2.83 (s, 2H, CH_2), 3.53 (s, 3H, CH_3), 8.14-8.86 (m, 3H, Ar-H), 11.55 (s, 1H, NH); ^{13}C nmr (dimethylsulfoxide-d_6): δ 21.54, 22.28, 24.47, 24.97, 43.83,

119.66, 120.64, 127.34, 130.91, 131.14, 133.96, 138.36, 148.07, 150.80, 155.68, 157.015, 159.53.

Anal. Calcd. for: $C_{17}H_{15}N_5O_7S_3$: C, 41.04; H, 3.01; N, 14.08; S,19.31. Found: C,41.40; H,2.70; N, 13.80; S, 18.95.

N-[2-[(2,4-Dinitrophenyl)thio]-5,6-dimethyl-4-oxothieno[2,3-*d*]-pyrimidin-3(4*H*)-yl]methanesulfonamide(**13b**).

Same procedure adopted for compound **13a**. Form sulphonamide **8b** (0.12 g 0.43mmoles); crystallized from ethanol/water to give **13b** as a yellow powder; yield 45 %.; mp 193-95°dec.; ir: 3210 br (NH), 1697 (C=O), 1343 and 1158 (N-SO_2) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): δ 2.32(s, 3H, CH_3), 2.35(s, 3H, CH_3), 3.53 (s. 3H,CH_3), 8.14-8.91(m, 3H, Ar-H), 11.53(s, 1H, NH); ^{13}C nmr (dimethylsulfoxide-d_6): δ 13.01, 13.65, 44.27, 112.44, 120.77, 127.10, 130.01,131.58, 132.20, 138.41, 148.01, 149.23, 153.23, 162.16, 169.14.

Anal. Calcd. for $C_{15}H_{13}N_5O_7S_3$: C, 38.21; H, 2.76; N, 14.86; S, 20.38. Found: C, 38.10; H, 2.95; N, 14.60; S, 20.00.

N-[2-[(2,4-Difluorophenyl)thio]-4-oxo-5,6,7,8-hexahydro[1]-benzothieno[2,3-*d*]pyridin-3(4*H*)-yl]methanesulfonamide (**14a**).

To a stirred solution of sulphonamide **8a** (0.28 g, 0.85 mmoles) and potassium hydroxide (100 mg, 1.8 mmoles) in ethanol/water 1:1(40 ml) 2,4-difluoroiodobenzene (0.22 g, 0.11 ml, 98%, 0.85 mmoles, d=2.006) and powdery copper (50 mg) were added; the mixture was heated at reflux under stirring for 4 hours; after cooling to room temperature the mixture was filtered and the filtrate poured in water (200 ml); the resulting solution was filtered and acidified with hydrochloric acid to pH 3-4: the solid separated was collected, washed with water, dried and crystallized from ethanol/water to give **14a** as a white powder; yield 45 %; mp 235-36 °C; ir: 3250 (NH), 1687 (C=O), 1347 and 1156 (SO_2-NH) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.74 (s, 4H, 2xCH_2), 2.66 (s, 2H, CH_2), 2.81(s, 2H, CH_2), 3.33 (s, 3H, CH_3), 7.18-7.73 (m, 3H, Ar-H), 11.38 (s, 1H, NH); ^{13}C nmr (dimethylsulfoxide-d_6): δ 21.49, 22.23, 24.32, 24.91, 43.97, 105.12 (t, J=26.15 Hz), 112.68 (d, J=21.2 Hz), 130.88, 132.70, 138.77 (d, J=10.3) 139.21, 160.22, 161.50, 165.51, 166.50, 184.99, 188.44 .

Anal. Calcd. for $C_{17}H_{15}F_2N_3O_3S_3$: C, 46.04; H, 3.38; N, 9.48; S, 21.67. Found: 45.75; H, 3.55; N, 9.30; S, 21.50.

N-[2-[(2,4-Difluorophenyl)thio]-5,6-dimethyl-4-oxothieno[2,3-*d*]-pyrimidin-3(4*H*)-yl]methanesulfonamide (**14b**).

Same procedure adopted for compound **14a**. From sulphonamide **8b** (0.26 g, 0.85 mmoles); recrystallized from water/ dioxane to give **14b** as a white powder; yield 60 %; mp 257-59°; ir: 3206 (NH), 1689 (C=O), 1328 and 1149 (SO_2-NH) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): δ 2.30 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.36 (s, 3H, CH_3), 7.18-7.73 (m, 3H, Ar-H), 11.38 (s, 1H, NH); ^{13}C nmr (dimethylsulfoxide-d_6) : δ 12.52, 12.63, 48.20, 105.21 (t, J=25.6), 109.57, 112.80 (d, J=21.3), 117.88, 128.87, 129.86, 138.62 (d, J=10.2), 139.41, 155.97, 163.66, 170.97, 171.24.

Anal. Calcd. for $C_{15}H_{13}F_2N_3O_3S_3$: C, 43.16; H, 3.11; N, 10.07; S, 23.02. Found: C, 43.00; H,3.25; N, 9.90; S, 22.80.

N-[2-(Cyclohexylthio)-4-oxo-5,6,7,8-tetrahydro[1]benzothieno-[2,3-*d*]pyrimidin-3(4*H*)-yl]methanesulfonamide (**15a**).

A mixture of sulphonamide **8a** (0.21 g, 0.63 mmoles), cycloexyl iodide (0.146 g, 98%, 0.68 mmoles, 0.09 ml, d=1.625) and potassium carbonate (0.18 g) in dimethylformamide (2 ml) was heated at 80 °C under stirring for 10 hours; after cooling to room temperature the mixture was treated with hydrochloric acid and poured in water (100 ml): the resulting solid was collected, washed with water, dried and recrystallized from petroleum ether to give **15a** as a yellow powder; yield 45 % ; mp 106-08°dec. ; ir: 3205 (NH), 1695 (C=O), 1349 and 1155 (SO_2-NH) cm^{-1}; ^{1}H nmr(dimethylsulfoxide-d_6): δ 1.22-1.99 (m, 14 H, cycloexyl and 2xCH_2) , 2.71 and 2.82 (s, 2H, CH_2), 3.33 (s, 3H, CH_3), 3.40-3.80 (m, 1H, -S-CH), 11.03 (s, 1H, NH).

Anal. Calcd. for $C_{17}H_{23}N_3O_3S_3$: C, 49.39; H, 5.56; N, 10.16; S, 23.24. Found: C, 49.50; H, 5,35; N, 10.35; S, 23.00.

N-[2-(Cyclohexylthio)-5,6-dimethyl-4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl]methanesulfonamide (**15b**).

Same procedure adopted for compound **15a**. From sulphonamide **8b** (0.19 g, 0.63 mmoles); recrystallized from petroleum ether to give **15b** as a white powder; yield 35 %; mp127-30°dec.; ir: 3205 (NH), 1695 (C=O), 1350 and 1157 (SO_2-NH) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.22-1.99 (m, 10 H, cyclohexyl), 3.29 (s, 3H, CH_3), 3.33 (s, 3H, CH_3), 3.40 (s, 3H, CH_3), 3.40-3.80 (m, 1H, -S-CH), 11.56 (s, 1H, NH).

Anal. Calcd. for $C_{15}H_{21}N_3O_3S_3$: C, 46.51; H, 5.42; N, 10.85; S, 24.80. Found: C, 46.30; H, 5.55; N, 10.70; S, 25.10.

REFERENCES AND NOTES

[1] A. Santagati, M. Modica, M. Santagati,V. Cutuli, D. Amore and A. Caruso, *Farmaco, Ed. Sc*., **50**, 605 (1995).

[2] A. Santagati, G. Granata, A. Marrazzo and M. Santagati, *Arch. Pharm. Pharm. Med. Chem*., **336**, 429 (2003).

[3] D. L. DeWitt, *Mol. Pharmacol*., **55**, 625, (1999).

[4] G. Dannhart and W. Kiefer, *Eur. J. Med. Chem*., **36**, 109 (2001).

[5] H. Wamhoff and L. Lichtenthaler, *Chem. Ber*., **111**, 2297 (1978).

[6] H. Wamhoff, Advances in Heterocyclic Chemistry, Vol. **38**, A. R. Katritzky, ed. Academic Press, Orlando, 1985, pp 299-368.

[7] J. A. Mitchell, P. Akarasereenont, C. Thiemerman, R. J. Flower, J. R. Vane, *Proc. Natl. Acad. Sci USA*, **90** 11693 (1994).

[8] M. Biava, G. C. Porretta, A. Cappelli, S. Vomero, F. Manetti, M. Botta, L. Sautebin, A. Rossi, F. Makovec, and M. Anzini, *J. Med. Chem*., **48**, 3428 (2005).

[9] K. Gewald, E. Schinke and H. Bottcher, *Chem. Ber*., **99**, 94 (1966).

[10] F. Kienzle, A. Kaiser and R. E. Minder, *Helv. Chim. Acta*, **66**, 148 (1983).

[11] A. Santagati, M. Santagati and M. Modica, *Heterocycles*, **36**, 1315 (1993)

[12] F. Russo, M. Santagati, A. Santagati, A. Caruso, S. Trombatore and M. Amico Roxas, *IlFarmaco Ed. Sc*., **38**, 762 (1983).

[13] A. Santagati, M. Modica and M. Santagati, *J. Heterocyclic Chem*., **31**, 1141 (1994).

[14] M. Modica, M. Santagati and A. Santagati, *J. Heterocyclic Chem*., **38**, 973 (2001).