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Pyridazines. Part 31[1]: Synthesis and Antiplatelet Activity of 4,5-Disubstituted-6-phenyl-3(2*H*)-pyridazinones

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This paper describes the synthesis and the antiplatelet activity of a series of 4,5-disubstituted-6-phenyl-3(2*H*)-pyridazinones. Some of these compounds show a dose-dependent activity and were found to be more active than their 5-substituted analogues.

Key words pyridazinone; platelet; platelet aggregation; platelet aggregation inhibitor

The homeostatic system is designed to maintain fluid blood flow under physiological conditions and to react rapidly to form a clot at sites of vascular damage. Nevertheless, the intravascular formation of platelet aggregates is an important pathogenic factor in widespread cardiovascular diseases, such as myocardial and cerebral circulatory disorders, venous thromboses, cardiac and cerebral infarcts and arteriosclerosis, as well as contributing to the risk of embolism in surgery on the heart and blood vessels. In these fields, the discovery of aggregation-inhibiting drugs has led to a new approach to therapy and prophylaxis.[2,3] The active compounds hitherto available for this function, such as acetylsalicylic acid, sulfinpyrazone and dipyridamole, have a relatively low activity and, consequently, extended therapy is unsatisfactory and the search for promising new drugs in this therapeutic area is of great interest. One of the most studied targets in this field is represented by the PDE III inhibitors, which have received a great deal of attention in recent years, not only because of their antiplatelet properties but also on the basis that they could be used as cardiotonic agents due to the absence of safe and orally effective ionotropic agents for the treatment of congestive heart failure.

In this regard a considerable number of 3(2*H*)-pyridazinones and their 4,5-dihydroderivatives with anti-aggregating properties have been described.[4,5] Zardaverine and Pimobendan are probably the most representative examples of this class of compounds.

As part of our ongoing medicinal chemistry project aimed at exploring the platelet inhibitory activity of a series of 5-substituted-6-aryl-3(2*H*)-pyridazinones,[6—10] we recently described the potent antiplatelet activity of compounds in this series along with some preliminary pharmacological studies. These studies reveal that the substituent in the 5-position not only determines the potency of the antiplatelet activity (quantitative aspect) but also the mechanism of action (qualitative aspect).[9,10] These results have prompted us to synthesise and evaluate the antiplatelet activity of several 4,5-disubstituted-6-phenyl-3(2*H*)-pyridazinones in order to determine the pharmacological profile induced by the introduction of a substituent at position 4 of this system.

Chemistry

The general synthetic strategy used to prepare the 4,5-disubstituted pyridazinones **3** is shown in Charts 1 and 2. The precursors in these routes are the 5-substituted pyridazinones **1**[11] and **2**[12] and the strategy is based on the modification of the reactivity at the 4-position caused by the electronic effect of the neighbouring group. Some preliminary results related to the compounds studied here as antiplatelet agents have been published in communication form.[13,14]

As part of our detailed study into the structure/reactivity relationships in these series of compounds we recently described a new synthetic route to 4,5-disubstituted-3(2*H*)-pyridazinones based on the high reactivity of aldehyde **1** toward the cyanide anion.[13] This route affords compounds bearing a cyano group at position 4 of the heterocyclic ring. The synthetic exploitation of this transformation (involving the cyanohydrin intermediate **1a**) has allowed the efficient preparation of several new derivatives (Chart 1).

Bearing this result in mind, we have then studied several transformations that involve the use of cyanohydrin **1a** as an intermediate. One of the best known of these procedures is the preparation of carboxylic acids by oxidation of cyanohydrins. The selective action of silver oxide (obtained by addition of sodium hydroxide to silver nitrate) on the hydroxyl group at position 5 of cyanohydrin **1a** provided an excellent preparative method to obtain 5-carboxy-4-cyano-6-phenyl-3(2*H*)-pyridazinone **3a** as a crystalline solid in 88% yield. This step occurs through nucleophilic attack of the hydroxyl ion on the acylcyanide intermediate. Another important procedure involving cyanohydrin formation is the one-pot three-step transformation of α,β-unsaturated aldehydes into the

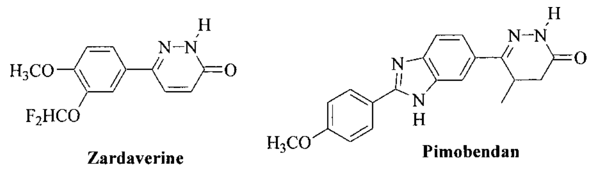

Fig. 1. Examples of Pharmacologically Useful 3(2*H*)-Pyridazinones

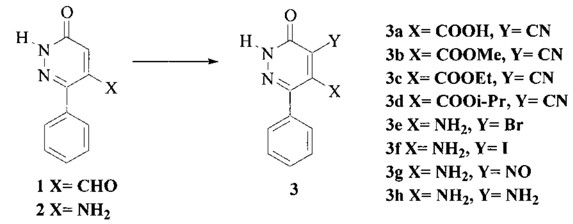

Fig. 2. 4,5-Disubstituted-6-phenyl-3(2*H*)-pyridazinones **3**

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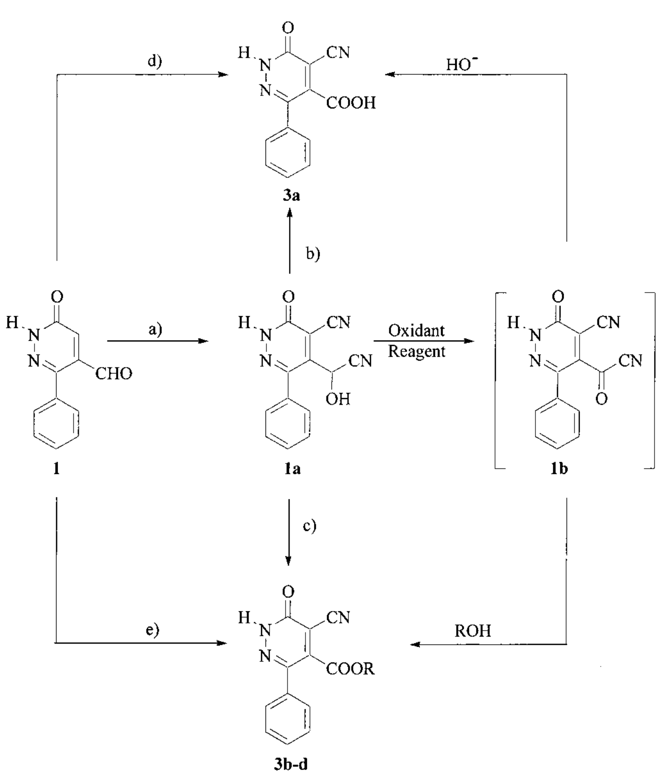
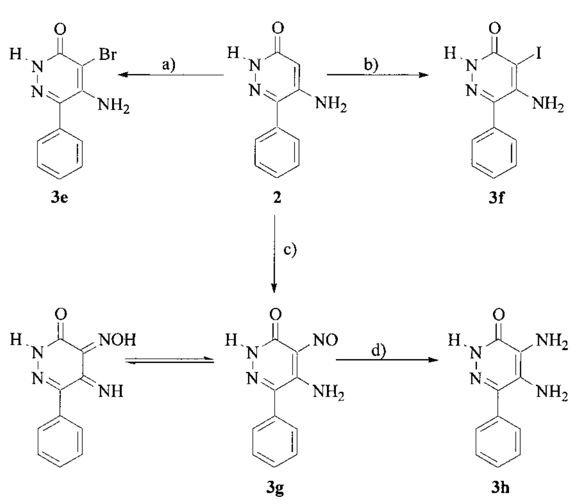


Reagents: (a) NaCN/EtOH, (b) AgNO$_3$/NaOH, (c) MnO$_2$/ROH, (d) (i) NaCN/EtOH (ii) AgNO$_3$/NaOH, (e) (i) NaCN/ROH, (ii) MnO$_2$.

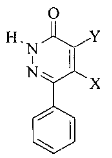
Chart 1



Reagents: (a) Br$_2$/AcOH, (b): I$_2$/dioxane, (c) NaNO$_2$/AcOH, (d) H$_2$/Pt$_2$O.

Chart 2

Table 1. Antiplatelet Activity of the 4,5-Disubstituted-6-phenyl-3(2*H*)-pyridazinones **3**



Compound	X	Y	IC$_{50}$ (mM)
3a	COOH	CN	0.71
3b	COOMe	CN	—
3c	COOEt	CN	1.10
3d	COO^{i}Pr	CN	0.53
3e	NH$_2$	Br	*a)*
3f	NH$_2$	I	—
3g	NH$_2$	NO	0.17
3h	NH$_2$	NH$_2$	*b)*
2	NH$_2$	H	—
4	COOH	H	—
Milrinone	—	—	0.0047

a) Precipitate at the studied doses. *b)* Induces platelet aggregation. —=Inactive.

corresponding esters by treatment with manganese dioxide and sodium cyanide.[15,16] The formation of the ester group takes place without isolation of the cyanohydrin, which is oxidised to an activated acylcyanide that, in turn, undergoes alcoholysis by the solvent. On the basis of these references, we performed the oxidation of cyanohydrin **1a** with manganese dioxide in different alcohols, to obtain the corresponding 5-alkoxycarbonyl-4-cyano-6-phenyl-3(2*H*)-pyridazinones **3b**—**d** in high yields as yellow crystalline solids. Is interesting to note that compounds **3a**—**d** can be prepared in one-pot procedures (Chart 1).

During the spectroscopic characterisation (^{1}H-, ^{13}C-NMR) of different 5-amino-3(2*H*)-pyridazinones[12] we have observed the nucleophilic character generated at C$_4$ produced by the push–pull effect operating in these compounds. In accordance with these observations, enaminone **2** readily reacted with different electrophiles to afford 5-amino-4-substituted-3(2*H*)pyridazinones **3e**—**h**. Thus, compound **2** was easily halogenated in the 4-position by treatment with bromine in acetic acid or iodine in dioxane to afford compounds **3e** and **3f**, respectively. In turn, nitrosation of **2** gives 5-amino-4-nitroso-6-phenyl-3(2*H*)-pyridazinone (**3g**) as a deep-red solid in excellent yield. Catalytic hydrogenation of the nitroso group in **3g** gave high yields of the diamine **3h**.

The 3(2*H*)-pyridazinones **3a**—**h** were fully characterised by their analytical and spectroscopic data. The IR spectra of compounds **3** showed the presence of NH and carbonyl groups (3380—3280 cm^{-1}, 1680 cm^{-1}) respectively. The structures of these compounds was confirmed by the absence of the H$_4$ signal in the ^{1}H-NMR spectra. Further experiments are currently in progress because these compounds constitute versatile intermediates to prepare other 4,5-disubstituted- and 4,5-heterofused-pyridazinones.

Results and Discussions

The platelet aggregation inhibitory activities of the 4,5-disubstituted-3(2*H*)-pyridazinone derivatives described here were examined on washed human platelets using the turbidimetric method of Born[17] and thrombin as inducer of platelet aggregation. The results of these experiments are summarised in Table 1.

Several of the studied pyridazinones showed antiplatelet activity, although in each case it was lower than the reference compound milrinone. A few of the compounds studied inhibit platelet aggregation in a dose-dependent manner. Comparison of these results with the antiplatelet activity of the 5-substituted-6-phenyl-3(2*H*)-pyridazinones (**4**, **2**) (Table 1) shows that the introduction of a substituent at position 4 of these compounds produces an increase in the platelet inhibitory activity; this effect is particularly significant in compounds **3a**, **3c**, **3d** and **3g**. For the cyanoesters **3b**—**d**, a slight increase in activity was observed within the series due to the modification of the alkoxy group in the ester function; the isopropyl derivative **3d** is the most active. Curiously, the diamine **3h** has the opposite activity and actually induces platelet aggregation.

As can be observed from the results in Table 1, the most potent compound within this series is the nitroso derivative **3g**. Bearing in mind the fact that nitroso derivatives are fatal in medical applications,[18] we are now preparing new compounds bearing substituents that can mimic the nitroso group (*e.g.* CHO, COR).

Although the antiplatelet activity of these derivatives is only modest, the real value of the data in Table 1 is to provide useful information to complete our overview of the SAR and mechanistic pharmacological studies of these series'.[9,10] These studies are still in progress and will be published in due course.

Experimental

Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 1640 FTIR spectrophotometer. ^{1}H-NMR spectra were obtained on Bruker WM250 and AM300 Hz spectrometers using tetramethylsilane as the internal standard (chemical shifts are in δ values, J in Hz). Mass spectra were determined on a Varian MAT-711 instrument. Elemental analyses were performed on a Perkin-Elmer 240B apparatus at the Microanalysis Service of the University of Santiago de Compostela. The progress of the reactions was monitored by thin layer chromatography with 2.5 mm Merck silica gel GF 254 strips, and the purified compounds each showed a single spot; unless otherwise stated iodine vapour and/or UV light were used for detection. Chromatographic separations were performed on silica gel columns by flash chromatography (Kieselgel 40, 0.040—0.063 mm).

Preparation of Washed Platelets Human platelet concentrates from blood anticoagulated with citrate-phosphate-dextrose were obtained from the Centro de Transfusión de Galicia (Santiago de Compostela, Spain). Platelets were purified by sedimentation through a discontinuous metrizamide gradient. For this purpose 8 ml of platelet concentrate was layered onto a 10%/25% (1 ml/1 ml) metrizamide gradient and centrifuged at 1000×***g*** for 20 min. The resulting platelet band was recovered, diluted to 8 ml with washing buffer (NaCl, 140 mM; KCl, 5 mM; trisodium citrate, 12 mM; glucose, 10 mM; sucrose, 12.5 mM; pH 6) and centrifuged again at 1000×***g*** for 20 min. Finally, the platelet band recovered from this step was resuspended in a modified Tyrode-HEPES buffer (HEPES, 10 mM; NaCl, 140 mM; KCl, 3 mM; MgCl, 0.5 mM; $NaHCO_3$, 5 mM; glucose, 10 mM; pH 7.4) to afford a concentration of 3—3.5 10^{-8} platelets/ml. The calcium concentration in the extracellular medium was 2 mM.

Platelet Aggregation Studies Platelet aggregation was measured using a dual channel aggregometer (Chrono-log, Havertown, PA, U.S.A). Each test compound was incubated with washed platelets at 37 °C for 5 min. Stimulus was then added to induce platelet aggregation, and the light transmission was monitored over a 5 min period. Platelet aggregation is expressed as the maximum change in light transmission during this period, with a 100% value being obtained when only stimulus, and not compound, was added.

4-Cyano-5-(1′-cyanohydroxymethyl)-6-phenyl-3(2*H*)-pyridazinone (1a) To a stirred solution of aldehyde **1** (0.10 g, 0.5 mmol) in ethanol (10 ml) was added, in several portions, sodium cyanide (0.07 g, 0.15 mmol) and the stirring was maintained for 1 h. The reaction mixture was poured into ice and then extracted with ethyl acetate. The organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give an oily residue that was purified by column chromatography (hexane/ethyl acetate, 1 : 1). Subsequent recrystallization from isopropanol afforded the cyanohydrin **1a** as yellow prisms (0.12 g, 90%). mp 145.5—147.0 °C (dec.). IR (KBr): ν_{max} cm^{-1} 3808—3065, 2230, 1663, 1590. ^{1}H-NMR: δ_H (300 MHz, DMSO-d_6): 3.21 (br s, 1H, OH), 4.10 (br s, 1H, CH), 7.55—7.48 (5H, m, Aromatics), 13.94 (1H, br s, NH, deuterium oxide exchangeable). HR-MS of sodium salt of **1a** *m/z* Calcd for $C_{13}H_7N_4$ NaO (M^+): 274.0467, Found: 274.0491.

5-Carboxy-4-cyano-6-phenyl-3(2*H*)-pyridazinone (3a) To a stirred solution of aldehyde **1** (0.20 g, 1.0 mmol) in ethanol (30 ml) was added slowly sodium cyanide (0.14 g, 3.0 mmol) and the stirring was maintained for 15 min at room temperature. To this suspension was added silver nitrate (0.50 g, 3.0 mmol) and the pH of the mixture was increased to 12 by the addition of 10% sodium hydroxide. The reaction mixture was stirred for 24 h at room temperature, diluted with 30 ml of dichloromethane and filtered through silica. The filtrate was treated with 10% hydrochloric acid and then extracted with dichloromethane. The extracts were dried (Na_2SO_4) and the solvent was removed under reduced pressure to afford a solid that was purified by recrystallization from isopropanol (0.20 g, 80%). mp 253—255 °C. IR (KBr): ν_{max} cm^{-1} 3000, 2235, 1688, 1648, 1589. ^{1}H-NMR: δ_H (300 MHz, DMSO-d_6): 7.17 (1H, br s, deuterium oxide exchangeable OH), 7.43 (5H, m, Aromatics), 13.47 (1H, br s, deuterium oxide exchangeable, NH). $C_{12}H_7N_3O_3$ requires C, 59.75; H, 2.92; N, 17.43, Found: C, 59.86; H, 3.14; N, 17.76.

5-Alkoxycarbonyl-4-cyano-6-phenyl-3(2*H*)-pyridazinones 3b-d, General Procedure To a stirred solution of aldehyde **1** (0.20 g, 1.0 mmol) in the corresponding alcohol (30 ml) was added slowly sodium cyanide (0.14 g, 3.0 mmol) and the stirring was maintained for 15 min at room temperature. To this suspension was added activated manganese dioxide (1.2 g, 12.9 mmol) and the stirring was maintained for 15 min at room temperature. The reaction mixture was diluted with dichloromethane and filtered through silica gel. The solvents were removed under reduced pressure and the resulting solid purified by recrystallization from isopropanol.

4-Cyano-5-methoxycarbonyl-6-phenyl-3(2*H*)-pyridazinone (3b) (0.24 g, 95%). mp 190—191 °C. IR (KBr): ν_{max} cm^{-1} 2230, 1735, 1668, 1588. ^{1}H-NMR: δ_H (300 MHz, DMSO-d_6): 3.76 (1H, s, OCH_3), 7.40 (2H, m, Aromatics), 7.50 (3H, m, Aromatics), 14.63 (1H, br s, NH, deuterium oxide exchangeable). $C_{13}H_9N_3O_3$ requires C, 61.17; H, 3.55; N, 16.46, Found: C, 61.24; H, 3.55; N, 16.56.

4-Cyano-5-ethoxycarbonyl-6-phenyl-3(2*H*)-pyridazinone (3c) (0.25 g, 95%). mp 181—183 °C. IR (KBr): ν_{max} cm^{-1} 2230, 1720, 1666, 1569. ^{1}H-NMR: δ_H (300 MHz, DMSO-d_6): 1.02 (t, J=6.9 Hz, 3H, CH_3), 4.24 (q, J=6.9 Hz, 2H, OCH_2), 7.45 (2H, m, Aromatics), 7.48 (3H, m, Aromatics), 14.35 (1H, br s, NH, deuterium oxide exchangeable). $C_{14}H_{11}N_3O_3$ requires C, 62.45; H, 4.12; N, 15.61, Found: C, 62.81; H, 4.03; N, 15.78.

4-Cyano-5-isopropyloxycarbonyl-6-phenyl-3(2*H*)-pyridazinone (3d) (0.26 g, 93%). mp 184—186 °C. IR (KBr): ν_{max} cm^{-1} 2233, 1731, 1669, 1587. ^{1}H-NMR: δ_H (300 MHz, DMSO-d_6): 1.11 (d, J=6.0 Hz, 6H, 2×CH_3), 5.04 (m, J=6.0 Hz, 1H, OCH), 7.49 (5H, m, Aromatics), 14.34 (1H, br s, NH, deuterium oxide exchangeable). $C_{15}H_{13}N_3O_3$ requires C, 63.59; H, 4.62; N, 14.83, Found: C, 63.71; H, 4.57; N, 14.96.

5-Amino-4-bromo-6-phenyl-3(2*H*)-pyridazinone (3e) To a mixture of 5-amino-6-phenyl-3(2*H*)-pyridazinone **2** (0.70 g, 3.7 mmol) and sodium acetate trihydrate (0.66 g, 4.8 mmol) in acetic acid (15 ml) at 0 °C was slowly added a solution of bromine (0.65 ml, 4.4 mmol) in acetic acid (3 ml). The resulting mixture was stirred at room temperature for 45 min and then added to ice/water. The precipitated solid was collected by filtration. Further purification was carried out by recrystallization from isopropanol to give **3e** as a white solid (0.91 g, 92%). mp 323—325 °C (dec.). IR: ν_{max} cm^{-1} 3422—3185 (NH_2), 1643 (CO), 1578 (Aromatics). ^{1}H-NMR: δ_H (300 MHz, DMSO-d_6): 6.04 (2H, br s, NH_2, deuterium oxide exchangeable), 7.48 (5H, m, Aromatics), 12.64 (1H, br s, NH, deuterium oxide exchangeable). $C_{10}H_8BrN_3O$ requires C, 45.13; H, 3.03; N, 15.79, Found: C, 45.21; H, 3.17; N, 15.72.

5-Amino-4-iodo-6-phenyl-3(2*H*)-pyridazinone (3f) To a solution of 5-amino-6-phenyl-3(2*H*)-pyridazinone **2** (0.20 g, 1.06 mmol) in dioxane (15 ml) was added iodine (0.27 g, 2.13 mmol) and 0.75 M nitric acid (1.5 ml). The mixture was heated under reflux for 12 h, the solvent was removed under reduced pressure and the resulting residue was poured into ice to afford a solid, which was purified by recrystallization from ethanol (0.27 g, 81%). mp 254—256 °C (dec.). IR: ν_{max} cm^{-1} 3500—3200 (NH_2), 1639 (CO), 1581 (Aromatics). ^{1}H-NMR: δ_H (300 MHz, DMSO-d_6): 5.93 (2H, br s, NH_2, deuterium oxide exchangeable), 7.55 (5H, m, Aromatics), 12.57 (1H, br s, NH, deuterium oxide exchangeable). $C_{10}H_8IN_3O$ requires C, 38.36; H, 2.58; N, 13.42, Found: C, 38.72; H, 2.47; N, 13.48.

5-Amino-4-nitroso-6-phenyl-3(2*H*)-pyridazinone (3g) To a solution of 5-amino-6-phenyl-3(2*H*)-pyridazinone **2** (0.50 g, 2.7 mmol) in a 1 : 1 mixture of acetic acid/water (25 ml) at 65 °C was added slowly a solution of sodium nitrite (0.36 g, 5.3 mmol) in water (4 ml). The reaction mixture was stirred at 65 °C during 2 h and the solid that precipitated was collected by filtration. Further purification was carried out by recrystallization from a 1 : 1 mixture of ethyl acetate/methanol to give **3g** as a red solid (0.45 g, 80%). mp 238—240 °C (dec.). IR: ν_{max} cm^{-1} 3422—3185 (NH_2), 1633 (CO), 1588 (Aromatics). ^{1}H-NMR: δ_H (300 MHz, DMSO-d_6): 3.38 (2H, br s, NH_2, deuterium oxide exchangeable), 7.40 (3H, m, Aromatics), 7.90 (2H, m, Aromatics), 12.39 (1H, br s, NH, deuterium oxide exchangeable). HR-MS *m*/*z* Calcd for $C_{10}H_8N_4O_2$ (M^+): 216.1963, Found: 216.1952.

4,5-Diamino-6-phenyl-3(2*H*)-pyridazinone (3h) To a solution of 5-amino-4-nitroso-6-phenyl-3(2*H*)-pyridazinone (**3g**) (0.25 g, 1.1 mmol) in a 1 : 1 mixture of methanol/ethyl acetate was added a catalytic amount of platinum (IV) oxide. The reaction mixture was stirred at room temperature under a hydrogen atmosphere for 24 h. The mixture was filtered through silica, the solvents were removed under reduced pressure and the resulting residue was purified by recrystallization from isopropanol (0.14 g, 60%). mp 189—191 °C (dec.). IR: $\nu_{\max}$ cm^{-1} 3457 (NH_2), 1636 (CO), 1585 (Aromatics). ^{1}H-NMR: δ_H (300 MHz, DMSO-d_6): 5.63 (2H, br s, NH_2, deuterium oxide exchangeable), 7.60 (3H, m, Aromatics), 7.65 (2H, m, Aromatics), 12.63 (1H, br s, NH, deuterium oxide exchangeable). $C_{10}H_{10}N_4O$ requires C, 59.39; H, 4.98; N, 27.70, Found: C, 59.29; H, 4.77; N, 28.13.

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