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α-Ketoamino acid ester derivatives as promising MAO inhibitors

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ABSTRACT

α-Ketoamino acid ester 2-[2-(2-acetamidophenyl)-2-oxoacetamido] and 2-[4-(2-(2-acetamidophenyl)-2-oxoacetamido)benzamido] derivatives were synthesized via the ring opening of *N*-acetylisatin under mild conditions. These compounds were then examined for their capacity to inhibit monoamine oxidase (MAO). The inhibition profile was found to be competitive for compounds **4d**, **6a**, **6b** and **6f,** which showed MAO-A selectivity. Observation of the docked positions of these compounds revealed interactions with many residues previously reported to have an effect on the inhibition of the enzyme. Our findings indicate that the members of this family of α-ketoamino acid esters are promising MAO inhibitors.

Monoamine oxidase A and B (MAO-A and -B) are flavin adenine dinucleotide (FAD)-containing enzymes, which are found in the outer mitochondrial membranes of neuronal and glial cells, among others[1] particularly abundant in the liver and brain.[2] These FAD-dependent enzymes are responsible for regulation and metabolism of major monoamine neurotransmitters, such as serotonin (5-OH tryptamine), nor-adrenaline, and dopamine. They are also involved in the biodegradation of exogenic amines, such as benzylamine, tyramine, MPTT, MPP^+, and a Parkinsonian syndrome-producing neurotoxin.[1] They catalyze the oxidative deamination of a range of endogenous and exogenous monoamines.[3] The two mammalian isoforms of MAO-A and -B are encoded by two different genes[4] and distinguished by distinct substrate specificities and sensitivities to selective inhibitors.[5] Thus, MAO-A is selectively inhibited by clorgyline and preferentially metabolizes serotonin, whereas MAO-B is inhibited by L-deprenyl and preferentially uses benzylamine and phenylethylamine as substrates. Selective MAO-A inhibitors are used in clinical practice as antidepressants and anxiolytics, while MAO-B inhibitors are used to slow down the progression of Parkinson's disease and of symptoms associated with Alzheimer's disease. Earlier MAO inhibitors introduced into clinical practice for the treatment of depression were abandoned due to adverse side-effects, such the 'cheese effect', which is characterized by hypertensive crises.[6]

For this reason, the interest of many research groups has been devoted to MAO as a therapeutic target.[7,8] Among the compounds studied as MAO inhibitors, heterocyclic hydrazines and hydrazides are prevalent. *N*′-Propan-2-ylpyridine-4-carbohydrazide, under the trade names of ipronid, iprozid, marsilid, propilniazida and rivivol, was the first modern antidepressant to be introduced into the market.

In the course of our ongoing studies aimed at the synthesis of heterocyclic compounds of potential pharmaceutical relevance,[9–11] here we focused on a novel family of α-ketoamino acid ester derivatives as MAO inhibitors.

The rational design of these compounds was based on hybrid structure of known inhibitors and previous reported substituted pyridazine-1-yl acetic acid derivatives **I** which were established as selective monoamine oxidase-A inhibitors.[11] The aim of the

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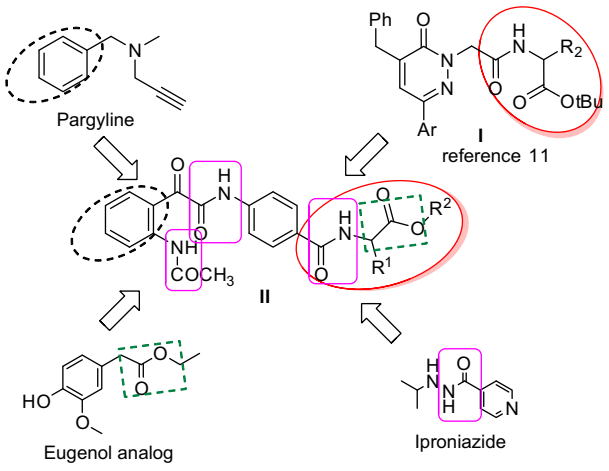


Figure 1. Planned modification and newly designed MAO inhibitors.

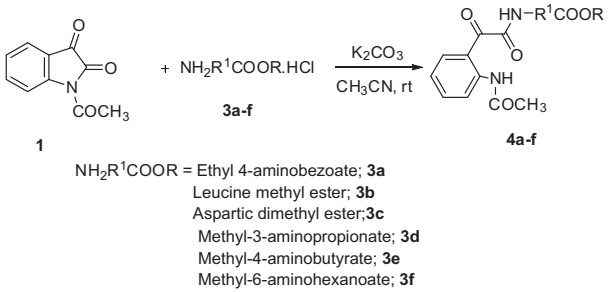
present study was tailoring MAO-A inhibitors considering some factors responsible for selectivity against A isoform[12] which are (i) the presence of electron-rich aromatic moieties (e.g., pargyline), (ii) the presence of amide functionality (e.g., Iproniazid[13]), (iii) the presence of ethoxycarbonyl methylene group (e.g., Eugenol analog[14]), (iv) the presence of amino acid moiety (**I**),[11] Figure 1. Moreover α-ketoamino moiety was included to study the effect of such molecular variation on MAO inhibitory activity.

α-Ketoamides are compounds of interest in organic chemistry and are present in many pharmaceutical compounds.[15–18] In parallel, their application in medicinal chemistry has fueled the development of several synthetic methods.[19–30] The synthesis of an α-ketoamide fragment could be achieved by the ring opening of *N*-acetylisatin **1** by attacking of an amine at C2-carbonyl group of *N*-acetylisatin.[31–39] Recently, Cheah et al.[39] reported the synthesis of *N*-glyoxylamide peptide mimics from the reaction of *N*-acetylisatin with α-amino esters. The reaction was carried out in DCM/H_2O (2:1) in the presence of saturated $NaHCO_3$, giving yields ranging from 61% to 98%. They claimed that the lower yield in some cases is due to the formation of glycoxalic acid derivative **2** (Scheme 1).

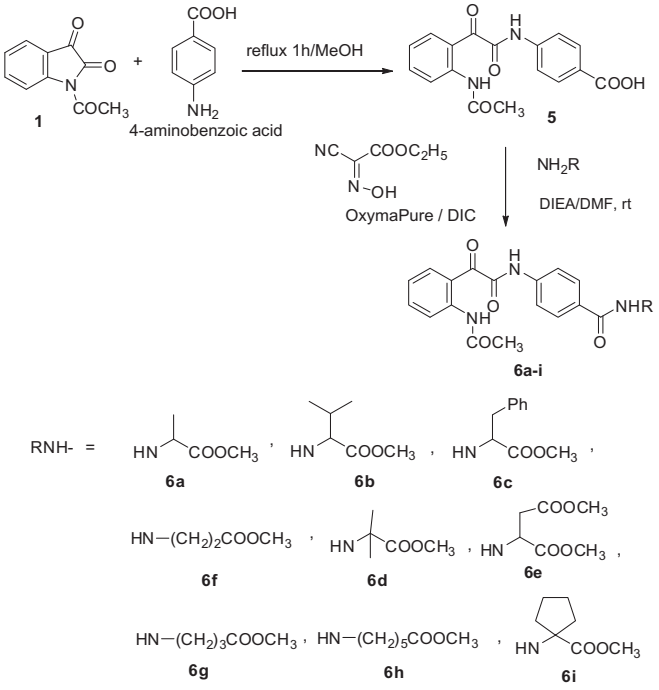
Later, our group,[40] reported the synthesis of α-ketoamides using CH_3CN and K_2CO_3 in place of DCM-H_2O/$NaHCO_3$. Herein we report on the synthesis of α-keto amino acid ester and (4-[2-(2-acetylaminophenyl)-2-oxo-acetylamino]benzoyl amino acid ester derivatives and their capacity to inhibit monoamine oxidase (MAO).

The α-ketoamino acid ester derivatives **4a–f** were prepared by the reaction of L-amino acid ester hydrochloride **3a–f** with *N*-acetylisatin **1**, following the reported method.[41] The reaction was performed in CH_3CN and K_2CO_3 at rt to afford the products **4a–f** in 80–92% yield (Scheme 2).[41] The structures of all the synthesized compounds were confirmed by IR and NMR (^{1}H NMR and ^{13}C NMR) and were in agreement with the reported data.[40,42]

The 4-aminobenzoic acid derivatives **6a–i** were prepared by the reaction 4-aminobenzoic acid with *N*-acetylisatin **1** using conventional heating for 1 h in MeOH as a solvent to afford 4-(2-



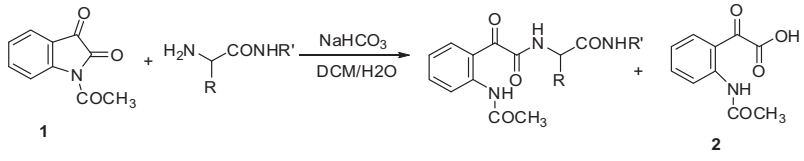
Scheme 2. Reaction of *N*-acetylisatin with amino acid ester·HCl.



Scheme 3. Synthesis 4-[2-(2-acetylaminophenyl)-2-oxo-acetylamino] benzoyl amino acid ester derivatives **6a–i**.

(2-acetamidophenyl)-2-oxoacetamido)benzoic acid **5** (Scheme 3). Compound **5** was then coupled with several L-amino acid esters using OxymaPure/DIC[43–47] in DMF as a solvent to afford the products **6a–i** in excellent yield and purity (Scheme 3).[48] The structure of all the compounds synthesized was confirmed by IR and NMR (^{1}H NMR and ^{13}C NMR) and were in agreement with the reported data.[49]

The final compounds **4a–4f** and **6a–6i** were evaluated for MAO-A inhibitory activity in vitro following the method described by Undenfriend et al.[50] The method involves the determination of MAO-A activity of rat liver mitochondria[51] using clorgyline as irreversible time-dependent reference standard. The test compounds or reference standard were preincubated for 60 min with enzymes



Scheme 1. Synthesis of *N*-glyoxylamide peptide.

before the addition of the corresponding substrate to ensure fair comparison. Furthermore, the synthesized compounds were tested to determine their selectivity for MAO-A and MAO-B in the presence of the specific substrate serotonin or benzylamine, respectively. Bovine brain mitochondria were isolated following Basford.[52] The compounds were tested to determine their activity toward MAO-A and -B, following methods of Matsumoto et al.[53] and Basford.[52] The MAO-A and -B results, expressed as IC_{50}, and also the selectivity index are given in Table 1. Compound **6b** showed MAO-A inhibitory activity (% MAO-A inhibition = $2.8 \times 10^{-9} \pm 0.11$) comparable to the standard clorgyline (% MAO-A inhibition = $2.9 \times 10^{-9} \pm 0.12$) while **4d**, **6a**, and **6f** showed lower inhibition activity than this compound. These three compounds showed greater capacity to inhibit MAO-A than MAO-B. Compound **6b** is the most selective compound as MAO-A inhibitor, it showed remarkable selectivity. Compounds **4d** and **6f** are comparable to clorgyline in their selectivity as MAO-A inhibitors. In an attempt to rationalize the MAO-A inhibitory activity observed for **6b**, we performed molecular modeling and conformational alignment studies. Molecular docking studies further contribute to unveiling the various interactions between the ligands and enzyme active sites.

MOE (Molecular Operating Environment)[54] docking studies of the inhibitors were performed using the crystal structure of human MAO-A (PDB ID: 2BXR). Docking of **6b** into the MAO-A active site (Fig. 2) revealed several molecular interactions were considered to be responsible for the observed affinity. For example five hydrogen bond interactions were observed, 3 hydrogen bond interactions with ARG51, one with ALA68, and one with TYR69. In addition, 16 hydrophobic interactions were observed with GLY20, GLY22, SER24, GLY49, GLY50, ARG51, THR52, GLY66, GLY67, ALA68, TYR69, ALA272, PRO274, TYR407, TYR444, MET445.

Consequently, these observations provide a good explanation for the potent inhibitory activity of compound **6b**. From the above-mentioned data **6b** may provide a starting point for the design of unique compounds with high affinity and selectivity for MAO-A.

The most active compounds **4d**, **6a**, **6b**, and **6f** were further evaluated for oral acute toxicity in male mice using the reported methods.[55,56] The results indicated that the compounds were nontoxic and well tolerated by the experimental animals up to 250 mg/kg, although no mortality was recorded at this concentration.

Table 1
Effect of some α-ketoamino acid ester derivatives on the activity of MAO-A and MAO-B[a]

Compound	MAO-A IC_{50} (M)	MAO-B IC_{50} (M)	Selectivity inhibition index (SI)[b]
1	$5.6 \times 10^{-8} \pm 0.13$	$3.8 \times 10^{-4} \pm 0.28$	0.678×10^{4}
4a	$8.3 \times 10^{-8} \pm 0.12$	$9.9 \times 10^{-4} \pm 0.32$	1.192×10^{4}
4b	$7.2 \times 10^{-8} \pm 0.22$	$4.8 \times 10^{-4} \pm 0.11$	0.666×10^{4}
4c	$5.8 \times 10^{-8} \pm 0.52$	$4.6 \times 10^{-4} \pm 0.22$	0.793×10^{4}
4d	$3.3 \times 10^{-8} \pm 0.22$	$9.9 \times 10^{-4} \pm 0.14$	3.000×10^{4}
4e	$6.2 \times 10^{-8} \pm 0.14$	$9.5 \times 10^{-4} \pm 0.18$	1.532×10^{4}
4f	$6.8 \times 10^{-8} \pm 0.44$	$5.9 \times 10^{-4} \pm 0.12$	0.867×10^{4}
6a	$3.6 \times 10^{-8} \pm 0.21$	$7.3 \times 10^{-4} \pm 0.28$	2.027×10^{4}
6b	$2.8 \times 10^{-9} \pm 0.11$	$4.3 \times 10^{-4} \pm 0.40$	1.537×10^{5}
6c	$3.6 \times 10^{-8} \pm 0.38$	$2.8 \times 10^{-4} \pm 0.62$	0.777×10^{4}
6d	$4.3 \times 10^{-8} \pm 0.13$	$3.6 \times 10^{-4} \pm 016$	0.837×10^{4}
6e	$6.0 \times 10^{-8} \pm 0.26$	$7.9 \times 10^{-4} \pm 0.14$	1.316×10^{4}
6f	$1.8 \times 10^{-8} \pm 0.28$	$5.3 \times 10^{-4} \pm 0.16$	2.944×10^{4}
6g	$4.9 \times 10^{-8} \pm 0.16$	$5.9 \times 10^{-4} \pm 0.11$	1.204×10^{4}
6h	$9.3 \times 10^{-4} \pm 0.28$	$7.8 \times 10^{-4} \pm 0.26$	0.838×10^{4}
6i	$4.8 \times 10^{-8} \pm 0.18$	$3.8 \times 10^{-4} \pm 0.38$	0.791×10^{4}
Clorgyline	$2.9 \times 10^{-9} \pm 0.12$	$9.8 \times 10^{-5} \pm 0.16$	3.379×10^{4}

[a] The results were expressed as mean ± S.E.M. Data were analyzed by one-way variance. The Student's *t* test for unpaired observations was used. *P* value = <0.001 and the significant number of experiments was 6.
[b] SI-MAO-B IC50/MAO-A IC50.

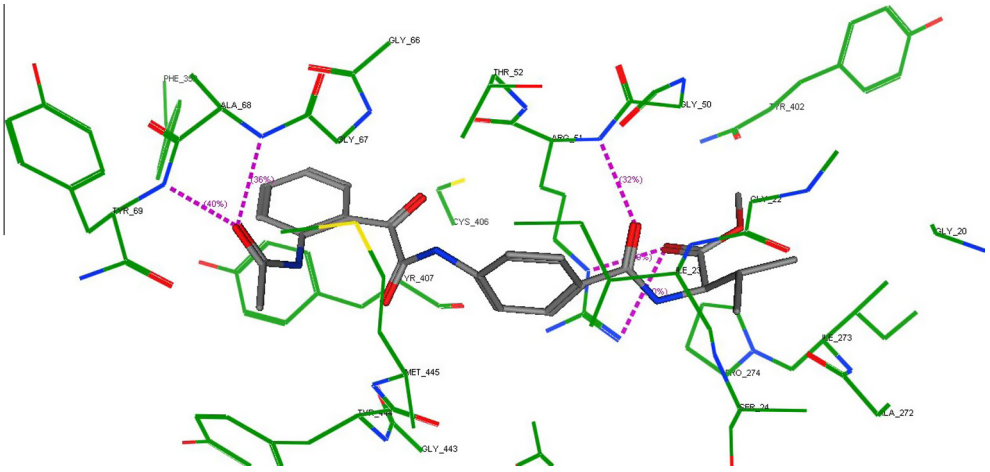


Figure 2. 3D view from a molecular modeling study of a minimum-energy structure of the complex of **6b** (stick) docked in the active site of MAO-A (PDB ID: 2BXR). Dashed lines depict hydrogen bond interactions. Viewed using a Molecular Operating Environment (MOE) module.

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Moreover, these compounds were tested for toxicity when administered through parenteral route.[57] All the test compounds were nontoxic up to 100 mg/kg. We conclude that the synthesis and biochemical evaluation of the newly synthesized α-ketoamino acid ester derivatives will contribute to the design of a novel class of reversible MAO-A inhibitors with an excellent therapeutic window.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.11.007.

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41. *General Method for the synthesis of* ***4a–f***:
Amino acid ester (12 mmol) and K_2CO_3 (1.66 g, 12 mmol) were added to the solution of *N*-acetylisatin **1** (1.89 g, 10 mmol) in CH_3CN (50 mL) with intensive stirring. This mixture was stirred at room temperature overnight. The reaction mixture was then filtered and washed with 10 mL of acetonitrile. The solvent was removed under vacuum to dryness, and the crude product was recrystallized from dichloromethane–hexane to afford the pure product.
Compound **4d** was obtained as an off-white solid, mp 90–92 °C; yield 87%. IR (cm^{-1}): 3288, 3124, 1741, 1672, 1607. ^1H NMR ($CDCl_3$): δ 2.18 (s, 3H, $COCH_3$), 2.64 (t, 2H, CH_2CH_2CO), 3.67 (q, 2H, $NHCH_2$), 3.70 (s, 3H, $COOCH_3$), 7.09 (t, 1H), 7.45 (s, 1H, NH), 7.57 (t, 1H), 8.28 (d, 1H), 8.62 (d, 1H), 10.93 (s, 1H, NH). ^{13}CNMR ($CDCl_3$): δ 25.5, 33.5, 35.1, 52.1, 118.6, 120.7, 122.6, 134.4, 136.6, 142.2, 163.1, 169.4, 172.6, 191.9. Anal. Calcd for $C_{14}H_{16}N_2O_5$: C, 57.53; H, 5.52; N, 9.58. Found: C, 57.74; H, 5.30; N, 9.72.
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48. *Synthesis of 4-(2-(2-acetamidophenyl)-2-oxoacetamido)benzoic acid* ***5***[46]: *N*-Acetylisatin and 4-aminobenzoic acid were refluxed in methanol as a solvent in the presence of glacial acetic acid (2–3 drops) for 1 h. After cooling, the solid product was filtered, washed with cold methanol, and then dried under vacuum to afford the product in a pure form. The product was obtained as a pale yellow powder, mp: 238–240 °C; yield 86% IR (cm^{-1}): 3270, 1679, 1601. ^1H NMR (DMSO-d_6): δ 1.99 (s, 3H, $COCH_3$), 7.25–7.30 (m, 2H), 7.62–7.68 (m, 2H), 7.90 (d, 2H), 7.95 (d, 2H), 10.55 (s, 1H, NH), 10.99 (s, 1H, NH), 13.00 (br s, 1H, COOH); ^{13}C NMR (DMSO-d_6): δ 22.8, 118.7, 121.0, 123.1, 124.3, 125.4, 129.6, 130.3, 132.9, 136.8, 141.2, 161.00, 166.1, 168.2, 188.1. Anal. Calcd for $C_{17}H_{14}N_2O_5$: C, 62.57; H, 4.32; N, 8.59. Found: C, 62.31; H, 4.57; N, 8.73.
General method for the synthesis of ***6a–i***: Acid **5** (1 mmol), Oxyma (1 mmol), and DIC (1 mmol) were mixed in DMF (5 mL) at 0 °C. The reaction mixture was stirred for 5 min at 0 °C to pre-activate the acid and generate the active ester. DIEA (1 mmol) was added, followed by (1 mmol) amino acid ester. The reaction mixture was stirred at 0 °C for 1 h and at room temperature overnight. The mixture was diluted with ethyl acetate (50 mL) and extracted with 1 N HCl (2 × 10 mL), 10% $NaHCO_3$ (2 × 10 mL), and saturated NaCl (2 × 10 mL). The organic phase was dried over anhydrous $MgSO_4$ and filtered, and the solvent was removed under vacuum. The residue was recrystallized from dichloromethane–hexane to afford the pure product. All the spectral data were in a good agreement with the reported data.[46]
Compound **6a** was obtained as a white powder, mp: 174–176 °C; yield 88%. IR (cm^{-1}): 3288, 3124, 1741, 1672, 1607. ^1H NMR (DMSO-d_6): δ 1.41 (d, 3H, $CHCH_3$), 2.00 (s, 3H, $COCH_3$), 3.65 (s, 3H, $COOCH_3$), 4.48 (m, 1H, $NHCHCH_3$), 7.45 (t, 1H), 7.64 (d,1H), 7.68 (d, 2H), 7.89 (m, 4H, Ar), 8.73 (s, 1H, NH), 10.55 (s, 1H, NH), 10.92 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 17.3, 24.2, 49.9, 52.1, 120.0, 122.4, 124.4, 126.4, 128.9, 129.0, 131.6, 134.21, 139.4, 142.2, 162.3, 166.2, 169.5, 173.8, 190.0. Anal. Calcd for $C_{21}H_{21}N_3O_6$: C, 61.31; H, 5.14; N, 10.21. Found: C, 61.52; H, 5.37; N, 10.41.
Compound **6b** was obtained as a white powder, mp: 178–180 °C; yield 81%. IR (cm^{-1}): 3293, 1747, 1679, 1634, 1608. ^1H NMR ($CDCl_3$): δ 1.00 (t, 6H, CH (CH_3)$_2$), 1.59 (m, 1H, $CHCH(CH_3)_2$), 2.27 (s, 3H, $COCH_3$), 3.78 (s, 3H, $COOCH_3$), 4.78 (m, 1H, CH), 6.61 (d, 1H), 7.17 (t, 1H), 7.64 (t, 1H), 7.78 (d, 1H), 7.86 (d, 2H), 8.50 (d, 1H) 8.64 (d,1H), 8.99 (s, 1H, NH), 10.79 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 18.1, 19.1, 25.0, 31.0, 52.4, 57.6, 119.4, 119.8, 120.9, 122.8, 128.4, 130.6, 134.3, 136.8, 140.0, 142.1,160.6, 166.5, 169.4, 172.8, 190.8. (C=O). Anal. Calcd for $C_{23}H_{25}N_3O_6$: C, 62.86; H, 5.73; N, 9.56. Found: C, 62.59; H, 5.97; N, 9.44.
Compound **6f** was obtained as a white powder, mp: 154–156 °C, yield 88%. IR (cm^{-1}): 3295, 1742, 1666, 1635, 1608. ^1H NMR (DMSO-d_6): δ 1.99 (s, 3H, $COCH_3$), 2.60 (t, 2H, CH_2), 3.49 (q, 2H, CH_2), 3.61 (s, 3H, CH_3), 7.29–7.85 (m, 8H, Ar), 8.51 (s, 1H, NH), 10.55 (s, 1H, NH), 10.90 (s, 1H, NH). ^{13}C NMR (DMSO-d_6); δ 23.9, 34.1, 36.1, 52.0, 119.9, 122.4, 124.4, 125.6, 128.6, 130.4, 131.6, 134.3, 138.2, 141.1, 162.4, 166.3, 169.5, 172.4, 189.6. Anal. Calcd for $C_{21}H_{21}N_3O_6$: C, 61.31; H, 5.14; N, 10.21. Found: C, 61.15; H, 5.42; N, 10.50.

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