Design, Synthesis and Inhibitory Activities of 8-(Substituted styrol-formamido)phenyl-xanthine Derivatives on Monoamine Oxidase B

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The design and synthesis of two series of 8-(substituted styrol-formamido)phenyl-xanthine derivatives are described. Their *in vitro* monoamine oxidase B (MAO-B) inhibition were tested and the effect of substituents on the N-7, phenyl and the substituted positions are discussed. It was observed that compound 9b displayed significant MAO-B inhibition activity and selectivity, fluorine substitution plays a key role in the selectivity of MAO-B inhibition, and the styrol-formamido group at position-3′ may enhance the activity and selectivity of 8-phenyl-xanthine analogues. These results suggest that such compounds may be utilized for the development of new candidate MAO-B inhibitors for treatment of Parkinson's disease.

Key words xanthine derivative; monoamine oxidase B inhibition activity; substituent effect

Parkinson's disease (PD) is a neurodegenerative disorder characterized pathologically by a marked loss of dopaminergic nigrostriatal neurons and clinically by disabling movement disorders.[1)] Currently, the therapy of PD is largely focused on dopamine replacement strategies with the dopamine precursor levodopa and dopamine agonist drugs. Although these strategies are highly effective in controlling the disease at the early stages, drug-related complications are associated with long-term treatment. The inadequacies of dopamine replacement therapy have prompted the research of alternative drug targets.[2—5)]

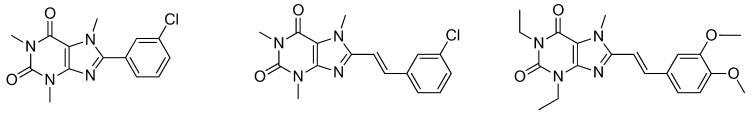
Monoamine oxidase (MAO) is an integral protein of the outer mitochondrial membrane, and exist in two forms, namely, monoamine oxidase A (MAO-A) and MAO-B. MAO-A preferentially deaminates aromatic monoamines such as the neurotransmitters serotonin (5-HT), noradrenaline (NA), andadrenaline (A); MAO-B is one of the most important key enzymes in metabolic pathways *in vivo* and plays mainly three roles: firstly, MAO-B decomposes dopamine into 3,4-dihydroxyphenyl acetic acid and homovanillic acid, meanwhile producing the neurotoxic H_2O_2. Secondly, MAO-B inactivates p-phenylethylamine which has the ability to stimulate secrection and reuptake function of dopamine. Thirdly, in the precence of MAO-B, catalyzed oxidation of 1-methyl-4-phenyl-1,2,3,6-terahydropyridine (MPTP) to intermediate 1-methyl-4-phenyl-2,3-dihydropyridin-1-ium ($MPDP^+$) and then to 1-methyl-4-phenylpyridin-1-ium (MPP^+)with neurotoxicity occurs. Together these suggest that inhibiting the action of MAO-B can not only reduce the degradation of dopamine and reuptake, improving the concentration of dopamine in the brain, but also lower levels of H_2O_2, and MPP^+ to enhance neuroprotective properties.[6—12)]

Recently, xanthine and its derivatives were reported to produce little or no inhibition of MAO-B. In the last decade, when introducing phenyl at position-8, 8-phenyl xanthine exhibited MAO-B inhibition activity *in vitro* with a inhibition constant (K_i) value of 86.2 μM.[2)] Petzer *et al.* found a series of 8-styryl xanthine analogues exhibiting more powerful inhibition of MAO-B than 8-phenyl xanthine analogues (CSC K_i=100 nM; KW6002 K_i=27 nM), indicating the styryl group which had been considered the main active group of xanthine analogues in inhibition of MAO-B[1,13—15)] (Fig. 1). And studies of CSC interaction with the protein pocket of MAO-B found that the caffeine group may embed one hole, and the styryl group into the another hole.[16)]

Taking advantage of above information, we speculated that condensation products of styrol-formamido group with 8-phenyl xanthine might exhibit potent MAO-B inhibition activity (Fig. 2). Therefore, two series of 8-(substituted styrol-formamido)phenyl-xanthine derivatives, totally 14 compounds, were prepared and their *in vitro* inhibition against MAO-B were determined. These compounds were also designed to examine the role of different substitutes in the N-7, phenyl and substituted positions of phenyl. It is hoped that continued research will lead to the development of new lead compounds from 8-phenyl xanthine as effective MAO-B inhibitors for PD.

Results and Discussion

Chemistry The structure and general synthesis of analogues designed are shown in Chart 1. The 8-(substituted styrol-formamido)phenyl-xanthine analogues were prepared in high yield according to the procedure previously reported for the preparation of (*E*)-8-styrylcaffeinyl analogues.[1,17—19)] The key starting materials for the procedure,



8-(3-chlorophenyl)-1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione CSC KW-6002

Fig. 1. Chemical Structures of Well-Known MAO-B Inhibitors

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1,3-dimethyl-5,6-diaminouracil **4**, were allowed to react with the appropriate carboxylic acid **8a**—**g** in the presence of *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimidehydrochloride (EDAC). The resulting amidyl intermediates underwent ring closure when heated under reflux in aqueous sodium hydroxide to yield the corresponding 1,3-dimethyl-8-sustituted-7*H*-xanthinyl analogues **9a**—**g**. **9a**—**g** were selectively 7*N*-methylated with an excess of iodomethane and potassium carbonate to yield 1,3,7-trimethyl-8-sustituted xanthinyl analogues **10a**—**g**. Following crystallization from a suitable solvent, the structures and purity of all compounds were verified by mass, IR, and ^{1}H-NMR spectrometry. The *trans–trans* geometry about the conjugated ethenyl π-bonds of the compounds were confirmed by proton–proton coupling constants 15.6 Hz for the olefinic proton signals.

Biological Activity On the base of MAO, kynuramine dihydrobromide was catalytically oxidized to fluorescence 4-hydroxyquinoline, and the fluorescence intensity detected indirectly to obtain the MAO activity. Our compounds had various degrees of MAO inhibition activity *in vitro*, according to MAO activity indirectly determined by the activity of compounds on MAO. The relationship between the K_i and IC_{50} is described by Cheng–Prusoff equation $K_i=IC_{50}/(1+[S]/K_m)$.

As shown in Tables 1 and 2, all of the 8-(substituted styrol-formamido)phenyl-xanthine analogues were found to exhibit MAO-B inhibition activity, especially **9b**, **9f**, and **10f**, which showed a better selectivity with the inhibition rate of 30.9, 9.6, 17.6 to 0% of MAO-A inhibition, respectively. This indicates that fluorine substitution plays a key role in the selectivity of MAO-B inhibition (except for **10b**). In the two series of compounds, the inhibition rate of MAO-B were: **9a** (20.2%)>**9e** (10.6%), **9b** (30.9%)>**9f** (9.6%), **9c** (33.5%)>**9g** (21.2%), **10a**(25.2%)>**10e** (3.4%), **10b** (32.6%)>**10f** (17.6%) and **10c** (18.5%)>**10g** (8.3%), indicating the styrol-formamido group at position-3′ may enhance the selectivity more than at position at 4′. Distinguishing between the two series of compounds, 7*N*-methylated had no obvious influence on the selectivity. Focussing only on the MAO-B inhibition activity, none of the compounds had significant differences, except for **10e**. **9b**, **9c**, **10b** yield better activities, with K_i values of 3.42, 3.58, and 3.68, respectivity. Coincidentally, the K_i values were **9a** (5.94)<**9e** (11.32), **9b** (3.42)<**9f** (12.50), **9c** (3.58)<**9g** (5.66), **10a** (4.76)<**10e** (34.30), **10b** (3.68)<**10f** (6.82) and **10c** (6.48)<**10g** (14.46), indicating the styrol-formamido group at position-3′ may enhance the inhibition more than at position at 4′. 7*N*-Methylated had no obvious influence on the inhibition.

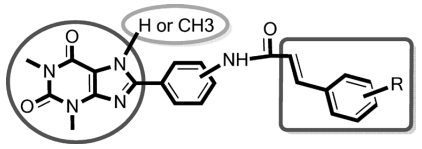
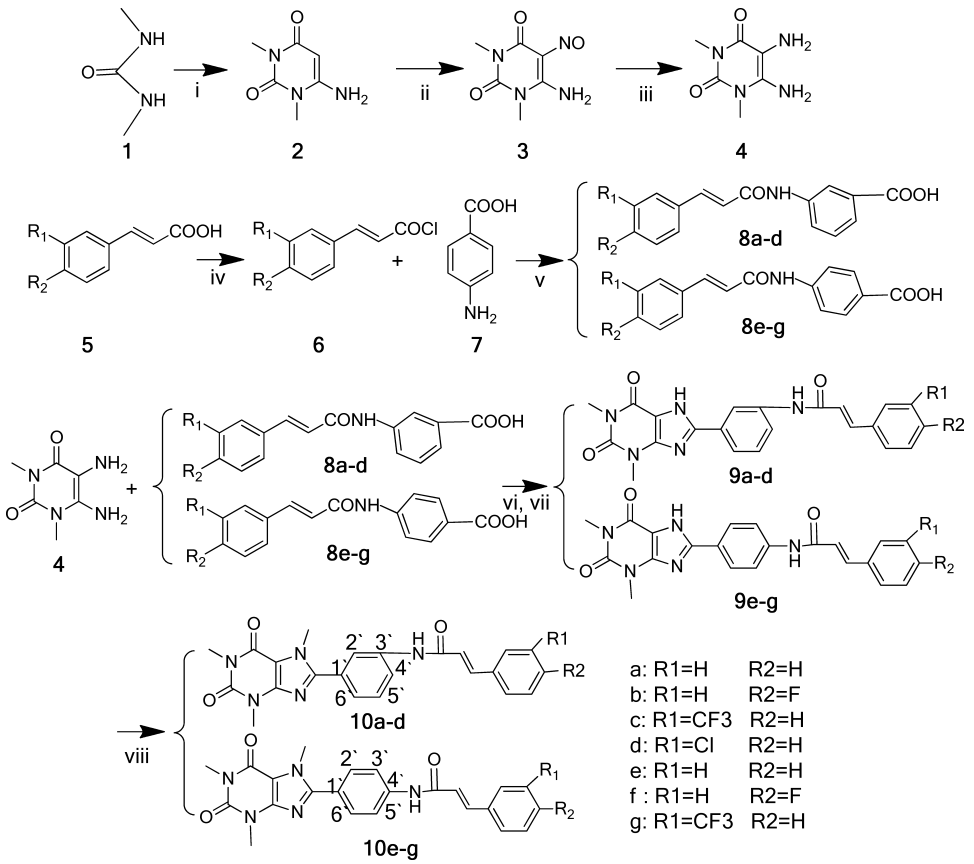


Fig. 2. Design of New MAO-B Inhibitors Based on the Pharmacophore



Reagents and conditions: (i) $NCCH_2COOH$, acetic anhydride; (ii) $NaNO_2$, CH_3COOH/H_2O; (iii) $Na_2S_2O_4$, NH_4OH; (iv) $SOCl_2$; (v) triethylamine, CH_2Cl_2; (vi) EDC·HCl, 1,4-dioxane/H_2O; (vii) NaOH, reflux; (viii) CH_3I, K_2CO_3, DMF.

Chart 1. General Route for the Synthesis of 8-(Substituted styrol-formamido)phenyl-xanthine Analogues

Table 1. Inhibition Rate of All Tested Compounds on MAO

Compounds	Inhibition rate (%)[a), b)]	
	MAO-A	MAO-B
9a	3.8±1.22	20.2±1.01
9b	0±0.66	30.9±0.97
9c	47.2±1.62	33.5±1.48
9d	16.5±1.51	7.1±0.88
9e	27.8±0.94	10.6±1.08
9f	0±0.44	9.6±0.74
9g	8.0±1.23	21.2±0.85
10a	2.8±0.41	25.2±1.22
10b	12.9±1.05	32.6±0.76
10c	14.3±1.14	18.5±0.85
10d	29.0±1.26	29.8±1.56
10e	27.5±0.81	3.4±0.67
10f	0±0.32	17.6±1.45
10g	19.8±1.15	8.3±1.04

a) Data represent mean values of at least three separate experiments. *b*) Percentage inhibition at an inhibitor concentration of 1 mM.

Table 2. The K_i and IC_{50} Values for the Inhibition of MAO-B by All Tested Compounds

Compounds	Exp. IC_{50} (μM)[a), b)]	K_i value (μM)[a), c)]
9a	29.70±1.22	5.94
9b	17.10±2.24	3.42
9c	17.91±1.08	3.58
9d	84.50±1.12	16.9
9e	56.60±2.46	11.32
9f	62.50±2.08	12.5
9g	28.3±2.34	5.66
10a	23.8±1.68	4.76
10b	18.40±1.14	3.68
10c	32.43±2.32	6.48
10d	20.13±3.06	4.02
10e	176.4±4.08	34.3
10f	34.09±2.18	6.82
10g	72.29±3.24	14.46

a) Data represent mean values of at least three separate experiments. *b*) The IC_{50} values were experimentally determined by fitting the rate data to the one site competition model incorporated into the Prism software package. c) The K_i values were calculated from the experimental IC_{50} values according to the equation by Cheng and Prusoff: $K_i=IC_{50}/(1+[S]/K_m)$.

Conclusion

C-8 substituted xanthine analogues have been reported for the therapy of PD through inhibiting MAO-B, so the rational design and synthsis of the 8-(substituted styrol-formamido)-phenyl-xanthine analogues presented here may also be useful for finding more advanced compounds for anti-PD. In this paper, two series of 8-(Substituted styrol-formamido)phenyl-xanthine analogues, totalling 14 compounds, were designed, synthsized, and evaluated for the inhibition of MAO-B. It was observed that **9b** showed remarkable MAO-B inhibition activity and excellent selectivity. We can conclude that in the structure of 8-phenyl xanthine, styrol-formamido group at position 3′ may enhance the selectivity and activity on MAO-B inhibition, and fluorine substituted compounds have clear selectivity.

Experimental

Chemistry Melting points were determined on a XRC-1 melting apparatus. ^{1}H-NMR spectra were recorded on a Bruker AC 600 spectrometer. The chemical shifts are presented in terms of ppm with tetramethylsilane (TMS) as the internal reference. IR were measured on a Bruker Vector22 spectrometer. Electron-spray ionization mass spectra in positive mode (ESI-MS) were recorded on a Esquire HCT spectrometer. The purity of all novel compounds was checked by TLC and ^{1}H-NMR. All reactions were monitored by TLC on pre-coated Silica Gel F254 plates (purchased from Qingdao Marine Chemical Factory, China) with detection by UV. All reagents used were of analytical grade. *m*-Aminobenzoic acid, *p*-aminobenzoic acid, cinnamic acid, and substituted cinnamic acid were purchased from Wuhan Yuancheng Co., Ltd. Other reagents were purchased from Wenzhou Jiutai Chemical Reagent Co., Ltd., China.

General Procedure of 5,6-Diamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione 4[20] To a stirred solution of 1,3-dimethylurea (40 mmol) **1** in acetic anhydride (30 mL) was added cyanoacetic acid (44 mmol), and the resulting mixture was stirred overnight at 70°C. The reaction mixture was concentrated, and the resulting oily residue was diluted with H_2O (40 mL) and treated with 5 N NaOH (15 mL). The precipitate thus formed was collected by filtration, washed with cold water, and purified by recrystalization from MeOH/H_2O to give 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione as a light-yellow solid **2** (5.70g). Compound **2** (25.8 mmol) was stirred in 50% aquesous AcOH solution (160 mL) at 75°C for 30 min until the reaction mixture became homogeneous. Once the reaction mixture was homogeneous, the temperature was reduced to 50°C, and sodium nitrite (51.6 mmol) was added in small portions. After the completion of the addition, the resulting mixture was cooled to room temperature and stirred for 1 h. The resulting precipitate was collected by filtration, washed with water, and dried to obtain 6-amino-1,3-dimethyl-5-nitrosopyrimidine 2,4(1*H*,3*H*)-dione **3** (4.64 g). A suspension of compound **3** (6.0 mmol) was stirred in 14.5% NH_4OH (40 mL) at 70°C for 30 min until the reaction mixture became homogeneous. The temperature was reduced to 50°C, and $Na_2S_2O_4$ (18.0 mmol) was added in small portions. During the addition, the red solution changed to yellow-green and to light yellow, and the solution was stirred at room temperature for another 30 min. The volume of the reaction mixture was reduced to half and cooled in an ice bath for 1 h, and the precipitate was collected by filtration, followed by washing with a small amount of water. Compound **4**, 5,6-diamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione, was collected as a white solid (0.69 g).

General Procedure of 3-(Substituted styrol-formamido)-benzoic Acid and 4-(Substituted styrol-formamido)benzoic Acid Cinnamic acid **5a**—**d** (24 mmol) and fresh thionyl chloride (26 mmol) were heated for 30 min, then rotary evaporated to remove excessive thionyl chloride to obtain a faint yellow needle crystal cinnamonyl chloride **6a**—**d**. Aminobenzoic acid **7a**, **b** (0.02 mmol) were dissolved in dichloromethane (60 mL) and added appropriate triethylamine to be dissolved, stirred for 30 min at room temperature, then 10 mL of dichloromethane containing cinnamonyl chloride slowly dropped **6a**—**d** (0.02 mmol) into the reaction mixture. After the completion of the dropping, a white precipitate was formed and stirred

for 2 h, collected by filtration, and crystallized from acetate to obtain white crystal substituted styrol-formamidobenzoic acid (**8a**—**g**).

General Procedure of 1,3-Dimethyl-(*E*)-8-substituted styrol-formamido-7*H*-xanthinyl Analogues (9a—g)[1,17—19] To a solution of 1,3-dimethyl-5,6-diaminouracil **4** (3.50 mmol) and EDAC (5.11 mmol) in dioxane–H_2O (1 : 1, 40 mL), the appropriate substituted styrol-formamidobenzoic acid **8a**—**g** (3.81 mmol) was added. The pH of the suspension was adjusted to 5 with 2 M aqueous hydrochloric acid and stirring was continued for an additional 8 h. The reaction mixture was neutralized with 1 M aqueous sodium hydroxide, cooled to 0°C, and the resulting precipitate was collected by filtration. The crude product was dissolved in 40 mL aqueous sodium hydroxide (1 M)–dioxane (1 : 1) and heated for 8 h at 90—105°C. The reaction mixture was cooled to 0°C, acidified to a pH of 4 with 4 M aqueous hydrochloric acid, and the precipitate was collected by filatration. This was follwed by crystallization from dimethyl sulfoxide (DMSO) to obtain pure 1,3-dimethyl-(*E*)-8-substituted styrol-formamido-7*H*-xanthinyl analogues (**9a**—**g**).

(*E*)-*N*-(3-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)cinnamamide (**9a**): Yield 46.0%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.28 (3H, s, $-CH_3$), 3.52 (3H, s, $-CH_3$), 6.91 (1H, d, J=15.6 Hz, –CH=C), 7.48 (3H, m, –ArH), 7.61 (1H, d, J=15.6 Hz, –CH=C), 7.61 (1H, m, -ArH), 7.71 (2H, d, J=9 Hz, -ArH), 7.86 (2H, d, J=9 Hz, -ArH), 8.44 (1H, s, –ArH), 10.45 (1H, s, –NH–C=O), 13.93 (1H, s, –NH–C). IR (KBr) cm^{-1}: 3152.94, 3364.71, 1648.98, 1683.39, 1696.52, 2902.28, 2984.39. ESI-MS *m*/*z*: 402 $[M+H]^+$.

(*E*)-*N*-(3-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)-3-(4-fluorophenyl)acrylamide (**9b**): Yield 41.0%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.28 (3H, s, $-CH_3$), 3.52 (3H, s, $-CH_3$), 6.80 (1H, d, J=15.6 Hz, –CH=C), 7.30 (2H, d, J=8.4 Hz, –ArH), 7.47 (1H, m, –ArH), 7.62 (1H, d, J=15.6 Hz, –CH=C), 7.71 (2H, d, J=8.4 Hz, –ArH), 7.86 (2H, m, –ArH), 8.43 (1H, s, –ArH),10.42 (1H, s, –NH-C=O), 13.91 (1H, s, –NH-C). IR (KBr) cm^{-1}: 3159.09, 3368.35, 1649.87, 1683.15, 1693.41, 2900.72, 2988.10. ESI-MS *m*/*z*: 420 $[M+H]^+$.

(*E*)-*N*-(3-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)-3-(3-trifluoromethyl)phenyl)acrylamide (**9c**): Yield 43.2%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.27 (3H, s, $-CH_3$), 3.52 (3H, s, $-CH_3$), 6.98 (1H, d, J=15.6 Hz, –CH=C), 7.48 (1H, m, –ArH), 7.70 (1H, d, J=15.6 Hz, –CH=C), 7.72 (1H, m, –ArH), 7.77 (1H, d, J=7.8 Hz, –ArH), 7.87 (2H, m, –ArH), 7.94 (1H, d, J=7.8 Hz, –ArH), 7.99 (1H, s, –ArH), 8.43 (1H, s, –ArH), 10.47 (1H, s, –NH–C=O), 13.92 (1H, s, –NH–C). IR (KBr) cm^{-1}: 3162.65, 3305.87, 1649.85, 1683.12, 1693.71, 2900.73, 2988.02. ESI-MS *m*/*z*: 470 $[M+H]^+$.

(*E*)-3-(3-Chlorophenyl)-*N*-(3-(1,3-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)acrylamide (**9d**): Yield 45.5%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.28 (3H, s, $-CH_3$), 3.52 (3H, s, $-CH_3$), 6.91 (1H, d, J=15.6 Hz, –CH=C), 7.48 (3H, m, –ArH), 7.61 (1H, d, J=15.6 Hz, –CH=C), 7.61 (1H, m, –ArH), 7.71 (1H, s, –ArH), 7.86 (2H, d, J=8.4 Hz, –ArH), 8.44 (1H, s, –ArH), 10.45 (1H, s, –NH–C=O), 13.93 (1H, s, –NH–C). IR (KBr) cm^{-1}: 3209.12, 3327.49, 1654.88, 1697.36, 1700.12, 2900.72, 2987.79. ESI-MS *m*/*z*: 436.4 $[M+H]^+$.

N-(4-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)cinnamamide (**9e**): Yield 48.1%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.27 (3H, s, $-CH_3$), 3.51 (3H, s, $-CH_3$), 6.91 (1H, d, J=15.6 Hz, –CH=C), 7.45 (3H, m, –ArH), 7.61 (1H, d, J=15.6 Hz, –CH=C), 7.65 (2H, d, J=7.2 Hz, –ArH), 7.84 (2H, d, J=8.4 Hz, –ArH), 8.12 (2H, d, J=8.4 Hz, –ArH), 10.46 (1H, s, –NH–C=O), 13.72 (1H, s, –NH–C). IR (KBr) cm^{-1}: 3157.26, 3291.24, 1625.77, 1645.29, 1706.03, 2900.70, 2987.95. ESI-MS *m*/*z*: 402 $[M+H]^+$.

(*E*)-*N*-(4-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)-3-(4-fluorophenyl)acrylamide (**9f**): Yield 43.7%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.27 (3H, s, $-CH_3$), 3.51 (3H, s, $-CH_3$) 6.79 (1H, d, J=15.6 Hz, –CH=C), 7.30 (2H, m, –ArH), 7.62 (1H, d, J=15.6 Hz, –CH=C), 7.72 (2H, m, –ArH), 7.83 (2H, d, J=9 Hz, –ArH), 8.11 (2H, d, J=9 Hz, –ArH), 10.45 (1H, s, –NH–C=O), 13.72 (1H, s, –NH–C). IR (KBr) cm^{-1}: 3157.26, 3291.24, 1645.29, 1689.47, 1706.03, 2900.70, 2987.95. ESI-MS *m*/*z*: 420 $[M+H]^+$.

(*E*)-*N*-(4-(1,3-Dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)acrylamide (**9g**): Yield 45.5%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.28 (3H, s, $-CH_3$), 3.52 (3H, s, $-CH_3$), 6.99 (1H, d, J=15.6 Hz, –CH=C), 7.72 (1H, m, –ArH), 7.73 (1H, d, J=15.6 Hz, –CH=C), 7.79 (1H, d, J=7.8 Hz, –ArH), 7.80 (1H, d, J=7.8 Hz, –ArH), 7.85 (2H, d, J=8.4 Hz, –ArH), 8.02 (1H, s, –ArH), 8.14 (2H, d, J=8.4 Hz, –ArH), 10.52 (1H, s, –NH–C=O), 13.76 (1H, s, –NH–C). IR (KBr) cm^{-1}: 3183.19, 3347.31, 1651.08, 1685.82, 1698.23, 2900.67, 2988.12. ESI-MS *m*/*z*: 470 $[M+H]^+$.

General Procedure of 1,3,7-Trimethyl-(*E*)-8-substituted styrol-formamido-7*H*-xanthinyl Analogues (10a—g)[17—19] Iodomethane (0.40 mmol) was added to a stirred suspension of (0.20 mmol) 1,3-dimethyl-(*E*)-8-substituted styrol-formamido-7*H*-xanthinyl analogues **9a**—**g** and potassium carbonate (0.50 mmol) in *N*,*N*-dimethylformamide (10 mL). Stirring was continued at 60°C for 1 h, and the insoluble materials were removed by filtration, and sufficient water was added to the filtrate to precipitate the product collected by filtration. This was followed by crystallization from DMSO to obtain pure 1,3,7-trimethyl-(*E*)-8-substituted styrol-formamido-7*H*-xanthinyl analogues **10a**—**g**.

(*E*)-*N*-(3-(1,3,7-Trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)cinnamamide (**10a**): Yield 48.6%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.26 (3H, s, $-CH_3$), 3.48 (3H, s, $-CH_3$), 4.03 (3H, s, $-CH_3$), 6.84 (1H, d, J=15.6 Hz, –CH=C), 7.45 (3H, m, –ArH), 7.52 (2H, d, J=9 Hz, –ArH), 7.61 (1H, m, –ArH), 7.63 (1H, d, J=15.6 Hz, –CH=C), 7.88 (2H, d, J=9 Hz, –ArH), 8.18 (1H, s, –ArH), 10.47 (1H, s, –NH–C=O). IR (KBr) cm^{-1}: 3340.88, 1630.65, 1650.21, 1697.77, 2900.70, 2970.84, 2988.27. ESI-MS *m*/*z*: 416 $[M+H]^+$.

(*E*)-3-(4-Fluorophenyl)-*N*-(3-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)acrylamide (**10b**): Yield 40.5%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.26 (3H, s, $-CH_3$), 3.48 (3H, s, $-CH_3$), 4.03 (3H, s, $-CH_3$), 6.78 (1H, d, J=15.6 Hz, –CH=C), 7.31 (2H, d, J=8.4 Hz, –ArH), 7.53 (2H, m, –ArH), 7.63 (1H, d, J=15.6 Hz, –CH=C), 7.72 (2H, d, J=8.4 Hz, –ArH), 7.88 (1H, m, –ArH), 8.17 (1H, s, –ArH), 10.46 (1H, s, –NH–C=O). IR (KBr) cm^{-1}: 3334.45, 1644.66, 1685.44, 1700.84, 2897.96, 2971.43, 2988.73. ESI-MS

m/z: 434 $[M+H]^+$.

(*E*)-3-(3-(Trifluoromethyl)phenyl)-*N*-(3-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)acrylamide (**10c**): Yield 44.6%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.27 (3H, s, $-CH_3$), 3.49 (3H, s, $-CH_3$), 4.04 (3H, s, $-CH_3$), 6.97 (1H, d, J=15.6 Hz, –CH=C), 7.54 (2H, m, –ArH), 7.71 (2H, m, –ArH), 7.72 (1H, d, J=15.6 Hz, –CH=C), 7.78 (1H, d, J=7.8 Hz, –ArH), 7.95 (1H, d, J=7.8 Hz, –ArH), 8.02 (1H, s, –ArH), 8.19 (1H, s, –ArH), 10.52 (1H, s, –NH–C=O). IR (KBr) cm^{-1}: 3325.81, 1650.12, 1681.99, 1698.04, 2900.76, 2971.86, 2987.78. ESI-MS *m/z*: 484 $[M+H]^+$.

(*E*)-3-(3-Chlorophenyl)-*N*-(3-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)acrylamide (**10d**): Yield 44.2%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.26 (3H, s, $-CH_3$), 3.48 (3H, s, $-CH_3$), 4.03 (3H, s, $-CH_3$), 6.89 (1H, d, J=15.6 Hz, –CH=C), 7.53 (3H, m, –ArH), 7.55 (1H, m, –ArH), 7.61(1H, d, J=15.6 Hz, –CH=C), 7.62 (1H, d, J=8.4 Hz, –ArH), 7.71 (1H, s, –ArH), 7.87 (1H, d, J=8.4 Hz, –ArH), 8.18 (1H, s, –ArH), 10.49 (1H, s, –NH–C=O). IR (KBr) cm^{-1}: 3327.72, 1651.64, 1681.32, 1694.79, 2900.71, 2971.80, 2984.12. ESI-MS *m/z*: 450.6 $[M+H]^+$.

N-(4-(1,3,7-Trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)cinnamamide (**10e**): Yield 42.3%, mp>300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.26 (3H, s, $-CH_3$), 3.47 (3H, s, $-CH_3$), 4.02 (3H, s, $-CH_3$), 6.86 (1H, d, J=15.6 Hz, –CH=C), 7.44 (1H, m, –ArH), 7.47 (2H, d, J=7.2 Hz, –ArH), 7.64 (1H, d, J=15.6 Hz, –CH=C), 7.65 (2H, d, J=7.2 Hz, –ArH), 7.82 (2H, d, J=8.4 Hz, –ArH), 7.90 (2H, d, J=8.4 Hz, –ArH), 10.50 (1H, s, –NH–C=O). IR (KBr) cm^{-1}: 3381.99, 1630.44, 1646.59, 1695.01, 2900.72, 2971.43, 2987.84. ESI-MS *m/z*: 416 $[M+H]^+$.

(*E*)-3-(4-Fluorophenyl)-*N*-(4-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)acrylamide (**10f**): Yield 40.7%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.26 (3H, s, $-CH_3$), 3.47 (3H, s, $-CH_3$), 4.02 (3H, s, $-CH_3$), 6.80 (1H, d, J=15.6 Hz, –CH=C), 7.30 (2H, m, –ArH), 7.64 (1H, d, J=15.6 Hz, –CH=C), 7.71 (2H, m, –ArH), 7.81 (2H, d, J=8.4 Hz, –ArH), 7.89 (2H, d, J=8.4 Hz, –ArH), 10.49 (1H, s, –NH–C=O). IR (KBr) cm^{-1}: 3509.77, 1655.61, 1682.25, 1699.39, 2971.82, 2902.28, 2984.39. ESI-MS *m/z*: 434 $[M+H]^+$.

(*E*)-3-(3-(Trifluoromethyl)phenyl)-*N*-(4-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)phenyl)acrylamide (**10g**): Yield 46.3%, mp >300°C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.28 (3H, s, $-CH_3$), 3.53 (3H, s, $-CH_3$), 4.03 (3H, s, $-CH_3$), 6.87 (1H, d, J=15.6 Hz, –CH=C), 7.47 (3H, m, –ArH), 7.63 (1H, d, J=15.6 Hz, –CH=C), 7.64 (2H, d, J=8.4 Hz, –ArH), 7.87 (2H, d, J=8.4 Hz, –ArH), 8.04 (1H, s, –ArH), 10.43 (1H, s, –NH–C=O). IR (KBr) cm^{-1}: 3462.90, 1662.08, 1688.43, 1709.03, 2900.68, 2971.82. ESI-MS *m/z*: 484 $[M+H]^+$.

Biology ICR mice (20 g) were euthanized and the brain tissues were rapidly frozen (−80°C) until analysis. Mouse brain MAO activity was measured following the procedure described previously with a slight modification.[21—23] Briefly, the brain tissues were homogenized with phosphate buffer (pH 7.4, 0.05 M, 4 mL). For lysis of the membranes, the tissue homogenate was treated with 20% Triton X-100 (0.4 mL), and phosphate buffer (pH 7.4, 2.5 mL), which was then mixed with the tissue homogenate (0.2 mL) to get tube A, with the same method to obtain tube B. Tube A was added 3.1 μL deprenyl (0.0023 g+10 mL dd H_2O, 1 mM) to inhibit MAO-B; tube B was added 3.1 μL clorgyline (0.0031 g+10 mL dd H_2O, 1 mM) to inhibit MAO-A. Drug solutions in DMSO, added to the reaction mixture at six different final concentrations ranging from 0.5 to 10 mM. The mixture was preincubated at 37°C for 15 min. Then kynuramine dihydrobromide (2.19 mM, 30 μL) was added to the reaction mixture (final concentration 22 μM) as substrate. Samples were then incubated at 37°C for 30 min once more. After incubation, the reaction was terminated by adding perchloric acid (5 M, 0.2 mL). After cooling and centrifugation at 1500 ***g*** for 10 min, an aliquot of the supernatant (0.5 mL) was added to NaOH (1 M, 2.5 mL). The fluorescence intensity was detected with 315 nm excitation and 380 nm emission wavelengths using a fluorescence spectrometer. The concentration of 4-hydroxyquinoline was estimated from a corresponding standard fluorescence curve of 4-hydroxyquinoline. MAO activity (U/mg prot) was calculated as=fluorescence÷0.01÷3 h÷sample protein (mg); Inhibition rate of MAO=(MAO inhibition activity/tool drug inhibition activity)×100%, to MAO-A the tool drug was clorgyline, and deprenyl for MAO-B, with inhibition activity of 100%.

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References

1) Petzer J. P., Steyn S., Castagnoli K. P., Chen J. F., Schwarzschild M. A., Van der Schyf C. J., Castagnoli N., *Bioorg. Med. Chem.*, **11**, 1299—1310 (2003).
2) Pretorius J., Malan S. F., Castagnoli N. Jr., Bergh J. J., Petzer J. P., *Bioorg. Med. Chem.*, **16**, 8676—8684 (2008).
3) Dauer W., Przedborski S., *Neuron*, **39**, 889—909 (2003).
4) Schwarzschild M. A., Agnati L., Fuxe K., Chen J. F., Morelli M., *Trends Neurosci.*, **29**, 647—654 (2006).
5) Dauer W., Przedborski S., *Neuron*, **39**, 889—909 (2003).
6) Gentili F., Pizzinat N., Ordener C., Marchal-Victorion S., Maurel A., Hofmann R., Renard P., Delagrange P., Pigini M., Parini A., Giannella M., *J. Med. Chem.*, **49**, 5578—5586 (2006).
7) Pålhagen S., Heinonen E., Hägglund J., Kaugesaar T., Mäki-Ikola O., Palm R.; Swedish Parkinson Study Group, *Neurology*, **66**, 1200—1206 (2006).
8) Di Santo R., Costi R., Roux A., Artico M., Befani O., Meninno T., Agostinelli E., Palmegiani P., Turini P., Cirilli R., Ferretti R., Gallinella B., La Torre F., *J. Med. Chem.*, **48**, 4220—4223 (2005).
9) Gökhan-Kelekçi N., Koyunoğlu S., Yabanoğlu S., Yelekçi K., Ozgen O., Uçar G., Erol K., Kendi E., Yeşilada A., *Bioorg. Med. Chem.*, **17**, 675—689 (2009).
10) Cohen G., Spina M. B., *Ann. Neurol.*, **26**, 689—690 (1989).
11) Kitani K., Minami C., Isobe K., Maehara K., Kanai S., Ivy G. O., Carrillo M. C., *Mech. Ageing Dev.*, **123**, 1087—1100 (2002).
12) Youdim M. B., Riederer P. F., *Neurology*, **63** (7 Suppl. 2), 32—35 (2004).
13) Chiba K., Trevor A., Castagnoli N. Jr., *Biochem. Biophys. Res. Commun.*, **120**, 574—578 (1984).
14) Koga K., Kurokawa M., Ochi M., Nakamura J., Kuwana Y., *Eur. J. Pharmacol.*, **408**, 249—255 (2000).
15) Gołembiowska K., Dziubina A., Kowalska M., Kamińska K., *Neurotox. Res.*, **15**, 155—166 (2009).

16) Toprakçí M., Yelekçi K., *Bioorg. Med. Chem. Lett.*, **15**, 4438—4446 (2005).
17) Vlok N., Malan S. F., Castagnoli N. Jr., Bergh J. J., Petzer J. P., *Bioorg. Med. Chem.*, **14**, 3512—3521 (2006).
18) Suzuki F., Shimada J., Shiozaki S., Ichikawa S., Ishii A., Nakamura J., Nonaka H., Kobayashi H., Fuse E., *J. Med. Chem.*, **36**, 2508—2518 (1993).
19) van den Berg D., Zoellner K. R., Ogunrombi M. O., Malan S. F., Terre'Blanche G., Castagnoli N. Jr., Bergh J. J., Petzer J. P., *Bioorg. Med. Chem.*, **15**, 3692—3702 (2007).
20) Kalla R. V., Elzein E., Perry T., Li X., Palle V., Varkhedkar V., Gimbel A., Maa T., Zeng D., Zablocki J., *J. Med. Chem.*, **49**, 3682—3692 (2006).
21) Xu Y., Ku B. S., Yao H. Y., Lin Y. H., Ma X., Zhang Y. H., Li X. J., *Pharmacol. Biochem. Behav.*, **82**, 200—206 (2005).
22) Xu Y., Wang Z. C., You W. T., Zhang X. H., Li S., Barish P. A., Vernon M. M., Du X., Li G., Pan J., Ogle W. O., *Eur. Neuropsychopharmacol.*, **20**, 405—413 (2010).
23) La Regina G., Silvestri R., Artico M., Lavecchia A., Novellino E., Befani O., Turini P., Agostinelli E., *J. Med. Chem.*, **50**, 922—931 (2007).