# Monoamine Oxidase and Succinate Dehydrogenase Inhibitory Properties of Substituted Mercaptoquinazolones



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Twelve 6-substituted-2-(1'-mercaptoacetyl-3'-arylurea)-3-phenyl-4-quinazolones were synthesized by condensation of 6-substituted anthranilic acids and aryl isothiocyanates followed by reaction with chloroacetyl arylurea. These compounds were characterized by their sharp melting points and elemental analyses. All compounds were evaluated for their enzyme inhibitory activity. It was found that all substituted quinazolones at a final concentration of $2 \times 10^{-4}$ *M* inhibited *in vitro* monoamine oxidase and succinate dehydrogenase activity of rat brain homogenates and the degree of inhibition ranged from 11-77% and 25-53%, respectively.

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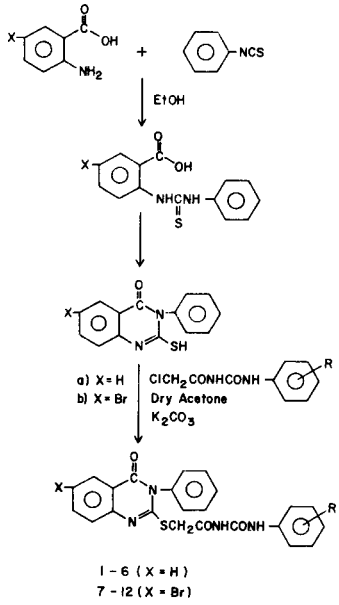
The broad spectrum of biological activity including central nervous system depressant activity associated with substituted 4-quinazolones (1,5) prompted synthesis of 6-substituted 2-(1'-mercaptoacetyl-3'-arylurea)-3-phenyl-4-quinazolones. It is well established that central nervous system depressants affect certain metabolic processes in brain (6). Monoamine oxidase, responsible for oxidative deamination of pharmacologically active biogenic amines, plays an important role in the central nervous system depressant activity (7), and inhibitors of monoamine oxidase have been shown to possess anticonvulsant activity (8). It has also been observed that inhibition of respiratory enzymes reflects depression of the central nervous system activity. These observations prompted synthesis and evaluation of 6-substituted-2-(1'-mercaptoacetyl-3'-arylurea)-3-phenyl-4-quinazolones for their ability to inhibit monoamine oxidase and succinate dehydrogenase activity of rat brain homogenates. Various steps involved in the synthesis of these substituted mercaptoquinazolones are outlined in Scheme 1.

Twelve new 6-substituted 2-(1'-mercaptoacetyl-3'-arylurea)-3-phenyl-4-quinazolones were synthesized by refluxing 6-substituted-2-mercapto-3-phenyl-4-quinazolones, with chloroacetyl arylurea in dry acetone and potassium carbonate. All the compounds were characterized by their sharp melting points and elemental analyses.




## EXPERIMENTAL

All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes with a partial immersion thermometer and are uncorrected.

6-Substituted-2-mercapto-3-phenyl-4-quinazolones (I).

A mixture of phenyl isothiocyanate (6 ml) and 6-substituted anthranilic acids (16 g) in absolute ethanol (70 ml) was refluxed for 6 hours. The product was washed with ethanol containing sodium hydroxide (10%, w/v), reprecipitated with hydrochloric acid, filtered, washed several times with water, dried, and recrystallized from ethanol, mp, a = 190°, b = 295° (9).

6-Substituted-2-(1'-mercaptoacetyl-3'-arylurea-3-phenyl-4-quinazolones (II).

A mixture of 6-substituted-2-mercapto-3-phenyl-4-quinazolones (0.01 mole), 0.01 mole of chloroacetyl aryl substituted urea (11), and anhydrous potassium carbonate (0.015 mole) was refluxed in dry acetone for 3-4 hours. The mixture was filtered hot and the filtrate was concentrated and cooled. The solid mass which separated out was collected by filtration, washed several times with cold water, and recrystallized from alcohol. The physical constants of various substituted quinazolones are recorded in Table I.

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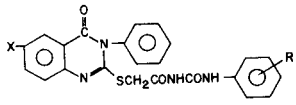
Table I

Physical Constants of 6-Substituted 2-(1'-Mercaptoacetyl-3'-arylurea)-3-phenyl-4-quinazolones

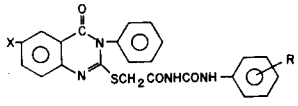



| Sl No. | Substitution X | Substitution R | Molecular formula | M.p. °C | % Carbon Calcd. | % Carbon Found | % Hydrogen Calcd. | % Hydrogen Found | % Nitrogen Calcd. | % Nitrogen Found |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | H | H | $C_{23}H_{18}N_4O_3S$ | 180 | 64.18 | 63.98 | 4.18 | 4.12 | 13.02 | 12.98 |
| **2** | H | *o*-$CH_3$ | $C_{24}H_{20}N_4O_3S$ | 185 | 64.86 | 64.56 | 4.50 | 4.65 | 12.61 | 12.66 |
| **3** | H | *m*-$CH_3$ | $C_{24}H_{20}N_4O_3S$ | 203 | 64.86 | 64.76 | 4.50 | 4.49 | 12.61 | 12.51 |
| **4** | H | *o*-$OCH_3$ | $C_{24}H_{20}N_4O_4S$ | 215 | 62.66 | 62.80 | 4.34 | 4.44 | 12.17 | 12.10 |
| **5** | H | *m*-$OCH_3$ | $C_{24}H_{20}N_4O_4S$ | 256 | 62.66 | 62.99 | 4.34 | 4.30 | 12.17 | 12.14 |
| **6** | H | *p*-Cl | $C_{23}H_{17}ClN_4O_3S$ | 210 | 59.40 | 59.31 | 3.65 | 3.56 | 12.05 | 12.06 |
| **7** | Br | H | $C_{23}H_{17}BrN_4O_3S$ | 154 | 54.22 | 54.44 | 3.33 | 3.32 | 11.00 | 10.91 |
| **8** | Br | *o*-$CH_3$ | $C_{24}H_{19}BrN_4O_3S$ | 220 | 55.06 | 54.98 | 3.63 | 3.56 | 10.70 | 10.63 |
| **9** | Br | *m*-$CH_3$ | $C_{24}H_{19}BrN_4O_3S$ | 240 | 55.06 | 55.04 | 3.63 | 3.59 | 10.70 | 10.71 |
| **10** | Br | *o*-$OCH_3$ | $C_{24}H_{19}BrN_4O_4S$ | 170 | 53.44 | 53.63 | 3.52 | 3.42 | 10.38 | 10.24 |
| **11** | Br | *m*-$OCH_3$ | $C_{24}H_{19}BrN_4O_4S$ | 120 | 53.44 | 53.14 | 3.52 | 3.56 | 10.38 | 10.43 |
| **12** | Br | *p*-Cl | $C_{23}H_{16}BrClN_4O_3S$ | 230 | 50.78 | 50.81 | 2.94 | 3.02 | 10.20 | 10.19 |

All the melting points are taken in open capillaries and are uncorrected.

Table II

Biochemical Studies of 6-Substituted 2-(1'-Mercaptoacetyl-3'-arylurea)-3-phenyl-4-quinazolones




| Sl. No. | Substituted X | Substituted $X_1$ | Inhibition of Enzyme Activity, % Monoamine Oxidase | Inhibition of Enzyme Activity, % Succinate Dehydrogenase |
|---|---|---|---|---|
| **1** | H | H | 42.6 | 52.8 |
| **2** | H | *o*-$CH_3$ | 51.3 | 31.0 |
| **3** | H | *m*-$CH_3$ | 28.2 | 52.8 |
| **4** | H | *o*-$OCH_3$ | 59.8 | 36.0 |
| **5** | H | *m*-$OCH_3$ | 11.3 | 38.3 |
| **6** | H | *p*-Cl | 62.8 | 35.0 |
| **7** | Br | H | 46.1 | 37.5 |
| **8** | Br | *o*-$CH_3$ | 59.6 | 48.1 |
| **9** | Br | *m*-$CH_3$ | 58.5 | 25.0 |
| **10** | Br | *o*-$OCH_3$ | 77.1 | 28.1 |
| **11** | Br | *m*-$OCH_3$ | 40.0 | 46.6 |
| **12** | Br | *p*-Cl | 73.6 | 46.6 |

All the compounds were tested at a final concentration of $2 * 10^{-4}$ *M*. Values reported here are mean value of two separate experiments done in duplicate.

Biochemical Studies.

Adult albino rats weighing approximately 100-150 g were killed by cervical dislocation. Brains were dissected out, cleaned of adhering tissue and blood and kept in ice-bath till homogenization. Brains were homogenized in 0.25 *M* sucrose using Potter-Elvehjem homogenizer under cold conditions. The enzyme inhibitory activity of substituted quinazolones was determined using brain homogenates as the source of these enzymes.

Determination of Monoamine Oxidase Activity.

The activity of the enzyme monoamine oxidase was determined spectrophotofluorometrically using kynuramine as the substrate (10). The 4-hydroxyquinoline formed by oxidative deamination of kynuramine undergoes spontaneous cyclization and the concentration of the end product is then measured for determination of the monoamine oxidase activity.

The reaction mixture in a final volume of 2 ml contained 1 ml phosphate buffer (0.5 *M*, *p*H 7.4), suitable amount of the enzyme preparation, 20.0 $\mu$g of kynuramine, test compounds in a final concentration of $2 \times 10^{-4}$ *M*, and water. The reaction was started by the addition of kynuramine after a preincubation period of 10 minutes at 37° in a water bath. After an incubation of 30 minutes the reaction was stopped by the addi-

tion of 1 ml of trichloroacetic acid (10%, w/v). The precipitated proteins were separated by centrifugation at 700 x g for 10 minutes. To 1 ml of the clear supernatant was added 2 ml of 1 *N* sodium hydroxide and fluorescence was measured at the activating light of 315 nm and excitation wavelength of 380 nm using Aminco Bowman Spectrophotofluorometer.

Determination of Succinate Dehydrogenase Activity.

Succinate dehydrogenase activity was determined using sodium succinate as the substrate according to the method of Slater and Bonner (12). The reaction mixture in a final volume of 2 ml contained 0.4 ml phosphate buffer (0.5 *M*, *p*H 7.2), 0.4 ml of potassium ferricyanide (0.01 *M*), 0.2 ml of sodium cyanide (0.1 *M*), suitable amount of brain homogenate, 0.01 ml of sodium succinate (0.1 *M*), test compounds and water. The reaction was started by the addition of sodium succinate after a preincubation period of 10 minutes at 37° in a water bath. After further incubation of 30 minutes, the reaction was stopped by the addition of 2 ml of trichloroacetic acid (10%, w/v). The contents were then centrifuged at 700 x g for 10 minutes and optical density of the clear supernatant was observed at 400 nm using Hitachi Perkin-Elmer UV-VIS Spectrophotometer.

All 6-substituted-2-(1′-mercaptoacetyl-3′-arylurea)-3-phenyl-4-quinazolones were found to inhibit the activity of rat brain monoamine oxidase at a final concentration of $2 \times 10^{-4}$ *M* and the degree of inhibition ranged from 11-77% (Table II). Maximum inhibition was found when the bromine substituent was introduced at position 6 of the quinazolone moiety with methoxy group at *ortho*-position of the phenyl ring of urea moiety (**10**). On the other hand absence of substituents in the quinazolone moiety in compounds having *meta*-methoxy substituent in the phenyl ring of urea moiety were found to show minimum inhibition (**5**). Bromine substituted quinazolones having *ortho*-methyl and *meta*-methyl substituents in the phenyl ring of urea portion exhibited moderate activity while introduction of chlorine in the *para* position of the phenyl ring of urea showed promising activity of 73% (**12**). The unsubstituted quinazolone having various substituents in the phenyl ring of urea moiety exhibited either a moderate or low activity (**1-6**). Amongst these, only *para*-chloro and *ortho*-methoxy derivatives showed moderate activity (**4** and **6**). From these alkyl substituents, as far as their substitution positions are concerned, *ortho*-position showed greater activity as compared to the corresponding *meta*-substitution. Similarly amongst bromo-substituted quinazolones having alkyl substituents, particularly methoxy substituents in the phenyl urea moiety, the activity of **11** with *meta* position (40%) was increased to 77% in the *ortho*-position (**10**).

All substituted quinazolones inhibited rat brain succinate dehydrogenase activity at a final concentration of $2 \times 10^{-4}$ *M* and the degree of inhibition ranged from 25-53% (Table II). Maximum inhibition was observed with unsubstituted quinazolone (**1**) and unsubstituted quinazolone having *meta*-methyl substituent in the phenyl urea moiety (**3**). The remaining unsubstituted quinazolones with different substituents at phenyl ring of urea portion did not show any inhibitory property. Bromo-substituted quinazolone derivatives having *ortho*-methyl, *meta*-methoxy, and *para*-chloro substituents in the phenyl urea moiety exhibited a moderate inhibitory activity as compared with other bromo derivatives.

The monoamine oxidase inhibitory activity of these compounds thus cannot be correlated for their ability to inhibit succinate dehydrogenase. It is hoped that further studies dealing with the synthesis of other related structures carrying different substituents and determination of their effects on other enzyme systems may possibly reflect the biochemical basis for the monoamine oxidase inhibitory effect of these 6-substituted 2-(1′-mercaptoacetyl-3′-arylurea)-3-phenyl-4-quinazolones.

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