This invention relates to a novel class of heterocyclic compounds useful for selectively inhibiting elastase, selectively inhibiting chymotrypsin-like enzymes, selectively inhibiting trypsin-like enzymes, or for generally inhibiting serine proteases of all classes.
HETEROCYCLIC INHIBITORS OF SERINE PROTEASES

RELATED APPLICATIONS

This application is a divisional of Ser. No. 07/499,561, filed on Mar. 26, 1990, now U.S. Pat. No. 5,109,018, issued on Apr. 28, 1992, which in turn is a division of Ser. No. 07/215,994, filed on Jul. 7, 1988, now abandoned, which in turn is a continuation of Ser. No. 06/784,459, filed on Jun. 13, 1986, now abandoned, which in turn is a continuation of Ser. No. 06/642,995, filed on Aug. 20, 1984, now U.S. Pat. No. 4,596,822, issued on Jun. 24, 1986.

BACKGROUND OF THE INVENTION

This invention relates to a novel class of heterocyclic compounds useful for selectively inhibiting elastase, selectively inhibiting chymotrypsin-like enzymes, or for generally inhibiting serine proteases of all classes. Certain clinical symptoms found in pancreatitis, emphysema, rheumatoid arthritis, inflammation, and adult respiratory distress syndrome are believed to be caused by uncontrolled elastase in the affected tissues. Likewise, similar clinical symptoms found in the same diseases are believed to be caused by uncontrolled cathepsin G, mast cell chymase, and other chymotrypsin-like enzymes. In vitro, proteolysis by serine proteases of the elastase, chymotrypsin, and trypsin families is often a severe problem in the production, isolation, purification, transport and storage of valuable peptides and proteins.

It is an object of this invention to find a novel group of specific inhibitors for elastase, chymotrypsin, trypsin, and other serine proteases of similar substrate specificity, and serine proteases in general. Inhibitors are substances which reduce or eliminate the catalytic activity of enzymes. Chymotrypsin and chymotrypsin-like enzymes normally cleave peptide bonds in proteins and peptides where the amino acid residue on the carbonyl side of the split bond (P1 residue) is typically Trp, Tyr, Phe, Met, Leu or other amino acid residues which contain aromatic or large alkyl side chains. Elastase and elastase-like enzymes, on the other hand, cleave peptide bonds where the P1 amino acid residue is much smaller, typically Ala, Val, Ser, Leu and other similar amino acids. Trypsin-like enzymes hydrolyze peptide bonds where the P1 amino acid is Lys or Arg. All of the above enzymes have extensive secondary specificity and recognize amino acid residues removed from the P1 residue. The inhibitors of this invention would be useful for treating diseases such as pancreatitis, emphysema, rheumatoid arthritis, adult respiratory distress syndrome, and inflammatory diseases which involve destruction of tissue by serine proteases. In some cases, it would be more useful to utilize a specific elastase, trypsin or chymotrypsin-like enzyme inhibitor, while in other cases an inhibitor with broader specificity would be appropriate.

It is an object of this invention to find a novel group of specific inhibitors useful in vitro for inhibiting elastase, trypsin, chymotrypsin and other serine proteases of similar specificity, and for inhibiting serine proteases in general. Such inhibitors could be used to identify new proteolytic enzymes encountered in research. They could also be used in research and industrially to prevent undesired proteolysis that occurs during the production, isolation, purification, transport and storage of valuable peptides and proteins. Such proteolysis often destroys or alters the activity and/or function of the peptides and proteins. Uses would include the addition of the inhibitors to antibodies, enzymes, plasma proteins, tissue extracts, or other proteins which are widely sold for use in clinical analysis, biomedical research, and for many other reasons. For some uses a specific inhibitor would be desirable, while in other cases, an inhibitor with general specificity would be preferred.

DETAILED DESCRIPTION OF THE INVENTION

Certain substituted isocoumarins have been found to be excellent inhibitors of a number of serine proteases including human leukocyte elastase, porcine pancreatic elastase, bovine chymotrypsin, human cathepsin G, various human and bovine blood coagulation enzymes, human complement factor D, and several mammalian mast cell proteases. These compounds inhibit the serine proteases by acylating the active site serine residue and in some cases form an additional covalent bond. These structures may be used in vivo to treat diseases resulting from tissue destruction due to elastase, chymotrypsin, trypsin, and related enzymes. They may be used in vitro to prevent proteolysis that occurs during the production, isolation, purification, storage, and transport of peptides and proteins. The novel substituted isocoumarins and related heterocyclic analogs have the following structural formula:

wherein X is selected from the group consisting of O and S, Z is selected from the group consisting of H, halogen, C1-6 alkyl, C1-6 alkyl with an attached phenyl, C1-6 fluorinated alkyl, C1-6 alkoxyl, C1-6 alkoxyl with an attached phenyl, benzzyloxy, 4-fluorobenzyloxy, -(OCH2C6H4-R') (2-substituent), -(OCH2C6H4-R') (3-substituent), -(OCH2C6H4-R') (4-substituent), -(OCH2C6H3-R2')(2,3-substituents), -(OCH2C6H3-R2')(2,4-substituents), -(OCH2C6H3-R2')(2,5-substituents), -(OCH2C6H3-R2')(2,6-substituents), -(OCH2C6H3-R2')(3,4-substituents), -(OCH2C6H3-R2')(3,5-substituents), R' is selected from the group consisting of H, halogen, trifluoromethyl, NO2, cyano, methyl, methoxy, acetyl, carboxyl, OH, and amino, Y is selected from the group consisting of H, halogen, trifluoromethyl, methyl, OH, and methoxy, R is selected from the group consisting of H, OH, NH2, NO2, halogen, -(NH)-C(NH)-NH2, -(C(NH)NH2)-C1-6 alkoxyl, C1-6 fluorinated alkoxyl, C1-6 alkyloxyl, C1-6 alkyloxylamin, M-AA and M-NH, wherein AA is selected from the group consisting of alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine, tryptophan, glycin, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine,
argine, histidine, beta-alanine, norleucine, norvaline, alpha-amino butyric acid, epsilon-aminocaproic acid, citrulline, hydroxyproline, ornithine, and sarcosine, wherein M is selected from the group consisting of hydrogen, lower alkanoyl having 1 to 6 carbons, carboxyalkanoyl, hydroxylalkanoyl, aminoalkanoyl, benzene sulfonyle, tosyl, benzoyl, and lower alkyl sulfonyle having 1 to 6 carbons.


The following compounds are representative of the invention:

- 3-chloroisocoumarin
- 3,4-dichloroisocoumarin
- 3-ethoxy-4-chloroisocoumarin
- 3-isobutoxy-4-chloroisocoumarin
- 3-benzoyl-4-chloroisocoumarin
- 3-(4-fluorobenzoyloxy)-4-chloroisocoumarin
- 7-amino-3-methoxy-4-chloroisocoumarin
- 7-amino-3-ethoxy-4-chloroisocoumarin
- 7-amino-3-benzoyloxy-4-chloroisocoumarin
- 7-nitro-3-methoxy-4-chloroisocoumarin
- 7-nitro-3-ethoxy-4-chloroisocoumarin
- 7-nitro-3-benzoyloxy-4-chloroisocoumarin
- 7-nitro-3-(2-phenethoxy)-4-chloroisocoumarin
- 7-nitro-3-(2-phenethoxy)-4-chloroisocoumarin, and
- 7-(N-tosylphenylalaninylamino)-4-chloro-3-methoxyisocoumarin.

When R is H, NH₂, NO₂, X is O, and Y and Z are any of the noted groups, the isocoumarin structure is a general inhibitor for both human leukocyte (HL) elastase and bovine chymotrypsin. Although these substituted isocoumarins are slightly less effective toward porcine pancreatic (PP) elastase and cathepsin G, they are still capable of inhibiting these enzymes. The rate constants for inactivation of HL elastase, PP elastase, and chymotrypsin by 3-chloroisocoumarin (X=O, Z=Cl, and Y=H) have been measured and published, (Harper, Hemmi, and Powers, J. Amer. Chem. Soc. 105, pp 6518–6520 (1983)). This publication is incorporated herein by reference. The 3-chloroisocoumarin also inactivates rat mast cell proteases I and II and Streptomyces RGH by changing both R, Z, and Y. The structures with long alkoxy or benzyloxy substituents are best at reactive towards trypsin-like enzymes. Additional specificity of the blood coagulation proteases and other trypsin-like enzymes was 3,4-dichloroisocoumarin (Z=Y=Cl, R=H, X=O). Attachment of positively charged groups such as guanidine or amines in R or Z would make the inhibitor reactive towards trypsin-like enzymes. Additional specificity towards a particular protease could be achieved by the placing the proper aminoacid or peptide derivative on the 7-amino group. Thus the specificity or generality of the inhibition reaction can be controlled first, by choosing the appropriate inhibitor structure and second, by choosing the inhibitor concentration utilized.
The spontaneous hydrolysis rates of many of these substituted isocoumarins in buffer solution have measured and are summarized in Table III. The hydrolysis rates are dependent upon the composition of the buffer. In general, these isocoumarins are more stable in phosphate buffered saline (pH 7.4) than in Hepes buffer (pH 7.5). The most stable inhibitor studied was 7-amino-3-methoxy-4-chloroisocoumarin while the most unstable was 3,4-dichloroisocoumarin. In addition, 7-amino-3-methoxy-4-chloroisocoumarin is quite stable in an albumin (0.4 mg/ml) while 3,4-dichloroisocoumarin decomposed rapidly upon addition to albumin (0.4 mg/ml). The 3-alkoxy-4-chloroisocoumarins are intermediate in stability. These compounds are significantly more stable than other serine protease inhibitors such as aza-peptides and sulfonl fluorides.

<table>
<thead>
<tr>
<th>TABLE III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Half-lives for Spontaneous Hydrolysis of Substituted Isocoumarins in Buffer Solution</strong></td>
</tr>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>3-chloroisocoumarin</td>
</tr>
<tr>
<td>3,4-dichloroisocoumarin</td>
</tr>
<tr>
<td>3-ethoxy-4-chloroisocoumarin</td>
</tr>
<tr>
<td>3-benzyloxy-4-chloroisocoumarin</td>
</tr>
<tr>
<td>3-fluorobenzylxoy-isocoumarin</td>
</tr>
<tr>
<td>7-amino-3-methoxy-isocoumarin</td>
</tr>
<tr>
<td>4-chloroisocoumarin</td>
</tr>
</tbody>
</table>

*Spontaneous hydrolysis rates were measured spectrophotometrically by monitoring the decrease in absorbance due to the isocoumarin ring system (wavelength range: 353-325 nm) and these rates converted to half-lives using the first order rate law.

*Conditions were: 0.1 M Hepes buffer, 0.5 M NaCl, pH 7.5, 10% Me2SO, at 25°C. Rate constants were obtained by the incubation method unless otherwise noted. An aliquot of inhibitor was added to a solution of enzyme and aliquots removed with time and assayed for remaining enzymatic activity. The first order rate constants, K_{onset}, were obtained from plots of In(v/v) versus time. The units of K_{onset}[I] are M⁻¹·s⁻¹.*

It has been found that certain heterocyclic compounds will inhibit serine proteases such as elastase and chymotrypsin by blocking the active site of the enzyme. The compounds of this invention are 3-substituted-7-substituted (1H)2-benzopyran-1,4(3H)-diones. The unsubstituted (1H)2-benzopyran-1,4(3H)-dione has been prepared previously for other purposes (cf. Knott, J. Chem. Soc., pp 402-410 (1963)). The heterocyclic serine protease inhibitors of this invention have the following structural formula:

![Structural formula](image)

wherein

- R₁ is selected from the group consisting of H, halogen, C₁-6 alkyl, C₁-6 alkyl with an attached phenyl, C₁-6 fluorinated alkyl, C₁-6 alkoxy, C₁-6 fluorinated alkoxy, C₁-6 alkoxy with an attached phenyl, benzylxoy, 4-fluorobenzylxoy, -OCH₂C₆H₄R’ (2-substituent), -OCH₂C₆H₄R’ (3-substituent), -OCH₂C₆H₄R’ (4-substituent), -OCH₂C₆H₄R’ (2,3-substituents), -OCH₂C₆H₄R’ (2,4-substituents), -OCH₂C₆H₄R’ (2,5-substituents), -OCH₂C₆H₄R’ (2,6-substituents), -OCH₂C₆H₄R’ (3,4-substituents), -OCH₂C₆H₄R’ (3,5-substituents), -OCH₂C₆H₄R’ (3,6-substituents), and -OCH₂C₆H₄R’ (3,5-substituents),

- R₂ is selected from the group consisting of H, halogen, trifluoromethyl, NO₂, cyano, methyl, methoxy, acetyl, carboxyl, OH, and amino,

- R₃ is selected from the group consisting of H, OH, NH₂, NO₂, halogen, -NH-(C=NH)-, -C(NH)NH₂, C₁-6 alkyl, C₁-6 fluorinated alkoxy, C₁-6 alkoxy, and C₁-6 alkylamino.

The following novel compounds are representative of this invention:

- (1H)2-benzopyran-1,4(3H)-dione,
- 3-ethyl-(1H)2-benzopyran-1,4(3H)-dione,
- 3-propyl-(1H)2-benzopyran-1,4(3H)-dione,
- 3-phenyl-(1H)2-benzopyran-1,4(3H)-dione.

These structures inhibit serine proteases by forming relatively stable acyl enzymes. HL elastase (0.004 mM) is totally inactivated by 3-propyl-1H2-benzopyran-1,4(3H)-dione (0.143 mM) while chymotrypsin (0.002 mM) is totally inhibited by this compound at a concentration...
A short paper is being written. And the novel inhibitors can then be used in benzene any cases where such correlations are made.  

The solvent was removed and the residue purified benzyl sulfuric acid added. The reaction mixture was heated at.

The following examples are given to illustrate the by silica gel chromatography using benzene as the elution. 

The serine protease inhibitors of this invention would be useful in a variety of experimental procedures where proteolysis is a significant problem. Inclusion of these inhibitors in radioimmunoassay experiments would result in higher sensitivity. The use of these inhibitors in plasma fractionation procedures would result in higher yields of valuable plasma proteins and would make purification of the proteins easier. The inhibitors disclosed here could be used in cloning experiments utilizing bacterial cultures, yeast and E. coli and would result in a more easily purified cloned product in higher yield.

It is well known in the literature that in vitro activity of elastase inhibitors correlates with in vivo activity in animal models of emphysema. Thus the novel inhibitors described here should be useful for the treatment of

The few known compounds have R=H, which is not claimed in the present invention.

To use the above identified inhibitors in vitro, they are dissolved in an organic solvent such as dimethylsulfoxide or ethanol and are added to an aqueous solution containing the protease which is to be inhibited such that the final concentration of organic solvent is 25% or less. The inhibitors may also be added as solids or in suspension.

The serine protease inhibitors of this invention would be useful in a variety of experimental procedures where proteolysis is a significant problem. Inclusion of these inhibitors in radioimmunoassay experiments would result in higher sensitivity. The use of these inhibitors in plasma fractionation procedures would result in higher yields of valuable plasma proteins and would make purification of the proteins easier. The inhibitors disclosed here could be used in cloning experiments utilizing bacterial cultures, yeast and E. coli and would result in a more easily purified cloned product in higher yield. It is well known in the literature that in vitro activity of elastase inhibitors correlates with in vivo activity in animal models of emphysema. Thus the novel inhibitors described here should be useful for the treatment of emphysema. Elastase inhibitors have been used orally, by injection or by instillation in the lungs in animal studies (cf. Powers, Am. Rev. Respir. Dis., 127, s54-s58 (1983) and references cited therein). The inhibitors described above can be used by any of these routes. The article by Powers (Am. Rev. Respir. Dis., 127, s54-s58 (1983)) is incorporated herein by reference.

Several other diseases also involve tissue destruction by proteases such as elastase-like and chymotrypsin-like enzymes (cf. Powers, Ad. in Chem., 198, 347-367 (1982)). This article by Powers is incorporated herein by reference. The other diseases include pancreatitis, inflammation, and adult respiratory syndrome. Although correlations between in vitro activity of elastase and chymotrypsin inhibitors and in vivo activity in animal models have not yet been made for these diseases, it is likely that such correlations will be made shortly. And the novel inhibitors can then be used in any cases where such correlations are made.

The following examples are given to illustrate the invention and are not intended to limit it in any manner.

**EXAMPLE 1**

Preparation of 3-Benzylxoxy-4-Chloroisocoumarin

Homophthalic acid (0.5 g, 2.78 mmoles) was placed in benzyl alcohol (5 mL) and 2 drops of concentrated sulfuric acid added. The reaction mixture was heated at 60 to 80 degrees centigrade for 45 min, poured into 50 mL of ice-cold NaHCO₃ (4%) and washed with three portions of ethyl acetate (50 mL). The aqueous layer was acidified to pH 2 with concentrated HCl and allowed to stand overnight. The precipitate was filtered and dried to give benzyl 2-carboxyphenyl acetate (300 mg) as a white solid: Rₜ=0.7 (chloroform/methanol (9:1)). The benzyl 2-carboxyphenyl acetate (300 mg) was dissolved in benzene 7 mL and PCl₃ (474 mg, 2.28 mmoles) added. The reaction mixture was refluxed for 45 min and the benzene and POCl₃ removed by rotary evaporation. Approximately one-half of the residue was chromatographed on silica gel using benzene as the eluent to give 3-benzylxoxy-4-chloroisocoumarin (125 mg) as a pale yellow solid: mp 92 deg centigrade, Rₜ=0.65 (benzene). Anal. Calcd. for C₁₅H₁₁ClO₃C: C, 67.02; H, 3.48. Found: C, 66.83; H, 3.86.

**EXAMPLE 2**

Preparation of 3-(4-Fluorobenzylxoxy)-4-Chloroisocoumarin

Homophthalic acid (4.0 g) was added to a solution containing 4-fluorobenzyl alcohol (10 mL), benzene (10 mL) and H₂SO₄ (3 drops) and the mixture refluxed at 80 degrees centigrade for 2 h. The reaction mixture was diluted with ethyl acetate (125 mL) and washed with 4% NaHCO₃ (500 mL). The aqueous layer was acidified to pH 3 with concentrated HCl and the solution placed in the refrigerator overnight. The crystals which formed upon cooling with filtered, dissolved in methylene chloride, and dried over magnesium sulfate. The (4-fluorobenzyl) 2-carboxyphenyl acetate was collected from methylene chloride as a white solid (800 mg) and was used without further purification. The (4-fluorobenzyl) 2-carboxyphenyl acetate (0.8 g) was added slowly to a solution of PCl₃ (1.16 g) in benzene (10 mL) and the mixture refluxed at 80 degrees centigrade for 45 min. The benzene was removed, and the residue dissolved in diethyl ether, and filtered. The filtrate was concentrated and collected with isopropyl ether as pale yellow needles (500 mg): mp 127, IR (CH₂Cl₂) 1743 cm⁻¹. Anal. Calcd. for C₁₅H₁₁ClO₃C: 63.07; H, 3.31. Found: C, 62.82; H, 3.36.

**EXAMPLE 3**

Preparation of 3-Isobutyloxy-4-Chloroisocoumarin

Homophthalic acid (4.0 g) was placed in isobutanol (25 mL) and 3 drops of H₂SO₄ added. The reaction mixture was heated for 1 h at 100-110 degrees centigrade, poured into 120 mL of ethyl acetate, and washed with 4% NaHCO₃ (15 mL, x 5). The combined aqueous washes were acidified to pH 3 using concentrated HCl and the resulting precipitate collected. The precipitate was dried to give isobutyl 2-carboxyphenyl acetate as a white solid (1.8 g). The isobutyl 2-carboxyphenyl acetate (0.7 g) was placed in a mixture of PCl₃ (1.23 g) and benzene (20 mL) and the mixture heated at reflux for 30 min. The solvent was removed and the residue purified by silica gel chromatography using benzene as the eluent to give product as pale yellow needles (338 mg); mp 48, IR (CH₂Cl₂) 1740 cm⁻¹ Anal. Calcd.: for C₁₅H₁₃ClO₅: C, 61.79; H, 3.19. Found: C, 61.64; H, 3.22.

**EXAMPLE 4**

Preparation of 7-Nitro-3-Benzylxoxy-4-Chloroisocoumarin

4-Nitro-homophthalic acid (5.0 g) was suspended in benzyl alcohol (50 mL) and H₂SO₄ (3 drops) added. The
reaction mixture was heated at 70-80 degrees centi-
grade for 45 min, diluted into 100 mL of ethyl acetate, and washed with 1 L of 4% NaHCO₃. The aqueous layers were combined, acidified to pH 3 using concentrated HCl, and extracted with 500 mL of ethyl acetate. The ethyl acetate layer was dried over magnesium sulfate and concentrated under reduced pressure. The residue chromatographed on silica gel using 1% mixture of methylene chloride/tetrahydrofuran (1:1) and triethylamine (0.037 mL in 2 mL of methylene chloride) added dropwise with stirring. After stirring at 25°C for 2 h, the reaction solvents were removed and the residue dissolved in ethyl acetate. After washing with 10% citric acid (3×30 mL) and 4% NaHCO₃ (2×30 mL), the ethyl acetate layer was dried over magnesium sulfate and concentrated. The crude product was purified by silica gel chromatography using a 1% mixture of methanol in methylene chloride and the 7-(N-tosyl-phenylalanylamino)-4-chloro-3-methoxycoumarin (22 mg) collected from methanol/isopropyl ether as a pale yellow solid: mp 222°-224°C (dec); one spot on TLC, R₂=0.3; IR (nujol) 1750 cm⁻¹; mass spectrum m/e 517 (M+1). Anal. Calcd. for C₂₆H₂₃ClN₂O₆S: C, 57.94; H, 3.50; N, 5.92. Found: C, 57.94; H, 3.50; N, 5.92.

EXAMPLE 6
Preparation of 7-Amino-4-Chloro-3(2-Phenethoxy)
Isocoumarin

The 4-chloro-7-nitro-3(2-phenethoxy) isocoumarin (50 mg, 0.14 mmoles) was dissolved in absolute ethanol (50 mL) and hydrogenated using Pd-C (50 mg) for 2 h. After filtering over celite, the solvent was removed and the residue chromatographed on silica gel using methylene chloride as the eluent to give 7-amino-4-chloro-3(2-phenethoxy) isocoumarin (35 mg) as yellow plates: mp 105°-107°C; one spot on TLC, R₂=0.45; IR (CH₂Cl₂) 1745 cm⁻¹; mass spectrum m/e 315 (M+1). Anal. Calcd. for C₁₇H₁₄ClN₂O₅ C, 64.66; H, 4.47. Found: C, 64.56; H, 4.51.

EXAMPLE 7
Preparation of 7-(N-Tosyl-Phenylalanylamino)-4-Chloro-3-Methoxycoumarin

N-Tosyl-phenylalanine acid chloride (77 mg, 0.3 mmoles) and 7-amino-4-chloro-3-methoxycoumarin (50 mg, 0.2 mmoles) were suspended in a mixture of methylene chloride/tetrahydrofuran (1:1) and triethylamine (0.037 mL in 2 mL of methylene chloride) added dropwise with stirring. After stirring at 25°C for 2 h, the reaction solvents were removed and the residue dissolved in ethyl acetate. After washing with 10% citric acid (3×30 mL) and 4% NaHCO₃ (2×30 mL), the ethyl acetate layer was dried over magnesium sulfate and concentrated. The crude product was purified by silica gel chromatography using a 1% mixture of methanol in methylene chloride and the 7-(N-tosyl-phenylalanylamino)-4-chloro-3-methoxycoumarin (22 mg) collected from methanol/isopropyl ether as a pale yellow solid: mp 222°-224°C (dec); one spot on TLC, R₂=0.3; IR (nujol) 1750 cm⁻¹; mass spectrum m/e 517 (M+1). Anal. Calcd. for C₂₆H₂₃ClN₂O₆S: C, 57.94; H, 3.50; N, 5.92. Found: C, 57.94; H, 3.50; N, 5.92.

EXAMPLE 8
Preparation of 3-Propyl-1H-2-Benzopyran-1,4(3H)-dione

A solution of 3-bromo, 3-(1-bromobutyl)-1(3H)-isobenzofuranone (500 mg) in Me₂SO (15 mL) was added dropwise to a solution of 0.1M Hepes buffer (pH 7.5, 50 mL) containing 0.5M NaCl and Me₂SO (35 mL). The mixture was stirred at 25 degrees centigrade, diluted with water (100 mL) and extracted with ethyl acetate (50 mL,×2). The extracts were combined, washed with water (50 mL,×5), dried over magnesium sulfate, and concentrated under reduced pressure. The residue was chromatographed on silica gel using benzene as the eluent to give the product as a white solid (170 mg): mp 44-45 degrees centigrade; IR (nujol), 1730, 1700 cm⁻¹. Anal. Calcd. for C₁₇H₁₄O₂: C, 70.57; H, 5.92. Found: C, 70.55; H, 5.96.


What is claimed is:

1. A compound of the formula:

\[
R \square X \square Y \square Z
\]

wherein

X is selected from the group consisting of O, and S;
Z is selected from the group consisting of H, halogen, Cl-C6 alkyl with an attached phenyl, Cl-C6 fluorinated alkyl, Cl-C6 fluorinated alkoxy, Cl-C6 alkoxy with an attached phenyl, benzyl, 4-fluorobenzyl, -OCH2C6H4-R' (2-substituent), -OCH2C6H4-R' (3-substituent), -OCH2C6H4-R' (4-substituent), -OCH2C6C6.

H3-(R')2 (2,3-substituents), -OCH2C6C6.

H3-(R')2 (2,4-substituents), -OCH2C6C6.

H3-(R')2 (2,5-substituents), -OCH2C6C6.

H3-(R')2 (2,6-substituents), -OCH2C6C6.

H3-(R')2 (3,4-substituents), -OCH2C6C6.

H3-(R')2 (3,5-substituents), -OCH2C6C6.

R' is selected from the group consisting of H, halogen, trifluoromethyl, N02, cyano, methyl, methoxy, acetyl, carboxyl, OH, and amino;

Y is selected from the group consisting of H, halogen, trifluoromethyl, methyl, OH, and methoxy, and

R is selected from the group consisting of NH2 and NO2.

3. A compound of the formula:

wherein

X is selected from the group consisting of O, and S;

Z is selected from the group consisting of H, halogen, Cl-C6 alkyl with an attached phenyl, Cl-C6 fluorinated alkyl, Cl-C6 fluorinated alkoxy, Cl-C6 alkoxy with an attached phenyl, benzyl, 4-fluorobenzyl, -OCH2C6H4-R' (2-substituent), -OCH2C6H4-R' (3-substituent), -OCH2C6H4-R' (4-substituent), -OCH2C6C6.

H3-(R')2 (2,3-substituents), -OCH2C6C6.

H3-(R')2 (2,4-substituents), -OCH2C6C6.

H3-(R')2 (2,5-substituents), -OCH2C6C6.

H3-(R')2 (2,6-substituents), -OCH2C6C6.

H3-(R')2 (3,4-substituents), -OCH2C6C6.

H3-(R')2 (3,5-substituents), -OCH2C6C6.

R' is selected from the group consisting of H, halogen, trifluoromethyl, NO2, cyano, methyl, methoxy, acetyl, carboxyl, OH, and amino;

Y is selected from the group consisting of H, halogen, trifluoromethyl, methyl, OH, and methoxy, and

R is selected from the group consisting of NH2 and NO2.

4. A compound of the formula:

wherein

X is selected from the group consisting of O, and S;

Z is halogen;

Y is selected from the group consisting of H, halogen, trifluoromethyl, methyl, OH, and methoxy, and

R is selected from the group consisting of OCH3 and OH.

5. A compound of the formula:

wherein

R1 is selected from the group consisting of halogen, Cl-C6 alkyl, Cl-C6 alkyl with an attached phenyl, Cl-C6 fluorinated alkyl, Cl-C6 fluorinated alkoxy, Cl-C6 alkoxy with an attached phenyl, benzyl, 4-fluorobenzyl, -OCH2C6H4-R' (2-substituent), -OCH2C6H4-R' (3-substituent), -OCH2C6H4-R' (4-substituent), -OCH2C6C6.

H3-(R')2 (2,3-substituents), -OCH2C6C6.

H3-(R')2 (2,4-substituents), -OCH2C6C6.

H3-(R')2 (2,5-substituents), -OCH2C6C6.

H3-(R')2 (2,6-substituents), -OCH2C6C6.

H3-(R')2 (3,4-substituents), -OCH2C6C6.

H3-(R')2 (3,5-substituents), -OCH2C6C6.

R' is selected from the group consisting of H, halogen, trifluoromethyl, NO2, cyano, methyl, methoxy, acetyl, carboxyl, OH, and amino;

Y is selected from the group consisting of H, halogen, trifluoromethyl, methyl, OH, and methoxy, and

R is selected from the group consisting of NH2 and NO2.
—OCH₂C₆H₄—R' (2-substituent), —OCH₂C₆H₄—R' (3-substituent), —OCH₂C₆H₄—R' (4-substituent), —OCH₂C₆H₃—(R')₂ (2,3-substituents), —OCH₂C₆H₃—(R')₂ (2,4-substituents), —OCH₂C₆H₃—(R')₂ (2,5-substituents), —OCH₂C₆H₃—(R')₂ (2,6-substituents), —OCH₂C₆H₃—(R')₂ (3,4-substituents), —OCH₂C₆H₃—(R')₂ (3,5-substituents);

R' is selected from the group consisting of H, halo-
gen, trifluoromethyl, NO₂, cyano, methyl, methoxy, acetyl, carboxyl, OH, and amino;

R₂ is selected from the group consisting of H, OH, NH₂, NO₂, halogen, —NH—C(NH)—NH₂, —C(NH)NH₂, C₁–C₆ alkoxy, C₁–C₆ fluorinated alkoxy, C₁–C₆ alkyl, and C₁–C₆ alkyl amino.