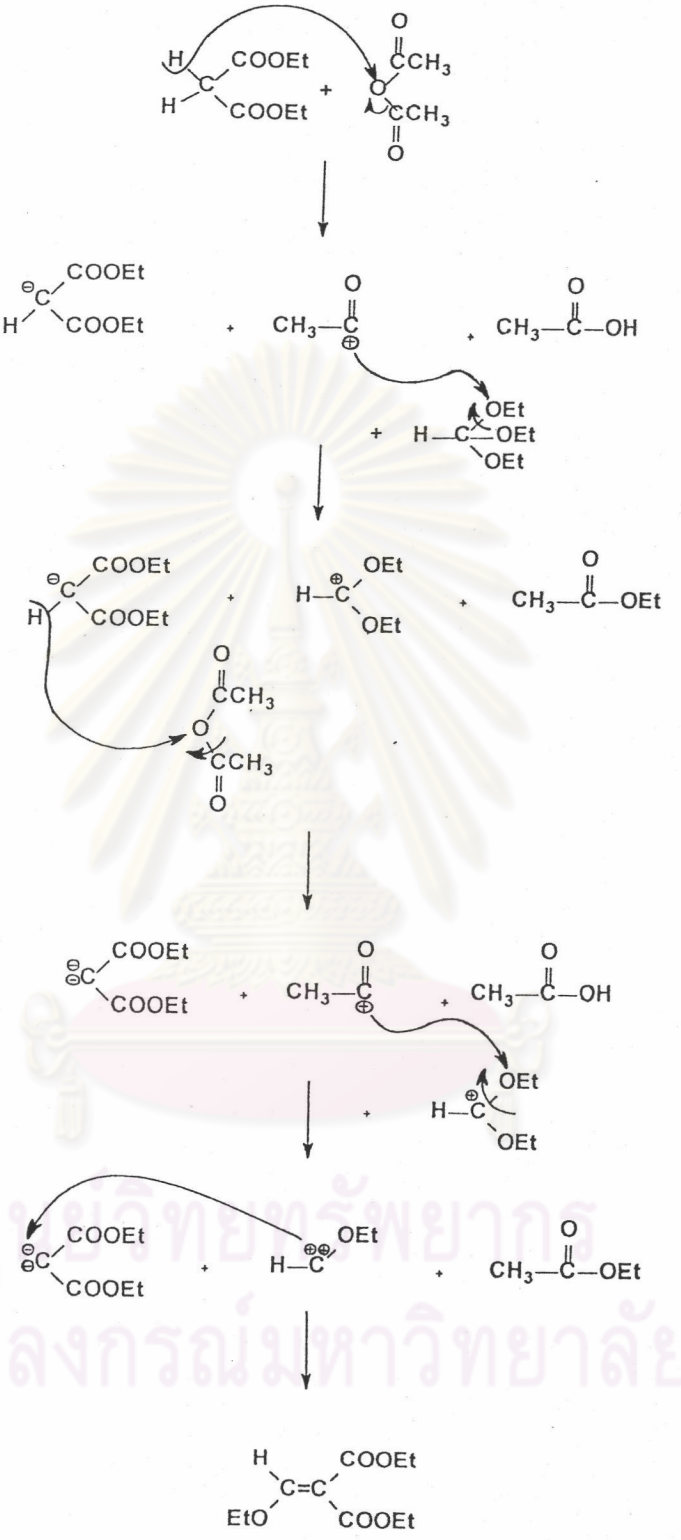
CHAPTER 4

RESULTS AND DISCUSSION

In this research, pyrazoloquinolone derivatives were synthesized. The quinoline ring was prepared according to the modified Gould-Jacobs cyclization. The 3-carboethoxy-4-hydroxyquinoline was converted to the intermediate 4-chloroquinoline-3-carboxylate by thionyl chloride. The synthesis of pyrazoloquinolone derivatives was readily obtained from treatment of 4-chloroquinoline-3-carboxylates with hyrazine. The overall reactions are discussed as followed:

Diethyl ethoxymethylenemalonate (XXVII)

Diethyl ethoxymethylenemalonate was prepared according to Claisen method (1897) by reaction of diethylmalonate with triethylorthoformate in acetic anhydride as proton and leaving group acceptor. The mechanism of the reaction was shown in Scheme 2.



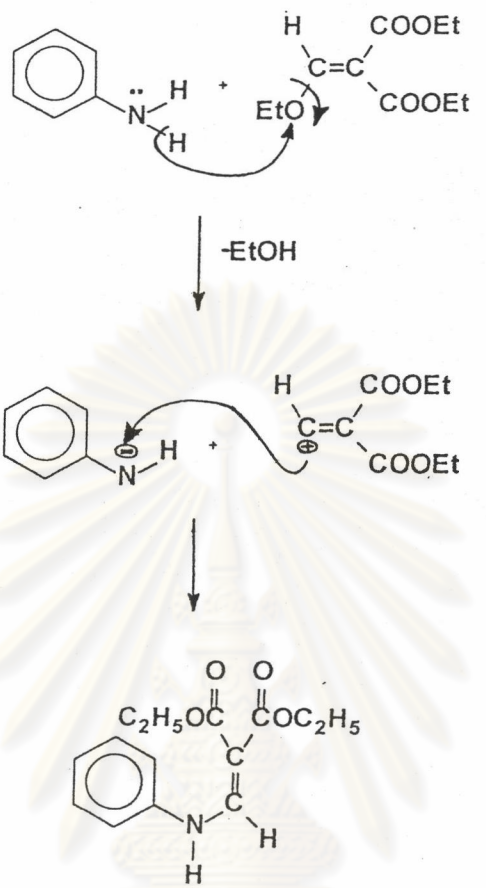
Scheme 2 Mechanism of the formation of diethyl ethoxymethylenemalonate

The IR spectrum of diethyl ethoxymethylenemalonate(XXVII) (Figure 1) showed a strong C=O stretching absorption peak at 1731 cm^{-1} , a peak between 1250-1290 cm^{-1} assigned for C-C-O stretching and a peak of C-O stretching vibration appeared at 1090 cm^{-1}. A peak at 1635 cm^{-1} represented C=C stretching vibration.

The ^{1}H-NMR spectrum of diethyl ethoxymethylenemalonate (XXVII) (Figure 2) showed peak at 1.28-1.45 ppm (multiplet, 9H) assigned for 3 methyl protons, the peak at 4.09-4.38 ppm (multiplet,6H) assigned for 3 methylene protons adjacent to oxygen and methyl group. The peak at 7.60 ppm (singlet, 1H) represented the olefinic methine proton.

Ethyl anilino-3-chloro-methylenemalonate (XXVIII-a)

The reaction of diethyl ethoxymethylenemalonate and aniline to form ethyl anilinomethylenemalonate took place readily even at room temperature. Anilinoacrylate product was used for further reaction without purification. The mechanism of this reaction was nucleophilic attack of aniline upon the alkenyl carbon of diethyl ethoxymethylenemalonate, resulted in the loss of ethoxy group to form ethanol and the corresponding ethyl anilinomethylenemalonate was obtained as shown:



Scheme 3 Mechanism of the formation of diethyl anilino-methylenemalonate

Reaction of 3-chloroaniline with diethyl ethoxymethylene-malonate occurred under refluxing condition and its mechanism of reaction was the same as above.

The IR spectrum of ethyl anilino-3-chloro-methylenemalonate (XXVIII-a)(Figure 3) exhibited characteristic peak of ester carbonyl group at 1682 cm^{-1} and a strong peak in the region 1253 cm^{-1} assignable to stretching vibration of C-O (ester). The peak at 1642 cm^{-1} could be

interpreted as C=C stretching vibration. The N-H stretching peak was found at 3282 cm^{-1} and N-H bending peak was found at 1615 cm^{-1}.

The ^{1}H-NMR spectrum of ethyl anilino-3-chloro-methylenemalonate (XXVIII-a) (Figure 4-5) exhibited the peaks at chemical shift 1.34, 1.38 ppm (2 triplets, 6H, J=7.2 Hz) for two methyl protons, the peaks at 4.26, 4.31 ppm (2 quartets, 4H, J=7.2 Hz) for two methylene protons adjacent to oxygen ester. The peak at 6.99-7.32 ppm represented four aromatic protons, while the one-proton doublet at 8.46 ppm represented methine proton adjacent to amino group and the peak of secondary amine proton showed up as one-proton doublet at 11.00 ppm.

Ethyl anilino 3-nitro-methylenemalonate (XXVIII-b)

3-Nitroaniline reacted with diethyl ethoxymethylenemalonate under refluxing condition with similar mechanism of reaction as that of the synthesis of ethyl anilino-3-chloromethylenemalonate.

The IR spectrum of ethyl anilino-3-nitro-methylenemalonate (XXVIII-b) (Figure 6) exhibited characteristic peak of ester carbonyl group at 1696 cm^{-1} and another strong peak at region 1261 cm^{-1} assignable to stretching vibration of C-O (ester). The peak at 1646 cm^{-1} could be interpreted as C=C stretching vibration while the N-H stretching

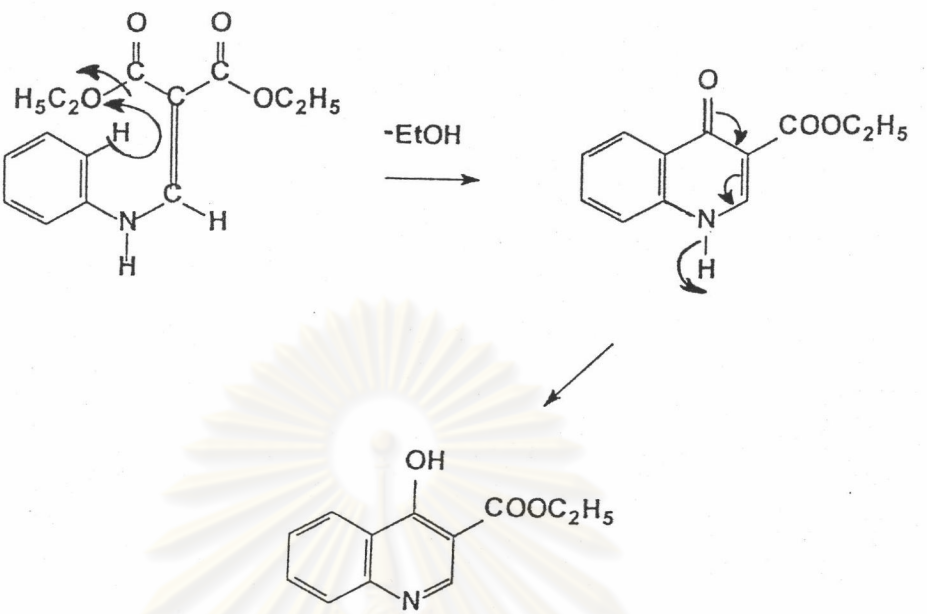
was found at 3310 cm^{-1} and N-H bending peak at 1608 cm^{-1}. The peak at 1536 and 1350 cm^{-1} represented N-O stretching vibration.

The ^{1}H-NMR spectrum of ethyl anilino-3-nitro-methylenemalonate (XXVIII-b) (Figure 7-8) exhibited the peaks at chemical shift of 1.35, 1.39 ppm (2 triplets, 6H, J=7.2 Hz) for two methyl protons, the peaks at 4.28, 4.33 ppm (2 quartets, 4H, J=7.2 Hz) for methylene protons adjacent to oxygen ester. The peaks between 7.43-8.00 ppm represented four aromatic protons, while the peak at 8.50 ppm (doublet, 1H) represented the methine proton coupled to the one-proton doublet of secondary amine at 11.17 ppm.

3-Carboethoxy-7-chloro-4-hydroxyquinoline (XXIX-a)

Ring closure reaction of ethyl anilino-3-chloro-methylenemalonate occurred at high temperature. Heating of this compound in boiling diphenyl ether readily afford the cyclization.

Delocalization of the electron of ethyl anilino-3-chloro-methylenemalonate gave an intermediate which could be converted to 3-carboethoxy-7-chloro-4-hydroxyquinoline by the loosing of ethanol since ethoxyl was better leaving group than hydroxyl group. The cyclization mechanism of ethyl anilino-3-chloro-methylenemalonate was as followed.



Scheme 4 Cyclization mechanism of ethyl anilinomethylene-malonate

The IR spectrum of 3-carboethoxy-7-chloro-4-hydroxyquinoline (XXIX-a) (Figure 9) showed carbonyl absorption peak at 1698 cm^{-1} and C-O ester stretching vibration peak at 1197 and 1354 cm^{-1}. The peak at 1621 cm^{-1} was from C=N stretching vibration. In addition, the region at 2906-3150 cm^{-1} represented C-H stretching vibration peak and C-H bending peak was found at 1464 cm^{-1}. The peak at 1378 cm^{-1} can be assigned for O-H bending vibration.

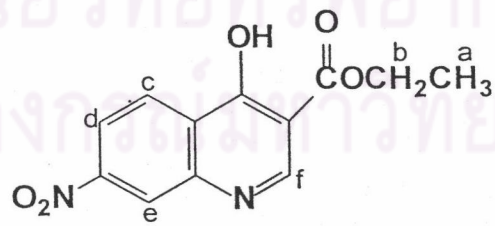
The ^{1}H-NMR spectrum of 3-carboethoxy-7-chloro-4-hydroxyquinoline (XXIX-a) (Figure 10-11) showed a peak at chemical shift of 1.28 ppm (triplet, 3H) assigned for methyl protons and another

peak at 4.22 ppm (quartet, 2H) assigned for methylene protons of the ester. The peak at 7.42 ppm was identified as the aromatic proton at position d (doublet of doublets, 1H, J=8.7, 2.1 Hz). The peak at 7.66 ppm could be assigned for the aromatic proton at position e (doublet,1H, J=2.1 Hz) and at 8.14 ppm (doublet, 1H, J=8.5 Hz) assigned for the aromatic proton at position c. The one-proton singlet at 8.56 ppm represented methine proton adjacent to nitrogen atom and the broad peak of hydroxy proton appeared at 12.24 ppm.

3-Carboethoxy-7-nitro-4-hydroxyquinoline (XXIX-b)

Ring closure reaction of ethyl anilino-3-nitromethylenemalonate proceeded at high temperature. Heating this compound in high boiling point solvent, especially diphenyl ether, facilitate the cyclization.

The mechanism of the reaction was similar to that of the preparation of 3-carboethoxy-7-chloro-4-hydroxyquinoline.



The IR spectrum of 3-carboethoxy-7-nitro-4-hydroxyquinoline (XXIX-b) (Figure 12) showed carbonyl absorption peak at 1694 cm^{-1} and C-O ester stretching vibration peaks at 1198 and 1297 cm^{-1}. The

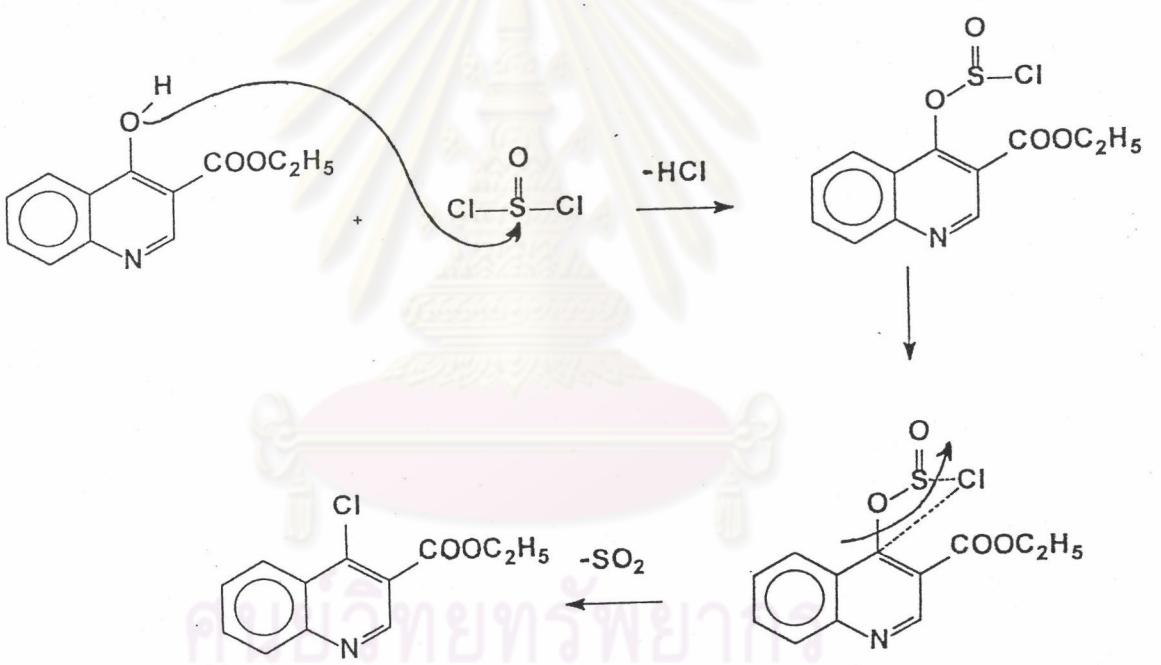
peak at 1635 cm^{-1} was from C=N stretching vibration. In addition, the region at 2890-3150 cm^{-1} represented C-H stretching vibration whereas C-H bending peak was found at 1468 cm^{-1}. The peak at 1381 cm^{-1} was ascribed to O-H bending vibration. Two peaks at 1567 and 1356 cm^{-1} were assigned for N-O stretching vibration.

The ^{1}H-NMR spectrum of 3-carboethoxy-7-nitro-4-hydroxyquinoline (XXIX-b) (Figure 13-14) showed a three-proton triplet at 1.29 ppm assigned for methyl protons of the ester moiety, whereas the two-proton quartet at 4.24 ppm could be assigned to the adjacent methylene protons. The peak at 8.12 ppm (doublet of doublets, 1H, J=8.8, 2.1 Hz) was identified as the aromatic proton at position d. The doublet at 8.36 ppm (1H, J= 8.8 Hz) was assigned to the aromatic proton at position c and another at 8.50 ppm (1H, J=2.1 Hz) to the proton at position e. The one-proton singlet at 8.71 ppm represented the aromatic methine proton adjacent to nitrogen atom and, finally the broad peak of hydroxy proton appeared at 12.57 ppm.

3-Carboethoxy-4,7-dichloroquinoline (XXX-a)

3-Carboethoxy-4,7-dichloroquinoline was prepared from chlorination of 3-carboethoxy-7-chloro-4-hydroxyquinoline using thionyl chloride as chlorinating agent. The phenolic hydroxy group was replaced with chlorine atom to produce 3-carboethoxy-4,7-dichloroquinoline. This synthetic procedure was performed according to the method by Kaslow and Clark (1953).

The possible mechanism of this chlorinaion involves the attack of the hydroxy group of 3-carboethoxy-7-chloro-4-hydroxyquinoline on the thionyl chloride to give an intermediate and a molecule of hydrogen chloride, which then combine to give the hydrochloride salt. This intermediate then undergoes nucleophilic aromatic substitution by chlorine atom. The process of chlorine substitution is facilitated by the presence of the positive charge at nitrogen atom and the final stage was the lost of sulfur dioxide. The mechanism was shown as followed:



Scheme 5 Mechanism of the chlorination of 4-hydroxyquinoline

The IR spectrum of 3-carboethoxy-4,7-dichloroquinoline (XXX-a) (Figure 15) showed peaks in the region 2869-3074 cm^{-1} assigned for aromatic and aliphatic C-H stertching. The strong peak at 1728 cm^{-1} was C=O stretching vibration peak of ester carbonyl whereas peaks at 1197

and 1228 cm^{-1} represented C-O (ester) stretching. In addition, the peak at 1582 cm^{-1} was identified as C=C stretching vibration and the one at 1472 cm^{-1} as aliphatic C-H bending peak.

The ^{1}H-NMR spectrum of 3-carboethoxy-4,7-dichloroquinoline (XXX-a) (Figure 16-17) showed characteristic peaks of ethyl ester group at 1.47 ppm (triplet, 3H) for methyl and 4.51 ppm (quartet, 2H) for methylene protons. The peak at chemical shift 7.65 ppm (doublet of doublets, 1H, J=9.1, 1.8 Hz) represented the aromatic proton at position d which also coupled to the aromatic proton at position e at 8.15 ppm (doublet, 1H, J=1.8 Hz) and position c at 8.35 ppm (doublet, 1H, J=8.9 Hz). The methine proton adjacent to nitrogen atom appeared as a singlet at 9.21 ppm.

3-Carboethoxy-4-chloro-7-nitroquinoline (XXX-b)

3-Carboethoxy-4-chloro-7-nitroquinoline was synthesized by the chlorination of 3-carboethoxy-7-nitro-4-hydroxyquinoline. The method and mechanism were the same as in the preparation of 3-carboethoxy-4,7-dichloroquinoline.

The IR spectrum of 3-carboethoxy 4-chloro-7-nitroquinoline (XXX-b) (Figure 18) showed peaks at region 2865-3102 cm^{-1} which accounted for aromatic and aliphhatic C-H stretching. The strong peak at 1737 cm^{-} was C=O stretching vibration peak of the ester carbonyl ,while

two peaks at 1210 and 1235 cm^{-1} represented C-O (ester) stretching. In addition, the peak at 1587 cm^{-1} was identified as C=C stretching vibration and the peak at 1471 cm^{-1} could be assigned to aliphatic C-H bending. Two peaks at 1533 and 1350 cm^{-1} represented N-O stretching vibration.

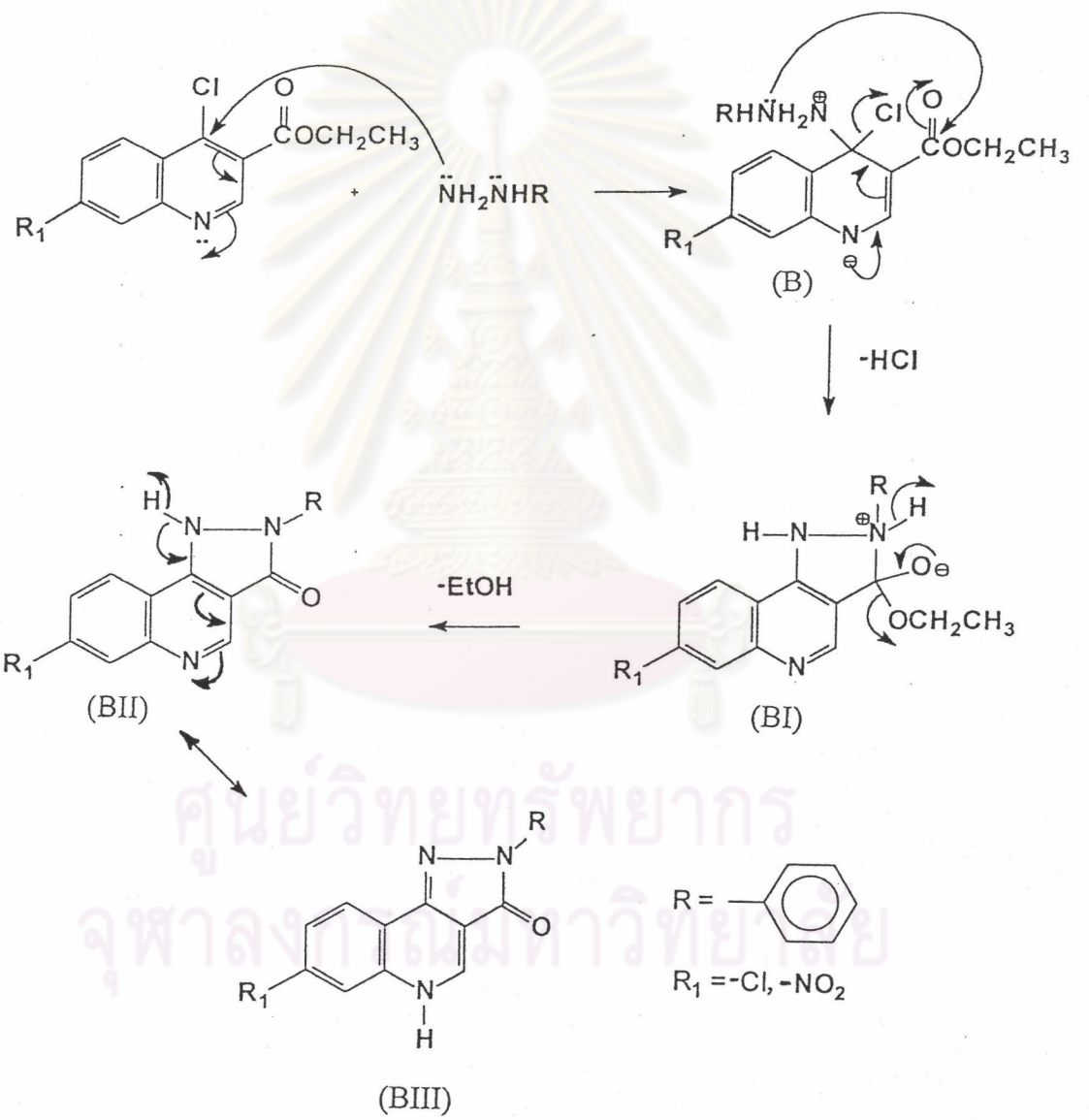
The ^{1}H-NMR spectrum of 3-carboethoxy-4-chloro-7-nitroquinoline (XXX-b) (Figure 19-20) exhibited characteristic ethyl ester peaks at chemical shifts of 1.49 ppm (triplet, 3H) for methyl protons and 4.54 ppm (quartet, 2H) for methylene protons. The peak at 8.46 ppm (doublet of doublets, 1H, J=9.2, 1.2 Hz) represented the aromatic proton at position d, ortho-coupled to the one-proton doublet at 8.59 ppm of the aromatic proton at position c (J=9.4 Hz), whereas the broad singlet at 9.03 ppm could be assigned to the aromatic proton at position e. The methine proton adjacent to nitrogen atom appeared as a singlet at 9.33 ppm.

Synthesis of pyrazoloquinolone derivatives

The reaction between 3-carboethoxy-4,7-dichloroquinoline and phenylhydrazine gave the desired pyrazoloquinolone derivatives.

This reaction involves nucleophilic aromatic substitution, with hydrazine serving as the nucleophile. Initially, the attack by amine group of hydrazine on 3-carboethoxy-4-chloroquinoline gives the intermediate

precursor (B). The carbonyl carbon of the ester is then attacked by the second nitrogen lone pair electrons to give pyrazoloquinolin-3-one intermediate (BI). The intermediate (BI) successively loses ethanol to form pyrazoloquinolin-3-one (BII) which can be tautomerized to give the target compound (BIII). The mechanism was shown as followed:



Scheme 6 Mechanism of the formation of pyrazoloquinolone derivatives

7-Chloro-2-phenylpyrazolo-[4,3-c]-quinolin-3-one (CU-736-09-01)

The synthesis of 7-chloro-2-phenylpyrazolo-[4,3-c]-quinolin-3-one (CU-736-09-01) was readily accomplished by heating a mixture of 3-carboethoxy-4,7-dichloroquinoline with phenylhydrazine at 130-150°C in xylene. The desired product (CU-736-09-01) precipitated in xylene and could be easily separated by filtration.

The mechanism of reaction was the same as above.

The IR spectrum of 7-chloro-2-phenylpyrazolo-[4,3-c]-quinolin-3-one (CU-736-09-01) (Figure 21) displayed the peak at 1616 cm^{-1} for C=O stretching vibration, at 1592 cm^{-1} for C=N stretching vibration, the peak at 1544 cm^{-1} for C=C stretching vibration.

The ^{1}H-NMR spectrum of 7-chloro-2-phenylpyrazolo-[4,3-c]-quinolin-3-one (CU-736-09-01) (Figure 22-23) showed peaks at chemical shifts of at 7.17, 7.43 and 8.18 ppm representing protons at position a (triplet, 1H), b (triplet, 2H) and f (doublet, 2H), respectively, of the phenyl moiety. The doublet of doublets at 7.56 ppm (1H, J=8.6, 2.0 Hz), represented the aromatic proton at position d which was meta-coupled to the proton at position c (doublet, 1H, J=2.1 Hz) at 7.72 ppm and ortho-coupled to the proton at position e at 8.20 ppm (doublet, 1H, J=8.6 Hz). The one-proton singlet at 8.75 ppm represented the proton adjacent to nitrogen atom of the pyridine system.

The 500 MHz HH COSY spectrum of CU-736-09-01 (Figure 25-26) showed the peak at 7.17 ppm, assignable to the proton at position a of the phenyl ring, vicinal coupled (J=7.3 Hz) to both protons at position b and also long range coupled (J=1.0 Hz) to another two protons at position f. The peak at 7.56 ppm, assigned to the proton at position d (H_d), exhibited cross peak with vicinal H_e proton (J=8.5 Hz) and long range coupled (J=2.1 Hz) with H_c at 7.72 ppm.

The 125 MHz ^{13}C-NMR spectrum of CU-736-09-01 (Figure 24) showed fourteen signals for sixteen carbons. Hence, there were two signals of representing equal carbon atoms of the phenyl ring. From the 125 Mhz CH-COSY spectrum (Figure 27-28), the signal at 118.66 ppm could be assigned to C-12 and C-16 and the one at 128.68 ppm to C-13 and C-15. The carbon signals at 118.87, 124.04, 124.10, 126.52, 139.96 and 161.43 ppm were assigned to C-5, C-14, C-8, C-7, C-3 and C-1, respectively. From calculation, the carbon at C-2, C-9, C-4, C-11, C-10 and C-6 should be assigned at chemical shift 106.68, 117.46, 134.24, 136.52, 139.93 and 142.33 ppm.

The EIMS spectrum of 7-chloro-2-phenylpyrazolo-[4,3-c]-quinolin-3-one (CU-736-09-01) was shown in Figure 29. The peak at m/e 295 represented the molecular ion. The fragments at m/e 77, 105, 127, 162, 176, 190, 266 and 267 were proposed in Scheme 7.

Scheme 7 Fragmentation pattern of 7-chloro-2-phenyl-pyrazolo-[4,3-c]-quinolin-3-one

7-Nitro-2-phenylpyrazolo-[4,3-c]-quinolin-3-one (CU 736-09-02)

The desired product (CU 736-09-02) was obtained by heating a mixture of 3-carboethoxy-4-chloro-7-nitroquinoline with phenyl-hydrazine at 130-150°C in xylene. 7-Nitro-2-phenylpyrazolo-[4,3-c]-quinolin-3-one precipitated in xylene which was then easily for separated by filtration.

The mechanism of the reaction was the same as in the preparation of 7-chloro-2-phenylpyrazolo-[4,3-c]-quinolin-3-one.

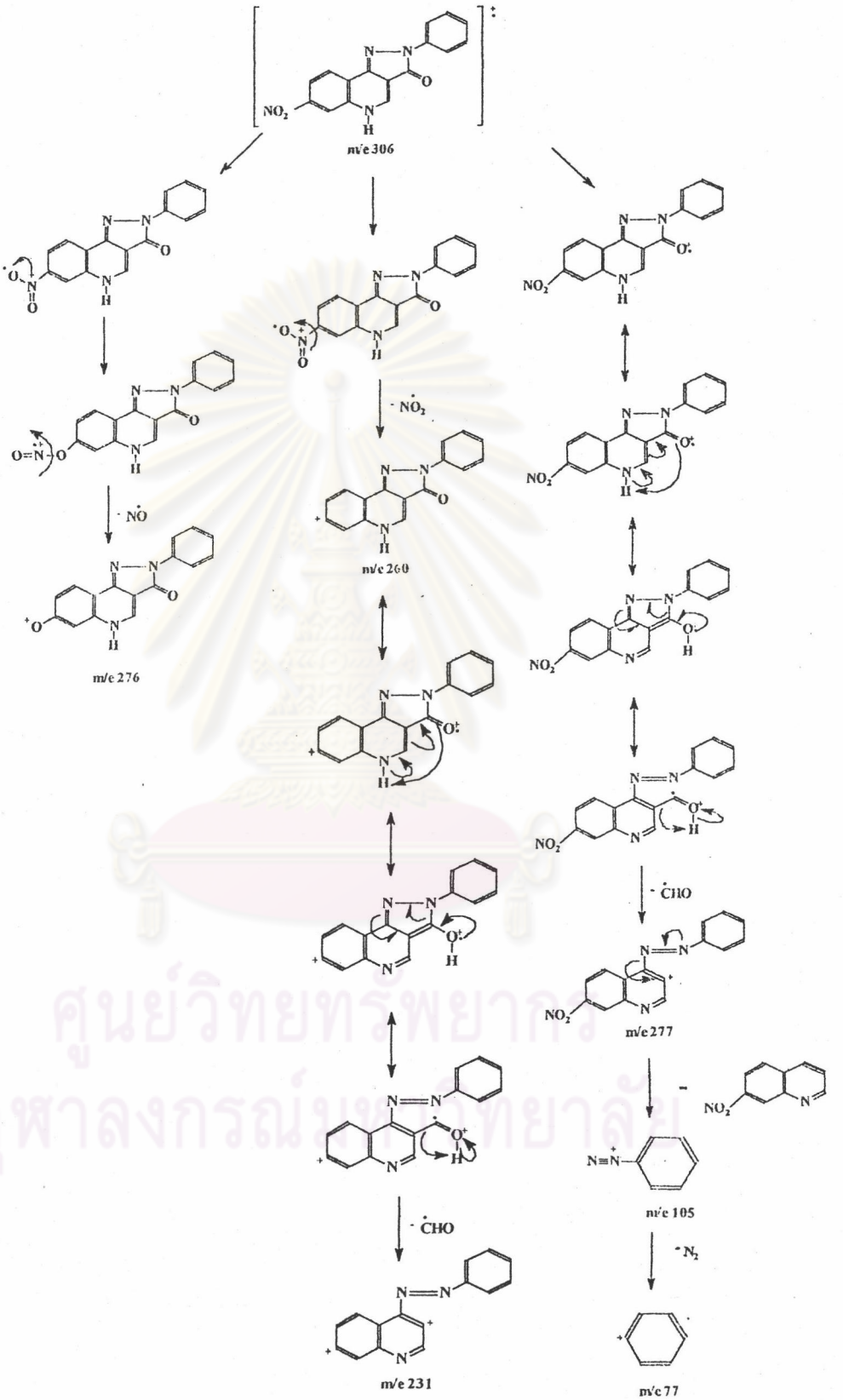
The IR spectrum of 7-nitro-2-phenylpyrazolo-[4,3-c]-quinolin-3-one (CU-736-09-02) (Figure 30) exhibited the peak at 1628 cm^{-1} for C=O stretching vibration, at 1594 cm^{-1} for C=N stretching vibration and at 1350 and 1526 cm^{-1} for N-O stretching vibration.

The ^{1}H-NMR spectrum of 7-nitro-2-phenylpyrazolo-[4,3-c]-quinolin-3-one (CU-736-09-02) (Figure 31-32) showed peaks at chemical shifts of at 7.20, 7.45 and 8.18 ppm representing protons at position a (triplet, 1H), b (triplet, 2H) and f (doublet, 2H), respectively, of the phenyl moiety. The doublet of doublets at 8.28 ppm (1H, J=8.8, 2.1 Hz), represented the aromatic proton at position d which was meta-coupled to the proton at position c (doublet, 1H, J=2.4 Hz) at 8.53 ppm and ortho-coupled to the proton at position e at 8.20 ppm (doublet, 1H, J=8.8 Hz). The one-proton singlet at 8.89 ppm represented the proton adjacent to nitrogen atom of the pyridine system.

The 500 MHz HH COSY spectrum of CU-736-09-02 (Figure 34-35) showed the peak at 7.20 ppm, assignable to the proton at position a of the phenyl ring, vicinal coupled (J=7.3 Hz) to both protons at position b and also long range coupled (J=1.2 Hz) to another two protons at position f. The peak at 8.28 ppm, assigned to the proton at position d (H_d), exhibited cross peak with vicinal H_e proton (J=8.8 Hz) and long range coupled (J=2.1 Hz) with H_c at 8.53 ppm.

The 125 MHz ^{13}C-NMR spectrum of CU-736-09-02 (Figure 33) showed fourteen signals for sixteen carbons. Hence, there were two signals of representing equal carbon atoms of the phenyl ring. From the 125 Mhz CH-COSY spectrum (Figure 36-37), the signal at 118.8 ppm could be assigned to C-12 and C-16 and the one at 128.7 ppm to C-13 and C-15. The carbon signals at 115.3, 120.4, 123.6, 124.4, 141.1, and 161.3 ppm were assigned to C-5, C-7, C-8, C-14, C-3 and C-1, respectively. From calculation, the carbon at C-2, C-9, C-4, C-11, C-10 and C-6 should be assigned at chemical shift 106.9, 123.4, 135.6, 139.7, 141.6 and 147.7 ppm.

The EIMS spectrum of 7-nitro-2-phenylpyrazolo-[4,3-c]-quinolin-3-one (CU-736-09-02) was shown in Figure 38. The peak at m/e 306 represented the molecular ion. The fragments at m/e 77, 105, 231, 260, 276 and 277 were proposed in Scheme 8.



Scheme 8 Fragmentation pattern of 7-nitro-2-phenyl-pyrazolo-[4,3-c]-quinolin-3-one