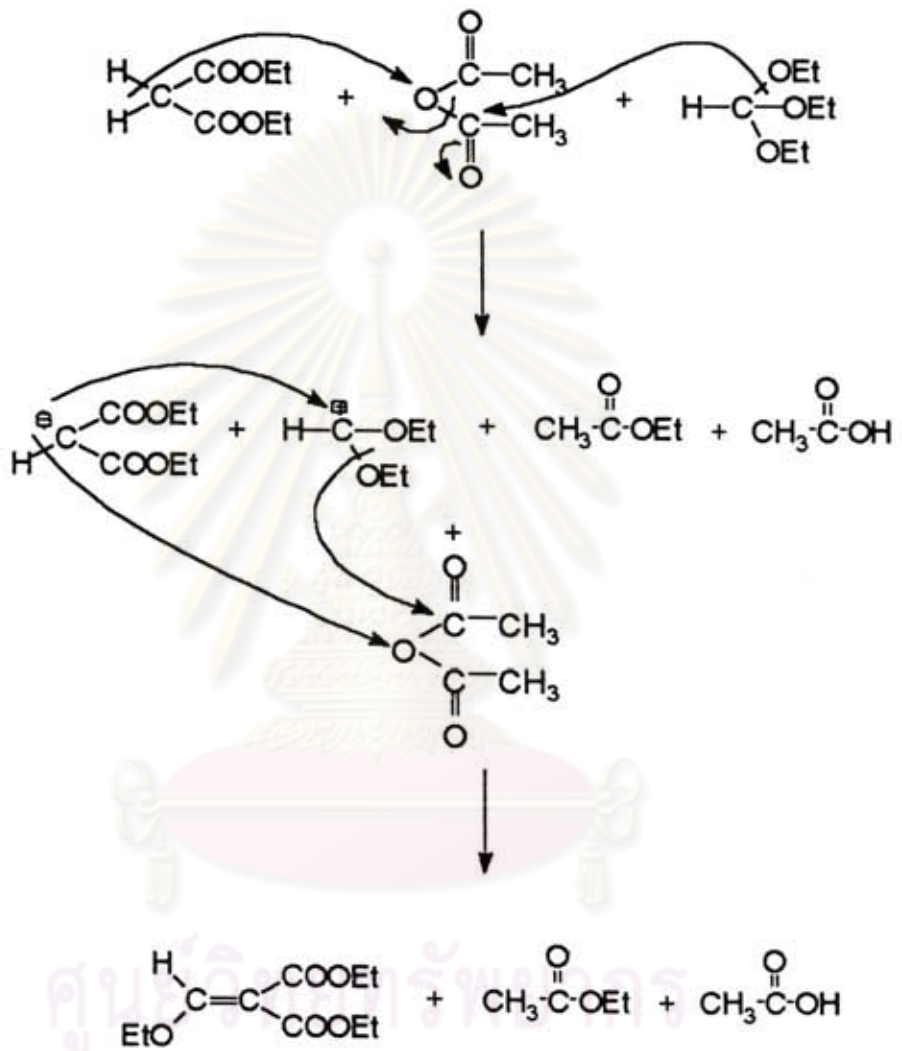
Chapter 4

Results and Discussion

From the experiment as shown in schme 1. , the 2- Thiooxpyrimido-[4,3-d]-quinoline4-one derivatives were synthesized here from several steps of reactions. Firstly the quinoline ring was prepared by the modified Gould-Jacobs cyclization. Secondly 3-Carboxyethoxy-4-hydroxyquinoline was converted to 4-Chloroquinoline-3-carboxylate which was intermediates of reaction by thionyl chloride or phosphorous oxychloride. Then the nucleophilic substitution of 4-Chloroquinoline-3-carboxylate with potassium thiocyanate in toluene produced 4-Isothiocyanatoquinoline-3-carboxylate which was the strong electrophile. Finally 2-thiooxopyrimido-[4,3-d]-quinoline-4-one derivatives were readily obtained from stirring 4-Isothiocyanatoquinoline-3-carboxylate with various amines. Each individual steps in the overall reactions are discussed as following.

Diethyl ethoxymethylenemalonate (XXXVIII).

Diethyl ethoxymethylenemalonate was prepared according to the method of Claisen in 1897 by reaction of diethylmalonate and active methylene compound, using triethylorthoformate in acetic anhydride as proton and leaving group acceptor. The mechanism of reaction was showed in scheme 2. .



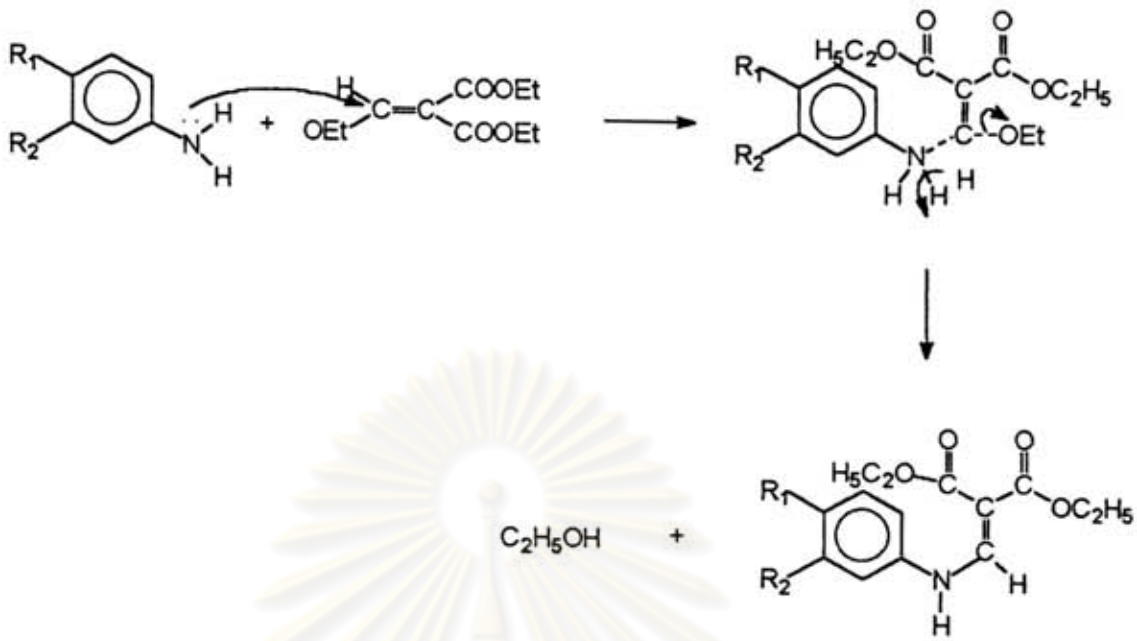
Scheme 2. The mechanism of the formation of diethyl ethoxymethylenemalonate.

The IR spectrum of diethyl ethoxymethylenemalonate (XXXVIII) (Figure 6.) showed a strong C=O stretching absorpsion peak at 1730 cm^{-1} , a peak between 1250-1290 cm^{-1} assigned for C-C(=O)-O stretching and a peak of C-O stretching vibration appeared at 1085 cm^{-1}. A peak at 1635 cm^{-1} represented C=C stretching vibration.

The ^{1}H-NMR spectrum of (XXXVIII) (Figure 7.) showed peak at 1.28-1.45 ppm (multiplet, 9H) assigned for 3 methyl protons, the peak at 4.09-4.38 (multiplet, 6H) assigned for 3 methylene protons adjacent to oxygen and methyl group. The peak at 7.60 ppm (singlet, 1H) represented methine proton.

Diethyl anilinomethylenemalonate (XXXX a)

The reaction of diethyl ethoxymethylenemalonate ester and aniline (XXXIX) to form diethyl anilinomethylenemalonate took place readily even at room temperature. The reactant ester should be a high degree of purity since usage of impure ester led to deep coloration and low yield in cyclization steps. Anilinoacrylates product was used for further reaction without purification. Mechanism of this reaction was nucleophilic attack of aniline upon the alkenyl carbon of diethyl ethoxymethylenemalonate which effected loosing of ethoxy group (leaving group) to from ethanol and corresponding to diethyl anilinomethylenemalonate as shown in Scheme 3. (R_1 , R_2 = H).



Scheme 3. Mechanism of the formation of diethyl anilinomethylenemalonate.

The IR spectrum of diethyl anilinomethylenemalonate (XXXX a) (Figure 8.) showed a strong C=O stretching absorption peak at 1720 cm^{-1}, the peak between 1200-1290 cm^{-1} assigned for C-O stretching vibration and a peak at 1618 cm^{-1} was for N-H bending vibration. The region of 2850-3030 cm^{-1} represented C-H stretching vibration.

The ^{1}H-NMR spectrum of (XXXX a) (Figure 9.) showed the peak at δ 1.21 ppm (multiplet, 6H) assigned for two methyl protons of ethyl ester, the peak at δ 4.11 ppm (multiplet, 4H) assigned for two methylene protons of ethyl ester. The peak at δ 7.14-7.32 ppm (multiplet, 5H) represented aromatic protons, the peak at δ 8.37 ppm (doublet, 1H) assigned for methine proton adjacent to amino group and the most down field signal at δ 10.69 ppm assigned for secondary amine proton (doublet, 1H).

Ethyl anilino-(3-chloro-4-fluoro)-methylenemalonate (XXXX b).

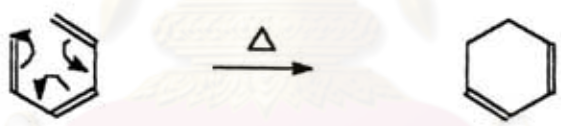
The substitute aniline reacted with diethyl ethoxymethylenemalonate was occured in refluxing condition and mechanism of reaction was shown in Scheme 3. (R_1 = F, R_2 = Cl).

The IR spectrum of Ethyl anilino-(3-chloro-4-fluoro) methylenemalonate (Figure 10.) exhibited characteristic peak of carbonyl ester group at 1720 cm^{-1} and strong peak at region of 1200-1290 cm^{-1} assigned for stretching vibration of C-O (ester). The peak at 1650 cm^{-1} could be interpreted as C=C stretching vibration, the N-H stretching peak was found at 3200-3450 cm^{-1} and N-H bending peak was at 1618 cm^{-1}.

The ^{1}H-NMR spectrum of (XXXX b) (Figure 11.) exhibited the characteristic proton of methyl and methylene of ester groups at δ 1.21 ppm (multiplet, 6H) and δ 4.05 ppm (multiplet, 4H), the two ester group was non equivalent so that methyl and methylene proton of each ester group showed peak at the different position. At δ 1.21 ppm, the two methyl proton showed two triplet which closed to each other. The aromatic hydrogen atom adjacent to the fluorine atom and a hydrogen atom which was meta coupling to fluorine atom was found at δ 7.3 ppm (doublet of doublet, 2H, J = 10.3 Hz, 7.7 Hz). The aromatic hydrogen atom adjacent to chlorine atom showed peak at δ 7.62 ppm (doublet, 1H, J = 6.9 Hz), This doublet peak was due to meta-coupling of proton with fluorine atom. The hydrogen attached to the olefinic carbon was coupling with hydrogen that attached to nitrogen atom, was showed at δ 8.18 ppm (doublet, 1H, J = 13.8 Hz). And proton of N-H presented at δ 10.51 ppm (broad, 1H, J = 13.5 Hz).

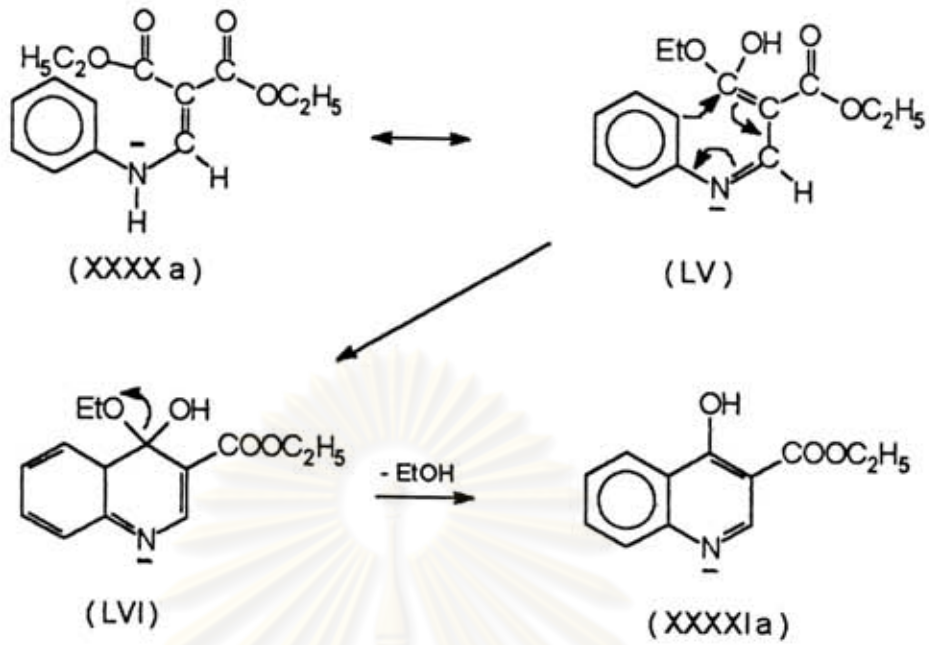
3-Carboethoxy-4-hydroxyquinoline (XXXXI a).

3-Carboethoxy-4-hydroxyquinoline was the intermediate which readily converted to the desired targets. It was synthesized by the method of Gould and Jacob (1939). The reaction was the cyclization of diethyl anilinomethylenemalonate at high temperature. The solvent that suitable for this reaction was Dowtherm A (diphenyl ether + biphenyl) or diphenyl ether since these solvent boil at temperature which was optimum for the cyclization and much less viscous and more easily to removed the product by filtration. The cyclization step may be rationalized as an orbital symmetry-allowed electrocyclic reaction. Enolization of diethyl anilinomethylenemalonate gave (LV), which could be considered as analogues to 1, 3, 5-hexatriene. This triene was known as thermal cyclization to give 1, 3-cyclohexadienes.



1, 3, 5 - hexatriene　　1, 3 - cyclohexadiene

Delocalization of electron of (LV) gave intermediate (LVI) and converted to 3-Carboethoxy-4-hydroxyquinoline by loosing of ethanol because OEt^- was the good leaving group than OH^-.



Scheme 4. Cyclization mechanism of diethyl anilinomethylenemalonate.

Three limitation which effect yield has been verified.

1. Dilution of diethyl anilinomethylenemalonate and solvent must be suitable (Riegel et al, 1946).

2. Temperature of reaction must be in appropiate range. If the temperature was too high, it was possibly to produced more side reaction. If the temperature was too low, the cyclization could not occur.

3. Purity of reactant effect to occur a lot of by product in the reaction and effect to separation of 4-Hydroxy quinoline.

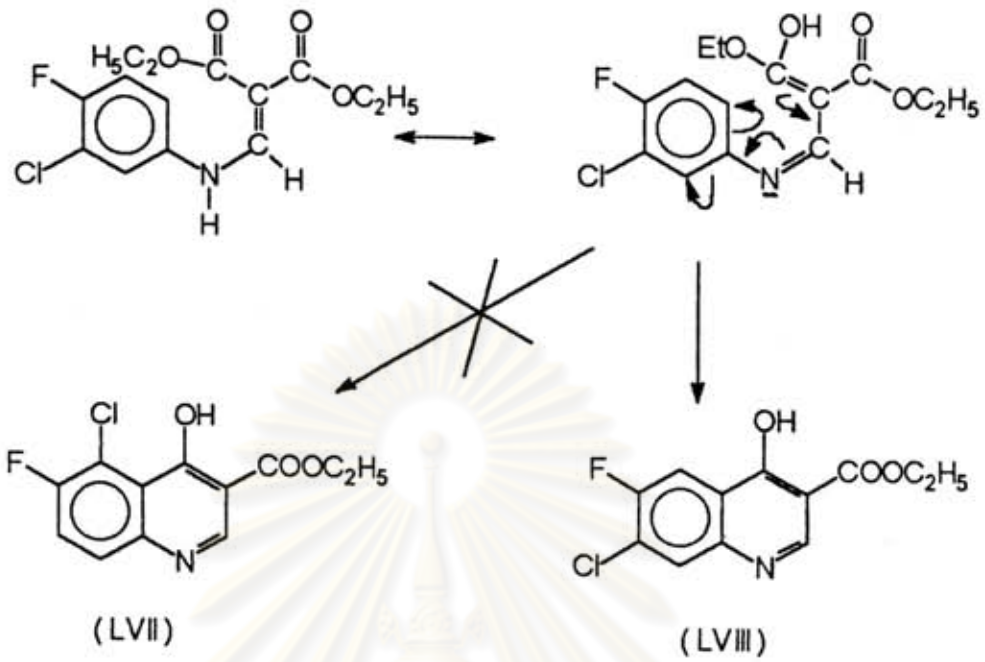
This compound was purified by recrystalization in DMF and identified by IR and ^{1}H-NMR. The IR spectrum of 3-Carboethoxy-4-hydroxy-quinoline (XXXXI a) (Figure 12.) showed C-H of aliphatic and aromatic stretching vibration peak in the region 2890-3150 cm^{-1}. Carbonyl of ester showed strong peak at 1700 cm^{-1}, peak at 1287 and 1198 cm^{-1} assigned for C-O stretching vibration of ester. The C=N stretching vibration was found at

1620 cm^{-1}. In addition, the O-H bending was the peak at 1380 cm^{-1} but O-H stretching was the broad peak at the same position of C-H stretching peak.

The ^{1}H-NMR of 3-Carboethoxy-4-hydroxyquinoline (Figure 13.) showed the peak at δ 1.28 ppm (triplet, 3H) which assigned for methyl protons of ester, the peak at δ 4.21 ppm (quartet, 2H) was for methylene protons adjacent to oxygen ester. The peak at δ 7.41 ppm (triplet, 1H) identified as aromatic proton at para position of nitrogen atom, the peak between chemical shift δ 7.59-7.75 ppm (multiplet, 2H) was the intersection of doublet of ortho to nitrogen atom and triplet of meta proton to nitrogen atom, the peak at δ 8.15 ppm (doublet, 1H) assigned for aromatic proton ortho to pyridine fused. The peak at δ 8.56 ppm (singlet, 1H) represented methine proton adjacent to nitrogen atom and the broad peak of hydroxy proton appeared at δ 12.3 ppm.

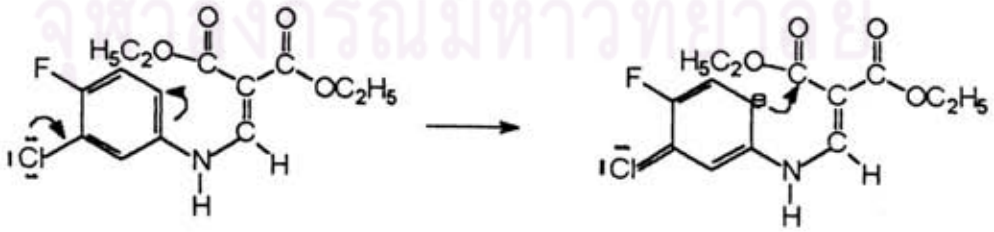
3-Carboethoxy-7-chloro-6-fluoro-4-hydroxy-quinoline (XXXXI b).

The ring closure reaction of ethyl anilino-(3-chloro-4-fluoro) methylenemalonate could be cyclized in two directions as ortho (LVII) or para (LVIII) position to chlorine atom. The condition used in this experiment gave the major compound that was condensed to para position with 85% yield as shown in scheme 5.. This could probably due to the steric hindance of chlorine atom. The cyclization process occured similar to the ring closure of 7-Chloro-4-hydroxy-quinoline (Price, Leonard and Reitsema, 1946). Compound (XXXXI b) could be assigned by the IR and ^{1}H-NMR spectrum.



Scheme 5. Cyclization mechanism of Ethyl anilino-(3-chloro-4-fluoro)-methylene malonate.

Occasionally, the production of (XXXXI b) was more easily occured than (XXXX a) which was considered due the effect of donating group at position 7. Delocalization of electron pair from 7-Chloro effected to more negative charge at para position of chlorine atom.



The IR spectrum of 3-Carboethoxy-7-chloro-6-fluoro-4-hydroxy-quinoline (Figure 14.) showed carbonyl absorption peak at 1700 cm^{-1} and C-O ester stretching vibration peak

appeared at 1180, 1370 cm^{-1}. The peak at 1610 cm^{-1} was C=N stretching vibration. In addition, the region at 2890-3150 cm^{-1} represented C-H stretching vibration peak and C-H bending peak was at 1464 cm^{-1}. The peak at 1382 cm^{-1} assigned for O-H bending vibration.

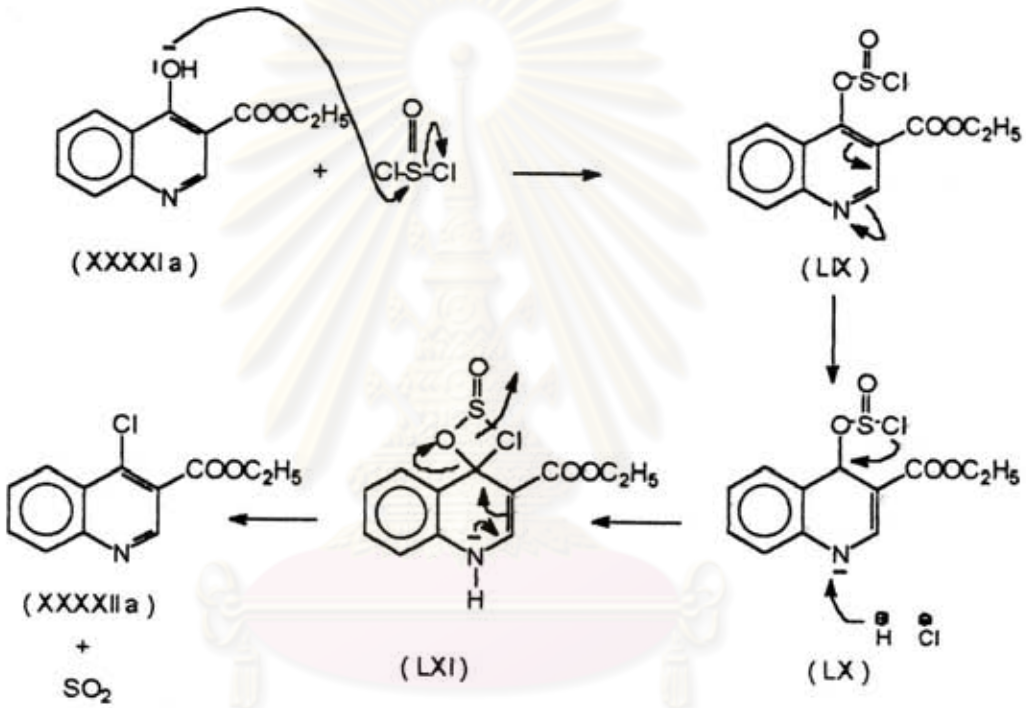
The ^{1}H-NMR spectrum for (XXXXI b) (Figure 15.) showed the peak at δ 1.38 ppm assigned for methyl protons of ester (triplet, 3H), the peak at δ 4.44 ppm assigned for methylene protons of ester (quartet, 2H). The chemical shift at δ 8.31 ppm identified as aromatic protons at ortho position to fluorine atom because Jvalue more than another one (doublet, 1H, J = 9.6 Hz) and at δ 8.49 ppm was proton at meta position to fluorine atom (doublet, 1H, J = 6.4 Hz). The down field peak at δ 9.17 ppm represented for methine proton which was adjacent to nitrogen atom in pyridine system. From ^{1}H-NMR in Figure 15. did not show a signal for O-H proton because oxygen attach proton could lose easily and exchange with solvent proton, or another reason could explained by the tautomerization of its molecule.

3-Carboethoxy-4-chloroquinoline (XXXXII a).

3-Carboethoxy-4-chloroquinoline was prepared from chlorination of compound (XXXXI a) using thionyl chloride or phosphorous oxychloride as chlorinating agent. The chlorine atom was nucleophilic replaced at 4-hydroxyl group to produce 3-Carboethoxy-4-chloroquinoline.

The possible mechanism of this chlorination involved the attack to hydroxyl group of chlorosulphite which was formed (LIX) (Scheme 5.). After that hydrogen ion from hydrochloric, which was occured from the reaction of (XXXXI a) and thionyl chloride,

combined to nitrogen of quinoline give hydrochloride salt (LX). The process of substitution of chlorine was facilitated by the presence of positive charge at nitrogen atom.



Scheme 6. Mechanism of chlorination of 3-Carboethoxy-4-hydroxyquinoline.

The intermediate (LX) decomposes *via* two sequentially formed ion pair species and the final process was the lost of sulfur.

Phosphorous oxychloride could react with 4-Hydroxy quinoline as showed in following equation.

The IR spectrum of 3-Carboethoxy-4-chloroquinoline (XXXXII a) (Figure 16.) showed C-H stretching vibration peak in the region of 2930-3070 cm^{-1}, strong peak at 1734 cm^{-1} assigned for C=O stretching of ester, peak at 1172 cm^{-1} and 1232 cm^{-1} represented C-O (ester) stretching vibration. Stretching vibration peak of C=C appeared at 1582 cm^{-1} and C-H (alkyl) bending vibration peak was at 1485 cm^{-1}.

The ^{1}H-NMR spectrum of (XXXXII a) (Figure 17.) showed the peak at δ 1.43 ppm (triplet, 3H) for methyl protons of ester, the peak at δ 4.47 ppm (quartet, 2H) assigned for methylene protons adjacent to oxygen ester. The peak at δ 7.66 ppm (triplet, 1H) represented aromatic proton at para-position to nitrogen atom, the peak at δ 7.80 ppm (triplet, 1H) for aromatic proton at meta-position to nitrogen atom, the peak at δ 8.10 ppm (doublet, 1H) assigned for proton ortho to fused pyridine ring and the proton which was ortho to the nitrogen atom presented at δ 8.36 ppm (doublet, 1H). And the last peak at δ 9.17 ppm (singlet, 1H) was the peak for methine proton adjacent to nitrogen atom in pyridine ring.

3-Carboethoxy-4,7-dichloro-6-fluoro-quinoline (XXXXII b).

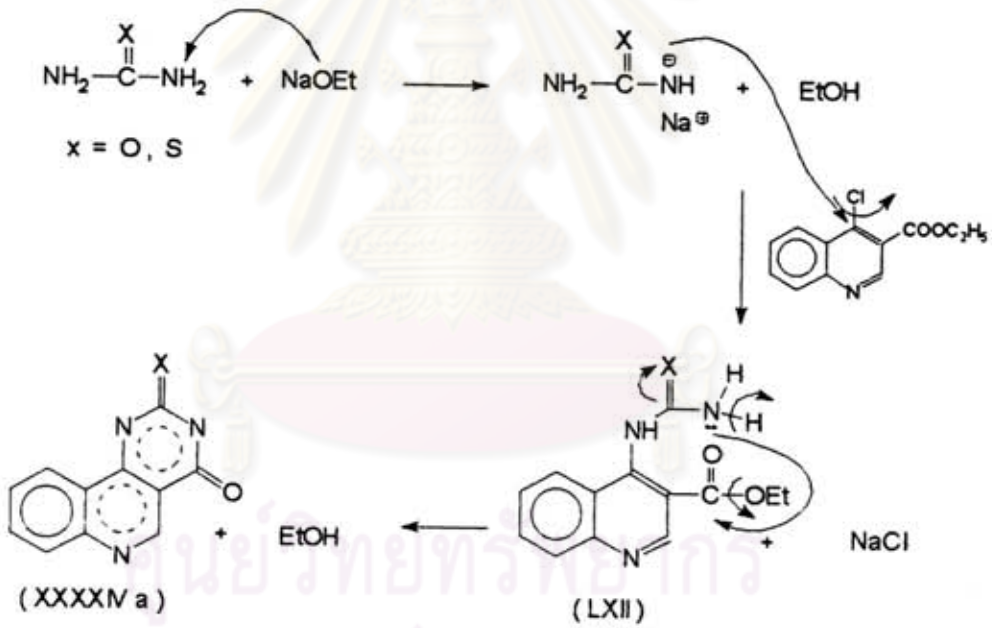
(XXXXII b) was synthesized by the chlorination of 3-Carbo ethoxy-7-chloro-6-fluoro-4-hydroxy-quinoline. The method and mechanism was the same as the preparation of (XXXXII a).

The IR spectrum of 3-Carboethoxy-4,7-dichloro-6-fluoro-quinoline (XXXXII b) (Figure 18.) showed the peak at region of 2934-3070 cm^{-1} assigned for C-H stretching of aromatic and aliphatic. The strong peak at 1729 cm^{-1} was C=O stretching vibration peak of carbonyl ester and peak at 1194 and 1230 cm^{-1} represented C-O (ester) stretching. In addition, the peak at 1581 cm^{-1} identified for C=C stretching vibration and peak at 1471 cm^{-1} assigned for C-H bending of aliphatic.

The ^{1}H-NMR spectrum of (XXXXII b) (Figure 19.) showed characteristic peak of ethyl ester group at δ 1.44 ppm for methyl protons (triplet, 3H) and δ 4.47 ppm for methylene protons (quartet, 2H). The peak at δ 8.14 ppm represented the aromatic proton (doublet, 1H, J = 9.6 Hz) which was ortho position to fluorine atom because Jvalue was in region of 6-10 Hz. The peak at δ 8.22 ppm (doublet, 1H, J = 6.4 Hz) assigned for aromatic proton adjacent to chlorine atom and meta to fluorine atom because Jvalue was in region of 4-8 Hz. The methine proton adjacent to nitrogen atom in pyridine appeared at chemical shift δ 9.15 ppm (single, 1H).

The synthesis of 2-Thiooxopyrimido-quinoline-4-one derivatives .

The 2-Thiooxopyrimido-[4,3-d]-quinoline-4-one derivatives were novel fused ring heterocyclic structure. The route for synthesis was designed in 3 different ways. Each reactions involved nucleophilic aromatic substitution. The first reaction was attemped by reacting 4-Chloroquinoline with thiourea for the synthesis of 2-Thiooxopyrimidoquinoline and urea for the synthesis of pyrimidoquinoline. Since urea and thiourea were considered to be low nucleophilicity compounds. Therefore treatment of urea derivatives in alkali solution may enhance the nucleophilicity.



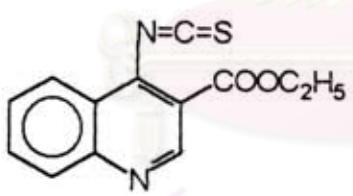
Intermediate (LXII) was auto cyclized with cabonyl of ester. From the experiment the target compound could not be obtained. It was considered that the ethoxide ion in this condition involved in the reaction.

The second reaction involved the conversion to 4 amino derivatives (Elderfield et.al , 1946) by reacting 4-Chloroquinoline with dry ammonia gas at high temperature or by

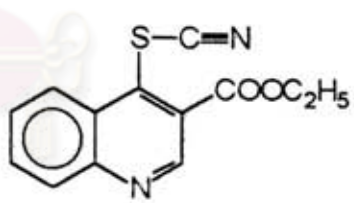
reacting 4-Chloroquinoline with phenylhydrazine to form the 4-Phenylhydrazine which was further reduced to the 4-amine derivatives or by reacting 4-Chloroquinoline with sodium azide to form the 4-azide which was further reduced to 4-amino derivatives. Once the 4-amino derivative was found, the cyclization with urea/thiourea or isothiocyanate could theoretically form the design compound. In this second reaction route, the desired compound was not successful.

The third reaction involved the conversion to the 4-Isothiocyanate derivatives which was subsequently reacted with the amine and this third route was proved to be the synthetic choice. The 3-Carboethoxy-4-chloro-quinoline was allowed to reacted with potassium thiocyanate in toluene under reflux condition for several days and yield the 3-Carboethoxy-4-isothiocyanatoquinoline.

The reaction of 4-Chloroquinoline and potassium thiocyanate may possibly theoretically result in either 4-Isothiocyanatoquinoline (LXIII) or 4-Thiocyanatoquinoline (LXIV) isomer or mixture of the two isomer (Esmail and Kurzer, 1975).

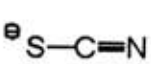


(LXIII)

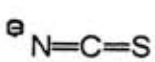


(LXIV)

The simultaneous production of the isomers had been interpreted in terms of the following mechanism. The thiocyanate ion exists as the mesomers thiocyanate ion (LXV) and isothiocyanate ion (LXVI).

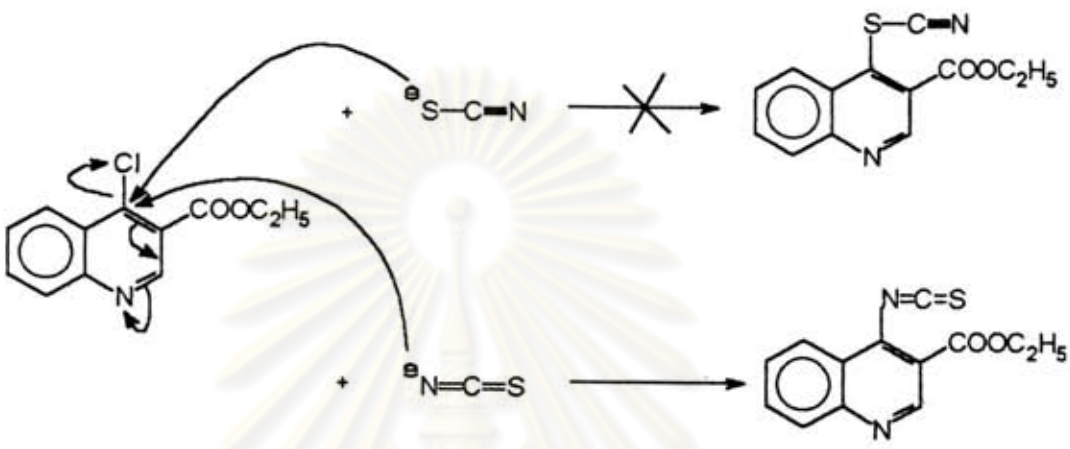


(LXV)



(LXVI)

Nucleophilic substitution of 4-Chloro-quinoline was equally possible at the S and N sites, giving rise to the (LXIII) or (LIV) as shown in scheme 7:



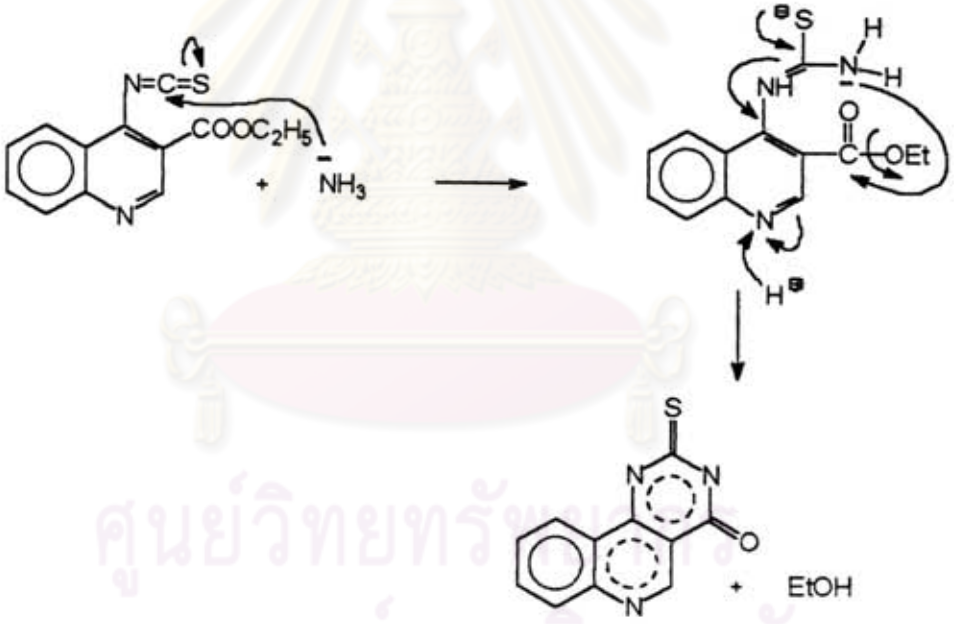
Scheme 7. Mechanism of nucleophilic substitution with potassium thiocyanate of 3-Carboethoxy-4-chloroquinoline.

Although the reaction of alkyl halide with metal thiocynate would result in the formation of a normal thiocyanate or isothiocyanate isomer. The formation of isothiocyanate did increase from primary to secondary to tertiary carbon derivatives and favoured in presence of aryl, ethylenic or carbonyl groups on the carbon atom at which substitution occurs. Either a reaction product was a thiocyanate or an isothiocyanate could readily be ascertained by infrared spectrophotometric (IR) analysis. The thiocyanate exhibit a strong, sharp band of -S-C=N stretching vibration beyond the normal range 2170-2136 cm^{-1}, while isothiocyanate exhibit a very strong and broad band at about 2150-2050 cm^{-1} (Biemann, 1985) .

The IR spectrum of product obtained from the reaction (Figure 20.) showed a very strong broad band at 2080 cm^{-1} which indicated that the Isothiocyanate substitution was occured and other peak such as C=O (ester) stretching was at 1740 cm^{-1}, C-O (ester)

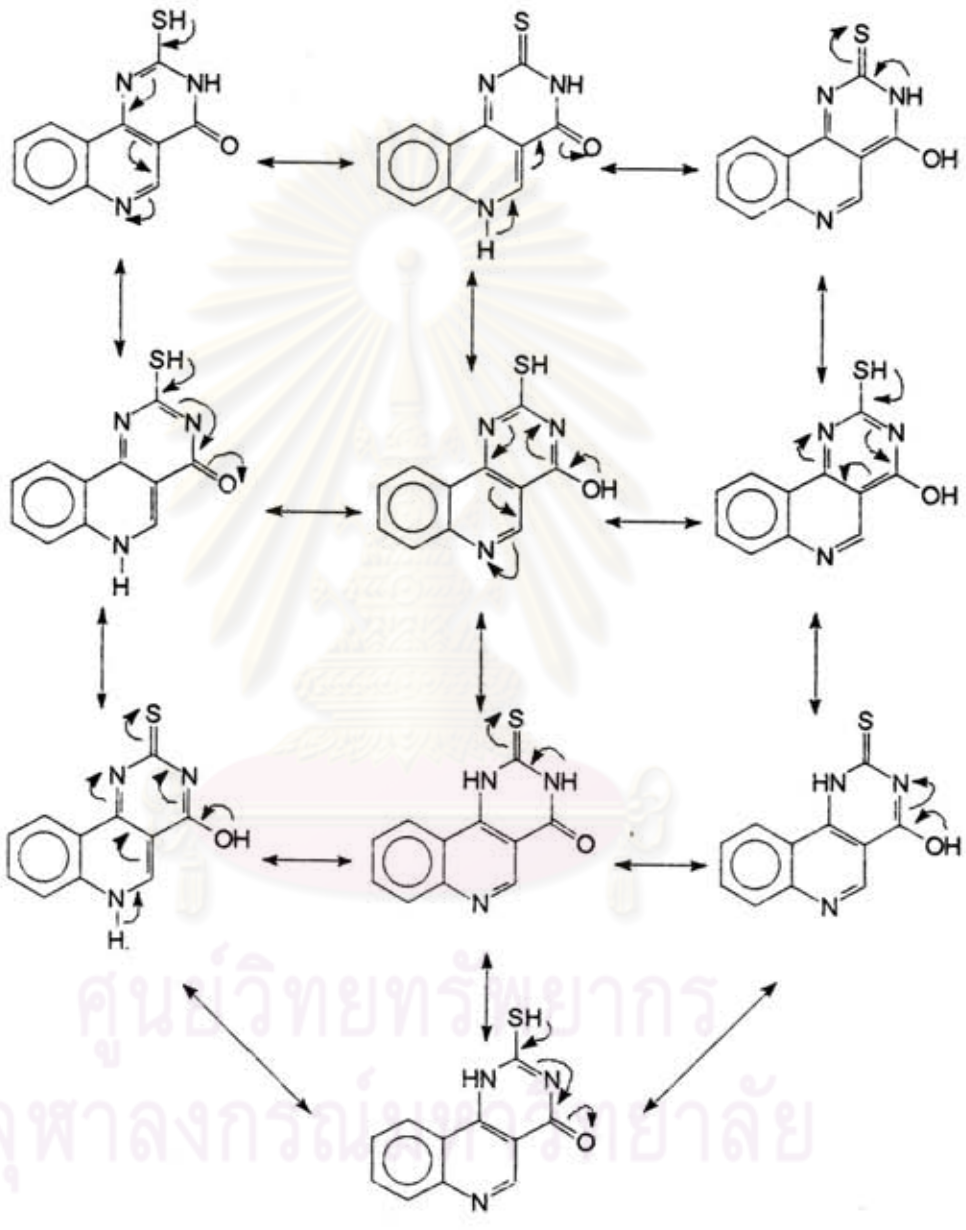
stretching peak assigned at 1172,1230 cm^{-1}, the peak at 1586 cm^{-1} identified for C=C stretching and the bending of C-H aliphatic showed peak at 1490 cm^{-1}, compound (XXXXIII a) was used without purification *in situ* for the subsequence reaction.

The subsequence reaction involved the nucleophilic attack to electrophilic carbon of isothiocyanate with concentrated ammonia solution. The cyclization process was occured simultaneously since NH_2 of thioamide was still a good nucleoplile that could react to carbon of carbonyl ester effected to lose ethanol from molecule. Scheme 8. represented the possible reactions :



Scheme 8. Possible reaction of 3-Carboethoxy-4-isothiocyanatoquinoline and ammonia solution.

The molecular structure of cyclization product can exist in several isomeric forms due to electron delocalize in the molecule. The posible isomer showed in scheme 9 :



Scheme 9. Possible isomer product of (XXXXIV) with ammonia solution.

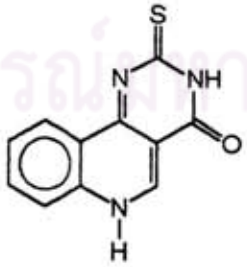
In this experiment, Infrared Spectrophotometer, Nuclear Magnetic Resonance (^{1}H-NMR) and Mass Spectrophotometer were used for identify structure of cyclization product's structure. IR spectrum (Figure 22.) showed broad peak at 2980-3260 cm^{-1} assigned for C-H stretching and N-H stretching. The strongest peak was C=O stretching vibration of amide appeared at wave number 1690 cm^{-1}, the peak at 1585 cm^{-1}, 1555 cm^{-1} was identified for C=C stretching of aromatic. The other peak at 1147 cm^{-1} assigned for N-C(=S)-N stretching. The information from IR spectrum only expressed the ester bond of the reactant was changed to be amide and the substitution of isothiocyanate carbon was occured.

More information of (XXXXIV a) was noted from ^{1}H-NMR in solvent DMSO-d_6 (Figure 23.) which showed the peak at δ 7.75 ppm assigned for aromatic proton at para position to nitrogen atom of pyridine ring (triplet, 1H). Another (triplet, 1H) at δ 7.96 ppm represented aromatic proton which was coupling to surrounding protons at meta position to nitrogen atom of pyridine ring. The peak at δ 8.07 ppm (doublet, 1H) identified as aromatic proton which was adjacent to nitrogen atom of pyridine ring because of the proton in this position could vary in range of δ 7.7-8.3 ppm due to the substitution of molecule. The last proton of aromatic peak presented at δ 8.96 ppm (doublet, 1H) which was the meta proton to nitrogen atom and nearest to pyrimidine ring. From the substitution at para position of pyridine ring effected to the position of aromatic proton that was adjacent to it. In this substance the adjacent aromatic proton was presented at move down field than another substitutes. The methine proton gave the peak at δ 9.15 ppm (singlet, 1H) and the down field peak at δ 12.92 ppm (broad, 1H) was for proton adjacent to nitrogen. In this molecule there were 2 proton which attach to nitrogen at nitrogen of quinolone ring and nitrogen between carbonyl and thiocarbonyl, be able to give down field broad peak. Due to nitrogen proton was the labile proton so that it might not be seen in the spectrum. However, from the later spectrum of another derivatives

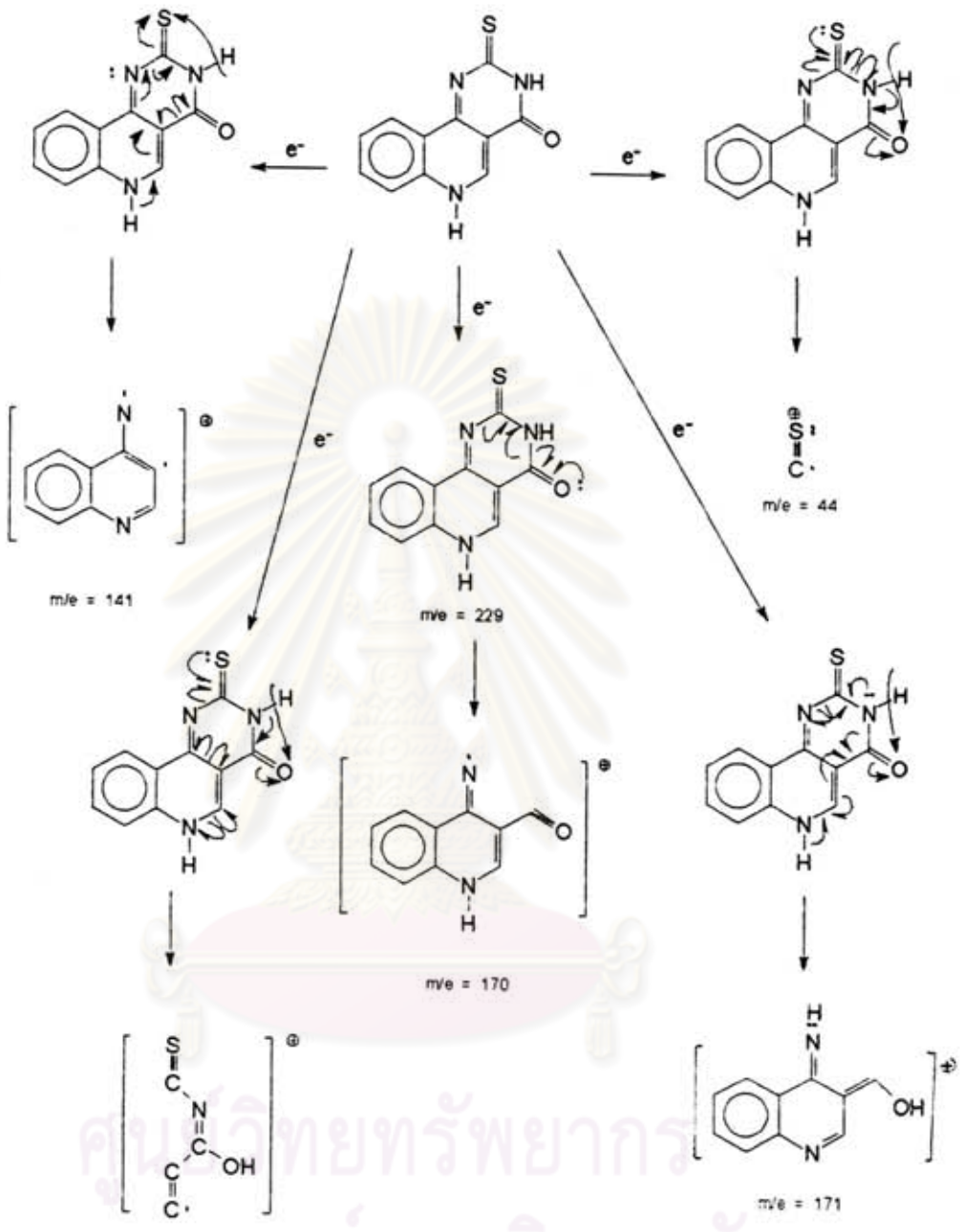
which had the substitution at nitrogen between carbonyl and thiocarbonyl still gave the broad peak for proton of quinolone ring at this position also. So this broad peak was proposed for quinolone proton and this spectrum did not show any proton adjacent to sulfur or oxygen.

The molecule of (XXXXIV a) had molecular weight = 229 according to the nitrogen rule. This structure was confirmed by mass spectrophotometry (Figure 24.). The base peak was at m/e = 229 which was also the molecular ion peak, M^+ peak was always accompanied by isotope peak which depended on the number, kind of the elements present and their natural isotopic distribution. (XXXXIV a) showed $(M+1)^+$ peak = 230, the relative abundance was approximate 14% and $(M+2)^+$ peak at m/e 231, the relative abundance was approximate 4.6%. Others fragment ions was characterized in scheme 10. .

In conclusion, (XXXXIV a) could be tautomerize into many type of structure. It might not be only in one form but from the IR spectrum showed C=S stretching vibration of thiourea part and from NMR spectrum gave signal of proton which attached to nitrogen of quinolone ring. So compound (XXXXIV a) should be the major structure as 2-Thiooxopyrimido [4,3-d]-quinoline-4-(3,6-H)-one.



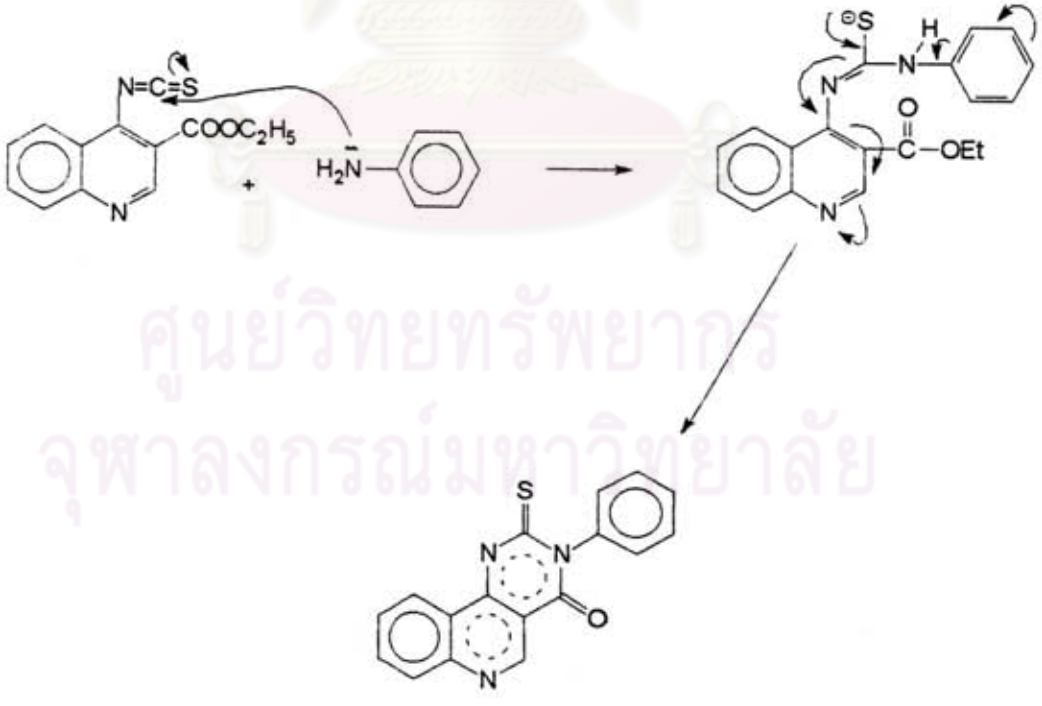
(XXXXIV a)



Scheme 10. The mechanism of the fragmentation reaction 2-Thiooxopyrimido-[4,3]-quinoline-4-one.

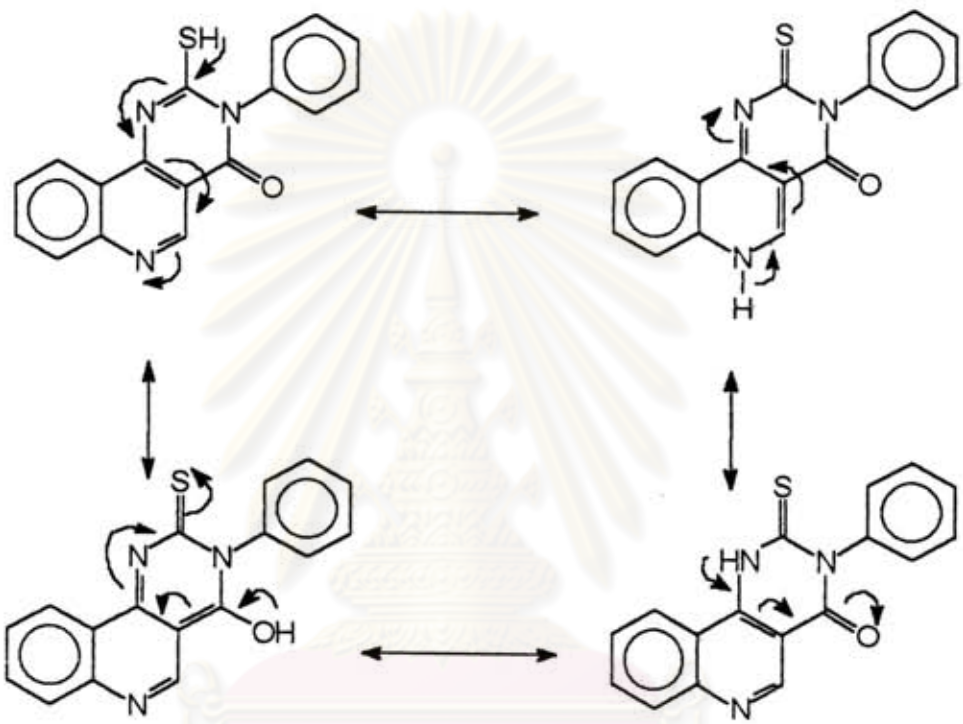
Synthesis of 3-Phenyl-2-thiooxopyrimido-[4,3-d]-quinoline-4-one (XXXXV a).

Compound (XXXXV a) was produced by react 3-Carboethoxy-4-isothiocyanato-quinoline with aniline. The mechanism of reaction was the same as ammonia, the carbon of isothiocyanate was attacked by lone pair electron of amine part. After that nitrogen still attack to carbonyl of ester, the cyclization process was occured. The expect mechanism was showed in scheme 11. . The nucleophilicity of aniline was less than ammonia so that this reaction took higher temperature and gave lower yield than reaction of ammonia. Another pathway of synthesis (XXXXV a) was accomplished by stired the 2-Thiooxopyrimido-[4,3-d]-quinoline-4-one (XXXXIV a) with phenyl iodide in base but this reaction expected to produce (XXXXV a) in lower yield due to the steric effect of phenyl group of amine.



Scheme 11. The possible mechanism of cyclization of (XXXXV a).

Each delocalization can also possible in this case and hence possible several isomeric form exist as shown in Scheme 12. .



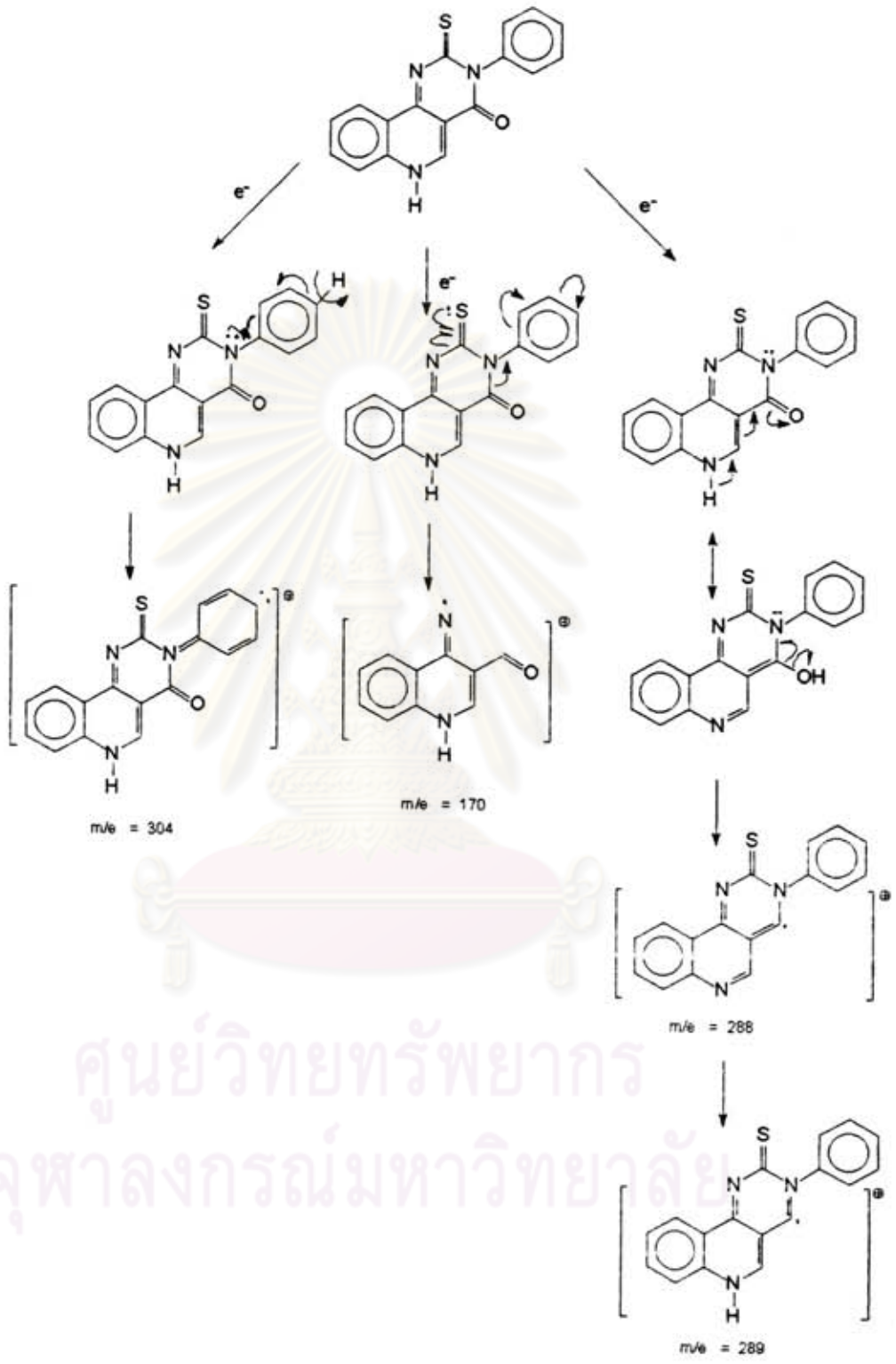
Scheme 12.

The IR spectrum of (XXXXV a) (Figure 28.) showed peak at region of 2960-3140 cm^{-1} assigned for C-H stretching vibration peak. The peak at 1720 cm^{-1} and 1650 cm^{-1} represented for C=O stretching of amide and C=N stretching vibration, respectively.

The ^{1}H-NMR of (XXXXV a) in solvent DMSO-d_6 (Figure 29.) found the peak at δ 7.41 ppm (doublet, 2H) which represented for protons at ortho-position of N-phenyl substitute group. Triplet (1H) at δ 7.48 ppm was proton at para-position of phenyl ring

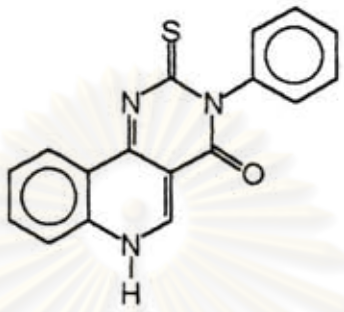
and meta-protons appeared at δ 7.54 ppm (triplet, 2H). The peak at δ 7.76 ppm identified for proton at para position to nitrogen atom of pyridine ring (triplet, 1H) which had less effect from environment. At δ 7.97 ppm (triplet,1H) was the peak of proton at meta position to nitrogen atom of pyridine ring. The peak at δ 8.10 ppm (doublet, 1H) assigned for ortho aromatic proton to nitrogen atom of pyridine ring and the peak at δ 8.80 ppm assigned for proton at meta position to nitrogen atom of pyridine. The peak of methine proton appeared at δ 9.17 ppm (singlet, 1H) and the broad peak at δ 12.35 ppm (1H) was for proton adjacent to nitrogen of pyridine ring. Thus, molecule of (XXXXV a) was the quinolone derivative, this information also supported by the broad peak of ^{1}H-NMR of (XXXXIV a) (Figure 23.) due to the proton adjacent to nitrogen of quinolone ring. Increase aromaticity by phenyl substitute at N-3 did not effect to chemical shift of quinolone protons so that the chemical shift of (XXXXIV a) and (XXXXV a) are closed to each other.

Additional confirmation of (XXXXV a) assignment was obtained by the identification of a mass spectral peak (Figure 30.) corresponding to molecular ion peak at m/e = 305. The isotope peak found $(M+1)^{+}$ at m/e 306 and $(M+2)^{+}$ at m/e 307 and the relative abundance was 24% and 6.2%, respectively. The electron donating activity of phenyl ring was increase possibility to loss of hydrogen atom at para-or ortho-position caused the peak at m/e = 304. The base ion peak at m/e 170 which was agreement with loss of phenyl isothiocyanate and fragment peak at m/e 288 and 289 due to the lost of hydroxyl group. The posible mechanism showed in scheme 13..



Scheme 13. The mechanism of the fragmentation reaction of 3-Phenyl-2-thiooxopyrimido-[4,3]-quinoline-4-one.

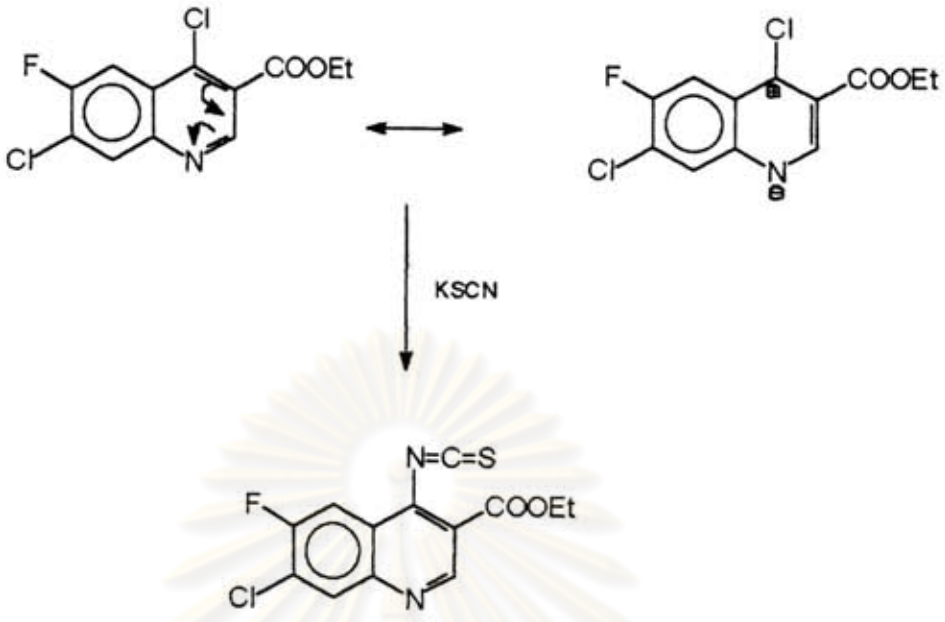
From IR spectrum that showed C=S stetching vibration peak of thiourea and signal proton of quinolone proton in NMR spectrum the major product of (XXXXV a) should be in form of 3-Phenyl-2-thiooxopyrimido-[4,3-d]-quinoline-4-(6H)-one.



(XXXXV a)

Synthesis of 8-Chloro-9-fluoro-2-thiooxopyrimido-[4,3-d]-quinoline-4-one (XXXXIV b).

The pathway of synthesis was the same as compound (XXXXIV a). Firstly the compound 3-Carboethoxy-7-chloro-6-fluoro-4-isothiocyanato quinoline (XXXXIII b) which had proved to be a valuable reagent for synthesis variety of heterocyclic compounds was prepared by reaction of 3-Carboethoxy-4,7-dichloro-6-fluoro-quinoline reflux with potassium thiocyanate in toluene for 3 days. Avoidance of nucleophilic solvents must be ascertained since chloride in compound (XXXXII b) was a good leaving group and sensitive to nucleophile attack. In addition, (XXXXII b) contains 2 chloro-substitution in molecule at position 4 and 7, the nucleophilic substiution of (XXXXII b) was approximately equal to that of (XXXXIII a). The expect mechanism was the substitution mostly occured to chloro at active site C-4. The localization of pair of electron N-1 promoted the loose of 4-chloro to produced major product 4-isothiocyanatoquinoline derivative.



The IR spectrum of (XXXXIII b) (Figure 21.) showed peak at wave number 2930-3070 cm^{-1} assigned for C-H stretching. The strong and broad peak of -N=C=S stretching appeared at 2100 cm^{-1} and the strong peak at 1760 cm^{-1} represented C=O (ester) stretching vibration peak. The C=C stretch of aromatic was at 1585 cm^{-1} and the medium peak of C-H aliphatic bending peak appeared at 1480 cm^{-1}. The C-O of ester showed 2 peaks at 1230 and 1196 cm^{-1}. And compound (XXXXIII b) was also as (XXXXIII a), it did not necessary to purify for another reaction.

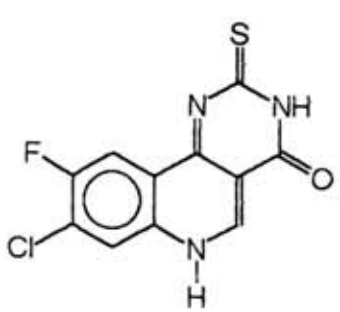
Further reaction of (XXXXIII b) with conc. ammonia solution in cool condition produced 7-Chloro-8-fluoro-2-thiooxopyrimido-[4,3-d]-quinoline-4-one. The mechanism of reaction expected to be the same as reaction of (XXXXIII a) with conc. ammonia solution. The product was confirmed by physical method to checked that the chloro substitution was still in molecule of target. The IR spectrum of (XXXXIV b) (Figure 25.) showed N-H stretching peak at 3417 cm^{-1} and C-H stretching was at the range of 2960-3200 cm^{-1}. The strong peak at 1670 cm^{-1} represented for C=O stretching of amide . The peak at wave number 1590 cm^{-1} and 1520 cm^{-1} assigned for C=C stretching. The C-F

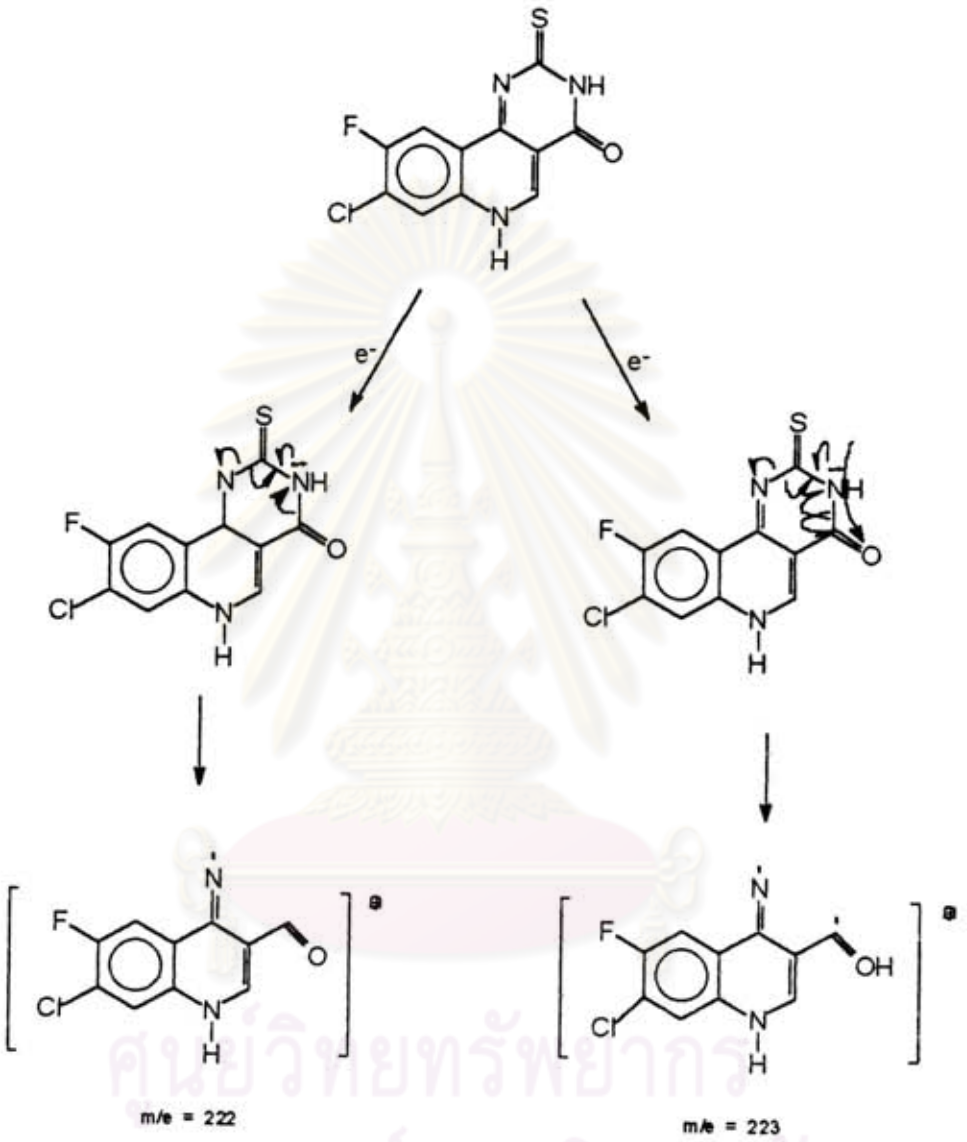
stretching appeared at 1175 cm^{-1}. The peak at 1133 cm^{-1} assigned for thioketone (N-C(=S)-N) stretching.

The ^{1}H-NMR of (XXXXIV b) in DMSO-d_6 (Figure 26.) showed two signals at δ 8.30 and 9.00 ppm for aromatic protons. At δ 8.30 ppm splited in doublet Jvalue = 7.2 Hz because of coupling with fluorine atom at meta position and at δ 9.00 ppm splited in doublet Jvalue = 10.5 Hz was the proton ortho coupling to fluorine atom. The singlet proton at chemical shift at δ 9.10 ppm was explained origin for proton adjacent nitrogen atom on pyridine system. The deshielding broad peak at δ 12.90 ppm was for proton that attached to nitrogen atom of quinolone ring as in (XXXXIV a) and (XXXXV a). The pyrimidine acidic proton which easily labiled did not observe in this spectra. Synthesis of (XXXXIV b) could be able to obtain ten possible tautomeric form the same as synthesis of (XXXXIV a).

Mass spectra of (XXXXIV b) (Figure 27.) was characterized the posible structure of molecule. The molecular ion peak and also the base peak was at m/e = 281. The isotope found $(M+1)^+$ and $(M+2)^+$ at m/e = 282 (relative abundance = 14%) and m/e = 283 (relative abundance = 37%) respectively. The fragment peaks at m/e = 222, 223 proposed as loss of thiocyanate group as from the molecule. If the molecule also loss of carbonyl, it showed the peak at m/e 193,194. The possible fragment exhibited in Scheme 14. .

From the IR and NMR data obtained the same as compound (XXXXIV a) , the structure of (XXXXIV b) was considered to exist as the following structural formed :

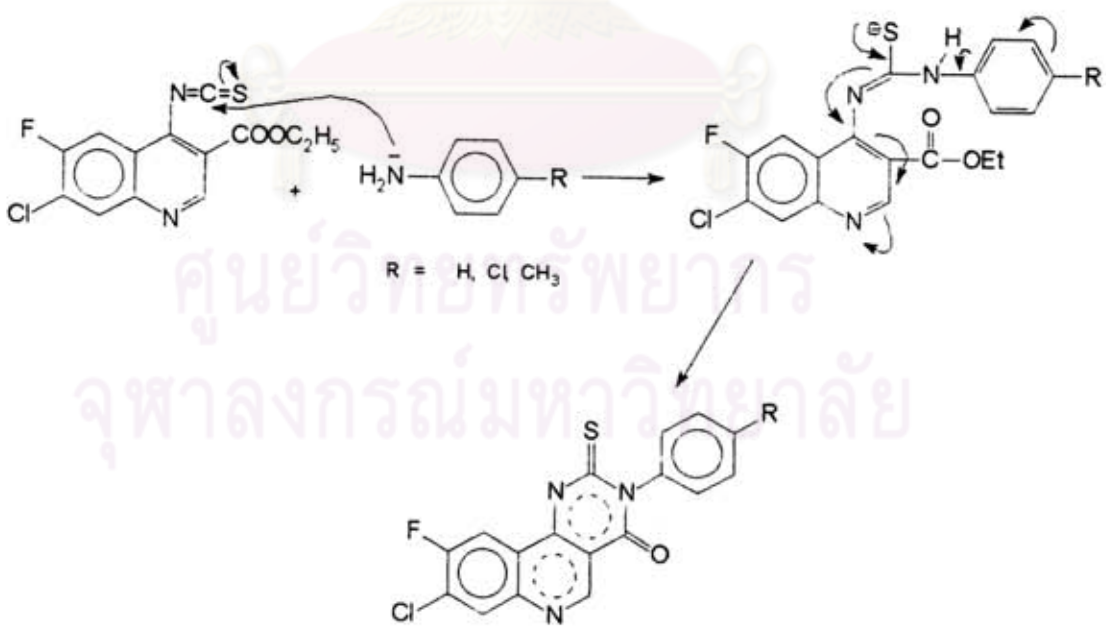




Scheme 14. The mechanism of the fragmentation reaction of 8-Chloro-9-fluoro-2-thiooxopyrimido-[4,3]-quinoline-4-one.

Synthesis of 3-Aryl-8-chloro-9-fluoro-2-thiooxopyrimido-[4,3-d]-quinoline-4-one derivatives (XXXXV b, XXXXVI - XXXXVII)

3-Carboethoxy-7-chloro-6-fluoro-4-isothiocyanatoquinoline could be condensed with aromatic amine derivatives in toluene for producing aryl derivatives at N-3. The mechanism of reaction was the same as synthesis of (XXXXV a). The aromatic amine derivatives which were used for the reaction were aniline, p-Chloro aniline and p-toluidine. The nucleophilicity of aromatic amine substituted were p-toluidine > aniline > p-Chloro aniline and correspond to the product yields. The rate of cyclization of thiourea intermediate of compound (XXXXIV) was faster than the disubstituted thiourea intermediate, due to the steric effects of their substituents. The tautomer forms of N-3 substituted product should be occured as compound (XXXXV a). The mechanism of reactions are shown in the following scheme:



Scheme 15. The possible mechanism of cyclization of 4-Isothiocyanatoquinoline with aryl amine and its derivatives.

The IR spectrum of 8-Chloro-9-fluoro-3-phenyl-2-thiooxopyrimido-[4,3-d]-quinoline-4-one (XXXXV b) (Figure 31.) represented strong peak of C=O (amide) stretching at 1700 cm^{-1}. The C=C stretching showed two peak at wave number 1580 cm^{-1} and 1550 cm^{-1}. At 1632 cm^{-1} expected to be C=N stretching vibration and at 1382 cm^{-1} assigned for C-N stretching vibration peak.

The ^{1}H-NMR of (XXXXV b) (Figure 32.) was runned in 2 systems for confirmation as show in table 1.

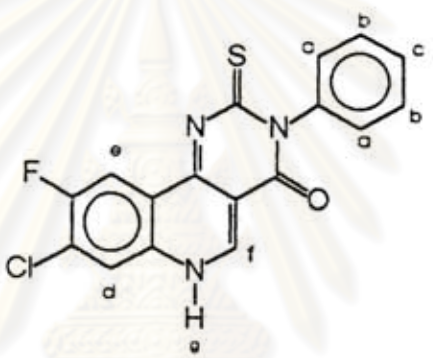
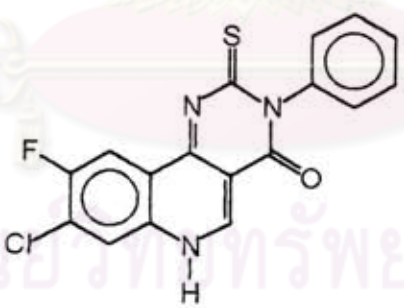


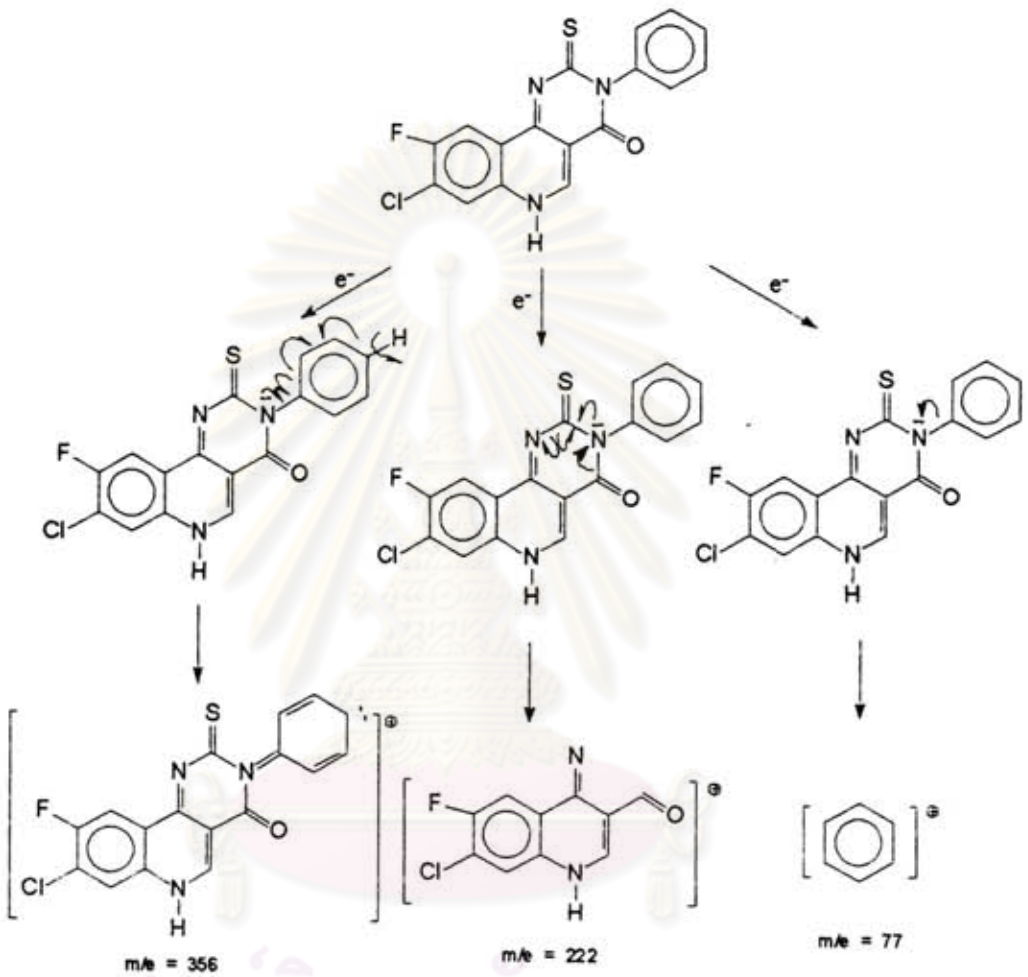
Table 1. Showed chemical shift of ^{1}H-NMR in 2 solvent systems.

proton at position	chemical shift (δ) in $CDCl_3$ +DMSO-d_6 (ppm) (Figure 32.)	chemical shift (δ) in DMSO-d_6 (ppm) (Figure 33.)
a	7.28 (doublet, 2H)	7.32 (doublet,2H)
b	-	7.51 (triplet, 2H)
c	7.50 (triplet, 1H)	7.43 (triplet, 1H)
d	8.22(doublet,1H,J=7.2)	8.37(doublet,1H,J=7.3)
e	9.07(doublet,1H,J=10.5)	9.18(doublet,1H,J=11)
f	9.28 (singlet, 1H)	9.16 (singlet,1H)
g	13.29 (broad, 1H)	13.33 (brood,1H)

The mass spectra (Figure 34.) also indicated the structure of (XXXXV b) was the quinolone derivatives. The molecular ion peak was found at m/e 357. The base ion peak was m/e 356 due to the lost of H atom. The isotope peak of $(M+1)^+$ and $(M+2)^+$ showed at m/e 358 and 359. The relative abundance of $(M+1)^+$ and $(M+2)^+$ from calculation was 20.8% and 37.1% respectively but from spectrum was 56.3% for $(M+1)^+$ and 38.2% for $(M+2)^+$. The relative abundance of $(M+1)^+$ peak was more than the calculation because it was combined $(M+1)^+$ peak and isotope (+2) of base peak $(M-1)^+$. The fragment peak was at m/e=77 for phenyl ring. The peak at 222 and 193 were also found for this compound. The possible fragment of molecule showed in scheme 16.

In conclusion the major form of compound (XXXXV b) was also expected in form of 8-Chloro-9-fluoro-3-phenyl-2-thiooxopyrimido-[4,3-d]-quinoline-4-(6H)-one.





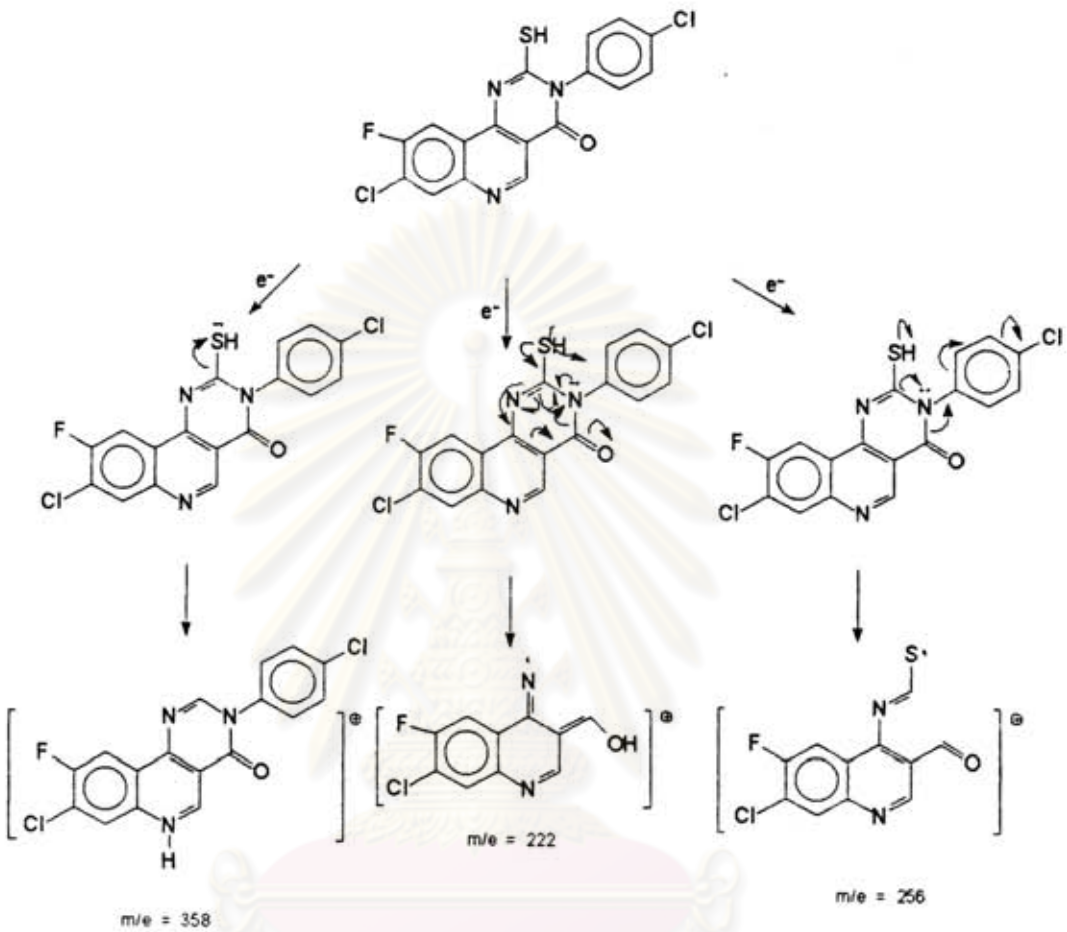
Scheme 16. The mechanism of the fragmentation reaction of 8-Chloro-9-fluoro-3-phenyl-2-thiooxopyrimido-[4,3-d]-quinoline-4-one.

The IR spectrum of 8-Chloro-9-fluoro-3-(4'-chloro-phenyl)-2-thiooxopyrimido-[4,3-d]-quinoline-4-one (XXXXVI) (Figure 35.) showed the peak of C-H strctching at 2930--3160 cm^{-1}. The strong peak at 1700 cm^{-1} represented for C=O stretching of amide. Th C=C stretching of aromatic appeared at 1580 cm^{-1} and 1545 cm^{-1}. At wave number 1632 cm^{-1} assigned for C=N stretching and the peak at 1450 cm^{-1} was C-N stretching vibration peak.

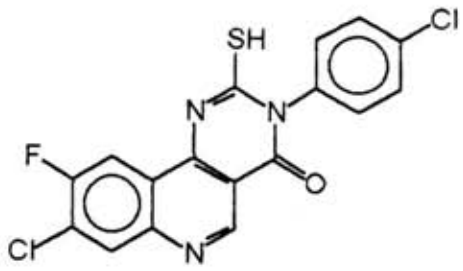
The ^{1}H-NMR of (XXXXVI) (Figure 36.) in DMSO-d_6 showed the phenyl proton peak at ortho position to pyrimidine ring at δ 7.22 ppm (doublet, 2H). and ortho protons peak of chloro at δ 7.51 ppm (doublet, 2H). The chemical shift δ 7.86 ppm (doublet, 1H,J=8.5Hz) represented proton adjacent to fluorine or meta position to nitrogen of pyridine ring. The meta proton of fluorine shifted to down field than ortho proton, presented at δ 8.32 ppm (doublet, 1H, J=7.0 Hz). The down field peak for methine proton appeared at δ 9.36 ppm (singlet, 1H).

Structure of compound (XXXXVI) was confirmed by MS (Figure 37.) which showed molecular ion peak and base peak at m/e 391. The high intensity fragment peak was found at m/e 390 cause by loss of hydrogen atom. The isotope peaks of $(M+1)^+$ and $(M+2)^+$ were at m/e 392 and m/e 393 which natural abundance at $(M+1)^+$ peak was the addition of isotope of M^+ and $(M-1)^+$ = 64.4% and at $(M+2)^+$ peak = 61.3%. The molecule of 11 could lost p-chloro aniline gave peak m/e at 265. The fragment peak at m/e 222 was also found (Scheme 17.).

From IR spectrum peak of C=S stretching was not found, the peak of proton which attached to nitrogen of quinolone ring in NMR spectrum was also disappeared and in MS spectrum was found peak at m/e 358 which was the loss of Thiol group. So the major form of compound (XXXXVI) should be, 8-Chloro-3-(4'-chloro phenyl)-9-fluoro-2-mercapto-pyrimido-[4,3-d]-quinoline-4-one, tautomer form.



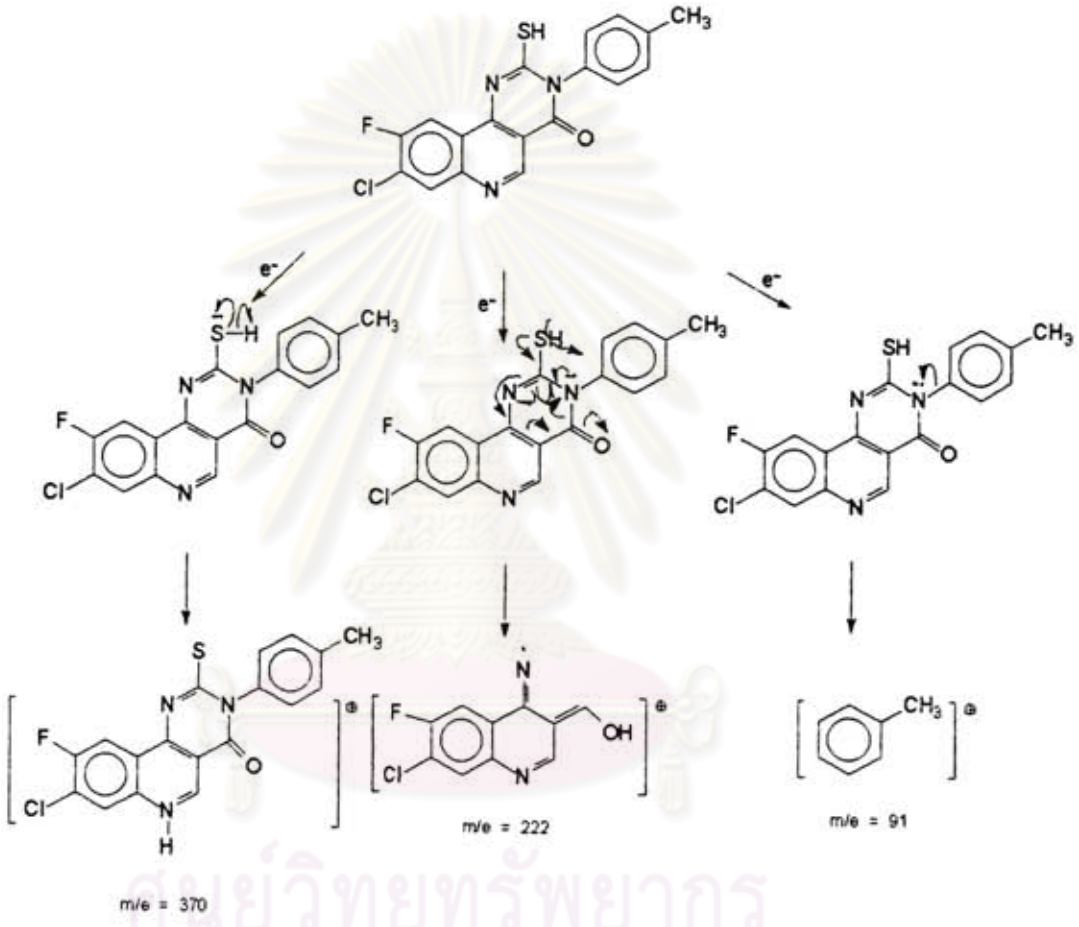
Scheme 17. The mechanism of the fragmentation reaction of 8-Chloro-9-fluoro-2-mercapto-3-(4'-chloro phenyl)-pyrido-[4,3-d]-quinoline-4-one.



(XXXXVI)

IR spectrum of 8-Chloro-9-fluoro-3-(4'-methyl phenyl)-2-thiooxopyrimido-[4,3-d]-quinoline-4-one (XXXXVII) (Figure 38.) showed peak of C-H stretching of aliphatic and aromatic at region 2860-3200 cm^{-1}. The strong peak of C=O (amide) stretcning appeared at 1698 cm^{-1} and C=C stretching showed 2 peak at 1580 and 1550 cm^{-1}. The peak at 1630 and 1445 cm^{-1} identified for C=N and C-N stretching, respectively.

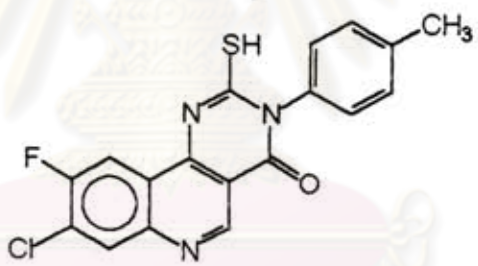
The ^{1}H-NMR (Figure 39.) showed the peak for aliphatic methyl group that attached to para position of phenyl ring appeared at δ 2.39 ppm (singlet, 3H). At chemical shift δ 7.18 ppm (doublet, 2H, J = 8 Hz) was for two equivalent phenyl protons at ortho position to pyrimidine ring or meta position to methyl group and at chemical shift δ 7.32 ppm (doublet, 2H, J = 8.3 Hz) assigned for meta protons to pyrimidine ring or ortho protons to methyl group. Another doublet peak at 8.37 ppm represented proton at meta position to fluorine atom (Jvalue=7.6 Hz.). The down field chemical shift appeared at δ 9.16 ppm (singlet, 2H) which one proton was identified for methine proton and the other expected to be the proton at ortho position to fluorine atom which disappeared in the spectrum. A proton in this did not found in the spectrum, it may be labile proton adjacent to nitrogen or proton adjacent to sulfur atom which represented at δ 2-4 ppm or equal to chemical shift of solvent.



Scheme 18. The mechanism of the fragmentation reaction of 8-Chloro-9-fluoro-2-mercapto-3-(4'-methyl phenyl)-pyrimido-[4,3-d]- quinoline-4-one.

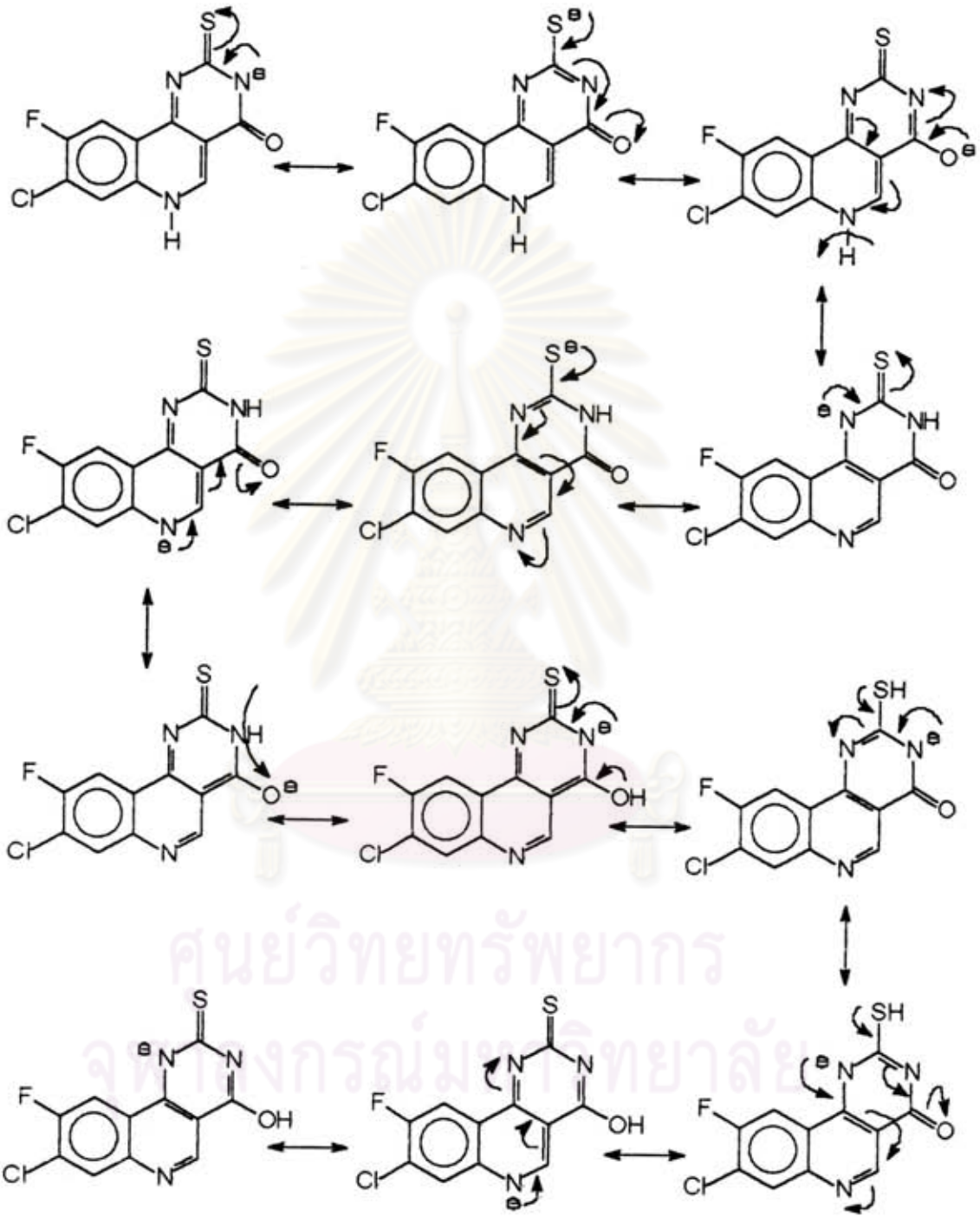
The exact structure was identified by mass spectrum (Figure 40.) which showed molecular ion peak at m/e =371. The base peak was at m/e 370 due to of lossing of hydrogen atom. The isotope peak appeared at m/e 372 and 373. At m/e=372 natural abundance was approximate 56% that was $(M+1)^+$ isotope and $((M-1)+2)^+$ isotope. The fragment peak found at m/e 193 and m/e 222. The peak appeared at m/e 44 due to loss of $[CS]^+$ and m/e 91 due to loss of $[C_7H_7]^+$ (Scheme 18.).

From interpletation of all spectrum the major form of (XXXXVII) should be as flollowing, 8-Chloro-9-fluoro-2-mercapto-3-(4'-methyl phenyl)-pyrimido-[4,3-d]-quinoline-4-one, tautomer form.



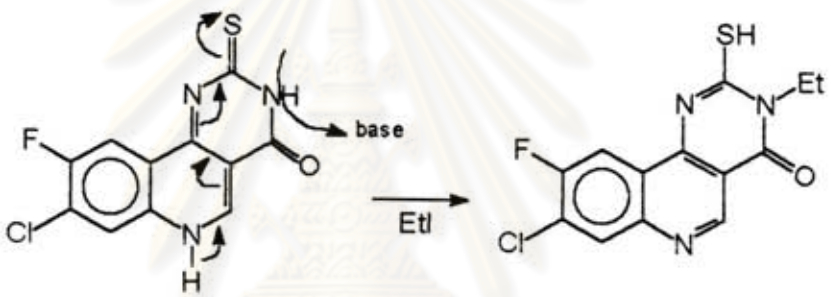
Synthesis of 8-Chloro-3-ethyl-9-fluoro-2-thiooxopyrimido-[4,3-d]-quinoline-4-one (XXXXVIII).

Compound (XXXXVIII) was synthesized by means of base catalyse nucleophilic substitution. The 7-Chloro-8-fluoro-2-thiooxopyrimido-[4, 3-d]-quinoline-4-one could be exist in twelve tautomeric from when reacted with sodium hydride as shown in scheme 19. .



Scheme 19.

Thus, the attempt to introduce ethyl substituent into thiopyrimidoquinoline series may produced a complicated mixture of products. From the experiment the ethylation products obtained was considered as the desired 3-ethyl-substitute under all experimental condition such as NaH and EtI, K_2CO_3 and EtI, 20% NaOH and EtI. However the product was in quinoline isomeric form, not the quinolone form. This phenomenon can be explained due to the proton at N-3 was the most acidic one and then could be easily loss by any kind of bases.



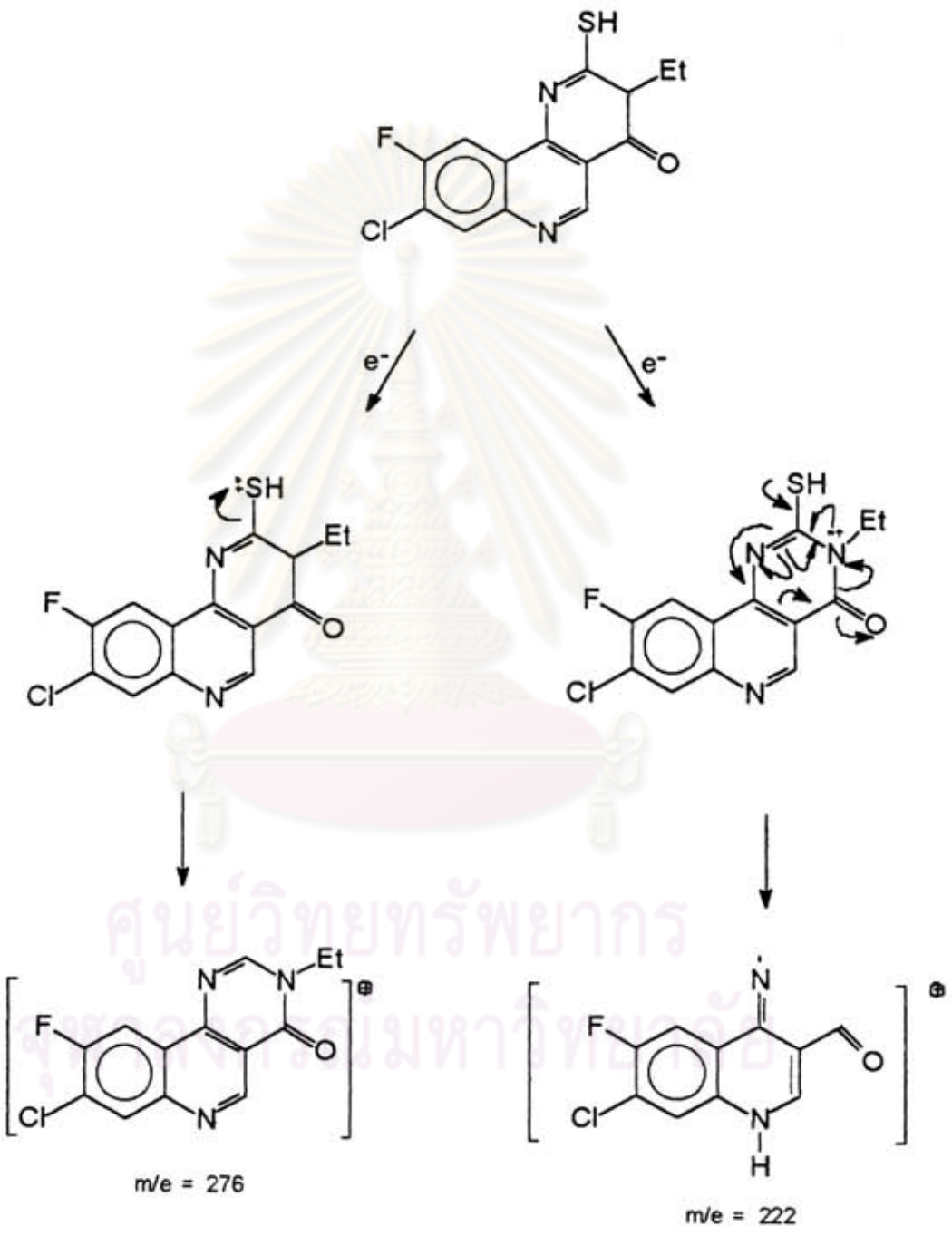
Another synthetic method for (XXXXVIII) was achieved by react (XXXXIII b) with ethylamine.

The IR spectrum of (XXXXVIII) (Figure 41.) showed peak at the range 3020-3100 cm^{-1} assigned for C-H stretching of aromatic and the range 2820-2950 cm^{-1} for C-H stretching of aliphatic. The broad peak at 2600-2800 cm^{-1} represented S-H stretching. The strongest peak of C=O (amide) stretching appeared at 1723 cm^{-1}. The peak at 1595 cm^{-1} and 1550 cm^{-1} assigned for C=C stretching. The bending of C-H appeared at 1456 cm^{-1}. The peak at 1180 cm^{-1} identified for C-F stretching and the peak at 1100-1150 cm^{-1} of N-C(=S)-N disappeared.

The ^{1}H-NMR of (XXXXVIII) (Figure 43.) in DMSO-d_6 at high temperature (because of the poor solubility) showed the peak at δ 1.45 ppm (triplet, 3H) assigned for

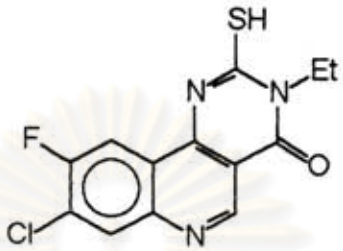
methyl proton of ethyl group and the peak at δ 3.40 ppm (quartet, 2H) for methylene proton of ethyl group. If the methylene proton appeared between δ = 3.00-4.00 ppm, the ethyl group could expect to attach with oxygen or nitrogen of amide. The aromatic proton found at δ 8.29 ppm (doublet, 1H, Jvalue = 7.1 Hz) so that it was identified for proton meta to fluorine atom. The ortho proton to fluorine found at δ 8.50 ppm (doublet, 1H, J = 9.8 Hz) and the singlet peak represented methine proton at quinoline ring.

The mass spectra of (XXXXVIII) confirmed the structure as describe as following (Figure 44.). The peak at m/e 309 was the molecular ion peak and the base peak, too. The isotope peak found at m/e =310 for $(M-1)^+$ and m/e = 311 for $(M+2)^+$ and the relative abundance was 19.4% and 37.7% respectively. The fragment ions peak showed at m/e 276 from losing of SH. This compound also found the peak at m/e 222,195. The propose fragment showed as following Scheme :



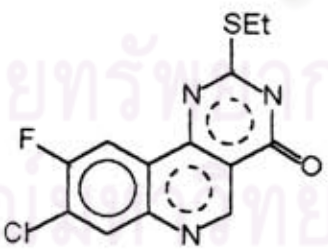
Scheme 20. The mechanism of the fragmentation reaction of 8-Chloro-3-ethyl-9-fluoro-2-mercapto-pyrimido-[4,3-d]-quinoline-4-one.

As the result, structure of (XXXXVIII) should be 8-Chloro-3-ethyl-9-fluoro-2-mercaptopyrimido-[4,3-d]-quinoline-4-one.



A minor product which was also separated form this reaction was 8-Chloro-2-ethylthio-9-fluoro-pyrimido-[4,3-d]-quinoline-4-one 10% yield.

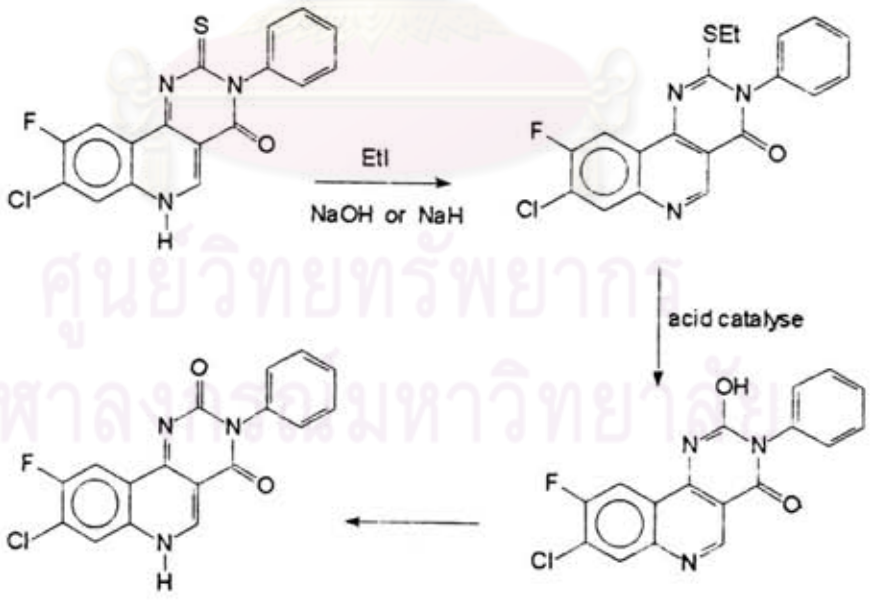
It was identified by IR spectrum (Figure 42.) which showed the peak of C-H stretching of aromatic at the range 3100-3200 cm^{-1} and the range 2820-2950 cm^{-1} for aliphatic C-H stretching. The strong peak at 1735 cm^{-1} assigned for C=O stretching and C=N stretching was the strongest peak at 1680 cm^{-1}. The possible structure of compound is:



Synthesis of 8-Chloro-9-fluoro-3-phenyl-pyrimido-[4,3-d]-quinoline-2,4-diones (IL).

The production of (IL) expected to react 3-Carboethoxy-4,7-dichloro-6-fluoro-quinoline (XXXXII b) with potassium cyanate (KOCN) to from 4-isocyanate derivatives and further react with amine series like the thio-derivatives. The other method was direct change of 8-Chloro-9-fluoro-3-phenyl-2-thiooxpyrimido-[4,3-d]-quinoline-4-one at 2-thiocarbonyl to be 2-carbonyl which can be done in two way.

1. Prepared the alkylthioether compound by alkylation at sulfur of thiocarbonyl and the reactive alkyl sulfide was replaced to hydroxyl group or its tautomer (carbonyl) by acid hydrolysis. the reaction showed in the follow reaction (Charn et al, 1993 ; Purkayaetha et al, 1990).



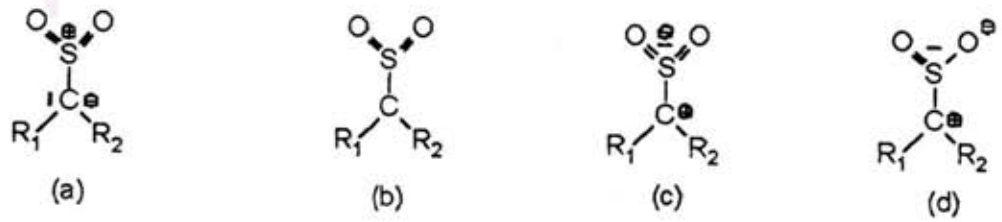
Scheme 21 .

2. Thiocarbonyl was oxidized to be carbonyl by alkaline hydrogen peroxide (Looney-Dean et al, 1984). this reaction gave compound (IL) in practically quantitative yield. The differences between carbonyl and thiocarbonyl group are explicable on differences in polarizability, size and electronegativity of heteroatom (Table 2.) (Opitz, 1967).

Table 2. Electronegativity according to.

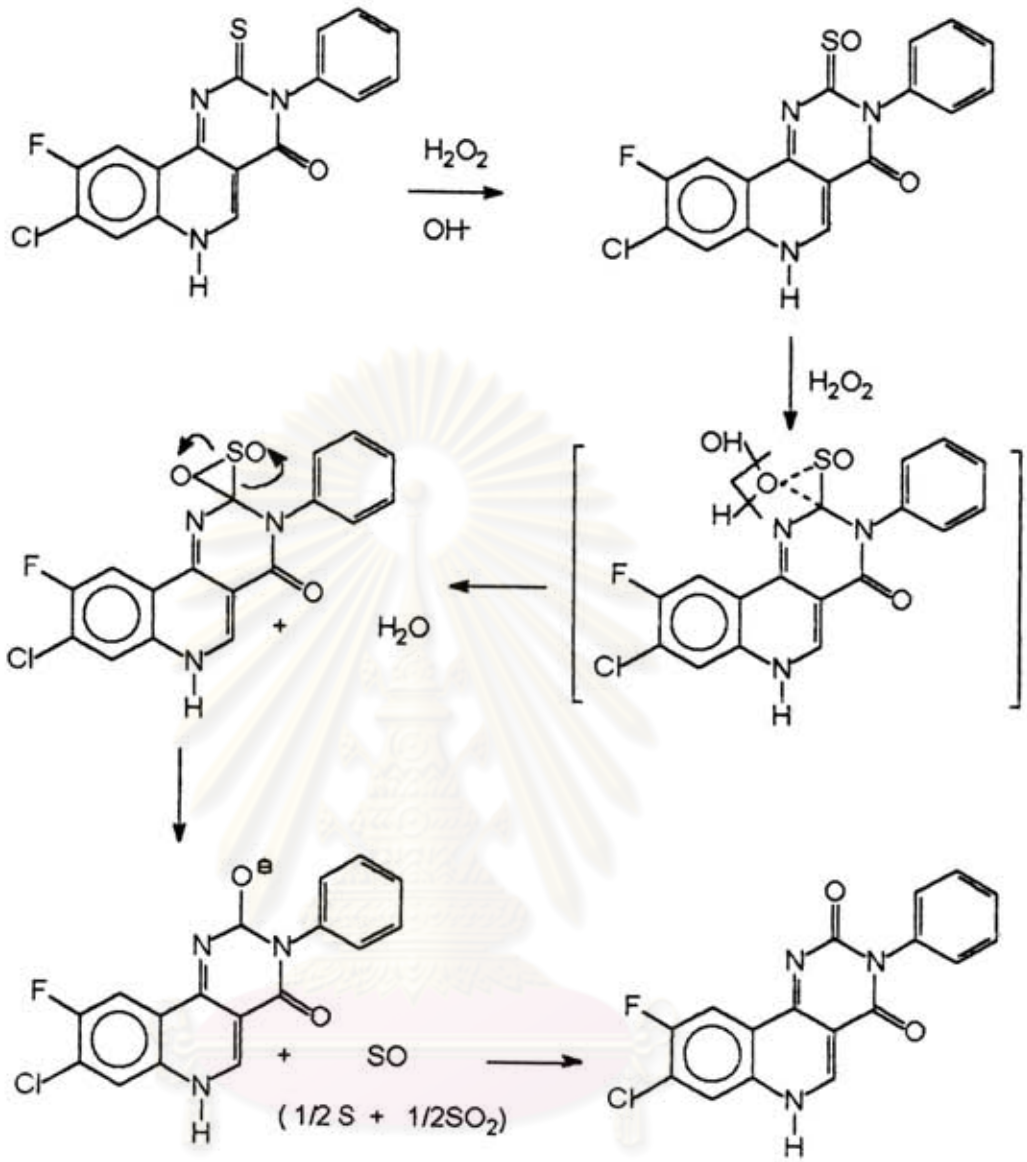
	Pauling	Mulliken	Allred and Rochow
C	2.60	2.63	2.50
O	3.50	3.17	3.50
S	2.60	2.41	2.44

It was suggest that S atom in case of thiocarbonyl may even carry a partial positive charge, in contrast the O atom in the carbonyl carry a partial negative charge. The proposed mechanism was start by hydrogen peroxide in the first portion was reacted with (XXXXV b) to from S-oxide or S,S-dioxide compound which more electronegativity O atom on the S atom would increase the carbonyl reactivity. Electron pair was delocalized into 4 types (a,b,c,d). A 1,3 dipolar formulation such as



(a) (b) (c) (d)

structure d was possible, in which the oxygen has taken over the negative charge.

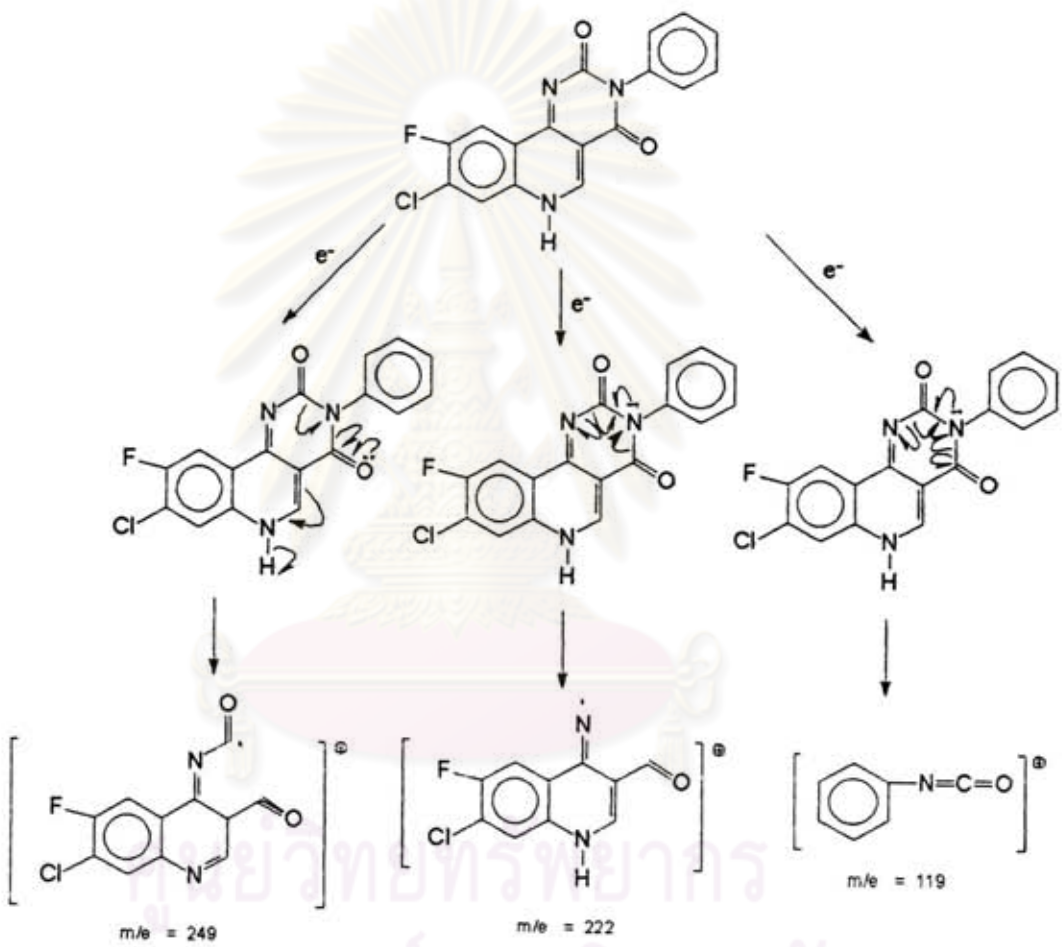


Scheme 22.

The product was identified by IR spectrum (Figure 45.) which showed peak at region 2800-3240 cm^{-1} assigned for C-H stretching vibration. The carbonyl stretching represented at 1730 and 1660 cm^{-1} of amide. The peak of C=N stretching was at 1570 cm^{-1}. The peak of N-(C=S)-N stretching at the range of 1100-1200 cm^{-1} disappeared.

The ^{1}H-NMR of (IL) in DMSO-d_6 (the compound has poor solubility in DMSO-d_6; thus, to increase solubility the experiment was performed at 80°c) (Figure 46.) showed the peak at δ 7.38 ppm (doublet, 2H) assigned for ortho protons of phenyl. Meta protons of phenyl were at δ 7.53 ppm (triplet,2H) and the para proton was at δ 7.45 ppm (triplet, 1H). The proton which was meta coupling to fluorine showed at δ 8.27 ppm (doublet,1H,J = 7.0 Hz). Proton ajacent to fluorine appeared at δ 8.76 ppm (doublet, 1H,J = 10.7Hz). The proton for methine group with connected to nitrogen of quinolone ring presented at δ 9.12 ppm (singlet, 1H). Down field peak at δ 12.21 ppm (broad, 1H) assigned for proton adjacent to nitrogen of quinoline ring. The comparable of ^{1}H-NMR of (XXXXV b) and (IL) showed in Figure 47. .

The MS used for corroborate the structure of (IL) (Figure 48.) which showed the molecular ion peak at m/e 341 and showed $(M+1)^+$, $(M+2)^+$ isotope peak. The fragment peak was at m/e 340 cause by loss of one hydrogen atom. The peak at m/e 249 reasoned for break at aniline and the fragment peak at m/e 222 died to further break of CO part. The possible fragment of (IL) showed in scheme 23. .



Scheme 23. The mechanism of the fragmentation reaction of 8-Chloro-9-fluoro 3-phenyl-2-pyrimido-[4,3-d]-quinoline-4-one.