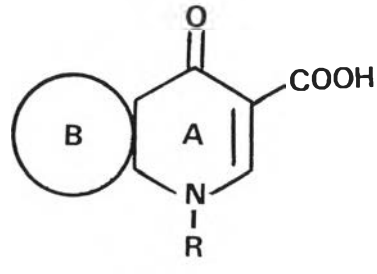
CHAPTER II

HISTORY AND APPROACH

Quinolones (1-substituted 1,4-dihydro-4-oxo-pyridine -3-carboxylic acid moiety).



XXXVII

The general structure of quinolones is structurally characterized by the combination of 1-substituted 1,4-dihydro-4-oxo-pyridine-3-carboxylic acid moiety (A) with a second aromatic or heteroaromatic ring (B), represented by structure (XXXVII). In addition, both nalidixic acid and enoxacin are aza (nitrogen) substituted at position 8 of the quinolone, making them 8-azaquinolones or 1,8-naphthyridines. Oxolinic acid is further distinguished by a 6,7-methylenedioxy substituent. Nalidixic acid is the prototype of naphthyridine class, and oxolinic acid is the prototype of the quinolone class.

Mechanisms of Action and Spectra of Activity of the quinolones

The primary target of nalidixic acid and oxolinic acid and all quinolones is DNA gyrase (topoisomerase II), (Crumplin, Keenwright and Hirst, 1984). This essential bacterial enzyme, was discovered in *Escherichia coli* (Gellert, 1981). DNA gyrase has a variety of activities including the introduction of negative superhelical twists into double-stranded DNA and the catenation or decatenation of two duplex DNA circles interlocked like links in a chain. It acts by introducing a double-strand break into the DNA, passing another DNA duplex through the break, and supertwising and catenation-decatenation activities of DNA gyrase. Quinolones preferentially and rapidly inhibit DNA synthesis, suggesting interference with movement of the DNA replication fork, in addition causes single strand nicking and limited destruction of the bacterial chromosome, induces the SOS DNA repair system of *E. coli*, and stimulates synthesis of the bacterial heat-shocked proteins (Krueger and Walker, 1984).

Little is known about the mechanisms of bacterial resistance to the quinolones. All the mutations confirming quinolone resistance located on the bacterial chromosome. Neither plasmids encoding quinolone resistance nor transfer of quinolone resistance in the clinical setting has yet been described (Burman, 1977).

Quinolones in general are very active against the enteric gram-negative *bacilli* and *cocci*, including *Neisseria gonorrhoeae*. The drugs are also active against other gram-negative bacteria, including *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, but are less active against species of *Pseudomonas* other than *P. aeruginosa*. The drugs have excellent activity against bacteria pathogens of the gastrointestinal tract including *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Campylobacter jejuni*, and *Vibrio* spp..

Potential Clinical Uses and Toxicities of the quinolones

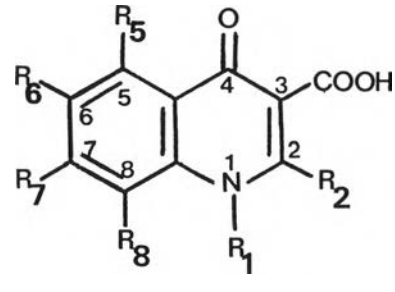
The antimicrobial activity and pharmacology of the quinolones suggest potential clinical uses in addition to treatment of urinary tract infections and gonorrhea. In

general the quinolones might be considered in bacterial infection in patients for whom use of other drugs such as ß-lactams or aminoglycosides is contraindicate and in infections with multiple resistant bacteria. Specific additional uses might include treatment of : (i) prostatitis; (ii) severe gastroenteritis likely to be of bacterial etiology before identification of a pathogen; (iii) selective decontamination of the gastrointestinal tract of neutropenic patients; (iv) pneumonia caused by gram-negative bacilli when oral therapy is desirable; (v) upper respiratory tract colonization with methicillin-resistant *Staphylococcus aureus* when indicated; (vi) serious infections caused by methicillin-resistant staphylococci when vancomycin cannot be used; and (vii) gram-negative bacillary osteomyeitis.

The adverse effect of the quinolones are limited to the previously cited studies, generally using norfloxacin or enoxacin. Additional potential toxicities include (i) the development of crystalluria with use of high doses of quinolones, (ii) central nervous system toxicities (iii) cartilage toxicity as has been seen in some animals treated with nalidixic acid.

Structure-Activity Relationships of the Quinolones

Systemic modification studies on nalidixic acid have produced compounds with increased potency and spectrum and have greatly enhanced the therapeutic application of quinolones. The mechanisms by which these substitutions enhance antimicrobial activity are for the most part unknown, but it seems plausible that many of the substitutions affect interaction with DNA gyrase.



Position 1 Earlier studies indicated that substitution at the N-1 position is important to be antibacterial activity (Albrecht, 1977). Quantitative structure-activity relationship analysis of a set of N-1-allyl and -alkyl derivatives suggested that the length of the substituent along the axis of the bond between the substituent and the parent molecule corresponding approximately to an ethyl group (0.42 nm) (Fujita, 1984;

Position 2 Very few modifications have been explored at position 2. Cinoxacin which has a nitrogen atom in position 2 has improved pharmacokinetic properties, however, it is less active *in vitro* than oxolinic acid. Benzothiazolo [3,2-a] quinolones posses good antibacterial activities. These derivatives have a sulfur atom positioned at C-2 ; the substituent is part of a ring system annealed to the benzene moiety. No clinical study on these compounds have been reported.

Position 3 In generally, the 4-carbonyl group are considered necessary for the binding of quinolones to DNA gyrase (Schentag and Domagala, 1985). Classical studies have produced no active quinolone with a significant modification of the C-3 carboxylic acid group, with the exception of groups which are converted *in vivo* back to a carboxylic acid group (Pesson, De Lajudie, and Antoine, 1971). For example, replacement of the 3-carboxylic acid group of norfloxacin by a formyl group produced a compound with low antibacterial activity. However, the compound exhibited the increased activity *in vivo*, owing to its rapid conversion into the 3-carboxylic acid group. A recent attempt to replace the 3-carboxylic acid group with a carboxylic mimicking compound 1-H-

Verloop, Hoogenstraaten, and Tipker, 1976). Most of the marketed quinolones such as norfloxacin, pefloxacin, and enoxacin have an ethyl group at the N-1 position. The discovery of potent quinolones with N-1 phenyl (Fernandes, Claibone, Pihuleac et al., 1985) and N-1-cyclopropyl (Wise, Andrews, and Edward, 1983) substitutions indicated that with respect to a N-1 substituent in addition to steric bulk, there are other factors, such as electronic donation and ideal spatial effects (Domagala et al., 1988) that also have a great influence on their biological activities. The cyclopropyl group is by far the optimal group by virtue of its favorable combination of steric, and through-space electronic interactions of ciprofloxacin having a N-1 cyclopropyl substituent. Fluorophenyl has excellent pharmacokinetic properties (Granneman, 1986).

The introduction of a t-butyl group at N-1 produced quinolones with enhanced activity against gram-positive bacteria, with a minor reduction of activity against gram-negative bacteria (Bouzard et al., 1989). This is another recent result which indicated that a steric bulk factor at N-1 alone cannot account for optimal biological activity.

tetrazol-5-yl, resulted in a total loss of antibacterial activity. Notwithstanding, a 62824 in which the 3-carboxylic acid group of ciprofloxacin has been replaced by a bioisostere-fused isothiazolo ring is more potent (Fernandes, Claiborne et al., 1988).

Position 4 A few development at position 4 have been reported none of them are biologically active (Drake et al, 1946; Shah and Coats, 1977).

Position 5 Limited investigations have been done on the C-5 position with substituents, such as nitro, amino, halo, and alkyl groups. The C-5 amino substitution may enhance absorption or tissue distribution. Although some reports have suggested that substitution at the C-5 position reduces antibacterial activity (Jack, 1986). The 5-aminoquinolones have been reported (Domagala, Hagen et al., 1988) to have *in vitro* antibacterial activity far superior to that of ciprofloxacin. The amino group at the C-5 position in the 6,8-difluoroquinolone series may enhance potency.

Position 6 Of the various C-6 substituents (H, F, Cl, Br, CH_3, $COCH_3$, and NO_2) the addition of a fluorine atom resulted in a dramatic increase in antibacterial potency (Koga et al., 1980). The fluoro group at position C-6 seems to improve both the DNA gyrase complex binding and cell penetration of the corresponding derivatives compare with no substitution at the C-6 position (Domagala, Hanna, et al., 1986). Because of such an enhancement of antibacterial potency, nearly all of the recently synthesized quinolones carry a C-6 fluorine substituent.

Position 7 Modifications at the C-7 position of the quinolone molecule have been extensively studied. In general, the nature of C-7 substituent have been divided into three groups (Domagala, Hanna et al., 1986). The first group contains quinolones with small or linear-like C-7 substituents (H, Cl, $CH_3NH_2CH_2NH$, CH_3NH, HO-N=CH).. It would appear that all the small substituents lack appreciable binding to the gyrase-DNA complex. The second group of C-7 substituents constitute the medium-size of five- and six-membered rings (pyrrolidinyl, pyrrolyl, thiazolidinyl, thiomopholinyl, and piperazinyl) have good antibacterial activities. Further minor substitutions on

the second group is comprised of the third group, for examples, the substitution of methyl at C-4 position of the piperazinyl group enhance the gram-positive antibacterial activity of the parent compound and the substitution of a 3-aminopyrrolidin-1-yl group at the C-7 position generally enhances the overall spectrum of activity. However, these substitution may make the products less water soluble at pH 7.4 and may cause absorption problems in human (Wentland et al., 1984).

Position 8 Among many investigated modifications at the C-8 position were replacement of C-8 with nitrogen atom or substitution with halogen atom (Cl, F). Ofloxacin, having an oxygen substituent at the C-8 cyclized with ethylene bridge to N-1 position forming morpholine-like ring system is somewhat more potent than norfloxacin *in vitro* (Sata et al., 1982). A number of naphthyridines in which C-8 is replaced by a nitrogen atom have excellent activity *in vitro* and *in vivo*. In general, however with similar substitutions at N-1, C-5, C-6 and C-7, napthyridine analogs are less active *in vitro* than their quinolone counterparts. This inferior *in vitro* activity is overcome by better absorption to enhance *in vivo* activity.

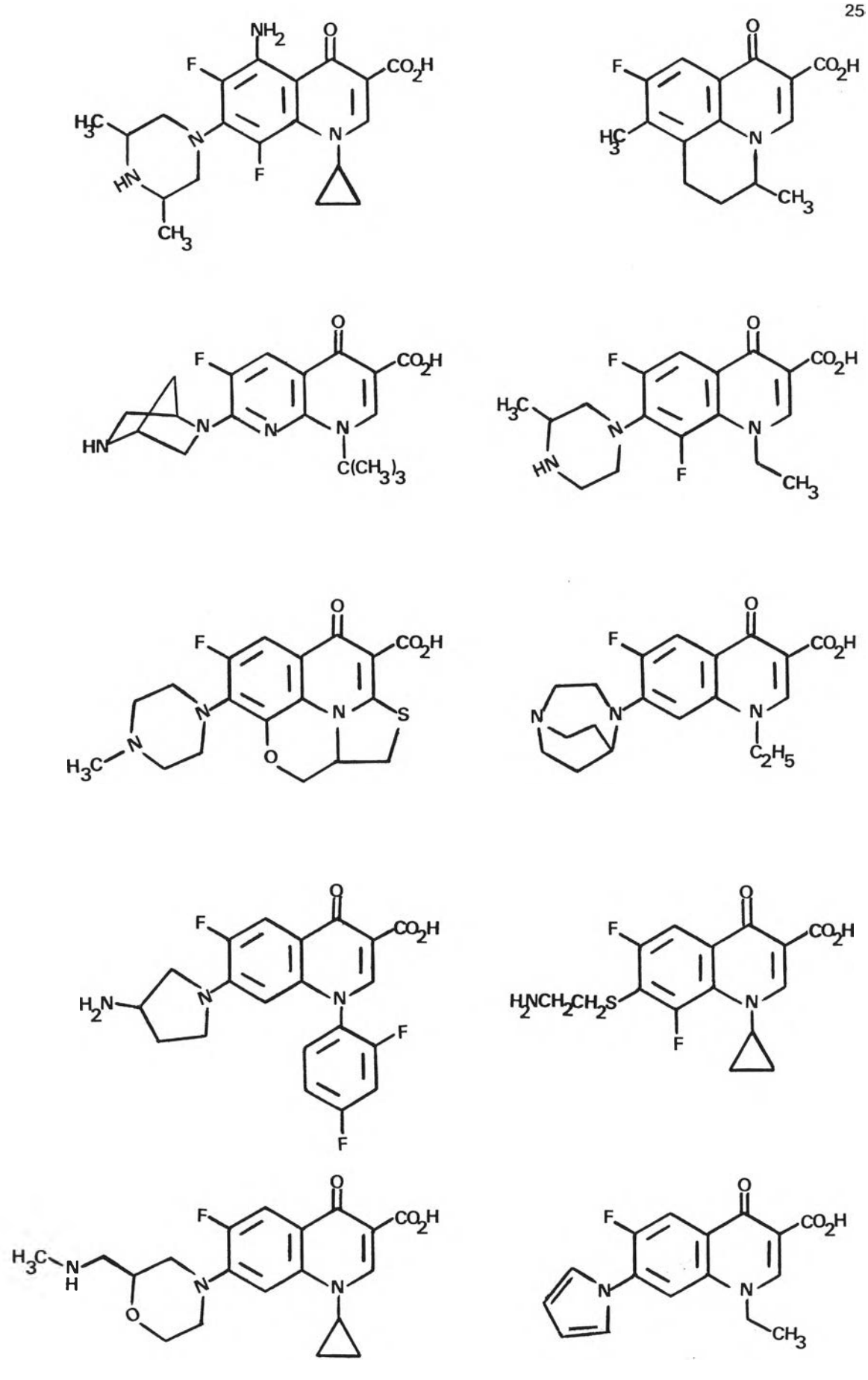


Figure 1 The chemical structures of various 7-substituted quinolones.

Ring-closured synthesis of quinoline derivatives

Utilization of classical route to quinoline structures involving the cyclization of ethylanilino-methylenemalonate (suggested by Gould and Jacob, 1939) will be the process of ring-closure for two main structures of quinoline derivatives, namely :

a. 4-Hydroxyquinolinecarboxylates.

b. N-Alkyl-1,4-dihydro-4-oxo-3-quinoline carboxylates (quinolones).

a. Synthesis of 4-Hydroxyquinolinecarboxylates

The reactions are comprised of two stages. The first involves a condensation reaction between aniline and diethylethoxymethylenemalonate. The second stage of the reaction is followed by the cyclization of diethylanilinomethylene -or nuclear substituted anilinomethylenemalonate to obtain 4-hydroxyquinoline carboxylates.

In 1887, Conrad and Limpach first prepared 4-hydroxy-quinaldine by condensation of aniline with acetoacetic ester at room temperature followed by cyclization at 250°C. Limpach forty-four years later improved the yield from about 30 to 90 - 95% by the use of mineral oil as a diluent in the cyclization step (Gould and Jacobs, 1939).

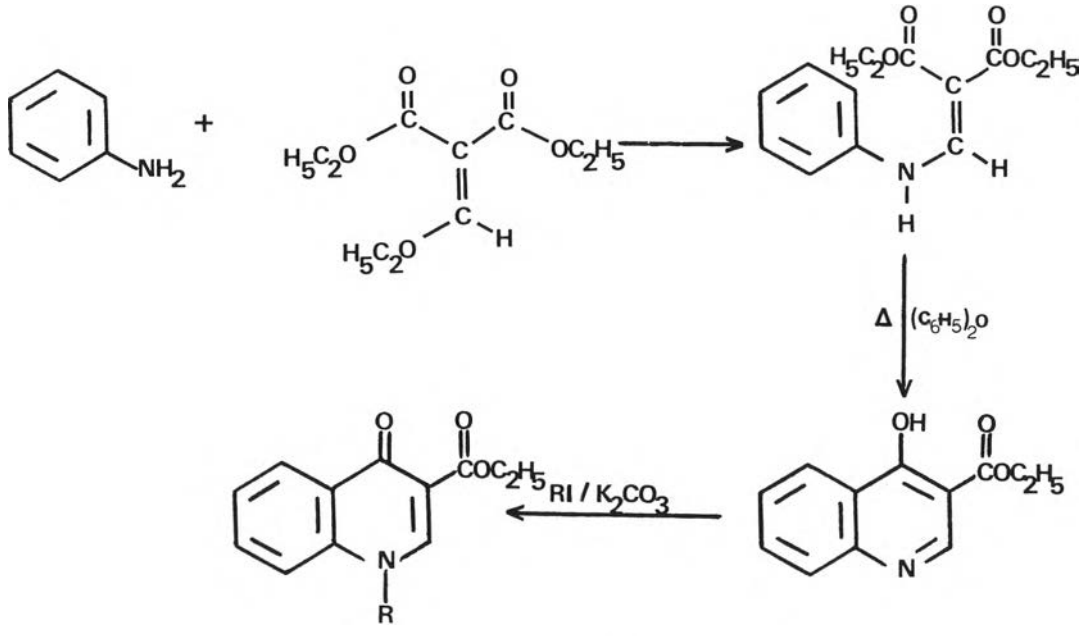
The original Limpach cyclization which was the thermal cyclization, as utilized by Gould and Jacobs involved adding to ß-aryl-amino-crotonate from two to ten time its weight of mineral oil preheated to 250° - 290°C and then heating the solution at 240° - 250°C for fifteen to twenty minutes. It has been found that both diphenyl ether and Dowtherm (biphenyl and diphenyl ether) are far superior as a cyclization medium. These solvents, boil at a temperature which is optimum for the cyclization, are much less viscous and more easily removed from the product by filtration and furthermore the product formed is much less darkening. The volume of solvent required for cyclization of various amine varied considerably (Price and Roberts, 1946).

Diethyl ethoxymethylenemalonate was prepared by the developed method of Claisen by heating a mixture of triethyl orthoformate, malonic ester, and acetic anhydride with a catalytic amount of zinc chloride.

$$CH_2(CO_2C_2H_5)_2 + HC(OC_2H_5)_3 + 2(CH_3CO)_2O \xrightarrow{ZnCl_2}$$

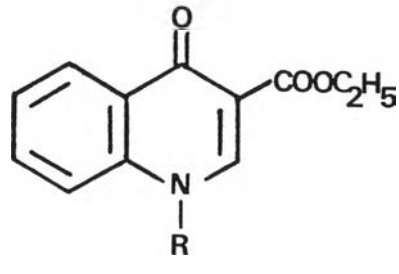
$$C_2H_5OCH = C(CO_2C_2H_5)_2 + 2CH_3CO_2C_2H_5 + 2CH_3CO_2H$$

The reaction of diethyl ethoxymethylenemalonate with aromatic amines to form anilinomethylenemalonate takes place readily even at room temperature (Price and Roberts, 1946). Claisen carried out such reaction, simply by heating molecular equivalents of aniline and diethyl ethoxymethylenemalonate on the steam-bath for a short peroid, the anilino-ester readily crystallizing on cooling in ice. Other nuclear-substituted anilinomethylenemalonates were similary obtained by heating the appropriate intermediates on the steam-bath (Duffin and Kendall, 1948).



b. Synthesis of 1-alkyl-1,4-dihydro-4-oxo-3-quinolinecarboxylates (quinolones)

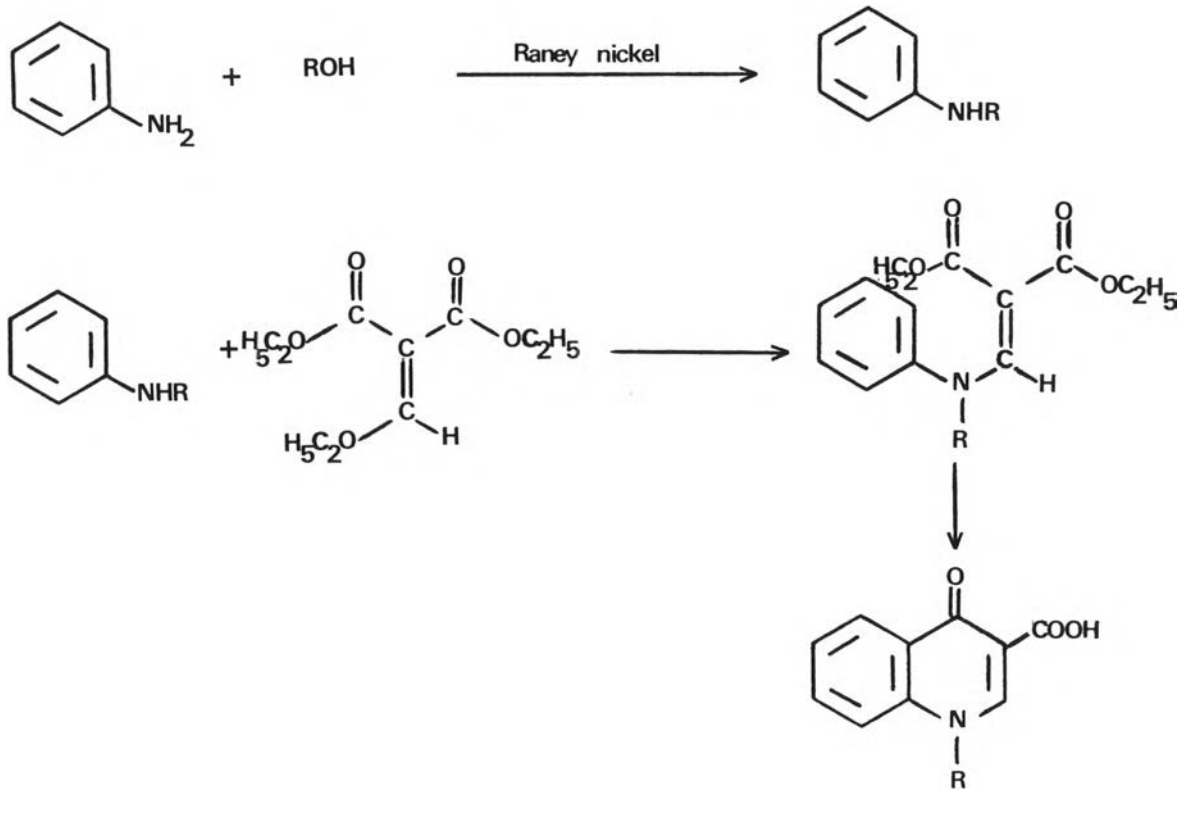
Generally, 1-alkyl -1,4-dihydro -4-oxo-3-quinoline carboxylates were obtained by base catalized alkylation of 4-hydroxy-3-quinolinecarboxylates with excess of alkyl halides.



Kaminsky and Meltzer (1968) found that N-alkylanilinomethylenemalonates cyclized smoothly to afford the desired 1-alkyl-1,4-dihydro-4-oxo-3-quinoline-carboxylates. N-Alkyl anilino were prepared by extending the Raney nickel catalyzed which was discovered by Mozingo et al. (1944) and developed independently, by Rice and Kohn (1955). Ainsworth (1956) has described the alkylation reaction of aniline with a variety of aliphatic alcohols in the presence of Raney nickel catalyst and N-alkylanilines were allowed to react with diethyl ethoxymethylenemalonate to obtain N-alkyl anilinomethylenemalonates.

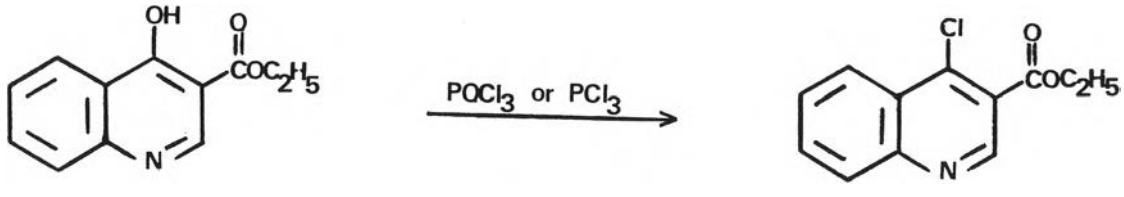
The cyclization of N-alkylanilinomethylenemalonates, which was performed successfully to 1-alkyl-1,4-dihydro--4-oxo3-quinolinecarboxylate (quinolones) by thermal cyclization in Dowtherm. Among other cyclizing agents reported by Nakanishi, Yokobe, and Tsuda (1969), was polyphosphoric acid (PPA, which prepared from ortho phosphoric acid and phosphorus pentoxide) by heating at 110 - 120^{o}C but decreased yield was obtained at elevated temperature. Polyphosphate ester (PPE; which prepared from phosphorus pentoxide in ether and chloroform) and boron trifluoride could replace PPA successfully in the

same cyclization. Bandiwala and Desai (1953) used the mixture of acetic anhydride and sulfuric acid in cyclization reaction for the preparation of 2-substituted-4-quinolinols. Heating N-alkylanilinomethylenemalonate with phosphorus pentoxide in boiling benzene or nitrobenzene (Fieser & Fieser) resulted in the partial cleavage of the malonate and N-alkylaniline being separated in addition to some of cyclized ester. Other variations of cyclizing agents, for example, zinc chloride in acetic anhydride and acetic acid or a molten aluminium chloride, had virtually no better effect.

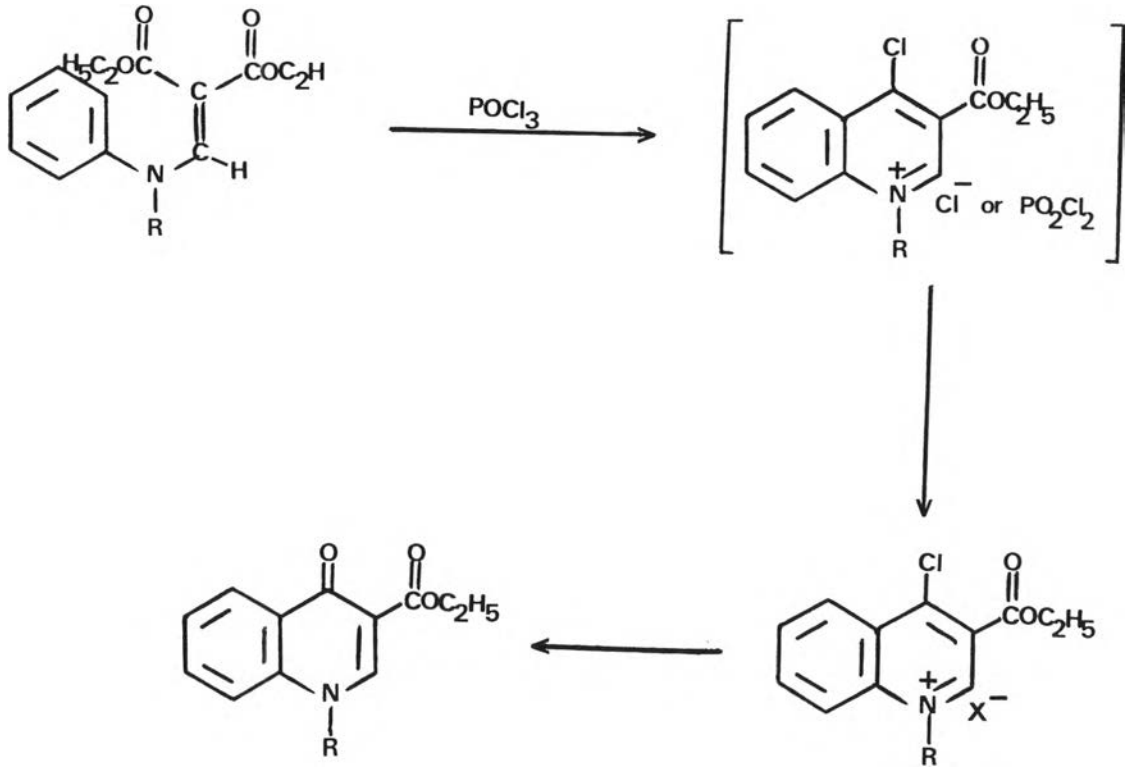


Synthesis of 4-Chloroquinolinecarboxylates

The 4-chloroquinoline reported in literature had been for the most part, prepared by the reaction of 4-hydroxy-3-quinolinecarboxylate with phosphoryl trichloride or phosphorous oxychloride (Kaslow and Clark, 1953). Price and Roberts have reported the preparation of 4,7-dichloroquinoline.



Nevertheless, 1-alkyl anilinomethylenemalonate was heated with phosphorus oxychloride to give 1-alkyl-4-chloro-3-carboethoxy quinolinium salt which was hydrolyzed readily to ethyl 1-alkyl-1,4-dihydro-4-oxo-3-quinolinecarboxylates without forming 4-chloro-3-quinoline-carboxylate (Agui et al., 1971).



The method of chlorination by means of the other reagents was thionyl chloride. In this synthesis a 4-chloroquinoline was required and made in substantial quantity as an intermediate which will be followed by condensation with amine.

Nucleophilic Substitution Reaction : utilization for preparation of target compounds

$$R : X + :Z \longrightarrow R : Z + :X^-$$

$$:Z = OH^-, OR^-, NH_3, CN^-, \text{etc.}$$

$$X = F, Cl, Br, I$$

A typical reaction nucleophilic substitution of aryl halides is employed. Halogen is substituted by bases such as OH^-, OR^-, NH_3, CN^-, etc. yielding alcohol, ether, amines or nitriles. The aryl halides do undergo nucleophilic substitution readily if the aromatic ring contains, in addition to halogen, other properly placed electron-withdrawal groups, such as NO_2 or CN, located ortho or para to halogen. It is causes activation.

The reaction of unactivated aryl halides with strong bases or at high temperatures. Replacement of halogens by amino groups may be accomplished using ammonia or amines if the halogen is activated (i.e. at 2 or 4 position, or at a site ortho or para to an electron withdrawing substituents). Since nucleophilic attack on

the ring is assisted when the heteroatom is positively changed, amino dehalogenations are catalysed by acids (Banks, 1944), Lewis acids like zinc chloride and cupric sulphate. Hydrazine and arylamine also react readily with 2- and 4-halogenopyridines, (which are weaker nucleophiles) may require higher temperatures or a catalyst : such as copper bronze (Tarbell et al., 1948). The reactivity of alkyl halides follow the sequence R-I > R-Br > R-Cl > R-F (Morrison & Bord, 1987).