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Jaroslav Šebestík

Biomedical Applications of Acridines

Derivatives, Syntheses, Properties and Biological Activities with a Focus on Neurodegenerative Diseases

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Jan Ježek
Academy of Sciences
Institute of Organic Chemistry
and Biochemistry
Prague
Czech Republic

Jaroslav Šebestík
Academy of Sciences
Institute of Organic Chemistry
and Biochemistry
Prague
Czech Republic

Jan Hlaváček
Academy of Sciences
Institute of Organic Chemistry
and Biochemistry
Prague
Czech Republic

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To memory of the late Dr. Dušan Drahoňovský, who contributed to the concept of human friendly science. He served as a very kind and good teacher of organic chemistry at Charles University, Prague. When we had started to study organic synthesis at University of Pardubice, Dušan's nickname was Acridine, because he had been devoted to carry out acridine synthesis since high school age.

Acknowledgements

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Contents

Acronyms

The three-letter code used for amino acids follows the rules of the IUPAC-IUB Commission on Biochemical Nomenclature. [1] The one-letter code used for amino acids follows the rules of the IUPAC-IUB Joint Commission on Biochemical Nomenclature. [2] These articles show also how to read formulas of peptides used in this work. [3] Selected abbreviations are commonly used especially for description of acridines in peptide conjugates and neurodegenerative disorders:

AAAcr	9-(p-aminomethylphenylamino)acridine-4-carboxylic acid
Aβ	amyloid β
AC04	5-(acridin-9-yl-methylidene)-3-(4-methyl-benzyl)-thiazolidine-2,4-dione
Acd	β-(9,10H-acridon-2-yl)alanine
AChE	Acetylcholinesterase
ACMA	9-amino-6-chloro-2-methoxyacridine
Acr	Acridin-9-yl
ACRAMTU	1-[2-(acridin-9-yl-amino)ethyl]-1,3-dimethylthiourea
Acr4CA	4-(2-methylaminoethylaminocarbonyl)-acridin-9-yl
AD	Alzheimer's disease
ADE	Amyloid degrading enzymes
AHMA	3-(9-acridinylamino)-5-hydroxy-methylaniline
ALS	Amyotrophic lateral sclerosis
AO	Acridine orange
Aol	3,6-bis-(dimethylamino)acridin-9-yl
APP	Amyloid precursor protein
BACE	β-secretase
BBB	Blood–brain barrier
BChE	Butyrylcholinesterase
BRACO19	*N*,*N'*-(9-(4-(dimethylamino)phenylamino)acridine-3,6-diyl) bis (3-(pyrrolidin-1-yl)propan-amide) trihydrochloride
BSE	Bovine spongiform encephalopathy

C-1305	5-dimethylaminopropylamino-8-hydroxytriazolo-acridinone
C-1748	9-(2'-hydroxyethylamino)-4-methyl-1-nitroacridine
CJD	Creutzfeldt–Jacob disease
Cl-DACA	*N*-(2-(dimethylamino)ethyl)-7-chloro-acridine-4-carboxamide
CMA	10-carboxymethyl-9-acridone
CTAC	cetyltrimethylammonium chloride
CWD	Chronic wasting disease
CYP3A4	Cytochrome P450 3A4
DABPA	9-(4-(1,2-diamine)benzene-*N*-1-phenyl)acridine
DACA	*N*-(2-(dimethylamino)ethyl)-acridine-4-carboxamide
DAPA	9-[3-(dimethylamino)propylamino]acridine
DFT	Density functional theory
DLB	Dementia with Lewy bodies
DPEPhos	bis[(2-diphenylphosphino)phenyl] ether
dppf	1,1'-ferrocenediyl-bis(diphenylphosphine)
FITU	Fluorescein 5-((amino)thiocarbonyl)
FFI	Familial fatal insomnia
FRDA	Friedreich's ataxia
FTLD	Fronto-temporal lobar degeneration
GPI	Glycophosphatidylinositol
GSS	Gerstmann–Sträussler–Scheinker syndrome
HD	Huntington's disease
hAChE	Human acetylcholinesterase
hBChE	Human butyrylcholinesterase
hMAO-B	Human monoamine oxidase B
HpβCD	hydroxypropyl-β -cyclodextrin
HuPrP106-126	Human prion peptide 106-126
hSIRT	Human sirtuin
Hsp90	Heat shock protein 90
IP	*Intraperitoneal*
IV	*Intravenous*
mAChE	Mouse acetylcholinesterase
MBAA	6-chloro-2-methoxy-*N*-(2-methoxybenzyl)acridin-9-amine
MCI	Mild cognitive impairment
MDR	Multidrug resistance
7-MEOTA	7-methoxy-9-amino-1,2,3,4-tetrahydroacridine
MSA	Multiple system atrophy
NEP	Neprilysin
NIID	Neuronal intranuclear inclusion disease
OHA	1,2,3,4,5,6,7,8-octahydroacridin-9-amine
PCB	Polychlorinated biphenyls
PCET	Proton-coupled electron transfer
PD	Parkinson's disease
P-gp	P-glycoproteine
Phthac	4,5-bis-(phenylthiomethyl)acridine

PPA	Polyphosphoric acid
PPAB	Poly(propargyl acridiniumbromide)
PPQB	Poly(propargyl quinoliniumbromide)
PrP	Prion protein
PrP^{C}	Cellular PrP
PrP^{res}	Proteainase K resistant PrP
PrP^{Sc}	Scrapie PrP
PrP^{sen}	Proteainase K sensitive PrP
PZA	Pyrazoloacridine
QSAR	Quantitative structure-activity relationships
Qui	6-chloro-2-methoxyacridin-9-yl
RAFT	Regioselectively addressable functional template
RCSB PDB	Research Collaboratory for Structural Bioinformatics Protein Databank
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
$rPrP^{Sc}$	Proteainase K resistant PrP^{Sc}
RT-QuIC	Real time-quaking-induced conversion
SBMA	Spinal and bulbar muscular atrophy
SCA	Spinocerebellar ataxias
$sPrP^{Sc}$	Proteainase K sensitive PrP^{Sc}
Tac	1,2,3,4-tetrahydroacridin-9-yl
TEOF	Triethyl orthoformate
TSE	Transmissible spongiform encephalopathy
vCJD	Variant Creutzfeldt–Jakob disease

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Chapter 1
Introduction

Abstract Acridines interact with both nucleic acids and proteins. The targeting of these biopolymers is broadly applied in cancer therapy and gene delivery. Interestingly, due to direct interactions of acridines with various enzymes, acridines can be suitable drugs for treatment of neurodegenerative diseases, inflammation, immunological disorders, protozoal diseases, etc. General introduction to the field of acridines is provided including scope and limitations of the field. Some important structures, which are used as drugs, are highlighted.

Acridines are heteroaromatic compounds (Figs. 1.1 and 2.1) that were applied for treatment of many diseases since the beginning of 20th century [1]. Namely, aminoacridines possessing higher solubility in physiological liquids were used as antiprotozoal and antibacterial drugs. The growth of transplanted tumor was suppressed by acriflavine (mixture of 3,6-diamino-10-methylacridinium chloride and 3,6-diaminoacridine) in the thirties [2]. In the past, anti-tumor activity of acridines was attributed to their interactions with nucleic acids; however, the more recent studies favor an explanation based on direct interaction of acridines with biologically important proteins [3–10]. According to search in RCSB PDB database conduced in August 2015, there were 48 structures containing acridine skeleton and 30 structures containing 1,2,3,4-tetrahydroacridine, respectively. From these 78 structures only 32 correspond to acridine binding to nucleic acids including one complex between amsacrine-nucleic acids and enzyme topoisomerase II. Thus, more than half of the structures represents direct binding of acridines to proteins.

The development of human society has incorporated acridine drugs to daily life of millions of individuals worldwide [6, 10–28]. For selected acridine drugs see Fig. 1.1. Due to high toxicity, compounds **1**–**4** are applied only as local antiseptic agents. *m*-Amsacrine (**6**) is very active against malignant lymphomas and acute leukemia. Asulacrine (**7**), which combines the substructures of *m*-amsacrine (**6**) and DACA (**5**), has improved pharmacokinetic profile and broader scope of applications against leukemia, Lewis lung tumors, and many solid tumors. Nitracrine (**8**) serves for treatment of mammary and ovarian tumors. BRACO19 (**9**) is powerful binder of quadruplexes. Pt-ACRAMTU (**10**) is mimicking anticancer cisplatin; however, it has different mode of action. Quinacrine (**11**) can be viewed as a universal medicine for

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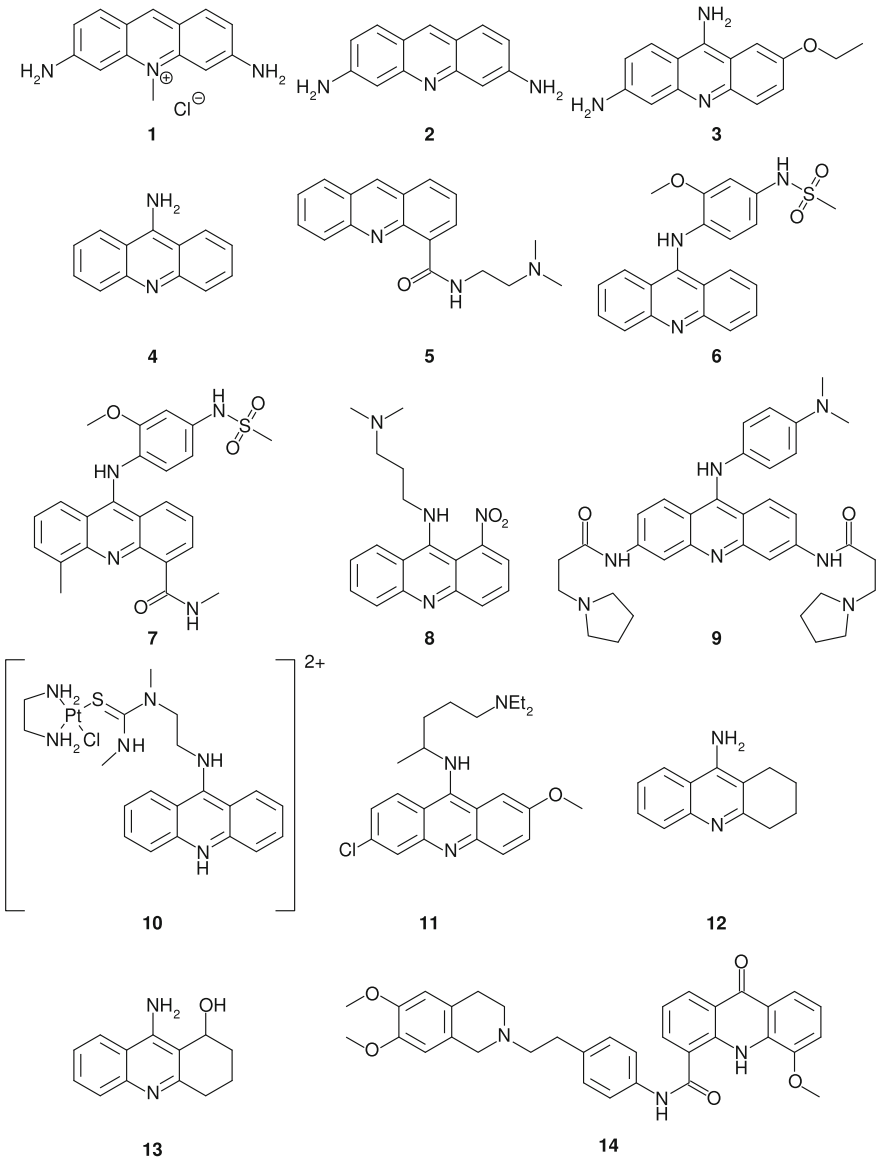

Fig. 1.1 Selected acridines used as antiseptic (**1**–**4**), anticancer (**5**–**10**), trypanocidal (**11**), neuroprotective (**11**–**13**), and transport-enhancing (**14**) drugs, respectively [4, 6, 11]. Acriflavine is a mixture of **1** and proflavine (**2**). Ethacrine (**3**). Aminacrine (**4**). DACA (**5**). *m*-Amsacrine (**6**). Asulacrine (**7**). Nitracrine (**8**). BRACO19 (**9**). Pt-ACRAMTU (**10**). Quinacrine (**11**). Tacrine (**12**). Velnacrine (**13**). Elacridar (**14**)

many diseases such as rheumatic arthritis, lupus erythrematosus, malaria resistant to chloroquine, the tapeworm infections (*Taenia saginata*), Chagas disease, epilepsy (refractory petit mal), and prion infections. Another acridine useful for treatment of neurodegenerative diseases, which has partially hydrogenated ring, is tacrine (**12**). It penetrates blood–brain barrier (BBB) and serves for treatment of Alzheimer's disease (AD). Velnacrine (**13**) served as less toxic analogue of tacrine with almost the same activity. Elacridar (**14**) is a strong inhibitor of the ABC transporters MDR-1 (P-gp) and BCRP. The bioavailability of cytotoxic antitumor drugs is enhanced by elacridar. Moreover, the levels of anti-HIV drugs in the brain and CNS are much higher after administration of elacridar. Rational drug design based on the concept of privileged structures was applied on acridine and quinoline scaffolds including dendrimers in study of neurodegenerative and protozoan diseases [8].

The aim of this book is an update and extension of the previous review [4] with focus to acridine–protein interactions and use of acridines for treatment of neurodegenerative diseases. However, since acridines are potent binders of nucleic acids including quadruplexes, chapter describing this topic cannot be omitted.

Owing to immense amount of published references, we are apologizing to all authors, whose papers are not included.

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Chapter 2
Nomenclature

Abstract Acridines have been known since 1870. Thus, their nomenclature has developed for long time. There are many numbering systems from which we have selected two most important ones. Moreover, plenty of trivial names exist and one has to be very careful when searching in the information databases.

This chapter provides a brief introduction for diving into the deep sea of acridine compounds without drowning, but will not show all possible names of acridines, which is far beyond the scope of the book.

Acridine was discovered and named by Graebe and Caro in 1870, when they investigated aromatic fractions of coal tar and found some basic impurity accompanying anthracene [1]: They named the new substance as "Acridin because of the sharp and biting effect that it exerts on the skin" [1] (from Latin acer = sharp, pungent, or acrid). This strong physiological effect is observable almost immediately after a drop of chloroform solutions of acridine touches a skin and persists for several minutes. The name "Acridin" (German) was imported to many languages with variations of c-k (Czech) and in some with suffixes -e (English, French) or -a (Spanish, Portuguese, Italian).

In past, the acridine ring was numbered differently than it is nowadays (Fig. 2.1) (cf. one of formers [2–5] and current [5–8] numbering). The current numbering reflects the locant order the same as was coined by Graebe [9], who applied it during understanding of 9-acridone structure. However, the numbers for carbon shared by more cycles were introduced later [5]. Thus, careful inspection of formerly published chemical structures is necessary in order to avoid confusion. For example, the most important 9-aminoacridines were called 5-aminoacridines in former works.

Concerning the numbering of acridine substituents, sometimes wrong labels are also used in literature. *m*-Amsacrine (**6**) can be named 4'-(9-acridinylamino) methanesulfon-*m*-anisidide [10], but when 1'-methanesulfonamide group is removed the priority of substituents is altered and 1' locant becomes 4' [11]. Sometimes priority of substituents is not followed and e.g. 5-hydroxymethyl is wrongly named 4-hydroxymethyl despite higher priority of carboxamide group [12].

Since the acridines are in use for more than a century, many of them have more than one trivial name [4, 6, 7, 13]: e.g. quinacrine (**11**) can be also named mepacrine,

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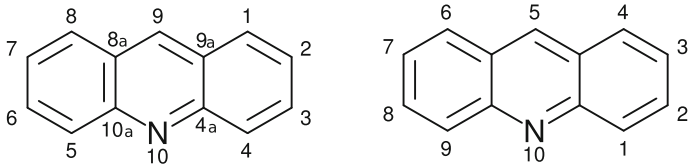

Fig. 2.1 Current (**15**) and former (**16**) numbering of acridine ring

and atabrine or atebrine. Other names for tacrine (**12**) are THA, cognex, tenakrin, or romotal [14–16]. Velnacrine (**13**) can be named 1-acridinol, 1-hydroxytacrine, and HP029 [17, 18]. Ledakrin can serve as a name for nitracrine (**8**) [19]. Thus, one has to be very careful conducing database-based search of acridines.

Some biologically active acridines are usually available not only under their trivial names but also under code numbers. Many examples can be given: GF120918 or GG918 can be named elacridar (**14**) [20] (PubChem CID: 119373); CI-921 stands for asulacrine (**7**) [12, 21, 22] (PubChem CID: 107924) ; NSC 601316 or XR 5000 is DACA (**5**) [23, 24] (PubChem CID: 107805); aminacrine (**4**) (PubChem CID: 7019); amsacrine (**6**) (PubMed CID: 2179); velnacrine (**13**) has number HP029 (PubChem CID: 3655); etc. Moreover different pharmaceutical forms have different numbers such as free proflavine (**2**) (PubChem CID: 7099); its salts: proflavine sulfate – isoflav (PubChem CID: 11111); proflavine hydrochloride (PubChem CID: 197873); proflavine hemisulfate (PubChem CID: 15741); etc.

Sometimes trivial name contained "acridine" despite the structure lacks the acridine ring i.e. acridine red is derivative of xanthene.

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Chapter 3
Syntheses

Abstract Modern syntheses of acridine scaffolds together with the conventional ones are described, thus the reader can easily select the appropriate one for a desired compound. For preparation of acridines, precursors with more aromatic/alicyclic rings are usually assembled using various catalysis. Then, electrophilic condensation led to formation of acridine ring. Modern syntheses are based on Buchwald–Hartwig amination, hypervalent iodine chemistry, iodocyclization, Rh(III)-catalyzed tandem reactions, and recyclable polymeric acido-basic catalysis. Usually, substituents are introduced by selection of appropriate precursors during the ring formation. Because the acridines readily undergo aromatic nucleophilic substitution at *C9* position, various derivatives are prepared by reaction with nucleophiles from *C9*-activated acridines. At positions 3 and 6, the aromatic electrophilic substitutions occurred; however, overreactions are possible. Many building blocks for peptide synthesis bearing acridine unit are available. The synthesized peptide conjugates can serve as drugs, gene delivery systems, imaging agents, etc. Furthermore, acridines with chelating groups can be used as rigid ligands for organometallic catalysis.

3.1 Syntheses of Acridine Ring and Its Precursors

Retrosynthetic scheme describes ways towards basic precursors of substituted acridines derivatized at position 9 (Fig. 3.1). Acridines substituted at position 9 are readily available by aromatic nucleophilic substitutions (Fig. 3.1, transformation i. and vi.). The acridine derivative (**17**) is obtained by direct reaction of nucleophile with activated acridine (**18**) [1] or reaction of strong nucleophile with *C9* unsubstituted acridine (**21**) in the presence or absence of hydride ion scavenger – Chichibabin like reaction [1–5]. However, the use of very strong bases makes the Chichibabin reaction harsh for a wide spectrum of functional groups. When X is chlorine, the 9-chloroacridines (**18**) are the most common precursors of 9-alkoxy-, 9-alkylthio-, 9-amino-, and 9-cyanoacridines [6–14]. Recently, the reaction of chloroacridines with sulfur nucleophiles was investigated by combination of computational and kinetic approaches [15]. Other important precursors of 9-substituted acridines are 9-alkoxyacridines, where the leaving group is very mild acid compatible with many

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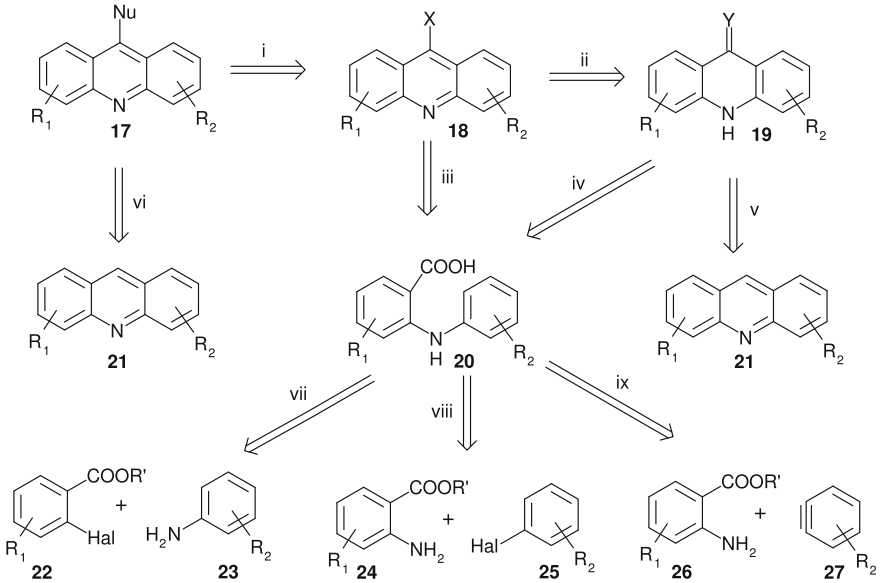

Fig. 3.1 Retrosynthetic scheme providing basic precursors of 9-substituted acridines. X stands for F, Cl, Br, I, NO_2, N_2^+, OR, SR, and NHR, respectively. Y represents O and S. Hal is F, Cl, Br, I. R_1 and R_2 are desired substituents sometimes with appropriate protection. Descriptions of individual transformations are only illustrative for more details see also text: *i*. HNu. *ii*., *iii*. POX_3, reflux. X = Cl, Br. *iv*. H_2SO_4 or PPA 100 °C, or P_4S_{10} dehydration. *v*. S_8, 220 − 230 °C. *vi*. NaNu, $K_3[Fe(CN)_6]$. *vii*, *viii*. 1. Ullmann–Jourdan reaction (Cu powder, K_2CO_3, pyridine, amyl alcohol, reflux) or Buchwald–Hartwig amination ($Pd(OAc)_2$, DPEPhos), 2. NaOH (optional). *ix* DCE, 80 °C

acid sensitive linkers [16]. 9-Heterocyclic substituted acridines can serve as activated acridines, as well [17–19]. Nucleophilic substitution at *C9* position is sometimes reversible, thus groups such as alkoxy, alkylthio, alkylamino are more or less interchangeable [20–27].

Alternative way to syntheses of *N9*-alkyl derivatives of acridines is a reductive amination of commercially available 9-aminoacridine [28, 29] using $NaBH_3CN$ with 1% AcOH/MeOH. Another approach is based on reaction of 9-aminoacridine with arenes activated for S_NAr such as *o* and *p*-nitrofluorobenzenes [28], *o* and *p*-halopyridines, and *o*-halopyrimidines [29] using Cs_2CO_3 as a base and DMF as a solvent at 90 °C. In some cases, the 9-aminoacridine can react with haloquinone electrophiles under the same conditions. However, when the reaction with quinones is carried out in refluxing EtOH in the absence of base, it provides more general route towards acridino-quinone hybrids [29].

N9-acylacridines can be prepared by reaction of acyl chlorides with 9-aminoacridine [30]. The prepared acridin-9-yl acrylate was used for syntheses of fluorescent polymers. Other acylations of 9-aminoacridine were described with DIC/HOBt/NMM [31, 32] and DCC/HOBt [33]. Aminoacridines can be also derivatized with isothiocyanates to corresponding thioureas [34].

Treatment of *N*-phenylanthranilic acids (**20**) or (thio)acridones (**19**) with $POCl_3$ led to activated 9-chloroacridines (**18**) [6–11]. During the reaction of the acid, firstly, the corresponding acridone is formed which is subsequently chlorinated. Activated 9-methylthioaciridines (**18**) can be prepared from corresponding thioacridones (**19**) with methyliodide [35]. Other activated compounds (**18**) are prepared by nucleophilic substitution. In some cases, the activation with $POCl_3$ can be carried out "one pot" with subsequent nucleophilic substitution, e.g. with 1,2,4-triazole [18]. The synthesis of chloroacridines as well as their conjugation with amines can be accelerated by microwave irradiation [36].

9-Acridones (**19**) are prepared by dehydration of *N*-phenylanthranilic acids (**20**) with H_2SO_4 or polyphosphoric acid [7, 37]. If *in-situ* prepared 9-chloroacridines are hydrolyzed during workup, dehydration can be carried out with $POCl_3$, as well [38]. Thioacridones are available by dehydration with P_4S_{10} via route iv (Fig. 3.1). However, when some acridine precursor is available, e.g. acridine orange (AO) (see also Fig. 3.21, compound **101**), it can be oxidized by elemental sulfur to thioacridone (**19**) via route v [35]. Similarly to pyridine chemistry, acridine-*N*-oxides underwent rearrangement to 9-acridinyl (thio)acetate upon treatment with acetyl anhydride or acetyl thioanhydride [39, 40]. These esters decomposed to corresponding acridones or thioacridones. Other syntheses of acridones and thioacridones have been recently reviewed [41, 42].

Acridones may be reduced with Al amalgam and subsequent reoxidation with $FeCl_3$ [43–45] provides acridines unsubstituted at *C9* position. This reaction tolerates various groups such as carboxylic acid.

The Lehmstedt–Tanasescu reaction enables the synthesis of acridone **30** and its derivatives from 2-nitrobenzaldehyde (**28**) and aromatic components **29** [46–48] (Fig. 3.2) or from benzo[c]isoxazoles (anthranil derivatives) [49].

Acridines and phenanthrolines have been synthesized from β-chlorovinyl aldehydes and various aniline derivatives. Many valuable substituents including ketone, nitro or amino groups at the heterocyclic core were incorporated into the acridine ring [50].

Acridine reacts with aliphatic carboxylic acids to 9-alkylacridans under photochemical decarboxylative conditions [51]. Illumination of δ-(9-acridyl)valeric acid or ε-(9-acridyl)caproic acid by UV light affords spiro compounds, 9,9-tetramethylene- and 9,9-pentamethyleneacridan, respectively. Broadening of the reaction to quinoline as a substrate yields 2- and 4-alkylquinolines and 4-alkyl-1,2,3,4-tetrahydroquinolines. Analogous reaction of isoquinoline gives 1-alkylisoquinolines.

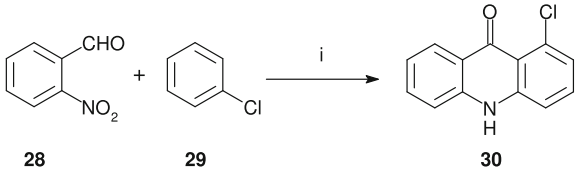

Fig. 3.2 Lehmstedt–Tanasescu acridone synthesis [46–48]. *i*. $NaNO_2$, H_2SO_4

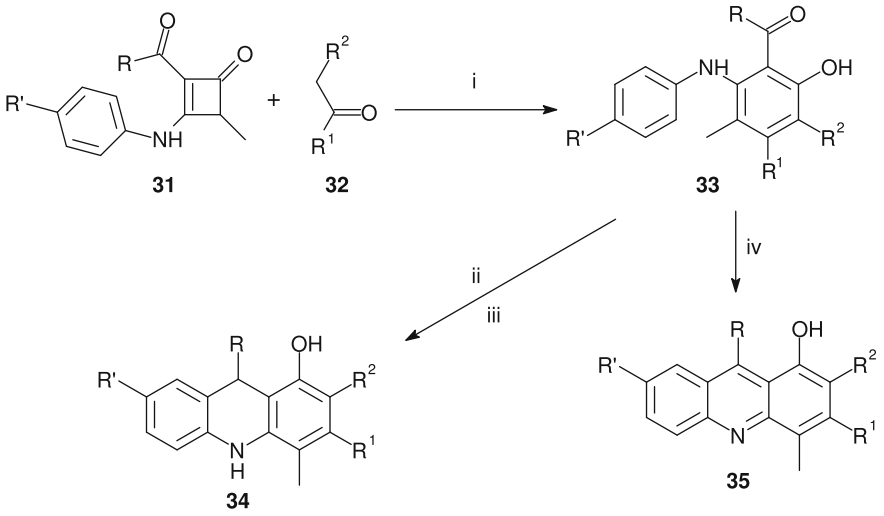

Fig. 3.3 Regiospecific [4 + 2] cycloaddition as an alternative route towards polysubstituted *N*-phenylanthranilic acids and acridines [64]. *i*. LDA/THF 80–140 °C. *ii*. $NaBH_4$/MeOH. *iii*. $BF_3.Et_2O$. *iv*. $In(OTf)_3$/DCE reflux

N-Phenylanthranilic acids (**20**) are available via three main routes by Ullmann–Jourdan reaction (routes vii and viii) [37, 38, 52–56], by Buchwald–Hartwig amination (routes vii and viii) [57, 58], and by reaction with thermochemically generated benzyne (route ix (Fig. 3.1)) [59]. The Ullmann–Jourdan reaction is limited to electron rich anilines and electron poor benzoic acids, otherwise Buchwald–Hartwig amination is recommended [60]. The Ullmann–Goldberg reaction can be accelerated by ultrasonication of the reaction mixture [61]. Recently, direct transformation of aromatic amines to anthranilic acids was described using Pd-catalysis [62]. Another example of Pd-catalysis is direct amination of benzoic acids [63].

A benzannulation approach for synthesis of *N*-phenylanthranilic acids proceeding via a regiospecific [4 + 2] cycloaddition of easily accessible cyclobutenones and active methylene ketones was developed [64] (Fig. 3.3). Applying this strategy, persubstituted anilines and phenols having up to six different functional groups on the benzene ring were obtained. This base-accelerated benzannulation reaction is completely regiocontrolled. In one step, many functional groups are introduced into the benzene ring. These polysubstituted *N*-phenylanthranilic acids (**33**) served as building blocks for syntheses of highly substituted acridine derivatives (**34** and **35**).

Formal [3 + 3] annulations of aromatic azides and aromatic imines or azobenzenes provided acridines and phenazines, respectively [65]. This is a cascade process, which is Rh(III)-catalyzed, consisting of amination, intramolecular electrophilic aromatic substitution and aromatization. Acridines are directly synthesized from aromatic aldehydes using in situ generated imines with benzylamine as catalyst.

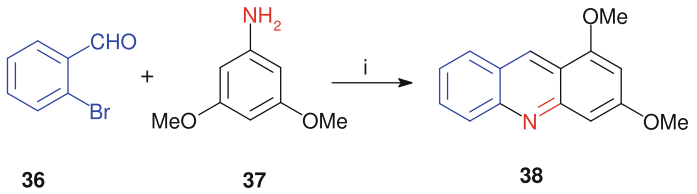

Fig. 3.4 Tandem Buchwald–Hartwig coupling and cyclization reaction [66]. *i*. $Pd_2(dba)_3$ (2.5 mol %), dppf (5 mol %), NaOtBu (2 eq), toluene, reflux, 12 h

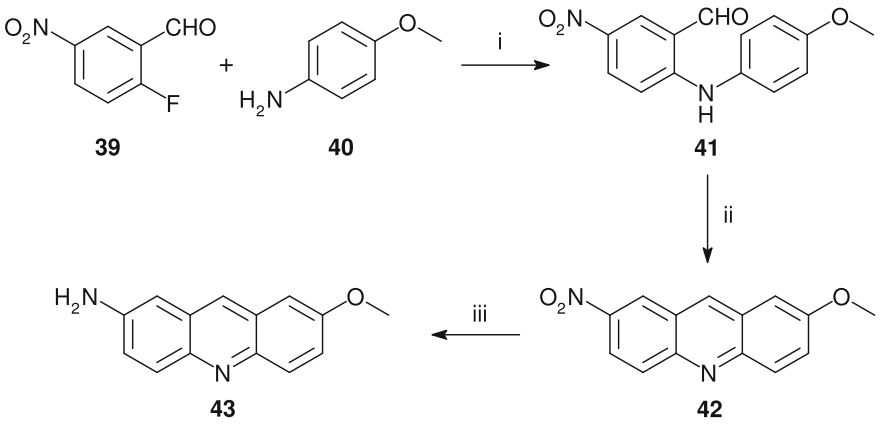

Fig. 3.5 Synthesis of 2-aminoacridines [67]. *i*. Et_3N, DMSO, 120 °C. *ii*. TFA, 80 °C, 24h (83% over two steps). *iii*. Pd/C, H_2, MeOH, DCM, 0 °C → 20 °C

Synthesis of series of acridines via a facile and efficient approach by the tandem coupling/cyclization of substituted 2-bromobenzaldehydes (**36**) and anilines (**37**) was described [66] (Fig. 3.4). The reaction can be easily completed by a catalytic amount of $Pd_2(dba)_3$ and diphosphine ligand dppf, giving a broad variety of substituted symmetrical and unsymmetrical acridines carrying multifunctional substituents as well as the unusual 1,2,3,4-tetrahydroacridine in good to excellent yields (up to 99%). The cyclization of less electron-rich anilines requires the promotion by Lewis acid like $AlCl_3$. Antileukemic agent DACA can be easily obtained in just two simple steps using this method.

Anilines react with 2-fluoro-4-nitrobenzaldehyde (**39**) affording suitable precursors **41** for acridine formation (Fig. 3.5). The acridine ring **42** is closed with TFA at elevated temperature. Careful hydrogenolysis of nitro group provides 2-aminoacridines **43** [67].

Derivatives of anthranilic acid can be constructed from arynes, where three component reactions among aryne, amine, and CO_2 occur [68]. Very reactive arynes, generated by elimination of TMS-OTf, can convert hydrazones to acridones or acridinium salts [69]. Generated arynes reacted with electron-deficient acridines in presence of dimethyl phosphite via 9,10-addition to heterocyclic system (Fig. 3.6) [70].