Phenazine Natural Products: Biosynthesis, Synthetic Analogues, and Biological Activity

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Contents

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1. Introduction

The medicine man of prehistoric time knew of the healing capacity of leaves, oils, extracts, roots, etc. from the surrounding flora. A widely acknowledged example of natural medicine from ancient times is willow bark (*Salicis cortex*), which was used as an analgesic and antifebrile by early Indian and Middle Eastern civilizations. The active substance therein was later identified as salicylic acid. Accordingly, recipes described in ancient texts have both potential and actual value for treatment of human diseases today,[1] and plant extracts as well as fermentation broths from soil bacteria are still widely recognized to provide efficient drugs. In addition, natural products serve as leads in the development of new synthetic drugs. Of all drug entities launched in the period from 1983 to 1994, as many as 60% of new anti-infective and anticancer drugs were derived from natural products, and this trend seems to continue.[2−4]

The history of modern antibiotics began in 1928, when Alexander Fleming discovered penicillin in a mold culture. A decade later, this active substance was produced and developed into the first useful antibiotic by Howard Walter Florey and Ernst Boris Chain.[5] For many years, the discovery and production of penicillin remained one of the most important medicinal accomplishments for humankind. However, the imminent emergence of resistance among pathogenic bacteria has prompted researchers to search continuously for new antibiotics. Traditionally, the major source of commercial natural antibiotics has been secondary metabolites produced by soil bacteria. However, recent discoveries from therapeutically rather unexplored sea-living species have revealed new natural products with intriguing effects.[6] Both soil habitants and marine microorganisms are known to produce phenazine natural products with antibiotic effects, and this class of metabolites presents great potential for the discovery of new anti-infective agents.

More than 6000 phenazine-containing compounds have been identified and reported during the past century, including compounds arising from early synthetic attempts and phenazine−transition metal complexes of interest in physical and electrochemical research. Several hundreds of the known phenazines possess biological activities, including antimicrobial effects, and are either isolated from natural sources

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Jane B. Laursen was born in Aarhus, Denmark, in 1975. She received her M.Sc. degree in chemistry from the Technical University of Denmark in 2000 and her Ph.D. degree in bioorganic and medicinal chemistry in 2003, also from the Technical University of Denmark. During her Ph.D. studies she worked with design and parallel synthesis of natural product-based libraries of new potential antibiotics, directed by Professor John Nielsen. A class of phenazine-based metabolites formed the basis for this design and synthesis. She is presently pursuing a research career with one of the world's leading pharmaceutical companies, AstraZeneca, in Mölndal, Sweden.

John Nielsen was born in Holbæk, Denmark, in 1960. He received his M.Sc. degree in organic chemistry from the University of Copenhagen in 1984 and subsequently his Ph.D. degree in bioorganic chemistry in 1987, also from the University of Copenhagen. His thesis, entitled "Solid- and solution-phase synthesis of oligonucleotides and their analogues", was completed under the supervision of Prof. O. Dahl and the late Prof. Ole Buchardt. From 1987 to 1988, he was a postdoctoral fellow with Prof. M. H. Caruthers at the University of Colorado at Boulder, working on oligonucleotide phosphorodithioate analogues, and he returned to a faculty position at the University of Copenhagen in 1988. From 1990 to 1992, he worked as a research chemist at DAKO A/S (now DakoCytomation A/S), and then he moved back to the United States as a senior research associate with Professors R. A. Lerner, S. Brenner, and K. D. Janda at The Scripps Research Institute (TSRI) in La Jolla. At TSRI, he was instrumental in the implementation of DNA-encoded combinatorial chemistry. In 1994, John Nielsen returned to a tenured position as an associate professor at the chemistry department at the Technical University of Denmark in Kgs. Lyngby, and continued his research interest in natural products and combinatorial chemistry. In 2002, he was promoted to full professor of bioorganic chemistry at The Royal Veterinary and Agricultural University in Frederiksberg inside Copenhagen. His current research interest remains in biologically active compounds, natural products, and combinatorial chemistry.

or synthetically constructed. This review will deal exclusively with a representative selection of these biologically significant phenazines, their natural occurrence, their biosynthesis, the design and synthesis of analogues, and their biological function and possible mode of action. In particular, phenazines produced by streptomycetes will be reviewed. Furthermore, generally recognized methods for phenazine synthesis will be reviewed. The present review should be considered as a presentation of highlights in the discovery of natural and synthetic phenazine compounds of biological significance and not as a comprehensive coverage of the literature on phenazines in general.

2. Phenazine Natural Products

Phenazine natural products are isolated as secondary metabolites primarily from *Pseudomonas*, *Streptomyces*, and a few other genera from soil or marine habitats. The biological properties of this class of natural products include antibiotic, antitumor, antimalaria, and antiparasitic activities. The role of phenazine pigments as antibiotics and virulence factors has been briefly reviewed recently,[7] and the biochemistry of phenazine production was reviewed in 1986.[8]

2.1. Pseudomonads

The Gram-negative pathogen *Pseudomonas* was known as the first and for several years also considered as the only genera of bacteria to produce phenazine pigments. The blue pyocyanin (**3**, Figure 1) and the green chlororaphine (**2**, Figure 1), both produced by *Pseudomonas aeruginosa*, were isolated in the late 19th century; this was followed by isolation of the purple iodinin (**6**, Figure 1) from *Pseudomonas aureofaciens* in 1938.[8] Phenazines produces by *Pseudomonas* have been reviewed earlier[9,10] and will be summarized only briefly in the following.

Phenazines isolated from *Pseudomonas* strains (e.g., *aeruginosa*, *aureofaciens*, *fluorescens*, and *cepacia*) are mostly simple hydroxyl- and carboxyl-substituted structures (Table 1), often C_2-symmetric,

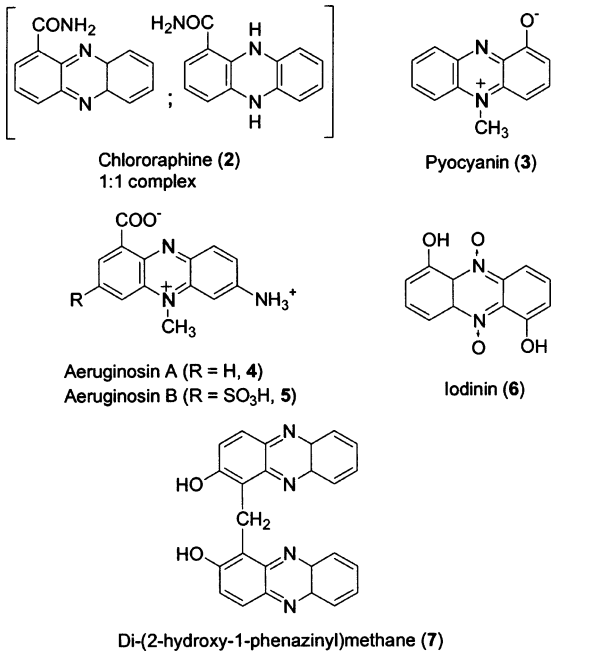
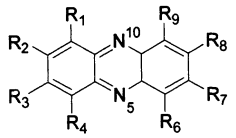


Figure 1. Other phenazines produced by *Pseudomonas* sp.

Table 1. Simple Phenazine Compounds Produced by *Pseudomonas* sp[9,10]



1a-1u

compd	R_1	R_2	R_3	R_4	R_6	R_7	R_8	R_9	trivial name
1a	OH								hemipyocyanin
1b		OH							
1c	OH				OH				
1d	OH						OH		
1e		OH	OH						
1f		OH	OH			OH			
1g	OH						NH_2		
1h	COOH								tubermycin B
1i	COOH	OH							
1j	COOH			OH					
1k	COOH				OH				
1l	COOH							OH	
1m	COOH	OH	OH						
1n	COOH	OH			OH				
1o	COOH	OH						OH	
1p	COOH	OH	OH	OH					
1q	COOH				COOH				
1r	COOH	OH	OH		COOH	OH			
1s	$COOCH_3$				$COOCH_3$				
1t	$COOCH_3$			OH	$COOCH_3$				
1u	$COOCH_3$			OH	$COOCH_3$			OH	

like **1c**, **1q**, **1s**, and **1u**.[8−13] Phenazine-1-carboxylic acid, also known as tubermycin B[14] (**1h**) because of its antibiotic activity against *Mycobacterium tuberculosis*, and phenazine-1,6-dicarboxylic acid (**1q**) are believed to be metabolic precursors for other phenazines, as discussed in the section on "Biosynthesis of Phenazines" (section 3).[15,16]

Besides the already-mentioned chlororaphine (**2**, a 1:1 complex of phenazine-1-carboxamide and its 5,10-dihydro derivative; Figure 1)[15,16] and iodinin (**6**, 1,6-dihydroxyphenazine-5,10-dioxide; Figure 1), other phenazines produced by pseudomonads include aeruginosins A (**4**) and B (**5**), isolated from *P. aeruginosa* and *P. iodina*, and the dimeric structure (**7**), di(2-hydroxy-1-phenazinyl)methane, isolated from a pigmentation mutant of *P. aureofaciens* (Figure 1).[8−10,17−21]

Tubermycin B (**1h**) and chlororaphine (**2**) efficiently inhibited growth of *Bacillus cereus* (MIC < 0.5 μg/mL) but showed only modest antibiotic activity against *Micrococcus luteus* and *Staphylococcus aureus* (MIC > 5 μg/mL).[15] Iodinin (**6**) exhibited great antibiotic activity against Gram-positive bacteria, several actinomycetes, and some fungi but none against Gram-negative bacteria. 1,6-Dihydroxyphenazine (**1c**) exhibited modest activity against Gram-positive bacteria and actinomycetes but was not active against Gram-negative strains or fungi.[21] Pyocyanin (**3**) displayed broad-spectrum bactericidal effects and is believed to protect the pyocyanin-producing organism against competing microbes.[22]

2.2. Streptomycetes

Several of the simple phenazines described above are also produced by the Gram-positive actinomycete, *Streptomyces* (e.g., **1h**, **3**, and **6**), but in the following we present metabolites that are uniquely produced by this genera. Common for these metabolites is a more complex structure, often comprising one or more *C*-substituents on the phenazine core. The first phenazine isolated from streptomycetes was the antibiotic griseolutein (**13**/**14**, Figure 2), reported in 1951 by Umezawa et al.[23] In recent years, *Streptomyces* species (e.g., *griseolutein*, *luteogriseus*, *antibioticus*, and *prunicolor*) have been the natural source of numerous diverse and complex phenazines, including some 5,10-dihydrophenazines with biological significance. In the following, the occurrence and activity of these phenazines will be summarized in subclasses of similar structural characteristics.

2.2.1. Simple Phenazines

Like pseudomonads, *Streptomyces* strains produce simple hydroxyl- and carboxyl-substituted phenazines along with methoxy- and methylester derivatives (Table 2), including several C_2-symmetric compounds (**8b** and **8e**). The weak antibiotics phencomycin (phenazine-1,6-dicarboxylic acid monomethyl ester, **8k**; Table 2) and its 5,10-dihydro methyl ester were isolated from soil and marine *Streptomyces* strains, respectively.[24,25] The common phenazine precursor phenazine-1,6-dicarboxylic acid (**1q**), also isolated from *Streptomyces* sp., was reported to inhibit the free radical (superoxide, $O_2^{\bullet-}$)-producing enzyme, xanthin oxidase.[26] Mycomethoxins A and B (1,2,6-trimethoxyphenazine, **8f**, and 6-methoxyphenazine-1-carboxylic acid methyl ester, **8h**; Table 2), isolated from *S. luteoreticuli*, showed potent antibacterial activity against pathogenic mycobacteria, including some resistant strains.[8]

Phenacein (**8g**, Table 2), isolated from *S. tanashiensis-zaomyceticus*, and the pentasubstituted

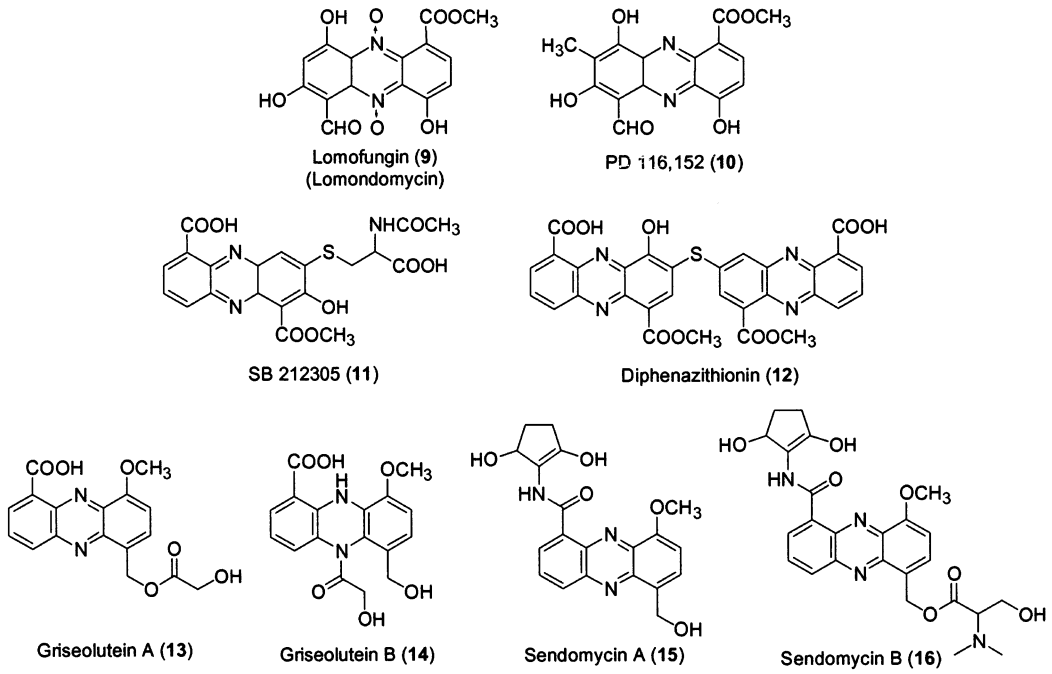
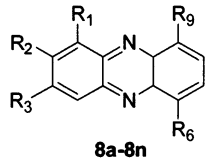


Figure 2. Some phenazines isolated from *Streptomyces* sp.

Table 2. Selected Simple Phenazine Compounds Produced by *Streptomyces* sp.[8]



compd	R_1	R_2	R_3	R_6	R_9	trivial name
8a	OH					hemipyocyanin
8b	OH			OH		
8c	OH			OCH_3		
8d	OCH_3					
8e	OCH_3			OCH_3		
8f	OCH_3	OCH_3		OCH_3		mycomethoxin A
8g	COOH		OH	OH		phenacein
8h	COOH			CH_2OH		mycomethoxin B
8i	COOH			CH_2OH	OH	
8j	COOH			CH_3	OCH_3	
8k	COOH			$COOCH_3$		phencomycin
8l	COOH	OH		$COOCH_3$		SB 212021
8m	$COOCH_3$					
8n	$COOCH_3$			OCH_3		

di-*N*-oxide, lomofungin (**9**, Figure 2), isolated from the soil habitant *S. lomondensis*, have different pharmacological targets. They were reported to inhibit angiotensin-converting enzyme (ACE), a target for treatment of hypertension, with IC_{50} values of 0.13 and 16 μM, respectively. The mechanism of action was believed to involve chelation with Zn^{2+} in the active site of the enzyme, and the hydrophilic 1- and 6-substituents were crucial for activity. Lomofungin (**9**) showed moderate antibiotic activity against both Gram-positive and Gram-negative bacteria, while phenacein (**8g**) was less active.[27−30]

2.2.2. More Complex Phenazines

More highly substituted phenazines include aldehydes, thioethers, ester and amides, which besides being antibiotic exhibit radical scavenging and antitumor activity.

The hexasubstituted phenazine PD 116,152 (**10**, Figure 2) resembled lomofungin (**9**, Figure 2) in structure and was found to have great inhibitory activity (MIC 0.46 μg/mL) against *Streptococcus pneunomiae* but insignificant or no activity at all against other screened bacteria and fungi. However, cytotoxicity was observed against L1210 lymphocytic leukemia and human colon adeno-carcinoma (HTC-8) cells with IC_{50} values of 0.52 and 0.71 μg/mL, respectively; moderate in vivo antitumor activity was observed against P388 lymphocytic leukemia cells.[31,32]

The trisubstituted phenazine SB 212021 (**8l**, Table 2) and its thioether analogue, SB 212305 (**11**, Figure 2), isolated from an unidentified streptomycete, showed only limited antibacterial activity. However, they were shown to inhibit zinc-dependent metallo-β-lactamase from *Bacillus cereus* by chelation of the active site with IC_{50} of 50−68 μM.[33] Another sulfur-

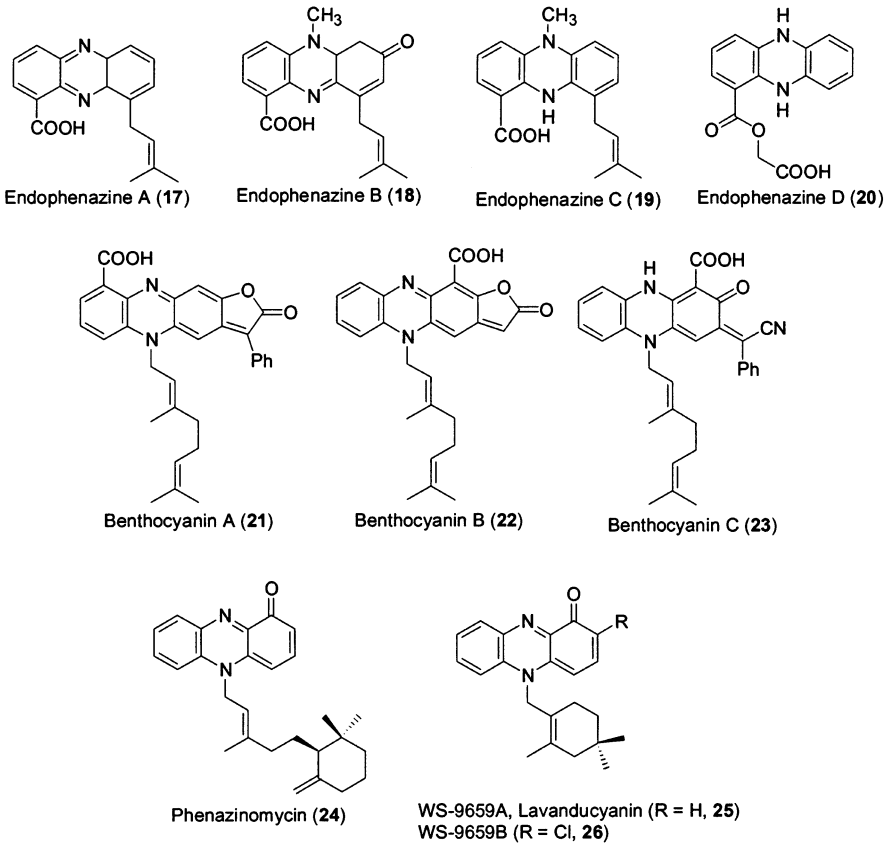


Figure 3. Terpenoid phenazines and related derivatives isolated from *Streptomyces* sp.

containing phenazine, diphenazithionin (**12**, Figure 2), was isolated from *S. griseus* ISP 5236 and shown to inhibit lipid peroxidation because of its strong antioxidant activity.[34]

Derivatives of 6-(hydroxymethyl)phenazine-1-carboxylic acid are present in several isolates. Hydroxyacetic acid derivatives griseoluteins A (**13**) and B (**14**, Figure 2) were isolated from *S. griseoluteus* and possessed antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria.[35−40] The enol amide sendomycin A (**15**, Figure 2) and its *N*,*N*-dimethylserine ester, sendomycin B[41] (**16**, also known as senacarcin A or DC-59A; Figure 2), were isolated from *S. endus* subsp. *aureus* DO-59. They possessed broad spectrum antibiotic activity and proved carcinostatic toward sarcoma 180 cells.[8,42]

2.2.3. Terpenoidal Phenazines

A common structural feature of several phenazines from *Streptomyces* is isoprenylated *C*- or *N*-side chains. These terpenoid phenazines were isolated from several different strains; besides potentially similar biosynthetic pathways, no obvious pattern in their biological activity could be derived from the literature.

C-Isoprenylated endophenazines A−C (**17**−**19**) were isolated along with endophenazine D (**20**, Figure 3) and tubermycin B (**1h**) from *S. anulatus*, a symbiotic actinomycete living in the intestines of millipedes, beetles, and wood lice. It has not been established whether the antibiotic compounds are of importance for their symbiotic life forms. Endophenazine A (**17**) and D (**20**) showed good antibiotic activity against Gram-positive bacteria and some fungi, while endophenazine B (**18**) and C (**19**) were void of activity. Endophenazine D (**20**) and tubermycin B (**1h**) showed herbicidal effect against *Lemna minor* and inhibited growth of the fungus *Botrytis cinerea*, while none of the isolated compounds inhibited the growth of Gram-negative bacteria.[43,44]

The *N*-monoterpenoids benthocyanin A−C (**21**−**23**, Figure 3) were isolated from *S. prunicolor* and shown to be powerful radical scavengers. Benthocyanin B (**22**) and C (**23**) inhibited lipid peroxidation induced by free radicals in rat liver microsomes with IC_{50} values of 0.16 and 0.29 μg/mL. These activities were 30−70 times as strong as that of vitamin E, whereas benthocyanin A was twice as active as vitamin E. Benthocyanin B (**22**) and C (**23**) were also reported to inhibit rat erythrocyte hemolysis with IC_{50} values of 0.56 and 1.30 μg/mL, respectively.[45−47]

Another *N*-isoprenylated compound, phenazinomycin (**24**, Figure 3), was isolated from a *Streptomyces* strain and exhibited activity against murine tumors in vivo and cytotoxicity against adriamycin-resistant P388 leukemia cells.[48] Related *N*-substituted monoterpene analogues WS-9659A (lavanducyanin, **25**) and B (**26**, Figure 3) were isolated from *Streptomyces* sp. CL190 and 9659 and showed antitumor activity against P388 and L1210 cancer cells ($IC_{50} \approx 0.1$ μg/mL). They were also shown to be competitive inhibitors of testosterone 5α-reductase in rat, dog, and human prostates, lavanducyanin (**25**) being the most potent with IC_{50} values of 0.5−0.8 μM.[49−51] Carbohydrate-containing aestivopheonin A, B, and C (**38**−**40**) and phenazoviridine (**41**) are also isoprenylated

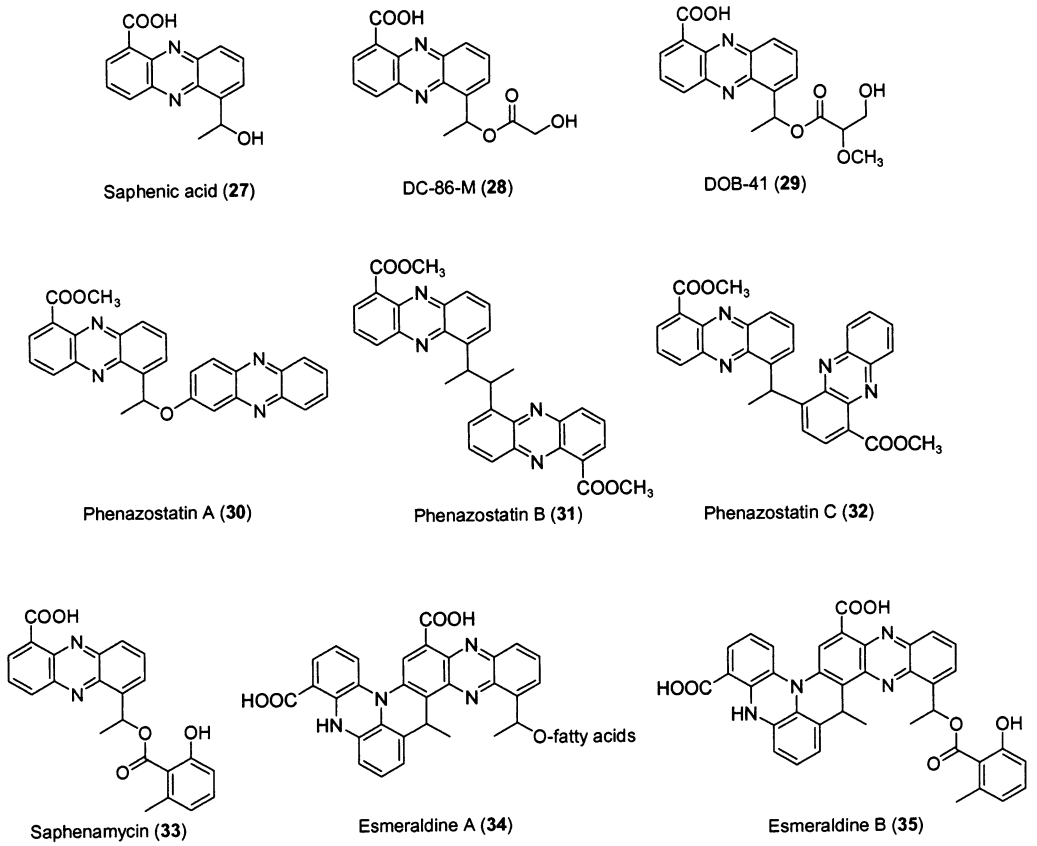


Figure 4. Saphenic acid derived isolates from *Streptomyces* sp.

(Figure 5; see section 2.2.5 on "Carbohydrate-Containing Phenazines").

2.2.4. Phenazines Derived from Saphenic Acid

The chiral 6-(1-hydroxyethyl)phenazine-1-carboxylic acid, saphenic acid (**27**, Figure 4), is present in phenazine metabolites from several *Streptomyces* strains, and its stereochemistry will be commented on in the section on "Chirality" (section 3.4). Besides this common "scaffold", the saphenic acid-derived metabolites have diverging structures, including antibiotic activity in the mono-phenazine derivatives **28**, **29**, and **33** (Figure 4) and radical scavenging activity of the di-phenazines **30**−**32** (Figure 4). *S. antibioticus* produces saphenic acid (**27**), its methyl ester, 6-acetyl-1-phenazine carboxylic acid, seven different fatty acid esters of saphenic acid, and saphenamycin (**33**, Figure 4). The latter was also isolated from *S. canaries* and showed high antibiotic activity, especially against Gram-positive bacteria (MIC <0.001−0.35 μg/mL). The ED_{50} value of saphenamycin (**33**) against eukaryotic tumor cell lines was 0.6 μg/mL, and the compound had a modest life-prolonging effect on mice with leukemia cell implants. Furthermore, saphenamycin (**33**) was reported to act as a mosquito larvacide and to improve feed efficiency in ruminants when administered in 0.5−1 mg/kg body weight of the animal.[36,52,53] Esmeraldines A and B (**34** and **35**, Figure 4) were isolated from the same *S. antibioticus* and could formally be derived from two molecules of saphenic acid. They had no antibacterial effect. However, the ED_{50} value against eukaryotic tumor cell lines was 0.4 μg/mL for esmeraldine B (**35**).[52,54]

The 3-hydroxy-2-methoxypropionic acid ester with unknown stereochemistry, DC-86-M (**28**, Figure 4), was isolated from the soil habitant, *S. luteogriseus*. It was strongly active against Gram-positive bacteria and weakly active against Gram-negative bacteria (MIC < 0.1−0.3 and 25−100 μg/mL, respectively) and had an LD_{50} against murine tumors below 25 mg/kg (administered i.p.).[55,56] The hydroxyacetic acid ester analogue, DOB-41 (**29**, Figure 4), also isolated from soil, was very active against Gram-positive bacteria (MIC 0.1−0.78 μg/mL) and showed moderate effect on the longevity of mice with implanted murine lymphatic P388 leukemia cells.[57]

Phenazostatins A, B, and C (**30**−**32**, Figure 4), diphenazines all structurally derived from saphenic acid, were isolated from *Streptomyces* sp. 833 as racemates and mixtures of possible diastereomers. They proved to be free radical scavengers; the C_2-symmetric dimer, phenazostatin B (**31**), was an inhibitor of phosphodiesterase, while all phenazostatins protected neuronal cells from glutamate toxicity with EC_{50} of 0.33−0.37 μM without any cytotoxic effect. The inhibition of glutamate toxicity was 2 times higher than that of idebenone, which is used as a brain-protecting agent in, e.g., senile dementia. All phenazostatins possessed antioxidant activity by a dose-dependent inhibition of lipid peroxidation induced by free radicals in rat liver microsomes, with EC_{50} values 6−10 times higher than of vitamin E.[58−60]

2.2.5. Carbohydrate-Containing Phenazines

Carbohydrate-containing phenazine natural products are rare, and only a few examples exist, all

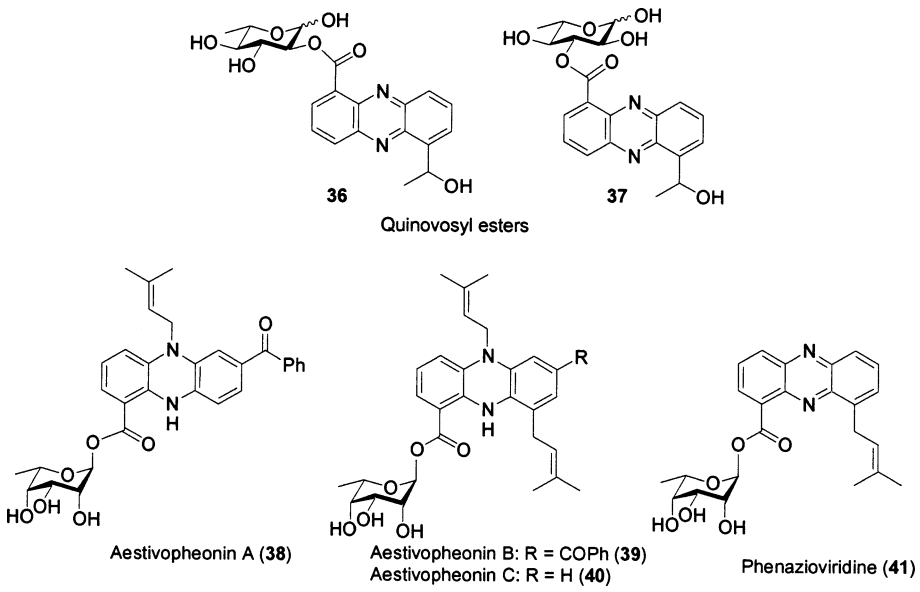


Figure 5. Carbohydrate-containing phenazines isolated from *Streptomyces* sp.

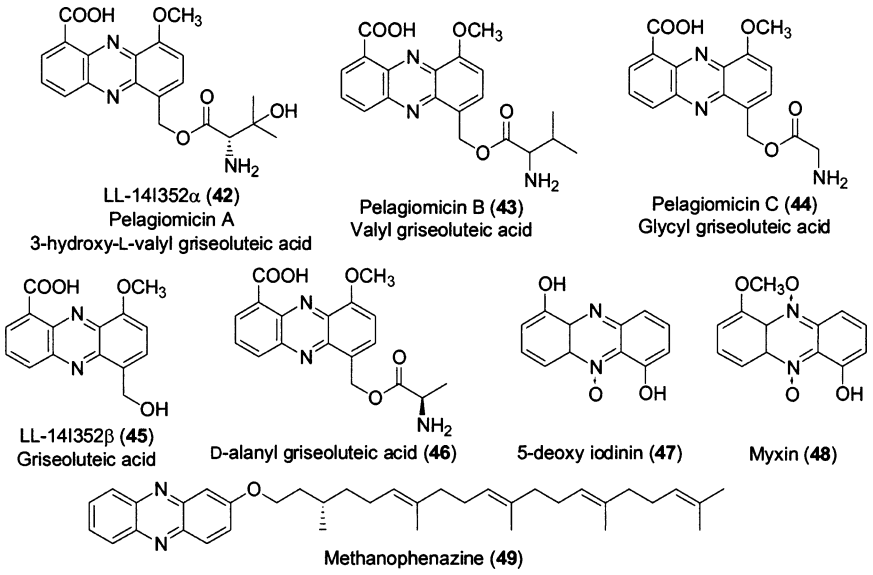


Figure 6. Phenazines isolated from other bacteria.

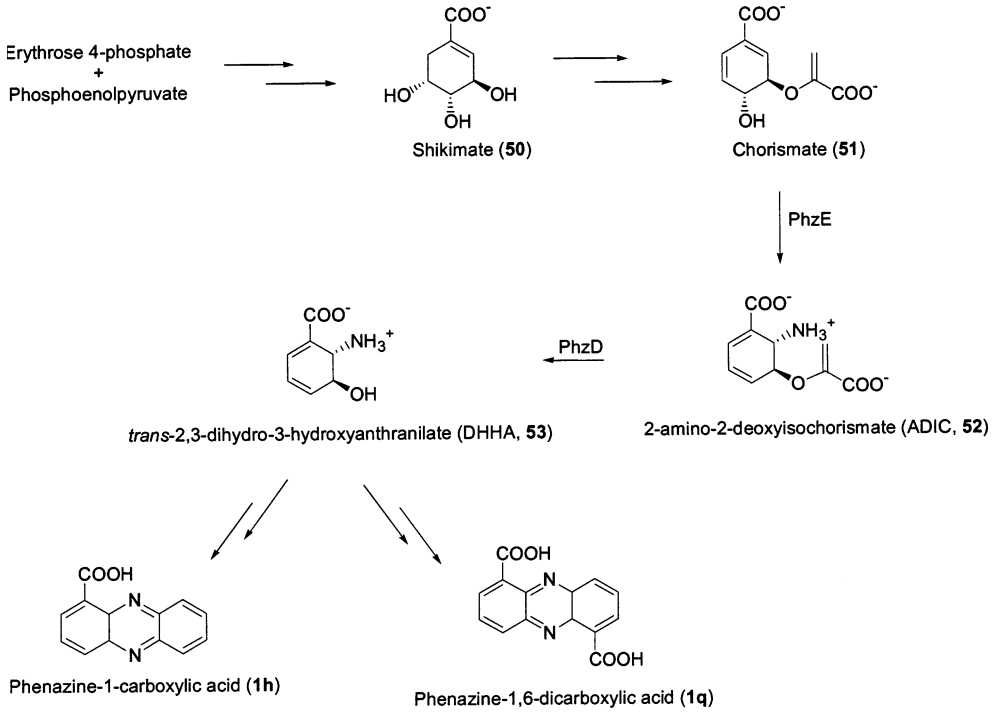
derived from 6-deoxy-L-glycopyranosides and exhibiting antibacterial activity. Additionally, isoprenylated derivatives showed radical scavenging abilities. 2-*O*- and 3-*O*-L-quinovosyl (6-deoxy-α-L-glucopyranosyl) esters of saphenic acid were isolated from a marine *Streptomyces* sp. as a mixture of α- and *β*-anomers (**36** and **37**, Figure 5). The α-anomers showed modest activity against a broad range of Gram-negative and Gram-positive bacteria, the 2-*O*-ester being the most active overall (MIC ≈ 4 *μ*g/mL). No cytotoxicity or antitumor activity was observed.[61]

Four 6-deoxy-α-L-talopyranosyl esters, aestivopheonins A, B, and C (**38**−**40**) and phenazoviridine (**41**, Figure 5), are other examples of carbohydrate-containing phenazines produced by *Streptomyces*. Phenazoviridine (**41**), isolated from *Streptomyces* sp. HR04, was the first glycoconjugated phenazine with antioxidant activity. It showed strong inhibitory activity against lipid peroxidation in rat brain homogenate, stronger than that of known antioxidants and radical scavengers, and also good protective activity against acute hypoxia in mice.[62] The aestivopheonins (**38**−**40**) were isolated from *S. purpeofuscus* and were likewise shown to have antioxidative activity, i.e., neuronal cell-protecting activity by inhibition of L-glutamate toxicity.[63]

2.3. Miscellaneous Genera

A few examples of other phenazine-producing species exist. The structure of the metabolites of these other species resembles that of the simple phenazines produced by *Pseudomonas* and *Streptomyces*, except for the lipid-like methanophenazine **49** produced by *Methanosarcina mazei* Gö1 (Figure 6). Amino acid esters of griseoluteic acid, pelagiomicins A−C (**42**−**44**, Figure 6), were isolated from the halophile Gram-negative marine bacterium *Pelagiobacter variabilis* collected from a macroalga. 3-Hydroxyvaline-derived pelagiomicin A (**42**) showed strong antibiotic activity against both Gram-negative and Gram-positive strains (<0.04−5.2 *μ*g/mL) but none against eukaryotes like yeast. It also proved cytotoxic

Scheme 1. Proposed General Biosynthetic Pathway of Common Phenazine Precursors, Phenazine-1-carboxylic Acid and Phenazine-1,6-dicarboxylic Acid[13,66]



against HeLa, BALB3T3, and BALB3T3/H-ras cell lines with IC_{50} values from 0.04 to 0.7 μg/mL and showed weak antitumor activity against P388 leukemia cells.[64] Pelagiomicin A (**42**) and griseoluteic acid (**45**, Figure 6) were isolated from another unidentified halophile marine bacterium living near a tunicate in the Pacific Ocean. The 3-hydroxyvaline analogue **42** proved 40-fold more active in antimicrobial assays than the parent griseoluteic acid **45**, indicating that the amino acid residue may enhance the compound's ability to penetrate bacterial membranes. None of the griseoluteic acid derivatives exhibited any binding to serum or DNA; however, they inhibited DNA, RNA, and protein synthesis. Cytotoxicity and antitumor activity were also observed in vitro, but lack of in vivo activity and high toxicology were observed for pelagiomicin A.[64,65] D-Alanyl griseoluteic acid (**46**) was isolated from *Vibrio* strains and *Erwinia herbicola* and exhibited antibiotic activity against a range of Gram-positive and Gram-negative bacteria as well as a n *Erwinia* pathogen.[66]

Other noteworthy phenazines that resemble simple metabolites produced by *Pseudomonas* include the mono-oxide derivative (**47**) of iodinin isolated from *Burkholderia phenazinium*[8,67] and *Waksmania aerata*[21] and the di-*N*-oxide, myxin (1-hydroxy-6-methoxyphenazine-5,10-di-*N*-oxide, **48**; Figure 6), isolated from a *Sorangium* species. Myxin showed in vitro inhibition of DNA and RNA synthesis and reduction of the protein synthesis. It was shown that myxin (**48**) affected the rate terms for incorporation of CTP and GTP into RNA but not for incorporation of ATP and UTP by interaction with CG base pairs in an intercalation fashion.[68] The first and so far only report on phenazines isolated from archaea was the recent isolation of methanophenazine (**49**) from the cytoplasmic membranes of *Methanosarcina mazei* Gö1 (Figure 6).[69] This unique terpenoid phenazine ether, produced by a methanogenic archaea, shows no resemblance to previous phenazine natural products and is believed to play an important role in membrane-bound electron transfer in the methanogenic pathway.[70]

3. Biosynthesis of Phenazines

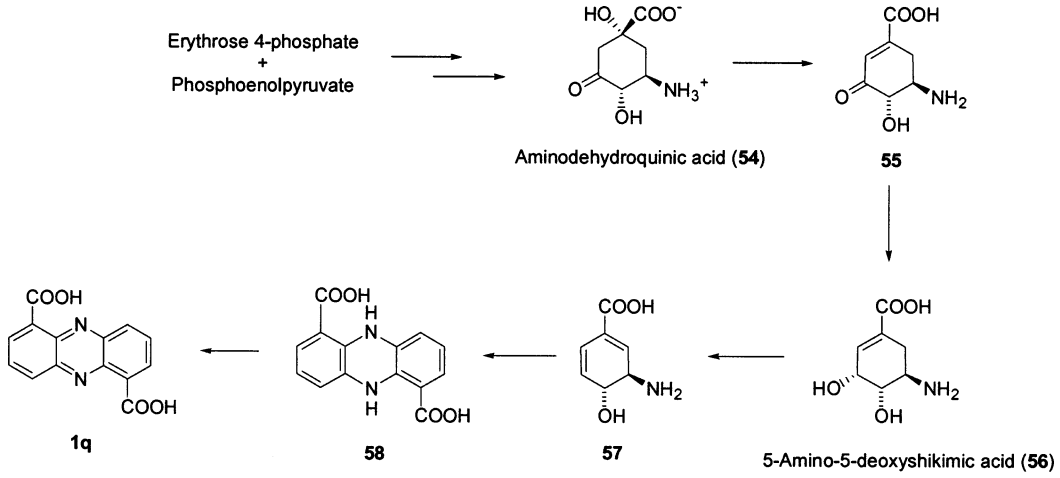
Phenazine natural products are believed to be secondary metabolites derived from a mutual primary metabolite. Most of the investigation of phenazine biosynthetic pathways has been carried out in *Pseudomonas* strains, but results from studies of *Streptomyces* support the belief that these species have similar biosynthetic pathways. Accordingly, it has been shown that phenazine-1,6-dicarboxylic acid (**1q**) and phenazine-1-carboxylic acid (**1h**) are precursors for more complex phenazine metabolites.

3.1. Shikimic Acid Pathway

Extensive studies support the hypothesis that these phenazine precursors are derived from the shikimic acid pathway, as outlined in Scheme 1, with chorismic acid (**51**) as the most probable branch point intermediate. Shikimic acid (**50**) is converted to chorismic acid (**51**) in known transformations that are part of the common aromatic amino acid biosynthetic pathway. The transformation from chorismic acid (**51**) to the phenazine precursors has been discussed and investigated through intensive biochemical studies; so far, no intermediates have been identified and little is known about the genetic origin and details of the phenazine biosynthesis.[9,71−73]

The amino group from glutamine is enzymatically transferred by PhzE, the phenazine synthesis-specific

Scheme 2. Proposed Alternate Pathway in the Biosynthesis of Phenazine-1,6-dicarboxylic Acid[75,79]



enzyme, in a Michael-type addition to chorismic acid giving rise to 2-amino-2-deoxyisochorismate (ADIC, **52**; Scheme 1). Though questioned in early reports,[9,73,74] it is believed that another specific enzyme, PhzD, catalyzes the ether hydrolysis that converts ADIC to *trans*-2,3-dihydro-3-hydroxyanthranilic acid (DHHA, **53**; Scheme 1). Two molecules of DHHA (**53**) can be converted into phenazine-1-carboxylic acid (**1h**) in several more poorly characterized biosynthetic transformations. One mechanism recently proposed[11] is oxidation of DHHA (**53**) to the corresponding ketone, which in solution would isomerize spontaneously to the more stable enol tautomer, 3-hydroxyanthranilic acid. The latter is known not to be an intermediate, and it is therefore believed that the enzyme stabilizes the otherwise unstable ketone that then self-condenses to form the phenazine. From this mechanism one should expect the formation of phenazine-1,6-dicarboxylic acid (**1q**), which is observed in *Streptomyces* strains,[75] *Pseudomonas aeruginosa*,[7,10] and *P. fluorescens*.[71,76,77] However, in studies of other *Pseudomonas* strains, a decarboxylation is observed during dimerization, affording phenazine-1-carboxylic acid (**1h**) as the condensation product.[11,19]

In the aromatic amino acid biosynthesis, chorismic acid (**51**) is converted into anthranilic acid, which would be a potential precursor providing both nitrogens for the ring as well as the aromatic system of phenazines. However, anthranilic acid and other proposed intermediates like quinic acid, tryptophan, tyrosine, and phenylalanine have been questioned on the basis of studies of mutants of phenazine producing organisms with blocked catabolism of these various possible intermediates.[10,78]

3.2. Alternate Pathways

In radiolabeled shikimate feeding experiments in *Streptomyces antibioticus*, Floss and co-workers observed only a little incorporation of shikimate (**50**) in esmeraldic acids and hence turned to alternate pathways. Earlier experiments had shown that aminodehydroquinic acid (**54**) was a precursor for antibiotics produced in actinomycetes.[79] Analogously, Floss predicted that the same precursor could lead to 5-amino-5-deoxyshikimic acid (**56**), which upon dehydration, self-condensation, and oxidation could lead to the formation of phenazine-1,6-dicarboxylic acid (**1q**, Scheme 2).[75] No further evidence for this pathway has been presented.

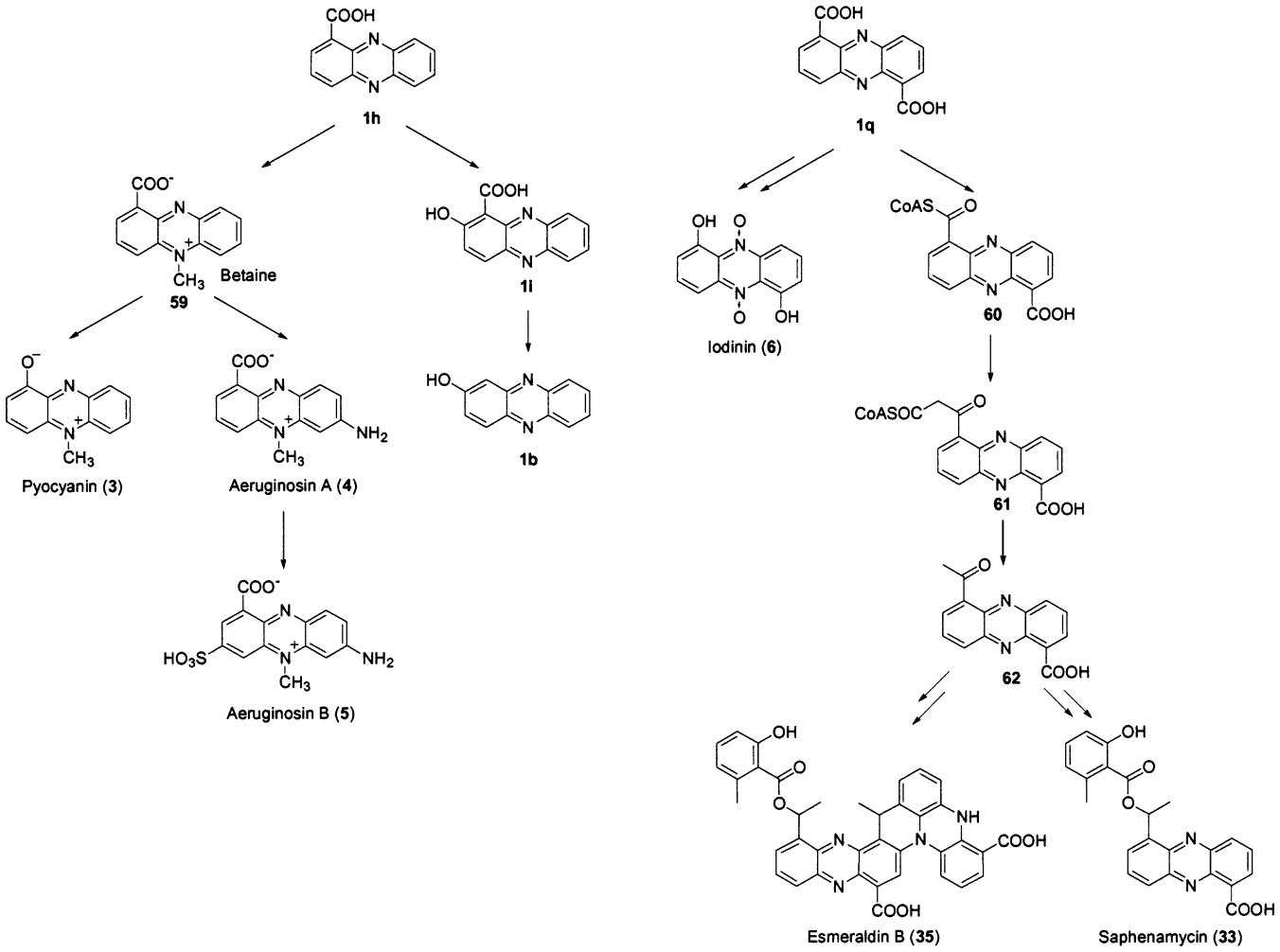
3.3. Phenazine-Modifying Pathways

Final modifications of the common precursor phenazines are specific to each phenazine-producing species. In general, hydroxyl groups can be introduced into the ring at any position, starting from phenazine-1-carboxylic acid (**1h**), as shown in ^{2}H-labeling experiments. Likewise, *N*-methylated or *N*-oxide derivatives are formed at late stages in the biosynthesis.[9] The majority of the simple and often symmetrically substituted phenazine derivatives isolated from *Pseudomonas* can be derived directly from the common precursors by methylation, hydroxylation, decarboxylation, *N*-oxidation, or other classical biosynthetic transformations.[78] Recently, the phenazine-modifying gene *phzH* was identified in the biosynthesis of phenazine-1-carboxamide, which is present in chlororaphine (**2**) in *P. chlororaphis*. The PhzH protein showed homology with amidotransferases, indicating that PhzH catalyzes a transamidation that converts phenazine-1-carboxylic acid to phenazine-1-carboxamide.[80]

It would be obvious to suggest that C_2-symmetric phenazines are formed by dimerization of appropriately substituted 6C-fragments. However, this finds no precedence in the literature. More complex structures, especially carbon-substituted phenazines isolated from *Streptomyces* strains, involve more sophisticated and yet unknown transformations.[8,9]

Studies of pyocyanin (**3**) production in *Pseudomonas* strains have shown that the biosynthetic transformations from phenazine-1-carboxylic acid (**1h**) to pyocyanin (**3**) are highly consistent in fluorescent *Pseudomonas* sp.[10,12] First, the 5-methylphenazine-1-carboxylic acid betaine (**59**) is formed by methylation of the 5-position on phenazine-1-carboxylic acid, catalyzed by SAM-dependent (*S*-adenosyl-L-methionine) methyltranferase, and subsequently an NADPH-dependent flavoprotein monooxygenase catalyzes the hydroxylative decarboxylation (Scheme 3).[20] 5-Methylphenazine-1-carboxylic acid betaine (**59**) is also

Scheme 3. Proposed Biosynthetic Routes to Selected Phenazine Natural Products[10,12,13,20,72,75,82,167]



believed to be an intermediate in the biosynthesis of aeruginosins A (**4**) and B (**5**, Scheme 3).[7] The biosynthesis of iodinin (**6**) in *Brevibacterium iodinum* involves hydroxylative decarboxylation as well, now from the dicarboxylic acid precursor **1q** (Scheme 3).[74] Another study of the iodinin (**6**) biosynthesis in *B. iodinum* revealed incorporation of phenazine-1-carboxylic acid (**1h**), but this does not exclude the existence of phenazine-1,6-dicarboxylic acid (**1q**) as a precursor.[81]

2-Hydroxyphenazine-1-carboxylic acid (**1i**) and 2-hydroxyphenazine (**1b**) are believed to be derived sequentially from phenazine-1-carboxylic acid (**1h**) via known biotransformation reactions, presumably via an arene oxide intermediate (Scheme 3). In *Pseudomonas aureofaciens*, an NADPH-dependent reductase is responsible for the hydroxylation to form **1i**, whereas the subsequent decarboxylation to give **1b** occurs spontaneously and nonenzymatically.[13,72]

The biosynthesis of saphenic acid (**27**) and derivatives thereof is based on addition of a one-carbon unit to phenazine-1,6-carboxylic acid (**1q**, Scheme 3). The transfer of a methyl group from C2 of acetate is a well-known biosynthetic transformation and occurs when the thioester of acetyl coenzyme A is converted by acetyl-CoA carboxylase to malonyl-CoA. Malonyl-CoA undergoes a decarboxylative Claisen condensation with a mono-CoA thioester of phenazine-1,6-dicarboxylic acid (**60**) to form **61**. Subsequent thioester hydrolysis and decarboxylation affords the one-carbon-elongated 6-acetylphenazine-1-carboxylic acid (**62**). Reduction of ketone **62** leads to formation of saphenic acid (**27**), presumably in an enantioselective manner. Details of further derivatization into dimeric derivatives or esters like **33** and **35** are less well-known.[75,82]

3.4. Chirality

Several optically active phenazines isolated from *Streptomyces* are derivatives of natural amino acids or natural terpenes, allowing an unambiguous determination of absolute stereochemistry. However, the secondary hydroxyl of saphenic acid (**27**) induces possible chirality in all derivatives thereof. Recently, we reported the absolute configuration of (+)- and (−)-saphenic acid as determined by X-ray crystallography of enantiomers obtained by resolution of a synthetic racemate. Both enantiomers of saphenamycin (**33**) were synthesized and, by comparison with the reported optical rotations of isolated samples, we suggested that naturally occurring saphenic acid (**27**) and saphenamycin (**33**) isolated from *Streptomyces* were the *R*-enantiomers.[83] This was the first report on determination of the absolute stereochemistry of saphenic acid (**27**) or derivatives thereof. Isolation of racemic saphenic acid derivatives from natural sources has been reported (e.g., phenostatins and esmeraldi-

nes **30**−**35**), and explained by postsynthetic racemizations either already in the host or during isolation. However, the racemization during isolation is unlikely since our recent report showed that no isomerization of enantiomers of saphenic acid esters could be detected under acidic or basic conditions.[83] Conversely, racemization in the host could be realized by reduction of the enantiopure saphenic acid to its 5,10-dihydro derivative and subsequent reversible elimination of water to form an achiral alkene species that upon rehydration and oxidation can give rise to both enantiomers. The achiral species itself can also be a precursor to enantiomerically impure products like esmeraldines (**34** and **35**).[75]

4. Mode of Action

In Nature, phenazines are formed by cells that have stopped dividing and are metabolized slowly. Phenazine metabolites have no obvious function for cell growth, i.e. no importance as energy sources or reserve substances of any kind. A general rationale of this secondary metabolite production is that it prevents the excessive accumulation of primary metabolites in the end of a growth phase by synthesis and excretion of innocuous end products. The lack of obvious metabolic functions of phenazines has led to several hypotheses on their physiological role in Nature. It has been shown that phenazine-producing organisms survive longer in their natural environment compared to non-phenazine-producing species. Therefore, it is likely that the phenazine production, due to the apparent antibiotic activity, serves to protect the producing organism and its habitat against other microorganisms and microbial competitors, and thus improves the living conditions for the host organism.[7−9,36] An example is the production of phenazine-1-carboxylic acid (**1h**) in *Pseudomonas fluorescens*, required to suppress a bacterial wheat root disease ("take-all" disease) and secure the survival of the host in the rhizosphere.[66,84]

It is also likely that phenazines represent bacterial virulence factors to their producing host organisms; i.e., they support and facilitate the development and continuation of the disease. Cystic fibrosis (CF) patients are prone to becoming infected with the opportunistic pathogen *P. aeruginosa*, which will produce pyocyanin (**3**) and 1-hydroxyphenazine (**1a**) during the infection, often seen as a blue-green discoloration of the mucus. This discoloration of the infected area is also seen in *P. aeruginosa* infections in the nails, the so-called "green nail syndrome", where dye formation serves as the diagnostic marker. The phenazines are believed to play a role in the tissue damage observed in chronic lung diseases like CF. They are also known to down-regulate the ciliary beat frequency of respiratory epithelial cells via a reduction in cAMP and ATP, to stimulate mucus secretion in epithelial cells by altering the calcium concentration in the cytosol by inhibition of plasma membrane Ca^{2+}-ATPase, and to induce death in human neutrophils (leukocytes). These and other functions of the virulence factors will lead to prolonged inflammation and escalation of the disease.[7,8,18−20,36,71]

The design of synthetic phenazines ideally strives to improve known biological activities like antibiotic, antitumor, antimalaria, and antiparasitic activities, which are results of cell growth inhibition or even cell death. The physiological function leading to this activity can be inhibition/control of DNA, RNA, and protein synthesis as well as disruption of energy-requiring membrane-associated metabolic processes. Possible modes of action thus include DNA interaction (intercalation or groove binding), topoisomerase interaction, and the role of phenazines as antioxidants or charge-transferring molecules,[72] as will be discussed in the following.

4.1. Polynucleotide Interaction

The planar, aromatic phenazine core has structural similarity to known intercalators, e.g., acridines, phenoxazines (actinomycins), daunomycin, chromomycin, and ethidium bromide, and several studies of the interactions between phenazines and DNA/RNA have been made. In 1971, Hollstein et al.[87] reported the study of the interaction of the *Pseudomonas*-produced antibiotics pyocyanin (**3**), iodinin (**6**), and myxin (**48**) with various polynucleotides. A change in the UV/vis absorption spectrum of the phenazine in the presence of calf thyme DNA as well as GC- and AT-rich double-stranded oligonucleotides compared to a spectrum of the pure phenazine would reveal any interaction. A $\pi-\pi$ interaction between the planar aromatic phenazines and the base pairs leading to intercalation was observed as hypochromic (lowered absorbance) and bathochromic (red, higher wavelength) shifts. In all cases, no binding to single-stranded DNA was observed. The binding to double-stranded DNA and RNA varied: all the studied phenazines showed DNA interaction with strong association constants in the 10^4-10^6 M^{-1} range, comparable to those of actinomycins and ethidium bromide. Phenazine-1-carboxamide (**2**) and pyocyanin (**3**) did not show any base specificity in the binding, while myxin (**48**) interacted most strongly but not exclusively with GC-rich regions. The latter specificity could be explained by electrostatic attraction between myxin and polar regions in the GC base pair. Myxin (**48**), iodinin (**6**), and pyocyanin (**3**) all inhibited DNA template-controlled RNA synthesis, either by blocking the template (DNA intercalation), binding to RNA polymerase, or binding to a ribonucleoside 5′-triphosphate. However, myxin (**48**) and iodinin (**6**) exhibited by far the strongest inhibitory effect. Myxin (**48**) affects the rate terms for polymerization, hence slowing down the incorporation of CTP and GTP into RNA but not of ATP and UTP,[68,87] and has been used as a chemotherapeutic in veterinary applications as the copper complex "cuprimyxin".[8]

Inhibition of DNA-dependent RNA synthesis in the absence of detected DNA intercalation has been observed for lomofungin (**9**), which has been shown to block the transcription complex at the initiation state as well as during elongation. Here, chelation to divalent metal cations (Mn^{2+}, Zn^{2+}, and Mg^{2+}), analogous to the mechanism for inhibition of ACE, accompanied by a direct interaction with RNA poly-

merase A, may be involved in the inhibition of RNA synthesis.[7,27,88]

4.2. Topoisomerase Inhibition

Topoisomerases I and II are enzymes responsible for topological changes in the DNA strand during cell division (translation and transcription) comprising catenation, decatenation, supercoiling, uncoiling, etc. Proliferating cells, like cancer cells, contain large concentrations of topoisomerases, which therefore serve as obvious therapeutic targets in cancer treatment. Drugs with topoisomerase II inhibition properties (e.g., etoposide and doxorubicin) are widely used in chemotherapy, whereas topoisomerase I inhibitors (e.g., camptothecin) have proven clinically useful in the treatment of colon cancer.[89] Topoisomerase inhibition has not been reported for any of the naturally occurring phenazines but has been intensively pursued in synthetic analogues (**67**−**71**, see section 5.2 on "Carboxamides").

Mutagenesis problems have been encountered in antitumor agents of the acridine-based DACA class (see **66**−**68**, Figure 8) that target topoisomerase II. They are potential cytotoxins and mutagens since cytotoxicity and mutagenesis most likely are mediated by the same mechanism, namely the formation of a "cleavable complex" between DNA and topoisomerase II.[90] These severe side effects of chemotherapy are being addressed by ongoing research taking advantage of the pH difference between malignant and nonmalignant cells. Co-administration of traditional cytotoxins and drugs that pH-dependently bind to DNA or topoisomerase II without inducing the DNA strand cleavage may prevent the cytotoxic effect in normal, nonmalignant cells.[91]

Synthetic dual topoisomerase inhibitors like phenazine-1-carboxamide derivatives (**67** and **68**) have the obvious advantage of functioning in more than one receptor and at more than one point in the cell cycle and could address multidrug resistance and other resistance issues.[92]

4.3. Radical Scavenging

It is believed that free radicals are involved in the development and progression of a wide range of serious human diseases. Oxygen-delivered free radicals are known to induce irreversible damage to neuronal cells in diseases like Parkinson's disease and perhaps dementia, atherosclerosis, and cerebral traumas or strokes. They are also believed to be involved in inducing carcinogenesis, aging of cells, asthma, renal failure, rheumatoid arthritis, and inflammation.[34,62,93] Natural antioxidants like vitamins C and E have multiple physiological functions but are insufficient in the treatment of free-radical-induced diseases. Therefore, efficient synthetic radical scavengers or antioxidants are needed to reduce these types of damage, including the irreversible loss of neural tissue.[47]

In normal cells, free radicals are continuously produced by metabolic enzymatic processes in the mitochondria as a part of energy production. Likewise, the body utilizes free radicals in immune responses toward incoming pathogens and in degeneration of toxins. However, overproduction of radicals or other dysfunctions that cause accumulation of radicals inside the cell exceeding the capacity of cellular dismutases ultimately leads to cell death.[13,94] Iron plays a key role in the initiation and propagation of free oxygen radicals and is one of the most efficient producers of free radicals. Other sources of free radicals include activation of xanthine oxidase, monoamine oxidase (MAO), and phagocytosis.[93] Inhibition of these will have the same effect as a radical scavenger.

Lipid peroxidation is one of many free-radical-induced pathophysiological processes. It leads to peroxidative disintegration of cells and organellar membranes. The antioxidant or radical scavenging character of the phenazines can therefore be confirmed by measuring their ability to inhibit lipid peroxidation in liver microsomes.[47,58]

During brain ischemia, L-glutamate is released, and this overstimulation of the glutamate receptor causes production of oxygen radicals through intercellular cascades. Ultimately, this leads to neuronal cell death, and it has been reported that free radical scavengers, including aestivophoenins A (**38**) and B (**39**), benthocyanins B (**22**) and C (**23**), as well as phenostatin A (**30**) and C (**32**) effectively inhibit these brain injuries.[47,58] Common for these radical scavengers is substitution by unsaturated isoprenyl chains or an enhanced aromatic area like **32**, although not all isoprenylated derivatives are radical scavengers.

The antibiotic activity of simpler phenazines can be explained by the opposite function, namely generation of free radicals.[95] One such example is pyocyanin (**3**), which can accept a single electron and form a stabilized anion radical. In earlier hypotheses, it was believed that this anion radical underwent redox cycling, leading to increased production of the toxic superoxide ($O_2^{\bullet -}$) and H_2O_2. Thereby the production of radicals exceeds the degenerating capacity of cellular superoxide dismutase, and radicals accumulate in the cell, leading to cell injury or even death.[7,8,13,94,96] Baron et al. questioned this hypothesis when they found no correlation between the level of dismutase activity and resistance toward pyocyanin (**3**). Instead, it was suggested that the pyocyanin radical was further stabilized by complexation with divalent metal cations in the physiological media, like Mg^{2+}. The zwitterionic properties of this complex would allow passage over cell membranes. When inside the cell, the phenazine radical can accept an electron and thereby interrupt electron transport and respiratory flow in the cell, essential for energy-requiring, membrane-bound metabolic processes such as active transport.[18,22]

4.4. Charge Transfer

Charge transfer is an uncommon feature in phenazines, which might be ascribed to the large electron-rich chromophore, and is observed in only a few examples. Early studies showed that 2-hydroxyphenazine (**1b**) and its reduced form, 5,10-dihydro-2-hydroxyphenazine, can act as artificial electron carriers for the enzyme systems in the cytoplasmic membrane

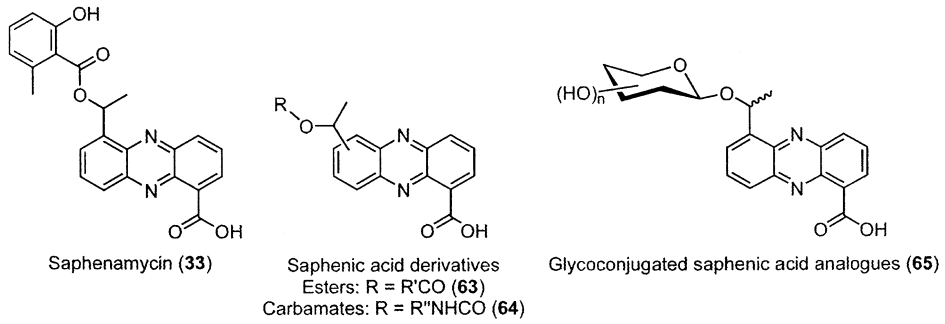


Figure 7. Saphenic acid derivatives.

in archaea, more specifically in the metabolic pathway to produce methane in methanogenic archaea.[69] Recently, other phenazines were shown also to be involved in this electron transport involved in methanogenesis.[70] A central intermediate in the methanogenic pathway is the co-enzyme methyl-S-CoM, which is reductively demethylated to methane catalyzed by methyl-CoM-reductase. The transformation of the liberated CoB-SH and CoM-SH to the heterodisulfide CoB-S-S-CoM delivers the two electrons required for demethylation. An electron carrier is responsible for the transport of electrons either from a membrane-bound hydrogenase in the presence of molecular hydrogen or from coenzyme $F_{420}H_2$ dehydrogenase to the heterodisulfide reductase. This reductase then catalyzes the reduction of the heterodisulfide in an energy-conserving step that regenerates CoM-SH and CoB-SH. It has been shown that methanophenazine (a hydrophobic $C_{25}H_{43}$ alkyl ether of 2-hydroxyphenazine, **49**) acts as this electron carrier in the methanogenic archaea *Methanosarcina mazei* Gö1, analogous to ubiquinone in mitochondria and bacteria. The electron transport is membrane-bound, and the structure of **49** allows for anchoring into the membrane, mediating electron transfer between the membrane-bound enzymes.[70,97]

5. Synthetic Phenazine Analogues

Natural product phenazines with interesting biological effects have prompted the development of synthetic analogues with tailor-made properties similar to or even better than those of the natural compound. The following section is divided into sections on the basis of structure, therapeutic use, and mode of action.

5.1. Saphenic Acid Derivatives

Saphenic acid (**27**) is a core structure of most antibiotics isolated from *Streptomyces* sp. and is therefore an obvious starting point for analoguing. A simple chloroacetic acid ester analogue of DC-86-M (**28**) was shown to possess equally potent antibiotic activities as the parent natural metabolite but no antitumor activity.[55] When saphenamycin (**33**, Figure 7) was reported for the first time in the early 1980s, several simple aliphatic and aromatic ester analogues were synthesized and tested for biological activity (Figure 7). Generally, the activity of aliphatic esters was negligible compared to that of their aromatic counterparts. Removal of the 2-hydroxy substituent on the benzoic acid in saphenamycin also led to significant loss in activity; however, an analogue without the 6-methylsubstituent was equally potent toward microorganisms as its parent saphenamycin, while a 10-fold loss of activity against tumor cell lines was observed.[98]

More recently, analogues of saphenamycin have been pursued by us. An efficient solid-phase methodology was developed for the synthesis of benzoic acid ester analogues (**63**). Halogenated and benzyloxy derivatives were screened against a broad range of pathogens, and one very potent analogue was identified as the 3-chloro analogue of saphenamycin. This analogue proved to be as potent as the parent antibiotic against all screened pathogens.[99] In the exploration of a second generation of ester analogues (**63**) and a new class of aromatic carbamates (**64**), we have identified new derivatives of saphenic acid and its 1,7-regioisomer with very potent antibiotic activity.[100] Recently, we reported the first synthetic glycoconjugated analogues **65** resembling quinovosyl esters **36** and **37**. We found that the quinovose derivative showed moderate inhibiting activity toward topoisomerase II.[101]

5.2. Carboxamides

In the late 1980s, Denny and co-workers reported the finding of a very potent dual topoisomerase I/II inhibitor and antitumor agent, an acridine-4-carboxamide, DACA (**66**, Figure 8), which was taken through to phase II clinical trials as an anticancer agent.[102,103] However, due to its fast metabolism and low human responses, further development of DACA was abandoned in 2001.[104,105] In later years, the search for new potent antitumor agents with improved properties has been inspired by DACA, and the syntheses and structure−activity relationships (SARs) of phenazine-based analogues (**67**, Figure 8) have been reported.

Denny and Baguley have pursued direct phenazine analogues and benzofused phenazine analogues of DACA (**68**, Figure 8). The SAR of a series of synthetic monosubstituted phenazine DACA analogues (**67**) with varying position (2, 3, 4, 6, 7, 8, and 9) as well as electronic and lipophilic character (Cl, CH_3, OCH_3) of the substituents was explored. Moving the attachment point of the amide side chain from the 1-position to the 2-position led to total loss of activity in all cases. Introduction of a 2-substituent led to loss of activity, presumably because of steric interaction with the 1-position side chain. At all other positions,

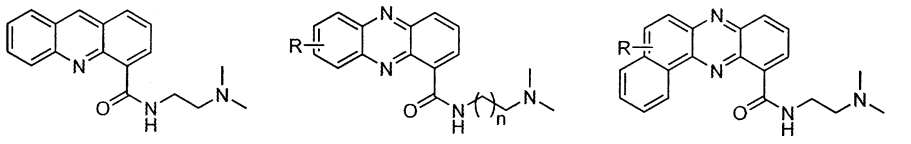


Figure 8. Acridine analogue DACA and synthetic phenazine analogues.[89,107]

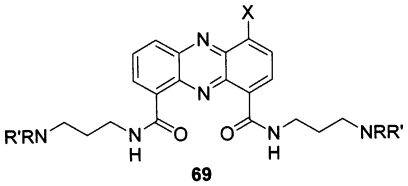


Figure 9. Dicationic DNA-binders.[111]

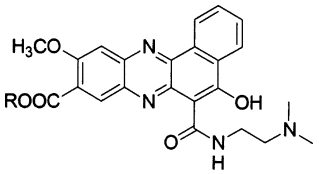


Figure 10. Intercalators NC-182 and NC-190.[112−114]

Cl-substituted derivatives were the most active, followed by OCH_3 and CH_3, though only small differences were observed. Introduction of 6-, 7-, and 8-substituents on the phenazine led inevitably to a loss of activity. Analogues substituted in the 9-position showed the largest increase in activity, 10−20-fold, compared to the unsubstituted analogue. Increasing the length of the side chain and increasing the size of the amino substituents led to a significant decrease in potency.[106] Generally, 8,9-benzofused phenazines (**68**) were 10-fold more potent that the 3,4- and 6,7-benzofused analogues. Substituents in the 4-position were tolerated to a large degree, including various electronic characters (however, they should be rather small), whereas substituents in the 9-position were restricted to be small, and those in the 10-position had to be small as well as to have hydrogen-bond-donating character. The amino side chain showed steric constraints at the terminus and no toleration of hydrophilic substituents along the chain. However, hydrophobic amino side-chain substituents of limited size, such as methyl, improved the potency. A chiral analogue with an α-methyl substituent revealed some chirality dependence; a 4-fold difference in enantiomer potency was observed. The *R*-enantiomer was recently taken into preclinical development and shows more than 70% oral bioavailability in female mice.[89] Reduction of the chromophore with one ring to the corresponding quinoxazoline system led to a dramatic loss of inhibitory potency. The mechanism of this class of topoisomerase inhibitors is still unknown. However, intercalation in the minor groove, complemented by hydrogen bonding to N7 of guanine in the major groove of guanine-rich regions, has been proposed.[89,90,104,106−110]

Antimalarial agents of the same class have been reported. Although they showed only limited antimalarial activity, they were strong DNA-binders. They have two cationic side chains enhancing the major groove binding (**69**, Figure 9).[111]

Development of antitumor agents NC-182 (**70**) and NC-190 (**71**) is a result of variation of substituents on the phenazine core together with a 3,4-benzofused system (Figure 10).[112−114] A hydroxyl group ortho to the carboxamide will, as suggested by Denny et al., form a hydrogen bond to the amide. Experiments have shown that the binding modes and activities for the two analogues are very similar. Both NC-182 (**70**) and NC-190 (**71**) are potent antitumor compounds at a level comparable with high-activity compounds of the monomeric Denny series (**67**).

A DNA unwinding assay showed that NC-190 (**71**) had intercalating activity that appeared to be weaker than those of ethidium bromide and adriamycin, whereas NC-182 (**70**) was a potent intercalator with the same binding abilities as daunomycin but showed no base specificity. Intercalative and electrostatic interactions dominated at low and high drug concentrations, respectively.

NC-190 (**71**) affected the DNA strand-passing activity of DNA topoisomerase II; it inhibited the decatenation activity of purified topoisomerase II by stabilization of the topoisomerase II−DNA cleavable complex but had only a weak inhibitory effect against topoisomerase I. NC-190 (**71**) induced growth inhibition, protein-linked DNA breaks, and DNA fragmentation in cultured HL-60 cells in a dose-dependent manner. These results demonstrate a good correlation between growth inhibition, topoisomerase II-dependent DNA cleavage, and DNA fragmentation induced by NC-190 (**71**). They also indicate that the mechanism by which NC-190 (**71**) exhibits antitumor activity may be the inhibition of topoisomerase II.

5.3. Biscarboxamides

Denny and co-workers[92,110,115−118] reported the discovery of potent bisintercalators, diamide analogues of the DACA series and the corresponding phenazine series (**73**), inspired by the structure of the known bisintercalator **72** (Figure 11).

When the chromophore was extended from a monomeric structure (**67**) to its dimeric analogue (**73**), variable but significant gains in potency were observed. In some cases a 20−30-fold increase in potency could be obtained for the dimeric compounds.[110] Substituents on the phenazine core were

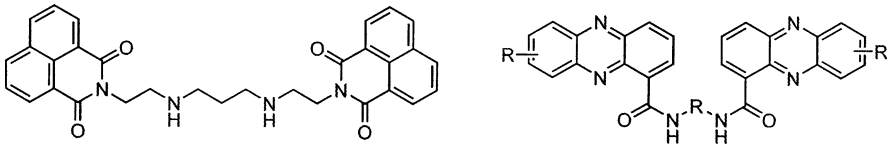


Figure 11. Potential bisintercalators. LU79553, a known bisintercalator that is currently in clinical trials. Dimeric phenazine analogues of varying structure.[89,107]

investigated, and as for the monomeric analogues, 9-substitution with a small lipophilic group (CH_3 or Cl) was preferred and led to a significant improvement in potency, up to 60-fold; large substituents in that position were disfavored.[92,115] 6,9-Dimethyl- and 6-chloro-substituted analogues also had great potency. On the other hand, 3-, 4-, 7-, and 8-substituents did not affect the activity.[115] Sterically demanding and hydrophilic groups were abandoned prior to synthesis due to SAR studies on the related acridine analogues. 2-Substitution led to loss in activity, as was the case for the monomers.[115]

The linker region between the two chromophores has been studied extensively. When changing from monocationic to biscationic linkers, a 30-fold increase in potency was observed toward human cancer cell lines, while the potency against murine cell lines was unaltered.[117] Symmetric and unsymmetric dimers were studied. No significant characters of the unsymmetric dimers were observed; their potency was, as anticipated, between that of the corresponding two symmetric dimers.[117] Variations in the linker flexibility, structure, and length were also studied.[116] Potency tended to decrease with increasing linker length. No decrease was observed with a higher degree of rigidity (introduction of piperazine moiety). Additionally, a steady decrease in potency was observed when the size of *N*-alkyl substituents in the linker moiety was increased. The most active linker variation was observed when the middle linker moiety was extended with one carbon in addition to methylation of amines.[116] A linker length exceeding 16 Å led to a significant drop in activity. Likewise, a small, rigid linker was inactive, while a larger, more rigid linker gained activity, showing some restriction to minimum length and flexibility.[116]

The mechanisms of action of these analogues are not fully known; however, side chains were proved to interact through hydrogen-bonding to guanine residues in the major groove. In several cases, experiments indicated that there was a lower degree of topoisomerase II interaction and more topoisomerase I and DNA interaction when compared to the corresponding monomeric analogues.[116] QSAR analyses on three phenazine and acridine dimer series confirmed the theory that two different receptor interactions were involved, i.e. that phenazines did not only intercalate but also did interact with topoisomerase I and/or II enzymes.[118]

5.4. Covalent DNA Derivatives

The use of phenazines as DNA-binding molecules is likely to be accompanied by a lack of specificity toward the desired site on DNA. Tethering to oligonucleotides predetermines the position of the chromophore and minimizes the risk of unspecific binding. Oligonucleotides and chemically modified analogues thereof are potentially useful in regulation of cell growth at the gene level in the treatment of viral infections and genetic diseases; the antisense technique involves specific binding to messenger RNA, inhibiting translation; the antigene approach involves binding to the major groove of double-stranded DNA and consequent prevention of transcription. The antigene approach involves formation of triplex structures, which are normally physiologically very unstable. Introduction of DNA-binding molecules (groove-binders or intercalators) like phenazines is a way to promote triplex formation and enhance their stability.[119,120]

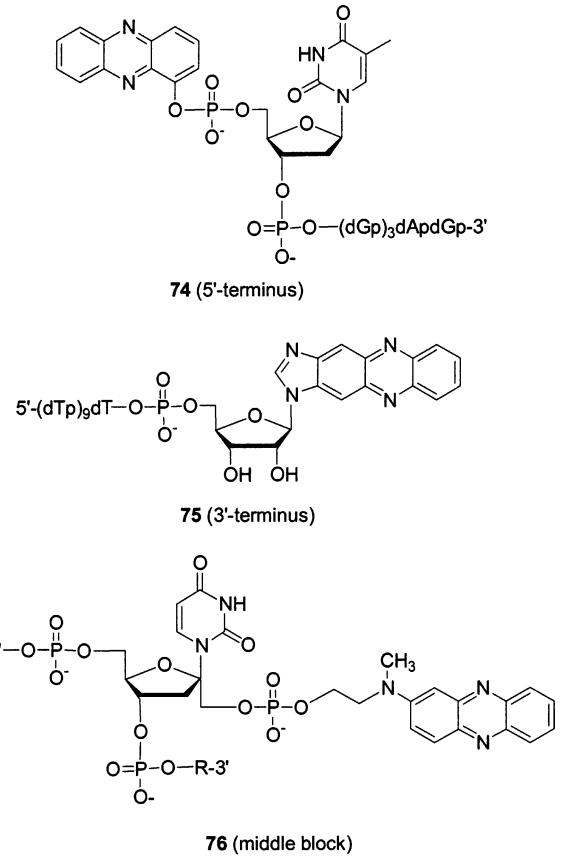


Figure 12. 5′- and 3′-terminal phenazine-containing oligonucleotides and a modified nucleotide.[119−122]

The stabilizing effect of introduction of 2-aminophenazine into duplex- and triplex-forming oligonucleotides as a function of positioning and linking of the chromophore has been investigated by Chattopadhyaya and co-workers. Figure 12 shows examples of two modified oligonucleotides (**74** and **75**) and a modified nucleotide ready for oligomerization (**76**). Generally, duplex and triplex stabilization via

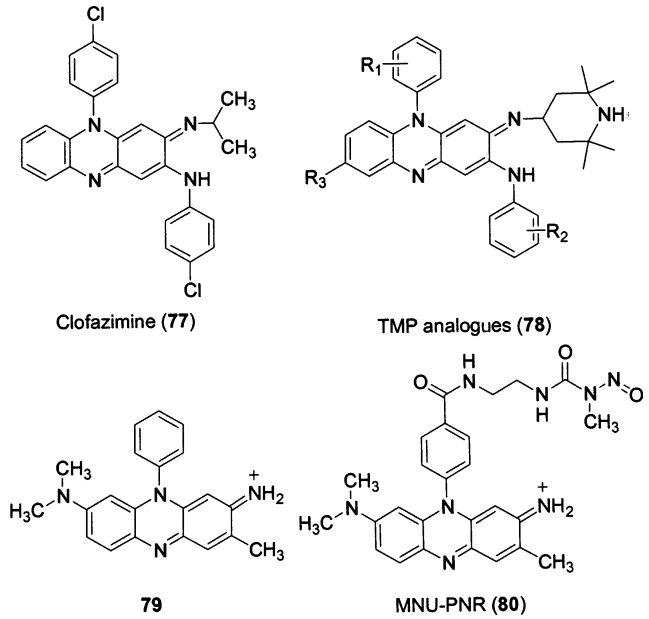


Figure 13. Clofazimine and tetramethylpiperidine (TMP) analogues.[124−127]

a strong intercalating effect was observed when phenazines were attached to the 5′-terminus of the oligonucleotides. Phenazine employment at both ends of the oligonucleotide provided even more stable duplex and triplex structures.[119,120] A strong stabilizing effect from intercalation was also observed when 1-hydroxyphenazine (**74**) and phenazole (**75**) were incorporated into oligonucleotides at the 5′- and 3′-end, respectively.[121,122]

Generally, the oligonucleotides were synthesized by automation, and the corresponding phenazine-modified phosphoramidates were synthesized in traditional ways.[123]

5.5. Other DNA-Binding Analogues

The antimicrobial agent clofazimine (**77**, Figure 13) was originally reported in 1957 as a potent antituberculosis agent and has been subject to further development for potential tuberculosis and leprosy treatment.[124] Clofazimine (**77**) was observed to stimulate release of reactive oxidants by polymorphonuclear leukocytes (PMNs), which was believed to be the mechanism for its antibiotic effect. Several approaches to improve the activity have been studied by O'Sullivan and co-workers (Figure 13).[124−127] Monobromoaryl-, dichloroaryl-, and trichloroaryl-substituted tetramethylpiperidine (TMP) analogues (**78**) proved superior in activity against normal and multidrug-resistant *Mycobacterium tuberculosis* strains compared to the parent clofazimine. Introduction of a chlorosubstituent in the 7-position on the phenazine core also improved the activity.[124,125,128] Substituents other than TMP or isopropyl on the 2-imine on the phenazine core led to diminished activities.[126]

Another member of the same class is phenyl natural red (**79**, Figure 13), a known DNA binder with specificity for GC-rich regions, used for staining in biological systems. The specificity arises mainly from electronic factors facilitated by groove binding of the 5-*N*-phenyl residue in addition to intercalation of the phenazine core.[127] Sequence-directed methylation of DNA was attempted via a nitroso derivative of phenyl neutral red (**80**, MNU-PNR). This analogue was also shown by CD, UV/vis, and viscosity studies to intercalate into DNA from the major groove. Methylation of the 7-position of guanine residues in the major groove was observed to be 5-fold preferred over methylation at residues in the minor groove. This is most likely a result of directing the methylating agent via intercalation of the phenazine core from the major groove.[129]

DNA cleavage could be achieved by conjugating a known DNA-cleaving compound, an analogue of netropsin, covalently to an intercalating phenazine. Netropsin is an antibiotic agent that generates oxygen radicals capable of causing DNA breaks, preferably in AT-rich regions. The hybrid molecule was found to attach in a bimodal way as anticipated; the phenazine moiety intercalated, whereas the netropsin analogue moiety was bound in the minor groove. The netropsin-induced DNA cleavage was observed to occur with approximately 10 base pairs distance, or the equivalent of one turn of the helix. This indicated that the site of attack was determined by the overall structure of the DNA rather than that of the primary base sequence.[130]

6. Synthesis of Phenazines

The limited commercial availability of substituted phenazines indicates that their synthesis presents a challenge for the synthetic chemist. To date, no efficient and generally applicable synthesis of substituted phenazines exists. Many synthetic methods are reported, most of them having severe limitations to localization and electronic character of the substituents. In general, substitutions on the phenazine core are introduced on the building blocks prior to phenazine formation. In the present section we outline the most commonly used methods, as depicted in Figure 14, and their scopes and limitations are discussed.

6.1. Wohl−Aue Procedure

In 1901, one of the first methods for preparation of phenazines was reported by Wohl and Aue. They discovered that anilines and nitrobenzenes form phenazines or phenazine-*N*-oxides upon heating to 200 °C in the presence of a strong base (Scheme 4).[131] The starting materials were generally readily available; however, yields were modest and significant amounts of byproducts, primarily consisting of azacompounds, were formed under the harsh reaction conditions.

Bahnmüller et al. have applied the Wohl−Aue procedure for the synthesis of ketal-protected natural product saphenic acid. Heating anthranilic acid and ethylene ketal-protected 3-nitroacetophenon in the presence of KOH and sand to 200 °C afforded saphenic acid (**27**) in very low yield (less than 5%).[98]

6.2. Bamberger−Ham Procedure

In 1911, Bamberger and Ham discovered that two molecules of 4-substituted nitrosobenzene fuse under

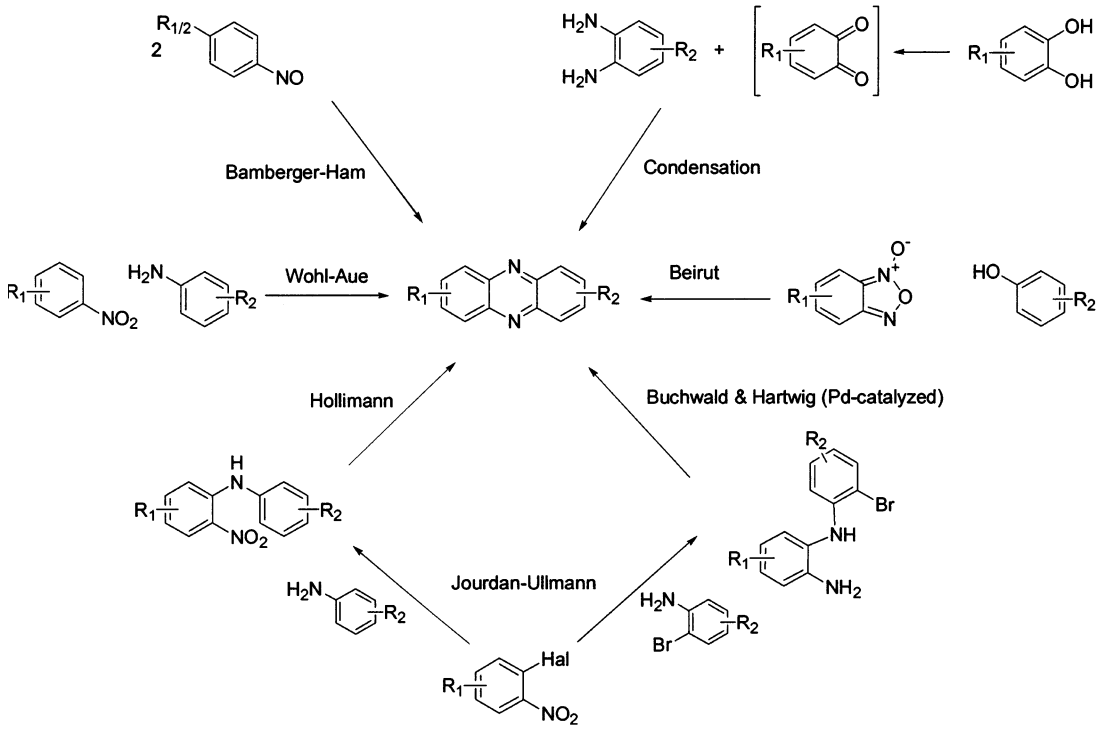
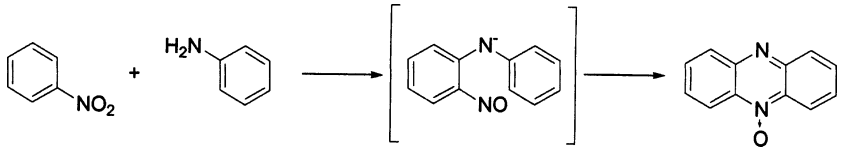
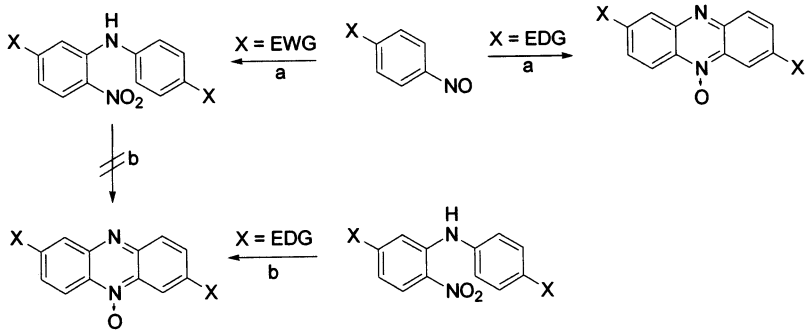


Figure 14. Overview of phenazine syntheses.

Scheme 4. Wohl−Aue Reaction: Heating to 200 °C in Sand or Strong Base (KOH)



Scheme 5. Bamberger−Ham Procedure[a]



[a] EGW, electron-withdrawing group (e.g., COOR); EDG, electron-donating group by the resonance effect (e.g., halide, methyl). (a) Concentrated H_2SO_4; (b) oleum.

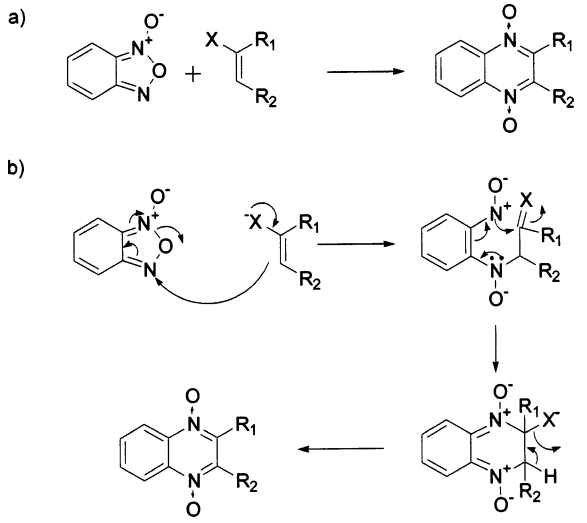
acidic conditions to give the 2,7-disubstituted phenazine-*N*-oxides in moderate yields via a diphenylhydroxylamine intermediate (Scheme 5).[132] The substitutions are limited to electron-donating (by the resonance effect) para-residues (e.g., halide and methyl); a competing reaction transforms nitrosobenzenes with electron-withdrawing substituents to the corresponding 2-nitrodiphenylamines, which were too stable to react under strongly acidic conditions that were known to convert 4,5′-dibromo-2-nitrodiphenylmine to the corresponding phenazine.[133]

Accordingly, both the Wohl−Aue and Bamberger−Ham procedures have received limited attention in the literature due to poor yields, harsh conditions, and severe limitations in terms of substrates, i.e., in the possible substitutions on the phenazine core.

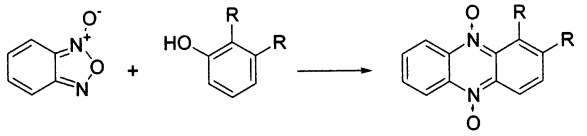
6.3. Beirut Reaction

In the mid 1960s, Issidorides and Haddadin addressed some of the limitations in substitution met in earlier reported procedures by developing a synthesis of heteroaromatic *N*-oxides that, upon reduction, afford heteroaromatic compounds like phenazine.[134] The Beirut reaction, named after its origin from the capital of Lebanon, involves condensation of benzofurazan oxide (benzo[1,2,5]oxadiazole-1-oxide) and, e.g., enamines, dienes, aldehydes, α,β-unsaturated ketones, or enolates to form heterocycles

Scheme 6. Beirut Reaction: (a) General Reaction and (b) General Mechanism



Scheme 7. Synthesis of Phenazine-5,10-dioxide via the Beirut Reaction



of the general structure of quinoxaline 1,4-dioxides (Scheme 6a) via the general mechanism depicted in Scheme 6b. The method was recently reviewed by the originators[135] and by others,[136] and this section will provide a short summary of the procedure exclusively for phenazine structures, even though the scope covers the synthesis of a large variety of heteroaromatic *N*-oxides.

Dehydrative condensation of benzofurazan oxide with phenolic enolates affords phenazine di-*N*-oxides (Scheme 7). The condensation proceeds under mild conditions ($NaOH/H_2O$, H_2O, $MeOH/RNH_2$, SiO_2/CH_3CN at room temperature) and can be applied to a broad range of substituted nucleophiles of varying oxidation levels (phenolates, resorcinolates, hydroquinones, and benzoquinones). A few limitations have been encountered for the phenol, and these include insufficient reactivity of dioxalane-protected *p*-formyl phenol and decarboxylation of free carboxylates at even mild reaction conditions ($NaOH/H_2O$, 60 °C).[137] Nucleophilic attack on the benzofurazan will occur from the para position in ortho- and meta-substituted phenolates, whereas para-substituted phenols will attack from the ortho position. Subsequently, elimination of H_2O or ROH will take place if possible; otherwise, elimination of H_2 will give the phenazine.[138]

However, limitations in the substitution pattern of the benzofuranzan oxide moiety exist: 5/6-mono- or disubstituted benzofurazan oxides react rapidly under mild conditions, whereas 4/7-mono- and disubstituted substrates react significantly slower or do not react at all. This is explained by the repulsive effect of the 4(7)-substituent and the *N*-oxide in the forming product. Hence, the Beirut reaction is well-suited for the synthesis of a broad range of substituted phenazines, but limitations occur when phenazines with 1,6-, 1,9-, or 1,4,6-substitution patterns are desired.[135]

In Table 3are listed substrates, products, conditions, and yields for selected Beirut reactions.[139,140]

The hitherto presented procedures afford mono- or di-*N*-oxides that are easily deoxygenated by treatment with $Na_2S_2O_4$,[141] $Na_2S_2O_3$,[138,142,143] PCl_3,[135] $TiCl_4$[144] or by catalytic hydrogenation (complex hydrides, dissolving metals, H_2-Pd/C).[145] Generally, reduction with dithionite gives higher yields, while phosphorus trichloride reduction proceeds faster.[141]

6.4. Condensation Procedure

Another early approach to the synthesis of phenazines was the condensation of substituted catechols (*o*-hydroxyphenol) and benzene-1,2-diamine neat or in the presence of PbO_2, first reported in 1886.[146] The synthesis of the natural antibiotic pyocyanin from 3-methoxycatechol and benzene-1,2-diamine using this PbO_2-catalyzed procedure was reported by Surrey.[147] The mechanism is believed to proceed via a dione intermediate upon hydrogen abstraction (Scheme 8).[147]

A recent approach using 1,2-cyclohexyldione derivatives was reported by Denny and co-workers[115] for the synthesis of tetrahydrophenazines that, upon oxidation, afford phenazines with the desired substitution pattern. In particular, halogenated phenazines like 4-chlorophenazine-1-carboxylic acid (**87**) that are unsuited for aromatic substitution approach (see below) can be synthesized this way (Table 4, entry a).[115] The same group of researchers used substituted naphthoquinones and 4,5,6-substituted 2,3-diaminobenzoic acids in a regioselective condensation reaction to form multisubstituted benzo[*a*]-phenazines (**88**) in hydrochloric ethanol in good yields (Table 4, entry b).[89] Another variation includes reaction of a hydroxyiminooxo acid and benzenediamine, giving the corresponding 6,8-disubstituted benzo[*a*]-phenazine (**89**) that was inaccessible by any other known methods (Table 4, entry c).[108]

The regioselectivity of these condensations was controlled by the relative functional reactivity of the electrophilic dione species and steric effects of the nucleophilic amino groups. A solid-phase approach was reported by Feldman et al. in 1999 on a benzoyl-functionalized Wang resin, giving 1-methoxyphenazine-3-carboxylic acid methyl ester in 14% yield.[148]

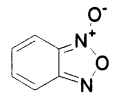
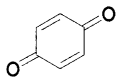
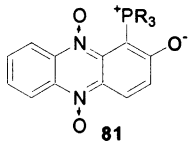
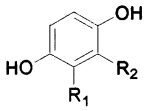
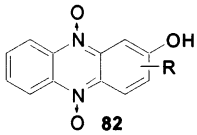
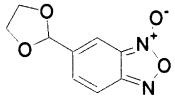
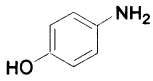
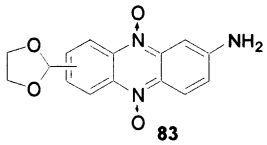
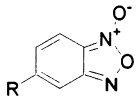
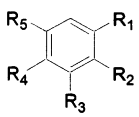
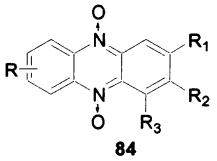
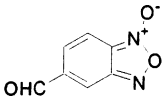
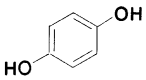
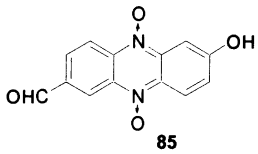
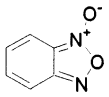
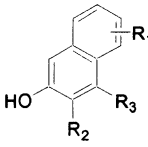
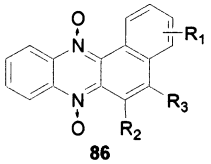
6.5. Diphenylamine Procedure

The most cited strategy for phenazine synthesis goes via 2-nitrodiphenylamine which, upon reductive ring closure, affords the desired phenazine.

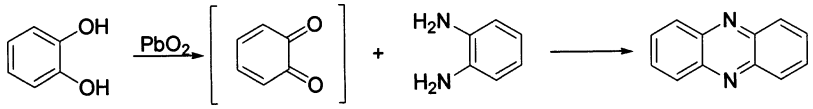
6.5.1. Diphenylamine Formation

Nucleophilic substitution of a 2-nitrophenyl halide with an aniline affords the corresponding 2-nitrodiphenylamine. The reaction conditions depend on the substitution pattern on the reagents and include normal base catalysis[149−151] or additional activation by copper metal, often referred to as Jourdan−Ullmann conditions (K_2CO_3, copper-bronze, neat or

Table 3. Substrates and Products for Selected Beirut Reactions[108,137−140,144]

Benzofuranzan oxide	Nucleophile	Beirut reaction product	Reaction conditions	Yield
	 PR_3 R = *i*-C_3H_7, *c*-C_6H_{11}, Ph	 **81**	EtOH, 20-30°C	40-62%
	 R_1 = H, Cl, *t*-C_4H_9, CH_3, OCH_3, Br, $COCH_3$, CN, COOH; R_2 = H, NO_2, $COCH_3$, CH_3	 **82**	$NaOH/H_20$, r.t.	54-89%
		 **83**	mixture of isomers, 8/7 = 2:1	
 R = H, Cl, OCH_3	 R_1 = OH, NH_2, OCH_3, O-Alkyl; R_2 = H, OH, O-Alkyl, OCH_3, COOH, $COCH_3$; R_3 = H, OH, CH_3; R_4 = H, OH; R_5 = H, OH, OCH_3	 **84**	THF/NH_3, r.t NaOMe/MeOH, reflux KOH/MeOH, r.t. to reflux	5-82%
		 **85**	$NaOH/EtOH/H_20$, r.t.	93%
	 R_1 = H, NO_2, NH_2, OCH_3, OH, $NHCOCH_3$, $NHSO_2CH_3$; R_2 = COOH, NH2; R_3 = SO_3Na	 **86**	$NaOH/H_2O$, 28°C	31-88%

Scheme 8. General Scheme for Phenazine Synthesis by Condensation of Catechol or 1,2-Cyclohexyldione with Benzene-1,2-diamine



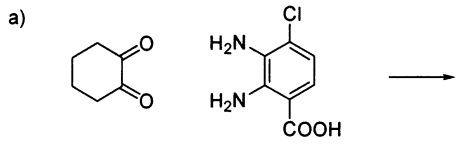
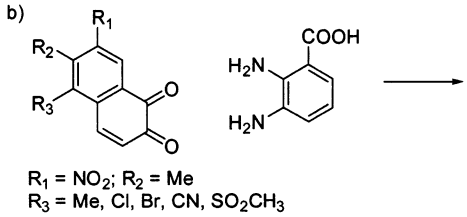
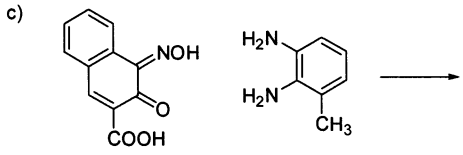
H_2O).[86] Variations of Ullmann's reaction conditions have been reported by Denny and co-workers, who successfully applied anhydrous conditions implying CuCl/Cu, *N*-alkylmorpholine as a base, and high-boiling solvents such as butanediol.[104,106,115] This method was generally high-yielding except for very electron-deficient amines (8-aminoisoquinoline)[104] or sterically hindered anilines (e.g., 2-chloro-6-fluoroaniline).[106] In some cases, copper catalysis led to disubstitution (2,6-dichloroaniline)[106] or had no effect on yields or reactivity;[152] these couplings were carried out in the absence of metal catalyst.

6.5.2. Cyclization of Diphenylamines

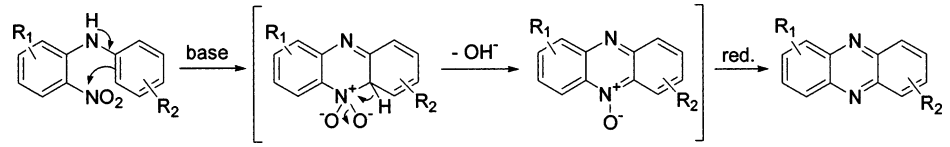
Waterman and Vivian presented the first reports on cyclization of 2-nitrodiphenylamines to form phenazines promoted by oxygen-abstracting agents like metallic Fe or Pb and ferrous oxide (formed in situ by heat decomposition of ferrous oxalate).[153] Limitations of the substitution of the diphenylamines occurred when 2′-chlorinated and 2′- or 4′-brominated 2-nitrophenylanilines were partly dehalogenated under harsh deoxygenating conditions (heating to 220−300 °C with ferrous oxalate and granulated lead).[86] 2-Halo-7/8-alkoxyphenazines[149] and 2-halo/methyl-7-alkoxy/halo-6-methyl-8-methyl/phenyl phenazines[150] were prepared in 25−60% yield by this method. 2-Nitrodiphenylamines with 2′-amino and 2′-alkoxy substituents underwent elimination upon cyclization, leading to the deamino- and dealkoxy phenazines,[150,151] and the presence of cyano substituents led to significantly lower yields.[151]

Other approaches reported by Cross et al.[154] include cyclization in alkaline solutions (KOH in xylene or

Table 4. Variations on the Catechol Theme[89,108,115]

Dione	Diamine	Reaction product	Reaction conditions	Yield
a) 		**87**	i) MeOH, reflux ii) DDQ/benzene	33%
b)  $R_1 = NO_2$; R_2 = Me R_3 = Me, Cl, Br, CN, SO_2CH_3		**88**	EtOH, conc. HCl, reflux	31-76%
c) 		**89**	AcOH, conc. HCl 100°C	66%

Scheme 9. General Mechanism for Reductive Cyclization of 2-Nitrodiphenylamines To Form Phenazines



decaline; 30−48% yield), highly acidic solution (10−20% oleum; 5−80% yield; goes via a 2′-sulfonated intermediate that eliminates SO_3 and water), or redox conditions (KOH/hydrazine in EtOH with Raney Ni or Ru/C; 38−76% yield). The regioselectivity of acid-catalyzed reactions is poor; 1,8- and 2,7-dichlorophenazine *N*-oxides are obtained in equal amounts from acidic cyclization.[154]

The most commonly used conditions for the transformation of diphenylamines to phenazines in recent years comprise a reductive alkaline environment like $NaBH_4$ and $NaOH/H_2O$ or NaOEt/EtOH[115,155] and were first developed by Holliman and co-workers in their synthesis of griseolutein A (**13**) and methyl diacylgriseolutein B.[35] The method was discovered when the authors observed formation of phenazines upon heating of certain 4-amino-2-nitrodiphenylamines in an aqueous base. Addition of an external reducing agent improved the phenazine formation to up to 80%. Reduction of the intermediary *N*-oxide (Scheme 9) was believed to arise from the *p*-phenylenediamine system. Yields vary to a large degree upon the substitution pattern and are, e.g., reduced by 50% when changing the substrate from 4′-amino-6-carboxy- to 4′-amino-4-carboxy-2-nitrodiphenylamine.[35]

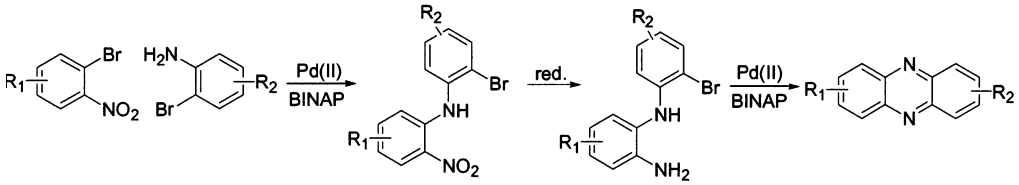
A byproduct from the reductive ring closure was often the corresponding 2-aminodiphenylamine, arising from direct reduction of the nitro group without ring formation.[106] Competing nucleophilic aromatic substitution by this new aniline of a displaceable ortho substituents normally leads to byproduct formation but can also be used to induce regioselectivity. Cyclization of 2′-chloro- or 2′-bromo-2-nitrodiphenylamine afforded a mixture of unsubstituted phenazine and 9-halosubstituted phenazine, favoring the former, whereas introduction of a more easily displaceable 2′-fluoro substituent increased the amount of the unsubstituted phenazine.[106,156] The yield of 9-substituted phenazines with displaceable groups in the ortho position, e.g., methoxy groups, could be improved by introduction of an additional ortho substituent that facilitates ring closure and gives rise to only one possible ring-closed product.[106] Ring-closing of 3′-substituted 2-nitrodiphenylamines favored formation of 6-substituted phenazines over 8-substituted phenazines.[106]

Observed limitations include partial decarboxylation during ring closure in the synthesis of phenazine-1,9-dicarboxylic acid[111,157] as well as poor yield and very low reactivity of ketal-protected ketone diphenylamine in the cyclization step of saphenic acid synthesis. The deprotected ketone was cyclized and reduced in one step, affording the desired saphenic acid in satisfactory yields.[152]

6.6. Palladium Catalysis

The application of transition metal catalysis for aryl amination was reported and developed primarily by the groups of Buchwald and Hartwig in the late 1990s and presented a mild and broadly applicable method for amination of aryl bromides and chlorides by use of palladium(II) and BINAP, tol-BINAP, or

Scheme 10. Phenazine Synthesis by Sequential Palladium(II)-Catalyzed Aryl Amination[45]



$^{t}Bu_3P$ as ligand.[158−164] Poor yields in the reductive cyclization method—when competitive cyclization is possible—and also the need for milder reaction conditions in the cyclization step urged Emoto et al.[45] to report an alternative method based on sequential palladium-catalyzed aryl amination (Scheme 10). The substrate for the cyclization was 2-amino-2′-bromodiphenylamine, obtained by selective bromination and reduction of the well-known 2-nitrodiphenylamines or by coupling of 2-bromoaniline with a 1-bromo-2-nitrobenzene and subsequent reduction. Treatment of 2-bromo-2′-nitrodiphenylamine with catalytic amounts of palladium(II) and BINAP as ligand afforded the desired phenazines in good yields.[45]

6.7. Phenazine Modifications

Besides conventional substitutions and derivatizations of functional groups attached to the phenazine ring, the amount of reported modifications is limited. Oxidation with H_2O_2 or *m*CPBA gives the corresponding *N*-dioxides in good yields.[137]

Phenazines are strongly deactivated toward electrophilic substitution under standard conditions.[165] Only a few examples of aromatic nucleophilic substitution have been reported,[106] and a thorough coverage of aromatic electrophilic and nucleophilic substitutions is outside the scope of this review. Phenazines are also considered poor π-conjugative binders because of their anthracene-like character, which makes cross-coupling reactions like Heck, Stille, Suzuki, Sonogashira, etc. nontrivial.[166] However, Yamamoto cross-coupling from 2,7-dibromophenazine to form a homopolymer, palladium-catalyzed dehalogenative coupling using bis(pinacolato)-diboron as a condensation reagent, Suzuki coupling of 2,7-dibromophenazine with the 1,3-propanediol diester of 1,4-phenylenediboronic acid, and Heck reaction of 2,7-dibromophenazine with 1,4-divinylbenzene catalyzed by palladium diacetate were performed in reasonable yields.[166]

7. Perspectives and Concluding Remarks

The diversity of structures as well as biological targets of phenazine natural products and synthetically designed analogues thereof is immense. Continual discoveries of new phenazine natural products indicate that a large resource of metabolites with biological significance is still to be disclosed in Nature, especially from marine environments. Little is yet known about the physiological function of phenazines in their natural environment, which leaves behind unanswered questions about mode of action and requirements for activity of natural and synthetic phenazine derivatives. An actual general structure−activity relationship analysis is difficult to derive from the literature since a large number of diverse targets have been used in the evaluation of biological activity. However, radical scavenging and DNA−topoisomerase binding activity seem to be dominating features. Small phenazine molecules that bind to DNA might lack binding selectivity and show general toxicity. However, the fact that several of the naturally occurring phenazine antibiotics are not cytotoxic and do not exhibit growth-inhibiting activity against eukaryotic cells in vitro brings hope that this class may function as potential anti-infective or anticancer agents. The design of new analogues could be based on a combination of the flat aromatic intercalative phenazine and structural characteristics of groove-binders or specific enzyme-binders and might potentially lead to selective and high-affinity binders targeting DNA and DNA−enzyme complexes. Nevertheless, after more than a century of phenazine synthesis, synthetic design is still limited by low-yielding phenazine formations and incompatibility of certain substitution patterns with existing synthetic methods. Transition metal catalysis of the phenazine cyclization could potentially address some limitations of the conventional methods in the future.

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9. Abbreviations

ACE	angiotensin-converting enzyme
ADIC	2-amino-2-deoxyisochorismate
AT	adenine-thymine
ATP	adenosine triphosphate
BINAP	2,2′-bis(diphenylphosphino)-1,1′-binaphthyl
cAMP	cyclic adenosine monophosphate
CD	circular dichroism
CF	cystic fibrosis
CoA and M	coenzymes A and M
CTP	cytidine triphosphate
DDQ	dichlorodicyanoquinone
DHHA	*trans*-2,3-dihydro-3-hydroxyanthranilate
DNA	deoxyribonucleic acid
ED_{50}	effective dose 50
$F_{420}H_2$	flavin-derived electron-donating coenzyme in the methanogenic pathway (reduced form)
GC	guanine-cytosine
GTP	guanosine triphophate
IC_{50}	inhibitory concentration 50
LD_{50}	lethal dose 50

MIC	minimum inhibitory concentration
*m*CPBA	*meta*-chloroperbenzoic acid
MAO	monoamine oxidase
MNU−PNR	*N*-methyl-*N*-nitrosourea derivative of phenyl neutral red
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
PhzD and E	enzymes involved in phenazine biosynthesis
PMN	polymorphonuclear leukocyte
QSAR	quantitative structure−activity relationship
RNA	ribonucleic acid
SAM	*S*-adenosyl-L-methionine
SAR	structure−activity relationship
^{t}Bu	*tert*-butyl
TMP	2,2,6,6-tetramethylpiperidine
tol	toluene
UTP	uridine triphosphate
UV/vis	ultraviolet/visible

10. References and Notes

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