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Synthesis and Characterisation of Novel 3-Chloropiperidine Derivatives as DNA Alkylating Agents

Kumulative Dissertation zur Erlangung des Grades „Doktor der Naturwissenschaften“ am Fachbereich Biologie und Chemie der Justus-Liebig-Universität Gießen

vorgelegt von

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Kurzzusammenfassung

Anfang des 21. Jahrhunderts begann in den entwickelten Staaten ein epidemiologischer Paradigmenwechsel, in dem die Relevanz von nicht-übertragbaren Krankheiten gegenüber den infektiösen Krankheiten deutlich zunahm. Schätzungen der *World Health Organization* (WHO) rechneten für das Jahr 2020 mit 19,3 Millionen neuen Diagnosen bösartiger Tumore. Daraus resultiert ein nie dagewesenes Verlangen nach neuen Medikamenten für die onkologische Therapie. Bis in die 1960er Jahre wurden zur Behandlung von Tumoren hauptsächlich die Radiotherapie und die Chirurgie angewendet. Die Chemotherapie gewann danach deutlich an Bedeutung, da Radiotherapie und Chirurgie nur mit eingeschränkter Wirkung bei den bis dahin unterschätzten Mikrometastasen therapeutisch genutzt werden konnte. Bis heute hat die Forschung an chemotherapeutischen Medikamenten eine Reihe an Wirkstoffklassen hervorgebracht, wie z.B. DNA-Alkylanzien, Platinierungs-Reagenzien, Topoisomerase Inhibitoren, Antimetabolite, Interkalationsverbindungen oder die Taxane, wobei die DNA-Alkylantien die älteste dieser Verbindungsklassen darstellt.

Die erste therapeutische Anwendung der DNA-Alkylanzien gelang im Jahr 1942 den US-Amerikanern *Gilman* und *Philips*. Sie verabreichten Mechlorethamin, ein Derivat des Senfgases, an Patienten, die an *Morbus Hodgkin* erkrankt waren. Hierbei stellten sie fest, dass der Wirkmechanismus die Alkylierung eines wichtigen zellulären Bestandteils beinhalten muss, obwohl die Struktur der DNA von *Franklin*, *Watson* und *Crick* noch nicht aufgeklärt war. Infolgedessen, wurden über die Jahre zahlreiche Derivate der sogenannten Stickstoff-Loste hergestellt, von denen einige auch heute noch therapeutische Anwendung finden (z.B. Melphalan, Chlorambucil und Cyclophosphamid). Da die pharmazeutisch aktive 2-Chloroethyl-Einheit sowohl in Stickstofflosten als auch in der Natur in der Verbindung *593A* zu finden ist, wird in der Arbeitsgruppe Göttlich der detaillierte Wirkmechanismus untersucht. Die *593A* strukturell verwandten Bis-3-chloropiperidine und monofunktionalen 3-Chlorpiperidine zeigten in Resultaten von *in vitro* Zellstudien vielversprechende DNA-Alkylierungseigenschaften. Einige Derivate waren hierbei sogar aktiver gegenüber der *HCT-15 colon*, *Ovarian 2008* und *BxPC-3* Tumorzelllinien als die Referenzverbindung Chlorambucil.

Die vorliegende Arbeit untersucht die Struktur-Wirkungs-Beziehung der 3-Chlorpiperidine mit einem speziellen Fokus auf den geminalen-Disubstituenten-Effekt. Hierbei wurde eine lineare Korrelation zwischen dem inneren Winkel des C5-Substituenten und der Bildung der reaktiven Aziridinium-Zwischenstufe beobachtet. Dies impliziert, dass die Beschleunigung der Bildung des Aziridinium-Ions durch die Winkelkontraktion stattfindet (Thorpe-Ingold-Effekt), anstatt durch eine höhere Population an reaktiven Rotameren (Reactive-Rotamer-Effekt). Des Weiteren konnte in der Arbeit eine effiziente, elektroorganische Methode zur Darstellung von 3-Chlorpiperidinen entwickelt werden. Dies stellt einen substanziellen Fortschritt dar, da hierbei auf konventionelle Oxidationsmittel in stöchiometrischer Menge verzichtet werden kann, was die Atomökonomie des Prozesses im Vergleich zu existierenden Prozessen deutlich steigert. Wir konnten zudem zeigen, dass 3-Chlorpiperidine über ihre antineoplastischen Eigenschaften hinaus auch anthelminthische Eigenschaften besitzen. Sie sind somit als neue Leitstruktur für die Entwicklung von Medikamenten gegen Schistosomiasis von Interesse.

Abstract

With the advent of the 21st century, an epidemiological transition occurs in most developed nations, in which infectious diseases are gradually superseded by noncommunicable diseases such as cardiovascular disease and the neoplastic diseases. With an estimated 19.3 million new cases of malign neoplasm in 2020, there is an ever more increasing desire for novel therapeutic compounds and methods in oncology. Surgery and radiotherapy had dominated this medical domain until the 1960s, when cure rates plateaued at about 33% due to the presence of heretofore underrated micrometastases. Using chemotherapy in combination with radiotherapy and surgery proved to be the best strategy to tackle these more advanced malign neoplasms. Consequently, different important classes of chemotherapeutics emerged over the years, including alkylating agents, platinating agents, topoisomerase inhibitors, antimetabolites, intercalating agents and taxanes, of which the alkylating agents represent the oldest class of clinically used chemotherapeutics.

The first documented therapeutic use of alkylating agents occurred in 1942, when *Gilman* and *Phillips* administered mechlorethamine, a structural derivative of sulfur mustard, to patients suffering from Hodgkin's lymphoma. Even though the structure of DNA had yet to be elucidated by *Franklin*, *Watson* and *Crick*, *Gilman* and *Phillips* correctly proposed a mechanism considering alkylation of a vital cellular constituent. Over the years, scientists tried to develop milder and more selective analogues of the so-called nitrogen mustards, which resulted in numerous compounds that are used clinically to this date, e.g. melphalan, chlorambucil and cyclophosphamide. The *Göttlich* group, inspired by nature, aimed to create simplified analogues of the naturally occurring nitrogen mustard *593A*, an antibiotic with antineoplastic properties. Unlike previous synthetic nitrogen mustards, this compound features a 2-chloroethyl moiety confined in a piperidine ring, potentially offering a milder alternative to clinically used nitrogen mustards. The resulting *bis*-3-chloropiperidines and 3-chloropiperidines showed promising DNA alkylating capabilities, with certain derivatives being more active in cell studies towards the *HCT-15 colon*, the *ovarian 2008* and the *BxPC-3* pancreatic cancer cell line than reference compound chlorambucil.

This work aims to elucidate the structure-activity-relationship (SAR) with a special focus on the geminal disubstituent effect in the formation of bicyclic systems, which could yield milder drugs in the future. Thereby, we observed a linear correlation between the inner angle of the C5-substituents in the piperidine ring and the rate constant of aziridinium ion formation, which suggests the presence of a classical *Thorpe-Ingold-effect* instead of a *Reactive-Rotamer-effect*. We furthermore developed an efficient, electroorganic protocol for the synthesis of 3-chloropiperidines, which resembles a substantial improvement in atom economy compared to existing methods as stoichiometric amounts of conventional oxidants can be avoided in the cyclization step. Biological assays revealed that *bis*-3-chloropiperidines show activity against the parasitic trematode *Schistosoma mansoni* and resemble a novel scaffold that can be considered in anthelmintic drug discovery.

Preface

This work resembles a cumulative dissertation which was prepared between November 2021 and February 2025 in the working group of Prof. Dr. Richard Göttlich at the Justus-Liebig-University in Giessen. Hereafter, the structure, synthesis and the application of novel 3-chloropiperidine derivatives is explored.

In the first chapter, the theoretical background of the work will be discussed with a special focus on the nitrogen mustards and their origin. This is followed by state-of-the-art knowledge on the geminal disubstituent effect, electroorganic synthesis and anthelmintic therapy. At the end of each of these three subchapters, the key motivations and the essentials of our work will be presented.

Detailed findings can then be assessed from the reprinted publications in chapter three. The respective supporting information is available at the publishers' website.

I want to close this work afterwards with a few warm words towards the people who supported me during my work over the past few years.

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1. Introduction

1.1. Chemotherapy

Chemotherapy uses small molecules to cure neoplastic and infectious diseases. This idea originated in the early 20th century, when *Paul Ehrlich* developed salvarsan **1** (figure 1). This mixture of arsenic compounds could be applied to treat patients infected by syphilis, a sexually transmitted disease originating from the bacterium *Treponema pallidum*.[1] In the following decades it became evident that this idea could be transferred to further pathogens including bacteria[2], viruses[3], fungi[4], and helminths[5]. One remarkable advancement occurred accidentally in the First World War, when physicians observed leukopenia, the deficiency of white blood cells, in patients with previous exposition to mustard gas, or *bis*-(2-chloroethyl) sulfide **2**.[6] Others concluded that similar compounds to mustard gas could be used to treat certain leukemia types which are accompanied with a surplus of white blood cells.[7] More broadly, this sparked the hope that small molecules could be used to treat neoplastic diseases, which feature the uncontrolled multiplication of cells, in general. However, due to its severe toxicity[8], sulphur mustard could never be used clinically.

It was later theorised by *Gilman* that replacing the sulphur in mustard gas with nitrogen would result in a less electrophilic compound.[7] This would in turn lead to less severe cytotoxic effects compared to the parent compound and render therapeutic application viable. In 1942, the first clinical trial started to investigate the therapeutic potential of the nitrogen containing derivative of mustard gas, Mechlorethamine **3**. The conductors, *Gilman* and *Philips*, observed tumour mass regression in patients with Hodgkin's lymphoma, a malign neoplasm of the lymphatic system. They correctly proposed that the cytotoxic effects were due to alkylation of a vital cellular constituent. Even though the detailed structure of DNA had not been elucidated by *Franklin*, *Watson* and *Crick* yet, *Gilman* could already identify that the application of nitrogen mustards resulted in a large amount of lesions in the chromosomes, which mainly consist of DNA. As a direct consequence to the successful therapeutic application of Mechlorethamine, numerous nitrogen mustards were synthesised by others to improve the pharmacological properties, which resulted in antineoplastic drugs like chlorambucil **5**, cyclophosphamide **6**, melphalan **8** and estramustine **10**.[9] These anti-proliferate drugs are in clinical use until now, even though different types of antineoplastic drugs emerged afterwards, including antimetabolites, anti-microtubules and topoisomerase inhibitors (figure 1).[10] Nevertheless, understanding the mode of action exerted by nitrogen mustards requires extensive research as this could result in novel drugs with less severe adverse effects and better pharmacokinetic properties. Thereby, an important goal is to circumvent common resistance mechanisms against nitrogen mustards and implement prodrug strategies to improve the selectivity of these compounds. In the following chapter, the mode of action of nitrogen mustards and some implemented prodrug strategies will be discussed.

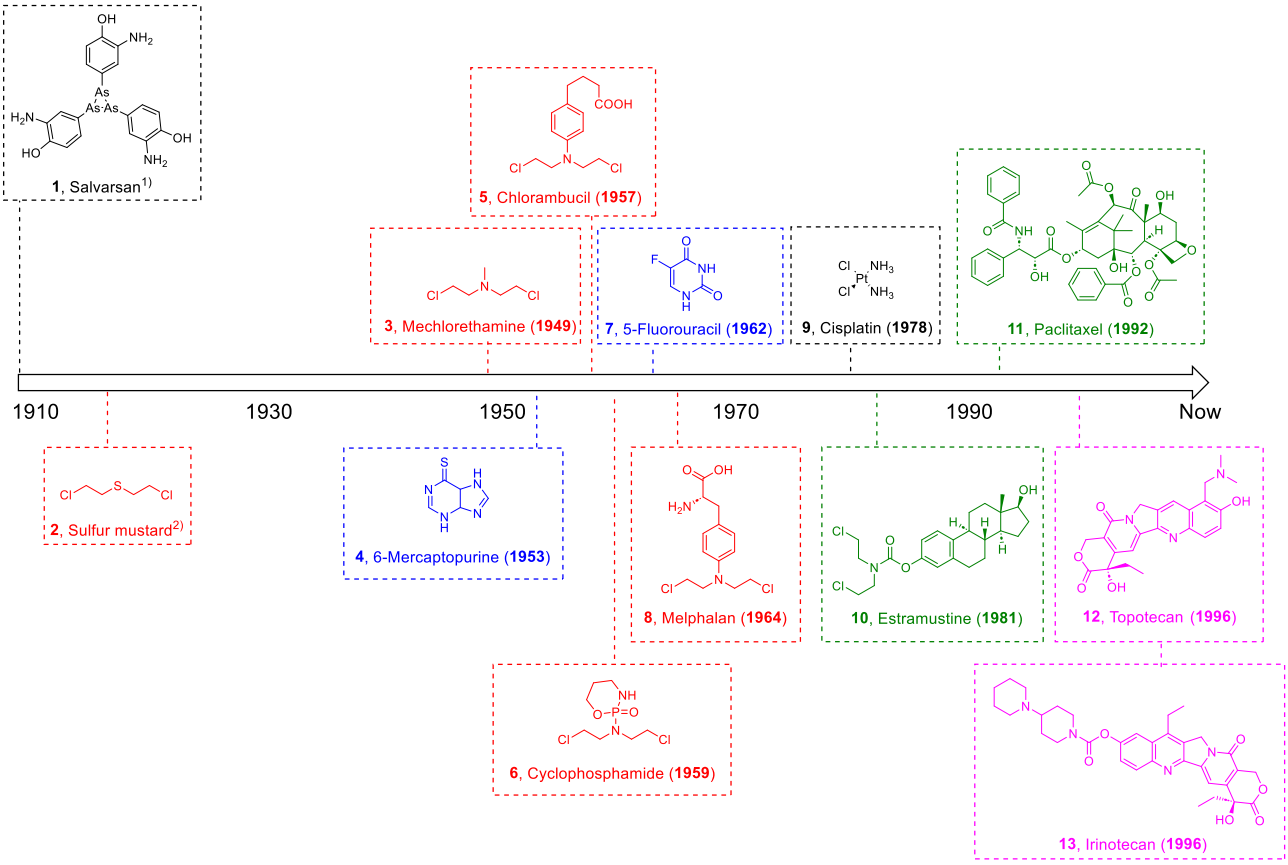


Figure 1: Development of antineoplastic agents starting from the first chemotherapeutic drug *Salvarsan*. A selection of different classes of antineoplastic agents is displayed (red: Alkylating agents; blue: Antimetabolite; green: Antimicrotubule; purple: Topoisomerase inhibitor) with the respective year of approval by the *Food & Drug Administration* (FDA) in apprentices. [1)] Salvarsan is a mixture of triaminotrihydroxyarsenobenzene and pentaaminopentahydroxyarsenobenzene. First introduction to the market is implicated (1910). [2)] The year of first deployment as a warfare agent is implicated (1917).[9,11]

1.2. Nitrogen Mustards

The mode of action of nitrogen mustards consists of two conceptual parts. The first part is the intramolecular formation of a reactive aziridinium ion *via* nucleophilic substitution (figure 2).[7] The second part is the covalent binding of this intermediate to the nucleophilic centres on DNA nucleobases, mainly the N^7 of guanine and to a lesser degree the N^3 of adenine.[12] This results either in mono-alkylation of the DNA strand or crosslinking. Crosslinking can tether the same strand (intra-strand crosslinking) or two opposing strands (inter-strand crosslinking). In particular, nitrogen mustards predominantly form inter-strand crosslinks over intra-strand crosslinks. As soon as the DNA is alkylated, transcription and therefore replication of the DNA helix is prohibited as the passage of polymerases is blocked due to the geometrical distortion. Consequently, cell division is intermittently prohibited.[13] Nevertheless, the alkylated DNA can still be repaired by repair mechanisms of the cell. Among them are the base excision repair (BER) and nucleotide excision repair (NER) mechanisms. Thereby, the BER mechanism can merely repair the damage caused by mono-alkylation, while the NER mechanism is able to also repair crosslinked DNA.[14] If DNA repair does not occur timely, depurination or deglycosylation is likely, resulting in double strand cleavage of DNA, which is irreversible. Also hydrolytic

DNA cleavage could occur, as the guanine N^7 alkylation increases the electrophilicity of the adjacent position.[15] The induced double strand breaks can ultimately lead to programmed cell death (apoptosis) *via* the so-called mitochondrial pathway by triggering the upstream (initiator) caspase-9. This upstream caspase consequently activates the downstream (effector) caspases 3 and -7. The caspases 3 and -7 are then able to cleave multiple vital cellular proteins, which triggers a range of apoptotic events and may therefore be the origin of the cytotoxic properties of nitrogen mustards.[16] However, the distinct role of each caspase is not fully understood yet.[17]

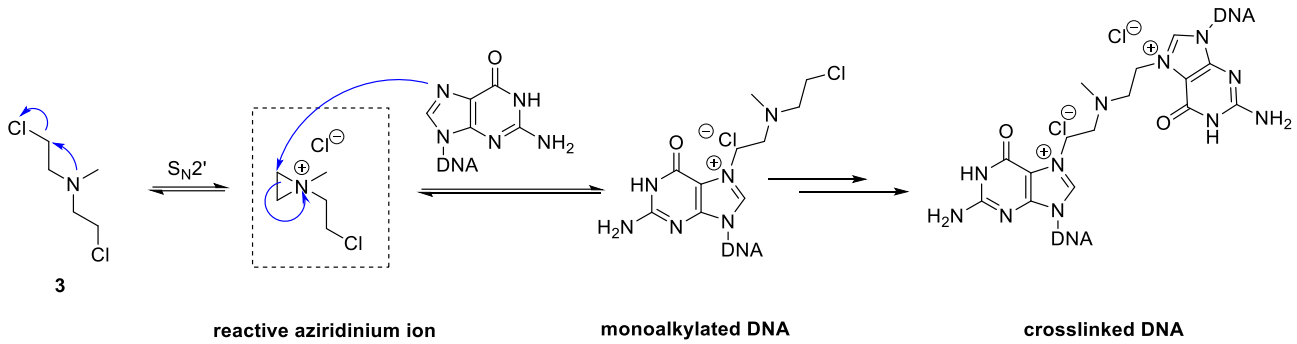
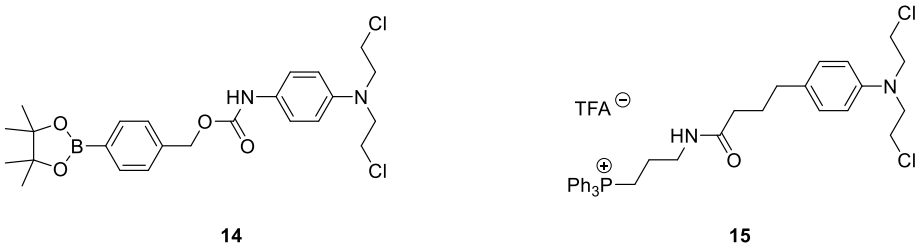


Figure 2: Mode of action of 3-chloropiperidine derivatives.

The cytotoxic profile of **3** resembled a large limitation for its therapeutic scope, as high cytotoxicity is commonly accompanied with severe adverse effects.[18] This encouraged the search for novel nitrogen mustards with better pharmacological properties. Chlorambucil **5**, which was developed in the early 1950s and retrieved approval by the FDA in 1957, incorporates a different *N*-substitution than **3**.[19] This serves two purposes: Firstly, the aryl substituent renders the antineoplastic agent less electrophilic overall. Secondly, the side chain incorporates a carboxylic acid group, which results in better solubility and therefore enables oral administration. Two years after the introduction of **5**, cyclophosphamide **6** was approved by the FDA and remains an important drug in the repertoire of physicians to this date. Thereby, the major advancement was that **6** is delivered as a prodrug to the liver. There, **6** is initially hydroxylated by mixed-function oxidase and is subsequently tautomerised by cytochrome P450 to yield aldophosphamide. Aldophosphamide is degraded to urotoxic acrolein and the bifunctional, active phosphoramide mustard by *β*-elimination. The active phosphoramide can then function as an alkylating agent.[9,20] Another major advancement resembled the development of Melphalan **8**, which has increased pharmacokinetic properties compared to previous nitrogen mustards. By implementing a phenylalanine moiety into the nitrogen mustard, the phenylalanine transport mechanism can be used to deliver the antineoplastic agent into the cell. Melphalan gained approval by the FDA in 1964 and is used to this date as well.[21]

Neverthless, the non-specific reactivity of nitrogen mustards was still accompanied with significant adverse effects, prompting researchers to add targeting groups to the side chain.[9] The development of estramustine **10** partially solved this problem by introducing an estradiol moiety into the nitrogen mustard, thereby delivering the drug to hormone-dependent tumour cells.[22] Recently, *Chen* et al. leveraged the different microenvironment of tumour cells with **14** to deliver nitrogen mustards more selectively by using the higher concentration of reactive oxygen species (ROS) of specific tumour cells (figure 3). Thereby, they measured GI_{50} values below 1 µM for a variety of tumour cell lines of leukemia,

lung, central nervous system, melanoma, renal and prostate. It was previously reported that these employed cell lines have a higher concentration of ROS, which facilitated that the prodrug could be converted efficiently.[23] Moreover, efforts were made by *Millard* et al. to leverage the higher intrinsic mitochondrial membrane potential of cancer cells (figure 3). Mitochondria are a particularly sensitive target for alkylating agents as mitochondria lack a functioning NER mechanism. By modification of the sidechain of chlorambucil with a triphenylphosphonium group, they retrieved **15**. This compound exerted an 80 fold increase of cell kill in a panel of breast and pancreatic cancer cell lines compared to the parent compound **5**.[24]

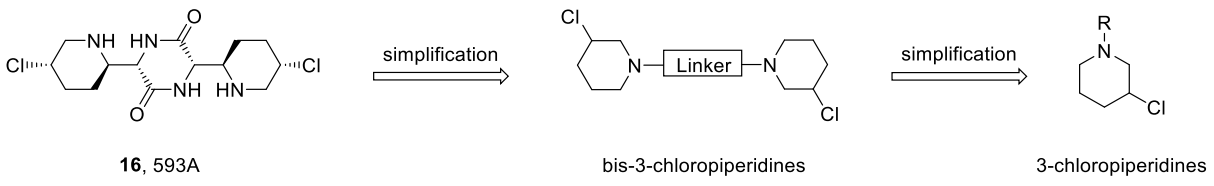


14 **15**

Figure 3: Novel nitrogen mustards derived from chlorambucil that target the increased amount of ROS in some cancer cells (left) and the increased mitochondrial membrane potential of some cancer cells (right).

The concepts described above aim to incrementally refine a lead structure to increase bioavailability and selectivity. In the past, simplifying natural products while retaining their activity proved to be an important strategy to develop drugs as well.[25] In regards to nitrogen mustards, the starting point for such a development could be the naturally occurring antibiotic 593A **16** (figure 4). Antibiotic 593A was first isolated in 1970 by *Gittermann* from a fermentation broth of *Streptomyces griseoluteus*. Thereby, he could prove the natural occurrence of nitrogen mustards. In addition to its antibiotic activity, **16** inhibits the growth of the L1210 lymphocytic leukemia, Krebs 2 murine tumour systems and the rat Walker 256 carcinosarcoma.[26] *Brockmann* et al. could also show that **16** exhibits activity against lines of *L1210* lymphocytic leukemia, which show resistance towards cyclophosphamide.[27] Nonetheless, due to its adverse effects, e.g. damage to the bone marrow, therapeutic application was not possible.[28]

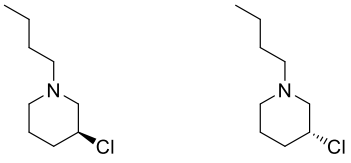
The synthetic preparation of **16** remains challenging and only one total synthesis has been published to this date by *Fukuyama* in 1979.[29] Especially because of the exhibited activity against tumours resistant to the commonly used antineoplastic agent cyclophosphamide, *Göttlich* et al. suspected a secondary mode of action (in addition to DNA alkylation) and subsequently synthesised simplified derivatives for further investigation (figure 4).[30]



16, 593A bis-3-chloropiperidines 3-chloropiperidines

Figure 4: Construction of simplified analogues of *593A*.

The structurally simplified *bis*-3-chloropiperidines (figure 4) showed activity in various cell-based assays, with certain derivatives being more active towards the *HCT-15* colon, the ovarian *2008* and the *BxPC-3* pancreatic cancer cell line than reference compound **5**.[31,32] Leveraging decatenation assays of kinetoplast DNA, *Sosic* et al. could show that *bis*-3-chloropiperidines are able to inhibit the human topoisomerase IIα (hTopoIIα), which is a well-known target that is commonly overexpressed in tumours.[33] Monofunctional 3-chloropiperidines were found to exert DNA alkylating capability, particularly against pancreatic cancer cells. Thereby, *Carraro* et al. showed that the stereochemistry of monofunctional 3-chloropiperidines modulates the activity *in vitro*. They found that the compound **17** was more cytotoxic in cell assays than its enantiomer **18** even though it was less active in DNA cleavage assays (figure 5). This again indicated that there are different modes of action involved besides DNA alkylation that influence the activity of 3-chloropiperidines.



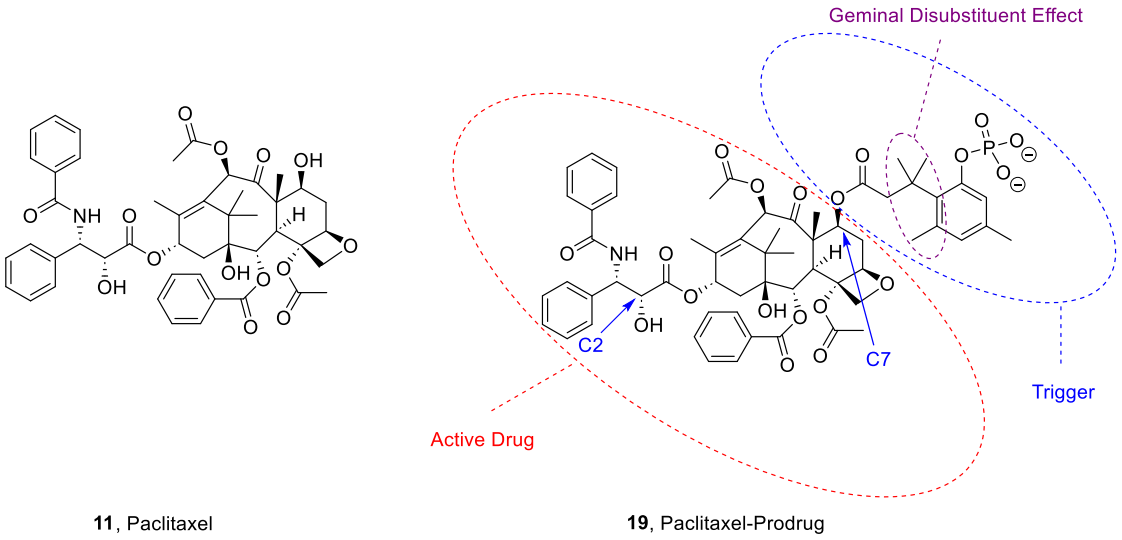
17 **18**

Figure 5: Enantiomers **17** and **18** that showed different activity *in vitro*.

Carraro et al. found that compounds **17** and **18** differ in their cellular permeation behaviour using the parallel artificial membrane permeation assay (PAMPA). Contrary to *bis*-3-chloropiperidines, monofunctional 3-chloropiperidines generally showed poor inhibition of hTopoIIα in decatenation assays, ruling out this mode of action.[34] In addition to elucidating the mode of action of mono- and bifunctional 3-chloropiperidines, the *Göttlich* group made efforts to understand the kinetics of the formation of the intermediate, reactive aziridinium ion. Thereby, the intermediate aziridinium ion could be isolated by abstraction with silver salts with weakly coordinating anions and subsequent single crystal XRD analysis. This confirmed the boat-like bicyclic structure of the reactive intermediate. The reactivity of this intermediate could then be investigated *via* NMR kinetic studies. This revealed that the derivative methylated in the geminal position exhibited a higher rate of solvolysis compared to the unmethylated analogue, which was attributed to the geminal disubstituent effect (chapter 1.3). Moreover, *Helbing* et al. showed that the formation of the reactive aziridinium ion is the rate-determining step of the reaction sequence. *Helbing* et al. concluded that the geminal disubstituent effect could potentially be used to fine-tune the reactivity of 3-chloropiperidines.[35] Leveraging the geminal disubstituent effect, milder alternatives to existing DNA alkylants could be obtained, which was our motivation to gain further understanding of the geminal disubstituent effect in bicyclic systems.

1.3. The Geminal Disubstituent Effect

The substitution of two hydrogens on a carbon chain tethering two reactive centres by two larger substituents generally increases the rate of cyclisation. This remarkable effect has been termed the geminal disubstituent effect and already found its application in cancer therapy - Especially for the release of the anti-microtubule agent paclitaxel **11** (taxol) from its prodrug (figure 6). [36–39]



11, Paclitaxel **19**, Paclitaxel-Prodrug

Figure 6: Antiproliferate drug paclitaxel (**11**, left) and the administered prodrug (**19**, right).

Paclitaxel is a natural compound that was first isolated from the western yew *Taxus brevifolia* and shows activity against the *L-1210*, *P-388* and *P-1534* leukemia types. Also, inhibition of solid tumours, such as the WM-256 carcinosarcoma was shown previously.[36] As **11** displays low solubility in aqueous media, the prodrug **19** was prepared, which can be degraded *in vivo* by enzymes to regenerate the parent drug. The introduction of a highly methylated hydroxyphenylpropionic acid at C2 or C7 of paclitaxel ensures the water solubility. After activation by a phosphatase, lactonisation can occur which releases the active compound **11**. The lactonization thereby must be facilitated by the geminal dialkyl effect through the introduction of three methyl groups.[37] Otherwise lactonisation is too slow and the parent drug cannot be generated in a timely manner (half-life >200h).[38]

For over one hundred years, the exact origin of the geminal disubstituent effect has remained unclear and is still under vehement discussion.[39] The most relevant theories for its origin are illustrated in figure 7. The first hypothesis was published in 1915 by *Thorpe*, *Ingold* and *Beesley*. They proposed that the introduced alkyl groups repulse each other, which in turn lowers the internal angle *β* and therefore brings the reactive centres closer together in accordance with *Baeyer's* theory of strain.[40] Since the rate increase of cyclisation was significantly higher than expected from small angular differences, alternative explanations emerged soon afterwards.[41] In 1960, *Bruice* and *Pandit* attributed the large rate increases to an elevated population of reactive rotamers, which was later doubted by *Parill* and *Dolata*, as they proposed that *Bruice* and *Pandit's* hypothesis conflicts with the Curtin-Hammett principle.[42,43] Also in 1960, *Allinger* and *Zalkow* determined thermodynamic data for the solvolysis reaction of organic halides,

through which they aimed to get insights on the origin of the geminal disubstituent effect. They concluded that alkyl substituents reduce the increase of gauche interactions in the transition state. This in turn lowers the transition state enthalpy of the ring closure compared to the unsubstituted derivative. They could also show that entropy has a significant contribution, whereby they propose that alkyl groups restrict internal rotations in acyclic compounds, which lowers the entropy, so that the loss of entropy is less pronounced in the cyclised product.[44] In 1972, *Milstien* and *Cohen* introduced a concept, which they termed '*stereopopulation control'*. Their idea involved the restriction of rotational freedom of molecules to favour reactive rotamers. They attribute this to *van-der-Waals* strain and electrostatic repulsion. For this, they examined the rates of lactonisation of hydrocoumarinic acid derivatives. They found that both alkyl substitutions on the side chain and on the aromatic ring significantly increase the rate of lactonisation. The most favourable observation included a rate increase by a factor of 10^{11} compared to the unmethylated parent compound. Because of this increase, they termed this observation explicitly as a *trimethyl-lock-effect*, which then found its application in compound **19**.[45] *Winans* and *Wilcox* doubted their hypothesis and suggested that the reason for the rate increase could be conventional steric effects, based on empirical force field calculations. They concluded that the relief of steric strain upon lactone formation was significant, which indicated that conventional steric effects play a major role in the rate enhancements of substituted hydrocoumarinic acids, rendering van-der-Waals strain and electrostatic repulsion merely secondary effects and less significant than *Milstien* and *Cohen* originally postulated.[46] The influence of ring size on the geminal disubstituent effect has also been determined by *Galli* et al through kinetic investigations with *ω*-bromocarbonic acids. They concluded that the geminal disubstituent effect plays a major role in small and medium sized rings while it diminishes in larger ring systems.[47] *Eberson* and *Welinder* also found that geminal methylation increases the equilibrium constant for cyclisation and therefore also influences the thermodynamics of a cyclisation.[48] In 1994, *Parill* and *Dolata* attributed the rate increase to reductions in the transition state enthalpy rather than reactive rotamer populations, which they supported by computations with the WIZARD program package. They termed this explanation the '*facilitated transition hypothesis'*.[43,49] Although many of the above articles either disregard the *Thorpe-Ingold-effect* or the *Reactive-Rotamer-effect*, it is reasonable to say that both of these explanations are likely to contribute to the rate enhancements of cyclisations observed upon geminal disubstitution. In this regard, we assumed that 3-chloropiperidines could offer valuable insight on the magnitude of the rate increase induced by the angle dependent Thorpe-Ingold effect. This is the case, because the already cyclic structure of 3-chloropiperidines spatially fixes the reactive centres. In open-chain systems the reacting centres move closer together by a magnitude of several angstroms by the formation of reactive rotamers prior to the cyclisation. In contrast, even if the formation of the reactive aziridinium ion proceeds *via* reactive rotamers (e.g. the twist-boat conformation), the decrease in distance between the reacting centres is limited by the induced ring strain. The rate increase of aziridinium ion formation in 3-chloropiperidines consequently cannot be explained by the original hypothesis of *Bruice* and *Pandit*.

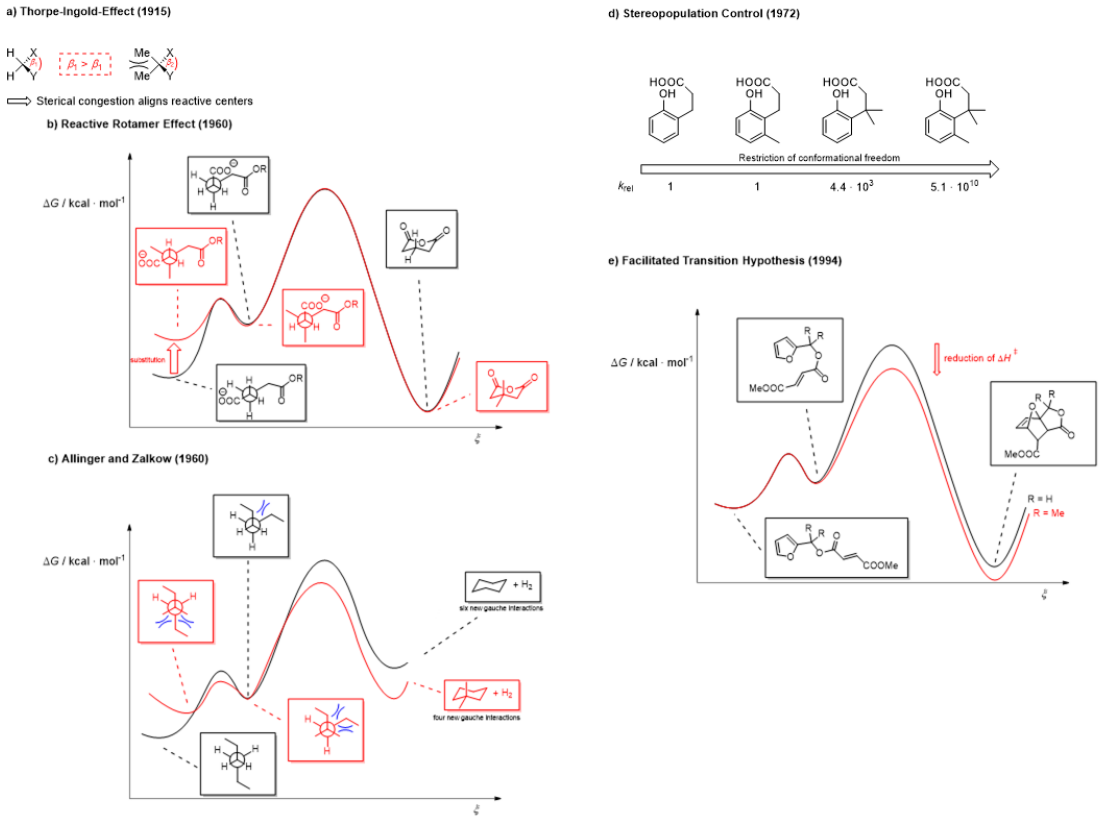


Figure 7: Hypotheses on the origin of the geminal disubstituent effect. For illustration, the Gibbs free energies in the potential energy surfaces were referenced on the corresponding reactive-rotamer.

We assumed that a linear correlation between the internal angles β_i (figure 8) and the relative rate constant of solvolysis would unveil the magnitude in rate increase induced by the Thorpe-Ingold-effect.

Therefore, we subjected five differently substituted 3-chloropiperidines **20**-**24** (figure 8) to solvolysis experiments in deuterated methanol (MeOH-d4), which we quantified via nuclear magnetic resonance spectroscopy (NMR) with dibenzyl ether as an internal standard. We observed a relative rate constant k_{rel}(**23**) = 1.56 for the solvolysis of **23** compared to the unsubstituted parent compound **22**. The diphenylated compound **24** exerted an even higher rate constant for solvolysis with k_{rel}(**24**) = 2.38. Conversely, introducing strained ring systems in the geminal position reduced the rate of solvolysis. Thereby, for the cyclopropyl and cyclobutyl substituted compounds **20** and **21** we determined k_{rel}(**20**) = 0.53 and k_{rel}(**21**) = 0.64 respectively, which is to the best of our knowledge the first deceleration of an examined reaction *via* the Thorpe-Ingold-effect. Contrary to that, in an earlier investigation by *Jung*[50], introduction of cyclopropyl and cyclobutyl moieties in geminal position lead to an increase in the rate of cyclisation, which indicates that this deceleration could be limited to bicyclic ring formations.

To put the kinetic data into relation with the internal angles β_i we obtained single crystals of the hydrochloric acid salts of the respective 3-chloropiperidines by slow vapor diffusion and subjected them to XRD analysis. The internal angles β_i and the relative rate constant show a good linear correlation

(R^2_{XRD} = 0.98), which supports the hypothesis that the modulation of the rate constant is dominated by a classical, angle dependent Thorpe-Ingold-effect. In contrast, *Jung* et al. observed a rate increase in comparison to the unsubstituted system, when they employed a geminal cyclopropyl and cyclobutyl substitution in an open chain system in a model *Diels-Alder* reaction.[51] Density functional theory[52] (DFT) calculations employing the three-fold corrected *Perdew-Burke-Ernzerhof*-type hybrid functional PBEh-3c[53] with the corresponding def2-mSVP basis set support the linear correlation of the inner angles β_i and the rate constant of solvolysis, albeit with a lower coefficient of determination ($R^2_{PBEh\text{-}3c}$ = 0.87).

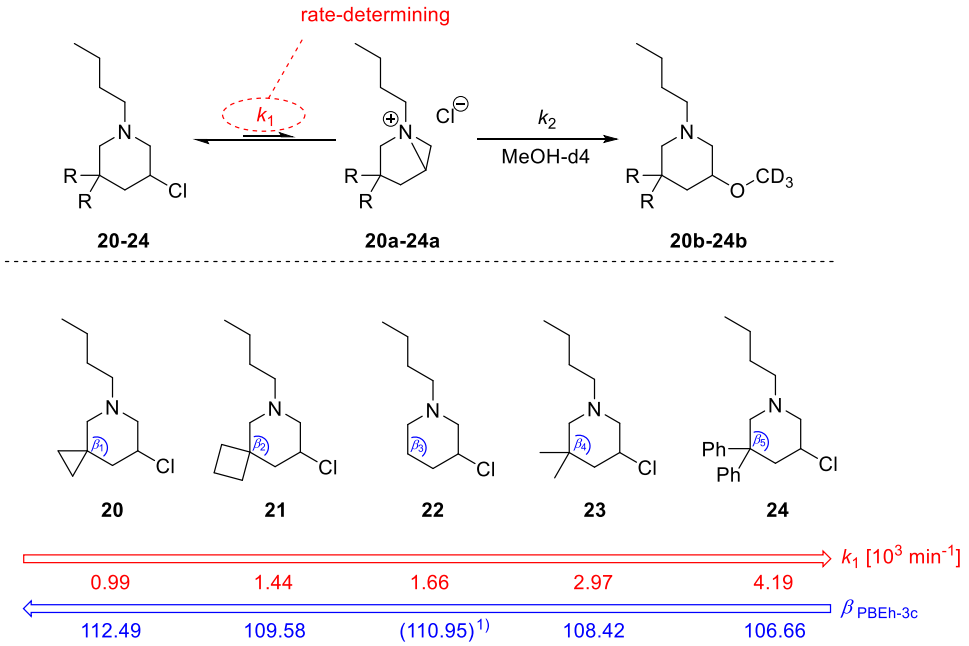


Figure 8: Kinetic studies on the formation of the reactive aziridinium intermediate in C5-substituted 3-chloropiperidines. 1) The unsubstituted derivative resembles an outlier in the trend.

The above-mentioned data implicate that the *Thorpe-Ingold*-effect in bicyclic systems could be used to refine the reactivity of 3-chloropiperidines as DNA alkylants. We aim to gather more information about the influence of the Thorpe-Ingold-effect in bicyclic ring formation in the future and what impact this could possibly have on the results of DNA cleavage assays or *in vitro* cell studies. To achieve this, we planned to synthesize more derivatives with different substitution patterns (figure 9). However, we deemed the synthesis of all the different permutations with existing synthetic protocols as inefficient, so we aimed to optimise the synthetic protocol to obtain 3-chloropiperidines first. In the next two chapters, I will give a brief overview of the possible synthetic strategies to form a 3-chloropiperidine cycle and how we aimed to add a time efficient and atom economic method to the toolkit of 3-chloropiperidine synthesis.

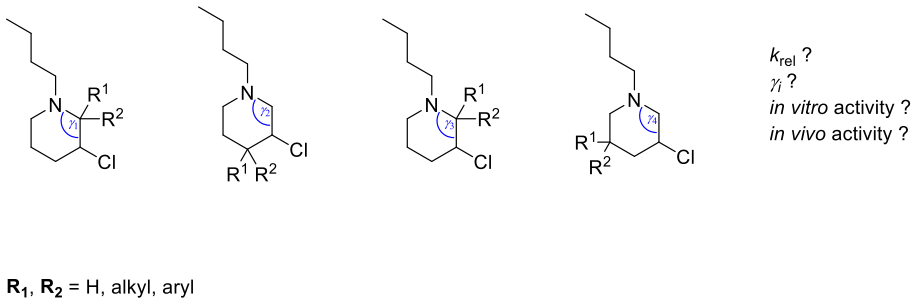


Figure 9: How does the substitution pattern on 3-chloropiperidines influence the activity *in vitro* and *in vivo*?

1.4. Conventional synthesis of 3-Chloropiperidines

Unsubstituted 3-chloropiperidines can be synthesised conveniently and enantioselectively leveraging the chiral pool. Thereby, L-proline **25** can be reduced to L-prolinol **26** using lithium aluminium hydride. After functionalisation of the nitrogen using aliphatic or benzylic halides, the pyrrolidine ring can be expanded *via* an aziridinium intermediate using thionyl chloride (figure 10), which affords the respective R-enantiomer **28**. The S-enantiomer is analogously accessible starting from D-proline.[34]

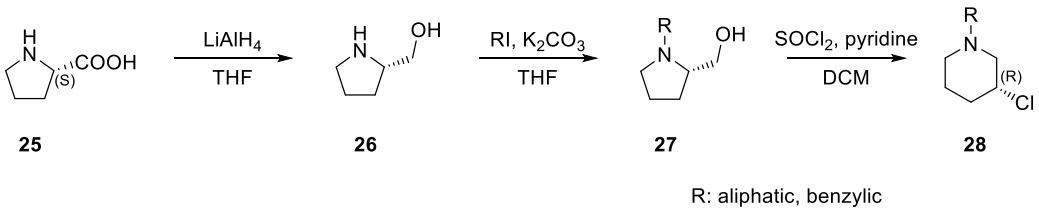


Figure 10: Synthesis of C5-unsubstituted 3-chloropiperidines by ring expansion of *L*-prolinol.

If alkyl substitution on the piperidine cycle is desired, the respective 3-chloropiperidine can be accessed through the respective 4-pentenylamines/4-pentenals and subsequent cyclisation. The general synthetic strategy is illustrated for bis-3-chloropiperidines in figure 11, but the strategy is also applicable to monofunctional 3-chloropiperidines.[30,32,34,54] Either the unsaturated, secondary amine precursors can be generated by reductive amination (figure 11, pathway a and a') or nucleophilic substitution with a primary amine and a benzylic halide. The resulting 3-chloropiperidine precursor can then be cyclised with various strategies.

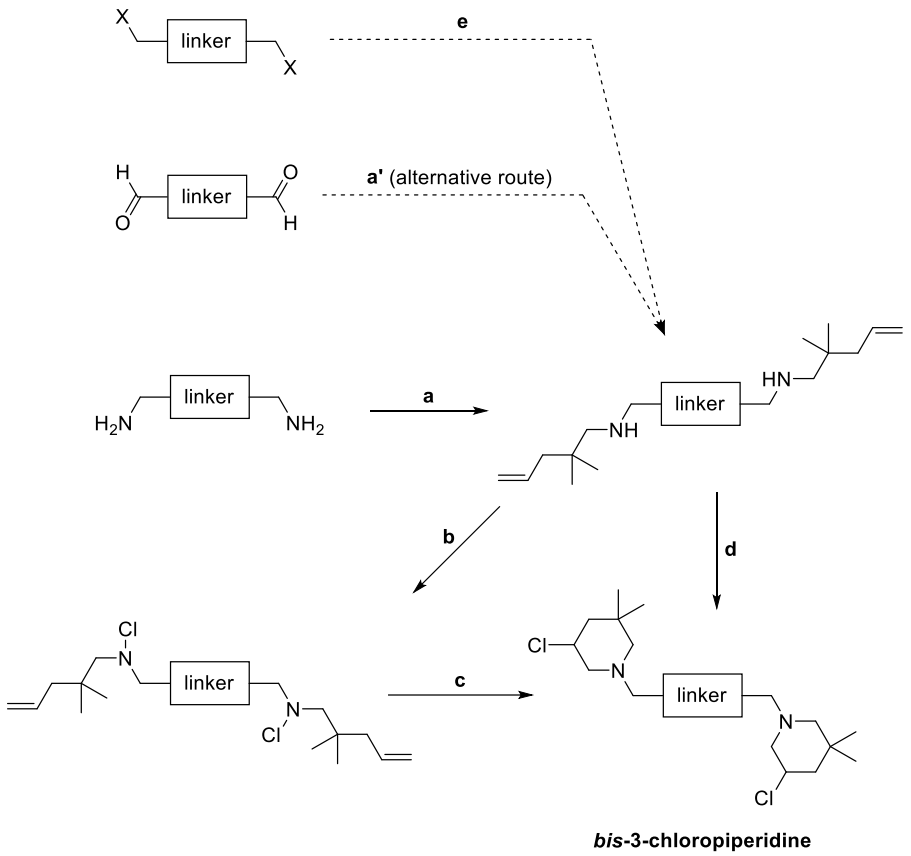


Figure 11: Synthetic methods for the preparation of *bis*-3-chloropiperidines. (a) 2,2-dimethylpent-4-enal, acetic acid, $NaBH(OAc)_3$, DCM, 0°C to rt, o.n.; (a') 2,2-dimethylpent-4-enylamine, acetic acid, $NaBH(OAc)_3$, DCM, 0°C to rt, o.n.; (b) NCS, DCM, 0°C to rt, 3h; (c) TBAI, $CHCl_3$, 60°C, 2h; (d) $CuCl_2 \cdot 2\ H_2O$, THF, rt, o.n.; (e) NaH, 2,2-dimethylpent-4-enylamine, THF, 0°C to rt, o.n.

Initially, stepwise procedures were published in which an oxidant in stoichiometric quantity is used to chlorinate a *δ*,*ε*-unsaturated amine. Cyclisation can then be facilitated by strong acid-[55], transition metal-[56] or iodide-[57,58] catalysis. Later, procedures were published, which intended to form the 3-chloropiperidine directly from the *δ*,*ε*-unsaturated amine in a one-step procedure. For example, a procedure was published by *Liu*[59,60] et al. in 2013, which cyclises the unsaturated amine employing copper(II) chloride (figure 12). In 2014, *Li* et al. published a method to form 3-chloropiperidines directly from *δ*,*ε*-unsaturated amines using catalytic iodine as an oxidant with potassium persulfate as a re-oxidant and lithium chloride as a chloride source.[61] The mechanistic proposals for these two direct ring closures are depicted in figure 12.

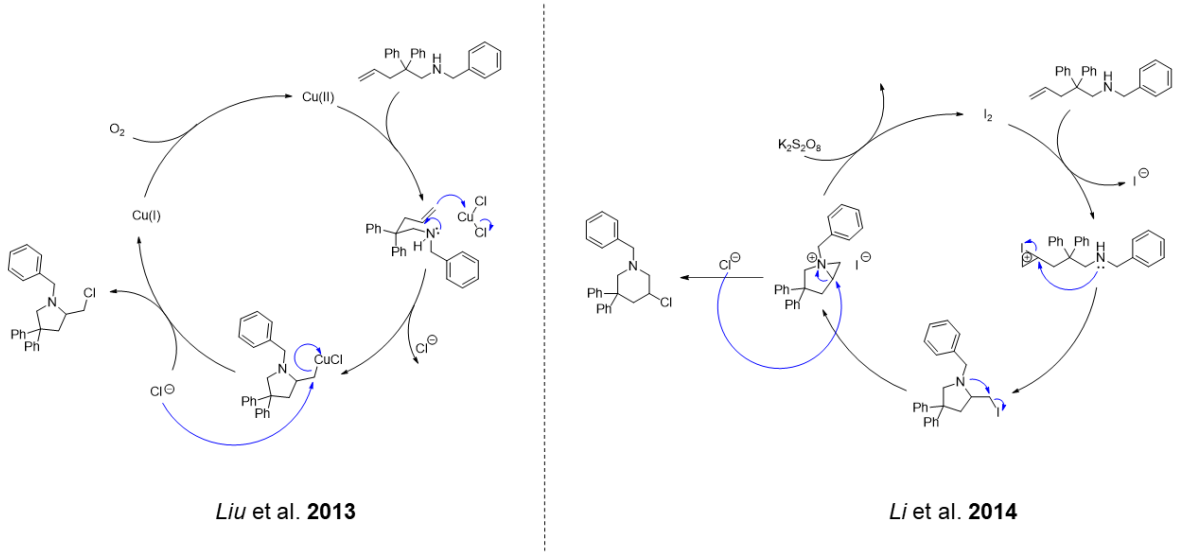


Figure 12: Mechanism of the direct cyclisation of 3-chloropiperidines.

All the cyclisation procedures stated above employ a conventional oxidant in stoichiometric quantity. This manifests in a low atom economy, which is a problem that we intended to overcome leveraging electroorganic synthesis.

1.5. Electroorganic Synthesis of 3-Chloropiperidines

The development of a more sustainable and responsible chemical industry is considered increasingly in recent years.[62,63] Especially the advancements in photochemistry[64], catalysis[65] and electrochemistry[62,66] mirror the global efforts for an environmentally benign future. The general advantages of green chemistry have been previously summarised in the twelve principles of green chemistry by *Paul Anastas* and *John Warner* (figure 13).[67]

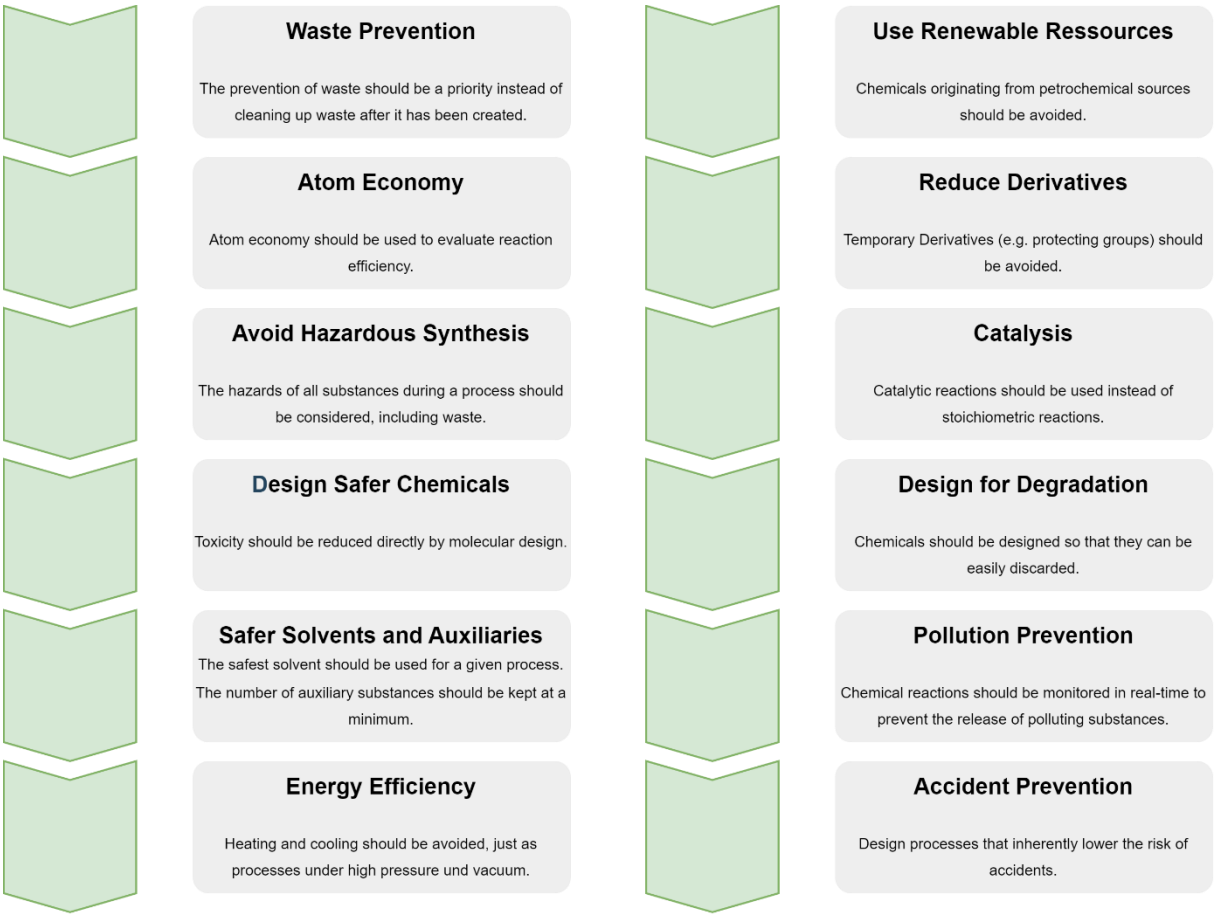


Figure 13: The twelve principles of green chemistry as described by *Paul Anastas* and *John Warner*.

Especially one subfield of electrochemistry, electro-organic synthesis, has relived a renaissance in recent years. Electro-organic synthesis uses electrical energy to drive reactions, which offers numerous benefits over conventional synthetic methods. On the one hand, electro-organic synthesis typically operates at ambient temperature and pressures, thereby reducing energy consumption. On the other hand, the atom economy is improved, if substrates are directly oxidised or reduced *via* electron transfer at the working electrode or *via* a (catalytic) redox mediator. This minimises waste and is also a benefit for safety as potentially hazardous reagents (e.g. bromine) can be formed *in situ* at a controlled rate. Electrochemical anodic oxidation of halides is frequently used to halogenate substrates such as ketones, olefins or amines, which is a concept that we intended to apply to synthesise 3-chloropiperidines.[68] In the recent past, various methods for the formation of aziridines[69] and pyrrolidines[70] were already published, which allows the efficient production of those synthetic building blocks. A particularly similar method to our problem was published in 2021 by *He* et al. (figure 14).[70] Therein, they developed an efficient synthesis of iodomethylpyrrolidines from non-activated aminoalkenes in an undivided cell under galvanostatic conditions. Also brominated derivatives were readily available with their published procedure.

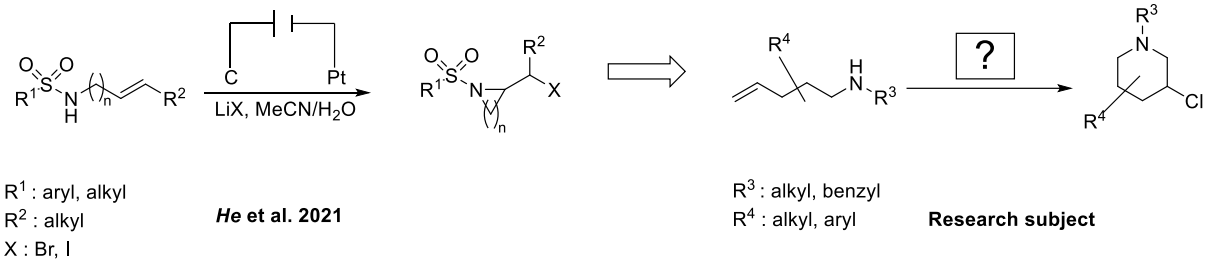


Figure 14: Cyclization of unsatured N-sulfonamides under electroorganic conditions as described by *He* et al. (right) and the transformation we aimed to achieve (left).

Electro-organic chlorinations, however, resemble a more challenging task than brominations and iodinations. This is the case, because chloride requires a higher cell potential[71] to be oxidised. Moreover, chlorine can degas from the reaction vessel, which would lower the faradaic efficiency of the overall process. We therefore used an indirect approach of oxidizing the substrate first with tetrabutyl ammonium iodide as a redox mediator with a subsequent *Finkelstein*-type chloride exchange. Due to the low solubility of metal halides in organic media, we prepared chloride ions in situ by cathodic cleavage of organohalides, a method that works particularly well for platinum and nickel electrodes and has been used before.[72] As an initial guess for the electroorganic synthesis of 3-chloropiperidines, we chose tetrabutyl ammonium iodide as an iodide source and dichloromethane as a chloride source, both because of their good miscibility with organic solvents and their affordability. In a first experiment without optimized process parameters, we could transform our model substrate with a yield of 74 %. Given the high terminal voltage of 3.9 V for this process, we started introducing chloride ions in the form of the hydrochloric acid salt of the original compound. This way, the atom economy of the process could be improved further, as the cathodic formation of hydrocarbons was replaced by the formation of dihydrogen. After optimisation of the process parameters such as electrode material, concentration and terminal voltage, sixteen 3-chloropiperidines could be synthesised with yields of up to 95 %. Thereby, functional groups such as esters, ethers and nitriles were tolerated. Akin to previous methods, the geminal disubstituent effect seemed to enhance the yields slightly corresponding to the internal angle.[58,59] After testing the scope of the process, we aimed to elucidate the underlying mechanism with control experiments that ultimately indicated a radical mechanism. In the future, the discussed synthetic protocol could be used to efficiently synthesise novel 3-chloropiperidines for the screening in tumour cell assays and other pathogens. In this work we performed an initial screening for cross-reactivity, in which we tested twenty-one derivatives for bioactivity against bacteria, fungi and helminths.

1.6. Cross-reactivity of Antineoplastic Agents

It is not uncommon that chemotherapeutic agents exhibit cross-reactivity, as implicated with the antineoplastic and antibacterial profile of **16** addressed above. Another very prominent example for cross-reactivity is the benzimidazole structure (figure 16). Due to its wide range of biological activity, the benzimidazole moiety is often regarded to as a privileged structure in heterocyclic chemistry. The diverse biological activities of the benzimidazole scaffold include antiparasitic, antimicrobial, antiviral, antifungal, analgesic, anti-inflammatory and antineoplastic activities.[73] This diversity has inspired us to

evaluate whether the therapeutic potential of bis-3-chloropiperidines also extends to infectious diseases originating from bacteria, fungi or helminths. In the related study (chapter 3.3), we could show that bis-3-chloropiperidines with an aromatic linker system could be a valuable motif to target diseases originating from parasitic helminth infection.

1.6.1. Anthelmintic Therapy

Novel anthelmintic drugs are crucial for treating helminthiasis and schistosomiasis. In the case of schistosomiasis caused by the parasitic flatworm *Schistosoma mansoni* (*S. mansoni*), pathogenesis is initiated by adult worms migrating to the mesenteric veins and laying eggs that can in turn migrate to the liver. The symptoms are thereby not caused by the worms themselves, but by the body's reaction to the eggs.[74] The typical lifecycle of *S. mansoni* is depicted in figure 15.[75]

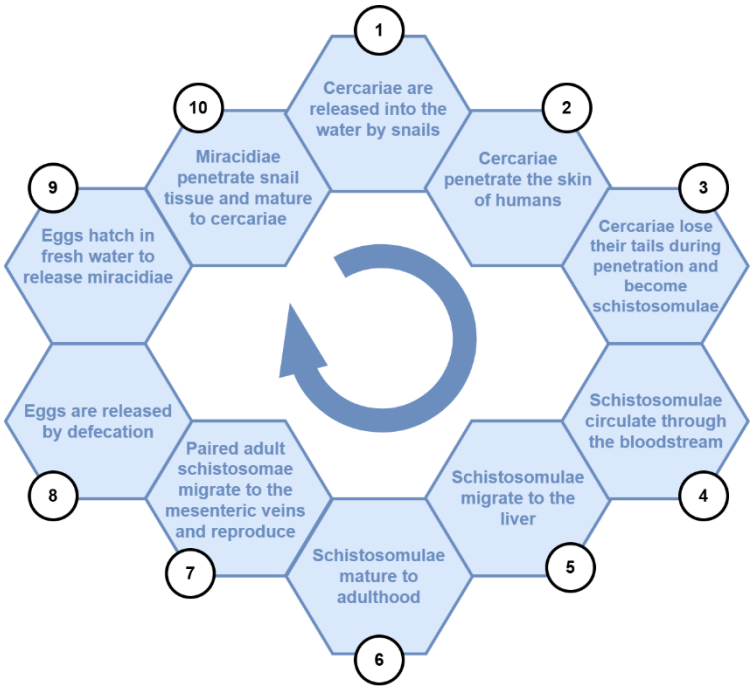


Figure 15: Life cycle of *Schistosoma mansoni*.

Even though the infections can sometimes be asymptomatic, the symptomatic cases usually include manifestations such as fever, cough, abdominal pain, diarrhoea, hepatosplenomegaly (simultaneous enlargement of liver and spleen), and eosinophilia (increase of certain leukocytes).[76] Such parasitic infections account for significant economic losses by infecting agriculturally important animals.[77] Morbidity and death in humans exacerbate this problem, particularly in developing countries.[78] Data from 2019 reveal that about 236 million people worldwide required mass drug administration to counteract schistosomiasis.[79] Despite this, only a limited number of anthelmintic drugs are available for treatment. This includes the before mentioned benzimidazoles, macrocyclic lactones, cyclic octadepsipeptides and aminoacetonitrile derivatives (figure 16).[77] For schistosomiasis induced by *S. mansoni*, the solitary treatment option is the drug praziquantel (**29**, figure 16). It remains the only drug

available, as no alternative antischistosomal agents were developed after praziquantel was developed in the 1970s.[80] Moreover, the advent of resistances against common anthelmintic agents, especially praziquantel, resemble a motivation for the development of novel lead structures to establish alternative treatment options.[81]

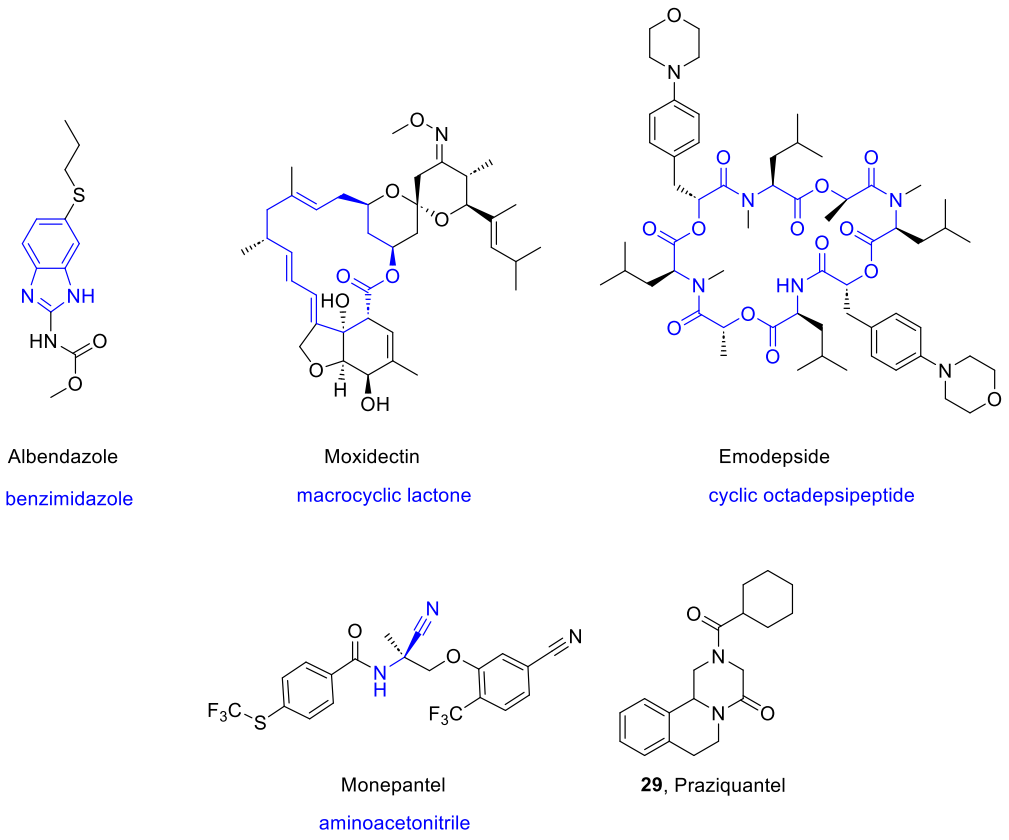


Figure 16: Common anthelmintic drugs. The compound class has been marked blue.

1.6.2. *Caenorhabditis Elegans* as a Model System for *Schistosoma Mansoni*

The complex environment of parasitic species in their host-based habitat complicates quantitative measurements that ultimately seek to find novel therapeutic agents.[82] As a result, the free-living nematode *Caenorhabditis elegans* (*C. elegans*) was considered already as a model screening system to identify new anthelmintic compounds.[77,83] The free-living nematode is considerably easier to cultivate than parasitic trematodes such as *S. mansoni*, which enables screening with a higher throughput.[84] Significant progress in this area was achieved by *Burns*[77], who showed that compounds active against *C. elegans* are likely to be effective against parasites of the same phylum (*nematoda*), which incorporated *Cooperia onchophora*, a parasite of cattle and *Haemonchus contortus*, a parasite of sheep. However, up to this date, no anthelmintic drugs on the market have originated from assays based on the *C. elegans* model system, eventhough most anthelmintic drugs on the market are active against *C. elegans*.[85] In general, the transferability of results from *C. elegans* studies to studies with further taxonomic relatives such as trematodes remains uncertain.

1.6.3. *Bis*-3-Chloropiperidines as a Novel Motif for Anthelmintic Drug Discovery

To test whether 3-chloropiperidines exert cross-reactivity like the above addressed benzimidazoles, we initially tested twenty-one 3-chloropiperidine derivatives against three microbial indicator strains, which included *Escherichia coli*, *Staphylococcus aureus* and *Septoria tritici*. Generally, 3-chloropiperidines exerted insufficient activity against those microbial indicator strains to be considered for further optimisation as the minimal inhibitory concentration (MIC) was too high (> 64 µM). Thereafter, we tested the same 3-chloropiperidines for activity against two *C. elegans* strains (N2, DC19) and *S. mansoni*. This served multiple purposes. For one, *C. elegans* is a member of the phylum nematoda, which also contains parasitic members, such as *ascaris lumbricoides*, *trichuris trichiuria* and *necator americanus*, which all infest the intestines of humans.[86] Therefore, even if *C. elegans* studies are not applicable to further taxonomic relatives such as *S. mansoni*, our results may still be transferable to parasitic worms of the phylum *nematoda*, which could be tested in future studies. However, the primary objective was to determine whether *C. elegans* serves as an effective predictor system for activity against the parasitic trematode *S. mansoni*. Our secondary aim was to deduce a structure activity relationship (SAR), which could be used in the future to refine the 3-chloropiperidine system towards activity against schistosomiasis. In addition to that, we determined the cytotoxicity of the compounds against the canine kidney cell line *MDCK II*. Overall, we observed the best performance for bifunctional 3-chloropiperidines containing an aromatic linker system. Trifunctional 3-chloropiperidines also exerted activity while monofunctional 3-chloropiperidines did not exert any acitivity against *C. elegans* or *S.mansoni* (figure 17). At a concentration of 20 µM we found eight compounds to be active against *S. mansoni*. Decreasing the concentration to 10 µM, however, resulted in only three compounds still being active. Out of these three compounds, especially **38** led to complete separation of worm pairs after three days at a concentration of 20 µM and to a lesser extent already at 10 µM after one day. Fast separation of the worm pairs is especially important in the treatment of schistosomiasis. This is the case, because Schistosoma are, unlike most other trematodes, not hermaphroditic. Female Schistosoma need their male counterparts for their reproductive maturation and can even devolve into an immature state if separated from their male counterparts. Thereby, production of the pathology causing eggs also ceases.[74] Due to the fast action of **38**, which is especially important in antischistosomal activity, we tested a triplicated dilution series against the *MDCKII* cell line, from which we could determine an IC_{50} value of 70.2 µM. This cytotoxicity resembles a therapeutic window with an approximately seven-fold stronger effect against *S. mansoni* than cell viability reduction.

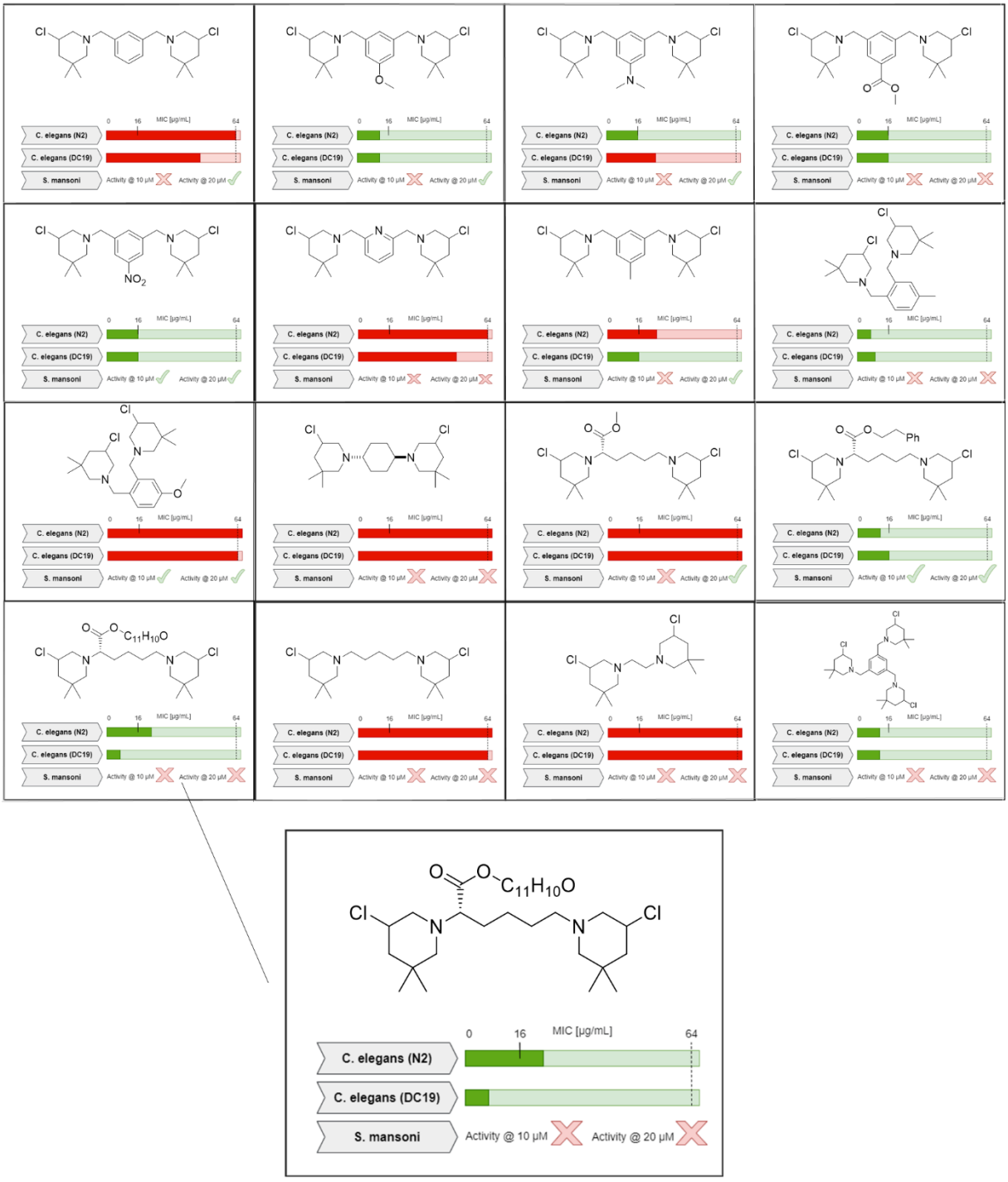


Figure 17: Anthelmintic activity of bis-3-chloropiperidines.

Nevertheless, at the tested concentrations of 20 µM and 10 µM, none of the compounds exerted 100% lethality of *S. mansoni*. Therefore, we still consider their anthelmintic properties as rather weak and further optimisation is required to render them applicable. However, we could show that the transferability from *C. elegans* studies to *S. mansoni* studies is generally good. Thereby, 56% of compounds that were active against the N2 strain of *C. elegans* and were also effective against *S. mansoni*. In addition to that, we observed a high degree of specifity against *S. mansoni*, with 33% of

compounds that were active against *S. mansoni* not being effective against any of the two investigated *C. elegans* strains. Ultimately, we could find four compounds (**31**, **34**, **38** and **40**) that balanced cytotoxicity and activity the best and could be used as a starting point for further optimisation and *in vitro* screening.

1.7. Summary and Future Directions

In the present work, significant advancements were made in understanding the structure, synthesis and bioactivity of 3-chloropiperidines in relation to helminth infection. By synthesising five distinct monofunctional 3-chloropiperidines with different geminal substitutions, we quantified the influence of the geminal substitution on the solvolysis rate constant of 3-chloropiperidines. This allowed us to correlate the change of the rate constant with the internal angles β_i, determined by SC-XRD and precise DFT calculations using the PBEh-3c hybrid functional. We discovered a linear correlation between the internal angles β_i and the solvolysis rate constant, suggesting the presence of a classical, angle-dependent Thorpe-Ingold-effect rather than a rate change due to reactive rotamers. For the first time, we successfully synthesised cyclopropyl compound **20** and cyclobutyl compound **21**, both of which decelerated the solvolysis compared to the unsubstituted compound **22**. Since the *Göttlich* group discovered that the geminal dimethylated compound was more active than the unsubstituted compound **22** through in vitro testing, it is necessary to verify if this trend persists during *in vivo* testing. An inverse trend might also be observed, as more reactive compound could be degraded prematurely by intracellular nucleophiles. If this proves to be accurate, introducing cyclopropyl rings at the geminal position could be an effective strategy to fine-tune the activity of 3-chloropiperidines as DNA alkylating agents.

Furthermore, we developed a novel synthetic method to access 3-chloropiperidines with a convenient, highly atom-economic procedure. By leveraging electro-organic synthesis, we successfully synthesised sixteen 3-chloropiperidines with good to excellent yields. Thereby, we demonstrated that various degrees of steric bulk were tolerated. Additionally, we showed that nitriles, esters and (silyl-)ethers are well tolerated. The original procedure employs a nickel cathode and a graphite anode. Moving forward, the process should be reoptimised to use two graphite electrodes, which would render the reaction entirely metal-free. We were also able to gather valuable information about the mechanism of the process. Control experiments confirmed that the mechanism likely proceeds *via* radicals, as the addition of a radical scavenger resulted in a significant decrease of yield. Overall, the atom-economy is significantly improved compared to existing processes, as it avoids the use of stoichiometric oxidants such as NCS. In particular, the only theoretical by-product is environmentally benign dihydrogen, which is generated at the cathode. In future applications within our laboratory, the scope of the reaction can be explored further, particularly concerning the synthesis of bis-3-chloropiperidines, which have been identified as an intriguing motif for anthelmintic drug design in the present work.

In the respective work, we were able to test the general chemotherapeutic potential of twenty-one 3-chloropiperidine derivatives for certain infectious diseases. Although the activity against the three employed microbial indicator strains (*Escherichia Coli*, *Staphylococcus aureus* and *Septoria tritici*) was insufficient to justify further optimisation, the activity against *C. elegans* (N2) and *S. mansoni* proved to

be promising. Overall, we could identify four compounds that exerted activity against *S. mansoni* and five that exerted activity against *C. elegans*, all of which exhibited no or low cytotoxicity, as evaluated in assays with the MDCKII canine liver cell line. The transferability of *C. elegans* assays to *S. mansoni* assays proved to be good, with 56% of compounds active against *C. elegans* (N2) also being active against *S. mansoni*. However, since none of the compounds exhibited full lethality against *S. mansoni*, even after seven days and a concentration of 20 µM, the anthelmintic activity of bis-3-chloropiperidines relative to the commonly applied drug praziquantel (**29**, figure 16) is still low. Future research could focus on enhancing the pharmacokinetic properties of bis-3-chloropiperidines starting from the four most promising derivatives **31**, **34**, **38** and **40**. This could adjust the *in vivo* activity to match that of the only drug against Schistosomiasis induced by *S. mansoni*, praziquantel, against which resistance is currently emerging.

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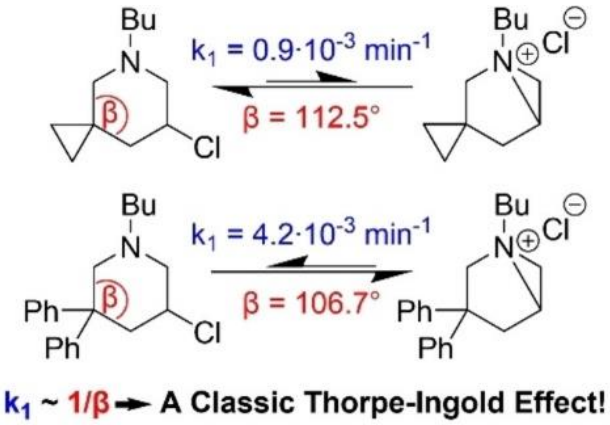
3. Publications

3.1 Separation of the Thorpe-Ingold and Reactive Rotamer Effect by Using the Formation of Bicyclic Aziridinium Ions



Abstract

A classic, angle dependent Thorpe-Ingold effect in the formation of bicyclic aziridinium ions is presented. Kinetic studies, backed up by SC-XRD and DFT calculations, reveal a linear correlation of the internal angle β with the rate constant k_1. Increased angles result in decreased rate constants and vice versa. The cyclic structure of the examined 3-chloropiperidines thereby excludes contribution of the reactive rotamer effect towards cyclization.

Reference

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Tim Helbing and Michael Kirchner contributed equally to this work.

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Separation of the Thorpe–Ingold and Reactive Rotamer Effect by Using the Formation of Bicyclic Aziridinium Ions

Tim Helbing[+],[a] Michael Kirchner[+],[a] Jonathan Becker,[b] and Richard Göttlich*[a]

The geminal dialkyl effect is widely used in organic synthesis to promote cyclization reactions, although the exact origin of its rate enhancement remains unclear. In the present study, we demonstrate the applicability of this effect for the intramolecular formation of bicyclic aziridinium ions and assign it to angle contractions provided by introduction of sterically demanding substituents. Due to the cyclic structure of the examined 3-chloropiperidines the acceleration of this ring closure cannot be explained by an increased population of reactive gauche rotamers and therefore agrees with the original Thorpe-Ingold theory. Furthermore, introduction of strained aliphatic rings resulted in internal angle expansions and were accompanied by a preliminary decrease of rate constants for the investigated aziridinium ion formation. These results lead to a linear correlation between internal angle and relative reaction rate, supported by computational and X-ray crystallographic structural data.

Introduction

Intramolecular reactions can be accelerated by the introduction of a *gem*-substituent group in the carbon chain connecting two reactive centres. This is commonly known as the *gem*-disubstituent or *gem*-dialkyl effect.[1,2] Thorpe, Ingold and Beesley offered the first explanation of this rate enhancement, which was later termed the "Thorpe-Ingold effect". According to their theory, the angle α between two alkyl substituents is enlarged by increased steric repulsion compared to the unsubstituted homologue. Consequently, the opposite angle β is decreased to relieve steric strain moving the two reactive centres closer together, thus promoting cyclization (Figure 1a).[3] In 1960, Allinger and Zalkow concluded that the increased number of *gauche* interactions in *gem*-disubstituted acyclic compounds, compared to the corresponding cyclic system, reduces the enthalpy of activation $\Delta H^{\neq}$.[4] Furthermore, substitution restrains the rotational entropy in the open chain precursor more than in the cyclic product, resulting in a favourable entropy of activation $\Delta S^{\neq}$ for the cyclization of *gem*-disubstituted compounds. Two years later, Schleyer calculated the contribution of the angle compression to the *gem*-dialkyl effect and mentioned that the small changes of the β angle cannot solely explain the experimentally observed enhancements of rate constants up to several orders of magnitude.[5] Therefore, Bruice and Pandit developed a different theory and associated the rate enhancements with an increased population of reactive *gauche* rotamers, which was later entitled the "reactive-rotamer effect".[6] In these conformations, the two reactive centres are arranged properly for cyclization (Figure 1b). This concept is similar to the later developed theory of near attack conformers (NACs) by Bruice and Lightstone,[7–10] for which experimental evidence was given lately.[11] NACs represent ground state conformers, which are similar to the transition state in terms of geometry and orientation of the reactive centres, while still being separated by roughly 3 Å to ensure that bond formation as well as bond breaking has not occurred yet. This separation also represents the critical distance known from the spatiotemporal hypothesis of Menger,[12–17,18] which correlates distance between reactive

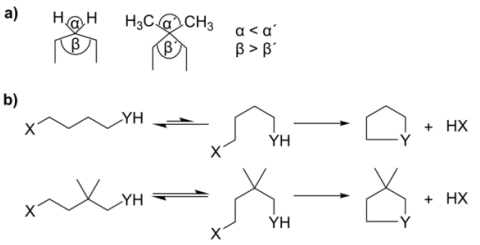

Figure 1. a) Compression of the β ' angle by introduction of geminal methyl groups known as the "Thorpe-Ingold effect". b) Increase in the population of a reactive *gauche* rotamer, which readily undergoes cyclization, by *gem*-methylation known as the "reactive-rotamer effect".

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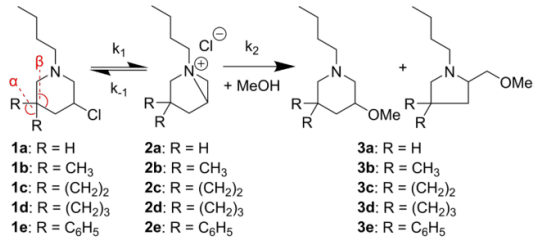
centres with the reaction rate. The concept postulates that a reaction is accelerated by several orders of magnitude, if the reactive moieties are kept in proximity, less than the critical distance, for a sufficient time. The aforementioned theories, along with many others, have also been extensively discussed to explain the severe rate accelerations achieved in enzyme catalysed reactions.[19]

Although several articles have been published to support or discourage either the Thorpe-Ingold or the reactive rotamer effect, this subject remains challenging since both effects contribute to rate enhancements provided by *gem*-disubstitution and are therefore difficult to distinguish. For instance, Parrill and Dolata showed that there is no linear correlation between the population of reactive rotamers and the relative reaction rates, suggesting a "facilitated transition" hypothesis instead.[20] Contrarily, Jung and Gervay gave evidence for the reactive rotamer effect by examination of intramolecular Diels-Alder experiments, demonstrating an increase in rate constants even if strained rings, like cyclobutane or cyclopropane, were used as *gem*-substituents.[21] The small angles of these cyclic systems should lead to larger internal angles and would therefore retard the reaction if valency deviation effects were the dominant factors in the examined reaction. Several other theories have been published, for instance "stereopopulation control"[22–24] and "relief of ground-state strain",[25,26] both discussed as the origin of the "trimethyl lock effect". This conformational restriction, which represents a combination of the "buttressing effect"[27] and the *gem*-disubstituent effect, provides extreme rate enhancements and was first observed for the cyclization of methylated hydrocoumarinic acid derivatives.[22–26,28] Moreover, a reduction of ring strain energy by *gem*-disubstitution of small ring systems has been discussed.[29] Although, the detailed origin of rate enhancements by *gem*-disubstitution is still up for discussion, there are numerous examples of successful applications of the geminal dialkyl effect leading to accelerated reactions, increased yields and selectivity in cyclization reactions[2,30] as well as in ligand design.[31]

Results and Discussion

In recent kinetic studies with substituted 3-chloropiperidines we noticed an enhanced reaction rate for the 5,5-dimethyl derivative **1 b** compared to the corresponding unsubstituted compound **1 a**.[32] The reaction of these compounds with nucleophiles proceeds *via* the highly electrophilic bicyclic aziridinium intermediate **2**, which is rapidly consumed by nucleophilic attack (Scheme 1). The observed increase in reaction rate is assumed to be the result of a *gem*-dialkyl effect, since a bicyclic system is obtained in the rate-determining formation of the aziridinium intermediate (k_1), for which the steady state approximation ($k_2 \gg k_{-1}$) can be applied. However, this rate acceleration cannot be explained by an increased population of reactive *gauche* conformers, since the cyclic structure of the 3-chloropiperidines already fixes the orientation of the reactive centres towards each other. Another explanation



Scheme 1. Reaction mechanism of the examined 3-chloropiperidines **1 a-e**, a mixture of the piperidine and pyrrolidine methyl ethers **3 a-e** are formed by methanolysis of the intermediate bicyclic aziridinium ions **2 a-e**.

would therefore be an angle contraction of the internal β angle, representing a classic Thorpe-Ingold effect, which could be studied separated from the reactive rotamer effect. If the hypothesis holds true for our 3-chloropiperidine system, it might be considered as a useful tool to modulate their reactivity as alkylating agents.[33] This would be of particular interest, since *gem*-dimethyl substitution has already been used to successfully improve the reactivity, stability or synthesis of various medical agents.[34]

In this study, we report for the first time a rate enhancement *via* distinct angle dependant Thorpe-Ingold effect for an intramolecular reaction in an already cyclic system. Furthermore, a preliminary deceleration of the examined solvolysis reaction, by introduction of strained cycloalkyl substituents, was observed. In the following, we will show a linear correlation between the internal β angle, provided by single crystal XRD and DFT[35] calculations, and the relative rate constant k_{rel} (with respect to the unsubstituted compound **1 a**) observed in ^{1}H-NMR kinetic experiments of the 3-chlorpiperidines **1 a-e**. Furthermore, we demonstrate an analogous tendency for the experimentally determined Gibbs free energies of activation ΔG^{+}. These results demonstrate, to the best of our knowledge, the first kinetic study about the contribution of the Thorpe-Ingold effect towards cyclization reactions separated from the reactive rotamer effect. Therefore, the novel *spiro* compounds **1 c** and **1 d** as well as the diphenyl derivative **1 e** were synthesized from the corresponding unsaturated amines **4 c-e**, which can be obtained by allylation and reduction of the corresponding nitriles, as described in literature.[36] The amine precursors were then alkylated by imine formation and subsequent reduction, followed by simultaneous *N*-chlorination and cyclization using copper(II)chloride[37,38] (Scheme 2). The synthesis of the 3-chloropiperidines **1 a** and **1 b** has already been described in our previous work.[39,40]

The reactivity of the obtained 3-chloropiperidines **1 a-e** against methanol as an explorative nucleophile was examined by ^{1}H-NMR kinetic solvolysis experiments. The compounds were dissolved in methanol-d_4, containing dibenzylether as an internal standard. The NMR tubes were heated to 50 °C for 7.5 h (except **1 e**, which was incubated for only 2.5 h) in an oil bath and withdrawn for the measurements after an appropriate time. The consumption of the starting material, following the

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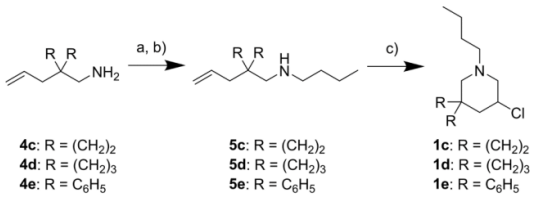

Scheme 2. Synthesis of 3-chloropiperidines **1 c-e**. a) *n*-butyraldehyde, $MgSO_4$, DCM, RT; b) $NaBH_4$, MeOH, 0 °C to RT; c) $CuCl_2 \cdot 2\ H_2O$, THF, RT (**1 c-d**) or $CuCl_2$, THF, RT to reflux (**1 e**).

mechanism depicted in Scheme 1, was monitored (Figures S1-S5) and the anticipated mixture of piperidine and pyrrolidine methyl ethers **3 a-e** was confirmed by GC/MS (see supplementary Experimental Section). The observed regioselectivity in favour of the six-membered piperidine compound is in accordance with several other experimental and theoretical studies on ring expansion of bicyclic aziridinium ions.[41] Sterically demanding substituents in C5 position therefore result in a higher fraction of the thermodynamic piperidine product, compared to the corresponding unsubstituted compound. The first order rate constants of the examined solvolysis reactions were calculated by plotting the natural logarithm of the integral of the corresponding starting material 3-H signal *ln(3-H)* (except **1 e**, where an overlapping CH_2 signal was included in the integral) against the reaction time *t* (Table S1). As expected, we observed an increase in reactivity of the dimethyl derivative **1 b** ($k_{rel} = 1.56$) compared to the unsubstituted compound **1 a**. The acceleration for the diphenyl derivative **1 e** ($k_{rel} = 2.38$) was even stronger, resulting in complete consumption of the starting material after approximately five hours. We also recognized a decreased reaction rate for compounds **1 c** and **1 d** in comparison to compound **1 a**. This could be due to the strained cyclic substituents, as observed for the intramolecular catalytic activity of cycloalkyl-substituted malonic acid derivatives.[42] The decrease in reaction rate was more prominent for the cyclopropyl compound **1 c** ($k_{rel} = 0.53$) than for the cyclobutyl derivate **1 d** ($k_{rel} = 0.64$), suggesting a direct correlation of the internal β angle and the rate constant in our 3-chloropiperidine system, as originally proposed by the valency deviation theory of Thorpe and Ingold.[43] To show the robustness and reproducibility of our kinetic method, we repeated the kinetic measurements at 50 °C for all compounds, performing the reaction at this temperature in the NMR spectrometer while also taking ^{1}H-NMR spectra more frequently (representative ^{1}H-NMR spectra shown in Figure S8) and obtained nearly the same rate constants (Table 1).

To confirm the assumed angle distortion by introduction of different substituents in 5-position, we crystallized several 3-chloropiperidine derivatives as their corresponding hydrochloride salts and analysed their structure *via* single crystal XRD. The respective angles α and β (as depicted in Scheme 1), obtained from the crystal structures of the HCl salts of compounds **1 a**, **1 c** and **1 e** (see Tables S2–S7 and S21–32) are summarized in Table 1. Since a crystal structure of the dimethylated compound **1 b** could not be obtained, the angles of the crystal structures of compounds **6 a** and **6 b** (see Tables S8–S20), which represent the *N*-benzyl substituted derivatives of **1 a** and **1 b**, were added. A similar effect resulting from *gem*-dimethylation can be assumed for the *N*-benzyl and *N*-butyl substituted derivatives, considering that the reaction of **1 a** and **1 b** as well as **6 a** and **6 b** with 2'-desoxyguanosine in aqueous solution provided similar relative rate constants in our previous work (k_{rel}(**1 b**/**1 a**) = 4.67; k_{rel}(**6 b**/**6 a**) = 4.08).[32] In addition, we obtained a relative rate constant of $k_{rel} = 1.66$ in a separate kinetic study of **6 a** and **6 b** in methanol-d_4 at 50 °C (see Figure S6), which is comparable to the values of the respective *N*-butyl compounds reported in Table S1 and Table 1. Furthermore, we investigated the structure of the examined 3-chloropiperidine derivatives computationally using the orca program package (version 5.0.3.)[44] on the PCM(methanol)-PBEh-3c[45,46] level of theory, containing the corresponding modified def2-mSV(P) basis set[46,47] and confirmed the lowest energy conformers using the Crest program package.[48] The experimentally obtained as well as the calculated angles α and β shown in Table 1 are in good agreement among each other, considering that the crystal structures were obtained from the corresponding hydrochloride salts. Moreover, both values show a good linear correlation ($R^2_{XRD} = 0.98$ and $R^2_{DFT} = 0.87$) between the internal β angle and the relative rate constant k_{rel} (Figure 2). An analogous correlation cannot be observed for the exocyclic α angle (Figure S9), which is not surprising since α and β show no direct linear relationship either (Figure S10). Nonetheless, introduction of substituents containing small α angles lead to

Table 1. Rate constants k_1 and relative rate constants k_{rel} for the solvolysis reactions of 3-chloropiperidines **1 a–e** in methanol-d_4. Bond angles α and β in compounds **1 a–e** derived from single crystal XRDs (hydrochloride salts), with their corresponding ESD-values in brackets, and DFT calculations. Activation parameters for compounds **1 a–e** derived from the respective Arrhenius and Eyring plots. N/A = not available

	k_1 [10^{-3} min^{-1}][a]	k_{rel}	α (XRD) [°]	α (DFT) [°]	β (XRD) [°]	β (DFT) [°]	E_a [kcal/mol]	ΔH^{+}_{298} [kcal/mol]	ΔS^{+}_{298} [cal/(K*mol)]	ΔG^{+}_{298} [kcal/mol]
1 a	1.66 ± 0.05	1	107.9[b] 107.9[b,c]	107.07	111.8 (3) 112.07 (14)[c]	110.95	18.03 ± 1.00	17.41 ± 1.00	−17.30 ± 3.21	22.56 ± 1.96
1 b	2.97 ± 0.12	1.79	109.1 (2)[c]	109.12	109.35 (19)[c]	108.42	17.51 ± 0.91	16.88 ± 0.91	−17.62 ± 2.89	22.13 ± 1.77
1 c	0.99 ± 0.01	0.54	60.06 (11)	60.30	113.28 (13)	112.49	17.11 ± 0.62	16.49 ± 0.62	−21.17 ± 2.00	22.80 ± 1.22
1 d	1.44 ± 0.04	0.87	N/A	88.47	N/A	109.58	16.94 ± 1.01	16.31 ± 1.01	−21.36 ± 3.24	22.68 ± 2.35
1 e	4.19 ± 0.13	2.52	110.2 (2)	107.57	107.54 (12)	106.66	17.28 ± 1.01	16.66 ± 1.02	−17.94 ± 3.25	22.01 ± 1.98

[a] Samples were incubated at 50 °C directly in the NMR spectrometer. [b] $\angle_{HC5H}$ values derived geometrically from the positions of non-hydrogen substituents of C5. [c] Values obtained from the corresponding hydrochloride salts of the *N*-benzyl derivates **6 a** and **6 b** (see Tables S8–S20).

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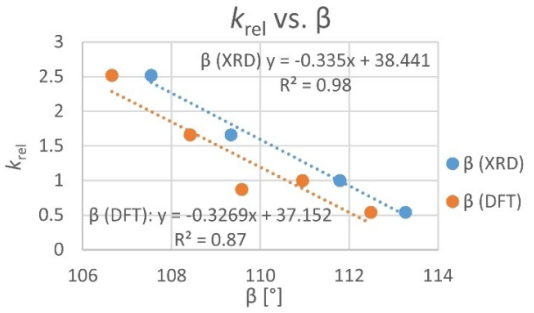

Figure 2. Linear correlation between the relative rate constants k_{rel} and the internal β angle, obtained by single crystal XRD and DFT calculations.

an increase of β and therefore retard the reaction, resulting in a lower relative rate constant, and *vice versa*, as originally proposed by Thorpe and Ingold. The obtained differences in rate constants are considerably smaller compared to reported systems in which the reactive rotamer effect is present,[2] but still show that angular distortion is capable of affecting the reaction rates. The change in the internal β angle is further accompanied by a slight change of the attack angle and distance of the nucleophilic nitrogen towards the electrophilic C3 centre, resulting from the geometrical distortion. Both are important factors to consider in a possible correlation following the spatiotemporal hypothesis.[12–17] The attack angle in the unsubstituted 3-chloropiperidine **1a** was calculated to be 142.85° (depicted in Figure S11), while the cyclopropyl substituted derivative **1c** performs the backside attack from an angle of 142.70° and the more reactive diphenyl compound **1e** attacks the leaving group C3 carbon from an angle of 143.28° (for other attack angles see Table S1). On the other hand, the cyclobutyl compound **1d** does not follow this trend, as its attack angle of 143° is closer to the angle of 180° typical for S_N2 nucleophilic substitutions, but its reactivity is lower compared to compound **1a**. Moreover, the changes in NC3 distance (Table S1) compared to the unsubstituted compound **1a** (2.428 Å) are very small ($\Delta r_{max.} = 0.02$ Å), while still following a tendency similar to the NC3Cl attack angles. The nitrogen atom of the more reactive diphenyl compound **1e** is a little closer to the leaving-group C3 carbon (2.411 Å), while the distance for the cyclopropyl derivative **1c** was calculated to be 2.432 Å. Again, the less reactive cyclobutyl derivate **1d** represents an exception (2.422 Å). When also considering the mean absolute deviation of 0.009 Å for small first and second row molecules calculated by the PBEh-3c method,[46] we assumed that these small differences are no dominant factors in the examined solvolysis reaction of the substituted 3-chloropiperidines **1a–e**.

To understand the observed effect in more detail we also determined the activation parameters for compounds **1a–e**. Therefore, ^{1}H-NMR solvolysis experiments were performed between room temperature (22.5 °C) and 60 °C (oil bath temperature). The resulting rate constants (see Figures S12–S16) were then used to determine E_a (Arrhenius plots: Figures S17–S21) and $\Delta H^{\neq}$ as well as $\Delta S^{\neq}$ (Eyring plots: Figures S22–S26), while $\Delta G^{\neq}$ was calculated from these values (Table 1). The Gibbs free energies of activation $\Delta G^{\neq}$ obtained from the Eyring plots reflect the general tendency of reactivity we observed in the initial kinetic experiments. Even though the differences in Gibbs free energies of activation $\Delta\Delta G^{\neq}$ are smaller than expected from the relative rate constants, which can be attributed to the experimental error of roughly 2 kcal/mol. Interestingly all activation enthalpy $\Delta H^{\neq}$ and activation energy E_a values are decreased when introducing substituents in 5-position, including the strained cycloalkyl moieties present in compounds **1c** and **1d**. In addition, the substitution of compounds **1b** and **1e** with more bulky groups just slightly increases the activation entropy $\Delta S^{\neq}$ compared to the unsubstituted homologue **1a**, accordingly their respective rate enhancements are almost entirely attributed to lower enthalpies of activation $\Delta H^{\neq}$. However, this is not the case for the cyclopropyl and cyclobutyl compounds **1c** and **1d**. Apparently the decrease in $\Delta H^{\neq}$ is overcompensated by a firmly negative entropy of activation $\Delta S^{\neq}$, resulting in higher Gibbs free energies of activation $\Delta G^{\neq}$ and therefore lower rate constants. The strained cycloalkyl moiety most likely further restricts the already constrained transition state by additional reduction of degrees of freedom. Similar effects on the activation parameters (lowering of $\Delta H^{\neq}$ and $\Delta S^{\neq}$) of cycloalkyl-substituted compounds have been reported by Jung and Gervay,[21] although in the examined system a strong decrease in $\Delta H^{\neq}$ resulted in small rate enhancements. The authors associated this with the presence of the reactive rotamer effect. However, this effect is not applicable in the reaction of our cyclic system, thus the related decrease in $\Delta H^{\neq}$ is lacking and cannot compensate the firmly negative $\Delta S^{\neq}$. As a result, the reaction is slowed down by the leftover angle distortion effect. Various studies discussed the entropic contribution in the accelerated intramolecular anhydride formation of substituted glutarates,[7–9,49] but most of them focus on conformational effects (*via* the NAC approach), which are not applicable in our rigid cyclic system. This is supported by the fact, that we observed only small changes in the entropy of activation $\Delta S^{\neq}$ for the *gem*-dimethyl and the *gem*-diphenyl derivatives (**1b** and **1e** respectively) in comparison to the unsubstituted compound **1a**. In addition, the determined Gibbs free energies of activation $\Delta G^{\neq}$ show a linear correlation with the internal β angle (see Figure S27), just as the aforementioned relative rate constants k_{rel} (Figure 2). Generally, our investigations support the presence of a classic Thorpe-Ingold effect separated from conformational effects, such as the reactive rotamer effect, in the examined solvolysis reaction of *gem*-disubstituted 3-chloropiperidines.

Conclusion

In conclusion, we investigated the original, angle dependant Thorpe-Ingold effect, excluding the otherwise competing reactive rotamer effect, by ^{1}H-NMR kinetic and structural analysis of the substituted 3-chloropiperidine derivatives **1a–e**. We could show a rate enhancement by introduction of bulky *gem*-

disubstituents in 5-position compared to the unsubstituted compound **1a** as well as a first-time deceleration of the examined reaction for the novel *spiro* derivatives **1c** and **1d**, containing cyclopropyl and cyclobutyl rings respectively. The introduction of these strained cyclic substituents resulted in an enlargement of the internal β angle, confirmed by single crystal XRD and DFT calculations. In contrast, the internal angle decreases with increasing steric demand of 5-substituents, demonstrated by the *gem*-dimethyl compound **1b** and the *gem*-diphenyl derivative **1e**. As a bicyclic system is obtained in the rate determining aziridinium ion formation, a *gem*-disubstituent effect can be assumed for the investigated solvolysis reaction (MeOH) of our 3-chloropiperidines. Yet, the observed acceleration and deceleration of rate constants cannot be explained by the reactive rotamer effect, since the orientation of the reactive centres is already locked in the cyclic piperidine system. Furthermore, the obtained relative rate constants k_{rel} as well as the experimentally determined Gibbs free energies of activation $\Delta G^{\neq}$ show good linear correlations with the internal β angle. Taken all of these results together, there is strong evidence for the presence of a classic, angle dependent Thorpe-Ingold effect separated from the reactive rotamer effect in the formation of bicyclic aziridinium ions. Although these angular distortion effects might be overcompensated by conformational effects in open-chain substrates, cyclic compounds seem to be essentially affected by the internal angle between the reactive centres. Consequently, this effect can be used as another useful approach to tune the reactivity of 3-chloropiperidines as DNA alkylating agents.

Experimental Section

All solvents were purified by distillation prior to use, in case of anhydrous solvents AcroSeal™ bottles from ACROS Organics™ were used. Commercially available reagents were used as supplied if not stated different. Synthesis using anhydrous solvents were carried out under Schlenk conditions. For purification by flash column chromatography silica gel 60 (Merck) was used. ^{1}H and ^{13}C NMR spectra were recorded at the Bruker Avance II 400, the Avance III 400 and the Bruker Avance II 200 "Microbay" spectrometer (^{1}H at 400 MHz and 200 MHz; ^{13}C at 101 MHz) in deuterated solvents. ^{1}H-NMR kinetic experiments were carried out at the Bruker Avance II 200 "Microbay" spectrometer (^{1}H at 200 MHz) and the Bruker Avance III 600 spectrometer (^{1}H at 600 MHz). ^{1}H and ^{13}C chemical shifts were determined by reference to the residual solvent signals. High-resolution ESI mass spectra were recorded in methanol using a ESImicroTOF spectrometer (Bruker Daltonics) in positive ion mode. All GC/MS spectra were recorded at the Aligent 5977B GC/MSD instrument equipped with a 7820 A GC System. All elemental analysis (CHN) were performed on a Thermo FlashEA–1112 series instrument. NMR spectra of the synthesized compounds **1c–e**, **4c–e** and **5c–e** as well as the synthetic procedures for the known primary amines **4c–e**[37,38] and their corresponding nitrile precursors are included in the Supporting Information. The synthesis of compounds **1a** and **1b** has been described elsewhere.[39,40]

General procedure for synthesis of secondary amines (5c–e)

To a solution of the corresponding primary amine **4c–e** in DCM (0.5 mL/mmol) freshly distilled *n*-butyraldehyde (1.1–1.4 eq.) as well as $MgSO_4$ (approx. 5–10 g) were added. The mixture was stirred at room temperature for 2 h, filtered afterwards and the solvent was removed under reduced pressure. The residue was dissolved in MeOH (0.5 mL/mmol) and $NaBH_4$ (1.5–2 eq.) was added at 0 °C. The mixture was stirred at room temperature for 16–18 h. The reaction was then quenched by the addition of 20% $NaOH_{(aq)}$ and DCM was added. The phases were separated and the aqueous phase was extracted with DCM (3x). The combined organic extracts were washed with brine, distilled water as well as brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was obtained, which was used in the next step without further purification.

N-[(propenyl)cyclopropyl]methylbutanamine (5c)

Was prepared according to the general procedure from [(Propenyl)cyclopropyl]methanamine **4c** (0.47 g, 4.25 mmol) yielding the title compound **5c** as a colorless oil (0.45 g) which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.85–5.77 (m, 1H, CH_2=C*H*-CH_2), 5.09–5.00 (m, 2H, CH_2=CH-CH_2), 2.62–2.57 (m, 2H, NH-CH_2-CH_2-CH_2-CH_3), 2.48 (s, 2H, CH_2-NH), 2.13 (dt, J = 7.1, 1.3 Hz, 2H, CH_2=CH-CH_2), 1.51–1.44 (m, 2H, CH_3-CH_2-CH_2-CH_2-NH), 1.37–1.28 (m, 3H, N*H* and CH_2-CH_3), 0.92 (m, 3H, CH_3), 0.44–0.34 (m, 4H, $(CH_2)_2$) ppm; ^{13}C NMR ($CDCl_3$, 101 MHz): δ = 136.50 (CH_2=CH-CH_2), 116.37 (CH_2=*C*H-CH_2), 56.14 (CH_2-*C*H_2-NH), 49.77 (*C*H_2-NH), 39.26 (CH_2=CH-*C*H_2), 32.09 (CH_3-CH_2-*C*H_2-CH_2-NH), 20.63 (CH_3-*C*H_2), 19.58 (C_q), 14.03 (CH_3), 10.10 ($(CH_2)_2$) ppm; HRMS (ESI): *m/z* calcd for $C_{11}H_{21}N^+$: 168.1747, found: 168.1750 $[M+H]^+$.

N-[(propenyl)cyclobutyl]methylbutanamine (5d)

Was prepared according to the general procedure from crude [(Propenyl)cyclobutyl]methanamine **4d** (1.02 g) yielding the title compound **5d** as a colorless oil (1.60 g) which was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.77–5.68 (m, 1H, CH_2=C*H*-CH_2), 5.03–4.96 (m, 2H, CH_2=CH-CH_2), 2.59–2.52 (m, 2H, NH-CH_2-CH_2-CH_2-CH_3), 2.51 (s, 2H, CH_2-NH), 2.18 (d, J = 7.3 Hz, 2H, CH_2=CH-*C*H_2), 1.78–1.69 (m, 6H, $(CH_2)_3$), 1.46–1.36 (m, 2H, CH_3-CH_2-CH_2-CH_2-NH), 1.33–1.19 (m, 3H, N*H* and CH_2-CH_3), 0.87–0.83 (m, 3H, CH_3) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ = 135.47 (CH_2=CH-CH_2), 116.77 (CH_2=*C*H-CH_2), 57.53 (CH_2-CH_2-NH), 50.52 (CH_2-NH), 42.11 (CH_2=CH-*C*H_2), 41.77 (C_q), 32.15 (CH_3-CH_2-*C*H_2-CH_2-NH), 29.70 ($(CH_2)_3$), 20.61 (CH_3-*C*H_2), 15.43 ($(CH_2)_3$), 14.16 (CH_3) ppm; HRMS (ESI): *m/z* calcd for $C_{12}H_{24}N^+$: 182.1903, found: 182.1903 $[M+H]^+$.

N-butyl-2,2-diphenylpent-4-en-1-amine (5e)

Was prepared according to the general procedure from 2,2-Diphenyl-4-penten-1-amine **4e** (2.90 g, 9.95 mmol) yielding the title compound **5e** as a colorless oil (2.79 g) which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.29–7.25 (m, 4H, Ar-*H*), 7.20–7.17 (m, 6H, Ar-*H*), 5.43–5.34 (m, 1H, CH_2=C*H*-CH_2), 5.04–4.93 (m, 2H, CH_2=CH-CH_2), 3.19 (s, 2H, CH_2-NH), 3.01 (d, J = 7.1 Hz, 2H, CH_2=CH-*C*H_2), 2.52 (m, 2H, NH-*C*H_2-CH_2-CH_2-CH_3), 1.37–1.31 (m, 2H, NH-CH_2-*C*H_2-CH_2-CH_3), 1.27–1.19 (m, 3H, N*H* and CH_2-CH_3), 0.84 (t, J = 7.3 Hz, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 101 MHz): δ = 147.10 (arom. C_q), 135.18 (CH_2=CH-CH_2), 128.23 (arom. *C*H), 128.06 (arom. *C*H), 126.04 (arom. *C*H), 117.65 (CH_2=CH-CH_2), 56.16 (CH_2-NH), 50.25 (CH_3-CH_2-CH_2-NH), 50.24 (CH_2=CH-*C*H_2), 41.86 (C_q), 32.15 (CH_3-CH_2-*C*H_2-NH), 20.50 (CH_3-*C*H_2), 14.11 (CH_3) ppm; HRMS (ESI): *m/z* calcd for $C_{21}H_{28}N^+$: 294.2216, found: 294.2216 $[M+H]^+$. These data are consistent with published data.[50]

General procedure for synthesis of 3-chloropiperidines (1 c–d)

To a solution of the corresponding secondary amine **5 c–d** in THF (2 mL/mmol) copper(II)chloride dihydrate (1.2–1.6 eq.) was added. The mixture was stirred at room temperature for 16–18 h and a second portion of copper(II)chloride dihydrate (1.2–1.6 eq.) was added and the mixture was stirred at room temperature for another 18–48 h. Afterwards, the reaction was quenched by the addition of conc. $NH_{3(aq)}$ and DCM was added. The phases were separated and the aqueous phase was extracted with DCM (3x). The combined organic extracts were washed with distilled water as well as brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was obtained, which was purified by flash column chromatography.

N-butyl-5-cyclopropyl-3-chloropiperidine (1 c)

Was prepared according to the general procedure from crude *N*-[(propenyl)cyclopropyl]methylbutanamine **5 c** (53 mg) and purified by flash column chromatography (R_f = 0.12 (pentane/TBME 20 : 1)) yielding the title compound **1 c** as a colorless oil (38 mg, 0.19 mmol; 50 % from **4 c**). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 4.13 (ddd, *J* = 14.6, 10.7, 4.2 Hz, 1H, C*H*-Cl), 3.22 (dd, *J* = 12.0, 3.0 Hz, 1H, CH_2), 2.51–2.25 (m, 3H, CH_2), 2.17 (t, *J* = 10.7 Hz, 1H, CH_2), 2.07–1.84 (m, 2H, CH_2-CH_2-CH_2-CH_3), 1.53–1.23 (m, 5H, CH_2 and CH_2-CH_2-CH_2-CH_3), 0.90 (t, *J* = 7.1 Hz, 3H, CH_3), 0.56–0.31 (m, 4H, $(CH_2)_2$) ppm; ^{13}C NMR ($CDCl_3$, 101 MHz): δ = 61.67 (CH_2), 61.13 (CH_2), 57.88 (CH_2), 54.87 (CH–Cl), 44.30 (CH_2), 34.27 (CH_2), 29.01 (CH_2), 22.48 (CH_2), 20.82 (CH_2), 18.10 (CH_2), 14.20 (CH_2), 14.12 (CH_3), 13.09 ((CH_2)$_2$), 9.64 ((CH_2)$_2$) ppm; HRMS (ESI): *m/z* calcd for $C_{11}H_{21}ClN^+$: 202.1357, found: 202.1357 $[M+H]^+$; elemental analysis calcd (%) for $C_{11}H_{21}Cl_2N$: C 55.47, H 8.89, N 7.44; found: C 55.78, H 8.83, N 7.64.

N-butyl-5-cyclobutyl-3-chloropiperidine (1 d)

Was prepared according to the general procedure from crude *N*-[(propenyl)cyclobutyl]methylbutanamine **5 d** (226 mg) and purified by flash column chromatography (R_f = 0.29 (DCM/acetone 50 : 1)) yielding the title compound **1 d** as a colorless oil (82 mg, 0.38 mmol; 33 % from **4 d**). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.90 (td, *J* = 10.9, 5.7 Hz, 1H, C*H*-Cl), 3.08 (d, *J* = 7.9 Hz, 1H, CH_2), 2.79 (d, *J* = 11.5 Hz, 1H, CH_2), 2.42–2.25 (m, 3H, CH_2), 1.99 (t, *J* = 10.9 Hz, 1H, CH_2), 1.95–1.67 (m, 8H, $(CH_2)_3$ and CH_2-CH_2-CH_2-CH_3), 1.50–1.38 (m, 3H, CH_2 and CH_2-CH_2-CH_3), 1.32 (h, *J* = 7.5 Hz, 2H, CH_2-CH_3), 0.91 (t, *J* = 7.3 Hz, 3H, CH_3) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ = 62.87 (CH_2), 61.90 (CH_2), 57.91 (CH_2), 53.95 (CH–Cl), 47.20 (CH_2), 39.98 (CH_2), 31.54 ((CH_2)$_3$), 31.17 (CH_2), 29.11 (CH_2), 20.71 (CH_2), 15.94 (C_q), 14.14 (CH_3) ppm; HRMS (ESI): *m/z* calcd for $C_{12}H_{24}ClN^+$: 216.1514, found: 216.1514 $[M+H]^+$; elemental analysis calcd (%) for $C_{12}H_{24}Cl_2N$: C 57.14, H 9.19, N 5.55; found: C 57.17, H 9.11, N 5.78.

N-butyl-5,5-diphenyl-3-chloropiperidine (1 e)

To a solution of the secondary amine **5 e** (440 mg, 1.34 mmol) in THF (25 mL) copper(II)chloride (180 mg, 1.34 mmol, 1.0 eq.) was added. The mixture was stirred at room temperature for 3 h and was then heated to reflux for another 22 h. Afterwards, the reaction was quenched by the addition of conc. $NH_{3(aq)}$ and DCM was added. The phases were separated and the aqueous phase was extracted with DCM (3x). The combined organic extracts were washed with distilled water as well as brine and dried over $MgSO_4$ The solvent was removed under reduced pressure and the crude product was obtained, which was purified by flash column chromatography (R_f = 0.37 (pentane/TBME 50 : 1)) yielding the title compound **1 e** as a colorless solid (198 mg, 0.60 mmol; 45 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.43–7.39 (m, 2H, Ar-*H*), 7.31–7.24 (m, 4H, Ar-*H*), 7.20–7.14 (m, 4H, Ar-*H*), 3.90–3.78 (m, 1H, C*H*-Cl), 3.63 (d, *J* = 12.1 Hz, 1H, CH_2), 3.25 (dd, *J* = 10.2, 3.9 Hz, 1H, CH_2), 2.96 (d, *J* = 12.3 Hz, 1H, CH_2), 2.46 (t, *J* = 7.4 Hz, 2H, CH_2-CH_2-CH_2-CH_3), 2.34 (t, *J* = 12.2 Hz, 1H, CH_2), 2.23–2.14 (m, 2H, CH_2), 1.56 (ddt, *J* = 20.6, 13.1, 6.4 Hz, 2H, CH_2-CH_2-CH_2-CH_3), 1.37 (h, *J* = 7.4 Hz, 2H, CH_2-CH_3), 0.96 (t, *J* = 7.3 Hz, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 147.72 (arom. *C*H), 145.39 (arom. *C*H), 128.72 (arom. *C*H), 128.47 (arom. *C*H), 128.21 (arom. *C*H), 126.58 (arom. *C*H), 126.47 (arom. C_q), 126.05 (arom. C_q), 62.58 (CH_2), 61.80 (CH_2), 58.01 (CH_2), 53.96 (CH–Cl), 48.30 (CH_2), 46.24 (CH_2), 28.87 (CH_2), 20.82 (CH_2), 14.18 (CH_3) ppm; HRMS (ESI): *m/z* calcd for for $C_{21}H_{27}ClN^+$: 328.1827, found: 328.1826 $[M+H]^+$; elemental analysis calcd (%) for $C_{21}H_{27}Cl_2N$: C 69.23, H 7.47, N 3.84; found: C 68.88, H 7.58, N 3.82.

Crystallographic data: Deposition Numbers 2153718 (for **1 a**), 2153719 (for **6 b**), 2153720 (for **6 a**), 2153721 (for **1 c**), and 2154152 (for **1 e**) contain the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service < url

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Angle distortion · Geminal disubstituent effect · Kinetics · Structure-Activity relationship · Thorpe-Ingold-Effect

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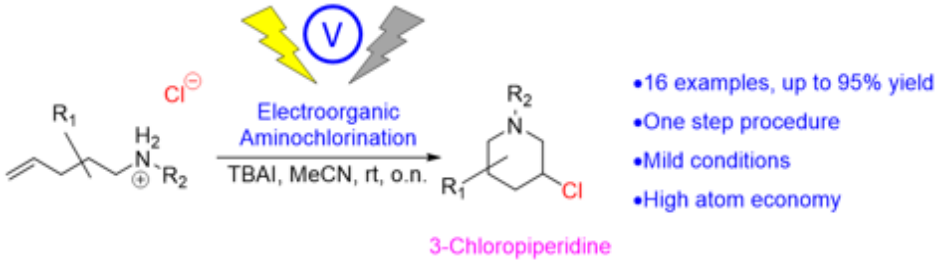
3.2 Synthesis of 3-chloropiperidines by iodide-mediated electrolysis



Abstract

Herein we report the cyclization of *N*-pentenylamines and their corresponding hydrochloric acid salts to 3-chloropiperidines under ambient conditions in an electroorganic fashion with tetrabutylammoniumiodide (TBAI) as a redox catalyst. Through iodide mediated oxidation, the unsaturated amine can be cyclized in an environmentally benign way without the need for conventional oxidants in stoichiometric quantity. A wide variety of 3-chloropiperidine derivatives were obtained in yields of up to 95% in an undivided cell. Mechanistic investigation indicates a radical pathway for this transformation.

Reference

Michael Kirchner, Yana Dubinina and Richard Göttlich, *Organic Chemistry Frontiers* **2023**, 10, 4631-4636. (DOI: 10.1039/D3QO00687E)

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Synthesis of 3-chloropiperidines by iodide-mediated electrolysis†

Michael Kirchner, Yana Dubinina and Richard Göttlich *

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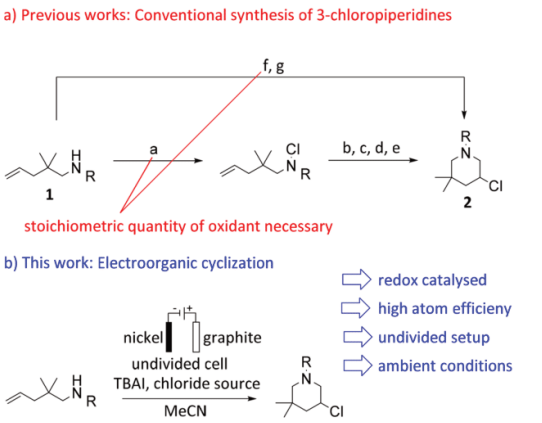
Herein we report the cyclization of *N*-pentenylamines and their corresponding hydrochloric acid salts to 3-chloropiperidines under ambient conditions in an electroorganic fashion with tetrabutylammoniumiodide (TBAI) as a redox catalyst. Through iodide mediated oxidation, the unsaturated amine can be cyclized in an environmentally benign way without the need for conventional oxidants in stoichiometric quantity. A wide variety of 3-chloropiperidine derivatives were obtained in yields of up to 95% in an undivided cell. Mechanistic investigation indicates a radical pathway for this transformation.

Introduction

The utilization of electroorganic synthesis as a scalable, cost-effective and environmentally benign alternative to conventional synthesis has yielded many accomplishments in recent years.[1–3] Extending the broad use of electroorganic chemistry to the synthesis of arylic and aliphatic organohalides could be a significant step to support a sustainable ecological footprint. In particular, aryl and alkyl halides are motifs frequently found in pharmacologically active compounds.[4–6] Nitrogen mustards, which share the alkyl halide motif, have been derived from the chemical warfare agent sulfur mustard and have emerged as valuable therapeutic agents for the treatment of cancer.[7,8] Moreover, their cyclic derivatives, 3-chloropiperidines, have shown DNA alkylating properties in recent studies, which could make them an addition to the toolbox of chemotherapy.[9] We therefore took great interest in finding efficient synthetic methods for the 3-chloropiperidine moiety so that a large variety of derivatives can be screened for their pharmacological properties. Conventional synthetic methods have already been developed in the past (Scheme 1), commonly utilizing oxidants in stoichiometric quantity to chlorinate an unsaturated amine followed by cyclization *via* a strong acid,[10] transition metal catalysis[11–14] or iodide catalysis.[15,16] The cyclization *via* free radicals has been reported as well.[17] In addition, a direct cyclization of unsaturated amines to 3-chloropiperidines was reported by Liu[18] using copper(II) chloride and by Li[19] using catalytic iodine, potassium persulfate and LiCl as a chloride source. Naturally, stoichiometric amounts of an oxidant in conventional methods manifest in a very low atom economy.[20] The habitual use of transition metals in common 3-chloropiperidine syntheses complements this with an additional ecological problem.[21] Electroorganic synthesis generally substitutes conventional oxidants and reductants with electric current as an inexpensive and safe alternative.[22] Commonly, iodide has been used as a redox mediator to oxidize substrates in a consecutive bulk reaction.[23,24] This has resulted in a wide variety of methods for cyclization reactions, especially for the formation of aziridines[25–27] and pyrrolidines.[28] Recently, Yin and coworkers[28] offered an iodine mediated approach for the efficient cyclization of non-acti-



Scheme 1 Intramolecular cyclization of unsaturated amines to 3-chloropiperidines. Conditions: (a) *N*-chlorosuccinimide (1 equivalent), DCM, rt; (b) TBAI (0.1 equivalent), $CHCl_3$, 50 °C;[15,16] (c) $Pd(PPh_3)_4$, THF, rt;[11] (d) acetic acid, 60 °C;[10] (e) CuCl, THF, rt;[29] (f) $CuCl_2{\cdot}2\,H_2O$, THF, rt;[18] (g) I_2 (2 mol%), $K_2S_2O_8$ (1.1 equivalents), LiCl (3 equivalents), 30 °C, THF.[19]

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vated aminoalkenes to iodomethylpyrrolidines. Their mechanistic proposal includes the electrophilic addition of iodine to a double bond with a consecutive ring closure to form the respective iodomethylpyrrolidine scaffold.

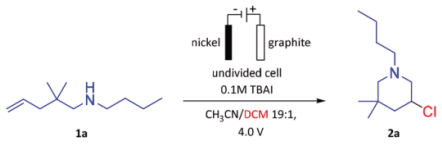
In contrast to iodation and bromination, electroorganic chlorination is a more challenging task. For one, because chloride requires a higher voltage[30] to be oxidized and also because chlorine readily degases from the reaction mixture resulting in a low faradaic efficiency of the process. The lower reactivity of chlorine with double bonds also remains problematic for certain applications.[31] We thought a valid solution could be intermediate iodation followed by a consecutive halogen exchange with a suitable chloride source. Even though common inorganic halide sources such as alkali metal halides are barely soluble in organic anhydrous solvents, halides can be prepared *in situ* by cathodic cleavage of alkyl halides.[32–35] The cleavage of dichloromethane (DCM) in particular leads to the formation of hydrocarbons such as methane, chloroform and isomeric butenes by recombination of methylene radicals on the cathode surface with a mechanism similar to the Fischer–Tropsch reaction.[32,36] For systems that tolerate acidic conditions, hydrogen halides are also a viable option. Inspired by previous studies, we developed an electroorganic protocol to sustainably access 3-chloropiperidines using either DCM or HCl as a chloride source.

Results and discussion

Optimization of the reaction conditions

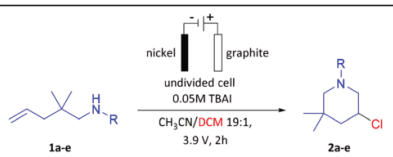
As unsaturated amines with geminal alkyl groups tend to cyclize faster due to the geminal-disubstituent-effect,[37,38] we selected **1a** as a model substrate to perform the intramolecular chloroamination. We chose TBAI as a redox catalyst, because of its high solubility in common organic solvents. As a chloride source, DCM was initially chosen due to its miscibility with acetonitrile in any quantity. In a first experiment, we could detect the formation of **2a** *via* GC-MS using the conditions depicted in Scheme 2. Using dichloromethane as a chloride source proved to be beneficial initially as the theoretical byproducts of chloride formation are gaseous hydrocarbons that leave the reaction mixture upon generation. Unfortunately, dichloromethane is only known to be cleaved at high voltages at nickel, copper, silver and platinum cathodes.[32] Our trials to decrease the cell potential to achieve a greener process therefore ended swiftly, as a reduction below a cell potential of 3.9 V resulted in the cessation of product formation. Even though we found little room for optimization with dichloromethane as a chloride source, yields of up to 74% were achieved for the cyclization of model system **1a** to 3-chloropiperidine derivative **2a** (Table 1). Comparable yields were achieved with four other substrates of different steric bulk and functionality (Table 1, entries 2–5).

To circumvent the problem of requiring high voltages and to achieve higher electrochemical efficiency, we investigated HCl as a different chloride source by using the respective hydrochloric acid salts as substrates. In addition to a possible reduction in the necessary cell potential, this would also open up the possibility of attempting the conversion under metal free conditions as the cathodic cleavage of dichloromethane is to the best of our knowledge only known for metal electrodes.[32] This is supported by the fact that we could not observe any conversion when substituting a graphite rod cathode in our initial conditions depicted in Scheme 2. Changing the original cathodic half-reaction to dihydrogen generation increases the atom economy and decreases the overall toxicity of the process, simultaneously forming a potentially useful byproduct. Contrary to our initial expectations, the solubility of the ionic substrate in acetonitrile did not impair our procedure, so 72% yield could be obtained for the conversion of **3a** to **2a** in a first experiment with non-optimized conditions (Table 2, entry 1). With the novel chloride source at hand, we screened different reaction conditions to improve the yield obtained from the process (Table 2). Our optimization revealed that lowering the electrolyte concentration increases the yield (Table 2, entry 3). Lowering the substrate concentration decreased the yield (Table 2, entry 4). In addition to that, the general scalability of the reaction was investigated. Unfortunately, attempting the reaction on a 50% bigger scale resulted in a reduced yield of 56% (Table 2, entry 5). Notably, the reaction also worked with moderate yields when employing other electrodes as the cathode. When employing a graphite cathode, and therefore operating under metal-free conditions, we reached 57% yield (Table 2, entry 6). The use of a copper rod electrode led to 50% yield (Table 2, entry 7). Heating up



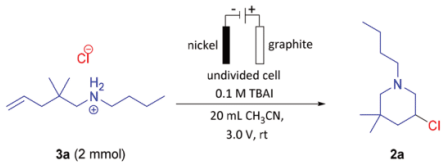
Scheme 2 Initial attempt to synthesize **2a** electroorganically from **1a**.

Table 1 Electroorganic cyclization of **1a–e** to **2a–e** with DCM as a chloride source (scale < 2.2 mmol). For detailed documentation of the cell parameters, the reader is referred to the ESI†



Entry	R	Yield [%]
1 (**1a** to **2a**)	Bu	74
2 (**1b** to **2b**)	Bn	48
3 (**1c** to **2c**)	Allyl	74
4 (**1d** to **2d**)	*t*Bu	63
5 (**1e** to **2e**)	$(CH_2)_3OTBDMS$	71

Table 2 Optimization of electroorganic cyclization of **3a** to **2a**



3a (2 mmol) **2a**

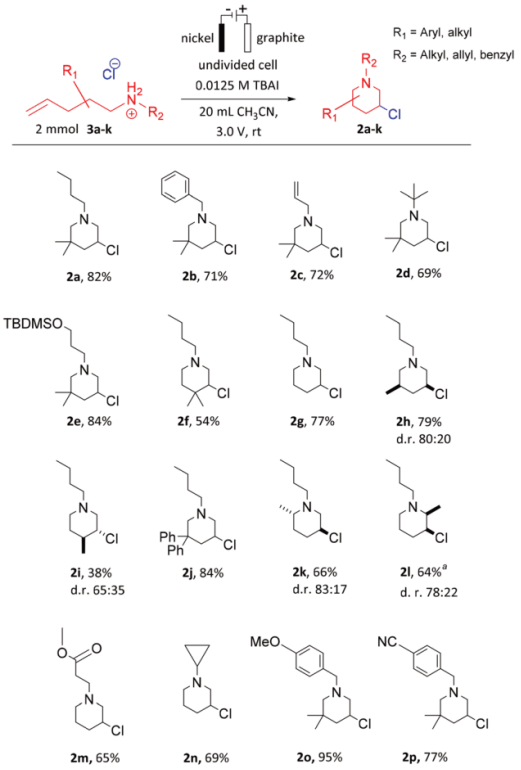
Entry	Deviation from standard conditions	Yield [%]
1	None	72
2	DMF as solvent	0
3	0.0125 M TBAI	82
4	30 mL acetonitrile	64
5	20 mmol **3a**, 200 mL acetonitrile	66
6	Graphite cathode	57
7	Copper cathode	50
8	0.0125 M TBAI, 50 °C	82
9	1.0 V	13
10	0.0125 M KI	82
11	No electric current	0

the reaction to 50 °C during electrolysis showed no influence on the yield (Table 2, entry 8).

Lowering the potential difference to 1.0 V resulted in a big drop in yield to 13% (Table 2, entry 9). Furthermore, we could show that potassium iodide can be readily substituted as a catalyst, as no decrease in yield was thereby detected (Table 2, entry 10). Lastly, we conducted a control experiment to show that the reaction was indeed electrochemical in nature. In line with our expectations, no conversion was observed in the absence of electric current (Table 2, entry 11). Owing to the results described above, we conclude that the optimal reaction conditions call for using a cell potential of 3.0 V, an undivided cell equipped with a nickel rod cathode and a graphite rod anode and a 12.5 mM solution of TBAI as a redox catalyst in acetonitrile. For a more extensive optimization table, the reader is referred to the ESI.†

Scope

With these optimized conditions for the electroorganic cyclization we worked out the scope for the reaction (Scheme 3). Gratefully, our method was successfully amenable for a wide range of different unsaturated amines. Aromatic moieties (**2b**) were tolerated by the employed standard conditions, although with some sacrifice in yield (down to 71%). The cyclizations of **2c** and **2d** offered similar yields of 72% and 69%, respectively, showing that additional primary alkenes and large steric bulk are tolerated as well. It is well known by us and others that the yield in cyclization reactions benefits greatly from geminal disubstitution.[37] Compounds **2a** and **2g** suggest that the geminal-disubstituent-effect is not crucially important to obtain a good yield when applying our conditions. Thereby, the difference in yield was merely 5%, even though we expect reactive rotamers to be more populated in **3a** than in **3g**. We attempted the cyclization of several compounds (**2h**, **2i**, **2k**, **2l**) with tertiary carbon centers to investigate whether the exerted radical stabi-

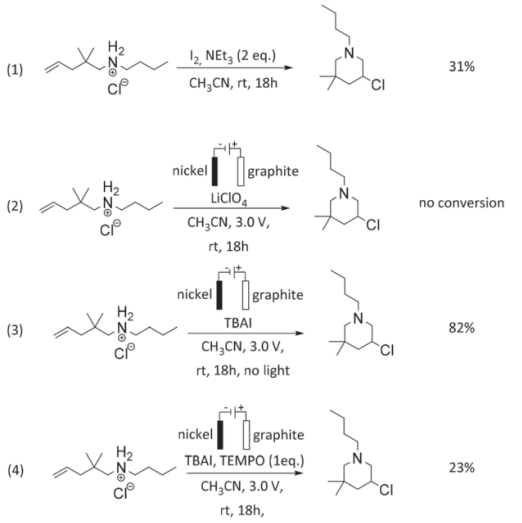


Scheme 3 Scope of the investigated electroorganic transformation from **3a–p** to **2a–p**. Diastereomeric ratio was determined *via* NMR. The major diastereomer was determined by comparison with literature NMR data.[16,17] [a] 1.36 mmol of **3l** were used as the starting material.

lization of a tertiary carbon center could impair the reaction. As Scheme 3 reveals, the conversion to **2h** and **2k** proceeded smoothly, returning 79% and 66% yields, respectively. Substituting a methyl group in the allylic position, however, resulted in a large reduction in yield. This could indicate an [1,2]H-shift, forming a more stable tertiary radical, paired with steric congestion around the reacting double bond. Through the synthesis of **2m**, **2o** and **2p**, we could show that esters, ethers and nitriles were tolerated. Therein, we obtained 65%, 95% and 77% yields, respectively. The tolerance of a cyclopropyl moiety (**2n**) indicates that the reaction does not proceed *via* an aminyl radical. We attempted to synthesize four- and seven-rings with the presented method, but unfortunately only uncharacterized side-products were obtained. The presented conditions also did not tolerate amides. For more information the reader is referred to the ESI.†

Mechanistic investigation

To gain deeper insight into the mechanism of the reaction, we conducted a few control experiments (Scheme 4). First, we tried to distinguish between a radical and a polar mechanism.

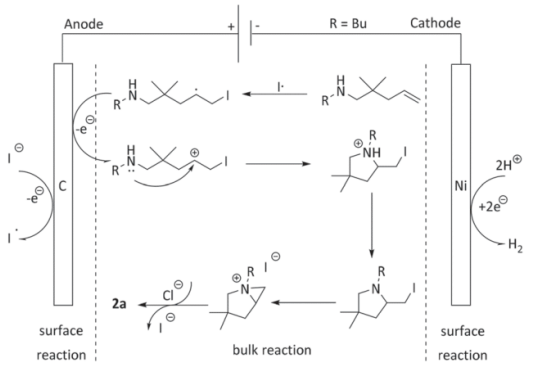


Scheme 4 Control experiments.

For this, we added iodine to a solution of hydrochloride salt **3a** in acetonitrile and two equivalents of triethylamine (Scheme 4, reaction (1)). If the mechanism were to proceed *via* electrophilic addition of iodine to the double bond with subsequent ring closure, one should expect a yield comparable to that of the electroorganic transformation. The same holds true for the direct oxidation of the amine to the iodoamine, which is expected to undergo cyclization spontaneously.[16] Only 31% yield was obtained in this experiment, proving that these are not valid mechanistic pathways for the given electroorganic transformation.

A second control experiment (Scheme 4, reaction (2)) also indicated that iodide is necessary as an electrochemical mediator as no product was formed when lithium perchlorate was employed as a supporting electrolyte. We therefore focused our attention on possible radical mechanisms. In recent literature, a mechanism for aziridine ring closure involving the photolysis of tetrabutylammoniumtriiodide ($TBAI_3$) has been discussed.[26,39] As $TBAI_3$ is in equilibrium with TBAI and electrochemically generated I_2, we suspected that our reaction could be promoted by $TBAI_3$. In the proposed mechanism, $TBAI_3$ is excited by visible light to generate iodine radicals ($I^{\bullet}$) and iodine radical anions ($I_2^{\bullet-}$), which then function as radical promoters in the electrochemical aziridination reaction. To investigate this possibility for our systems, we used our standard conditions for the transformation of **3a** to **2a** under exclusion of visible light. However, we could not detect a reduction in yield under these conditions (Scheme 4, reaction (3)). We therefore conclude that the reaction proceeds *via* either direct oxidation at the anodic surface or oxidation by iodide radicals formed at the anode.

Owing to prior cyclovoltammetric experiments by Yin and coworkers,[31] the direct oxidation of the amine to an aminyl radical cation seems unlikely, indicating that TBAI indeed acts as a redox mediator in the observed transformation. Also, a fourth control experiment (Scheme 4, reaction (4)) validated that a radical mechanism seems likely as the employment of TEMPO as a radical trapping agent led to a large decrease in yield. On the basis of the observations described above, we propose the mechanism shown in Scheme 5 for the electrochemical formation of 3-chloropiperidines. Initially, the oxidation of iodide to iodine radicals at the anode is likely. Afterwards, an addition of iodine radicals to the unsaturated amine could take place. Then, the resulting alkyl radical could be oxidized at the anode to the respective carbocation. This carbocation then cyclizes to the respective iodomethylpyrrolidonium cation. After proton abstraction, the iodomethylpyrrolidine could form 3-chloropiperidine *via* nucleophilic attack of a chloride anion on the reactive aziridinium intermediate. Simultaneously, iodide is regenerated and reenters the catalytic cycle.



Scheme 5 A plausible radical mechanism.

Conclusions

In summary, we presented an efficient strategy for the electroorganic synthesis of 3-chloropiperidines. The described methods offer good yields for various substrates with different magnitudes in steric bulk. We furthermore showed that the synthesis of the 3-chloropiperidine motif is possible under metal free conditions when employing our electroorganic protocol. Control experiments suggest that the formation of 3-chloropiperidines likely proceeds *via* a radical mechanism. The application of the herein discussed chemistry under metal-free conditions will be developed further in our laboratory in the future.

Author contributions

Michael Kirchner: writing – original draft (lead) and investigation (lead). Yana Dubinina: investigation (supporting).

Richard Göttlich: writing original draft (supporting) and project administration (lead).

Conflicts of interest

The authors declare no conflict of interest.

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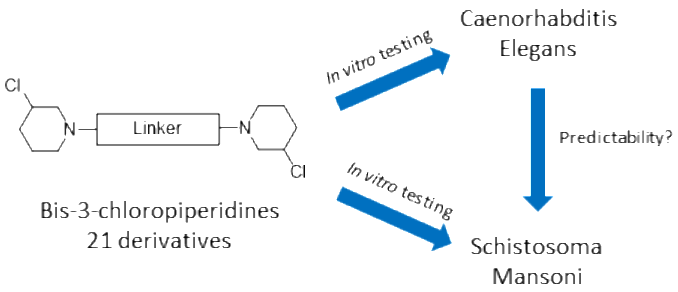
3.3 Bis-3-chloropiperidines: a novel motif for anthelmintic drug design



Abstract

We report the *in vitro* screening of bis-3-chloropiperidine derivatives against the free-living *Caenorhabditis elegans* and the parasitic *Schistosoma mansoni*. 56% of compounds active against *C.elegans* were also active against *S. mansoni*.

Reference

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Michael Kirchner and Michael Marner contributed equally to this work.

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Bis-3-chloropiperidines: a novel motif for anthelmintic drug design†

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Parasites account for huge economic losses by infecting agriculturally important plants and animals. Furthermore, morbidity and death caused by parasites affect a large part of the world population, especially in economically weak regions. Anthelmintic drugs to tackle this challenge remain scarce and their efficiency becomes increasingly endangered by the advent of drug resistance development. In the present study, we assessed the anthelmintic potential of bis-3-chloropiperidines, a family of compounds which have already demonstrated antiproliferative activity against various cell lines. We synthesized and tested the activity of 21 bis-3-chloropiperidine derivatives against two strains of the free-living nematode *Caenorhabditis elegans* (N2 and DC19) and the parasitic flatworm *Schistosoma mansoni*. Overall, bifunctional chloropiperidines featuring an aromatic linker performed best against the tested indicator organisms and could be considered for future optimization efforts. Ultimately, out of the 21 analyzed bis-3-chloropiperidines, four derivatives (**2**, **5**, **9** and **11**) reduced vitality parameters against *S. mansoni* and five the motility of *C. elegans* (**2**, **4**, **5**, **13**, **21**) while exhibiting no or low cytotoxicity.

1 Introduction

Schistosomiasis and soil-transmitted helminthiases are among the most common infectious diseases and pose a major challenge in veterinary and human medicine. The World Health Organization (WHO) estimated that 1.5 billion people are infected by soil-transmitted helminths such as *Ascaris lumbricoides*, *Trichuris trichiura* or *Necator americanus*.[1] At the same time, helminths like *Haemonchus contortus* cause immense economic losses in livestock by infecting in particular small ruminants like sheep, goats and young cattle.[2]

In 2022, more than 0.25 billion people required preventive chemotherapy, while several millions suffered from severe morbidity as a consequence of infections with the blood fluke(s) *Schistosoma* sp. Since the introduction of praziquantel (PZQ) in the 1980s, no alternative treatment option for schistosomiasis was developed. Hence, besides vector control and the improvement of sanitation and water safety, strategies to fight neglected tropical diseases (NTD) in low- and middle-income countries (LMIC) are the development of multiplex diagnostics accompanied with new treatment approaches.[3] The situation is further aggravated by anthelmintic resistance (AR) to the already limited number of efficacious drugs. For each major anthelminthic compound class, widespread resistance emerged within parasitic nematodes.[4,5] Likewise, reduced efficacy of the only antischistosomal drug PZQ was reported.[6] Thus, research and development towards reproposed or new anti-infective agents is urgently needed.[7–10] This urgency has already been manifested in the emergence of some promising lead scaffolds including organic peroxides[11] and avermectins.[12]

Many licenced anthelmintic small-molecules exhibit several biological activities including antimicrobial and anti-tumour.[13] Hence, in a first step of anthelmintic drug discovery, the activity profile of a candidate compound is optimized to balance potential conflicting attributes such as parasite efficacy and *in vitro* cytotoxicity.[14]

In this study we set out to investigate the anthelmintic potency of a series of bis-3-chloropiperidines analogs inspired by natural product antibiotic 593A. Initially, Antibiotic 593A was isolated from *Streptomyces griseoluteus* in 1970 by Gittermann[15] followed by total synthesis by Fukuyama[16] in 1980. Besides the antibacterial activity, 593A proved to be active against solid tumors and leukemia, most importantly against cancer cells that were resistant against the commonly used antiproliferative

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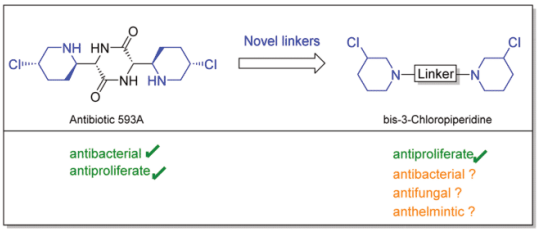

Fig. 1 Preparation of simplified analogs of antibiotic 593A could yield novel pharmacologically active compounds.

drug cyclophosphamide.[17] Interestingly, our simplified compounds as well showed activity in various cell-based assays.[18–20] It was demonstrated that *bis*-3-chloropiperidines form an electrophilic and highly reactive aziridinium ion, which can be attacked by nucleophilic agents such as nucleobases or intracellular thiols.[21,22]

Compared to clinically used nitrogen mustards, the reactive moiety is congested in a ring system, which could enhance DNA interactions *in vivo*.[23] Moreover, Sosic *et al.* could show that next to DNA-alkylation, bis-3-chloropiperidines have a second mode of action and serve as an inhibitor of the human topoisomerase II α.[24] Through previous studies, we could also find that aromatic and L-lysine linkers can significantly increase the activity of bis-3-chloropiperidines *in vitro*.[18,19,25] Also geminal dimethylation proved to be a valid strategy to increase the activity of these compounds *in vitro*.[20] Despite these intriguing findings, this series of compounds was, to the best of our knowledge, never screened for further applications (Fig. 1). Hence, in this study, we focused our research efforts to investigate whether these compounds might represent a starting point for an antimicrobial or anthelmintic drug design. First, we investigated the bioactivity of mono-, bi- and trifunctional 3-chloropiperidine agents in antibacterial and antifungal assays, followed by cytotoxicity evaluation in mammalian cell cultures. To analyse anthelmintic activity, we used the free-living nematode *Caenorhabditis elegans* (*C. elegans*) as a surrogate for the parasitic nematodes. Besides the wildtype *C. elegans* N2, we used strain DC19 in our assays. *C. elegans* DC19 combines an enhanced cuticle permeability with low fitness consequences. It is thus regarded as a drug sensitive screening strain with enhanced drug target accessibility. Out of our results we deduced an anthelmintic structure–activity relationship of 3-chloropiperidines and investigated the predictive value of *C. elegans* models for *Schistosoma mansoni* activity.

2 Results and discussion

2.1 Chemistry

The synthesis of 3-chloropiperidines **1**, **2**, **4**, **6**, **8**, **10–14** and **16–21** (Fig. 3) has been described in recent literature.[19,20,23,25–27] This leaves compounds **3**, **5**, **7**, **9** and **15** as novel compounds the synthesis of which we will describe hereinafter (Fig. 2). Compound **3** was prepared in a five-step sequence from commercially available dimethyl-5-aminoisophthalate. In the first step, two methyl groups were introduced *via* reductive amination in a yield of 86%. The respective aldehyde was then prepared by initial reduction with lithium aluminium hydride (LAH) with subsequent *Swern*-oxidation, which was accomplished in yields of 85% and 84% respectively. The 3-chloropiperidine moiety was then formed through reductive amination with 2,2-dimethyl-pent-4-enylamine and consecutive *in situ* chlorination/cyclization with copper(II)–chloride, following a procedure of Liu[27] *et al.* The final product **3** was thereby obtained in a yield of 14% over two steps. Formation of the 3-chloropiperidine moiety by initial *N*-chlorination of the secondary amine with *N*-chlorosuccinimide (NCS) and successive cyclization with tetrabutylammonium iodide (TBAI)[28] did not yield any product, presumably due to oxidation of the reactive dimethyl aniline moiety.

Compound **5** was prepared from 5-nitroisophthalic acid. The carbonic acid was first selectively reduced with $NaBH_4$ to the alcohol under activation with $BF_3 \cdot Et_2O$ in a yield of 96%. The obtained alcohol was readily reoxidized to the aldehyde by activated MnO_2 in acetonitrile in a yield of 63%. The secondary amine was then formed by reductive amination with 2,2-dimethylpent-4-enylamine and could be readily oxidized by NCS without further purification. Afterwards, catalytic cyclization with TBAI[28] provided **5** in a yield of 73%. The synthesis of **7** was attempted starting from 5-methylisophtalic acid. Thereby, the acid was reduced to the respective alcohol with LAH. The alcohol was then readily brominated with PBr_3 in a yield of 67% over two steps. Then, the azide was formed by nucleophilic substitution using sodium azide, which was followed by Staudinger reduction to yield the respective amine in a yield of 40% over two steps. Step-wise reductive amination with 2,2-dimethylpent-4-enal was then leveraged to avoid multiple substitutions on one nitrogen center. This gave a yield of 45% of the respective secondary amine. This compound could again be cyclized with copper(II) chloride in THF[27] in a yield of 20%. Electroorganic cyclization[29] did not lead to any conversion, presumably as the respective hydrochloride was insufficiently soluble in acetonitrile.

Compound **9** was prepared in a nine-step sequence from commercially available 4-nitrophthalonitrile. In the first step, the nitro-group was replaced with a methoxy-group by nucleophilic aromatic substitution. As the direct reduction to the diamine with LAH or Red-Al resulted merely in partial reduction, we introduced the amine functionality over a step-wise way. Thereby, the crude residue was first subjected to alkaline hydrolysis which yielded the respective carbonic acid in a yield of 47% over two steps. Reduction with LAH afforded the respective alcohol in 84% yield. Consecutive bromination with PBr_3 afforded the intermediate bromide in a yield of 76%. The obtained bromide could now be converted to the respective amine by nucleophilic substitution and consecutive *Staudinger* reduction, which resulted in a yield of 64%. Imine formation with 2,2-dimethylpent-4-enal and reduction again afforded the secondary amine in a good yield of 61%, which could then be precipitated quantitatively as the hydrochloride salt with ethereal hydrochloric acid. The hydrochloric acid salt could then be cyclized under electroorganic conditions[29] to yield compound **9** with a yield of 9%.

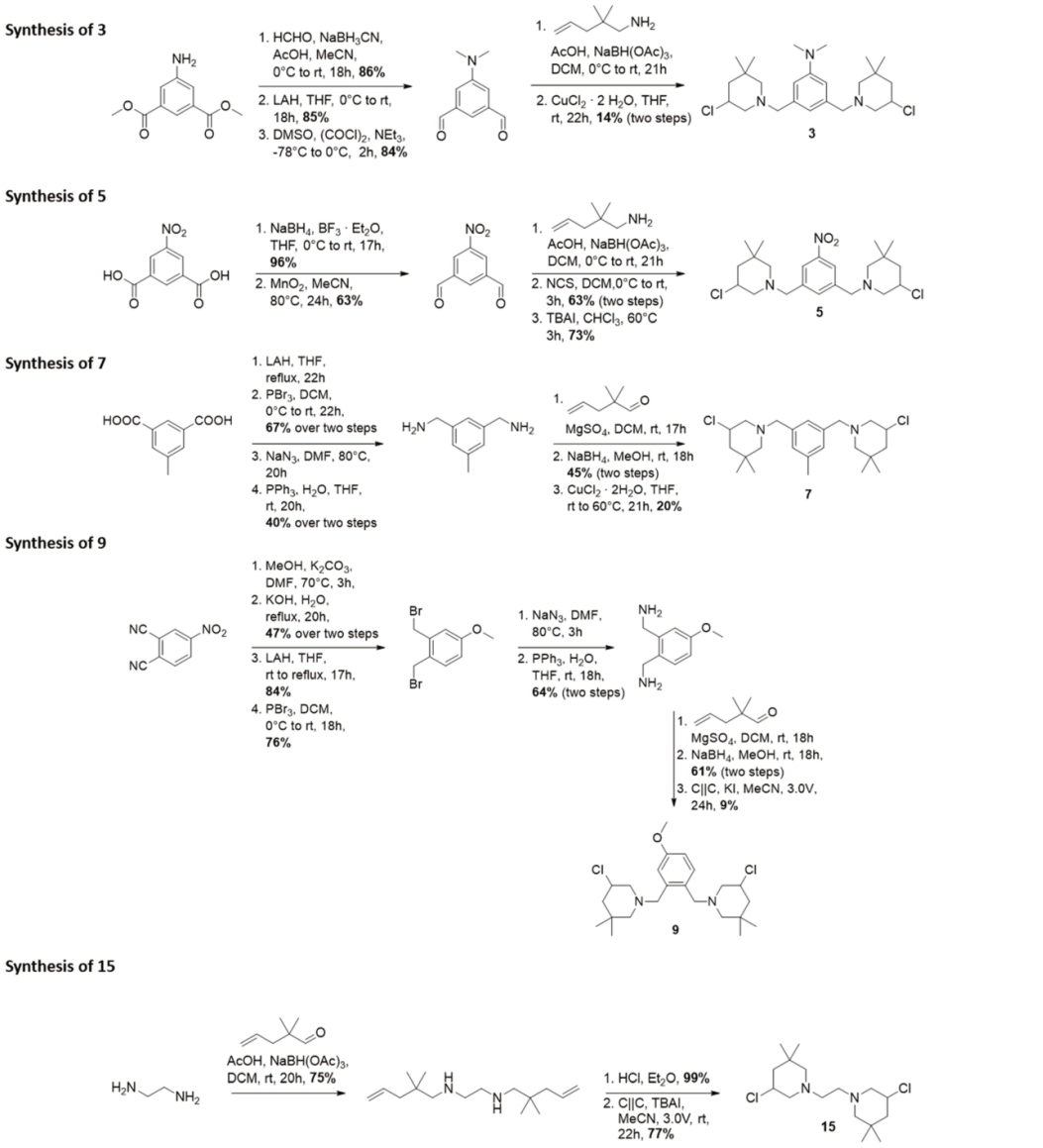

Fig. 2 Synthesis of novel bis-3-chloropiperidines **3**, **5**, **7**, **9** and **15**.

Compound **15** was prepared from commercially available ethylene diamine, which was first reductively aminated with 2,2-dimethylpent-4-enal to yield 75% of the corresponding secondary amine. The free amine was then precipitated quantitatively as the hydrochloride salt with ethereal hydrochloric acid. The hydrochloride salt was then cyclized in an electro-organic,[29] metal-free fashion to form **15** in a yield of 77%. For synthetic details, the reader is referred to the ESI.† With these novel compounds at hand, we tested the antimicrobial and anthelmintic activity.

2.2 Antimicrobial and anthelmintic activity tests

The minimum inhibitory concentration (MIC) of the synthesized compounds was initially determined against three microbial indicator strains (*Escherichia coli* ATCC35218, *Streptococcus aureus* ATCC33592, *Septoria tritici* MUCL45408).

Only compounds **8** and **13** showed weak activity against *S. tritici* (64–32 and 16 μg mL^{-1} respectively), while the remaining derivatives were inactive (64 or >64 μg mL^{-1}) against the selected indicator strain at the tested concentrations.

Table 1 Screening results of the activity tests against *E. coli* (Ec), *S. aureus*, *S. tritici* (St), *C. elegans* (N2 and DC19) and a canine kidney cell line (MDCK II). Positive controls were chosen according to the nature of the test strain. For bacteria: rifampicin (RIF), tetracycline (TET), gentamicin (GEN). For St: tebuconazole (TEB), amphotericin B (AMP), nystatin (NYS). *C. elegans*: ivermectin (IVR) and MDCKII cells: ionomycin (ION). MIC: minimum inhibitory concentration given in μg mL; MMIC: minimum motility inhibitory concentration given in μg mL^{-1}. Cytotoxicity at high dose of 100 μM against MDCKII either "+" (toxic) or "−" (not toxic). Compounds that resulted in a cell viability of less than 80% relative to the DMSO control were categorized as cytotoxic. nd: not determined

	MIC [μg mL^{-1}]			MMIC [μg mL^{-1}]		
ID	Ec	Sa	St	N2	DC19	MDCKII
1	>64	>64	64	64	64–32	+
2	>64	>64	64	16–8	16–8	−
3	>64	>64	>64	16	32–16	+
4	>64	>64	>64	16	16	−
5	>64	>64	>64	16	16	−
6	>64	64	>64	64	64–32	+
7	>64	>64	>64	32–16	16	+
8	>64	>64	64–32	8	8–4	+
9	>64	>64	>64	>64[a]	64	+
10	>64	>64	>64	>64	>64	+
11	>64	>64	>64	>64[a]	>64	−
12	>64	>64	>64	16–8	16	+
13	>64	>64	16	32–16	8–4	−
14	>64	>64	>64	>64	64	+
15	>64	>64	>64	>64	>64	+
16	>64	>64	>64	>64	>64	−
17	>64	>64	>64	>64	>64	−
18	>64	>64	>64	>64	32	+
19	>64	>64	>64	>64	>64	−
20	>64	>64	>64	>64	>64	−
21	>64	>64	>64	16–8	16–8	−
22	>64	>64	>64	>64	>64	−
RIF	4	>64	nd	nd	nd	nd
TET	4–2	32	nd	nd	nd	nd
GEN	1	0.5	nd	nd	nd	nd
TEB	nd	nd	>0.03	nd	nd	nd
AMP	nd	nd	0.125	nd	nd	nd
NYS	nd	nd	0.25	nd	nd	nd
IVR	nd	nd	nd	0.005	0.005	nd
ION	nd	nd	nd	nd	nd	+

[a] Some worms were underdeveloped at 64–32 μM.

To evaluate the anthelmintic potential of compounds **1–22**, we chose the nematode *C. elegans* as a model system for parasitic nematodes. In addition, we screened the compound series against the obligate internal parasitic trematode *S. mansoni* and determined cytotoxicity against the canine kidney cell line (MDCK II, Table 1) at a high dose of 100 μM and subsequently determined the CC_{50} for prioritized compounds (Table 2).

The cuticle of *C. elegans* strain DC19 is known to be more permeable for small molecules compared to the wildtype N2.[30] DC19 is thereby considered an informative drug-sensitive test organism. In our assays, we determined the minimum mobility inhibitory concentration (MMIC) as a proxy for anthelmintic activity. For most of our 3-chloropiperidines derivatives however, the susceptibility of the two *C. elegans* stains was similar, indicating that the moderate activity is not influenced by the cuticle structure. In contrast, MMICs of some small molecule anthelmintics such as albendazole, mebendazole or PF-1022A were lower for DC19 (Fig. S1†).

Table 2 Summary of prioritized compounds and their effects on tested nematodes and MDCKII cells. Compounds exhibiting moderate effect on *C. elegans* strains N2 and DC19 (MMIC ≤20 μg mL^{-1}) and/or inflicting phenotypic changes in *S. mansoni* assays, while not showing reduced MDCKII cell viability at a high dose of 100 μM were subject of CC_{50} determination against the same cell line. MMIC: minimum motility inhibitory concentration. Det: detachment of suckers, Mot: motility reduction, Sep: Pair separation. Compare Table 1 and Fig. 4

ID	MMIC [μg mL^{-1}] N2 and DC19	Effect on *S. mansoni*	MDCKII CC_{50} [μM]
2	16–8	Det, Sep, Mot, Lethal	83
4	16	—	205
5	16	Det, Sep, Lethal	>1000
9		Det, Sep, Lethal	70
11		Det, Sep	123
13	8–4	—	>1000
21	16–8	—	>1000

In general, we observed that mono-functional agents (**16–20**) did not have any effect on the test organisms (MIC and MMIC >64 μg mL^{-1}) and canine kidney cells, while compounds featuring two or three 3-chloropiperdine building blocks showed a range of activities.

Inactivation of the reactive moiety, by exchange of the chlorine atom in the piperidine scaffold with a hydroxyl group, eliminated the *C. elegans* mobility inhibition (comparison **2** and **22**). This suggests, that the observed activity of *e.g.* of compound **2** is indeed related to the previously mentioned reactive aziridinium ion formation, a reaction requiring the electrophilic β-carbon.

The results of the bifunctional 3-chloropiperidines indicate a strong influence of the linker moiety on the bioactivity against our surrogate models N2 and DC19 (Table 1). Derivatives with an aliphatic linker such as a cyclohexane moiety (**10**) or a linear, aliphatic chain (**14** and **15**) seem to have weak activity (MMIC 64 μg mL^{-1} and above), while substitution of the chain with aromatic ester groups increased potency to 16 μg mL^{-1} (**12**) and 8–4 μg mL^{-1} (**13**) against DC19. This effect was not observed for the methyl ester derivative **11**. Compounds featuring an aromatic linker performed better against *C. elegans*. However, the data indicate, that the substitution pattern of the employed aromatic linker is crucial for activity. Switching from a meta-substituted aromatic system to an *ortho*-substituted aromatic compound lowered the MMIC from 64 μg mL^{-1} (**1**) to 8 μg mL^{-1} (**8**). Furthermore, substitution of the benzylic linker with a pyridinylic linker (**6**) also resulted in activity.

By comparison of compound **1** with **2–5**, we concluded that a second meta-substitution of the linker might be beneficial for activity. We observed that a linker featuring electron withdrawing or electron donating substituents increases the anti-nematode activity of the compounds (compounds **2–5**). Comparison of MMICs of compound **1**, **2** and compound **7**, a bis-3-chloropiperidine featuring a 5-methylbenzene linker with

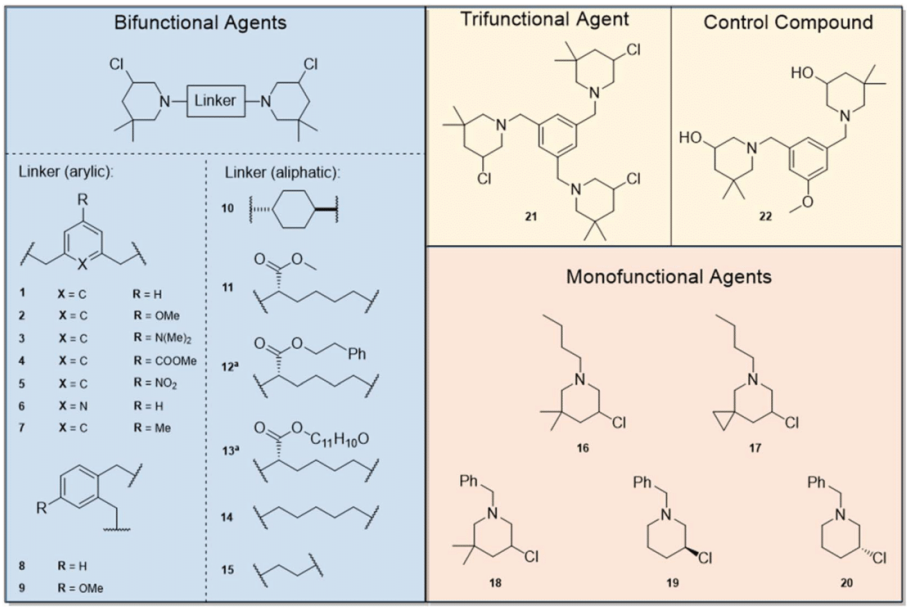

Fig. 3 Synthesized mono-, bi and trifunctional 3-chloropiperidine agents. [a]aliphatic linker with aromatic side-chain.

intermediate electron density compared to **1** and **2**, supports this hypothesis (**1** = 64–32 μg mL; **7** = 32–16 μg mL^{-1} and **2** = 16–8 μg mL^{-1}). A compound with a third 3-chloropiperidine moiety (**21**) in meta-position exhibits the same degree of activity as **2** with only two active moieties (16–8 μg mL^{-1}).

Compound **9** combines the structural elements of **2** and **8**, which both showed moderate MMICs in our initial tests. However, the combination of an ortho-substituted aromatic linker (like **8**) substituted with an additional methoxy group (like **2**) did exert comparable activity against N2 or DC19 (64 μg mL^{-1} compared to 16–8 and 8–4 μg mL^{-1} respectively). Overall, compounds **2–5**, **7–8**, **12–13** and **21** were observed to exhibit the strongest *C. elegans* mobility inhibitory activity of <20 μg mL^{-1}. Of these, compounds **2**, **13** and **21** did not exhibit cytotoxic properties. Subsequently, we tested whether compounds with nematocidal activity display a broader activity also against parasitic flatworms. To this end, *in vitro* tests against the blood fluke *S. mansoni* were conducted (Fig. 4). The worms were incubated in presence of 10 and 20 μM of compounds **1–22** and phenotypic vitality parameters (separation of worm pairs, detachment of suckers from the bottom of the well and weakening of body movements) were assessed as previously described.[31] Compounds reaching 100% effect strength in one of the parameters within 7 days were considered active. Reduction of motility is considered most relevant for anti-schistosomal drug candidates.[32] In addition, loss of sucker activity would make parasites drifting off from their host habitat (mesenteric veins) and with pair separation, the production of pathology-causing eggs eventually ceases.[33] From the herein investigated 3-chloropiperidines, 56% of *C. elegans* active derivatives (MMIC ≤20 μg mL^{-1}) also showed an effect on *S. mansoni* (compounds **2**, **3**, **5**, **7**, **12**) whereas four compounds (**4**, **8**, **13** and **21**) were *C. elegans*- and three (**1**, **9**, **11**) *Schistosoma*-specific. For some molecular targets *e.g.* glutamate and GABA chloride channels (ivermecin, abamectin) and β-tubulin (albendazole, mebendazole) the *C. elegans* assays results were transferable to *Schistosoma*, while for others *e.g.* acetylcholin mimetic (levamisole) the *C. elegans* MMIC was not predicative (Fig. S1†).

Overall, none of the compounds caused 100% lethality at the tested concentrations, which means their antischistosomal activity can be considered rather weak. However, at 20 μM, nine compounds were found active while at 10 μM, activity remained only for three. At 10 μM, only **5** and **12** led to full sucker detachment of *Schistosoma* worm pairs, while the other parameters remained unaltered by the treatment (Fig. 4). Compound **9** led to the separation of all worm pairs after 3 days and to a lesser extent already at day 1 at 10 μM.

Looking at effects in more detail, we did not observe any changes in the vitality parameters of *Schistosoma* as a response to monofunctional 3-chloropiperidines exposure, which is in agreement with our *C. elegans* data. While the trifunctional derivative (**21**) exited mobility inhibitory activity in N2 and DC19, both did not affect *Schistosoma*.

Similarly, the bifunctional compounds **8** and **13**, which showed the strongest mobility inhibition against *C. elegans* DC19 (8–4 μg mL^{-1} *e.g.*, 17–8.5 μM and 12.5–6.3 μM respectively), did not inflict any phenotypic changes in *Schistosoma* at 20 μM in comparison to the untreated control.

Despite that, exposure to bifunctional 3-chloropiperidines with an aromatic linker strongly affected vitality parameters in *Schistosoma* at 20 μM. We observed that the efficacy of these

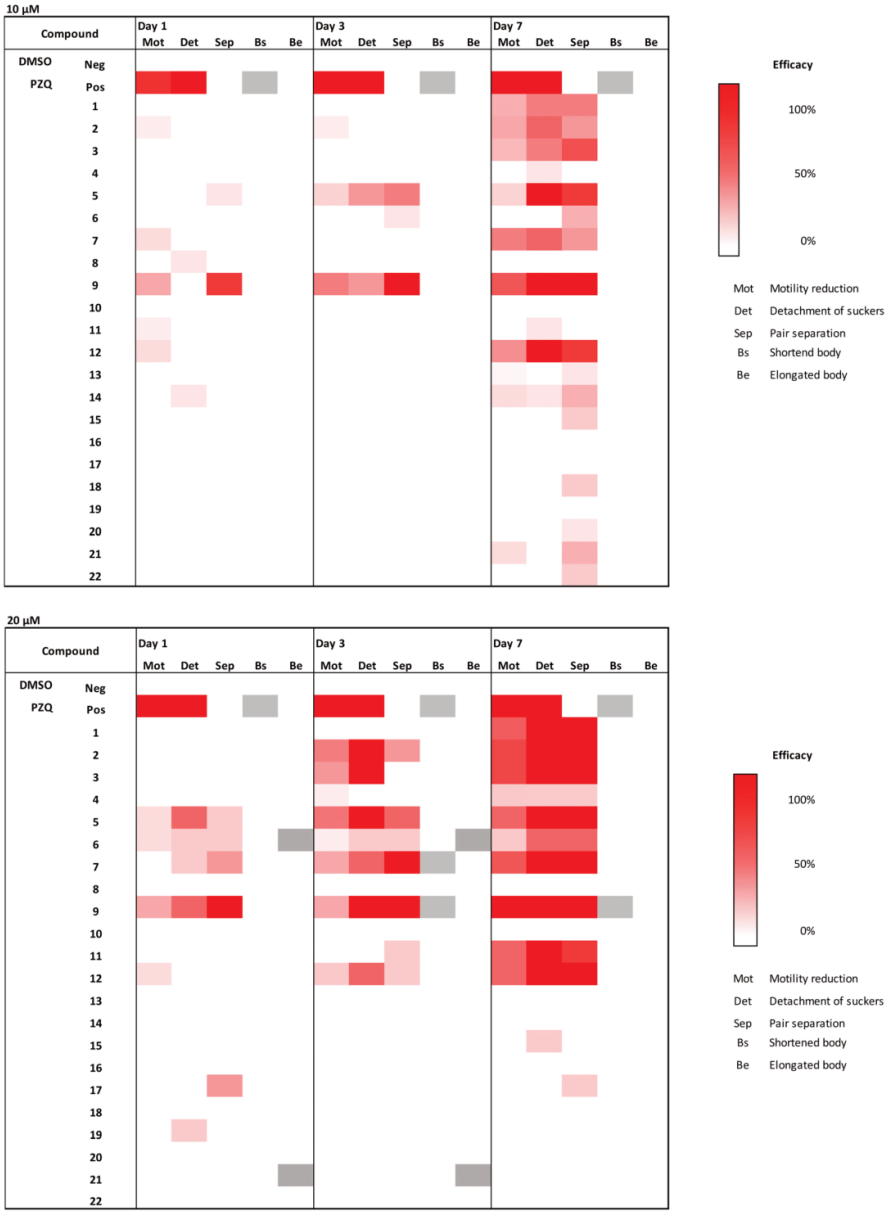

Fig. 4 Activity of 3-chloropiperidine derivatives against *S. mansoni* at a concentration of 10 µM and 20 µM. Phenotypic readout (Mot: motility reduction, Det: detachment of suckers, Sep: pair separation, Bs: shortened body, Be: elongated body) was done after 1, 3 and 7 days. Efficacy is expressed weak (white) and strong (red). Positive control (Pos): *Praziquantel* PZQ. Hatched: not determined. Mean values of two independent experiments are shown.

compounds increased over time. In that sense, we observed detachment of suckers and pair separation of all worm pairs treated with compounds **1–3**, **5**, **7**, **9**, **11** and **12** at 20 µM (Fig. 4) after 7 days incubation. Most strikingly, exposure to compounds **2**, **3** and **9** led to sucker detachment already after three days and lethal effects after seven days. This indicates that the activity of the 3-chloropiperidines against *S. mansoni* might be correlated with the electron density in the aromatic system, as compound **4** with a low electron density in the aromatic system exerted no activity against *S. mansoni*. On the other hand, the strong activity of nitro-substituted system **5** surprised us, as it can be considered active at 10 µM, even though the

implemented aromatic system is very electron-poor. However, the CC_{50} for **5** (Table 2) was significantly higher than for **2**, **9** and **11**, which could indicate that they have a different mode of action. Overall, three compounds (**1**, **5**, **12**) stopped motility almost completely (motility score 1–1.2). Fast action of compounds is an important parameter for defining anti-schistosomal activity, considering a rather short exposure of parasites to initial drug concentrations within the mesenteric veins.[34] After a short treatment period of one day, only methoxylated compound **9** was active, causing complete worm pair separation (as observed at 10 μM). This indicates that the position of the methoxy-group on the arylic system might be crucial for good activity. Therefore, it could prove valuable to investigate other arylic substitution patterns for their biological activity (such as the two possible 1,2,3-substitution patterns). As the three methoxylated 3-chloropiperidines **2**, **9** and **11** were all active at 20 μM, a correlation between activity and the count of methoxy groups might also be likely.

The cytotoxicity assessment revealed that from the nine compounds, which were seen to reduce vitality parameters of *S. mansoni*, five (**1**, **3**, **7**, **9** and **12**, see Table 1 and Fig. 4) were cytotoxic at 100 μM. However, due to the fast action of compound **9** at 10 μM, we additionally tested a triplicated dilution series of this compound against MDCK-II. Based on the dose–response curve (Fig. S3†) a CC_{50} value of 70 μM was determined, indicating a 7-fold stronger effect on *Schistosoma* pair separation as on cell viability reduction (Table 2 and Fig. S3†). Compounds **2** (lethal effect at 20 μM), **5** (sucker detachment at 10 μM), **11** (detachment of suckers and pair separation at 20 μM) showed even weaker cytotoxic effects (CC_{50} of 83 μM (**2**), >1000 μM (**5**) and 123 μM (**11**)), leaving them, in addition to compound **9**, as potential starting points for anti-*Schistosoma* drug design.

3 Conclusions

This study highlighted the anthelmintic potential of bis-3-chloropiperidines. Herein, we demonstrated that the activity against *C. elegans* and *S. mansoni* is very dependent on the employed linker system. Generally, bifunctional compounds featuring an aromatic linker performed better. Further studies towards the molecular target(s) and mode of action in nematodes should be conducted to understand the true value of the presented group of compounds with regard to anthelmintic drug design. Despite that, we could show that the transferability from the *C. elegans* to *Schistosoma* assays is generally good (56%). However, we also observed a high degree of *Schistosoma* specificity (33%). Ultimately, we could find nine compounds that reduced one or more vitality parameters of the parasitic flatworm *S. mansoni* of which compounds **2**, **5**, **9** and **11** are balancing cytotoxicity and anti-parasitic activity the best.

Author contributions

M. Kirchner performed the synthesis and analytics and prepared the draft. M. Marner performed the biological studies and prepared the draft. T. Kramer assisted with the synthesis and analytics. F. Mühlemeyer, J. Eichberg and M. Oberpaul assisted with the biological studies. S. Haeberlein assisted in preparing the draft and with the biological studies. R. Göttlich assisted in preparing the draft and administered the project.

Conflicts of interest

The authors declare no conflict of interest.

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3.4 Further Co-Authored Publications

1. Design, synthesis and antimycobacterial activity of imidazo[1,5-a]quinolines and their zinc-complexes, Michael Marner, Niclas Kulhanek, Johanna Eichberg, Kornelia Hardes, Michael Dal Molin, Jan Rybniker, Michael Kirchner, Till F. Schäberle and Richard Göttlich, *RSC Medicinal Chemistry* **2024**, 15, 1746-1750. (DOI: 10.1039/D4MD00086B)

2. Synthesis and Optical and Theoretical Characterization of Imidazo[1,5-a]isoquinolines and Imidazo[1,5-a]quinolines , Carina Rössiger, Thomas Oel, Pascal Schweitzer, Olesia Vasylets, Michael Kirchner, Ajlin Abdullahu, Derck Schlettwein and Richard Göttlich, *European Journal of Organic Chemistry* **2024**, e202400298. (DOI: 10.1002/ejoc.202400298)

3. Characterization and Optimization of the Photoluminescent Properties of Imidazo[1,5-a]quinolines, Niclas Kulhanek, Kateryna V. Borysova, Michael Kirchner, Klaus Müller-Buschbaum and Richard Göttlich, European Journal of Organic Chemistry **2024,** e202400783. (DOI: 10.1002/ejoc.202400783)

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