(Yang, H., et al. *ACS Chem. Biol.* **2016**, *11*, 1823−1826) has found that the structure of the macrocycle is important for good activity but activity can be retained with modifications to the linear tail. Analogues can be synthesised by firstly using solution-phase synthesis to make large quantities of the macrocycle, initially substituting arginine for the rare non-proteinogenic amino-acid enduracididine. Solid-phase synthesis is used to form heptapeptides to couple to the macrocycle to make a series of analogues, which can then be tested for activity.

Synthesis of enduracididine has also been carried out, allowing total synthesis using the same method as for analogues (Figure 1).

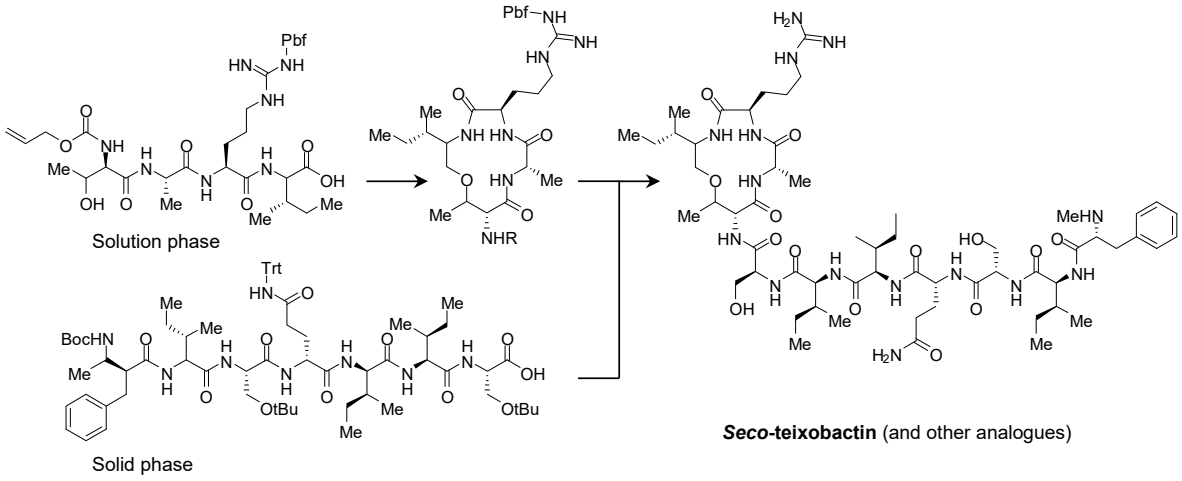

Figure 1. Outline of the synthesis of teixobactin analogues.

5.15. Probing Imidazotetrazine Prodrug Activation Mechanisms (P22)

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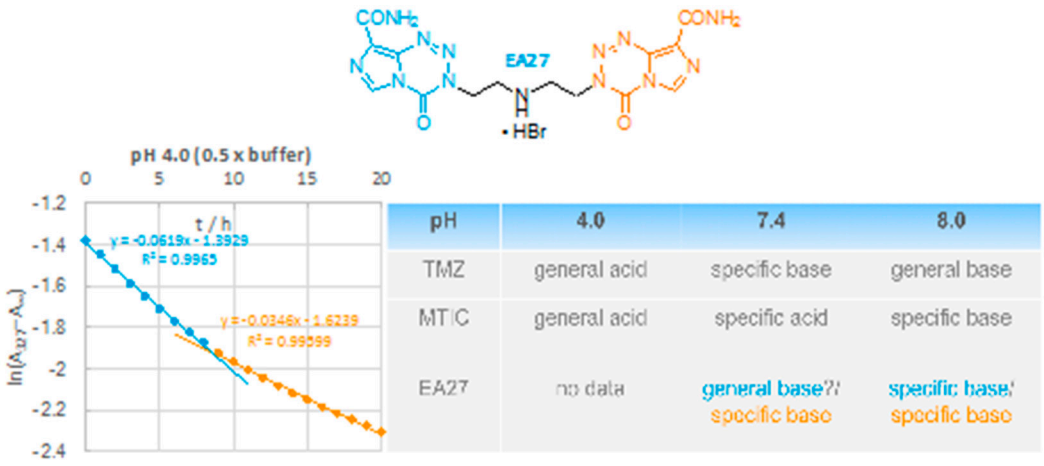
The archetypal prodrug of the imidazotetrazine class is the anticancer agent temozolomide (TMZ). The prodrug activation kinetics of TMZ show an unusual pH dependence: it is stable in acid and rapidly hydrolyses in alkali (Denny, B.J., et al. *Biochemistry* **1994**, *33*, 9045–9051). The incipient drug MTIC has the opposite properties—relatively stable in alkali but unstable in acid. In this study, the mechanism of prodrug activation was probed in greater detail to determine whether the reactions are specific or general acid or base catalysed. Three prodrugs and drugs were investigated, TMZ, MTIC and the novel dimeric imidazotetrazine EA27. Hydrolysis in a range of citrate-phosphate buffers (pH 8.0, 7.4, 4.0) was measured by UV spectrophotometry.

Reaction of TMZ and MTIC obeyed single-phase, pseudo-first order kinetics (Figure 1). EA27 was more complex, showing biphasic but approximately pseudo-first order kinetics, Figure. General acid or base catalysis indicates that protonation or deprotonation is the rate-limiting step (rls). In biological milieu, the nature and concentration of other acidic or basic solutes may affect the prodrug activation reaction. In contrast, specific acid or base catalysis indicates that protonation or deprotonation occurs before the rls, so catalysis depends only on the local concentration of hydroxide or hydronium ion (i.e., pH) so the reaction kinetics are not expected to change appreciably in a biological medium.



pH	4.0	7.4	8.0
TMZ	general acid	specific base	general base
MTIC	general acid	specific acid	specific base
EA27	no data	general base?/ specific base	specific base/ specific base

Figure 1. EA27 (top), example hydrolysis data (L) and the prodrug activation mechanisms determined (R).

The difference in reactivity between the tetrazines of the symmetrical dimer EA27 is surprising. This suggests a sequential mechanism where the hydrolysis of one imidazotetrazine slows the rate of the second within the same molecule, even though the two are FIVE atoms apart and not conjugated. The switch of mechanism (especially at pH 8) between TMZ and EA27 implies a role for the intramolecular secondary amine at the rate-limiting step.

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5.16. Mechanisms of Action of Silane-Substituted Anti-Cancer Imidazotetrazines (P23)

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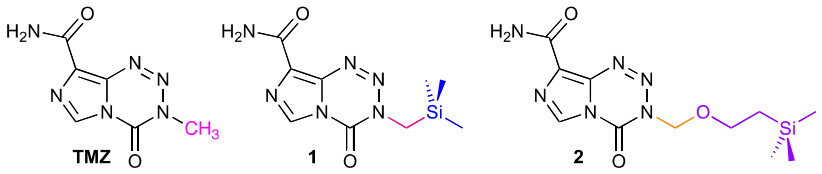
Silane-substituted imidazotetrazines **1**,**2** were investigated for their activity as anticancer prodrugs related to temozolomide (TMZ). The TMS-derivative **1** showed an activity profile against TMZ susceptible and resistant cell lines very similar to TMZ; in contrast, the SEM-derivative **2** showed activity irrespective of MGMT expression or MMR deficiency (Table).

Probing the prodrug activation mechanism by NMR kinetic studies determined that the TMS compound **1** follows a reaction pathway and time-course very similar to temozolomide. ^{1}H-NMR spectra of the reaction mixture showed considerable incorporation of deuterium into the final alkylation products of the reaction (methanol and methyl phosphate) as had previously been shown for temozolomide (Wheelhouse, R.T., et al. *Chem. Commun*. **1993**, *15*, 1177–1178). The SEM-derivative **2** reacted more rapidly than TMZ or TMS-derivative **1**. Somewhat surprisingly, the silane remained intact throughout the experiment and the observed reaction was the hydrolysis of the imidazo-tetrazine to ultimately release formaldehyde hydrate and 2-TMS-ethanol.

In conclusion, TMS-derivative **1** is a diazomethane precursor with prodrug activation mechanism, kinetics and anti-cancer activity in vitro similar to TMZ. In contrast, the SEM derivative **2** was more rapidly hydrolysed, a precursor of 2-TMS-ethanol and had activity in vitro different from TMZ. 2-TMS-ethanol was previously reported as a non-toxic compound in mice (Voronkov, M.G., et al. *Dokl. Akad. Nauk SSSR* **1976**, *229*, 1011–1013) and is known as a substrate for alcohol dehydrogenase (Zong, M.-H., et al. *Appl. Microbiol. Biotechnol*. **1991**, *36*, 40–43) and as a modest inhibitor of acetylcholinesterase (Aberman, A., et al. *Biochim. Biophys. Acta* **1984**, *791*, 278–280; Cohen, S.G., et al. *J. Med. Chem*. **1985**, *28*, 1309–1313).



Activity of TMZ, compounds **1** and **2** against a panel of TMZ susceptible and resistant cell lines

Compound	GI_{50} Value ± SD (μM)		
	U373 V	**U373 M**	**HCT 116**
TMZ	51.9 ± 7.4	302 ± 56	291 ± 4.9
1	61.0 ± 6.0	>500	233 ± 80
2	36.1 ± 2.9	29.1 ± 2.5	32.3 ± 10

6. Conclusions

The meeting was a success in its primary aims of bringing young researchers together to exchange scientific ideas and experiences. New collaborations, formal and informal, were forged. Reflecting the high standard of oral and poster presentations, prizes were awarded to young medicinal chemists. Dr. Amit Nathubhai (University of Bath, Bath, UK) received the award for the best submitted oral presentation, Isabelle Lengers (University of Münster, Münster, Germany) for the best poster with flash oral presentation (sponsored by the Society of Chemical Industry Young Chemists Panel, London, UK). Mike Kenny (University of Bath, Bath, UK) and Alexia Ville (Université d'Angers, Angers, France) received awards for best posters (Sponsored by the Royal Society of Chemistry Liverpool Local Section, Liverpool, UK).

The 26th Annual Meeting of GP2A is scheduled for 13–15 June 2018 in Asnelles-sur-Mer (Normandie, France), as a joint meeting with the 32nd Journées Franco-Belges de Pharmacochimie.

With the election of a new President and new Committee for GP2A, the organisation looks forward to developing and expanding its activities, fostering greater collaborations between member laboratories and expanding into more countries in Western Europe.

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Conflicts of Interest: The authors declare no conflict of interest.