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2-NITROIMIDAZOL-5-YLMETHYL AS A POTENTIAL BIOREDUCTIVELY ACTIVATED PRODRUG SYSTEM: REDUCTIVELY TRIGGERED RELEASE OF THE PARP INHIBITOR 5-BROMOISOQUINOLINONE

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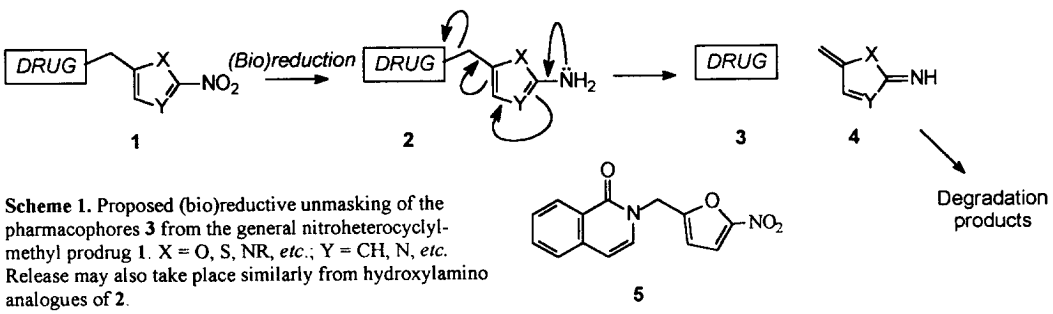
Abstract: 5-Chloromethyl-1-methyl-2-nitroimidazole reacted efficiently with the anion derived from 5-bromo-isoquinolin-1-one to give 5-bromo-2-((1-methyl-2-nitroimidazol-5-yl)methyl)isoquinolin-1-one. Biomimetic reduction effected release of the 5-bromoisoquinolin-1-one. The 2-nitroimidazol-5-ylmethyl unit thus has potential for development as a general prodrug system for selective drug delivery to hypoxic tissues.

Introduction

Owing to the primitive state of the tumour vasculature, most solid tumours have regions with acute or chronic hypoxia[1,2]. In these hypoxic tissues, viable cells are relatively resistant to radiotherapy and to many chemotherapeutic strategies[1,2]. Much effort has been expended on development of bioreductively activated cytotoxins for selective therapy of this tissue and of various prodrugs to deliver drugs selectively to tumours[3-6]. 1-Substituted-2-nitroimidazoles are selectively retained in hypoxic tumour tissue by reductive metabolism[7-9]. It is only recently that attention has been focussed on exploiting the physiological difference in concentration of O_2 between normal and hypoxic tumour tissue by design of biologically inactive prodrug systems which, upon selective bioreduction in hypoxic tissue, would *release* known therapeutic drugs only in that tissue. This would improve greatly the selectivity of biodistribution of such agents. Denny has described[10,11] such prodrugs as comprising Trigger, Linker and Effector



Scheme 1. Proposed (bio)reductive unmasking of the pharmacophores **3** from the general nitroheterocyclylmethyl prodrug **1**. X = O, S, NR, *etc.*; Y = CH, N, *etc.* Release may also take place similarly from hydroxylamino analogues of **2**.

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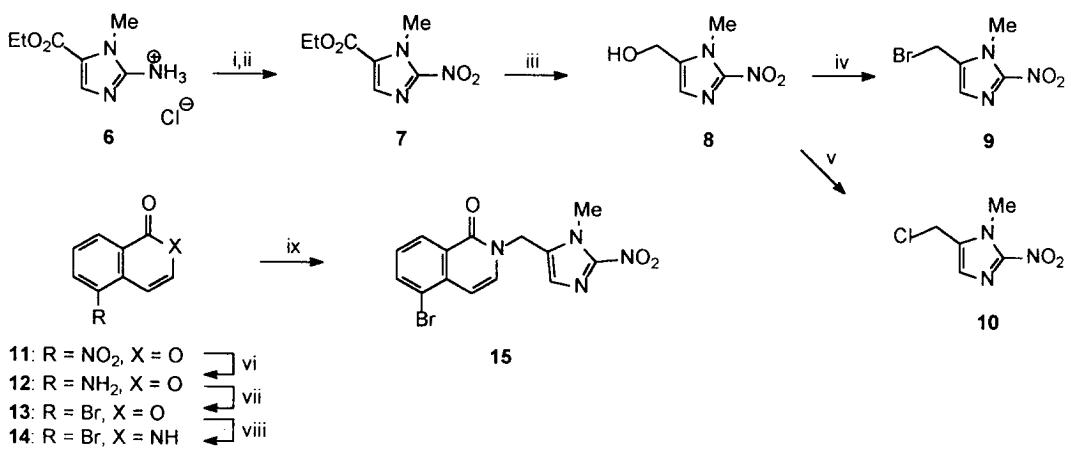
units. In our previous papers[12,13], we reported a potential general reductively activated prodrug system for delivery of isoquinolinones (*e.g.* **5**), amines and diols, in which 2-nitrofuran was used as the redox-sensitive Trigger. Others have investigated indolequinones in this way[14,15]. The proposed mechanism for reductively triggered drug release is shown in Scheme 1 (X = O, Y = CH). The redox potentials of 2-nitrofurans are relatively high (E^1_7 = -325 mV for a 5-nitrofuran-3-carboxamide)[4] for this application; those of 2-nitroimidazoles are more appropriate for selective bioreduction in hypoxic tumour tissue (E^1_7 = -389 mV for 1-alkyl-2-nitroimidazoles)[4]. We demonstrate here for the first time the potential of the 2-nitroimidazol-5-ylmethyl unit (Scheme 1: X = NR, Y = N) as a (bio)reductively cleaved masking group for a pharmacophore of importance as a poly(ADP-ribose)polymerase (PARP) inhibitor. Inhibition of PARP diminishes repair of DNA damaged by radiation; thus PARP inhibitors act as radiosensitisers.

Chemical Synthesis

The aminoimidazole ester **6** was diazotised and treated with nitrite ion in the presence of Cu to give the 2-nitroimidazole **7** in 53% yield (Scheme 2). The ester was reduced selectively by lithium borohydride, affording the nitroimidazole-methanol **8**[16]. In initial preparations of the target nitroimidazolylmethylisoquinolinone **15**, it was planned to use the corresponding 5-bromomethyl-2-nitroimidazole **9** as the alkylating electrophile; this was prepared[17] by Mitsunobu-like reaction of the alcohol **8** with Ph_3PBr_2 (prepared *in situ* from Ph_3P and Br_2). However, this proved to be less effective than the corresponding 5-chloromethyl-2-nitroimidazole **10**. This was synthesised[18] by the reaction of **8** with methanesulfonyl chloride in pyridine, mesylation of the alcohol being followed by displacement of the leaving group with chloride ion.



Scheme 2. Synthesis of the nitroimidazolylmethylisoquinolinone **15**. *Reagents and yields*: i, $NaNO_2$, aq. HBF_4; ii, $NaNO_2$, Cu, 53%; iii, $LiBH_4$, THF, 57%; iv, Ph_3PBr_2, DMF, 17%; v, MsCl, pyridine, 70%; vi, H_2, Pd/C, THF, 99%; vii, $NaNO_2$, aq. H_2SO_4, KBr, CuBr, 35%; viii, NH_3, $MeOCH_2CH_2OH$, Δ, 71%; ix, $LiN(SiMe_3)_2$, **10**, THF, DMF, 85%.

We have previously reported[12] the synthesis of the potent PARP inhibitor 5-bromoisoquinolinone **14** (IC_{50} < 270 nM)[19,20] by Curtius rearrangement of *E*-3-(2-bromophenyl)propenoic acid at 275°C and cyclisation of the intermediate isocyanate *in situ*. This process is not readily amenable to large-scale preparations of **14** and a new route to this isoquinolinone was developed. Catalytic hydrogenation of 5-nitroisocoumarin **11**[21] gave the aminoisocoumarin **12** in excellent yield[22]. This represented a considerable improvement over the procedure reported by Somei *et al.*[23] who used $TiCl_3$ as the reductant. Diazotisation and Sandmeyer reaction with bromide ion[24] afforded the previously unknown 5-bromoisocoumarin **13**. Treatment with ammonia in boiling 2-methoxyethanol[25] replaced the isocoumarin oxygen, giving **14**.

Isoquinolin-1-ones are readily deprotonated by strong non-nucleophilic bases and the resulting anions can be benzylated efficiently at nitrogen[12]. The anion of **14** was formed readily with lithium hexamethyldisilazide but the reaction with the bromomethylnitroimidazole **9** was low yielding. In contrast, reaction[26] with the corresponding chloromethylnitroimidazole **10** gave the target prodrug **15** in 85% yield.

Reductively Triggered Release

In our previous studies[12,13] of reductively activated release from 5-nitrofuran-2-ylmethyl prodrugs, two reductant systems were used to convert the nitrofuran to the aminofuran, mimicking bioreduction in hypoxic tissue. Both of these, sodium borohydride / palladium / aqueous methanol and tin (II) chloride were initially investigated as selective reductants for the nitro group of the prodrug **15**. HPLC[27] was used to follow the reduction and release processes, using UV detection at 326 nm (**8**: λ_{max} = 326 nm; **14**: λ_{max} = 297, 328 (weak) nm; **15**: λ_{max} = 297, 326 nm).

Treatment of **15** with excess $NaBH_4$ and palladium on carbon[28] in aqueous propan-2-ol caused complete consumption of **15** within 10 min, as demonstrated by HPLC[27]. A peak corresponding to the bromoisoquinolinone **14** was observed with retention time (RT) = 5.5 min, along with peaks at RT = 4.5 min and RT = 4.9 min. The HPLC trace after a reaction time of 2.5 h was similar. When, as a control, the bromoisoquinolinone **14** was treated with the $NaBH_4$ / Pd / aq. Pr^{i}OH system, it was converted almost quantitatively to the peak with RT = 4.9 min, indicating that this was a product of reduction of the delivered drug **14**. This material was shown by HPLC comparison and by NMR to be the 5-unsubstituted isoquinolinone **17**. Interestingly, a similar, although less efficient, reductive debromination of **14** was observed on treatment with Pd/C alone in aq. Pr^{i}OH; this reduction may be effected by H_2 adsorbed onto the metal surface during manufacture. Thus, although **17** is released cleanly from **15** by this biomimetic reduction system, this system also carries out further (but non-biomimetic) degradation of the "delivered drug" **14**. However, these studies suggested that **14** had been released upon reduction of **15**.

Reduction of the nitro group of **15** with tin (II) chloride[29] was complete in less than 5 min, as shown by HPLC[27]. Six new peaks were observed, mostly at shorter HPLC retention times (*i.e.* more polar). Similar patterns of peaks were observed after reaction times of 1 h and 2 h. However, no peak corresponding to **14** was present at any of these reaction times. A control experiment, treatment of **14** with $SnCl_2$, indicated that this material was completely unaffected by the reagent. Thus it is likely that the nitroimidazole has been reduced to the aminoimidazole **16a** (or the hydroxylaminoimidazole **16b**) by the $SnCl_2$ but that the tin has prevented release by complexation as a Lewis acid.

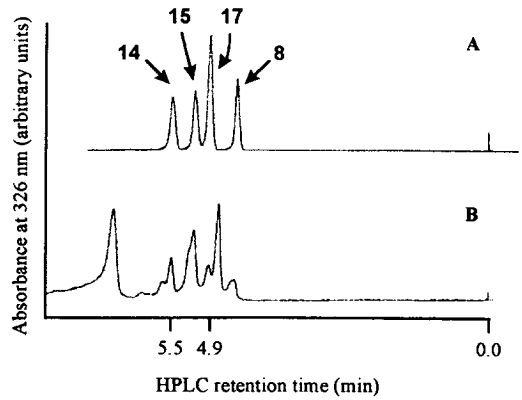


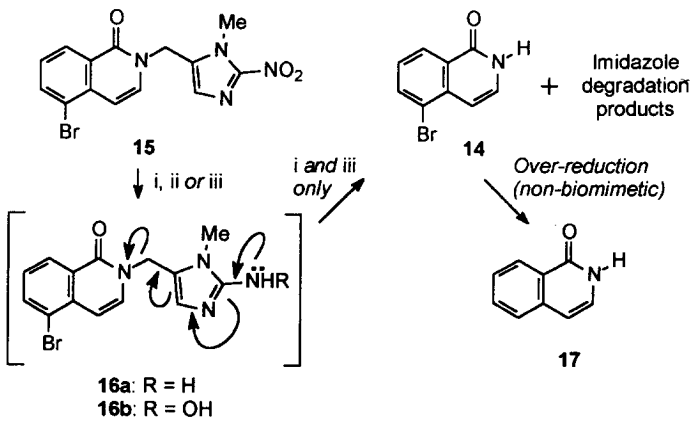
Figure 1. Typical HPLC chromatograms from the reductively triggered drug release study.
A: Synthetic standards; B: **15** + Zn / NH_4Cl (24 h).

Varghese and Whitmore[30] have used a zinc / ammonium chloride system as a mimic for bioreduction of misonidazole (1-(2-hydroxy-3-methoxypropyl)-2-nitroimidazole), noting that it produced both the aminoimidazole and the hydroxylaminoimidazole. This is appropriate for the present study, since both the imidazole-NH_2 and the imidazole-NHOH are likely products of bioreduction of the nitroimidazolylmethyl prodrugs. Control experiments showed that **15** was unaffected by zinc alone and by ammonium chloride alone. The "delivered drug" **14** was reduced only slowly by the Zn / NH_4Cl system, giving small amounts of **17** (HPLC[27] RT = 4.9 min) and very minor amounts of other materials. Varghese and Whitmore[30] noted that reduction of misonidazole was complete in 17 min. Treatment of **15** with the Zn / NH_4Cl system[31] showed consumption of this prodrug after 20 min, with release of a small quantity of drug **14**. Major peaks were observed at short retention times, probably due to reduction products of **15** from which the bromoisoquinolinone had not yet been released. Release of **14** was greater at 1 h. After 1 d and 2 d, peaks corresponding to **14** and its known degradation products (including **17**) were strongly evident, along with peaks corresponding to a degradation product of the reduced nitroimidazole unit (Figure 1). The provenance of the latter was shown by treatment of an appropriate control nitroimidazole (the alcohol **8**) with the Zn / NH_4Cl system. Figure 1 shows a typical chromatogram from this Zn / NH_4Cl reductively triggered release study.

Conclusions

In this *Letter*, we have described the synthesis of a potential prodrug **15** of 5-bromoisoquinolin-1-one **14**, a potent inhibitor of poly(ADP-ribose)polymerase and thus of DNA repair. In this prodrug, the critical secondary amide pharmacophore is masked with 2-nitroimidazol-5-ylmethyl. Release of the drug **14** has been demonstrated in two chemical systems which mimic bioreduction. Scheme 3 shows the proposed mechanism for this release. Reduction of

the nitroimidazole gives the corresponding amine **16a** and / or the hydroxylamine **16b**. Now the increased electron-density can cause expulsion of the leaving group (the bromoisoquinolinone **14**), according to the electron flow shown. We believe that this is the first literature report of a 2-nitroimidazole, a heterocycle which is known to be bioreduced selectively in hypoxic tumour tissue, to be used in this way.



Scheme 3. Reductive release of 5-bromoisoquinolinone **14** from the prodrug **15**. *Reagents*: i, $NaBH_4$, Pd/C, Pr^iOH, H_2O; ii, $SnCl_2$, MeOH; iii, Zn, NH_4Cl, MeOH.

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References and Notes

1. Vaupel, P.; Kallinowski, F.; Okunieff, P. *Cancer Res.* **1989**, *49*, 6449.
2. Okunieff, P.; Hoekel, M.; Dunphy, E. P.; Schlenger, K.; Knoop, C.; Vaupel, P. *Int. J. Radiat. Oncol. Biol. Phys.* **1993**, *26*, 631.
3. Kennedy, K. A.; Teicher, B. A.; Rockwell, S.; Sartorelli, A. C. *Biochem. Pharmacol.* **1980**, *29*, 1.
4. Naylor, M. A.; Stephens, M. A.; Cole, S.; Threadgill, M. D.; Stratford, I. J.; O'Neill, P.; Fielden, E. M.; Adams, G. E. *J. Med. Chem.* **1990**, *33*, 2508.
5. Jenkins, T. C.; Naylor, M. A.; O'Neill, P.; Threadgill, M. D.; Cole, S.; Stratford, I. J.; Adams, G. E.; Fielden, E. M.; Suto, M. J.; Steir, M. J. *J. Med. Chem.* **1990**, *33*, 2603.
6. Sinhababu, A. K.; Thakker, D. R. *Adv. Drug Delivery Rev.* **1996**, *19*, 241.
7. Maxwell, R. J.; Workman, P.; Griffiths, R. J. *Int. J. Radiat. Oncol. Biol. Phys.* **1989**, *16*, 925.
8. Wood, P. J.; Scobie, M.; Threadgill, M. D. *Int. J. Radiat. Biol.* **1996**, *70*, 587.
9. Swenson, D. H.; Laster, B. H.; Metzger, R. L. *J. Med. Chem.* **1996**, *39*, 1540.
10. Tercel, M.; Wilson, W. R.; Anderson, R. F.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 1084.
11. Denny, W. A.; Wilson, W. R.; Hay M. P. *Br. J. Cancer* **1996**, *74*, S32.
12. Berry, J. M.; Watson, C. Y.; Whish, W. J. D.; Threadgill, M. D. *J. Chem. Soc., Perkin Trans. 1*, **1997**, 1147.
13. Mahmud, N. P.; Garrett, S. W.; Threadgill, M. D. *Anti-Cancer Drug Design*, **1998**, *13*, 655.
14. Jaffar, M.; Everett, S. A.; Naylor, M. A.; Moore, S. G.; Ulhaq, S.; Patel, K. B.; Stratford, M. R. L.; Nolan J.; Wardman, P.; Stratford, I. J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 113.
15. Naylor, M. A.; Swann, E.; Everett, S. A.; Jaffar, M.; Nolan J.; Robertson, N.; Lockyer, S. D.; Patel, K. B.; Dennis, M. F.; Stratford, M. R. L.; Wardman, P.; Adams, G. E.; Moody, C. J.; Stratford, I. J. *J. Med. Chem.* **1998**, *41*, 2720.
16. Cavalleri, B.; Breccia, A.; Lancini, G. C. *J. Heterocycl. Chem.* **1972**, *9*, 979.
17. Br_2 (53 mg, 330 μmol) was added to **8** (50 mg, 320 μmol) and Ph_3P (90 mg, 343 μmol) in DMF (1 mL) and the mixture was stirred for 16 h. Evaporation and preparative TLC (EtOAc / hexane 1:1) gave **9** (12 mg, 17%), pale yellow crystals: mp 84-87°C (decomp.); NMR (400 MHz, $CDCl_3$) δ 3.98 (3 H, s, NMe), 4.41 (2 H, s, CH_2), 7.13 (1 H, s, imidazole 4-H); MS (EI) *m/z* 220.9562 (M) ($C_5H_6{}^{81}BrN_3O_2$ requires 220.9623), 218 (M) ($C_5H_6{}^{79}BrN_3O_2$ requires 218.9643), 140 (M - Br).

18. $MeSO_2Cl$ (50 mg, 480 μmol) was stirred with **8** (50 mg, 320 μmol) in pyridine (1.0 mL) for 3 h. The evaporation residue, in $CHCl_3$, was washed (aq. H_2SO_4, aq. $NaHCO_3$). Drying and evaporation gave **10** (39 mg, 70%), pale yellow solid: mp 94-96°C; NMR ($CDCl_3$) δ 4.00 (3 H, s, NMe), 4.55 (2 H, s, CH_2), 7.20 (1 H, s, imidazole 4-H); MS (EI) *m/z* 177.0131 (M) ($C_5H_6{}^{37}ClN_3O_2$ requires 177.0119), 175.0158 (M) ($C_5H_6{}^{35}ClN_3O_2$ requires 175.0149), 140 (M - Cl).
19. Watson, C. Y.; Whish, W. J. D.; Threadgill, M. D. *Bioorg. Med. Chem.* **1998**, *6*, 721.
20. Watson, C. Y. PhD Thesis, University of Bath, **1997**.
21. Matsui, T.; Sugiura, T.; Nakai, H.; Iguchi, S.; Shigeoka, S.; Takada, H.; Odagaki, Y.; Nagao, Y.; Ushio, Y.; Ohmoto, K.; Iwamura, H.; Yamazaki, S.; Arai, Y.; Kawamura, M. *J. Med. Chem.* **1992**, *35*, 3307.
22. 5-Nitroisocoumarin **11**[21] (2.98 g, 15.6 mmol) was treated with H_2 and Pd/C (10%, 370 mg) in THF (44 mL) and aq. HCl (2 M, 8 mL) for 6 h. Filtration (Celite®) and evaporation gave a residue which, in CH_2Cl_2, was washed (aq. $NaHCO_3$). Drying and evaporation gave **12** (2.49 g, 99%), yellow crystals: mp 185-187°C; (lit.[23] mp 194-195°C); NMR ($CDCl_3$) δ 4.00 (2 H, brs, NH_2), 6.44 (1 H, dd, J = 8.0, 0.5 Hz, 4-H), 7.02 (1 H, dd, J = 8.0, 1.2 Hz, 6-H), 7.26 (1 H, d, J = 8.0 Hz, 3-H), 7.32 (1 H, t, J = 8.0 Hz, 7-H), 7.76 (1 H, ddd, J = 8.0, 1.2, 0.5 Hz, 8-H).
23. Somei, M.; Karasawa, Y.; Shoda, T.; Kaneko, C. *Chem. Pharm. Bull.* **1981**, *29*, 249.
24. $NaNO_2$ (1.07 g, 15.5 mmol) in H_2O (5.0 mL) was added to **12** (2.49 g, 15.5 mmol) in aq. H_2SO_4 (2 M, 80 mL) at < 5°C. KBr (3.65 g, 31 mmol) and CuBr (4.41 g, 31 mmol) were added. The mixture was stirred for 2 h and was extracted (EtOAc). Evaporation, chromatography (EtOAc / hexane 1:6) and recrystallisation (EtOAc / hexane) gave **13** (1.22 g, 35%), white crystals: mp 113-115°C; NMR ($CDCl_3$) δ 6.87 (1 H, dd, J = 6.0, 0.7 Hz, 4-H), 7.23 (1 H, d, J = 6.0 Hz, 3-H), 7.39 (1 H, t, J = 7.9 Hz, 7-H), 7.95 (1 H, dd, J = 7.9, 1.1 Hz, 6-H), 8.29 (1 H, ddd, J = 7.9, 1.1, 0.7 Hz, 8-H); MS (FAB) *m/z* 226.9534 (M) ($C_9H_5{}^{81}BrO_2$ requires 226.9531), 224.9551 (M) ($C_9H_5{}^{79}BrO_2$ requires 224.9551). Analysis: C, 48.0; H, 2.26. $C_9H_5BrO_2$ requires C, 48.04; H, 2.24%.
25. Compound **13** (601 mg, 2.7 mmol) was boiled under reflux in 2-methoxyethanol (50 mL) saturated with NH_3 for 8 h. Evaporation and recrystallisation (MeCN) gave **14** (428 mg, 71%), white crystals: mp 220-222°C (lit.[12] mp 242-244°C); NMR ($(CD_3)_2SO$) δ 6.66 (1 H, d, J = 7.9 Hz, 4-H), 7.35 (1 H, dd, J = 8.1, 7.7 Hz, 7-H), 7.42 (1 H, d, J = 7.9 Hz, 3-H), 8.03 (1 H, d, J = 7.7 Hz, 6-H), 8.21 (1 H, d, J = 8.1 Hz, 6-H), 11.55 (1 H, brs, NH).
26. $LiN(SiMe_3)_2$ (1.0 M in THF, 340 μL, 340 μmol) was stirred with **14** (58 mg, 260 μmol) in DMF (1.0 mL) for 2 h. **10** (37 mg, 210 μmol) in DMF (1.0 mL) and NaI (2 mg) were added and the mixture was stirred for 2 d. The evaporation residue, in EtOAc, was washed (H_2O, brine). Drying, evaporation and chromatography (EtOAc / hexane 1:2) gave **15** (66 mg, 85%), pale yellow solid: mp 208-210°C; NMR ($CDCl_3$) δ 3.99 (3 H, s, NMe), 5.23 (2 H, s, CH_2), 6.93 (1 H, dd, J = 8.4, 0.5 Hz, isoquinoline 4-H), 7.14 (1 H, d, J = 8.4 Hz, isoquinoline 3-H), 7.23 (1 H, s, imidazole 4-H), 7.38 (1 H, t, J = 8.1 Hz, isoquinoline 7-H), 7.93 (1 H, dd, J = 8.1, 1.1 Hz, isoquinoline 6-H), 8.39 (1 H, ddd, J = 8.2, 1.1, 0.5 Hz, isoquinoline 8-H); MS (EI) *m/z* 363.9983 (M) ($^{12}C_{14}H_{11}{}^{81}BrN_4O_3$ requires 363.9994), 361.9999 (M) ($^{12}C_{14}H_{11}{}^{79}BrN_4O_3$ requires 362.0015), 347, 345, 318, 316, 140; Found: C, 46.4; H, 3.09; N, 15.0. $C_{14}H_{11}BrN_4O_3$ requires C, 46.30; H, 3.05; N, 15.43%.
27. HPLC analysis was performed with a Kromasil 10C18 semi-preparative column and a JASCO PU-986 preparative pump using methanol as eluant with flow rate 5 mL min^{-1} with UV detection at 326 nm by a JASCO UV-975 detector. An injection volume of 20 μL was used.
28. $NaBH_4$ (2 mg) was stirred with the substrate (**8**, **14**, or **15**) (5 mg) and Pd / C (10%, 5 mg) in Pr^iOH (2.0 mL) and water (0.04 mL). At the time points, aliquots (100 μL) were removed, filtered (glass wool) and analysed by HPLC[27].
29. $SnCl_2$ (1.3 mg) was stirred with the substrate (**8**, **14**, or **15**) (0.5 mg) in MeOH (1.0 mL). At the time points, aliquots (100 μL) were removed, filtered (glass wool) and analysed by HPLC[27].
30. Varghese, A. J.; Whitmore, G. F. *Cancer Res. 1980*, **40**, 2165.
31. Zn dust (10 mg) was stirred with the substrate (**8**, **14**, or **15**) (0.5 mg) and NH_4Cl (0.5 mg) in MeOH (0.95 mL) and water (0.05 mL). At the time points, aliquots (100 μL) were removed, filtered (glass wool) and analysed by HPLC[27].