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Synthesis and evaluation of 9-aminoacridines derived from benzyne click chemistry

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ABSTRACT

A small set of 9-aminoacridine-3- and 4-carboxamides were synthesized efficiently using the benzyne/azide click chemistry. The products bind to duplex DNA but have different antitumour activity in the HL60 cell line.

DNA intercalators have found great utility as antitumour agents, from natural products such as doxorubicin and actinomycin D to synthetic agents such as mitoxantrone and amsacrine.[1] Amsacrine (**1**) is an acridine that binds to DNA and inhibits the enzyme topoisomerase II.[1] It has formed the prototype for a series of new agents that have had varying success in clinical development. Possibly of most interest are the threading agents.[2] These are intercalators in which one substituent is located in one groove of the DNA and another is threaded through the helix and resides in the other groove. Denny and Wakelin have carried out extensive studies of these agents, particularly modelled on the 9-aminoacridine-4-carboxamides, which have consistently been shown to have potent antitumour activity.[3] Their mode of action has been suggested to involve topoisomerase inhibition.[4] Crystallographic studies[5,6] have recently demonstrated that the acridines bind with the 9-amino substituent in the minor groove and the carboxamide in the major groove. Our own studies[7] of a bisintercalator that crosslinks duplexes showed that the 4-carboxamide makes H-bonding interactions with the O6 or N7 of G and that the 9-amino substituent protrudes from the minor groove.

Simple models of these interactions would suggest that a 9-aminoacridine-3-carboxamide would not interact so well with DNA, with the 3-substituent less favourably positioned within the groove. However, studies by Ferguson and co-workers have shown that acridine-3-carboxamides also have potential as antitumour agents.[8] Previous studies on these compounds suggested that although they bound to duplex DNA, they had no antitumour activity.[3] The more recent work showed that the antitumour activity is cell-line specific and that acridine-3-carboxamides such as **2** have topoisomerase inhibitory activity in pancreatic cell lines.[9] This work demonstrates that the acridine-carboxamides still have much to offer as potential therapeutic agents and herein, we describe the rapid synthesis of a small set of acridine compounds (Fig 1, compounds **3**–**6**) via a novel route involving benzyne click chemistry.

At its most basic, click chemistry involves simple routes to novel compounds using effective reactions with little or no chromatography.[10] Larock recently described the use of a benzyne generated in situ as one of the coupling partners in a click reaction with an azide.[11] This generates the benzotriazole structure, a fairly common motif in medicinal chemistry. This reaction has also been exploited by others,[12] most recently by Moses and co-workers in a further development of their aromatic azide-based click methodology.[13] In this case, the benzyne is generated in a similar manner to the original benzotriazole route, first described in 1964,[14] whereas in the Larock chemistry the same molecule is generated via CsF-mediated TMS-elimination.

Prior to investigation of the click reaction in the context of acridine chemistry, the synthesis of the set of substrates would require formation of the 9-(2-azidoethyl)aminoacridine-4-carboxamide from the 9-chloroacridine and azidoethylamine. 2-Azidoethylamine was originally accessed in the literature via heating of 2-bromoethylamine with sodium azide[15] so it seemed likely that the molecule was relatively stable (although care should be taken when handling low molecular weight azides and this led us to seek an alternative, milder approach to the synthesis). Commercially available Boc-ethanolamine **7** was converted to the mesylate **8**,

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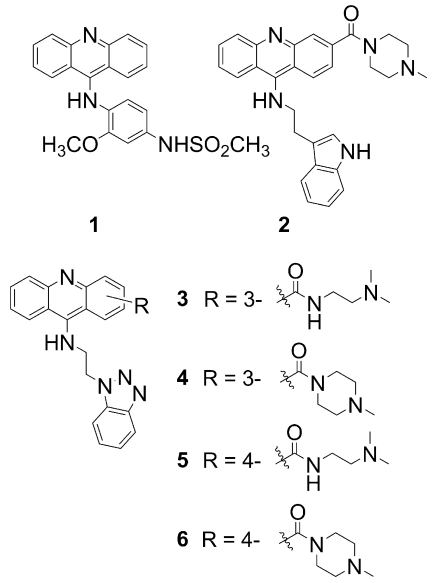
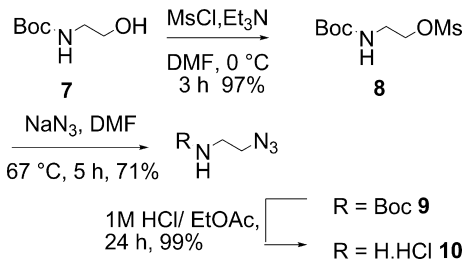


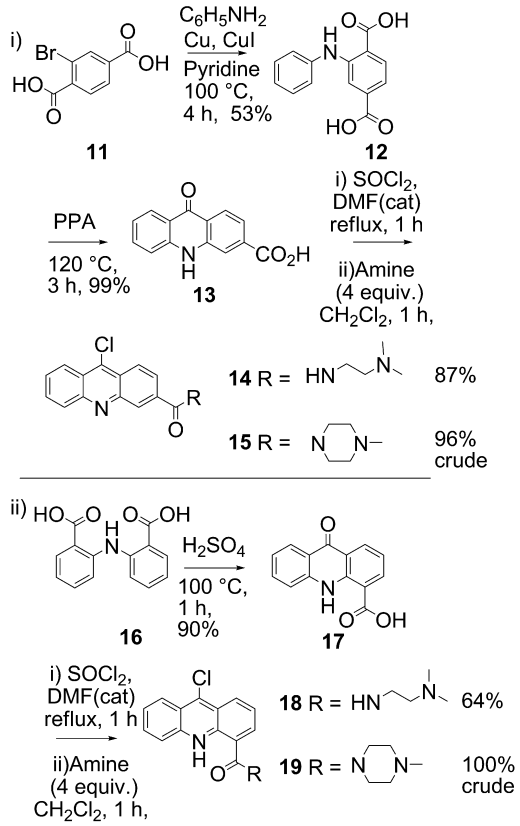
Figure 1. Compound **2** has been shown to have activity against topoisomerase I. Compounds **3**–**6** were synthesised in this study.

which then smoothly underwent direct substitution with sodium azide to give the Boc-protected azide **9**. Removal of the amine protecting group under standard anhydrous conditions gave the azidoethylamine as the hydrochloride salt **10** (Scheme 1).

9-Aminoacridine carboxamides substituted in the 3- and 4-position can be readily accessed via reported methods (Scheme 2).[3,16] For example, aniline reacted with 2-bromoterephthalic acid **11** under Jourdan–Ullmann conditions (Cu, CuI, Py, 100 °C, 53%) to give 2-phenylamino terephthalic acid **12** which was cyclised in essentially quantitative yield to give 9(10*H*)-acridone-3-carboxylic acid **13** (PPA, 120 °C, 99%). Conversion to the 9-chloroacridine-3-carboxamides **14** and **15** by treatment with thionyl chloride under reflux followed by rapid addition of an excess of the required amine in dichloromethane yielded the desired products (Scheme 2). Compound **14** was purified by column chromatography whereas **15** has been reported in the literature to be unstable, a property confirmed in our hands and as such was used in the next step without further purification.[16] For the 4-carboxamides, cyclisation of the commercially available 2,2′-iminodibenzoic acid **16** with sulfuric acid gave acridone-4-carboxylic acid **17** which was then converted to 9-chloroacridine-4-carbonyl chloride using thionyl chloride with catalytic DMF. Selective reaction of the acid chloride involving rapid addition of the required amine along with triethylamine in cold CH_2Cl_2 gives the target carboxamides **18** and **19**. Compound **19** again proved to be unstable and was used without further purification. The amines to form the carboxamide were selected on the basis of previous work. Thus the 2-(dimethylamino)ethylamine side chain has previously been used extensively by us and others and



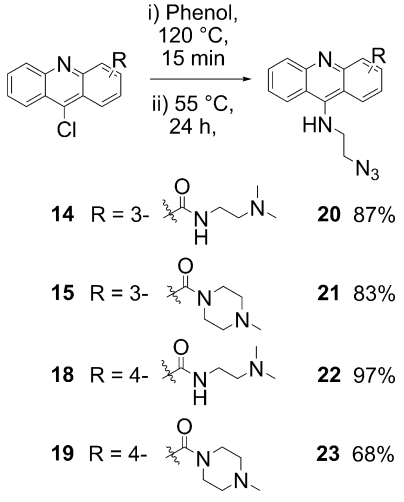
Scheme 1. Synthesis of 2-azidoethylamine **10**.



Scheme 2. Synthesis of (i) 9-chloroacridine-4-carboxamides and (ii) 3-carboxamides.

consistently generates compounds with good cytotoxicity and DNA binding characteristics. The piperazine sidechain was used by Ferguson and co-workers and had good activity in a cell line selective fashion.

The substrates for the click reaction were generated through an in situ reaction with the 9-phenoxyacridine, generated by, for example, heating **14** to 110 °C in phenol for 15 min (Scheme 3). Cooling to 55 °C was followed by addition of **10** and stirring at this temperature for 24 h. Compound **20** was obtained in excellent yield as the hydrochloride salt following purification and the structure



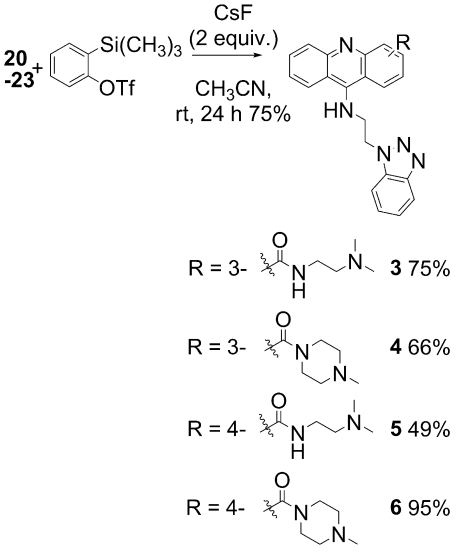
Scheme 3. Synthesis of 9-(2-azidoethyl)aminoacridine carboxamides.

was verified by ^{1}H NMR, IR and high resolution mass spectrometry. Compounds **21**–**23** were prepared in a similar fashion from the respective phenoxyacridines in good to excellent yield.[17]

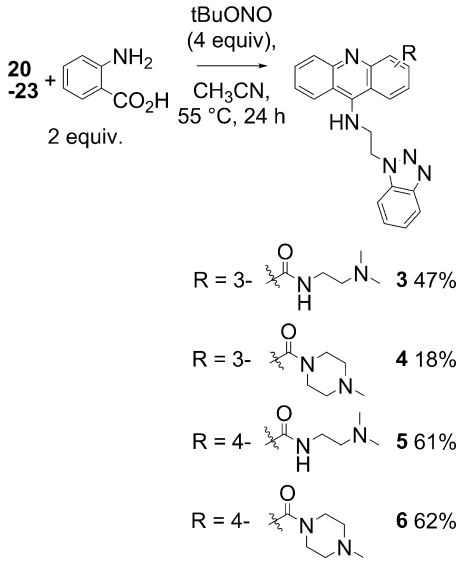
Initial studies focussed on the Larock click chemistry involving reaction of *o*-(trimethylsilyl)phenyltriflate with CsF to generate the benzyne in situ, which then reacts directly with the azide. The optimum conditions described by the original authors involved stirring at room temperature in CH_3CN and reaction times of 18–24 h. Gratifyingly, all of the azide substrates reacted under these conditions to give the benzotriazole products **3–6** in good yield (Scheme 4). All of the compounds generated were extremely polar and purification by column chromatography under normal phase conditions was not possible. However, it was found that basification of the reaction mixture with $NaHCO_3$, followed by extraction into CH_2Cl_2 and removal of solvent gave an oil that could then be converted into the HCl salt (1.25 M HCl/CH_3OH), which was found to be >90% pure as assessed by proton NMR. This purification of the target compounds led us to discount the similar Feringa methodology[12] for this reaction, which uses an excess of one of the substrates and would probably require further purification, although this later proved not to be a problem with the Moses chemistry (see below).

The use of anthranilic acid as a starting material for the synthesis was also attractive, as the number of substituted analogues of these compounds that are commercially available or easily accessed is greater than that for the *o*-(trimethylsilyl)phenyltriflates. With this in mind, the Moses methodology for synthesis of similar compounds was also briefly investigated (Scheme 5). The optimised conditions for this reaction involved heating to reflux in acetonitrile for 15 h with 2 equiv of anthranilic acid and 2 equiv of *t*-BuONO.[13] In fact, under these conditions with our substrates, excessive decomposition was observed. The reaction failed to progress at room temperature. Ultimately, we found that the use of 2 equiv of the anthranilic acid and four of *t*-BuONO gave the required products in fair to good yields, although in the case of **4** there was a substantial decrease in product obtained. Work-up was exactly as for the Larock conditions, basification and extraction into CH_2Cl_2 and gave products of similar purity on acidification.

Compounds **3–6** were assessed initially for their antitumour activity against the human leukaemia cell line HL60 and the results are shown in Table 1. None of the click chemistry products had antitumour activity higher than the parent azide **20**–**23** and the piperidine side chain (compounds **4**, **6**, **21**, **23**) clearly leads to a



Scheme 4. Larock click chemistry to generate compounds **3–6**.[18]



Scheme 5. Moses click chemistry to generate the compounds **3–6**.

Table 1
Cytotoxicity and DNA binding constants for compounds **3–6** and **16–19**

Compounds	Cytotoxicity IC_{50}[a] (μM)	C_{50} (μM)[b]	Log K_{app}
3	262.4	12.1	6.54
4	na	26.2	6.20
5	23.4	30.0	6.14
6	na	93.2	5.65
20	5.4	17.6	6.37
21	45.7	>200	—
22	0.72	13.6	6.49
23	52.0	>200	—

[a] Carried out in HL60 cell line (na = not active).
[b] Competitive ethidium bromide displacement assay.

further decrease in antitumour activity. This decrease in antitumour activity could be a consequence of a decrease in DNA binding affinity. Initially, we assessed the relative binding affinity of the various agents via an ethidium displacement assay at a single concentration (2 μM) for each agent. None of the click compounds **3–6** showed any appreciable displacement at this concentration, whereas the 9-(2-azidoethyl)aminoacridine-4-carboxamides **20–23** all had significant effects. Titration of the agents against ethidium bromide gave C_{50} values and K_{app} values as shown in Table 1.[19]

The antitumour activity of these compounds against HL60 cells is clearly not directly related to their DNA binding affinity, as compounds with similar log K_{app} values varied from low micromolar activity to inactive. Interestingly, it is clear from these results that a change in carboxamide substitution pattern from the 3- to the 4-position has little effect on DNA binding affinity and that the structure of the side chain has a much greater impact. For three of the four piperidine compounds (**6**, **21**, **23**) there is a significant decrease in DNA binding affinity, with no binding detected for the azide derivatives **21** and **23**. However, these same compounds maintain antitumour activity, whereas **4** and **6**, which have measurable binding affinity, have no antitumour effect in HL60 cells. This suggests that either DNA binding is not required for biological activity of acridine carboxamides or that the compounds with different side chains are acting through different mechanisms.

As the acridines are intrinsically fluorescent, we briefly and qualitatively investigated their uptake in HL60 cells to see if this

may have affected their antitumour activity (data not shown). All compounds were taken up by the cells and this is unlikely to explain the total lack of activity of the 3-carboxamides. If the acridine-4-carboxamides are inhibitors of topoisomerase II, as has previously been reported, then it seems likely that the piperidine substituted analogues are unable to exert an effect on this enzyme, at least in these cell lines.

In summary, benzyne click chemistry is an important addition to the arsenal of reactions available to the medicinal chemist. We have shown that it can be applied to the synthesis of threading intercalators that bind to DNA with reasonable affinity and have up to low micromolar antitumour activity. The reasons for differences in the activity of the different compounds are still under investigation but do not seem to correlate with DNA binding affinity or cellular uptake. This may suggest that these compounds vary in their ability to inhibit topoisomerases, as described by Ferguson and co-workers, and we are currently investigating this potential mode of action. This is also the first description of the azide-substituted threading intercalators and these compounds are currently being studied for their ability to undergo click reactions under the usual Sharpless conditions.

Acknowledgements

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17. Compound **12**: ν_{max}/cm^{-1} 3275 (NH), 2936 (CH_2/CH_3), 2819 (CH_2/CH_3), 2094 (N_3), 1643 (CO). δ_H (300 MHz; CDCl,) 8.32 (1H, d, J = 1.5 Hz, H-4), 8.13 (2H, t, J = 9.3 Hz, H-1 + H-8), 7.93 (1H, d, J = 8.7 Hz, H-5), 7.78 (1H, dd, J_1 = 1.8 Hz, J_2 = 9.0 Hz, H-2), 7.69–7.63 (1H, m, H-6), 7.12–7.36 (2H, m, H-7 + NH), 3.95 (2H, t, J = 5.4 Hz, CH_2N_3), 3.65–3.55 (4H, m, CONHCH_2 + NHCH_2), 2.56 (2H, t, J = 5.9 Hz, CH_2N(CH_3)$_2$), 2.29 (6H, s, N(CH_3)$_2$). m/z (ES+) 378.2035 $[M+H]^+$ $C_{20}H_{24}N_7O$ requires 378.2037; compound **13**: ν_{max}/cm^{-1} 3273 (NH), 2935 (CH_2/CH_3), 2790 (CH_2/CH_3), 2095 (N_3), 1628 (CO). δ_H (300 MHz; $CDCl_3$) 8.13 (1H, d, J = 9.0 Hz, H-8), 8.09 (1H, d, J = 9.0 Hz, H-1), 7.89 (2H, br s, H-4 + H-5), 7.62 (1H, t, J = 7.5 Hz, H-6), 7.37–7.30 (2H, m, H-7 + H-2), 3.92 (2H, t, J = 5.6 Hz, CH_2N_3), 3.83 (2H, br s, $CONCH_2$), 3.59 (2H, t, J = 5.6 Hz, $NHCH_2$), 3.51 (2H, br s, $CONCH_2$), 2.49 (2H, br s, C$H_2$$NCH_3$), 2.35 (2H, br s, C$H_2$$NCH_3$), 2.30 (3H, s, NCH_3). m/z (ES+) 390.2035 $[M+H]^+$ $C_{21}H_{24}N_7O$ requires 390.2037; compound **14**: ν_{max}/cm^{-1} 3313 (NH), 2935 (CH_2/CH_3), 2856 (CH_2/CH_3), 2095 (N_3), 1638 (CO). δ_H (300 MHz; MeOD) 8.54 (1H, d, J = 6.6 Hz, H-1), 8.36 (1H, dd, J_1 = 1.4 Hz, J_2 = 8.7 Hz, H-3), 8.21 (1H, d, J = 8.7 Hz, H-8), 7.87 (1H, d, J = 8.1 Hz, H-5), 7.66 (1H, t, J = 8.4 Hz, H-6), 7.37–7.32 (2H, m, H-2 + H-7), 3.92 (2H, t, J = 6.3 Hz, CH_2N_3), 3.69 (2H, t J = 6.3 Hz, CONHCH_2), 3.60 (2H, t, J = 5.7 Hz, $NHCH_2$), 2.70 (2H, t, J = 6.5 Hz, CH_2N(CH_3)$_2$) 2.39 (6H, s, N(CH_3)$_2$). m/z (ES+) 378.2039 $[M+H]^+$ $C_{20}H_{24}N_7O$ requires 378.2037; compound **15**: ν_{max}/cm^{-1} 3323 (NH), 2936 (CH_2/CH_3), 2799 (CH_2/CH_3), 2096 (N_3), 1608 (CO). δ_H (500 MHz; CH_3OD) 8.34 (1H, dd, J_1 = 1.0 Hz, J_2 = 8.5 Hz, H-1), 8.24 (1H, d, J = 8.5 Hz, H-8), 7.94 (1H, d, J = 8.5 Hz, H-5), 7.65–7.61 (2H, m, H-3 + H-6), 7.37–7.32 (2H, m, H-2 + H-7), 3.96 (2H, br s, $CONCH_2$), 3.92 (2H, t, J = 5.5 Hz, CH_2N_3), 3.58 (2H, t, J = 5.5, $NHCH_2$), 3.20 (2H, br, s, $CONCH_2$), 2.65 (2H, br s, CH_2NH), 2.29 (5H, s, NCH_3 + CH_2NH). m/z (ES+) 390.2032 $[M+H]^+$ $C_{21}H_{24}N_7O$ requires 390.2037.
18. Compound **3**: ν_{max} /cm^{-1} 3253 (NH), 3034 (CH), 2935 (CH_2/CH_3), 1637 (CO). δ_H (500 MHz; MeOD) 8.57 (1H, d, J = 9.0 Hz, H-1), 8.54 (1H, d, J = 8.5 Hz, H-5), 8.15 (1H, d, J = 1.0 Hz, H-4), 8.01 (1H, t, J = 7.5 Hz, H-7), 7.96 (2H, d, J = 8.5 Hz, benzyl), 7.87 (1H, d, J = 9.0 Hz, H-8), 7.73–7.71 (1H, m, H-2), 7.64–7.59 (2H, m, H-6 + benzyl), 7.49 (1H, app q, J = 7.5 Hz, benzyl), 4.36 (2H, t, J = 5.5 Hz, CH_2N_3), 4.31 (2H, t, J = 6.3 Hz, CH_2N(CH_3)$_2$), 3.96 (2H, t, J = 5.5 Hz, $NHCH_2$), 3.78 (6H, s, N(CH_3)$_2$), 3.69 (2H, t, J = 6.3 Hz, CONHCH_2). m/z (ES+) 454.2345 $[M+H]^+$ $C_{26}H_{28}N_7O$ requires 454.2350; compound **4**: ν_{max}/cm^{-1} 3349 (NH), 3024 (CH), 2937 (CH_2/CH_3), 2915 (CH_2/CH_3), 1635 (CO). δ_H (500 MHz; MeOD) 8.66 (1H, d, J = 8.5 Hz, H-1), 8.56 (1H, d, J = 8.5 Hz, H-5), 8.04–8.01 (1H, m, H-7), 8.00 (1H, d, J = 1.0 Hz, H-4), 7.96 (2H, d, J = 8.0 Hz, benzyl), 7.89 (1H, d, J = 8.5 Hz, H-8), 7.74 (1H, t, J = 7.8 Hz, benzyl), 7.68 (1H, d, J = 7.0 Hz, benzyl), 7.66–7.63 (2H, m, H-2 + H-6), 4.68 (2H, br s, CH_2), 4.58 (2H, br s, CH_2), 4.38 (2H, t, J = 5.5 Hz, CH_2N_3), 4.22 (2H, br s, CH_2), 3.97 (2H, t, J = 5.5 Hz, $NHCH_2$), 3.74 (2H, br s, CH_2), 3.63 (3H, s, NCH_3). m/z (ES+) 466.2347 $[M+H]^+$ $C_{27}H_{28}N_7O$ requires 466.2350; compound **5**: ν_{max}/cm^{-1} 3359 (NH), 3039 (CH), 2935 (CH_2/CH_3), 1621 (CO). δ_H (500 MHz; MeOD) 8.71 (1H, d, J = 8.0 Hz, H-1), 8.55 (1H, d, J = 8.5 Hz, H-5), 8.19 (1H, d, J = 7.5 Hz, H-3), 8.04 (1H, t, J = 7.8 Hz, H-7), 7.99 (2H, d, J = 8.5 Hz, benzyl), 7.94 (1H, d, J = 8.5 Hz, H-8), 7.64 (1H, t, J = 7.5 Hz, H-6)), 7.58–7.53 (2H, m, H-2 + benzyl), 7.41 (1H, t, J = 7.5 Hz, benzyl), 4.39–4.34 (4H, m, CH_2N(CH_3)$_2$ + CH_2N_3), 3.97 (2H, t, J = 5.0 Hz, $NHCH_2$), 3.80 (6H, s, N(CH_3)$_2$), 3.75 (2H, t, J = 6.0 Hz, CONHCH_2). m/z (ES+) 454.2354 $[M+H]^+$ $C_{26}H_{28}N_7O$ requires 454.2350; compound **6**: ν_{max}/cm^{-1} 3358 (NH), 3020 (CH), 2987 (CH_2/CH_3), 2945 (CH_2/CH_3), 1630 (CO). δ_H (500 MHz; MeOD) 8.63 (1H, d, J = 8.0 Hz, H-1), 8.51 (1H, d, J = 8.5 Hz, H-5), 8.04 (2H, br d, J = 8.0 Hz, H-8 + H-3), 7.98–7.93 (3H, m, H-7 + benzyl), 7.74 (1H, t, J = 7.5 Hz, benzyl), 7.66 (1H, t, J = 7.0 Hz, benzyl), 7.61–7.56 (2H, m, H-6 + H-2), 4.67 (2H, br s, CH_2), 4.54 (2H, br s, CH_2), 4.35 (2H, s, CH_2N_3), 4.23 (2H, br s, CH_2), 4.08 (2H, br s, CH_2), 3.97 (2H, s, $NHCH_2$), 3.65 (3H, s, NCH_3). m/z (ES+) 466.2353 $[M+H]^+$ $C_{27}H_{28}N_7O$ requires 466.2350.
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