

Full Length Research Paper

Design, Synthesis and biological evaluation of novel acridine-polyamine conjugates against prostate cancer

Hildebert B. Maurice^{1*}, Rodger Phillips² and Nazira Karodia³

¹School of Pharmacy, St. John's University of Tanzania, P.O. Box 47 Dodoma Tanzania.

²Yorkshire Cancer Research, University of Bradford, West Yorkshire, BD7 1DP, UK.

³School of Chemistry and Forensic Sciences, University of Bradford, West Yorkshire, BD7 1DP, UK.

Accepted 4 May, 2009

Prostate cancer is the most common cause of cancer death in men, aged 85 and over. Androgen receptor, a single polypeptide with three functional domains is very important during initiation and progression of the disease. In this study, a DNA intercalating agent, acridine is linked to the testosterone via a polyamine linker to obtain a compound with trifunctional characteristics, where the acridine intercalates the DNA, the polyamine linker binds the phosphoryl groups of the DNA backbone and the testosterone moiety binds into the AR ligand binding domain, with which its DNA binding domain is bound already to the DNA. This trifunctional compound and related derivatives have been synthesized and tested against androgen dependent- and androgen independent- prostate cancer cell lines and they have demonstrated to be cytotoxic at the micromolar concentrations.

Key words: Polyamines, Testosterone, Prostate cancer, Acridine, Synthesis.

INTRODUCTION

Prostatic adenocarcinoma is the most common cause of cancer death in men, aged 85 and over (Crawford, 2009). The tumour is androgen dependent, due to the presence of androgen receptor (AR). AR is a single polypeptide with three functional domains; an amino terminal domain and a DNA binding domain linked by a hinge region to a ligand binding domain. Although these domains are different, they influence each other's activity. Androgens, mainly testosterone, binds the AR through the ligand binding domain which consequently binds to the DNA to start the expression of the proteins important for biological functions of the organism (Nahoum and Bourguet, 2007). Polyamines are small amines containing aliphatic molecules, which are water-soluble. Polyamines are positively charged at physiological pH, with amino groups separated by 3 or 4 carbon atoms. They are found ubiquitously in all living cells and the most common naturally occurring are putrescine, spermidine and spermine (Figure 1). These polyamines are biosynthesized mainly in most cell types where they are necessary for optimal growth and replication of all cells by influen-

cing transcriptional and translational stages of protein synthesis and by altering intracellular levels of free calcium, hence suggesting their roles as intracellular messengers and stabilizers of conformations of nucleic acids (Pegg, 1998). Cellular polyamines originate mainly from intracellular biosynthesis (endogenous) or by uptake via transporters from the extracellular environment (exogenous). Amongst the three naturally occurring polyamines, spermine has a higher cellular uptake in prostate cancer cell lines than in other types of malignancies (Srinath et al. 2002). Spermine blocks prostatic tumour proliferation *in vitro* and prevents subcutaneous tumour progression *in vivo* (Cheng et al., 2001). These effects, mainly active transport, DNA binding and high cellular uptake of spermine in prostate cells, make it an important tool in drug design for prostate cancer chemotherapy. Acridine is a tricyclic planar compound and its derivatives intercalate DNA. This intercalation inhibits DNA replication and translation which eventually leads to cellular death which is enhanced by the presence of a side chain such as alkyl chains and polyamines at C-9. However, intercalation alone cannot account for the pronounced cytotoxicity shown by these amino acridine derivatives but rather the formation of DNA-protein cross-links and DNA breaks (Filipski et al., 1977) by block topoisomerase I and II,

*Corresponding author. E-mail: hildebert1@yahoo.com. Tel: +255 769593540.

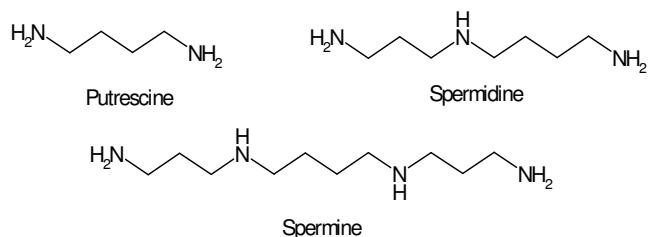


Figure 1. Naturally occurring polyamines.

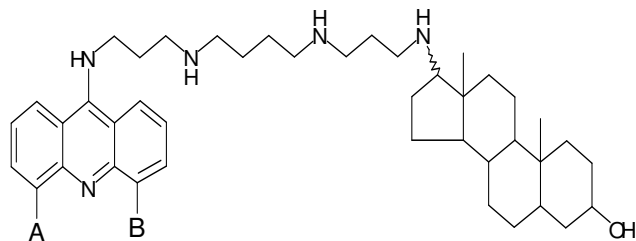


Figure 2. Designed trifunctional compound.

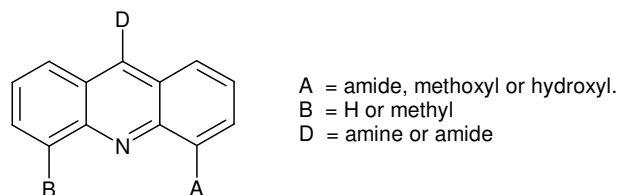


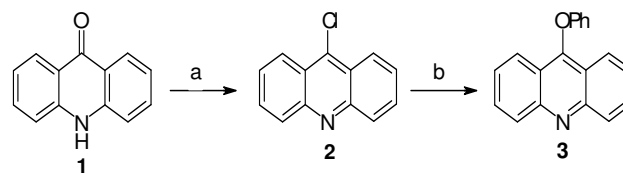
Figure 3. Acridine derivatives synthesized.

enzymes responsible for DNA cleavage during helix formation. The target compounds comprised of three parts: acridine and its derivatives linked to a polyamine and the third fragment comprised of an androgen were synthesized. It was expected that the planar structure of the acridine would interact with DNA base pairs, while the polyamine was expected to bind to the negatively charged phosphate DNA backbone and also to bind DNA bases. It was anticipated that when the androgen moiety is attached to the acridine-spermine conjugate, binds to the AR receptors to form a complex, the whole molecule will then be pulled onto the DNA where it will bind strongly thus inhibiting further biological activities (Figure 2).

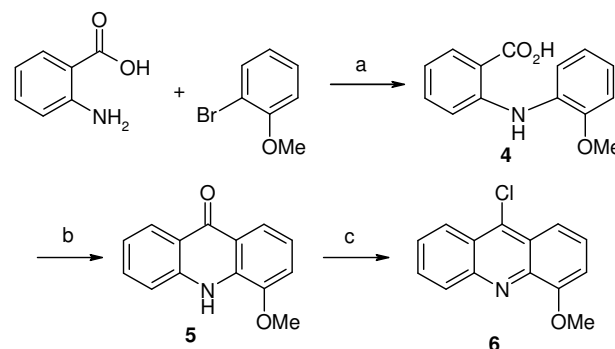
METHODOLOGY

Synthesis

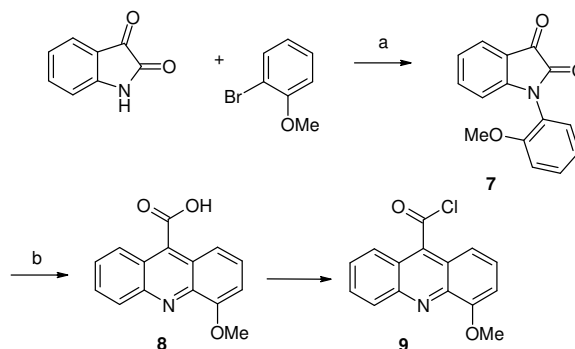
Several acridine derivatives were synthesized using different approaches depending on the nature and position of various functional groups. Selected Acridine derivatives are shown in Figure 3. The synthesis started with a commercially-available unsubstituted acridone 1 that was converted to the chloride 2 at C-9



Scheme 1. a) SOCl_2 , DMF 120°C, 96%. b) f1 20°C, 5 h, 95%.



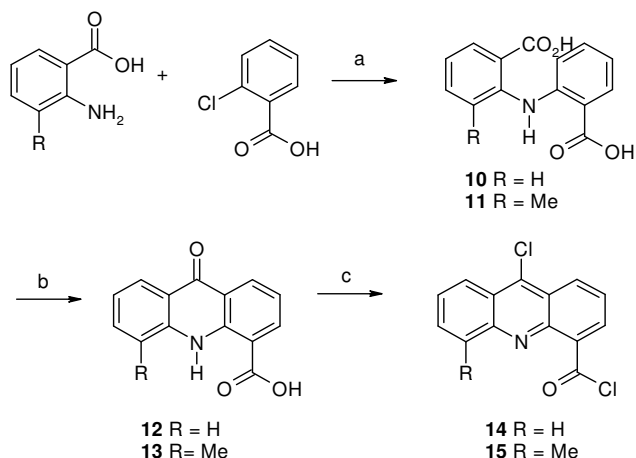
Scheme 2. a) Cu, CuO, K_2CO_3 , 145°C, 60%. b) polyphosphoric acid, 120°C, 67%. c) SOCl_2 , 120°C, 84%.



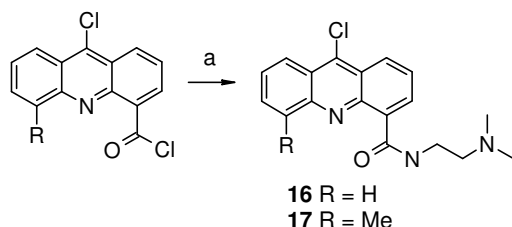
Scheme 3. a) CuO, DMF, 120°C, 24 h, 56% b) NaOH, H_2O , 100°C, 6 h, 86%, c) SOCl_2 , 120°C, 2h

by chlorination with thionyl chloride. Due to the instability of the chloride it had to be converted into a phenoxy derivative 3 which resulted in a stable compound that could be stored for a longer time (Scheme 1).

The synthesis of a methoxy substituted acridone began with the reaction of anthranilic acid and bromoanisole in the presence of copper and copper oxide as catalysts. The reaction was heated at 145°C and a relatively good yield was obtained when a black amorphous solid was purified using a large volume of methanol containing ammonia in the column chromatography. Cyclization of the resulting acid 4 was accomplished by heating the reactant with polyphosphoric acid for about an hour after which the reaction mixture was poured into hot water and cooled for 24 h to produce acridone 5 as a yellow precipitate that was pure and ready for further reaction. This methoxyacridone 5 was chlorinated using thionyl chloride (Scheme 2). The chloride 6 was reacted immediately with a protected polyamine. For the synthesis of methoxyacridine-9-carboxylic acid, (Scheme 3) isatin was reacted with bromoanisole in DMF with CuO as a catalyst to give product 7



Scheme 4. a) DIPEA, DMF, Cu(OAc)₂, 12 h, 120 °C, 75%. b) polyphosphoric acid, 120°C, 3 h, 70%. c) i) SOCl₂, 80°C 1 h, 95% ii) N, N-dimethylethylene diamine, CH₂Cl₂, 0°C.

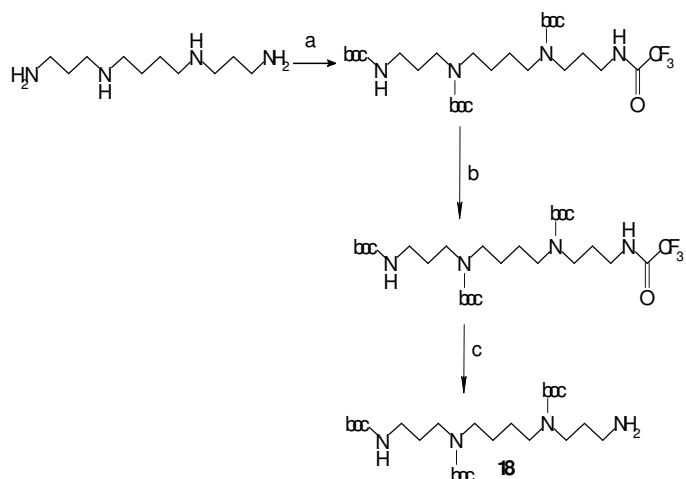


Scheme 5. a) 0°C, dichloromethane, N, N-dimethylethylene diamine, 64% overall.

as a dark brown solid (Keshava et al., 2005). Cyclization to give the 4-methoxyacridine-9-carboxylic acid **8** was successfully achieved by dissolving the product **7** in aqueous sodium hydroxide solution and then heating (Allais, 1982). The desired product **8** was isolated with an excellent yield. 4-Methoxyacridine-9-carboxylic acid **8** was reacted with thionyl chloride to obtain an acyl chloride **9**, which was reacted in further steps without isolation.

The other set of acridine derivatives prepared was those with a carboxamide substituent at C-4 with or without another group at C-5. The precursors for this reaction were chlorobenzoic acid and aminobenzoic acid or its methyl derivative. Anthranilic acid was reacted with 2-chlorobenzoic acid using cupric acetate and DIPEA as catalysts. The diacid **10** was obtained as a black powder that was purified by recrystallization. Cyclization was achieved by heating **10-11** in PPA. The resulting acridonic acids **12-13** were bright yellow in colour and isolated with the good yields of 60 and 78% respectively in comparison with literature yields of 70 and 80% respectively (Atwell, 1987). The PPA was removed by pouring the hot reaction mixture into vigorously stirred boiling water and the product precipitated out upon cooling (Scheme 4). Heating the acids **12-13** with thionyl chloride resulted in chlorination at C-9 and formation of an acyl chloride. Once the products **14-15** were obtained, they were reacted directly with the N, N-dimethylethylene diamine in the next step to obtain **16-17** (Scheme 5).

Unreacted amine and by-products were removed by washing the reaction mixture with water. The organic phase was recovered and evaporation of the solvent gave the product as an orange solid. This was used in subsequent reactions without further purification due to its instability. Boc protection was carried out using a one-



Scheme 6. a) Ethyl TFA, -78°C THF, CH₃OH, 30 min. b) di-*tert*-butyldicarbonate, CH₃OH, 30 min. c) NH₃.

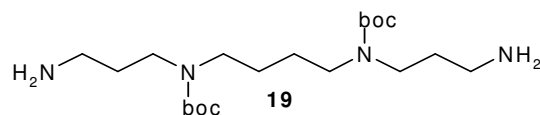
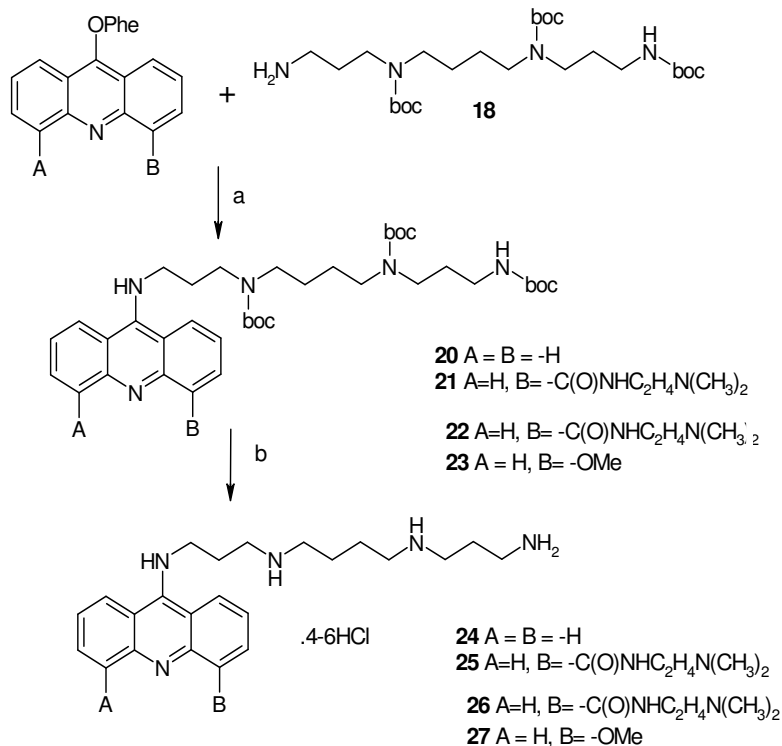


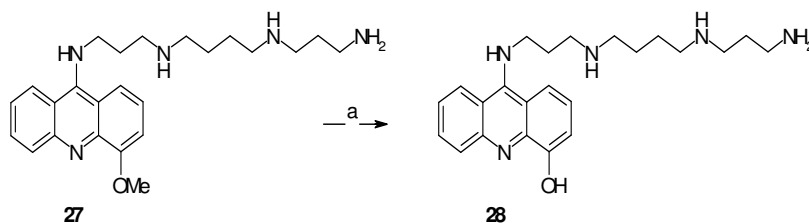
Figure 4. di-Bocspermine.

pot procedure, whereby one of the primary amino groups was first protected with the trifluoroacetyl group by reaction with ethyl trifluoroacetate (Geall et al., 2000). The reaction was started at -78°C to limit acylation to one primary amine. Ethyl trifluoroacetate was added drop wise over a period of 30 min. to a solution of the acid. After this stage, the temperature was raised to 0°C after which a solution of di-*tert*-butyldicarbonate was added drop wise over 30 min. Aqueous ammonia was added to increase the pH to 11 to enable the cleavage of the trifluoroacetyl group and generate tri-protected spermine **18**. Purification was done by column chromatography to give the pure product as colourless oil in 40% yield (Scheme 6). Di-Boc spermine **19** (Figure 4) was prepared by a modifying the method whereby the equivalents of ethyl trifluoroacetate were doubled and the cleavage of the N-trifluoroacetyl groups was facilitated by aqueous lithium hydroxide rather than ammonia. Linking a protected spermine to an acridine is relatively straight forward and very good yields were obtained. 9-Chloroacridine **2** as well as 9-phenoxyacridine **3** was used in subsequent amination of C-9.

The synthesis of the amine was carried out by heating a protected amine with an activated acridine derivative **2** or **3** in phenol at about 120°C overnight. Phenol was removed by washing a dichloromethane solution of the reaction mixture with an aqueous solution of sodium hydroxide. The organic phase was recovered, evaporated and the residue was subjected to column chromatography. These tri-Boc spermine-acridine conjugates were isolated as yellow viscous oils in high yields. Deprotected spermine-acridine conjugates **24-27** were prepared by dissolving the N-protected derivatives **20-23** in dichloromethane and adding excess TFA. Purification of these deprotected amines was carried out using column chromatography using DOWEX, a cation exchange resin, as a stationary phase and isopropanol and water as mobile phase for a gravity column. The deprotected residue was dissolved in 50:50 water and isopropanol and then adsorbed onto the resin. Hydrochloric acid was passed through the column in the eluotropic



Scheme 7. a) Phenol, 120°C, 16 h, 75%. b) CH₂Cl₂, TFA, 1 h, 80%.



Scheme 8. a) NaEtSH, 100°C, 3 h, 76%.

series of 1, 2, 2.5 and 4M. On evaporation of the solvents, the yellow hydrochloride salts 24-27 were obtained, and these products were polar (Scheme 7). The hydroxyl deprotection of the methoxyacridine conjugate 27 was executed successfully by reacting the conjugate with sodium ethanethiolate at a temperature of 100°C for 3 h in DMF (Scheme 8) and compound 31 was obtained in an excellent yield (Linderberg et al., 2004).

Amide linked conjugates

The route followed was the conversion of the acid functionality to the acyl chloride 33 by the reacting it with thionyl chloride. The resulting acyl chloride was reacted immediately with protected spermine 18 in the presence of catalytic quantities of DIPEA for 2 h, to avoid the deprotection of tri-Boc-spermine by hydrochloric acid generated (Scheme 10). Deprotection of the amide conjugates 34 and 35 was accomplished using the standard conditions and purification was done on silica gel (Scheme 9).

Di-Boc-spermine 19 and androsterone 33 (Figure 5) were

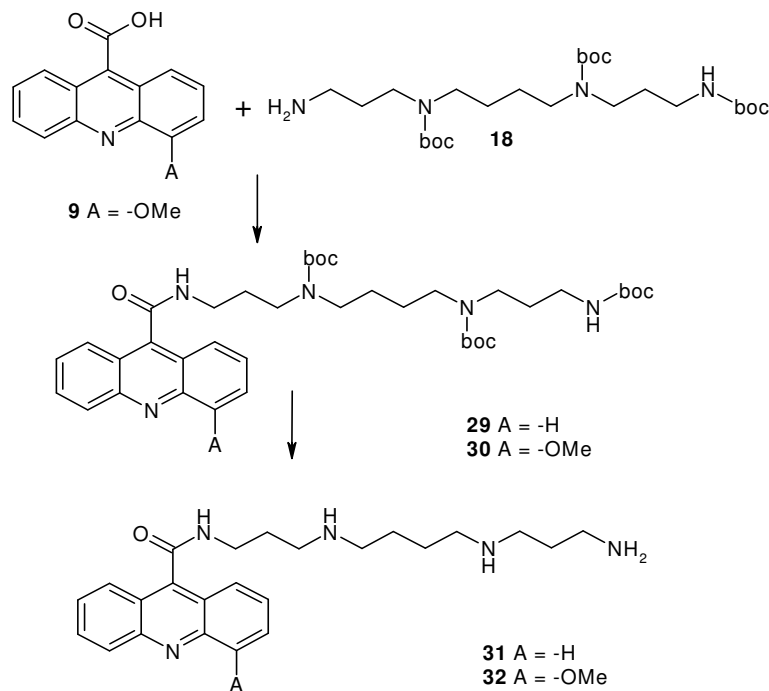
reacted to in molar equivalents limited to a 1:1 ratio to ensure reaction exclusively at one primary amino group while sparing the other for further reaction with the acridine intermediates.

Borane-Pyridine (BAP) was used as a reducing agent (Rastogi et al., 2006) which worked very well to produce 34 in a yield of 60%.

The free amine of the androsterone-di-Boc-spermine conjugate 34 was reacted successfully with phenoxy acridine 3. The work up was done as in previous experiments of that nature to obtain the trifunctional 35 as yellow-coloured oil (Scheme 11). Deprotection of 35 was done as usual with TFA and purification by recrystallization by warming the residue with acetonitrile and leaving the mixture undisturbed overnight gave product 36 as a yellow solid which was recrystallized from ethanol (Scheme 11).

Cytotoxicity testing

The cell lines employed in this study were LNCaP and PC3 cells, both of which are derived from human prostatic sarcoma. Both cell lines were routinely maintained as monolayer cultures in RPMI



Scheme 9. a) Coupling agent (DCC, HOBt, or EDAC).

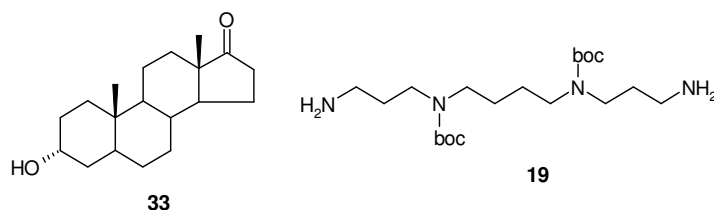


Figure 5. Androsterone and di-Bocspersmine.

1640 medium supplemented with 10% foetal calf serum, sodium pyruvate (2mM), L-glutamine (2mM), penicillin/streptomycin (50 IUml⁻¹/50µgm⁻¹) and buffered with HEPES (25mM). Both cell lines were routinely maintained at 37°C in a humidified atmosphere containing 5% CO₂.

All test compounds were dissolved in DMSO at 10mg/ml and stored at -20°C. Chemosensitivity was assessed using MTT assay as follows:

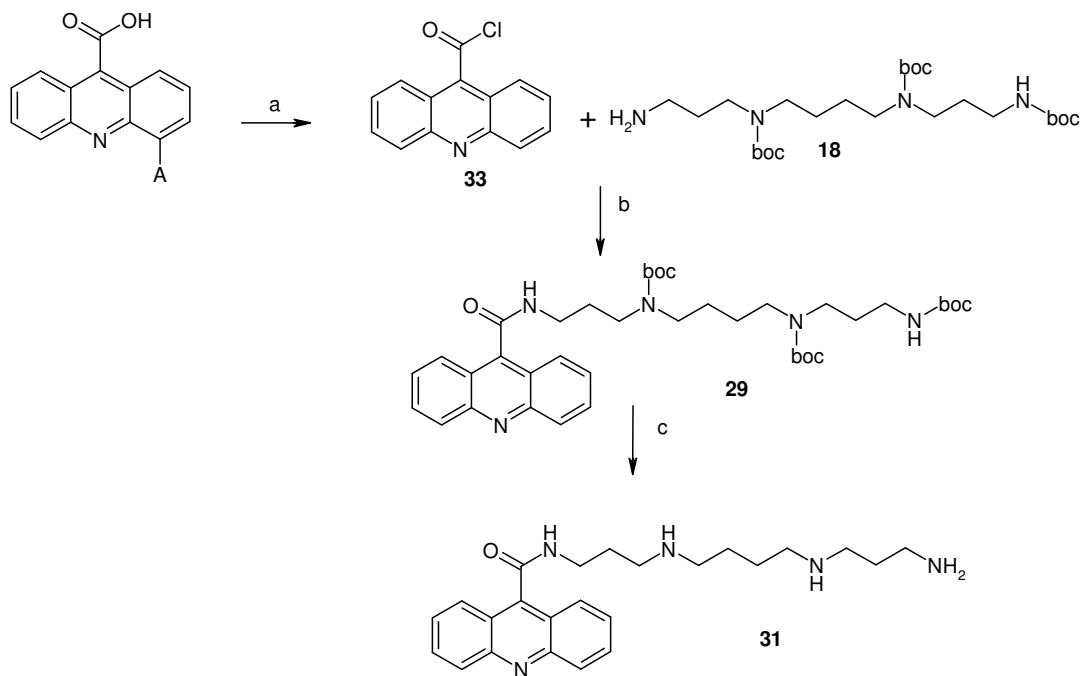
Cells were seeded into 96 well plates at 1×10^3 cells per well (with the exception of the first lane of the plate which contained media only that served as the blank for spectrophotometry) in a volume of 100 µl complete RPMI 1640 medium. A range of drug dilutions were prepared at 2 times the final concentration desired and 100 µl of each drug concentration added to the plate (8 wells per drug concentration). The highest drug concentration tested was 10 µg/ml and the final DMSO concentration was 0.1% in all cases. Following 6 day incubation at 37°C, 20 µl of MTT (5 mg/ml) was added to each well and incubated for a further 4 h at 37°C. Media plus MTT (200 µl) was carefully removed from each well and 150 µl of the DMSO was added to each well to dissolve formazan crystals. The absorbance of the resulting solution was determined at 540 nm using an ELISA plate reader and the results were expressed in terms of percentage survival as follows:

$$\% \text{ Survival} = \frac{(\text{Absorbance of treated wells} - \text{blank})}{(\text{Absorbance of control well} - \text{blank})} \times 100$$

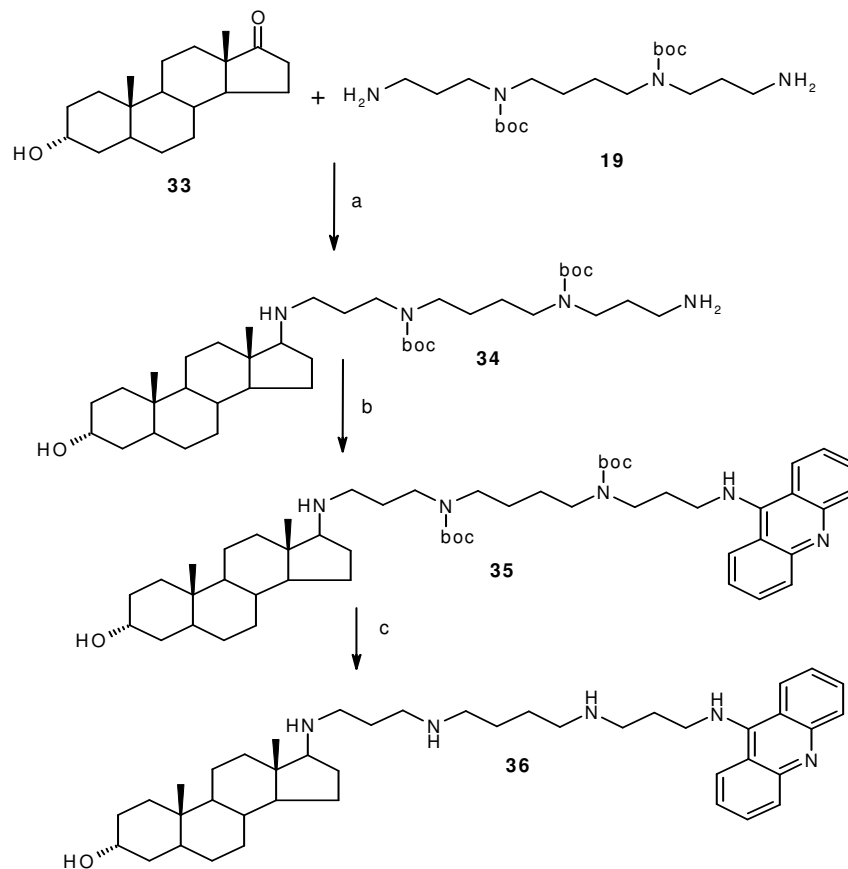
Each assay was repeated in triplicate and dose response curves constructed by plotting % survival against drug concentration. IC₅₀ values were determined which is defined as the concentration of a drug required to kill 50% of cells.

RESULTS AND DISCUSSION

Towards the synthesis of functionalized trifunctional compounds, several intermediates were made. The synthesis of substituted acridones was initiated from their respective precursors and the amination step using Johnson-Ullman reaction proceeded smoothly. Also, the cyclization reactions involving the heating and use of PPA had some separation problems for some acridones due to the high viscosity of the acid. According to the literature, PPA was supposed to be removed by pouring the hot reaction mixture into vigorously stirred boiling



Scheme 10. a) SOCl_2 , 80°C , 1 h, 95%. b) CH_2Cl_2 , DIPEA, rt, 2 h, 50%



Scheme 11. a) BAP, dry, THF/MeOH, RT, 12 h, Acetic acid, 60%. b) 9-Phenoxyacridine, phenol, 120°C , 70%. c) TFA, CH_2Cl_2 , 2 h, RT, 90%

water and the product was expected to precipitate out upon cooling. This however, produced low yields in the range of 25% at first because not all the PPA reacted with water and, due to its high viscosity, it could not be filtered through sintered glass thus trapping the product in an oily mixture. The workup was modified and a dilute solution of ammonia in methanol and dichloromethane was added to the reaction mixture to produce ammonium phosphates which crystallized out, leaving the acridones in solution. For acridones 12 and 13, the presence of a ketone peak at 177.5 ppm and upfield shift of a carboxylic acid signal at 170.0 ppm in the ^{13}C NMR spectrum was a confirmation. The ^1H NMR spectrum showed signals for the aromatic hydrogens at around 8-7 ppm and a broad singlet for the carboxylic acid peak was at 7.37 ppm. The methyl substituted acridone 13 had a similar ^{13}C NMR spectrum with signals in the expected chemical shift regions. The signal appeared at 175.8 ppm, the carboxylic acid functionality peak was at 171.4 ppm and the signal for the methyl group was at 19.4 ppm. Analysis of the ^1H NMR spectrum showed the carboxylic acid proton at 12.00 ppm while the methyl protons were at 2.34 ppm. The double chlorination of the ketone at C-9 and the carboxylic acids 12-13 at C-4 was carried out at the same time using thionyl chloride. Because the two functional groups reacted at different rates it was necessary to ensure that both the ketone and the acid functionalities had reacted before the reaction was quenched. An optimum reaction temperature was found because overheating resulted in decarboxylation of the starting material or the resultant product thus producing several by-products and 14-15 obtained were reacted directly with the *N,N*-dimethylethylene diamine to obtain carboxamides at C-4 at 0°C to preserve the chloride at C-9 where a polyamine had to be attached in subsequent step. The protection of the secondary amino groups in spermine was easily effected using the procedure from the literature in a very good yield (Geall et al., 2000). The ^1H NMR spectrum confirmed the presence of the Boc groups with a characteristic tertiary butyl signal at 1.42 ppm which integrated to 27 hydrogens. The methylene protons appeared in the region of 3.24-3.35 ppm. ^{13}C NMR analysis also confirmed the structure of 18 showing a distinctive signal at 28.7 ppm for the methyl carbons of the boc group and a carbamate signal at 155.5 ppm.

The amine conjugates of the acridines were synthesized by heating the tribocspermine with the derivatized acridines and very good yields were obtained. Deprotection of spermine-acridine conjugates was rapidly carried out using TFA. Previously documented methods using a hydrobromic acid/acetic acid mixture was abandoned because acetic acid could neither be eliminated *in vacuo* or by phase separation (Kunze, 1996; Zheng et al., 2000). The purification of the resultant conjugates could not be done on conventional column chromatography material such as silica gel or alumina because the polyamine moiety strongly adhered to the silica and

attempts to purify them on alumina resulted in a continuous streak making separation impossible. DOWEX, a cation exchange resin, which is widely used in the separation of peptides was adopted as a stationary phase and isopropanol and water as mobile phase for a gravity column. This afforded pure hydrochloride salts of the conjugates. The NMR spectra of all three compounds showed the absence of the Boc groups. As expected, the signals for the NH and NH_2 were not observed since D_2O was used as the solvent. The signals for the aromatic protons were observed in the expected region downfield. The hydroxyl deprotection of the methoxyacridine conjugate 28 was attempted using several methods. Demethylation attempts were made using boron tribromide (Spence et al., 2003) and pyridine hydrochloride salts (Kirkiacharian et al., 2002) under various conditions without success. The methoxy derivative 27 was successfully demethylated by reaction with sodium ethanethiolate. This reaction proceeded at a temperature of about 100°C for 3 h in DMF and this method is preferred since it does not affect other functional groups present in the molecule (Linderberg et al., 2004). Synthesis of the amide-linked acridine-spermine conjugates using coupling agents such as DCC, EDAC and PyBOP with the addition of catalytic amounts of HOBt was not successful even under stringent dry conditions suggested in the literature. Due to the problems encountered with the coupling agent method, the acid functionality was converted to the acyl chloride with thionyl chloride which was further reacted immediately with protected spermine in the presence of catalytic quantities of DIPEA. This method was uncomplicated and was complete within a short period of time with a relatively good yield of product even though it had to be stopped within 2 h, to avoid the generation of hydrochloric acid which may deprotect both tri-Boc-spermine and the spermine-acridine conjugate leading into the formation of by-products, including a *bis*-acridine-spermine conjugate. For the synthesis of the trifunctional compounds, the first strategy was to join two precursors namely androsterone 33 and dibocspermine 19.

The molar equivalents of di-Boc-spermine and androsterone 33 to limit reaction on single amino group exclusively were reductively aminated by using Borane-Pyridine (BAP) (Rastogi et al. 2006) which worked very well to produce the product in sufficient quantities. The ^1H NMR spectrum showed the presence of the Boc groups as well as a crowded region around 0.7-2.1 ppm, characteristic for the androgen moiety. Similarly, the aliphatic region of the ^{13}C NMR spectrum was busy, and all the expected signals were present. The free amine of the androsterone-di-Boc-spermine conjugate 34 was reacted successfully with phenoxy acridine 3 to obtain uncontaminated trifunctional target molecule. Both ^{13}C and ^1H NMR spectroscopy confirmed the structure of the product. The signals for the aromatic ring appeared at 8.31, 8.12 and 7.53 in the ^1H NMR spectrum and there

Table 1. *In vitro* cytotoxic activity against LNCaP and PC3 cell lines

Compounds	AR+ LNCaP IC ₅₀ (μM)	AR- PC3 IC ₅₀ (μM)
27	1.51 (0.00275)	1.00
28	0.6 (0.00112)	3.04
32	>10	>10
25	0.18	0.98
26	0.36	2.04
36	0.6	1.01

were peaks in the region 3.35-1.75 ppm corresponding to the polyamine. The signals for the steroid were overlapping between 0.64-2.10 ppm. The ¹³C NMR spectrum contained peaks at 154 ppm for the carbonyl of the protecting group and there were five aromatic ring signals around 151.6-116.1 ppm. The signals for the steroid were in the expected region of the spectrum. Deprotected product by TFA 35 was purified by recrystallization whereby the residue was warmed with acetonitrile and left overnight to give product 49 as a yellow solid which was recrystallized from ethanol. MS, NMR, and IR analysis were consistent with the structure. Cytotoxicity studies were carried out and results are as they appear in Table 1

Cytotoxic studies

Two cell lines were selected for study; the LNCaP cell lines which are androgen sensitive and PC3 that are androgen insensitive. Table 1 displays the activity of the 6 compounds screened. All six compounds 27, 28, 32, 25, 26 and 36 showed activity against both cell lines. Compounds that have close chemical structure have demonstrated structural relationship to their cytotoxic activities. Compound 27 and 28 which only differ by having a methoxy and hydroxyl substituent respectively, have shown this relationship. The removal of a methoxyl group increased activity by about 3-fold. This might be contributed by the hydrophilicity added by the methoxy group cleavage. Compound 27 displayed superior activity on PC3 than LNCaP while 28 showed a 5-fold decrease in activity in the androgen independent cell line PC3. Compounds 25 and 26 also showed this kind of relationship. The presence of the methyl group at C-5 halved the activity on androgen dependent cell lines. The same compounds had their activity depressed five folds on the androgen independent cell lines. This trend is in agreement with that observed for compound 28.

However, compound 36 as expected, showed potency on LNCaP and this potency was lowered by 1.67 folds on androgen independent cells PC3. This trend may suggest that hydrophilic substituents are favourable while hydrophobic are detrimental for the activity. The addition of an androgen moiety at the acridine-spermine conjugate, which made the 36, produced the activities that

were equally potent and similar to 28.

Conclusion

In this study, six compounds have been designed and synthesized and one of them was trifunctional. These compounds were all tested against androgen dependent and androgen independent prostate cancer cell lines. All of them, with the exception of one compound, were equally cytotoxic to both cell lines and have shown to be effective inhibitors of prostate cancer cells *in vitro*. The trifunctional compound was active against androgen dependent cell lines, while that activity was halved to androgen-independent cells. Efforts are underway to understand the structure- activity relationship to obtain the most optimal compounds.

REFERENCES

- Allais AGJ, Poittevin A, Nedelec L, Chiffot L (1982). *Eur. J. Med. Chem. Chim. Ther.* 17: 371-382.
- Atwell GJ (1987). *J. Med. Chem.* 30(4): 664-669.
- Cheng LL, Wu Cl. (2001). "Non-destructive quantitation of spermine in human prostate tissue samples using HRMAS 1H NMR spectroscopy at 9.4 T." *FEBS Letters* 494(1-2): 112-116.
- Crawford ED (2009). "Understanding the Epidemiology, Natural History, and Key Pathways Involved in Prostate Cancer." *Urology* 73(5, Supplement 1): S4-S10.
- Filipski J, Marczyński B (1977). "Interactions of some nitro-derivatives of substituted 9-aminoacridine with DNA." *Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis* 478(1): 33-43.
- Geall AJ, Taylor RJ (2000). "Synthesis of cholesteryl polyamine carbamates: pK(a) studies and condensation of calf thymus DNA." *Bioconjugate Chemistry* 11(3): 314-326.
- Keshava C, Divi RL (2005). "Induction of CYP1A1 and CYP1B1 and formation of carcinogen-DNA adducts in normal human mammary epithelial cells treated with benzo[a]pyrene." *Cancer Letters* 221(2): 213-224.
- Kirkiacharian S, Thuy DT (2002). "Structure-activity relationships of some 3-substituted-4-hydroxycoumarins as HIV-1 protease inhibitors." *Il Farmaco* 57(9): 703-708.
- Kunze T (1996). "Phosphono analogues of glutathione as new inhibitors of glutathione S-transferases." *Archiv Der Pharmazie* 329(11): 503-509.
- Linderberg MT, Moge M (2004). "Claisen Condensation as a Facile Route to an -Alkoxy-cinnamate: Synthesis of Ethyl 2-Ethoxy-3-(4-hydroxyphenyl)propanoate." *Org. Process Res. Dev.* 8(6): 838-845.
- Nahoum V, Bourguet W (2007). "Androgen and estrogen receptors: Potential of crystallography in the fight against cancer." *Int. J. Biochem. Cell Biol.* 39(7-8): 1280-1287.
- Pegg AE (1998). "An encyclopedia of polyamines." *Trends in*

- Biochemical Sciences 23(9): 366-367.
- Rastogi S, Dogra RKS (2006). "Skin tumorigenic potential of aflatoxin B1 in mice." *Food and Chemical Toxicology* In Press, Corrected Proof 44(5): 670-677.
- Spence JD, Raymond AE (2003). "Condensations of N-arylhydroxylamines for the preparation of 5,5'-di-tert-butyl-2,2'-dihydroxydiphenylamine." *Tetrahedron Letters* 44(4): 849-851.
- Srinath P, McQuarrie SA (2002). "Comparative uptake of polyamines by prostate and non-prostate cancer cell lines." *Nuclear Med. Biol.* 29(4): 497-503.
- Zheng G, Potter WR (2000). "Photosensitizers related to purpurin-18-N-alkylimides: A comparative in vivo tumoricidal ability of ester versus amide functionalities." *Bioorganic & Medicinal Chemistry Letters* 10(2): 123-127.