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Solvent-free synthesis of heteroannulated carbazoles: A novel class of anti-tumor agents

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ABSTRACT

A series of novel carbazole analogues that hold pyrido, isoxazolo, pyrimido and pyrazolo templates were designed and synthesised in good yield by grinding conditions. All the synthesised compounds were screened for their anti-tumor activity and displayed enviable selective growth inhibition on HeLa cell line compared to AGS cell line. Among these compounds, compound 2-(3′,4′-diethoxy-benzylidene)-6-methyl-2,3,4,9-tetrahydro-carbazol-1-one,6-chloro-2-(3′,4′-diethoxy-benzylidene)-2,3,4,9-tetrahydro-carbazol-1one, 2-(3′,4′-diethoxy-benzylidene)-8-methyl-2,3,4,9-tetrahydro-carbazol-1-one, 3-(3′,4′-diethoxyphenyl)-7-methyl-4,5-dihydro-10*H*-isoxazolo[3,4-*a*]carbazole, 7-chloro-3-(3′,4′-diethoxyphenyl)-4,5-dihydro-10*H*-isoxazolo[3,4-*a*]carbazole, 4-(3′,4′-diethoxyphenyl)-2-ethoxy-8-methyl-6,11-dihydro-5*H*-pyrido[2,3-*a*]carbazole-3-nitrile, 8-chloro-4-(3′,4′-diethoxyphenyl)-2-ethoxy-6,11-dihydro-5*H*-pyrido[2,3-*a*]carbazole-3-nitrile, 4-(3′,4′-diethoxyphenyl)-2-ethoxy-10-methyl-6,11-dihydro-5*H*-pyrido[2,3-*a*]carbazole-3-nitrile were found to have promising anti-tumor properities with reference to the standard ellipticine against the HeLa cancer cell line. All these intermediates showed IC_{50} outranged the standard ellipticine. The same compounds showed moderate activity against AGS cancer cell lines. The efforts were undertaken to optimize potency and selectivity of this class of compounds.

1. Introduction

In the last few decades, reports on carcinoma cases have shown a drastic increase, although there have been significant advancements in cancer treatment. Changes in lifestyle and environment are regarded as the leading causes, resulting in over 10 million reported cases globally in year 2001 [1]. Two of the most common type of cancer in females are cervical and breast cancer, with the mortality rate for cervical cancer in the United States in 2006 being 37%. So there is a strong need for the establishment of new chemo preventives and the discovery of new drugs to combat these carcinomas [1,2]. Carbazoles constitute an important class of alkaloids displaying a wide variety of biological activities [3] and their analogues are also widely used as building blocks for new organic materials [4]. Accordingly, syntheses of simple carbazoles and modified carbazoles have been extensively studied [5,6]. Benzodihydro[*a*]carbazoles (BDHC) and pyrido carbazoles have been reported as starting compounds for the synthesis of various drugs and possess important biological, pharmacological and medicinal activities [7–14]. They are associated with anticancer, antimicrobial and antifungal activities [13,15,16]. Many carbazole derivatives especially that bearing chloro groups are important for the creation of promising new anti-tumor agents [17]. The combination of pharmacopores may provide a synergisitic effect to improve the activity and reducing the risk of side effect. The carbazole back bone has been chosen because it possess better inhibition properties among the other nitrogen containing alkaloids. This adds considerable support in favor of carbazole as core moiety for new potential anti-tumor compounds. Based on the previous findings and in continuation of our interest in the synthesis of bioactive heterocycles, including a promising anti-tumor agents, the present study includes the green synthesis of some carbazoles that bearing, isoxazolo, pyrimido, pyrazolo and pyrido moiety and to evaluate their *in vitro* antitumor activities against two human tumor cell-lines HeLa and AGS.

2. Results and discussion

2.1. Chemistry

A central underpinning aim of our research was to develop an alternative solvent free green synthetic route for the synthesis of cyclised hetero annulated carbazoles with potential improvements

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in yield. In this paper, we describe the synthesis of benzylidene derivatives of carbazole having ethoxy groups at C_3' & C_4' by solvent free conditions. The higher yields, mild reaction conditions, easy isolation and purification make the eco-friendly procedure an attractive alternative to the existing methods for the synthesis of carbazole analogues. Nowadays, organic chemists are vigorously taking on the challenge of developing green synthetic methodologies to meet the criteria of sustainable, environmentally conscious development. Green chemistry has emerged as an important area of chemistry and has achieved outstanding progress towards the development of green reaction processes [18]. As a result, solvent-free synthetic methods have attracted much interest not only for laboratory synthesis but also in chemical industry, because of reduced pollution, lower costs, mild conditions, and ease of purification. Recently, practical procedures in the absence of solvents have been accomplished for greener and cleaner syntheses [18–23]. As the typical representative of solvent-free reactions, the grinding technique has been widely used in organic synthesis [24–30].

In earlier work from our laboratory, we have reported the synthesis of hetero annulated carbazoles [31–35]. In order to show the accessibility of the present work, we compared this method (*grinding technique*) with the results obtained using solvent (Table 1), which showed that grinding is the most efficient technique with respect to the reaction time, temperature and exhibited broad applicability in terms of yields. The reason for the efficiency of the current procedure might be due to an enhanced second-order reaction rate resulting from ultimately high concentration of reactants in the absence of solvent. We are able to obtain the cyclised product in a single step using this method. The yield of the products obtained using solvent conditions were low when compared to the yield obtained by grinding conditions. The yield of the product obtained, using solvent and without solvent conditions were compared in Table 1.

Here we have synthesized 3′,4′-diethoxy-benzylidene derivatives by mixed aldol condensation of 2,3,4,9-tetrahydro-1*H* carbazol-1-ones with 3,4-diethoxy benzaldehyde by using solvent and solvent free conditions and the yields of the product were compared which was given in Table 1. Consequently, the formation of diethoxy-benzylidene derivatives was identified as a key precursor in affording the corresponding panel of isoxazolo, pyrido, pyrimido and pyrazolocarbazoles Scheme 2. Further the3′,4′-diethoxy-benzylidene derivatives were reacted with hydroxylamine hydrochloride, malononitrile, hydrazine hydrate, and guanidine nitrate to give the corresponding cyclised heteroannulated isoxazolo, pyrido, pyrazolo and pyrimido carbazoles respectively. The synthetic routes were shown in the Scheme 1. The same procedure was repeated for other derivatives also (see Fig. 1).

2.2. Biology

The ability of the fifteen newly synthesized hetero annulated carbazoles to inhibit cell growth was evaluated by means of *in vitro* assay performed against two human tumor cell lines namely HeLa (cervix adeno carcinoma) and AGS cancer cell lines (human stomach cancer). The ellipticine was used as a standard which showed IC_{50} value 4.12 μM for Hela and IC_{50} value 7.33 μM for AGS cancer cell lines. The obtained results (Table 2) revealed that the compounds exhibited variable degrees of inhibitory activity toward the two tested human tumor cell lines. The cytotoxic activity results revealed that majority of the synthesized compounds exhibited potent anticancer activity against HeLa cell-line and moderate activity against AGS cancer cell lines which is represented in Table 2. These results indicate that all the synthesized carbazole analogues depicted selective inhibition against HeLa cancer cell line. All the compounds exhibited varying inhibitory concentrations depending on the kind of substituent present. Furthermore, the inhibitory activity was governed by the nature and position of the substituent. The cytotoxicity of all the three intermediates showed IC_{50} outranged the standard ellipticine (IC_{50} value 4.12 μM) against HeLa. The 3′,4′-diethoxy-benzylidene derivatives intermediates showed selective toxicity against HeLa cancer cell line. Among the intermediates, as for activity against HeLa cell line, the very highest cytotoxic activity was displayed by compound 6-chloro-2-(3′,4′-diethoxy-benzylidene)-2,3,4,9-tetrahydro-carbazol-1-one which showed the percentage viability IC_{50} at 0.37 μM compared with the corresponding cyclised analog was due to the presence of an electron withdrawing chloro group at C_6 position, whereas, the next highest cytotoxic activity was displayed by compound 2-(3′,4′-diethoxy-benzylidene)-6-methyl-2,3,4,9-tetrahydro-carbazol-1-one which showed the percentage viability IC_{50} at 0.80 μM and moderate inhibitory activity was also demonstrated by compound 2-(3′,4′-diethoxy-benzylidene)-8-methyl-2,3,4,9-tetrahydro-carbazol-1-one.

Out of the products formed, the pyrido, pyrazolo, isoxazolo, pyrimido substituted carbazoles that bearing chloro group at the 7 and 8th position [**7-chloro-**3-(3′,4′-diethoxyphenyl)-4,5-dihydro-10*H***-**isoxazolo[3,4-*a*]carbazole**, 8-chloro**-4-(3′,4′-diethoxyphenyl)-2-ethoxy-6,11-dihydro-5*H*-pyrido[2,3-*a*]carbazole-3-nitrile**,** 7-chloro-3-(3′,4′-diethoxyphenyl)-2,4,5,10-tetrahydropyrazolo-5*H*-[3,4-*a*]carbazole & **8-chloro**-4-(3′,4′-diethoxyphenyl)-10-methyl-11*H*-pyrimido-[4,5-*a*]carbazole-2-amine] showed a preferential growth inhibitory activity against the two cell lines than the corresponding methyl substituted analogues. Among these, the compound **8-chloro**-4-(3′,4′-diethoxyphenyl)-2-ethoxy-6,11-dihydro-5*H*-**pyrido**[2,3-*a*]carbazole-3-nitrile which holds the pyrido substituted moiety showed IC_{50} value 1.02 μM against HeLa was found to exhibit significant activity. This may be due to the presence of electron withdrawing cyano group at C_3 position. In this series, the next highest toxicity was shown by **7-chloro**-3-(3′,4′-diethoxyphenyl)-4,5-dihydro-10*H*-**isoxazolo**[3,4-*a*]carbazole which carries the isoxazolo group was endowed with IC_{50} value 1.30 μM against HeLa. The next most active compound was the one which holds the pyrimido group at the C_1 position (**8-chloro**-4-(3′,4′-diethoxyphenyl)-11*H*-**pyrimido**-[4,5-*a*]carbazole-2-amine) with the IC_{50} value 4.51 μM against HeLa. In this series, the least activity was shown by the one which carries the pyrazolo moiety (**7-chloro**-3-(3′,4′-diethoxyphenyl)-2,4,5,10-tetrahydro**pyrazolo**-5*H*-[3,4-*a*]carbazole) with the IC_{50} value 10.05 μM against HeLa cancer cell line.

Between the cyclised products, that bearing methyl group at the 7 and 8th position (3-(3′,4′-diethoxyphenyl)-**7-methyl**-4,5-dihydro-10*H*-isoxazolo[3,4-*a*]carbazole**,** 4-(3′,4′-diethoxyphenyl)-2-ethoxy-**8-methyl**-6,11-dihydro-5*H*-pyrido[2,3-*a*]carbazole-3-nitrile, 3-(3′,4′-diethoxyphenyl)-**7-methyl**-2,4,5,10-tetrahydropyrazolo-5*H*-[3,4-*a*]carbazole & 4-(3′,4′-diethoxyphenyl)-**8-methyl**-11*H*-pyrimido-[4,5-*a*]carbazole-2-amine), the highest toxicity was obtained with the pyrido substituted moiety, 4-(3′,4′-diethoxyphenyl)-2-ethoxy-**8-methyl**-6,11-dihydro-5*H*-**pyrido**[2,3-*a*]-carbazole-3-nitrile with IC_{50} value 1.50 μM against HeLa. In this series, the second highest toxicity was shown by the compound 3-(3′,4′-diethoxyphenyl)-**7-methyl**-4,5-dihydro-10*H*-**isoxazolo**-[3,4-*a*]carbazole with the IC_{50} value 2.73 μM against HeLa cancer cell line. The compound substituted with the pyimido group at the C_1 position (4-(3′,4′-diethoxyphenyl)-**8-methyl**-11*H*-**pyrimido**-[4,5-*a*]carbazole-2-amine) was found to be next highly active compound with the IC_{50} value 5.08 μM against HeLa. In this series, the least activity was shown by the intermediate that is substituted with the pyrazolo moiety (3-(3′,4′-diethoxyphenyl)-**7-methyl**-2,4,5,10-tetrahydro**pyrazolo**-5*H*-[3,4-*a*]carbazole) with the IC_{50} value 18.97 μM against HeLa.

The compounds that bearing methyl group at the 9 and 10th position (3-(3′,4′-diethoxyphenyl)-**9-methyl**-4,5-dihydro-10*H*-