European Journal of Medicinal Chemistry 92 (2015) 236–245

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthetic modification of hydroxychavicol by Mannich reaction and alkyne–azide cycloaddition derivatives depicting cytotoxic potential

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ARTICLE INFO

Article history:
Received 15 September 2014
Received in revised form
24 December 2014
Accepted 26 December 2014
Available online 31 December 2014

Keywords:
Hydroxychavicol
Cytotoxicity
Piper betel
Click chemistry
Mannich reaction

ABSTRACT

Here we report the design, synthesis and lead optimization of hydroxychavicol (**1**) a high yielding metabolite ubiquitously present in the *Piper betel* leaves with the significant cytotoxic activity. This is the first report to describe the synthetic strategies of two distinct series of hydroxychavicol by Mannich reaction (**2**–**10**) and alkyne–azide cycloaddition (**11**–**20**). Furthermore, all the synthesized derivatives along with parent compound were evaluated for their *in-vitro* cytotoxic and antiproliferative potential in several distinct cancers cell lines. Among all, the Mannich reaction derived molecules **6**, **8** and **10** displayed more potent cytotoxic activities with IC_{50} value in a range from 3 to 9 μM, which were 7–10 fold more potent than **1** against five human cancer cell lines *viz*. HL-60, Mia PaCa-2, MCF-7, HEP G2 and SK-N-SH. Our results describe an efficient synthetic approach used to evaluate the structure activity relationship of **1** and its derivative in search of potential new anticancer agents.

1. Introduction

Natural Products metabolites are the richest source for the discovery of novel chemical entities which are the boon for chemists and physicians for new drugs [1–3]. Due to the unique and vast chemical diversity natural products are recognized as privileged scaffolds because they interact with biological macromolecules, especially proteins [4,5]. As experimental fact, natural product-based screening libraries routinely yield higher hit rates than synthetically-produced small molecule libraries [6]. Nature product to be an inexhaustible resource of novel and effective anticancer agents and remain unchallenged by other alternative drug discovery approaches [7]. A recent review by Newman and Cragg shown that 74.8% of small molecules used for cancer are from non-synthetic origin [8]. While, semi-synthetic modification of naturally-occurring bioactive phytochemical has leads to the most successful anticancer drugs such as paclitaxel from 10-deacetylbaccatin III, etoposide from podophyllotoxin, irinotecan/topotecan from camptothecin and many more [9]. Over the past few decades, increased interest has been seen to treat cancer. Therefore, researchers are on a continuous pursuit to design and produce novel, safe and effective therapeutic agents [10].

Our intensive research focus on isolation and structural modifications of bioactive metabolite from medicinal plants [11,12] to achieve their new biochemical knowledge enthuse us to isolate the hydroxychavicol (**1**), a well-known phenolic from *Piper betel* leaves in several grams quantity. The leaves of *P. betel* extensively been used as betel quid chewing from time immoral in India, Taiwan and many other Southeast Asian countries. The broad heart shape of the leaf makes it ideally suited for assembling areca nut and lime paste on its surface and to be folded into a 'betel quid' to exerts refreshing test [13].

Hydroxychavicol (**1**) is the highly functionalized molecule, belongs to relatively new class (allylbenzene) of natural compounds and having characteristic odor of *P. betel* leaves having remarkable therapeutic potential. In addition, **1** is reported to possess antimutagenic and anti-carcinogenic activity [14,15] with several other biological activities like antimicrobial, antioxidant, anti-inflammatory and xanthine oxidase inhibitory effect [16,17]. The exact mechanism of **1** as anticancer agent is still remaining uncertain but importance of selected modification to the aromatic core for the development of SAR and pharmacophore determination is highlighted recently [18].

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http://dx.doi.org/10.1016/j.ejmech.2014.12.047
0223-5234/

Here we designed and synthesized the Mannich reaction based scaffold to introduce aminoalkyl substituent and we also optimize the synthetic condition for alkyne–azide cycloaddition derivatives of **1** in good to excellent yields (68–85%) which provides a suitable method into a molecule and serves as promising templates for lead optimization [19]. The insertion of Mannich side chain withstands a large diversity of functional groups and hence it has been witnessing a continuous growth in the field of organic synthesis [20]. As a result, Mannich derivatives exhibit better activity than the parent molecule **1** due to the presence of Mannich side chain increases the solubility and hence the bioavailability of the drug molecule. Furthermore, our structure-activity relationship studies showed that antiproliferative effects were markedly enhanced 3–10 folds by insertion of aminoalkyl substituent in five distinct human cancer cell lines the most promising activities were proclaimed in **6**, **8** and **10** (Table 1). Due to high therapeutic potential of **1**, we explore the chemistry and biological activities by its structural modifications and synthesised the distinct derivatives of **1** for the development of a SAR and pharmacophore determination for identification of unknown target of this class of compounds.

2. Results and discussion

2.1. Synthesis of Mannich based aminoalkyl derivatives of *1*

Mannich reaction is the most fundamental way to introduce the CC-CC bond formation in organic synthesis [21]. Herein, we describe the synthesis of aminoalkyl derivative of Hydroxychavicol (**1**) by Mannich reaction via condensation of substituted cyclic secondary amine in presence of formaldehyde afforded compounds **2**–**10** in (Scheme 1). Before evaluating the biological activity, we characterized each molecule with the help 1D and 2D spectroscopic data. The ^{1}H NMR spectral data of all the derivatives (**2**–**10**) showed characteristic signal between δ 3.68–3.72 and in ^{13}C NMR spectrum a new signal appeared between δ 61.5–65.8 due to the presence of methylene (CH_2), which confirm insertion of aminoalkyl moiety in **1**. Furthermore the insertion of aminoalkyl moiety were authenticated by ^{13}C NMR spectrum of synthesized derivatives, where chemical shift underwent a downfield shift and appeared at δ 122.5–120.35 (C-3) as compared to chemical shift at δ 115.2 (C-6) in parent compound **1**. Aminoalkylation at C-3-position was further ascertained by HMBC correlation study of compound **5** (Fig. 1), an aromatic singlet at δ 6.39 (H-4) correlated with δ 61.01(C-1″) and others correlation between δ 3.74 (H-1″) proton with δ119.75 (C-4), 120.50 (C-3) and 142.34 (C-2) confirmed the insertion of amino alkyl group at C-3 position of **5**. The remaining other signal resonated at δ 39.63 (C-1′) correlate with two aromatic singlets at δ 6.39 (H-4) and 6.70 (H-6) also authenticate that reaction did not took place either at C-4 or C-6, in that case C-1′ of **5** would show only one correlation with these aromatic protons depending on site of insertion.

2.2. Synthesis of alkyne–azide cycloaddition derivatives of *1*

While the compounds in second series were synthesized by selective *O*-propargylation at the C-1-Hydroxyl group of **1** afforded the intermediate **11** which were further, coupled with different substituted of azide by employing facile click chemistry [22,23] to get distinct derivatives of 1,2,3-triazol-4-yl-hydroxychavicol (**12**–**20**). The ^{1}H NMR signals appeared at δ 4.8–5.1 confirmed the condensation of propargyl bromide. The signals appear at δ 58.8–61.3 attributed to carbon of the active methylene inserted after *O*-propargylation of **1**. ^{1}H NMR of all the triazole derivatives (**12**–**20**) has shown a characteristic singlet for triazole proton in the range δ 8.1–8.27. In addition to that, ^{13}C NMR spectra exhibit characteristic signals for triazole ring carbon at δ 119.8–121.6 and 146.7–148.2 respectively. The synthetic yields, melting points, molecular weight and NMR spectra of all the compounds are described in the experimental section.

For SAR studies, compounds of both the series were evaluated for *in vitro* cytotoxicities against five human cancer cell lines. As evident from the Table 1, compounds containing phenolic substituent on piperazine (**6**), benzyl substituted piperidine (**8**) and simple piperazine derivative (**10**) showed the highest cytotoxic potential. Whereas, triazoles derivatives of **1** showed decrease in activity. The increased activity of Mannich derivatives over triazole derivatives of **1** was probably due to change of pharmacophore, which facilitate binding of ligand in the receptor pocket.

Table 1
Growth inhibition% of **1** and its derivatives by using MTT assay (72 h).

Entry	HL-60		Mia PaCa-2		MCF-7		HEPG2		SK-N-SH	
	30 μM	50 μM	30 μM	50 μM	30 μM	50 μM	30 μM	50 μM	30 μM	50 μM
1	88.8	92.4	41.7	42.8	36.9	44.1	27.2	29.3	34.2	71.7
2	89.8	90.5	54.4	67.5	33.5	48.9	2.1	6.5	49.6	86.1
3	91.1	91.8	91.5	90.6	41.7	54.2	57.6	69.7	74.7	84.2
4	42.1	53.4	46.8	69.5	22.8	35.1	1.5	6.3	24.7	30.8
5	87.9	89.2	86.2	75.1	42.8	47.1	46.1	65.9	53.8	54.6
6	90.3	93.5	88.7	89.9	77.7	79.7	72.8	82.8	69.2	79.5
7	37.7	43.3	34.1	38.6	26.2	32.3	4.7	5.4	21.9	49.4
8	90.6	94.4	88.9	90.9	70.9	86.3	70.2	84	78.8	83.4
9	39.4	52.1	54.4	71.8	21.4	33.3	2.5	2	15.3	40.6
10	92.5	94.4	82.8	85.6	69.2	74.2	64.2	73	65.3	69.9
11	30.7	34.2	27.4	30.3	23.7	15.8	1.5	5.6	11.8	88.4
12	29.8	65.5	42.9	58.8	42.3	56.5	15.2	30.2	31.1	68.9
13	38.3	40.5	40.1	53.7	40.3	35.6	14.8	16.2	41.9	58.1
14	31.7	47.1	39.6	52.1	34.5	40.4	4.2	8.5	12.9	87.1
15	32.8	41.4	41.1	49.8	37.3	38.1	1.2	2.5	44.1	55.9
16	20.8	31.8	39.3	45.9	35.7	35.6	1.5	12.1	37.5	62.5
17	22.5	30.9	26.6	41.6	31.9	33.4	2.3	3.4	21.2	78.8
18	22.1	25.6	43.3	47.3	19.4	29.1	8.9	9.5	38.3	61.7
19	21.3	38.9	62.3	88.2	38.8	57.2	15.4	49.9	31.5	68.5
20	38.7	43.2	46.6	57.7	58.3	34.3	2.5	4.7	10.2	42.8

Data are the mean ± SD of three independent experiments performed.