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Tyrosine-Derived Novel Benzoxazine Active in a Rat Syngenic Mammary Tumor Model of Breast Cancer

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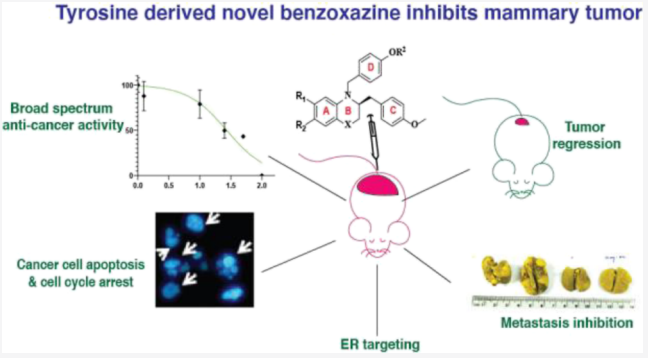
ABSTRACT: In continuing efforts of improving benzoxazepine derivatives as an anti-breast cancer agent, a new chemical entity, benzoxazine, was designed from scaffold morphing. Structure−activity relationship studies revealed that H, −OMe, $-CF_3$, and −F were well tolerated on R_1 and R_2 positions of ring **A**, and R^2 as $-CH_2CH_2N(CH_2)_4$ (*N*-ethyl pyrrolidine) and $-CH_2CH_2N-(CH_2)_5$ (*N*-ethyl piperidine) chains on ring D increased activities (**Series B**, Figure 3). **13d** selected as a lead compound (IC_{50}: 0.20 to 0.65 μM) induces apoptosis, cell cycle arrest, and loss of mitochondrial membrane potential in breast cancer cells. Compound **13d** was formulated into **13d−f** using cyclodextrin to improve its solubility for a pharmacokinetic, *in vivo* efficacy study. Both **13d** and **13d−f** regressed tumor growth at concentrations of 5 and 20 mg/kg better than tamoxifen without any mortality in a rat syngenic mammary tumor model. Collectively, our data suggest that tyrosine-derived novel benzoxazine **13d** could be a potential lead for the treatment of breast cancer and hence deserve further in-depth studies.



INTRODUCTION

Breast cancer is one of the most frequently diagnosed life-threatening diseases in women and more than 1 million women are diagnosed with breast cancer every year, accounting for 10% of all new cancers and 23% of all female cancer cases. In 2019, more than 1.7 million new cancer cases are expected to be diagnosed worldwide.[1] In India, the prevalence of this cancer is estimated around 2.5 million, with over 0.8 million new cases and 0.5 million deaths occurring each year.[2] In the current decades, drug discovery has been strongly focused on the development of drugs acting against a specific biological target with high potency and selectivity.[3,4] This prototype is based on the lock and key model proposed by Fischer and has led to numerous successful drugs in the market.[5]

Estrogen receptors are the most common type of hormone receptors in breasts and are the most important promoter of breast cancer that could directly induce cell propagation and tumor growth. To block estrogen action in breast cancer, various therapeutic approaches are available, which includes Selective Estrogen Receptor Modulator (SERM) like Tamoxifen[6] and aromatase inhibitor.[7] However, the therapies, which target estrogen often, develop endocrine resistance and these are not effectual in estrogen insensitive breast cancers.[8] The tissue selective activity of anti-estrogens is quite interesting and, in this regard, the activities of tamoxifen and raloxifene at different organs deserve mentioning. Tamoxifen possessing estrogen-like effects in the uterus functions as an antagonist in the breast tissue while it acts as an agonist on the bone in post-menopausal women.[9] On the other hand, raloxifene acts as an antagonist in both breast and uterine tissues but is agonist in the bone.[10] Centchroman, having an antagonistic profile in the breasts and uterus, acts as an agonist on the bone.[11] Several other molecules like bazedoxifene acetate, lasofoxifene, pipendoxifene, and so forth are in various stages of development as an estrogen antagonist or modulators.[12] Although generally these compounds display improved selectivity but optimal tissue selectivity has not yet been demonstrated. As such, there is still a need for additional diversity and new chemical entities to allow for the exploration of improved tissue selectivity in breast cancer.

In our effort toward searching new pharmacophores as anti-breast cancer agents, the benzoxathine core {Figure 1A} has been reported to exhibit potent anti-breast cancer activity.[13] The

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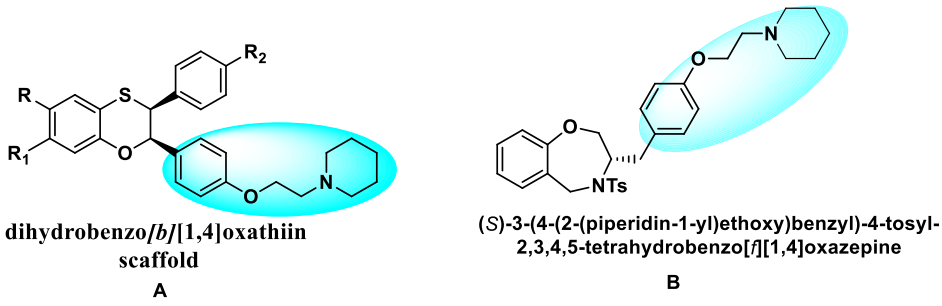

Figure 1. Structure of benzoxathine and benzoxazepine derivatives.

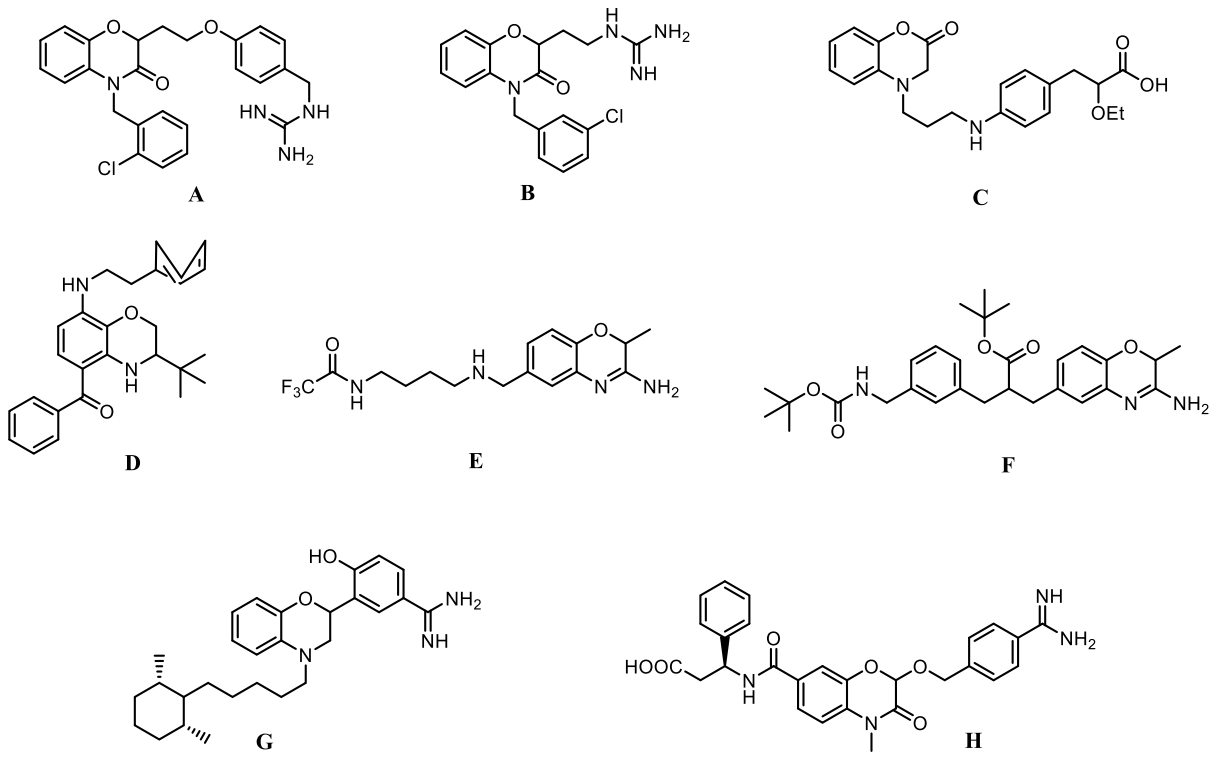

Figure 2. Structure of biologically active molecules containing a benzoxazine core.

novel benzoxathine core containing ERα selective SERM displays the low-nanomolar binding affinity and sub-nanomolar functional activity along with imparting cytotoxicity in ER/PR +-and ER/PR-independent cell lines.

From the last decade our group is working on the development of new pharmacophores as anti-breast cancer agents and toward this objective we have reported phenanthrene-based compounds containing amino chains that have promising activity with a lower IC_{50} against the ER +ve MCF-7 cell line.[14] Our group is also working on the advance of new and efficient protocols for the synthesis of amino acid-based molecules in quest for steroidomimetics.[15] Benzoxazepines {Figure 1B} are well-known pharmacophores in medicinal chemistry showing promising activity against various diseases such as antipsychotic, central nervous system along with an anticancer profile against breast cancer cells. In this context, we reported amino acid-derived benzoxazepines toward accessing nonsteroidal architectures as anti-tumor agents.[16]

Benzoxazine derivatives also represent one of the most important classes of organic molecules. Among several 1,2-, 1,4-, and 1,3-benzoxazine rings, 1,4-benzoxazines, in particular, are of significant interest and have been extensively studied because of their profound biological activities.[17] For example, 1,4-benzoxazine derivatives exhibit activity against infections, heart diseases, diabetes, neurodegenerative, inflammatory, autoimmune and cardiovascular disorders.[18,19] A literature survey identified several 1,4-benzoxazine- and 1,4-benzoxazinone-based compounds in the development phase as potential new drugs (Figure 2).[20] "**A**" and "**B**" are inhibitors of the bacterial histidine protein kinase, 1,4-benzoxazine derivative "**C**" possesses peroxisome proliferator-activated receptor α (PPARα) and PPARγ agonist activity and could be used in treating diabetes, hyperlipidemia, and other diabetic complications. French investigators recently introduced new 8-arylalkylamino-1,4-benzoxazine neuroprotectants "**D**" and Schering has disclosed 1,4-benzoxazines "**E**" and "**F**" as inhibitors of nitric oxide synthase (NOS), which are potential drugs for treating neurodegenerative, inflammatory, autoimmune, and cardiovascular disorders. 1,4-Benzoxazinone "**G**" inhibits the coagulation serine proteases factor Xa, thrombin and factor VIIa, and compound "**H**" possesses a dual antithrombotic action, exhibiting both thrombin inhibitory and fibrinogen receptor antagonistic activities. Apart from benzoxazine derivatives, some other skeletons showed anti-breast cancer

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Research paper

New Spisulosine Derivative promotes robust autophagic response to cancer cells

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ABSTRACT

Therapy resistance by evasion of apoptosis is one of the hallmarks of human cancer. Therefore, restoration of cell death by non-apoptotic mechanisms is critical to successfully overcome therapy resistance in cancer. By rational drug design approach, here we try to provide evidence that subtle changes in the chemical structure of spisulosine completely switched its cytotoxic function from apoptosis to autophagy. Our most potent molecule (**26b**) in a series of 16 synthesized derivatives showed robust autophagic cell death in diverse cancer cells sparing normal counterpart. Compound **26b** mediated lethal autophagy induction was confirmed by formation of characteristic autophagic vacuoles, LC3 puncta formation, upregulation of signature autophagy markers like Beclin and Atg family proteins. Altogether, we have detected novel autophagy inducer small molecule which can be tested further for drug discovery research.

1. Introduction

Cancer is one of the most serious life-threatening diseases in the world. During past several years, huge efforts have been made by researchers but the search of effective clinical approaches for the treatment of cancer is still a tough challenge. Chemotherapy using anticancer agents is another valuable option for the cancer treatment apart from surgery, immunotherapy, and radiotherapy [1].In the last few years, it is now very common in the developed countries [2]. So, the development of novel anticancer agents is a highly active research field and has achieved considerable attention from chemists [3]. Presently, most of the drugs in clinical trials for cancer treatment are natural products or pharmacophores derived from natural product demonstrating their fewer side effects, thus the impact of natural products upon anticancer drug discovery and design is very inspiring [4,5]. However, in the initial phase of these active natural or synthetic compounds, poor solubility is one of the major issues [6]. The poor prognosis of colon cancer and poor sensitivity to current therapeutics, associated with resistant to apoptosis, urge the search of new drugs which induce cancer cell death by some other mechanisms [7].

Long-chain α-amino-alcohols i.e. sphingoid-type bases are considered as the principal backbone of the more complex sphingolipids and ceramides [8–10[8a,b,9,10]](Fig. 1). Ceramides show its mechanism of action through cellular signaling and activating various protein kinase cascades whereas sphingolipids show diverse biological activities such as antitumor [11–13], immunostimulatory, and immunosuppressive [14], neuronal proliferation [15a,b] and protein kinase activity variation. Sphingosine is also a sphingoid base, which is a derivative of sphinganine **2** [16]. Over the past decade, 1-deoxysphingoid bases have received increased attention from synthetic chemists due to their important biological activity [17]. Among them, spisulosine **3** has received the most attention.

(*2S,3R*)-2-Amino-3-octadecanol (spisulosine or ES-285) is a marine derived bioactive compound isolated from the North Arctic clam *Spisula polynyma* [18] by Rinehart et al. This compound is known for its biological activity to inhibit cell proliferation with an IC_{50} of 1 μM in the prostate tumor PC-3 and LNCaP cell lines [19]

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Abbreviations

br s	broad signal
rt	room temperature
mp	melting point
THF	tetrahydrofuran
DCM	dichloromethane
MeOH	methanol
K_2CO_3	potassium carbonate
$NaHCO_3$	sodium bicarbonate
MOM-Cl	chloromethyl methyl ether
MsCl	methanesulfonyl chloride
Et_3N	triethylamine
Boc anhydride	di-*tert*-butyl dicarbonate
$LiAlH_4$	lithium alumininumtetrahydride
TBDPS-Cl	tert-Butyldiphenylchlorosilane

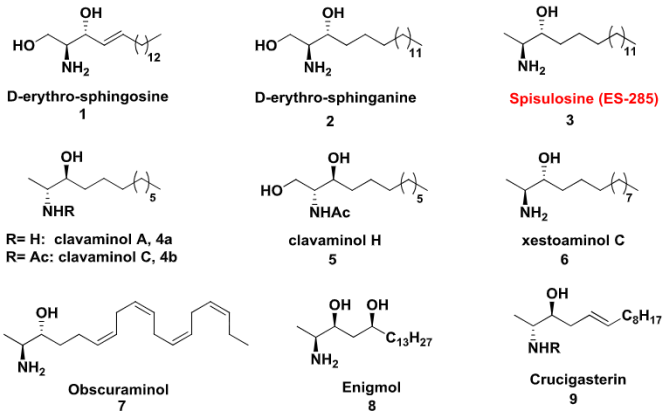

Fig. 1. Structures of several natural sphingoid bases.

and is responsible for loss of actin stress fibres [20].The mechanism of action of spisulosine is still hypothetical. Ceramide signaling and GTP binding protein (RHO protein) regulating actin stress fibers have been proposed as a potential molecular target [21].Although, spisulosine was primarily developed as a novel anticancer agent but due to its poor clinical outcomes it was discontinued from phase I in 2008 [22,23].ES-285 showed a tolerable safety profile at doses up to 128 mg/m^2. In a number of clinical studies of various compounds of natural origin and especially with some marine agents, drug-related neurotoxicity has been a common problem [24–26]. In the phase I program of ES-285, the neurological disorders like dizziness, headache, sensory or motor neuropathy, neuropathic pain, aphasia, and decreased level of consciousness was detected. One patient died after the first administration of the ES-285 when treated at 200 mg/m^{2}for 24-h i.v. infusion following drug related central neurotoxicity. Other toxicities included phlebitis, nausea, fatigue, and fever. The pharmacokinetic studies revealed high volume of distribution and long elimination half-life, which led to discontinuation of this compound. Due to an unfavorable risk/profit balance and absence of clinically significant antitumor efficacy in the whole clinical program, recruitment of patients to spisulosine clinical trials was discontinued. The seriousness of the neurological toxicity experienced by one patient supported this decision [27].

However, this compound is structurally similar to other related 1-deoxysphingoids with remarkable cytotoxic properties, such as obscuraminols [28], clavaminols [29],crucigasterins [30], and xestoaminols [31], (Fig. 1). This close structural relationship makes this type of compound as a prominent lead for anticancer oriented drug discovery programs.In literature, various methods are reported for the total synthesis of this natural product 32–41[32a,b,33,38–41], in which chiral amino acid, carbohydrate, garner aldehyde and various achiral substrates like palmityl alcohol, palmitaldehyde, pentadec-1-yne etc have been used as an starting material (Fig. 2).

However, only few reports of analogs of spisulosine are present in the literature. So far, only Delgado et al. [42],Bittman et al. [43] and Dauban et al. [44] have synthesized spisulosine analogs (10–19) and evaluated for biological activity in order to evaluate the ceramides synthase activity in whole cells (Fig. 3) [45].Bittman et al. found that analogue **10** shows IC_{50} = 27.8 ± 3.2 μM against sphingosine kinase (SphK1 isoform) and analogue**11** was found to be a nonselective SphKs inhibitor.Delgado et al. have synthesized **3** and its analogs such as stereoisomers **12**, **13** and **14**, and dehydrospisulosines**15**, **16**, **17** and **18** in order to analyse the ceramides synthase activity in whole cells (Fig. 3). Only three compounds (spisulosine **3,** 3-epimer **14** and 4,5-dehydrospisulosine **18)** were found to be most active for further screening. Recently, Dauban et al. have synthesized spisulosine **3** and its 3-fluoro derivative **19**and screened for biological evaluation in three malignant cells (KB, HCT-116, HL-60). As expected, only compound **3** exhibited cytotoxicity (IC_{50} in the 100 nM range).

Although much synthetic and biological effort in the field of 1-deoxysphingoid bases and their related compounds have been done, but many challenges in this research still remain. All of these natural compounds are an interesting source of inspiration for both the organic community and biomedical research, and further intensive studies in this area could provide a new insight into the design and mechanism of actionof the sphingolipid related anticancer agents. Inspired from these analogs and their biological activity, we have synthesized a new derivative of spisulosine which induces cancer cell death by autophagy. Although the role of autophagy in cancer is very complex, various pharmacological agents with different antineoplastic properties have been shown to induce autophagic activity resulting in massive death of cells in some cancer types.For example in renal cell, carcinomas are refractory to standard therapies, but are sensitive for autophagy induced cell death [46]. Apoptosis resistance is one of the major causes of chemoresistance, and an important challenge in the treatment of cancer is the development of therapies that overcome chemoresistance. Thus activation of autophagy in apoptosis resistant cancer could potentially provide a way to induce cell death and impede malignant growth.

1.1. Rationale of the design

The simple 1,2-amino alcohols have been observed to show a rare type of bioactivity such as anti-proliferative and cytotoxic activities along with their ability to influence the sphingolipids biosynthesis and metabolism. Because of their wide applications in biological field, some of these sphingosine related natural products have been selected as lead molecule for the design of new anticancer agents [47].

Structural analysis of all the sphingoid type bases revealed that 1,2 amino alcohol (the body region) is highly preserved. From literature survey, we hypothesized that the poor pharmacokinetic profile perhaps arises from the strong intra-molecular hydrogen bonding between two adjacent amino and hydroxyl functionalities present in spisulosine skeleton which may be responsible for its long elimination half-life. Thus, the free amino and hydroxyl group were protected to observe the effect of substitution on water

F. No. ND/CT/22/000017
Government of India
Directorate General of Health Services
Central Drugs Standard Control Organization
(New Drugs Division)

FDA Bhawan, Kotla Road
New Delhi – 110002
Dated:

02 MAY 2022

To

The Director,
CSIR-Central Drug Research Institute, Lucknow,
Sector 10, Jankipuram Extension, Sitapur Road,
Lucknow-- 226031, Uttar Pradesh

Sub.: Application for grant of permission to conduct Phase III clinical trial entitled, "Phase 3, Randomized, Open label, Multicentre, Repurpose, comparative trial of Efficacy, Safety and Tolerability of Almitrine and Ifenprodil vs standard care of therapy in the patients of hypoxemia of Acute Lung Injury (ALI) due to infections including patients of Covid-19".

Sir,

This is with reference to your application on the above mentioned subject. Your proposal was examined in consultation with the meeting to examine COVID-19 related proposal under accelerated approval process made in its meeting held on 20.04.2022, wherein you have presented the Phase III clinical trial proposal before the committee.

After detailed deliberation, the committee recommended that applicant should conduct Phase II trial in COVID-19 patients with statistically significant sample size and provide supportive documents including published literature for the Phase II trial for efficacy in COVID -19 patients as proof of concept.

Accordingly, applicant should submit revised protocol for Phase II CT to CDSCO for further review by the committee.

The recommendation of the Committee has been considered by this office.

You are therefore, requested to submit revised protocol for Phase II Clinical trial alongwith published supportive literature as recommended by the committee to this office for taking further necessary action in the matter.

Yours faithfully,

(Dr. V.G. Somani)
Drugs Controller General (India)