Original Research Article

Synthesis, anti cancer and anti fungal studies of new phenyl ethylene derivatives

Abstract: *Synthesis of 4-[Ethyl(4-methoxy-a-methylphenethyl)amino]butyl veratrate and their derivatives (4a-4f) are reported in this communication. Products were characterized by ^{1}HNMR, Carbon (^{13}C) NMR and Mass spectral data. Synthesized compounds of 4a-4f were screened for anti cancer and anti fungal studies and their results are presented. The main intermediate (3) was prepared from 4-methoxy phenyl acetone (1) using reductive amination with aqueous ethylamine in the presence of Sodium borohydride to get the compound (2). Further dehydrohalogenation of (2) with 4-bromobutanol and potassium carbonate at 80°C resulted in compound (3) which on further dehydration with p-toluene sulfonic acid under reflux conditions gave 4a to 4f.*

"Keywords": Anti cancer, Antifungal, Synthesis, 4-Methoxy phenyl acetone, derivatives and characterization

Introduction

Phenyl ethylamine derivatives were found with very good biological activities viz., anticancer, antifungal. In addition some of the phenyl ethylamine derivatives also act as a musculotropic antispasmodic agent with a direct action on the smooth muscle of the gastrointestinal tract (GIT) especially phenyl ethyl amine and ester are inhibiting the peristaltic reflex of the guinea-Pigilem and inhibiting sphincter of odi etc.

Besides the synthetic phenyl ethylamine and derivatives are also of great value in our daily life, Keeping in view, the author focused on the synthesis of phenyl ethylene derivatives. These scaffolds are being subjected to biological screenings like antifungal and anti cancer activities

Material and Methods:

Preparation of phenyl ethylene derivatives from 4-methoxy phenyl acetone (1) to final compounds (4a- 4f) used Chemicals, reagents and solvents are LR grade. Data interpretation used SA-Varian 400 MHz NMR for analysis of ^{1}HNMR and ^{13}C NMR. Chemical shifts values are reported in ∂(PPM) and deteriorated solvents $CDCl_3$and DMSO. Trimethyl silane (TMS) as a reference standard for NMR. The Multiplicity of spectra identified by following: Singlet (s), Doublet (d), Triplet (t), quarter (q), multiplet (m), broad (br), Doublet of doublet (dd).

The ESI/MS experiments were performed on aVelos Pro ion trap mass spectrometer from Thermo Scientific (San Jose, CA, U.S.A.). Elemental analysis for C, H and N used instrument vario EL.

Basic reaction completion in Laboratory identification used thin layer chromatography (TLC)

Performed anticancer studies in Stellixir Bio tech PVT Ltd, Bangalore. and Hele celline and MCF7 from NCCS, PUNE

Comment [MF1]: HELA

Comment [MF2]: CELLLINE

Anti fungal studies from Department of environmental science, GITAM institute of science. Visakhapatnam.

Results and Discussion:

The compounds are prepared by using the synthetic scheme shown below. The main intermediate (3) was prepared from 4-methoxy phenyl acetone (1) using reductive amination with aqueous ethylamine in the presence of Sodium borohydride to get the compound (2). Further dehydrohalogenation of (2) with 4-bromobutanol and potassium carbonate at 80°C resulted compound (3) which on further dehydration with p-toluene sulfonic acid under reflux conditions gave 4a to 4f. Products are characterized by ^{1}HNMR , ^{13}C NMR and Mass spectral data.[1-4]

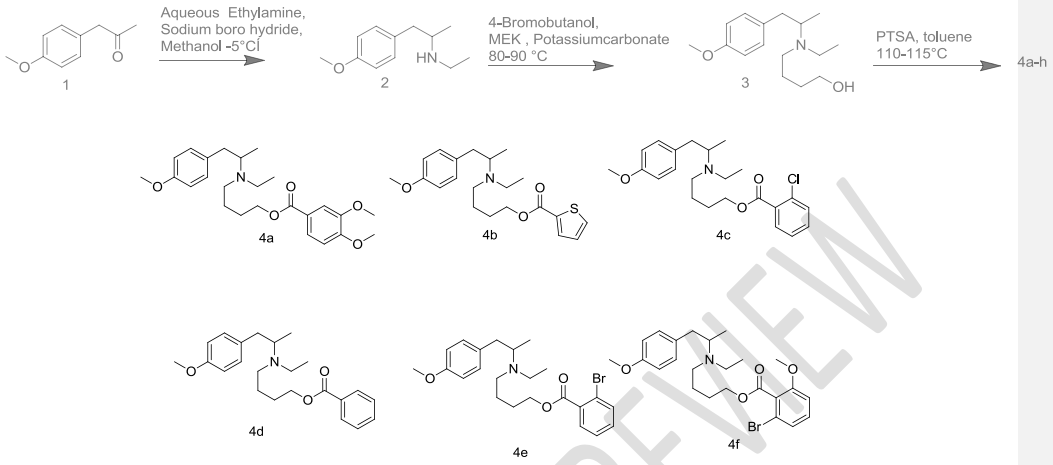
Anti Cancer activity:

MCF-7: MCF 7 is a human breast cancer cell line with estrogen, Progesterone and glucocorticoid receptors. It is useful for the in-vitro breast studies because they retained several ideal characteristics particular to mammary epithelium such as the processing of estrogen, in the form of Estradiol. It is the first human responding breast cancer cell line. It proves to be a suitable model cell line for breast cancer investigation worldwide. MCF 7 allows the researchers to use this cell line for bringing more light into breast cancer pathogenesis and treatment protocols through reliable in-vitro assay

Hela cells: Refer to a line of cells belonging to a strain that has been continuously cultured since 1951. Compared to other human cells, Hela cell were the only survive in-vitro. Hela cells are a common source of cross contamination with other cell line and suspected to cause of numerous instances of cell-line misidentification. Genome of Hela cell is highly unstable. Although these were the first cells that could be easily shared and multiplied in a lab setting.
Johns Hopkins has not expected any profited from the discovery or distribution of HeLa cells and he offered HeLa cells freely and widely for scientific research. Over the past years, Hela cell line has contributed to many medical breakthroughs, from research on the effects of zero gravity in outer space and the development of polio and COVID-19 vaccines, to the study of leukemia, the AIDS virus and cancer worldwide.

Antifungal activity: Aspergillus candidus is a common contaminant of grain dust and causes respiratory disease in human. The species is widely distributed in nature and grows on vegetation in the later stages of decay. *Aspergillus niger* is fungi, it is also common contaminated foods grapes, apricots, onions, and peanuts. It cause a disease called Black mold. *Aspergillus niger* is less likely to cause human disease than some other *Aspergillus* species. In extremely rare instances, humans may become ill, but this is due to a serious lung disease.

Synthetic pathway for title compound (4a-4f) Scheme-1



Experimental:

Preparation of N-Ethyl-1-(4-methoxyphenyl) propan-2-amine (2)

A Stirred solution of 4-methoxy phenyl acetone **1** (10.0 g, 0.16 mol) in methanol (60.0 mL, 6.0t), at room temperature, slowly add the aqueous ethylamine (25.0 mL, 2.5 t) into the mass then the reaction mass was Cooled to -3± 2°C. At the same temperature slowly add sodium borohydride portion wise (3.0 g, 0.01 mol) after that reaction mass was maintained for 1hr. Charge water (200.0 mL) and toluene (50.0 mL) into reaction mass, stired the reaction mass for 5 to 10 min and separated the toluene layer and water layer. After distillation of toluene layer to get the required N-Ethyl-1-(4-methoxyphenyl) propan-2-amine **2** was obtained.

Comment [MF3]: ,add slowly

Comment [MF4]: correct

Comment [MF5]: add slowly

Comment [MF6]: stirred

^{1}H NMR (DMSO d_6, 400 MHz):7.08 (s, 2H), 6.84 (s, 2H), 2.99 (s, 3H), 2.76-2.36 (m, 5H), 0.99-0.90 (m, 6H)Mass (M+H): 194.2, Weight : 8.0g Purity: NLT 95.0

Preparation of 4-(N-Ethyl-N-(1-(4-methoxyphenyl) propan-2-yl) amino) butan-1-ol (3)

A Stirred solution of N-Ethyl-1-(4-methoxyphenyl) propan-2-amine **(2)** 7.5 g, 4-bromobutanol (22.0 g) in toluene (30.0 mL), The resulting mixture was heated to 80±5°C for 24 to 30 hrs. After completion of reaction maintenance cool the reaction mass to 30±5°C then charge 10 mL of water into the mass and stir for 10 min. Separated the toluene layer and aqueous layer followed by distillation of toluene layer to get the 4-(N-Ethyl-N-(1-(4-methoxyphenyl) propan-2-yl)amino) butan-1-ol.

^{1}H NMR (DMSO d_6, 400 MHz):7.08-7.06 (d, 2H), 6.82-6.80 (d,2H), 4.51-4.49 (m, 1H), 3.70 (s, 3H), 3.36 (t, 3H), 2.88-2.83 (m, 1H), 2.73-2.68 (m, 1H), 2.43-2.29 (m, 4H), 1.38-1.37 (m, 4H), 0.95(t, 3H), 0.83-0.82 (d, 3H)

Mass (M+H): 266.2

General procedure for the synthesis of title compounds 4(a-h):
Dehydration of compound 4 and corresponding acid in-presence of PTSA and toluene at 110± 5°C for 20-24 hrs. After maintenance cool the reaction mass and charge the water and stir for 10 min and separate the organic layer distill the toluene completely. The o obtained crude by column chromatography eluting with 5-8 % DCM in ethyl acetate furnished the title compounds 4(a-h)

4-(Ethyl(1-(4-methoxyphenyl)propan-2-yl)amino)butyl 3,4-dimethoxybenzoate hydrochloride (4a)
^{1}H NMR ($CDCl_3$, 400 MHz):12.1-12.2 (brs, 1H), 7.68 (dd, 1H J 1.6),7.53 (d, 1H J 1.6), 7.15 (d, 1H J 0.8), 6.88 (d, 1H J 8.4), 6.83 (d, 2H, J 8.4), 4.37 (t, 2H J 6.4), 3.93 (s, 6H), 3.78 (s, 3H), 3.56 (d, 2H J 10.8), 3.22-3.08 (m,4H), 2.54 (t, 1H), 2.14-2.13 (m, 2H), 1.89 (t, 2H), 1.56-1.54 (m, 3H), 1.24 (d, 3H J 6)

^{13}C NMR: 9.88, 12.02, 21.19, 26.20, 36.20, 44.63, 45.95. 54.89, 55.72, 59.43, 63.07, 110.45, 111.62, 113.87, 122.02, 123.30, 127.68, 129.77, 148.31, 152.78, 158.40, 165.91.

Mass: M.Wt:429.2 (M+H: 430.2)
4-(Ethyl(1-(4-methoxyphenyl)propan-2-yl)amino)butyl thiophene-2-carboxylate (4b) :

^{1}HNMR :($CDCl_3$ 400 MHz): 7.83 (d, 1H J 7.2), 7.45-7.41 (m, 2H), 7.34-7.32 (m, 1H), 7.13 (d, 2H J 8.4), 6.82 (d,2H J 8.4), 4.38 (t, 2H J 6), 3.77 (s, 3H), 3.49-3.37 (m, 2H), 3.13-2.92 (m 4H), 2.54-2.48 (m, 1H), 2.06-2.00 (m, 2H), 1.90-1.85 (m, 2H), 1.42 (t, 3H J 8), 1.16 (d, 2H J 8).

Mass: M.Wt: 375 (M+1: 376)

4-(Ethyl(1-(4-methoxyphenyl)propan-2-yl)amino)butyl 2-chlorobenzoate (4c)

^{1}HNMR($CDCl_3$, 400 MHz): 8.04 (dd,2H J 8), 7.55 (t, 1H),7.43 (t, 2H), 7.06 (d, 2H J 8), 6.79 (d, 2H J 8), 4.29 (t, 2H), 3.76 (s,3H), 2.97-2.92 (m, 1H), 2.85-2.80 (m, 1H), 2.60-2.47 (m, 4H), 2.37-2.34 (m, 1H), 1.80-1.69 (m,2H), 1.59-1.53 (m, 2H), 1.051 (t, 3H J 8), 0.91 (d, 3H J 8).

Mass: M.Wt: 404 (M+2: 406)

4- (Ethyl(1-(4-methoxyphenyl)propan-2-yl)amino)butyl benzoate(4d)
^{1}HNMR, (CDCl3, 400 MHz): 7.91 (d, 1H J 8), 7.23-7.22 (m, 1H), 7.13-7.06 (m, 2H), 6.83-6.79 (m, 3H), 6.73-6.71 (m,1H), 4.38 (t, 1H), 3.75-3.70 (m, 4H), 3.61-3.46 (m, 4H), 3.12-3.00 (m, 2H), 2.60-2.49 (m, 1H), 2.06-2.01 (m, 3H), 1.42 (t, 3H), 1.25-1.016 (m, 3H).

Mass: M.Wt: 369 (M+1: 370)

4-(Ethyl(1-(4-methoxyphenyl)propan-2-yl)amino)butyl 2-bromobenzoate (4e)

^{1}HNMR ($CDCl_3$, 400 MHz): 7.80 (dd, 1H, J 2), 7.65 (dd, 1H J 1.2), 7.40-7.28 (m, 2H), 7.14 (d, 2H), 6.83 (d, 2H), 4.4 (t, 2H), 3.78 (s, 3H), 3.56-3.51 (m, 2H), 3.23-2.96 (m, 4H), 2.57-2.51 (m, 1H), 2.18-2.06 (m, 2H), 1.92-1.877 (m, 2H), 1.48 (t, 3H), 1.21 (d, 3H).

(^{13}C NMR , CDCl3): 10.43, 12.55, 21.81, 26.26, 36.73, 45.37, 49.40, 55.16, 59.45, 64.41, 114.09, 121.27, 127.25, 128.33, 130.20. 131.27, 132.03, 132.57, 134.14, 158.56, 166.14

Mass: M.Wt: 448 (M+2: 450)

4-(ethyl(1-(4-methoxyphenyl)propan-2-yl)amino)butyl 2-bromo-6-methoxybenzoate (4f)

^{1}HMR ($CDCl_3$, 400 MHz): 7.51 (d, 1H J 8), 7.29 (d, 1H), 7.13 (d, 2H), 6.90-6.87 (m, 1H), 6.83-6.81 (d, 2H J 0.8), 4.38 (t, 2H), 3.80 (s, 3H), 3.77 (s, 3H), 3.52-3.49 (m, 1H), 3.41-3.38 (dd, 1H), 3.19-2.96 (m, 4H),2.55-2.49 (m, 1H),2.10-2.03 (m, 2H), 1.91-1.86 (m, 2H), 1.44 (t, 3H), 1.18-1.17 (d, 3H).

(^{13}C NMR , CDCl3)10.72, 12.67, 22.10, 26.25, 36.89, 45.24, 49.39, 55.14, 55.61, 59.59, 64.65, 111.43, 114.03, 114.38, 116.21, 118.81, 128.71, 130.19, 132.76, 134.85, 158.47, 158.52, 166.04

Mass: 478 (M+2: 480)

Anti cancer activity: Anti Cancer activity of newly synthesized phenyl ethylene derivatives and calculated IC 50 values against MCF7 and HeLa celline are tabulated in Table 1. All the compounds are found to be showing good anti cancer activity . Some of the compounds 4d, 4e, and 4f showed high potential and remaining compounds showed moderate potential values.

Comment [MF7]: cell line

Table -1 List of compounds

S.No.	Compound name	Hele IC 50 (µG/mL)	MCF7 IC 50 (µG/mL)
1	4a	29.51	25.97
2	4b	38.07	22.41
3	4c	28.7	31.05
4	4d	44.5	37.18
5	4e	46.5	40.18
6	4f	67.1	55.01

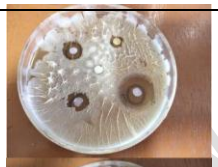
Antifungal Activity: The antifungal activities with zone of inhibition of all the newly synthesized 2-phenyl ethylene derivatives were evaluated in vitro against a wide variety of fungal species such as *Aspergillus niger* and *Aspergillus candidus*. All the compounds exhibits antifungal activity less than the reference standard *Pencillin G*. compound 4a and 4e exhibits good antifungal activity than remaining compounds.[5-8]

Table -2 Fungal species zone of inhibition

S.No	Name of the compound	Fungal species zone of inhibition (mm)	
		Aspergillus niger	Aspergillus candidus
1	4a	25	28
2	4b	12	14
3	4c	9	8
4	4d	10	8

5	4e	18	25
6	4f	14	12

Table : 3 Report On Antibiotic Assay With Fungi

S.No	SAMPLE NAME	GROWTH OF INHIBITION	PHOTO
1.	**4a, 4b, 4c,**	All have shown the zone of inhibition but 4a has shown very high zone of inhibition to *Aspergillus niger* and *Aspergillus candidus*	
2.	**4d, 4e, 4f**	All have shown the zone of inhibition but 4e has shown very high zone of inhibition to *Aspergillus niger* and *Aspergillus candidus*	

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Comment [MF9]: correct

Conclusion :

In the present study, we have reported the synthesis of 4-[Ethyl(4-methoxy-a-methyl phenethyl)amino]butyl veratrate and their novel derivatives were developed as a broad spectrum anticancer, antifungal agent. Results are suggest the utility of the these novel series against cancer and fungal, Still needed more studies to optimize these molecule to get the potent inhibitor, and our study is in progress towards development of new derivatives of the Skelton and will report in the due course.

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Comment [MF10]: