RESEARCH ARTICLE

Synthesis, Pharmacological Study of 3,5-Di- Substituted Isoxazole Derivatives as Anticancer Drug Against Breast Cancer Cell Line Followed by Computational Study

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ABSTRACT

Objective: Cancer has remained a challenge to the healthcare system due to changes in eating habits and lifestyle, tremendous side effects of chemotherapy, and resistance to existing treatment due to mutations, which creates difficulty in identifying specific targets.

Chemistry and Methodology: In this study, equimolecular amounts of reagents were used to synthesize substituted isoxazoles from alpha-beta unsaturated intermediates.

Result: Compounds were collected in varying amounts of yield. Spectroscopic data supports the formation of compounds.

Compound Id shows GI50 of 46.3 µg/mL against MDA-MB-231.

Conclusion: The effective synthesis of novel derivatives has opened up exciting new avenues for medicinal research; one of these compounds has moderate anticancer properties.

Keywords: Heterocycles, Isoxazole, Anticancer activity.

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INTRODUCTION

Cancer is one of the prime reasons for death in the world, noted for nearly 10 million mortalities in 2020.[1] The reported cancer incidence in India in 2022 was 19–20 lakh, whereas the actual number of cases is expected to be 1.5 to 3 times higher. A study titled "Call for Action: Delivering excellent cancer treatment accessible and cost-effective to Indian Population" was undertaken by FICCI and EY.[2,3] Therefore, there is a necessity for the discovery by doing revision on developed agents and improvement of novel antitumor drug molecules which could be more efficient, less toxic and would be targeted one with promising biological effect.[4,5] In today's scenario, nitro heterocyclic ring like isoxazole has gained much therapeutic importance in antibiotics and is yet to be explored as a hybrid motif fused with various regents as an anticancer agent. Hence it is aimed to synthesize newer substituted isoxazole derivatives.[6]

MATERIAL AND METHODS

Proton nuclear magnetic resonance (^{1}H-NMR) spectra:Brooker 400, 500 spectrometers. IR spectra obtained on Brooker spectrophotometer. The electrothermal melting point analyzer was used to determine the uncorrected melting points. Data from a Waters TSQ70 Spectrometer in the United States was used to generate the mass spectra.[7,8]

Method

Step I: In a cold environment, chalcone derivatives were made by swirling an equal amount of aldehyde and ketone derivatives in 40 to 70% basic alcohol.[9,10]

Step II: Equimolar quantity of chalcones and hydroxylamine hydrochloride were irradiated with microwave energy at 70 to 80°C, 210 W for 5 to 20 minutes to give novel isoxazole derivatives.[11,12] After the reaction has cooled, the cold water added to the liquid. Recrystallization of the product done after filtration and drying of the precipitate (Scheme 1, Table 1).[13,14]

Anticancer Activity

Cells were sown into 96-well plates and maintained at the specified temperature and pressure according to the standard methodology. Conditions (370°C, 5% CO_2, and 95% humidity)

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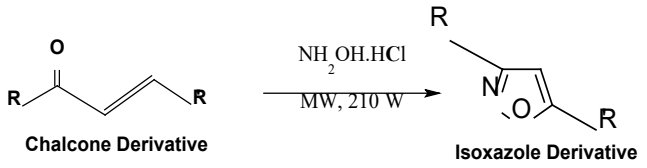
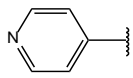
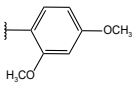
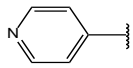
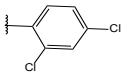
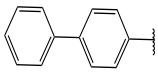
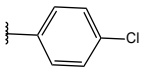
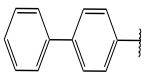
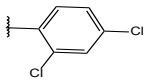
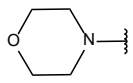
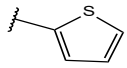
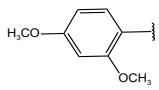
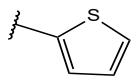
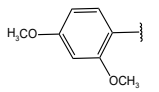
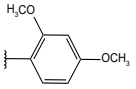
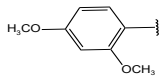
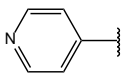


Scheme 1: Synthesis of isoxazole derivatives

Table 1: Substituents[15,16]

S. No	*R*	*R'*	*Mol. formula*	*Mol. Weight*
Ia			$C_{16}H_{14}N_2O_3$	282.29396
Ib			$C_{14}H_8Cl_2N_2O$	291.13212
Ic			$C_{21}H_{14}ClNO$	331.79496
Id			$C_{21}H_{13}Cl_2NO$	366.24002
Ie			$C_{11}H_{12}N_2O_2S$	236.29018
If			$C_{15}H_{13}NO_3S$	287.33362
Ig			$C_{19}H_{19}NO_5$	341.35786
Ih			$C_{16}H_{14}N_2O_3$	282.29396

were maintained by microplate overnight. A 1-mg/mL stock solution of the substance under test was kept refrigerated. A portion of the frozen concentrates was also used to make drug dilutions with concentrations of 100, 200, 400, and 800 µg/mL. Drug concentrations were 10, 20, 40, and 80 µg/mL, respectively, reached by adding 10 µL of each solution to 90 µL of media now current in a micrometre well. An additional 48 hours of plate incubation was completed. The cells are fixed in place by adding 10% TCA and then incubating at 4°C for 60 minutes. Step two involves discarding the supernatant, washing by tap water 5 times, and then letting it air dry. Then, 50 µL of a sulphorhodamine B solution containing 0.4% w/v in 1% CH_3COOH was added to cell and left to incubate at ambient temperature for 20 minutes.[17,18]

Docking Study

In-silico docking was carried out by using auto dock tool byusing EGFR target having PDB id 1M17 selected for the study.[19,20] The common methodology followed by doing a proper literature review of the Auto dock procedure.[21,22] Binding energies were calculated by generating energy-minimized confirmations. Prior to that, target preparation was done. Finally, the Ramachandran plot is drawn.[23,24]

Table 2: Physical properties

S. No.	*Mol Formula*	*Yield (%)*	*Reaction time in microwave*	*RF Value*	*M.P. (°C)*
1	$C_{16}H_{14}N_2O_3$	56	18 minutes	0.56	152–154
2	$C_{14}H_8Cl_2N_2O$	64	10 minutes	0.8	150–152
3	$C_{21}H_{14}ClNO$	52	22 minutes	0.65	164–168
4	$C_{21}H_{13}Cl_2NO$	50	20 minutes	0.69	160–162
5	$C_{11}H_{12}N_2O_2S$	66	12 minutes	0.71	148–150
6	$C_{15}H_{13}NO_3S$	48	15 minutes	0.60	156–158
7	$C_{19}H_{19}NO_5$	53	17 minutes	0.59	168–170
8	$C_{16}H_{14}N_2O_3$	55	20 minutes	0.57	166–168

RESULTS

Physical findings after synthesis are specified in Table 2.

Characterisation

4-[5-(2,4-dimethoxyphenyl)isoxazol-3-yl]pyridine (Ia)

IR: 2930-3050 (CH str), 1500–1600(C=C str), 1403 (C=N str) 1300 (CH ben.),1015 (C-O str), 640 (C-O isoxa).

^{1}H NMR (CHCl3) 7.74–7.70 (m, 2H), δ 8.67 – 8.63 (m, 2H), 6.77 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.66 (d, *J* = 8.3 Hz, 1H), 6.48 (d, *J* = 2.5 Hz, 1H), 3.88 (s, 3H), 3.82 (s, 3H). M/Z:282.10

*4-[5-(2,4-dichlorophenyl) isoxazol-3-yl] pyridine (*Ib*)*

IR: 3110 (CH str aromtic), 1680 (C=N pyridine), 1470 (C=C str), 1110 (C-O ring str),680 (C-Cl str).

NMR:δ 8.67 – 8.63 (m, 2H), 7.74–7.69 (m, 3H), 7.53 (d, *J* = 2.2 Hz, 1H), 7.35 (dd, *J* = 8.2, 2.0 Hz, 1H). M/Z: 292.0

3-biphenyl-4-yl-5-(4-chlorophenyl) isoxazole (Ic*)*

IR: 3070 (CH str),1570 (C=C str)1610 (C=N str), 750 (C-Cl str).

NMR- δ 7.67 – 7.62 (m, 4H), 7.62 – 7.56 (m, 4H), 7.47–7.41 (m, 5H), 7.40–7.34 (m, 1H). M/Z :331.08

*3-biphenyl-4-yl-5-(2,4-dichlorophenyl) isoxazole (*Id*)*

IR: 3050 (CH str),1550(C=C str)1653 (C=N str), 750(C-Cl str).

NMR: δ 7.71 (d, *J* = 8.3 Hz, 1H), 7.67 – 7.56 (m, 6H), 7.53 (d, *J* = 2.1 Hz, 1H), 7.47 – 7.41 (m, 2H), 7.40 – 7.33 (m, 2H). M/Z: 367.03

4-[5-(2-thienyl) isoxazol-3-yl] morpholine(Ie*)*

IR:3150 (CH str), 1600(C=C str), 1360 (CH bending), 1575 (C-N str), 1160(C-O bending).

NMR δ 7.45–7.38 (m, 2H), 7.10 (dd, *J* = 7.5, 4.9 Hz, 1H), 6.03 (s, 1H), 3.85 – 3.76 (m, 4H), 3.59 (ddd, *J* = 14.3, 6.0, 4.0 Hz, 2H), 3.50 (ddd, *J* = 14.3, 6.2, 4.0 Hz, 2H). M/Z: 236.06

*3-(2,4-dimethoxyphenyl)-5-(2-thienyl) isoxazole (*If*)*

IR:2740-3030 (Ch str), 1610 (CH str), 1630 (C=N str), 1040 (C-O str in morpholine).

NMR δ 7.69 (d, *J* = 8.9 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.14 – 7.09 (m, 1H), 7.02 (s, 1H), 6.81 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.54 (d, *J* = 2.4 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 3H). M/Z: 287.06

3,5-bis(2,4-dimethoxyphenyl)isoxazole(Ig)

IR 2810–3170 (CH str), 1648 (C=N str), 1130 (C-O Srt iso), 775(C-O methox)

NMR δ 8.69 – 8.65 (m, 2H), 7.74–7.68 (m, 3H), 6.81 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.54 (d, *J* = 2.5 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 3H). M/Z: 341.13

*4-[3-(2,4-dimethoxyphenyl)isoxazol-5-yl]pyridine(*Ih*)*

IR 2950–3050 (CH str), 1628 (C=N), 1130 (C-O Srt iso), 790 (C-O methox)

NMR δ 8.69 – 8.65 (m, 2H), 7.74–7.68 (m, 3H), 6.81 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.54 (d, *J* = 2.5 Hz, 1H), 3.86 (s, 3H), 3.82 (s, 3H). M/Z: 282.10

DISCUSSION

Derivatives were successfully synthesized from benzylideneacetophenone in moderate to good yield. IR, ^{1}H-NMR and mass spectra are in agreement with structural aspects of test compounds. The formation of isoxazole is confirmed on the basis of the same.

New derivatives tested for anti-neoplastic activity by SRB assay method.[24] Percent inhibition was calculated by performing three sets of the experiment at 10 to 80 µg/mL. An average value is taken from 3 sets and a growth curve is obtained by putting these values in the y-axis (Figure 1).

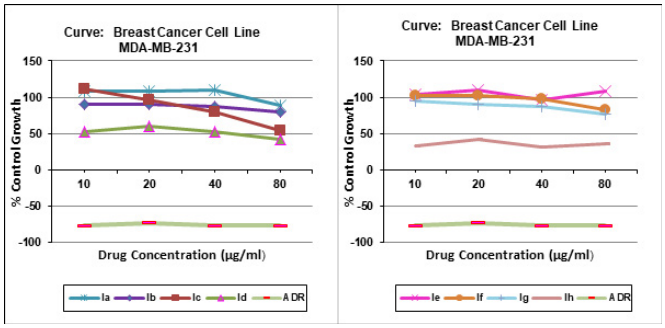


Figure 1: Growth curve of compounds with standard ADR

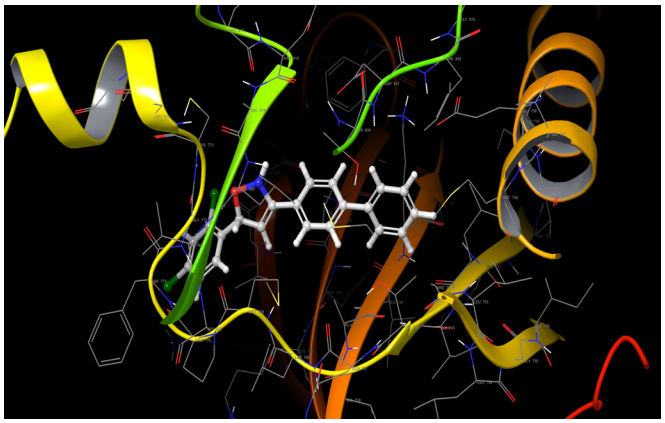


Figure 2: 3d Docking pose of Id

Anticancer Activity

Anti-neoplastic activity taken against MDA-MB 231 cell line. Compound 1d show the lowest GI50 value which is 46.3 µg/mL, calculated from the Figure 1. Id molecules have biphenyl group substituted in 3rd position of isoxazole, whereas o-p dichloro phenyl placed at 5th position of main ring, respectively. When docking interactions were studied, Id ligand molecules showed good binding than the doxorubicin drug. The quantity of amino acids involved in hydrophobic reactions is greater than doxorubicin. The same amino acid involved in hydrophobic interaction from standard drug and test molecule is Leu694A. Other amino acids are Val 702A, Ala 719, Lys 721, whereas hydrogen bonding is seen with amino acids Thr766A and Met769A of the target active site, which are different than that of doxorubicin indicated in Figure 2.

CONCLUSION

The compound was successfully synthesized using a microwave and tested for viability against the MDA-MB 231 cell line. Compounds with diphenyl and chloro substitution showed the lowest inhibitory concentration. Amongst eight derivatives, compound1d shows the moderate GI50 value (46.3 µg/mL). It has an electron-withdrawing group on 5th place of the target ring and diphenyl substitution on 3rd place of the ring. Molecular docking interactions show that these compounds have good binding in the target protein. Such compounds with other hydrophobic and electron-withdrawing substituents on the ortho para position of the isoxazole scaffold have better future scope for potential EGFR inhibitors.

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