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Effectiveness of N-[3-Chloro-2-(Substituted)-4-Oxazetidin-1-Yl]-4-(1h-Indol-3-Yl) Butanamide Derivatives as A-Amylase Inhibitors

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Different N-[3-Chloro-2-(substituted)-4-oxazetidin-1-yl] The cyclo condensation of Schiff bases with tri ethyl amine and chloroacetyl chloride has produced derivatives of 4-(1H-indol-3-yl) butanamide. Based on their spectral data, the structures of the recently synthesized chemicals have been determined. The inhibitory activity of α-amylase was assessed for each drug. Every compound under investigation showed notable activity that was on line with the industry norm.

Keywords: N-[4-hydroxy-3-methoxy phenyl)-4-oxazetidin-1-yl] -4-(1H-indol-3-yl) butanamide, inhibitor of α-amylase

INTRODUCTION

Commonly referred to as β-lactam, azetidin-2-ones are four-membered heterocyclic compounds with a wide range of physiological properties. Since the antibacterial qualities of cephalosporins, cephamycins, and penicillins were discovered, azetidin-2-one moiety research has been conducted intensively worldwide. N-[3-Chloro-2-(4-hydroxy-3-methoxy phenyl)-4-oxazetidin-1-yl] The β-lactam moiety is present in derivatives of 4-(1H-indol-3-yl) butanamide. Numerous azetidin-2-one derivatives have recently been shown to possess a variety of pharmacological properties, including antimicrobial (Keri et al., 2010), antitubercular, anti-inflammatory, anticancer, anti-HIV, anti-parkinsonian, anti-diabetic, and vasopressin V1a antagonist (Mehta et al., 2010).

Experimenting

Uncorrected melting points were measured in open capillaries. Thin layer chromatography with an 8:2 methanol:chloroform mobile phase was used to track the progress of the reactions. By exposing a dry plate in an iodine chamber, the location was visible. A Brucker-400 MHz spectrometer was used to measure 1HNMR spectra, and a JASCO FT/IR-140 spectrophotometer was used to record IR spectra in KBr disc. A Shimadzu device was used to record the mass spectra. The chemicals of reagent grade were bought before being used, after being further cleaned from commercial sources.

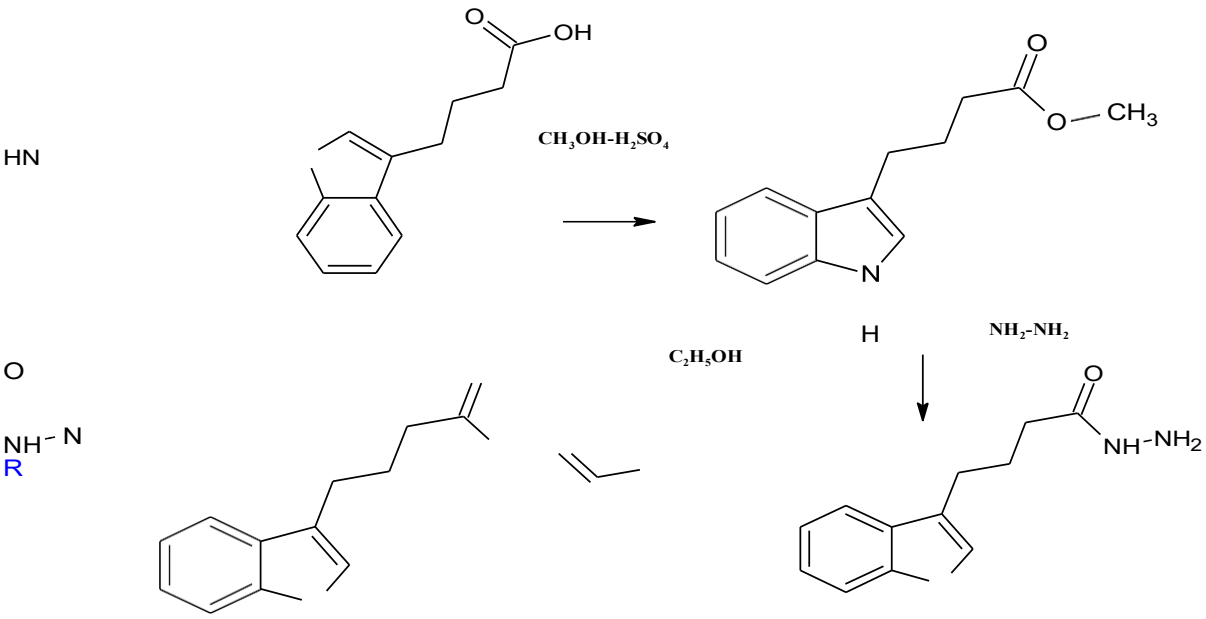
Indole-3-butyric acid ester synthesis Methanol (6.4 ml), dichloromethane (100 ml), concentrated sulfuric acid (5 drops), and 20.4 g (0.1 mole) of indole-3-butyric acid were refluxed for 5 hours before being cooled to 5°C. The items were added to 100 milliliters of ice water. To obtain the crude product, the organic layer at the bottom was separated, and dichloromethane was distilled out. The pure chemical that crystallizes from methanol was obtained through high vacuum distillation of this crude product. A single spot on the TLC plate was used to determine the indole-3-butyric acid ester's purity. Methanol:chloroform (3:1) was the solvent system employed (Holla et al., 2004; Mohan et al., 2004).

Indole-3-butyric acid hydrazine Synthesis

Hydrazine hydrate (99%-2 ml) was added in drops to the indole-3-butyric acid ester (21.7g; 0.1 mole) in ethanol (20 ml) while being constantly stirred. The mixture was then refluxed for four hours. The mixture was poured onto crushed ice once it had cooled. utilized a 3:1 ratio of methanol to chloroform (Govindarajan and Bhat, 2002).

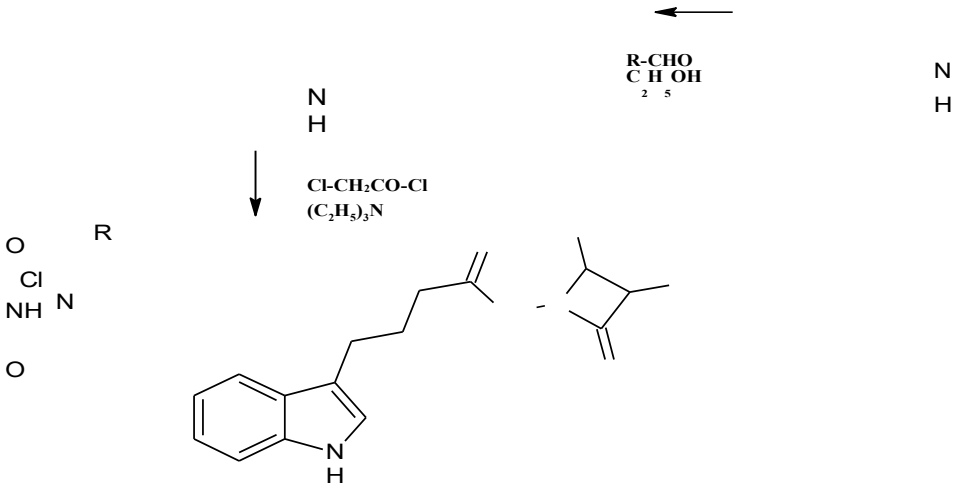
Manufacturing of Schiff's base .In a 100 ml beaker, 0.1 moles of indole-3-acid hydrazide and 0.1 moles of arylaldehyde were dissolved in 15 ml of ethanol and refluxed for two hours. After cooling the reaction mixture, the solid that had formed was filtered out, cleaned with cold ethanol, and then recrystallized from ethanol. A single spot on the TLC plate indicated the product's purity. Methanol:chloroform (3:1) was the solvent system in use (Udupi et al., 2000).

Scheme



Filtration, drying, and recrystallization of the isolated solid from methanol were performed. A single spot on the TLC plate was used to determine the compound's purity. The system of solvents

Substituted azetidin-2-one synthesis A 100 ml beaker was filled with a solution of Schiff base (0.01 moles) in DMF. Trimethylamine (1 ml) and chloroacetyl chloride (0.01 mole; 1.12 ml) were gradually added to it. After that, the mixture was put in a microwave oven set at 20% power for three to four minutes. Lastly, ice-cold water was used to dilute the mixture. Filtration was used to isolate the solid product, which was then recrystallized from ethanol. A single spot on the TLC plate indicated the product's purity. Methanol: chloroform (3:1) was the solvent system utilized (Bhat et al., 2007).



BIOLOGICAL ACTIVITY

α-amylase inhibitory activity

In a test tube, 1 ml of test drug solution is combined with 1 ml of 1% w/v soluble starch solution. Various concentrations of the test drug solution are ingested in 1 milliliter. To every tube, add 1 ml of the enzyme solution, and let it react at 25°C for three minutes. Each tube receives 1 milliliter of 3, 5-dinitro salicylic acid. A boiling water bath was used to heat the contents for ten to fifteen minutes. The production of maltose was determined by the conversion of 3, 5-dinitro salicylic acid to 3-
5-nitro salicylic acid amino acid. At 540 nm, this reaction—which results in a color shift from orange to red—is detected in comparison to the blank. The following formula was used to calculate the percentage of inhibition (Volgel HG, 2003).

$$\% \text{ inhibition} = \frac{\text{Control - test}}{\text{Control}} X100$$

RESULTS AND DISCUSSION

N-[3-Chloro-2-(Substituted)- 4-oxazetidin-1-yl] is the synthesis As specified in the plan, -4-(1H-indol-3-yl) butanamide derivatives were produced. A straightforward synthetic process was used to create the compounds, which began with indole-3 butyric acid. Figure 1 showed the general structure of the recently synthesized derivative. Table 1 provided the compounds' physical characteristics.

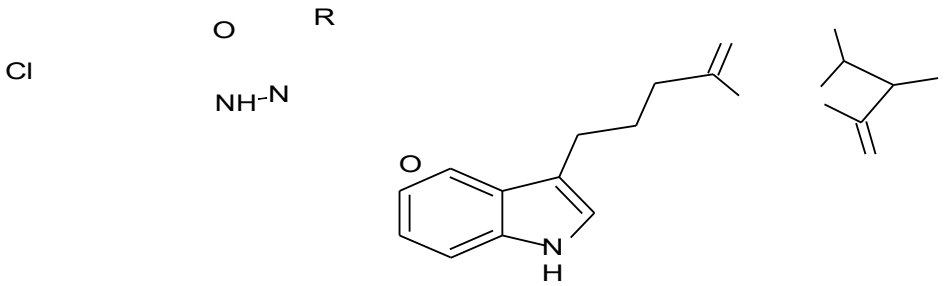


Fig 1: General structure of newly synthesized compounds

Table 1: Physical characterization of newly synthesized compounds

S. No.	Compound Code	R	Molecular Formula	Molecular Weight	Melting Point	% yield	R_f value
1	AZ1	3-$OCH_3$4-OHC_6H_5	$C_{22}H_{22}ClN_3O_4$	427.881	54	72%	0.58
2	AZ1	3-$NO_2C_6H_5$	$C_{21}H_{19}ClN_4O_4$	426.853	63	78%	0.80
3.	AZ3	4-Cl C_6H_5	$C_{21}H_{19}Cl_2N_3O_2$	416.300	76	88%	0.88
4.	AZ4	4-$OCH_3C_6H_5$	$C_{22}H_{22}ClN_3O_3$	411.881	87	63%	0.65
5.	AZ5	4-OHC_6H_5	$C_{21}H_{20}ClN_3O_3$	397.855	78	86%	0.78

Characterization of the newly synthesized compounds

UV, IR, H1NMR, and mass spectrum data have been used to establish the structures of planned and manufactured compounds. The distinctive peaks of recently produced chemicals were seen in IR spectra. The δ scale was used to report chemical changes. The molecular weight of the produced molecules was validated using mass spectral data.

N-[3-Chloro-2-(4-hydroxy-3-methoxy phenyl)-4-oxazetidin-1-yl]-4-(1H-indol-3-yl) butanamide (AZ_1). 752 (C–Cl); mp 54°C; Rf (0.58); λmax (295); IR (KBr) v (cm-1); 3367 (Ar-NH); 1595 (C=0); 1718 (β-lactam); 1310 (Sec. Amine); 1H NMR (CDCl3) δ 9.5 (-NH-); 7.0 to 8.0 (Ar-H); 6.8 (β lactam); 3.7 (-OCH3). Yield 72%.

N-[3-Chloro-2-(3-nitrophenyl)-4-oxazetidin-1-yl]-4-(1H-indol-3-yl) butanamide (AZ_2): 78% yield; mp 63°C; Rf (0.80); λmax (266); IR (KBr) v (cm-1); 3364 (Ar-NH); 1450 (NO2); 1590 (C=0); 1716 (β-lactam); 1305 (Sec. Amine); 749 (C–Cl); 1H NMR (CDCl3) δ 9.4 (-NH-); 8.4 (-OH); 7.0 to 8.0 (Ar-H); 6.8 (β lactam).

***In vitro* α-amylase inhibitory activity:**

N-[3-Chloro-2- (Substituted)-4-oxazetidin-1-yl] All of the recently synthesized The α-amylase inhibitory activity of 4-(1H-indole-3-yl) butanamide derivatives was assessed, and the percentage of inhibition for each concentration ranged from 50 µg/ml to 800 µg/ml. When compared to the control, the percentage of inhibition of the standard acarbose was also computed and found to be 91.58 percent at the

N-[3-Chloro-2-(4-Chlorophenyl)-4-oxazetidin-1-yl]-4-(1H-indole-3-yl) butanamide (AZ_3):88% yield; mp 76°C; Rf (0.88); λmax (285); IR (KBr) v (cm-1); 3372 (Ar-NH); 1592 (C=0); 1728 (β-lactam); 1306 (Sec. Amine); 755 (C–Cl); 1H NMR (CDCl3) δ 9.5 (-NH-); 8.3 (-OH); 7.0 to 8.0 (m, 13H, Ar-H); 6.7 (β lactam).

N-[3-Chloro-2-(4-methoxyphenyl)-4-oxazetidin-1-yl]- 4-(1H-indol-3-yl) butanamide (AZ_4): Yield 63%; mp 87°C; Rf (0.65); λmax (282); IR (KBr) v (cm-1); 3358 (Ar-NH); 1594 (C=0); 1731 (β-lactam); 1311 (Sec. Amine); 745 (C–Cl); 1H NMR (CDCl3) δ 9.4 (-NH-); 8.4 (-OH); 7.0 to 8.0 (Ar-H); 6.8 (β lactam); 3.7 (-OCH3).-[3-Chloro-2-(4-hydroxyphenyl)-4-oxazetidin-1-yl]- 4-(1H-indol-3-yl) butanamide (AZ_5):
86% yield; mp 78°C; Rf (0.78); λmax (290); IR (KBr) v (cm-1); 3368 (Ar-NH); 1590 (C=0); 1712 (β-lactam); 1302 (Sec. Amine); 754 (C–Cl); 1H NMR (CDCl3) δ 9.4 (-NH-); 8.4 (-OH); 7.0 to 8.0 (Ar-H); 6.8 (β lactam).

concentration of 800 g/ml. AZ3, one of the recently synthesized compounds, had strong α-amylase inhibitory efficacy at concentrations ranging from 50 µg/ml to 800 µg/ml, which is comparable to the usual medication acarbose. At each of the five doses, ranging from 50µg/ml to 800µg/ml, AZ5 and AZ2 demonstrated moderate α-amylase inhibitory action.
Table 2 provided the drugs' in vitro α-amylase inhibitory efficacy (% inhibition).

Table 2: Inhibitory activity of α-amylase in vitro (% inhibition)

50µg/ml, 100µg/ml, 200µg/ml, 400µg/ml, and 800µg/ml of compound

14.40 0.43 24.50 0.76 30.51 0.64 41.86 0.60 AZ1 9.18 0.64

AZ2 29.65 0.65 = 42.29 0.64 = 51.61 0.54 = 58.56 0.75 = 70.57 0.31

AZ3 38.24 0.56 48.66 0.65 59.48 0.72 71.09 0.59 89.49 0.70

10.59□**�**0.55 24.20±0.56 33.41±0.47 41.66±0.40 50.35±0.61 AZ4

AZ5 22.83₱0.89 33.05₱1.03 44.62₱0.46 51.76₱0.53 61.97₱1.12

40.73□**�**1.39 49.34±1.04 63.48±0.91 72.56±1.22 91.58±1.89 Regular Acarbose

The mean SEM of three parallel measurements is used to provide the percentage inhibition values.

FINAL RESULTS

The novel N-[3-Chloro-2- (Substituted) in this study -4-oxazetidin-1-yl -(1H-)

The in vitro α-amylase inhibitory activity of indol-3-yl) butanamide derivatives was assessed. The α-amylase in this family of chemicals is promising. inhibitory action and are successfully employed as lead compounds in the creation of new drugs. To learn more about the pharmacological significance of this class of chemicals, more research is being conducted.

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The mean SEM of three parallel measurements is used to provide the percentage inhibition values.

CONCLUSION

The novel N-[3-Chloro-2- (Substituted) in this study -4-oxazetidin-1-yl] -4-(1H-

The in vitro α-amylase inhibitory activity of indol-3-yl) butanamide derivatives was assessed. The α-amylase in this family of chemicals is promising.
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All the newly synthesized N-[3-Chloro-2-(Substituted)-4-oxazetidin-1-yl] -4-(1H- indole-3-yl) butanamide derivatives were evaluated for their α-amylase inhibitory activity and the percentage of inhibition for all the concentrations ranging from 50 µg/ml to 800 µg/ml was calculated. The percentage of inhibition of the standard acarbose was also calculated and it was found to be 91.58 % at the concentration 800 µg/ml comparing with control. Among the newly synthesized compounds AZ3 showed high α-amylase inhibitory activity at 50µg /ml to 800µg/ml concentration comparable with standard drug acarbose. AZ5 and AZ2 showed moderate α-amylase inhibitory activity at all the five concentrations ranging from 50µg/ml to 800µg/ml.

In vitro α-amylase inhibitory activity (% inhibition) of the compounds was given in table 2.

Table 2: In vitro α-amylase inhibitory activity (% inhibition)

Compound	50µg/ml	100µg/ml	200µg/ml	400µg/ml	800µg/ml
AZ1	9.18±0.64	14.40±0.43	24.50±0.76	30.51±0.64	41.86±0.60
AZ2	29.65±0.65	42.29±0.64	51.61±0.54	58.56±0.75	70.57±0.31
AZ3	38.24±0.56	48.66±0.65	59.48±0.72	71.09±0.59	89.49±0.70
AZ4	12.59±0.55	24.20±0.56	33.41±0.47	41.66±0.40	50.35±0.61
AZ5	22.83±0.89	33.05±1.03	44.62±0.46	51.76±0.53	61.97±1.12
Standard Acarbose	40.73±1.39	49.34±1.04	63.48±0.91	72.56±1.22	91.58±1.89

% inhibition values are given as mean ±SEM of three parallel measurements

CONCLUSION

In the present study, new N-[3-Chloro-2-(Substituted) -4-oxazetidin-1-yl] -4-(1H-indol-3-yl) butanamide derivatives were synthesized and evaluated for in vitro α- amylase inhibitory activity. This class of compounds is having promising α-amylase inhibitory activity and effectively utilized as lead molecules for drug development. Further studies on this class of compounds are in progress for getting more information on pharmacological importance.

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