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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ABSTRACT

Various N-[3-Chloro-2-(substituted)-4-oxazetidin-1-yl]-4-(1H-indol-3-yl) butanamide derivatives have been synthesized by the cyclo condensation of Schiff bases with tri ethyl amine and chloro acetyl chloride. The structures of the newly synthesized compounds have been established on the basis of their spectral data. All compounds were evaluated for a-amylase inhibitory activity. All compounds investigated exhibited significant activity, comparable to that of the standard used.

Keywords: *N-[3-Chloro-2-(4-hydroxy-3-methoxy phenyl)-4-oxazetidin-1-yl]-4-(1H-indol-3-yl) butanamide, a-amylase inhibitor.*

INTRODUCTION

Azetidin-2-ones are four membered heterocyclic compounds, commonly known as β-lactam, having a wide variety of physiological functions. The research on azetidin-2-one moiety has done extensively throughout the world as a result of the discovery of the antibacterial properties of penicillins, cephalosporins and cephamycins. N-[3-Chloro-2-(4-hydroxy-3-methoxy phenyl)-4-oxazetidin-1-yl]-4-(1H-indol-3-yl) butanamide derivatives contain β-lactam moiety. Recently, many azetidin-2-one derivatives were reported to have various pharmacological activities such as antimicrobial (Keri *et al.*, 2010), antitubercular, anti-inflammatory, antitumor, anti-HIV, anti parkinsonian, anti-diabetic and vasopressin V1a antagonist (Mehta *et al.*, 2010).

Experimental

Melting points were taken in open capillaries and are uncorrected. Progress of reactions was monitored by thin layer chromatography using methanol: chloroform (8:2) as mobile phase. The spot was visualized by exposing dry plate in iodine chamber. IR spectra were recorded in KBr disc on a JASCO FT/IR-140 spectrophotometer and ^{1}HNMR spectra was measured on a Brucker-400 MHz spectrometer. The Mass spectra were recorded on a Shimadzu instrument. The reagent grade chemicals were purchased from the commercial sources and further purified before use.

Synthesis of Indole-3-butyric acid ester

A mixture of 20.4 g (0.1 mole) of indole-3-butyric acid, methanol (6.4ml), dichloromethane (100ml) and concentrated sulphuric acid (5 drops) was refluxed for 5 hrs and cooled to 5°C. The contents were poured into ice water (100 ml). The organic layer at the bottom was separated and dichloromethane was distilled off to get the crude product. The high vacuum distillation of this crude product afforded the pure compound which is crystallized from methanol. The purity of the indole-3-butyric acid ester was established by single spot on the TLC plate. The solvent system used was methanol: chloroform (3:1) (Holla *et al.*, 2004; Mohan *et al.*, 2004).

Synthesis of Indole -3-butyric acid hydrazine

To the indole-3-butyric acid ester (21.7g; 0.1 mole) in ethanol (20 ml), hydrazine hydrate (99% - 2 ml) was added in drops with constant stirring and the mixture was refluxed for 4 hours. After cooling, the solution was poured on to crushed ice. The solid separated was filtered, dried and recrystallized from methanol. The purity of the compound was established by single spot on the TLC plate. The solvent system

used was methanol: chloroform (3:1) (Govindarajan and bhat, 2002).

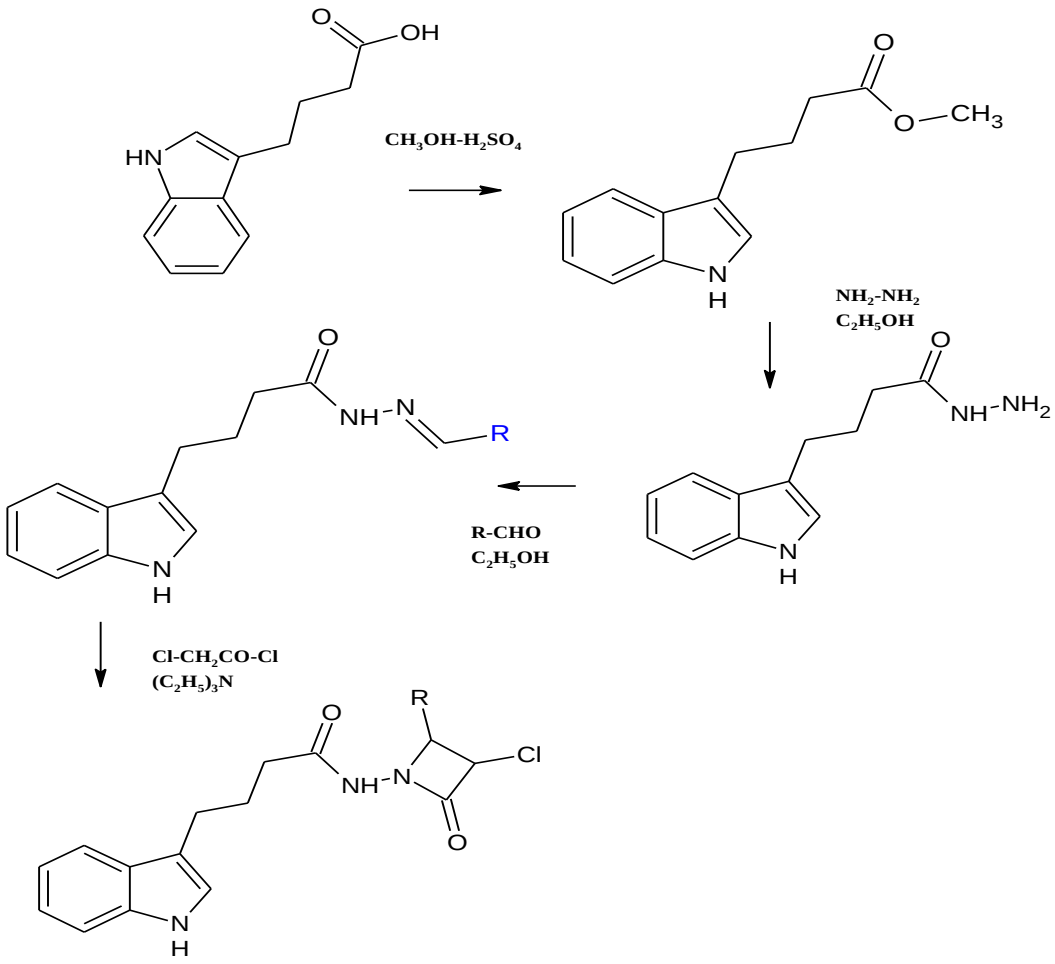
Synthesis of Schiff's base

Indole-3-acid hydrazide (0.1 mole) and arylaldehyde (0.1 mole) were dissolved in ethanol (15ml) in a 100 ml beaker and refluxed for 2 hrs. The reaction mixture was cooled and the solid formed was separated by filtration, washed with cold ethanol and recrystallised from ethanol. Purity of the product was established by single spot on the TLC plate. The solvent system used was methanol:chloroform (3:1) (Udupi *et al.,* 2000).

Synthesis of substituted azetidin-2-ones

A mixture of Schiff base (0.01 moles) in DMF was taken in a 100 ml beaker. To it chloroacetylchloride (0.01 mole; 1.12ml) and trimethylamine (1ml) were added slowly. Then the mixture was placed inside a microwave oven for about 3-4 minutes at a power level of 20%. Finally the mixture was diluted with ice cold water. The solid product obtained was separated by filtration and recrystalized from ethanol. Purity of the product was established by a single spot on the TLC plate. The solvent system used was methanol: chloroform (3:1) (Bhat *et al.*, 2007).

Scheme



BIOLOGICAL ACTIVITY

α-amylase inhibitory activity

1 ml of 1% w/v soluble starch solution is mixed with 1 ml of test drug solution in a test tube. 1 ml of the test drug solution is taken in different concentrations. To each tube add 1 ml of enzyme solution and left to react for 3 minutes at 25°C. 1 ml of 3, 5-dinitro salicylic acid is added on each tube. The contents were heated for 10 to 15 minutes on a boiling water bath. The generation of maltose was quantified by the reduction of 3, 5-dinitro salicylic acid to 3-

amino 5-nitro salicylic acid. This reaction (corresponding to color change from orange to red) is measured at 540 nm against the blank. The percentage of inhibition was determined by using the following formula (Volgel HG, 2003).

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

RESULTS AND DISCUSSION

The synthesis N-[3-Chloro-2-(Substituted)-4-oxazetidin-1-yl]-4-(1H-indol-3-yl) butanamide derivatives were done as outlined in the scheme. Compounds were synthesized by a simple synthetic pathway starting form indole -3 butyric acid. General structure of the newly synthesized derivative was given in **fig 1**. Physical characterizations of the compounds were given in **table 1**.

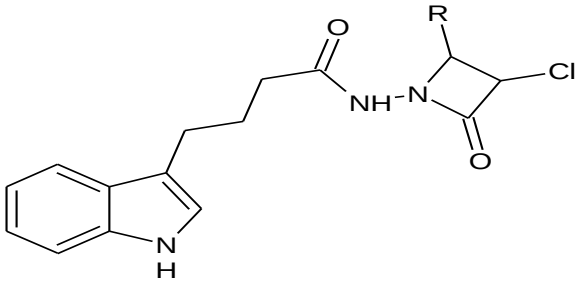


Fig 1: General structure of newly synthesized compounds

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

The structures of designed and synthesized compounds have been established on the basis of UV, IR, H^1NMR and Mass spectral data. IR spectra showed the characteristic peaks of newly synthesized compounds. Chemical shifts were reported on δ scale. Mass spectral data confirmed the molecular weight of the synthesized compounds.

N-[3-Chloro-2-(4-hydroxy-3-methoxy phenyl)-4-oxazetidin-1-yl]-4-(1H-indol-3-yl) butanamide (AZ_1). Yield 72%; mp 54°C;Rf (0.58); λ_{max} (295);IR (KBr) ν (cm-1); 3367 (Ar-NH); 1595 (C=0); 1718 (β-lactam); 1310 (Sec. Amine); 752 (C–Cl); ^{1}H NMR ($CDCl_3$) δ 9.5 (-NH-); 7.0 to 8.0 (Ar-H); 6.8 (β lactam); 3.7 (-OCH3).

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

N-[3-Chloro-2-(4-Chlorophenyl)-4-oxazetidin-1-yl]-4-(1H-indole-3-yl) butanamide (AZ_3): Yield 88%; mp 76°C;Rf (0.88); λ_{max} (285); IR (KBr) ν (cm-1); 3372 (Ar-NH); 1592 (C=0); 1728 (β-lactam); 1306 (Sec. Amine); 755 (C–Cl); ^{1}H 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); λ_{max} (282);IR (KBr) ν (cm-1); 3358 (Ar-NH); 1594 (C=0); 1731 (β-lactam); 1311 (Sec. Amine); 745 (C–Cl); ^{1}H NMR (CDCl3) δ 9.4 (-NH-); 8.4 (-OH); 7.0 to 8.0 (Ar-H); 6.8 (β lactam); 3.7 (-OCH3).

N-[3-Chloro-2-(4-hydroxyphenyl)-4-oxazetidin-1-yl]- 4-(1H-indol-3-yl) butanamide (AZ_5): Yield 86%; mp 78°C;Rf (0.78); λ_{max} (290);IR (KBr) ν (cm-1); 3368 (Ar-NH); 1590 (C=0); 1712 (β-lactam); 1302 (Sec. Amine); 754 (C–Cl); ^{1}H NMR (CDCl3) δ 9.4 (-NH-); 8.4 (-OH); 7.0 to 8.0 (Ar-H); 6.8 (β lactam).

Biological activity

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

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 AZ_3 showed high α-amylase inhibitory activity at 50µg /ml to 800µg/ml concentration comparable with standard drug acarbose. AZ_5 and AZ_2 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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