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Designing, Synthesis and Characterization of Novel Substituted Pyrazol-Azetidin-2-One/Thiazolidin-4-One Derivatives for their Antimicrobial Activity

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ABSTRACT

This research work includes the synthesis of (3-hydroxy-4-methoxyphenyl)-3-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl) ethylidene)amino)thiazolidin-4-one(VIIa-f)/3-chloro-4-(substitutedphenyl)-1-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol1yl)ethylidene) amino) azetidin-2-one(VI a-f) having a pyrazol ring, obtained from the reaction of hydrazine hydrate with chalcone along with athiazolidinone ring/azetidinonemoeity, procured after the cyclization of a schiff base by thioglycolic acid/Chloroacetyl chloride. The structure of the synthesized compounds has been established on the basis of elemental analysis, UV-Vis absorption spectroscopy, IR, 1HNMR, and mass spectral studies. The in vitro antimicrobial screening of all the novel compounds was done against S.aureus, E.coli, P.aeruginosa and B. subtilis. The activity of VIb, VIf, VId and VIIa, VIIf, VIIdVIIe compounds exhibited excellent activity while the rest showed moderate to good activity against the tested microbes.

Keywords: Chloroacetyl chloride, Thioglycoolic acid, Antimicrobial, Aromatic aldehyde, Hydrazine Hydrate, Acetophenone.

INTRODUCTION

Compounds having two different heterocyclic ring are challenging precursor models for the preparation of medicinally useful bioactive scaffolds [1,2]. A voluminous documentation exists elucidating the diverse presence of the heterocycle in nature. The heterocycle are estimated to be present in over two third of all the naturally existing compounds. The versatile functional and structural attributes associated with the heterocycle have added to their ever expanding applications in the industry as well as in biological system [3].The presence of hetero atoms like nitrogen, sulfur and oxygen are largely responsible for the innate diversity exhibited by these compounds whether in natural or artificial molecular scaffold.

Chalcones are frequently employed as precursor molecular motifs in the synthetic preparation of heterocyclic pyrazoles and related moieties manifesting certain biological properties such as antibacterial, anti-inflammatory, antifungal and analgesic which are of significant importance in the drug industry [4,7].

Azetidinones moieties are recognized as the structural backbone unit in penicillin and many other related antibiotics [8] possessing interesting microbial activities [9-10]. It has been observed that β-lactam, having chloro group at 3 position shows profound anti-tubercular, anticonvulsant, anti-inflammatory and antimicrobial activities [11-13]. They are also engaged as potent enzyme inhibitors apart from their intense effect on the central nervous system (CNS) [14,15].

As per the large body of documented evidence available Thiazolidinedione (TZD) is acknowledged as one of the most important heterocyclic ring system of Thiazole having a wide range of therapeutic action and when formulated with other heterocyclic compound exhibits enormous functional and biological diversities [16].

The importance and prominence of pyrazoles and Azetidinones/Thiazolidinedione (TZD) moieties [17,18] in drug discovery have continually attracted chemist worldwide in their pursuit for novel compounds with good antimicrobial activity, demanding their synthesis in short reaction times. In continuation of our quest to design and synthesize heterocyclic compound with better yields and biological activities we herein report the synthesis of novel heterocyclic compound having pyrazole and Azetidinones/Thiazolidinedione [19,20] (TZD) moieties.

EXPERIMENTAL SECTION

Materials and Methods

All melting points (m.p.) were determined in open capillaries on Jindal melting point apparatus and were uncorrected. The purity of the compounds was routinely checked by thin layer Chromatography (TLC) using silica gel G (Merck). The ^{1}HNMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO on Bruker NMR spectrophotometer at 300 MHz using teteramethyalsilane(TMS) as an internal reference and chemical shift value (δ) were given in part per million(ppm). The instruments used are: Jasco FTIR-470 spectrophotometer (KBr) with diffuse reflectance method; MS-JEOL SX102 Mass spectroscopy by using Argon/Xenon (6Kv, 10 mA) as the FAB gas and m-nitro benzyl alcohol (NBA) as the matrix. UV spectrums of the sample were carried out on double beam UV spectrophotometer.

Synthesis of (E)-3-(o-nitrophenyl)-1-phenylprop-2-en-1-one(I)

A (0.1 mole) of acetophenone and (0.1 mole), of o-nitrobenzaldehyde was stirred in ethanol (50 ml). Aqueous potassium hydroxide solution (15ml, 30%) was then added to it. The mixture was refluxed on a water bath for 10.00 hrs. The reaction was monitored by thin-layer chromatography (TLC) with the methanol- chloroform. After completion, the reaction mixture was poured into crushed ice and acidified with dilute hydrochloric acid. The chalcone precipitates out as solid. It is then filtered and crystallized from ethanol.

Yield 78 %; m.p. 124-125°C; Anal. Calcd for $C_{15}H_{11}O_3N$ (MW=253.25),: C 71.14, O 18.95 N 5.53found: C 71.10, O 18.89, N 5.48;.IR (KBr,cm-1): 1740(C=O),1625(CH=CH),

Synthesis of 1-(5-(o-nitrophenyl)-3-phenyl-4,5-dihydro-1H -pyrazol-1-yl) ethanone(II)

A (0.02 mole) of chalcone, (E)-3-o-nitrophenyl)-1-phenylprop-2-en-1-one, in acetic acid (20 ml) and 0.02 mole of hydrazine hydrate in absolute EtOH (30 ml) was refluxed for 8-10 hrs on a water bath. After completion of the reaction, the solution was concentrated by distillation under reduce pressure. Upon cooling, solid mass was obtained, dried and purified by recrystallization from methanol. Yield 73 %; m.p. 132-133°C; Anal. Calcd for C_{17} H_{15} N_3 O_3 (MW=309.32): C 66.01, O 15.52, N 13.58 found: C 65.96, O 15.4, N 13.51; IR (KBr,cm^{-1}): 1624(C=N), 1633 (C=C-H), 2335(C-N).

Synthesis of (substituted benzylidene hydrazine(III a-f)

Hydrazine Hydrate (0.01mol), aryl aldehyde (0.01 mol),and 25 mL of absolute ethyl alcohol were refluxed for 10-12 hrs on a water bath in the presence of glacial acetic acid (1 ml). Excess solvent was removed under reduced pressure. The solid obtained was washed with cold water several times and purified by recrystallization from methanol. Characterization data of the compounds thus synthesized, are given as:
IIIa.5-(hydrazonomethyl)-2-methoxyphenol (3-OH,4-OCH3-Benzaldehyde): Yield 68 %; m.p. 94-95°C; Anal.Calcd for $C_8H_{10}N_2O_2$ (MW=166.18),: C 57.82, N 16.86, O 19.26, found: C 57.78, O 19.21, N 1680.

IIIb.(4-chlorobenzylidene)hydrazine (*p*-Chlorobenzaldehyde)

Yield 98 %; m.p. 116-117 °C; Anal. Calcd for $C_7H_7N_2Cl$(MW=154.60),: C 54.38, N 18.12, found: C 54.33, N 18.

IIIc.4-(hydrazonomethyl)phenol (p-Hydroxybenzaldehyde)

Yield76 %; m.p. 120-121°C; Anal.Calcd for $C_7H_8N_2O$(MW=136.15),: C 61.74, O 11.75, N 20.58 found: C61.68, O 11.71, N 20.49.

IIId.(2-nitrobenzylidene)hydrazine(O-nitro benzaldehyde)

Yield 82 %; m.p. 184-185 °C; Anal.Calcd for $C_7H_7N_3O_2$ (MW=306.79),: C 50.91, N 25.44 O 19.38 found: C 50.88; N 25.38, O 19.31.

IIIe.2-(hydrazonomethyl)pheno (o-Hydroxybenzaldehyde)

Yield 68 %; m.p. 136-137°C; Anal.Calcd for $C_7H_8N_2O$ (MW=136.15),: C61.75, N 20.58, O 11.75, found: C 61.69, O 11.68, N 20.47.

IIIf. 4-(hydrazonomethyl)-2-methoxyphenol (4-OH,3-OCH3-Benzaldehyde)

Yield 77 %; m.p. 111-112°C; Anal.Calcd for $C_8H_{10}N_2O_2$ (MW=166.18),: C 57.82, N 16.86, O 19.26, found: C 57.79, O 19.19, N 16.86.

Synthesis of 1-amino-3-chloro-4-(substituted phenyl) azetidin-2-one (IVa-f)

(0.01) mole of compound (IIIa-f) in dixon (50 ml) was added to chloroacetyl chloride (0.01 mole) and triethylamine (0.01 mole) at 0^{o}C with stirring. The reaction mixture was left at room temperature for 3 hours and then refluxed for 10 hrs. Excess solvent was distilled off and the residue was poured into crushed ice and recrystallized.Characterization data of the compounds thus synthesized, are given as:

IVa.1-amino-3-chloro-4-(3-hydroxy-4-methoxyphenyl)azetidin-2-one

Yield: 68%. M.p.:133-134°C; Anal.Calcd for C_{10} $H_{11}N_2$ O_3Cl(MW=242.66),: C 49.50, N 11.54, O 19.78found: C 49.42, N 11.47, O 19.66.
IR (KBr) (ν_{max} in cm^{-1}): 3035 ν(Ar-H), 1754 (C=O, monocyclic β-lactam), 3427 (3-OH,3-hydroxyphenyl), 2348(N-N), 3431(N-Hstretch), 1678 (OCH_3,p-methoxyphenyl),765(C-Cl,β- lactam),1527(C=C,Ar);^{1}H NMR (δ in ppm) ($CDCl_3$):4.88 (s, 1H, Ar-OH), 6.95-7.10 (m, 3H, Ar_H), 5.14 (d, 1H, Cl-CH), 4.81(d,1H,N-CH-R of β-lactam)3.38 (s,3H,Ar-OCH_3).

IVb.1-amino-3-chloro-4-(4-chlorophenyl)azetidin-2-one

Yield: 58%. M.p.:152-153°C; Anal.Calcd for $C_9 H_8N_2 O Cl_2$ (MW=231.08),: C 46.78, N 12.12, O06.92 found: C 46.63, N 12.07, O 6.88.; IR (KBr) (ν_{max} in cm^{-1}): 2340(N-N), 3429(N-H stretch),2970 (C-H), 745 (C-Cl stretch)
3041 v(Ar-H), 1751 (C=O,monocyclicβ-lactam), 763(C-Cl,β- lactam),1527(C=C,Ar);^{1}H NMR (δ in ppm) ($CDCl_3$):6.70-7.45(m, 4H, Ar_H), 5.28 (d, 1H, Cl-CH), 4.83(d,1H,N-CH-R of β-lactam).

IVc.1-amino-3-chloro-4-(4-hydroxyphenyl)azetidin-2-one

Yield: 70%. M.p.:131-132°C; Anal.Calcd for $C_9 H_9 N_2 O_2 Cl$(MW=212.63),: C 50.84, N 13.17, O 115.05 found: C 50.77, N 13.11, O 15.01.; IR (KBr) (ν_{max} in cm^{-1}): 3430 (p-OH,p-hydroxyphenyl) 3044 v(Ar-H), 1762 (C=O, monocyclic β-lactam), 3425 2345(N-N), 3440(N-Hstretch,778(C-Cl,β- lactam),1543 (C=C,Ar);^{1}H NMR (δ in ppm) ($CDCl_3$):4.74 (s, 1H, Ar-OH), 6.78-7.62 (m, 4H, Ar_H), 5.32 (d, 1H, Cl-CH), 4.91(d,1H,N-CH-R of β-lactam).

IVd.1-amino-3-chloro-4-(2-nitrophenyl)azetidin-2-one

Yield: 74%. M.p.:149-150°C; Anal.Calcd for $C_9 H_8 N_3 O_3Cl$(MW=241.63),: C 44.74, N 17.39, O 19.86 found: C 44.71, N 17.35, O 19.78.; IR (KBr) (ν_{max} in cm^{-1}): 2970 (C-H) 3044 v(Ar-H), 1750 (C=O, monocyclic β-lactam),783(C-Cl, β-lactam),1522(C=C,Ar),2340(N-N), 3441(N-H stretch), 1520 (N=O str.asym),1330(N=O str,sym);^{1}H NMR (δ in ppm) ($CDCl_3$):6.84-7.52 (m, 4H, Ar_H), 5.26 (d, 1H, Cl-CH), 4.97(d,1H,N-CH-R of β-lactam).

IVe.1-amino-3-chloro-4-(2-hydroxyphenyl)azetidin-2-one

Yield: 58%. M.p.:162-163°C; Anal.Calcd for $C_9 H_9 N_2 O_2 Cl$(MW=212.63),: C 50.84, N 713.17, O 15.05 found: C 50.77, N 13.13, O 15.02.; IR (KBr) (ν_{max} in cm^{-1}):3040 v(Ar-H), 1760 (C=O, monocyclic β-lactam), 3430 (o-OH,o-hydroxyphenyl),2340(N-N), 3430(N-H stretch), ,775(C-Cl,β- lactam),1533(C=C,Ar);^{1}H NMR (δ in ppm) ($CDCl_3$):4.72 (s, 1H, Ar-OH), 6.73-7.30 (m, 4H, Ar_H), 5.30 (d, 1H, Cl-CH), 4.88(d,1H,N-CH-R of β-lactam).

IVf.1-amino-3-chloro-4-(4-hydroxy-3-methoxyphenyl)azetidin-2-one

Yield: 72%. M.p.:110-111°C; Anal.Calcd for $C_{102} H_{11}N_2 O_3 Cl$(MW=242.66),: C 49.50, N 11.54, O 19.78 found: C 49.44, N 11.47O 19.77.; IR (KBr) (ν_{max} in cm^{-1}):3045 v(Ar-H), 1758 (C=O, monocyclic β-lactam), 3434 (3-OH,3-hydroxyphenyl),2338(N-N), 3429(N-Hstretch), 1685 (OCH_3,p-methoxyphenyl),785(C-Cl, β-lactam),1532(C=C,Ar);^{1}H NMR (δ in ppm) ($CDCl_3$):4.82 (s, 1H, Ar-OH), 6.93-7.66 (m, 3H, Ar_H), 5.27 (d, 1H, Cl-CH), 4.86(d,1H,N-CH-R of β-lactam)3.28 (s,3H,Ar-OCH_3).

The synthesis 3-amino-2-(substituted Phenyl)thiazolidin-4-one (Va-f)

(0.01) mole of compound (IIIa-f) and thioglycollic acid (0.01 mole) containing traces of zinc chloride (0.1 gm) in dimethylformamide (DMF) was heated under reflux for 4 hours. It was poured into crushed ice and stirred vigorously. Solidification occurred after fifteen minutes. It was filtered off and washed with cold water. Recrystallization from ethanol gave analytically pure sample. The compounds of this category were presented as:

Va.3-amino-2-(3-hydroxy-4-methoxyphenyl)thiazolidin-4-one

Yield: 72%. M.p.:188-189°C; Anal.Calcd for $C_{10}H_{11}N_2 O_3 S$(MW=240.28/),: C 49.99, N11.66, O 19.98 found: C 49.928, N 11.59, O 19.94.; IR (KBr) (ν_{max} in cm^{-1}):1652 (tert amide C=O), 3605 (Ar-OH), 1079 (C-S-C), 3070 (Aromatic C-H str.), 1588 (C=C skeletal), 2343(N-N), 3445(N-Hstretch),3430 (3-OH,3-hydroxyphenyl), 1690 (OCH_3,p-methoxyphenyl;^{1}H NMR (δ in ppm) ($CDCl_3$):4.66 (s, 1H, Ar-OH), 7.10-7.56 (m, 3H, Ar-H), 4.65 (brs, 1H, s, replaceable-OH), 3.22 (s, -N-CHS-R 1H,), 3.44 (s, 2H, O=CCH_2-S).

Vb.3-amino-2-(4-chlorophenyl)thiazolidin-4-one

Yield: 67%. M.p.:136-137°C; Anal.Calcd for $C_9 H_9N_2 O Cl S$(MW=228.78),: C 47.27, N 12.27, O07.00 found: C 47.22, N 12.19., O 06.98.; IR (KBr) (ν_{max} in cm^{-1}):1655 (tert amide C=O),1074 (C-S-C), 3072(Aromatic C-H str.), 1580 (C=C skeletal), 2343(N-N), 3445(N-Hstretch), 737(C-Cl stretch);^{1}H NMR (δ in ppm) ($CDCl_3$):6.60-7.75 (m, 4H, Ar-H), 3.28 (s, -N-CHS-R1H,), 3.41 (s, 2H, O=CCH_2-S).

Vc.3-amino-2-(4-hydroxyphenyl)thiazolidin-4-one

Yield: 58%. M.p.:190-191°C; Anal.Calcd for $C_9 H_{10}N_2 O_2 S$(MW=210.25),: C 59.41, N 03.32, O 15.25 found: C 59.36, N 13.28, O 15.19.;IR (KBr) (ν_{max} in cm^{-1}):1660(tert amide C=O),1070 (C-S-C), 3078(Aromatic C-H str.), 1582 (C=C skeletal), 2348(N-N), 3443(N-Hstretch), 737(C-Cl stretch), 3445 (p-OH,p-hydroxyphenyl);^{1}H NMR (δ in ppm) ($CDCl_3$):6.90-7.78(m, 4H, Ar-H),3.33 (s, -N-CHS-R1H,), 3.44 (s, 2H, O=CCH_2-S),4.70 (brs, 1H, s, replaceable-OH).

Vd.3-amino-2-(2-nitrophenyl)thiazolidin-4-one

Yield: 56%. M.p.:176-177°C; Anal.Calcd for $C_9 H_9 N_3 O_3 S$(MW=239.25),: C 45.18, N 17.56, O 20.06 found: C 45.13, N 17.51, O 20.01.;IR (KBr) (ν_{max} in cm^{-1}): 16850(tert amide C=O),1080 (C-S-C), 3075(Aromatic C-H str.), 1590 (C=C skeletal), 2352(N-N), 3443(N-Hstretch), 747(C-Cl stretch), 1540 (N=O str.asym),1342(N=O str,sym);^{1}H NMR (δ in ppm) ($CDCl_3$):6.72-7.66(m, 4H, Ar-H),3.42(s, -N-CHS-R1H,), 3.48 (s, 2H, O=CCH_2-S).

Ve.3-amino-2-(2-hydroxyphenyl)thiazolidin-4-one

Yield: 65%. M.p.:128-129°C; Anal.Calcd for $C_9H_{10}N_2O_2S$(MW=210.25),: C 51.41, N 13.32, O 15.25 found: C 15.38, N 13.27, O 15.19.;IR (KBr) (ν_{max} in cm^{-1}): 1685(tert amide C=O),1080 (C-S-C), 3080(Aromatic C-H str.), 1575 (C=C skeletal), 2338(N-N), 3435(N-Hstretch), 745(C-Cl stretch), 3438 (o-OH,o-hydroxyphenyl);^{1}H NMR (δ in ppm) ($CDCl_3$):6.70-7.71(m, 4H, Ar-H),3.42(s, -N-CHS-R1H,), 3.52 (s, 2H, O=CCH_2-S),4.74 (brs, 1H, s, replaceable-OH).

Vf.3-amino-2-(4-hydroxy-3-methoxyphenyl)thiazolidin-4-one

Yield: 70%. M.p.:130-131°C; Anal.Calcd for $C_{10}H_{12}N_2O_3S$ (MW=240.28),: IR (KBr) (ν_{max} in cm^{-1}): 1670(tert amide C=O),1085 (C-S-C), 3073(Aromatic C-H str.), 1586(C=C skeletal), 2352(N-N), 3448(N-Hstretch), 744(C-Cl stretch), 3440 (p-OH,p-hydroxyphenyl),1690 (OCH_3,p-methoxyphenyl;^{1}H NMR (δ in ppm) ($CDCl_3$):6.65-7.60(m, 3H, Ar-H),3.40 (s, -N-CHS-R1H,), 3.39 (s, 2H, O=CCH_2-S),4.68 (brs, 1H, s, replaceable-OH),3.35(s,3H,Ar-OCH_3).

Synthesis of 3-chloro-4- (substituted phenyl) -1- ((1- (5- (2-nitrophenyl) -3 -phenyl-4, 5 – dihydro-1 H-pyrazol-1-yl) ethylidene) amino) azetidin-2-one (VIa-f)

The target compounds were synthesized by taking (0.02) mole of compound II and (0.02) mole of 1-amino-3-chloro-4-(substituted phenyl) azetidin-2-one (IVa-f) in pyridine (30 ml) were heated under reflux on a heating mantel for 6 hrs. Consequently, the reaction mixture was added to ice cold water (100 ml). A solid started to separate out which was allowed to settle down for 1 hr. It was filtered off and washed successively with water after drying in a vacuum desiccator. The clean final compound was thus obtained.

Characterization data of the compounds thus synthesized are given in as

VIa.3-chloro-4-(3-hydroxy-4-methoxyphenyl)-1-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)azetidin-2-one

Yield: 72%. M.p.:120-121°C; Anal.Calcd for $C_{27}H_{24}N_5O_5Cl$ (MW=533.96),: C 60.73, N 13.12, O 14.98found: C 60.68, N 13.07, O 14.86.; IR (KBr) (ν_{max} in cm^{-1}): 2970 (C-H) 3044 v(Ar-H), 1750 (C=O,monocyclicβ-lactam),783(C-Cl,β- lactam),1522(C=C,Ar),2340(N-N), 1628 (C=N), 1136 (C-N str.), 3430 (3-OH,3-hydroxyphenyl), 1677 (OCH_3,p-methoxyphenyl), ;^{1}H NMR (δ in ppm) ($CDCl_3$):6.60-7.52 (m, 13H, Ar_H), 5.28(d, 1H, Cl-CH), 4.90(d,1H,N-CH-R of β-lactam), 2.82 (s,3H,Ar-OCH_3), 5.80 (s, 1H, Ar-OH); Mass: M^+ 533,412,457,335,295,242,124,105,70.

VIb.3-chloro-4-(4-chlorophenyl)-1-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)azetidin-2-one

Yield: 65%. M.p.:154-155°C; Anal.Calcd for $C_{26}H_{21}N_5O_3Cl_2$ (MW=522.38),: C 59.78,N 13.41, O 09.19found: C 59.71, N 13.35, O 09.11.; IR (KBr) (ν_{max} in cm^{-1}): 2975 (C-H) 3048 v(Ar-H),740(C-Cl stretch), 1760 (C=O,monocyclicβ-lactam),788(C-Cl,β- lactam),1532(C=C,Ar),2340(N-N), 1638 (C=N), 1136 (C-N str.),;^{1}H NMR (δ in ppm) ($CDCl_3$):6.60-7.82 (m, 14H, Ar_H), 5.22(d, 1H, Cl-CH), 4.88(d,1H,N-CH-R of β-lactam),2.85 (s,3H,N=C-CH_3), 3.24 (dd,1H, CH_2);Mass:M^+ 521,399,445,323,294,229,111,105,70.

VIc.3-chloro-4-(4-hydroxyphenyl)-1-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)azetidin-2-one

Yield: 58%. M.p.:163-164°C; Anal.Calcd for $C_{26}H_{22}N_5O_4Cl$ (MW=503.94),: C 61.97,N 13.90, O 12.70found: C 61.91, N13.88O 12.66.; IR (KBr) (ν_{max} in cm^{-1}): 3440 (p-OH,p-hydroxyphenyl), 2977 (C-H) 3048 v(Ar-H), 1768 (C=O,monocyclicβ-lactam),788(C-Cl,β-lactam),1541(C=C,Ar),2340(N-N), 1632 (C=N), 1126 (C-N str.),;^{1}H NMR (δ in ppm) ($CDCl_3$):6.70-8.60 (m, 14H, Ar_H), 2.61 (d,1H,N-CH-R of β-lactam),2.3 (s,3H,N=C-CH_3), 2.7 (dd,1H, CH_2);Mass:M^+ 503,427,381,305,295,211,105,93.

VId.3-chloro-4-(2-nitrophenyl)-1-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)azetidin-2-one

Yield: 60%. M.p.:123-124°C; Anal.Calcd for $C_{26}H_{21}N_6O_5Cl$ (MW=532.94),: C 58.60, N 15.77, O 15.01found: C 58.55, N 15.71, O 14.96; IR (KBr) (ν_{max} in cm^{-1}):2960 (C-H) 3048 v(Ar-H),1530 (N=O str.asym),1338(N=O str,sym), 1778 (C=O,monocyclicβ-lactam),785(C-Cl,β-lactam),1548(C=C,Ar),2344(N-N), 1636 (C=N), 1129 (C-N str.),;^{1}H NMR (δ in ppm) ($CDCl_3$):6.66-7.72 (m, 14H, Ar_H), 5.31(d, 1H, Cl-CH), 4.81(d,1H,N-CH-R of β-lactam),2.90 (s,3H,N=C-CH_3), 3.33 (dd,1H, CH_2);Mass: M^+ 531,455,511,335,295,241,123,105.

VIe.3-chloro-4-(2-hydroxyphenyl)-1-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)azetidin-2-one

Yield: 75%. M.p.:115-116°C; Anal.Calcd for $C_{26}H_{22}N_5O_4Cl$ (MW=503.94),: C 61.97,N 136.90, O 12.70found: C 61.92, N 13.83, O 12.63.; IR (KBr) (ν_{max} in cm^{-1}):2972 (C-H) 3048 v(Ar-H),1548 (N=O str.asym),1333(N=O str,sym), 1759 (C=O,monocyclicβ-lactam),778(C-Cl,β-lactam),1555(C=C,Ar),2344(N-N), 1641 (C=N), 1132 (C-N str.),3438 (o-OH,o-hydroxyphenyl) ;^{1}H NMR (δ in ppm) ($CDCl_3$):6.64-7.76 (m, 14H, Ar_H), 5.41(d, 1H, Cl-CH), 4.90(d,1H,N-CH-R of β-lactam),2.88 (s,3H,N=C-CH_3), 3.38 (dd,1H, CH_2),5.77 (s, 1H, Ar-OH) ;Mass: M^+ 503,427,381,305,294,211,105,93.

VIf.3-chloro-4-(4-hydroxy-3-methoxyphenyl)-1-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)azetidin-2-one

Yield: 62%. M.p.:161-162°C; Anal.Calcd for $C_{27}H_{24}N_5O_5Cl$ (MW=533.96),: C 60.73 N 13.12, O 14.98found: C 60.67, N 13.07O 14.93.; IR (KBr) (ν_{max} in cm^{-1}): 2965 (C-H) 3044 v(Ar-H), 1760 (C=O,monocyclicβ-lactam),780(C-Cl,β- lactam),1532(C=C,Ar),2342(N-N), 1628 (C=N), 1140 (C-N str.), 3441(4-OH,4-hydroxyphenyl), 1670 (OCH_3,3-methoxyphenyl), ;^{1}H NMR (δ in ppm) ($CDCl_3$):6.71-7.62 (m, 13H, Ar_H), 5.32(d, 1H, Cl-CH), 4.86(d,1H,N-CH-R of β-lactam), 2.88 (s,3H,Ar-OCH_3), 5.78 (s, 1H, Ar-OH); Mass:M^+ 533,412,335,295,242,124,105.

Synthesis of (3–hydroxyl–4–methoxyphenyl)–3- ((1 - (5 - (2 – nitro phenyl) – 3 – phenyl – 4 , 5 - dihydro-1H-pyrazol-1-yl) ethylidene) amino) thiazolidin-4-one (VIIa-f)

The target compounds were synthesized by taking (0.02 mole) of compound II and (0.02) mole of 3-amino-2-(3-hydroxy-4-methoxyphenyl) thiazolidin-4-one (IVa-f)in pyridine (30 ml). The reactants were heated under reflux on a heating mantel for 6 hrs. Consequently, the reaction mixture was added to ice cold water (100 ml). A solid started to separate out which was allowed to settle down for 1 hr. It was filtered off and washed successively with water. After drying in vacuum desiccator, The clean final compound was obtained. Characterization data of the compounds thus synthesized, are given below:

VIIa.2-(3-hydroxy-4-methoxyphenyl)-3-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)thiazolidin-4-one

Yield: 70%. M.p.:158-159°C; Anal.Calcd for C_{270} $H_{25}N_5O_5$ S (MW=531.58),: C 61.00,N 13.17, O 15.05found: C 60.96, N 13.12, O 15.01.; IR (KBr) (ν_{max} in cm^{-1}):1655 (tert amide C=O), 3615 (Ar-OH), 1072(C-S-C), 3075 (Aromatic C-H str.), 1576 (C=C skeletal),1636 (C=N), 1129 (C-N str.),3428(3-OH,3-hydroxyphenyl), 1660(OCH_3,p-methoxyphenyl);^{1}H NMR (δ in ppm) ($CDCl_3$):6.58-7.75 (m, 13H, Ar-H), 4.75 (brs, 1H, s, replaceable-OH), 3.12 (s, -N-CHS-R1H,), 3.36 (s, 2H, O=CCH_2-S), 2.92(s,3H,N=C-CH_3), 3.20 (dd,1H, CH_2),3.72(d,1H,NCH-R),2.91(s,3H,Ar-OCH_3).
Mass:M^+ 521,455,409,333,295,239,224,123,103.

VIIb.2-(4-chlorophenyl)-3-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)thiazolidin-4-one

Yield: 68%. M.p.:198-199°C; Anal.Calcd for C_{26} $H_{22}N_5$ O_3 S Cl (MW=520),: C 60.05,N 13.47, O 09.23found: C 60.02, N 13.39, O 09.19.; IR (KBr) (ν_{max} in cm^{-1}):1678 (tert amide C=O),1088 (C-S-C), 3086 (Aromatic C-H str.), 1570 (C=C skeletal),1638 (C=N), 1131 (C-N str.), 575 (C-Cl); ^{1}H NMR (δ in ppm) ($CDCl_3$):6.70-7.78 (m, 14H, Ar-H), 3.23 (s, -N-CHS-R1H,), 3.36 (s, 2H, O=CCH_2-S), 2.95(s,3H,N=C-CH_3), 3.32 (dd,1H, CH_2),3.89(d,1H,NCH-R).Mass:M^+ 519,443,397,321,294,227,213,111.

VIIc.2-(4-hydroxyphenyl)-3-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)thiazolidin-4-one

Yield: 76%. M.p.:180-181°C; Anal.Calcd for C_{26} $H_{23}N_5$ O_4 S (MW=501.56),: C 62.26,N 13.96, O 112.76found: C 62.18, N 13.88, O 12.71. IR (KBr) (ν_{max} in cm^{-1}):1665 (tert amide C=O), 3620 (Ar-OH), 1074 (C-S-C), 3078(Aromatic C-H str.), 1580(C=C skeletal),1631 (C=N), 1136 (C-N str.);^{1}H NMR (δ in ppm) ($CDCl_3$):4.55 (s, 1H, Ar-OH), 6.66-8.76 (m, 14H, Ar-H), 2.73 (brs, 1H, s, replaceable-OH), 3.21 (s, -N-CHS-R1H,), 2.9 (s, 2H, O=CCH_2-S), 2.77(s,3H,N=C-CH_3), 3.21 (dd,1H, CH_2),3.80(d,1H,NCH-R);Mass:M^+ 501,424,379,303,295,209,195,103,93.

VIId.2-(2-nitrophenyl)-3-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)thiazolidin-4-one

Yield: 55%. M.p.:1710-172°C; Anal.Calcd for C_{26} $H_{22}N_6$ O_5 S (MW=530.56),: C 58.86,N 15.84, O 15.08found: C 58.81, N 15.78, O 15.01.; IR (KBr) (ν_{max} in cm^{-1}):1643 (tert amide C=O), 3624(Ar-OH), 1066 (C-S-C), 3082 (Aromatic C-H str.), 1580 (C=C skeletal),1630 (C=N), 1125 (C-N str.).1528 (N=O str.asym),1330(N=O str,sym).;^{1}H NMR (δ in ppm) ($CDCl_3$):6.61-7.70 (m, 13H, Ar-H),3.29 (s, -N-CHS-R 1H,), 3.39 (s,2H, O=CCH_2-S), 3.11 (s,3H,N=C-CH_3), 3.10 (dd,1H,CH_2), 3.73 (d,1H,NCH-R); Mass:M^+ 529,453,409,333,294,2396,223,123,103.

VIIe.2-(2-hydroxyphenyl)-3-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)thiazolidin-4-one

Yield: 68%. M.p.:130-131°C; Anal.Calcd for C_{26} $H_{23}N_5O_4$ S (MW=501.56),: C 62.26,N 13.96, O 12.76found: C 62.22, N13.88, O12.67; IR (KBr) (ν_{max} in cm^{-1}):1680 (tert amide C=O), 3630 (Ar-OH), 1060(C-S-C), 3070 (Aromatic C-H str.), 1582 (C=C skeletal),1644 (C=N), 1138 (C-N str.);^{1}H NMR (δ in ppm) ($CDCl_3$):4.60 (s, 1H, Ar-OH), 6.68-7.89 (m, 14H, Ar-H), 4.66 (brs, 1H, s, replaceable-OH), 3.38(s, -N-CHS-R1H,), 3.31(s, 2H, O=CCH_2-S), 2.90(s,3H,N=C-CH_3), 3.22 (dd,1H, CH_2),3.73(d,1H,NCH-R);Mass:M^+ 501,425,379,303,295,209,194.103,94.

VIIf.2-(4-hydroxy-3-methoxyphenyl)-3-((1-(5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethylidene)amino)thiazolidin-4-one

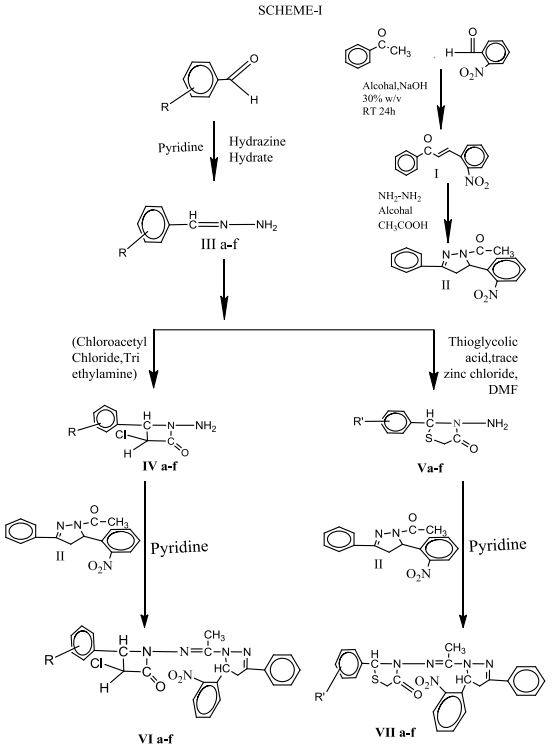
Yield: 70%. M.p.:176-177°C; Anal.Calcd for C_{27} $H_{25}N_5O_5S$ (MW=531.58),: C 61.00,N 13.17,O 15.05found: C 60.92, N13.11, O 15.01.;IR (KBr) (ν_{max} in cm^{-1}): 3446(3-OH,3-hydroxyphenyl), 1678(OCH_3,p-methoxyphenyl).1677 (tert amide C=O), 3628(Ar-OH), 1066 (C-S-C), 3075 (Aromatic C-H str.), 1574(C=C skeletal),1642(C=N), 1132 (C-N str.);^{1}H NMR (δ in ppm) ($CDCl_3$):2.80(s,3H,Ar-OCH_3), 6.65-7.80 (m, 13H, Ar-H), 4.64 (brs, 1H, s, replaceable-OH), 3.33(s, -N-CHS-R1H,), 3.30(s, 2H, O=CCH_2-S), 2.81(s,3H,N=C-CH_3), 3.26 (dd,1H, CH_2),3.80(d,1H,NCH-R);Mass:M^+ 531,455,409,333,295,240,225,123,103.

The potency of the synthesized compounds were tested against some gram positive (*Staphylococcus aureus, Bacillus subtilis*) and gram negative bacteria (*E. coli, and Pseudomonas aeruginosa*) using routine antimicrobial test. All the test microorganism were grown in different identical sets ofLB media containing variable concenterations of the compound(VIa-f, VIIa-f).Growth of the pathogenic test microbes was measured after 24 hrs by recording the optical density of the solution at 600nm. The optical density versus compound concenteration growth curve was plotted for each set to get the minimum inhibitory concenteration (MIC) values of the tested compounds against the pathogenic gram positive and gram negative bacteria. In a conical tube containing 10 ml of LB media, *Staphylococcus aureus,Bacillussubtilis, E.coli and Pseudomonas aeruginosa,*were incubated for 8 h at 37°C and their growth were measured by recording the optical density of the solution at 600 nm. 100 µl of the bacterial suspension is added into each conical tube containing the test compound with final concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, 0.98, 0.49 and 0.244 µg ml^{-1} in 10 ml of LB media, respectively. The parallel control experiment was done by adding the same 100 µl of the bacterial suspension to conical tube containing 10 ml of LB media alone. The growth of bacteria for each conical tube was measured by recording the optical density of the solution at 600 nm after 24 hour time. The optical density versus compound concentration was plotted together. The drop in optical density at respective antibacterial compound concentration was taken as the MIC of that compound.

RESULTS AND DISCUSSIONS

The synthesized compounds were evaluated for their potency against the test microorganism (*Staphylococcus aureus, Bacillus subtilis, E.coli and*

Pseudomonas aeruginosa. All the synthesized compound showed moderate to excellent activity. Compound VIb VIId were the most effective against the tested pathogens while the rest displayed moderate to good activity. Compound VIb showed high potency against all the test organism but was most effective against *B.subtilis* (12.5 µg/mL). Compounds VIId, VIIe, VIIf showed excellent results against *P.aeruginosa* showing MIC values ranging from(12.5-50 µg/mL)with VIIe being most effective of all exhibiting an MIC value of (12.5 µg/mL). In Gram positive bacteria strains, compounds VIb, VIe, VIf, VIIa, VIIc, and VIId showed good activity (12.5-50 µg/mL) against *S.aureus* and *B.subtilis* where asIn Gram negative bacteria strains compounds VIc,VId, VIe, VIIe and VIIf showed tremendous potency v (12.5-50 µg/mL) against *P.aeruginosa* and *E.coli* compared with Ampicillin. All other compounds show moderate activity activity against the test microbial strains (Table 1) (Scheme 1)



Scheme 1: Synthesis and characterization of novel Substituted pyrazol-azetidin-2-one/thiazolidin-4-one derivatives for their antimicrobial activity

Table 1: In Vitro Anti-Microbial Activity.

S.No.		Gram positive		Gram negative	
	R	***B.subtilis***	***S.aureus***	***E.coli***	***P. aeruginosa***
VIa.	*3*-OH,4-OCH_3-Benzaldehyde	50	>100	>100	50
VIb.	*p*-Chlorobenzaldehyde	12.5	50	50	50
VIc.	*p*-Hydroxy benzaldehyde	>100	>100	50	50
VId.	*O*-nitro benzaldehyde	50	50	12.5	25
VIe.	*o*-Hydroxy benzaldehyde	50	>100	>100	25
VIf.	*4*-OH,3-OCH_3-Benzaldehyde	25	50	>100	50
VIIa.	*3*-OH,4-OCH_3-Benzaldehyde	25	12.5	100	50
VIIb.	*p*-Chlorobenzaldehyde	100	50	50	100
VIIc.	*p*-Hydroxy benzaldehyde	50	25	50	100
VIId.	*O*-nitro benzaldehyde	12.5	50	100	25
VIIe.	*o*-Hydroxy benzaldehyde	100	50	50	12.5
VIIf.	*4*-OH,3-OCH_3-Benzaldehyde	50	100	50	25

CONCLUSION

In the present work all the synthesized novel heterocyclic products were prepared using simple procedures which involved less reaction time, affordable, cost effective reagents avoiding hazardous and toxic chemicals. The Compound VIbVIId exhibited tremendous potencies against the tested pathogens while the rest displayed moderate to good activity. Compound VIb showed high potency against all the test organisms but was most effective against *B.subtilis* (12.5µg/mL). Compounds VIId, VIIe, VIIf showed excellent results against *P.aeruginosa* showing MIC values ranging from (12.5 µg/mL-50 µg/mL) with VIIe being most effective of all exhibiting an MIC value of (12.5 µg/mL). Thus the above synthesized compounds hold promise as novel chemotherapeutic agents.

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