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Synthesis of Some New Pyridazino-Pyrimidinone Derivatives and Evaluation of their Antimicrobial Activity

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Abstract

A series of pyridazino-pyrimidinone derivatives[V-a to V-l] has been synthesized using synthetic procedure of Bianchi method, an improved method of Friedal-Craft acylation reaction and nucleophilic addition of indole/methyl indole followed by cyclo-condensation of the resulting adduct to give corresponding pyridazinone[1]. These derivatives were biologically evaluated for their antimicrobial activity.

Keywords: pyridazin-3(2H)-one, pyridazine, biologically active, γ-Keto acid, derivatives, antimicrobial, microbial resistant.

Introduction

The inhibition of microbial growth under standardized condition may be utilized for antimicrobial activities of the compound. The antimicrobial activities are based upon the comparative study of the inhibition of growth of microorganism by measuring inhibition zone of the synthesized derivative to be examined with that produced by the known concentration of the standard preparation having a known activity. In recent decades, the appearance of multidrug resistance against many antibiotics is a reason of great concern. Antimicrobial resistance happens when microorganisms (such as bacteria, fungi, viruses, and parasites) change when they are exposed to antimicrobial drugs (such as antibiotics, antifungal, antiviral, antimalarials, and anthelmintics). Microorganisms that develop antimicrobial resistance are sometimes referred to as “superbugs”. As a result, the medicines become ineffective and infections persist in the body, increasing the risk of spread to others. Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. AMR is an increasingly serious threat to global public health that requires action across all government sectors and society. Without effective antibiotics, the success of major surgery and cancer chemotherapy would be compromised. The cost of health care for patients with resistant infections is higher than care for patients with non-resistant infections due to longer duration of illness, additional tests and use of more expensive drugs. In 2016, 490 000 people developed multi-drug resistant TB globally, and drug resistance is starting to complicate the fight against HIV and malaria, as well[2].

In recent years, there has been an increased interest in pyridazinone derivatives. Pyridazinone-3(2H)-one derivatives represent one of the most active class of compounds possessing a wide spectrum of biological activity ranging from cardiovascular properties, anti-inflammatory, antidiabetic, analgesic, anti AIDS, anticancer, antimicrobial, anxiolytics, p38 MAP Kinase inhibitor, indicators, antiulcer, antisecretory and anticonvulsant activities[3]. Pyridazinone containing compounds are the group of heterocyclic compounds that are known for their wide range of biological activities from the early period. More-over, since the early 80's it was proven that pyridazinone derivatives possess various biological activities including platelet anti-aggregation[4] and antidepressant and tranquilizing[5].

Pyridazinone are the oxo derivatives of pyridazine. Pyridazinone hold considerable interest relative to the preparation of organic intermediates and physiologically active compounds[6]. In recent years a great deal of work have been directed to the organic synthesis of pyridazines. These nitrogen heterocyclic compounds are of biological importance and therefore, design and strategy for their synthesis is important. In order to explore the activity associated with the structural moiety $NHNHCONH_2$, various aryl-substituted pyridazinone derivatives were synthesized and their antimicrobial activities were tested. Moreover, compounds containing indole and its derivatives are reported to exhibit anti-fungal, antibacterial, antiphage, antiproliferative, anticholinergic, antiviral, antihypertensive antitumor activities[7].

Material and Method

All the chemicals used for the experimental work were commercially procured from chemicals unit like Hi Media, Loba Chemicals, Qualigens, S.D. Fine Chemoicals, E.Merck, CDH and Samar Chemicals. The solvents and reagents were of the AR grade and some were LR grade and are of adequate purity. The melting point of organic compound was determined by Thiel's Melting point tube method. Purity of the compounds was checked by the Silica G (60-120mesh) used for analytical chromatography (TLC) using Benzene: Ethanol (9:1) and Toluene: Ethyl Formate:Formic acid (5:4:1) as solvents. IR spectra were recorded Perkin Elmer (KBr disc). The method used for the present study was agar disc diffusion method and strains of pathogenic microorganism used *Staphylococcus aureus* (MTCC-96), *Escherichia Coli* (MTCC-443) and *Candida Albicans*(MTCC-183).

Aryl-substituted-4-oxo-but-2-enoic acid (1-a-1-f) was synthesized by adding a mixture of aryl- substituted compounds (0.08 mol) and maleic anhydride (0.08 mol) to magnetically stirred suspension of aluminum chloride (0.275 mol) in DCM at a temperature of 20°C. the reaction mixture is stirred for about 8 hours at 25°C and allowed to stand overnight. The mixture was then poured onto crushed ice containing concentrated HCl and stirred for 30 min. The precipitate obtained was crystallized from toluene-hexane and are dried.

Aryl-substituted-4-oxo-but-2-enoic acid(1-a-1-f) was added aryl compound(Indole/Methyl Indole) in dry benzene and the reaction mixture was then refluxed for 6 hours and product is

recrystallized from benzene to give aryl-substituted-(1*H*-Indole/Methylindole)-3-yl-4-oxo-butyric acid(II-a-II-l). To the stirring mixture of compounds (II-a-II-l) was added hydrazine hydrate (10mmol) in presence of dry benzene..The resulting mixture was refluxed fro 6 hours and was recrystallized from benzene to give Aryl-substituted-(1*H*-Indole/Methylindole)-3-yl-4,5-dihydro-2H-pyridazinone(III-a-III-l). Allowed the reaction mixture of compounds III-a-III-l with $POCl_3$ under reflux for 3 hours. Poured the mixture onto crushed ice, filtered, washed well with water and recrystallized with benzene to give Aryl-substituted-4-yl-1*H*-Indole/ Methylindole)-3-chloro-pyridazine(IV-a-IV-l). The final product V-a to V-l was synthesized by adding anthranilic acid (1mmol) to solution of Aryl–substituted-3-chloropyridazine-4-yl-1*H*-Indole/Methyl Indole(1mmol) in dry benzene and the reaction mixture was refluxed for 6 hour. The product obtained was separated after concentration and cooling was recrystallized from benzene. The compounds were characterized by IR spectral data.

Biological Study

Antimicrobial activities (antibacterial and antifungal) of all the compounds V-a to V-l were screened against bacteria such as *Staphylococcus aureus* (MTCC-96), *Escherichia Coli* (MTCC-443) and against human pathogenic fungus *Candida Albicans* (MTCC-183) using agar disc diffusion method. Ciprofloxacin for bacteria and fluconazole for fungus were as reference drugs. Sterilized cork bore with 1.3 cm outer diameter was used to cut cups in the petridish contains the seeded agar media. The solution of synthesized compound (0.5 ml)was added aseptically to the cups. The dishes with the bacterial culture were incubated at 37°C for 24 hours. Whereas the dishes with fungal culture were kept at room temperature for 72 hours to facilitate growth. The zone of inhibition were observed then measured and compared that of standard. The microbial growth inhibitions were shown in Table-III.

Results and Discussion

Here we have synthesized a series of aryl-substituted-4-(2-methyl-1H-indol-1-yl)-10H-pyridazino [6,1-b]quinazolin-10-one and reported their antibacterial and antifungal activities. The reaction sequence leading to the formation of different title compounds is outlined in reaction scheme. Substituted aryl compounds as a starting material was synthesized by Friedal-Craft's acylation of aromatic heterocyclic with maleic anhydride in the presence of $AlCl_3$ (I-a to I-f). This on refluxing with indole or methyl indole in dry benzene for 6 hours to give Aryl-substituted-(1H-Indole/MethylIndole)-3-yl-4-oxo-butyric acid(II-a to II-l). This on reaction with hydrazine hydrate yielded Aryl-substituted-(1H-Indole/MethylIndole)-3-yl-4,5-dihydro-2H-pyridazinone(III-a to III-l) followed by reaction with Phosphorous oxy chloride to yield the compounds(IV-a to IV-l). The resulting compounds were then refluxed for 6 hours with anthranilic acid in the presence of dry benzene to give compounds (V-a to V-l). All the compounds V-a to V-l were screened for antimicrobial activity and evaluated by agar disc diffusion method using *Staphylococcus Aureus*-Gm(+)ve bacteria, *Escherichia Coli*-

Gm(-)ve bacteria and *Candida Albicans*(Fungus). Out of the twelve compounds subjected to antimicrobial screening, four compounds showed significant activity against Gm(+) ve microorganisms, three of them shows activity against Gm(-)ve microorganism while all the five compounds shows significant antifungal activity.. Of the test compounds tested, compound V-a, V-b and V-g shows more area in the zone of inhibition for all the test microorganisms. Compound V-a was found more active than compound V-b. All the compounds reported in the Table-III shows antifungal activity. The antimicrobial activity of the test compounds were may be due to presence of bromine and chlorine group in the test compounds. Rest showed significantly low or no antimicrobial activity.

Reaction Scheme

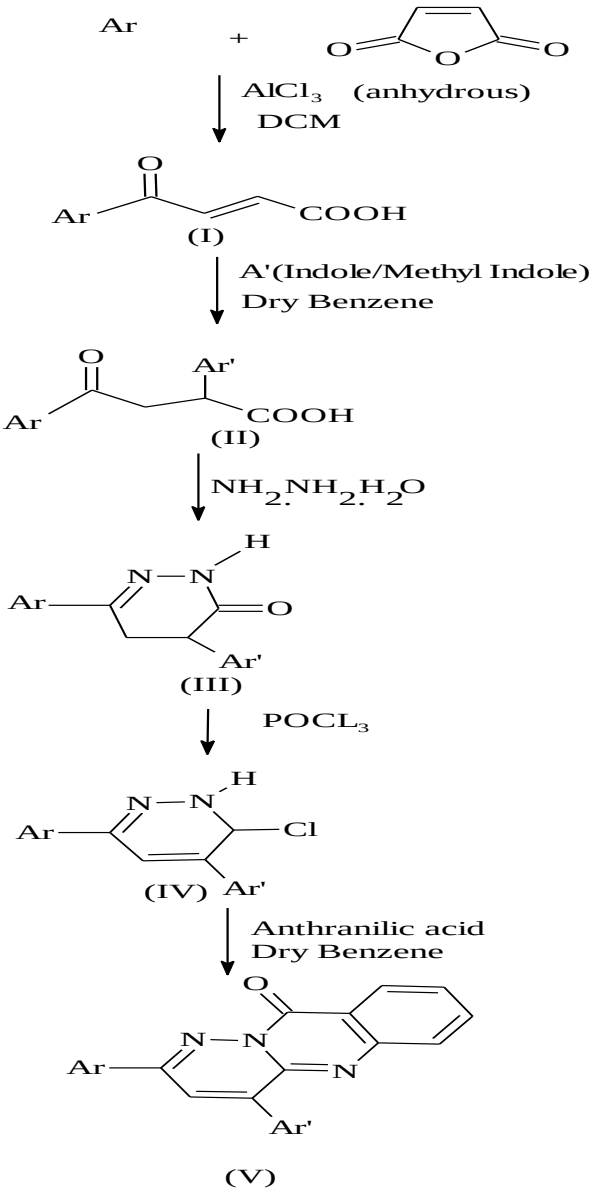

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Table 1.Physical Data of the Compounds Synthesized-(V)

COMP.	Ar/ Ar'	Molecular Formula	Molecular weight	m.p.°C	% yield	R_f
V-a	Bromobenzene/ Indole	$C_{25}H_{15}BrN_4O$	467.32	196-198	52.32	0.4
V-b	2-chloro toluene/ Indole	$C_{26}H_{17}ClN_4O$	436.9	115-116	46.18	0.32
V-c	Biphenyl/ Indole	$C_{31}H_{20}N_4O$	464.52	130-131	44.97	0.5
V-d	Naphthalene/ Indole	$C_{29}H_{18}N_4O$	438.48	191-192	43.91	0.37
V-e	Benzanilide/ Indole	$C_{32}H_{21}N_5O_2$	507.54	144-145	46.6	0.47
V-f	Acetanilide/ Indole	$C_{27}H_{19}N_5\ O_2$	445.51	112-113	41.72	0.61
V-g	Bromobenzene/ Methylindole	$C_{26}H_{17}BrN_4O$	471.92	117-118	57.9	0.56
V-h	2-chloro toluene/ Methylindole	$C_{27}H_{19}$ ClN_4O	450.93	128-129	41.45	0.62
V-i	Biphenyl/ Methylindole	$C_{32}H_{23}N_4O$	478.56	177-178	57.2	0.34
V-j	Naphthalene/ Methylindole	$C_{30}H_{20}N4O$	452.52	180-181	54.32	0.35
V-k	Benzanilide/ Methylindole	$C_{33}H_{23}N_5O_2$	521.64	119-121	53.33	0.57
V-l	Acetanilide/ Methylindole	$C_{27}H_{20}N_5O_2$	416.47	167-168	49.87	0.54

Table 2.Spectral Data of the Compounds Synthesized-(V)

COMP.	IR(cm^{-1})
V-a	1659.5(C=O) str of pyridazinone, 1476.9 (C=N) str, 3488.4(N-H)str of indole, 765.8(p-substituted benzene),
V-b	1669.8,1459.2, 3470.0, 666.1(C-Cl),
V-c	1657.2, 1510.2, 3487.1,1189.2(N-N)
V-d	1667.4, 1648.1(C=C), 3474.9,1189.2(N-N)
V-e	1669.2, 1498.1, 3461.4
V-f	1669.1, 1485.9, 3470.5,1157.0
V-g	1658.7, 1476.2, 3490.0, 560.0(C-Br)
V-h	1670.5, 1459.9, 3463.8, 1459.9
V-i	1670.5, 1459.9, 3491.7,1459.9
V-j	1659.0, 1578.0, 3490.7
V-k	1671.2, 1482.8, 3471.8,
V-l	1485.9(C-N), 3469.2, 1669.1(C-H) STR OF CH_3.

Table 3.Screening Result of Antimicrobial Activity

S. No.	Compound	Activity at 100µ/ml concentration		
		Gm(+) ve	Gm(-) ve	Fungus
		S.aureus	*E.Coli*	*C. Albicans*
1	V-a	++	++	++
2	V-b	++	++	++
3	V-v	+	+	++
4	V-g	++	++	++
5	V-h	++	+	++

[++ means more active; + means moderately active]

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