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AN ANALYSIS UPON CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF NEW PYRAZOLO-PYRIDAZINE DERIVATIVES: SYNTHESIS AND ANTIMICROBIAL ACTIVITIES

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An Analysis upon Characterization and Biological Activity of New Pyrazolo-Pyridazine Derivatives: Synthesis and Antimicrobial Activities

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Abstract – Several new pyrazolo-pyridazine derivatives (4a-h) were synthesized through multi-step synthesis and evaluated for their antimicrobial activities. In the first step, 6-phenyl-2, 3, 4, 5-tetrahydropyridazin-3-one (2) was prepared by reacting 4-(4-chlorophenyl)-4-oxobutanoic acid (1) with hydrazine hydrate. Then, aryl-aldehydes were reacted with 2 to furnish pyridazinone derivatives (3a-g). Finally, pyridazinones (3a-h) were reacted with hydrazine hydrate to furnish the title compounds (4a-h). The newly synthesized compounds were evaluated for their in vitroantibacterial and antifungal activities against six microbial strains. Compounds 4d, 4e and 4f exhibited significant antibacterial action, whereas compounds 4c and 4d showed potential antifungal activity. Compound 4d, 5-(4-Chlorophenyl)-3-(4-fluorophenyl)-3,3a,4,7-tetrahydro-2H-pyrazolo[3,4-c]pyridazine, emerged as lead compound having broad spectrum of antimicrobial action.

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INTRODUCTION

Pyridazine, a six membered nitrogen containing heterocyclic ring, plays an important role in pharmaceuticals particularly in the field of medicinal chemistry. Pyridazine ring is a part of the structures of a number of drugs available in the market like Cadralazine, Minaprine, Hydralazine, Pipofezine, etc (Figure 1). Pyridazine derivatives found to possess important biological activities including antibacterial, antifungal, anti-tubercular, anticonvulsant, antihypertensive, analgesic and anti-inflammatory. Another heterocycle, pyrazole, is an example of five-membered nitrogen containing heterocyclic ring systems. Pyrazole derivatives have also been reported to possess potential biological activities including antibacterial and antifungal actions.

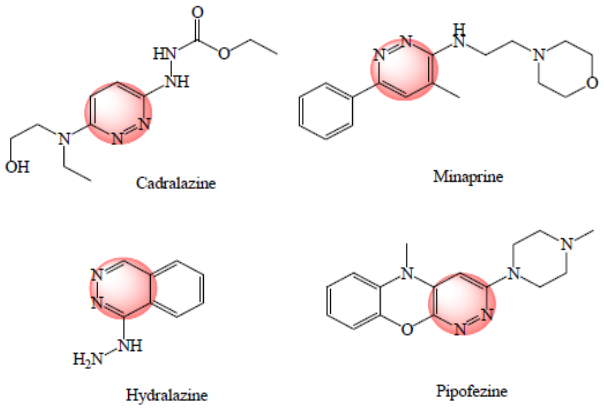

Figure 1: Pyridazine Bearing Drugs.

In view of the antimicrobial activities associated with pyridazine and pyrazole derivatives, it was thought worthwhile to study their fused derivatives as antibacterial and antifungal agents. Thus, several pyrazolopyridazine derivatives (4a-h) have been synthesized and evaluated for their antibacterial and antifungal actions against six microbial strains.

Pyridazine compounds are commonly used as anticancer, antituberculosis, antihypertensive, antifungal, or antimicrobial agents due to their intense biological activity. They have a rapid systemic

effect on the plants and are active at very low concentration. Some of the investigated pyridazine derivatives have chemical structures related to those of the phytohormones. In addition, similar chemical structures occur in living cells, being involved in various biochemical reaction pathways. Some new synthesized pyridazine derivatives were used in many research fields due to their structure, stability and reactivity and their tendency to form stable yields with important biological properties. On the other hand, as a rule, a cytotoxic effect of higher concentrated compounds is correlated with their stimulation effect at lower concentrations. We synthesized new pyridazine derivatives and cycloadducts derived from the pyridazine moiety in our group, still our concern is related to their possible toxicity on the environment. Therefore, this paper reports on the biological activity of some pyridazine derivatives on wheat germination and seedling growth. Higher plants are suitable for such research being recognized as excellent indicators of cytogenetic and mutagenic effects of some chemicals and are applicable for the detection of environmental noxious compounds.

The germination tests are very simple, little time consuming, cheap and, therefore, could be ideal methods for testing the biological activity of some new synthesized compounds. Because some of the investigated compounds might have a stimulatory action on germination, we searched for suitable agents among the pyridazine structures to improve seed germination, that could be of paramount importance for genetic conservation work in the gene banks.

Pyridazine ring is a part of the structures of a number of drugs available in the market like Hydralazine, Minaprine, Cefozopran, Pipofezine, etc. Pyridazine derivatives have been reported to possess various pharmacological activities including antibacterial, antifungal, anti-tubercular, anticonvulsant8-10, anhiypertensive11, analgesic and anti-inflammatory. Similarly, pyrazole (five-membered nitrogen containing heterocyclic ring) derivatives also show potential biological activities including antimicrobial actions. In view of the antimicrobial activities exhibited by pyridazines and pyrazoles, it was thought worthwhile to study their fused derivatives as potential antibacterial and antifungal agents. Therefore, a series of pryrazolo-pyridazine derivatives have been synthesized and evaluated for their antibacterial and antifungal activities against some selected microbes.

Our literature survey showed that the chemistry of fused pyrazolo[3,4-*d*]pyrimidine derivatives has drawn great attention due to their pharmacological importance and their structural resemblance to purines. In fact, several pyrazolo[3,4-*d*]pyrimidine derivatives demonstrated significant antimicrobial and cytotoxic activities.

On the other hand, the literature reveals that several methods have been described for the elaboration of substituted pyrazolo [3,4-*d*] pyrimidines. Among the already known routes to the fused pyrazolopyrimidine scffold, the most commonly used strategy involves a preliminary transformation of aminopyrazole-carbonitrile derivatives into the corresponding imidates followed by a subsequent ring-closure into pyrazolo [3,4-*d*] pyrimidines upon treatment with hydrazine.

Therefore, taking into account all these above-described data, we report here our recent work on the synthesis of a new family of fused heterocyclic compounds using a nucleophilic addition-cyclization reaction on 5-aminopyrazole-4-carbonitriles with formamide and a series of cyclic anhydrides.

Prompted by the varied biological activities of pyrazolo [3,4-*d*] pyrimidine derivatives, we also described the synthesis of pyrazolo [3,4-*d*] pyrimidine-4-amines by condensation of imidate 7b with various aromatic primary amines. Some new synthesized compounds were evaluated for their antibacterial activity using microdilution tests against some strains of bacteria.

Pyrimidine is the most important and essential diazine which is useful for all types of life processes on the mother earth. It occupies a great role in the heterocyclic compounds because of its therapeutic, pesticidal, insecticidal and fungicidal actions. It also possesses good pharmacological properties. Pyrimidines have a unique place and have contributed significantly to biological and medicinal fields. Pyrimidine ring system containing substituent‟s six member ring exhibits anti-cancer and herbicidal activities. In recent years, pyrimidine derivatives have received significant attraction due to their miscellaneous possibilities of biological actions mostly anti-tubercular, anti-malerial, anti-inflammatory, calcium channel blockers and other miscellaneous activities. A pyrimidine derivative has been found to be a potent anti-viral agent against HIV type 1 *in vitro* and to decrease mortality and opportunistic infections in patients with AIDS and pyrimidine ring also forms the nucleus hitherto a new non-nucleoside reverse transcriptase inhibitor active against HIV. The synthetic potency of some pyrimidine derivatives with their nitrogen nucleophile examined as an opportunistic route for the synthesis of amino-pyrimidine and other pyrazole derivatives and they were synthesized recently and tested their anti-microbial and anti-fungal activities. Pyrimidine derivative like amino-pyridimine shows the biodynamic characteristics prepared by condensing the chalcones with guanidine carbonate in methanol and some kind of nitrates also reported. Almost all the derivatives of pyrimidine based chalcones demonstrated excellent anti-microbial activities.

LITERATURE REVIEW

The comprehensive literature survey must require in any research work. In the science stream, the past studies and experience will be considered for the future work that is more effective for the research.

Kolyamshin O. A., and Danilov V. A. (2004) have suggested a few numbers of N-aryl- 2-(4-alkyl-1-piperazinyl) and N-aryl- 2-(2-benzimidazolyl) succinimides were synthesized and proved as anti-convulsant, anti-arrhythmic and other types of biological activities. Some of these are prepared by the reaction of N-aryl maleimides with an equimolar amount of diethylamine, piperidine, and morpholine given the equivalent N-aryl-2-dialkylamino-succinimides by using piperidine and morpholine.

Azizian J. et al (2006) reported the cyclization of spiro pyrimido-pyrazine derivatives. The intermediate chalcones were condensed by acetophenone in alkaline medium. Then the next spiro compounds like pyrazine and isatin pyrazolines were prepared by hydrazine hydrate. Several compounds were prepared by cyclization in solvent free conditions.

Willy B. and Muller T. J. J. (2008) have reported the heterocyclic analogues such as pyrazoles, pyrimidines, isoxazoles and substituted furans. These are prepared by multi-component synthesis which was catalyzed by Pd-Cu from alkynones. The modified coupling synthesis like Sonogashira was easily applied for acid chlorides and alkynes. All the heterocycle generics were fabricated by cyclo-addition, cyclo-condensation multi-component synthesis.

Wang Y. S. et al. (2008) have forwarded that the glutarimide compounds possess a variety of biological activities and also prepared by different cyclic imides. Several kinds of 4, 5-disubstituted-3-sulfonyl glutarimides were synthesized from α-sulfonyl acetamide and ethyl α,β-disubstituted acrylate esters through stepwise superficial (3+3) annulations nearly specified. An intermediary key pyridin-2-one was used for the synthesis of end product i.e. mappicine ketone.

Rajput A. P., Rajput S. S. (2009) reported the microbial screenings of seven membered heterocyclic compounds. These are highly reactive chemical compounds usually shows excellent biological behavior. Some of the heterocyclic compounds like pyrazolone provide significant anticancer activities such as colon, stomach, lung and liver cancer. Seven membered pyrazoline derivatives gave fine and reasonable antimicrobial potencies against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aurous strains*.

Solankee A. et al. (2009) developed the different substituted chalcones of s-triazinyl based pyrazoline and amino-pyrimidine derivatives namely 2,4–Bis-ethylamino-6-[4- {3-(substituted-phenyl-1or2-furanyl)-2-propenone-1-yl}-phenyl-amino]-s-triazine treated by ketones with several substituted aromatic aldehydes. Further treated with hydrazine hydrate and guanidine nitrate which give pyrazolone and amino-pyrimidine compounds.

Al-Azzawi A. M. and Hassan A. S. (2011) have prepared the citraconimide four step series connected to benzothiazole and sulfonimado groups. N-phenyl-citraconimic acid obtained from citraconamic anhydride and aniline. Then phenyl citraconimides, phenyl sulfonyl chloride to different substituted benzothiazoles were prepared. The synthesized final compounds were screened on gram +ve, gram -ve bacteria and *Candida albican* fungi strains.

Sid A. et al (2011) both the researches prepared Phenyl pyrazoline derivatives were prepared by using conventional method. Initially, the chalcones were prepared by the mixture of acetophenone and benzaldehyde in presence of ethanolic NaOH. Afterwards the synthesized chalcones and hydrazine hydrate with formic acid were refluxed for 24 hrs which forms appropriate pyrazoline compounds.

Baseer M. A. et al. (2011) have developed the novel pyrazoline five membered derivatives from piperazines. These novel pyrazoline analogous were designed from piperazine chalcones with hydrazine hydrate in the methanolic alkaline media within few hrs which gives excellent yield. Almost all new pyrazolines were checked for their antimicrobial potencies.

Nofal Z. M. et al. (2011) have suggested that the pyrimidine which signifies the wide range of compounds having significant interest due to their broad range of biological, agricultural and pharmacological activities. Some pyrimidine derivatives like diazotized and cyclo-condensation of hydrazono-pyrimidines and its substituted derivatives synthesized by using aminopyrimidin-5-carboxaldehyde compounds.

Sharma V. and Sharma K. V. (2011) described that the chemistry of pyrimidine derivatives inspected ever since previous times because of their pharmaceutical relationship along with different pharmacological characteristics. About 24 numbers of different substituted 2-amino-4, 6-diaryl-pyrimidine strings were synthesized by the reaction of chalcones and guanidine carbonate in the DMF solvent.

Joshi V. D. et al (2012) have synthesized various pyrazoline derivatives from chalcones by conventional method. Initially the intermediate chalcones were prepared by substituted acetophenones and benzaldehyde in the presence of 40% ethanolic NaOH. Then pyrazolines were refluxed by chalcones. *In vitro* antimicrobial activities were recorded of all the pyrazoline series.

Babu N. M., Sharma L. and Madhvan V. (2012) have synthesized and reported some novel pyrrolidine compounds. The pyrrolidine derivatives were prepared via benzocaine and succinic

anhydride to benzoic acid, benzoate and then benzamide by using acetic anhydride, THF, pyridine, sodium acetate and ethanol solvents. All newly synthesized derivatives were screened against antibacterial and antifungal activities on *Aspergillus flavus* and *Aspergillus niger*.

Rasool et al (2013) have synthesized some pyrazoline derivatives derived from cyclic imides by using synthetic pathways. Cyclic imides as starting compounds were prepared by the condensation of amino-acetophenone and succinic anhydride in presence of acetic acid. Then intermediate chalcones were prepared by cyclic imides and substituted aromatic aldehydes in ethanolic KOH. Pyrazoline were prepared in the last step viz chalcones and hydrazine-hydrate refluxed in ethanol for 12 hrs which is exposed in the reaction. Antibacterial and anticancer activities were screened of all the final chalcones and pyrazolines.

NEW PYRIDAZINES CONTAINING IMIDAZOLIDINE MOIETY

Nitrogen-containing heterocyclic compounds are one of the most fruitful and extensively developing fields of heterocyclic chemistry. These compounds exhibit various kinds of biological activities. During the past decades increasing interest in the synthesis and biological activities of pyridazine derivatives has been observed. Pyridazine compounds have been reported to possess varied biological activities such as antimicrobial, antihypertensive, anticancer, antiinflammatory and antifungal activities. These facts have prompted us to synthesize some novel pyridazine derivatives. Recently, pyridazinone nucleus has been extensively studied in the search for new and selective medicinal agents as drugs acting on the cardiovascular system. Furthermore, a number of thienopyridazines have been claimed to possess interesting biological and pharmacological activities such as, anticancer., Pyridazines can also be used as novel therapeutic agents to target Alzheimer's disease and other neurodegenerative diseases like Parkinson.

Recent studies indicate pyridazine derivatives can be used in the treatment of dermatitis, prostate cancer, and dry eye disorders. Moreover, pyridazines are pharmaceutically acceptable acid-addition salts that are used as an active component in cardio tonic compositions to increase cardiac contractilit.

Imidazolidines, a saturated imidazole (tetrahydroimidazole), have been reported to have important biological activities including potential α-adrenergic receptor agonist, antimicrobial,antiparasitic, oral hypoglycaemic, antiarrhythmic, anticonvulsant, anti-inflammatory and analgesic.

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

Antibacterial activity -

Antibacterial activity of the compounds was determined by adopting cup plate method. In this method, sample solution diffuses from a vertical cylinder or a cavity through the solidified agar layer of a Petri dish in a manner that growth of the added microorganism is prevented entirely in a circular area or a zone around the cylinder or cavity containing a solution of the sample if the added sample possesses antibacterial activity. For determining antibacterial activity, freshly prepared liquid agar medium (35mL/Petri dish) was transferred into the Petri dishes (8Petri dishes/sample) and allowed the medium to solidify. Then, the 200µL-standardized culture (99 mL Nutrient broth media + 1mL culture) of organism was spread on each Petri dish by L-shaped spreader. With the help of the borer (5 mm), three bores were made in each plate. The synthetic compounds diluted with dimethyl sulfoxide (DMSO) atthree different concentrations (50, 100, and 200µg/mL) were added to each well separately.

The Petri dishes were kept aseptically for approximately 4 to 5 h for diffusion of the sample. Following diffusion, all the Petri dishes were incubated for 24 h at a temperature of 37º C. After the stipulated period of 24 h, the activity of compounds in terms of zone of inhibition was observed against two *Gram positive*:

Staphylococcus aureus (*S. aureus*) and *Micrococcus luteus* (*M. luteus*), and two *Gram negative* microbial strains; *Escherichia coli* (*E. coli)* and *Klebsiella pneumoniae* (*K. pneumoniae*). Antibacterial activity of the synthesized compounds is reported in Table 1.

Compd	Concentration (µg/mL)	Zone of inhibition (in mm)			
		Gram positive		*Gram negative*	
		S. aureus	*M. luteus*	*E. coli*	*K. pneumoniae*
4a	50	-	-	-	-
	100	-	11	-	9
	200	11	12	13	12
4b	50	-	-	-	-
	100	12	10	-	13
	200	13	14	14	16
4c	50	-	-	-	-
	100	12	-	-	12
	200	15	17	12	14
4d	50	-	-	-	-
	100	13	-	14	12
	200	15	18	14	17
4e	50	-	-	-	-
	100	-	-	16	17
	200	17	18	18	19
4f	50	-	-	-	-
	100	15	-	17	-
	200	17	18	19	18
4g	50	-	-	-	-
	100	14	-	-	17
	200	16	14	18	15
4h	50	-	-	-	-
	100	-	-	15	13
	200	10	14	18	16
Ampicillin	50	24	22	27	24
Chloramphenicol	50	23	27	25	22

Table 1: Antibacterial activity of the title compounds (4a-g).

Antifungal activity-

The Sabouraud agar medium (dextrose 4%, peptone 1%, and agar 1.5%) was used for determining antifungal activity of the compounds. The medium was prepared and sterilized in an autoclave for 15 min at 15 psi. Then, it was aseptically transferred into sterilized Petri plates. After a duration of 2 h, the two fungal strains; *Candida albicans* (*C. albicans*) and *Cryptococcus neoformans* (*C. neoformans*) were inoculated on the surface of Petri plates separately. Following this, the cups of approximately 6mm in diameter were made in the Sabouraud agar medium using sterilized cup borer under aseptic conditions. Then 0.1mL of each standard (100µg/mL) and test compounds (100, 250 and 500 µg/mL) prepared by dissolving in DMSO was added into cups. Following addition of solutions, these Petri plates were incubated for 48 h at a temperature of 28±2º C and then growth and zones of inhibition (in mm) were recorded. The antifungal activity of synthesized compounds is tabulated in Table 2.

Compd	Concentration (µg/mL)	Zone of inhibition (in mm)	
		C. albicans	*C. neoformans*
4a	100	-	-
	250	7	5
	500	9	7
4b	100	-	-
	250	13	10
	500	16	14
4c	100	8	9
	250	13	12
	500	18	20
4d	100	7	9
	250	14	16
	500	19	20
4e	100	-	-
	250	8	10
	500	13	15
4f	100	-	-
	250	9	6
	500	10	9
4g	100	-	-
	250	10	8
	500	11	12
4h	100	-	-
	250	8	8
	500	10	12
Fluconazole	100	24	28
Griseofulvin	100	23	26

Table 2: Antifungal activity of the title compounds (4a-g).

CONCLUSION

In conclusion, seven new fused heterocyclic derivatives, 3-substituted-phenyl-5- (4-chlorophenyl)-3, 3a, 4, 7- tetrahydro - 2*H*- pyrazolo [3,4-c] pyridazine (4a-h), were synthesized and evaluated for their antimicrobial actions against four bacterial and two fungal strains. From the antimicrobial data, it could be concluded that compounds bearing fluoro, hydroxyl or methoxy group (4d, 4e and 4f) were highly active against all the four bacterial strains, whereas compound bearing electron withdrawing group, chloro or fluoro (4c & 4d) exhibited potential antifungal activity. Compound 4d emerged as lead compound. The data of antimicrobial screening showed the antibacterial and antifungal potential of the pyrazolo-pyridazine derivatives.

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