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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF CERTAIN NEW PYRAZOLE DERIVATIVES

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ABSTRACT

A new series of substituted pyrazole derivatives were synthesised by 1,3,4-benzoxazinone with active hydrogen atoms of an amino group by conventional synthetic methods to form quinazolinone nucleus. These are further cyclized to prepare different pyrazoles by the reaction with appropriate cyclising agents like acetyl acetone. All the reactions are monitored by TLC technique and chemical tests as applicable. The structures of these compounds have been established by means of IR, proton NMR, Mass spectral analysis and elemental analysis. The compounds have been evaluated to determine their anti-tubercular profile and also were evaluated for antifungal and antimicrobial activity.

Key words: Pyrazoles, Quinazolines, NMR, Anti-tubercular, Antimicrobial activity.

INTRODUCTION

Pharmacologically quinazolinones[1,2] are among the most important classes of heterocyclic compounds displaying a wide variety of biological and pharmacological activities like antibacterial[3,4], anti-inflammatory[5-7], analgesic[6-8], antimalarial[9], anthelmintic, neuroleptic, antitubercular, platelet anti-aggregating, antifungal, anticancer, antiviral, CNS depressant activity, antiparkinson, bronchodilator etc. Recently, several scientists have

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elucidated that quinazolinone system possesses the variable sites like position 2 and 3, which can be suitably modified to yield potent chemotherapeutic and pharmacotherapeutic agents.

Further, it was observed from the literature that certain five member heterocyclic compounds possess interesting biological activity. Among those, pyrazole and their fused derivatives are known to exhibit diverse biological activities and important applications in pharmaceutical industries. Several biological activities of pyrazole derivatives such as, antimicrobial[10-18], anti-inflammatory[12,13], anticancer[19,20], antitumor[21], COX-2 inhibitors[22], antimalarial and anti-tubercular[23,24] have been reported by several research groups. Hence, based on the above mentioned reviews, we present herein, the synthesis of some new structural hybrids of nitrogen heterocycles comprising Quinazolinones and Pyrazoles and evaluation for their antimicrobial and anti-tubercular activities.

EXPERIMENTAL

Materials

Melting points were determined in open capillary tubes and were found uncorrected. IR spectra were recorded on FT-IR spectrometer (Perkin Elmer) using KBr disc method. ^{1}H NMR spectra were recorded on ^{1}H FT-NMR (Brucker AMX 500 MHz) spectrometer in DMSO. The compounds were analyzed for elemental analysis and the percentages of elements were found to be very near that of the calculated values.

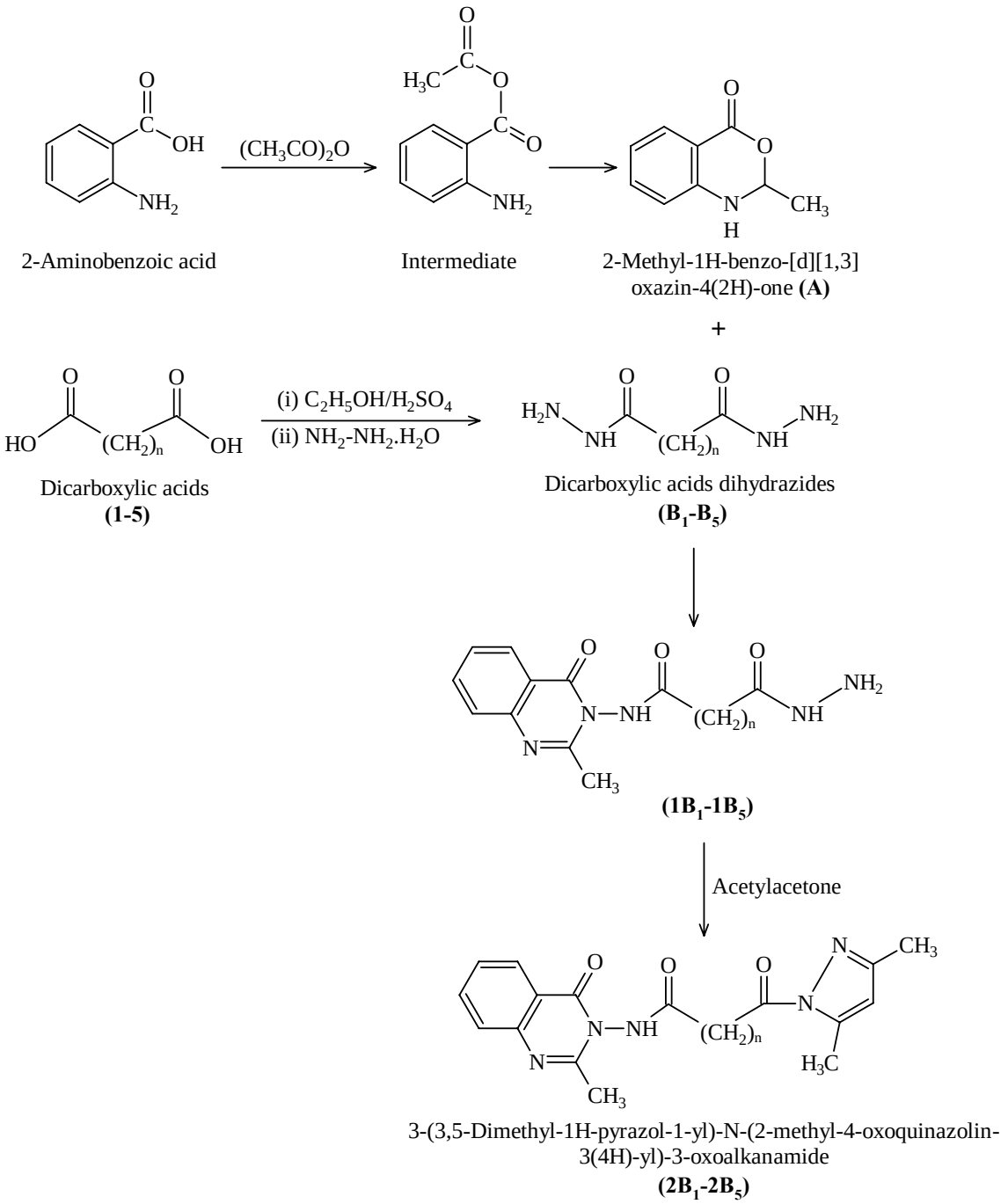
Synthetic methods

Step 1: Preparation of 2-methyl 1, 3, 4-benzoxazinone (A)

A mixture of anthranilic acid (0.12 moles) and acetic anhydride (0.2 moles) with few drops of pyridine was refluxed for 3 hr. The reaction mixture was filtered, washed and recrystallised from absolute ethanol, to get the crystals of 2-methyl 1,3,4-benzoxazinone (A).

Step 2: Preparation of acid hydrazide (B_1-B_5)

The acid (0.1 moles) and absolute ethanol (50 mL) were taken with a few drops of conc. H_2SO_4 and was refluxed for 6 hrs. The reaction mixture was concentrated by distilling off the excess of ethanol under reduced pressure. The ester obtained was used for the preparation of hydrazide directly. The ester (0.1 moles) was dissolved in anappropriate quantity of ethanol and to this hydrazine hydrate (0.2 moles) was added. The reaction mixture was refluxed for 12-18 hrs. Excess of ethanol was distilled off under reduced pressure. It was then poured into ice-cold water and the solid obtained was filtered and dried. It was recrystallized from aqueous ethanol.


(CH3CO)2O
2-Aminobenzoic acid
Intermediate
2-Methyl-1H-benzo-[d][1,3] oxazin-4(2H)-one (A)
+
(i) C2H5OH/H2SO4
(ii) NH2-NH2.H2O
Dicarboxylic acids (1-5)
Dicarboxylic acids dihydrazides (B1-B5)
(1B1-1B5)
Acetylacetone
3-(3,5-Dimethyl-1H-pyrazol-1-yl)-N-(2-methyl-4-oxoquinazolin-3(4H)-yl)-3-oxoalkanamide
(2B1-2B5)

Scheme 1

Step 3: Preparation of the 3-hydrazinyl-N-(2-methyl-4-oxoquinazolin-3(4H)-yl)-3-oxo substituted amide ($1B_1$-$1B_5$)

0.1 moles of 2-methyl-benzoxazinone (A) and 0.1 moles of acid hydrazide (B_1-B_5) in the presence of glacial acetic acid was taken in 50 mL of ethyl alcohol and refluxed in an anhydrous condition for 8 hr. The reaction mixture was cooled to room temperature and filtered the product and separated. It was dried and recrystallised from absolute ethanol. The physical data of compounds are given in Table 1.

Table 1: Physical data of compounds [$1B_1$-$1B_5$]

Compd.	n	Molecular formula	Molecular weight	Melting point (^oC)	R_f Value	Yield (%)
$1B_1$	0	$C_{11}H_{11}N_5O_3$	261.26	121	0.75	60.23
$1B_2$	1	$C_{12}H_{13}N_5O_3$	275.26	106	0.62	59.75
$1B_3$	2	$C_{13}H_{15}N_5O_3$	289.26	117	0.79	70.64
$1B_4$	3	$C_{14}H_{17}N_5O_3$	303.26	111	0.67	61.89
$1B_5$	4	$C_{15}H_{19}N_5O_3$	317.26	162	0.59	63.71

n: Number of alkyl substituent's in structure, R_f: relative factor

Step 4: Preparation of 3-(3,5-dimethyl-1H-pyrazol-1-yl)-N-(2-methyl-4-oxoquinazolin-3 (4H)-yl)-3-oxoalkanamide ($2B_1$-$2B_5$)

Take 0.1 moles of the product ($1B_1$-$1B_5$) with 0.1 mol of acetyl acetone and reflux for 6 hr in the presence of acetic acid (10 mL). After cooling the reaction mixture was poured into ice cold water the powder obtained filter the product and dried it then recrystallized with ethanol. It gives the title compounds ($2B_1$-$2B_5$) (Table 2).

Table 2: Physical data of compounds ($2B_1$-$2B_5$)

Compd.	n	Molecular formula	Molecular weight	Melting point (^oC)	R_f Value	Yield (%)
$2B_1$	0	$C_{16}H_{15}N_5O_3$	448	186	0.72	64.23
$2B_2$	1	$C_{17}H_{17}N_5O_3$	472	212	0.62	61.75
$2B_3$	2	$C_{18}H_{19}N_5O_3$	476	228	0.75	62.64

Cont…

Compd.	n	Molecular formula	Molecular weight	Melting point (^oC)	R_f Value	Yield (%)
$2B_4$	3	$C_{19}H_{21}N_5O_3$	500	240	0.67	62.89
$2B_5$	4	$C_{20}H_{23}N_5O_3$	504	256	0.59	59.77

n: number of alkyl substituent's in structure, R_f: relative factor

RESULTS AND DISCUSSION

Some of the synthesized compounds were characterized by TLC, Melting point, IR and ^1H NMR and Mass spectral analysis. Analysis indicated by the symbols of the elements is very close to the theoretical values. The titled derivatives have been evaluated for anti-tubercular activity against *Mycobacterium tuberculosis* H37 Rv using Microplatealamar blue dye assay (MABA). The minimum inhibitory concentration (MIC) was determined for each of the samples. The first line anti-tubercular drug Isoniazid (INH) was used as a reference standard. The results are tabulated in Table 3.

Table 3: Data showing the results of anti-tubercular activity of compounds of series ($2B_1$-$2B_5$) against *Mycobacterium tuberculosis* H_{37} RV

Compd.	Concentration in µg/mL									
	100	50	25	12.5	6.5	3.125	1.6	0.8	0.4	0.2
$2B_1$	S	S	S	S	R	R	R	R	R	R
$2B_2$	S	S	S	S	S	S	R	R	R	R
$2B_3$	S	S	S	S	R	R	R	R	R	R
$2B_4$	S	S	S	S	R	R	R	R	R	R
$2B_5$	S	S	S	S	S	S	S	S	R	R
INH	S	S	S	S	S	S	S	S	S	S

INH: Isoniazid hydrazide, S: significant, R: reduced

The literature survey prompted us to evaluate the synthesized compounds also for their anti-fungal activity and antibacterial activity. The anti-fungal activities for the entirely synthesised compounds were evaluated against the fungi *Candida albicans* and *Aspergillusniger*. The antibacterial activity was also carried out against a panel of

gram-positive and gram-negative bacteria namely, *Staphylococcus aureus*, *Enterococcus fecalis*, *Klebsiellapneumoniae and Escherichia coli*. MIC for each of the compound was determined. Clotrimazole and Ciprofloxacin were used as reference standards for the study and the results are tabulated in Table 4. The compounds evaluated for anti-tubercular activity have shown to possess excellent anti-tubercular potency. Particularly the compound 2B5 obtained as anadipic acid derivative (n = 4) is emerged as most potent anti-tubercular agent by posing the least MIC of 0.8 µg/mL. The compound $2B_2$ has also shown to possess anexcellent anti-tubercular property by having MIC of 3.125 µg/mL. The other compounds $2B_1$, $2B_3$ and $2B_4$ have exhibited significant anti-tubercular properties at MIC of 12.5 µg/mL.

Table 4: Data showing the results of antifungal and antibacterial activity studies of compounds of the series ($2B_1$-$2B_5$) by MIC method

Compound	Minimum Inhibitory Concentration (MIC in µg)					
	Antifungal activity			Antibacterial activity		
	C. albicans	***A. niger***	***S. aureus***	***E. fecalis***	***E. coli***	***K. pneumoniae***
$2B_1$	50	50	6.25	50	50	100
$2B_2$	25	12.5	6.25	50	50	100
$2B_3$	25	25	50	50	50	100
$2B_4$	50	12.5	R	R	R	R
$2B_5$	50	50	50	50	50	100
Clotrimazole (Anti-Fungal)	0.2	0.2	-	-	-	-
Ciprofloxacin (Anti-Bacterial)	-	-	0.2	0.2	0.2	0.2

Table 5: Spectral analysis of synthesised compounds

Compd.	IR (KBr) ν (cm^{-1})	^{1}H NMR (DMSO) δ in ppm
$1B_1$	3474 (NH_2), 3335 (-NH), 2893 (C-H stretching of $–CH_2$, asymmetric & symmetric), 1675 (C=O of –CONH and C=O of ring), 1619 (C=N), 1590, 1537 (C=C ring stretching), 1487 (C-N), 1420 (C-H bending of -CH_2 asymmetric & symmetric, 1250 (C-O bending)	2.00 (3H, s, 3H of $–CH_3$), 6.50 (2H, s, NH_2), 6.72-8.62 (4H, m, 4H of ring), 11.09 (2H, s, 2H of 2 CONH)

Cont…

Compd.	IR (KBr) ν (cm^{-1})	^{1}H NMR (DMSO) δ in ppm
$1B_3$	3475 (NH_2), 3073 (-NH), 3074 (Ar-CH stretching), 2993, 2823 (C-H stretching of –CH_3, -CH_2 group), 1684 (C=O & C=O of ring), 1620 (N-H), 1596 (C=N), 1562 (C=C ring stretching), 1489 (C-N), 1489, 1422 (C-H bending of –CH_3 –CH_2 group), 1252 (C-O bending)	1.83 (3H, s, CH_3), 2.11 (4H, s, 2 CH_2), 46.48 (2H, s, NH_2), 6.50-8.47 (5H, m, 5H of ring), 9.79 (1H, s, CONH), 11.1 (1H, s, CONH attached to ring)
$2B_3$	3208 (N-H stretching of NH of CONH), 3040 (Ar-CH stretching, the bond of C-H stretching of CH_3 & CH_2goups are poorly resolved), 1670, 1599 (C=O of CONH), 1599, 1486 (C=C ring stretching), 787 (substituted Ar-ring)	1.13 (3H, s, 3H of CH_3), 1.75 (6H, s, 6H of 2 × CH_3 of pyrazole ring), 2.1-4.07 (4H, m, 4H of 2 × CH_2), 7.1-7.7 (5H, m, 5H of Ar & heterocyclic protons), 9.78 (1H, s, 1H of NH of CONH)
$2B_5$	3208 (N-H stretching of NH of CONH), 3040 (Ar-CH stretching), 2950, 2872 (C-H stretching of CH_3 both asymmetric & symmetric carbonyl group around 1680 is not properly resolved and exits as a weak bond), 1599,1492 (C=C), 1429, 1366 (C-H bonding of CH_3 both asymmetric & symmetric), 1338 (C-N), 1297 (C-O), 899 (Substituted Ar-ring)	1.1 (3H, s, 3H of CH_3 of quinazolinone), 7.4 (6H, s, 6H of 2 × CH_3 of pyrazole ring), 1.75-4.06 (8H, m, 8H of 4 × CH_2), 7.11-8.45 (5H, m, Ar- & heterocyclic protons), 12.3 (NH, s, NH of CONH)

CONCLUSION

Finally, in conclusion, a series of pyrazole derivatives were synthesized successfully using multistep processes. The newly synthesized title compounds were spectroscopically characterized and were subjected to anti-fungal, antibacterial and anti-tubercular activities.

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