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Synthesis of some ethoxyphthalimide derivatives of pyrazoloisoxazoles and pyrazolopyrimidines and their antimicrobial and anticancer screening

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Synthesis of 4-arylidene-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**IIIa-d**) has been achieved by the condensation reaction between 5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**I**) and 4-substituted benzaldehydes (**IIa-d**). Ethyl acetoacetate and hydrazine hydrate in absolute alcohol undergo cyclization reaction to give (**I**). 4-Arylidene-5-methyl-2,4-dihydro-3*H*-pyrazol-3-ones (**IIIa-d**) have been converted to corresponding ethoxyphthalimide derivatives (**IVa-d**) by treatment with phthalimidoxyethyl bromide (**A**). 1-N-Ethoxyphthalimido-3-methyl-4-(4-substituted benzylidene) pyrazol-5-one (**IVa-d**) has been reacted with hydroxylamine hydrochloride and guanidine nitrate separately to yield ethoxyphthalimide substituted pyrazolo[3,4-c]isoxazoles (**Va-d**) and pyrazolo[3,4-d]pyrimidines (**VIa-d**) respectively. All the compounds have been characterized by elemental and spectral analysis mainly IR, ^{1}H NMR and mass spectroscopy. Synthesized compounds have also been screened for various biological activities *viz.* antibacterial, antifungal, antiviral and anticancer.

Keywords: Ethoxyphthalimide, pyrazoloisoxazoles, pyrazolopyrimidines, antimicrobial, anticancer

Many natural and synthetic products containing heterocyclic rings as isoxazoles[1] were reported to possess varied pharmacological activities. Isoxazoles are of considerable interest on account of their intriguing structural, chemical and biological properties. Among the wide variety of heterocycles that have been explored for developing pharmaceutically important molecules, isoxazoles[2] have played a dynamic role in the medicinal chemistry. Formation of isoxazoles from open chain hydroxy methylketones and hydroxylamine is well known[3]. Study of isoxazole derivatives is of considerable current interest as a result of their important biological and biophysical properties *i.e.* herbicidal[4], antitumor, antipsychotic, anticoagulant, antimicrobial[5] and antagonist. Isoxazoline derivatives elicit wide variety of biological activities as bactericides, fungicides, insecticides[6], analgesic and antipyretic agents and antioxidant[7], *etc*. The chemistry of isoxazoles has been reviewed and the importance of such heterocycles and their derivatives in medicinal chemistry is recognized as antiviral, bactericidal[8], anticancer[9], analgesic, antitubercular[10] and anti HIV[11] agents.

Pyrimidines are heterocyclic compounds, which serve as both biomimetic and reactive pharmacophores and many are key elements with potential biological activities. The chemistry of pyrimidines and their derivatives has been studied for over a century due to their diverse medicinal properties[12,13]. It has been associated with various medicinal applications *viz*. antitumor[14], immunodialator[15], anti tuberculosis, and radio protective. Condensed pyrazoles are biologically interesting compounds and their chemistry has received considerable attention. Pyrazolines are considered as useful synthon in organic reactions[16]. Synthetic method of these compounds involves the base catalyzed aldol condensation reaction of carbonyl compounds to give chalcones which upon cyclization reaction with hydrazine afford 2-pyrazolines[17].

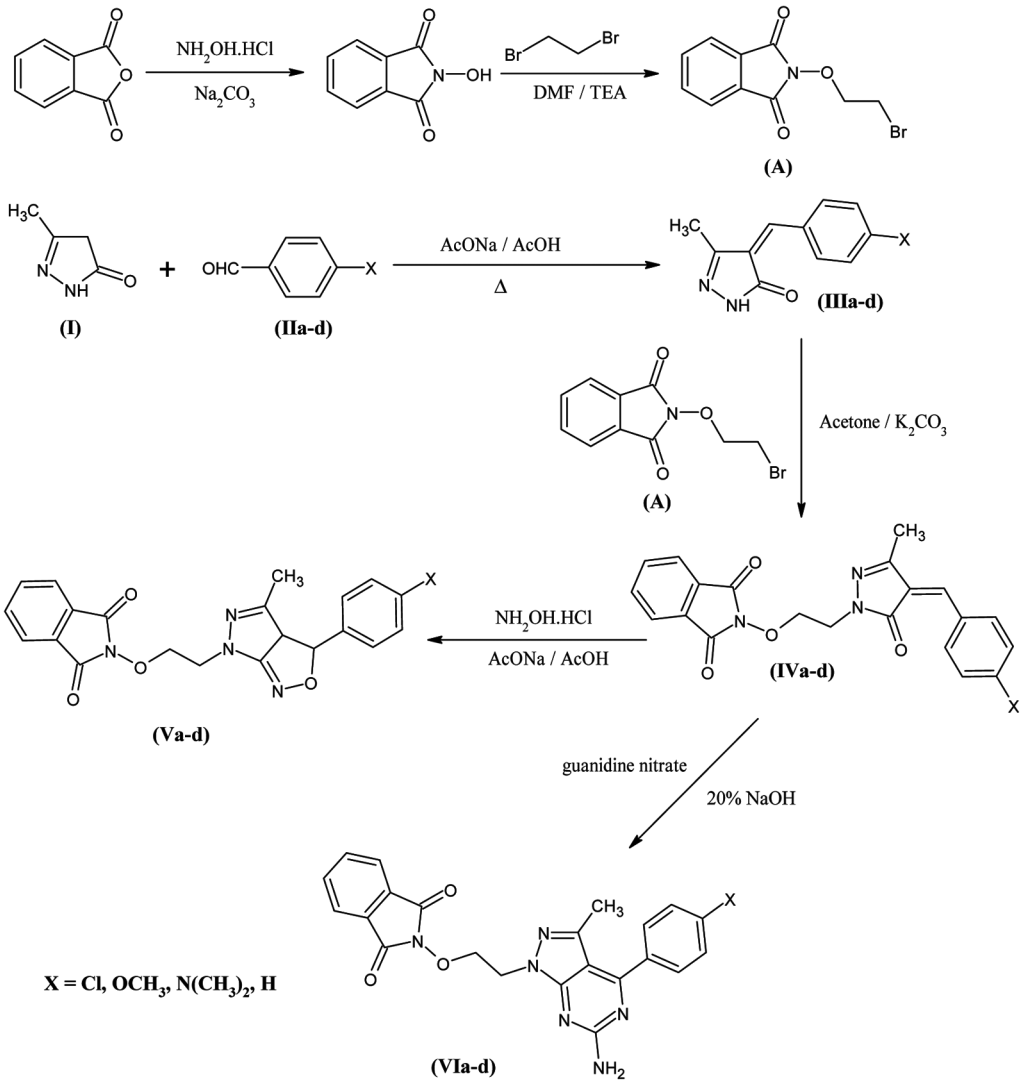
A considerable number of pyrazolo[3,4-d]pyrimidines[18] are known to be bio-active and display antitumor, antiviral and antipyretic, anti-inflammatory, analgesic, anti-arthritic, antitumor[19], antiviral, antidepressant, anticonvulsant[20], antimicrobial[21], anticancer[22], hepatoprotective, antifungal[23], antihyperglycemic, antiproliferative[24] activities, *etc*.

Isoxazoles and their condensed derivatives form an important class of cyclic compounds with various

biochemically interesting properties and pharmacologically significant activities. In recent years, many researchers have studied the role of pyrazoles and pyrimidines as chemotherapeutic agents. Encouraged by these facts and in continuation of our work on alkoxyphthalimide derivatives[25-28], we have synthesized some pyrazoloisoxazolines and pyrazolopyrimidines bearing ethoxyphthalimide moiety in anticipation of expected interesting biological activities. New combinational molecules bearing various heterocycles with alkoxyphthalimide moiety, having N-O linkage were expected to show many folds higher pharmacological activities than their respective precursors. Synthetic route of the newly synthesized compounds is depicted in Scheme I. Physical characterization data and elemental analysis of synthesized compounds are given in Table I.

Results and Discussion

In the present work an attempt has been made to undertake the synthesis of ethoxyphthalimide derivatives of some pyrazoloisoxazoles (**Va-d**) and pyrazolopyrimidines (**VIa-d**) from a series of reactions. The starting compound, 5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**I**) was prepared by the reaction between ethyl acetoacetate and hydrazine hydrate in absolute alcohol. Condensation reaction of compound (**I**) with 4-substituted benzaldehydes (**IIa-d**) in the presence of sodium acetate as a base afforded 4-arylidene-5-methyl-2,4-dihyro-3*H*-pyrazol-3-one (**IIIa-d**). Their identities were



Scheme I

Table I — Physical characterization data and elemental analysis of synthesized compounds

Compd	X	Mol. Formula	Mol. Wt.	m.p. (°C)	Yield (%)	Found/ Calcd (%) N
I	–	$C_4H_6N_2O$	98	222	92	28.50/28.57
IIIa	Cl	$C_{11}H_9N_2OCl$	220	156	75	12.71/12.72
IIIb	OCH_3	$C_{12}H_{12}N_2O_2$	216	168	76	12.81/12.96
IIIc	$N(CH_3)_2$	$C_{13}H_{15}N_3O$	229	176	69	18.07/18.34
IIId	H	$C_{11}H_{10}N_2O$	189	172	73	15.00/15.05
IVa	Cl	$C_{21}H_{16}N_3O_4Cl$	409	141	68	10.21/10.26
IVb	OCH_3	$C_{22}H_{19}N_3O_5$	405	130	70	10.16/10.37
IVc	$N(CH_3)_2$	$C_{23}H_{22}N_4O_4$	418	150	63	13.23/13.39
IVd	H	$C_{21}H_{17}N_3O_4$	375	170	65	11.08/11.20
Va	Cl	$C_{21}H_{17}N_4O_4Cl$	424	152	61	13.02/13.20
Vb	OCH_3	$C_{22}H_{20}N_4O_5$	420	146	63	9.79/10.00
Vc	$N(CH_3)_2$	$C_{23}H_{23}N_5O_4$	433	166	56	16.01/16.16
Vd	H	$C_{21}H_{1B}N_4O_4$	390	173	59	14.21/14.35
VIa	Cl	$C_{22}H_{17}N_6O_3Cl$	448	>300	64	18.58/18.76
VIb	OCH_3	$C_{23}H_{20}N_6O_4$	444	286	66	18.81/18.91
VIc	$N(CH_3)_2$	$C_{24}H_{23}N_7O_3$	457	>300	55	21.39/21.44
VId	H	$C_{22}H_{18}N_6O_3$	414	291	59	20.06/20.28

confirmed by means of IR and ^{1}H NMR spectral analysis. An intense band between 3435-3396 cm^{-1} for NH stretching appeared in IR and ^{1}H NMR signal for CH of C=CH-Ar at δ 6.1-6.3 and for NH at δ 8.0-8.3 confirmed the formation of (**IIIa-d**)**.** Treatment of (**IIIa-d**) with phthalimidoxyethyl bromide (**A**) in acetone using K_2CO_3 as a base afforded 1-N-ethoxyphthalimido-3-methyl-4-(4-substituted benzylidene) pyrazol-5-one (**IVa-d**). This was characterized by disappearance of the band in the region of 3430-3390 cm^{-1} due to NH functionality and appearance of a new band for C=O of CO-N-CO at 1776-1698 cm^{-1} in IR spectra. Appearance of new signals of alkyl side chain also helped in assigning the structure of (**IVa-d**). Formation of (**IVa-d**) was also supported by the positive fluorescence test; characteristic for the CO-N-CO group. Compounds (**IVa-d**) were used as common precursor for the targeted pyrazoloisoxazoles (**Va-d**) and pyrazolopyrimidines (**VIa-d**). 6-N-Ethoxy phthalimido-4-methyl-3-(4-substituted phenyl)-3,3a-dihydropyrazolo[3,4-c] isoxazole (**Va-d**) were obtained by the cyclization reaction of (**IVa-d**) with hydroxylamine hydrochloride in the presence of sodium acetate. Their ^{1}H NMR spectra were conclusive is assigning the structures. Two doublets at δ 4.2 and 3.9 (for **Va**) were attributed to the CH-CH group of pyrazoloisoxazole rings and ^{1}H NMR spectra was devoid of singlet for C=CH-Ar, which confirmed the cyclization. Further confirmation of structure of (**Va**) was obtained by recording its mass spectrum. It gave the molecular ion peak at 424 and 426 (M+2 peak) corresponds to the molecular formula $C_{21}H_{17}N_4O_4Cl$.

In an another reaction, (**IVa-d**) were converted to 2-amino-7-N-ethoxyphthalimido-4-(4-substituted phenyl)-5-methyl pyrazolo[3,4-d]pyrimidines (**VIa-d**) by the treatment with guanidine nitrate in 20% NaOH. The spectral data were clearly in favour of the proposed structure. IR showed intense band in the 3430-3350 cm^{-1} region, corresponds to NH_2 group, confirming the occurrence of ring closure in the form of aminopyrimidine ring. The ^{1}H NMR spectra substantiated the results of the IR analysis and exhibited a singlet at δ 6.78 for NH_2 protons. Compounds (**VIa-d**) were also verified by their elemental analysis and MS fragmentation pattern data. The measured values were consistent with the corresponding calculated ones.

Experimental Section

For characterization of compounds FTIR IR RX1 Perkin-Elmer spectrophotometers (for IR) and Bruker DRX-300 MHz spectrometer ($CDCl_3$) (for NMR) and a MICROMASS QUATTRO II triple quadrupole mass spectrometer having a JASCOPU-980 HPLC pump (for mass) connected were used.

Synthesis of phthalimidoxyethyl bromide[25], A

N-Hydroxyphthalimide (16.3g, 0.1 mol) was dissolved in dimethyl formamide (120 mL) and 1,2-dibromoethane (37.5g, 28 mL, 0.2 mol) and triethylamine (0.02 mol) were added to it slowly. This reaction mixture was allowed to stand at RT with occasional stirring, until the red colour of the solution

had turned colourless (18 hrs.). The precipitate of triethyl-ammonium bromide was then filtered. The filtrate was diluted with ice cold water (800 mL) and the solid precipitated was filtered off. This precipitate was washed with petroleum ether (b.p. 40-60°C) to remove excess of dibromoethane and then recrystallized by ethanol. Product yield 52%, m.p. 79°C.

Synthesis of 5-methyl-2,4-dihydro-3*H*-pyrazol-3-one, I

In a conical flask ethyl acetoacetate (0.1 mol) and hydrazine hydrate (0.2 mol) in ethanol (20 mL) were mixed with drop wise addition of hydrazine hydrate coupled with stirring. The reaction is exothermic in nature, so the temperature raised during the reaction was maintained at 60°C at which a crystalline solid separated out. Stirring was continued for 1 hr. at RT after that cooled in an ice bath to complete the crystallization. Finally the crystals were washed with ice cold ethanol.

Synthesis of 4-(4-chlorobenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one, IIIa: In a round bottomed flask, 5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (I, 0.01 mol), 4-chloro-benzaldehyde (IIa, 0.01 mol) and anhydrous sodium acetate (0.02 mol) were taken, the solid was dissolved in acetic acid and refluxed for 10 hrs. The reaction mixture was then filtered and the filtrate was poured on crushed ice. The product was crystallized from ethanol. IR (KBr): 3412 (N-H str.), 3080 (C-H str., Ar-H), 2952 (C-H str., CH_3), 1718 (C=O str.), 1612 (C=N str.), 728 (C-Cl str.); ^{1}H NMR ($CDCl_3$): δ 8.0 (m, 4H, Ar-H), 6.2 (s, 1H, =CH), 7.03-7.62 (s, 1H, NH), 1.8 (s, 3H, CH_3).

Compounds (**IIIb-d**) were also synthesized by similar method using appropriate reactants with required change in reflux time.

4-(4-Methoxybenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one, IIIb: IR (KBr): 3435 (N-H str.), 3076 (C-H str., Ar-H), 2964 (C-H str., CH_3), 1690 (C=O str.), 1602 (C=N str.), 1098 cm^{-1} (C-O str.); ^{1}H NMR ($CDCl_3$): δ 8.2 (m, 4H, Ar-H), 6.1 (s, 1H, =CH), 7.2-7.5 (s, 1H, NH), 3.7 (s, 3H, OCH_3), 1.7 (s, 3H, CH_3).

4-(4-Dimethylaminobenzylidene)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one, IIIc: IR (KBr): 3410 (N-H str.), 3069 (C-H str., Ar-H), 2950 (C-H str., CH_3), 1685 (C=O str.), 1601 cm^{-1} (C=N str.); ^{1}H NMR ($CDCl_3$): δ 8.2 (m, 4H, Ar-H), 6.1 (s, 1H, =CH), 7.3-7.6 (s, 1H, NH), 3.27 (s, 6H, $N(CH_3)_2$), 1.9 (s, 3H, CH_3).

4-Benzylidene-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one, IIId: IR (KBr): 3396 (N-H str.), 3062 (C-H str., Ar-H), 2946 (C-H str., CH_3), 1705 (C=O str.), 1599 cm^{-1} (C=N str.); ^{1}H NMR ($CDCl_3$): δ 8.1 (m, 5H, Ar-H), 6.3 (s, 1H, =CH), 7.2-7.6 (s, 1H, NH), 1.8 (s, 3H, CH_3).

Synthesis of 4-(4-chlorobenzylidene)-1-N-ethoxyphthalimido-3-methyl pyrazol-5-one, IVa: A mixture of compound (**IIIa**, 0.01 mol), phthalimidoxyethyl bromide (**II**, 0.01 mol) and K_2CO_3 (0.02 mol) as base in acetone was refluxed for 20 hrs, cooled and poured on crushed ice. The product obtained was filtered and further recrystallised from ethanol. IR (KBr): 3056 (C-H str., Ar-H), 2938, 2846 (C-H str., CH_2), 1756, 1714 (C=O str.), 1602 (C=N str.), 1350 (N-O str.), 1075 (C-O str.), 728 cm^{-1} (C-Cl str.); ^{1}H NMR ($CDCl_3$): δ 7.2-7.8 (m, 8H, Ar-H),6.6 (s, 1H, =CH), 4.8 (t, 2H, OCH_2), 3.66 (t, 2H, NCH_2), 1.7 (s, 3H, CH_3). Similarly (**IVb-d**) were prepared with minor modification in reaction time.

4-(4-Methoxybenzylidene)-1-N-ethoxyphthalimido-3-methyl pyrazol-5-one, IVb: IR (KBr): 3072 (C-H str., Ar-H), 2932 (C-H str., CH_2), 1755, 1722 (C=O str.), 1603 (C=N str.), 1375 (N-O str.),1080 cm^{-1} (C-O str.); ^{1}H NMR ($CDCl_3$): δ 7.3-7.6 (m, 8H, Ar-H), 6.7 (s, 1H, =CH), 4.72 (t, 2H, OCH_2), 3.79 (t, 2H, NCH_2), 3.6 (s, 3H, OCH_3), 1.9 (s, 3H, CH_3).

4-(4-Dimethylaminobenzylidene)-1-N-ethoxyphthalimido-3-methyl pyrazol-5-one, IVc: IR (KBr): 3067 (C-H str., Ar-H), 2940 (C-H str., CH_2), 1748, 1716 (C=O str.), 1605 (C=N str.), 1380 (N-O str.), 1092 cm^{-1} (C-O str.); ^{1}H NMR ($CDCl_3$): δ 7.0-7.88 (m, 8H, Ar-H), 6.4 (s, 1H, =CH), 4.79 (t, 2H, OCH_2), 3.65 (t, 2H, NCH_2), 3.27 (s, 6H, $N(CH_3)_2$), 2.00 (s, 3H, CH_3).

4-Benzylidene-1-N-ethoxyphthalimido-3-methyl pyrazol-5-one, IVd: IR (KBr): 3068 (C-H str., Ar-H), 2946, 2848 (C-H str., CH_2), 1776, 1698 (C=O str.), 1605 (C=N str.), 1380 (N-O str.),1100 cm^{-1} (C-O str.); ^{1}H NMR ($CDCl_3$): δ 7.1-7.5 (m, 8H, Ar-H), 6.8 (s, 1H, =CH), 4.82 (t, 2H, OCH_2),3.55 (t, 2H, NCH_2), 1.9 (s, 3H, CH_3).

Synthesis of 3-(4-chlorophenyl)-6-N-ethoxyphthal-imido-4-methyl-3,3a-dihydropyrazolo[3,4-c]isoxazole, Va: Anhydrous sodium acetate (0.01 mol) was dissolved in hot acetic acid.

Compound (**IVa** 0.01 mol) was taken in absolute alcohol (10 mL) and to it hydroxylamine hydrochloride (0.01 mol) in absolute alcohol (10 mL) was added. The solution of sodium acetate in acetic acid was transferred to this reaction mixture and refluxed for 10 hrs, which was poured into ice cold water, the solid obtained was filtered and recrystallised from DMF. IR (KBr): 3080 (C-H str., Ar-H), 2922 (C-H str., CH_2), 1789, 1728 (C=O str.), 1597 (C=N str.), 1375 (N-O str.), 1087 (C-O str.), 702 cm^{-1} (C-Cl str.); ^1H NMR ($CDCl_3$): δ 7.32-8.31 (m, 8H, Ar-H), 4.48 (t, 2H, OCH_2), 4.23 (d, 1H, CHO), 3.9 (d, 1H, CH), 3.64 (t, 2H, NCH_2), 2.08 (s, 3H, CH_3); MS: *m/z* 424 $[M]^{+\cdot}$, 426 $[M+2]^{+\cdot}$, 385,319, 321, 280, 221, 190, 182, 165, 99, 74.

Compounds (**Vb-d**) are prepared in a similar manner with a minor change in reflux time.

3-(4-Methoxy phenyl)-6-N-ethoxyphthalimido-4-methyl-3,3a-dihydropyrazolo[3,4-c]isoxazole, Vb: IR (KBr): 3050 (C-H str., Ar-H), 294 (C-H str., CH_2), 1780, 1730 (C=O str.), 1600 (C=N str.), 1360 (N-O str.), 1080 cm^{-1} (C-O str.); ^1H NMR ($CDCl_3$): δ 7.16-7.80 (m, 8H, Ar-H), 4.72 (t, 2H, OCH_2), 4.22 (d, 1H, CHO), 3.9 (d, 1H, CH), 3.79 (t, 2H, NCH_2), 3.58 (s, 3H, OCH_3), 2.10 (s, 3H, CH_3); MS: *m/z* 420 $[M]^{+\cdot}$, 381, 317, 315, 276, 217, 190, 182, 161, 95, 71.

3-(4-Dimethylaminophenyl)-6-N-ethoxyphthalimido-4-methyl-3,3a-dihydropyrazolo[3,4-c]isoxazole, Vc: IR (KBr): 3020 (C-H str., Ar-H), 2960 (C-H str., CH_2), 1756, 1730 (C=O str.), 1601 (C=N str.), 1355 (N-O str.), 1065 cm^{-1} (C-O str.); ^1H NMR ($CDCl_3$): δ 7.00-7.89 (m, 8H, Ar-H), 4.79 (t, 2H, OCH_2), 4.25 (d, 1H, CHO), 4.00 (d, 1H, CH), 3.65 (t, 2H, NCH_2), 2.89 (s, 6H, $N(CH_3)_2$), 2.06 (s, 3H, CH_3); MS: *m/z* 433 $[M]^{+\cdot}$, 394, 330, 328, 289, 230, 190, 182, 174, 108, 83.

3-Phenyl-6-N-ethoxyphthalimido-4-methyl-3,3a-dihydropyrazolo[3,4-c]isoxazole, Vd: IR (KBr): 3088 (C-H str., Ar-H), 2980 (C-H str., CH_2), 1786, 1730 (C=O str.), 1590 (C=N str.), 1364 (N-O str.), 1072 cm^{-1} (C-O str.); ^1H NMR ($CDCl_3$): δ 7.23-7.79 (m, 9H, Ar-H), 4.82 (t, 2H, OCH_2), 4.25 (d, 1H, CHO), 3.98 (d, 1H, CH), 3.55 (t, 2H, NCH_2), 2.09 (s, 3H, CH_3); MS: *m/z* 390 $[M]^{+\cdot}$, 351, 287, 285, 246, 205, 190, 182, 131, 65, 40.

Synthesis of 2-amino-4-(4-chloro phenyl)-7-N-ethoxyphthalimido-5-methyl pyrazolo[3,4-d]pyrimidines, VIa: 3-(4-Chloro phenyl)-6-N-ethoxyphthalimido-4-methyl-3,3a-dihydropyrazolo[3,4-c]isoxazole (**IVa**, 0.01mol) and guanidine nitrate (0.01mol) were dissolved in absolute alcohol and refluxed for 1 hr. Then 10% NaOH solution was added to the reaction mixture and refluxing was continued for 8 hrs. Reaction mixture was cooled and poured on crushed ice to get light brown coloured product of **VIa**, which was recrystallised from DMF. IR (KBr): 3430, 3380 (N-H str., NH_2), 2924 (C-H str., CH_2), 1790, 1725, 1686 (C=O str.), 1591 (C=N str.), 1378 (N-O str.), 1090 (C-O str.), 702 cm^{-1} (C-Cl str.); ^1H NMR ($CDCl_3$): δ 7.13-8.12 (m, 8H, Ar-H), 6.78 (s, 2H, NH_2), 4.48 (t, 2H, OCH_2), 3.64 (t, 2H, NCH_2), 2.06 (s, 3H, CH_3); MS: *m/z* 448 $[M]^{+\cdot}$, 450 $[M+2]^{+\cdot}$, 386, 344, 324, 254, 240, 220, 208, 195, 182, 140, 115, 99.

Similarly (**VIb-d**) were prepared with minor modification in concentration of NaOH and reflux time.

2-Amino-4-(4-methoxy phenyl)-7-N-ethoxyphthalimido-5-methyl pyrazolo[3,4-d]pyrimidines, VIb: IR (KBr): 3432, 3350 (N-H str., NH_2), 3025 (C-H str., Ar-H), 2928 (C-H str., CH_2), 1789, 1720 (C=O str.), 1590 (C=N str.), 1380 (N-O str.), 1085 cm^{-1} (C-O str.); ^1H NMR ($CDCl_3$): δ 7.17-7.79 (m, 8H, Ar-H), 6.50 (s, 2H, NH_2), 4.49 (t, 2H, OCH_2), 3.84 (t, 2H, NCH_2), 3.59 (s, 3H, OCH_3), 2.09 (s, 3H, CH_3); MS: *m/z* 444 $[M]^{+\cdot}$, 386, 340, 324, 250, 244, 191, 182, 136, 111, 95.

2-Amino-4-(4-dimethylamino phenyl)-7-N-ethoxyphthalimido-5-methyl pyrazolo[3,4-d]pyrimidines, VIc: IR (KBr): 3415, 3355 (N-H str., NH_2), 3030 (C-H str., Ar-H), 2970 (C-H str., CH_2), 1780, 1715 (C=O str.), 1601 (C=N str.), 1350 (N-O str.), 1072 cm^{-1} (C-O str.); ^1H NMR ($CDCl_3$): δ 7.17-8.51 (m, 8H, Ar-H), 6.52 (s, 2H, NH_2), 4.55 (t, 2H, OCH_2), 3.80 (t, 2H, NCH_2), 3.15 (s, 6H, $N(CH_3)_2$), 2.10 (s, 3H, CH_3); MS: *m/z* 457 $[M]^{+\cdot}$, 386, 353, 324, 2363, 240, 229, 217, 204, 182, 149, 124, 108.

2-Amino-4-phenyl-7-N-ethoxyphthalimido-5-methyl pyrazolo[3,4-d]pyrimidines, VId: IR (KBr): 3415, 3375 (N-H str., NH_2), 3052 (C-H str., Ar-H), 2980 (C-H str., CH_2), 1785, 1724 (C=O str.), 1602 (C=N str.), 1386 (N-O str.), 1070 cm^{-1} (C-O str.); ^1H NMR ($CDCl_3$): δ 7.60-7.80 (m, 9H, Ar-H), 6.64 (s, 2H, NH_2), 4.50 (t, 2H, OCH_2), 3.62 (t, 2H, NCH_2), 2.04 (s, 3H, CH_3); MS: *m/z* 414 $[M]^{+\cdot}$, 386, 324, 310, 240, 220, 186, 182, 174, 161, 106, 65.

Antimicrobial Screening

Experimental details

Bacterial strains used for the present investigation are one gram positive *B. subtilis* and three gram

negative *P. mirabilis*, *E. coli* and *K. pneumoniae*. Two standard drugs were used for comparative study *viz*. ciprofloxacin and cefuroxime. *Candida albicans* (MTCC227) and *Aspergillus fumigatus* (MTCC2550) were used for the antifungal screening of compounds. Amphotericin B was used as standard drug.

Method

Cup or Well method[30] was used for carrying out antimicrobial screening. Nutrient agar medium was used to culture the microbes which was sterilized by autoclaving at 15 psi and 121°C for twenty minutes. The nutrient agar medium was poured in petri dishes and spread plate method[31] was used in which 0.2 mL suspension of organism were inoculated in petri dishes. In the nutrient medium, around four wells of 11 mm diameter were made and filled with 500 ppm solution of testing compound in DMF. Similarly other wells were made for standard drugs and filled with standard concentration[32]. These Petri plates were incubated at 37°C in an incubator. Zone of inhibition were examined after 24-48 hrs.

Results and Discussion

Results of antibacterial activity are summarized in Table II. Zone of inhibition was measured in mm. Activity index of all the synthesized compounds was also calculated for all bacterial strains against standard drugs.

The data in the table reveals that most of the compounds show significant antibacterial as well as antifungal activity against the all tested organism. All the compounds exhibited strong activity against *P. mirabilis* as compared to standard. Most of the compounds have been found to show poor to moderate inhibition against *E. coli.* Compounds displayed promising antibacterial activity against *P. mirabilis* and *K. pneumoniae*. Similarly (**Va**) exhibited strong inhibition against all pathogenic bacteria. The rests of the compounds showed poor to moderate activity as compared to the standards used.

By the keen observation of Table II, it can be concluded that all the compounds show stronger activity against both the fungal strains as compared to the

Table II — Antibacterial Activity (500 ppm) Zone of inhibition (mm) (activity index)*

Compd	Code	*P. mirabilis*	*K. pneumoniae*	*E. coli*	*B. subtilis*	*Candida albicans*	*Aspergillus fumigatus*
Va	1	14 (1.00)^{A} (1.07)^{B}	13 (1.08)^{A} (2.16)^{B}	9 (0.75)^{A} (0.81)^{B}	14 (1.27)^{A}	25 (2.08)^{C}	18 (3.00)^{C}
Vb	2	15 (1.07)^{A} (1.15)^{B}	11 (0.91)^{A} (1.83)^{B}	8 (0.66)^{A} (0.72)^{B}	12 (1.09)^{A}	22 (1.83)^{C}	18 (3.00)^{C}
Vc	3	13 (0.92)^{A} (1.00)^{B}	10 (0.83)^{A} (1.66)^{B}	7 (0.58)^{A} (0.63)^{B}	14 (1.27)^{A}	21 (1.75)^{C}	19 (3.16)^{C}
Vd	4	15 (1.07)^{A} (1.15)^{B}	8 (0.66)^{A} (1.33)^{B}	8 (0.66)^{A} (0.72)^{B}	10 (0.90)^{A}	21 (1.75)^{C}	20 (3.33)^{C}
VIa	5	16 (1.14)^{A} (1.23)^{B}	11 (0.91)^{A} (1.83)^{B}	10 (0.83)^{A} (0.90)^{B}	13 (1.18)^{A} (2.16)^{C}	21 (1.75)^{C}	20 (3.33)^{C}
VIb	6	16 (1.14)^{A} (1.23)^{B}	9 (0.75)^{A} (1.5)^{B}	8 (0.66)^{A} (0.72)^{B}	11 (1.00)^{A}	22 (1.83)^{C}	22 (3.66)^{C}
VIc	7	19 (1.35)^{A} (1.46)^{B}	13 (1.08)^{A} (2.16)^{B}	6 (0.50)^{A} (0.54)^{B}	12 (1.09)^{A}	18 (1.50)^{C}	19 (3.16)^{C}
VId	8	16 (1.14)^{A} (1.23)^{B}	12 (1.00)^{A} (2.00)^{B}	9 (0.75)^{A} (0.81)^{B}	10 (0.90)^{A}	17 (1.41)^{C}	21 (3.50)^{C}
Standard	A	14	12	12	11	–	–
	B	13	6	11	NA	–	–
	C	–	–	–	–	12	6

* Activity index = Inhibition area of the sample/inhibition area of the standard.
A = Ciprofloxaci
B = Cefuroxime
C = Amphotericin B

standard drug used. Especially against *A. fumigatus* three fold or four folds activity was observed than Amphotericin B. Compound (**Va**) showed powerful inhibition on *C. albicans*. All the final compounds exhibited pronounced inhibition on *A.fumigatus*. Compounds (**Va**) and (**VIb**) were found to be the most potent against both *C.albicans* and *A. fumigatus*.

Finally it can be tentatively concluded that heterocyclic compounds containing phthalimidoxy moiety are strong antifungal agents.

Anticancer Activity

Anticancer activity was tested against proliferation of murine leukemia cells and human T-lymphocyte cells. Ethoxyphthalimido pyrazoloisoxazole (**Va**) was found to be the best anticancer agent. It showed the inhibition against all three tested cell lines at less than 20 µg/mL concentration. Results of the anticancer activity have been depicted in Table III.

Antiviral activity

Viruses are a connecting link between nonliving and living organisms, possessing both living and nonliving characteristics like absence of metabolic enzyme machinery and protein synthesizing system as well as presence of a host cell dependent machinery of multiplication.

Cytotoxicity and antiviral activity of synthesized compounds in HEL, HeLa and Vero cell cultures

Results of the antiviral[33] assay in HEL, HeLa and Vero cells with the 8 compounds have been presented in Table IV, Table V, Table VI and Table VII. No specific antiviral effects (*i.e.* minimal antivirally

Table III — Inhibitory effects of synthesized compounds on the proliferation of murine leukemia cells and human T-lymphocyte cells

Compd	*IC_{50} (µg/mL)		
	L1210/0	Molt4/C8	CEM/0
Va	18 ± 0	20 ± 0	18 ± 1
Vb	> 200	> 200	144 ± 13
Vc	119 ± 7	112 ± 18	104 ± 15
Vd	56 ± 1	75 ± 5	44 ± 25
VIa	20 ± 0	22 ± 2	18 ± 1
VIb	> 200	> 200	> 200
VIc	58 ± 5	58 ± 9	34 ± 14
VId	110 ± 4	138 ± 87	84 ± 7

*50% inhibitory concentration

Table IV — Cytotoxicity and antiviral activity of synthesized compounds in HEL cell cultures

Compd	Minimum cytotoxic conc.[a] (µg/mL)	Minimum inhibitory concentration[b] (µg/mL)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK^- KOS ACV^r
Va	≥20	>20	>20	>20	>20	>20
Vb	100	>20	12	>20	>20	20
Vc	>100	>100	>100	>100	>100	>100
Vd	>100	>100	>100	>100	>100	>100
VIa	>100	>100	>100	>100	>100	>100
VIb	>100	>100	>100	>100	>100	>100
VIc	20	>4	>4	>4	>4	>4
VId	20	>4	>4	>4	>4	>4
Brivudin (µM)	>250	0.08	10	6	>250	50
Ribavirin (µM)	>250	10	>250	150	>250	>250
Acyclovir (µM)	>250	0.16	0.24	>250	>250	150
Ganciclovir (µM)	>100	0.032	0.096	>100	>150	12

[a]Required to cause a microscopically detectable alteration of normal cell morphology
[b]Required to reduce virus-induced cytopathogenicity by 50%.

Table V — Cytotoxicity and antiviral activity of ethoxyphthalimidopyrazoloisoxazole in HEL cell culture (Exp.-2)

Compd	Minimum cytotoxic conc.[a] (µg/mL)	Minimum inhibitory concentration[b] (µg/mL)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK^- KOS ACV^r
Va	100	20	20	>20	>20	20
Brivudin (µM)	>250	0.08	150	6	>250	10
Ribavirin (µM)	>250	>250	>250	150	>250	250
Acyclovir (µM)	>250	0.4	0.4	>250	>250	150
Ganciclovir (µM)	>100	0.032	0.032	>100	>100	4

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.

Table VI — Cytotoxicity and antiviral activity of final compounds in HeLa cell cultures

Compd	Minimum cytotoxic conc.[a] (μg/mL)	Minimum inhibitory concentration[b] (μg/mL)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytical virus
Va	100	>20	>20	12
Vb	>100	>100	>100	>100
Vc	>100	>100	>100	>100
Vd	>100	>100	>100	>100
VIa	100	>20	12	>20
VIb	>100	>100	>100	>100
VIc	4	>0.8	>0.8	>0.8
VId	>100	>100	>100	>100
Brivudin (μM)	>250	>250	>250	>250
(S)-DHPA (μM)	>250	250	>250	>250
Ribavirin (μM)	>250	30	150	10

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.

Table VII — Cytotoxicity and antiviral activity of final synthesized compounds in Vero cell cultures

Compd	Minimum cytotoxic conc.[a] (μg/mL)	Minimum inhibitory concentration[b] (μg/mL)				
		Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
Va	100	>20	>20	>20	>20	>20
Vb	100	>20	>20	>20	>20	>20
Vc	100	>20	>20	>20	>20	>20
Vd	≥100	>100	>100	>100	60	>100
VIa	>100	>100	>100	>100	60	100
VIb	≥100	>100	>100	>100	60	>100
VIc	20	>4	>4	>4	>4	>4
VId	>100	>100	>100	>100	>100	>100
Brivudin (μM)	>250	>250	>250	>250	>250	>250
(S)-DHPA (μM)	>250	150	>250	>250	>250	>250
Ribavirin (μM)	>250	250	250	>250	>250	150

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.

effective concentration) were noted for any of the compounds evaluated against any of the viruses, except for compound (**Va**) against respiratory syncytical virus and (**VIa**) against coxsackie B4 virus. These tests were subject of second experiment. For (**Va**), a second experiment was carried out against HSV and this experiment confirmed activity of (**Va**) against HSV but only at a concentration that was 5 fold below the cytotoxic threshold.

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