Full Paper

Synthesis and Antimicrobial Study of Novel 1-Aryl-2-oxo-indano[3,2-*d*]pyrido/pyrimido[1,2-*b*]pyrimidines

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A series of 2-(arylidene)-indan-1, 3-diones **1** were prepared by Knoevenagel reaction between indane-1,3-dione and the appropriate araldehydes. The α,β-enones **1** have been used as a component of Michael addition with equimolar amounts of 2-aminopyridine/2-aminopyrimidine to give novel heterocyclic systems **4** and **5**, respectively. They are reported here for the first time. The structures of the newly synthesized compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. All compounds were screened for their antibacterial and antifungal activities. Some of them showed promising activities.

Keywords: Antibacterial activity / Antifungal activity / 2-(Arylidene)-indan-1,3-diones / Pyrido-/pyrimido-pyrimidines

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Introduction

The high therapeutic properties of the compounds incorporating nitrogen heterocycles have encouraged the medicinal chemists to synthesize large numbers of novel therapeutic agents [1–4]. In addition, the applications of nitrogen heterocycles as toxic agents into the cell wall of pathogenic microorganism have evoked considerable attention during the last twenty years [5–7].

Several fused heterocyclic systems, incorporating a pyrimidine ring in their structures, play important roles as analgesic [8], antihypertensive [9], antiviral [10], anti-inflammatory [11], antioxidant [12], antiplatelet [13], and hepatoprotective [14] agents. Pyrimidopyrimidines, an important class of nitrogen-containing heterocycles, occupy a unique place in medicinal chemistry owing to their wide spectrum of clinical applications [15–16]. Many analogues of pyrimido-[4,5-*d*]pyrimidine have been reported to display a substantial level of inhibitory action against the tyrosine-kinase domain of epidermal growth factor receptor [17] and have potential applications in cancer therapy [18–19]. A perusal of the literature has revealed manifold implications of pyridopyrimidines *e.g.,* pyrido-[1,2-*b*]pyrimidine systems show antifungal, herbicidal, and anti-asthmatic properties [20–22], while pyrido-[2,3-*d*]pyrimidines were found to have strong biological activities [23–24]. Therefore, it was thought of interest to design a system which combines bio-labile nuclei; pyridine, pyrimidine, and indane fused together in a molecular framework to see their additive effect on antimicrobial power.

The toxicity, side effects, and resistance of common pathogens to standard drugs play important roles in treatment failure [25–26]. Therefore, searching for new antimicrobial agents with specific activity, possibly acting through mechanism, which are distinct from those of well-known classes is of prime interest.

The above facts coupled with our desire to develop efficacious antimicrobial agents and in continuation of our work on fused heterocycles with biological interest [27–29], prompted us to device an efficient and convenient synthetic method of hitherto unknown and novel title compounds 1-aryl-2-oxo-indano-[3,2-*d*]-pyrido / pyrimido-[1,2-*b*]pyrimidines **4** and **5**. The antibacterial and antifungal results of these newly synthesized compounds are reported in this paper.

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Abbreviation: minimum inhibitory concentration (MIC)

Results and discussion

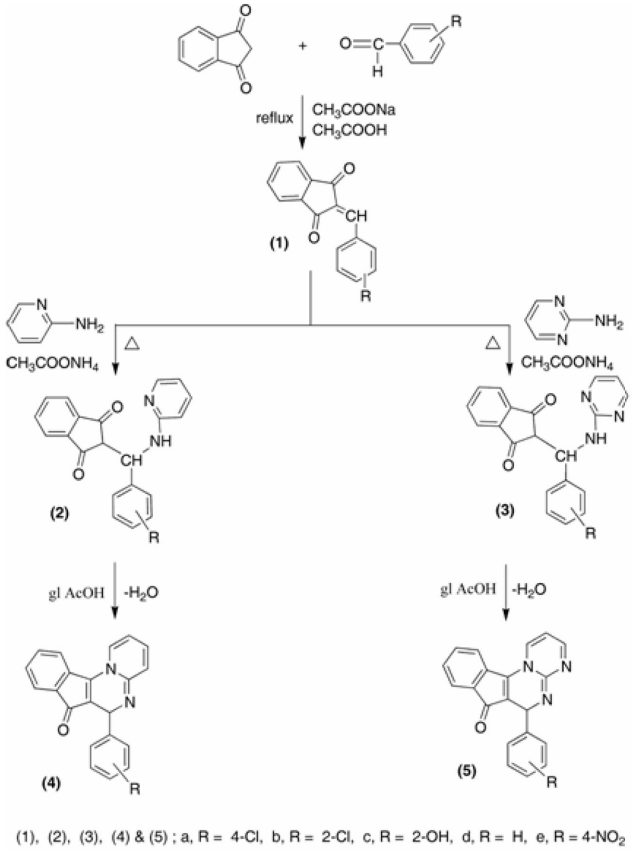
Chemistry

The newly designed compounds have been synthesized as given in Scheme 1. The required 2-(arylidene)-indan-1,3-diones **1** were obtained by Knoevenagel reaction between indane – 1,3-dione and appropriate araldehydes in the presence of CH_3COOH/CH_3COONa in good yields. The enones **1** were used as valuable intermediates for the preparation of the title compounds. In fact, these compounds with an α,β-unsaturated ketone (-CH=CH-CO-) function in their structure are activated alkenes and have been used as a component of Michael addition. These arylidenes (0.004 mol), when condensed with 2-aminopyridine/2-aminopyrimidine (0.004 mol) in the presence of ammonium acetate (0.028 mol) for two hours, furnished the presumed Michael adducts **2** and **3**, respectively. These Michael adducts **2** and **3** have been isolated, characterized, and then subjected to cyclodehydration in the presence of glacial acetic acid to give the final products **4** and **5**. The structures of the final products **4** and **5** and the Michael adducts **2** and **3** have been confirmed by elemental and spectral data.

In the IR spectrum of compound **1a**, a stretching peak at 1675 cm^{-1} indicates an α,β-unsaturated carbonyl group and the arylidene C=C stretching was observed at 1610 cm^{-1}. The disappearance of a peak at 1610 cm^{-1} and shifting of the carbonyl peak to a higher frequency (1710 cm^{-1}) in **2a** indicates the absence of unsaturation at the α,β-position of the carbonyl group. The appearance of new peaks at 3260 cm^{-1} (N-H) and 1560 cm^{-1} (endo C=N) in **2a** further support its formation. In the IR spectrum of **4a**, the N-H peak disappeared, which indicates its cyclization from **2a**. The other prominent peaks are at 1705 cm^{-1} (C=O) and 1570 cm^{-1} (exo C=N).

The ^{1}H-NMR spectrum of **1a** shows a singlet at δ 4.9 due to olefinic proton, which disappears on reacting with 2-aminopyridine, indicates the formation of **2a**, in which this proton appeares as a multiplet at δ 4.3. The appearance of two other doublets at δ 3.2 (-HC-C=O) (J = 7.1 Hz) and δ 4.0 (N-H exchangeable with D_2O) further confirmed the formation of **2a**.

In the ^{1}H-NMR spectrum of **4a**, no signals for −HC-C=O and N-H protons were observed, but a new singlet at δ 3.1 appeared. It clearly indicates the cyclization of **2a** into **4a**. Further, support for the structures of **2a** and **4a** were obtained from the ^{13}C-NMR spectra. In the ^{13}C-NMR spectrum of **1a** nine signals including a signal of carbonyl carbon at δ 190.8 were observed, while on reaction with 2-aminopyridine, the ^{13}C-NMR showed thirteen signals with carbonyl carbon at δ 192.5, which corresponds to the carbon skeleton of **2a**. Similarly, the number of signals obtained in the ^{13}C-NMR spectrum of **4a**, were nineteen, the carbonyl carbon at δ 188.5 is in good agreement with its structure. A similar spectral pattern (IR, ^{1}H-NMR, and ^{13}C-NMR) was also observed in the formation of **3a** from **1a** and **5a** from **3a**, which are given in Experimental (section 4). The physical data of the compounds **1a – 5e** are given in Table 1



(1), (2), (3), (4) & (5) ; a, R = 4-Cl, b, R = 2-Cl, c, R = 2-OH, d, R = H, e, R = 4-NO_2

Scheme 1. Schematic diagram showing the synthesis of compounds **1 – 5**.

Antimicrobial activity

Antibacterial studies

The results of antibacterial testing revealed that all tested compounds showed moderate to good antibacterial activity. Compound **4b**, **4c**, **5b**, and **5c** showed very promising activity against *E. coli, S. Aureus*, and *B. Subtilis* at 6.25 μg/μL concentrations, while **4a**, **4e**, **5a**, **5e**, and **5d** are active against the *gram*-negative *Escherichia coli*, *Klebsiella pneumoniae, gram*-positive *Staphylococcus aureus* and *Bacillus subtilis* at 12.5 and 25 μg/μL concentrations, respectively, as compared with the standard drug ciprofloxacin. Compound **4d** is the least active in this series. The results of the antibacterial studies are given in Table 2.

Table 1. Physical data of compounds **1a–5e**.

Compound[a]	R	Mol. Formula	Mp. (°C)	Yield (%)
1a	4-Cl	$C_{16}H_9ClO_2$	150–152	70
1b	2-Cl	$C_{16}H_9ClO_2$	227–228	67
1c	2-OH	$C_{16}H_{10}O_3$	168–170	65
1d	H	$C_{16}H_{10}O_2$	117–119	71
1e	4-NO_2	$C_{16}H_9NO_4$	210	73
2a	4-Cl	$C_{21}H_{15}ClN_2O_2$	103–104	65
2b	2-Cl	$C_{21}H_{15}ClN_2O_2$	109–111	63
2c	2-OH	$C_{21}H_{16}N_2O_3$	131–133	61
2d	H	$C_{21}H_{16}N_2O_2$	121–123	67
2e	4-NO_2	$C_{21}H_{15}N_3O_4$	160–163	70
3a	4-Cl	$C_{20}H_{14}ClN_3O_2$	129	62
3b	2-Cl	$C_{21}H_{14}ClN_3O_2$	134	64
3c	2-OH	$C_{20}H_{15}N_3O_3$	141–143	59
3d	H	$C_{20}H_{15}N_3O_2$	117–119	69
3e	4-NO_2	$C_{20}H_{14}N_4O_4$	181	72
4a	4-Cl	$C_{21}H_{13}ClN_2O$	160–162	68
4b	2-Cl	$C_{21}H_{13}ClN_2O$	150–152	61
4c	2-OH	$C_{21}H_{14}N_2O_2$	227–229	59
4d	H	$C_{21}H_{14}N_2O$	203	65
4e	4-NO_2	$C_{21}H_{13}N_3O_3$	260–261	72
5a	4-Cl	$C_{20}H_{12}ClN_3O$	201–203	70
5b	2-Cl	$C_{20}H_{12}ClN_3O$	190 (dec.)[b]	62
5c	2-OH	$C_{20}H_{13}N_3O_2$	210–212	61
5d	H	$C_{20}H_{13}N_3O$	170–173	67
5e	4-NO_2	$C_{20}H_{12}N_4O_3$	242	70

[a] Compounds are within the range of ± 0.4% in elemental analysis.

[b] (dec.) = decomposition

Table 2. Antibacterial activities of compounds **4a–5e**.

Compound	*Escherichia coli* Zone of inhibition[a]/(MIC)	*Klebsiella pneumoniae* Zone of inhibition[a]/(MIC)	*Staphylococcus aureus* Zone of inhibition[a]/(MIC)	*Bacillus subtilis* Zone of inhibition[a]/(MIC)
4a	15 (12.5)	16 (6.25)	15 (12.5)	16 (12.5)
4b	18 (6.25)	17 (12.5)	20 (6.25)	21 (6.25)
4c	19 (6.25)	20 (6.25)	21 (6.25)	22 (6.25)
4d	10 (100)	12 (25)	13 (12.5)	11 (25)
4e	16 (12.5)	16 (12.5)	18 (6.25)	17 (12.5)
5a	15 (6.25)	15 (12.5)	16 (12.5)	14 (25)
5b	17 (6.25)	20 (6.25)	21 (6.25)	21 (6.25)
5c	19 (12.5)	21 (6.25)	22 (12.5)	23 (6.25)
5d	13 (25)	14 (12.5)	16 (12.5)	11 (50)
5e	16 (12.5)	15 (25)	16 (12.5)	18 (12.5)
Standard	21 (6.25)	23 (3.25)	25 (6.25)	24 (6.25)

[a] Zone of inhibition is expressed in mm; MIC values are given in brackets

Antifungal studies

The screening data of antifungal activity of these series of compounds shows moderate to good antifungal activity. The antifungal data reveal that all the ten compounds screened showed high antifungal power on all the tested fungi. The compounds **4b**, **4c**, **5b**, **5c**, and **5e** have antifungal activities which are quite comparable to the standard compound ketoconazole, tested under similar conditions. The most active compound is **5e**, the activity of which is even greater than ketoconazole. Particularly compounds **4b**, **4c**, **5b**, and **5e** emerged as very potent compounds of this investigation.

Table 3. Antifungal activities of compounds **4a–5e.**

Compound	*Candida albicans* Zone of inhibition[a]/(MIC)	*Sporotrichum schenkiv* Zone of inhibition[a]/(MIC)	*Aspergillus fumigatus* Zone of inhibition[a]/(MIC)	*Trichophyton rubrum* Zone of inhibition[a]/(MIC)
4a	14 (12.5)	13 (25)	14 (12.5)	12 (25)
4b	18 (6.25)	19 (6.25)	20 (6.25)	18 (12.5)
4c	12 (12.5)	18 (6.25)	20 (12.5)	19 (6.25)
4d	14 (25)	10 (50)	11 (50)	13 (25)
4e	9 (100)	12 (12.5)	14 (25)	11 (12.5)
5a	13 (25)	11 (12.5)	10 (25)	14 (12.5)
5b	16 (12.5)	18 (6.25)	20 (6.25)	17 (6.25)
5c	21 (6.25)	17 (6.25)	17 (6.25)	16 (12.5)
5d	10 (50)	11 (25)	10 (12.5)	11 (50)
5e	23 (6.25)	20 (12.5)	21 (12.5)	19 (6.25)
Standard	22 (6.25)	19 (12.5)	21 (6.25)	20 (6.25)

[a] Zone of inhibition is expressed in mm; MIC values are given in brackets

It is interesting to note that a minor alteration in the molecular configuration of the investigated compounds may have a pronounced effect on antimicrobial activity. Thus, compound **4b** and **5b** containing a chloro group at position-2 on the phenyl ring are more active than compounds **4a** and **5a** which also have a chloro group in the phenyl ring but at position-4. The presence of a hydroxy group at position-2 in the phenyl ring in **4c** and **5c** also impart much towards their antimicrobial activities. The results of the antifungal studies are listed in Table 3.

Conclusion

We have synthesized novel 1-aryl-2-oxo-indano-[3,2-*d*]-pyrido / pyrimido-[1,2-*b*]-pyrimidines **4** and **5** to evaluate their antimicrobial properties. From the antibacterial data it seems that pyrimido-[1,2-*b*]-pyrimidines pharmacophores **5** seems to be more potent than pyrido-[1,2-*b*]-pyrimidines **4**. The data also revealed that the presence of substituents in the phenyl group at C_1 exerted significant influence on the antimicrobial profile, *e.g.,* **4b**, **4c**, and **5b**, **5c** are more active in comparison to other compounds of their respective group. These compounds have either an OH- or Cl-group at position-2 in the phenyl ring. The presence of a Cl-group at position-4 in the phenyl ring does not impart much as compare to the 2-Cl and 2-OH substitution.

Therefore, it can be inferred that the presence of electron-donating groups (Cl, OH) at position-2 in the phenyl

ring works better for antimicrobial activity of this series of compounds than other groups and/or other positions. This work is important, since it offers the possibility to find new compounds being more efficacious drugs against bacteria and fungi; for them, a thorough investigation regarding the structure-activity relationship, toxicity, and their biological effects is essential. This could be helpful in designing more potent antimicrobial agents for therapeutic use.

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The authors have declared no conflict of interest.

Experimental

Chemistry

One typical procedure for each step has been described. Melting points (Mp.) were taken in open capillaries and are uncorrected. IR spectra were recorded in KBr on a Shimadzu 8201 PC Spectrophotometer (ν_{max} in cm^{-1}) (Shimadzu, Tokyo, Japan) and ^{1}H-NMR and ^{13}C-NMR spectra in DMSO-d_6 on a Bruker DRX – 300 (300 MHz) spectrometer (Bruker, Bioscience, Billerica, MA, USA) using TMS as a internal reference (chemical shifts in δ, ppm). The purity of compounds was checked by thin layer chromatography on silica gel plate using ether and ethyl acetate. The physical data of the compounds **1a – 5e** are given in Table 1.

*2-(Arylidene)indan-1,3-diones **1***

A mixture of indane-1,3-dione (0.01 mol), substituted benzaldehyde (0.01 mol) in glacial acetic acid (20 mL) and anhydrous sodium acetate (0.11 mol) was refluxed for four hours. The reaction mixture was cooled and poured into ice water. The resulting solid was filtered, washed with water, dried and recrystallized from aqueous ethanol.

*Compound **1a***

IR (KBr) ν_{max} (cm^{-1}): 3090 (aromatic C-H), 3020 (olefin C-H), 1675 (α,β-unsaturated C=O), 1610 (α,β-unsaturated C=C), 1595, 1450, 1411 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 7.28 – 8.50 (m, 8H, Ar H), 4.9 (s, 1H, olefinic proton). ^{13}C-NMR (DMSO-d_6) δ: 122.5, 127.8, 130.8, 132.3, 135.5, 141.5, 145.4, 152.3, 190.8.

*Compound **1b***

IR (KBr) ν_{max} (cm^{-1}): 3110 (aromatic C-H), 3047 (olefin C-H), 1685 (α,β-unsaturated C=O), 1615 (α,β-unsaturated C=C), 1605, 1455, 1418 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 7.32 – 8.40 (m, 8H, Ar H), 4.7 (s, 1H, olefinic proton).

*Compound **1c***

IR (KBr) ν_{max} (cm^{-1}): 3423 (-OH), 3050 (aromatic C-H), 3027 (olefin C-H), 1681 (α,β-unsaturated C=O), 1627 (α,β-unsaturated C=C), 1585, 1415, 1411 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 7.25 – 8.32 (m, 8H, Ar H), 4.3 (s, 1H, olefinic proton), 5.3 (s, 1H, -OH).

*Compound **1d***

IR (KBr) ν_{max} (cm^{-1}): 3089 (aromatic C-H), 3031 (olefin C-H), 1689 (α,β-unsaturated C=O), 1619 (α,β-unsaturated C=C), 1610, 1434, 1411 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 7.22 – 8.35 (m, 9H, Ar H), 4.5 (s, 1H, olefinic proton).

*Compound **1e***

IR (KBr) ν_{max} (cm^{-1}): 3076 (aromatic C-H), 3042 (olefin C-H), 1690 (α,β-unsaturated C=O), 1621 (α,β-unsaturated C=C), 1590, 1444, 1422 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 7.21 – 8.26 (m, 8H, Ar H), 4.5 (s, 1H, olefinic proton).

*2-[Substituted phenyl(pyridin-2-yl-amino)methyl]indan-1,3-diones **2***

A mixture of 2-(arylidene)indan-1,3-dione **1** (0.004 mol), 2-aminopyridine (0.004 mol), and fused ammonium acetate (0.028 mol) was fused for two hours. The reaction mixture was cooled and poured into ice water. The resulting solid was filtered, washed with water, dried, and recrystallized from aqueous ethanol.

*Compound **2a***

IR (KBr) ν_{max} (cm^{-1}): 3260 (N-H), 1710 (C=O), 1560 (endo C=N), 1580, 1450 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.5 – 7.2 (m, 8H, aromatic H), 7.3 – 7.7 (m, 4H, pyridine proton), 3.2 (d, 1H, -CH-C=O), 4.3 (m, 1H, methine proton adjacent to N-H), 4.0 (d, 1H, N-H, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6) δ: 50.8, 70.3, 120.2, 122.3, 125.4, 130.2, 133.6, 135.3, 137.8, 140.5, 150.2, 153.4, 192.5.

*Compound **2b***

IR (KBr) ν_{max} (cm^{-1}): 3250 (N-H), 1705 (C=O), 1551 (endo C=N), 1587, 1444 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.4 – 7.3 (m, 8H, aromatic H), 7.3 – 7.8 (m, 4H, pyridine proton), 3.1 (d, 1H, -CH-C=O), 4.5 (m, 1H, methine proton adjacent to N-H), 4.1 (d, 1H, N-H, exchangeable with D_2O).

*Compound **2c***

IR (KBr) ν_{max} (cm^{-1}): 3420 (-OH), 3249 (N-H), 1714 (C=O), 1546 (endo C=N), 1579, 1443 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.5 – 7.4 (m, 8H, aromatic H), 7.4 – 7.8 (m, 4H, pyridine proton), 3.1 (d, 1H, -CH-C=O), 4.4 (m, 1H, methine proton adjacent to N-H), 4.1 (d, 1H, N-H, exchangeable with D_2O), 5.7 (s, 1H, -OH).

*Compound **2d***

IR (KBr) ν_{max} (cm^{-1}): 3241 (N-H), 1706 (C=O), 1532 (endo C=N), 1568, 1493 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.7 – 7.3 (m, 9H, aromatic H), 7.4 – 7.9 (m, 4H, pyridine proton), 3.3 (d, 1H, -CH-C=O), 4.3 (m, 1H, methine proton adjacent to N-H), 4.0 (d, 1H, N-H, exchangeable with D_2O).

*Compound **2e***

IR (KBr) ν_{max} (cm^{-1}): 3252 (N-H), 1701 (C=O), 1529 (endo C=N), 1555, 1491 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.6–7.4 (m, 8H, aromatic H), 7.3–7.8 (m, 4H, pyridine proton), 3.2 (d, 1H, -CH-C=O), 4.4 (m, 1H, methine proton adjacent to N-H), 4.0 (d, 1H, N-H, exchangeable with D_2O).

*2-[Substituted phenyl(pyrimidin-2-yl-amino)methyl]indan-1,3-diones **3***

A mixture of 2-(arylidene)indan-1,3-dione **1** (0.004 mol), 2-aminopyrimidine (0.004 mol), and fused ammonium acetate (0.028 mol) was fused for two hours. The reaction mixture was cooled and poured into ice water. The resulting solid was filtered, washed with water, dried, and recrystallized from aqueous ethanol.

*Compound **3a***

IR (KBr) ν_{max} (cm^{-1}): 3290 (N-H), 1718 (C=O), 1573 (endo C=N), 1529, 1426 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.6–7.5 (m, 8H, aromatic H), 7.6–7.9 (m, 3H, pyrimidine proton), 3.4 (d, 1H, -CH-C=O), 4.4 (m, 1H, methine proton adjacent to N-H), 4.1 (d, 1H, N-H, exchangeable with D_2O). ^{13}C-NMR (DMSO-d_6) δ: 52.2, 75.6, 120.5, 128.6, 132.4, 135.6, 138.2, 142.3, 158.5, 163.2, 193.5.

*Compound **3b***

IR (KBr) ν_{max} (cm^{-1}): 3287 (N-H), 1721 (C=O), 1549 (endo C=N), 1540, 1456 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.4–7.5 (m, 8H, aromatic H), 7.5–7.8 (m, 3H, pyrimidine proton), 3.1 (d, 1H, -CH-C=O), 4.2 (m, 1H, methine proton adjacent to N-H), 4.0 (d, 1H, N-H, exchangeable with D_2O).

*Compound **3c***

IR (KBr) ν_{max} (cm^{-1}): 3271 (N-H), 1709 (C=O), 1546 (endo C=N), 1556, 1430 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.5–7.3 (m, 8H, aromatic H), 7.4–7.9 (m, 3H, pyrimidine proton), 3.2 (d, 1H, -CH-C=O), 4.2 (m, 1H, methine proton adjacent to N-H), 3.9 (d, 1H, N-H, exchangeable with D_2O), 5.4 (s, 1H, -OH).

*Compound **3d***

IR (KBr) ν_{max} (cm^{-1}): 3266 (N-H), 1716 (C=O), 1561 (endo C=N), 1573, 1446 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.4–7.3 (m, 9H, aromatic H), 7.4–7.8 (m, 3H, pyrimidine proton), 3.3 (d, 1H, -CH-C=O), 4.3 (m, 1H, methine proton adjacent to N-H), 3.9 (d, 1H, N-H, exchangeable with D_2O).

*Compound **3e***

IR (KBr) ν_{max} (cm^{-1}): 3254 (N-H), 1717 (C=O), 1556 (endo C=N), 1572, 1451 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.3–7.5 (m, 8H, aromatic H), 7.5–7.9 (m, 3H, pyrimidine proton), 3.2 (d, 1H, -CH-C=O), 4.4 (m, 1H, methine proton adjacent to N-H), 4.0 (d, 1H, N-H, exchangeable with D_2O).

*1-Aryl-2-oxo-indano[3,2-d]pyrido[1,2-b]pyrimidines **4***

A mixture of 2-[substituted phenyl(pyridin-2-ylamino)methyl]indan-1,3-dione **2** (0.004 mol) and glacial acetic acid (15 mL) was refluxed for two hours. The solvent was removed under vacuum distillation; the reaction mixture was cooled and poured into ice water. The resulting solid was filtered, washed with water, dried, and recrystallized from aqueous ethanol.

*Compound **4a***

IR (KBr) ν_{max} (cm^{-1}): 2865 (methine C-H), 1705 (C=O), 1570 (exo C=N), 1608, 1538, 1485 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.8–7.5 (m, 8H, aromatic H), 7.4–7.8 (m, 4H, pyridine proton), 3.1 (s, 1H, methine proton). ^{13}C-NMR (DMSO-d_6) δ: 58.2, 110.7, 118.2, 120.3, 126.5, 128.2, 129.2, 131.3, 134.4, 135.8, 138.6, 141.3, 145.2, 148.2, 155.3, 160.2, 161.4, 163.2, 188.5.

*Compound **4b***

IR (KBr) ν_{max} (cm^{-1}): 2900 (methine C-H), 1711 (C=O), 1545 (exo C=N), 1611, 1545, 1473 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.6–7.4 (m, 8H, aromatic H), 7.4–7.9 (m, 4H, pyridine proton), 3.2 (s, 1H, methine proton).

*Compound **4c***

IR (KBr) ν_{max} (cm^{-1}): 3425 (-OH), 2890 (methine C-H), 1719 (C=O), 1583 (exo C=N), 1603, 1540, 1480 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.7–7.4 (m, 8H, aromatic H), 7.4–7.9 (m, 4H, pyridine proton), 3.0 (s, 1H, methine proton), 5.5 (s,1H, -OH).

*Compound **4d***

IR (KBr) ν_{max} (cm^{-1}): 2905 (methine C-H), 1709 (C=O), 1580 (exo C=N), 1590, 1536, 1489 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.8–7.5 (m, 9H, aromatic H), 7.4–7.8 (m, 4H, pyridine proton), 3.2 (s, 1H, methine proton).

*Compound **4e***

IR (KBr) ν_{max} (cm^{-1}): 2900 (methine C-H), 1705 (C=O), 1578 (exo C=N), 1610, 1531, 1474 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.8–7.5 (m, 8H, aromatic H), 7.5–7.9 (m, 4H, pyridine proton), 3.1 (s, 1H, methine proton).

*1-Aryl-2-oxo-indano[3,2-d]pyrimido[1,2-b]pyrimidines **5***

A mixture of 2-[(aryl)-(pyrimidin-2-ylamino)methyl]indan-1,3-dione (**3**) (0.004 mol) and glacial acetic acid (15 mL) was refluxed for 2 hours. The solvent was removed under vacuum distillation; the reaction mixture was cooled and poured into ice water. The resulting solid was filtered, washed with water, dried, and recrystallized from aqueous ethanol.

*Compound **5a***

IR (KBr) ν_{max} (cm^{-1}): 2866 (methine C-H), 1712 (C=O), 1546 (exo C=N), 1566, 1475, 1449 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.7–7.6 (m, 8H, aromatic H), 7.4–7.8 (m, 3H, pyrimidine proton), 3.1 (s, 1H, methine proton). ^{13}C-NMR (DMSO-d_6) δ: 52.2, 109.8, 111.3, 122.2, 123.1, 126.5, 128.1, 129.4, 130.9, 134.3, 138.2, 140.1, 141.3, 145.8, 160.3, 163.4, 165.2, 190.2.

*Compound **5b***

IR (KBr) ν_{max} (cm^{-1}): 2905 (methine C-H), 1709 (C=O), 1560 (exo C=N), 1568, 1479, 1430 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.6–7.5 (m, 8H, aromatic H), 7.5–7.9 (m, 3H, pyrimidine proton), 3.2 (s, 1H, methine proton).

*Compound **5c***

IR (KBr) ν_{max} (cm^{-1}): 3430 (-OH), 2890 (methine C-H), 1719 (C=O), 1573 (exo C=N), 1559, 1471, 1449 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.7–7.6 (m, 8H, aromatic H), 7.6–7.9 (m, 3H, pyrimidine proton), 3.0 (s, 1H, methine proton), 5.6 (s, 1H , –OH).

*Compound **5d***

IR (KBr) ν_{max} (cm^{-1}): 2890 (methine C-H), 1720 (C=O), 1565 (exo C=N), 1545, 1469, 1434 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.6 – 7.4 (m, 9H, aromatic H), 7.4 – 7.9 (m, 3H, pyrimidine proton), 3.2 (s, 1H, methine proton).

*Compound **5e***

IR (KBr) ν_{max} (cm^{-1}): 2895 (methine C-H), 1708 (C=O), 1562 (exo C=N), 1561, 1474, 1434 (phenyl ring). ^{1}H-NMR (DMSO-d_6) δ: 6.5 – 7.3 (m, 8H, aromatic H), 7.4 – 7.8 (m, 3H, pyrimidine proton), 3.1 (s, 1H, methine proton).

Biological screening

Antibacterial studies

The newly synthesized heterocyclic compounds were screened *in vitro* for their antibacterial activity against *Escherichia coli* (ATCC-8739) and *Klebsiella pneumoniae* (ATCC 10031) as examples of *gram*-negative bacteria; *Staphylococcus aureus* (ATCC-8538) and *Bacillus subtilis* (PTCC-1023) as examples of *gram*-positive bacteria by the disc diffusion method [30]. Ciprofloxacin was used as a standard drug to compare the results.

Two-fold serial dilutions of the compounds were prepared in Müller – Hilton agar. One milligram of each test compound was dissolved in 100 μL DMSO to prepare stock solution. Further progressive double dilutions were performed from the stock solution to obtain the required concentrations of 10, 20, 25, 50, and 100 μg/μL. Petri dishes were inoculated with 1 to 5×10^4 colony forming units (cfu) and incubated at 37 ± 1°C for 26 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the test compound, which resulted in no visible growth on the plate.

A control test in triplicate was also performed with test medium supplemented with DMSO at the experimental dilutions. Zone of inhibition and MIC were noted. The results of antibacterial studies are given in Table 2.

Antifungal studies

The newly synthesized compounds were screened for their antifungal activity against four species of fungi viz. *Candida albicans* (MTCC 183), *Sporotrichum schenkiv* (MTCC 1152), *Aspergillus fumigatus* (MTCC 343), and *Trichophyton rubrum* (MTCC 296) in DMSO by serial dilution method [31]. Sabourands broth was prepared. Solution of the test compound (0.2 mL) was added to 1.8 mL of the seeded broth and this formed the first dilution. Subsequently, 1.0 mL of this solution was diluted further with a 1.0 mL of the seeded broth to give the second dilution and so forth until five such dilutions were obtained. A set of tubes containing only seeded broth and the solvent controls were maintained under identical conditions. The tubes were incubated at 28°C. After 96 h, the zones of inhibition and MIC values were noted.

A commercial drug ketoconazole was also tested under similar conditions to compare the results of tested compounds. The data for the antifungal studies are listed in Table 3.

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