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Design, synthesis and biological evaluation of N-(substituted benzylidene)-2/4-(1, 3 dioxoisoindolin-2-yl) alkanehydrazide

Annu Saini, Jyoti, Sweety Ranga, Aakash Deep and Rakesh Kumar Marwaha

Abstract
There is an urgent need to discover/develop antimicrobial agents having higher efficacy and potency against the rapidly growing antibiotic resistant strains of pathogenic micro-organism. A series of new Isoindole-1, 3-dione derivatives were synthesized via reaction of phthalic anhydride with two amino acids under fused conditions. Esterification of *N*-phthaloyl alkanoic acid derivatives with methanol in the presence of sulfuric acid yielded the corresponding esters, whereas, the esterification with methanol in presence of thionyl chloride gave *N*-phthaloyl alkanoic acid acetyl chloride. Both the esters and acetyl chlorides so formed produce the corresponding *N*-phthaloyl acid hydrazides on reaction with hydrazine hydrate and ethanol. Further reaction with different benzaldehydes yielded the corresponding substituted benzylidene phthalimide derivatives. The structures of the synthesized compounds were confirmed from their analytical and spectral data such as IR spectra and 1H-NMR spectra.
The antimicrobial activity of synthesized compounds determined by tube dilution method against various microorganisms indicated compounds, 9, 18 and 22 as most active ones. The synthesized compounds were also screened for *in vitro* antidiabetic activity by inhibition of *α*-amylase enzyme and antioxidant activity by DPPH assay method. The compounds 6 and 11 exhibited excellent *α*-amylase inhibitory activity whereas the compounds 10 and 22 found to possess good antioxidant activity.

Keywords: Phathalimide, *in vitro* evaluation, antimicrobial activity, α-amylase inhibitory activity, antioxidant activity

1. Introduction
Infectious and parasitic diseases are responsible for approximate 23% of total deaths worldwide and are the second leading cause of death according to the World Health Organization. Further, matter of relevance with the infectious diseases is their apparent resistance against the existing classes of antibiotics. Antibacterial resistance among various pathogenic microbial strains has arisen globally and has become a severe challenge for the medical researchers worldwide (Amin *et al.*, 2013) [2]. In order to overcome microbial resistance and build up effective therapies discovery of novel and potent antimicrobial agents is the finest way (Popiolek *et al.*, 2016; Venkateshwarlu *et al.*, 2014) [19, 25].
Diabetes mellitus is a major endocrine disorder now become one of the biggest threats to healthcare and all over the world it affects nearly 10% of the population (Bhutkar and Bhise *et al.*, 2012) [4], (Mahapatra *et al.*, 2010) [13]. Type 2 diabetes mellitus is characterized by progressive pancreatic β-cell dysfunction caused by chronic insulin resistance, high glucose level, less uptake of insulin-stimulated glucose by peripheral tissues (Eom *et al.*, 2016) [6], (Lupascu *et al.*, 2013) [11]. The final step in the digestive process of carbohydrates and the biosynthesis of the *N*-linked oligosaccharides on the enveloped glycoprotein is catalyzed by the Enzyme *α*-Glucosidase (Bian *et al.*, 2013) [3]. *α*-Glucosidase inhibitors (AGIs) therefore delays carbohydrate digestion and prolongs the overall carbohydrate digestion time, leading to reduced rate of glucose absorption and consequently blunt the postprandial plasma glucose level (Ali and Fathalla, 2009) [1].
Reactive oxygen species (ROS) and free radicals are considered to be implicated in a variety of pathological events, such as cancer and aging, liver chirrhosis, atherosclerosis, cancer, diabetes, ageing (Nezhawy *et al.*, 2009; Tadele *et al.*, 2017) [14, 23]. Oxidative stress results from the metabolic reactions involving oxygen causing a disturbance in the equilibrium status of antioxidant reactions in living organisms (Valko *et al.*, 2007) [24].

The prevention of oxidative stress related diseases has been tentatively achieved by the development of antioxidant compounds that are able to scavenge ROS (reactive oxygen species) and RNS (reactive nitrogen species), and thus avoid radical-induced oxidative damage. The main purpose of antioxidants is to neutralize free radicals by scavenging reactive oxygen species (Raghunath *et al*., 2012) [20].

Isoindole-1,3-diones, commonly known as phthalimides are the basic structural units in a variety of biologically essential compounds. For example, alkenyl-substituted phthalimides are present as key structural elements in several fungicides, metabolic drugs and functional materials (Pawlu *et al*., 2012) [17]. The most substantial pharmacological effects that have been reported for phthalimide derivatives are anti-microbial (Amin *et al.*, 2013) [2], anti-inflammatory (Rajasekaran *et al*., 2011) [21], anti-cancer (Kamal *et al*., 2006) [9], anti-oxidant (El-Gaby *et al*., 2000) [5], and analgesic activities (Pophale and Deodhar, 2010) [18].

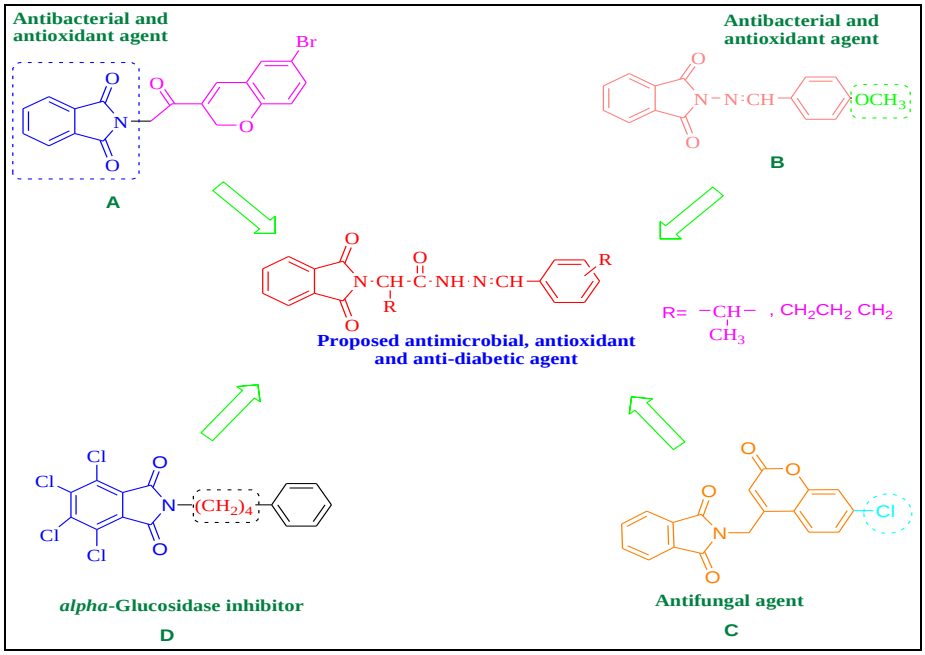
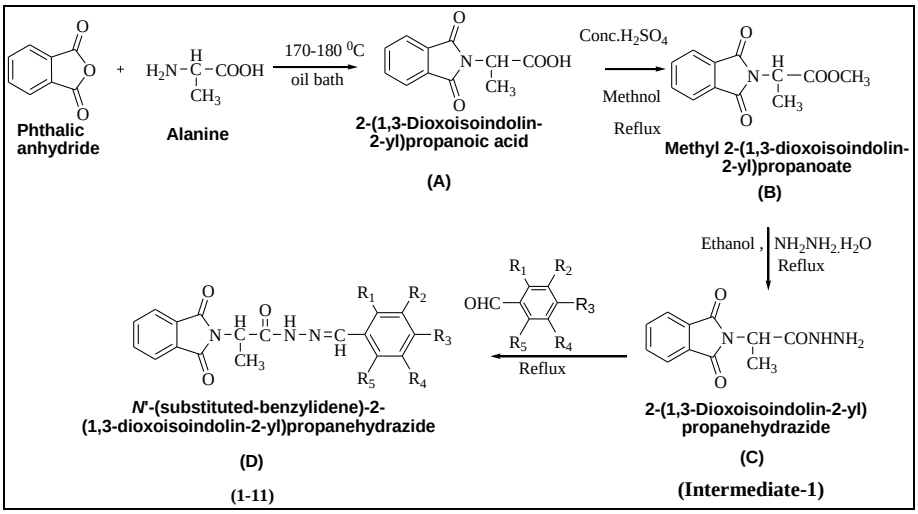


Fig 1: Rational for designing the proposed *N*-(substituted benzylidene)-2/4-(1, 3-di- oxoisoindolin-2-yl)-alkanehydrazide derivatives based on literature.

2. Results and Discussion

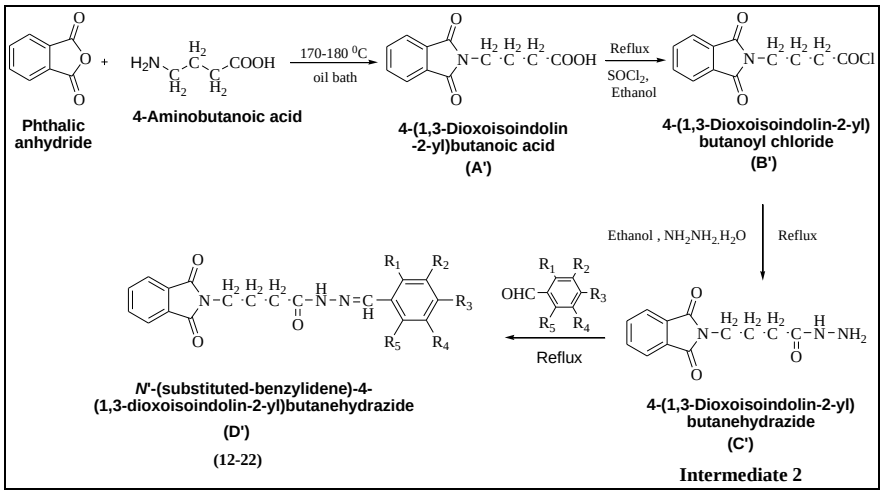
2.1 Chemistry

A new series of novel *N*-(substituted benzylidene)-2-(1, 3-dioxoisoindolin-2-yl) propanehydrazide and *N*-(substituted benzylidene)-4-(1,3-dioxoisoindolin-2-yl) butanehydrazide derivatives was synthesized using procedure outlined in Scheme-1 and Scheme-2, respectively.



Scheme 1: Synthesis of *N*-(substituted benzylidene)-2-(1, 3-dioxoisoindolin-2-yl) propane hydrazide derivatives (1-11).

1.	R_2,R_3,R_4,R_5=H; R_1=NO_2	7.	R_1,R_2,R_4,R_5=H; R_3=Cl
2.	R_1,R_3,R_4,R_5=H; R_2=NO_2	8.	R_1,R_2,R_4,R_5=H; R_3=N(C_2H_5)
3.	R_1,R_2,R_4,R_5=H; R_3=NO_2	9.	R_1,R_2,R_4,R_5=H; R_3=Br
4.	R_1,R_2,R_4,R_5=H; R_3=OH	10.	R_1,R_2,R_4,R_5=H; R_3=CHO
5.	R_1,R_4,R_5=H; R_2=OCH_3; R_3=OH	11.	R_1,R_5=H; R_2,R_3,R_4=OCH_3
6.	R_1,R_2,R_4,R_5=H; R_3=N(CH_3)$_2$		



Scheme 2: Synthesis of *N*-(substituted benzylidene)-4-(1, 3-dioxoisoindolin-2-yl) butane hydrazide derivatives (12-22).

12.	R_2,R_3,R_4,R_5=H; R_1=NO_2	**14.**	R_1,R_2,R_4,R_5=H; R_3=NO_2
13.	R_1,R_3,R_4,R_5=H; R_2=NO_2	**15.**	R_1,R_2,R_4,R_5=H; R_3=OH
16.	R_1,R_4,R_5=H; R_2=OCH_3; R_3=OH	**19.**	R_1,R_2,R_4,R_5=H; R_3=$N(C_2H_5)$
17.	R_1,R_2,R_4,R_5=H; R_3=$N(CH_3)_2$	**20.**	R_1,R_2,R_4,R_5=H; R_3=Br
18.	R_1,R_2,R_4,R_5=H; R_3=Cl	**21.**	R_1,R_2,R_4,R_5=H; R_3=CHO

Table 1: Physiochemical properties of synthesized compounds

S. No.	M. Formula	M. Wt.	M. pt. (˚C)	R_f value	% Yield
1.	$C_{18}H_{14}N_4O_5$	366.33	180-182	0.78	64.2
2.	$C_{18}H_{14}N_4O_5$	366.33	170-172	0.66	67.9
3.	$C_{18}H_{14}N_4O_5$	366.33	185-187	0.74	59.8
4.	$C_{18}H_{15}N_3O_4$	337.33	210-212	0.71	62.3
5.	$C_{20}H_{19}N_3O_5$	381.38	205-207	0.76	77.4
6.	$C_{20}H_{20}N_4O_3$	364.15	222-224	0.80	89.7
7.	$C_{18}H_{14}ClN_3O_3$	355.78	190-192	0.70	67.3
8.	$C_{22}H_{24}N_4O_3$	392.45	164-166	0.62	55.8
9.	$C_{18}H_{14}BrN_3O_3$	400.23	260-262	0.64	72.5
10.	$C_{19}H_{15}N_3O_4$	349.34	220-222	0.53	61.3
11.	$C_{21}H_{21}N_3O_6$	411.41	262-264	0.57	91.5
12.	$C_{19}H_{16}N_4O_5$	380.35	195-197	0.62	66.2
13.	$C_{19}H_{16}N_4O_5$	380.35	244-246	0.72	92.4
14.	$C_{19}H_{16}N_4O_5$	380.35	260-262	0.55	66.1
15.	$C_{19}H_{17}N_3O_4$	351.36	210-212	0.61	88.9
16.	$C_{21}H_{21}N_3O_5$	395.41	206-208	0.58	76.5
17.	$C_{21}H_{22}N_4O_3$	378.42	198-200	0.68	71.7
18.	$C_{19}H_{16}ClN_3O_3$	369.09	189-210	0.75	59.3
19.	$C_{23}H_{26}N_4O_3$	406.48	178-180	0.46	63.9
20.	$C_{19}H_{16}BrN_3O_3$	414.25	204-206	0.58	88.1
21.	$C_{20}H_{17}N_3O_4$	363.37	178-180	0.61	82.5
22.	$C_{22}H_{23}N_3O_6$	425.43	270-272	0.55	57.5

*TLC Mobile phase: Toulene: Methanol (6:4)

3. Experimental

Starting materials were obtained from commercial sources (Loba Chemie and Fluka Goldie) and were used without further purification. Completion of reaction was monitored and confirmed by thin layer chromatography prepared on glass plates by using silica gel G. Melting points were determined in open capillary tubes on a sonar melting point apparatus and reported uncorrected. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were determined by Bruker Top Spin 3.2 400 MHz NMR spectrometer in dimethylsulphoxide (DMSO) d^6 as solvent. NMR data of compounds is given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) of number of protons. Infra-red (IR) spectra were recorded on Bruker 12060280, Software: OPUS 7.2.139.1294 spectrophotometer by using attenuated total reflectance (ATR).

General procedure for the synthesis of *N*-(substituted benzylidene)-2/4-(1, 3-dioxoiso- indolin-2-yl)alkane hydrazide derivatives (scheme-1) and (scheme-2)

***Step 1: Synthesis of N- substituted (1, 3-dioxo- isoindolin-2-yl)alkanoic acid* (A)**

A mixture of phthalic anhydride (0.01 mol) and appropriate amino acids (alanine, 4-aminobutyric acid) was heated on an oil bath at 180-185 °C for 15 minutes with constant stirring. After that the reaction mixture was left undisturbed for 5 minutes. The reaction mixture was cooled until the liquid gets solidifies. The reaction mixture was monitored with Thin Layer Chromatography (TLC). The resulting residue was recrystallized from 10% ethanol.

Step 2: (a) ***Synthesis of 2-(1, 3-dioxoisoindolin-2-yl)propanoate*** **(B)**

2-(1,3-dioxoisoindolin-2-yl)propanoic acid (A) (0.01 mol) (Sheme-1) in methanol (30 ml) with conc. sulfuric acid (0.01 mol) were added drop wise in a round bottom flask and refluxed for 3-4 h. The reaction mixture was monitored, with TLC when reaction completed, the reaction mixture was then poured into cold water and the separated solid was filtered and recrystallized from ethanol (Nikalje *et al.*, 2015) [16].

(b) Synthesis of ***4-(1, 3-dioxoisoindolin-2-yl)butanoyl chloride*** **(B′)**

4-(1,3-dioxoisoindolin-2-yl)butanoic acid (A) **(Scheme-2)** (0.01 mol) in methanol (30 ml) with thionyl chloride (0.02 mol) were added drop wise in a round bottom flask and refluxed for 3-4 h. The reaction mixture was monitored, with TLC when reaction was completed, the reaction mixture was poured into cold water and the separated solid was filtered and recrystallized from ethanol.

Step 3: ***Synthesis of N-substituted-2/4-(1, 3-dioxoisoindolin-2-yl)alkanehydrazide (C)***

Methyl 2-(1,3-dioxoisoindolin-2-yl)propa- noate (B) (Scheme-1) and 4-(1,3-dioxoisoindolin-2-yl)butanoyl chloride (B′) (0.01 mol) in ethanol (30 ml) with hydrazine hydrate (0.02 mol) were added drop wise in a round bottom flask with rapid stirring and the mixture was refluxed for 5 h. The reaction mixture was monitored with TLC. The solid separated was filtered, washed with water and recrystallized from ethanol (Yassin *et al.*, 2014) [26].

4: ***Synthesis of N-substituted benzylidene)-2/4-(1,3-dioxoisoindolin-2-yl)hydrazide (D)***

N-substituted (1,3-dioxoisoindolin-2-yl)hydrazide (C) (0.01 mol) and substituted aromatic aldehydes (0.01 mol) in methanol (25 ml) with few drops of glacial acetic acid as a catalyst were added in round bottom flask and refluxed for 6-8 h. The completion of reaction was confirmed by TLC then added ice cold water with continuous shaking of mixture. The separated solid was filtered off and recrystallized from ethanol.

Spectral data of synthesized intermediates and final compounds (1-22)

- ***Intermediate 1: 2-(1,3-Dioxoisoindolin-2-yl)propanehydrazide: IR (ATR, cm^{-1})*****:** 1543 (C=C str., Ar), 3011 (C-H str., Ar), 1690 (C=O str., Ar), 1650 (C=O str., 2ºamide), 3216 (NH str., 2° amine), 3011 (C-H str., aliphatic), 1075 (C-N str.).
- ***Intermediate 2: 4-(1,3-Dioxoisoindolin-2-yl)butanehydrazide: IR (ATR, cm^{-1})*****:** 1515 (C=C str., Ar), 3100 (C-H str., Ar), 1745 (C=O str., Ar), 1657 (C=O str., 2ºamide), 3428 (NH str., 2° amine), 2994 (C-H str., aliphatic), 1028 (C-N str.).
- ***Compound 1: N′-(2-nitrobenzylidene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*****:** 1491 (C=C str., Ar), 3026 (C-H str., Ar), 1658(C=O str., 2ºamide), 3518 (NH str., 2ºamide), 2942 (C-H str., aliphatic), 1558 (NO_2 str.), 1551 (C=N str., N=CH), 1338 (C-N str.).
- ***Compound 2: N′-(3-nitrobenzylidene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*****:** 1463 (C=C str., Ar), 3072 (C-H str., Ar), 1679 (C=O str., Ar), 1651 (C=O str., 2ºamide), 3311 (NH str., 2ºamide), 2875 (C-H str., alliphatic), 1523 (NO_2 str.), 1654 (C=N str., N=CH), 1160 (C-N str.).
- ***Compound 3: N′-(4-nitrobenzylidene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*****:** 1402 (C=C str., Ar), 3085 (C-H str., Ar), 1741 (C=O str., Ar), 1693 (C=O str., 2ºamide), 3609 (NH str., 2ºamide), 2897 (C-H str., aliphatic), 1402 (NO_2 str.), 1515 (C=N str., N=CH), 1210 (C-N str.).
- ***Compound 4: N′-(4-hydroxybenzylidene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*****:** 1512 (C=C str., Ar), 3071 (C-H str., Ar), 2983 (C-H str., aliphatic), 1707 (C=O str., Ar), 1692 (C=O str., 2ºamide), 3559 (NH str., 2ºamide), 3644 (C-OH str., Ar-OH), 1674 (C=N str., N=CH), 1336 (C-N str.).
- ***Compound 5: N′-(3-ethoxy-4-hydroxybenzyli- dene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*****:** 1499 (C=C str., Ar), 3070 (C-H str., Ar), 2955 (C-H str., aliphatic), 1777 (C=O str., Ar), 1686 (C=O str., 2ºamide), 3545 (NH str., 2ºamide), 1585 (C=N str., N=CH), 1327 (C-N str.) 1028 (C-OCH_2CH_3), 3635 (OH str.).
- ***Compound 6: N′-(4-(dimethylamino)benzyli- dene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*****:** 1462 (C=C str., Ar), 3028 (C-H str., Ar), 1741 (C=O str., Ar), 1700 (C=O str., 2ºamide), 3645 (NH str., 2ºamide), 1652 (C=N str., N=CH), 2857 (C-H str., aliphatic), 1268 (C-N str.). ^{1}H NMR (DMSO, δ ppm): 6.75-7.64 (m, 8H, ArH), 7.66 (s, 1H, NH), 7.67 (s, 1H, CH=N), 6.79 (s, 1H, CH), 2.75 (s, 6H, CH_3), 2.74 (s, 3H, CH_3).
- ***Compound 7: N′-(4-chlorobenzylidene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*****:** 1464 (C=C str., Ar), 2988 (C-H str., Ar), 1680 (C=O str., Ar), 1622 (C=O str., 2ºamide), 3606 (NH str., 2ºamide), 2923 (C-H str., aliphatic), 1587 (C=N str., N=CH), 765 (C-Cl str., Ar-Cl), 1235 (C-N str.).
- ***Compound 8: N′-(4-(diethylamino)benzylidene)-2-(1,3-dioxoisoindolin-2-yl) propanehydrazide: IR (ATR, cm^{-1})*****:** 1521 (C=C str., Ar), 3036 (C-H str., Ar), 1796 (C=O str., Ar), 1694 (C=O str., 2ºamide), 3564 (NH str., 2ºamide), 2935 (C-H str., aliphatic) 1561 (C=N str.), 1229 (C-N str.).
- ***Compound 9: N′-(4-bromobenzylidene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*****:** 1538 (C=C str., Ar), 3060 (C-H str., Ar), 1680 (C=O str., Ar), 1659 (C=O str., 2ºamide), 3604 (NH str., 2ºamide), 2970 (C-H str., aliphatic), 1538 (C=N str., N=CH), 782 (C-Br str., Ar-Br), 1179 (C-N str.).
  ^{1}H NMR (DMSO, δ ppm): 7.84-7.90 (m, 8H, ArH), 7.88 (s, 1H, NH), 7.89M (s, 1H, CH=N), 1.22 (s, 3H, CH_3).
- ***Compound 10: N′-(4-formylbenzylidene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*****:** 1492 (C=C str., Ar), 3046 (C-H str., Ar), 1670 (C=O str., Ar), 1612 (C=O str., 2ºamide), 3563 (NH str., 2ºamide), 2893 (C-H str., aliphatic), 1562 (C=N str., N=CH), 1250 (C-CHO str.,), 1250 (C-N str.).
- ***Compound 11: N′-(3,4,5-trimethoxylbenzyli- dene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*****:** 1486 (C=C str., Ar), 3084 (C-H str., Ar), 1657 (C=O str., Ar), 1630 (C=O str., 2ºamide), 3638 (NH str., 2ºamide), 2901 (C-H str., aliphatic), 1559 (C=N str., N=CH), 1254 (C-O str., OCH_3), 1214 (C-N str.).
  ^{1}H NMR (DMSO, δ ppm): 6.79-7.79 (m, 6H, ArH), 7.08 (s, 1H, NH), 7.13 (s, 1H, CH=N), 4.75 (s, 1H, CH),

2.228s, 3H, CH_3) 3.87 (s, 9H, $(OCH_3)_3$).

- ***Compound 12: N'-(2-nitrobenzylidene)-4-(1,3-dioxoisoindolin-2-yl)butane hydrazide: IR (ATR, cm^{-1})*:** 1342 (C=C str., Ar), 3023 (C-H str., Ar), 1744 (C=O str., Ar), 1696 (C=O str., 2ºamide), 3622 (NH str., 2ºamide), 2883 (C-H str., aliphatic), 1523 (NO_2 str.), 1655 (C=N str., N=CH), 1071 (C-N str.).
- ***Compound 13: N'-(3-nitrobenzylidene)-4-(1,3-dioxoisoindolin-2-yl)butane hydrazide: IR (ATR, cm^{-1})*:** 1494 (C=C str., Ar), 3003 (C-H str., Ar), 1772 (C=O str., Ar), 1660 (C=O str., 2ºamide), 3545 (NH str., 2ºamide), 2886 (C-H str., aliphatic), 1599 (NO_2 str.), 1647 (C=N str., N=CH), 1344 (C-N str.). ^{1}H NMR (DMSO, δ ppm): 8.33-8.38 (m, 8H, ArH), 8.35 (s, 1H, NH), 8.37 (s, 1H, CH=N), 3.34 (t, 2H, CH_2), 1.23 (m, 2H, CH_2), 2.52 (t, 2H, CH_2).
- ***Compound 14: N'-(4-nitrobenzylidene)-4-(1,3-dioxoisoindolin-2-yl)butane hydrazide: IR (ATR, cm^{-1})*:** 1495 (C=C str., Ar), 3021 (C-H str., Ar), 1798 (C=O str., Ar), 1655 (C=O str., 2ºamide), 3623 (NH str., 2ºamide), 2963 (C-H str., aliphatic), 1598 (NO_2 str.), 1598 (C=N str., N=CH), 1014 (C-N str.).
- ***Compound 15: N'-(4-hydroxybenzylidene)-4-(1,3-dioxoisoindolin-2-yl)butane hydrazide: IR (ATR, cm^{-1})*:** 1505 (C=C str., Ar), 3058 (C-H str., Ar), 1613 (C=O str., Ar), 1690 (C=O str., 2ºamide), 3590 (NH str., 2ºamide), 2987 (C-H str., aliphatic), 3649 (O-H str., Ar-OH), 1563 (C=N str., N=CH), 1099 (C-N str.).
- ***Compound 16: N'-(3-ehoxy-4-hydroxybenzyli- dene)-4-(1,3-dioxoisoindolin-2-yl)butane hydrazide: IR (ATR, cm^{-1})*:** 1507 (C=C str., Ar), 3048 (C-H str., Ar), 1613 (C=O str., Ar), 1677 (C=O str., 2ºamide), 3659 (NH str., 2ºamide), 2891 (C-H str., aliphatic), 3559 (O-H str.,), 1044 (C-OCH_2CH_3 str., Ar) 1586 (C=N str., N=CH), 1034 (C-N str.).
- ***Compound 17: N'-(4-(dimethylamino)benzyli- dene)-4-(1,3-dioxoisoindolin-2-yl)butane hydrazide: IR (ATR, cm^{-1})*:** 1513 (C=C str., Ar), 3056 (C-H str., Ar), 1742 (C=O str., Ar), 1696 (C=O str., 2ºamide), 3619 (NH str., 2ºamide), 2858 (C-H str., aliphatic), 1651 (C=N str., N=CH), 1071 (C-N str.). ^{1}H NMR (DMSO, δ ppm): 6.77-7.89 (m, 8H, ArH), 7.66 (s, 1H, NH), 7.87 (s, 1H, CH=N), 3.01 (t, 2H, CH_2), 2.50 (m, 2H, CH_2), 2.52 (t, 2H, CH_2), 2.84 (s, 6H, CH_3).
- ***Compound 18: N'-(4-chlorobenzylidene)-4-(1,3-dioxoisoindolin-2-yl)butane hydrazide: IR (ATR, cm^{-1})*:** 1486 (C=C str., Ar), 3012 (C-H str., Ar), 1725 (C=O str., Ar), 1697 (C=O str., 2ºamide), 3568 (NH str., 2ºamide), 2868 (C-H str., aliphatic), 1550 (C=N str., N=CH), 625 (C-Cl str., Ar-Cl), 1078 (C-N str.). ^{1}H NMR (DMSO, δ ppm): 7.92-8.72 (m, 8H, ArH), 8.08 (s, 1H, NH), 8.09 (s, 1H, CH=N), 3.37 (t, 2H, CH_2), 2.50 (m, 2H, CH_2), 2.73 (t, 2H, CH_2).
- ***Compound 19: N'-(4-(diethylamino)benzyli dene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*:** 1466 (C=C str., Ar), 3128 (C-H str., Ar), 1690 (C=O str., Ar), 1600 (C=O str., 2ºamide), 3549 (NH str., 2ºamide), 2972 (C-H str., aliphatic) 1532 (C=N str.), 1084 (C-N str.).
- ***Compound 20: N'-(4-bromobenzylidene)-4-(1,3-dioxoisoindolin-2-yl) butanehydrazide: IR (ATR, cm^{-1})*:** 1492 (C=C str., Ar), 3043 (C-H str., Ar), 1690 (C=O str., Ar), 1667 (C=O str., 2ºamide), 3681 (NH str., 2ºamide), 2883 (C-H str., aliphatic), 1586 (C=N str., N=CH), 625 (C-Br str., Ar-Br), 1218 (C-N str.).
- ***Compound 21: N'-(4-formylbenzylidene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*:** 1489 (C=C str., Ar), 3091 (C-H str., Ar), 1658 (C=O str., Ar), 1658 (C=O str., 2ºamide), 3568 (NH str., 2ºamide), 2876 (C-H str., aliphatic), 1601 (C=N str., N=CH), 2876 (C-CHO str.,), 1077 (C-N str.).
- ***Compound 22: N'-(3,4,5-trimethoxylbenzyli- dene)-2-(1,3-dioxoisoindolin-2-yl)propane hydrazide: IR (ATR, cm^{-1})*:** 1508 (C=C str., Ar), 3084 (C-H str., Ar), 1680 (C=O str., Ar), (C=O str., 2ºamide), 3320 (NH str., 2ºamide), 2875 (C-H str., aliphatic),1598 (C=N str., N=CH), 709 (C-OCH_3 str.,), 1311 (C-N str.,).
  ^{1}H NMR (DMSO, δ ppm): 6.61-7.20 (m, 6H, ArH), 7.08 (s, 1H, NH), 7.15 (s, 1H, CH=N), 3.20 (t, 2H, CH_2), 2.30 (m, 2H, CH_2), 2.33 (t, 2H, CH_2), 3.94 (s, 9H, $(OCH_3)_3$).

Experimental (Biological Part)

3.1 Evaluation of antimicrobial activity

The antimicrobial activity of the novel *N*-(substituted benzylidene)-2/4-(1,3-dioxoisoindolin-2-yl)alkane hydrazide derivatives (1-22) against Gram-positive bacteria: *S. aureus*, *B. subtilis*,

Gram-negative bacteria: *E. coli*, *S. typhi*, *K. pneumonia* and fungal strains: *C. albicans*, *A. niger* was evaluated by using cefadroxil (antibacterial) and fluconazole (antifungal) as reference drugs. Test and standard dilutions were prepared in double strength nutrient broth I.P (for bacteria) or sabouraud dextrose broth I.P (for fungi) (Pharmacopoeia of India, 2007). The samples were incubated at 37$\pm$ 1 ºC for 24 h (bacteria), at 25 $\pm$ 1 ºC for 7 days (*A. niger*) and at 37$\pm$ 1 ºC for 48 h (*C. albicans*).

3.2 *In vitro* evaluation of *α*-amylase inhibitory activity

All the synthesized compounds were investigated for the *α*-amylase inhibitory activity by using *diastase* based on colorimetric method (Nickavar and Amin, 2010). 0.25 g of soluble potato starch was dissolved in 50 ml of 20 Mm phosphate buffer by heating for 15 minutes. 1 mg *diastase* (amylase enzyme) was mixed in 100 ml of 20 Mm phosphate buffer (pH 6.9) to obtain the enzyme solution. All the synthesized derivatives were dissolved in DMSO to give the different concentrations. The color agent was prepared by mixing 20 ml of 96 mM 3,5-dinitrosalicylic acid with 5.31 M sodium potassium tartrate in 8 ml of 2 Mm sodium hydroxide and 12 ml deionized water.

1 ml of enzyme solution was mixed with 1 ml of each synthesized derivatives and then incubated for 10 minutes at 25 ºC. 1 ml of this mixture was mixed with 1 ml of soluble potato starch solution in a tube and incubated for 10 minutes at 25 ºC. Then tube was closed after adding 1 ml of color reagent and placed into water bath for 15 minutes at 85 ºC. The reaction mixture was removed from water bath after 15 minutes. After cooling, the reaction mixture was diluted with 9 ml of distilled water and the absorbance was taken on 540 nm in UV-spectrophotometer. Blank solution was prepared by replacing the enzyme solution with buffer solution and absorbance was taken. Measurement of control was performed in identical manner by replacing the synthesized derivatives in 1 ml of DMSO. Acarbose solution was used as a standard drug (Kumar *et al*., 2013) [10].

Percentage inhibition of α-amylase was calculated by using following formula

% inhibition = [($A_{control}$ -A_{sample})/ $A_{control}$ ×100]
Where $A_{control}$ = Absorbance of the control
A_{sample} = Absorbance of the sample (1)

3.3 *In vitro* evaluation of antioxidant activity of newly synthesized compounds

The antioxidant activity of synthesized derivatives and the standard ascorbic acid was evaluated on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH)- free radical activity by modified method given below (15). DPPH assay is based upon the reaction of DPPH which act as free radical scavenger as it accepts hydrogen (H) from the scavenging molecule and is shown by color change from purple to yellow. The absorbance of the sample was absorbed spectrophotometrically and was utilized for the determination of the parameters for antioxidant properties (Mishra *et al*., 2012) [12].

3.3.1 Preparation of stock solution

Weighed accurately 10 mg of each synthesized compound and standard drug (*i.e*. ascorbic acid) and transfer each into 10 ml volumetric flask. Make up the volume up to
10 ml with methanol. The concentration of the stock solution was 1000 µg/ml.

3.3.2 Preparation of dilutions

4 dilutions of each sample and standard drug were prepared from the above stock solutions. From the above stock solution of sample and standard, 0.25 ml, 0.50 ml, 0.75 ml and 1 ml were pipetted out into 10 ml volumetric flask and in each methanol was added to make up the volume up to 10 ml, which resulted in different dilutions of sample and standard drug of 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml concentrations respectively.

3.3.3 Preparation of DPPH solution

The DPPH solution of 3 µg/ml concentration was prepared by dissolving 3.9432 mg of DPPH in 100 ml of methanol.

3.3.4 Screening of antioxidant activity

1 ml solution of each sample and standard drug of each concentration was mixed with 1 ml of DPPH solution separately. 1 ml of methanol and 1 ml of DPPH solution was mixed and used as a control. All these solution mixtures were kept under dark for 30 min and absorbance was measured at 517 nm. The absorbance was noted and % inhibition was calculated using the formula given below (Singh *et al*., 2015) [22].

% inhibition = [($A_{control}$ -A_{sample})/ $A_{control}$ ×100]
Where $A_{control}$ = Absorbance of the control
A_{sample} = Absorbance of the sample (2)

Table 2: Antimicrobial activity of newly synthesized *N*-(substituted benzylidene)-2/4-(1,3-dioxoisoindolin-2-yl)alkanehydrazide derivatives.

Comp.	Minimum inhibitory Concentration (µM/ml)*10^{-2}						
	Bacterial strains					Fungal strains	
	S. aureus	*B. subtilis*	*K. pneumonia*	*E. coli*	*S. typhi*	*A. niger*	*C.albicans*
1.	3.41	3.41	3.41	6.82	3.41	3.41	6.82
2.	3.41	6.82	3.41	3.41	3.41	3.41	6.82
3.	3.41	13.6	13.6	3.41	6.82	3.41	3.41
4.	3.70	3.70	3.70	3.70	7.41	3.70	1.85
5.	3.27	3.27	3.27	3.27	6.55	3.27	3.27
6.	3.43	3.43	3.43	3.43	3.43	6.86	3.43
7.	3.51	3.51	3.51	3.51	3.51	3.51	3.51
8.	3.18	3.18	3.18	3.18	3.18	3.18	3.18
9.	6.24	3.12	1.56	3.12	1.56	3.12	3.12
10.	3.57	6.37	3.57	3.57	3.57	6.37	3.57
11.	3.03	3.03	3.03	3.03	3.03	3.03	3.03
12.	13.1	3.28	3.28	3.28	1.64	3.28	3.28
13.	3.28	3.28	3.28	3.28	1.64	3.28	3.28
14.	6.57	3.28	3.28	3.28	3.28	3.28	6.57
15.	3.55	3.55	3.55	3.55	3.55	3.55	3.55
16.	3.16	3.16	3.16	3.16	3.16	3.16	3.16
17.	6.60	3.30	6.60	3.30	3.30	3.30	3.30
18.	3.38	1.69	3.38	3.38	3.38	3.38	1.69
19.	3.07	3.07	6.15	3.07	3.07	6.15	3.07
20.	3.01	3.01	3.01	3.01	3.01	3.01	3.01
21.	3.44	3.44	3.44	3.44	3.44	3.44	3.44
22.	2.93	2.93	2.93	2.93	2.93	2.93	2.93
Std.	3.43[a]	3.43[a]	3.43[a]	3.43[a]	1.72[a]	4.08[b]	4.08[b]

[a]Cefadroxil, [b]Fluconazole

Compound 9 ($MIC_{kp,\ st}$ =1.56×10^{-2}µM/ml) was found to be most active against *S. pneumonia* and *S. typhi*, compound 18 (MIC_{bs} =1.69×10^{-2}µM/ml and compound 22 ($MIC_{sa,\ ec}$ =1.69×10^{-2}µM/ml) was found to be most potent antibacterial agent against *S. aureus* and *E. coli*. Antifungal activity results indicated that the compound 22 (MIC_{an}=1.69×10^{-2}µM/ml) was found to be most active against *A. niger* and compound 18 (MIC_{ca} =1.69×10^{-2}µM/ml) was found to be most active against *C. albicans*. The compounds 9, 18 and 22 had high antibacterial potentials whereas compounds 18 and 22 had high antifungal potential among the synthesized compounds and may be taken as lead compounds for further development of novel antimicrobial agents.

Table 3: *In vitro* antidiabetic activity of newly synthesized derivatives.

Comp.	% Inhibition				IC_{50} (µg/ml)
S. No.	25(µg/ml)	50(µg/ml)	75(µg/ml)	100(µg/ml)	
1.	20.82	30.82	53.81	88.57	64.39
2.	20.61	43.27	59.24	87.47	59.45
3.	20.65	31.08	44.63	68.50	76.52
4.	23.55	48.10	65.41	89.25	54.87
5.	19.54	42.63	57.98	88.18	60.15
6.	27.29	42.95	60.09	98.71	50.63
7.	20.46	43.36	58.36	83.05	60.89
8.	21.86	42.15	58.73	89.38	59.07
9.	22.70	45.23	63.78	97.04	55.07
10.	37.81	42.40	57.86	84.18	53.52
11	27.35	53.45	73.25	88.57	49.45
12.	21.75	46.33	61.74	97.75	55.45
13.	27.26	43.91	58.22	90.28	56.48
14.	26.19	46.04	62.19	95.39	54.17
15.	18.86	39.67	54.17	86.81	62.88
16.	19.56	42.51	58.61	89.01	59.85
17.	22.08	46.52	59.78	93.32	57.02
18.	21.35	44.43	60.18	90.28	57.94
19.	18.54	38.86	52.07	80.17	65.96
20.	23.11	47.95	63.70	96.91	54.18
21.	16.49	47.32	65.87	99.53	55.70
22.	19.26	41.46	58.31	88.45	60.93
Acarbose	32.82	49.31	68.97	89.15	49.46

From the obtained results, it was found that the all synthesized derivatives showed good to moderate anti-diabetic activity. Among them compound 6 and 11 exhibited excellent anti-diabetic activity with IC_{50} values 50.63 µg/ml and 49.45 µg/ml, respectively when compared with acarabose as standard drug and may be used as lead for further study.

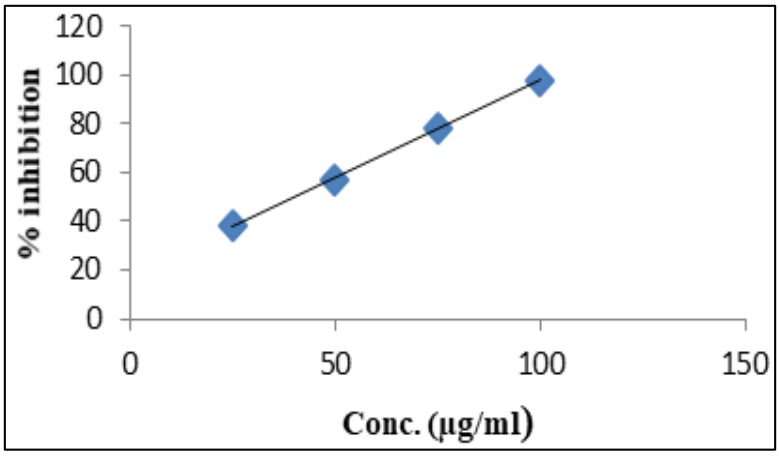


Fig 2: Standard curve of acarbose

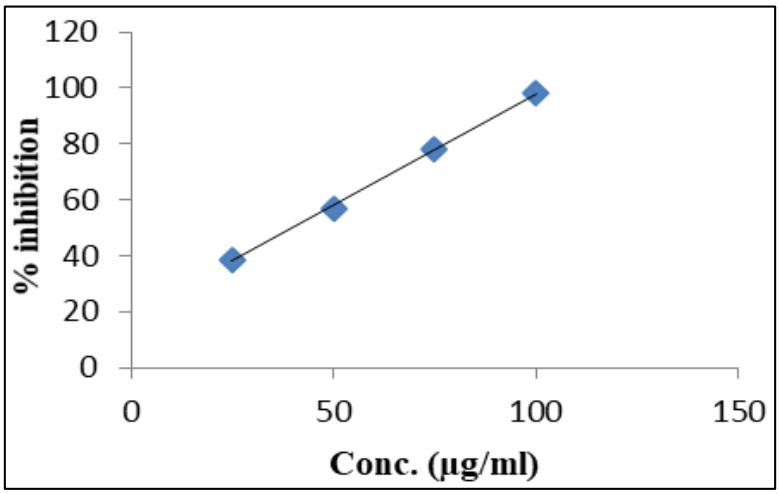


Fig 3: Standard curve of ascorbic acid

Table 4: Antioxidant activity of newly synthesized *N*-(substituted benzylidene)-2/4-(1, 3-dioxoisoindolin-2-yl)alkanehydrazide derivatives.

Comp.	% Inhibition				IC_{50} (µg/ml)
	25(µg/ml)	50(µg/ml)	75(µg/ml)	100(µg/ml)	
1.	21.94	42.60	57.17	86.26	60.10
2.	28.95	43.54	58.50	97.47	54.56
3.	18.81	42.40	57.86	94.18	59.08
4.	23.55	48.10	65.41	99.25	53.24
5.	19.54	42.63	71.98	88.18	56.63
6.	15.65	31.08	56.63	68.50	72.09
7.	20.46	43.36	58.36	93.05	58.41
8.	20.45	42.15	66.73	89.38	57.78
9.	25.70	45.23	62.78	97.04	63.44
10.	20.29	56.95	70.09	98.71	51.10
11	24.82	49.31	65.97	99.15	52.73
12.	21.75	46.33	61.61	97.75	55.62
13.	21.26	43.91	58.22	90.28	58.57
14.	22.69	46.08	62.19	95.39	55.87
15.	18.86	39.67	54.17	86.81	62.08
16.	19.56	42.51	58.61	89.01	60.32
17.	22.08	46.52	59.78	93.32	57.02
18.	21.35	44.43	60.18	80.28	60.49
19.	18.54	38.86	52.07	80.17	65.96
20.	23.11	47.95	63.70	96.91	54.64
21.	19.26	41.46	58.31	88.45	51.42
22.	22.49	55.32	68.87	99.53	51.14
Ascorbic acid	38.57	56.84	77.92	98.03	40.54

The compound 10 with IC_{50} value 51.10µg/ml had highest antioxidant activity among the synthesized compounds and may be taken as lead compound for further development of novel antioxidant agents as indicated in Table 4. The IC_{50} value of synthesized compounds was calculated from the graph plotted as percentage inhibition against concentration of compound (Fig 4, 5). Tests were carried out in triplicate using ascorbic acid as standard drug. Standard curve was plotted using different concentration of ascorbic acid.

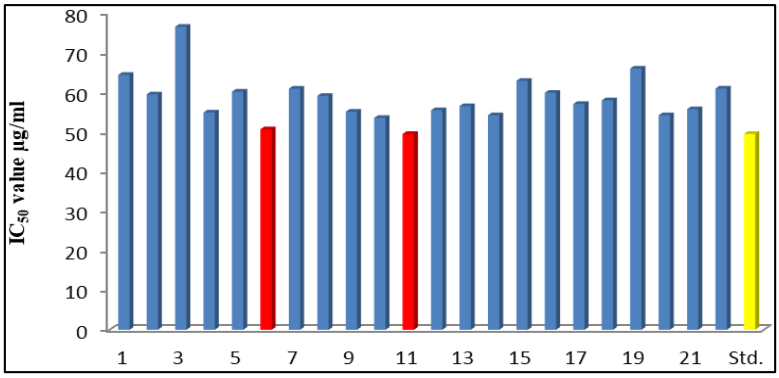


Fig 3: Shows the comparitive IC_{50} values of compounds (1-22) with Std. (Acarbose).
The columns (6 and 11) denote to most active compounds among the synthesized compounds (1-22) and yellow column denotes to activity of standard compound.

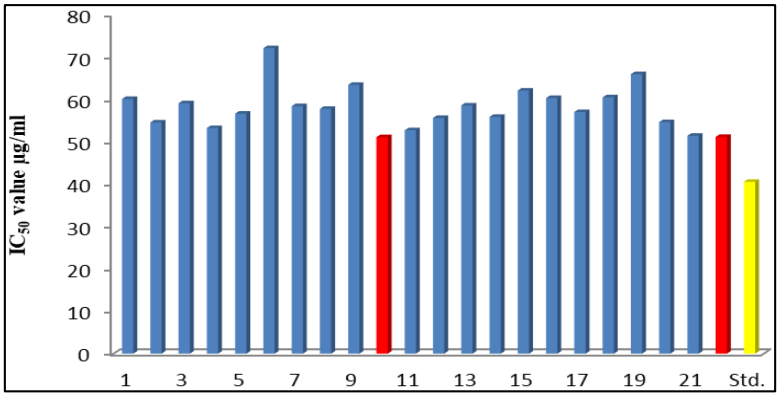


Fig 5: Comparitive IC_{50} values of compounds (1-22) with Std. (Ascorbic acid).
The columns (10 and 22) denote to most active compounds among the synthesized compounds (1-22) and yellow column denotes to activity of standard compound.

4. Structure Activity Relationship Studies

The structure activity relationship for antimicrobial and anticorrosive activities of synthesized *N*-(substituted benzylidene)-2/4-(1,3-dioxoisoindolin-2-yl)alkanehydrazide derivatives are indicated in Figure 6.

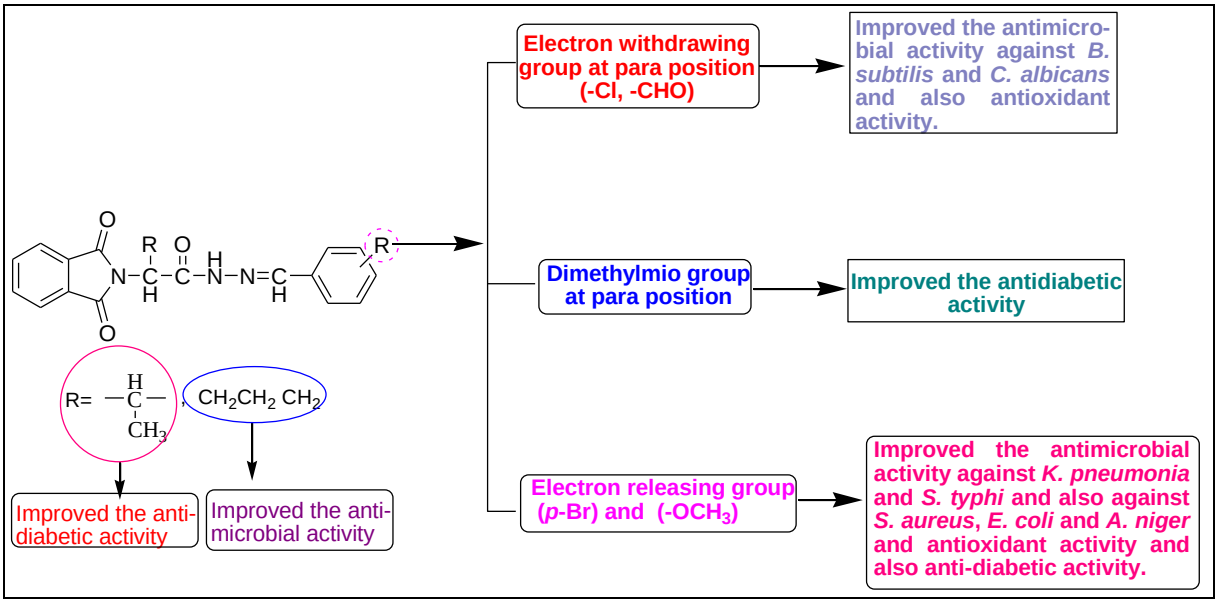


Fig 6: Structural requirements for the antimicrobial, antidiabetic and antioxidant activity of *N*-(substituted benzylidene)-2/4-(1,3-dioxoisoindolin-2-yl)alkanehydrazide derivatives.

5. Conclusion

A total 22 *N*-(substituted benzylidene)-2/4-(1,3-dioxoisoindolin-2-yl)alkanehydrazide derivat- ives were synthesized in the present series and evaluated for antimicrobial activity against different bacterial strains fungal strains using tube dilution method. The compounds 9, 18 and 22 had high antibacterial potentials whereas compounds 18 and 22 had high antifungal potential among the synthesized compounds. The compound 6 and 11 exhibited excellent anti-diabetic activity with lowest IC_{50} values better than acarabose used as standard. The compound 10 with exhibited highest antioxidant potential among the synthesized compounds. The synthesized compounds exhibiting best activity can be used as lead structures for further development of novel compounds in the respective categories.

6. Acknowledgement

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7. Conflict of interest

The authors declare no conflict of interest.

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