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Synthesis of some novel metformin Schiff's bases and its Antibacterial, Antifungal, Anti-inflammatory and Antioxidant activity

R. A. Shastri[1*], Satish B. Jadhav[2] & P. P. Joshi[3]

[1,3]Post Graduate Department of Chemistry, S.B.E.S. College of Science, Aurangabad-431001, Maharashtra, (India)
[2]Department of Chemistry R. B. Attal Arts, Science and Commerce College, Georai Dist.- Beed, Maharashtra, (India)

Email Id: shastriranjana@yahoo.com

Abstract : Some novel Schiff's bases of Metformin hydrochloride **(3a-k)** were synthesized by conventional method. Synthesized compounds were screened for antibacterial, antifungal, anti-inflammatory and antioxidant activity. Compounds **3b, 3c, 3d, 3g,** showed promising activity to *Bacillus subtilis, E. coli* and *C. albicans* whereas other samples showed mild to moderate activity. Compounds**3a, 3b, 3c, 3d, 3g, 3j** and **3k** caused complete inhibition of *Aspergillus niger Pencillium chrysogenum* and *Aspergillus Flavus* hence these compounds can be considered as fungicidal. Anti-inflammatory activity exists for compound **3d** having fluoro substitution whereas all other shows mild to moderate activity. Compounds **3b, 3d, 3g** showed best antioxidant activity. Activity report of Schiff's bases of metformin showed that they can be considered as new bioactive molecules that may serves as leads in the development of new pharmaceutical drugs.
Key-words : Metformin, antibacterial, antifungal, anti-inflammatory, antioxidant activity.

1. Introduction

Metformin is N,N- dimethylbiguanide, an oral anti-hyperglycemic agent which is used for treating non-insulin dependent diabetes.[1] Metformin improves liver sensitivity to insulin due to which it decreases the glucose production in liver and insulin absorption is increased. Hence metformin and its derivatives are used in the treatment of blood sugar management. Metformin has also been shown to have anticancer, antiageing effects; it also reduces cardiovascular diseases [2,3,4,5.]

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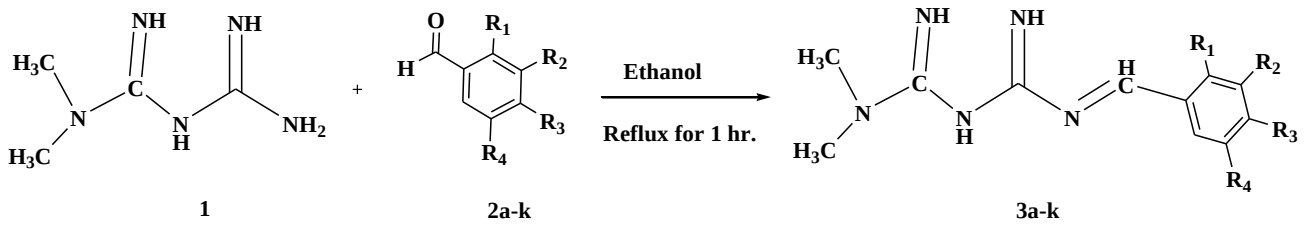
Diabetes is also called as hyperglycemia, in which your body does not produce enough insulin or cannot use insulin properly. Diabetes are of two types, Type 1 which is seen in infants and young when body does not make enough insulin, Type 2 which is seen in adults here body makes insulin, but cannot use it well. As a result of high sugar concentration in the blood. It causes damage to many systems/organs of body, especially hearty, kidney, nerves, micro and macro vascular system.

Hyperglycemia increases the lipid peroxidation[6] and oxidative stress[7,8]. Oxidative stress causes autoxidation of glucose due to which free radicles are generated, the balance between free radical generated and its elimination is imbalanced. These free radicals causes damage of cell by passing unpaired electron in cell due to which oxidation of cell component takes place. This leads to increase in variety of pathological conditions such as cancer, cardiovascular diseases, rheumatoid arthritis etc, and kidney damage. Free radicals contributes to kidney damage, filtering units present in kidney has tiny blood vessels, overtime high sugar causes the blood vessels to become narrow and albumin passes through kidney. To protect kidney from damage Nefrosaver, Nefrosaver forty is given. It's a combination of two medicines Taurine and acetylcysteine, both these drugs act as antioxidant which delay kidney damage progression in patients with diabetes and reduces the risk of kidney failure.

Diabetes for a long period causes damage of peripheral nerve[9] and reduces the blood flow due to which chances of infection increases, due to high sugar in blood and tissues, bacterial and fungal infection[10] appears.

Literature survey reveals that Schiff's bases of metfomin hydrochloride[11] have been synthesized having electron withdrawing group (ortho and p-nitro group), whereas report having electron releasing groups, halogens is not reported. The present attempt is to synthesize new metformin derivatives and evaluate for antibacterial, antifungal, anti-inflammatory and antioxidant activity. The results of these studies are presented in paper.



1 **2a-k** **3a-k**

Where:

3a- R_3- Cl **3b-** R_2- Cl **3c-** R_2, R_3, R_4- OCH_3 **3d-** R_3- F **3e-** R_2- NO_2 **3f-** R_1, R_2, R_3, R_4 -H

3g- R_2, R_4- OCH_3, R_3- OH **3h-** R_3- OH **3i-** R_3- NO_2 **3j-** R_3- OCH_3 **3k-** R_1- OH

Scheme-1

2. Experimental Methods

All melting points were determined in open capillary tubes and are uncorrected. The homogeneity of all the compounds were checked by TLC on silica gel coated plates. IR spectra were obtained in KBr on Perkin-Elmer FTIR Spectrophotometer.^{1}H NMR spectra were recorded in $CDCl_3$ on varian NMR spectrometer operating at 400 MHz. Chemical Shifts are expressed in δ w.r.t. TMS. Mass spectra were recorded on a MICRO MASS operating at 70 eV. Thin Layer Chromatography was performed with E Merck pre coated TLC plates, silica gel 60 $F_{254}$with layer thickness 0.25 mm. Metformin hydrochloride was purchased from Sigma Aldrich. All chemicals used were of LR grade.

2.1. General Procedure for the synthesis of Schiff's Bases of Metformin Hydrochloride (3a-k)

A mixture of 10mmole metformin hydrochloride and 10 mmole of substituted benzaldehyde were dissolved in 5 ml ethanol and2-3 drops of acetic acid were added refluxed for one hour. The reaction mixture was cooled and poured on cold water, filtered and recrystallized from aqueous ethanol.

2.2. Spectral analysis of compounds

(3a) Yield 85%; m. p. 214^{O}C : Elemental analysis Cal. for $C_{11}H_{14}ClN_5$; C, 52.49; H, 5.61; N, 27.82; found: C, 52.39; H, 5.58; N, 27.72; IR (KBr pellets Cm^{-1}): 3492 (N-H, secondary amine, stretching), 3121 (Aromatic C-H stretching), 1630 (C=N, imine); ^{1}H NMR (DMSO, 400 MHz) δ(ppm) 8.27 (1H, s, =CH), 8.10-7.54 (4H, m, Ar-H), 6.85 (2H, s, =NH), 3.32 (1H, s, -NH secondary amine), 3.13 (3H, s, -CH_3), 3.16 (3H, s, -CH_3);Mass (m/z): 251.72 (M+1).

(3b) Yield 90 %; m.p. 222^{O}C:Elemental analysis Cal. for$C_{11}H_{14}ClN_5$; C, 52.49; H, 5.61; N, 27.82; found:C, 52.39; H, 5.55; N, 27.70; IR (KBr pellets Cm^{-1}): 3490 (N-H, secondary amine, stretching), 3118(Aromatic C-H stretching),1632 (C=N, imine); ^{1}H NMR (DMSO, 400 MHz)) δ(ppm)8.26 (1H, s, =CH), 8.12-7.50 (4H, m, Ar-H), 6.82 (2H, s, =NH), 3.30 (1H, s, -NH secondary amine), 3.12 (3H, s, -CH_3), 3.16 (3H, s, -CH_3); Mass (m/z): 251.72 (M+1).

(3c) Yield 82%; m.p. 95^{O}C:Elemental analysis Cal. for$C_{14}H_{21}N_5O_3$; C, 54.71; H, 6.89; N, 22.79; found:C, 54.61; H, 6.91; N, 22.80;IR (KBr pellets Cm^{-1}): 3496 (N-H, secondary amine, stretching), 3116(Aromatic C-H stretching),1635 (C=N, imine),1190(-OCH_3);^{1}H NMR (DMSO, 400 MHz)) δ(ppm)8.20 (s, 1H, =CH), 8.10-7.55 (m, 2H, Ar-H), 6.80 (s, 2H, =NH), 3.85 (s, 6H, OCH_3), 3.70 (s, 3H, OCH3), 3.25 (1H, s, -NH secondary amine), 3.10 (3H, s, -CH_3), 3.14 (3H, s, -CH_3); Mass (m/z): 303.35 (M+1).

(3d) Yield 85%; m.p. 215^{O}C:Elemental analysis Cal. for$C_{11}H_{14}FN_5$; C, 56.16; H, 6.00; N, 29.77; found:C, 56.10; H, 6.12; N, 29.70; IR (KBr pellets Cm^{-1}): 3490 (N-H, secondary amine, stretching), 3127(Aromatic C-H stretching),1632 (C=N, imine); ^{1}H NMR (DMSO, 400 MHz)) δ(ppm)8.29 (1H, s, =CH), 8.14-7.52 (4H, m, Ar-H), 6.82 (2H, s, =NH), 3.30 (1H, s, -NH secondary amine), 3.10 (3H, s, -CH_3), 3.15 (3H, s, -CH_3);Mass (m/z): 235.26 (M+1).

(3e) Yield 87%; m.p. 202^{O}C:Elemental analysis Cal. for $C_{11}H_{14}N_6O_2$; C, 50.38; H, 5.38; N, 32.04; found:C, 50.35; H, 5.32; N, 32.02;IR (KBr pellets Cm^{-1}): 3497 (N-H, secondary amine, stretching), 3125(Aromatic C-H stretching),1630 (C=N, imine), 1532 (-NO_2); ^{1}H NMR (DMSO, 400 MHz)) δ(ppm) 8.28 (1H, s, =CH), 8.12-7.50 (4H, m, Ar-H), 6.80 (2H, s, =NH), 3.31 (1H, s, -NH secondary amine), 3.12 (3H, s, -CH_3), 3.18 (3H, s, -CH_3); Mass (m/z): 262.27 (M+1).

(3f) Yield 80%; m.p. 221^{O}C:Elemental analysis Cal. for$C_{11}H_{15}N_5$; C, 60.81; H, 6.96; N, 32.23; found:C, 60.83; H, 6.90; N, 32.23; IR (KBr pellets Cm^{-1}): 3490 (N-H, secondary amine, stretching), 3130(Aromatic C-H stretching),1640 (C=N, imine); ^{1}H NMR (DMSO, 400 MHz)) δ(ppm)8.25 (1H, s, =CH), 8.14-7.45 (5H, m, Ar-H), 6.75 (2H, s, =NH), 3.29 (1H, s, -NH secondary amine), 3.16 (3H, s, -CH_3), 3.22 (3H, s, -CH_3); Mass (m/z): 217.27 (M+1).

(3g) Yield 87%; m.p. 110^{O}C:Elemental analysis Cal. for $C_{13}H_{19}N_5O_3$; C, 53.23; H, 6.53; N, 23.88; found: C, 53.20; H, 6.51; N, 23.90; IR (KBr pellets Cm^{-1}): 3480 (N-H, secondary amine, stretching), 3390 (-OH), 3125 (Aromatic C-H stretching), 1642 (C=N, imine), 1145(-OCH_3); ^{1}H NMR (DMSO, 400 MHz)) δ(ppm)10.17 (1H, s, -OH), 8.22 (1H, s, =CH), 8.12 (1H, s, Ar-H), 8.35 (1H, s, Ar-H), 6.72 (2H, s, =NH),3.78 (s, 6H, 2x -OCH_3), 3.24 (1H, s, -NH secondary amine), 3.10 (3H, s, -CH_3), 3.18 (3H, s, -CH_3); Mass (m/z): 217.27 (M+1).

(3h) Yield 85%; m.p. 95^{O}C: Elemental analysis Cal. for $C_{11}H_{15}N_5O$; C, 56.64; H, 6.48; N, 30.02; found: C, 56.60; H, 6.42; N, 30.10; IR (KBr pellets Cm^{-1}): 3476 (N-H, secondary amine, stretching), 3382 (-OH), 3122 (Aromatic C-H stretching), 1642 (C=N, imine); ^{1}H NMR (DMSO, 400 MHz)) δ(ppm)10.10 (1H, s, -OH), 8.15 (1H, s, =CH), 8.20-7.40 (4H, m, Ar-H), 6.65 (2H, s, =NH),3.20 (1H, s, -NH secondary amine), 3.11 (3H, s, -CH_3), 3.14 (3H, s, -CH_3); Mass (m/z): 233.13 (M+1).

(3i) Yield 90%; m.p. 230^{O}C:Elemental analysis Cal. for $C_{11}H_{14}N_6O_2$; C, 50.38; H, 5.38; N, 32.04;found:C, 50.37; H, 5.30; N, 32.00; IR (KBr pellets Cm^{-1}): 3497 (N-H, secondary amine, stretching), 3120(Aromatic C-H stretching), 1630 (C=N, imine), 1530 (-NO_2); ^{1}H NMR (DMSO, 400 MHz)) δ(ppm)8.26 (1H, s, =CH), 8.11-7.52 (4H, m, Ar-H), 6.80 (2H, s, =NH), 3.30 (1H, s, -NH secondary amine), 3.11 (3H, s, -CH_3), 3.18 (3H, s, -CH_3); Mass (m/z): 262.27 (M+1).

(3j) Yield 81%; m.p. 210^{O}C:Elemental analysis Cal. for $C_{12}H_{17}N_5O$; C, 58.28; H, 6.93; N, 28.32;found:C, 58.28; H, 6.93; N, 28.32;IR (KBr pellets Cm^{-1}): 3480 (N-H, secondary amine, stretching), 3110(Aromatic C-H stretching),1625 (C=N, imine), 1185 (-OCH_3);^{1}H NMR (DMSO, 400 MHz)) δ(ppm) 8.22 (s, 1H, =CH), 8.16-7.50 (m, 4H, Ar-H), 6.80 (s, 2H, =NH), 3.75 (s, 3H, -OCH_3),3.21 (1H, s, -NH secondary amine), 3.09 (3H, s, -CH_3), 3.13 (3H, s, -CH_3); Mass (m/z): 247.30 (M+1).

(3k) Yield 87%; m.p. 218^{O}C: Elemental analysis Cal. for $C_{11}H_{15}N_5O$; C, 56.64; H, 6.48; N, 30.02; found: C, 56.60; H, 6.42; N, 30.12; IR (KBr pellets Cm^{-1}): 3474 (N-H, secondary amine, stretching), 3378 (-OH), 3125 (Aromatic C-H stretching), 1640 (C=N, imine); ^{1}H NMR (DMSO, 400 MHz)) δ(ppm)10.25 (1H, s, -OH), 8.11 (1H, s, =CH), 8.15-7.40 (4H, m, Ar-H), 6.63 (2H, s, =NH),3.20 (1H, s, -NH secondary amine), 3.10 (3H, s, -CH_3), 3.14 (3H, s, -CH_3); Mass (m/z): 233.13 (M+1).

3. Result and Discussion

In the present study Metformin schiffs bases were prepared with substituted benzaldehydeby modified conventional method.Present synthesis involves less quantity of solvent volume due to which interaction in reactants is maximum as a result, time period of reaction is reduced,which results the products in high yields.

The IR spectra of **3a-k** revealed the absence of carbonyl group of aldehyde and absence of primaryamino group stretching of metformin near 3266 $cm^{-1}$and 3290 $cm^{-1}$and existence of newly synthesized imine group near to 1625 cm^{-1} to1640 cm^{-1}, which confirms the formation of product. The IR spectra also exhibited a intact N-H band of metformin near 3490cm^{-1}indicating involvement of primary amino group in reaction.This is further supported by ^{1}H NMR group which showed singlet for new imine group near to δ8.3and the presence of aromatic protons in the region δ 8.00-7.6, the appearance of singlet for >C=NH group of metformin near δ6.7-6.8and for secondary amino group at δ3.2-3.3.The EI-MS of compounds **3a-k** revealed the existenceof their molecular ion peaks which were in accordance with the given structure.

4. Antifungal activity :

The compounds **3a-k** were screened for antifungal activity against *Aspergillus niger, Pencillium chrysogenum*, *Aspergillus Flavus* and *Fusarium moneliforme* by using Griseofulvin (100µg/ml) as reference standard and DMSO was used as control solvent, by poison plate method[12].The observed minimum inhibitory concentration (MIC) values for all synthesized compounds are presented in **Table 2**.The investigation of antifungal screening result indicates**3i** was found to be inactive towards all the strains of fungi ,whereas,**3h** was found active against *A. Flavus* and reduced growth for *F. Monoliforme*. Compounds **3b, 3c, 3d, 3f, 3g**,and **3j** causes complete inhibition of *F. Monoliforme*. Compound **3e** showed reduced growth to *Aspergillus niger & Pencillium chrysogenum* and inactive to *Aspergillus Flavus* and *F. Monoliforme.* It was found, compounds **3a,3b,3c,3d,3g,3j,**and**3k**caused complete inhibition of *Aspergillus niger Pencillium chrysogenum, Aspergillus Flavus* and *F. Monoliforme* hence these compounds can be considered as fungicidal.

Table-1. Antifungal screening results of the compounds **(3a-k)**

Compound	*Aspergillus niger*	*Penicillum chrysogenum*	*Aspergillus flavus*	*F. moneli*
3a	-ve	-ve	-ve	+ ve
3b	-ve	-ve	-ve	-ve
3c	-ve	-ve	-ve	-ve
3d	-ve	-ve	-ve	-ve
3e	RG	RG	+ ve	+ ve
3f	-ve	+ ve	-ve	-ve
3g	-ve	-ve	-ve	-ve
3h	+ ve	+ ve	-ve	RG
3i	+ ve	+ ve	+ ve	+ ve
3j	-ve	-ve	-ve	-ve
3k	-ve	-ve	-ve	+ ve
Griseofulvin	-ve	-ve	-ve	-ve
DMSO	+ ve	+ ve	+ ve	+ ve
+ ve no antifungal activity –ve no growth, antifungal activity observed RG Reduced growth				

5. Antibacterial activity:

The compounds **3a-k**were screened for antibacterial activity against *Bacillus subtilis, E. coli* and *C.albicans* by using *Pencilline* as reference standard DMSO was used as solvent control. The method employed was agar cup method[13]. The zones of inhibitions were measured in mm and shown in the **table No. 3 .**Compounds **3f, 3e** and **3i** showed mild activity towards all strains of bacteria, where as **3a, 3f, 3h, 3i, 3j** and **3k** showed moderate activity. Sample **3h** showed good activity to *E. Coli*and moderate activity to *Bacillus subtilis & Candida albican.***3b** has promising activity to *Bacillus subtilis, E. Coli.* and moderate activity towards *C. albicans.* Schiffs bases such as**3b, 3c, 3d, 3g** showed promising antibacterial activity to all strains of bacteria.

Table-2. Antibacterial screening results of the compounds **(3a-k)** (Zone of inhibition in mm)

Compound	*Bacillus subtilis*	*E. Coli*	*C. Albicans*
3a	18	12	14
3b	20	18	25
3c	22	16	34
3d	25	15	32
3e	10	09	20
3f	13	14	22
3g	21	15	31
3h	14	16	25
3i	11	10	18
3j	13	16	24
3k	15	11	25
Penicillin	30	20	40

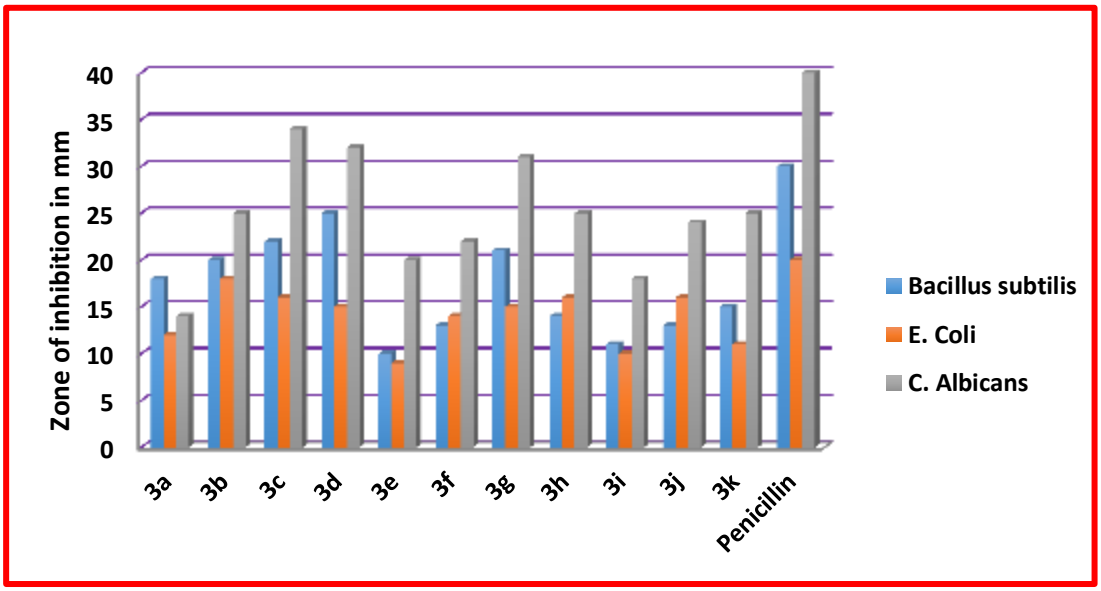


Fig-1 Flow Chart for Antibacterial activity

6. Anti-inflammatory Activity

The synthesized compounds were screened for anti-inflammatory activity by using inhibition of albumin denaturation technique which was studied according to Muzushima & Kabayashi[14] with slight modification. Ibuprofen was used as standard drug. Result of these study is presented in **Table no.4.**The investigation data of the compounds showed good anti-inflammatory activity exists only for compound **3d** having fluoro substitution whereas all other shows mild to moderate activity.

Table 3 In vitro Anti-inflammatory activity of synthesized compounds **(3a-k)**

Compounds	Mean absorbance value ± SEM	Inhibition of denaturation (in %)
Control	0.0780	-
Ibuprofen	0.149 ± 0.004	91.02
3a	0.102± 0.003	30.76
3b	0.108± 0.002	38.46
3c	0.112± 0.005	43.58
3d	0.128 ± 0.006	64.10
3e	0.018± 0.003	23.07
3f	0.115± 0.005	47.43
3g	0.108± 0.003	38.46
3h	0.101± 0.005	29.48
3i	0.016± 0.003	20.51
3j	0.106± 0.002	35.89
3k	0.096± 0.002	23.07

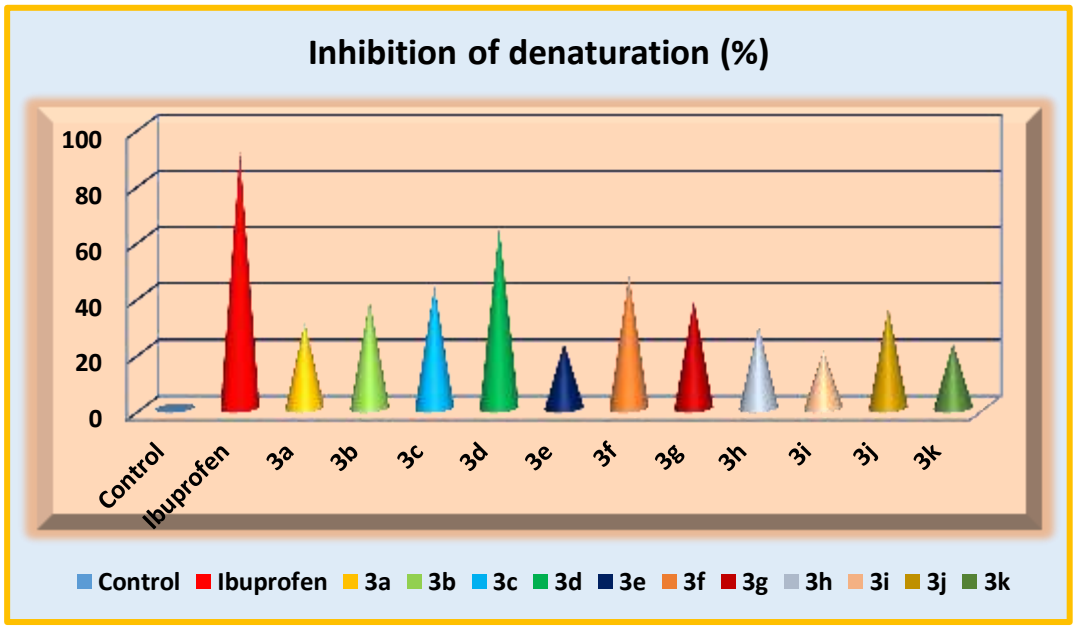


Fig-2 Flow Chart for Anti-inflammatory activity

7. Antioxidant Activity

Antioxidant activity for compounds **3a-K**was performed by Shimada method [15] which is based on the scavenging the DPPH radical and evaluated free radical scavenging activities of sample with reference to the standard ascorbic acid.Different concentration of test sample and ascorbic acid (10-100µg/ml) were prepared in methonal. Absorbance was measured at 517nm by taking test sample 1ml, 1 ml of ascorbic acid and 0.1ml of DPPH solution, the mixture was vigorously shaken and kept in dark for 30 minutes.It was found from IC_{50} value, synthesized compounds**3b,3d,3g** shows best antioxidant activity.**3a** and **3c** has less antioxidant activitywhereas all other synthesized compounds were as active as standard.

Table-4 Antioxidant activity of synthesized compounds **(3a-k)**

Compound Code	Concentration in µg/ml					IC_{50}**
	10	25	50	75	100	
Ascorbic acid	44.26	54.54	64.18	80.10	83.15	15.59
3a	6.34	10.08	16.11	37.38	57.17	95.29
3b	35.9	43.8	55.86	69.18	84.25	20.35
3c	2.76	7.22	12.53	41.78	49	100.95
3d	42.49	48.79	61.89	68.27	73.01	18.37
3e	39.02	43.41	49.36	51.62	57.86	60.82
3f	24.36	40.65	47.5	52.4	76.34	55.51
3g	30.2	45.27	62.52	69.87	86.6	19.84
3h	36.74	46.89	54.64	62.39	65.82	42.22
3i	40.43	41.71	50	62.39	76.55	41.71
3j	42.74	46.89	54.64	62.39	65.82	42.02
3k	39.74	43.89	50.64	60.59	63.42	43.45

**µgm/ml

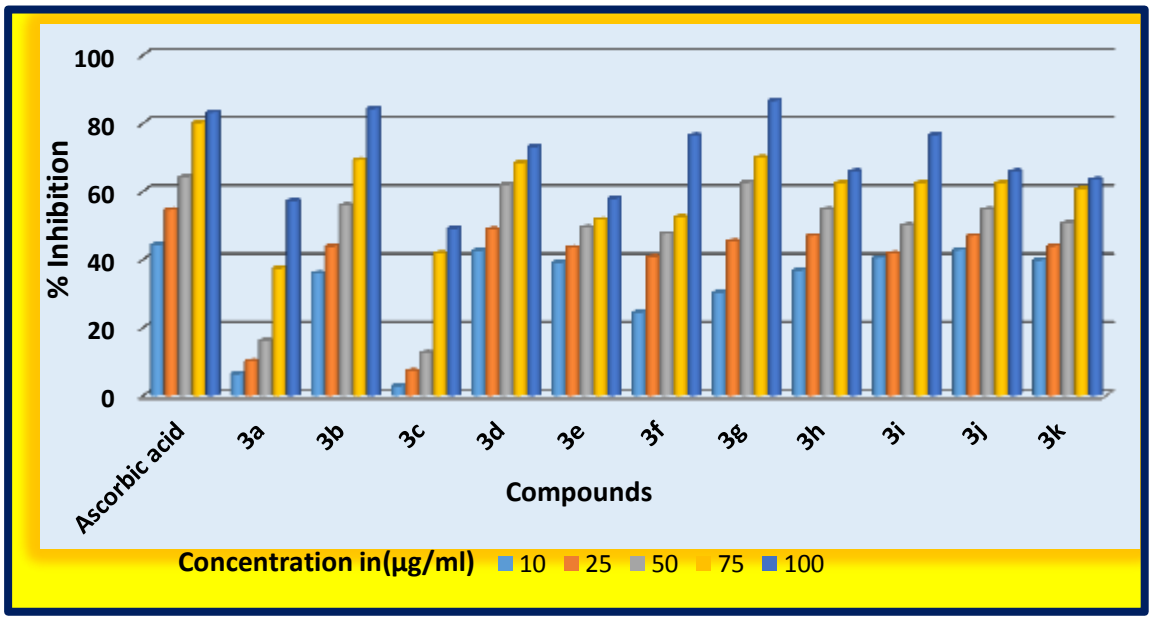


Fig-3 Flow Chart for Antioxidant activity

8. Conclusion

The synthesized **3a-k** all are novel derivatives of metformin.Compounds with electron releasing group such as methoxy and compounds having pharmacophors such as chloro, fluoro groups when present in moiety exhibited potent antibacterial and antifungal activity. Compounds having electron withdrawing group such as nitro group showed less active or inactive towards antibacterial and antifungal strains.Compounds without substitution in aromatic nucleus and with OH group showed moderate activity towards antifungal strains.Schiff's bases having *m*-chloro, *p*-fluoroand methoxy group showed promising activity. Compound **3d** with fluoro substituent showed good anti-inflammatory activity. compounds**3b, 3d, 3g** shows best antioxidant activity. .Study of antibacterial,antifungal,anti-inflammatory and antioxidant activity showed that newly synthesized Schiff's bases of metformin has promising activity,hence these could serve as effective free radical inhibitor or scavenger which will protect damage of organs from oxidative stress,at the same it can also be a weapon for fighting bacterial and fungal infections.Thus these molecules can act as drugs for controlling diabetes as well as preventing all complications resulting due to diabetes.

The present study of Schiff's bases showed that they can be considered as new bioactive molecules that may serves as leads in the development of new pharmaceutical research activities.

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