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Synthesis and evaluation of some new 2-benzamido-3-(3, 4–substituted phenyl) acrylohydrazides for their *in vitro* antitubercular, antibacterial and antifungal activities

Fathi H Assaleh[a*], Mohammed H Assaleh[b]

[a]*School of Basic Sciences, Libyan Academy of Postgraduate Studies, Janzour, Libya*
[b]*Faculty of Sciences, University of Zawia, Al-Zawia, Libya*

ABSTARCT

Present work was designed to synthesize some new acrylohydrazides and evaluate new 2-benzamido-3-(3, 4–substituted phenyl) acrylohydrazides for their *in vitro* antitubercular, antibacterial and antifungal activitivity. *Synthesis of 4-(3, 4–disubstituted) benzylidine-2-phenyloxazolin-5-ones**(2 a-g)*** *and 2-benzamido-3-(3, 4-substituted) phenyl acrylohydrazides (3a-g) were done.* A total of 14 compounds were synthesized, purified and characterized by UV, FT-IR, ^{1}H-NMR, ^{13}C-NMR, Mass (ESI/MS) spectral data. Compounds were screened for their *in vitro* antibacterial activity against *S.aureus* (Gram–positive bacteria), *E.coli, P.vulgaris, P.aeruginosa* (Gram–negative bacteria) and antifungal activity against *A. niger* and *C. albicans* by agar plate disc diffusion method at a concentration of 50µg/disc. The antitubercular screening was carried out against *M. tuberculosis H$_{37}$Rv* by Alamar Blue Assay method. It was found that 4-(3, 4-substituted) benzylidine-2-phenyl oxazol-5 (4H)-one **2(a-g)** derivatives displayed better activity against bacteria and fungi than mycobacteria. The conversion of intermediate **2(a-g)** into acrylohydrazides **3(a-g)** resulted in improved antitubercular activity, but still showed moderate to mild antibacterial and antifungal action. Compounds **2e, 2d, 3e, 3d,** possess common 4-hydroxy phenyl moiety and are structurally similar, it was admirable that they showed disparity in antitubercular activity with respect to the 3-alkoxy substitution in the same phenyl ring. It was concluded that substituted phenylacrylohydrazides exhibits considerable antitubercular activity and it may be related to partition coefficient of compounds.

Keywords: Acrylohydrazides, ^{1}H-NMR, ^{13}C-NMR, *in vitro* antitubercular, antibacterial, antifungal.

INTRODUCTION

Tuberculosis is accompanying AIDS or existing as multi-drug resistant tuberculosis (MDR-TB) or as extensively/extreme resistance tuberculosis (XTR-TB), where neither standard antitubercular drug, nor any of the regimen could be effective[1]. Because of ineffective remedy and risk in the current treatment, in 2020 the global burden of tuberculosis is estimated to be 2.3 million, of which 99% in developing countries (WHO, 1997). In 2000, the global alliance of WHO was established to accelerate the research in development of new antitubercular agents and to ensure their availability and affordability in high–epidemic countries. Under the guidance of global alliance and pharmaceutical companies, 20 new molecules and 40 DNA vaccines were developed with promising characteristics during *in vitro* and *in vivo* animal studies[2]. However those entities were not brought to the realization and precluded in future development. In addition to the present status of tuberculosis, present chemotherapy for bacterial and fungal infections is becoming worse with morbidity and mortality. It is due to high resistance to the current regimens and remain important health problems in developing countries such as the Indian sub-continent, part of South America and tropical part of Africa[3,4]. Along with the risk management of tuberculosis, few most

Author for Correspondence:
Fathi H Assaleh
School of Basic Sciences, Libyan Academy of Postgraduate Studies,
Janzour, Libya. Email: assaleh@academy.edu.ly

common antibacterial drugs such as, Amoxicillin, Norfloxacin, Erythromycin and Ciprofloxacin are known to possess severe side effects, toxicities and treatment failure[5, 6].

Reports revealed that both hydrazide[7-10] and oxazolinone[11,12] are attractive cores for many researchers, in design of chemotherapeutics due to their considerable antibacterial, antiviral, antimycobacterial, anti-cancer, anti-fungal, anthelmentic and insecticidal potentials. With the discovery of isonicotinic acid hydrazide (Isoniazid) in the treatment of tuberculosis, hydrazide core has became an attractive agent for all researchers in the field of synthetic and medicinal chemistry. Owing to the lack of suitable target, cell physiology and transduction mechanism in cell signaling process of mycobacterium, the modern drug discovery tool is still inactive in the area of antitubercular and antimicrobial drug research. Hence, a need for newer agents against mycobacteria, bacteria and fungi is essential and still continuing as interest. Based on above discussion and needy research in finding newer agents for chemotherapy, the present work was designed to synthesize some new acrylohydrazides.

MATERIALS AND METHODS

General

TLC tool was employed for the confirmation of reaction progress and product formation. Purity of compounds was authenticated by HPLC (Agilent LC) technique on C18 Column (250 mm x 4. 6 mm, 5 micron) using 75:25 % v/v of Acetonitrile and 0.2 % TEA (pH adjusted to 3.0 using OPA). Melting point and its range was determined by open capillary tube method using Kemi digital melting point apparatus. The chromophore of structure was supported by UVmax spectrum (Shimadzu UV-Visible Double beam Spectrometer, using ethanol as solvent), whilst functional nature of carbons and protons of the structure was assigned by IR (Jasco–510 FT-IR Spectrophotometer at 4 cm^{-1} using KBr pellet disc), ^{1}H-NMR (Varian VXR Unity at 400MHz using TMS as internal standard and DMSO-d_6/$CDCl_3$ as solvent), ^{13}C-NMR (Brukers UXNMR at 100MHz, using DMSO-d_6/$CDCl_3$ as solvent) (DMSO–d_6/$CDCl_3$), and mass spectral (Agilent LC/MS, positive mode, ESI) studies.

2.2 Synthesis

2.2.1 Synthesis of 4-(3, 4–disubstituted) benzylidine-2-phenyloxazolin-5-ones (2 a-g)

A mixture of benzoylglycine (0.01 mol), substituted benzaldehydes (0.01 mol), acetic anhydride (0.015 mol) and anhydrous sodium acetate (0.005 mol) were placed in Round bottom flask and refluxed on sand bath with constant shaking. Soon as the mixture has liquefied completely, the flask was transferred to a water bath and heated for 3 hrs. Then 25 ml of 95% of ethanol was added into the contents and allowed overnight under refrigeration. The solid mass separated out was filtered and washed with ice-cold alcohol and water. The crude product was recrystallized from rectified spirit and its homogeneity was confirmed by TLC using the eluent consist of toluene and ethyl acetate at the ratio of 70:30 % v/v and then followed by HPLC. The same procedure was adopted for all compounds.

Synthesis of 2-benzamido-3-(3, 4-substituted) phenyl acrylohydrazides (3a-g)

Compound **2(a-g)** (0.03 mol) was mixed with hydrazine hydrate (0.035 mol) in 25 ml of 95% ethanol and stirred for 3 hrs at RT. The deep yellow colored solid was precipitated out and then it was filtered, washed and crystallized from 50% ethanol. The crude product was recrystallized from rectified spirit and its homogeneity was confirmed by TLC using the eluents consist of n-hexan, ethyl acetate and methanol the ratio of 60:38:2 % v/v and followed by HPLC. The same procedure was adopted for all compounds.

Spectral data

(Z)-4-benzylidine-2-phenyl oxazolin–5 (4H)-one ***2a***: IR (KBr pellet, cm^{-1}): 3042, 3002, 2968, 1708, 1614, 1505, 1475 1391, 854, 759. ^{1}H-NMR (DMSO–d_6) : δ = 6.39 (1H, dd, =CH-, J = 12 Hz), 7. 20-7.81 cpx (10H, m, 10 H Phenyl). ^{13}C-NMR ($CDCl_3$): δ = 172.1(C=O), 154.6, 147.3, 144.8, 132.3, 131.9, 131.8, 130.3, 129.9 128.2, 127.2, 124.3, 116.2 (=CH) Mass (ESI/MS) : m/z 251 (M+H).

(Z)-4-(4-methoxy–benzylidine)-2-phenyl oxazolin–5 (4H)-one ***2b***: IR (KBr pellet, cm^{-1}): 3045, 3012, 2968, 2957, 1711, 1617, 1506, 1479 1391, 1213, 861, 753. ^{1}H-NMR (DMSO–d_6) : δ = 4.03 (3H, s, OCH_3), 6.44 (1H, dd, =CH-, J = 13 Hz), 7. 25-7.98 cpx (9H, m, Phenyl). ^{13}C-NMR ($CDCl_3$): δ = 173.2(C=O), 155.6, 147.4, 144.8, 133.3, 132.9, 131.9, 130.4, 139.0 128.9, 127.1, 126.8, 115.2 (=CH), 34.2 (-OCH_3) ,Mass (ESI/MS) : m/z 280 (M+H).

(Z)-4-(4-dimethyl amino–benzylidine)-2-phenyl oxazolin–5 (4H)-one ***2c***: IR (KBr pellet, cm^{-1}): 3043, 3010, 2971, 1713, 1614, 1509, 1462, 1233, 759 ^{1}H-NMR (DMSO–d_6) : δ = 3.91 (6H, s, $N(CH_3)_2$, 6.83 (1H, dd, =CH-, J = 12Hz), 7.33-7.78 cpx (9H, m, Phenyl). ^{13}C-NMR ($CDCl_3$): δ = 172.1 (C=O), 154.7, 144.8, 143.2, 133.3, 132.9, 131.6, 130.4, 129.4 128.3, 126.3, 125.1, 112.2 (=CH), 30.8 & 31.1 (-$N(CH_3)$. Mass (ESI/MS) : m/z 292 (M+H).

(Z)–4-(4-hydroxy-3-methoxy–benzylidine)-2-phenyl oxazolin–5 (4H)-one ***2d****:* IR (KBr pellet, cm^{-1}): 3514, 3033, 3022, 2978, 2976, 1714, 1609, 1516, 1480 1382, 1216, 1011, 753. ^{1}H-NMR (DMSO–d_6) : δ = 4.16 (3H, s, OCH_3), 6.52 (1H, dd, =CH-, J = 11 Hz), 7. 55-8.28 cpx (8H, m, Phenyl), 10.11 (1H, s, OH). ^{13}C-NMR ($CDCl_3$): δ = 174.3 (C=O), 157.5, 148.3, 132.9, 133.5, 131.4, 130.9, 129.5 128.4, 127.9, 126.8, 125.5, 117.9 (=CH), 30.3 (-OCH_3). Mass (ESI/MS) : m/z 296 (M^+).

(Z)-4-(4-hydroxy-3-ethoxy–benzylidin)-2-phenyl oxazolin–5 (4H)-one ***2e****:* IR (KBr pellet, cm^{-1}): 3498, 3022, 3010, 2962, 2899, 1719, 1624, 1501, 1465 1387, 1211, 1013, 876, 753. ^{1}H-NMR ($CDCl_3$) : δ = 2.14 (3H, t,-CH_3), 3.42 (2H, q,-CH_2), 6.98 (1H, dd, =CH-, J = 12 Hz), 7. 45-7.88 cpx (8H, m, Phenyl). ^{13}C-NMR ($CDCl_3$): δ = 168.3, 152.8, 146.3, 145.8, 132.3, 131.9,

131.9, 131.1, 129.8 128.1, 126.1, 125.1, 123.2, 111.9(=CH), 22.3 (-CH_2), 17.2(-CH_3). Mass (ESI/MS) : m/z 310 (M+H).

*(Z)-4-(4-hydroxy–benzylidin)-2-phenyl oxazolin–5 (4H)-one **2f**:* IR (KBr pellet, cm^{-1}): 3502, 3014, 3018, 2999, 1716, 1614, 1509, 1455 1377, 1201, 1036, 749. ^{1}H-NMR (DMSO–d_6) : δ = 5.35 (1H, dd, =CH-, J = 13 Hz), 7.55-8.08 cpx (9H, m, Phenyl). ^{13}C-NMR ($CDCl_3$): δ = 170.4, 150.7, 147.1, 146.7, 132.7, 132.2, 131.9, 130.9, 129.6 128.5, 127.2, 126.5, 124.9, 119.9 (=CH). Mass (ESI/MS) : m/z 310 (M+H).

*(Z)-4-(4-chloro–benzylidin)-2-phenyl oxazolin–5 (4H)-one **2g***: IR (KBr pellet, cm^{-1}): 3044, 3023, 2973, 2944, 1709, 1622, 1518, 1477 1389, 1213, 987, 861, 749, 653. ^{1}H NMR (DMSO–d_6) : δ = 5.44 (1H, dd, =CH-, J = Hz), 7. 55-8.13 cpx (9H, m, Phenyl). ^{13}C NMR ($CDCl_3$): δ = 174.2, 154.7, 146.1, 147.6, 135.8, 134.3, 133.8, 132.7, 131.3, 130.5,129.7 128.5, 127.4, 123.1, 116.3 (=CH). Mass (ESI/MS) : m/z 284 (M+H).

*(Z)-2-benzamido-3–phenyl acrylohydrazides **3a*** **:** IR (KBr pellet, cm^{-1}): 3342 and 3353 (coupled),3273, 3011, 2965, 2954, 1680, 1692, 1511, 1444 1321, 1201, 1123, 1012, 754. ^{1}H-NMR (DMSO–d_6) : δ = 6.11 (1H, s, NH_2), 5.92 (1H, dd, J = 10 Hz), 7. 15-8.18 cpx (11H, m, NH & Phenyl), 9.71(1H, s, NH). ^{13}C-NMR ($CDCl_3$): δ = 179.2 (C=O), 170.1(C=O), 143.4, 140.1, 139.8, 132.1, 132.7, 131.1, 130.9, 129.9 127.9, 127.1, 126.8, 120.1. Mass (ESI/MS) : m/z 299 (M+H_20), 281(M+H).

*(Z)-2-benzamido-3–(4-methoxy-phenyl acrylohydrazides **3b*** **:** IR (KBr pellet, cm^{-1}): 3344& 3351 (coupled),3271, 3012, 2968, 2952, 2943,1684, 1691, 1501, 1443 1322, 1204, 1123, 1012, 862, 754. ^{1}H-NMR (DMSO–d_6) : δ = 6.22 (1H, s, NH_2), 7.01 (1H, dd, J =11Hz), 7. 25-8.22 cpx (10H, m, NH & Phenyl), 9.79(1H, s, NH). ^{13}C-NMR ($CDCl_3$): δ = 178.3 (C=O), 170.4 (C=O), 139.5, 138.1, 137.9, 132.3, 132.9, 132.5, 130.7, 128.1 127.6, 126 126.8, 120.1, 34.5 (-OCH_3). Mass(ESI/MS) : m/z 312 (M+H).

*(Z)-2-benzamido-3–(4-dimethylamino-phenyl acrylohydrazides **3c*** **:** IR (KBr pellet, cm^{-1}): 3349 & 3355 (coupled), 3278, 3012, 2965, 2955, 2947,1683, 1690, 1511, 1442 1330, 1214, 1125, 1002, 856, 759. ^{1}H-NMR (DMSO–d_6) : δ = 3.8 (6H, s, $N(CH_3)_2$), 6.33 (1H, s, NH_2), 7.08 (1H, dd, J =10Hz), 7. 27-8.44 cpx (10H, m, NH & Phenyl), 9.90(1H, s, NH). ^{13}C-NMR($CDCl_3$): δ = 177.8(C=O), 170.3(C=O), 136.6, 134.2, 133.8, 132.3, 130.7, 129.1, 128.8, 126.2 125.7, 123.1, 122.7, 121.5, 29.4 and 29.2 (-$N(CH_3$). Mass (ESI/MS) : m/z 325(M+H).

*(Z)-2-benzamido-3–(4–hydroxy-3-methoxy-phenyl acrylohydrazides **3d*** **:** IR (KBr pellet, cm^{-1}): 3550, 3343 & 3354 (coupled),3272, 3011, 2969, 2954, 2938,1689, 1679, 1510, 1434 1321, 1211, 1124, 1011, 865, 762. ^{1}H-NMR (DMSO–d_6) : δ = 6.43 (1H, s, NH_2), 6.53 (1H, dd, J =11Hz), 7. 11-8.39 cpx (9H, m, NH & Phenyl), 9.41 (1H, s, NH), 11.2 (1H, s, OH). ^{13}C-NMR ($CDCl_3$): δ = 180.2 (C=O), 175.3 (C=O), 137.5, 135.3, 134.3 132.0, 131.3, 130.9, 129.9, 128.6 127.7, 126. 8, 124.5, 123.1, 31.6 (-OCH_3). Mass (ESI/MS) : m/z 312 (M+H).

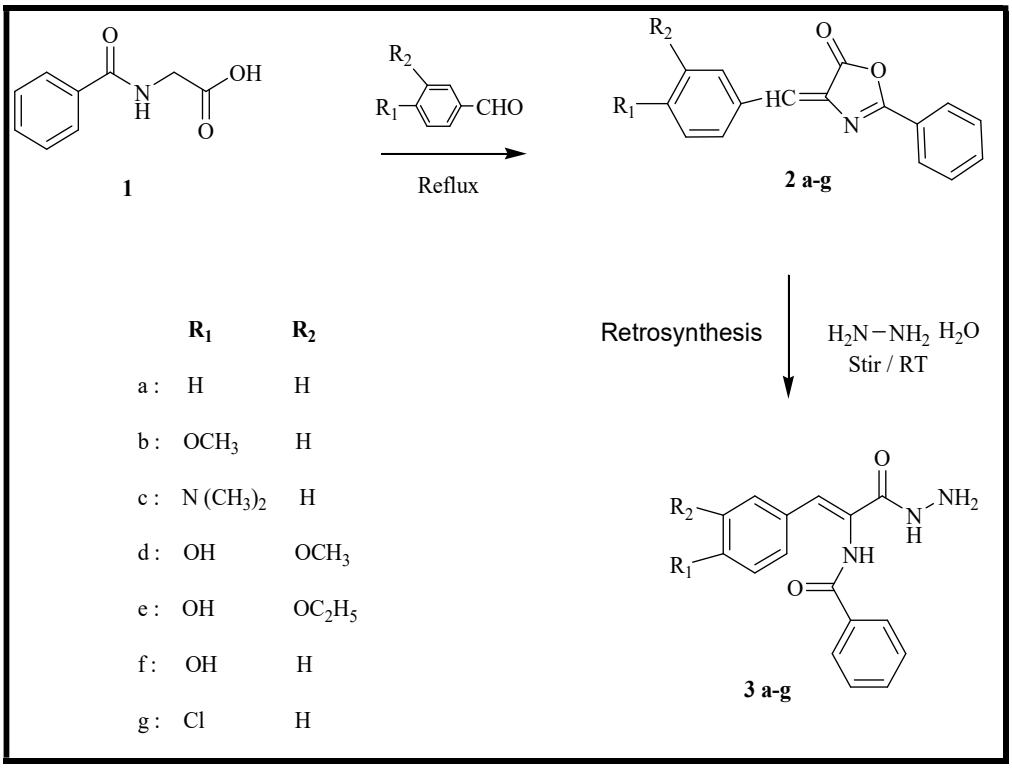
*(Z)-2-benzamido-3–(4–hydroxy-3-ethoxy-phenyl acrylohydrazides **3e*** **:** IR (KBr pellet, cm^{-1}): 3499, 3343& 3354 (coupled), 3272, 3011, 2969, 2954, 2938,1689, 1679, 1510, 1434 1321, 1211, 1124, 1011, 865, 762. ^{1}H-NMR (DMSO–d_6) : 2.33 (3H, t,-CH_3), 3.66 (2H, q,-CH_2), 6.43 (1H, s, NH_2), 6.73 (1H, dd, J =11Hz), 7. 11-8.39 cpx (9H, m, NH & Phenyl), 9.90 (1H, s, NH), 11.0 (1H, s, OH). ^{13}C-NMR ($CDCl_3$): δ = 181.3(C=O), 174.5(C=O), 134.7, 133.4, 132.9 132.1, 129.9, 128.7, 125.9, 125.1 124.8 123.7, 122.2, 121.8, 27.4 (-CH_2), 22.6 (-CH_3).Mass (ESI/MS) : m/z 342 (M+H).

*(Z)-2-benzamido-3–(4–hydroxy-phenyl acrylohydrazides **3f*** **:** IR (KBr pellet, cm^{-1}): 3488, 3333& 3344 (coupled), 3222, 3010, 2971, 2951 ,1680, 1672, 1505, 1444, 1311, 1201, 1104, 1001, 864, 759. ^{1}H-NMR ($CDCl_3$)) : 6.21 (1H, s, NH_2), 6.66 (1H, dd, J =11Hz), 7. 20-8.39 cpx (9H, m, NH & Phenyl), 9.62 (1H, s, NH), 10.9 (1H, s, OH). ^{13}C-NMR ($CDCl_3$): δ = 180.4 (C=O), 174.5(C=O), 135.8, 134.9, 134.2, 132.4, 133.7, 132.4, 130.3, 129.2 128.3 126.3, 126.2, 124.8. Mass (ESI/MS) : m/z 298 (M+H).

*(Z)-2-benzamido-3–(4–chloro-phenyl acrylohydrazides **3g*** **:** IR (KBr pellet, cm^{-1}): 3298& 3334 (coupled), 3210, 3009, 2987, 2963 ,1684, 1674, 1509, 1443, 1313, 1221, 1109, 1011, 908, 864, 763. ^{1}H-NMR ($CDCl_3$)) : 6.01 (1H, s, NH_2), 6.73 (1H, dd, J =11Hz), 7. 31-8.39 cpx (9H, m, NH & Phenyl), 9.90 (1H, s, NH). ^{13}C-NMR ($CDCl_3$): δ =181.6 (C=O), 173.8(C=O), 139.9, 138.8, 137.1, 136.5, 135.9, 134.3, 133.7, 133.0 131.9, 131.1, 132.8, 129.1. Mass (ESI/MS) : m/z 316 (M+H).

Antitubercular and antimicrobial activity

Compounds were screened for their *in vitro* antibacterial activity against *S.aureus* (Gram–positive bacteria), *E.coli, P.vulgaris, P.aeruginosa* (Gram–negative bacteria) and antifungal activity against *A. niger* and *C. albicans* by agar plate disc diffusion method[13] at a concentration of 50µg/disc. The antitubercular screening was carried out against *M. tuberculosis $H_{37}Rv$* by Alamar Blue Assay method[14]



Scheme 1. Synthesis of (Z)-4–(3,4-disubstituted benzylidine-2-phenyl oxazolin–5 (4H)-one 2 (a-g) and (Z)-2-benzamido-3-(3, 4-disubstituted phenyl) acrylohydrazides 3 (a-g).

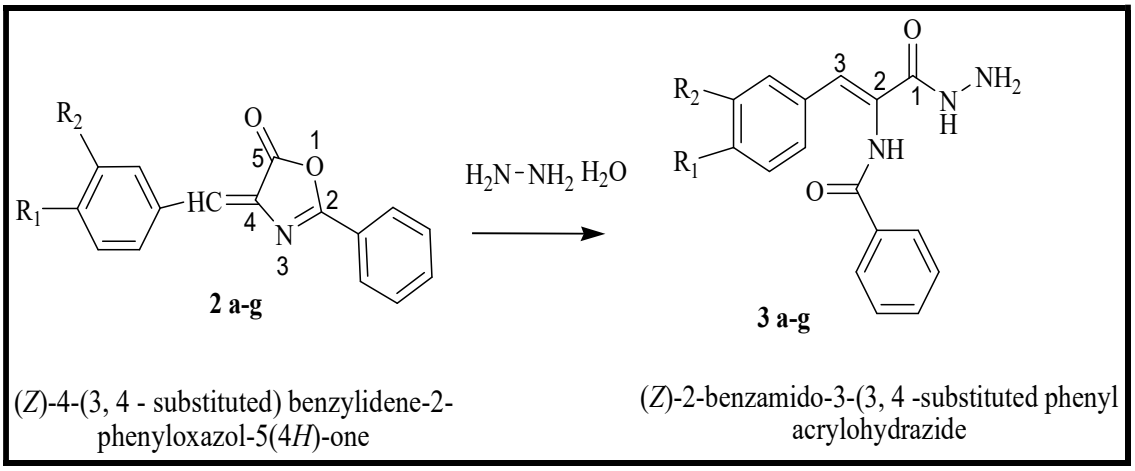


Fig: 1. Hydrazinolysis of oxazolinone

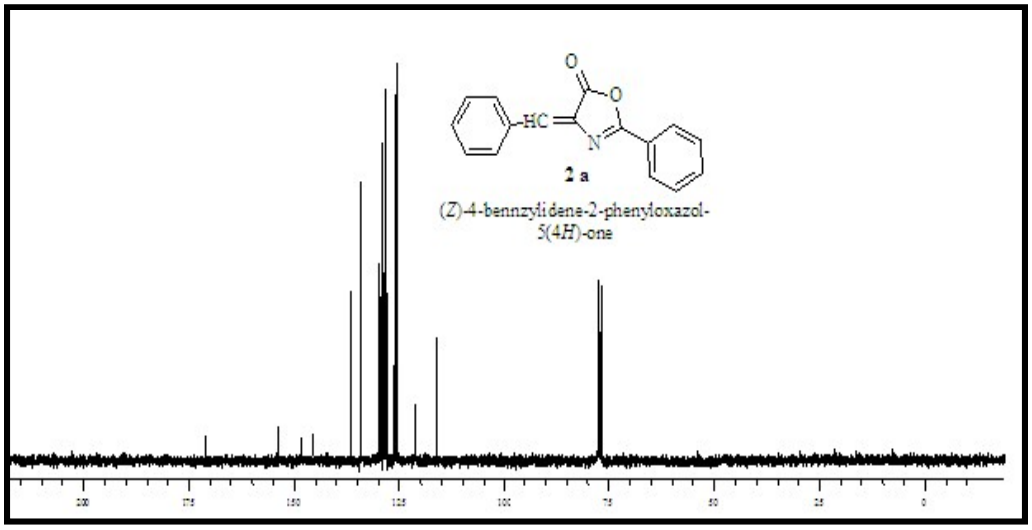


Fig: 2. ^{13}C-NMR Spectrum of Compound 2a

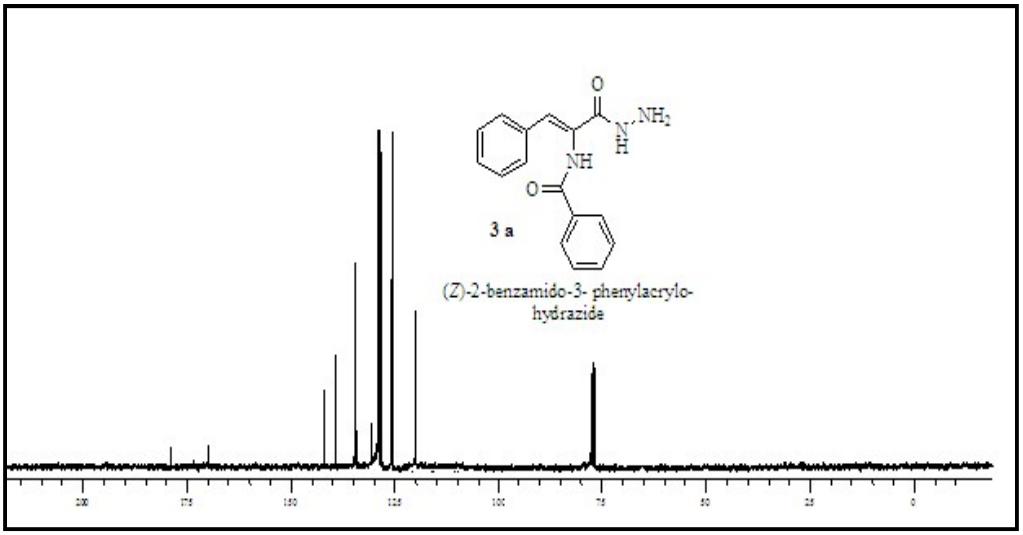


Fig: 3. ^{13}C-NMR Spectrum of Compound 3a

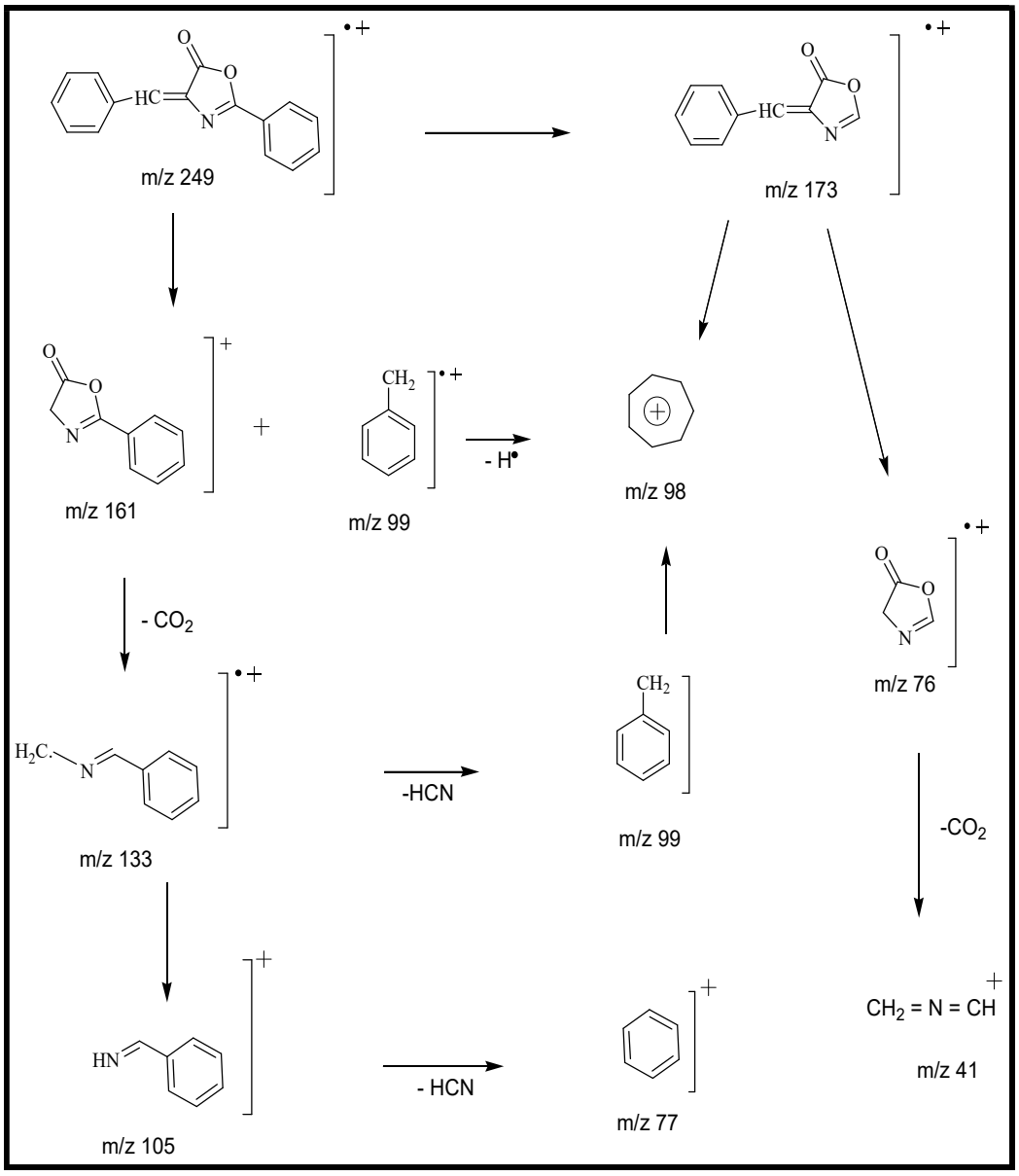


Fig: 4. Mass (ESI) Fragmentation of (Z)-4-benzylidine-2-phenyl oxazolin–5 (4H)-one (2a)

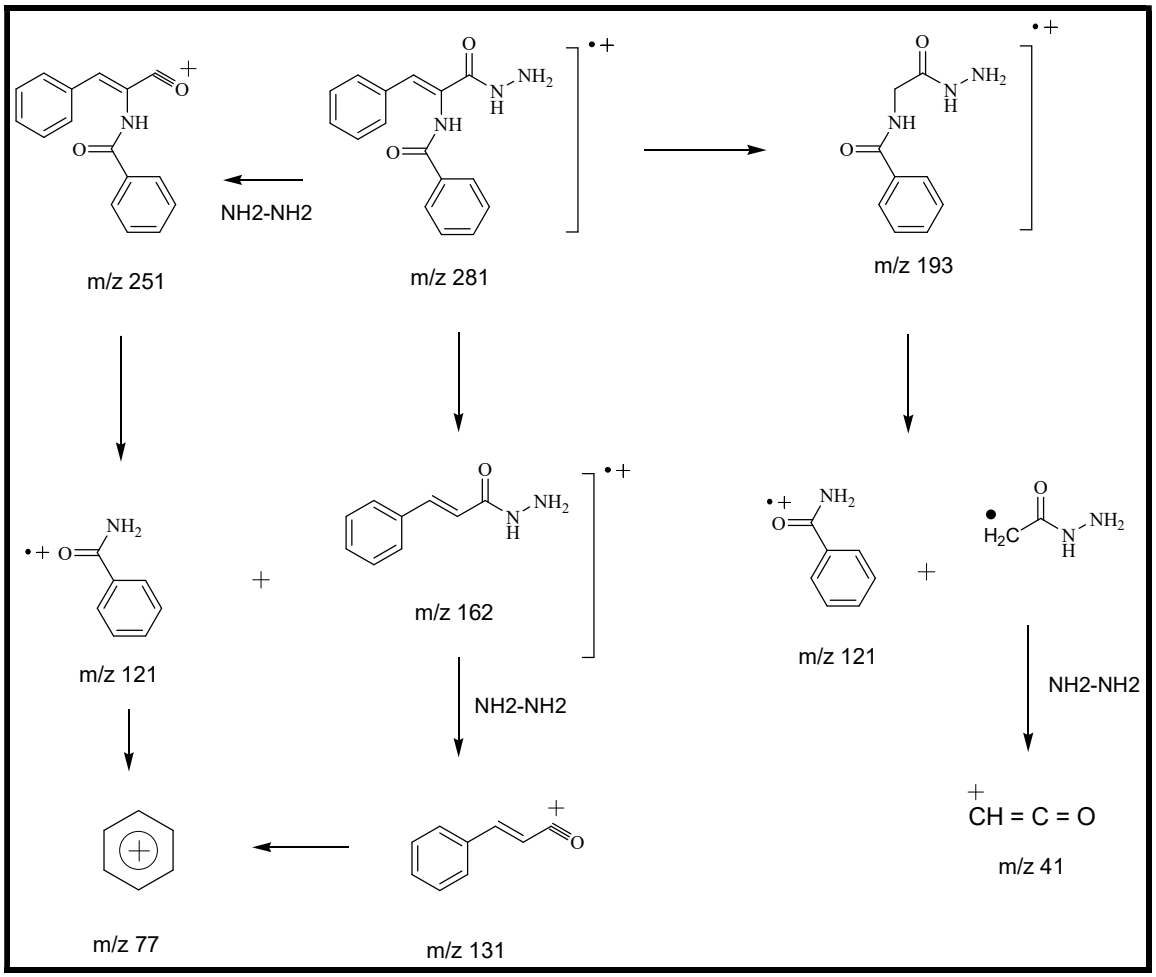


Fig: 5. Mass (ESI) Fragmentation of (Z)-2-benzamido-3–phenyl acrylohydrazides (3a)

Table 1. Physical data of the compounds (2a–g) and (3a–g)

Compound	Molecular Formula	Molecular Weight	Melting Point (°C)	% Yield	λ max (nm)	R_f[a] value	Log P[b]
2a	$C_{16}H_{11}NO_2$	249.271	293-94	63	447	0.588	3.37
2b	$C_{17}H_{13}NO_3$	279.297	233-34	47	457	0.504	3.25
2c	$C_{18}H_{16}N_2O_2$	292.339	200-01	56	494	0.421	3.66
2d	$C_{17}H_{13}NO_4$	295.296	>300	63	465	0.433	2.86
2e	$C_{18}H_{15}NO_4$	309.323	221-23	58	444	0.547	3.19
2f	$C_{16}H_{11}NO_3$	265.272	>300	43	463	0.443	2.98
2g	$C_{16}H_{10}ClNO_2$	283.715	220-21	49	412	0.442	3.91
3a	$C_{16}H_{15}N_3O_2$	281.316	279-80	61	367	0.732	1.32
3b	$C_{17}H_{17}N_3O_3$	311.342	222-23	43	356	0.757	1.19
3c	$C_{18}H_{20}N_4O_2$	324.384	99-100	54	385	0.679	1.61
3d	$C_{17}H_{17}N_3O_4$	327.342	>300	45	345	0.777	0.8
3e	$C_{18}H_{19}N_3O_4$	341.368	>300	56	341	0.665	1.14
3f	$C_{16}H_{15}N_3O_3$	297.315	>300	34	356	0.768	0.93
3g	$C_{16}H_{14}ClN_3O_2$	315.76	243-44	64	365	0.705	1.88

[a] Mobile phase: Ethylacetate: methanol : water (60:38:2).

[b]Log P values are calculated from Ultra chem. 8.0 by Crippens fragmentation method

Table 2. Antitubercular and antimicrobial activity of the synthesized compounds

Comp.	Antibacterial [a]				Antifungal [b]		*M. tuberculosis H37 Rv*
	S.aureus	*E.coli*	P.vulgaris	*P.aeruginosa*	*A. niger*	*C. albicans*	(MIC in µg/mL)
2a	21	22	21	25	22	25	10.12
2b	13	17	12	19	24	23	10.04
2c	23	26	27`	24	23	23	9.12
2d	21	23	14	15	14	15	8.33
2e	22	23	15	15	15	16	10.65
2f	20	24	26	25	13	10	7.58
2g	22	22	20	25	24	18	12.86
3a	16	16	18	18	16	10	3.32
3b	18	20	16	21	10	11	3.12
3c	14	15	11	18	21	13	2.54
3d	18	17	15	14	13	12	4.01
3e	19	18	16	13	11	10	3.39
3f	14	15	15	16	14	13	3.92
3g	20	23	22	20	09	08	2.78
Std[b]	24	25	28	23	26	28	0.02
SD	3.3321	3.6682	4.2426	4.3120	5.3580	5.5077	3.5973
CD	1.8112	1.9940	2.3062	2.3439	2.9126	2.9939	1.9554

[a] Values are the mean value of triplicate experiments reported as zone inhibition in millimeter at a test concentration of 50µg/disc. [b] Ciprofloxacin (10 µg/disc), Clotrimazole (10 µg/disc) and isoniazid were used as standard respectively for antibacterial, antifungal and antitubercular screening. DMSO was used as solvent blank and has shown no activity.
SD–Standard deviation; CD–critical difference at 5% level of significance.

RESULTS AND DISCUSSION

Chemistry

In the present communication, treatment of benzoyl glycine **1** with various aldehydes afforded 4–(3, 4–substituted) benzylidine-2-phenyl oxazolin–5 (4H)-one **2(a-g)** as intermediate which on hydrazinolysis with hydrazine hydrate yielded **3(a-g).** A total of 14 compounds were synthesized, purified and characterized by UV, FT-IR, ^{1}H-NMR, ^{13}C-NMR, Mass (ESI/MS) spectral data. Compounds were screened for *in vitro* antimicrobial activity against mycobacteria, bacteria and fungi.

Synthesis of the compounds was carried as outlined in scheme 1 and Figure 1. The starting compound benzoyl glycine **1** was prepared by treating benzoyl chloride and glycine in alkaline medium. The compound **1** was treated with substituted aldehydes in acidic condition, yielded 4–(4, 3-substituted) benzylidine-2-phenyl oxazol–5 (4H)-ones **2(a-g)** as intermediate. The cyclization reaction was confirmed by various spectral characteristics such as, appearance of IR absorption band at 1614 cm^{-1} (C=N), shift of IR absorption band for C=O (oxazolinone) to higher wave number around 1714 cm^{-1}, ^{13}C-NMR signal for benzylidine carbon (=CH) around 115 ppm and bathochromic shift of λ max in UV–spectrum. The deshielded doublet-doulet benzylidine ^{1}H-NMR proton (=CH) signal at around 6 ppm with J value of 14 Hz confirmed the 'Z' configuration. The intermediate **2(a-g)** under hydrazinolysis afforded 2-benzamido-3-(3, 4-substituted) phenylacrylohydrazide**s 3(a-g).** The product conversion was established by IR spectral evidence of coupled vibration of NH_2–stretching at 3324 and 3342 cm^{-1} and amide-CONH- at 1683 cm^{-1}. Further, structure was supported by presence of ^{13}C-NMR signal for C=O at 170 ppm. ^{13}C-NMR spectra for the representative compounds were shown in Figure 2 and 3. The molecular weight of the synthesized compounds was determined by ESI/MS in positive mode and compared with calculated value. Fragmentation patterns for prototype compounds were in good agreement with structures and shown in Figure 4 and 5. The yield of Synthesized compounds was in between 45-65% with the exception for **3f**. The appearance of intermediate compounds **2(a-g)** were yellow colour crystals where as acrylohydrazides **3(a-g)** appeared as light yellow to white crystalline substances. All other physical data of the compounds were summarized in table 1.

Antitubercular and antimicrobial activity

Result of *in vitro* antibacterial, antifungal and antitubercular screening against tested species were shown in table 2**.** It was noted that 4-(3, 4-substituted) benzylidine-2-phenyl oxazol-5 (4H)-one **2(a-g)** derivatives displayed better activity against bacteria and fungi than mycobacteria. The conversion of intermediate **2(a-g)** into acrylohydrazides **3(a-g)** resulted in improved antitubercular activity, but still showed moderate to mild antibacterial and antifungal action. The significant antitubercular activity of 2-benzamido-3-(3, 4-substituted phenyl) acrylohydrazide**s 3(a-g)** was observed with optimum lipophillicity value (log P) of 1-2 (calculated log P value shown in table 1). Neither increase nor decrease in log P value resulted in better activity. Compounds **3c** and **3g** have displayed as most

effective, with MIC values of 2.54 and 2.74µg/ml, respectively, and then followed by **3d** and **3e** with MIC values of 4.01 and 3.39 µg/ml against mycobacteria. Though compound **2d** and **2e** are structurally similar, the disparity in activity was noted against mycobacteria but not with bacteria and fungi. It was noted that substituted acrylohydrazides **3(a-g)** have demonstrated three times more potent antitubercular activity than intermediate oxazolinones **2(a-g).** 5% critical difference in statistical analysis (found 1.9554 against 1.731 for one tailed t' test) showed significant disparity in results of screening against compounds synthesized. Antitubercular activity of **3(a–g)** and **2 (a-g)** indicated the significance of basic core nucleus and their partition coefficient value in drug design. Compound **3c** and **3g** were predominantly potent than **3e** and **3d** and hereby stressing the importance of non polar functional substitution on phenyl system. Critical difference (CD) at 5% level of significance (95% confidence) showed the importance of substitution in phenyl group in antifungal activity (2.9956 against 1.731) than in antibacterial and antitubercular activity. In antibacterial activity, CD value was greater for Gram-negative bacteria than Gram-positive bacteria.

CONCLUSIONS

Structurally, compounds **2e, 2d, 3e, 3d,** possess common 4-hydroxy phenyl moiety and are structurally similar, it was admirable that they showed disparity in antitubercular activity with respect to the 3-alkoxy substitution in the same phenyl ring. It was concluded that substituted phenylacrylohydrazides exhibits considerable antitubercular activity and it may be related to partition coefficient of compounds. Further studies on these compounds with more chmical library, may be highly encouraged for finding newer antitubercular and antimicrobial agents.

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