In Vitro Leishmanicidal Activity of 3-substituted Isocoumarins: Synthesis and Structure activity Relationship

Khalid Mohammed Khan[a,*], Sumbul Ahmed[a], Zulfiqar Ali Khan[a], Zia-Ullah[a], Mubeen Rani[a], Muhammad Iqbal Choudhary[a] and Shahnaz Perveen[b]

[a]*H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi-75270, Pakistan;* [b]*PCSIR Laboratories Complex, Shahrah-e-Dr. Salimuz Zaman Siddiqui, Karachi-75280, Pakistan*

Abstract: Twenty-five 3-substituted isocoumarins were synthesized using cutting edge microwave-assisted technology in high yields. The syntheses of different isocoumarins were carried out in a single step by the direct condensation of homophthalic acid with aryol and acyl chlorides under the solvent-free conditions without any solid support. The structures of all the synthesized compounds were characterized using different spectroscopic techniques including UV, IR, ^{1}HNMR and EIMS and purity was confirmed by CHN analysis. All the synthesized compounds were tested for *in vitro* leishmanicidal activity. Compounds **3a**, **3b**, **3g**, **3l**, **3m**, **3r**, **3t**, **3w**, **3x**, and **3y** displayed potential *in vitro* leishmanicidal activity with IC_{50} values in the range of 0.56-84.38 µg/ml, whereas standard inhibitors amphotericine B have IC_{50} = 0.24 µg/ml. The compounds **3b**, **3g**, **3m**, **3t**, **3w**, **3x**, and **3y** having IC_{50} values 27.86, 28.88, 36.49, 34.37, 28.68, 0.89 and 0.56 µg/ml, respectively, were most active among the present series while remaining others were found less active. The compound **3x** and **3y** can act as potential lead molecules for further development of isocoumarin-based new drugs for the treatment of leishmaniasis.

Key Words: 3-Substituted isocoumarins, microwave-assisted synthesis, leishmanicidal activity.

1. INTRODUCTION

Protozoal diseases, such as leishmaniasis and trypanosomiasis are the cause of considerable mortality and morbidity throughout the world and affect millions of peoples every year. The leishmaniasis is classified on the basis of symptoms as cutaneous, visceral (Kala Azar), mucosal or mucocutaneous and diffused cutaneous forms [1,2].

Cutaneous leishmaniasis is a contagious skin disease [3, 4], caused by a parasitic protozoa (Kinetoplastida: Trypanosomatidae) which transmit as metacyclic flagellated promastigote forms from host to host by the bite of infected sand flies [5,6]. The bite of sand fly produces a hard boil on the skin which turns into a wound. The parasite of the leishmaniasis is found in rats, squirrels, mongooses, dogs and cats. When rodents dies, sand fly consumes the rodents blood which carrying the parasite of leishmaniasis and transmit it to human being. The sandfly appears from its habitation in summer, particularly in August, goes into dormant hibernation in the winter to lay eggs and breed only to reappear in February. These flies only appear after sunset from their holes and cracks of the muddy houses [7-12]. Moreover, this species has been isolated from patients with visceral disease or with post-Kala Azar dermal leishmaniasis [1,2].

The symptoms of different leishmaniases includes wound, fever, weight loss, anorexia, anxiety, change in hair color, abnormal growth and major dysfunctions of liver, spleen, bone marrow, lymph nodes, ulceration, nasal blockage, swelling of nose and lips with damage of soft tissues of oronasal cavity, dissemination of skin, thickening in plaques and multiplex nodules.

During Afghan war the refugees from Afghanistan introduced this skin disease in the north west frontier province of Pakistan. Some of the hilly areas of Larkana district *i.e.* Warah, Kamber and Shahdad Kot areas, bordering with Baluchistan and Kheirther mountains range became victim of the disease.

Unfortunately there is no effective and safe therapy for leishmanias is yet to be developed. In fact the clinically used drugs sufficient to combat this disease. Pentavalent antimonials, including sodium stibogluconate (Pentostam), urea stibamine, meglumine antimonate (Glucantime) are first line drugs while amphotericine B and pentamidine are used when primary treatment fails. All currently available drugs are associated with severe side effects, including chemical pancreatitis and cardiovascular toxicity. If used at high dosage, one has been shown to cause diabetes in more than 10% of cases. Second-line drugs trigger dangerous side effects, some of which can be lethal. Some treatments require injections while others need to be administered intravenously over a period of 15 to 30 days under close medical supervision [13]. The current medicinal therapies for leishmaniasis have known side effects, the high price of these drugs and long treatment courses make them practically ineffective. Since different forms of leishmaniasis developed resistance to many of these drugs, particularly HIV leishmania co-infected patients, therefore, research work for development of new and effective drugs with less side effects and low toxicity is an urgent task.

*Address correspondence to this author at the H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi-75270, Pakistan; Tel: +92-21-4824910; Fax: +92-21-4819018-19; E-mail: hassaan2@super.net.pk

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Isocoumarins and their structure variants exhibited diverse biological activities such as antibacterial, antifungal, antihypertensive antirheumatic and anticoagulant [14-16]. They also showed anticancer [17], antiulcer [18], antitumor [19], antiangiogenic activities [20], and used as immunomodulator, angiogenesis inhibitor [21], serine protease inhibitor, anticougulants, anti-inflammatory [22], and in vitro anti-HIV activity [23].

Recently we reported two new classes of compounds showing antileishmanial activities [24]. In an extension of our research work in search of medicinally important organic molecules, we initiated by keeping in mind the structure of amphotericin B which has a lactone ring in its skeleton. Isocoumarins have also a lactone ring which may act as pharmacophore and results of antileishmanial activity clearly proves our supposition.

2. RESULTS AND DISCUSSION

2.1. Chemistry

Twenty-five, 3-substituted isocoumarins were synthesized from aliphatic/aromatic acid chlorides (**2a-2y**) by using an improved microwave-assisted synthetic method [25]. The microwave-assisted synthesis of 3-substituted isocoumarins **3a-3y** has been achieved by direct condensation of acid chlorides **2a-2y** with homophthalic acid **1** (Scheme **1**).

This method appeared to be economical, solvent and solid support free with a wide range of applications. The advantage of this newly developed method over existing methods is that it completed in short time and produced high yields whereas classical method took 3-4 h to complete in average yields (Scheme **1**). The structures of all the synthesized compounds were determined by using different spectroscopic methods including UV, IR, ^{1}NMR and mass spectrometry. The purity of the compounds was confirmed by CHN analysis.

3. BIOLOGICAL ACTIVITY

Of the twenty-five 3-substituted isocoumarins tested for their in vitro anti-leishmanial activity, the compounds **3b**, **3g**, **3m**, **3t**, **3w**, **3x**, and **3y** exhibited anti-leishmanial activity with IC_{50} values of 27.86, 28.88, 36.49, 34.37, 28.68, 0.89 and 0.56 μg/ml, respectively, while remaining compounds **3a**, **3l** and **3r** displayed IC_{50} values of 52.70, 54.17 and 84.38 μg/ml, respectively, and were found to be less active. The compounds showing IC_{50} values greater than 100 μg/ml were considered to be in active. In this panel of twenty six compounds the 3-(4-fluorophenyl)isocoumarin (**3y**) was found to be most active against leishmaniasis with an IC_{50} values 0.56 μg/ml, however, 3-(3-fluorophenyl)isocoumarin (**3x**) possesses second highest degree (IC_{50} = 0.89 μg/ml) of antileishmanial activity.

Comparing the antileishmanial activities with structures of these compounds, it seems that the activities of 3-substituted isocoumarins are mainly dependent on C-3 substituent along with isocoumarin residue itself. Compound **3a** having an unsubstituted phenyl ring at C-3 of isocoumarin showed moderate activity (IC_{50} = 52.70 μg/ml) suggested that only phenyl group is not helpful in activity. The compounds having para substituted phenyl residue at C-3 of isocoumarin showed higher degree of activity which suggested that a suitably substituted phenyl residue at C-3 also takes part in activity. A para substitution enhance the activity where as ortho and meta substitutions decrease the activity, which is also obvious from the activity difference of compounds **3x** and **3y** (Table **1**). This trend of activity may be explained on the more linear geometry of para substituted phenyl ring. This linearity of molecule may allow the molecule for active diffusion into the cell membrane of parasite.

Compound **3g** having an undecanyl residue at C-3 found to be only active compound amongst the aliphatic C-3 substituent containing isocoumarin which suggested that the activity in this type of compounds mainly depend on the length of aliphatic chain at C-3 position (Table **1**).

Compounds **3c**-**3f**, **3h**-**3k**, **3n**-**3q**, **3s**, **3u** and **3v** were considered to be inactive (Table **1**). The complete loss of activity in these compounds is possibly due to the inappropriate C-3 residue on isocoumarin nucleus.

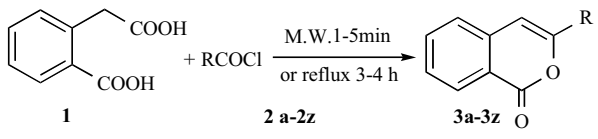
4. CONCLUSIONS

In the results of the present studies, twenty-five 3-substituted isocoumarins were screened and 3-(3-fluorophenyl) isocoumarin (**3x**) and 3-(4-fluorophenyl)isocoumarin (**3y**) were demonstrated excellent in vitro antileishmanial activities suggesting that suitably substituted phenyl residue at C-3 of isocoumarins along with isocoumarin moiety itself might be responsible for activity. Of this series, the most active compounds **3x** and **3y** may act as a potential lead molecule for the future research on 3-substituted isocoumarin-based antileishmanial agents.

5. EXPERIMENTAL SECTION

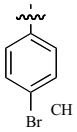
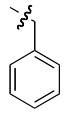
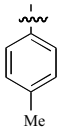
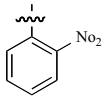
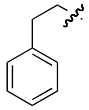
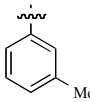
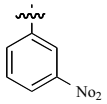
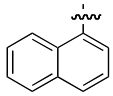
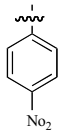
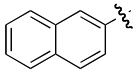
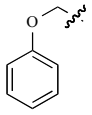
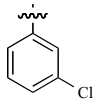
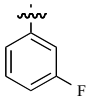
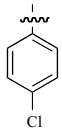
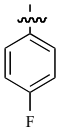
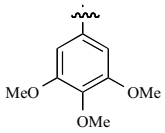
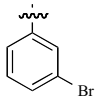
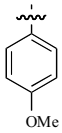
5.1. General Methods

The ultraviolet spectra were measured in chloroform on a Lambda 5 UV/Vis. spectrophotometer (Perkin-Elmer). IR spectra (KBr discs or MeOH) were recorded on a Bruker FT-IR IFS48 spectrophotometer. Melting points were determined on Büchi 535 melting point apparatus and are uncorrected. EI mass spectra data were recorded with various MAT 711 (70eV) spectrophotometers and data are tabulated as m/z. ^{1}H-NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 using Bruker AC (300 MHz and 400 MHz) spectrophotometer, respectively. Splitting patterns were as fol-



Scheme 1: Synthesis of 3-substituted isocoumarins.

Table 1. ***In Vitro*** **Antileishmanial Activity of 3-Substituted Isocoumarins 3a-3y**

Comp.	R	IC_{50} (mg/ml)	Comp.	R	IC_{50} (mg/ml)	Comp.	R	IC_{50} (mg/ml)
3a		52.70	**3j**		>100	**3s**		>100
3b		27.86	**3k**		>100	**3t**		34.37
3c		>100	**3l**		54.17	**3u**		>100
3d		>100	**3m**		36.49	**3v**		>100
3e		>100	**3n**		>100	**3w**		28.68
3f		>100	**3o**		>100	**3x**		0.89
3g		28.88	**3p**		>100	**3y**		0.56
3h		>100	**3q**		>100		Amphotericin B	0.24
3i		>100	**3r**		84.38			

lows: s (singlet), d (doublet), td (triplet of doublet), dd (double doublet), t (triplet), and m (multiplet). Chemical shifts are reported in δ (ppm) and coupling constants are given in hertz (Hz). The progress of all reactions was monitored by TLC, which was performed on 2.0 X 5.0 cm aluminum sheets pre-coated with silica gel $60F_{254}$ to a thickness of 0.25 mm (Merck). The chromatograms were visualized either under ultraviolet light (254-366 nm) or by iodine vapors. All the carboxylic acids are commercially available (Flulka, Aldrich).

5.2. General procedure for the syntheses of 3-substituted isocoumarins 3a-3y

Compound 3a

A mixture of homophthallic acid (1 g, 5.5 mmol) and acid chloride (22 mmol) was placed in a 25 mL conical flask cover with stopper. Microwave irradiation (MW domestic type oven 900 W with a frequency 2450 MHz, Dawlance, Pakistan) was applied for 90 seconds (three pulses each of 30 s). The completion of reaction was monitored by the TLC

analysis. The solvent system for TLC was hexane and ethyl acetate (7:3). The product was purified by column chromatography using hexane as eluent wherever necessary and crystallized by methanol. Representative physical and spectroscopic data of prepared compounds **3a-3y** is given below.

5.3. 3-Phenylisocoumarin (3a)

Colorless solid; Yield: 1.13 g (92%); M.p. = 80 ˚C; IR (KBr) ν_{max} 3025, 1734, 1614, 1550, 1461 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.30 (dd, 1H, *J* = 8.1, 1.4 Hz, H-8), 8.10 (dd, 1H, *J* = 8.1, 1.4 Hz, H-5), 7.84 (td, 1H, *J* = 8.1 Hz, 1.7 Hz, H-7), 7.63 (td, 1H, *J* = 8.1, 1.7 Hz, H-6), 7.36-7.52 (m, 5H, Ar-*H*′), 6.94 (s, 1H, H-4); UV (MeOH) λ_{max} (log ε): 228 (3.34) nm; EI-MS *m/z* (rel. abund. %): 222 (M^+, 100), 194 (75), 193 (5), 105 (68), 89 (48), 77 (89), 76 (5); Anal. Calcd. for $C_{15}H_{10}O_2$ (222.23): C, 81.07; H, 4.54; Found: C, 81.11; H, 4.51.

5.4. 3-(4-Methylphenyl)isocoumarin (3b)

Brown solid; Yield: 1.25 g (96%); M.p. = 125-128 ˚C; IR (KBr): ν_{max} 1735, 1608, 1475, 3074, 2973 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.15 (dd, 1H, *J* = 8.4, 1.5 Hz, H-8), 7.89 (d, 2H, *J* = 8.0 Hz, H-2′, H-6′), 7.76 (dd, 1H, *J* = 8.4, 1.5 Hz, H-5), 7.5 (td, 1H, *J* = 8.4, 1.8 Hz, H-7), 7.39 (td,1H, *J* = 8.4, 1.8 Hz, H-6), 7.25 (d, 2H, *J* = 8.0 Hz, H-3′, H-5′), 6.89 (s, 1H, H-4), 2.41 (s, 3H, -CH_3); UV (MeOH) λ_{max} (log ε); 228 (4.31) nm; EI-MS *m/z* (rel. abund. %): 236 (M^+, 100), 119 (89), 91 (37), 89 (16%); Anal. Calcd. for $C_{16}H_{12}O_2$ (236.26): C, 81.34; H, 5.12; Found: C, 81.37; H, 5.15.

5.5. 3-(3-Methylphenyl)isocoumarin (3c)

Brown solid; Yield: 1.27 g (97%); M.p. = 94 ˚C; IR (K-Br): ν_{max} 1724, 2925, 1486, 1529 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.13 (dd, 1H, *J* = 7.8, 1.4 Hz, H-8), 7.90-7.84 (m, 2H, H-5, H-6′), 7.52 (td, 1H, *J* = 7.8, 1.8 Hz, H-7), 7.46 (td, 1H, *J* = 7.8, 1.8 Hz, H-6), 7.40 (d, 1H, *J* = 7.8 Hz, H-5′), 7.36 (s, 1H, H-2′), 7.26 (d, 1H, *J* = 7.8 Hz, H-4′), 6.93 (s, 1H, H-4), 2.39 (s, 3H, CH_3); UV (MeOH) λ_{max} (log ε): 230 (4.39) nm; EI-MS *m/z* (rel. abund. %): 236 (M^+, 82), 208 (35), 119 (100), 118 (9), 91 (29); Anal. Calcd. for $C_{16}H_{12}O_2$ (236.26) : C, 81.34; H, 5.12; Found: C, 81.29; H, 5.10.

5.6. 3-(2-Methylphenyl)isocoumarin (3d)

Reddish brown solid, Yield: 1.28 g (98%); M.p = 165-66 ˚C; IR (KBr): ν_{max} 1728, 1464, 2930 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.48 (dd, 1H, *J* = 8.0, 1.4 Hz, H-8), 8.01 (dd, 1H, *J* = 8.0, 1.4 Hz, H-5), 7.91 (td, 1H, *J* = 8.0, 2.1 Hz, H-7), 7.69 (td, 1H, *J* = 8.0 , 2.1 Hz, H-6), 7.61 (d, 1H, *J* = 7.8 Hz, H-6′), 7.42-7.36 (m, 2H, H-4′,5′), 7.26 (d, 1H, *J* = 7.8 Hz, H-3′), 6.59 (s, 1H, H-4), 2.62 (s, 3H, CH_3); UV (MeOH) λ_{max} (log ε): 234 (4.48) nm; EI-MS *m/z* (rel. abund. %): 236 (M^+, 63), 208 (23), 119 (45), 118 (100), 91 (25), 76 (2). Anal. Calcd. for $C_{16}H_{12}O_2$ (236.26): C, 81.34; H, 5.12; Found: C, 81.30; H, 5.14.

5.7. 3-Nonanylisocoumarin (3e)

Yellow oil; Yield: 0.93 g (62%); IR (KBr): ν_{max} 1743, 2926, 2855,1460, 3010 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.11 (dd, 1H, *J* = 7.8, 1.2 Hz, H-8), 7.50 (td, 1H, *J* = 7.8, 1.8 Hz, H-7), 7.37 (td, 1H, *J* = 7.8, 1.8 Hz, H-6), 7.25 (dd, 1H, *J* = 7.8, 1.2 Hz, H-5), 6.84 (s, 1H, H-4), 2.27 (t, 2H, *J* = 7.4, $CH_2(CH_2)_7CH_3$), 1.25 (m, 14H, $CH_2(CH_2)_7CH_3$), 0.85 (t, 3H, *J* = 7.0 Hz, $CH_2(CH_2)_7CH_3$); UV (MeOH) λ_{max} (log ε): 229 (4.47) nm; EI-MS *m/z* (rel. abund. %): 272 (M^+, 4), 243 (8), 229 (5), 215 (4), 145 (10), 133 (100), 89 (20), 76 (18); Anal. Calcd. for $C_{18}H_{24}O_2$ (272.38): C, 79.37; H, 8.88; Found: C, 79.33; H, 8.84.

5.8. 3-Octylisocoumarin (3f)

Yellow oil; Yield: 0.86 g (60%); IR (KBr): ν_{max} 1723, 2954, 2925, 1605, 1437 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.11 (dd, 1H, *J* = 7.7, 1.2 Hz, H-8), 7.51 (td, 1H, *J* = 7.7, 2.1 Hz, H-7), 7.38 (td, 1H, *J* = 7.7, 2.1 Hz, H-6), 7.27 (dd, 1H, *J* = 7.7, 1.2 Hz, H-5), 6.86 (s, 1H, H-4), 2.33 (t, 2H, *J* = 7.5 Hz, $CH_2(CH_2)_6CH_3$), 1.26 (m, 12H, $CH_2(CH_2)_6CH_3$), 0.85 (t, 3H, *J* = 6.8 Hz, $CH_2(CH_2)_6CH_3$); UV (MeOH) λ_{max} log ε): 230 (3.60) nm; EI-MS *m/z* (rel. abund. %): 258 (M^+, 2), 145 (4), 141 (4), 117 (2), 118 (100), 76 (4). Anal. Calcd. for $C_{17}H_{22}O_2$ (258.35): C, 79.03; H, 8.58 Found: C, 79.07; H, 8.62.

5.9. 3-Undecylisocoumarin (3g)

Yellow oil; Yield: 1.33 g (80%); IR (KBr): ν_{max}1738, 2917, 3010, 1465 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.24 (dd, 1H, *J* = 7.7, 1.3 Hz, H-8), 7.63 (td,1H, *J* = 7.7, 2.2 Hz, H-7), 7.44 (td, 1H, *J* = 7.7, 2.2 Hz, H-6), 7.32 (dd, 1H, *J* = 7.7, 1.3 Hz, H-5), 6.22 (s, 1H, H-4), 2.39 (t, 2H, *J* = 7.1 Hz, H-1′, $CH_2(CH_2)_9CH_3$), 1.23-1.27 (m, 18H, $CH_2(CH_2)_9CH_3$), 0.85 (t, 3H, *J* = 6.5 Hz, $CH_2(CH_2)_9CH_3$); UV (MeOH) λ_{max} (log ε): 227 (3.76) nm; EI-MS *m/z* (rel. abund. %): EIMS *m/z* (%): 300 (M^+, 30), 257 (2), 243 (4), 145 (8), 118 (100), 117 (10), 76 (24); Anal. Calcd. for $C_{20}H_{28}O_2$ (300.43): C, 79.96; H, 9. 39, found: C, 79.92; H, 9.44.

5.10. 3-(2-Bromophenyl)isocoumarin (3h)

Brown solid; Yield: 1.58 g (95%); M.p. = 205 ˚C; IR (KBr): ν_{max} 3027, 1732, 1608, 1566, 1488, 1464 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): 8.19 (dd, 2H, *J* = 7.8, 1.4 Hz, H-8), 8.1 (dd, 1H, *J* = 7.8, 1.4 Hz, H-5), 7.93 (d, 2H, *J* = 7.6 Hz, H-5′,6′) 7.84 (td, 1H, *J* = 7.8, 2.2 Hz, H-7), 7.53 (td, 1H, *J* = 7.8, 2.2 Hz, H-6), 7.43 (d, 1H, *J* = 7.6 Hz, H-3′), 6.93 (s, 1H, H-4); UV (MeOH) λ_{max} (log ε): 232 (4.68), 264 (4.57) nm; EI-MS *m/z* (rel. abund. %): 302 (M + 2, 20), 300 (M^+, 22), 274 (24), 272 (27), 185 (100), 183 (96), 157 (36), 155 (39), 76 (34). Anal. Calcd. for $C_{15}H_9O_2Br$ (301.13): C, 59.83; H, 3.01; Found: C, 59.79; H, 3.05.

5.11. 3-(3-Bromophenyl)isocoumarin (3i)

Brown solid; Yield; 1.50 g (90%); M.p = 149 ˚C; IR (K-Br): ν_{max} 1747, 1608, 1566 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): 8.33 (dd, 1H, *J* = 7.9, 1.3Hz, H-8), 8.21 (dd, 1H, *J* = 7.8, 1.4 Hz, H-5), 8.04 (s, 1H, H-2′), 7.80 (td, 1H, *J* = 7.9, 1.9 Hz, H-7), 7.74 (td, 1H, *J* = 7.8, 1.9 Hz, H-6), 7.54-7.46 (m, 2H, H-6′,4′), 7.33 (t, 1H, *J* = 7.3 Hz, H-5′), 6.94 (s, 1H, H-4); UV (MeOH) λ_{max} (log ε): 226 (4.51), 310 (4.08) nm; EI-MS *m/z* (rel. abund. %): 302 (M + 2, 96), 300 (M^+, 100), 274 (34), 272 (37), 185 (52), 183 (56), 157 (41), 155 (44), 76 (31); Anal. Calcd. for $C_{15}H_9O_2Br$ (301.13): C, 59.83; H, 3.01; Found: C, 59.87; H, 3.04.

5.12. 3-(4-Bromophenyl)isocoumarin (3j)

Brown solid; Yield: 1.60 g (96%); M.p. = 135 ˚C; IR (KBr): ν $_{max}$ 3031, 1732, 1608, 1566, 3074 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.30 (dd, 1H, *J* = 7.8, 1.4 Hz, H-8), 7.96 (dd, 1H, *J* = 7.8, 1.4 Hz, H-5), 7.83 (d, 2H, *J* = 7.4 Hz, H-2′,6′), 7.58 (td, 1H, *J* = 7.8, 2.1 Hz, H-7), 7.43 (td, 1H, *J* = 7.8, 2.1 Hz, H-6), 7.28 (d, 2H, *J* = 7.4 Hz, H-3′,5′), 6.93 (s, 1H, H-4); UV (MeOH) λ_{max} (log ε): 239 (4.55) nm; EI-MS *m/z* (rel. abund. %): 302 (M +2, 94), 300 (M^+,100), 274 (41), 272 (44), 185 (14), 183 (17), 157 (56), 155 (59), 76 (29); Anal. Calcd. for $C_{15}H_9O_2Br$ (301.13): C, 59.83; H, 3.01; Found: C, 59.80; H,3.08.

5.13. 3-(2-Nitrophenyl)isocoumarin (3k)

Yellow solid; Yield: 0.96 g (65%); M.p. = 180 ˚C; IR (KBr): ν $_{max}$ 3033, 1724, 1521, 1440 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.43 (d, 1H, *J* = 7.7 Hz, H-3′), 8.29 (t, 1H, *J* = 7.7 Hz, H-5′), 8.19 (dd, 1H, *J* = 7.8, 1.4 Hz, H-8), 7.97 (d, 1H, *J* = 7.8, 1.4 Hz, H-5), 7.84 (d, 1H, *J* = 7.7 Hz, H-6′), 7.63 (td, 1H, *J* = 7.8, 2.2 Hz, H-7), 7.43 (td, 1H, *J* = 7.8, 2.2 Hz, H-6), 7.76 (d, 1H, *J* = 7.7 Hz, H-4′), 6.93 (s, 1H, H-4); UV (MeOH) λ_{max} (log ε): 206 (4.59), 261 (3.60) nm; EI-MS *m/z* (rel. abund. %): 267 (M^+, 100), 239 (19), 223 (16), 150 (25), 145 (29), 118 (9), 76 (8); Anal. Calcd. for $C_{15}H_9NO_4$ (267.23): C, 67.42; H, 3.39; N, 5.24; Found: C, 67.44; H, 3.41, N, 5.20.

5.14. 3-(3-Nitrophenyl)isocoumarin (3l)

Yellow solid; Yield: 1.43 g (97%); M.p. = 75 ˚C; IR (K-Br): ν $_{max}$ 3029, 1725, 1608, 1525, 1436, 1343 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.95 (s, 1H, H-2′), 8.55 (d, 1H, *J* = 7.8 Hz, H-4′), 8.47 (d, 1H, *J* = 7.8 Hz, H-6′), 8.33 (dd, 1H, *J* = 7.6, 1.2 Hz, H-8), 7.93 (dd, 1H, *J* = 7.6, 1.2 Hz, H-5), 7.55 (d, 1H, *J* = 7.8 Hz, H-5′), 7.65 (td, 1H, *J* = 7.6, 2.5 Hz, H-7), 7.45 (td, 1H, *J* = 7.6, 2.5 Hz, H-6), 7.09 (s, 1H, H-4); UV (MeOH) λ_{max} (log ε): 205 (4.55), 261 (3.74) nm; EI-MS *m/z* (rel. abund. %): 267 (M^+, 100), 239 (22), 223 (11), 150 (21), 145 (5), 118 (16), 76 (17). Anal. Calcd. for $C_{15}H_9NO_4$ (267.23): C, 67.42; H, 3.39; N, 5.24; Found: C, 67.40; H, 3.43; N, 5.27.

5.15. 3-(4-Nitrophenyl)isocoumarin (3m)

Yellow solid; Yield: 1.30 g (88%); M.p. = 82 ˚C; IR (K-Br): ν $_{max}$ 1721, 1604, 1525, 1439, 1347 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): 8.48 (d, 1H, *J* = 7.8 Hz, H-3′,5′), 8.41 (d, 2H, *J* = 7.8 Hz, H-2′,6′), 8.14 (dd, 1H, *J* = 7.6, 1.3 Hz, H-8), 8.01 (dd, 1H, *J* = 7.6, 1.3 Hz, H-5), 7.91 (td, 1H, *J* = 7.6, 1.8 Hz, H-7), 7.69 (td, 1H, *J* = 7.6, 1.8 Hz, H-6), 6.85 (s, 1H, H-4); UV (MeOH) λ_{max} (log ε): 203 (4.67), 258 (3.67) nm; EI-MS *m/z* (rel. abund. %): 267 (M^+, 22), 239 (10), 223 (9), 150 (100), 145 (19), 118 (48), 76 (27); Anal. Calcd. for $C_{15}H_9NO_4$ (267.23): C, 67.42; H, 3.39; N, 5.24; Found: C, 67.45; H, 3.37; N, 5.22.

5.16. 3-(2-Chlorophenyl)isocoumarin (3n)

Yellow solid; Yield: 1.32 g (93%); M.p. = 103-104 ˚C; IR (KBr): ν $_{max}$ 3027, 1725, 1606, 1460 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.13 (dd, 1H, *J* = 7.7, 1.3 Hz, H-8), 7.98 (dd, 1H, *J* = 7.7, 1.3 Hz, H-5), 7.79 (d, 1H, *J* = 7.6 Hz, H-6′), 7.52 (td, 1H, *J* = 7.7, 1.8 Hz, H-7), 7.58 (t, 1H, *J* = 7.6 Hz, H-5′), 7.45 (td,1H, *J* = 7.7, 1.8 Hz, H-6) 7.34 (dd, 1H, *J* = 7.6, 1.7 Hz, H-4′), 7.26 (d, 1H, *J* = 7.6 Hz, H-3′), 6.98 (s, 1H, H-4); UV (MeOH) λ_{max} (log ε): 221 (3.64) nm; EI-MS *m/z* (rel. abund. %): 258 (M + 2, 16), 256 (M^+, 50), 230 (15), 228 (46), 139 (100), 141 (35), 118 (46), 113 (11), 111 (31), 76 (18); Anal. Calcd. for $C_{15}H_9ClO_2$ (256.68): C, 70.19; H, 3.53; Found: C, 70.22; H, 3.50.

5.17. 3 (3-Chlorophenyl)isocoumarin (3o)

Yellow solid; Yield: 1.22 g (86%); M.p. = 118 ˚C; IR (KBr): ν $_{max}$ 3035, 1728, 1469, 1338 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.11 (dd, 1H, *J* = 7.8, 1.3 Hz, H-8), 8.05 (s, 1H, H-2′), 7.95 (dd, 1H, *J* = 7.8, 1.3 Hz, H-5), 7.57 (d, 1H, *J* = 7.9 Hz, H-6′), 7.51 (td, 1H, *J* = 7.8, 2.1 Hz, H-7), 7.39 (td, 1H, *J* = 7.8, 2.1 Hz, H-6), 7.34 (d, 1H, *J* = 7.9 Hz, H-4′), 7.28 (t, 1H, *J* = 7.9 Hz, H-5′), 6.90 (s, 1H, H-4); UV (Me-OH) λ_{max} (log ε): 227 (3.54) nm; EI-MS *m/z* (rel. abund. %): 258 (M + 2, 21), 256 (M^+, 64), 230 (14), 228 (43), 141 (28), 139 (86), 118 (100), 113 (17), 111 (39), 76 (4); Anal. Calcd. for $C_{15}H_9ClO_2$ (256.68): C, 70.19; H, 3.53; Found: C, 70.24; H, 3.49.

5.18. 3-(4-Chlorophenyl)isocoumarin (3p)

Yellow solid; Yield: 0.81 g (57%); M.p. = 165 ˚C; IR (KBr): ν $_{max}$ 3020, 1731, 1509, 1461 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.34 (dd, 1H, *J* = 7.8, 1.4 Hz, H-8), 8.14 (dd, 1H, *J* = 7.8, 1.4 Hz, H-5), 7.83 (d, 2H, *J* = 7.9 Hz, H-2′,6′), 7.51 (td, 1H, *J* = 7.8, 2.2 Hz, H-7), 7.38 (1H, td, *J* = 7.8, 2.2 Hz, H-6), 7.27 (d, 2H, *J* = 7.9 Hz, H-3′,5′), 6.88 (s, 1H, H-4); UV (MeOH) λ_{max} (log ε): 224 (3.76) nm; EI-MS *m/z* (rel. abund. %): 258 (M + 2, 16), 256 (M^+, 49), 228 (34), 230 (11), 141 (13), 139 (36), 118 (100), 113 (19), 111 (55), 76 (4); Anal. Calcd. for $C_{15}H_9ClO_2$ (256.68): C, 70.19; H, 3.53; Found: C, 70.26; H, 3.46.

5.19. 3-(3,4,5-Trimethoxyphenyl)isocoumarin (3q)

Brown solid; Yield: 1.68 g (97%); M.p. = 147 ˚C; IR (KBr): ν $_{max}$ 3074, 2973, 1737, 1608, 1475, 1264 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.1 (dd, 1H, *J* = 7.8, 1.2 Hz, H-8), 7.52 (td, 1H, *J* = 7.8, 2.2 Hz, H-7), 7.42 (td, 1H, *J* = 7.8, 2.2 Hz, H-6), 7.34 (s, 2H, H-2′,6′), 7.27 (dd, 1H, *J* = 7.8, 1.2 Hz, H-5), 6.27 (s, 1H, H-4), 3.90 (s, 9H, OCH_3); UV (Me-OH) λ_{max} (log ε): 226 (3.45) nm; EI-MS *m/z* (rel. abund. %): 312 (M^+, 12), 195 (22), 145 (5), 118 (100), 76 (16); Anal. Calcd. for $C_{18}H_{16}O_5$ (312.31): C, 69.22; H, 5.16 Found: C, 69.24; H, 5.14.

5.20. 3-(4-Methoxyphenyl)isocoumarin (3r)

Brown solid; Yield: 1.34 g (96%); M.p. = 136 ˚C; IR (KBr): ν $_{max}$ 3069, 2978, 1741, 1608, 1468, 1273 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.34 (dd, 1H, *J* = 7.8, 1.4 Hz, H-8), 8.1 (dd, 1H, *J* = 7.8, 1.5 Hz, H-5), 7.97 (d, 2H, *J* = 7.9 Hz, H-2′,6′), 7.51 (td, 1H, *J* = 7.8, 2.2 Hz, H-7), 7.38 (td, 1H, *J* = 7.8, 2.2 Hz, H-6), 7.26 (dd, 1H, *J* = 7.8 Hz, H-5), 6.91 (d, 2H, *J* = 7.9 Hz, H-3′,5′), 6.86 (s, 1H, H-4), 3.86 (s, 3H, O-CH_3); UV (MeOH) λ_{max} (log ε): 217 (4.48) nm; EI-MS *m/z* (rel. abund. %): 252 (M^+, 100), 224 (8), 145 (2), 135 (34), 118 (19), 76 (6); Anal. Calcd. for $C_{16}H_{12}O_3$ (252.26): C, 76.18; H, 4.79; Found: C, 76.15; H, 4.82.

5.21. 3-Benzylisocoumarin (3s)

Yellow semisold; Yield : 1.10 g (84%); IR (KBr): ν_{max} 1718, 1640, 1608, 1468, 1273 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.37 (dd, 1H, *J* = 8.0, 1.2 Hz, H-8), 8.22 (dd, 1H, *J* = 8.0, 1.2 Hz, H-5), 7.98 (td, 1H, *J* = 7.9, 1.8 Hz, H-7), 7.64 (td, 1H, *J* = 8.0, 1.8 Hz, H-6), 7.21-7.45 (m, 5H, Ar-*H*′), 3.83 (s, 2H, H-1′); UV (MeOH) λ_{max} (log ε): 228 (4.19), 276 (3.27) nm; EI-MS: *m/z* (rel. abund. %) : 236 (M^+), 208 (60), 117 (2), 105 (20); Anal. Calcd. for $C_{16}H_{12}O_2$ (236.26) : C, 81.34; H, 5.12 ; Found : C, 81.39 ; H, 5.10.

5.22. 3-(Ethylphenyl)isocoumarin (3t)

Brown solid; Yield : 1.11 g (80%) ; M.p. = 116 ˚C ; IR (KBr): ν_{max} 1737, 2919, 3011, 1464 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.37 (dd, 1H, *J* = 8.0, 1.2 Hz, H-8), 8.22 (dd, 1H, *J* = 8.0, 1.2 Hz, H-5), 7.98 (td, 1H, *J* = 7.9, 1.9 Hz, H-7), 7.64 (td, 1H, *J* = 8.0, 1.9 Hz, H-6), 7.21-7.45(m, 5H, Ar-*H*′), 2.94 (t, 2H, *J* = 6.5 Hz, H-1′, CH_2), 2.66 (t, 2H, *J* = 6.5 Hz, H-2′, CH_2); UV (MeOH) λ_{max} (log ε): 226 (4.11), 276 (3.36) nm; EI-MS *m/z* (rel. abund. %) : 250 (M^+, 2), 206 (12) , 117 (3), 104 (43), 91 (100) ; Anal. Calcd. for $C_{17}H_{14}O_2$ (250.29): C, 81.58; H, 5.64; Found : C, 81.61 ; H, 5.62.

5.23. 3-(1-Naphthyl)isocoumarin (3u)

Yellow solid; Yield: 0.99 g (66 %); M.p. = 115-118 ˚C; IR (KBr): ν_{max} 3032, 1720, 1616, 1512, 1487 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.12 (d, 1H, *J* = 8.6 Hz, H-8′), 9.04 (d, 1H, *J* = 8.6 Hz, H-2′), 8.42 (d,1H, *J* = 8.6 Hz, H-4′), 8.34 (dd, 1H, *J* = 7.8, 1.4 Hz, H-8), 8.14 (dd, 1H, *J* = 7.8, 1.4 Hz, H-5), 8.06 (td, 1H, *J* = 7.8, 2.1 Hz, H-7), 7.93 (d, 1H, *J* = 8.6 Hz, H-5′), 7.61 (t, 1H, *J* = 8.6 Hz, H-7′), 7.59-7.52 (m, 3H, H-3′,6,6′), 7.19 (s, 1H, H-4); UV (MeOH) λ_{max} (log ε): 284 (3.73), 216 (4.32) nm; EI-MS *m/z* (rel. abund. %): 272 (M^+, 29), 145 (9), 127 (100), 118 (4), 101 (8), 89 (7); Anal. Calcd. for $C_{19}H_{12}O_2$ (272.29): C, 83.81; H, 4.44 ; Found: C, 83.88 ; H, 4.48.

5.24. 3-(2-Naphthyl)isocoumarin (3v)

Yellow solid; Yield: 1.11 g (74%); M.p. = 123 ˚C; IR (KBr): ν_{max} 1709, 1520, 1487 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.69 (s, 1H, H-1′), 8.08 (dd, 1H, *J* = 8.1, 1.3 Hz, H-8), 7.97 (dd, 1H, *J* = 8.1, 1.3 Hz, H-5), 7.89 (m, 3H, H-4′,5′,8′), 7.64 (t, 1H, *J* = 7.8 Hz, 1.8 Hz, H-7′), 7.52-7.59 (m, 3H, H-3′, H-7, H-6′), 7.43 (td, 1H, *J* = 8.1, 2.2 Hz, H-6); 7.2 (s, 1H, H-4); UV (MeOH) λ_{max} (log ε): 288 (3.62), 214 (4.42) nm; EI-MS *m/z* (rel. abund. %): 272 (M^+, 10), 145 (2), 127 (100), 118 (6), 101 (11), 89 (19); Anal. Calcd. for $C_{19}H_{12}O_2$ (272.29): C, 83.81; H, 4.44; Found: C, 83.84; H, 4.41.

5.25. 3-(Phenoxymethyl)isocoumarin (3w)

Yellow solid; Yield: 0.93 g (67%); M.p. = 111 ˚C; IR (KBr): ν_{max} 1738, 1630, 1596, 1194 cm^{-1}; ^{1}H-NMR: (300 MHz, $CDCl_3$): 8.28 (d, 1H, *J* = 8.1 Hz, H-5), 8.17 (dd, 1H, *J* = 7.9, 1.4 Hz, H-5), 7.72 (dd, 1H, *J* = 7.9, 1.4 Hz, H-7), 7.55-7.40 (5H, m, Ar-*H*′), 7.31 (1H, t, *J* = 7.7 Hz, H-6), 6.96 (s, 1H, H-4), 4.35(2H, s, OCH_2); (MeOH) λ_{max} (log ε): 307 (2.51), 269 (3.63) nm; EI-MS *m/z* (rel. abund. %): 252 (M^+, 18), 208 (25), 118 (100), 104 (49), 76 (29); Anal. Calcd. For $C_{16}H_{12}O_3$ (252.26): C, 76.18; H, 4.79; Found: C, 76.24, H, 4.73.

5.26. 3-(3-Fluorophenyl)isocoumarin (3x)

Yellow semisolid; Yield: 0.86 g (65%); IR (KBr): ν_{max} 1724, 1615, 1594, 1228 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): 8.34 (dd, 1H, *J* = 7.9, 1.4 Hz, H-8), 8.15 (dd, 1H, *J* = 7.9, 1.4 Hz, H-5), 8.08 (t, 1H, *J* = 7.9 Hz, H-2′), 8.01 (d, 1H, *J* = 8.8 Hz, H-6′), 7.82 (t, 1H, *J* = 8.4 Hz, H-4′), 7.52 (td, 1H, *J* = 7.8, 1.8 Hz, H-7), 7.36 (td, 1H, *J* = 7.8, 1.8 Hz, H-6), 7.26 (t, *J* = 8.8 Hz, H-5′); 6.94 (s, 1H, H-4); (MeOH) λ_{max} nm (log ε): 227 (3.98); 309 (3.16); EI-MS *m/z* (rel. abund. %): 240 (M^+, 100), 196 (4), 123 (27), 118 (2), 104 (31), 76 (59); Anal. Calcd. for $C_{15}H_9FO_2$ (240.22): Found C, 75.00; H 3.78; Found C, 75.12; H 3.82.

5.27. 3-(4-Fluorophenyl)isocoumarin (3y)

Yellow semi solid; Yield: 1.01 g (80 %); IR (KBr): ν_{max} 1723, 1612, 1590, 1268 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.34-8.28 (m, 2H, H-3′,5′), 8.21 (dd, 1H, *J* = 7.9, 1.4 Hz, H-8), 8.14 (dd, 1H, *J* = 7.8, 1.4 Hz, H-5), 7.81 (td, 1H, *J* = 7.8, 2.2 Hz, H-7), 7.65 (td, 1H, *J* = 7.8, 2.2 Hz, H-6), 7.20-7.14 (m, 2H, H-2′,6′); 6.9 (s, 1H, H-4); (MeOH) λ_{max} nm (log ε): λ_{max} (log ε): 225 (3.98); 307 (3.16) nm; EI-MS *m/z* (% abund.): 240 (M^+, 40), 196 (38), 123 (6), 118 (100), 104 (31), 76 (18) ; Anal. Calcd. for $C_{15}H_9FO_2$ (240.22): C, 75.00; H, 3.78; Found: C 74.95; H 3.85.

IN VITRO LEISHMANICIDAL ACTIVITY

Leishmania major (MHOM/PK/88/DESTO) promastigotes, cultivated in bulk were aseptically be sedimented down at 300 rpm, counted with the help of Neubaver chamber under microscope and diluted with the fresh medium to a final concentration of $2x10^6$ parasites/mL. The compounds to be checked were dissolved to a final concentration of 1.0 mg in 0.1 mL of PBS (Phosphate Buffered Saline, pH 7.4 containing 0.5% MeOH, 0.5% DMSO).

In a 96-well microtiter plate, 90 µL of the parasite culture ($2x10^6$ parasites/mL) was added in different wells. Experimental compound (10 µL) was added in to culture and serially diluted so that minimum concentration of the compound is 0.1 µg/mL. 10 µL of PBS (Phosphate buffered saline), pH 7.4 (containing 0.5% MeOH, 0.5% DMSO) was added as negative control while glucantime, amphotericin B, pentamidine, and ampicilline to a final concentration of 1.0 mg/mL was added separately as positive control. The plate was incubated between 21-22 ˚C in dark for five days during which control organisms multiply six times. The culture was examined microscopically on an improved Neubaver chamber and IC_{50} values of compounds possessing antileishmanial activity were calculated [26]. All assays were run in triplicate.

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