Trop J Nat Prod Res, August 2018; 2(8):383-387 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Synthesis and Antimicrobial Evaluation of N,N'-(4-Nitro-1,2-phenylene)diamide Derivatives

Beatrice N. Iwuala[1*], James D. Habila[1], Mohammed S. Sallau[1]

Department of Chemistry, Ahmadu Bello University Zaria, Nigeria.

ARTICLE INFO

Article history:
Received 01 July 2018
Revised 18 July 2018
Accepted 23 July 2018
Published online 10 August 2018

ABSTRACT

In the search for new antibiotics, 4-nitro-1,2-phenylenediamide analogues were synthesized via nucleophilic addition/elimination reaction of carboxylic acid derivatives with 4-nitro-1,2-phenylenediamine. *In vitro* antimicrobial assay of the analogues was done using the conventional broth dilution method on seven selected clinical isolates. The results of the zones of inhibition showed that N,N'-(4-nitro-1,2-phenylene)diacetamide (**3a**) has zones of inhibition ranging from 08 mm to 12 mm while (N,N'-(4-nitro-1,2-phenylene)dibenzamide (**3c)** has zones of inhibition ranging from 12 mm to 21 mm. N,N'-(4-nitro-1,2-phenylene)bis(2,2,2-trifluoroacetamide) (**3d)** was the most active against all tested organisms with zones of inhibition ranging from 11 mm in *Pseudomonas aeruginosa* to 34 mm in Methicillin-resistant *Staphylococcus aureus* (MRSA). The minimum inhibition concentration (MIC) determination reveals that **3d** inhibits the growth of tested microbes at a concentration of 12.5 mg/mL with the exception of *Klebsiella pneumonia* in which the MIC was 25 mg/mL. The Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) results revealed that **3d** has the highest bactericidal effect on *Pseudomonas aeruginosa*, MRSA and *Escherichia coli* at a concentration of 12.5 mg/mL and was bacteriostatic/fungistatic against the rest of the organisms. The results observed clearly shows that molecules with electron withdrawing groups demonstrated better antibacterial activity.

Keywords: 4-nitro-1,2-phenylenediamine, carboxylic acid derivatives, antimicrobial activity and 4-nitro-1,2-phenylenediamide.

Introduction

Drug resistance has become a problem recently because the pace of novel antibiotics discovery has drastically reduced and opportunistic pathogens which gives rise to the case of invasive microbial infections has long been on the rise for over two decades now. The overuse of antibodies is increasing, not just because of bacterial resistance to antibiotic, but microbes have the potential to cause ineffectiveness of existing antibiotics.[1] Opportunistic microbial infection are most commonly seen in patients who are severely immuno-compromised due to underlying disease like leukemia, recently AIDS or patients who undergo cancer chemotherapy or organ transplantation.[2] Clinically, microbial resistances occurs in almost all of the major classes of antibiotics that is bacterial resistance of macrolide, quinolone, *β*-lactam and vancomycin has become a major worldwide health problem.[3] The existing available antimicrobial drugs used in the treatment of microbial infections has shown some drawbacks such as toxicity, resistance which may be due to prolonged period of administration, recurrence of the disease/infection due to the fact that most of these drugs are bacteriostatic/fungistatic and less bacteriocidal/fungicidal.[4] The amide functionality is the backbone of several organic compound and natural products with diverse biological and pharmacological features.[5, 6] Amides also play a role for medicinal chemists as amide derivatives are associated with broad spectrum of biological activities including anti-tuberculosis,[7] anticonvulsant,[8] analgesic-anti-inflammatory,[9] insecticidal,[10] antifungal,[11] and antitumor properties.[12] There is a need for discovery of new compounds which can be used clinically and has antibacterial and antifungal activities in which their mechanism of action are distinct from existing antimicrobial agents and do not show resistance compared to the existing antimicrobial agents.[13]

Materials and Methods

General experimental procedures

All chemicals were of analytical grade and purchased from Sigma-Aldrich (Germany) through Bristol Scientific Company and commercial suppliers. Analytical thin layer chromatography (TLC) was performed using aluminum plates coated with silica gel $60F_{254}$ (Merck). Spots were visualized using UV lamp or by saturation with Iodine vapour. NMR spectra were recorded using 400 MHz spectrometer at room temperature in DMSO-*d*6. The infrared spectra of the solid samples were recorded on Agilent FTIR 400s Fourier Transform Infra-Red Spectrophotometer. Melting point determination was carried out on Stuart Automatic Melting Point /SMP40/ apparatus.

Synthesis of N,N'-(4-Nitro-1,2-phenylene)diacetamide (3a)

An intimate mixture of Acetic anhydride (0.04 mol), 4-Nitro-1,2-phenylenediamine (0.02 mol) and 20 mL of toluene was stirred at room temperature for 20 minutes. The reaction was monitor with TLC until completion, the solvent was evaporated using rotary evaporator and the separated solid was filtered, washed with toluene and dried. The crude products were recrystallized from ethanol for further purification (Scheme 1).

*Corresponding author. E mail: iwualabeatrice@gmail.com
Tel: +2348153613465

Citation: Iwuala BN, Habila JD, Sallau MS. Synthesis and Antimicrobial Evaluation of N,N'-(4-Nitro-1,2-phenylene)diamide Derivatives. Trop J Nat Prod Res. 2018; 2(8):383-387. doi.org/10.26538/tjnpr/v2i8.3

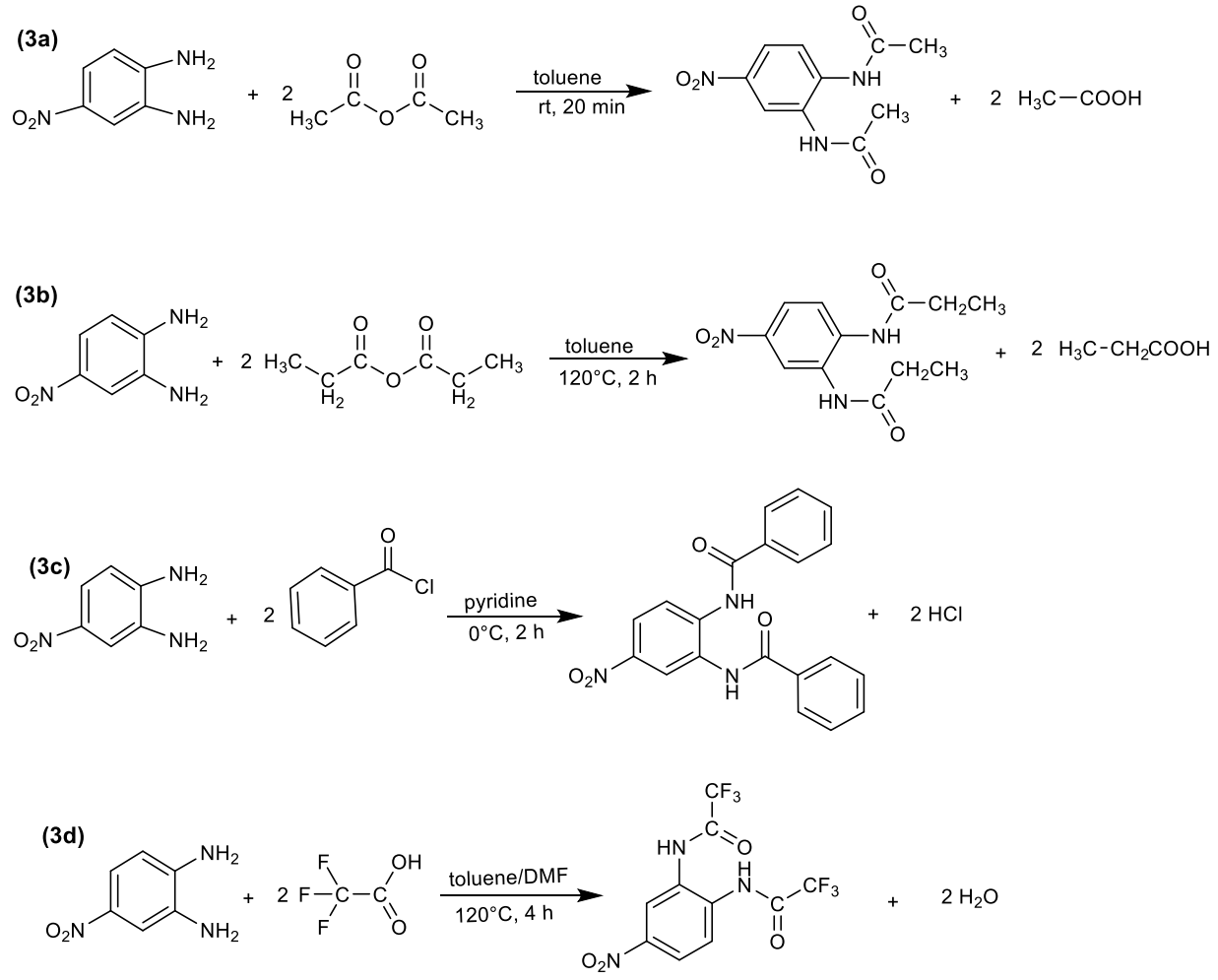
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Synthesis of N,N'-(4-Nitro-1,2-phenylene)dipropionamide (3b)
A mixture of propionic anhydride (0.04 mol), 4-Nitro-1,2-phenylenediamine (0.02 mol) and 20 mL of toluene was heated for 2 hours in an oil bath under reflux. The reaction was monitor with TLC until completion, at the end of this period, the heat was removed, and the reaction pot was further stirred for 30 minutes and cooled to room temperature, the solvent was evaporated using rotary evaporator. The separated solid was filtered, washed with toluene and dried under suction. The crude products were recrystallized using ethanol as the solvent, to obtain 5.199 g of milk colored solid (98% yeild). The equation of the reaction is shown in scheme 1.

Synthesis of N,N'-(4-Nitro-1,2-phenylene)dibenzamide (3c)
Benzoyl chloride (0.04 mol) was added to 4-Nitro-1,2-phenylenediamine (0.02 mol) in a round bottom flask. The flask was put into an ice bath to reduce the temperature of the mixture to 0°C. Pyridine (9.67 mL) was added to the mixture and the reaction was stirred for 20 minutes and filtered under suction. The reaction was monitor with TLC until completion. Further purification was done using column chromatography (hexane:ethylacetate 9:1), to obtain a greenish yellow solid (6 g, 83% yield). The equation of the reaction is shown in scheme 1.

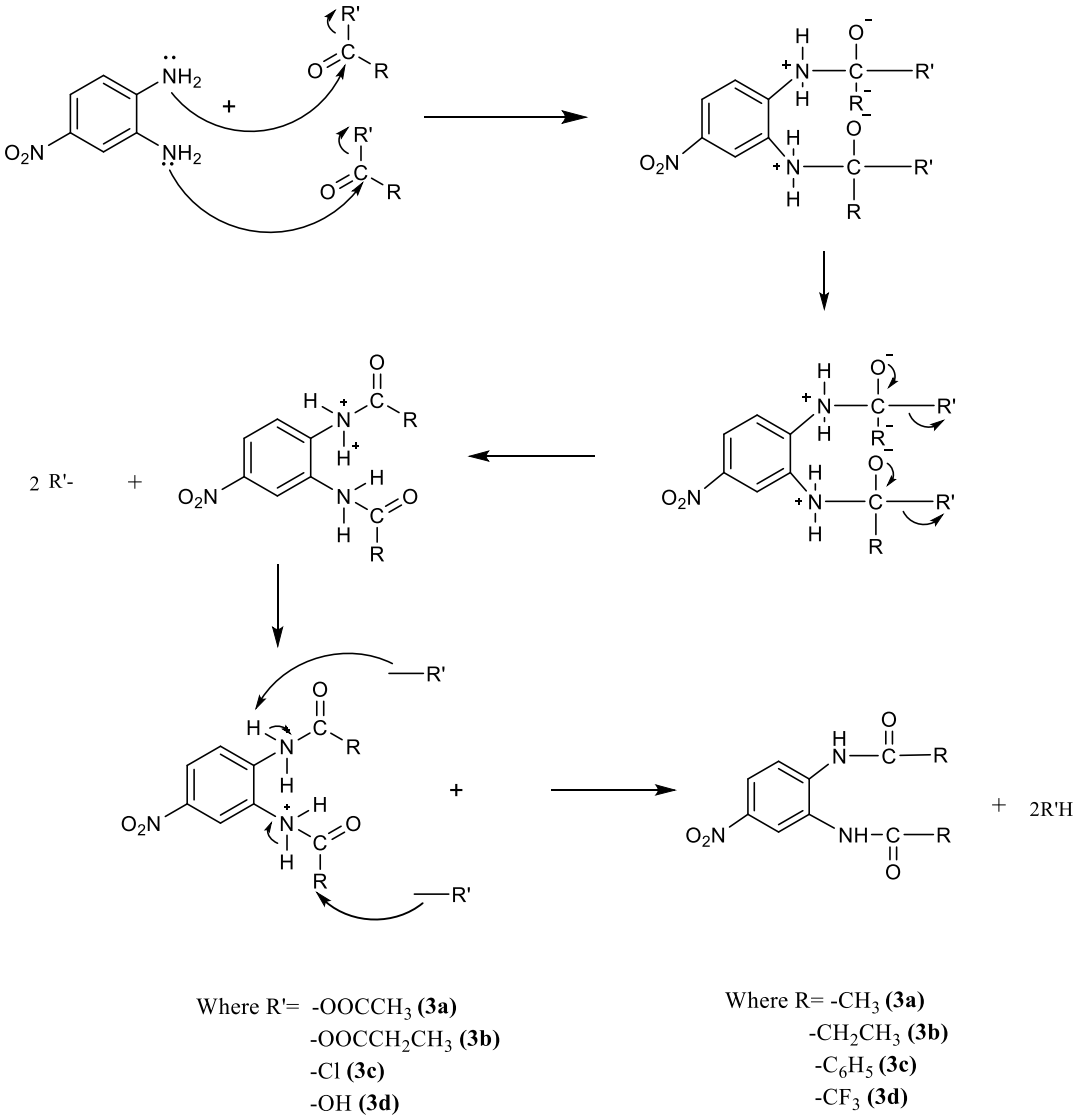
Synthesis of N,N'-(4-Nitro-1,2-phenylene)bis(2,2,2-trifluoroacetamide) (3d)
Trifluoroethanoic acid (0.04 mol) was added to 4-Nitro-1,2-phenylenediamine (0.02 mol) in a round bottom flask. Toluene (20 mL) was added to the mixture in the round bottom flask and a drop of DMF was also added to the mixture. Then, the reaction was refluxed for 4 hours. The reaction was monitor with TLC until completion using ethylacetate:hexane 4:6, the reaction was allowed to cool to room temperature and the mixture was poured into a cooled water. The precipitate was filtered under suction. Further purification was done using column chromatography (hexane:ethylacetate 8:2). The weight of the product was 5.53 g (80% yield). The equation of the reaction is shown in scheme 1.

Biological Assay
Clinical Isolates. The test compounds (3a, 3b, 3c and 3d) were tested on the following isolates, obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria, Nigeria (ABUTH): *Escherichia coli, Pseudomonas aeruginosa*, S*almonella typhi, Klebsiella pneumonia,* Methicillin Resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* and *Candida albicans*.



Scheme 1: Synthesis of 4-Nitro-1,2-phenylenediamide Derivative 3a, 3b, 3c and 3d.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)



Scheme 2: Mechanisms of the reactions.

Antimicrobial Susceptibility Test

The cork bore and diffusion method as reported by Karou *et al*.[14] was used to determine the antimicrobial activity of the test compounds. Pure cultures of the organism were inoculated on to Mueller Hinton Agar (MERCK) and incubated for 24 h at 38°C for bacteria and 48 h at 34ºC for fungi. About 5 discrete colonies were aseptically transferred using sterile wire loops into tubes containing sterile normal saline (0.9% NaCl) and were adjusted to a McFarland Standard turbidity of 0.5. The suspensions were then inoculated on the surface of sterile Mueller-Hinton Agar plates using sterile cotton swabs. A sterile 6 mm diameter Cork borer was used to make holes (wells) into the set of inoculated Mueller-Hinton Agar. The wells were filled with different concentration (100 and 200 mg/mL) of the test compounds. The plates were then incubated and the diameter of inhibition zone (mm) produced by the test compounds were taken after the incubation period. The minimum inhibitory concentration (MIC) was also investigated using the concentration (12.5, 25, 50, 100 and 200 mg/mL) of the synthesized compound and test tubes which showed no visible growth were recorded as the MIC.[15]

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The MBC/MFC was determined by aseptically inoculating aliquots of culture, from the minimum inhibition concentration (MIC) tubes that showed no growth, on sterile nutrient plates incubated at 37°C for 24 h and 34°C for 48 h for bacteria and fungi respectively. After which the plates were observed for colony growth, the MBC/MFC was recorded as the lowest concentration of compounds showing no bacterial/fungal growth.[16]

Results and Discussion

The compounds were synthesized adopting the chemical pathways outlined in Scheme 1. The reactions involved the formation of amides derivatives by nucleophilic addition and elimination reaction of 4-nitro-1,2-phenylenediamine with carboxylic acid derivatives. The reaction took place in toluene under reflux, where the amine attacks the carbonyl carbon with the elimination of $–OOCCH_3$, $–OOCCH_2CH_3$, HCl and H_2O (3a, 3b, 3c and 3d, respectively) as shown in the mechanisms of the reaction (Scheme 2).[17]

*N,N'-(4-nitro-1,2-phenylene)diacetamide (**3a**)***:*** was obtained as a pale yellow solid powder (94% yield). Its melting point was determined to be 189.6 - 191.9°C. The infra-red (IR ν_{max}, cm^{-1}) signal at 3261.4 indicates N-H stretch, signal at 3078.8 corresponds to C-H of Aromatic benzene, 1662.4 indicates a carbonyl carbon (C=O stretch of amide), 1587.8 - 1505.8 is for C=C of substituted benzene ring. The NMR spectral data showed that ^{13}C-NMR (100 MHz, DMSO-*d*6) : 170.11 (C-1', 1''), 143.55 (C-4), 137.50 (C-1), 130.44 (C-2), 124.37 (C-6), 120.58 (C-5), 114.41(C-3), 24.69 (C-2',2'') and ^{1}H-NMR (400 MHz, DMSO-*d*6) δ_H: 8.44 (s, 1H, H-3), 7.96 (d, 1H, H-6), 7.80 (d, 1H, *J* = 7.52 Hz, H-5), 7.18 (s, 2H, N-H), 2.10 (s, 6H, H-2', 2'').

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

*N,N'-(4-nitro-1,2-phenylene)dipropionamide (**3b**)***:*** was obtained as a milky solid powder (98% yield). Its melting point was determined to be 231.2 - 233.5°C. The infra-red (IR ν_{max}, cm^{-1}) showed signal at 3257.7 N-H stretch, signal at 3078.8 corresponds to C-H of Aromatic benzene, signal at 2981.9 corresponds to C-H of aliphatic, 1654.9 indicates a carbonyl carbon (C=O stretch of amide), 1587.8 - 1546.8 is for C=C of substituted benzene ring. The NMR spectral data showed that ^{13}C-NMR (100 MHz, DMSO-*d*6): 173.21 (C-1', 1''), 143.10 (C-4), 137.17 (C-1), 130.09 (C-2), 123.99 (C-6), 120.22 (C-5), 120.10 (C-3), 29.84 (*C*-2', 2''), 9.70 (C-3', 3'') and ^{1}H-NMR (400 MHz, DMSO-*d*6) δ_H: 9.55 (s, 1H, H-3), 8.46 (d, 1H, *J* = 10.16 H_Z, H-6), 7.97 (d,1H, *J* = 9.56 H_Z, H-5), 4.06 (s, 2H, N-H), 2.39 (q, 4H, *J* = 7.44, 7.84, 7.76 H_Z, H-1''), 1.04 (t, 6H, *J* = 14.04, 3.64 H_Z, H-2'').

*N,N'-(4-Nitro-1,2-phenylene)dibenzamide (**3c**)***:*** was obtained as a greenish yellow solid powder (83% yield). Its melting point was determined to be 200 - 202.2°C. The infra-red (IR ν_{max}, cm^{-1}) signal at 3384.4-3302.4 corresponds to N-H stretch, signal 3071.3 corresponds to C-H of Aromatic benzene, signal 1654.9 indicates a carbonyl carbon (C=O stretch of amide), signals 1580.4 - 1509.6 indicates C=C of substituted benzene ring. The NMR spectral data showed that ^{13}C-NMR (100 MHz, DMSO-*d*6): 166.48 (C-1',1''), 151.21 (C-1), 135.67 (C-4), 134.64 (C-2', 2''), 132.09 (C-5', 5''), 131.50 (C-2), 128.69 (C-4', 4''), 128.69 (C-6', 6''), 128.43 (C-7',7''), 128.43 (C-3', 3''), 124.10 (C-6), 121.65 (C-5), 114.24 (C-3) and ^{1}H-NMR (400 MHz, DMSO-*d*6) δ_H: 9.73 (s, 2H, N-H), 8.57 (s, 1H, H-3), 8.13 (d, 1H, H-6), 8.09 (dd, 4H, H-3', H-3'' H-7'and H-7''), 7.89 (d, 1H, *J* = 2.28 H_Z, H-5), 7.57 (t, 2H, *J* = 4.92, 6.08 H_Z, H-5', H-5''), 7.51 (dd, 4H, *J* = 5.24 H_Z, H-6', H-6'' H-4' and H-4'').

*N,N'-(4-Nitro-1,2-phenylene)bis(2,2,2-trifluoroacetamide) (**3d**)*: was obtained as a grey solid powder at 80 % yield. Its melting point was determined to be 105.6 – 107.6 °C. The infra-red (IR ν_{max}, cm^{-1}) signal at 3582.0 indicates N-H stretch, signal 3108.6 corresponds to C-H of Aromatic benzene, signal 1785.4 indicates a carbonyl carbon (C=O stretch of amide), 1520-1483.5 indicates C=C of substituted benzene ring. The NMR spectral data showed that ^{13}C-NMR (100 MHz, DMSO-*d*6): 171.87 (C-1', 1''), 144.34 (C-4), 141.07 (C-1), 138.71 (C-2), 119.87 (C-6), 117.55 (C-2', 2''), 116.39 (C-5), 114.87(C-3) and ^{1}H-NMR (400 MHz, DMSO-*d*6) δ_H: 8.67 (s, 2H, N-H), 8.48 (s, 1H, H-3), 8.14 (d, 1H, *J* = 7.28 H_Z, H-6), 7.79 (d, 1H, *J* = 6.8 H_Z, H-5).

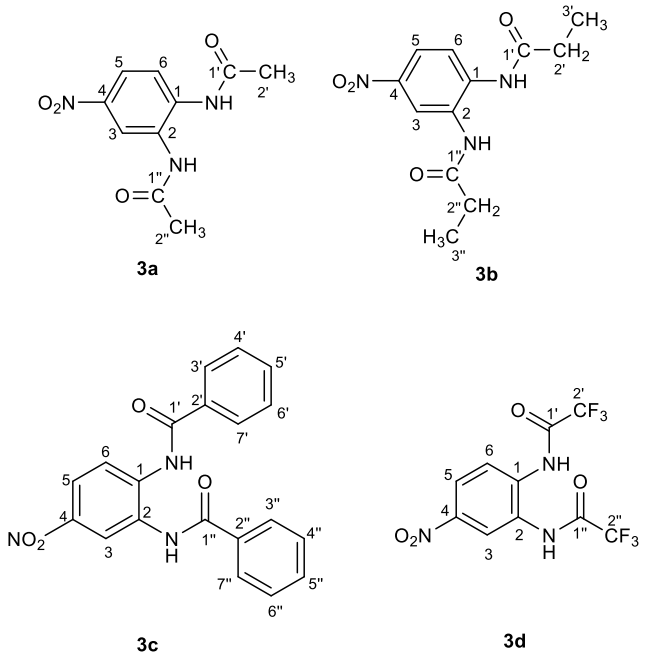


Figure 1: Chemical structures of the synthesized compounds.

All the newly synthesized compounds were evaluated for their *in vitro* antimicrobial activity against six bacterial and fungus strains (MRSA, *C. albicans*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, and *S. typhi*) by the conventional broth-dilution method[8] using standard drugs pefloxacin. The results of antimicrobial studies are presented in Table 1. In general, compounds **3a, 3c** and **3d** showed antibacterial and antifungal activity while compound **3b** shows no activity against all the test organisms. Compound **3d** had the best activity among the compounds tested, with zone of inhibition ranging from 11 to 34 mm on the test microbe.

Table 1: Antimicrobial Activities of the Compounds (Diameters of Zones of Inhibition in millimeters (mm).

Samples	Conc. mg/mL	*S. aureus*	*S. typhi*	*K. pneumoniae*	*E. coli*	MRSA	*P. aeruginosa*	*C. albicans*
3a	200	10	12	11	11	08	10	11
	100	08	09	00	10	07	09	08
3b	200	00	00	00	00	00	00	00
	100	00	00	00	00	00	00	00
3c	200	18	18	20	14	18	13	21
	100	15	16	17	12	15	12	18
3d	200	30	28	30	26	35	12	29
	100	28	25	27	24	34	11	28
Pefloxacin	30 µg/mL	19	20	25	28	17	24	-
Fluconazole	10 µg/mL	-	-	-	-	-	-	25

Key - = not determine.

Table 2: Minimum Inhibitory Concentration (MIC) in mg/mL.

Samples	*S. aureus*	*S. typhi*	*K. pneumoniae*	*E. coli*	MRSA	*P. aeruginosa*	*C. albicans*
3a	12.5	25.0	12.5	50.0	25.0	100.0	12.5
3c	50.0	50.0	50.0	12.5	12.5	12.5	50.0
3d	12.5	12.5	25.0	12.5	12.5	12.5	12.5

Table 3: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) in mg/mL.

Samples	*S. aureus*	*S. typhi*	*K. pneumoniae*	*E. coli*	*MRSA*	*P. aeruginosa*	*C. albicans*
3a	200.0	+	+	+	+	200	+
3c	+	+	+	+	200.0	200.0	+
3d	+	+	+	12.5	12.5	12.5	+

Key: + = growth Values = MBC/MFC (Bactericidal).

Minimum inhibitory concentration (MIC) results (Table 2) revealed that at concentration of 12.5 mg/mL, compound **3d** inhibited the growth of all the test organisms with the exception of *K. pneumoniae* which has MIC of 25 mg/mL. Compound **3a** shows MIC of 12.5 mg/mL for *K. pneumoniae, S. aureus* and *C. albicans* while **3c** inhibited the growth of *E. coli,* MRSA and *P. aeruginosa* at 12.5 mg/mL. The MBC results (Table 3) shows that compound **3d** was bactericidal at 12.5 mg/mL to *E. coli,* MRSA and *P. aeruginosa*.

Conclusion

In summary, the synthesis of new analogues of 4-nitro-1,2-phenylenediamide was accomplished. Among the four newly synthesized compounds, N,N'-(4-Nitro-1,2-phenylene)bis(2,2,2-trifluoroacetamide) (**3d**) was effective against all the tests organisms with the zone of inhibition ranging from 35 mm (MRSA) to 12 mm (*Psedomonas aeruginosa).*

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Jirka T, Hafner M. Estimating the economic costs of antimicrobial resistance: Model and Results 2014; 1-113.
2. Goker H, Kus C, Boykin, DW, Yildiz S, Altanlar N. Synthesis of some new 2-substituted-phenyl-1H-benzimidazole-5-carbonitriles and their potent activity against candida species. Bioorg Med Chem. 2002; 10:2589–2596.
3. He Y, Wu B, Yang J, Robinson D, Risen L, Ranken R, Blyh L, Sheng S, Swayze EE. 2-Piperidin-4-yl-benzimidazoles with broad spectrum antibacterial activities. Bioorg Med Chem Lett 2003; 13:3253–3256.
4. Fostel JM and Lartey PA. Emerging novel antifungal agents. Drug Discov Today. 2000; 5:25–32.
5. Silverman B. The organic chemistry of drug design and drug action, 2nd (Ed) Elsevier Academic Press. 2004; 86 p.
6. Lednicer D. Strategies for organic drug synthesis and design. John Wiley & Sons, New York. 1998.
7. Mohamed IH, Abdel-Samee MA, Nabil MY. Synthesis, X-ray structure and Pharmacological activity of some 6,6-disubstituted chromeno[4,3-b] and chromeno-[3,4-c]-quinolines. Arch Pharm Chem Life Sci. 2007; 340(8):396-399.
8. Nadeem S, Alam MS, Waquar A. Synthesis, anticonvulsant and toxicity evaluation of 2-(1H-indol-3-yl) acetyl-N-(substituted phenyl)hydrazine carbothioamides and their related heterocyclic derivatives. Acta Pharm. 2008; 58:445-454.
9. Galewicz-Walesa K and Pachuta-Stec A. The synthesis and properties of N-substituted amides of 1-(5-methylthio-1,2,4-triazol-3-yl)-cyclohexane-2-carboxylic acid. Medical Academy in Lublin. 2003; 9:118-125.
10. Graybill TL, Ross MJ, Gauvin BR, Gregory JS, Harris AL, Ator MA, Rinker JM, Dolle, RE. Synthesis and evaluation of azapeptide-derived inhibitors of serine and cysteine proteases. Bioorg Med Chem Lett. 1992; 2(11):1375-1380.
11. Mihaela M, Valeriu S, Lenuta P, Marcel P, Catalina L. Synthesis and antimicrobial activity of some new (sulfonamidophenyl)-amide derivatives of N-(4-nitrobenzoyl)-Phenylalanine. FARMACIA, 2008; 56(3) PMC 5609808 Freely accessible
12. Andre W, Iduna F, Gretel S, Felix K. Synthesis, Cleavage Profile and antitumor efficacy of anAlbumin- Binding Prodrug of Methotrexate that is cleaved by Plasmin and Cathepsine B. Arch Pharm Chem Life Sci. 2007; 340(8):389-395.
13. Khalafi-Nezhad A, Rad MNS, Mohabatkar H, Asrari Z, Hemmateenejad B. Design, synthesis, antibacterial and QSAR studies of benzimidazole and imidazole chloroaryloxyalkyl derivatives. Bioorg Med Chem 2005; 13:1931–1938.
14. Karou D, Savadogo A, Canini A, Yameogo S, Montesano C, Simpore J, Colizzi V, Traore AS. "Antibacterial activity of alkaloids from *Sida acuta*," Afr J Biotechnol. 2006; 5(2):195–200.
15. Vellokobia A, Kostalova D, Sochorova K. Isoquinoline alkaloid from Mahonia aquifolium stem bark active against Neisseria species. Folia Microbiol. 2001; 46:107-111.
16. Abdullahi MI, Musa AM, Haruna AK, Sule MI, Abdullahi MS, Abdulmalik MI, Akinwande Y, Abimiku AG, Iliya I. Antimicrobial Flavonoid Diglycosides from the leaves of Ochna scheinfurthiana (Ochnaceae). Nig J Pharm Sci. 2011; 10(2):1-7.
17. Pattabiraman VR and Bode JW. Rethinking amide bond synthesis. Nature 2011; 480:471-479.