Synthesis, Characterization and Preliminary Biological Evaluation of New 3 and 4-nitro Isoindoline-1, 3-dione/phthalimide Analogues

Sharad Sankhe¹ and Nitesh Chindarkar¹

¹Organic Research Laboratory, Patkar-Varde College of Science, Goregaon (West), Mumbai 400 062, India.

Authors’ contributions

This work was carried out in collaboration between both authors. Author SS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NC managed the literature searches, implemented the experiments, and managed the analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i631186

Editor(s):
(1) Dr. Rafik Karaman, Al-Quds University, Palestine.

Reviewer(s):
(1) Cyprian O. Onyeji, University of Nigeria, Nigeria.
(2) Hazem M. Shaheen, Damanhour University, Egypt.

Complete Peer review History: http://www.sdiarticle4.com/review-history/65952

Received 14 December 2020
Accepted 18 February 2021
Published 01 March 2021

Original Research Article

ABSTRACT

Aims: To synthesize new nitroisoindoline-1,3-diones analogues and evaluate their preliminary biological activities

Methodology: New isoindoline-1,3-diones analogues were synthesized by coupling phthalic anhydride derivatives with appropriate aromatic amines. Newly synthesized heterocyclic compounds were evaluated for their in vitro antibacterial activity against gram-positive bacterial strains and gram-negative bacterial strains. They were also tested for their in vitro antifungal activity against fungi strains. Determination of the preliminary antibacterial and antifungal activity were investigated using agar-dilution method. The structures of newly synthesized analogues were elucidated by ¹H and ¹³C-NMR techniques.

Results: Bioassay indicated that some of the newly synthesized isoindoline-1,3-dione analogues shows moderate biological activities.

Conclusion: Newly synthesized analogues can be used as antibacterial or antifungal agents on modifications.

*Corresponding author: E-mail: chindarkar.nitesh@gmail.com;
Graph 1. Graphical Abstract

Keywords: Biological activity; isoindoline-1,3-dione/phthalimide analogues; 3-nitro and 4-nitro phthalic anhydride; Primary aromatic amine.

1. INTRODUCTION

Phthalimides belong to the community of cyclic imides which are obtained by various organic synthetic processes, generally using phthalic anhydride as the precursor. Phthalimide is an important drug structural unit. Norcantharimide 1, phthalimide 2, N-substituted norcantharimide 3 are some examples of isoindole derivatives containing imide (-CO-N(R)-CO-) functional group [1] (Fig. 1).

These compounds and their N-substituted derivatives are interesting compounds due to their important biological properties in the field of synthetic organic chemistry and medicinal chemistry. For this reason, these molecules have been the object of several research centres studies worldwide. Isoindole and its N-substituted derivatives are of much important due to their optical [1], anti-HIV [2,3], antibacterial [3], antifungal [3], analgesic [3], vanxiolytic [3,4], anti-inflammatory [5], anticonvulsant [6], antitumor [3,7,8,9,10], antimicrobial activities [11,12] and their biological properties [13-17].

Cyclic imides such as phthalimides possess structural features which confer biologically potent and pharmaceutically very useful. Some marketed pharmaceutical products of isoindoline are reported in Table 1.

So, considering the efficacy of N-substituted derivatives of phthalimides, we decided to synthesize 8 new N-aryl phthalimides. All of them were tested for biological activities because the literature did not record such evaluation of these compounds against gram positive bacterial strains, gram negative bacterial strains and antifungal activity against fungi strains.

Fig. 1. The structure of norcantharimide 1, phthalimide 2 and N-substituted norcantharimide 3
Table 1. List for biological active some reported isoindoline analogues

| S.N. | Structure | Use/s                |
|------|-----------|----------------------|
| 1    | ![Structure](image1) | Fungicide            |
| 2    | ![Structure](image2) | Fungicide            |
| 3    | ![Structure](image3) | Antineoplastic antileprotic |
| 4    | ![Structure](image4) | Pesticide, insecticide and acaricide. |
| 5    | ![Structure](image5) | A-glucosidase inhibitor |
| 6    | ![Structure](image6) | Anti-candida activity |

2. MATERIALS AND METHODS

All chemicals required for the synthesis were purchased from commercial suppliers and were used without any further purification. Reactions were monitored by TLC analysis using silica gel plates (eluents petroleum ether – AcOEt in various proportions). Compounds were visualized by UV irradiation. $^1$H and $^{13}$C NMR spectra of all compounds were recorded on NMR spectrometer (600, 300 and 75 MHz). Chemical shifts are given in parts per million from TMS or deuterated solvent (DMSO-d$_6$, chloroform-d) as internal standard.

2.1 ISOINDOLINE-1, 3-DIONE ANALOGUES 3a-d and 4a-d: general procedure

The target compounds (3a-d and 4a-d) were synthesized by the condensation of an appropriate phthalic anhydride with an appropriate primary aromatic amine in refluxing glacial acetic acid for 2-3 hrs. The progress of the reaction was monitored using TLC. This reaction was then quenched in cold water. The crude product was filtered and washed several times with water and then dried.

2.2 3-nitro-2-(m-tolyl) isoindoline-1,3-dione (3a)

3-Nitrophthalic anhydride 1a (0.10 g, 0.52 mmol) and m-toluidine 2a (0.36 g, 0.52 mmol) which were refluxed in glacial acetic acid. Product 3a was obtained in yield 0.090 g (62%). $^1$H-NMR (500 MHz, CDCl$_3$): δ-2.41 (s, 3H), δ-7.20-7.24 (m, 3H), δ-7.39 (t, $J$ = 7.5 Hz, 1H), δ-7.97 (t, $J$ = 8 Hz, 1H), δ-8.14 (d, $J$ = 8 Hz, 1H), δ-8.21 (d, $J$ = 7.5 Hz, 1H). $^{13}$C-NMR (125 MHz, CDCl$_3$): δ-21.54 (CH), 123.58 (CH), 123.85 (CH), 127.37 (CH), 127.53 (C), 128.99 (CH), 129.23 (CH), 129.77 (CH), 130.93 (CH), 133.99 (C), 135.84 (C), 139.53 (C), 145.60 (C), 162.17 (C), 165.10 (C). Analysis for C$_{16}$$^\text{H}_{10}$$^\text{N}_2$$^\text{O}_4$ (282.25): Calculated: C, 63.83; H, 3.57; N, 9.92; O, 22.67. Found: C, 64.09; H, 3.27; N, 9.65; O, 22.20.
2.3 3-nitro-2-(o-tolyl) isodindole-1,3-dione (3b)

3-nitro Phthalic anhydride 1a (0.10 g, 0.52 mmol) and o-toluidine 2b (0.36 g, 0.52 mmol) which were refluxed in glacial acetic acid. Product 3b was obtained in yield 0.072 g (50%). 1H-NMR (500 MHz, CDCl3): δ-2.20 (s, 3H), δ-7.18-7.41 (m, 4H), δ-7.78 (t, J = 7.5 Hz, 1H), δ-8.16 (d, J = 8 Hz, 1H), δ-8.21 (d, J = 7.5 Hz, 1H). 13C-NMR (125 MHz, CDCl3): δ-18.18 (CH), 123.81 (CH), 129.03 (CH), 130.05 (CH), 131.45 (CH), 134.13 (C), 135.87 (C), 136.55 (C), 145.59 (C), 162. (C), 165.01 (C). Analysis for C_{15}H_{10}N_2O_4 (282.25): Calculated: C, 63.83; H, 3.09; N, 8.59; O, 29.72. Found: C, 64.15; H, 3.85; N, 9.55; O, 29.12.

2.4 Methyl 4-(3-nitro-1,3-dioxoisodindol-2-yl) (3c)

3-nitro phthalic anhydride 1a (0.50 g, 3.38 mmol) and 4-amino benzoate 2c (0.51 g, 3.38 mmol) which were refluxed in glacial acetic acid. Product 3c was obtained in yield 0.18 g (53%). 1H-NMR (300 MHz, DMSO): δ-3.93 (s, 3H), δ-7.60 (d, J = 8.7 Hz, 1H), δ-8.10 (s, 1H), δ-8.17-8.10 (m, 4H), δ-8.26 (d, J = 7.8 Hz, 1H). 13C-NMR (75 MHz, DMSO): δ-52.17 (CH), 126.44 (CH), 127.38 (C), 128.87 (CH), 129.56 (C), 130.03 (CH), 133.33 (CH), 135.22 (C), 136.33 (C), 145.07 (C), 161 (C), 163.21 (C), 164.10 (C). Analysis for C_{16}H_{11}N_2O_6 (326.26): Calculated: C, 58.90; H, 3.09; N, 8.59; O, 29.42. Found: C, 60; H, 3.45; N, 8.29; O, 29.12.

2.5 Ethyl 4-(3-nitro-1,3-dioxoisodindol-2-yl) (3d)

3-nitro phthalic anhydride 1a (0.20 g, 1.04 mmol) and ethyl 4-amino benzoate 2d (0.172 g, 1.04 mmol) which were refluxed in glacial acetic acid. Product 3d was obtained in yield 0.13 g (55%). 1H-NMR (600 MHz, CDCl3): δ-1.517 (t, J = 7.2 Hz, 3H), δ-4.513 (q, J = 7.2 Hz, 2H), δ-7.683-7.662 (m, 2H), 8.110 (d, J = 7.8 Hz, 1H), δ-8.306-8.275 (m, 2H), δ-8.334 (d, J = 1.2 Hz, 1H), δ-8.346 (d, J = 1.2 Hz, 1H). 13C-NMR (150 MHz, CDCl3): δ-14.30 (CH), 61.33 (CH), 119.33 (CH), 125.21 (CH), 125.79 (CH), 129.81 (C), 130.34 (CH), 130.55 (CH), 132.94 (C), 134.91 (C), 135.87 (C), 152.09 (C), 164.47 (C), 164.72 (C), 165.61 (C). Analysis for C_{17}H_{12}N_2O_6 (340.29): Calculated: C, 60; H, 3.55; N, 8.23; O, 28.21. Found: C, 60.25; H, 3.35; N, 8.49; O, 28.

2.6 4-nitro-2-(m-tolyl) isodindole-1,3-dione (4a)

4-nitro Phthalic anhydride 1b (0.50 g, 2.59 mmol) and m-toluidine 2a (0.28 g, 2.59 mmol) which were refluxed in glacial acetic acid. Product 4a was obtained in yield 0.48 g (73%). 1H-NMR (600 MHz, CDCl3): δ-2.20 (s, 3H), δ-4.513 (q, J = 7.2 Hz, 2H), δ-7.683-7.662 (m, 2H), δ-8.110 (d, J = 7.8 Hz, 1H), δ-8.306-8.275 (m, 2H), δ-8.334 (d, J = 1.2 Hz, 1H), δ-8.346 (d, J = 1.2 Hz, 1H). 13C-NMR (150 MHz, CDCl3): δ-14.30 (CH), 61.33 (CH), 119.33 (CH), 125.21 (CH), 125.79 (CH), 129.81 (C), 130.34 (CH), 130.55 (CH), 132.94 (C), 134.91 (C), 135.87 (C), 152.09 (C), 164.47 (C), 164.72 (C), 165.61 (C). Analysis for C_{17}H_{12}N_2O_6 (340.29): Calculated: C, 61; H, 3.55; N, 8.23; O, 28.21. Found: C, 60.25; H, 3.35; N, 8.49; O, 28.
2.9 Ethyl 4-(4-nitro-1,3-dioxoisindolin-2-yl) (4d)

4-nitro phthalic anhydride 1b (0.20 g, 1.036 mmol) and ethyl 4-amino benzoate 2d (0.086 g, 1.036 mmol) which were refluxed in glacial acetic acid. Product 4d was obtained in yield 0.23 g (62%). $^{1}$H-NMR (600 MHz, CDCl$_3$): δ-1.42 (t, $J$ = 7.2 Hz, 3H), δ-4.42 (q, $J$ = 7.2 Hz, 2H), δ-8.59 (dd, $J$ = 6.8, 2.1 Hz, 2H), δ-8.22-8.17 (m, 3H), δ-8.69 (d, $J$ = 2 Hz, 1H), δ-8.79 (s, 1H).

$^{13}$C-NMR (150 MHz, CDCl$_3$): δ 14.30 (CH), 61.33 (CH), 119.33 (CH), 125.21 (CH), 125.79 (CH), 129.81 (C), 130.34 (CH), 130.55 (CH), 132.94 (C), 134.91 (C), 135.87 (C), 152.09 (C), 164.47 (C), 164.72 (C), 165.61 (C). Analysis for C$_{17}$H$_{12}$N$_2$O$_6$ (340.29): Calculated: C, 60; H, 3.55; N, 8.23; O, 28.21. Found: C, 59.75; H, 3.85; N, 8.47; O, 28.45.

3. RESULTS AND DISCUSSION

The starting compound named 3-nitro phthalic anhydride (1a) was converted into 3-nitro-2-(m-tolyl) isoindoline-1,3-dione (3a) by the reaction with m-toluidine (2a) in glacial acetic acid for 2-3 hrs. The compounds (3b-d and 4a-d) were synthesized by using the same reaction conditions. The chemical structures of all newly synthesized compounds were confirmed by both elemental and spectral data.

3.1 Antibacterial and Antifungal Activity

All newly synthesized heterocyclic compounds were evaluated for their in vitro antibacterial activity against gram-positive bacterial strain (S. aureus) and gram-negative bacterial strain (E. Coli). They were also tested for their in vitro antifungal activity against fungi (A. brasiliensis) strain.

Determination of the preliminary antibacterial and antifungal activity were investigated using agar-dilution method. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around discs in mm (Table 3). Results show that compounds 3a and 3b show antimicrobial activity against E.Coli whereas compounds 3b and 4a shows antimicrobial activity against A.Brasiliensis. Compounds 3c, 3d did not comply specification requirements and compound 4c did not show any antimicrobial activity upto 1 g/100 μl.

![Scheme. Synthesis of new nitroisoindoline-1,3-dione analogues (3a-d and 4a-d)](image)

| Product Number | $R_1$ | $R_2$ | Yield (%) |
|----------------|-------|-------|-----------|
| 3a             | 3-NO$_2$ | 3-Me  | 62        |
| 3b             | 3-NO$_2$ | 2-Me  | 50        |
| 3c             | 3-NO$_2$ | 4-COOMe | 53       |
| 3d             | 3-NO$_2$ | 4-COEt  | 55       |
| 4a             | 4-NO$_2$ | 3-Me  | 73        |
| 4b             | 4-NO$_2$ | 2-Me  | 73        |
| 4c             | 4-NO$_2$ | 4-COOMe | 34       |
| 4d             | 4-NO$_2$ | 4-COEt  | 62       |
Two newly synthesized heterocyclic compounds were evaluated for their in vitro antibacterial activity against gram positive bacterial strains (*S.Aureus & S.Pyogenus*) and gram negative bacterial strains (*E.Coli & P.Aeruginosa*). They were also tested for their in vitro antifungal activity against fungi (*C.Albicans, A.Niger & A.Clavatus*) strain.

Determination of the preliminary antibacterial and antifungal activity were investigated using agar-diffusion method ('Broth Dilution Method'). It is one of the non-automated in vitro bacterial susceptibility tests. This classic method yields a quantitative result for the number of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in tubes. The main advantage of the 'Broth Dilution Method' for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

Both the zones of inhibition (mm) and minimum inhibitory/fungicidal concentration (MIC) (μg/ml) of the investigated compounds were recorded and compared with Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin as antibacterial and Nystatin, Greseofulvin as antifungal reference medications. DMSO was used as vehicle to get desired concentration of drugs to test upon Standard bacterial strains.

The investigation of antibacterial screening data is summarized in Table 4. Results show that MIC value less than that of standard drug were considered promising, results reveal that compound 4b exhibited higher activity (100 μg/mL) against *S. Aureus* while compound
compound 4d possessed pronounced activity against \( E.\ coll \).

4. CONCLUSION

It is concluded that new isoindoline-1,3-diones analogues were synthesized by coupling phthalic anhydride derivatives with appropriate aromatic amines. Newly synthesized heterocyclic compounds were evaluated for their in vitro antibacterial activity against gram-positive bacterial strain and gram-negative bacterial strain. Newly synthesized compounds show moderate antibacterial and antifungal activities.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It’s not applicable.

ETHICAL APPROVAL

It’s not applicable.

ACKNOWLEDGEMENT

The authors are thankful to Prof. INN Namboothiri, Dr. Suyog Marathe, Dr. Rajesh Kenny, SAIF IIT Mumbai, NSRT lab Gujarat, Microcare Lab Surat, University of Mumbai, Patkar College and A. P. Shah Institute of Technology, for support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tan A, Kizilkaya S, Gunduz B, Kara Y. Synthesis and optical properties of some isoindole-1,3-dione compounds: optical band gap, refractive index and absorbance band edge. Org. Commun. 2018;11:4:173-180.

2. Prado SR, Cechnel-Filho V, Campos-Buzzi, F, Correa R, Cadena SM, De Oliveira MB. Biological evaluation of some selected cyclic imides: Mitochondrial effects and in vitro cytotoxicity. Z. Naturforsch. C. 2004;59:663–672.

3. Sabastiyan A, Suvaikin MY. Synthesis, characterization and antimicrobial activity of 2-(dimethylaminomethyl) isoindoline-1,3-dione and its cobalt (II) and nickel (II) complexes. Adv. in Appl. Sci. Res. 2012;3:45-50.

4. Hassazadeh F, Rabbani M, Khodarahmi GA, Fasihi A, Hakimelahi GH, Mohajer M. Synthesis of phthalimide derivatives and evaluation of their anxiolytic activity. Res. Pharm. Sci. 2007;2:35-41.

5. Lima LM, Castro P, Machado AL, Fraga CA, Lugnier C, De Moraes, VL, Barreiro EJ. Synthesis and anti-inflammatory activity of phthalimide derivatives, designed as new thalidomide analogues. Bioorg. Med. Chem. 2002;10:3067-3073.

6. Abdel-Hafez AA. Synthesis and anticonvulsant evaluation of N-substituted isoindolinedione derivatives. Arch. Pharm. Res. 2004;27:495-501.

7. Kok SHL, Gambari R, Chui CH, Yuen, MCW, Lin E, Wong, RSM, Lau F.Y, Cheng GYM, Lam WS, Chan SH, Lam KH, Cheng CH, Lai PBS, Yu MWY, Cheung F, Tang JCO, Chan ASC. Synthesis and anticancer activity of benzothiazole containing Phthalimide on human carcinoma cell lines. Bioorg. Med. Chem. 2008;16:3626-3631.

8. Sami SM, Dorr RT, Alberts DS, Solyom, AM, Remers WA. Analogues of amonafide and azonafide with novel ring systems. J. Med. Chem. 2000;43:3067-3073.

9. Wang JJ, Liu TY, Yin PH, Wu CW, Chem YT, Chi CW. Adamantyl maleimide induced change in adhesion molecules and ROS are involved in apoptosis of human gastric cancer cells. Anticancer Res. 2000;20:3067-3073.

10. Li M, Sun W, Yang Y, Xu B, Yi W, Ma Y, Li Z, Cui J. In vitro anticancer property of a novel thalidomide analogue through inhibition of NF-κB activation in HL-60 cells. Acta Pharm. Sin. 2009;30:134-140.

11. Ching LM, Browne WL, Tchernegovski R, Gregory T, Baguley, BC, Palmer BD. Interaction of thalidomide, phthalimide analogues of thalidomide and pentoxyfiline with the anti-tumour agent 5, 6-
dimethylxanthenone-4-acetic acid: Concomitant reduction of serum tumour necrosis factor-alpha and enhancement of anti-tumour activity. British J. Can. 1998;78:336-343.

12. Singh J, Singha T, Naskar A, Kundu M, Harwansh RK, Mondal A, Ghosh T, Maity TK. Synthesis and anti-proliferative activity of some isoindoline-1, 3-dione derivatives against ehrlich’s ascites carcinoma bearing mice model. Pharmacologyonline, 2011;2:976-987.

13. Kose A, Bal Y, Kishali, NH, Sanli-Mohamed G, Kara Y. Synthesis and anticancer activity evaluation of new isoindole analogues. Med. Chem. Res. 2017;26(4):779-786.

14. Tan A, Bozkurt E, Kara Y. Investigation of solvent effects on photophysical properties of new aminophthalimide derivatives-based on methanesulfonate. J. Fluoresc. 2017;27:981-992.

15. Yedage SL, D’silva DS, Bhanage BM. MnO$_2$ catalyzed formylation of amines and transamidation of amides under solvent free conditions. RSC Adv. 2015;5:80441-80449.

16. Bach DH, Liu JY, Kim WK, Hong JY, Park, SH, Kim W, Qin SN, Luu TTT, Park HJ, Xu YN, Lee SK. Synthesis and biological activity of new phthalimides as potential anti-inflammatory agents. Bioorganic and Medicinal Chemistry. 2017;25:3396–3405.

17. Patel NB, Purohit AC, Rajani DP, Moo-Puc R, Rivera G. New 2-benzylsulfanyl-nicotinic acid based 1,3,4-oxadiazoles: Their synthesis and biological evaluation. European Journal of Medicinal Chemistry. 2013;62:677-687.