Heterocyclic Letters
Vol. 14| No.4|859-872|Aug-Oct|2024
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

DESIGN, SYNTHESIS AND *IN-VITRO* DEGRADATION STUDY OF AZO COMPOUNDS AS PRODRUGS OF 4-AMINO PYRIDINE

S. M. Koshti*, S. K. Girase, J. B. More

Department of Chemistry S. S. V. P.S's. L. K. Dr. P. R. Ghogrey Science College, Dhule (M. S.), India
**Corresponding author Email:smkoshti@rediffmail.com, smkoshti82@gmail.com*

ABSTRACT:

The aim of this study is to explore the drug release property of azo compounds. In the present work, 4-amino pyridine is diazotized to form diazonium salt and coupled with antibacterial agent's such as salicylic acid, thymol, and carvacrol to form the azo compounds. Then azo compounds were degraded *in-vitro* by using azoreductase enzyme secreted by *Pseudomonas aeruginosa* bacterial species. A parent drug 4-amino pyridine released after the degradation was confirmed by HPTLC technique by comparing the R_f value of released drug with pure 4-amino pyridine. The combined formulation of 4-amino pyridine with the antibacterial agent's salicylic acid, thymol and carvacrol as azo compounds provides a new tool as colon targeting agents as well as for neuromuscular dysfunction of colon.

KEY WORDS: 4-amino pyridine; azo compounds; degradation

INTRODUCTION:

4-Amino pyridine is used as potassium channel blocker, potent calcium channel activator, drug for chronic multiple sclerosis,used in the treatment of spinal cord injury[i-ii]. Sustained-released-4-amino pyridine (SR-AP) is a safe drug and easy for administration in the treatment of walking disabilities in patients with multiple sclerosis in 4-7 EDSS range[iii]. 4-Amino pyridine is used in treatment of Episodic Ataxia type-2 (EA-2)[iv], ataxia telangiectasia[v], prevent loss of dentate granule neurons[vi] and Alzheimer's disease[vii].

5-Amino salicylic acid is used as non-steroidal anti-inflammatory drug in the treatment of ulcerative colitis and Inflammatory Bowel Syndrome (IBS) in colon[viii]. The action of 5-amino salicylic acid is related to the modulation of inflammatory cytokine production[ix], reduction in transcriptional activity of NF-kB [x-xii] and helping to stop the biosynthesis of prostaglandin. Thymol has significant post antibacterial effect against some microorganisms[xiii]. Thymol expands dipalmitoylphosphatidylcholine monolayer by decreasing the surface elasticity of lipid layers due to which it incorporates in the lipid film[xiv,xv]. Carvacrol suppresses enzyme cyclooxygenase-2 appearance, which is primary cause of redness and swelling[xvi]. Carvacrol is accountable for the biological activities such as antimicrobial, antitumor, antimutagenic, antigenotoxic, analgesic, antispasmodic, anti-inflammatory, angiogenic, antiparasitic, antiplatelet, Ache inhibitory, antielastase, insecticidal, antihepatotoxic and hepatoprotective activities [xvii,xviii].

A prodrug can be used to get better drug delivery action, pharmacological activity and pharmacokinetics of a parent drug molecule. It is also used for lowering the toxicity of parent drug towards specific cells or tissue[xix, xx]. Though prodrug design is complicated, still it is more rational or quicker way than developing a new therapeutic drug molecule with desired absorption, distribution, metabolism, excretion and toxicity[xxi]. An azo based synthetic compounds shows versatile biological activity in drug discovery and drug release study such as antioxidant, cytotoxic activity, anthelmentic activity, Cholinesterase inhibitory effect, wound healing, anti-analgesic, and anti rheumatoidal[xxii, xxiii]. An azo compounds are metabolized in the colon, where it degrades into two aromatic amines by means of reduction of azo (-N=N-) bond using azo reductase enzyme which is secreted by colon microflora[xxiv, xxv]. The azo compounds are also used as great tool for development of colon targeting agents in which bacterial infection of colon can be minimized.

By considering the above properties of 4-amino pyridine, 5-Amino salicylic acid, thymol, and carvacrol, we have presented the synthesis of azo compounds of 4-Amino pyridine with salicylic acid, thymol and carvacrol (**Figure 1**) and the synthesized azo compounds were screened as prodrug of 4-amino pyridine.

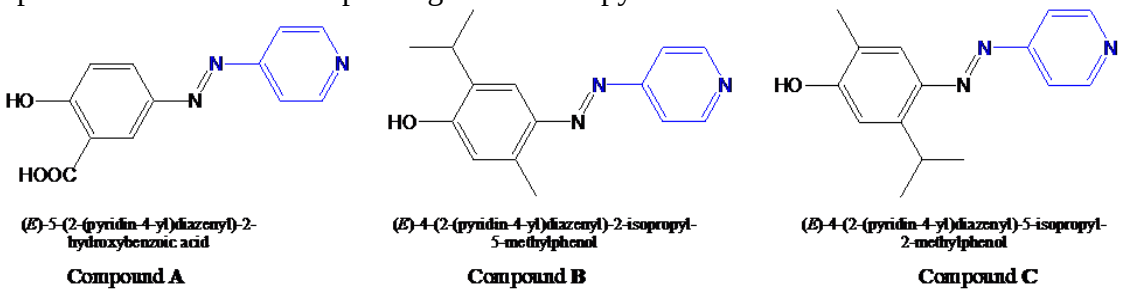


Figure 1: Structures of synthesized azo compounds of 4-amimo compound.

EXPERIMENTAL SECTION:

Pharmaceutical grade 4-amino pyridine were used, sodium nitrite, sodium hydroxide, Thymol, Salicylic acid and carvacrol from SD fine chemicals ltd. Mumbai, India. All other reagents and solvents were of analytical grade.

The compounds were characterized by IR, ^{1}H NMR and ^{13}C NMR. The melting points were determined by open capillary method and are uncorrected. The IR spectra were recorded on Perkin-Elmer spectrum-one FTIR instrument in the form of KBr pallet. The ^{1}H NMR and ^{13}C NMR were recorded in DMSO on a BRUKER AVANCE II 400 NMR spectrometer using TMS as an internal standard. The purity of synthesized azo compounds were checked by TLC. The crude products were recrystallized from ethanol.

GENERAL PROCEDURE FOR SYNTHESIS OF AZO COMPOUNDS (PRODRUGS OF 4-Amino Pyridine)[xxiv, xxv]:

4-Amino pyridine (0.94 g, 0.01mole) was mixed with conc. HCl (2.5 mL). To the resultant suspension crushed ice (25 gm) and $NaNO_2$ (2.5 mL, 4*N*) was added with constant stirring. Diazotization was carried out over 0.5 hr at 5^0C (Scheme-1) and then diazonium salt solution was added drop wise at 5^0 -10^0C to the alkaline solution of Salicylic acid. The coupling reaction mixture was stirred for 0.5 hr and the *p*H of the resultant mixture was adjusted to the *p*H 7 (Scheme-2). The formed azo colored compound was filtered, washes with water and dried. Crude products were recrystallized with ethanol as solvent.

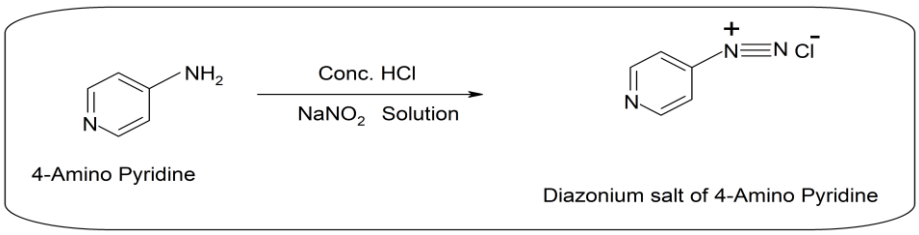
The melting points were determined by open capillary method and are uncorrected for pressure effects

2-hydroxy-5-[(*E*)-pyridin-4-yldiazenyl]benzoic acid (**Compound A):** M. P.:128°C; IR1383.01 cm^{-1}-C-N- , 1444.73 cm^{-1} -OH of –COOH, 1589.40 cm^{-1}-N=N-, 1620.26 cm^{-1}-C=N- of Py, 3093.92 cm^{-1} Ar -C-H, above 3200 cm^{-1} –OH, very broad; ^{1}HNMR(DMSO-d_6): δ 6.91 (d, 1H, Ar-H), 7.12 (d, 1H, Ar-H), 8.23 (s, 1H, Ar-H), 7.80 (dd, 2H, Ar-H of Pyridine), 8.43 (dd, 2H, Ar-H of Pyridine), 8.82(bs, 1H, of -OH of Salicylic acid), 10.98 (bs, 1H, of -COOH of Salicylic acid); ^{13}CNMR(DMSO-d_6): δ 112.76 (for Ar-CH of salicylic acid), 116.93 (for Ar-CH of salicylic acid), 118.89 (for Ar-CH of pyridine), 127.59 (for Ar-C of Salicylic acid bearing to -COOH), 128.71 (for Ar-CH of salicylic acid), 135.28 (for Ar-CH of pyridine), 144.10 (for Ar-C of salicylic acid bearing -N=N-), 161.23 (for Ar-C of salicylic acid bearing -OH), 171.95 (for -COOH).

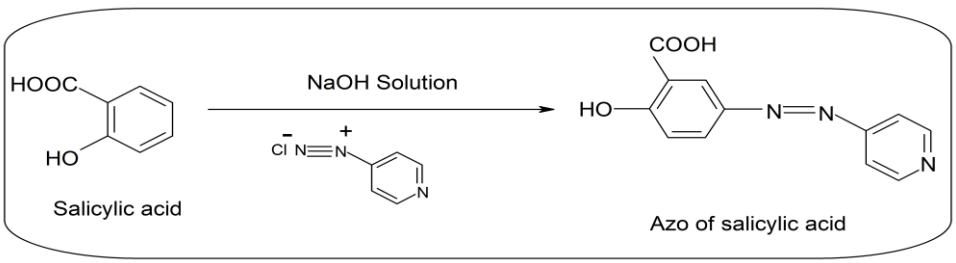
2-isopropyl-5-methyl-4-[(*E*)-pyridin-4-yldiazenyl]phenol (Compound B): M. P.:152°C; IR 1394.58 $cm^{-1}$of -C-N, 1512.24 cm^{-1}-N=N-, 1631.83 cm^{-1}-C=N- , 2958.90 cm^{-1}Aro -C-H, above 3200.00 cm^{-1}-OH broad; ^{1}HNMR(DMSO-d_6): δ 1.20 (d, 6H, two -CH_3), 2.18 (s, 3H, -CH_3 of thymol), 3.18 (m, 1H, isopropyl of thymol), 6.52 (s, 1H, aro. of thymol), 6.54 (s, 1H, aro. of thymol), 6.60 (bs, 1H, -OH of thymol), 6.94 (dd, 2H, Ar-H of pyridine), 8.95 (dd, 2H, Ar-H of pyridine); ^{13}CNMR(DMSO-d_6): δ 20.60 (for -CH_3 of thymol), 22.48 (for-CH_3 of isopropyl of thymol), 26.00 (for -CH of isopropyl of thymol), 115.48 (for aro.-CH= of thymol), 119.49 (for Aro. -CH= of pyridine), 125.44(for Aro. -CH= of thymol), 131.15 (for aro.-C of thymol attached to - CH_3), 135.09 (for aro. -CH= of pyridine), 139.09 (for aro. C of thymol attached to - N=N-), 140.01 (for aro. C of thymol attached to isopropyl group), 154.10 (for aro. C of thymol attached to -OH).

5-isopropyl-2-methyl-4-[(*E*)-pyridin-4-yldiazenyl]phenol (Compound C): M. P.:102°C, IR 1394.58cm^{-1} -C-N, 1512.24 cm^{-1}-N=N-, 1631.83 cm^{-1}-C=N-, 2958.90 cm^{-1} Aro -C-H, above 3200.00 cm^{-1} –OH, broad; ^{1}HNMR(DMSO-d_6): δ 1.15 (d, 6H, two -CH_3), 2.10 (s, 3H, -CH_3 of carvacrol), 3.61 (m, 1H, isopropyl of carvacrol), 6.68 (bs, 1H, -OH of carvacrol), 6.86 (s, 1H, aro.-CH= of carvacrol), 7.32 (s, 1H, aro. -CH= of carvacrol), 7.89 (dd, 2H, aro. -CH= of pyridine), 8.89 (dd, 2H, aro. -CH= of pyridine); ^{13}CNMR(DMSO-d_6): δ 16.02 (for -CH_3 of carvacrol), 24.16 (for-CH_3 of isopropyl of carvacrol), 32.57 (for -CH of isopropyl of carvacrol), 114.27 (for Aro. -CH= of carvacrol), 123.23 (for aro. -CH= of pyridine), 123.87 (for Aro. -C of carvacrol attached to - CH_3), 128.21 (for aro.-CH= of carvacrol), 138.37 (for -CH=, of pyridine), 153.17 (for aro. C of carvacrol attached to -OH).

Scheme-1 Synthesis of Diazonium Salt:



Scheme-2 The Coupling reaction :



In vitro azo reduction by Pseudomonas aeruginosa i.e. drug release studies[xxvi]

Pseudomonas aeruginosa was isolated from industrial effluent water samples collected from Disan Agro Ltd. Dhule (MS) India by spreading diluted sample from 10^{-5}dilutions over a sterile Cetrimide Agar plate (g L-1 Enzyme digest of Gelatine- 20g, Magnesium chloride- 1.4g, potassium chloride- 10g, Cetrimide (Cetyl tri methylammonium Bromide),- 0.3g, Glycerol- 10ml, pH- 7.2) and incubated for 24 hours at 37°C in an incubator.

The isolated *Pseudomonas aeruginosa* strain was tested for de-colorization activity against newly synthesized azo compounds (0.250gm/Ltr) in nutrient broth (gL-1 Peptic digest of animal-5gm, Sodium chloride-5gm, Beef extract 1.50gm, Yeast Extract- 1.50gm, pH- 7.4) by inoculating with loop full bacterial culture. These flasks were incubated at 37°C for 24 hrs. Un-inoculated flasks served as controls to assess the abiotic de-colorization. Optical densities values were measured spectrophotometrically at 426.8 nm, 430.8 nm and 435.6 nm respectively for the estimation involving de-colorization process.

Results and Discussion:

The synthesized azo compounds were screened as prodrug of 4-amino pyridine. For *in-vitro* study bacterium species that secretes azo reductase enzyme and inoculate them with newly synthesized azo compounds. Fig. 2 shows the flasks containing solutions of newly synthesized azo compounds before inoculation of bacteria and after 24 hours of inoculation of bacteria. The formation Precipitate indicates the growing of bacteria and degradation process is completed.

Fig 2. Flasks before degradation Flasks after degradation

In vitro azo reduction by Pseudomonas aeruginosa i.e. drug release studies[xxvi]

The synthesized azo compounds were reduced by *Pseudomonas aeruginosa (Please see Supporting Information for the detailed* **in vitro azo reduction by Pseudomonas aeruginosa***).*

After reduction of azo compounds into primary aromatic amines, that newly formed primary aromatic amines were identified by HPTLC technique by comparing with pure 4-amino pyridine as standard.

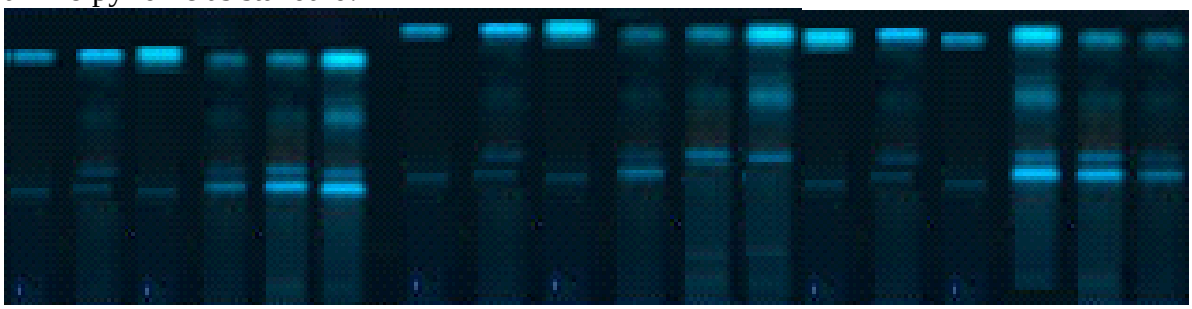


Fig 3. The chromatographic plate showing the distinct release of parent drug (last three in each image) for Compound A, Compound B and Compound C

Figure 4 shows the HPTLC spectra of pure parent drugs i.e. 4-amino pyridine. Figure 5, 6 and 7 shows the HPTLC spectra of newly synthesized azo compound A**,** compound **B,** and compound **C** respectively after inoculation. On comparison of HPTLC spectra of 4-amino pyridine those with the HPTLC spectra of **Compound A, B, and C**; it is found that the Rf value of pure 4-amino pyridine is at 0.43 which is well matched with Rf value of released drug from synthesized azo compound **A**, compound **B**, and Compound **C** after 24 hours' of inoculation of *Pseudomonas aeruginosa* bacterium species.

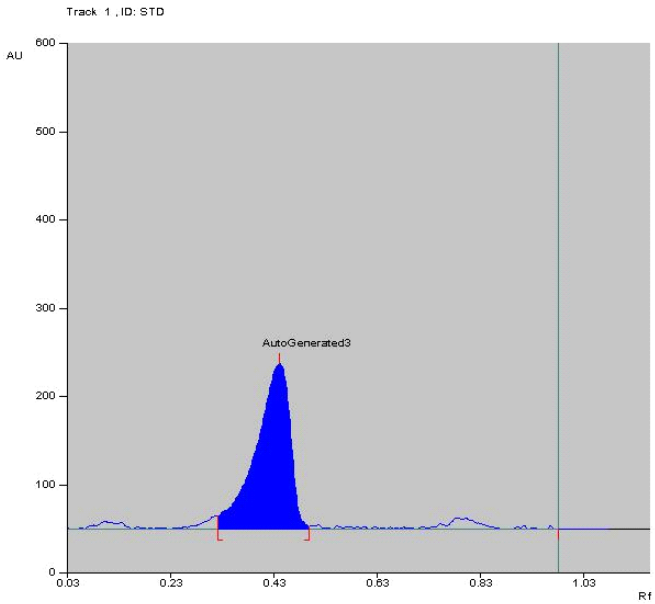


Fig 4: HPTLC spectrum of standard 4-aminopyridine

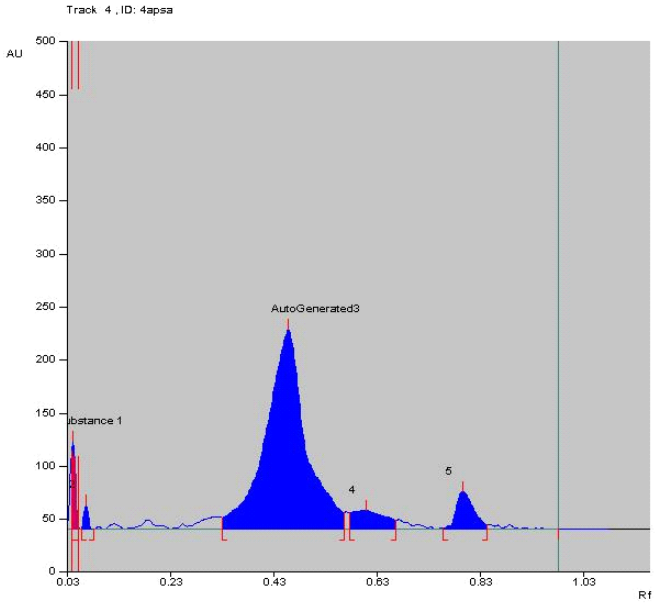


Fig 5: HPTLC spectrum of degraded azo compound A

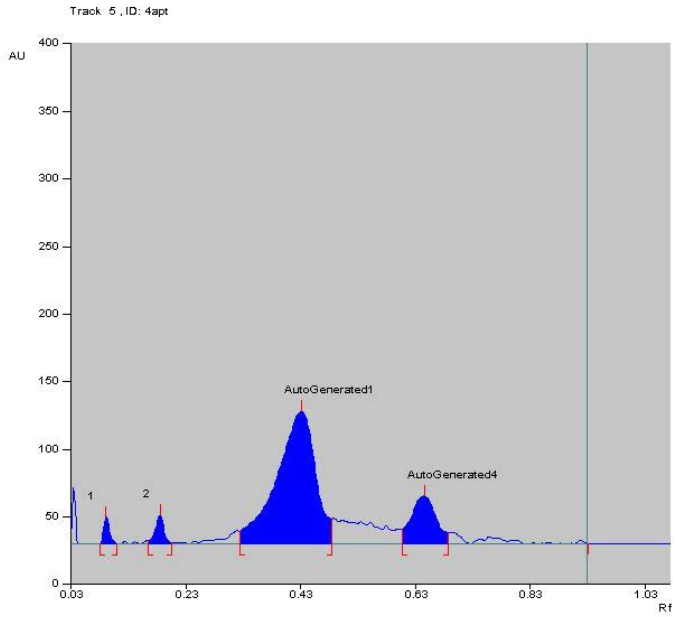


Fig 6: HPTLC spectrum of degraded azo compound B

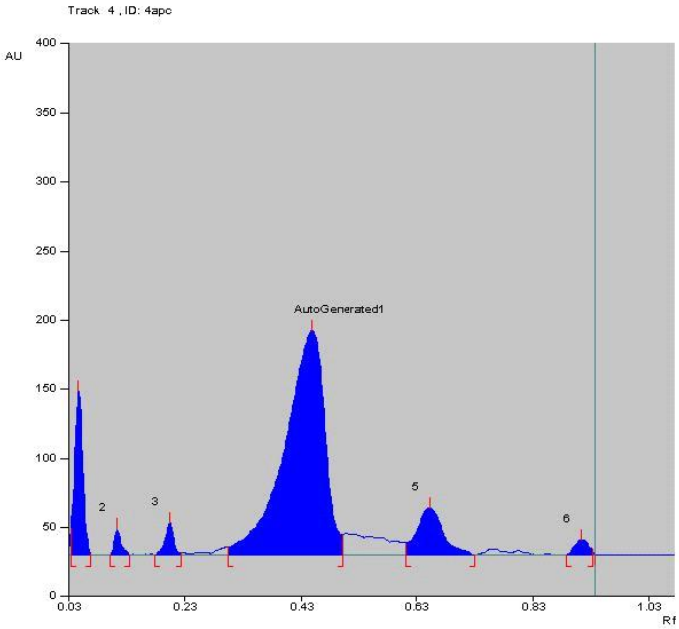


Fig 7: HPTLC spectrum of degraded azo compound C

Figure 8, 9, and 10 shows the comparative 3D HPTLC spectra; first three peak lines in each spectra are for pure 4-amino pyridine and next three peak lines for drug obtained after degradation of azo of compound A, compound B, and Compound C. According to Fig. 10, first three peak lines for 4-amino pyridine drug of concentration 3µl, 2µl, 1µl and next three peak lines for drug obtained after degradation of azo of compound C of concentration 4µl, 3µl, 2µl matches well.

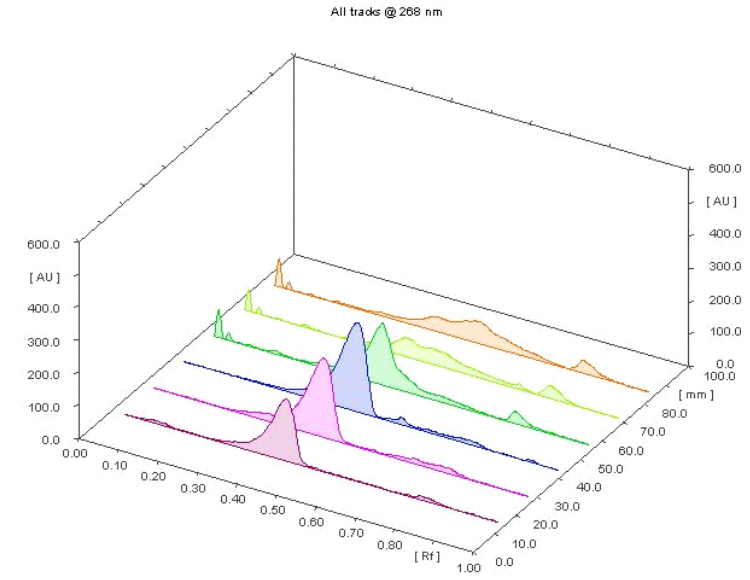


Fig 8. Comparable HPTLC spectrum of pure 4-Amino pyridine and drug release after degradation of azo of Compd A

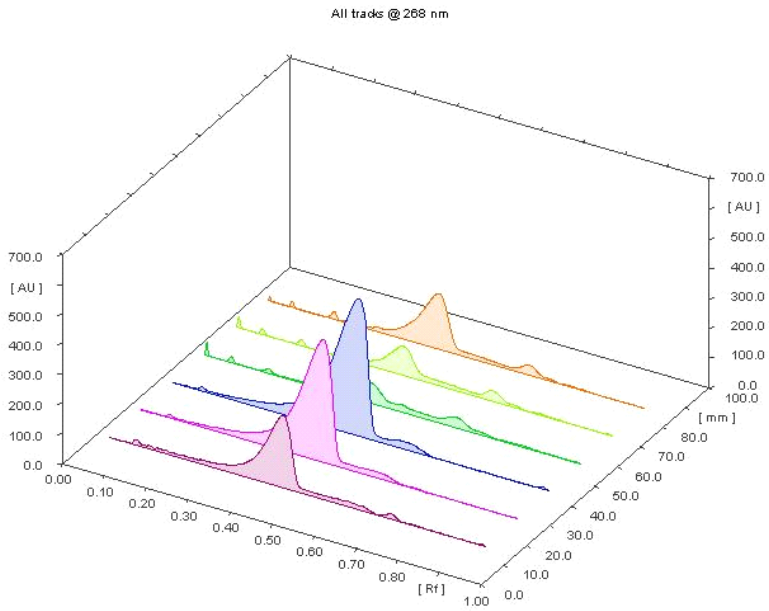


Fig 9. Comparable HPTLC spectrum of pure 4-Amino pyridine and drug release after degradation of azo of Compd B

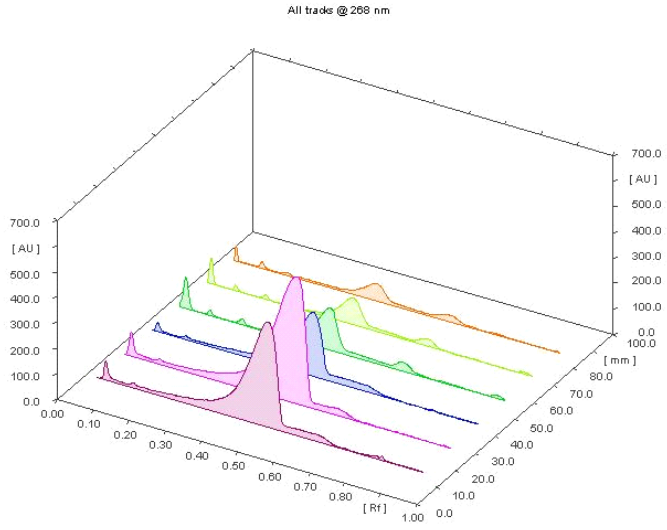


Fig 10. Comparable HPTLC spectrum of pure 4-Amino pyridine and drug release after degradation of azo of Compd C

Table No. 2 shows the Rf value of pure parent drug molecule i.e. 4-amino pyridine and Rf values of Compound A, Compound B and compound C after 24 hours of bacterial inoculation respectively.

Table 2: Comparable R_f values of pure 4-amino pyridine and drug released from azo compounds A, B and C.

Compound	R_f value of drug released	R_f value of pure 4-Amino pyridine
Compd A	0.43	0.43
Compd B	0.43	
Compd C	0.43	

Validation of method:

The developed method was validated in accordance with ICH guidelines.

Linearity and range: Linearity was found in the range of 267 to 933 ng/band for compound A, 233 to 667 ng/band for compound B and 159 to 464 ng/band for compound C. The released parent drug peak areas were calculated for each level and were shown in graph of plot of concentration (ng/band) v/s peak area in figure 24, 25, 26 respectively (Please see supporting information).

Accuracy: For accuracy purpose of this method, a standard addition method was employed. The known amount of parent drug was added at 3 different levels to degraded compound A, degraded compound B and degraded compound C and analysis was performed in triplicate at each level. The result of release of parent drug expressed in % release are shown in table 3 . The % of release for compound A, B and C were found to be nearly 97 % suggesting that there is no interference in the analysis.

Table 3: Accuracy result

Level of release in %	Release in %*	Release in %*	Release in %*
	Compound A	Compound B	Compound C
68 %	96.91	96.92	96.90
76 %	96.79	96.72	96.73
87 %	96.89	96.88	96.86

Note: * mean of three determination

Robustness: The effect of change of mobile phase composition (), in chamber saturation period (), in time of application to development (30 mins, 60 mins), in scanning time (30 mins, 60 mins), on peak areas, in R_f values were consider for robustness. It was seen that, in all mentioned factors has no significant change is observed (% RSD < 2 for peak area, change in R_f less than). Hence developed method was said to be robust.

UV absorption spectra of pure 4-amino pyridine (Fig. 11), azo compound A after 24 hours bacterial inoculation (Fig. 12), and overlain UV absorption spectra of pure 4-amino pyridine and azo compound A (Fig 13). From the overlain spectra (Fig 13); it is clearly observed that the –N=N- azo bond is cleaved enzymatically and there is a release of 4-amino pyridine. Similar observations (i.e degradation –N=N-) were observed from the UV spectra of azo compound B (Fig 14,15) and Compound C (Fig 16,17) after 24 hours bacterial inoculation (supporting information;Table 4).

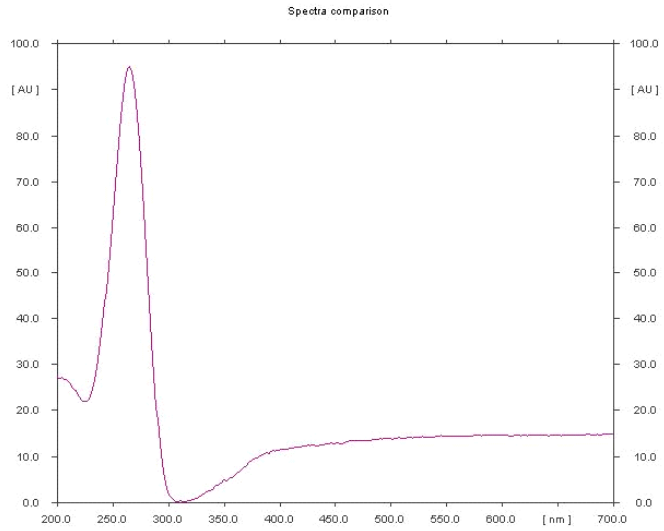


Fig 11: Absorption spectrum of standard 4-amino pyridine

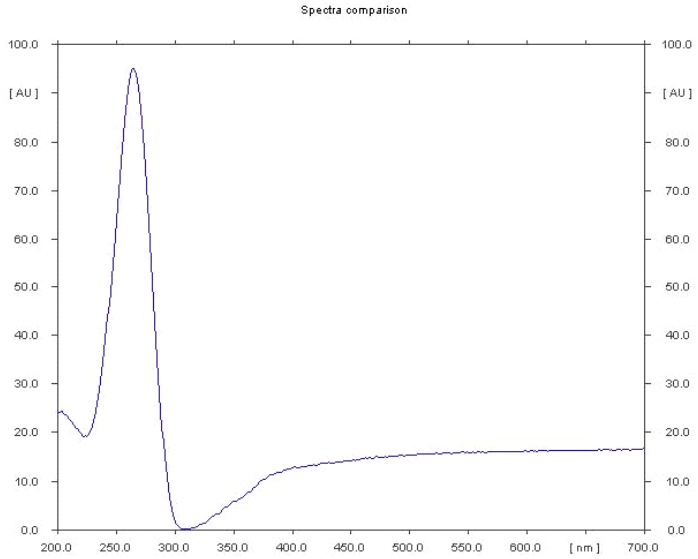


Fig 12: Absorption spectrum of degraded azo compound A

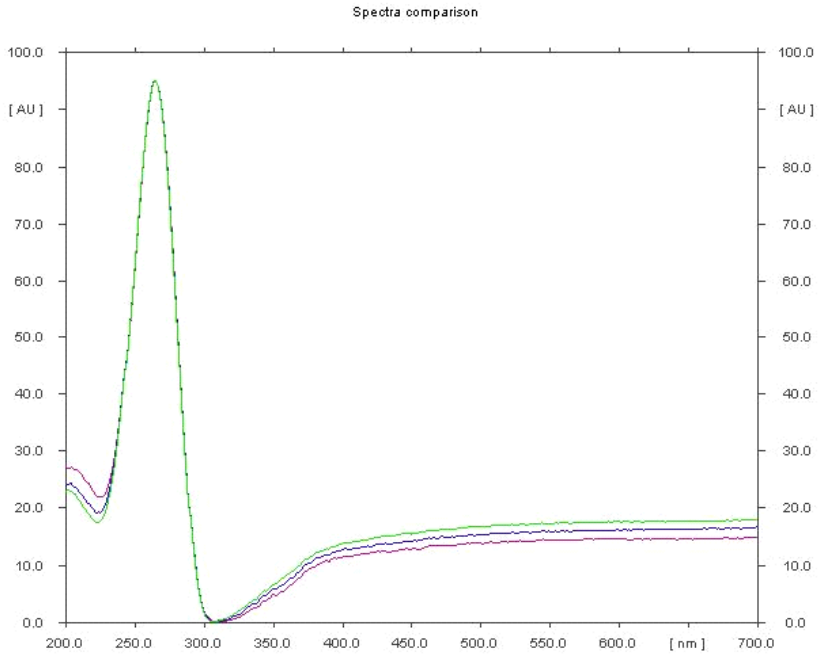


Fig 13. Overlainabsorption spectrum of pure 4-Amino pyridine and drug release after degradation of azo of Compd A

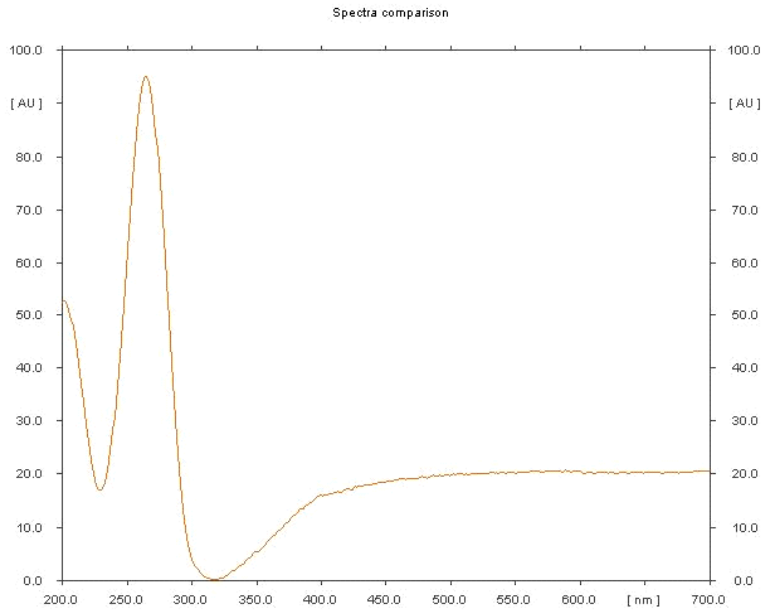


Fig 14: Absorption spectrum of degraded azo compound B

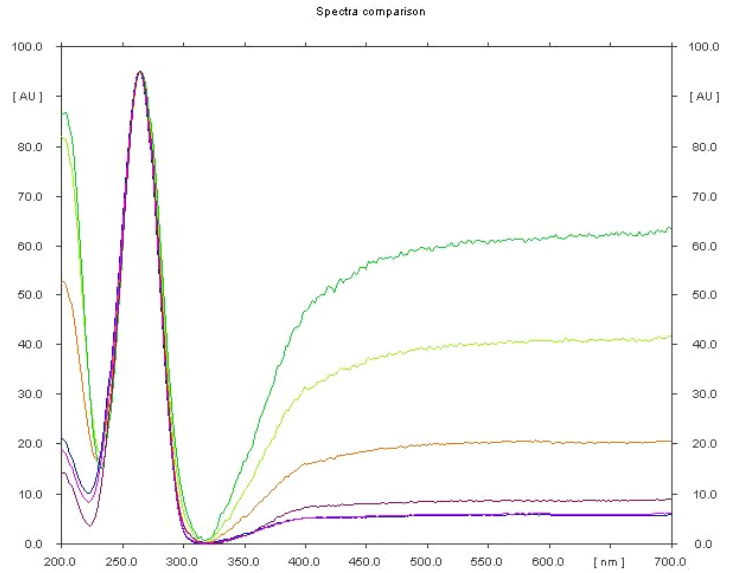


Fig 15. Overlain absorption spectrum of pure 4-Amino pyridine and drug release after degradation of azo of Compd B

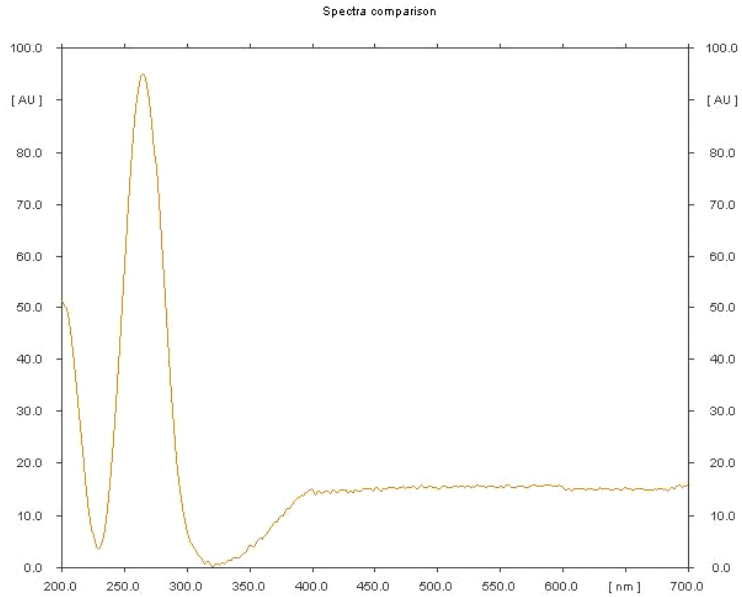


Fig 16: Absorption spectrum degraded azo compound C

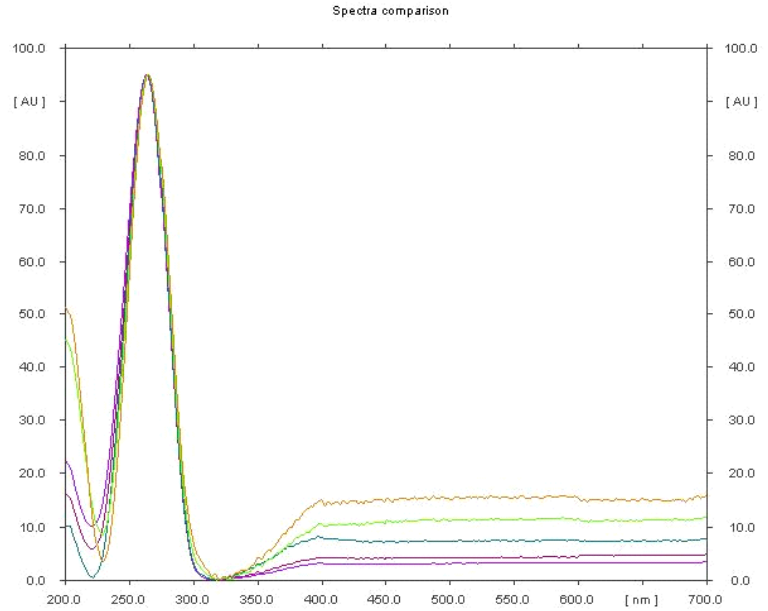


Fig 17. Comparable absorption spectrum of pure 4-Amino pyridine and drug release after degradation of azo of Compd C

The spectral absorption intensity of λ max at 426.8nm for compound A, λ max at 430.8 nm for compound B and λ max at 435.6 nm for compound C respectively which gets decreased as a function of time of inoculation of *Pseudomonas aeruginosa* bacterium species (Supporting Information; Table 5-7). It helps to conclude that the azo linkage (-N=N-) get breaking down as time gradually increases.

Conclusion

In the present research work, synthesis of azo compounds made from 4-amino pyridine and salicyclic acid, thymol, carvacrol is demonstrated. The azo compounds were degraded *in-vitro* by using azoreductase enzyme secreted by *Pseudomonas aeruginosa* bacterial species. The release of parent drug 4-amino pyridine was confirmed by HPTLC and UV-visible technique. It is expected that the combined formulation of 4-amino pyridine with the antibacterial agents salicylic acid, thymol, carvacrol as azo compounds provides a new tool as colon targeting agents as well as for neuromuscular dysfunction of colon. This prodrug approach strategy by using azo compounds can find the new varieties of application in the field of medicinal chemistry and pharmaceutical chemistry especially for colon targeting treatments.

Acknowledgement

We, the authors are very thankful to ISHITA DRUGS & PHARMACEUTICALS, AHMEDABAD, Gujarat, India for supply of good quality of sulfonamide drug compounds. We are also thankful to Sophisticated Analytical Instrumentation Facility, Punjab University, Chandigarh, (Punjab) India for giving the ^{1}H NMR and ^{13}C NMR scanning facility.
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

i Wu Z Z, Li D P, Chen S R, Pan H L; Aminopyridines potentiate synaptic and neuromuscular transmission by targeting the voltage-activated calcium channel beta subunit;*The Journal of biological chemistry;* 2009, **284(52)**, 36453.

ii Van Dieman H. A, Polman C H, Koetsier J C, Van Loenen A C, Nauta J J, Bertelsmann F W; 4-Aminopyridine in patients with multiple sclerosis: dosage and serum level related to efficacy and safety; *Clinical Neuropharmacology*, *1993*, **16 (3)**,195.

iii Jensen H B, Ravnborg M, Dalgas U, Stenager E; 4-Aminopyridine for symptomatic treatment of multiple sclerosis: A systematic review; *Therapeutic Advances in Neurological Disorders;* 2014, **7(2)**, 97.

iv Lohle M, Schrempf W, Wolz M, Reichmann H, Storch A: Potassium channel blocker 4-aminopyridine is effective in interictal cerebellar symptoms in episodic ataxia type 2—a video case report;*Movement Disorders*, 2008, **23 (9)**, 1314.

v Shaikh A G, Marti S, Tarnutzer A A, Palla A, Crawford T O, Zee D S, Straumann D; Effects of 4- aminopyridine on nystagmus and vestibuloocular reglex in ataxia-talengiectasia; *Journal of Neurology***,** 2013, **260 (11)**, 2728.

vi Franciosi S, Ryu J K, Choi H B, Radov L, Kim S U, McLarnori J G, *The Journal of Neurosciences***,** 2006, **26 (45)**, 11652.

vii Wesseling H, Agoston S; Effect of 4-amino pyridine in elderly patients with Alzheimer's disease; *The new England Journal of Medicine*; 1984, **310**, 988.

viii Garjani A, Davaran S, Rashidi M, Maleki N; Protective effects of some azo derivatives of 5- amino salicylic acid and their pegylated prodrugs on acetic –acid-induced rat colitis; *DARU: Journal of Pharmaceutical sciences;* 2003, **12 (1)**, 24.

ix Kaiser G C, Yan F, Polk DD B; Mesalamine blocks tumor necrosis factor growth inhibition and nuclear factor kappaB activation in mouse colonocytes; *Gastroenterology*, 1999, **116**, 602.

x EShron P, Ligumsky M, Rachmilewitz D, Zor U; *Gastroenterology;*1978, **75**, 638.

xi Hanumantharao K, LakshmanaRao A, Chandrasekhar KB, *International Journal of Research in Pharmacy and Chemistry*; 2013, **3 (2)**, 472.

xii Dorman H J D, Deans S G; Antimicrobial agents from plants: antibacterial activity of plant volatile oils*; Journal of Applied Microbiology;* 2000, **88(2)**, 308.

xiii Zarrini G, Bahari-Delgosha Z, Mollazadeh-Moghaddam K, Shahverdi A R; Antibacterial effect of thymol; *Pharmaceutical biology*; 2010, **48 (6)**, 633.

xiv Ferreira J N;*American Chemical society, Langmuir*; 2016, **32(13)**, 3234.

xv Carayon J, Tene N, Bonnfe E, Alayrargues J, Hoteir L, Armengaud C, Treilhou M; *Environmental Science and Pollution research***;** 2014, **21 (7)**, 4934.

xvi Hotta M, Nakata R, Katuskawa M, Horik, Takahashi S, Inoue H, *Journal of Lipid Research***;** 2010, **51 (1)**, 132.

xvii Lu Y, Wu C; Reduction of Salmonella enteric contamination on grape tomatoes by washing with thyme oil, thymol and carvacrol as compared with chlorine treatment; *Journal of food Protection*; 2010, **73(12)**, 2270.

xviii Baser K H; Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils; *Current pharmaceutical Design*; 2008, **14(29)**, 3106.

xix Suntres Z E, Coccimiglio J, Alipour M; The bioactivity and toxicological actions of carvacrol; *Critical Reviews in Food Science Nutrition*;2015, **55(3)**, 304.

xx Montellano P R O; Cytochrome P_{450}- activated prodrugs; *Future medicinal Chemistry*, 2013, **5(2)**, 213.

xxi Huttanen K M, Raunio H, Rautio J; Prodrugs—from serendipity to rational design; *Pharmacological Reviews*, 2011, **63 (3)**, 750.

xxii Tomczak E W, Gorecki L; Azo-dyes biological activity and synthetic strategy; *Chemik Science-Technique- Market;* 2012, **66 (12)**, 1298.

xxiii Sahoo J, Paidesetty S; Medicinal interest of azo-based organic compounds: A Review; *Asian Journal of Pharmaceutical and Clinical Research*; 2016, **9 (1)**, 33.

xxiv Koshti S M, Sonar J P, Sonawane A E, Pawar Y A, Nagle P S, Mahulikar P P, More D H; Synthesis and characterization of azo compounds containing thymol moiety;*Indian Journal of Chemistry***;** 2008,**47(B)**, 329.

xxv Swain S S, Paidesetty S K, Padhy R N; Antibacterial, antifungal and antimycobacterial compounds from cyanobacteria; *Biomedicine & Pharmacotherapy*; 2017, **88**, 181.

xxvi Vijaya P P, Aishwaryalakshmi R, Yogananth N, Ali M, Isolation, Purification and characterization of Oxygen Insensitive Azoreductase from Pseudomonas Aeruginosa and Bio- Degradation of Azo Dye -Methyl Red; *Journal of Advanced Laboratory Research in Biology*; 2012, **3(4)**, 285.

xxvii S. Koshti; Synthesis, Antimicrobial, Anthelmintic activity, Enzymatic degradation and Molecular docking studies of some azo compounds containing carvacrol moiety; *Asian journal of chemistry*; 2022, **34(12),** 3206.

Received on May 24, 2024.